segmental mobility in the random coil state and remains flexible even in the α-helix form, up to pD 13. The activation energy for the segmental motion of the main chain is of the order of 6 kcal/mol at pD 7, like poly(L-glutamic acid)[14] and poly[N^5-(3-hydroxypropyl)-L-glutamine][21] under similar conditions, confirming that in the random coil state the flexibility of polypeptides is nearly independent of the nature of side chains.[28]

The rotational isomerism and the jump rates of side-chain methylene group are quite different from those of a hydrocarbon chain attached to a macromolecule,[29,30] showing, in particular, for model I of segmental motion, which seems the most likely, a gradual decrease of the reorientational freedom from the main chain through the terminal group. In the present case the comparatively slow rotation of $(CH_2)_\epsilon$ may be explained by the high hydration degree of the adjacent ND_3^+ group, which has been evidenced by NMR.[31,32]

Acknowledgment. We are greatly indebted to Dr. H. R. Wyssbrod for his helpful comments and suggestions concerning the interpretation of vicinal coupling constants in terms of rotamer populations.

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Conformation of *cyclo*(L-Alanylglycyl-ϵ-aminocaproyl), a Cyclized Dipeptide Model for a β Bend. 1. Conformational Energy Calculations[1a]

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ABSTRACT: The cyclized peptide derivative *cyclo*(L-alanylglycyl-ϵ-aminocaproyl) contains three peptide groups. These are constrained to form a β bend because the distance between the C^α and C^ϵ atoms of the ϵ-aminocaproyl residue cannot exceed 5.04 Å, even when the alkyl chain is fully stretched. Therefore, this molecule serves as a model compound for bends. Its experimentally observed physical properties can be used as standards for the detection of the presence of bends in peptides. An analysis of the complete conformational space of this molecule has been carried out, using energy computation. The conformational space of the L-Ala-Gly dipeptide was mapped in a search for low-energy conformations which permit ring closure with the ϵ-aminocaproyl residue. A numerical search method was used to achieve ring closure. Locally stable conformations were found by energy minimization. Low-energy conformations occur only when all three peptide groups are in the trans conformation because the presence of even one cis peptide group raises the energy by at least 9.7 kcal/mol. Ten low-energy conformations of minimum energy were found. Two are type II bends, with relative energies 0.00 and 0.93 kcal/mol. Five are type I and III bends, with relative energies ranging from 0.74 to 1.59 kcal/mol. Three are type I′ and III′ bends, with relative energies ranging from 2.80 to 3.08 kcal/mol. These results suggest that the molecule exists predominantly as a type II bend, with small amounts of type I and III bend conformations present. This prediction was borne out by experimental measurements in solution and in the solid state (reported in two accompanying papers).

I. Introduction

Bends constitute one of the important local conformational features of proteins, along with α helices and extended chains.[2] About 17% of all dipeptide sequences in many proteins of known structure occur as bends or combinations of bends.[2,3] The geometrical features of bends have been characterized by Venkatachalam,[4] who described and classified bend conformations into types I, II, and III. A more general classification of bends into several additional types was introduced by Lewis et al.[5] They

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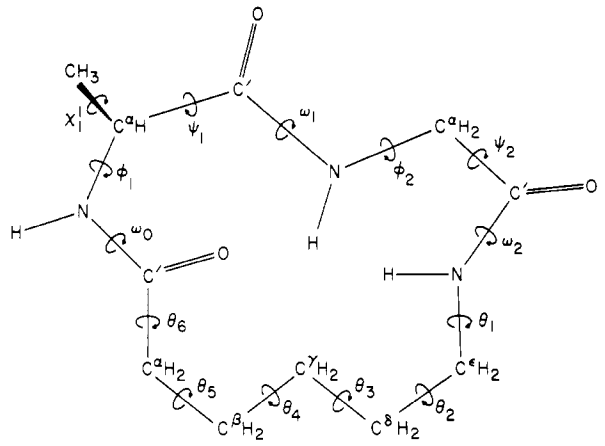

Figure 1. Structural formula of *cyclo*(L-Ala-Gly-Aca), with the definition of the dihedral angles used to describe the conformation of the molecule.

defined a bend formed by residues $i + 1$ and $i + 2$ if these two residues are not in an α-helical sequence of three or more residues and

$$R_3 \leq 7.0 \text{ Å} \quad (1)$$

where R_3 is the distance between the C^α atoms of residues i and $i + 3$. Zimmerman et al.[6] showed that the probability distribution of R_3 for all pairs of residues in 20 globular proteins, excluding pairs inside helices or extended chains, has two peaks, separated by a minimum at $R_3 \approx 7$ Å. Thus, bends can be distinguished from other nonrepeating structures in a natural way by using the criterion of eq 1. Bends were also characterized in terms of their overall shape by Rackovsky and Scheraga,[7] who used a differential geometric analysis.

The probabilities of formation of bends in oligopeptides and proteins were analyzed by Lewis et al.[5,8] and by other investigators.[3,6,9-21] These studies were based upon the concept[22] that short-range interactions are dominant in determining the conformations of various structures (including bends) in proteins.

The presence of bends in a variety of small open-chain and cyclic oligopeptides has been suggested in experimental investigations, using primarily various spectroscopic techniques.[23-35] The interpretation of experimental studies on oligopeptides and proteins, in terms of the presence of bends, is rendered difficult by the lack of well-defined experimental parameters which could be attributed uniquely to bends. There is one main problem regarding the establishment of criteria and parameters which can be used to indicate the presence of bends in a polypeptide chain. Open-chain oligopeptides used as models (as well as *larger* cyclic peptides) usually exist in solution as an ensemble of many conformations, only some of which are bends.[30,36] The observed properties represent an ensemble average over *all* conformations, not just bends. Theoretical conformational energy calculations on *N*-acetyl-*N'*-methylamides of various dipeptides predict a content of bends ranging from 0 to about 60%, depending on the sequence,[18-21,37] even when solvent is not taken into account. The total number of conformations of small cyclic oligopeptides is much more limited, but the presence of cis peptide links may complicate the analysis of observations.

In order to circumvent these difficulties, we have prepared and investigated cyclized dipeptide derivatives[38] which are constrained to exist as a bend because of the requirements of ring closure.[39] In this series of papers, we report on the cyclo-blocked alkane dipeptide *cyclo*(L-ala-nylglycyl-ε-aminocaproyl), shown in Figure 1 and abbreviated as *cyclo*(L-Ala-Gly-Aca). This molecule contains only two α-amino acid residues and three peptide groups. An alkyl chain (from C^α to C^ϵ) is used to close the ring because it contains no functional groups and it is optically inactive. Therefore, most observed properties of the molecule can be attributed to the terminally blocked dipeptide moiety. It was desired to use the shortest feasible ω-amino acid to close the ring, in order to keep the possible number of conformations small, without distorting the peptide groups significantly from planarity or forcing them into the cis conformation. Preliminary calculations and model building indicated that Aca satisfies these conditions.

Table I
Bond Lengths and Bond Angles of the Aca Residue Used in the Computations

Bond Lengths, Å			
$N-C^\epsilon$	1.453[a]	N-H	1.000[a]
$C^\alpha-C'$	1.530[a]	C-C	1.530[b]
$C'-N$	1.325[a]	C-H	1.090[b]
$C'=O$	1.230[a]		
Bond Angles, Deg			
$C^\alpha C'N$	115.0[a]	C'NH	124.0[a]
$OC'N$	124.5[a]	HNC^ϵ	115.0[a]
$C^\alpha C'O$	120.5[a]	CCC, NCC	111.0[b]
$C'NC^\epsilon$	121.0[a]	HCH	107.0[b,c]

[a] Selected to keep the geometry of the peptide group the same as in ECEPP.[44] [b] Selected in analogy with amino acid side chains in ECEPP.[44] [c] The two hydrogens of the CH_2 groups were generated symmetrically with respect to the CCC (or NCC) planes.

The distance R_3 between the C^α and C^ϵ atoms of the Aca residue cannot exceed 5.04 Å, the distance in a fully extended five-carbon aliphatic chain. Thus, the Ala-Gly dipeptide is forced into a bend, no other conformations being possible. Therefore, the compound can be used to characterize the properties of a bend. The spectroscopic parameters obtained for it can be applied to detect bends in future studies of peptides in solution.

In this paper, we report the conformational analysis of *cyclo*(L-Ala-Gly-Aca) by means of energy calculations. The next parts of the series[40,41] will describe the synthesis of the molecule and the results of NMR, CD, infrared, and Raman studies. Some of the results have been summarized in a preliminary report.[39] Studies on the related compounds *cyclo*(L-Ala-L-Ala-Aca) and *cyclo*(L-Ala-D-Ala-Aca) will be reported elsewhere.[42]

II. Methods

A. Definitions. The recommended nomenclature and conventions for polypeptide conformations are used.[43] The designation of the dihedral angles in the Aca residue is shown in Figure 1.

B. Bond Geometry. The bond lengths and bond angles used for the Ala and Gly residues and for the peptide groups are those of the standard geometry adopted in the ECEPP computer program, used in this laboratory,[44] with one exception. The original form of ECEPP used 1.00 Å for the C^α–H bond length.[44] Recent neutron diffraction studies on amino acid crystals[45,46] indicate that the C^α–H distance is 1.09 Å, as in other C–H bonds. The revised bond length was used in this work. The bond lengths and bond angles involving the C and H atoms in the Aca residue are shown in Table I.

C. Partial Charges. The values adopted for ECEPP were used for the Ala and Gly residues. The partial charges on the atoms of the Aca residue were determined by a CNDO/2 (ON) calculation[47] on several conformations

Table II
Partial Charges on Atoms of the Aca Residue

atom	charge[a]	atom	charge[a]
N	−0.344	C^{β}	−0.006
H	0.176	H^{β}	0.015
C^{ϵ}	0.081	C^{α}	−0.077
H^{ϵ}	0.010	H^{α}	0.023
C^{δ}	−0.033	C′	0.450
H^{δ}	0.021	O	−0.384
C^{γ}	−0.012		
H^{γ}	0.006		

[a] In electronic charge units (ecu).

of *cyclo*(L-Ala-Gly-Aca). The computed values were adjusted slightly for the sake of self-consistency,[47] i.e., to make the charges on atoms of the peptide groups the same as in ECEPP and to ensure charge neutrality of the molecule. The values used are shown in Table II.

D. Ring Closure. In a cyclic structure with fixed bond lengths and bond angles, containing n bonds around which rotation is possible, $n - 6$ of the n dihedral angles are independent variables.[48] The values of the six dependent variables are determined by the condition of ring closure. We chose the dihedral angles in the Ala-Gly moiety of the cyclic molecule as the independent variables so that the six dihedral angles inside the Aca residue, viz., θ_j (j = 1, ..., 6), became the dependent variables. This choice permits the mapping of the conformational space of the molecule in terms of the usual polypeptide diehedral angles and makes it easier to compare results obtained here with those from studies of open-chain oligopeptides. Programming is simplified if the dependent variables correspond to adjacent bonds. The number of independent variables is four (viz., ϕ_{Ala}, ψ_{Ala}, ϕ_{Gly}, ψ_{Gly}) when the peptide bonds are considered rigidly fixed (ω_i = 180 or 0° for i = 0, 1, 2) and seven when the ω_i's are variable. The value of the side-chain dihedral angle χ^1 of Ala was kept fixed at 60° throughout this work, thus eliminating an additional variable, because preliminary work indicated that this dihedral angle does not vary significantly in low-energy conformations.

Conformations with exact ring closure were generated by means of the method derived by Gō and Scheraga.[48] The algorithm used in our computer program[49] is based on section III of ref 48. The main concepts of the method are summarized here.[50] Equation numbers preceded by GS- refer to the paper by Gō and Scheraga.[48]

For a given choice of all independent variables, possible combinations of the dependent variables which permit the closure of the ring are searched for by numerical solution of eq GS-16 to GS-36. Equations GS-16 to GS-19 represent the condition of exact ring closure, i.e., that the initial and terminal atoms of a chain structure are superimposed on each other, with correct bond orientations. It was shown[48] that two of the dependent variables, say θ_5 and θ_6, can be expressed in terms of the four others (by means of eq GS-17 and GS-18). Therefore, four scalar equations, corresponding to the four components of the vector eq GS-16 and GS-17, can be solved for the four unknowns θ_1, θ_2, θ_3, and θ_4.

The four-equation system for the four unknowns is solved by the following numerical method. Three of the variables, θ_2, θ_3, and θ_4, are expressed as functions of the variable θ_1. This is done by means of eq GS-29, GS-33, GS-35, and GS-36. The existence of solutions of these equations requires the satisfaction of several necessary and sufficient conditions. The conditions are expressed by means of eq GS-27, GS-28, and GS-34. The conditions must be tested during the computation. It they are satisfied, eq GS-29, GS-33, GS-35, and GS-36 can be substituted into eq GS-20, which then becomes an algebraic equation in one variable, θ_1.

This equation must be solved numerically. Values of θ_1 are chosen in small increments, and the value of the left-hand side of eq GS-20 is determined for each choice of θ_1. If the sign of this quantity changes for two successive choices of θ_1, a solution to eq GS-20 must exist in that interval of θ_1. The solution is found by means of numerical interpolation, using binary partition.[51]

There may exist several solutions for ring closure with a given set of values of the independent variables.[48] In such a case, several values of θ_1 satisfy eq GS-20. All solutions are obtained by stepping through the possible range of θ_1. For each solution, the values of the other dependent variables, θ_2 to θ_6, are determined from eq GS-29, GS-36, GS-33, GS-17, and GS-18, respectively. The different solutions correspond to different branches of the potential-energy surface (i.e., the energy as a function of all independent variable dihedral angles), as discussed by Gō et al.[48,52-55]

The impossibility of ring closure is indicated by the violation of at least one of the conditions in eq GS-27, GS-28, or GS-34. Equation GS-27 is not satisfied if the distance between the ends of the open chain is too large or too small, so that the ring cannot be closed by any choice of θ_1. In this case, the given values of independent variables must be rejected. If either eq GS-28 or GS-34 is not satisfied, no ring closure is possible for the current value of θ_1 but there may be solutions for other values of θ_1 for the given set of values of the independent variables.

In principle, the entire range of $0° \leq \theta_1 < 360°$ should be searched for possible solutions of eq GS-20. The search can be shortened if it is known from preliminary studies or from information on possible constraints that certain ranges of θ_1 do not contain any solutions. In this case, the search can be confined to the remaining range(s). The sizes of the increments of θ_1 have to be chosen carefully. If the increment is very large, there is a possibility that two solutions are missed, occurring at two slightly different values of θ_1 within a given interval. A very small value of the increment requires many steps in the numerical prodcedure, increasing the computation time. An increment of 2.5° was used in this work. A more detailed description of search limits and intervals is given in the user's guide of the program.[49]

The method of Gō and Scheraga[48] was applied earlier to cyclic peptides composed of only α-amino acid residues.[52,55] The computer program used here is applicable to *exact* ring closure of any cyclic molecule. A similar procedure, derived recently and applied to a cyclic tetrapeptide, is based on a grid search of (ϕ,ψ) conformational space and an *approximate* numerical search for ring closure.[56]

E. Potential Energy Computation. Whenever the ring closure program locates one or more sets of θ_i's which allow exact ring closure for a given choice of the independent variables, a modified form of the computer program ECEPP is used to generate the atomic coordinates of all atoms (including the hydrogens and the alanyl side chain) and to compute the intramolecular potential energy of the conformation. The parameters characterizing the various semiempirical terms of the potential energy were those used in ECEPP.[44] No solvent was included in the calculations.

F. Uses of the Ring Closure Program. The ring closure program, combined with the energy program, can be used in two modes.

1. Determination of Ranges of the Independent Variables for Which the Ring Can Be Closed. A grid search is carried out over all or part of the multidimensional conformational space characterized by the independent variables. Ranges and increments are specified at the outset for each independent variable. The ring closure program is applied at each grid point. Whenever closed rings can be generated, the number of solutions, the dihedral angles, and the energy of each conformation are computed and printed. The range of dihedral angles to be used in the grid search may be limited, based on prior information. For example, only low-energy regions of the (ϕ,ψ) conformational maps for the Ala and Gly residues were considered here (section II.G.1).

2. Determination of Minimum-Energy Ring Conformations of the Cyclic Peptide. The programs for ring closure and energy computation are combined with a function minimizer algorithm. The potential energy is minimized as a function of the dihedral angles, subject to the condition that exact ring closure is maintained. Whenever the function minimizer routine changes the values of the dihedral angles used as independent variables, in the course of minimization, the ring closure program is entered as the next step, in order to find a set of the dependent variables which close the ring. If the ring cannot be closed, no energy is computed for the molecule. Instead, an arbitrary high value (e.g., 1.0×10^8 kcal/mol) is assigned to the energy. This forces the function minimizer algorithm to move away from conformations with no ring closure. If the ring can be closed, its energy is computed. If there are several possible solutions of the ring closure equations, i.e., if there exist several sets of dependent variables which allow the ring to be closed, the energy of each solution is determined, and only the solution of lowest energy is used further in the minimization procedure.

Energy minimization was carried out by using the algorithm of Powell.[57] The computation was terminated when the total conformational energy, expressed as a single-precision variable on the computer, did not change between consecutive iterations.

G. Selection Strategy for Obtaining Starting Conformations and Energy Minimization. In order to avoid excessive computation time, a screening procedure, consisting of several steps, was used to eliminate those regions of (ϕ,ψ) conformational space where there are no solutions for ring closure or where the energy is very high.

Steps 1–5 of the procedure described below were carried out with fixed values of the ω's (either 180 or 0°), and step 6 was done with variation of the ω's.

1. Elimination of High-Energy Conformations. High-energy regions of the (ϕ,ψ) maps for the *N*-acetyl-*N'*-methylamides of alanine and glycine do not have to be considered because cyclic structures formed from them would also be of high energy. Only regions of the conformational maps with $\Delta E \leq 5$ kcal/mol (relative to the lowest point of *each* map) were used in the subsequent steps. Maps for Ala and Gly residues flanked by two trans peptide groups were taken from ref 58. The conformational maps used for *cis*-containing peptides are shown and discussed in Appendix A.

2. Elimination of Dipeptide Conformations in Which the Distance between the Ends Is Too Long To Close the Ring. If the distance R_3 between the terminal methyl groups in the *N*-acetyl-*N'*-methylamide of a dipeptide exceeds 5.04 Å, i.e., the length of the fully stretched $-(CH_2)_5-$ chain, no ring closure is possible for this conformation of *cyclo*(L-Ala-Gly-Aca). All such dipeptide conformations can be eliminated. Violation of the constraint on R_3 would be detected by the ring closure program, as a violation of eq GS-27. It is more efficient, however, to eliminate many conformations by the use of the simple test described below, prior to the use of the ring closure program (step 3), because only limited ranges of ϕ and ψ in a dipeptide satisfy the requirement that $R_3 <$ 5.04 (cf. Table IV of ref 19).

Points were selected at 15 or 30° intervals in the low-energy (ϕ,ψ) regions of the terminally blocked Ala and Gly residues with fixed ω's (shown in Appendix A). A grid of test points was generated in the four-dimensional conformational space of *N*-acetyl-*N'*-methyl-L-alanylglycinamide by combining all points for Ala with all points for Gly. R_3 was computed at each grid point. Only points with $R_3 \leq 5.04$ Å were retained for the next step. Separate grid searches were carried out for each of the eight possible combinations of trans and cis peptide groups.

3. Use of the Ring Closure Program. The program was used first in the mode described in section II.F.1 to test those grid points of the four-dimensional Ala-Gly conformational space which were retained in step 2. In order to save computer time, a first cycle of testing was carried out by using points located at 30° intervals of the (ϕ,ψ) grids. This test eliminated large regions of conformational space in which ring closure is not possible because of violation of one or more conditions, as described in section II.D. In the second test cycle, grid points were selected at 15° intervals around each of the grid points accepted in the first cycle of testing. the use of this narrow grid served to define more closely the boundaries of the regions of conformational space in which the ring can be closed and to compute the energies of the cyclic molecule for many conformations for use in the next step.

4. Mapping of Conformational Space for the Cyclic Molecule. The results of the preceding step were used to construct energy contour maps along two-dimensional sections of the four-dimensional conformational space of the molecule. These sections represent the variation of the two dihedral angles (ϕ,ψ) of either the Ala or the Gly residue for fixed values of the dihedral angles (ϕ,ψ) of the other residue. The fixed values of (ϕ,ψ) were all the 30° grid points (section II.G.3) for which ring closure was possible, supplemented by several grid points at 15° intervals. This mapping locates low-energy regions of the four-dimensional conformational space in which ring closure is possible.

5. Energy Minimization with Fixed ω. The starting points were selected on the basis of the mapping described in step 4. The points were chosen wherever the mapping suggested that a minimum may occur nearby. Usually, several starting points were chosen around a suspected minimum in order to test whether they all lead to the same minimum. Several additional starting points were selected in other parts of the regions covered by the grids in order to make sure that no medium-energy minimum is missed. Energy minimization was carried out from these starting points, using the program in the mode described in section II.F.2, with respect to the four independent variables ϕ_1, ψ_1, ϕ_2, ψ_2.

6. Energy Minimization with Variable ω. A second set of energy minimizations was carried out, using the minima obtained in step 5 as starting points. All three peptide dihedral angles (ω_0, ω_1, and ω_2) were now allowed to vary, in addition to ϕ and ψ of Ala and Gly. These seven-variable minimizations are much more time-consuming than the minimizations described in step 5. In the course of the seven-variable minimizations, no large var-

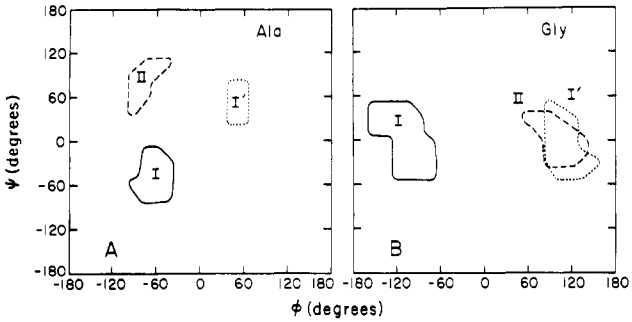

Figure 2. Conformational maps for the (A) Ala and (B) Gly residues in *cyclo*(L-Ala-Gly-Aca), indicating the combinations of low-energy regions for each residue within which the ring can be closed, with planar trans peptide groups. The Ala residue in regions I, II, and I′ can combine with the Gly residue in the corresponding regions to form a bend of type I (or III), type II, or type I′ (or III′), respectively. The ring cannot be closed or the energy is very high for conformations outside these regions.

iations of the values of the ω's occurred in low-energy conformations (see Results below). Therefore, it was felt that the grid tests for fixed ω, described above in steps 1–4, were sufficient and that no additional low-energy minima would be found if those steps were repeated with variable ω. The ring might be closed in some additional conformations, but only with large distortions of the peptide groups from planarity. Such conformations would have high energy because of the high torsional barrier for the peptide group. Therefore, they do not have to be considered.

The use of the strategy described in steps 1–6 required several separate computational steps, with intervening analyses of the intermediate results. It is preferable over a completely automated algorithm in which the ring closing and energy minimization program would have to be applied to a wide range of starting conformations because it saves considerable computer time. Intermediate results in several of the steps led to useful insights into the conformational behavior of *cyclo*(L-Ala-Gly-Aca).

III. Results

A. The Mapping of Conformational Space. The ring closure program was applied to *cyclo*(L-Ala-Gly-Aca) as described in section II.G.1–4. Low-energy conformations of the cyclic molecule can occur only in several very small regions of the four-dimensional Ala-Gly (ϕ_1, ψ_1, ϕ_2, ψ_2) conformational space.

1. Conformations Containing Only Trans Peptide Bonds. The cyclic molecule can exist in three distinct sets of low-energy conformations, corresponding approximately to bends of type I and III, type II, and type I′ and III′, respectively. Each set consists of the combination of a narrowly circumscribed low-energy region of the (ϕ,ψ) map for the Ala residue with a similarly small low-energy region of the (ϕ,ψ) map for the Gly residue (Figure 2). Within each region, there is some flexibility of choice; i.e., a given conformation of Ala in Figure 2A, with a particular (ϕ,ψ), can be combined with a range of Gly conformations within the appropriate region in Figure 2B, and vice versa. The three sets are separated from each other in the four-dimensional conformational space by regions in which the energy is very high and/or no ring closure is possible. Figure 2 is drawn for conformations with fixed planar peptide bonds (ω_i = 180°, for i = 0, 1, 2). The boundaries shown in the figure expand only slightly when the ω's are allowed to vary.

The largest of the three low-energy regions is that containing type I and III bends, denoted here as region I. The

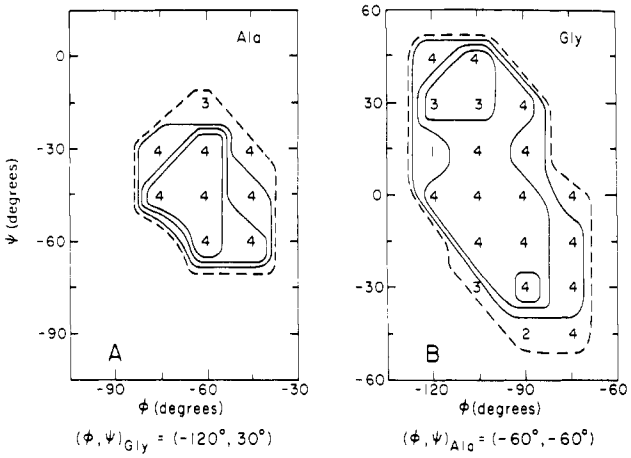

Figure 3. Representative examples of two-dimensional sections in the four-dimensional L-Ala-Gly conformational space of *cyclo*(L-Ala-Gly-Aca). (A) Conformational (ϕ_1,ψ_1) map for Ala, drawn with the conformation of Gly fixed at (ϕ_2,ψ_2) = (−120°, 30°). (B) Conformational (ϕ_2,ψ_2) map for Gly, drawn with the conformation of Ala fixed at (ϕ_1,ψ_1) = (−60°, −60°). The maps are drawn for planar trans peptide groups. The dashed lines enclose the low-energy regions in which ring closure is possible. The full lines are energy contours, corresponding to 2.5, 5.0, and 10.0 kcal/mol above the lowest point in each map. They are interpolated from the energy evaluations at grid points in 15° intervals of the variable dihedral angles. Numbers indicate the number of solutions of the ring closure equations at each grid point, as discussed in the text.

entire region cannot be represented easily in two dimensions, but its properties can be visualized in an approximate manner by plotting two-dimensional sections across this region, as described in section II.G.4. A representative pair of such sections is shown in Figure 3. They were chosen to lie near the minima occurring in the region. The analysis of several sections, similar to the ones shown in Figure 3, indicated that region I is subdivided into two low-energy valleys, characterized by positive and negative values of ψ_{Gly}, respectively, and separated by a saddle of moderate height (with an energy of about 3 kcal/mol above the minima) near $\psi_{Gly} \approx 0°$. These two valleys are seen clearly in the section shown in Figure 3B. In this particular section, the saddle occurs near $\psi_{Gly} \approx 15°$. The two valleys correspond to different signs of ψ_{Gly}. The corresponding structural difference is discussed below (section III.B).

Low-energy region I′, containing type I′ and III′ bends, is similar to region I, except that it is of higher relative energy and it is much narrower. It, too, is divided by a saddle around $\psi_{Gly} \approx 0°$ into two valleys, containing type I′ and III′ bends.

Region II, containing bends of type II, is divided in a similar fashion into two low-energy valleys. The saddle separating them is located near $\psi_{Gly} \approx 0°$ in this region, too.

Each of the low-energy valleys may contain one or several local minima (section III.B).

Usually, whenever ring closure is feasible at all for a given choice of the independent variables, it can be achieved by means of more than one set of the dependent variables θ_1, ..., θ_6, i.e., with several different conformations of the Aca residue. These conformations are obtained as different solutions of eq GS-20 (section II.D). As an illustration, the number of solutions is indicated in Figure 3 for the grid points chosen at 15° intervals of the variables. Most frequently, there exist four solutions. The number of solutions often decreases near the boundaries of the low-energy regions that are shown in Figure 2. The energy contours of Figure 3 show the energy of the lowest energy

solution at each of the points.

2. Conformations Containing One or More Cis Peptide Bonds. The regions of conformational space in which ring closure is feasible with cis peptide bonds, without severe atomic overlaps resulting in very high energies, are much narrower than those of the all-trans form. The mapping of these regions is discussed in Appendix B.

The presence of one or several cis peptide bonds raises the energy of *cyclo*(L-Ala-Gly-Aca) considerably. The lowest energy for any conformation with a cis peptide bond is at least 13 kcal/mol, relative to the lowest energy all-trans conformation, if the peptide groups are constrained to be planar (trans or cis), and at least 9.7 kcal/mol when the peptide groups are flexible, i.e., when the ω's are allowed to vary (sections III.B and III.C). These numbers are lower limits: the energies of most of the cis-containing conformations are much higher than these values (Appendix B).

B. Minimum-Energy Conformations with Variable ω's. These were obtained by means of the procedure described in section II.G.6.

1. Conformations Containing Only Trans Peptide Bonds. Ten minimum-energy conformations were found. Their energies and dihedral angles are listed in Table III. The lowest energy conformation (global minimum) is a type II bend. It is described in section IV. The energies of another type II bend and of five type I or III bends are very close to it, within 2 kcal/mol of the global minimum. On the other hand, the energies of the three type I′ and III′ bends are higher, about 3 kcal/mol above the global minimum. Thus, it is likely that only type II and type I or III bends are significant for this cyclic peptide.

The alkyl chain of the Aca residue generally is not in a fully staggered conformation. One of the four CH_2–CH_2 pairs, usually C^{γ}–C^{δ}, is nearly eclipsed in all conformations, except for the global minimum (cf. θ_3 in Table III). The low potential barrier ($U_0 \approx 3$ kcal/mol) for CH_2–CH_2 bonds permits the existence of such eclipsed conformations if this leads to a significant reduction of strain elsewhere in the cyclic molecule.

The four lowest energy conformations (1–4 in Table III) represent four distinct structures. They differ from each other in terms of the orientation of some peptide groups and, as a result, of the signs of dihedral angles next to these peptide groups.

It is well-known[4,5,18] that type II bends differ from type I (and III) bends by the opposite orientation of the peptide group between residues $i + 1$ and $i + 2$, in this case the Ala-Gly peptide group. The N–H bond of Gly points in the same direction as the C^{α}–C^{β} bond of the L-Ala residue in type I and III bends and in the opposite direction in type II bends. The altered orientation is reflected in the changed sign of ψ_1 and ϕ_2 when conformations 1 (a type II bend) and 2 (a type I bend) are compared. The two dihedral angles change by −133 and +171°, respectively. Differences of most of the other dihedral angles are small. An analogous relationship exists between conformation 3 (a type II bend) and conformation 4 (a type III bend).

Type I and III bends of the cyclic molecule can be differentiated from each other in a similar fashion by considering the orientation of the Gly-Aca peptide group. The N–H bond of Aca points in the same direction as the Ala C^{α}–C^{β} bond in the type III bend (conformation 4) but in the opposite direction in the type I bend (conformation 2). This is reflected in the changed signs of ψ_2 and θ_1. These dihedral angles differ in conformations 4 and 2 by −115 and +128°, respectively. The other dihedral angles do not differ by much.

Table III
Minimum-Energy Conformations of *cyclo*(L-Ala-Gly-Aca) with Trans Peptide Bonds

conformation no.	energy ΔE,[a] kcal/mol	bend type	dihedral angles, deg										code[b]
			ω_0	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ω_2	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6	
1[c]	0.00	II	171	−89	85	−176	81	74	−167	71	62	−160	157	−64	−71	CA*g$^+$g$^+$tg$^-$g$^-$
2	0.74	I	169	−76	−48	175	−108	62	−179	81	62	−117	165	−75	−83	ACg$^+$g$^+$a$^-$tg$^-$g$^-$
3	0.93	II	174	−85	74	−174	132	−62	173	−150	60	−120	167	−75	−82	CD*tg$^+$a$^-$tg$^-$g$^-$
4	1.07	III	174	−75	−55	179	−86	−53	175	−151	64	−114	173	−70	−78	AAtg$^+$a$^-$tg$^-$g$^-$
5	1.22	III	175	−78	−54	180	−87	−48	175	−156	63	−114	173	−69	−76	AAtg$^+$a$^-$tg$^-$g$^-$
6	1.25	III	179	−72	−45	176	−97	−58	177	−142	62	−114	172	−71	−81	AAa g$^+$a$^-$tg$^-$g$^-$
7	1.59	I	174	−78	−54	173	−92	67	−170	112	−71	169	−119	62	−136	ACa$^+$g$^+$ta$^-$g$^+$a$^-$
8	2.80	III′	−174	53	66	180	88	53	180	146	−64	116	−167	74	85	A*A*a$^+$g$^-$a$^+$tg$^+$g$^+$
9	2.96	I′	−167	51	58	179	113	−63	180	−85	−63	115	−159	80	95	A*D*g$^-$g$^-$a$^+$tg$^+$a$^+$
10	3.08	I′	−171	52	73	180	92	−67	172	−124	71	−162	123	−61	148	A*C*a$^-$g$^+$ta$^+$g$^-$a$^+$

[a] $\Delta E = E - E_0$, where $E_0 = 2.56$ kcal/mol, the computed energy of conformation 1. [b] Shorthand notation for the backbone conformation. Capital letters denote the backbone conformational regions of the Ala and Gly residues in terms of the code introduced in ref 58. Lower case letters denote the conformation of the Aca residue: g$^+$, t, and g$^-$ indicate staggered, gauche, and trans conformations having values of θ within ±30° of 60, 180, and −60°, respectively; a$^+$ and a$^-$ indicate eclipsed (anticlinal) conformations having values of θ within ±30° of 120 and −120°, respectively.[59] [c] There are two low-energy conformations corresponding to this set of Ala and Gly dihedral angles. The Aca dihedral angles of the second conformation are $(\theta_1, \ldots, \theta_6) = (121, -63, 155, -162, 63, -122°)$; i.e., it is described by the shorthand notation CA*a$^+$g$^-$tg$^+$a$^-$. Its energy is $\Delta E = 1.31$ kcal/mol.

The two type II bend conformations (1 and 3) bear the same relationship to each other as the type I and III bends: in each case, the Gly-Aca peptide group is oriented differently; i.e., ψ_2 and θ_1 have different signs. They differ by −136 and +139°, respectively. The orientation of the Aca NH group in conformation 1 is the same as in a type I bend (conformation 2).

The geometrical relationships between various bend types are discussed more fully elsewhere.[60] Here, it has to be noted that the differences in the orientation of peptide groups, described above, limit the possibilities of formation of hydrogen bonds in various bends. A bent Aca-N-H···O=C-Aca hydrogen bond (in a ten-membered structure) is possible only in conformations 1 or 2, but not in 3 or 4. A bent Gly-N-H···O=C-Aca hydrogen bond (in the C_7^{eq} conformation of the Ala residue) is possible only in type II bends (conformations 1 and 3). A bent Aca-N-H···O=C-Ala hydrogen bond (in the C_7^{eq} or C_7^{ax} conformation of the Gly residue) is possible only in conformations 2 and 3. The hydrogen bonds mentioned do not necessarily exist in these conformations: the O and H atoms have to be not only similarly oriented but also located near each other.

The other conformations of Table III are variants of the first four. The three type III bends (conformations 4–6) are very similar, with dihedral angles differing from each other by 14° or less. The three conformations apparently represent closely spaced local minima at the bottom of a potential energy valley because they do not merge upon energy minimization. The Ala-Gly conformation is very similar in the two type I bends (conformations 2 and 7). They differ from each other mainly by having a different Aca conformation.

The backbone conformations in type I′ (and III′) bends are enantiomers of those in type I (and III) bends.[4,5] Stable bends of these two types, in an L-Ala-Gly sequence, cannot be exact mirror images because of the presence of the chiral L-Ala residue. Table III indicates, however, that the backbones of conformations 8, 9, and 10 are very close to being mirror images of conformations 4, 2, and 7, respectively: all of the corresponding dihedral angles in the two groups are opposite in sign but similar in magnitude. The values of (ϕ,ψ) of Ala in conformations 8–10 are shifted so as to bring the Ala residue into the small low-energy region A* of the Ala (ϕ,ψ) map.[58]

In the case of the global minimum (conformation 1), two low-energy solutions of the equations of ring closure exist for the given Ala-Gly dihedral angles. They correspond to different conformations of the Aca residue (footnote c of Table III). Their energy difference is only 1.3 kcal/mol, so that it might be possible that a fraction of the molecules would exist in the second conformation. Nuclear magnetic resonance measurements[40] argue against this possibility. The $C^{\gamma}H_2$ group is positioned somewhat differently, so that a different H^{γ} atom points into the ring in the two conformations. The observed chemical shift of the H^{γ} inside the ring differs from that of the other H^{γ}, due to shielding.[40] Equilibration of the two forms would lead to splitting of the signals. The observed $^3J_{HC-CH}$ coupling constant for the C^{α}–C^{β} bond is not consistent with the 1.3 kcal/mol form. The energy of all other solutions of the ring closure equations is much higher (8.6 kcal/mol or more) for all ten conformations listed in Table III, so that they can be disregarded.

2. Conformations Containing One or More Cis Peptide Bonds. The energies of all minimum-energy conformations of this type are much higher than those of the conformations with trans peptide bonds. The results of the energy minimizations are tabulated in Appendix B. Summarizing the results, the relative energies of trans-trans-cis minima range from 9.7 to 13.9 kcal/mol, those of cis-trans-trans minima range from 11.9 to 13.5 kcal/mol, those of trans-cis-trans minima extend from 16.7 to 54.0 kcal/mol, those of minima with two cis peptide bonds range from 30.6 to 40.6 kcal/mol, and that of a minimum with three cis peptide bonds is 91.9 kcal/mol. These energies are very high. Therefore, *cyclo*(L-Ala-Gly-Aca) does not contain any detectable amount of any conformation with a cis peptide bond. The limited experimental evidence available to test this conclusion was discussed earlier.[39] It supports the theoretical result.

Table V
Predicted $^3J_{HN-C^{\alpha}H}$ Coupling Constants[a] of *cyclo*(L-Ala-Gly-Aca)

	coupling constant, Hz				
	Ala	Gly		Aca[b]	
lowest energy conformation (1)	8.2	7.6	6.9	7.8	5.8
averaged over the two type II conformations (1 and 3)	8.1	6.6	7.5	7.9	4.9
averaged over the five type I and III conformations (2, 4, 5, 6, and 7)	6.0	9.1	5.5	7.6	3.8
averaged over all conformations	7.3	7.5	6.8	7.8	4.5
observed[c]	7.1	7.1	5.0	~5	

[a] Calculated with the J vs. ϕ relation of ref 65. [b] $^3J_{HN-C^{\alpha}H}$. [c] In Me_2SO-d_6.[40]

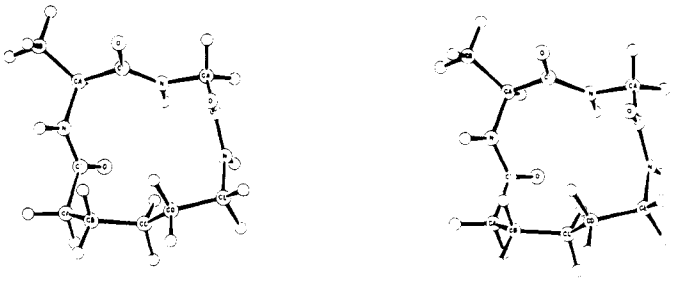

Figure 4. Stereoscopic drawing of the computed lowest energy structure of *cyclo*(L-Ala-Gly-Aca), a type II bend (see the first line of Table III).

C. Minimum-Energy Conformations Computed with Fixed ω's (Planar Peptide Bonds). As an intermediate step in the computation, energy minimization was carried out with ω_i (i = 0, 1, 2) fixed in the planar trans or cis form (section II.G.5). The results for the trans-trans-trans minima are shown in Appendix C. A comparison of the energies and dihedral angles with those in Table III shows that it is important to allow for the flexibility of the peptide group in energy minimization for this compound. The energy of every conformation is lowered by 0.9–4.1 kcal/mol when the ω's are allowed to vary, even in conformations in which the final values of the ω's do not differ much from 180°. Accordingly, this cyclic compound is somewhat strained, but the strain can be reduced by moderate twisting of the peptide groups out of planarity, without the necessity to introduce cis peptide bonds.

IV. Discussion

The conformational space of *cyclo*(L-Ala-Gly-Aca) is very limited, as shown in the discussion of the mapping (section III.A and Appendix B). The limitations arise from the

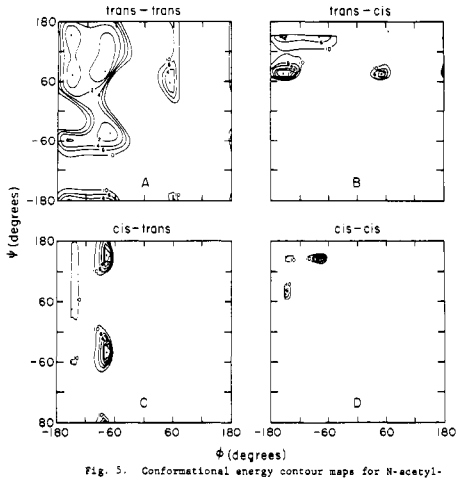

Fig. 5. Conformational energy contour maps for N-acetyl-N'-methyl-L-alanineamide for $\chi^1 = 60°$. The two peptide bonds are fixed in the states (A) trans-trans, (B) trans-cis, (C) cis-trans, (D) cis-cis. The contours are labeled with energy in kcal/mol above the minimum-energy point of each map: (A) $(\phi,\psi) = (-84°, 80°)$, $E_0 = -3.3$ kcal/mol, (B) (-153°, 77°), 8.6 kcal/mol, (C) (-77°, -41°), 10.2 kcal/mol, (D) (-77°, 149°), 25.4 kcal/mol. Locations of minima (obtained with fixed ω's) are indicated by filled circles.

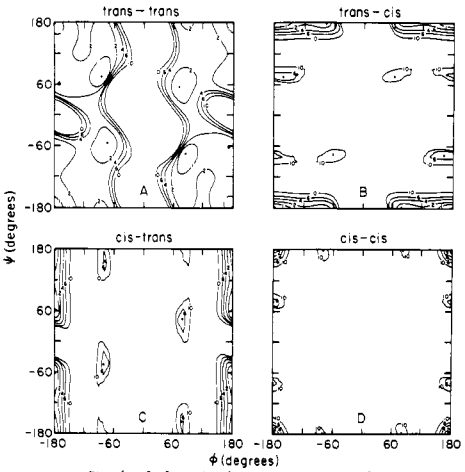

Fig. 6. Conformational energy contour maps for N-acetyl-N'-methylglycineamide. The two peptide bonds are fixed in the states (A) trans-trans, (B) trans-cis, (C) cis-trans, (D) cis-cis. The contours are labeled with energy in kcal/mol above the minimum-energy point of each map: (A) $(\phi,\psi) = (-83°, 77°)$, $E_0 = -4.8$ kcal/mol, (B) (180°, 180°), 2.0 kcal/mol, (C) (180°, 62°), 2.8 kcal/mol, (D) (180°, 180°), 9.1 kcal/mol. Locations of minima (obtained with fixed ω's) are indicated by filled circles.

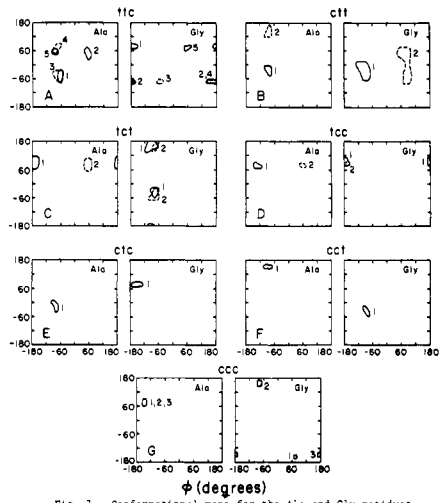

Fig. 7. Conformational maps for the Ala and Gly residues in cyclo(L-Ala-Gly-Aca), indicating the combinations of low- and medium-energy regions for each residue within which the ring can be closed with planar peptide groups. The state of the peptide groups is as follows: (A) trans-trans-cis, (B) cis-trans-trans, (C) trans-cis-trans, (D) trans-cis-cis, (E) cis-trans-cis, (F) cis-cis-trans, (G) cis-cis-cis. Within each pair of maps, conformations of the two residues within similarly labeled contours can be combined with each other to form the cyclic molecule. Outside these regions, the ring cannot be closed and/or the energy is very high.

APPENDIX A. CONFORMATIONAL ANALYSIS OF THE N-ACETYL-N'-METHYLAMIDES OF ALANINE AND OF GLYCINE, WITH TRANS AND CIS PEPTIDE GROUPS

(1) Conformational energy maps. Such maps have been computed earlier for both derivatives with trans peptides,[58] and for the terminally blocked glycyl residue with one or both peptide groups in the cis conformation.[16] In the case of alanine, only a hard-sphere map for the cis-cis form had been calculated before.[68] It was necessary to check whether the change in the C^β-H bond length (Sec. II.B) affects the energy contours.

The maps shown in Figs. 5 and 6 were generated using the ECEPP program.[44] The intramolecular potential energy was computed at 10° intervals of ϕ and ψ. The peptide groups were constrained to be planar, and χ^1 was fixed at 60°. No interactions with the solvent were included.

The maps for the trans-trans forms are identical with those computed earlier,[58] i.e., the change in the C^β-H bond length does not influence them (Figs. 5A and 6A). The introduction of a cis peptide group severely limits the width of the relatively low-energy regions on the (ϕ,ψ) conformational map, because of nonbonded repulsions in close atomic approaches. The energy of even the most favorable conformations is raised very much, as discussed below.

The presence of a cis peptide group preceding either the Ala or the Gly residue eliminates certain ranges of ψ on the maps, viz., $\psi < +150°$ and $\psi > +70°$ for Ala, $100° < |\psi| < 140°$ and $|\psi| < 70°$ for Gly (Figs. 5C and 6C). These regions are excluded because of close contacts between the N-terminal CH_3 group and the Ala CH_3 group, the Ala O atom, one of the Gly H^α atoms, or the Gly O atom, respectively.

In an analogous manner, the presence of a cis peptide group following both residues eliminates large ranges of ϕ on the maps, viz., $\phi > 150°$ and $\phi < 70°$ for Ala, $100° < |\phi| < 140°$ and $\phi < 70°$ for Gly (Figs. 5B and 6B). The high energy in these regions arises from close contacts between the C-terminal CH_3 group and the Ala CH_3 group, the Ala O atom and CH_3 group, the Gly H^α atoms, or the Gly O atom, respectively. The positions of the low-energy regions are similar to those computed earlier[69] for the trans-cis form of Ala and Gly in terminally blocked Ala-Pro and Gly-Pro with a cis peptide group preceding Pro and trans terminal peptide groups. The number and extent of low-energy regions is even smaller in the dipeptide than in Figs. 5B and 6B, because of the additional effect of the Pro neighbor.

The allowed conformational space is extremely small for the cis-cis dipeptides (Figs. 5D and 6D). Not only are all areas eliminated which were of high relative energy in the cis-trans and trans-cis maps, but several additional regions are of very high energy.

The contour lines in each of the individual parts of Figs. 5 and 6 show the points with energies 1 to 10 kcal/mol above the lowest point of each map, not above the global minimum (which occurs in the trans-trans map for both residues). The energies are very high in all cis-containing conformations, relative to the global minimum, as indicated in the legends of Figs. 5 and 6. Therefore, the width of the areas enclosed by the contour lines in each of the maps in parts B to D of both figures merely reflects the conformational freedom of that particular combination of trans and cis states.[16]

If the dihedral angles ω_0 and ω_1 are allowed to vary in order to relax the conformation at each (ϕ,ψ) point on the maps for cis-containing forms, there is some lowering of the energy at many points, by 1 to 3 kcal/mol on the Ala maps and by 0 to 1 kcal/mol on the Gly maps. Consequently, some of the energy contours would include a larger area. These changes would not alter the essential appearance of the maps, because large deviations of the peptide groups from planarity raise the energy significantly. The low-energy contours in the trans-trans maps (Figs. 5A and 6A) do not change noticeably when the ω's are allowed to vary.

(2) Minimum-energy conformations. Energy minimizations were carried out in the manner indicated earlier,[58] in order to find all local energy minima. In addition to ϕ_1, ψ_1 (and χ_1 for Ala), ω_0 and ω_1 were treated as variables of the minimization. The results are shown in Tables VI and VII. The given values of ΔE are relative energies, expressed with respect to the energy of the global minimum.

The results for the trans-trans forms of both peptides are virtually identical with those obtained earlier with fixed $\omega_0 = \omega_1 = 180°$, except for conformations with $\Delta E > 5.0$ kcal/mol (cf. Tables II and III of ref. 58). The very small differences in some ΔE's are due to the improvement in the C^β-H geometry (Sec. II.B). The ω's change by less than 1° during minimization. This result shows that the assumption of fixed planar peptide groups, frequently made in conformational energy calculations of open-chain peptides, is a good one for open-chain all-trans peptides.

On the other hand, the cis peptide group departs from planarity during energy minimization for most conformations. If the ω's are held fixed during minimization (not shown here), the energies of some minima remain several kcal/mol above those shown in Tables VI and VII. Thus, it is important to allow deviations from planarity in cis-containing conformations. All conformations of the terminally blocked Ala residue with a cis peptide group have $\Delta E > 10.8$ kcal/mol (Table VI), while those of the terminally-blocked Gly residue have $\Delta E > 6.8$ kcal/mol (Table VII). In other words, the cis peptide group is energetically very unfavorable, even if departures from planarity relieve some unfavorable atomic contacts.

Both peptide groups remain planar in the lowest-energy conformations of the terminally blocked Gly residue for every combination of trans and cis peptide groups (first entry for each combination in Table VII).[70] This indicates that the computed cis/trans equilibrium for glycine[16] is not affected significantly by the assumption of fixed peptide groups.

The departure of ω from planarity in some of the cis conformations of both peptides can amount to as much as 32°. The corresponding torsional energy may be as high as 3 kcal/mol. This increase in energy is compensated for by the decrease of unfavorable nonbonded interactions. Larger increases of ω would raise the torsional energy rapidly; therefore, they are not likely to occur.

Deviations of ω from planarity by as much as 33° were computed in some cis-containing conformations of cyclo(L-Ala-Gly-Aca) (Table VIII). Decreased repulsion did not result in sufficient lowering of the nonbonded energy, however, to populate these conformations to any significant extent.

APPENDIX B. CONFORMATIONS OF CYCLO(L-ALA-GLY-ACA) WITH CIS PEPTIDE BONDS

(1) The mapping of conformational space. There are seven forms in which the molecule contains one or more cis peptide bonds, viz., trans-trans-cis, cis-trans-trans, etc. (Table VIII). For each combination, the corresponding pair of maps, chosen from Figs. 5 and 6, was used to map those relatively low-energy regions of conformational space in which ring closure is possible [Sec. II.G(1)-(2)]. Ring closure was tested for all combinations of conformations of Ala and Gly which fall inside the regions with contours shown in Figs. 5 and 6 [Sec. II.G(3)-(4)]. There are very few combinations for which ring closure is possible. They are shown in Fig. 7 for each combination of cis and trans peptide groups, in the same manner as in Fig. 2 for the trans-trans-trans form. The regions with ring closure become progressively smaller as the number of cis peptide bonds in the molecule increases. Furthermore, the energies of even the most favorable conformations in the map become very high, as seen from Table VIII.

(2) Minimum-energy conformations. The results of energy minimizations, carried out with seven independent variable dihedral angles, are shown in Table VIII. This Table is a continuation of Table III. The energies of all cis-containing conformations are very high, with ΔE at least 9.7 kcal/mol and usually much higher.

APPENDIX C. MINIMUM-ENERGY CONFORMATIONS OF CYCLO(L-ALA-GLY-ACA) WITH PEPTIDE GROUPS CONSTRAINED TO BE PLANAR

This computation corresponds to step 5 in the computational procedure (Sec. II.G). The minimization was carried out with four independent variables, viz., ϕ_1, ψ_1, ϕ_2, and ψ_2. The minima for the all-trans form of the peptide are listed in Table IX. A comparison with Table III shows that the dihedral angles of conformations with fixed and variable peptide groups, respectively, generally do not differ much but that the energies are much lower when the peptide group is not constrained to be planar. The type II bend conformation (No. 1) which becomes the global minimum in the seven-variable minimization behaves differently in the two minimizations. It is the sixth lowest-energy conformation (with $\Delta E = 1.48$ kcal/mol) when the peptide groups are held fixed. Some of its dihedral angles change considerably during the seven-variable minimization, and the Aca residue adopts a different conformation.

All conformations with planar cis peptide groups are at least 13 kcal/mol higher in energy than conformation 4 of Table IX (the lowest-energy conformation of the Table).

Table IV

Cartesian Coordinates of the Computed Lowest-Energy Conformation of Cyclo(L-Ala-Gly-Aca)

Residue	Atom	X	Y	Z
		(Angstrom units)		
ALANINE	N	0.00	0.00	0.00
	HN	-0.41	0.90	-0.16
	CA	1.44	0.01	0.19
	HA	1.83	-0.93	-0.20
	CB	2.05	1.17	-0.59
	HB	1.83	1.05	-1.65
	HB	1.64	2.11	-0.24
	HB	3.13	1.17	-0.47
	C'	1.75	0.09	1.69
	O	1.97	1.17	2.22
GLYCINE	N	1.76	-1.08	2.33
	HN	1.64	-1.96	1.87
	CA	1.95	-1.14	3.76
	HA	2.32	-2.13	4.04
	HA	2.71	-0.42	4.06
	C'	0.64	-0.86	4.50
	O	0.47	0.21	5.08
AMINO-CAPROIC ACID	N	-0.25	-1.84	4.46
	HN	-0.04	-2.77	4.16
	CE	-1.63	-1.61	4.86
	HE	-1.65	-1.12	5.83
	HE	-2.14	-2.57	4.97
	CD	-2.37	-0.75	3.83
	HD	-1.87	0.21	3.73
	HD	-3.38	-0.55	4.18
	CC	-2.42	-1.45	2.47
	HC	-3.18	-2.21	2.48
	HC	-1.46	-1.95	2.27
	CB	-2.70	-0.45	1.35
	HB	-2.21	0.50	1.57
	HB	-3.77	-0.25	1.29
	CA	-2.20	-0.98	0.00
	HA	-2.50	-0.28	-0.79
	HA	-2.67	-1.94	0.27
	C'	-0.68	-1.14	0.00
	O	-0.17	-2.26	0.00

Table VI. Minimum-energy conformations of N-acetyl-N'-methyl-L-alaninеamide

Peptide bond conformation	Code[a]	Energy ΔE[b] (kcal/mol)	Dihedral angles[c] (deg) ω_0	ϕ_1	ψ_1	ω_1	χ_1
tt	C	0.00	-179	-84	80	179	61
	E	0.40	180	-154	153	180	59
	D	0.75	180	-151	73	180	58
	A	1.19	180	-74	-45	180	61
	G	1.63	180	-158	-58	179	54
	A*	2.42	180	54	57	180	65
	F*	5.07	179	64	-178	178	80
	G*	8.79	175	80	-63	180	87
tc	D	10.82	180	-154	71	18	58
	E	12.01	180	-156	161	-21	47
	F	12.84	180	-80	160	-21	55
	A*	13.39	-179	52	69	20	63
	F*	18.12	179	69	164	-27	86
ct	A	11.71	-22	-68	-43	-179	60
	F	12.36	-21	-68	152	180	62
	E	14.58	28	-164	157	180	70
	D	14.81	28	-164	60	180	66
	G	15.32	32	-166	-56	179	61
cc	F	24.51	-20	-69	161	-22	53
	D	25.20	30	-166	71	-17	65
	E	26.58	32	-166	161	-21	48

[a]Ref. 58.

[b]$\Delta E = E - E_0$, where E_0 = -3.32 kcal/mol for the first conformation listed.

[c]Only variable dihedral angles are listed; all others have a value of 180°.

Table VII. Minimum-energy conformations of N-acetyl-N'-methyl-glycineamide

Peptide bond conformation	Code[a]	Energy ΔE[b] (kcal/mol)	Dihedral angles[c] (deg) ω_0	ϕ_1	ψ_1	ω_1
tt	C	0.00	-179	-83	77	179
	E	0.82	180	180	180	180
	D	1.04	180	-173	63	180
	A	1.22	180	-73	-54	180
tc	E	6.82	180	180	180	0
	F	7.88	180	-83	179	-1
	D	9.89	180	-166	72	15
	A	11.42	178	-63	-73	-17
ct	D	7.64	1	180	-62	180
	E	7.84	0	180	180	180
	A	10.35	-19	-71	-45	180
	F	11.26	-17	-71	160	-179
cc	E	13.90	0	180	180	0
	D	15.66	-2	-179	72	16
	F	17.70	-17	-72	179	-1
	A	21.87	-27	-70	-71	-21

[a]Ref. 58.

[b]$\Delta E = E - E_0$, where E_0 = -3.62 kcal/mol for the first conformation listed.

[c]Only variable dihedral angles are listed; all others have a value of 180°.

Table VIII. Minimum-energy conformations of cyclo(L-Ala-Gly-Aca) with one or more cis peptide bonds

Conformation No.	Peptide bond conformation	Energy ΔE[a] (kcal/mol)	Dihedral angles (deg) ω_0	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ω_2
11	ttc	9.7	177	-83	77	-169	154	-68	-22
12		11.9	179	-85	69	-171	51	62	28
13		12.4	179	-80	-50	172	-53	-67	-20
14		13.9	180	-73	-53	170	-160	75	6
15		13.9	179	55	71	-169	149	-87	17
16	ctc	11.9	22	-93	-38	173	-68	-61	176
17		11.9	-27	-63	149	-170	84	-75	175
18		13.5	25	-89	133	-169	76	68	180
19	tcc	16.7	173	56	61	41	-101	-67	168
20		17.0	177	-156	88	50	-98	-40	-177
21		18.0	-178	-166	64	50	-111	132	180
22		34.0	-169	58	73	-1	-82	147	180
23	ccc	32.2	-174	-139	84	-3	-167	82	0
24		36.1	-168	47	81	-2	-154	76	9
25	ccc	30.6	33	-100	-33	171	-139	65	29
26	cct	40.6	0	-78	150	0	-84	-41	180
27	ccc	91.9	0	-138	75	0	84	-176	0

[a]$\Delta E = E - E_0$, where E_0 = 2.56 kcal/mol, the computed energy of conformation 1 in Table III.

Table IX. Minimum-energy conformations of cyclo(L-Ala-Gly-Aca) with trans peptide bonds, computed with peptide groups constrained to be planar[a]

Conformation No.[b]	Energy ΔE[c] (kcal/mol)	Dihedral angles[d] (deg) ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4	ψ_4	ϕ_5	ψ_6
1	4.10	-70	94	83	26	175	-68	141	-135	61	-142
2	3.33	-65	-40	-124	38	110	63	-112	154	-79	-90
3	4.01	-78	74	115	-28	-174	57	-125	153	-73	-80
4	2.62	-71	-57	-94	-34	-166	61	-117	166	-68	-79
5	2.67	-72	-57	-92	-33	-169	61	-117	165	-67	-80
6	5.33	-68	-44	-114	-39	-147	49	-115	173	-72	-77
7	5.16	-58	-56	-102	40	152	-62	147	-122	67	-155
8	3.71	55	58	97	44	154	-63	117	-162	71	88
9	4.67	54	45	125	-46	-103	-64	114	-150	80	96
10	4.89	51	66	99	-46	-151	64	-149	123	-64	154

[a]$\omega_{i-1} = 180°$, for i = 0, 1, 2.

[b]These numbers correspond to those in the first column of Table III. They indicate similar conformations in the two Tables.

[c]$\Delta E = E - E_0$, where E_0 = 2.56 kcal/mol, the computed energy of conformation 1 in Table III.

[d]Only variable dihedral angles are listed; all others have a value of 180°.

requirement to close the small ring and from steric interactions within each residue (Appendix A). As a result, it was possible to carry out a complete search of conformational space and to find all minimum-energy conformations of this molecule. Low-energy minima occur only when all three peptide bonds are in the trans conformation. The presence of one or several cis peptide bonds raises the energy by at least 10 kcal/mol.

Nonplanar distortions of the peptide bonds had to be allowed in order to find true minimum-energy structures. The need for allowing flexibility of peptide bonds in some conformational energy calculations has been pointed out earlier.[61] The effect of this degree of freedom is not significant in computations on open-chain peptides (except those containing proline) with trans peptide bonds,[16,58,62] but it has to be taken into account in compounds with cis peptide bonds[16,61] and in small cyclic peptides, as shown here.

The lowest energy conformation (no. 1 in Table III) is a type II bend. Its dihedral angles differ from those of the type II bend defined by Venkatachalam,[4,63,64] but the overall shape of the backbone is similar in the two cases. Conformation 1 is shown in Figure 4, and its coordinates are given in Table IV. This result agrees with the experimental measurements.[40,41] The latter indicate that the predominant conformation is a type II bend.

Conformation 1 contains a bent and stretched, weak C_7^{eq} Gly-N–H···O=C-Aca hydrogen bond in which the $H_{Gly}\cdots O_{Aca}$ distance is 2.6 Å and the $N_{Gly}\cdots O_{Aca}$ distance is 3.2 Å. The Aca NH group is somewhat further from the Aca C=O group: the corresponding H···O and N···O distances are 4.2 and 4.5 Å, respectively. This NH hydrogen, too, is shielded somewhat from the solvent. The lack of an Aca-N–H···O=C-Aca hydrogen bond is at variance with the results of the NMR and IR spectroscopic measurements, which suggest the presence of such a bond.[40,41] A slight rotation of the Gly-Aca peptide group would, however, bring the Aca NH group within hydrogen-bonding distance of the Aca C=O group. Mapping of conformational space indicated that such a rotation would cause only a small rise in energy. It is possible that interactions with the solvent could supply this energy.

The Aca C=O group is shielded in conformation 1. The other two C=O groups point outward. The Gly C=O is fully exposed. The Ala C=O is shielded only partially by the Ala CH_3 group. One of the hydrogens of the Aca $C^{\gamma}H_2$ group points into the center of the molecule and is strongly shielded from the solvent. All other alkyl hydrogens are exposed on the surface of the molecule. Shielding of the Ala C=O and of one Aca H^{γ} was observed in the NMR studies.[40] The observed nuclear Overhauser effects[40] exclude the presence of type I or III bends as major components.

The distances and relative exposures of peptide H and O atoms are similar in both computed type II conformations. It is not possible, however, to alter conformation 3 in order to form an Aca-N–H···O=C-Aca hydrogen bond, without changing it to conformation 1. Hence this conformation would be inconsistent with the infrared measurements in solution.[41] No NH and C=O groups are within hydrogen-bonding distance in the computed type I and III bend conformations, except for the higher energy

conformation 7. In all of them, the Ala and Gly NH and C=O groups point to the outside, but the Aca NH and C=O groups point to the inside and are not exposed to the solvent.

The analysis of the mapping of conformational space (section III.A) indicates that the energy barrier between the two type II bends is low, of the order of 3–4 kcal/mol. The barrier between the type I and III bends is similar. This suggests that the conformations indicated are easily interconvertible. On the other hand, conversion from the type II bends to the type I and III bends is difficult in this molecule without significant departures of peptide bonds from planarity and/or bond angle bending because the corresponding regions in conformational space are separated from each other by regions with high energy or regions in which ring closure is not possible.

Predicted $^3J_{HN-C^\alpha H}$ coupling constants can be calculated as Boltzmann averages, using the relationship between J and ϕ given by Bystrov[65] (Table V). Differences in the computed coupling constants for various types of bends exceed the uncertainty[65] (±0.7 Hz) of the relationship. Although it would appear that a comparison of these predictions with measured coupling constants can help to decide about the presence of various bend conformations, it must be kept in mind that some of the computed J values are very sensitive to changes of ϕ. Within the range of ϕ_{Ala} shown in Table III, a change of only 5° in ϕ can alter the computed J by about 1 Hz. Such changes would reduce the differences between the observed coupling constants and the values computed for conformation 1 to less than ±0.7 Hz. Solvent interactions might cause deviations of this magnitude from the computed values of the dihedral angles.

Based on the values of ΔE in Table III, the Boltzmann statistical weights of the type II, type I (+III), and type I′ (+III′) conformations at T = 298 K are 0.63, 0.36, and 0.01, respectively. While the type II bend conformations are favored energetically, the energy differences are small. Therefore, the conformational energy calculations presented here indicate that the molecule may exist as a mixture of mostly type II with some type I (+III) bends.

No interactions with the solvent were included in the computation. Hydration changes the relative energies of some conformations of *N*-acetyl-*N*′-methylamides of amino acids and dipeptides by as much as 1.0–1.5 kcal/mol.[66,77] Solvation energy differences of this magnitude would be sufficient to shift the distributions strongly in favor of one of the two bend types.

Experiments[40,41] indicate that the predominant conformation in solution is a type II bend, with a conformation similar to that of the global minimum computed here. A minor component (up to about 35%) of type I (+III) bends is also suggested by the experiments. The amount of this component is solvent and temperature dependent. Thus, observations and the theoretical computation give fully consistent results.

The substitution of chiral residues for Gly would change the relative energies of various bend types. The energies of the type II, I′, and III′ bends are very high for *cyclo*(L-Ala-L-Ala-Aca). This compound must therefore exist in the form of a type I or type III bend. On the other hand, the latter types of bends are of high energy for *cyclo*(L-Ala-D-Ala-Aca), so that this compound must exist as a type II bend, with possibly a very small amount of type I′ bend. A theoretical and experimental investigation of these two compounds will be reported later.[42]

The use of the three compounds not only provides useful model systems to establish the experimental parameters characterizing the presence of bends in general but also yields parameters which can distinguish different types of bends from each other.[40,41]

Miniprint Material Available: Full-size photocopies of Appendices A–C, Tables IV and VI–IX, and Figures 5–7 (15 pages). Ordering information is given on any current masthead page.

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Conformation of *cyclo*(L-Alanylglycyl-ϵ-aminocaproyl), a Cyclized Dipeptide Model for a β Bend. 2. Synthesis, Nuclear Magnetic Resonance, and Circular Dichroism Measurements[1]

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ABSTRACT: *cyclo*(L-Alanylglycyl-ϵ-aminocaproyl) has been synthesized by means of classical solution techniques. The Ala-Gly moiety of this molecule is constrained to form a β bend because of the closure of the ring by the five-carbon alkyl chain of the ϵ-aminocaproyl residue. ^{13}C NMR spectroscopic measurements indicate the absence of rapid internal segmental motion. Reorientation takes place by means of anisotropic rotational diffusion. ^1H NMR spectroscopic measurements show that the molecule has little flexibility and that its predominant backbone conformation is a type II β bend, with a strongly bent NH···O=C bond in the ϵ-aminocaproyl residue. Circular dichroism measurements confirm this conclusion. In addition, they indicate the presence of a minor component in a type I (and III) bend conformation. The distribution between these bend types depends on solvent and on temperature. The results are consistent with infrared and Raman spectroscopic data and with conformational energy calculations. Thus, the cyclized molecule can serve as a model for β bends, especially since the dihedral angles of the model compound fall in the range observed for β bends in proteins. The open-chain analogues Ac-L-Ala-Gly-NHMe and Boc-L-Ala-Gly-Aca-OMe exist as ensembles of conformations in solution, but with significant amounts of type II β-bend structures in the ensemble. This confirms earlier theoretical studies which predicted a high probability of the L-Ala-Gly dipeptide to form type II bends.

I. Introduction

This paper is part of a series[3,4] which reports studies of the conformational properties of *cyclo*(L-alanylglycyl-ϵ-aminocaproyl), abbreviated *cyclo*(L-Ala-Gly-Aca). The Ala-Gly dipeptide, flanked by two peptide groups, must be in a β-bend conformation because of the steric constraint of the $(CH_2)_5$ chain in the Aca residue used to cyclize the molecule. See Figure 1 of paper 1[3] for nomenclature for the Aca methylene groups.

Experimental investigations of small open-chain and cyclic oligopeptides, using primarily various spectroscopic techniques, led to results that have been interpreted in

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