Modular Design of Synthetic Protein Mimics. Crystal Structures, Assembly, and Hydration of Two 15- and 16-Residue Apolar, Leucyl-Rich Helical Peptides

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Abstract: Two 15- and 16-residue peptides containing α-aminoisobutyric acid (Aib) have been synthesized, as part of a strategy to construct stereochemically rigid peptide helices, in a modular approach to design of protein mimics. The peptides Boc-(Val-Ala-Leu-Aib)$_4$-OMe (I) and Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe (II) have been crystallized. Both crystals are stable only in the presence of mother liquor or water. The crystal data are as follows. I: $C_{78}H_{140}N_{16}O_{19}\cdot 2H_2O$, $P2_1$, a = 16.391 (3) Å, b = 16.860 (3) Å, c = 18.428 (3) Å, β = 103.02 (1)°, Z = 2, R = 9.6% for 3445 data with $|F_o| > 3\sigma(F)$, resolution 0.93 Å. II: $C_{74}H_{133}N_{15}O_{18}\cdot 7.5H_2O$, $C222_1$, a = 18.348 (5) Å, b = 47.382 (11) Å, c = 24.157 (5) Å, Z = 8, R = 10.6% for 3147 data with $|F_o| > 3\sigma(F)$, resolution 1.00 Å. The 15-residue peptide (II) is entirely α helical, while the 16-residue peptide (I) has a short segment of 3_{10} helix at the N terminus. The packing of the helices in the crystals is rather inefficient with no particular attractions between Leu-Leu side chains, or any other pair. Both crystals have fairly large voids, which are filled with water molecules in a disordered fashion. Water molecule sites near the polar head-to-tail regions are well determined, those closer to the hydrophobic side chains less so and a number of possible water sites in the remaining "empty" space are not determined. No interdigitation of Leu side chains is observed in the crystal as is hypothesized in the "leucine zipper" class of DNA binding proteins.

Several distinct approaches are being considered for the design and construction of synthetic polypeptides, which mimic the folding motifs observed in proteins.[1] In the first approach the 20 genetically coded amino acids are used and a choice of sequence is based on a knowledge of the propensities of specific sequences to fold in a predictable manner.[2] This method has been used most recently to construct synthetic four-helix motifs,[3] which have been characterized by circular dichroism and hydrodynamic properties in solution.[3a,b,c] The use of covalently linked templates for organizing the spatial arrangement of synthetic peptides has also been explored.[4] A third approach, which is being developed in these laboratories, is also a modular approach,[5] based on the ability of specific non-protein amino acids to direct polypeptide chain folding, by virtue of their unique stereochemical properties. In particular, the use of α-aminoisobutyric acid (Aib)[6,7] to construct stereochemically rigid helices is being systematically investigated.[8] The scheme illustrated in Figure 1 envisages modular assembly of conformationally rigid helices into super-secondary structures like the α,α motif by means of intervening, flexible linker sequences. This approach may be divided into three distinct steps: (i) the synthetic construction and unambiguous structural characterization of the helical modules; (ii) the design and synthesis of appropriate linker peptides, a problem that is being approached by a computer-based analysis of linker sequences occurring in α,α motifs identified in crystalline proteins; (iii) the covalent assembly of the modules and the linker peptides, and introduction of features to promote specific helix orientations.

In this report we describe the characterization in crystals of two apolar helical peptides of 15 and 16 residues in length:

Boc-(Val-Ala-Leu-Aib)$_4$-OMe (I)

Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe (II)

The sequences have been chosen to maintain a relatively low Aib content (I, 25%; II, 20%) and to be completely apolar to facilitate studies in organic solvents, in order to avoid the influence of hydrophobic interactions on the folding process, at this stage of synthetic design. The distribution of Leu residues in II is reminiscent of the arrangements observed in the dimer-forming leucine zipper region of a class of DNA binding proteins.[9] The pairs Leu^3/Leu^{10} and Leu^7/Leu^{14} are seven residues apart in the sequence in II. In the leucine zipper proteins such heptad repeats occur in succession four or five times and the stabilization of dimers is expected to be due to interdigitation of leucine side chains in a helical coiled-coil motif.[9,10] Peptides I and II provide an opportunity to examine attractive interactions between leucyl side chains in helical peptides. The crystal structures of I and II demonstrate stable helical conformations over the entire length of the molecules. No interdigitation or particular association between Leu side chains has been observed. Large voids occur

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(1) DeGrado, W. F. *Adv. Protein Chem.* **1988**, *39*, 51-124.
(2) (a) DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, *243*, 622-628. (b) Richardson, J. S.; Richardson, D. C. In *Protein Engineering*; Oxender, D. L., Fox, C. F., Eds.; Alan R. Liss Inc.: New York, 1987; pp 149-163. (c) Urry, D. W. *Res. Dev.* **1988**, *30*, 72-76. (d) Moser, R.; Thomas, R. M.; Gutte, B. *FEBS Lett.* **1983**, *157*, 247-251. (e) Moser, R.; Frey, S.; Munger, K.; Hehlgans, T.; Klauser, S.; Langen, H.; Winnacker, E.; Metz, R.; Gutte, B. *Protein Eng.* **1987**, *1*, 339-343.
(3) (a) Regan, L.; DeGrado, W. F. *Science* **1988**, *241*, 976-978. (b) Ho, S. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1987**, *109*, 6751-6758. (c) DeGrado, W. F.; Regan, L.; Ho, S. P. *Cold Spring Harbor Symp. Quant. Biol.* **1987**, *52*, 521-526. (d) Hecht, M. H.; Richardson, D. C.; Richardson, J. S.; Ogden, R. *J. Cell. Biochem.* **1989**, *13A*, 86. (e) Kaumaya, P. T. P.; Berndt, K. D.; Heidorn, D. B.; Trewhella, J.; Kezdy, F. J.; Goldberg, E. *Biochemistry* **1990**, *29*, 13-23.
(4) Mutter, M.; Vuilleumier, S. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 535-554.
(5) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Biochemistry* **1989**, *28*, 6696-6701.
(6) (a) Abbreviations used: Aib, α-aminoisobutyric acid; Boc, (*tert*-butyloxy)carbonyl. (b) For definitions of torsional angles, see: IUPAC-IUB Commission on Biochemical Nomenclature. *Biochemistry* **1970**, *9*, 3471-3479. (c) All chiral amino acids are of the L configuration.
(7) (a) Prasad, B. V. V.; Balaram, P. *CRC Crit. Rev. Biochem.* **1984**, *16*, 307-347. (b) Toniolo, C.; Benedetti, E. *ISI Atlas Sci.: Biochem.* **1988**, *1*, 225-230.
(8) (a) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Biopolymers* **1990**, *29*, 1835-1845. (b) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Proteins: Struct. Funct. Genet.* **1990**, *7*, 62-73. (c) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, H.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 765-769.
(9) (a) Landschulz, W. H.; Johnson, P. F.; McKnight, S. L. *Science* **1988**, *240*, 1759-1764. (b) O'Shea, E. K.; Rutkowski, R.; Kim, P. S. *Science* **1989**, *243*, 538-542. (c) Kouzarides, T.; Ziff, E. *Nature* **1988**, *336*, 646-651.
(10) Vinson, C. R.; Sigler, P. B.; McKnight, S. L. *Science* **1989**, *246*, 911-916.

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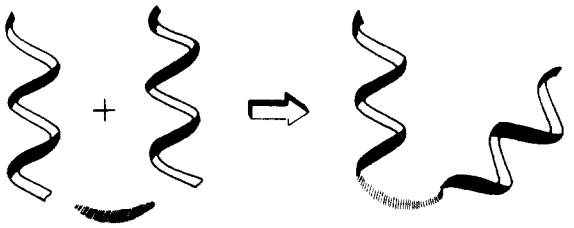

Figure 1. Schematic illustration of the modular or "Meccano set" approach to construction of an α,α motif.

between helices, which are filled by water, providing insights into hydration and the occurrence of water in hydrophobic cavities.

Experimental Procedures

Both peptides I and II were synthesized by conventional solution-phase procedures by a fragment condensation approach using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in dichloromethane or dimethylformamide.[11] The final peptides were purified by high-performance liquid chromatography on a reverse-phase Lichrosorb RP-18 column (8 mm × 250 mm; particle size, 10 μm) using isocratic elution (95% MeOH in H_2O; flow rate, 1.5 mL min^{-1}). Repetitive injections of ~5 mg per run were carried out. Crystals were grown by slow evaporation from a CH_3OH/H_2O solution.

The crystals of both peptides were stable only in contact with mother liquor, and each was sealed in a thin-walled capillary for X-ray data collections. Crystals of peptide II were particularly fragile and had to be handled with extreme care. A crystal of II needed an unusually large amount of mother liquor sealed with it in a capillary to maintain its viability. To prevent the crystal from sliding inside the capillary, the temperature was lowered to 2 °C with a cold nitrogen stream for the data collection.

For each crystal, X-ray diffraction data were measured on an automated four-circle diffractometer with a graphite monochromator. Three reflections used as standards, monitored after every 97 measurements, remained constant to within 3% in the two data sets. Pertinent diffraction data are listed in Table I.

Both structures were solved by a vector search procedure in the Patsee computer program[12] contained in the SHELX84 package of programs (MicroVAX version of SHELXTL system of programs, Siemens Instruments, Madison, WI). The model used for the vector search consisted of 43 backbone atoms from the Boc-Aib-(Val-Ala-Leu-Aib)$_3$-OMe structure.[5] After rotation and translation to the correct position in the cell, the remainder of the atoms in both molecules were found with the partial structure procedure.[13]

Full-matrix, anisotropic least-squares refinement was performed on the C, N, and O atoms after which hydrogen atoms were placed in idealized position, with C–H of 0.96 Å, and allowed to ride with the C or N atom to which each was bonded for the final cycles of refinement. The thermal factor for the hydrogen atoms was fixed at U_{iso} = 0.125. Four water sites, each at 0.5 occupancy were found in crystal I. The sites for W(1A) and W(1B) are mutually exclusive as well as the sites for W(2A) and W(2B). In crystal II, 13 water sites were identified in difference maps and remained stable in the least-squares refinement, 3 sites with full occupancy and 10 sites with occupancies 0.4–0.6. Among the 10 sites with partial occupancy, only 5 are occupied in any one asymmetric unit because of mutual exclusivity. There may be additional disordered sites present in a few remaining voids in the cell of crystal II, but this cannot be substantiated because of weak and diffuse electron density distributions in the difference maps. Fractional coordinates for peptides I and II are listed in Tables II and III, respectively. Conformational angles are listed in Table IV.

Results

The Helix. Peptides I (16 residues) and II (15 residues) differ only by the presence of Aib[8] in I and its removal in II. The stereodiagrams in Figure 2a and b show the helical structures of both peptides. In I, where the Leu residue occurs in every fourth position, the side chains of Leu[7], Leu[11], and Leu[15] are extended to the left side of Leu[3] of the helix whereas the side chain of Leu[3] (near the top) projects forward, as viewed in Figure 2a. The removal of Aib[8] from I, to yield peptide II, results in the extension to the left of the side chains of all four Leu residues, i.e., Leu[3], Leu[7], Leu[10], and Leu[14] (Figure 2b). Furthermore, the side chains of Val[1], Val[5], Val[8], and Val[12] are all extended to the right side of the helix.

Table I. Diffraction Data for Boc-(Val-Ala-Leu-Aib)$_4$-OMe·2H_2O (I) and Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe·7.5H_2O (II)

	I	II
empirical formula	$C_{78}H_{140}N_{16}O_{19}\cdot 2H_2O$	$C_{74}H_{133}N_{15}O_{18}\cdot 7.5H_2O$
color/habit	colorless/fragment	colorless/plate
crystal size, mm	0.25 × 0.50 × 0.15	0.65 × 0.30 × 0.15
space group	$P2_1$	$C222_1$
cell parameters		
a, Å	16.391 (3)	18.348 (5)
b, Å	16.860 (3)	47.382 (11)
c, Å	18.428 (3)	24.157 (5)
β, deg	103.02 (1)	
vol, Å^3	4962.4 (1.4)	21001 (9)
Z	2	8
mol wt	1606.09 + 36.03	1520.98 + 135.12
density, g/cm	1.099	1.048
$F(000)$	1784	7208
radiation	Cu Kα	Cu Kα, λ = 1.5418 Å
temp, °C	ambient	2
mounted in capillary	yes	yes
2θ range, deg	1–112	1–100
resolution, Å	0.93	1.00
scan type	θ–2θ	θ–2θ
scan speed, deg/min	7–13 (variable)	29°
index ranges		
h	−18 to 18	0–19
k	0–19	0–48
l	0–20	0–24
independent reflcns	6762	5909
obsd reflcns [$\|F_o\| > 3\sigma(F)$]	3445	3147
final R indices (obsd data), %	R 9.6	R 10.6
	R_w 9.2	R_w 10.7
goodness of fit	1.5	12.2
data to parameter ratio	7.2:1	6.7:1
largest diff ratio, e Å^{-3}	0.29	0.36
largest diff hole, e Å^{-3}	−0.33	−0.31

The helical structure of the backbone is quite similar in the two peptides. A least-squares fit of backbone atoms N(3)–O(14) in I with N(2)–O(13) of II yields a root mean square deviation of 0.28 Å. Peptide II is completely α-helical with 12 5→1 hydrogen bonds (Table V), while peptide I has 11 5→1 hydrogen bonds and 2 4→1 hydrogen bonds (3_{10} helix) near the N terminus (Table V). A partial transition from α to 3_{10} helix, particularly at the N terminus, is not unusual, even for the same peptide in different polymorphs[16] or for multiple independent molecules in the same cell.[8c] The conformations obtained earlier[8b] for the hepta- and octapeptide fragments, Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe and Boc-(Val-Ala-Leu-Aib)$_2$-OMe, are largely similar to that observed for the corresponding segments in the longer peptides I and II.

Head-to-Tail Hydrogen Bonding. Apolar helical peptides utilize head-to-tail hydrogen bonding to form continuous columns.[5,8,14-16,18] Crystal structure information for helical peptides with polar side chains is not extensive; therefore, a general statement about head-to-tail hydrogen bonding cannot be made at this time. For the apolar helices, the head-to-tail hydrogen bonding motifs vary from three direct NH···O=C bonds between the first three NH moieties in one helix and the last three C=O moieties from another helix with the two helices in perfect register with each other[8c] to hydrogen bonding where the NH and O=C moieties from the separate helices are mediated by water or alcohol

(11) Balaram, H.; Sukumar, M.; Balaram, P. *Biopolymers* **1986**, *25*, 2209–2223.
(12) Egert, E.; Sheldrick, G. M. *Acta Crystallogr.* **1985**, *A41*, 262–268.
(13) Karle, J. *Acta Crystallogr.* **1968**, *B24*, 182–186.
(14) Karle, I. L.; Flippen-Anderson, J. L.; Sukumar, M.; Balaram, P. *Int. J. Pept. Protein Res.* **1988**, *31*, 567–576.
(15) Karle, I. L.; Flippen-Anderson, J. L.; Sukumar, M.; Balaram, P. *Int. J. Pept. Protein Res.* **1990**, *35*, 518–526.
(16) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Biopolymers*, in press.

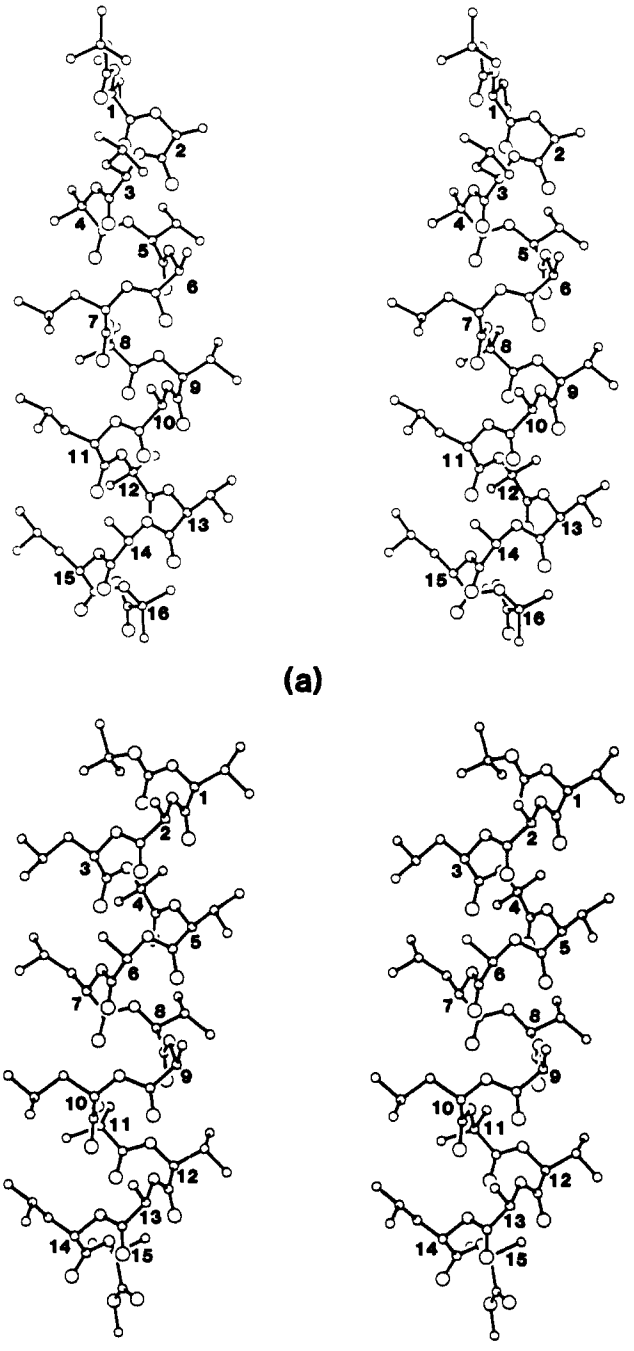

Figure 2. (a) Stereodiagram of Boc-(Val-Ala-Leu-Aib)$_4$-OMe (peptide I). (b) Stereodiagram of Boc-(Val-Ala-Leu-Aib)-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe (peptide II). In each diagram, the C^α atoms are numbered. The helices are oriented so that the Leu side chains are extended to the left and the Val side chains are extended to the right.

molecules.[15] The latter motif is present to an extreme in peptide II.

The stereodiagram in Figure 3 depicts the lower portion of one helical molecule II placed over an upper portion of another helical molecule II with numerous water molecule sites (labeled W) near the head-to-tail region. The N(1)H···O(13) hydrogen bond is the only direct hydrogen bond between the two separate helices. The N(2)H moiety forms a hydrogen bond with W(2), which in turn participates as a donor in hydrogen bonds with O(13) and O(15) of the upper peptide and as a receptor in a hydrogen bond with W(1). The N(3)H moiety forms a hydrogen bond with W(1), which in turn is a donor to W(2) and W(8A). Water molecule W(8A) forms another hydrogen bond with W(5A) which, in succession, is a donor to a hydrogen bond with O(12) of the upper

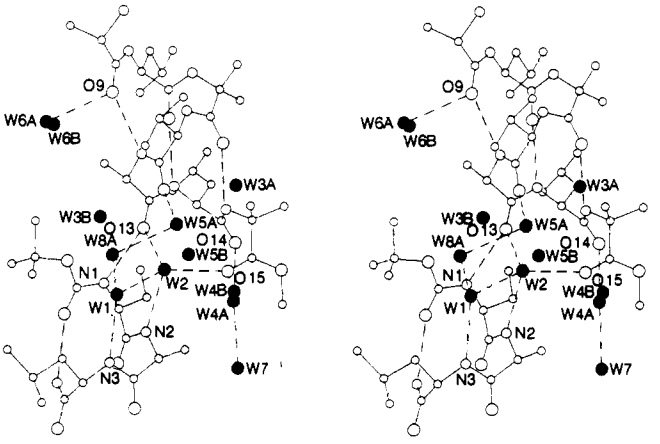

Figure 3. Stereodiagram depicting the head-to-tail region of peptide II with 12 of the water sites shown as black dots with W(n) labels. Only the bottom portion of the top peptide helix and the top portion of the bottom peptide helix are shown. Hydrogen bonds are indicated by dashed lines. Water molecules W(nA) and W(nB) occur only at ~50% occupancy.

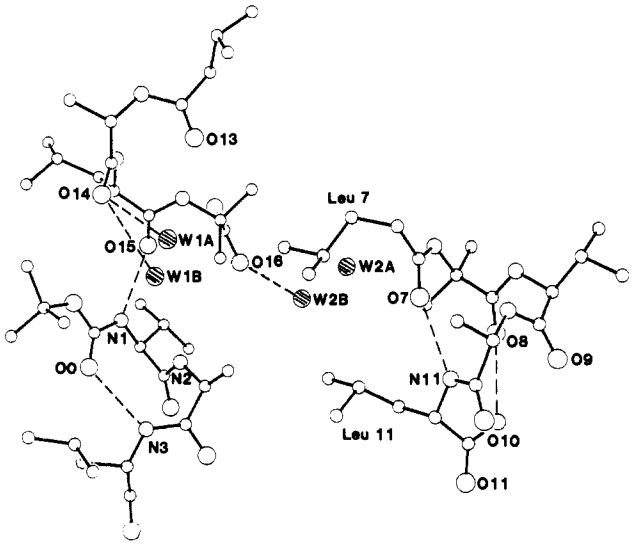

Figure 4. Water molecules [W(n)] in the head-to-tail region of peptide I. Fragments of three neighboring peptide molecules are shown. Hydrogen bonds are indicated by dashed lines. Each of the water sites has ~50% occupancy.

peptide. Water molecules W(1), W(2), and W(7) [where W(7) lies on a symmetry element] occur at full occupancy, whereas the remaining water sites are occupied only half the time. Those water sites labeled with the same number, such as W(6A) and W(6B), are mutually exclusive; that is, if one site is occupied, the other site must be empty. Some of the water molecules, especially W(3A) and W(3B), occur in voids between the peptides and are not close enough for any hydrogen bonding. There undoubtedly are other water sites that are occupied to a lesser extent but could not be definitely substantiated in our experiment.

In the crystal of peptide I, the water structure is much simpler, as shown in Figure 4, where fragments of three surrounding peptide molecules are shown. Water sites W(1A) and W(1B), each with ~50% occupancy on the average throughout the crystal, have full occupancy for one or the other with one site unoccupied in any particular region of the crystal lattice. The same pertains to water sites W(2A) and W(2B). There is only one direct hydrogen bond, head-to-tail, between peptide molecules, N(1)H···O(15). The N(2)H moiety does not participate in any hydrogen bonding and N(3)H forms an internal N(3)H···O(0) bond of the 3_{10} helix type. W(1A) or W(1B) is a donor in a hydrogen bond with O(14), and W(2B) is a donor to a hydrogen bond with O(16). W(2A) is at least 3.9 Å from any other C or O atom. Obviously, the underutilized hydrogen-bonding potential of the water mol-

Table II. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$)

	x	y	z	U(eq)[a]		x	y	z	U(eq)[a]
C(1)	4260 (8)	5986 (8)	1165 (7)	74 (1)	N(9)	1498 (6)	3411 (6)	7884 (6)	67 (1)
C(2)	3328 (7)	6156 (8)	926 (8)	96 (1)	$C^{\alpha}(9)$	1787 (7)	3579 (8)	8690 (7)	57 (1)
C(3)	4718 (8)	6277 (8)	590 (7)	89 (1)	C'(9)	1310 (7)	4265 (7)	8955 (7)	50 (1)
C(4)	4614 (9)	6413 (8)	1897 (8)	128 (1)	O(9)	1092 (5)	4220 (6)	9532 (5)	73 (1)
O	4438 (5)	5145 (5)	1246 (5)	54 (1)	$C^{\beta}(9)$	2721 (7)	3729 (8)	8837 (7)	66 (1)
C'(0)	4130 (7)	4680 (8)	1711 (6)	47 (1)	$C^{\gamma 1}(9)$	3212 (8)	3017 (9)	8684 (1)	94 (1)
O(0)	3629 (5)	4918 (6)	2076 (5)	75 (1)	$C^{\gamma 2}(9)$	3078 (8)	3933 (9)	9666 (7)	103 (1)
N(1)	4448 (6)	3945 (6)	1746 (6)	53 (1)	N(10)	1210 (6)	4889 (6)	8511 (6)	56 (1)
$C^{\alpha}(1)$	4103 (8)	3336 (8)	2112 (7)	75 (1)	$C^{\alpha}(10)$	780 (8)	5588 (7)	8729 (7)	57 (1)
C'(1)	4177 (8)	3508 (8)	2941 (8)	73 (1)	C'(10)	−120 (7)	5384 (7)	8824 (7)	47 (1)
O(1)	3686 (6)	3260 (6)	3298 (6)	114 (1)	O(10)	−373 (6)	5661 (6)	9326 (5)	72 (1)
$C^{\beta}(1)$	4540 (8)	2575 (8)	1989 (8)	94 (1)	$C^{\beta}(10)$	721 (8)	6246 (9)	8205 (8)	115 (1)
$C^{\gamma 1}(1)$	4295 (10)	1853 (9)	2438 (9)	174 (1)	N(11)	−550 (6)	4924 (6)	8278 (5)	43 (1)
$C^{\gamma 2}(1)$	4333 (9)	2341 (9)	1213 (9)	164 (1)	$C^{\alpha}(11)$	−1383 (7)	4716 (8)	8340 (5)	55 (1)
N(2)	4861 (6)	3913 (7)	3267 (5)	86 (1)	C'(11)	−1434 (7)	4124 (7)	8950 (6)	41 (1)
$C^{\alpha}(2)$	5034 (9)	4119 (10)	4039 (7)	130 (1)	O(11)	−1975 (5)	4229 (6)	9297 (5)	67 (1)
C'(2)	4307 (8)	4505 (10)	4231 (7)	98 (1)	$C^{\beta}(11)$	−1910 (8)	4398 (8)	7591 (7)	71 (1)
O(2)	4194 (6)	4432 (7)	4883 (5)	112 (1)	$C^{\gamma}(11)$	−2070 (8)	4954 (9)	6938 (8)	101 (1)
$C^{\beta}(2)$	5763 (9)	4338 (13)	4378 (8)	325 (1)	$C^{\delta 1}(11)$	−2538 (10)	5660 (10)	7060 (8)	169 (1)
N(3)	3775 (7)	4988 (7)	3768 (5)	80 (1)	$C^{\delta 2}(11)$	−2577 (10)	4555 (9)	6236 (7)	140 (1)
$C^{\alpha}(3)$	3103 (7)	5388 (8)	3966 (7)	68 (1)	N(12)	−857 (6)	3573 (6)	9073 (5)	47 (1)
C'(3)	2420 (7)	4832 (9)	4076 (6)	61 (1)	$C^{\alpha}(12)$	−787 (7)	3011 (8)	9646 (7)	58 (1)
O(3)	2027 (5)	5012 (6)	4535 (4)	74 (1)	C'(12)	−726 (7)	3484 (9)	10369 (8)	73 (1)
$C^{\beta}(3)$	2740 (8)	6079 (8)	3426 (6)	71 (1)	O(12)	−1117 (5)	3333 (6)	10881 (5)	74 (1)
$C^{\gamma}(3)$	3266 (8)	6791 (9)	3436 (8)	98 (1)	$C^{\beta 1}(12)$	−6 (7)	2503 (8)	9756 (7)	73 (1)
$C^{\delta 1}(3)$	3430 (11)	7181 (10)	4179 (9)	195 (1)	$C^{\beta 2}(12)$	−1550 (7)	2468 (8)	9535 (7)	76 (1)
$C^{\delta 2}(3)$	2863 (10)	7343 (9)	2818 (9)	166 (1)	N(13)	−180 (5)	4093 (6)	10508 (5)	46 (1)
N(4)	2290 (6)	4191 (7)	3661 (5)	66 (1)	$C^{\alpha}(13)$	−48 (6)	4577 (7)	11194 (7)	54 (1)
$C^{\alpha}(4)$	1697 (9)	3623 (9)	3734 (7)	89 (1)	C'(13)	−811 (9)	5081 (8)	11248 (8)	79 (1)
C'(4)	1878 (9)	3324 (8)	4569 (8)	79 (1)	O(13)	−1088 (6)	5103 (7)	11814 (5)	98 (1)
O(4)	1312 (6)	3208 (6)	4926 (5)	98 (1)	$C^{\beta}(13)$	750 (8)	5104 (9)	11228 (7)	93 (1)
$C^{\beta 1}(4)$	1733 (9)	2900 (8)	3238 (7)	99 (1)	$C^{\gamma 1}(13)$	1500 (7)	4579 (9)	11372 (7)	106 (1)
$C^{\beta 2}(4)$	787 (8)	3953 (8)	3548 (7)	102 (1)	$C^{\gamma 2}(13)$	837 (8)	5685 (10)	11869 (7)	126 (1)
N(5)	2662 (7)	3155 (7)	4843 (5)	81 (1)	N(14)	−1178 (6)	5472 (6)	10598 (6)	56 (1)
$C^{\alpha}(5)$	3033 (9)	2837 (8)	5609 (7)	89 (1)	$C^{\alpha}(14)$	−1886 (8)	5985 (8)	10581 (8)	76 (1)
C'(5)	2939 (8)	3444 (9)	6201 (8)	82 (1)	C'(14)	−2664 (8)	5529 (9)	10754 (8)	76 (1)
O(5)	2638 (7)	3303 (7)	6733 (5)	123 (1)	O(14)	−3151 (6)	5860 (7)	11055 (6)	119 (1)
$C^{\beta}(5)$	3904 (10)	2539 (9)	5725 (8)	136 (1)	$C^{\beta}(14)$	−2080 (8)	6438 (8)	9863 (8)	95 (1)
$C^{\gamma 1}(5)$	3853 (10)	1867 (10)	5177 (9)	191 (1)	N(15)	−2809 (6)	4813 (7)	10429 (6)	68 (1)
$C^{\gamma 2}(5)$	4280 (9)	2296 (11)	6477 (8)	209 (1)	$C^{\alpha}(5)$	−3524 (7)	4339 (7)	10461 (8)	65 (1)
N(6)	3188 (7)	4184 (8)	6068 (5)	83 (1)	C'(15)	−3475 (9)	3851 (8)	11145 (8)	84 (1)
$C^{\alpha}(6)$	3116 (8)	4809 (9)	6603 (8)	113 (1)	O(15)	−4079 (6)	3538 (6)	11288 (6)	99 (1)
C'(6)	2215 (8)	4943 (8)	6604 (8)	77 (1)	$C^{\beta}(15)$	−3778 (8)	3844 (8)	9765 (9)	109 (1)
O(6)	2081 (5)	5108 (7)	7258 (5)	93 (1)	$C^{\gamma}(15)$	−4040 (8)	4334 (8)	9014 (8)	86 (1)
$C^{\beta}(6)$	3494 (9)	5489 (9)	6382 (8)	145 (1)	$C^{\delta 1}(15)$	−4762 (9)	4869 (9)	8959 (8)	124 (1)
N(7)	1606 (7)	4941 (8)	5998 (6)	94 (1)	$C^{\delta 2}(15)$	−4182 (9)	3785 (9)	8344 (8)	129 (1)
$C^{\alpha}(7)$	767 (8)	4984 (8)	6059 (7)	79 (1)	N(16)	−2667 (7)	3778 (7)	11575 (6)	84 (1)
C'(7)	465 (8)	4374 (8)	6500 (7)	81 (1)	$C^{\alpha}(16)$	−2498 (10)	3347 (10)	12307 (9)	118 (1)
O(7)	5 (6)	4579 (6)	6945 (5)	88 (1)	C'(16)	−2882 (10)	2549 (11)	12140 (10)	132 (1)
$C^{\beta}(7)$	223 (7)	5062 (10)	5264 (7)	106 (1)	O(16)	−3221 (8)	2240 (9)	12646 (7)	171 (1)
$C^{\gamma}(7)$	−702 (11)	5165 (10)	5191 (8)	184 (1)	$C^{\beta 1}(16)$	−1554 (9)	3207 (10)	12519 (8)	142 (1)
$C^{\delta 1}(7)$	−1245 (10)	5015 (11)	4408 (8)	193 (1)	$C^{\beta 2}(16)$	−2905 (10)	3797 (10)	12881 (9)	179 (1)
$C^{\delta 2}(7)$	−900 (10)	5989 (11)	5429 (9)	212 (1)	O(17)	−2825 (7)	2166 (7)	11546 (7)	121 (1)
N(8)	676 (7)	3609 (7)	6402 (6)	91 (1)	C(17)	−3237 (10)	1373 (10)	11413 (9)	154 (1)
$C^{\alpha}(8)$	438 (8)	2987 (8)	6856 (8)	79 (1)	W(1A)[b]	2651 (13)	973 (12)	7268 (11)	199 (1)
C'(8)	705 (8)	3136 (7)	7695 (7)	57 (1)	W(1B)[b]	3626 (12)	407 (12)	7402 (10)	199 (1)
O(8)	213 (5)	3022 (6)	8069 (5)	81 (1)	W(2A)[b]	8491 (12)	2007 (12)	4573 (11)	177 (1)
$C^{\beta 1}(8)$	889 (9)	2258 (9)	6619 (8)	111 (1)	W(2B)[b]	7400 (12)	2491 (12)	4271 (11)	177 (1)
$C^{\beta 2}(8)$	−525 (8)	2824 (9)	6596 (7)	102 (1)					

[a] Equivalent isotropic U defined as one-third of the trace of orghogonalized U_{ij} tensor. [b] Occupancy ~0.5.

ecules, as well as the free N(2)H moiety, indicates that additional water sites with low occupancy can be present in the head-to-tail region.

Helix Aggregation. Completely parallel packing of apolar peptides with 8–16 residues has been established for a number of peptides.[16-19] Some of the peptides that aggregate in a parallel fashion in one polymorph aggregate also in an antiparallel fashion in a polymorph grown from a different solvent.[8a,15] The common motif in the aggregation of apolar helical peptides in crystals is the formation of continuous columns by head-to-tail hydrogen bonding and the assembly of the columns into sheets with *all* helix axes in the *parallel* mode. The differences in packing occur in the mode of assembly of the sheets: parallel, antiparallel, or skewed.[8a,15-17] In crystal I (space group $P2_1$), the sheets of parallel helices are related by the 2-fold screw axis perpendicular to the helix axes, resulting in an antiparallel assembly as shown in Figure 5. In Figure 6 (space group $C222_1$), a sheet of parallel helices with all N termini at the top is shown for crystal II. The sheets of helices above and below the one shown are rotated by 180°, thus making an antiparallel arrangement in the direction into the page.

(17) Terwilliger, T. C.; Eisenberg, D. *J. Biol. Chem.* **1982**, *257*, 6016–6022.
(18) Karle, I. L.; Flippen-Anderson, J. L.; Sukumar, M.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5087–5091.
(19) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 299–303.

Table III. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$)

	x	y	z	U(eq)[a]		x	y	z	U(eq)[a]
C(1)	7589 (15)	6012 (7)	1352 (11)	107 (4)	N(9)	8060 (11)	3200 (4)	2109 (8)	72 (4)
C(2)	8168 (17)	5947 (7)	933 (12)	147 (4)	$C^{\alpha}(9)$	7937 (13)	2951 (4)	2483 (10)	58 (4)
C(3)	7406 (22)	6339 (6)	1360 (15)	213 (4)	C′(9)	7362 (13)	2757 (6)	2221 (9)	62 (4)
C(4)	6895 (19)	5872 (8)	1309 (15)	204 (4)	O(9)	7346 (12)	2508 (3)	2283 (7)	100 (4)
O	7902 (10)	5980 (3)	1927 (7)	92 (4)	$C^{\beta}(9)$	7720 (18)	3082 (5)	3033 (9)	109 (4)
C′(0)	8104 (16)	5738 (5)	2100 (11)	75 (4)	N(10)	6743 (9)	2889 (4)	1979 (8)	58 (4)
O(0)	8032 (10)	5504 (4)	1878 (7)	88 (4)	$C^{\alpha}(10)$	6203 (13)	2720 (5)	1734 (10)	63 (4)
N(1)	8424 (10)	5772 (3)	2587 (7)	56 (3)	C′(10)	6457 (14)	2553 (6)	1255 (11)	68 (4)
$C^{\alpha}(1)$	8777 (11)	5535 (4)	2869 (8)	47 (4)	O(10)	6362 (7)	2292 (3)	1235 (6)	59 (3)
C′(1)	8370 (12)	5277 (4)	2984 (8)	37 (4)	$C^{\beta}(10)$	5545 (13)	2927 (5)	1556 (11)	76 (4)
O(1)	8512 (7)	5040 (3)	2844 (6)	53 (3)	$C^{\gamma}(10)$	4896 (16)	2751 (7)	1304 (14)	112 (4)
$C^{\beta}(1)$	9259 (13)	5631 (4)	3385 (9)	58 (4)	$C^{\delta 1}(10)$	4505 (13)	2572 (8)	1704 (12)	136 (4)
$C^{\gamma 1}(1)$	9674 (14)	5380 (5)	3626 (11)	101 (4)	$C^{\delta 2}(10)$	4392 (18)	2970 (8)	1088 (16)	177 (4)
$C^{\gamma 2}(1)$	9819 (11)	5880 (5)	3288 (11)	84 (4)	N(11)	6875 (10)	2692 (4)	853 (8)	59 (4)
N(2)	7791 (10)	5347 (3)	3317 (7)	57 (3)	$C^{\alpha}(11)$	7215 (11)	2536 (5)	384 (8)	45 (4)
$C^{\alpha}(2)$	7399 (13)	5117 (5)	3530 (9)	73 (4)	C′(11)	7654 (16)	2286 (5)	569 (11)	77 (4)
C′(2)	7009 (12)	4947 (5)	3081 (9)	51 (4)	O(11)	7562 (9)	2050 (3)	345 (7)	69 (3)
O(2)	6950 (8)	4683 (3)	3094 (6)	60 (3)	$C^{\beta 1}(11)$	7711 (14)	2743 (5)	99 (11)	85 (4)
$C^{\beta}(2)$	6851 (14)	5208 (5)	3957 (10)	99 (4)	$C^{\beta 2}(11)$	6541 (12)	2454 (5)	−25 (11)	67 (4)
N(3)	6702 (9)	5096 (3)	2654 (7)	45 (3)	N(12)	8124 (11)	2328 (4)	1010 (8)	63 (4)
$C^{\alpha}(3)$	6392 (12)	4927 (4)	2190 (9)	57 (4)	$C^{\alpha}(12)$	8581 (14)	2099 (5)	1190 (11)	75 (4)
C′(3)	6891 (12)	4764 (5)	1873 (8)	47 (4)	C′(12)	8177 (13)	1869 (4)	1488 (10)	53 (4)
O(3)	6749 (8)	4512 (3)	1755 (6)	64 (3)	O(12)	8334 (10)	1622 (3)	1412 (7)	83 (4)
$C^{\beta}(3)$	5976 (12)	5146 (4)	1831 (8)	50 (4)	$C^{\beta}(12)$	9234 (18)	2235 (7)	1524 (17)	165 (4)
$C^{\gamma}(3)$	5570 (14)	4999 (6)	1337 (10)	87 (4)	$C^{\gamma 1}(12)$	9699 (18)	2405 (6)	1149 (13)	129 (4)
$C^{\delta 1}(3)$	5323 (17)	5239 (7)	903 (15)	172 (4)	$C^{\gamma 2}(12)$	9595 (25)	1995 (12)	1784 (17)	324 (4)
$C^{\delta 2}(3)$	4956 (14)	4807 (5)	1481 (10)	87 (4)	N(13)	7592 (11)	1950 (4)	1772 (8)	67 (4)
N(4)	7549 (9)	4886 (3)	1751 (7)	50 (3)	$C^{\alpha}(13)$	7150 (15)	1721 (5)	2023 (11)	81 (4)
$C^{\alpha}(4)$	8109 (11)	4726 (5)	1469 (9)	48 (4)	C′(13)	6752 (11)	1532 (5)	1605 (9)	47 (4)
C′(4)	8259 (10)	4458 (4)	1737 (10)	42 (4)	O(13)	6652 (10)	1280 (3)	1705 (6)	72 (3)
O(4)	8282 (7)	4227 (3)	1507 (5)	48 (3)	$C^{\beta}(13)$	6587 (13)	1870 (4)	2404 (10)	67 (4)
$C^{\beta 1}(4)$	8752 (10)	4906 (4)	1463 (9)	55 (4)	N(14)	6517 (9)	1661 (4)	1149 (8)	54 (4)
$C^{\beta 2}(4)$	7891 (14)	4669 (6)	865 (10)	98 (4)	$C^{\alpha}(14)$	6057 (14)	1495 (5)	721 (9)	67 (4)
N(5)	8385 (9)	4473 (3)	2311 (7)	48 (3)	C′(14)	6623 (12)	1349 (5)	310 (10)	50 (4)
$C^{\alpha}(5)$	8551 (13)	4229 (5)	2628 (9)	62 (4)	O(14)	6371 (9)	1147 (3)	43 (7)	80 (4)
C′(5)	7981 (12)	4019 (5)	2629 (9)	54 (4)	$C^{\beta}(14)$	5588 (13)	1684 (6)	434 (10)	75 (4)
O(5)	8083 (9)	3750 (3)	2553 (7)	69 (3)	$C^{\gamma}(14)$	5010 (19)	1814 (7)	737 (16)	152 (4)
$C^{\beta}(5)$	8754 (13)	4323 (5)	3223 (8)	58 (4)	$C^{\delta 1}(14)$	4580 (19)	1631 (8)	1094 (16)	186 (4)
$C^{\gamma 1}(5)$	8781 (17)	4075 (6)	3571 (11)	131 (4)	$C^{\delta 2}(14)$	4588 (15)	2005 (7)	450 (13)	131 (4)
$C^{\gamma 2}(5)$	9486 (13)	4491 (5)	3221 (12)	94 (4)	N(15)	7291 (10)	1451 (4)	277 (7)	53 (4)
N(6)	7272 (10)	4114 (4)	2714 (7)	54 (3)	$C^{\alpha}(15)$	7748 (15)	1356 (6)	−154 (10)	74 (4)
$C^{\alpha}(6)$	6680 (12)	3907 (5)	2710 (8)	54 (4)	$C^{\beta 1}(15)$	8532 (15)	1478 (6)	−67 (13)	111 (4)
C′(6)	6570 (10)	3742 (4)	2194 (7)	33 (4)	C′(15)	7843 (13)	1017 (4)	−140 (12)	65 (4)
O(6)	6414 (8)	3496 (3)	2221 (6)	57 (3)	O(15)	7936 (12)	901 (3)	283 (6)	96 (4)
$C^{\beta}(6)$	5972 (17)	4083 (5)	2874 (11)	130 (4)	$C^{\beta 2}(15)$	7513 (18)	1475 (6)	−730 (13)	132 (4)
N(7)	6661 (10)	3898 (3)	1721 (7)	50 (4)	O(16)	7931 (11)	914 (4)	−650 (6)	85 (4)
$C^{\alpha}(7)$	6585 (13)	3751 (6)	1192 (11)	81 (4)	C(16)	8007 (19)	612 (4)	−662 (11)	118 (4)
C′(7)	7134 (13)	3545 (5)	1055 (10)	62 (4)	W(1)	6310 (14)	5697 (4)	2765 (8)	135 (4)
O(7)	7022 (10)	3313 (3)	859 (7)	77 (4)	W(2)	7240 (10)	5908 (4)	3617 (7)	89 (4)
$C^{\beta}(7)$	6523 (14)	3970 (5)	720 (9)	65 (4)	W(3A)[b]	891 (33)	1644 (13)	346 (26)	187 (7)
$C^{\gamma}(7)$	5830 (13)	4143 (6)	695 (13)	97 (4)	W(3B)[b]	5771 (33)	3248 (13)	−122 (25)	173 (7)
$C^{\delta 1}(7)$	5885 (20)	4367 (6)	221 (10)	137 (4)	W(4A)[b]	869 (24)	4396 (9)	−39 (19)	152 (7)
$C^{\delta 2}(7)$	5144 (13)	3988 (6)	675 (13)	118 (4)	W(5A)[b]	9704 (24)	1310 (10)	1262 (19)	172 (7)
N(8)	7818 (10)	3605 (4)	1237 (7)	58 (4)	W(6A)[b]	1769 (32)	2198 (14)	1686 (26)	216 (7)
$C^{\alpha}(8)$	8390 (14)	3401 (5)	1213 (12)	84 (4)	W(6B)[b]	2289 (33)	2234 (15)	1550 (26)	201 (7)
C′(8)	8300 (12)	3144 (4)	1562 (10)	52 (4)	W(4B)[b]	58 (27)	4318 (10)	27 (20)	128 (7)
O(8)	8392 (3)	2892 (3)	1437 (7)	66 (3)	W(7)[b]	6512 (32)	0 (73)	0 (73)	331 (7)
$C^{\beta}(8)$	9162 (16)	3536 (6)	1292 (11)	90 (4)	W(5B)[b]	9656 (29)	1056 (13)	1057 (23)	136 (7)
$C^{\gamma 1}(8)$	9357 (14)	3744 (6)	832 (15)	141 (4)	W(8A)[b]	157 (60)	1059 (20)	2345 (48)	494 (7)
$C^{\gamma 2}(8)$	9788 (12)	3328 (6)	1345 (14)	118 (4)	W(8B)[b]	879 (49)	1380 (21)	2373 (42)	381 (7)

[a] Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor. [b] Occupancy ~0.5.

The packing of the hydrocarbon side chains in crystals of both peptides (Figures 5 and 6) is quite inefficient with considerable space around them. Nearest approaches between C···C in neighboring molecules are rarely closer than 3.9 Å and generally considerably larger than 4.1 Å. There is no particular attraction between specific types of side chains. The voids between helices are especially apparent in the crystal of peptide II (Figure 6). The large amount of water in peptide II is ordered [W(1) and W(2)] in the head-to-tail region where each water molecule participates in several hydrogen bonds with the NH and C=O moieties of the peptides and becomes less ordered [(W(4), W(5), W(6)] when only one W···O hydrogen bond or a W···W hydrogen bond is formed. Several apparent water sites [W(3A), W(3B), W(8B)] are not within any hydrogen bond distance or van der Waals distance of any other atom, an indication that there probably are other water sites filling the hydrophobic holes.

Discussion

The helical conformations determined for I and II suggest that long stable helices can be observed in peptides with relatively low Aib content. II, in fact, contains a central stretch of six contiguous non-Aib residues. The results underscore the dominant role of Aib residues in nucleating and stabilizing helical folding patterns in peptides. Both helices are straight, cylindrical structures, in contrast to the curved helix observed in a peptide of similar length containing internal Pro residues.[18] The observed structures

Table IV. Torsion Angles[a] (deg)

residue	ϕ	ψ	ω	χ^1	χ^2
		Boc-(Val-Ala-Leu-Aib)$_4$-OMe			
Val(1)	−61.7[b]	−33.0	−179.5	173.7, −66.9	
Ala(2)	−50.9	−34.5	−176.7		
Leu(3)	−68.4	−32.8	177.6	−69.8	−62.4, 172.9
Aib(4)	−55.5	−46.7	−178.2		
Val(5)	−64.3	−48.8	179.6	−61.5, 175.1	
Ala(6)	−64.5	−39.8	172.9		
Leu(7)	−57.2	−44.4	176.2	−176.6	−164.1, 73.1
Aib(8)	−53.1	−43.7	179.0		
Val(9)	−66.0	−45.1	−178.3	−63.4, 178.5	
Ala(10)	−58.2	−44.0	179.8		
Leu(11)	−71.8	−35.4	176.1	−62.3	−61.4, 178.6
Aib(12)	−54.5	−48.3	−178.2		
Val(13)	−67.1	−44.9	−178.2	−69.4, 172.6	
Ala(14)	−61.3	−41.4	174.2		
Leu(15)	−82.3	−14.9	176.6	−61.2	−60.6, 173.2
Aib(16)	+54.4	+40.7[c]	−179.1[d]		
		Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe			
Val(1)	−55.5[b]	−60.2	−171.6	−174.2, −49.6	
Ala(2)	−65.5	−38.5	173.7		
Leu(3)	−64.8	−40.3	175.0	−176.4	−166.1, 66.5
Aib(4)	−51.9	−50.0	−178.9		
Val(5)	−61.8	−45.8	179.2	168.8, −67.2	
Ala(6)	−60.3	−39.5	−178.2		
Leu(7)	−67.7	−34.0	−169.4	−69.6	176.0, −54.8
Val(8)	−64.5	−45.1	178.4	−63.0, 172.6	
Ala(9)	−58.9	−42.7	180.0		
Leu(10)	−62.4	−46.8	174.1	−177.0	69.0, −172.0
Aib(11)	−51.6	−46.0	−177.4		
Val(12)	−72.5	−30.6	174.3	−66.4, 169.4	
Ala(13)	−67.8	−34.4	−176.5		
Leu(14)	−87.3	−20.0	−169.1	−66.3	−46.7, 176.5
Aib(15)	−57.5	148.2[c]	−179.0[d]		

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, and ω) and about bonds of the amino acid side chains (χ^1 and χ^2) are described in ref 6b. esd's are ~1.5° for peptide I and ~2.5° for peptide II. [b] C′(0), N(1), Cᵅ(1), C′(1). [c] N(*f*), Cᵅ(*f*), C′(*f*), O(OMe) where *f* ≡ final residue. [d] Cᵅ(*f*), C′(*f*), O(OMe), C(OMe) where *f* ≡ final residue.

Table V. Hydrogen Bonds in Peptide I

type	donor	acceptor	N···O, Å	H···O,[a] Å	angle C=O···N, deg
head-to-tail	N(1)	O(15)[b]	2.817	1.87	140
	N(2)				
4→1	N(3)	O(0)	3.076	2.17	131
	N(4)	O(1)	2.973	2.27	125
	N(5)				
5→1	N(6)	O(2)	3.047	2.11	156
	N(7)	O(3)	2.931	1.98	155
	N(8)	O(4)	3.197	2.27	146
	N(9)	O(5)	3.133	2.20	161
	N(10)	O(6)	2.999	2.06	154
	N(11)	O(7)	2.865	1.92	162
	N(12)	O(8)	2.971	2.07	152
	N(13)	O(9)	3.052	2.13	155
	N(14)	O(10)	2.954	2.04	150
	N(15)	O(11)	2.909	2.00	160
	N(16)	O(12)	3.181	2.49	146
water-to-peptide	W(1A)[c]	O(14)[d]	3.02		
	W(1B)[c]	O(14)[d]	3.20		
	W(2A)[c]				
	W(2B)[c]	O(16)[b]	2.97		

[a–d] See corresponding footnotes in Table VI.

comprise approximately four helical turns in each case and permit segregation of Leu residues on one face of the helices. These structures are therefore of some interest in evaluating the role of projecting side chains in mediating helix association. This is a problem of interest in the case of the leucine zipper motif seen in a class of DNA binding proteins.[9,10] Peptide II indeed has two pairs of Leu residues spaced seven positions apart and may be, in principle, a model for the leucine zipper structure. However, since Leu[3], Leu[7], Leu[10], and Leu[14] all project from one side of

Table VI. Hydrogen Bonds in Peptide II[f]

type	donor	acceptor	N···O, Å	H···O,[a] Å	angle C=O···N, deg
head-to-tail	N(1)	O(13)[e]	2.95	2.02	155
peptide-to-water	N(2)	W(2)	2.94	1.99	
	N(3)	W(1)	2.95	2.03	
5→1	N(4)	O(0)	3.07	2.14	158
	N(5)	O(1)	2.99	2.04	161
	N(6)	O(2)	2.91	1.97	154
	N(7)	O(3)	2.91	1.97	165
	N(8)	O(4)	3.13	2.20	157
	N(9)	O(5)	2.82	1.88	163
	N(10)	O(6)	2.99	2.07	151
	N(11)	O(7)	2.95	2.00	157
	N(12)	O(8)	2.90	1.96	161
	N(13)	O(9)	2.95	2.07	146
	N(14)	O(10)	3.01	2.15	166
	N(15)	O(11)	2.89	2.25	158
water-to-peptide or water	W(1)	W(8A)[c]	2.90		
	W(1)	W(2)	2.85		
	W(2)	O(13)[e]	2.80		
	W(2)	O(15)[e]	2.68		
	W(4A)[c]	O(14)	2.73		
	W(4B)[c]	O(14)	3.27		
	W(5A)[c]	O(12)	2.94		
	W(5A)[c]	W(8A)[c]	2.99		
	W(6A)[c]	O(9)	3.32		
	W(6B)[c]	O(9)	3.18		
	W(7)	W(4A)[c]	3.10		

[a] The H atoms were placed in idealized positions with the N–H distance equal to 0.96 Å. [b] Symmetry equivalent $1 + x, y, -1 + z$ to coordinates listed in Table II. [c] Occupancy ~50%. A pair of disordered water oxygens [W(*n*A) and W(*n*B)] cannot coexist. [d] Symmetry equivalent $-x, -{}^1/_2 + y, 2 - z$ to coordinates listed in Table II. [e] Symmetry equivalent $1{}^1/_2 - x, {}^1/_2 + y, {}^1/_2 - z$ to coordinates listed in Table III. [f] Water oxygens W(3A), W(3B), and W(8B) occur in large holes without any hydrogen bond distances.

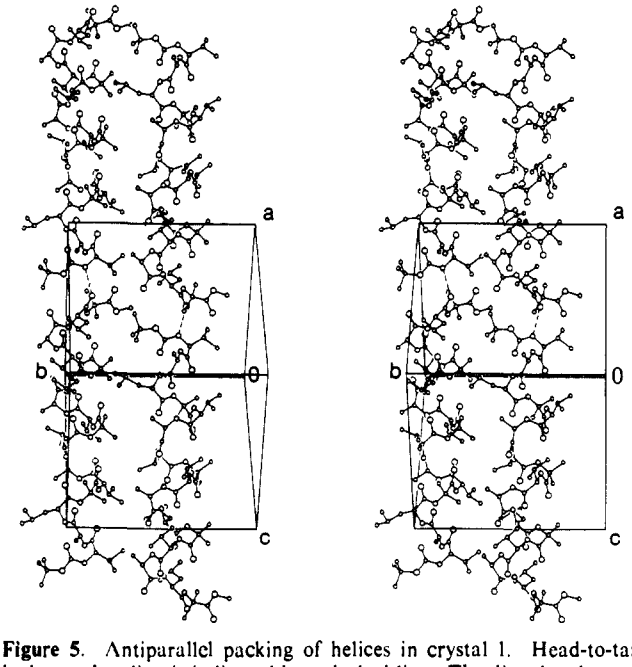

Figure 5. Antiparallel packing of helices in crystal I. Head-to-tail hydrogen bonding is indicated by a dashed line. The disordered water molecules in the head-to-tail region are omitted (see Figure 4). The molecules in the sheets (or layers) perpendicular to the page aggregate with all the helices in a parallel mode. The *b* axis (2-fold screw axis) is horizontal.

the helix in II, they cannot interdigitate with those from a second similar helix in the same manner as proposed for the leucine zipper. The observed packing arrangement in II does not lead to any interdigitation of the Leu side chains. Instead, the hydrocarbon side chains are loosely packed, with large voids developing between helices. In a more direct comparison with II, interdigitation of leucyl side chains had been established in the crystal structure of Boc-(Aib-Ala-Leu)$_3$-Aib-OMe, a hydrophobic decapeptide that

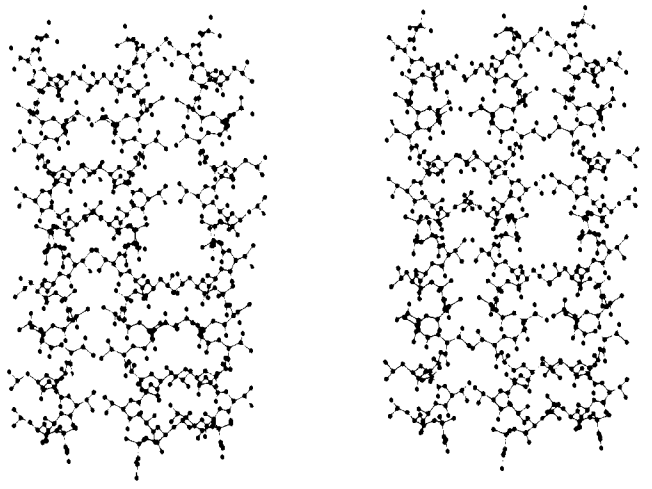

Figure 6. A sheet of parallel helices in crystal II with all N termini pointing upward (six molecules are drawn). Sheets of helices above and below the one shown are rotated by 180° to give an antiparallel arrangement. Water molecules in the head-to-tail region are omitted (see Figure 3). Axial directions of the cell are $a\rightarrow$, $b\uparrow$, and c up from the page.

had been rendered amphipathic by the insertion of water molecules into the helix.[19] In this case the leucyl side chains occur in every third position, therefore Leu[3] and Leu[6] are adjacent to each other on the helix. Since there is not sufficient space between adjacent leucyl side chains for others to penetrate in the same "plane", the interdigitating leucyl side chains from the neighboring molecule are offset, perpendicular to the view in Figure 3 of ref 19.

The loose, inefficient packing in the crystals of peptide II results in the occurrence of several water molecules in completely hydrophobic cavities, with at least some appearing to be uninvolved in hydrogen bonding. Presumably, the presence of water in hydrophobic holes does lead to some enthalpic stabilization by means of dipole-induced dipole (London) interactions with neighboring alkyl side chains.[20] These structures provide a high-resolution glimpse of water molecules in totally hydrophobic environments and may be of use in modeling trapped water in the apolar interior of globular proteins.[21,22] A recent analysis of protein crystal structures reveals that water molecules in the vicinity of apolar side chains lie predominantly at van der Waals contact distances, but most of these have a primary, shorter contact with a neighboring atom.[22] The unusual amount of water associated with the apolar peptide II is reminiscent of the antifreeze polypeptides (AFP) found in the blood of arctic fishes, which often are characterized by a very high alanine content and a high α-helical structure.[23] The first report on a crystal structure of an antifreeze polypeptide, at 2.5 Å, shows that the 36-residue peptide forms a single helix.[24] The water content and placement within the AFP crystal was not determined at this time.

The structures of I and II in crystals demonstrate that the incorporation of a few Aib residues permits the synthetic construction of largely α-helical structures. The length of the helices compares well with that found for helical segments in globular proteins.[25] Future studies will focus on the covalent linking of such helical modules and in the development of cylindrical peptide helices as a scaffold for the controlled orientation of functional residues and binding cavities.

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Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and H atom coordinates (23 pages); observed and calculated structure factors for peptides I and II (47 pages). Ordering information is given on any current masthead page.

(20) Burley, S. K.; Petsko, G. A. *Adv. Protein Chem.* **1988**, *39*, 125-189.

(21) Matsumura, M.; Wozniak, J. A.; Dao-Pin, S.; Matthews, B. W. *J. Biol. Chem.* **1989**, *264*, 16059-16066.

(22) Thanki, N.; Thornton, J. M.; Goodfellow, J. M. *J. Mol. Biol.* **1988**, *202*, 637-657.

(23) Ananthanarayanan, V. S.; Hew, C. L. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 685-689.

(24) Yang, D. S. C.; Sax, M.; Chakrabarty, A.; Hew, C. L. *Nature* **1988**, *333*, 232-237.

(25) Barlow, D. J.; Thornton, J. M. *J. Mol. Biol.* **1988**, *201*, 601-619.

Unified Synthesis of Vinylsilanes and Silylated Butadienes. Nickel-Catalyzed Olefination and Silylolefination of Dithioacetals[1]

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Abstract: A simple unified reaction has been developed in the syntheses of various vinylsilanes and silylated butadienes by the nickel-catalyzed coupling of dithioacetals with appropriate Grignard reagents. Reactions of 2-aryl-2-(trimethylsilyl)dithianes with MeMgI or cyclopropyl Grignard reagent give in good yields 1-aryl-1-(trimethylsilyl)styrenes and 1-aryl-1-(trimethylsilyl)butadienes, respectively. Reactions of these substrates with Me_3SiCH_2MgCl afford 1,2-bis(silyl)styrenes. Allylic dithioacetals react with Me_3SiCH_2MgCl furnishing the synthesis of 1-(trimethylsilyl)butadienes. Reactions of 1-(trimethylsilyl)-substituted allylic dithioacetals with MeMgI or Me_3SiCH_2MgCl give internal (trimethylsilyl)butadienes or bis(trimethylsilyl)butadienes in satisfactory yields. Treatment of allylic orthothioester with Me_3SiCH_2MgCl under similar conditions provides a facile synthesis of compounds having both vinyl- and allylsilane functionalities in one step.

The synthetic utility of vinylsilanes is rich.[4] Various literature procedures are known for the preparation of this functionality,[4-7] but there seems to be no general method for the synthesis of different kinds of vinylsilanes and silylated butadienes. Occa-

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