Table I

CF_3COOH	3.0	4.0	6.8	7.3	8.0	10.0
T_c (° C)			25	32	37	>45
f_H^{max}	0.72	0.63		0.56	0.52	0.06[a]

[a] Measured at 45°.

cause of a similar mixture of solvents used. In both cases viscosity minima are found in the region of low helicity, but their intensities appear to be very different.

The dependence of molecular dimensions as a function of the degree of helicity in the transition region was treated theoretically by Nagai[7] and by Ptitsyn and Skvortsov.[8,17] The theoretical curves can pass through a minimum, their shape depending on the degree of cooperativity of the transition and on the degree of polymerization. Also long-range interactions cannot be neglected.[17] Taking into account the comparable degrees of polymerization of $(BzlGlu)_n$ in the paper of Teramoto[6] and of $(CbzLys)_n$ in our work it follows that the difference in the viscosity behavior of both polymers must be due to either a different degree of cooperativity of the transition or some influence of long-range interactions. The cooperativity parameters σ of both polypeptides have been determined by several authors[10,18] and that of $(CbzLys)_n$ found to be lower than that of $(BzlGlu)_n$, *i.e.* the transition of $(CbzLys)_n$ is more cooperative. This finding is at variance with the appearance of a deeper viscosity minimum for $(CbzLys)_n$ as well as with the low slope of the $[\eta] - f_H$ function in the region $0.1 \leq f_H \leq 0.6$.

The influence of the long-range interaction on molecular dimensions can be expressed by the expansion coefficient α.[19] If α is pH or solvent composition dependent, it can affect considerably the $[\eta]$ *vs.* f_H function and consequently the position of the viscosity minimum.[15,17] The relative independence of f_H in the region $0.1 \leq f_H \leq 0.6$ (which can be due to either small σ or high α) and the presence of a deep viscosity minimum outside this region seem to indicate strong long-range interactions of $(CbzLys)_n$ in the system. In this connection the findings of Buděšinský and Bláha[20] could be of importance. These authors have found by nmr spectroscopy that in solutions of model compounds having the benzylcarbonyl-protective group and containing trifluoroacetic acid a strong interaction occurs between the trifluoroacetic acid molecules and one of the urethane oxygen atoms. In these solutions a fast exchange with the NH proton has been observed resulting in the decoupling of the adjacent methylene proton. The behavior of $(CbzLys)_n$ in the mixed solvents of trifluoroacetic acid–$CHCl_3$ is assumed to be consistent with the suggestion of a protonated urethane oxygen. The partially protonated side chain of the $(CbzLys)_n$ could explain the marked difference in the long-range interactions between the $(CbzLys)_n$ and $(BzlGlu)_n$ which follows from the viscosity behavior of both polymers in solutions containing strong organic acids.

(17) A. M. Skvortsov and O. B. Ptitsyn, *Mol. Biol.*, 2, 710 (1968).
(18) F. Gaskin and J. T. Yang, *Biopolymers*, 10, 631 (1971).
(19) G. Hagnauer and W. G. Miller, *Biopolymers*, 9, 589 (1970).
(20) M. Buděšínský and K. Bláha, in preparation.

Calculation of the Conformation of *cyclo*-Hexaglycyl[1]

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ABSTRACT: A systematic search of the minimum-energy conformations (MEC's) of *cyclo*-hexaglycyl has been carried out under the assumption of rigidity of bond lengths and bond angles, and of planarity of the trans peptide groups. Conformations of this cyclic chain molecule with one of the possible symmetries, C_6, S_6, C_3, I, and C_2, are generated exactly with a computer using mathematical methods developed previously. All 24 MEC's with symmetries are found, located, and characterized. Fourteen of them are stabilized by intramolecular hydrogen bonds; they can be classified into six types depending on the intramolecular hydrogen-bonding network. Conformations with I symmetry, which exist in a crystal of this molecule, are found to be similar to some of the MEC's with I symmetry. However, the small differences between the observed and calculated conformations indicate the important role played by intermolecular interactions in the crystal of this molecule. Many of the MEC's are consistent with those inferred from nmr and ir spectroscopic data from solution.

There is presently considerable interest in the conformations of cyclic hexapeptides. For these, *cyclo*-hexaglycyl is of particular importance because it may be regarded as a reference molecule in the sense that differences in backbone conformation of *cyclo*-hexaglycyl and other cyclic hexapeptides can be interpreted in terms of the influence of the side chains of the non-glycyl residues. The purpose of this paper is to report the results of a systematic search of the minimum-energy conformations (MEC's) of *cyclo*-hexaglycyl under the assumption of fixed bond lengths and bond angles and planar trans peptide groups. This molecule can assume conformations with C_6, S_6, C_3, I, and C_2 symmetries as well as conformations with no symmetry. In this paper, we limit ourselves to conformations having one of these possible symmetries, which can be generated exactly using the mathematical

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Table I
Values of Coefficients $a_{\alpha(i)\alpha(j)}$ in (kcal Å^6)/mol

Type	H^α	C	N	O	H^N
H^α	47	128	125	124	47
C	128	370	366	367	128
N	125	366	363	365	125
O	124	367	365	367	56
H^N	47	128	125	56	47

Table II
Values of Coefficients $b_{\alpha(i)\alpha(j)}$ in (cal Å)^{12}/mol × 10^6

Type	H^α	C	N	O	H^N
H^α	4.5	38	27	25	4.5
C	38	286	216	205	38
N	27	216	161	153	27
O	25	205	153	145	2.42
H^N	4.5	38	27	2.42	4.5

methods described elsewhere.[3] The MEC's, located either by computing complete energy maps (in the cases of C_6, S_6, C_3, and I symmetries) and/or by carrying out systematic energy minimization (in the case of I and C_2 symmetries), are compared with experimental results.

I. Energy Parameters

The energy parameters used in this paper are the same as the ones employed elsewhere[4] in the calculation of the conformational energy of *cyclo*-(Gly_3Pro_2). In the energy expression employed, the parameters for the nonbonded interaction between pairs of atoms participating in the formation of a hydrogen bond (*i.e.*, the amide hydrogen atom and the acceptor oxygen atom) were modified so as to account for the energy of the hydrogen bond.[4,5] This hydrogen-bond energy has a rather weak directional dependence, and makes a considerable contribution to the total conformational energy even when the NH···O "bond angle" (vertex at the hydrogen) varies from linearity by as much as 90°. In fact, such "bent" hydrogen bonds are found to exist in some of the MEC's reported in this paper.

The total conformational energy E_{tot} is taken as a sum of three contributions, torsional energy E_{tor}, nonbonded interaction energy E_{nb}, and electrostatic interaction energy E_{es}.

$$E_{tot} = E_{tor} + E_{nb} + E_{es} \quad (1)$$

where

$$E_{tor} = (U_\psi/2)\sum_{i=1}^{6} \cos 3\psi_i - (U_\phi/2)\sum_{i=1}^{6} \cos 3\phi_i \quad (2)$$

with $U_\phi = 0.58$ kcal/mol and $U_\psi = 0.2$ kcal/mol.

$$E_{nb} = \sum_{i<j}[b_{\alpha(i)\alpha(j)}/r_{ij}^{12} - a_{\alpha(i)\alpha(j)}/r_{ij}^{6}] \quad (3)$$

$$E_{es} = c\sum_{i<j} e_{\beta(i)}e_{\beta(j)}/r_{ij} \quad (4)$$

and the summations extend over all pairs of atoms whose interatomic distance r_{ij} is a function of the variable dihedral angles ϕ and ψ, expressed according to a recently adopted convention.[6] In eq 3, the type of ith atom [which is any one of H^α (hydrogen on C^α), C, N, O, or H^N (hydrogen on N)] is designated as $\alpha(i)$, and the values of $a_{\alpha(i)\alpha(j)}$ and $b_{\alpha(i)\alpha(j)}$ are given in Tables I and II, respectively. In eq 4, the value of the coefficient c is taken to be 83.0 (kcal Å)/mol, which is equivalent to a choice of dielectric constant D of 4.0; the electronic partial charges of each atom $e_{\beta(i)}$ are given in Table III.

Table III
Electronic Partial Charge $e_{\beta(i)}$ of the ith Atom of Type $\beta(i)$ in Electronic Unit

$\beta(i)$	$e_{\beta(i)}$
C^α	0.000
H^α	0.051
C′	0.318
O	−0.422
N	−0.202
H^N	0.204

Table IV
Cartesian Coordinates[a] of Atoms in the (2i − 1)th Local Coordinate System (CH_2 Unit)

	Ångstrom Units		
Atom	x	y	z
N_i[b]	0.000	0.000	0.000
$C^\alpha{}_i$	1.470	0.000	0.000
$H^{\alpha 1}{}_i$	1.803	−0.471	0.817
$H^{\alpha 2}{}_i$	1.803	−0.471	−0.817
$C'{}_i$[b]	1.980	1.443	0.000

[a] The C–H bond distance is taken as 1 Å. [b] Nonproper atoms, *i.e.*, those not belonging to the (2i − 1)th unit; they are included in this Table because they have constant coordinates with respect to the (2i − 1)th local coordinate system.

Table V
Cartesian Coordinates[a] of Atoms in the 2ith Local Coordinate System (CONH Unit)

	Ångstrom Units		
Atom	x	y	z
$C^\alpha{}_i$[b]	0.000	0.000	0.000
$C'{}_i$	1.530	0.000	0.000
O_i	2.169	−1.063	0.000
N_{i-1}	2.067	1.206	0.000
H_{i-1}	1.522	2.045	0.000
$C^\alpha{}_{i-1}$[b]	3.519	1.436	0.000

[a] These coordinates correspond to a planar trans peptide unit with Pauling–Corey geometry.[7] [b] Nonproper atoms.

II. Generation of Conformations and Energy Minimization

The conformations of *cyclo*-hexaglycyl with symmetry are generated by the procedure described elsewhere.[3] In subsection A, we discuss some general considerations which are common to all symmetries, and in the subsequent subsections we describe those aspects which are specific to each type of symmetry.

A. General Considerations. In this cyclic molecule, there are 12 dihedral angles ϕ_i and ψ_i[6] around which rotation can occur, with i = 1, 2, ···, 6. The molecule contains two kinds of units (in the sense defined in A[3]), *viz.*, $-CH_2-$ and -CONH-, both of which have a plane of sym-

(3) N. Gō and H. A. Scheraga, *Macromolecules*, 6, 273 (1973); this paper will be referred to hereinafter as A, with equation number cited as (A-x).
(4) N. Gō and H. A. Scheraga, *Macromolecules*, 3, 188 (1970).
(5) D. Poland and H. A. Scheraga, *Biochemistry*, 6, 3791 (1967).
(6) IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, 9, 3471 (1970).

metry in which all backbone atoms lie. If we designate these two types of unit by F_1 and F_2, respectively, then the molecule may be expressed as

$$\text{—}(F_1F_2)_6\text{—}$$

where $F_1 = F_1^*$ and $F_2 = F_2^*$ (F_i^* being a mirror image of F_i as defined in paper A). According to the theory developed in paper A, this molecule may have C_6, S_6, C_3, I, C_2, and C_1 symmetries (C_1 meaning no symmetry), and all cases except C_1 will be treated in this paper.

We assign 12 local coordinate systems with serial numbers from 1 to 12, one to each unit in the molecule, *i.e.*, $(CH_2)_1$, $(CONH)_2$, $(CH_2)_3$, etc. In the CH_2 coordinate systems (odd indices) and in the (CONH) systems (even indices), the cartesian coordinates of the atoms are those given in Tables IV and V, respectively. These coordinates, based on Pauling–Corey geometry,[7] were used in earlier calculations on a cyclic peptide.[4]

If a given point in space is expressed by a position vector $\mathbf{r}_i$ with respect to the ith local coordinate system, the relations between the $\mathbf{r}_i$'s of adjacent coordinate systems are given[3] by

$$\mathbf{r}_{2i-2} = \mathbf{T}_\alpha\mathbf{R}(\phi_i + \pi)\mathbf{r}_{2i-1} + \mathbf{p}_0 \quad (5)$$

$$\mathbf{r}_{2i-1} = \mathbf{T}_\beta\mathbf{R}(\psi_i)\mathbf{r}_{2i} + \mathbf{p}_1 \quad (6)$$

where

$$\mathbf{R}(\theta) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & -\sin\theta \\ 0 & \sin\theta & \cos\theta \end{bmatrix} \text{ with } \theta \equiv \phi_i + \pi \text{ or } \psi_i \quad (7)$$

$$\mathbf{T}_\alpha = \begin{bmatrix} \cos\alpha & -\sin\alpha & 0 \\ \sin\alpha & \cos\alpha & 0 \\ 0 & 0 & 1 \end{bmatrix} \text{ with } \alpha = 9.0° \quad (8)$$

$$\mathbf{T}_\beta = \begin{bmatrix} \cos\beta & -\sin\beta & 0 \\ \sin\beta & \cos\beta & 0 \\ 0 & 0 & 1 \end{bmatrix} \text{ with } \beta = 70.5° \quad (9)$$

$$\mathbf{p}_0 = \begin{bmatrix} 2.067 \\ 1.206 \\ 0.000 \end{bmatrix} \quad (10)$$

$$\mathbf{p}_1 = \begin{bmatrix} 1.470 \\ 0.000 \\ 0.000 \end{bmatrix} \quad (11)$$

using the new convention[6] for the nomenclature of angles, etc.

According to the theory developed previously,[3] the number of independent variables in conformations with C_6, S_6, C_3, I, and C_2 symmetries is 0, 1, 2, 3, and 4, respectively. Table VI summarizes the choices of the sets of independent variables adopted here, and the relationships among the values of the dependent and independent variables in the molecule. The ranges of the independent variables in which the conformational energy should be examined are also shown. These ranges are determined by the following two considerations. (a) Because this molecule consists of units, each of which is enantiomorphic to itself, a conformation which is a mirror image of any conformation having a certain symmetry is also an allowed conformation with the same symmetry. The conformational energies of these two structures are the same, and the dihedral angles in one of the structures are obtained by reversing the signs of the dihedral angles in the other. Therefore, only one of them has to be examined. This property is used to limit the range of study to the one in which the value of the first variable is in the range of $[0,\pi]$. (b) Because the molecule is a cyclic homopolymer, structures that can be obtained by shifting the values of ϕ_i and ψ_i by one residue are, in fact, the same structure. This property is used to limit the range of study to structures in which the absolute value of the first ϕ_i is not larger than the absolute value of other ϕ_i's, *i.e.*, we designate the residue with the smallest absolute value of ϕ as the first one. The case of I symmetry is special because a set of angles that are not consecutive are taken as the independent variables. The reason why this special treatment was adopted in the case of I symmetry is explained in subsection E of this section.

B. Case of C_6 Symmetry. In this case, the matrix **U** of eq A-7 is given by **AXBY**, where $\mathbf{A} = \mathbf{T}_\alpha$, $\mathbf{B} = \mathbf{T}_\beta$, $\mathbf{X} = \mathbf{R}(\phi_1 + \pi)$ and $\mathbf{Y} = \mathbf{R}(\psi_1)$. The vector **p** of eq A-8 becomes the following constant vector.

$$\mathbf{p} = \begin{bmatrix} 2.067 + 1.470\cos\alpha \\ 1.206 + 1.470\cos\alpha \\ 0 \end{bmatrix} \quad (12)$$

From these quantities, those in eq A-39 and A-40 (c_{ij} in these equations are elements of a matrix defined by eq A-36) can be calculated as functions of an unknown ϕ_1. If the left-hand side of eq A-42 is written as $f(\phi_1)$, the graph of $f(\phi_1)$ is given in Figure 1, for the range of ϕ_1 given in Table VI.

Since the value of $f(\phi_1)$ never vanishes, no conformation of *cyclo*-hexaglycyl with C_6 symmetry can exist.[8] It should be noted that this conclusion is based solely on geometric and not energetic criteria.

C. Case of S_6 Symmetry. The matrices **E** and **Z** in eq A-66 are $\mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta$ and $\mathbf{R}(\psi_1)$, respectively. Hence, the quantities in eq A-69 are given as functions of an independent variable ϕ_1. The value of ψ_1 is given by eq A-71, for the range satisfying the inequality of eq A-70. It turns out that the inequality is satisfied for all values of ϕ_1, so that there are always two conformations with S_6 symmetry for any value of ϕ_1. The conformational energies were computed for each of these conformations and are shown in Figure 2. Four MEC's appear in the curve of Figure 2, and are named as shown. The characterization of these MEC's will be given later. It should be mentioned that these four conformations are *local* MEC's among those conformations with S_6 symmetry, *i.e.*, the conformational energy will get larger for any small conformational change as long as such a conformational change preserves the S_6 symmetry. However, it does not necessarily imply that the conformational energy should get larger for any small symmetry-breaking conformational change. Any conformations which have S_6 symmetry also have C_3 and I symmetry. Therefore, any MEC's in the S_6 space (one-dimensional space corresponding to conformations with S_6 symmetry) appear again in the C_3, I, and C_1 spaces (two-, three- and six-dimensional spaces corresponding to conformations with the C_3, I, and C_1 symmetries, respectively). The possibility that an energy min-

(7) L. Pauling, "Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 282.

(8) When $\tau(NC^\alpha C')$ is allowed to deviate from the tetrahedral value, conformations with C_6 symmetry can be formed, as was found by Ramakrishnan and Sarathy.[9]

(9) C. Ramakrishnan and K. P. Sarathy, *Int. J. Protein Res.*, 1, 103 (1969).

Table VI
Choice of Independent Variables and Their Range of Variation

Symmetry	Independent Variables	Dependent Variables	Symmetry Relations	Range of Variation
C_6	None	ϕ_1, ψ_1	$\phi_1 = \phi_2 = \phi_3 = \phi_4 = \phi_5 = \phi_6$ $\psi_1 = \psi_2 = \psi_3 = \psi_4 = \psi_5 = \psi_6$	$0 \leq \phi_1 \leq \pi$ $-\pi < \psi_1 \leq \pi$
S_6	ϕ_1	ψ_1	$\phi_1 = -\phi_2 = \phi_3 = -\phi_4 = \phi_5 = -\phi_6$ $\psi_1 = -\psi_2 = \psi_3 = -\psi_4 = \psi_5 = -\psi_6$	$0 \leq \phi_1 \leq \pi$ $-\pi < \psi_1 \leq \pi$
C_3	ϕ_1, ψ_1	ϕ_2, ψ_2	$\phi_1 = \phi_3 = \phi_5, \phi_2 = \phi_4 = \phi_6$ $\psi_1 = \psi_3 = \psi_5, \psi_2 = \psi_4 = \psi_6$	$0 \leq \phi_1 \leq \pi, \phi_1 \leq \lvert\phi_2\rvert$ $-\pi < \psi_1, \psi_2 \leq \pi$
I	ϕ_1, ϕ_2, ϕ_3	ψ_1, ψ_2, ψ_3	$\phi_1 = -\phi_4, \phi_2 = -\phi_5, \phi_3 = -\phi_6$ $\psi_1 = -\psi_4, \psi_2 = -\psi_5, \psi_3 = -\psi_6$	$0 \leq \phi_1 \leq \pi, \phi_1 \leq \lvert\phi_2\rvert, \lvert\phi_3\rvert$ $-\pi < \psi_1, \psi_2, \psi_3 \leq \pi$
C_2	$\phi_1, \psi_1, \phi_2, \psi_2$	ϕ_3, ψ_3	$\phi_1 = \phi_4, \phi_2 = \phi_5, \phi_3 = \phi_6$ $\psi_1 = \psi_4, \psi_2 = \psi_5, \psi_3 = \psi_6$	$0 \leq \phi_1 \leq \pi, \phi_1 \leq \lvert\phi_2\rvert, \lvert\phi_3\rvert$ $-\pi < \psi_1, \psi_2, \psi_3 \leq \pi$

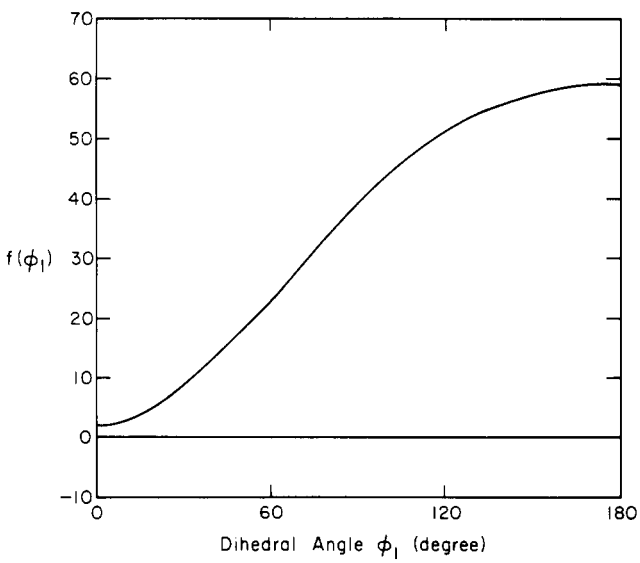

Figure 1. Graph of $f(\phi_1)$, showing that no conformation of *cyclo*-hexaglycyl with C_6 symmetry exists.

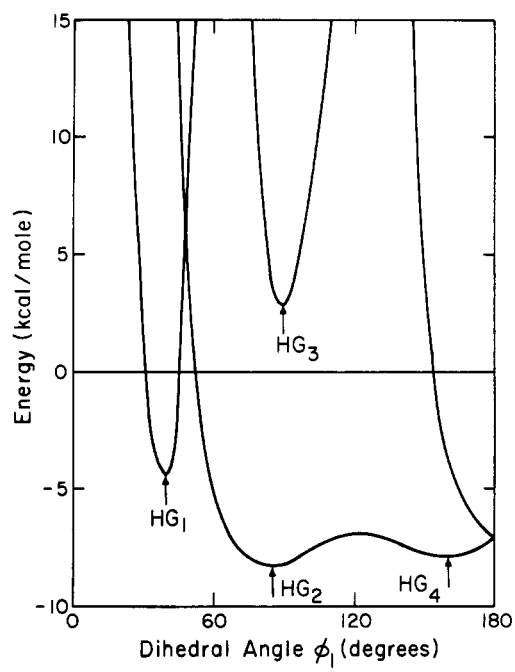

Figure 2. Conformational energy of *cyclo*-hexaglycyl with S_6 symmetry as a function of the independent variable ϕ_1. Four energy minima are marked. The two curves which cross at $\phi_1 = 180°$ extend smoothly into the region where ϕ_1 is negative.

imum in the S_6 space is a saddle point in a larger C_3, I, or C_1 space cannot be excluded. However, as will be described below, all four energy minima in the S_6 space were also found to be energy minima in the C_3 and I spaces. Because conformations with C_1 symmetry (*i.e.*, no symmetry) were not explored in this paper, it is not known whether the energy minima (obtained in this paper under the assumption of one of the symmetries, S_6, C_3, I, or C_2) are energy minima in the full six-dimensional space.

D. Case of C_3 Symmetry. In this case, there are four variable dihedral angles in a symmetry unit. The calculation of the quantity on the left-hand side of eq A-42 is straightforward, where $\alpha', \cdots, \dot{\gamma}''$ in eq A-42 are given by eq A-39 and A-40, and c_{ij} and p_i in eq A-39 and A-40 are the i,j element of a matrix **C** defined by eq A-36 [or, in this case, $\mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_1)\mathbf{T}_\alpha\mathbf{R}(\phi_2 + \pi)\mathbf{T}_\beta$], and the ith component of the vector **p** defined by eq A-19 (or, in this case, **p** is given by eq 13). Figure 3 shows the graph of

$$\mathbf{p} = \mathbf{p}_0 + \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{p}_1 + \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_1)\mathbf{p}_0 + \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_1)\mathbf{T}_\alpha\mathbf{R}(\phi_2 + \pi)\mathbf{p}_1 \quad (13)$$

the left-hand side of eq A-42 for fixed ϕ_1 and ψ_1 (taken arbitrarily as 120 and 60°, respectively) and varying ϕ_2. In Figure 3 there are two solutions of eq A-42. Similarly, two solutions of eq A-42 were always found to exist for any set of values of ϕ_1 and ψ_1. The values of ϕ_2 and ψ_2 were determined by eq A-42 and A-41, respectively. Only conformations whose dihedral angles fall in the range given in Table VI (*i.e.*, $0 \leq \phi_1 \leq \pi$ and $\phi_1 \leq |\phi_2|$) are taken, and those conformations whose dihedral angles fall outside this range are not considered for the reason stated in section IIA. The energies for the conformations within these ranges were calculated and plotted in Figure 4. Because there are two possible conformations for any set of values of ϕ_1 and ψ_1, the energy map consists of two portions (designated as a and b in Figure 4). The range of variation of ϕ_1 is $0 \leq \phi_1 \leq \pi$ as given in Table VI. The energy map is not given in the whole range of $0 \leq \phi_1 \leq \pi$ and $-\pi \leq \psi_1 \leq \pi$, because of the other condition $\phi_1 \leq |\phi_2|$. On the boundary of the map in Figure 4, the relation $\phi_1 = |\phi_2|$ holds. In fact, on the boundary shown by ——— the relation $\phi_1 = \phi_2$ holds (but ψ_1 not equal to ψ_2). On the boundary shown by ——— the relation $\phi_1 = -\phi_2$ (and also $\psi_1 = -\psi_2$) holds. Conformations corresponding to the boundary shown by —·—, in fact, have S_6 symmetry and they are nothing but the ones generated and studied before in section IIC. Any conformations with S_6 symmetry also have C_3 symmetry. Therefore, any conformations found under the assumption of S_6 symmetry must appear again under the treatment assuming C_3 symmetry. In fact, they appeared on the boundary in Figure 4. All the MEC's obtained in section IIC were also found to corre-

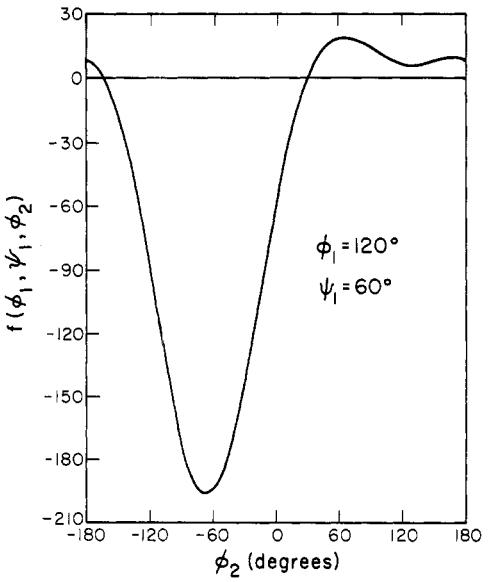

Figure 3. Graph of $f(\phi_1,\psi_1,\phi_2)$ for fixed ϕ_1 and ψ_1 (taken arbitrarily as 120 and 60°, respectively) and varying ϕ_2. The two solutions of the equation $f(\phi_1, \psi_1, \phi_2) = 0$ correspond to the two conformations of *cyclo*-hexaglycyl with C_3 symmetry.

spond to MEC's in the expanded two-dimensional space corresponding to C_3 symmetry. They are marked by a plus sign in the map and designated by the names shown. Three more minima (HG_5, HG_6, and HG_7) were found besides those on the boundary. The locations of these minima were determined precisely by calculating the energies at finer grids in the vicinity of the regions of the minima. The characterization of these MEC's will be given later.

E. Case of I Symmetry. In this case, there are six variable dihedral angles in a symmetry unit. At the beginning of this study, three consecutive angles ϕ_1, ψ_1, and ϕ_2 were chosen as the independent variables, and the other three in the symmetry unit, ψ_2, ϕ_3, and ψ_3, were determined by solving the algebraic equations worked out in paper A.[3] When this choice of independent variables was made, sometimes no solutions were found for the other three angles, and sometimes one or two solutions were found, depending on the values of the three independent angles. However, in the course of the study, it was found, that, if the nonconsecutive angles ϕ_1, ϕ_2, and ϕ_3 are chosen as the independent variables, there are always two solutions for the other three angles ψ_1, ψ_2, and ψ_3 in the symmetry unit. Therefore, the calculation of the conformational energy was performed for this choice of independent angles. In this case, the matrices in eq A-54 are as follows: $\mathbf{A} = \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta$, $\mathbf{X} = \mathbf{R}(\psi_1)$, $\mathbf{B} = \mathbf{T}_\alpha\mathbf{R}(\phi_2 + \pi)\mathbf{T}_\beta$, $\mathbf{Y} = \mathbf{R}(\psi_2)$, $\mathbf{C} = \mathbf{T}_\alpha\mathbf{R}(\phi_3 + \pi)\mathbf{T}_\beta$, $\mathbf{Z} = \mathbf{R}(\psi_3)$. The values of the dependent variables ψ_1, ψ_2, and ψ_3 were determined by straightforward application of eq A-62, A-63, and A-64. The fact that there are always two solutions for ψ_1, ψ_2, and ψ_3 for any value of ϕ_1, ϕ_2, and ϕ_3 corresponds to the fact that eq A-61 is always satisfied when the matrices **A**, **B**, and **C** have the form stated above. The range of variation of the independent angles is the one given in Table VI. The conformational energies were calculated for both conformations at each of 9066 grid points of a three-dimensional cubic lattice of 10° spacing within the above range. The minima were located roughly on this grid. Then the energy was minimized in the continuous space by starting from these rough minimum-energy grid points in order to locate the energy minima more precisely. A generalized partan method[10] was used for this minimization. Twelve local minima were located, four of

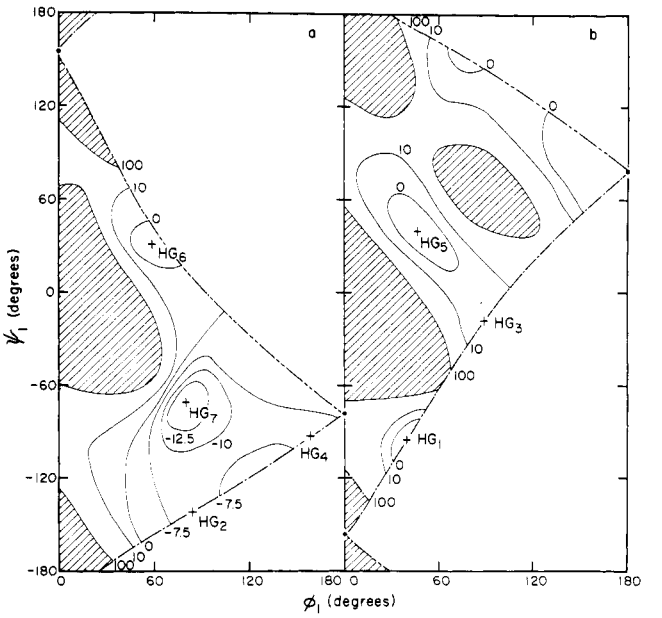

Figure 4. Conformational energy (in kcal/mole) of *cyclo*-hexaglycyl with C_3 symmetry as a function of the independent variables ϕ_1 and ψ_1. There are always two conformations with C_3 symmetry for any value of (ϕ_1, ψ_1); these are shown in a and b. However, the energy is given only for the range of variation shown in Table VI. Seven energy minima are marked. The regions where the energy exceeds 100 kcal/mol are shaded.

Table VII
Number of Conformations that Converged to Each of the Nine Local Minima

Local Energy Min	No. of Conformations That Converged to Min
HG_{16}	50
HG_{17}	22
HG_{18}	51
HG_{19}	53
HG_{20}	43
HG_{21}	76
HG_{22}	14
HG_{23}	14
HG_{24}	21

which were found on the boundary of the region defined in Table VI; they again correspond to conformations found under the assumption of S_6 symmetry. Thus, all energy minima in the S_6 space also turn out to be energy minima in the I space. The other eight minima were found *within* the range (*i.e.*, not on the boundary) given in Table VI. These minima were designated HG_8 to HG_{15}. The characterization of the corresponding conformations will be given later.

F. Case of C_2 Symmetry. In this case, there are six variable dihedral angles in a symmetry unit, four of which are independent. We chose these as ϕ_1, ψ_1, ϕ_2, and ψ_2 as indicated in Table VI. Then the matrices and vectors of eq A-20 to A-23 are as follows

$$\mathbf{A} = \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_1)\mathbf{T}_\alpha\mathbf{R}(\phi_2 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_2)\mathbf{T}_\alpha \quad (14)$$

$$\mathbf{B} = \mathbf{T}_\beta \quad (15)$$

$$\mathbf{a} = \mathbf{p}_0 + \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{p}_1 + \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_1)\mathbf{p}_0 + \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_1)\mathbf{T}_\alpha\mathbf{R}(\phi_2 + \pi)\mathbf{p}_1 + \mathbf{T}_\alpha\mathbf{R}(\phi_1 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_1)\mathbf{T}_\alpha\mathbf{R}(\phi_2 + \pi)\mathbf{T}_\beta\mathbf{R}(\psi_2)\mathbf{p}_0 \quad (16)$$

$$\mathbf{b} = \mathbf{p}_1 \quad (17)$$

(10) R. A. Scott, G. Vanderkooi, R. W. Tuttle, P. M. Shames, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, 58, 2204 (1967).

Table VIII
Characterization of 24 Local Energy Minima

Symmetry	HG_1 S_6	HG_2 S_6	HG_3 S_6	HG_4 S_6	HG_5 C_3	HG_6 C_3	HG_7 C_3	HG_8 I	HG_9 I	HG_{10} I	HG_{11} I	HG_{12} I
ϕ_1[a]	39.22	83.68	89.13	159.37	46.54	59.44	79.02	37.28	45.93	48.19	51.28	64.22
ψ_1	−94.78	−141.70	−17.81	−92.91	39.64	31.38	−71.12	47.62	53.84	52.81	44.67	179.36
ϕ_2	−39.22	−83.68	−89.13	−159.37	−159.33	71.27	−167.48	−161.54	−153.20	66.97	88.24	72.42
ψ_2	94.78	141.70	17.81	92.91	44.53	166.35	163.64	42.14	−71.00	41.63	−51.00	−74.86
ϕ_3	39.22	83.68	89.13	159.37	46.54	59.44	79.02	76.01	−173.84	−155.42	−65.68	−153.93
ψ_3	−94.78	−141.70	−17.81	−92.91	39.64	31.38	−71.12	−65.96	−60.45	169.24	171.91	−56.61
ϕ_4	−39.22	−83.68	−89.13	−159.37	−159.33	71.27	−167.48	−37.28	−45.93	−48.19	−51.28	−64.22
ψ_4	94.78	141.70	17.81	92.91	44.53	166.35	163.64	−47.62	−53.84	−52.81	−44.67	−179.36
ϕ_5	39.22	83.68	89.13	159.37	46.54	59.44	79.02	161.54	153.20	−66.97	−88.24	−72.42
ψ_5	−94.78	−141.70	−17.81	−92.91	39.64	31.38	−71.12	−42.14	71.00	−41.63	51.00	74.86
ϕ_6	−39.22	−83.68	−89.17	−159.37	−159.33	71.27	−167.48	−76.01	−173.84	155.42	65.68	153.93
ψ_6	94.78	141.70	17.81	92.91	44.53	166.35	163.64	65.96	60.45	−169.24	−171.91	56.61
E_{tot}[b]	−4.51	−8.24	2.77	−7.89	−5.70	−3.46	−14.52	−9.82	−6.53	−13.00	−14.25	−11.19
E_{tor}	−0.96	−0.82	−0.43	−0.91	−0.72	−1.34	−0.72	−0.46	−0.52	−0.71	−0.63	−0.61
E_{nb}	−12.37	−16.64	−5.34	−14.55	−16.67	−17.43	−18.61	−16.31	−17.31	−20.65	−20.68	−17.78
E_{es}	8.81	9.21	8.55	7.56	11.69	15.32	4.80	6.96	11.30	8.36	7.05	7.20

[a] Angles in degrees. [b] Energies in kcal/mol. E_{tot} = total conformational energy, E_{tor} = torsional energy, E_{nb} = nonbonded interaction

The dependent angles, ϕ_3 and ψ_3, are determined by a straightforward application of eq A-31 and A-32. The calculation was performed at 2160 grid points in the range of Table VI in the four-dimensional space of ϕ_1, ψ_1, ϕ_2, and ψ_2. The value of the independent angles at the grid points were chosen either as $\phi_1 = \pi i/3$, $\psi_1 = \pi j/3$, $\phi_2 = \pi k/3$, $\psi_2 = \pi l/3$, or $\phi_1 = (\pi/3)(i + \frac{1}{2})$, $\psi_1 = (\pi/3)(j + \frac{1}{2})$, $\phi_2 = (\pi/3)(k + \frac{1}{2})$, $\psi_2 = (\pi/3)(l + \frac{1}{2})$, where i, j, k, l are integers. Equation A-30 was found to be satisfied at about one-fourth of these grid points. Then the conformational energy was minimized by starting from each of the two conformations obtained at each of the grid points where eq A-30 was satisfied. The process of minimization can be visualized as a point moving in a four-dimensional space corresponding to ϕ_1, ψ_1, ϕ_2, and ψ_2. As the minimization proceeds, the points in the four-dimensional space sometimes approach and hit the border between the regions in which eq A-28 has solutions and has no solutions. If it occurred, such a conformation was discarded. After four cycles of energy minimization using the partan method and discarding conformations which hit the border, 352 temporary energy minima were obtained. As the energy minimization was carried further, 8 of the 352 conformations hit the border and were discarded. The remaining ones converged into nine different local minima. They are designated as HG_{16} to HG_{24}. The number of conformations that converged to each of the final local MEC's are shown in Table VII. From the fact that all of the local MEC's that were obtained were reached at least 14 times (on the average 38 times), it can almost surely be said that *all* local minima in the four-dimensional energy surface were found. The characterization of the MEC's thus obtained will be given in the next section.

III. Results and Discussion

From the foregoing, it is clear that all MEC's with S_6, C_3, or I symmetry have been found, and quite sure that all MEC's with C_2 symmetry have been located. These conformations are characterized in Table VIII and shown in Figure 5. The representations of HG_{18}, HG_{19}, HG_{20}, HG_{21}, and HG_{23} in Figure 5 are of the mirror images of the corresponding conformations whose dihedral angles are given in Table VIII. The total conformational energies and their components are also given in Table VIII. It can be seen that E_{tor} makes a minor contribution to E_{tot}, and thus plays a very minor role in determining the MEC. On the other hand, both E_{nb} and E_{es} are very important for determining the relative stabilities of the MEC's.

In Table IX we have listed the pairs of amide hydrogen atom and carbonyl oxygen atom whose interatomic distances are less than 2.45 Å, and can therefore be regarded as participating in hydrogen bonds. Only three types of hydrogen bonds $i \rightarrow j$ between an ith amide H and jth carbonyl O are found to occur in this molecule, *viz.*, those in which j is i, $i - 2$, or $i - 3$, depending on the relative positions of the amino acid residues to which the H and O atoms belong. Such hydrogen bonds lead to the formation of five-, seven-, and ten-membered rings, and are referred to as (C_5),[11] (C_7),[11] and (β bend)[12] conformations for $j = i$, $i - 2$, and $i - 3$, respectively. These pairs of H and O atoms are connected by dashed lines in Figure 5 in those cases where the interatomic distance is less than 2.45 Å. When only hydrogen bonds of the types $i \rightarrow i - 2$ and $i \rightarrow i - 3$ are considered, there are six types of networks of intramolecular hydrogen bonds that can exist in this molecule, as shown schematically in Figure 6. The networks in those MEC's with intramolecular hydrogen bonds are classified as one of these six types, and are so indicated in Table IX.

The four MEC's with S_6 symmetry are shown in Figure 5, as viewed along the symmetry axis. None of these four are very stable, *i.e.*, they do not have very low conformational energies. Conformation HG_1 is stabilized by weak type $i \rightarrow i - 2$ hydrogen bonds (with a long O···H distance of 2.50 Å). In conformations HG_2 and HG_4 the chains are rather extended, with a low-energy barrier between them (see Figure 2). Conformations intermediate between HG_2 and HG_4 (*e.g.*, on the curve in Figure 2, where ϕ_1 is in the range of ~100 to ~140°) can still have S_6 symmetry with their C=O and N-H bonds almost parallel to the symmetry axis (see Figure 5). Such conformations of *cyclo*-hexaglycyl are not stable compared to conformations that have lower symmetry than S_6. However, it may be possible that such conformations of some other cyclohexapeptides [*e.g.*, *cyclo*(LDLDLD), where L and D are L- and D-amino acids, respectively, with bulky

(11) M. Avignon and P. V. Huong, *Biopolymers*, 9, 427 (1970).
(12) C. M. Venkatachalam, *Biopolymers*, 6, 1425 (1968).

HG_{13} I	HG_{14} I	HG_{15} I	HG_{16} C_2	HG_{17} C_2	HG_{18} C_2	HG_{19} C_2	HG_{20} C_2	HG_{21} C_2	HG_{22} C_2	HG_{23} C_2	HG_{24} C_2
65.11	65.37	73.48	47.54	54.42	54.80	55.54	61.04	62.26	64.69	70.24	71.54
−85.56	−150.36	46.59	41.35	48.76	−103.88	45.13	−92.34	−93.66	−85.71	73.22	45.52
−157.91	−86.11	83.89	133.20	63.97	−86.83	91.17	−69.03	−95.61	−155.66	85.30	84.15
48.37	60.69	165.61	−53.37	34.29	55.14	−57.11	−43.62	48.34	46.29	−62.36	168.45
−167.56	170.01	76.00	167.42	−151.31	175.19	−58.11	160.87	68.80	−163.50	−162.42	72.48
173.40	−172.82	−150.12	−167.46	99.77	62.23	96.56	−174.40	−177.95	122.67	169.87	55.94
−65.11	−65.37	−73.48	47.54	54.42	54.80	55.54	61.04	62.26	64.69	70.24	71.54
85.56	150.36	−46.59	41.35	48.76	−103.88	45.13	−92.34	−93.66	−85.71	73.22	45.52
157.91	86.11	−83.89	133.20	63.97	−86.83	91.17	−69.03	−95.61	−155.66	85.30	84.15
−48.37	−60.69	−165.61	−53.37	34.29	55.14	−57.11	−43.62	48.34	46.29	−62.36	168.45
167.56	−170.01	−76.00	167.42	−151.31	175.19	−58.11	160.87	68.80	−163.50	−162.42	72.48
−173.40	172.82	150.12	−167.46	99.77	62.23	96.56	−174.40	−177.95	122.67	169.87	55.94
−15.17	−13.53	−8.77	−11.79	−12.11	−16.26	−21.76	−16.17	−14.27	−15.00	−12.09	−6.78
−0.86	−0.79	−0.71	−0.01	−1.05	−0.96	−0.83	−1.11	−0.60	−1.11	−0.47	−0.61
−18.87	−19.02	−18.81	−19.04	−21.11	−20.82	−24.72	−20.48	−20.40	−18.99	−19.95	−20.89
4.56	6.28	10.75	7.26	10.05	5.51	3.79	5.41	6.74	5.09	8.33	14.71

energy, E_{es} = electrostatic interaction energy.

side chains] are more stable. Then a crystal of such molecules might consist of stacked rings stabilized by interring hydrogen bonds.

There are seven MEC's with C_3 symmetry, four of which also have S_6 symmetry and have already been discussed. The remaining three conformations are also shown in Figure 5, as viewed along the symmetry axis. Of these three conformations, the last one (HG_7) is stabilized by three type $i \rightarrow i - 2$ and three type $i \rightarrow i$ hydrogen bonds, and has a fairly low conformational energy.

There are twelve MEC's with I symmetry, four of which also have S_6 symmetry and have already been discussed. The remaining eight conformations are also shown in Figure 5. Most of them are stabilized by hydrogen bonds as indicated in Table IX and Figures 5 and 6. It is of interest to note that HG_{12} can be obtained roughly from HG_{11} by rotating the first and fourth peptide units of HG_{11} [the ith peptide unit being the -CONH- group which links the ith and $(i + 1)$th residues] around the lines joining C^{α}_1 and C^{α}_2, and C^{α}_4 and C^{α}_5, respectively, and by renaming the ith residue of the conformation thus obtained as the $(i + 1)$th residue. A similar relation also exists between MEC's HG_{13} and HG_{14}.

Comparison of these results with the crystal structure of *cyclo*-hexaglycyl reveals some interesting facts about the role of intermolecular interactions in the crystal. Karle and Karle[13] studied the crystal of the hemihydrate of this molecule by X-ray analysis, and found all peptide groups to be planar trans. A unit cell of this crystal contains eight of these cyclic molecules and four water molecules. Four of the eight have the same conformation (which we shall designate here as conformation I), and are stacked on top of each other in the unit cell, *i.e.*, conformation I is repeated four times in the z direction as illustrated in Figure 2 of ref 13. The other four have different conformations (which we shall designate here as conformations II, II′, III, and IV) and are stacked on top of each other in the crystal in the order II, IV, II′, and III. The conformations I, III, and IV have I symmetry while those of II and II′ have no symmetry. The dihedral angles in these conformations were calculated by Boedefeld and Schellman[14] from Table III of ref 13, and are shown in Figure 7. In this figure, the values of (ψ_i, ϕ_{i+1}) are plotted and connected by arrows [pointing from (ψ_i, ϕ_{i+1}) to (ψ_{i+1}, ϕ_{i+2})] for the five conformations shown.[15] Three important points emerge from these plots. (a) Conformations II and II′ are exactly mirror images of each other. (b) The thick lines in the plots for conformations II, II′, III, and IV are almost identical to each other. This means that the values of $(\psi_1, \phi_2, \psi_2, \phi_3)$ and $(\psi_4, \phi_5, \psi_5, \phi_6)$ are almost the same in all four conformations II, II′, III, and IV. Only (ψ_3, ϕ_4) and (ψ_6, ϕ_1) differ in these four conformations. The parts of the molecule whose conformations are determined by the dihedral angles $(\psi_1, \phi_2, \psi_2, \phi_3)$ and $(\psi_4, \phi_5, \psi_5, \phi_6)$ have identical conformations in II, II′, III, IV, and these parts have a relative geometrical arrangement in the unit cell such that they repeat themselves in the z direction, as illustrated in Figure 3 of ref 13. The crystal itself is not invariant with respect to this ¼ translation in the z direction because of the different conformations in the parts of the chain determined by (ψ_3, ϕ_4) and (ψ_4, ϕ_1); thus, there is a pseudotranslational symmetry in *cyclo*-hexaglycyl.[13] Since translational invariance is a general characteristic of a crystal, the existence of this pseudosymmetry implies that intermolecular interactions play an important role in keeping the values of $(\psi_1, \phi_2, \psi_2, \phi_3)$ and $(\psi_4, \phi_5, \psi_5, \phi_6)$ the same in the four conformations. Conformation IV can be obtained from conformation II by rotating one peptide group around a line connecting two C^{α} atoms. It is remarkable that this can be done without affecting the conformation of the rest of the molecule. In a similar manner, conformations II′, III and II can be obtained from conformations IV, II′, and III, respectively. (c) Conformations I and III are very similar to each other. The dihedral angles of MEC's HG_{11} and HG_{15} are plotted in the same way in Figure 8, from which it can be seen that conformations I and III are close to MEC HG_{11}[16] and conformation IV is close to MEC HG_{15}.

At the present time, we cannot determine whether or not there are any MEC's close to conformations II and II′,

(13) I. L. Karle and J. Karle, *Acta Crystallogr.*, 16, 969 (1963).
(14) E. Boedefeld and J. A. Schellman, private communication.
(15) It should be noted that this is not the usual plot of (ϕ_i, ψ_i).
(16) The crystal structure of *cyclo*-(Gly_4Ala_2) was also determined by Karle *et al.*[17] This molecule crystallizes in an orthorhombic cell with one molecule per asymmetric unit (four molecules per unit cell) and only one conformation for the molecule. The backbone conformation of this structure is also very similar to that of MEC HG_{11}.
(17) I. L. Karle, J. W. Gibson, and J. Karle, *J. Amer. Chem. Soc.*, 92, 3755 (1970).

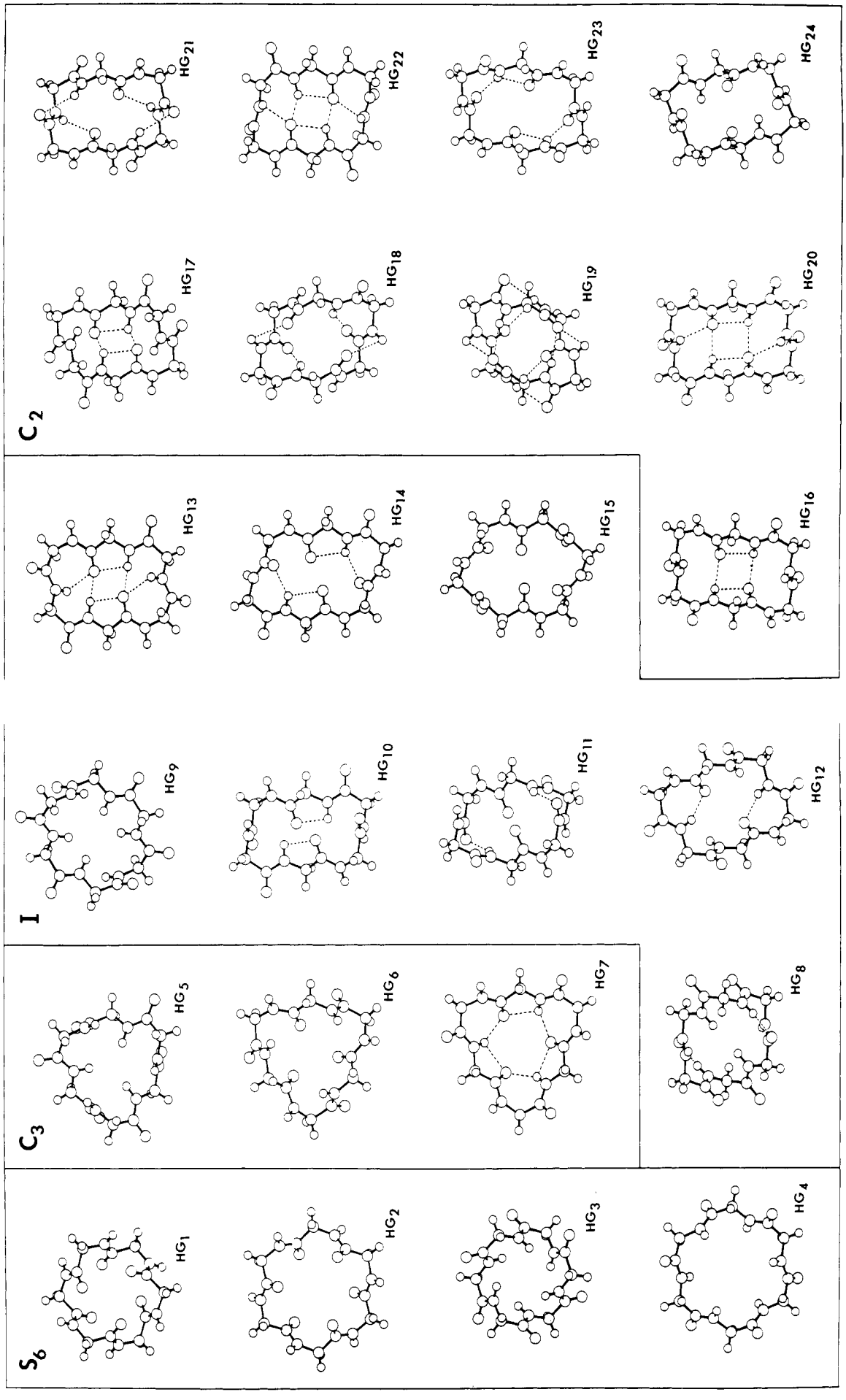

Figure 5. Twenty-four minimum-energy conformations of *cyclo*-hexaglycyl.

Table IX
Intramolecular Hydrogen-Bond Formation in Minimum Energy Conformation

Type of H-Bond Network[a]	HG_7 A	HG_8 B	HG_{10}	HG_{11} B	HG_{12} B	HG_{13} D	HG_{14} B	HG_{16} C	HG_{17} C	HG_{18} B	HG_{19} F	HG_{20} D	HG_{21} E	HG_{22} D	HG_{23} B
Short H^N–O distance[b] $(i \rightarrow j)$[c]	2.22	1.89	2.25	2.17	1.97	2.20	2.20	2.21	2.32	2.12	2.26	2.23	2.38	2.15	2.22
	(2→2)	(4→2)	(3→3)	(3→1)	(3→1)	(3→3)	(3→3)	(3→3)	(6→3)	(3→1)	(3→1)	(3→3)	(5→3)	(5→3)	(3→3)
	(4→4)	(1→5)	(6→6)	(6→4)	(6→4)	(6→6)	(6→6)	(6→6)	(3→6)	(6→4)	(6→4)	(6→6)	(2→6)	(2→6)	(6→6)
	(6→6)														
						2.15	2.12	2.21			2.13	2.33	2.42	2.23	2.11
	2.04					(5→3)	(3→1)	(6→3)			(5→2)	(5→3)	(3→1)	(6→3)	(3→1)
	(4→2)					(2→6)	(6→4)	(3→6)			(2→5)	(2→6)	(6→4)	(3→6)	(6→4)
	(6→4)														
	(2→6)					2.29						2.10			
						(6→3)						(6→3)			
						(3→6)						(3→6)			

[a] The types of networks of hydrogen bonds are illustrated in Figure 6. [b] Distances in Angstrom units. [c] $(i \rightarrow j)$ indicates that the interatomic distance between the amide H atom of the ith residue and the carbonyl O atom of the jth residue is less than 2.45 A.

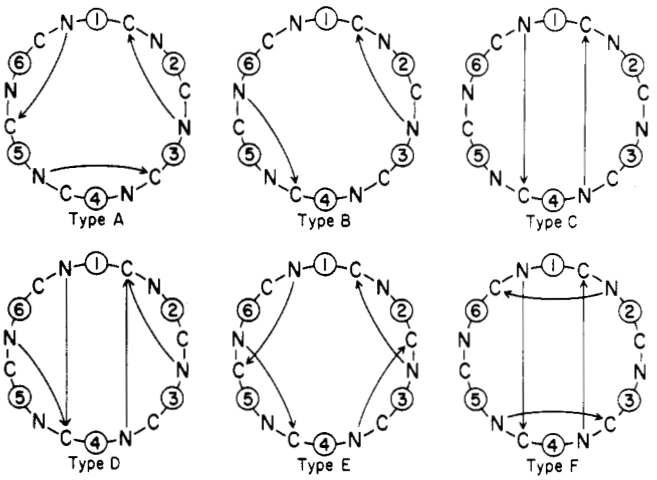

Figure 6. Types of networks of intramolecular hydrogen bonds.

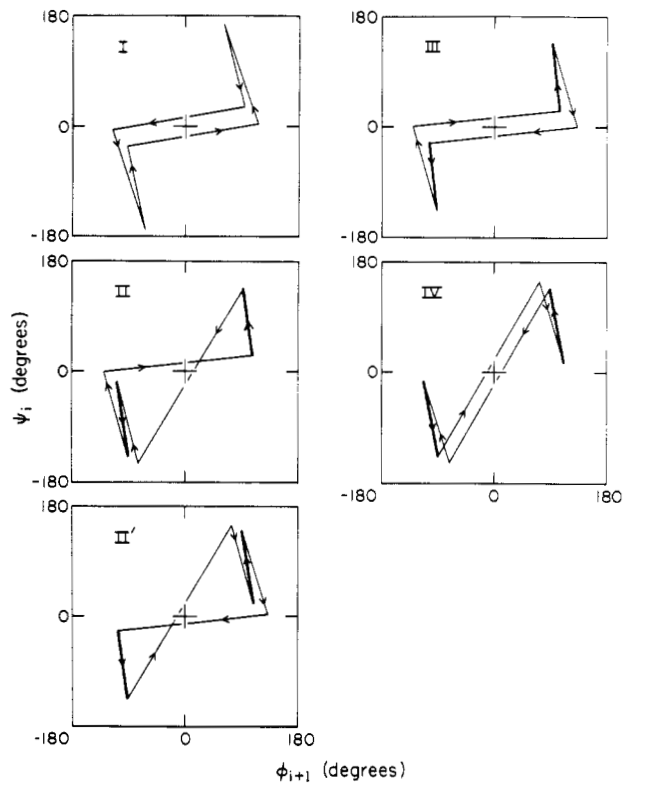

Figure 7. Plots of the dihedral angles in five different conformations of *cyclo*-hexaglycyl in its hemihydrate crystal.[13,14] The values of (ψ_i, ϕ_{i+1}) for each peptide unit are plotted. The arrows run in the direction from (ψ_i, ϕ_{i+1}) to (ψ_{i+1}, ϕ_{i+2}).

since we have not yet examined conformations of *cyclo*-hexaglycyl without any symmetry. The deviations of conformations I, III, and IV from the MEC's HG_{11} and HG_{15} obtained in this paper are to be attributed to: (i) intermolecular interactions in the crystal (especially hydrogen bonds, which are reported[13] to be quite numerous), and (ii) a possible inadequacy of the conformational energy function used here. As already discussed, intermolecular interactions seem to play a very important role in determining the conformation of *cyclo*-hexaglycyl in the crystal. Since there is extensive intermolecular hydrogen bonding, the closeness of conformations I, III, and IV to some of the MEC's obtained here is remarkable, and may indicate that our conformational energy functions are adequate. The conformational energy of HG_{15}, which is close to conformation IV, is not very low (see Table VIII). Therefore, the energy of conformation IV is also not very

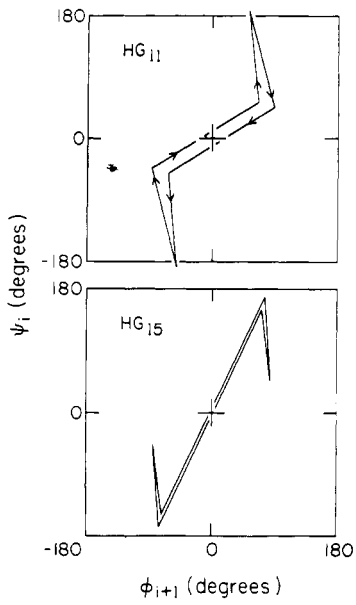

Figure 8. Plot of dihedral angles of MEC's HG_{11} and HG_{15}.

low, when isolated. However, its existence in the crystal again must arise because of intermolecular interactions. Conformation I of *cyclo*-hexaglycyl and the crystal conformation of *cyclo*-Gly_4Ala_2) are reported[13,17] to have a pair of type $i \rightarrow i - 3$ intramolecular transannular hydrogen bonds. In the MEC HG_{11}, to which the above two conformations are very close, there is instead a pair of type $i \rightarrow i - 2$ hydrogen bonds. The distances between the H and O atoms in the $i \rightarrow i - 2$ and $i \rightarrow i - 3$ positions are 2.17 and 2.56 Å, respectively. Therefore, it can be concluded that there is also a pair of weak type $i \rightarrow i - 3$ hydrogen bonds in HG_{11}. In fact, it seems that there is a pair of bifurcated hydrogen bonds, each of which is a type $i \rightarrow i - 3$ and $i \rightarrow i - 2$ bifurcated hydrogen bond with the H atom in common.

There are nine MEC's with C_2 symmetry, all but one of which (HG_{24}) are stabilized by hydrogen bonds, as indicated in Table IX and Figures 5 and 6. Conformations HG_{16} and HG_{17}, both of which have a pair of type $i \rightarrow i - 3$ hydrogen bonds, are similar to each other, but with an energy barrier between them (since they do not converge into each other). The MEC HG_{19} also has a pair of type $i \rightarrow i - 3$ hydrogen bonds. The local regions of the molecule around the hydrogen-bonded units are very similar in HG_{16} and HG_{19}. The difference in the overall shapes arises from the way in which two hydrogen-bonded conformations are joined at the third and sixth C^α's in HG_{16} and at the second and fifth C^α's in HG_{19}, respectively. MEC's HG_{13}, HG_{20}, and HG_{22} have a type D intramolecular hydrogen-bond network (see Table IX and Figures 5 and 6). While HG_{20} and the mirror image of HG_{22} may look very similar to each other, there is a difference, *viz.*, the axial and equatorial positions occupied by the hydrogen atoms attached to the α carbons are interchanged at four residues in these two conformations. The simultaneous existence of types $i \rightarrow i - 2$ and $i \rightarrow i - 3$ hydrogen bonds in these conformations should be noted, since the simultaneous existence of such a hydrogen-bonding network has been stated to be impossible.[12,18] This difference arises from the different criteria used to judge whether a hydrogen bond is formed. The criterion used in this paper has a weaker directional dependence (*i.e.*, the energy does not rise rapidly with departure from linearity) than the one used elsewhere.[12,18] The overall shapes of MEC's HG_{16} and HG_{23} are somewhat similar,[19] the difference lying in the way that the hydrogen bonds are formed. The conformation of the lowest energy (HG_{19}) is interesting. It has a type F network of intramolecular hydrogen bonds (see Table IX and Figures 5 and 6). This conformation is stabilized by these hydrogen bonds and also by (mainly electrostatic) interactions between the following four pairs of amide H and carbonyl O atoms (with interatomic distances indicated in parentheses): $4 \rightarrow 1$, $1 \rightarrow 4$ (2.81 Å) and $5 \rightarrow 3$, $2 \rightarrow 6$ (2.46 Å). If we regard these interactions as weak hydrogen bonds, then this conformation has four sets of bifurcated hydrogen bonds.

The conformations of *cyclo*-hexaglycyl with C_2 symmetry were also studied theoretically by Ramakrishnan and Sarathy,[9,20] who generated conformations of three linked peptide units with a type $i \rightarrow i - 3$ hydrogen bond, linked two identical sets of tripeptide units to form a cyclic hexapeptide, and then tilted them about the line joining the linking C^α's so that the value of the bond angles τ at the linking C^α's lay within 108 and 112°. In this way, they generated a number of conformations and calculated their conformational energies, four of which (which they designated[20] A, A′, B, and B′) had low energies. Their A, A′, and B′ are very similar to HG_{20}, and to the mirror images of HG_{19} and HG_{16}, respectively, and their B is similar to the mirror image of their A′. In fact, the following relations are found to exist between the dihedral angles in Table I of ref 20

$$\phi_i(A') + \phi_{i+3}(B) \simeq 360° \quad (18)$$

and

$$\psi_i(A') + \psi_{i+3}(B) \simeq 360° \quad (19)$$

We did not find any more MEC's that are similar to HG_{19}. This means that their conformation B is not a different MEC, at least for the energy function employed in this paper. In the present paper, two more MEC's (HG_{17} and HG_{22}) in addition to HG_{16}, HG_{19} and HG_{20} are found to have a pair of type $i \rightarrow i - 3$ intramolecular hydrogen bonds. Their failure to find these conformations may be attributed to their use of 10° grid points to generate a number of conformations.

On the basis of nmr and ir spectroscopic measurements, *e.g.*, on

$$\text{—(Gly-Pro-Gly)}_2\text{—}$$

Schwyzer *et al.*[21-23] have proposed a conformation, which has subsequently been inferred to exist in solutions (in various solvents) of a number of cyclic hexapeptides.[24] In this structure, the amide hydrogens are classified into groups, one with two hydrogens involved in intramolecular hydrogen bonds and one with four hydrogens which are more exposed to the solvent. More specifically, Schwyzer *et al.* proposed a cyclic conformation consisting of almost antiparallel chains held together by a pair of type $i \rightarrow i - 3$ transannular hydrogen bonds. Of the 24 MEC's, six conformations (HG_{13}, HG_{16}, HG_{17}, HG_{19}, HG_{20}, and HG_{22}) have a pair of type $i \rightarrow i - 3$ hydrogen bonds. All of these but HG_{19} have very extended almost antiparallel chains. The lowest energy conformation HG_{19} also has two groups of hydrogen atoms, one set of two which is involved in bifurcated hydrogen bonds (in the sense discussed above) in which the hydrogen atoms are in common, and a second

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set of four hydrogen atoms which are involved in bifurcated hydrogen bonds in which the oxygen atoms are in common. Conformations of type B (HG_8, HG_{11}, HG_{12}, HG_{14}, HG_{18}, and HG_{23}) also have a pair of intramolecular hydrogen bonds. It does not seem possible to decide from the present experimental data[24] which one of these MEC's or a mixture of them is realized in solution.

The calculations carried out in this paper should be regarded as the first step of a process to determine the most stable conformation of an isolated molecule in solution.[4,25,26] The second and third steps of the process require the determination of the conformational entropy and the introduction of the flexibility of bond lengths and bond angles in the molecule, as was done in the conformational analysis of *cyclo*-(Gly_3Pro_2).[27,28] As was found in ref 28, the relative conformational energy may change when flexibility is introduced. The effect of the introduction of flexibility would not be as strong as observed for *cyclo*-(Gly_3Pro_2) because *cyclo*-hexaglycyl is a bit less crowded than *cyclo*-(Gly_3Pro_2). However, the possibility of interchange of the relative order of stability by introduction of flexibility cannot be excluded. Therefore, the order of stabilities of the MEC's obtained in this paper should be regarded as tentative. However, the conformations themselves should not change too much when flexibility is introduced.

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Investigation of the Cis and Trans Isomers of Sarcosylsarcosine by Nuclear Magnetic Resonance Spectroscopy and Conformational Energy Calculations[1]

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ABSTRACT: The proton nuclear magnetic resonance spectrum of the dipeptide sarcosylsarcosine in D_2O shows the presence of both the cis and trans isomeric forms of the peptide group; *i.e.*, the rate of interconversion between the two forms is slow on the nmr time scale so that the resonance peaks of the α-CH_2 and N-CH_3 protons each exhibit different chemical shifts in the two forms. The peaks for the N-terminal N-CH_3 protons are the most easily resolved, and the ratio of the areas for these protons in the cis and trans forms represents the equilibrium constant for the interconversion. The temperature dependence of this equilibrium constant leads to an enthalpy change of 610 ± 90 cal/mol, with the trans isomer having the lower enthalpy. Conformational energy calculations are carried out, and the low-energy conformations of both the cis and trans peptide are found. These calculations account for the observed occurrence of only the cis conformation in the crystalline state and also identify the forces responsible for the occurrence of *both* cis and trans conformations in aqueous solution.

In this paper, we consider some of the forces which affect the preference for the cis or trans conformation of the dipeptide sarcosylsarcosine in the crystalline state and in aqueous solution.

In the absence of constraints such as those imposed by the presence of small rings (as in diketopiperazines) the peptide group -CONH- in polypeptides and proteins appears to exist in the planar trans conformation.[3,4] The planarity of the peptide group, as well as the high barrier to rotational interconversion between the cis and trans forms,[5-7] can be directly related to the partial double bond character of the C′-N bond.[3,4,8] However, it is difficult to understand by theoretical methods[8] the origin of the preference for the cis or trans forms, in terms of electronic effects. When the amide nitrogen is methylated, as in proline or sarcosine, the cis ⇄ trans equilibrium is shifted sufficiently toward the cis form, as, *e.g.*, in poly(L-proline)[7] and polysarcosine,[9] so that it is possible to detect the cis form experimentally. On the other hand, according to molecular orbital calculations,[8] the barriers to rotation about the C′-N bond in *N*′-methyl- and *N*,*N*-dimethylacetamide are nearly equivalent, but striking ste-

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