The Crystal Structure of Pivaloyl-D-prolyl-L-prolyl-L-alanyl-*N*-methylamide

By **C. Mohana Kumaran Nair** and **Mamannamana Vijayan,*** Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

Pivaloyl-D-prolyl-L-prolyl-L-analyl-*N*-methylamide (I), $C_{19}H_{32}N_4O_4$, crystallizes in the orthorhombic space group $P2_12_12_1$ with four molecules in a unit cell of dimensions a = 9.982 (1), b = 10.183 (3), c = 20.746 (2) Å. The structure has been refined to R 0.048 for 1 745 observed reflections. All the peptide bonds in the molecule are *trans* and both the prolyl residues are in the C^{γ}-*exo*-conformation. The molecule assumes a highly folded conformation in which a Type II′ DL bend is followed by a Type I LL bend, both stabilised by intramolecular 4 ⟶ 1 hydrogen bonds. This conformation, which has been observed for the first time, is of interest in relation to the structure of gramicidin S.

PROLYL residues play an important role in determining the conformation of proteins,[1,2] peptides, and peptide antibiotics.[3] As part of a programme aimed an exploring the conformational preferences of proline-containing peptides of either all L- or mixed L- and D-sequences, the crystal structure of pivaloyl-D-prolyl-L-prolyl-L-alanyl-*N*-methylamide (I) is reported here. It may be mentioned that the conformation of the molecule is of particular interest in relation to those of gramicidin S, which contains the sequence D-Phe-L-Pro-L-Val and its analogues.

EXPERIMENTAL

The compound, obtained by Dr. P. Balaram and Mr. Y. V. Venkatachalapathi as a by-product in the synthesis of the all-L-isomer, was crystallised from chloroform–light petroleum.[4] The space group was determined to be $P2_12_12_1$ from oscillation and Weissenberg photographs. The density was measured by flotation in potassium iodide solution in water.

Crystal Data.—Pivaloyl-D-prolyl-L-prolyl-L-alanyl-N-methylamide (I), $C_{19}H_{32}N_4O_4$, M = 380.1. Orthorhombic, $P2_12_12_1$, a = 9.982 (1), b = 10.183 (3), c = 20.746 (2) Å, U = 210 8.8 Å^3, D_m = 1.207 g cm^{-3} (by flotation), D_c = 1.196 g cm^{-3}, Z = 4. Cu-K_α radiation; μ(Cu-K_α) = 6.19 cm^{-1}.

The intensity data were collected from a specimen of approximate dimensions 0.6 × 0.4 × 0.2 mm on a computer controlled CAD-4 diffractometer employing a ω—2θ scan up to a maximum Bragg angle of 60° using graphite monochromated Cu-K_α radiation. Of the 2 058 reflections measured in this range, 1 988 reflections which had positive intensities were used in the initial stages of the analysis. Only 1 745 reflections having $I > 2\sigma(I)$ were used in the final refinement cycles. The intensities were corrected for Lorentz and polarisation factors but not for absorption.

The structure was solved by direct methods by use of MULTAN [5] and was refined by the block-diagonal SFLS method using the locally modified version of a program originally written by Professor R. Shiano. In the final cycles the non-hydrogen atoms were given anisotropic temperature factors and the hydrogen atoms, located from a difference Fourier map, isotropic temperature factors. The refinement was terminated at R 0.048 when all the least squares shifts were much smaller than the corresponding estimated standard deviations. The weighting function used in the final cycle had the form [6] $1/(a + bF_o + cF_o^2)$ where a = 1.650 0, b = −0.033 2, and c = 0.005 3. The scattering factors for the non-hydrogen atoms and the hydrogen atoms were taken from refs. 7 and 8, respectively. The computations were performed on IBM 360/44 and DEC-1090 computers. The final positional parameters of the non-hydrogen atoms are given in Table 1. The thermal parameters of all the atoms, the positional parameters of hydrogen atoms, and the observed and the calculated structure factors are given in Supplementary Publication No. SUP 22885 (16 pp.).†

TABLE 1

Final positional coordinates (× 10^4) of non-hydrogen atoms, with standard deviations in parentheses

Atom	x	y	z
C(1)	2 361(6)	3 197(6)	−2 082(3)
C(2)	829(6)	1 318(5)	−2 135(2)
C(3)	−58(6)	3 440(5)	−1 740(2)
C(4)	1 179(4)	2 550(4)	−1 747(2)
C(5)	1 479(4)	2 112(4)	−1 053(2)
O(1)	1 196(3)	997(2)	−873(1)
N(1)	2 009(3)	2 955(3)	−621(1)
C(6)	2 404(5)	4 348(4)	−685(2)
C(7)	2 381(5)	4 842(5)	−2(3)
C(8)	2 809(5)	3 670(5)	392(2)
C(9)	2 136(4)	2 503(4)	50(2)
C(10)	3 010(3)	1 277(4)	87(2)
O(2)	4 077(2)	1 220(2)	−210(1)
N(2)	2 587(3)	286(3)	462(1)
C(11)	1 374(5)	235(5)	865(2)
C(12)	1 700(6)	−830(6)	1 353(2)
C(13)	2 548(6)	−1 782(5)	984(2)
C(14)	3 397(4)	−906(4)	525(2)
C(15)	3 683(4)	−1 623(4)	−98(2)
O(3)	4 618(3)	−2 399(3)	−122(1)
N(3)	2 833(3)	−1 418(3)	−589(1)
C(16)	2 999(5)	−2 075(4)	−1 207(2)
C(17)	1 600(5)	−2 330(5)	−1 504(2)
C(18)	3 897(5)	−1 354(5)	−1 680(2)
O(4)	4 104(5)	−1 824(4)	−2 205(2)
N(4)	4 433(4)	−217(4)	−1 492(2)
C(19)	5 330(6)	529(6)	−1 899(3)

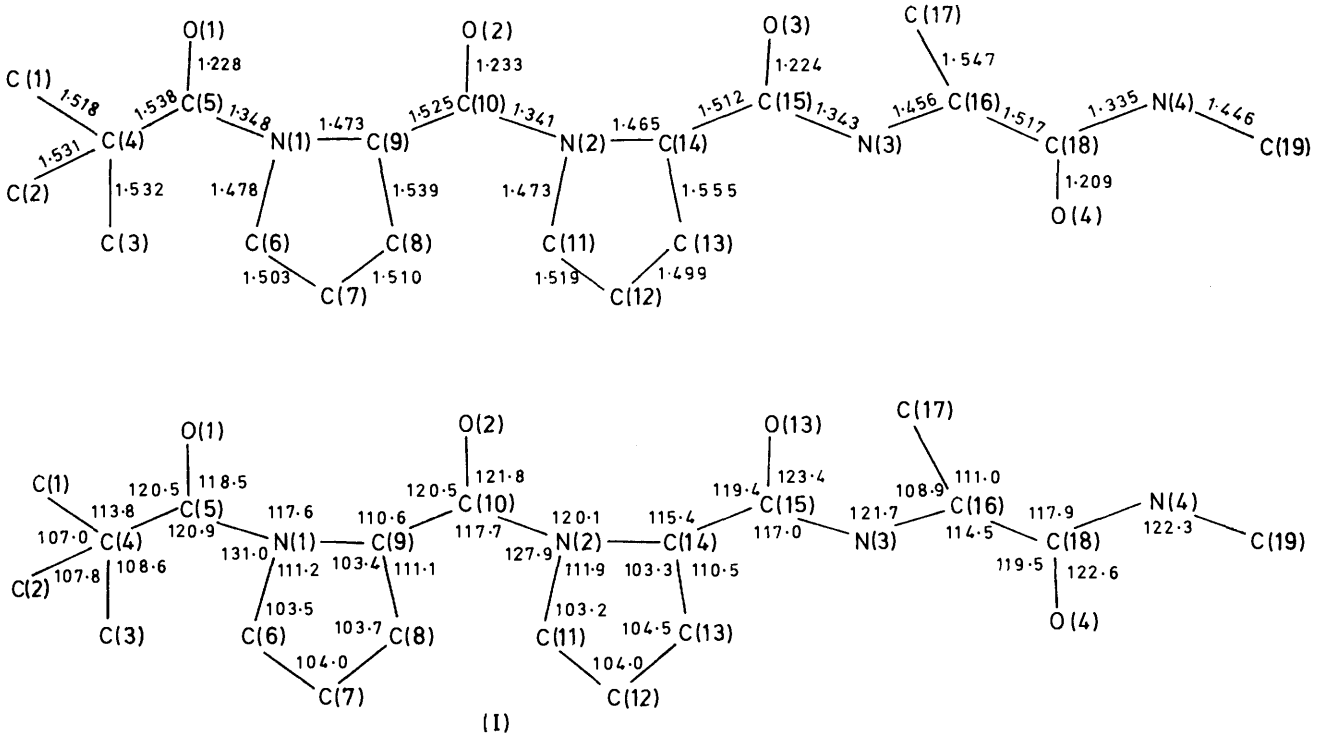
DISCUSSION

Bond Lengths and Valency Angles.—The bond lengths and angles in the molecule (Figure 1), except angles C(5)–N(1)–C(6) and C(4)–C(5)–N(1), have values comparable with those found in other peptides containing

† For details of Supplementary Publications, see Notice to Authors No. 7, in *J.C.S. Perkin II*, 1979, Index issue.

prolyl residues.[9] The observed values of C(5)–N(1)–C(6) and C(4)–C(5)–N(1) are however larger than those found in similar structures. These angles presumably open out to accommodate the bulky pivaloyl group. The C^{α}–C–N angle C(9)–C(10)–N(2) in the peptide group that links the two consecutive prolyl residues has a value from planarity, with the values of the dihedral angle [14] ω varying from 173.8 to −178.9° (Table 2).

Prolyl residues are endowed with some conformational flexibility on account of the different puckerings the pyrrolidine rings can adopt. As can be seen from Table 3, the prolyl residues in the present structure assume the



(I)

FIGURE 1 Bond lengths and valency angles in molecule (I). Mean estimated standard deviations for bond lengths and angles are 0.006 Å and 0.3°, respectively

greater than that found in normal peptide groups [10,11] presumably due to the steric interactions between the adjacent prolyl side chains. This feature is found to occur when two consecutive prolyl residues are on the same side of the main chain as in t-pentyloxycarbonyl-L-prolyl-L-prolyl-L-proline.[12]

Peptide Geometry and Pyrrolidine Ring Conformation.—X–Pro peptide bonds can adopt *cis*- or *trans*-geometry.[9] All peptide bonds in the present structure are, however, *trans*. The first tertiary amide bond in the molecule is

TABLE 2

Main chain dihedral angles (°)

	$i = 1$	$i = 2$	$i = 3$	$i = 4$
ω_i	173.8	−178.2	−178.9	178.4
ϕ_i		59.1	−58.6	−88.1
ψ_i		−135.6	−23.2	−0.1

locked into the *trans*-geometry presumably due to the presence of the bulky pivaloyl group.[13] The ready formation of a hydrogen bond between the carbonyl group of D-Pro residue and the amino-group of the terminal methylamide function apparently stabilises the observed *trans*-geometry of the D–Pro–L–Pro bond. The peptide groups do not exhibit appreciable deviations

C_s–C^{γ} *exo*-conformation.[15] In the D-residue C^{γ} deviates from the mean plane of the other four atoms in the pyrrolidine ring by 0.552 Å. The corresponding value in the L-residue is 0.541 Å. In both residues, C^{γ} and the CO group lie on the opposite sides of their mean plane.

TABLE 3

Deviation of atoms of prolyl residues from the respective least squares planes

Atomic group	Atom	Deviation from the least squares plane (Å)
D-Pro	N(1)	0.0389
	C(6)	−0.0245
	C(7)	−0.5524 *
	C(8)	0.0225
	C(9)	−0.0370
	C(10)	1.1447 *
L-Pro	N(2)	0.0171
	C(11)	−0.0107
	C(12)	−0.5405 *
	C(13)	0.0097
	C(14)	−0.0161
	C(15)	1.1288 *

* These atoms were not included in the mean plane calculations.

Main Chain Conformation.—A perspective view of the

molecule is shown in Figure 2. The main chain dihedral angles [14] which define the conformation of the molecule are given in Table 2. These values indicate that the molecule has a Type II' D-Pro,L-Pro β-bend followed by a Type I L-Pro,L-Ala β-bend. The observed ϕ, ψ angles in these bends are close to the corresponding theoretical

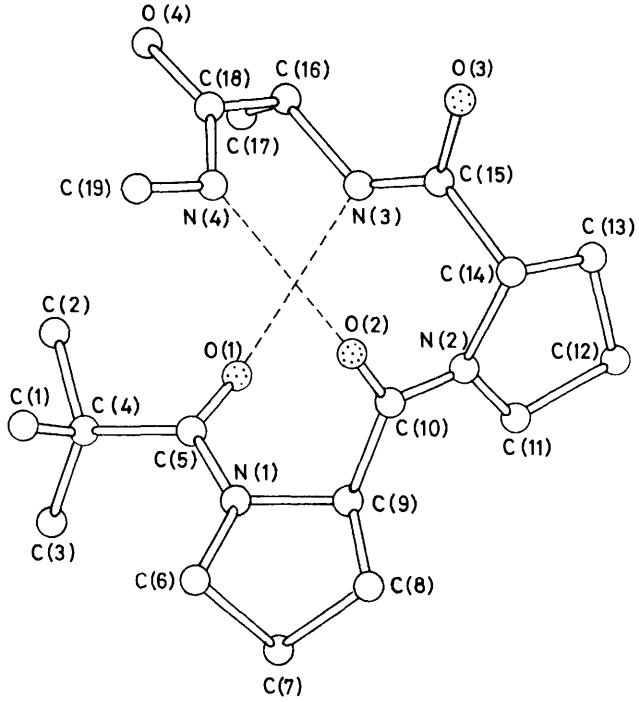


FIGURE 2 Perspective view of the molecule

values,[16] namely, $\phi_{i+1} = 60°$, $\psi_{i+1} = -120°$ and $\phi_{i+2} = -80°$, $\psi_{i+2} = 0$ for a Type II' bend and $\phi_{i+1} = -60°$, $\psi_{i+1} = -30°$ and $\phi_{i+2} = -90°$, $\psi_{i+2} = 0°$ for a Type I bend. Both the bends are stabilised by 4 ⟶ 1 N–H · · · O hydrogen bonds, which, in fact, are the only hydrogen bonds in the structure. The parameters of this hydrogen bonds are given in Table 4.

β-Bends with L- or glycyl residues at the corners are of great importance in the three dimensional structure of proteins [17] whereas LD- and DL-bends have been known to occur in peptide and depsipeptide antibiotics.[3,17] Therefore a number of *X*-ray crystallographic studies

TABLE 4

Hydrogen bond parameters, with standard deviations in parantheses

O(1) · · · N(3)	3.011(4) Å
O(2) · · · N(4)	3.055(4)
O(1) · · · N(3) · · · H(N3)	18(3)°
O(2) · · · N(4) · · · H(N4)	14(3)°

have been carried out recently to define the possible geometrical features of β-bends at the atomic resolution. The present structure contains two bends, one of the DL-type and the other of the LL-type. All the DL-bends observed so far in crystal structures [18] are of Type II'; so is that in the present structure. The latter, however, provides the first example of a bend with prolyl residues at both corners. A majority of the L-Pro-L-X bends observed in peptide structures are of Type I.[19-23] Type II L-Pro-L-Ala and L-Pro-Gly bends are, however, observed in *N*-isobutyl-L-prolyl-L-alanylisopropylamide [24] and *N*-acetyl-L-prolyl-glycyl-L-phenylalanine,[25] respectively. The L-Pro-L-Ala bend in the present structure is of Type I.

Consecutive Type III β-bends give rise to a 3_{10} helix. Such consecutive bends have been observed in some peptides containing the unusual optically inactive amino-acid α-aminoisobutyric acid.[26,27] The present structure provides the first description of two consecutive β-bends in a peptide not containing α-aminoisobutyric acid. It is also noteworthy that neither of the bends is of Type III.

Relevance to the Structure of Gramicidin S.—The sequence D-Phe-L-Pro-L-Val occurs twice in the structure of the cyclic decapeptide antibiotic gramicidin S, and it is of interest to examine the observed conformation of (I) with sequence D-Pro-L-Pro-L-Ala in relation to that of gramicidin S. The structure of gramicidin S in its crystals consists of a two-strand anti-parallel β-sheet, each stand made up of a Val-Orn-Leu sequence, looped at each end by a D-Phe-L-Pro bend.[28] It is interesting to explore the other possible conformations of this important antibiotic. The conformation of the biologically active gramicidin S analogue bis-*N*-methyl-leucyl-gramicidin S, as determined by n.m.r. studies in methanol,[29] is significant in this context. This analogue, without the leucine NH groups, cannot obviously form the β-sheet of the type found in the crystal structure of gramicidin S itself. The n.m.r. data for the analogue are compatible with a structure incorporating consecutive Type II' D-Phe-L-Pro and Type I L-Pro-L-Val bends. The occurrence in the present structure of a similar arrangement involving DL- and LL-bends establishes the need for considering such conformations in D–L–L sequences with L-Pro as the central residue, when exploring the biologically active conformations of gramicidin S and its analogues.

It has been reported [30] that gramicidin S retained some antimicrobial activity when D-Phe was replaced by D-Ala or Gly while no activity was observed when it was replaced by L-Ala or α-aminoisobutyric acid. A Type II' β-bend can be formed only if its first corner is occupied by a D or glycyl residue. Therefore the above experimental observation shows that the preference of a Type II' β-bend of the kind observed in the present structure, and postulated in the structure of bis-*N*-methyl-leucylgramicidin S, is perhaps a requirement for retaining the biological activity of the antibiotic.

Crystal Structure.—The arrangement of molecules in the crystal is shown in Figure 3. The crystal structure is devoid of intermolecular hydrogen bonds and is stabilised by van der Waals interactions.

We thank Dr. P. Balaram and Mr. Y. V. Venkatachalapathi for supplying the samples used in this investigation

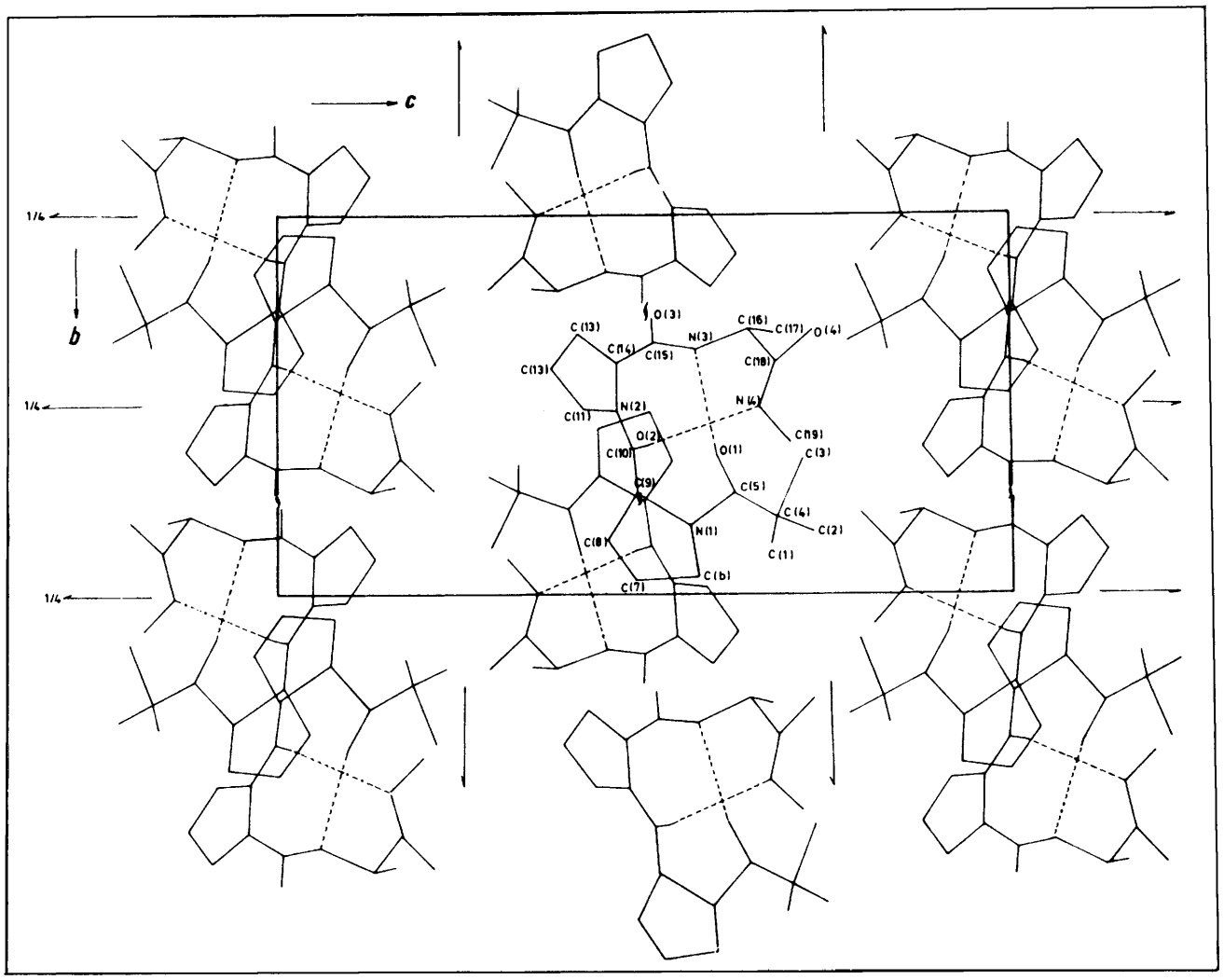


FIGURE 3 The crystal structure of the molecule as viewed along the *a* axis. Hydrogen bonds are shown by broken lines

and for many discussions. Financial assistance from the University Grants Commission, India, and the Indian National Science Academy is acknowledged.

[0/779 *Received*, 21*st May*, 1980]

REFERENCES

[1] P. Y. Chou and G. D. Fasman, *J. Mol. Biol.*, 1977, **115**, 135.
[2] P. Y. Chou and G. D. Fasman, *Ann. Rev. Biochem.*, 1978, **47**, 251.
[3] I. L. Karle in ' Peptides: Chemistry, Structure and Biology,' eds. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, Michigan, 1975, pp. 139—143.
[4] C. M. K. Nair, M. Vijayan, Y. V. Venkatachalapathi, and P. Balaram, *J.C.S. Chem. Comm.*, 1979, 1183.
[5] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.
[6] D. W. J. Cruickshank, A. Bujosa, F. M. Lovell, and M. R. Truter, in ' Computing Methods and the Phase Problem in *X*-Ray Analysis,' eds. R. Pepinsky and J. M. Robertson, Pergamon, Oxford, 1961, p. 45.
[7] D. T. Cromer and J. T. Waber, *Acta Cryst.*, 1965, **18**, 104.
[8] R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.
[9] D. F. Detar and N. Luthra, *J. Amer. Chem. Soc.*, 1977, **99**, 1232.
[10] R. B. Corey and L. Pauling, *Proc. Roy. Soc.*, 1953, **B141**, 10.
[11] M. Vijayan, in ' Handbook of Biochemistry and Molecular Biology, Proteins, Vol. II,' ed. G. D. Fasman, C.R.C. Press, Cleveland, 1976, p. 742.
[12] G. Kartha, T. Ashida, and M. Kakudo, *Acta Cryst.*, 1974, **B30**, 1861.
[13] H. Nishihara, K. Nishihara, T. Uefuji, and N. Sakota, *Bull. Chem. Soc. Japan*, 1975, **48**, 553.
[14] IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, 1970, **9**, 3471.
[15] T. Ashida and M. Kakudo, *Bull. Chem. Soc. Japan*, 1974, **47**, 1129.
[16] S. S. Zimmerman and H. A. Scheraga, *Biopolymers*, 1977, **16**, 811.
[17] R. Chandrasekharan, A. V. Lakshminarayanan, U. V. Pandya, and G. N. Ramachandran, *Biochim. Biophys. Acta*, 1973, **303**, 14.
[18] I. L. Karle, *J. Amer. Chem. Soc.*, 1975, **97**, 4379.
[19] A. D. Rudko and B. W. Low, *Acta Cryst.*, 1975, **B31**, 713.
[20] T. Ueki, T. Ashida, M. Kakudo, Y. Sasada, and Y. Katsube, *Acta Cryst.*, 1969, **B25**, 1840.
[21] T. Ueki, S. Bando, T. Ashida, and M. Kakudo, *Acta Cryst.*, 1971, **B27**, 2219.
[22] C. Lecomte, A. Aubry, and J. Protas, *Acta Cryst.*, 1974, **B30**, 1992.
[23] T. Ashida, I. Tanaka, T. Shimonishi, and M. Kakudo, *Acta Cryst.*, 1977, **B33**, 3054.
[24] A. Aubry, J. Protas, G. Boussard, and M. Marraud, *Acta Cryst.*, 1977, **B33**, 2399.
[25] S. K. Brahmachari, T. N. Bhat, V. Sudhakar, M. Vijayan,

R. S. Rapaka, R. S. Bhatnagar, and V. S. Ananthanarayanan, *J. Amer. Chem. Soc.*, in the press.
[26] R. Nagaraj, N. Shamala, and P. Balaram, *J. Amer. Chem. Soc.*, 1979, **101**, 16.
[27] N. Shamala, R. Nagaraj, and P. Balaram, *J.C.S. Chem. Comm.*, 1978, 996.
[28] S. E. Hull, R. Karlsson, P. Main, M. M. Woolfson, and E. J Dodson, *Nature*, 1978, **275**, 206.
[29] N. G. Kumar, N. Izumiya, M. Miyoshi, H. Sugano, and D. W. Urry, *J. Amer. Chem. Soc.*, 1975, **97**, 4105.
[30] M. Kawai and U. Nagai, *Biopolymers*, 1978, **17**, 1549.