# Helix Packing of Leucine-Rich Peptides: A Parallel Leucine Ladder in the Structure of Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe 

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#### Abstract

The packing of peptide helices in crystals of the leucine-rich decapeptide Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-AibOMe provides an example of ladder-like leucylleucyl interactions between neighboring molecules. The peptide molecule forms a helix with five $5 \rightarrow 1$ hydrogen bonds and two $4 \rightarrow 1$ hydrogen bonds near the $\mathbf{C}$ terminus. Three head-totail NH $\cdot \operatorname{O}=\mathbf{C}$ hydrogen bonds between helices form continuous columns of helices in the crystal. The helicial columns associate in an antiparallel fashion, except for the association of Leu . . . Leu side chains, which occurs along the diagonal of the cell where the peptide helices are parallel. The peptide, with formula $\mathrm{C}_{56} \mathrm{H}_{102} \mathrm{~N}_{10} \mathrm{O}_{13}$, crystallizes in space group $P 2_{1} 2_{1} 2_{1}$ with $Z=4$ and cell parameters $a=$ $16.774(3) \AA, b=20.032(3) \AA$ and $c=20.117(3) \AA$; overall agreement factor $R=10.7 \%$ for 2014 data with $\left|F_{\text {obs }}\right|>\mathbf{3 \sigma}(F)$; resolution $1.0 \AA$.


Key words: X-ray diffraction analysis, hydrogen bonds, peptide conformation, $3_{10} / \alpha$-helix transition, antiparallel helix packing, leucyl-leucyl interaction

## INTRODUCTION

Interactions between closely packed helices in proteins are often mediated by close contacts between apolar side chains. In fibrous proteins such as tropomyosin, a regular heptad repeat of bulky apolar residues results in coiled-coil models, ${ }^{1}$ which utilize the knobs in holes packing arrangement. ${ }^{2}$ Recent hypothetical models for the dimeric "leucine zipper" class of transcription regulatory factors also envisaged efficient interdigitation of Leu side chains on two neighboring parallel helices with a net supercoiling over a short segment of the polypeptide chains. ${ }^{3,4}$ Thus far, the "leucine zipper" structure has not been characterized in crystals; however, a recent crystal structure analysis of GCN4-p1 shows that a member of this class forms a coiled coil with leucyl side chains abutting each other at the same level. ${ }^{5}$ Also, a recent structure of $E$. coli seryl-tRNA synthetase provides an unique example of a pair of
long, antiparallel helices, which are held together by apolar, interhelical interactions between leucine(iso) leucine residues, which butt against one another, forming a "leucine ladder." ${ }^{6}$ Crystal structure analyses at high resolution of leucine-rich peptides in this laboratory have so far revealed several instances of different side-by-side interactions of Leu residues, ${ }^{7,8}$ which may provide additional models and parameters for protein structures.

This work describes the structure of the decapeptide Boc-Aib- $\propto 5$ qLeu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe 1, which provides an example of a distorted ladder-like packing of leucyl side chains. Furthermore, the packing of helices in the crystal results in a checkerboard array of antiparallel helices, rather than a hexagonal array observed in several other peptides, where there are four antiparallel and two parallel neighbors around each helix. ${ }^{17,19,20,22}$ The effect on the backbone conformation of replacing Val with the larger Leu residue can also be assessed by comparing this structure withBoc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-AibOMe. ${ }^{9}$

## EXPERIMENTAL

The decapeptide 1 was synthesized and purified as previously described. ${ }^{10}$ Crystals were grown a number of years ago from $\mathrm{CH}_{3} \mathrm{OH} / \mathrm{H}_{2} \mathrm{O}$ mixture and allowed to dry. X-ray data were collected from a tiny plate ( $0.14 \times 0.15 \times 0.05 \mathrm{~mm}$ ) at $-40^{\circ} \mathrm{C}$ maintained by a cold stream of $\mathrm{N}_{2}$ gas from a liquid $\mathrm{N}_{2}$ reservoir. A four-circle automated diffractometer with a graphite monochromator was used with $\mathrm{CuK} \alpha$ radiation. The $\theta-2 \theta$ scan technique was used with a $2.0^{\circ}$ scan, variable scan speed of $7^{\circ} / \mathrm{min}$ to $14 / \mathrm{min}$, and $2 \theta_{\text {max }}$ $=105^{\circ}$, although the number of reflections measured with intensities greater than $3 \sigma(F)$ were only $1 / 5$ of the available reflections in the shell with $1.0-$

[^0]$1.1 \AA$ resolution and very few at resolutions better than $1.0 \AA$. The total number of unique reflections measured was 4,299 , of which, 2,014 had intensities $>3 \sigma(\mathrm{~F})$. Three reflections monitored after every 100 measurements remained constant within $3 \%$ during the data collection. Lorentz and polarization corrections were applied to the data. The space group is orthorhombic $\mathrm{P} 2_{1} 2_{1} 2_{1}$ with $\mathrm{a}=16.774(3) \AA, \mathrm{b}=$ $20.032(3) \AA, \mathrm{c}=20.117(3) \AA, \mathrm{V}=6759.6 \AA^{3}$, and Z $=4$. There is no cocrystallized solvent. The calculated density is $1.104 \mathrm{~g} / \mathrm{cm}^{3}$, based on a molecular weight of 1123.51 for $\mathrm{C}_{56} \mathrm{H}_{102} \mathrm{~N}_{10} \mathrm{O}_{13}$.
The structure was solved by direct phase determination ${ }^{11}$ using the random-tangent formula procedure in the SHELXTL computer program ${ }^{12}$ to find a fragment, followed by partial structure development to locate the remaining atoms. ${ }^{13}$ After several cycles of least-squares refinement, H atoms were placed in idealized positions on the C and N atoms and allowed to ride with the atom to which they are bonded during the remainder of the full-matrix, least-squares refinement. However, due to the relative paucity of data from which to determine anisotropic thermal parameters of each atom, only those three terms along the axial directions were used and the three cross terms were set equal to zero. Under these conditions, the ratio of observed data (>3q) to refined parameter is 4.2 to 1 . The final agreement factors for the 2014 data $>3 \sigma(\mathrm{~F})$ were $\mathrm{R}=0.107$ and $\mathrm{R}_{\mathrm{w}}=0.098$ (where $\mathrm{w}=\left[\boldsymbol{\sigma}^{2}(\mathrm{~F})+\mathrm{abs}(\mathrm{g}) \mathrm{F}^{2}\right]^{-1} ; \mathrm{g}=$ 0.0010 ). The final difference map was featureless, with maximum excursions of +0.52 and $-0.43 \mathrm{e} / \AA^{3}$. There were no cocrystallized solvent molecules.
Fractional coordinates for the $\mathrm{C}, \mathrm{N}$, and O atoms are listed in Table I. Bond lengths and angles (e.s.d.s. $\sim 0.03 \AA$ for bonds and $\sim 1.8^{\circ}$ for angles) do not show significant or systematic differences from expected values.*

## RESULTS

Conformation of Peptide
The present molecule, shown in Figure 1, forms a helix with five $5 \rightarrow 1$ type hydrogen bonds and two $4 \rightarrow 1$ type hydrogen bonds near the C terminus (Table II). The analog peptide in which all five Leu residues were replaced with Val residues (molecule A in ref. ${ }^{9}$ ) forms an almost ideal $\alpha$-helix with seven $5 \rightarrow 1$ hydrogen bonds. A least-squares fit of all the backbone atoms in the two molecules gives an r.m.s. deviation of $0.42 \AA$, with the largest deviations of $0.80 \AA$ at and near $\mathrm{C}^{\alpha}(6)$.
A comparison of the $\phi$ and $\psi$ torsional angles for the backbone, ${ }^{14}$ listed in Table III for the Leu pep-
tide and plotted in Figure 2 for both the Leu and Val peptides, shows that the largest differences occur for Leu (5) ( $\phi=-79^{\circ}, \psi=-29^{\circ}$ ) as compared to Val (5) $\left(\phi-66^{\circ}, \psi=-38^{\circ}\right) .{ }^{9}$ The backbone rotations in Leu (5) swing carbonyl $\mathrm{O}(5)$ away from $\mathrm{N}(9)$ so that $\mathrm{O}(5)$ does not participate in any hydrogen bonding (Fig. 1). It is at this point that a helix transition from $\alpha$ to $3_{10}$ - takes place in the Leu peptide.

In this peptide there are three adjoining Leu residues at positions 5, 6, and 7. Another example of a peptide with three continguous Leu residues is Boc-(Leu-Leu-Leu-Aib) ${ }_{2}-\mathrm{OBz} \ell .^{15}$ In that case, the average $\phi, \psi$ values for the first group of three Leu residues are $-62^{\circ},-46^{\circ}$ (expected), and $-72^{\circ},-35^{\circ}$, somewhat extreme for the second group of three. A Leu residue in the second set of three with the most extreme $\phi, \psi$ values ( $-76^{\circ},-33^{\circ}$ ) is associated with a kink in the $\alpha$-helix and with a long $\mathrm{N} \cdots \mathrm{O}$ distance of $3.36 \AA$ for the expected $5 \rightarrow 1$ hydrogen bond. The contiguity of three bulky Leu residues does not appear per se to be the cause of the distortion in the helix. An examination of many leucyl-rich helical peptides, in which the Leu residue is not adjacent to another Leu residue, shows that the $\phi$ and $\psi$ values for Leu residues often are extreme (large negative $\phi$ and small negative $\psi)^{7}$ as compared to idealized values ${ }^{16}$ of $\phi=-65^{\circ}, \psi=-41^{\circ}$ for $\alpha$-helices. Similar data for helices containing other residues with bulky side chains, specifically the Phe residue, are being derived.

## Head-to-Tail Hydrogen Bonds

Helical peptides, containing about seven or more residues, form continuous columns in crystals by head-to-tail hydrogen bonding. ${ }^{7,17,18}$ There are various motifs for head-to-tail hydrogen bonds, including mediation by solvent molecules. In the present crystal, there is a good register between $O(8), O(9)$, and $\mathrm{O}(10)$ at the bottom of one helix with $\mathrm{N}(1) \mathrm{H}$, $\mathrm{N}(2) \mathrm{H}$, and $\mathrm{N}(3) \mathrm{H}$, respectively, at the top of another helix, so that the helical backbone appears to be continuous (see Fig. 4). A similarly good register for three direct NH $\cdots \mathrm{O}=\mathrm{C}$ head-to-tail hydrogen bonds occurs in the Val analog. ${ }^{9}$ Crystals of both the Leu and the Val analogs are anhydrous.

## Packing of Helices

Helical peptides pack in a crystal in an all-parallel fashion or in an antiparallel fashion, rarely skewed. A number of examples have been found when the same peptide packs in both an all parallel
*Supplementary material consisting of bond lengths and angles, anisotropic thermal parameters, and coordinates for H atoms are deposited in the Cambridge Crystallographic Data file. Observed and calculated structure factors are available from I.L.K. and J.L. F.-A.

TABLE I. Atomic Coordinates ( $\times 10^{4}$ ) and Equivalent Isotropic Displacement Coefficients ( $\AA^{2} \times 10^{3}$ )

|  | x | y | z | U(eq)* |
| :---: | :---: | :---: | :---: | :---: |
| C(1) | 3226(14) | 6937(12) | 8320(11) | $45(2)$ |
| C(2) | 3803(13) | 7284(10) | 7865(10) | 45(2) |
| C(3) | 3540(11) | 6850(11) | 9016(10) | 38(2) |
| C(4) | 2401(12) | 7258(11) | 8339(12) | 55(2) |
| 0 | 3068(8) | 6252(7) | 8071(7) | 33(2) |
| $\mathrm{C}^{\prime}(0)$ | 3647(15) | 5788(12) | 7992(12) | 46(2) |
| $\mathrm{O}(\mathrm{O})$ | 4363(9) | 5924(8) | 7958(9) | 60(2) |
| $\mathrm{N}(1)$ | 3357(9) | 5189(8) | $7939(8)$ | 25(2) |
| $\mathrm{C}^{\alpha}(1)$ | 3806(13) | 4595(12) | 8060(10) | 34(2) |
| $\mathrm{C}^{\prime}(1)$ | 4518(13) | 4547(11) | 7542(11) | $32(2)$ |
| $\mathrm{O}(1)$ | 5216(8) | 4477(8) | 7692(7) | 42(2) |
| $\mathrm{C}^{\mathbf{\beta}}(11)$ | 3294(13) | 3965(11) | 7982(13) | 68(2) |
| $\mathrm{C}^{\mathrm{B}}(12)$ | 4217(12) | 4597(12) | 8756(11) | 58(2) |
| $\mathrm{N}(2)$ | 4271(10) | 4524(9) | 6908(9) | 35(2) |
| $\mathrm{C}^{\alpha}(2)$ | 4835(11) | 4389(10) | 6385(10) | 25(2) |
| $\mathrm{C}^{\prime}(2)$ | 5440(11) | 4980(12) | 6290(10) | $24(2)$ |
| $\mathrm{O}(2)$ | 6143 (8) | 4871 (7) | 6248(7) | 39(2) |
| $\mathrm{C}^{\mathrm{B}}(2)$ | 4433(14) | 4195(10) | 5727(10) | 39(2) |
| $\mathrm{C}^{\curlyvee}(2)$ | 4021(11) | 3517(11) | 5749(10) | 32(2) |
| $\mathrm{C}^{\mathbf{8}}$ (21) | 3494(14) | 3451(11) | 5128(12) | 69(2) |
| $\mathrm{C}^{\mathbf{8}}(22)$ | 4584(17) | 2944(13) | 5832(14) | 102(3) |
| $\mathrm{N}(3)$ | 5087(10) | 5574(9) | 6219(8) | 27(2) |
| $\mathrm{C}^{\alpha}(3)$ | 5598(12) | 6176(14) | 6124(10) | 46(2) |
| $\mathrm{C}^{\prime}(3)$ | 6278(11) | 6162(12) | 6654(11) | 35(2) |
| $\mathrm{O}(3)$ | 6977(8) | 6253(9) | 6514(7) | 45(2) |
| $\mathrm{C}^{\mathbf{\beta}}$ (31) | 5920(12) | 6175(11) | 5440(10) | 39(2) |
| $\mathrm{C}^{\boldsymbol{\beta}}(32)$ | 5057(13) | 6819(12) | 6238(12) | 63(2) |
| $\mathrm{N}(4)$ | 6031(9) | 6098(8) | 7294(7) | 22(2) |
| $\mathrm{C}^{(4}$ (4) | 6593(11) | 6172(10) | 7872(10) | 20(2) |
| $\mathrm{C}^{\prime}(4)$ | 7230(13) | 5656(11) | 7801(10) | 23(2) |
| $\mathrm{O}(4)$ | 7959(8) | 5763(7) | 7863(8) | 42(2) |
| $\mathrm{C}^{\mathbf{\beta}}(41)$ | 6108(11) | 5972(11) | 8487(9) | $32(2)$ |
| $\mathrm{C}^{\boldsymbol{\beta}}(42)$ | 6898(12) | 6842(10) | 7914(11) | 41(2) |
| $\mathrm{N}(5)$ | 7005(9) | 4995(9) | 7698(7) | 24(2) |
| $\mathrm{C}^{\alpha}(5)$ | 7551(12) | 4433(10) | 7750(11) | 30(2) |
| $\mathrm{C}^{\prime}(5)$ | 8088(15) | 4338(10) | $7141(12)$ | 39(2) |
| O(5) | 8729(10) | $4082(8)$ | 7181(8) | $62(2)$ |
| $\mathrm{C}^{\beta}(5)$ | 7105(12) | 3815(10) | 7951(11) | 37(2) |
| $\mathrm{C}^{\mathbf{\delta}}$ (5) | 6790(16) | 3819(12) | 8650(12) | 61(2) |
| $\mathrm{C}^{\mathbf{8}}$ (51) | 6162(14) | 3271(13) | 8785(13) | 91(2) |
| $\mathrm{C}^{\mathbf{8}}$ (52) | 7344(16) | 3813(16) | 9197(11) | 118(3) |
| N (6) | 7746(9) | 4554(9) | 6572(8) | 33(2) |
| $\mathrm{C}^{\alpha}(6)$ | 8218(12) | 4538(11) | 5965(10) | 28(2) |
| $\mathrm{C}^{\prime}(6)$ | 8861(12) | 5073(12) | 6025(10) | 31(2) |
| O (6) | 9553(8) | 4993(8) | 5839(7) | $43(2)$ |
| $\mathrm{C}^{\mathrm{B}}$ (6) | 7732(12) | 4589(10) | 5318(10) | 37(2) |
| $\mathrm{C}^{\gamma}(6)$ | 7174(12) | 3987(10) | 5228(10) | 29(2) |
| $\mathrm{C}^{\mathbf{s}}$ (61) | 7645(14) | $3337(10)$ | 5105(10) | 50(2) |
| $\mathrm{C}^{\mathbf{s}}$ (62) | 6628(14) | 4127(13) | 4657(11) | 70(2) |
| $\mathrm{N}(7)$ | 8622(9) | 5687(8) | 6245(7) | 19(2) |
| $\mathrm{C}^{\mathbf{\alpha}}(7)$ | 9190(11) | 6204(9) | 6357(10) | 18(2) |
| $\mathrm{C}^{\prime}(7)$ | 9833(13) | 6051(11) | 6839(11) | 31(2) |
| O(7) | 10536(9) | 6278(7) | 6757(8) | 52(2) |
| $\mathrm{C}^{\mathrm{B}}$ (7) | 8837(15) | 6911(12) | 6491(13) | 71(2) |
| $\mathrm{C}^{\gamma}(7)$ | 8456(17) | 7252(15) | 5929(17) | 104(3) |
| $\mathrm{C}^{\mathbf{\delta}}$ (71) | 9266(18) | 7602(13) | 5554(17) | 114(3) |
| $\mathrm{C}^{\text {s }}$ (72) | 8044(22) | 7919(17) | 6268(17) | 141(3) |
| $\mathrm{N}(8)$ | 9619(10) | 5675(8) | $7350(8)$ | 28(2) |
| $\mathrm{C}^{\alpha}(8)$ | 10234(13) | 5461(11) | 7859(12) | 40(2) |
| $\mathrm{C}^{\prime}(8)$ | 10928(12) | 5112(11) | 7520(11) | 29(2) |
| $\mathrm{O}(8)$ | 11574(8) | $5077(8)$ | 7833(7) | 47(2) |
| $\mathrm{C}^{\mathrm{B}}(81)$ | 9795(11) | 4995(11) | 8309(10) | 34(2) |
| $\mathrm{C}^{\boldsymbol{\beta}}(82)$ | 10509(13) | 6062(10) | 8257(12) | $56(2)$ |
| N(9) | 10788(9) | 4745(8) | 6969 (8) | $20(2)$ |
| $\mathrm{C}^{\alpha}(9)$ | 11441(12) | 4392(10) | 6637(10) | 24(2) |
| $\mathrm{C}^{\prime}(9)$ | 12024(14) | 4886(12) | $6341(11)$ | $37(2)$ |
| O 9 ) | 12755(7) | 4716(7) | 6276 (7) | ${ }^{33(2)}$ |
| $\mathrm{C}^{\mathrm{B}}$ (9) | 11100(12) | 3968(10) | 6096(9) | $28(2)$ |
| $\mathrm{C}^{\gamma}(9)$ | 10551(17) | 3406(13) | 6365(14) | $77(2)$ |
| $\mathrm{C}^{\mathbf{\delta}}$ (91) | 10151(14) | 3056(13) | 5779(13) | $77(2)$ |
| $\mathrm{C}^{\mathbf{8}}(92)$ | 10960(19) | 2943 (16) | 6740(17) | $176(3)$ |
| $\mathrm{N}(10)$ | 11781(10) | $5458(8)$ | 6110 (7) | $19(2)$ |
| $\mathrm{C}^{\mathbf{\alpha}}(10)$ | $12263(14)$ | 5982(12) | $5865(10)$ | 38(2) |
| $\mathrm{C}^{\prime}(10)$ | 12902(12) | 5713(10) | $5381(10)$ | $20(2)$ |
| $\mathrm{O}(10)$ | 13610(8) | 5870(8) | 5376 (8) | 46(2) |
| $\mathrm{C}^{\mathrm{B}}(101)$ | 12694(15) | 6313(13) | 6416 (11) | $80(2)$ |
|  | 11786(14) | ${ }^{65509(12)}$ | - ${ }_{489888(7)}$ | $60(2)$ <br> $37(2)$ |
| $\mathrm{C}(11)$ | 13092(13) | $5113(11)$ | 4364(9) | 39(2) |

*Equivalent isotropic $U$ defined as one-third of the trace of the orthogonalized $U_{i j}$ tensor.


Fig. 1. Conformation of Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe. Hydrogen bonds are indicated by dashed lines. The number zero is at the position of an O atom in the Boc group.


Fig. 2. A comparison of the conformational angles $\phi$ and $\psi$, about $\mathrm{N}-\mathrm{C}^{\alpha}$ and $\mathrm{C}^{\alpha}-\mathrm{C}^{\prime}$, respectively, for the Leu analog (present molecule) and the Val analog $\triangle$ (molecule A of ref. ${ }^{9}$ ) having the same sequence except for replacing Val with Leu residues. A helix reversal takes place at the terminal Aib(10) in both molecules (not shown). The $\phi(10), \psi(10)$ values for the Leu and Val analogs, respectively are $+47^{\circ},+54^{\circ}$ and $+50^{\circ},+44^{\circ}$.


Fig. 3. Antiparallel packing arrays in crystals of helical peptides. The tops of the helices are labelled $N$ for the $N$-terminus and C for the C-terminus. A. Hexagonal array. B. Checkerboard array in present molecule where the axial directions are $\leftarrow \mathrm{y}$ and $\downarrow \mathrm{z}$. The Leu . . . Leu contacts are between parallel molecules along
the diagonals, see, e.g., the top center molecule and the right center molecule (also shown in a different orientation in Fig. 4A), where approaches of $3.9 \AA$ between $\mathrm{C}^{\delta}$ atoms are indicated by dotted lines.

TABLE II. Hydrogen Bonds*

| Type |  |  |  |  | Angle, deg. |
| :--- | :---: | :---: | :---: | :---: | :---: |

*Hydrogen atoms were placed in idealized position with $\mathrm{N}-\mathrm{H}=0.96 \AA$. Atom $\mathrm{O}(5)$ does not participate in any hydrogen bonding; the $\mathrm{N}(8) \cdots \mathrm{O}(5)$ distance is $3.54 \AA$ and the $\mathrm{N}(9) \cdots \mathrm{O}(5)$ distance is $3.72 \AA$.
${ }^{+}$These atoms are related to the coordinates listed in Table I by the symmetry operation $-1+x, y, z$.
TABLE III. Torsion Angles (deg.)*

| Residue | $\phi\left(\mathrm{N}-\mathrm{C}^{\alpha}\right)$ | $\psi\left(\mathrm{C}^{\alpha}-\mathrm{C}^{\prime}\right)$ | $\omega\left(\mathrm{C}^{\prime}-\mathrm{N}\right)$ | $\chi^{1}\left(\mathrm{C}^{\alpha}-\mathrm{C}^{\beta}\right)$ | $\chi^{2}\left(\mathrm{C}^{\beta}-\mathrm{C}^{\gamma}\right)$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Aib 1 | $-63^{\dagger}$ | -60 | -172 |  |  |
| Leu 2 | -68 | -52 | 180 | -69 | $168,-66$ |
| Aib 3 | -47 | -54 | -171 |  |  |
| Aib 4 | -60 | -51 | -168 |  |  |
| Leu 5 | -79 | -29 | 175 | -69 | $165,-66$ |
| Leu 6 | -70 | -46 | 176 | -64 | $-68,170$ |
| Leu 7 | -58 | -36 | 177 | -70 | $-84,172$ |
| Aib 8 | -55 | -33 | -179 |  |  |
| Leu 9 | -67 | -33 | 175 | -64 | $173,-65$ |
| Aib 10 | +47 | $+54^{\ddagger}$ | $175^{\S}$ |  |  |

*The torsion angles for rotation about bonds of the peptide backbone ( $\phi, \psi$ and $\omega$ ) and about bonds of the amino acid side chains ( $\chi$ ) are described in ref. 14. E.s.d.'s are $\sim 1.8^{\circ}$.
${ }^{\dagger} \mathrm{C}^{\prime}(0), \mathrm{N}(1), \mathrm{C}^{\alpha}(1), \mathrm{C}^{\prime}(1)$.
${ }^{\ddagger} \mathrm{N}(10), \mathrm{C}^{\alpha}(10), \mathrm{C}^{\prime}(10), \mathrm{O}(\mathrm{OMe})$.
${ }^{8} \mathrm{C}^{\alpha}(10), \mathrm{C}^{\prime}(10), \mathrm{O}(\mathrm{OMe}), \mathrm{C}(\mathrm{OMe})$.
motif and in an antiparallel motif in different polymorphs that have been crystallized from different solvents. ${ }^{19,20,21}$ In the antiparallel motif, the helices have usually been found to pack in a hexagonal array with four antiparallel and two parallel nearest neighbors ${ }^{17,19-22}$ as illustrated in Figure 3A. In the present crystal, the helix packing is antiparallel but with an approximately square array with four nearest neighbors antiparallel and four parallel neighbors along the diagonals at somewhat greater distances (Fig. 3B). However, the side chains of Leu(2), Leu(6), and Leu(9) are extended along a diagonal direction of the cell, as shown in Figures 3B and 4A, and a very orderly Leu . . . Leu noncovalent interaction ensues between parallel neighbors related by a two-fold screw axis, with nearest approach Leu(2) $\cdots \operatorname{Leu}(6)=3.88 \AA$ and $\operatorname{Leu}(2) \cdots \operatorname{Leu}(9)=3.93 \AA$. Most other nearest approaches between C . . C atoms in neighboring molecules in other directions are in the range of 3.8 to $4.1 \AA$. The leucyl side chains do not interdigitate but do butt up against each other in a staggered fashion.

## DISCUSSION

The use of Aib residues permits the synthetic construction of peptide helices with appreciable conformational stability, which crystallize readily. ${ }^{23,24}$ Systematic X-ray diffraction studies of these peptide helices has yielded a great deal of accurate and useful information on subtle conformational effects of substituents and on packing modes of the cylindrical structures. ${ }^{7}$ The example of peptide 1 presented in this study reveals several interesting features.

The larger side chain of the leucyl residue causes subtle changes in the shape of an $\alpha$-helix backbone as compared to more ideal helices formed with valyl and alanyl residues with smaller side chains. The distortions in the helix are manifested by sizeable departures from ideal conformational angles, by long distances ( $>3.2 \AA$ ) between the NH and $\mathrm{C}=0$ moieties expected to form hydrogen bonds, and by transitions from $\alpha$-helices to $3_{10}$-helices. It is not clear whether the helix adjusts to steric hindrance in the molecule itself caused by the bulkier side


Fig. 4. Association of Leu . . . Leu groups between parallel helices in Boc-ULUULLLULU-OMe (present molecule, $\mathrm{U} \equiv \mathrm{Aib}$, upper A) and between antiparallel helices in Boc-UALAUULALU$\mathrm{OMe}^{29}$ (lower B). Three molecules of each peptide are shown in stereo. Side chains in leucyls 2, 6, and 9 in A, and 3 and 7 in B are stippled. Only normal van der Waals' distances are observed for closest approaches: in $A, C^{\delta 1}(2) \cdots C^{\delta 1}(6)=3.88 \AA$ and $C^{\delta 2}(2)$
$C^{\delta 1}(9)=3.93 \AA$; in $B, C^{8}(7) \cdots C^{81}(3)=3.98 \AA, C^{8}(7) \cdots$ $C^{\delta 2}(3)=4.00 \AA, C^{\beta}(3) \cdots C^{\delta 1}(7)=4.16 \AA$ and $C^{\beta}(4) \cdots C^{\delta 2}(7)$ $=4.07 \AA$. The leucyl side chains in $A$ are staggered in the vertical direction; those in B are staggered in both the vertical direction and perpendicular to the page. Interhelical (helix axis to helix axis) distances are $13.8 \AA$ in $A$ and $10.8 \AA$ in $B$. The packing motifs in $A$ and $B$ are different than in a coiled-coil (with twofold symmetry) where the interhelix distance is $-9.3 \AA$ and the closest approaches are between $\mathrm{C}^{\beta}$ atoms of leucyl side chains at the same level. ${ }^{5}$
chains, or to distortions caused by the extended leucyl side-chains in finding a low energy packing arrangement with neighboring molecules.
The packing mode in the present case has each peptide molecule surrounded by four nearest antiparallel neighbors, whereas four more distant molecules are aligned in the same direction. This checkerboard arrangement is in contrast to the hexagonal
array generally observed in these types of peptides. ${ }^{17,19-22}$ In contrast to the antiparallel or skewed association of helices often found in proteins, ${ }^{25,26}$ apolar helical peptides containing 10 or more residues crystallize frequently with all the helix axes pointing in the same (or nearly the same) direction. ${ }^{21,22,23,27}$ For some peptide molecules, when several polymorphic crystal types have been grown from various solvents, the same peptide crystallizes in a parallel mode in one polymorph and in an antiparallel mode in another polymorph with apparent equal ease. ${ }^{19,20,21,27}$

The interactions between projecting Leu side chains on adjacent parallel helices related by a twofold screw axis are mediated by close contacts between the two $\delta$-methyl groups of Leu(2) with one methyl group each of Leu(6) and Leu(9) of a symmetry related molecule. This leads to a ladder-like arrangement, with the Leu … Leu contacts forming distorted rungs (Fig. 4A). Although Leu(2) and Leu(9) are spaced by seven residues, interdigitation has not been observed. The bulky Leu side chain at position 6 impedes closer approach of the helices. An example of antiparallel helix packing with Leu . . . Leu interactions in a peptide is shown in Figure 4B for Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-AibOMe. ${ }^{29}$ The helix axes are much closer, $10.8 \AA$ in Figure 4B compared to $13.8 \AA$ in Figure 4A. The packing of leucyl groups is also much closer. They are staggered in the vertical direction as well as in the direction pointed into the page. In Figure 4B, the close contacts between leucyl groups is between $\mathrm{C}^{\beta}$ $\cdots \cdot \mathrm{C}^{\delta \prime}$ atoms rather than between $\mathrm{C}^{\delta} \cdot \cdots \mathrm{C}^{\delta,}$ as in Figure 4A. In each example, the helix axes are essentially straight and the angle between the axes of adjacent helices is $0^{\circ}$ or $180^{\circ}$.

Two recent crystal structure analyses of proteins with heptads of leucyl groups have shown both an antiparallel ladder and a parallel coiled-coil. In $E$. coli. seryl-tRNA synthetase, ${ }^{6}$ there is a long helical arm compsed of a pair of antiparallel helices that are separated by an interaxis distances of $8.8 \AA$ to 10.5 $\AA$. The helices are slightly twisted but do not form a coiled-coil. The abutting leucyl side chains from each helix are on the same level but turn away from each other in opposite directions, with the appearance that the closest approaches are between $\mathrm{C}^{\beta} \ldots$ $\mathrm{C}^{\beta^{\prime}}$ atoms. A coiled-coil structure, determined for the synthetic 33 -residue GCN4-p1 ${ }^{5}$, shows an approximate twofold rotation axis between a pair of helices with an average interaxis distance of $9.3 \AA$. The leucyl side chains, occurring on the same level on separate helices also have closest contacts between $\mathbf{C}^{\beta} \cdots \mathbf{C}^{\beta^{\prime}}$ atoms, with the $\mathbf{C}^{\delta}, \mathrm{C}^{\boldsymbol{\delta}^{\prime}}$ atoms turned away from each other.

In the above examples, four variants of Leu . . . Leu interactions between parallel or antiparallel helices have been described briefly. Other variants will undoubtedly be found. So far the packing motifs
are tighter in the proteins than in the peptides, with close approaches between $\mathrm{C}^{\beta} \cdots \mathrm{C}^{\beta^{\prime}}$ of the leucyls rather than $\mathrm{C}^{\delta} \cdots \mathrm{C}^{\delta^{\prime}}$ or $\mathrm{C}^{\beta} \cdots \mathrm{C}^{\delta^{\prime}}$. Loose packing between leucyl side chains has often been observed in other Leu-rich peptides. ${ }^{7,28}$ Interdigitation of leucyl groups in a popularly named "leucine zipper" motif has not been observed so far. However, parallel zipper formation with interdigitation in helical peptide crystals has been observed in the case of very bulky and extended side chains as in the decapeptide Boc-Aib-Glu(OBzl)-Leu-Aib-Ala-Leu-Aib-Ala-Lys(Z)-Aib-OMe. ${ }^{30}$ In that case the very large protrusions on the cylindrical helical structures, with a small intervening side chain, leucyl in this case, permit close interdigitation between parallel helical columns. ${ }^{30}$

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