Calculation of the Conformation of *cyclo*-Hexaglycyl. 2. Application of a Monte-Carlo Method[1]

Nobuhiro Gō[2a] and Harold A. Scheraga*[2b]

Department of Chemistry, Cornell University, Ithaca, New York 14853.
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ABSTRACT: A Monte-Carlo method is used in a conformational energy analysis of *cyclo*-hexaglycyl. This method simulates, in an approximate way, the real process in which the molecule changes its conformation in solution. If the conformation is trapped in a local potential energy minimum for a given finite number of steps of the Monte-Carlo run, the minimum is defined as a good one; otherwise it is designated as a poor one. It is shown that such poor minima exist for this molecule and that the Monte-Carlo method can alter the conformation away from such minima. It is also found that many good minima (at least 81 and surely more) exist for this molecule. A nearly complete list of minimum-energy conformations (MEC's) with low conformational energy is obtained. All conformations with and without symmetry, that exist in a hemihydrate crystal of this molecule, are each found to be very similar to one of the good-minimum low-energy MEC's. The Monte-Carlo method is also used to examine the nature of the MEC's with symmetry that were located in a previous paper. Fourteen of the 24 MEC's with symmetry are found to be good minima. The other ten either are poor minima or correspond to a saddle point in the full six-dimensional conformation space.

I. Introduction

Studies of the conformation of *cyclo*-hexaglycyl are particularly interesting because this molecule may be regarded as a reference for other cyclic hexapeptides. 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. It was from this point of view that a calculation of the conformation of *cyclo*-hexaglycyl was started in the first paper of this series.[3]

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 paper I,[3] a systematic search was made of the minimum-energy conformations (MEC's) with one of these possible symmetries under the assumption of fixed lengths and bond angles and planar trans peptide groups. According to the mathematical theory developed to generate exactly closed rings having symmetric conformations,[4] the number of independent variables in this molecule is 0, 1, 2, 3, or 4 when it assumes conformations with C_6, S_6, C_3, I, or C_2 symmetry, respectively. The MEC's were located[3] by computing complete energy maps (for C_6, S_6, C_3, and I symmetries) and/or by carrying out systematic energy minimization (for I and C_2 symmetries).

The study in paper I was incomplete in two respects. First, MEC's with no symmetry were not explored. A *cyclo*-hexaglycyl molecule with fixed bond lengths and bond angles and planar trans peptide groups has six degrees of freedom when it assumes conformations with no symmetry;[5] i.e., a general conformation of a *cyclo*-hexaglycyl molecule corresponds to a point in a six-dimensional space. A conformation that has one of the above five possible symmetries corresponds to a point in a subspace of fewer dimensions. No studies of MEC's without symmetry were made in paper I because of difficulties in carrying out systematic energy minimization in a six-dimensional space. Second, the local stabilities of the MEC's with one of the above five symmetries were not examined; a conformation is regarded as locally stable if restoring forces return it to the original conformation after any possible small conformational fluctuations in directions that either break or preserve the symmetry. In other words, a conformation of the *cyclo*-hexaglycyl molecule is locally stable only if it corresponds to an energy minimum in the full six-dimensional space. The MEC's located in paper I are energy minima in subspaces of lower dimensionality but are not necessarily energy minima in the full six-dimensional space. In this sense, the stabilities of the MEC's located in paper I are yet to be examined.

In the present paper, we apply the Monte-Carlo method devised by Metropolis et al.[6] to the conformational analysis of this cyclic molecule. This method has been applied previously to numerous problems in physical chemistry and in polymer chemistry. In this method, random numbers are used to give probabilities of successive conformational transitions in such a way that, in the long run, every conformational state appears with a probability proportional to that determined by the Boltzmann expression. The conformational energy sometimes increases in the course of the calculation, which is in contrast to the monotonic decrease in energy that occurs in the usual energy minimization procedures. This method was shown[7] to simulate the time course (approximately) in a system undergoing an isothermal process, if the transition probabilities are chosen properly.

Because of this characteristic of the Monte-Carlo method, a typical computer run may be expected to proceed as follows. An initial conformation (chosen arbitrarily in most cases) usually has a high conformational energy. The gradient of the conformational energy is also usually large. Therefore, the conformational energy most surely will decrease at first. It may then approach a local minimum. If the local minimum is shallow, the conformation will soon escape from it and will move to a part of the conformational space with even lower conformational energy. Because of this property, this Monte-Carlo method is expected to be one of the possible tools to overcome the multiple-minimum problem. If the local minimum is very deep, the conformation will be trapped in it practically, and the conformational energy will fluctuate in the potential well of the local minimum. This situation corresponds to a long lifetime of a metastable conformation of the real molecule under investigation. If the computer run is carried out long enough, the conformational energy eventually will rise high enough to escape from the local minimum. However, because of the finiteness of the time of the computer run, some local minima practically may appear to be stable.

From this anticipated behavior of the Monte-Carlo run, we may classify local minima as poor and good ones. A good local minimum is defined as one which appears stable (practically) in the Monte-Carlo run described above; otherwise the minimum is a poor one. This classification may have experimental significance in that the lifetimes of conformations corresponding to the defined computed good local minimum may be observable experimentally.

There are three objectives in the present paper. (1) To test the practical applicability of the Monte-Carlo method of Metropolis et al. in escaping from local minima in the conformational energy analysis of a molecule with several degrees

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of freedom. (2) To assess the stabilities of the MEC's reported in paper I. This can be done by starting the Monte-Carlo run in the full six-dimensional space from an MEC obtained in a space of lower dimensionality (corresponding to a conformation with a certain symmetry). If the original MEC is a saddle point in the full six-dimensional space, the conformational energy will decrease readily. Otherwise, it is either a poor or good local minimum, depending on whether the conformation eventually leaves the potential well of the local minimum. (3) To obtain as complete a list as possible of the good local minima of *cyclo*-hexaglycyl.

II. Geometry and Energy Parameters

The geometry used in this paper (basically that of Pauling and Corey[8]) is the same as that used in paper I. The energy parameters used in this paper are also the same as those used in paper I, instead of the more recently revised ones[9] in use in this laboratory. This choice is dictated by the need to compare the results in this paper with those in paper I. However, in order to see the effect of a different choice of energy parameters, the conformational energies are also calculated (for the good local minima) with the recently adopted[9] energy parameters (ECEPP parameters).

An exactly closed ring conformation of the backbone of a *cyclo*-hexaglycyl molecule with no symmetry may be generated by the mathematical method developed previously.[5] In this method, three consecutive pairs of (ϕ,ψ) dihedral angles (say $\phi_i,\psi_i,\phi_{i+1},\psi_{i+1}$, and ϕ_{i+2},ψ_{i+2}) are treated as independent variables. The other three pairs of (ϕ,ψ)'s (viz., $\phi_{i+3}\psi_{i+3}$, ϕ_{i+4},ψ_{i+4}, and ϕ_{i+5},ψ_{i+5}) are dependent variables that are determined by solving a set of algebraic equations for a given set of values of the independent variables. This set of algebraic equations has zero or a finite number (up to eight, as found empirically[5]) of solutions for the six unknown (i.e., dependent) dihedral angles.

$$\begin{aligned} \phi_{i+j}^{(k)} &= \phi_{i+j}^{(k)}\,(\phi_i,\psi_i,\phi_{i+1},\psi_{i+1},\phi_{i+2},\psi_{i+2}) \\ \psi_{i+j}^{(k)} &= \psi_{i+j}^{(k)}\,(\phi_i,\psi_i,\phi_{i+1},\psi_{i+1},\phi_{i+2},\psi_{i+2}) \\ j &= 3, 4, \text{ and } 5;\ k = 1, 2, \ldots, l \end{aligned} \tag{1}$$

Here, l is the number of solutions of the set of algebraic equations (in one particular subspace of a given set of values of the independent variables) and k is a serial number (a branch number) specifying each of the different solutions. The values of the dependent variables (i.e., solutions of eq 1) change continuously (for a given value of k) for continuous changes of the values of the independent variables. The number of solutions l is generally found (empirically;[5] see also Figure 4 of ref 5) to be an even number. The six-dimensional space of the independent variables may be divided into subspaces, in each of which l is constant, but with l possibly differing from one subspace to another. As we cross a border between two subspaces, two branches of solutions (i.e., two different values of k) merge into one and then disappear (or, if the border is crossed in the opposite direction, a new branch emerges and then becomes a pair of new branches); in some cases, a branch may pass continuously from one subspace to another. Since any surface between two six-dimensional subspaces is a five-dimensionl one, the border is a five-dimensional surface in the six-dimensional space; since, e.g., two solutions merge into one on the border, there is an odd number of different solutions of the algebraic equations on the border.

For later use, we define the l conformations whose dihedral angles are given by eq 1 as *conjugate* to each other; i.e., all solutions in a given subspace are said to be *conjugate* to each other. Therefore, since all conformations in a given subspace have the same values of the independent variables, the conformations of half of the molecule in two conjugate conformations are identical, and the conformations of the other half are different.

An arbitrary conformation of the *cyclo*-hexaglycyl molecule can be specified by assigning either a set of values of twelve dihedral angles $(\phi_1,\psi_1,\ldots,\phi_6,\psi_6)$ or a set of values of six independent variables plus a branch number to specify a set of values of six dependent variables $(\phi_i,\psi_i,\phi_{i+1},\psi_{i+1},\phi_{i+2},\psi_{i+2};k)$. The latter method of designation defines a set of l "branch" spaces (corresponding to the l solutions for a given set of the independent variables). Each set of dependent variables (solution of eq 1 for the given set of independent variables) corresponds to a point in a branch space, there being only one point in each branch space; thus, any conformation of the *cyclo*-hexaglycyl molecule is represented by a point in one of the branch spaces of the six-dimensional conformational space. This is a convenient representation with which to discuss changes of independent variables during the course of the computations (see next paragraph).

When we apply either the Monte-Carlo or energy-minimization method, as described in the next section, we generate a series of conformations. It often happens that, during the course of a change of conformation in the computations, a point in one branch space of the six-dimensional conformational space hits the five-dimensional border and tries to move into a different subspace (where the value of l differs). It is necessary that the computations should be continued on a branch in the new subspace that is the smooth continuation of the old branch; i.e., the solutions must not jump from one branch (value of k) to another when the border is crossed in going from one subspace to another. However, it is not an easy computational task to establish the correspondence between branches in adjoining subspaces. The following method of "rotation" of the independent variables was employed to avoid this difficulty. Let $(\phi_i,\psi_i,\phi_{i+1},\psi_{i+1},\phi_{i+2},\psi_{i+2};k)$ be the conformation just prior to hitting the border. This conformation is represented by a point very close to the border, and we calculate the values of its 12 dihedral angles $(\phi_i,\psi_i,\phi_{i+1},\psi_{i+1},\ldots,\phi_{i+5}\psi_{i+5})$. Then we change the variables originally designated as the independent ones by "rotating" them from $(\phi_i,\psi_i,\phi_{i+1},\psi_{i+1}\phi_{i+2}\psi_{i+2})$ to $(\phi_{i+1},\psi_{i+1},\phi_{i+2},\psi_{i+2},\phi_{i+3},\psi_{i+3})$ and look for a branch number k' such that conformation $(\phi_{i+1},\ldots,\psi_{i+3};k')$ is identical with the above conformation specified by $(\phi_i,\ldots,\psi_{i+5})$. Now, since the conformation of the molecule will move in a new set of subspaces, the conformation $(\phi_{i+1},\ldots,\psi_{i+3};k')$, in general, will not be close to a border in the six-dimensional space of the independent variables $(\phi_{i+1},\ldots,\psi_{i+3})$. Thus, the generation of new conformations can be continued *on this same branch* of the six-dimensional conformational space. If it hits a border again, we again "rotate" the independent variables to $(\phi_{i+2},\ldots,\psi_{i+4})$, etc.

A similar problem of hitting a border was encountered in paper I,[3] when studying the conformations of *cyclo*-hexaglycyl with C_2 symmetry, by energy minimization. During the course of such computations in paper I, those conformations that hit a border were discarded. (As will be seen in section IVB, it appears that we did not lose any MEC's when such conformations were discarded.) In the present paper, however, we saved such conformations by "rotating" the independent variables.

Because the glycyl residue itself has a plane of symmetry, and because all residues in this cyclic hexapeptide are identical, 12 conformations are related to each other by the following two types of equivalences: A renumbering equivalence between two conformations a and b, if they satisfy the relation

$$(\phi_i{}^a,\psi_i{}^a) = (\phi_{i+k}{}^b,\psi_{i+k}{}^b) \quad (2)$$

$i = 1, 2, \ldots, 6$; k = an arbitrary integer

and a mirror-image equivalence between two conformations a and b, if they satisfy the relation

$$(\phi_i{}^a,\psi_i{}^a) = (-\phi_i{}^b,-\psi_i{}^b) \quad (3)$$

$$i = 1, 2, \ldots, 6$$

The 12 conformations are separated into two groups, each consisting of six conformations. The six conformations in each group are related to each other by the renumbering equivalence, and each conformation in one group has its mirror-image-equivalent conformation in the other group. All 12 conformations that are related to each other by eq 2 and 3 should be considered as the same one. Of these 12 equivalent conformations, the one that satisfies the relations

$$\pi \geq \phi_1 \geq 0$$

$$|\phi_i| \geq \phi_1 \qquad (i = 2, \ldots, 6) \quad (4)$$

and

$$\pi \geq \psi_i \geq -\pi \quad (5)$$

is usually taken as the representative one.

It often is necessary to judge whether two conformations are similar or not. Two conformations are considered to be similar when the distance between them, defined below, is small. Let $d(\theta^a,\theta^b)$ be the difference between two dihedral angles θ^a and θ^b in molecular conformations a and b, respectively. If $\pi \geq (\theta^a - \theta^b) > -\pi$, $d(\theta^a,\theta^b)$ is defined as $(\theta^a - \theta^b)$. If, however, $\pi \geq (\theta^a - \theta^b) > -\pi$ is not satisfied, an integral multiple of 2π is added to or subtracted from $(\theta^a - \theta^b)$, so that the result satisfies the above inequality, and this result is then defined as $d(\theta^a,\theta^b)$. The two conformations a and b are chosen so that the distance between them corresponds to the minimum value of $\text{Max}\{d(\phi_i{}^a,\phi_i{}^c), d(\psi_i{}^a,\psi_i{}^c); i = 1,2, \ldots .6\}$, where c runs over all 12 conformations equivalent to b. By selecting a and b in this manner, the same result is obtained no matter which of the 12 equivalent conformations is chosen as conformation a. In other words, we examine the dihedral angle with the largest deviation (for all 12 equivalent conformations) and select the pair with the smallest value of $d(\theta^a,\theta^b)$ to decide whether the two conformations are similar or not. By using the largest deviation in the above criterion, we avoid having to judge that two conformations (in which only one or a few dihedral angles differ considerably) are similar. Two such conformations would be more dissimilar than two conformations in which a larger number of dihedral angles differ by smaller amounts.

III. Monte-Carlo and Energy-Minimization Methods

Two different procedures are applied here to obtain the MEC's, a Monte-Carlo and an energy-minimization method.

A. Monte-Carlo Method. The basic idea of this method is to simulate the real process (in which a molecule in solution undergoes conformational changes) by generating a series of conformations stochastically. The method is described elsewhere[6,7] and we summarize it briefly here as it is applied to the present problem.

Let C_i be the ith conformation in the Markov chain of generated conformations, and let $E(C_i)$ be its conformational energy. To initiate the $(i + 1)$th step, a test conformation $C_i{}^*$ is generated from C_i by changing the values of each of the six independent dihedral angles randomly within a range of ±10°. In solving the set of algebraic equations (eq 1) to obtain the values of the six dependent dihedral angles, care is taken that the solution remain in the same branch;[10] i.e., the value of k must be the same for C_i and $C_i{}^*$. Then $E(C_i{}^*)$ is calculated. If ΔE, taken as $E(C_i{}^*) - E(C_i)$, is negative the test conformation $C_i{}^*$ is taken as the $(i + 1)$th conformation C_{i+1}. If $\Delta E \geq 0$, then $C_i{}^*$ is taken as the $(i + 1)$th conformation C_{i+1} only with a probability[11] of $\exp(-\Delta E/k_B T)$, where k_B is the Boltzmann constant and T is the absolute temperature. If $C_i{}^*$ is rejected,[11] then C_i is taken as C_{i+1}; i.e., the molecule remains in the old conformation. This completes the $(i + 1)$th step of the Markov chain.

As shown by Metropolis et al.,[6] any conformation of the molecule will appear in the Markov chain with a probability proportional to that given by the Boltzmann expression, if the chain is long enough. As discussed elsewhere,[7] the conformations in this chain can be regarded as having been sampled at approximately equal time intervals in a real process in which the molecule undergoes conformational changes in solution.

In this paper, the value of $k_B T$ was taken as 0.417×10^{-13} ergs/molecule (0.6 kcal/mol) in most of the calculations.

B. Energy-Minimization Method. The method of Powell[12] was used for energy minimization. In essence, it involves construction of conjugate directions without calculations of derivatives of the function to be minimized.

Minimization was terminated when either of the following two conditions was satisfied: (a) the conformational energy changed by less than 0.01 kcal/mol between any two successive iterations, or (b) each of the independent variables changed by less than 0.5° between two successive iterations.

C. Combination of the Two Methods. These two methods were used separately or in concert in the following manner.

They were usually used in concert in order to locate a minimum exactly; i.e., the Monte-Carlo method was used first and then the energy-minimization method (within the potential well reached by the Monte-Carlo procedure) was applied. Starting from an appropriately chosen conformation (see section IV), a chain of conformations was generated by the Monte-Carlo method until the energy could not be lowered by 200 additional steps in the chain. Then the conformation of lowest energy was taken as the starting conformation for subsequent energy minimization. However, if the conformation reached at the end of the Monte-Carlo conformation was similar (within 30.0°; in the sense defined in section II) to one of the previously located MEC's (from this paper), the energy-minimization calculation was omitted. Generation of 100 conformations in the Monte-Carlo method took about 20 s with an IBM 370/168 computer. Most of this computation time was used for calculating the conformational energy. The ring closure and generation of atomic coordinates require a minor fraction of this time.

The two methods were used separately when it was desired to test the power of the Monte-Carlo method[6] to reach low-energy MEC's. For this purpose, 17 starting conformations, not necessarily close to minimum-energy ones, were chosen, as described in section IVB, and their energies were minimized. In a separate set of calculations, the procedure described in the previous paragraph (i.e, the combined Monte-Carlo and energy-minimization methods) was applied to the same 17 starting conformations. The results were then compared (see section IVB).

IV. Results and Discussion

A. Stabilities of Minimum-Energy Conformations with Symmetry. Monte-Carlo runs were carried out twice for $k_B T$ = 0.6 kcal/mol and once for $k_B T$ = 0.3 kcal/mol by starting from each of the 24 MEC's with symmetry (HG_1–HG_{24}) that were located in paper I,[3] in order to see if they are stable MEC's in the full six-dimensional conformational space. The results are summarized in Table I. An MEC is classified as a "good minimum" if the conformation remains in the vicinity

Table I
Nature of Minimum-Energy Conformations with Symmetry

MEC	Symmetry	Conform. energy,[a] kcal/mol	Good min.	Poor min. or not a min.	No. of times this min. was reached[b]
HG_1	S_6	−4.51		+	0
HG_2	S_6	−8.24		+	0
HG_3	S_6	2.77		+	0
HG_4	S_6	−7.89		+	0
HG_5	C_3	−5.70		+	0
HG_6	C_3	−3.46		+	0
HG_7	C_3	−14.52	+		0
HG_8	I	−9.82		+	0
HG_9	I	−6.53	+		0
HG_{10}	I	−13.00	+		2
HG_{11}	I	−14.25	+		0
HG_{12}	I	−11.19	+		0
HG_{13}	I	−15.17	+		2
HG_{14}	I	−13.53	+		0
HG_{15}	I	−8.77		+	0
HG_{16}	C_2	−11.79		+	0
HG_{17}	C_2	−12.11	+		0
HG_{18}	C_2	−16.26	+		2
HG_{19}	C_2	−21.76	+		5
HG_{20}	C_2	−16.17	+		2
HG_{21}	C_2	−14.27		+	0
HG_{22}	C_2	−15.00	+		2
HG_{23}	C_2	−12.09	+		1
HG_{24}	C_2	−6.78	+		0

[a] This is the conformational energy obtained for the structure with symmetry, in paper I.[3] [b] When starting with 166 conformations (see section IVB) in the combined Monte-Carlo plus energy-minimization procedure.

of the starting conformation for 200 steps in all runs at the two temperatures. Otherwise, it is classified as a "poor minimum or not a minimum". MEC's HG_8 and HG_{15} appeared to be "good minima" in Monte-Carlo runs at the lower temperature, k_BT = 0.3 kcal/mol, but they changed to "poor minima" at the higher temperature. Therefore, they are classified in Table I as a "poor minimum or not a minimum". In the Monte-Carlo runs for other MEC's that are not classified as a "good minimum", it was found that the conformation tends to remain longer in the vicinity of the starting conformation at the lower temperature than at the higher temperature (this finding suggests the possible use of an artificially high temperature in this Monte-Carlo method to get out of local minima).

A more standard method for discussing stabilities is the examination of the matrix of second derivatives at each extremum; if the matrix is positive definite, then the conformation at the extremum is a stable one. We did not use this method in this paper because it requires a nontrivial amount of computation. This definition of stability depends only on the properties of the conformational energy surface close to the minimum. In contrast to this, the definition of a "good minimum" in the present paper reflects, to some extent, the properties of the surface farther from the minimum; e.g., the minimum may exist in a very shallow trough, from which the conformation can "escape" in a Monte-Carlo calculation.

It is difficult to distinguish between a "poor minimum" and "not a minimum" by the present Monte-Carlo method. The conformational energy can increase in the initial steps of a Monte-Carlo run started from a saddle point if the directions along which the conformational energy increases occupy most of the solid angle of the conformational space around the saddle point. If this happens, it is operationally difficult to distinguish this behavior from that which would be observed in a shallow minimum.

Because of this difficulty, a "poor minimum" and "not a minimum" are classified together in one category in this paper. Because the definition of a "good minimum" is operational, and because the operation is a stochastic one, the distinction between a "good minimum" and the others is also not a clear-cut one. We might classify the MEC's differently in Table I, if we were to repeat the Monte-Carlo calculations using different random numbers. Nevertheless, the definition of a "good minimum", used here, is very useful if its operational meaning is properly understood.

In the last column of Table I, we have listed the number of times that the combined procedure (Monte-Carlo plus energy minimization), described in more detail in section IVB, converged to each of the MEC's with symmetry. Of course, those MEC's classified as a "poor minimum or not a minimum" were not found by this method. The greater the number of times that the combined procedure reached a "good minimum" MEC, and stayed in its vicinity, the lower is the probability that the MEC is a saddle point with a narrow pass leading downward away from it; i.e., the statistical significance of the "good minimum" is enhanced.

Among the MEC's in Table I that are not "good minima", HG_2, HG_4, and HG_5 very likely are saddle points, because the conformational energy is decreased within a few (viz., 15) steps. HG_{21} is classified as a "poor minimum or not a minimum" in Table I. In section IVD, evidence is given that HG_{21} *is* a minimum in the full six-dimensional space; therefore, HG_{21} is a "poor minimum". However, HG_{21} is a good minimum in the four-dimensional space corresponding to conformations with C_2 symmetry because, as reported in Table VII of paper I,[3] it was reached more times than any of the other conformations (HG_{16}–HG_{24}) with C_2 symmetry. It is interesting that this conformation turns out to be a "poor minimum" in the full six-dimensional space.

B. Minimum-Energy Conformations without Imposition of a Condition of Symmetry. Two types of initial conformations were chosen, to which the combination of the Monte-Carlo and energy-minimization methods was applied. The first type consisted of all conformations conjugate to each of the 24 MEC's with symmetry (HG_1–HG_{24}).[3] Excluding the 24 for which the computations were described in section IVA, it turned out that there were 198 of them, and their conformational energies were calculated. More or less arbitrarily, we chose 86 of the 198 conjugate conformations for further consideration. All eight conformations conjugate to HG_{11} and all nine conjugate to HG_{15} are included in these 86. The remaining 69 were chosen mainly from those with low conformational energies.

This choice of these initial conformations was motivated by the appearance of Figure 7 of paper I,[3] in which the values of (ϕ_{i+1},ψ_i) are plotted for five different conformations, I, II, II′, III, and IV, of *cyclo*-hexaglycyl in its hemihydrate crystal.[13] Conformations III and II differ by only one pair of dihedral angles (ϕ_{i+1},ψ_i), the remaining five pairs of dihedral angles being almost the same in each conformation. This means that conformation II can be generated from conformation III by rotating one peptide plane around the axis connecting the two C^α atoms at each end of the peptide plane. To describe this situation, we define this pair of conformations III and II as *rotation linked*. Similarly, conformations II and IV, IV and II′, and II′ and III, respectively, are also rotation-linked. Conformations III and IV are very close to the symmetrical MEC's, HG_{11} and HG_{15}, respectively, and it was suspected that conformations II and II′, which have no symmetry and are mirror images of each other, might be close to MEC's with no symmetry. One purpose (besides the desire to test the power of the Monte-Carlo method) of choosing all 17 conformations conjugate to HG_{11} or HG_{15} (these being the 17 conformations referred to in section IIIC) was to search for

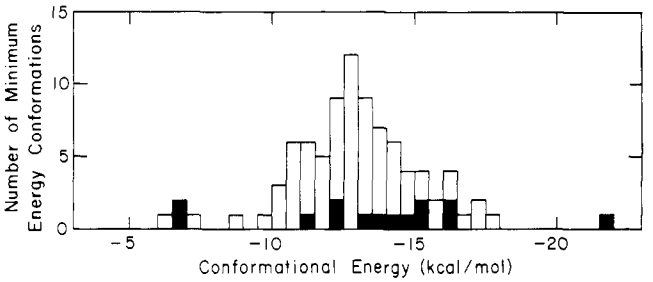

Figure 1. Distribution of conformational energies of "good" minimum-energy conformations. The 14 "good minima" with symmetry of Table I and the 74 additional ones without symmetry are included in this diagram: (■) MEC's with symmetry; (□) MEC's with no symmetry.

these possible MEC's with no symmetry. The purpose of choosing a fairly large number (viz., 69) of other conformations conjugate to those in the set of HG_1–HG_{24} was to try to locate "good minima" which are rotation linked to these MEC's with symmetry, with an expectation that the number of such "good minima" is large.

The second type of initial conformations was chosen by generating values of the six independent dihedral angles $(\phi_1,\psi_1,\phi_2,\psi_2,\phi_3,\psi_3)$ randomly within an interval between $-\pi$ and $+\pi$ and testing whether a ring could be closed in the *cyclo*-hexaglycyl molecule for this set of values of the independent variables. On the average, the ring could be closed for one out of every 15 sets of randomly generated values of the six dihedral angles. Whenever such a set was found, one of the l mutually conjugate conformations was chosen randomly as one of the initial conformations (where l is the number of solutions of the algebraic equations for the six dependent dihedral angles). In all, 80 initial conformations were generated by this random generation method. Thus, the total number of initial conformations, of both types, was 166.

As shown in Table I, 16 (the sum of the numbers in the last column) of the 166 initial conformations converged to 7 MEC's with symmetry. Five of these 16 initial conformations were chosen by the second type of procedure, i.e., by random choice of the independent dihedral angles. In paper I,[3] we calculated the complete conformational energy maps for conformations with one of C_6, S_6, C_3, and I symmetries and, hence, obtained all MEC's with one of these symmetries. However, in the case of C_2 symmetry, we employed a method of systematic energy minimization. From the multiplicity of each MEC arrived at in the energy minimization, it was concluded that it was fairly certain that all MEC's with C_2 symmetry were obtained. The fact that all MEC's with symmetry arrived at in the present calculations are limited to those found previously supports our contention in paper I that we located all MEC's with symmetry. It also indicates that we did not lose any MEC's in the process of discarding conformations at a border (the problem discussed in section II).

The remaining 150 of the 166 initial conformations converged to 74 different MEC's with no symmetry. Since these MEC's were obtained by the Monte-Carlo method, they are by definition "good minima". The histogram in Figure 1 shows the distribution of their conformational energies. Because the choice of the initial conformations, in general, was rather arbitrary, and because additional good MEC's would be expected to exist (as discussed in the next paragraph), this distribution does not have much significance, except that it indicates that (a) the conformational energy (−21.76 kcal/mol) of HG_{19} with C_2 symmetry is distinctively low and (b) there are many good MEC's with energies around −13 kcal/mol.

Table II summarizes the number of times that each minimum was arrived at in this study. This table includes the seven

Table II
Number of Times Each Minimum Was Arrived at in This Study

No. of times an MEC was reached	No. of MEC's	No. of times an MEC was reached	No. of MEC's
1[a]	42[a]	5	2
2	18	6	1
3	10	7	2
4	5	8	1

[a] For example, 42 minima were reached once, 18 twice, etc.

MEC's (with symmetry) of Table I that were obtained in the Monte-Carlo/Energy-Minimization procedure and the 74 MEC's with no symmetry. Now we must ask whether the 81 minima of Table II constitute a complete set of the "good minima". In order to be sure that these 81 minima are the complete set, and to know whether 166 is a large enough number to find all of them, each of the MEC's should be arrived at at least several times. However, in Table II, we see that about half of the MEC's are arrived at only once. This means that the 81 MEC's are far from being a complete set. We suspended our efforts to achieve a complete set because it was realized (from the data of Table II) that the complete set is a rather large one, and because such a large set would contain many high-energy MEC's that would not be likely to be observed. In any event, one conclusion from this study is that there is a very large number (probably a few hundred) of good MEC's with no symmetry for the *cyclo*-hexaglycyl molecule.

However, if we focus our attention on conformations with conformational energies lower than −15 kcal/mol, we find that all of them but one were arrived at more than once (see Table III). The fact that these conformations were arrived at many times (except in one case) indicates that the list of good MEC's with low conformational energy is nearly complete. If another low-energy MEC had existed, it is likely that it would have been arrived at in the computations.

Many of the good MEC's were found to be rotation linked to one or more of HG_1–HG_{24}, as was expected. A few such examples are illustrated in Figure 2. MEC M_{67} can be obtained from HG_{21} by rotating one peptide plane from (ϕ_{i+1},ψ_i) = (68.80°,48.34°) to (−184.15°,−49.66°) and by adjusting the other (ϕ_{i+1},ψ_i) dihedral angles only slightly. [We retain the value of −184.15° here, even though it does not correspond to the standard convention (e.g., see eq 4 and 5), in order to emphasize the similarity of the location of this point of M_{67} to the corresponding point of M_{27} in Figure 2.] MEC M_{27} can be obtained from M_{67} essentially by variation of (ϕ_{i+1},ψ_i), from (−76.44°,−100.63°) to (135.45°,41.79°). The conformational energies of HG_{21}, M_{67}, and M_{27} are −14.27, −15.50, and −12.90 kcal/mol, respectively. MEC M_{145} can be obtained either from HG_{23} by changing (ϕ_{i+1},ψ_i) from (−162.42°,−62.36°) to (63.79°,64.98°), or from HG_{24} by changing (ϕ_{i+1},ψ_i) from (71.54°,55.94°) to (−168.16°, −57.63°). The conformational energies of HG_{23}, M_{145}, and HG_{24} are −12.09, −11.13, and −6.78 kcal/mol, respectively. It is interesting to see that hitherto unrelated MEC HG_{23} and HG_{24} are thus related through MEC M_{145} with no symmetry. Another such example is given in Figure 3, from which it can be seen that MEC M_{166} is rotation linked both to HG_{11} and HG_{15}. The conformational energies of HG_{11}, M_{166}, and HG_{15} are −14.25, −12.13, and −8.77 kcal/mol, respectively. What is more important is that these three MEC's, HG_{11}, M_{166}, and HG_{15}, are very similar to conformations III, II, and IV, respectively. The latter three are found in a crystal of this molecule.[13] In Table I, HG_{15} is classified as a "poor minimum

Table III
Characterization of the 15 Lowest Good Minimum-Energy Conformations in Increasing Order of Energy

Min. energy conform[a]	HG_{19}	M_{57}	M_{138}	M_{34}	M_{111}	M_{136}	HG_{18}	HG_{20}	M_{39}	M_{152}	M_{67}	M_{118}	HG_{13}	M_{125}	HG_{22}
ϕ_1[b]	55.54	46.30	52.89	47.69	60.30	51.03	54.80	61.04	53.87	69.78	57.38	68.23	65.11	57.04	64.69
ψ_1	45.13	−102.20	−105.72	−115.92	−95.71	47.51	−103.88	−92.34	−98.87	−85.92	−100.62	−151.81	−85.56	−137.51	−85.71
ϕ_2	91.17	−84.90	−59.00	−81.64	−72.02	90.07	−86.83	−69.03	−91.99	175.83	−76.44	−72.81	−157.91	−84.53	−155.66
ψ_2	−57.11	52.10	−44.96	67.06	−42.65	−49.96	55.14	−43.62	46.57	63.42	−49.66	81.12	48.37	61.55	46.29
ϕ_3	−58.11	−176.06	−77.61	178.92	−81.54	−54.55	175.19	160.87	−171.35	179.16	175.85	−176.65	−167.56	−177.40	−163.50
ψ_3	96.56	59.55	171.74	−156.74	164.23	95.43	62.23	−174.40	174.09	−88.65	171.72	−108.41	173.40	149.95	122.67
ϕ_4	55.54	68.69	−57.19	−73.70	−62.01	65.20	54.80	61.04	−57.86	−81.09	67.32	−122.69	−65.11	−67.35	64.69
ψ_4	45.13	−88.44	92.35	77.42	98.67	−84.58	−103.88	−92.34	96.79	66.11	−85.14	79.99	85.56	82.82	−85.71
ϕ_5	91.17	−87.30	94.98	89.15	77.88	−142.82	−86.83	−69.03	59.54	74.33	−93.94	82.86	157.91	161.95	−155.66
ψ_5	−57.11	−45.77	−48.26	−51.21	37.04	39.58	55.14	−43.62	44.02	−76.84	48.38	−60.60	−48.37	−47.91	46.29
ϕ_6	−58.11	−76.82	−74.59	−64.28	−165.39	−159.56	175.19	160.87	−161.62	−74.64	67.53	−68.36	167.56	−169.47	−163.50
ψ_6	96.56	73.63	73.37	85.66	63.05	39.58	62.23	−174.40	67.87	164.10	−160.13	83.78	−173.40	147.82	122.67
E_{tot}[c]	−21.76	−17.86	−17.50	−17.32	−16.58	−16.46	−16.26	−16.17	−16.03	−15.92	−15.50	−15.34	−15.17	−15.10	−15.00
N_{reach}[d]	5	3	7	5	6	3	2	2	4	4	4	1	2	3	2
$E_{tot,ECEPP}$[c]	36.11	39.56	40.48	40.24	36.66	42.23	40.10	37.55	40.61	37.01	39.76	39.06	37.84	38.94	40.28
Order of[f] $E_{tot,ECEPP}$	1	16	33	25	2	60	22	5	36	3	19	13	6	10	27

[a] Good MEC's designated as HG are those given in Table VIII in paper I. They have either C_2 symmetry (HG_{18}, HG_{19}, HG_{20}, and HG_{22}) or *I* symmetry (Hg_{13}). Those designated as M have no symmetry. Subscripts on M have no significance other than to indicate the order in which they were obtained in the computation. [b] Dihedral angles in degrees. [c] Total conformational energies in kcal/mol for the same potential function used in paper I. MEC's are arranged in ascending order of E_{tot}. [d] Number of times that this MEC was reached. [e] Total conformational energies in kcal/mol for the ECEPP energy function. [f] Number indicating where the value of $E_{tot,ECEPP}$ appears in ascending order.

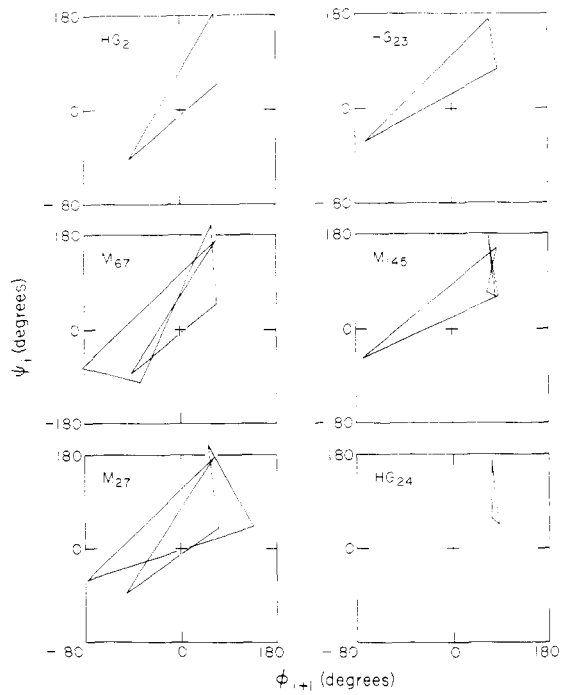

Figure 2. Plots of the dihedral angles of *rotation-linked* MEC's of *cyclo*-hexaglycyl. The values of (ϕ_{i+1},ψ_i) for each peptide unit are plotted. Conformations HG_{21} and M_{67}, M_{67} and M_{27}, HG_{23} and M_{145}, and M_{145} and HG_{24}, respectively, are rotation linked.

or not a minimum". The fact that conformation IV, which is very similar to HG_{15}, is observed in a crystal implies that HG_{15} is a shallow minimum and not a saddle point.

Transitions between rotation-linked MEC's of *cyclo*-hexaglycyl were not observed within the rather small number of steps employed in this study. It is interesting to note that this type of transition, in which an amide plane is flipped, was actually observed in a recent study of the dynamics of folded proteins by McCammon et al.[14]

There are five different conformations in a hemihydrate crystal[13] of this molecule, which we designated in paper I as I, II, II′, III, and IV. Conformations II and II′ are mirror images of each other. Conformation I is very similar to conformation III. Therefore, every conformation in this crystal is now found to be very close to a good MEC. The slight differences between HG_{11}, M_{166}, and HG_{15}, on the one hand, and III (and I), II (and II′), and IV, on the other hand, may be attributed to intermolecular interactions in the crystal. However, it is interesting to note that the global minimum energy conformation, HG_{19}, is not observed in this crystal. In fact, all three MEC's, HG_{11}, M_{166}, and HG_{15}, found in this crystal do not have very low conformational energies. This indicates that conformations of molecules of this size existing in its crystal must be close to an MEC, but not necessarily to an MEC with very low conformational energy. The choice from a long list of MEC's appears to be dominated by the existence of favorable intermolecular interactions. this last statement is supported, at least in the case of *cyclo*-hexaglycyl, by the fact that there are many intermolecular hydrogen bonds in its crystal.[13]

C. Effect of the Use of New Energy Parameters. In order to see the effect of a different choice of energy parameters, the conformational energies (of the 74 good MEC's with no symmetry and the 14 good MEC's with symmetry) were calculated also with the recently adopted (ECEPP) parameters.[9] The geometry of the glycyl residue in the ECEPP formulation is also slightly different from the one used in this paper. However, we did not change the geometry but changed only the parameters for the conformational energy. This

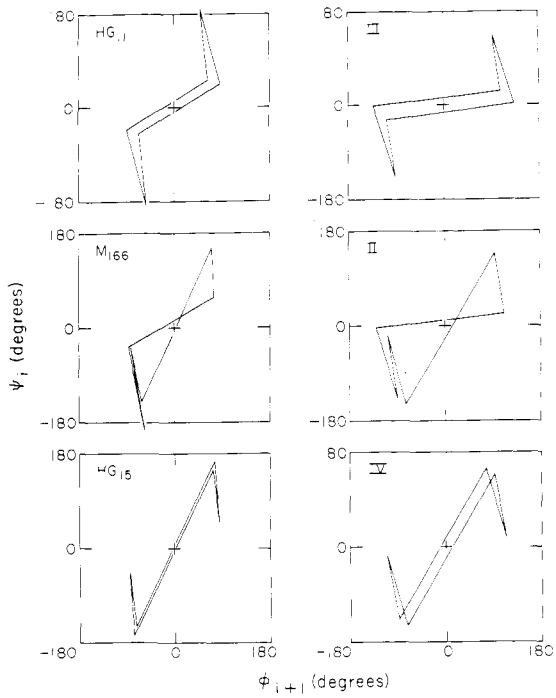

Figure 3. Plots of the dihedral angles of *rotation-linked* MEC's HG_{11}, M_{166}, and HG_{15} and of the three conformations III, II, and IV, found in the hemihydrate crystal of *cyclo*-hexaglycyl.[13] MEC's HG_{11}, M_{166}, and HG_{15} are very similar to conformations III, II, and IV, respectively.

procedure was adopted because the difference in geometry is very small, and it would have required a nontrivial amount of work to rewrite the program for exact ring closure, if the geometry were changed.

The global minimum energy conformation, with the energy parameters used here and in paper I, is also the global minimum with the ECEPP energy parameters. The ECEPP conformational energy is 36.11 kcal/mol. The large positive value arises mainly from the electrostatic contribution. The numbers of MEC's in 1-kcal/mol intervals are 2, 4, 5, 10, 23, 14, 8, 10, 6, and 6. The first two MEC's are in the 36.0–37.0-kcal/mol interval. The 40.0–41.0-kcal/mol interval has the largest number (23) of MEC's. The next-to-the-last interval corresponds to 44.0–45.0 kcal/mol, and the last one corresponds to MEC's with conformational energies larger than 45.0 kcal/mol. The ECEPP conformational energies for the 15 conformations given in Table III are also included in that table. The ECEPP conformational energies (of the 74 good MEC's with no symmetry and the 14 good MEC's with symmetry) were arranged in ascending order, and these order numbers are also given in Table III. It is interesting that, except for the fourth one, all MEC's up to the sixth lowest one are found in this table. The fourth lowest MEC with ECEPP parameters is HG_7 in Table VIII of paper I. This MEC was the 18th lowest MEC, with the parameters used in paper I. We conclude that low-energy conformations with the parameters of paper I also tend to be low-energy conformations with the ECEPP parameters.

The ECEPP conformational energies of MEC's HG_{11}, M_{166}, and HG_{15} are 43.54, 42.11, and 41.49 kcal/mol, respectively, which again are not very low. Therefore, the role of intermolecular interactions, pointed out in the last paragraph of section IIIB, also holds for the ECEPP energy parameters.

In the above discussion, we focused on the ECEPP conformational energies calculated for conformations determined with the earlier set of energy parameters. To be more precise, energy minimization should have been carried out with the ECEPP energy parameters. However, the relative values of the energies of different local MEC's would be expected to be affected more than their locations by a change in energy parameters. Since the purpose of carrying out this calculation with ECEPP parameters was not to make a systematic comparison of the two sets of energy parameters but simply to check how sensitive the conclusion of this paper is to a different choice of energy parameters, it is gratifying to observe that even the *relative* values of the conformational energies are reasonably well maintained.

Table IV
Conformational Energies of Initial and Final Conformations Obtained by Two Different Methods

Initial conform.	Energy[a] of initial conform.	Energy[a] of final MEC: Monte-Carlo method followed by energy min.	Energy[a] of final MEC: Direct application of energy min.
HG_{11}-1	−5.54	−14.34	−14.34
HG_{11}-2	−5.14	−13.22	−12.72
HG_{11}-3	279.35	−14.33	−21.76
HG_{11}-4	−4.50	−16.46	−13.03
HG_{11}-5	−9.55	−12.90	−10.02
HG_{11}-6	52.68	−14.34	−14.13
HG_{11}-7	−7.49	−14.34	−14.34
HG_{11}-8	27.41	−14.34	−14.25
HG_{15}-1	−9.36	−14.58	−11.36
HG_{15}-2	157.46	−15.10	−10.27
HG_{15}-3	−6.86	−16.58	−11.66
HG_{15}-4	7.56	−12.82	−10.95
HG_{15}-5	−1.27	−21.76	−15.50
HG_{15}-6	276.23	−12.13	−15.92
HG_{15}-7	16.38	−14.93	−14.27
HG_{15}-8	1.83	−21.76	−21.76
HG_{15}-9	8.88	−17.32	−21.76

[a] In kcal/mol.

D. Assessment of the Power of the Monte-Carlo Method. An advantage of the Monte-Carlo method of Metropolis et al.,[6] as applied to conformational energy analysis, is its ability to get out of shallow local minima. If a computer run is carried out for an infinitely long time, it is in principle possible to get out of all local minima and arrive at the global minimum. However, in practice, a computer run must be terminated within a finite time. In the present study, a computation was terminated if it was trapped in a minimum for 200 steps, and such a minimum was defined as a "good minimum". Whether a minimum is good or not depends on whether the average number of steps in which it is trapped is or is not larger than a cutoff number of steps. The fact that 81 good MEC's were located in this study, and still more are expected to exist, means that the cutoff number of 200 steps was not large enough to restrict the good minima to a limited number (including the global minimum); i.e., to reduce the number of good MEC's, a cutoff number of more than 200 steps would be required.

However, there is some evidence to indicate that, even with this rather small cutoff number of 200 steps, a few shallow local minima were screened out by this Monte-Carlo method. For the purpose of assessing the power of the Monte-Carlo method the combined procedure (Monte Carlo followed by energy minimization) was compared to energy minimization by itself. This test was carried out on the 17 conformations conjugate to either HG_{11} or HG_{15}. The energies of the initial and final conformations obtained in these calculations are summarized in Table IV. In three out of 17 cases (HG_{11}-1, HG_{11}-7, and HG_{15}-8), the same MEC's were arrived at by both methods. In 11 out of the remaining 14 cases, the combined method arrived at MEC's with lower conformational energy than the MEC's arrived at by direct application of the energy-minimization procedure. In the remaining three cases (HG_{11}-3, HG_{15}-6, and HG_{15}-9), direct application of energy

minimization led to MEC's with lower conformational energy. The number of initial conformations (17 in this test) may not be large enough to enable definitive conclusions to be drawn. However, the results summarized in Table IV support the conclusion that the Monte-Carlo method, as applied in this study, has an ability to skip over some shallow minima.

One of the MEC's (HG_{15}-7), obtained by direct application of the energy-minimization procedure to the nine conformations conjugate to HG_{15}, is the same as HG_{21}, which was classified as a "poor minimum or not a minimum" in Table I; also, as shown in Table I, this MEC was not reached in any of the calculations on the 166 starting conformations. However, the fact that HG_{21} was reached by energy minimization (from HG_{15}-7) means that it is in fact a minimum, and not a saddle point, in the six-dimensional space. Therefore, HG_{21} is a clear example of a "poor minimum". Even with a small cutoff number of 200 steps, some of the local minima become poor minima (such as HG_{21}) and can be skipped over by the Monte-Carlo method.

In the application of this Monte-Carlo method to lattice models of proteins,[7] computations were performed up to as many as 10^6 steps, and, in certain types of models, the native conformation corresponding to the global minimum could be reached. The power of the Monte-Carlo method depends very much on the computationally feasible number of steps and on the average number of steps in which the molecule is trapped in local minima.

V. Conclusions

The Monte-Carlo method of Metropolis et al.[6] has been applied to the calculation of the conformation of *cyclo*-hexaglycyl. The basic idea of this method, as applied to conformational-energy analysis, is to simulate the real process in which a molecule undergoes conformational changes in solution. If the computations can be carried out long enough, it is in principle possible to move out of any local minimum and finally reach the global minimum. However, in practice, the power of this Monte-Carlo method is determined by the ratio of the practically feasible number of computational steps to the average number of conformational changes required to move out of a local minimum in which the molecule is trapped. In the present study, the Monte-Carlo run was terminated if the conformations in 200 consecutive steps did not have lower energies than that of the previous conformation. Even with this rather small number of cutoff steps, some of the local minima of *cyclo*-hexaglycyl were found not to be deep enough to trap the conformation. Such shallow minima were termed "poor minima". If the local minimum was deep enough to trap the conformation for 200 steps, then it was defined as a "good minimum". It was found that there exist many (at least 81 and surely more) "good minima" and that some "poor minima" could be screened out.

The Monte-Carlo method was also applied here to examine the nature of the MEC's with symmetry that were found in paper I.[3] These MEC's could be saddle points in the full six-dimensional space because they were located as minima in subspaces of lower dimensionality, corresponding to conformations with symmetry. Fourteen of the 24 MEC's with symmetry were found to be "good minima". The other 10 are either "poor minima" or "not minima".

Finally, a nearly complete list of the MEC's with low conformational energies was obtained by reaching each of them several times with the Monte-Carlo method. The conformational characteristics of these MEC's were described. There is a relation, defined here as a *rotation link*, between many pairs of good MEC's. Two conformations are rotation linked if one of them can be obtained from the other by rotating only one peptide plane around the axis connecting the two C^α atoms at each end of the peptide plane without affecting the conformation of the other part of the molecule too much. One interesting example is MEC M_{166}, which is rotation linked to two different MEC's, HG_{11} and HG_{15}. These three conformations, HG_{11}, M_{166}, and HG_{15}, are very similar to conformations III, II, and IV, respectively, found[13] in a hemihydrate crystal of *cyclo*-hexaglycl.

References and Notes

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(10) Since C_i is known, the value of k is known. If the value of l for C_i* is the same as that for C_i, then the appropriate value of k is selected. However, if the value of l is not the same, this means that a border is crossed. In such a case, the independent variables are rotated, and a new C_i* is generated, until the value of l is the same for C_i and C_i*, in which case the appropriate value of k can be selected.
(11) The value of $\exp(-\Delta E/k_B T)$ is compared with a randomly generated number between 0 and 1. If the random number is smaller than $\exp(-\Delta E/k_B T)$, C_i* is taken as C_{i+1}. Otherwise, C_i is retained, and the procedure is repeated.
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