# Conformation of *cyclo*(L-Alanylglycyl-ϵ-aminocaproyl), a Cyclized Dipeptide Model for a β Bend. 3. Infrared and Raman Spectroscopic Studies[1]

**F. R. Maxfield,[2a] J. Bandekar,[2b] S. Krimm,[2b] D. J. Evans,[2c] S. J. Leach,[2c] G. Némethy,[2a] and H. A. Scheraga*[2a]**

*Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853, Biophysics Research Division and Department of Physics, The University of Michigan, Ann Arbor, Michigan 48109, and Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Victoria 3052, Australia.* *Received August 7, 1980*

ABSTRACT: Infrared and Raman spectra of *cyclo*(L-alanylglycyl-ϵ-aminocaproyl) have been obtained in the solid state and in solution. The spectra have been analyzed with the aid of a normal mode analysis, carried out on computed low-energy conformations of the molecule. The results of this analysis and spectra of model compounds can be used to interpret the effects of conformation on vibrational frequencies. In the solid state, the spectra are found to be consistent only with a type II β bend. A type II bend is the predominant conformation in solution as well. The presence of *large* amounts of other low-energy bend conformations (of bend types I, III, I′, and III′) in solution is clearly ruled out as being inconsistent with one or several of the observed vibrational frequencies. Small amounts of type I and III bends may be present in aqueous solution. This result agrees with the conclusions reached from theoretical conformational energy calculations and from NMR and CD spectroscopic measurements, reported in the two preceding papers of this series. The spectra of the open-chain dipeptide analogue *N*-acetyl-*N*′-methyl-L-alanylglycinamide differ quantitatively from those of the cyclic molecule, and they indicate considerably more conformational flexibility in the open-chain peptide.

## I. Introduction

We have investigated[4-6] the conformational properties of *cyclo*(L-alanylglycyl-ϵ-aminocaproyl), abbreviated *cyclo*(L-Ala-Gly-Aca). In this cyclized molecule, the Ala-Gly dipeptide, flanked by two peptide groups, exists as a β bend because it is constrained by the $(CH_2)_5$ chain in the Aca residue. Thus, *cyclo*(L-Ala-Gly-Aca) is a good model compound for the study of observed properties of β bends. The rationale for choosing this molecule has been outlined in the first paper of the series.[5] It was also shown there, by means of conformational energy calculations, that the molecule can exist at most in a few bend conformations. The most favorable of these conformations was predicted to be a type II bend. The prediction of the theoretical analysis is confirmed by experimental studies. Nuclear magnetic resonance and circular dichroism measurements were described in the preceding paper.[6] In this paper, we present infrared and Raman spectra of *cyclo*(L-Ala-Gly-Aca) in the solid state and in solution. The spectra are analyzed by means of normal mode calculations and are compared with spectra of model compounds. All experimental results reported here and earlier[6] show that the molecule exists predominantly as a type II bend. Infrared and Raman spectra are also shhown for the open-chain analogue *N*-acetyl-*N*′-methyl-L-alanylglycinamide, abbreviated Ac-L-Ala-Gly-NHMe.

Infrared and Raman spectroscopy have long been used for studying polypeptide conformation. Both of these methods can provide information about the conformation of polypeptides in solution and in the solid state. Recently, detailed normal mode calculations have been combined with experimental studies to yield force fields, used to calculate the vibrational spectra of polypeptides.[7-9] The refined force fields have been applied to the prediction of the characteristic amide frequencies of β bends[10,11] and have been tested successfully in the calculation of the frequencies of specific β bends in insulin[12] and in an oligopeptide known from X-ray crystallography to contain a β bend.[3] The general predictions[10,11] have also been used successfully in analyzing the β bends in enkephalin[13] and in prolyl oligopeptides.[14] These studies have confirmed the usefulness of refined force fields, which include transition dipole coupling[15,16] in the amide I and II modes, for predicting the conformations of β bends.

The N–H stretching region of infrared spectra has been used in many studies to examine hydrogen bonds and other interactions involving the N–H portion of the amide group.[17-19] The frequencies in this region can also be interpreted in terms of the conformation of peptides, based on spectra of model compounds. Infrared spectra in this region have been used previously to study oligopeptides which might contain β bends.[20,21]

## II. Methods

The synthesis of the peptides has been described elsewhere.[6]

**Spectroscopic Measurements.** Solution IR spectra were obtained on a Perkin-Elmer Model 521 spectrometer, using KBr cells with a path length of 5 cm. Concentrations between $5 \times 10^{-5}$ and $5 \times 10^{-4}$ M in chloroform were used. The peptides were dried in vacuo over $P_2O_5$ for 24 h before use. The chloroform (spectral grade, Fisher Scientific) was shaken over aluminum oxide (Aloxide Woelm Basic, activity grade I, ICN Pharmaceuticals) for 2 days and then passed through a column of aluminum oxide (15 × 3.5 cm) into dark bottles under a stream of nitrogen. Solutions were prepared immediately, and the spectra were always recorded within 3 h after the drying of the chloroform.

Solution Raman spectra were obtained with an instrument described previously.[22] The 488.0-nm line of an argon ion laser was used at a power of 300 mW. The instrumental resolution was 4 $cm^{-1}$. Aqueous solutions of *cyclo*(L-Ala-Gly-Aca) were at a concentration of 0.03 M, and those of Ac-L-Ala-Gly-NHMe were at 0.12 M. N-Deuterated peptides were prepared by dissolving the compounds in a large excess of $D_2O$, allowing the solution to stand overnight, lyophilizing the solution, and redissolving the peptide in $D_2O$.

Solid-state IR spectra of *cyclo*(L-Ala-Gly-Aca) (which contained about 10% of $^{13}C$ in the Ala C=O group) were obtained on a Perkin-Elmer Model 180 spectrometer, using KBr disks with 0.5% by weight of the peptide. N-Deuterated samples were prepared by dissolving ~5 mg of the sample in 10 $cm^3$ of $D_2O$ for 2 days and then lyophilizing the solution. Although complete deuteration was difficult to achieve, a satisfactory exchange was obtained after three successive treatments.

Solid-state Raman spectra were obtained by using an instrument described previously.[23] The 514.5-nm line of an argon ion laser was used at a power of 380 mW. The instrumental resolution was about 4 $cm^{-1}$.

**Normal Mode Vibration Calculations.** The calculations were carried out for the ten low-energy computed conformations[5] of *cyclo*(L-Ala-Gly-Aca). These conformations contain trans

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Table I
Parameters Characterizing the Theoretically Computed[a] Minimum-Energy Conformations of *cyclo*(L-Ala-Gly-Aca) with Trans Peptide Bonds

| conformation no.[a] | energy $\Delta E$,[b] kcal/mol | bend type | calcd max splitting ($cm^{-1}$) of amide I modes, with transition dipole coupling: absent | present |
|---|---|---|---|---|
| 1 | 0.00 | II | 10 | 11 |
| 2 | 0.74 | I | 7 | 10 |
| 3 | 0.93 | II | 10 | 45 |
| 4 | 1.07 | III | 12 | 21 |
| 5 | 1.22 | III | 11 | 21 |
| 6 | 1.25 | III | 10 | 18 |
| 7 | 1.59 | I | 15 | 21 |
| 8 | 2.80 | III' | 13 | 53 |
| 9 | 2.96 | I' | 18 | 57 |
| 10 | 3.08 | I' | 11 | 63 |

[a] The numbering corresponds to that used in Table III of ref 5. [b] $\Delta E = E - E_0$, where $E_0$ is the computed energy of conformation 1. See ref 5.

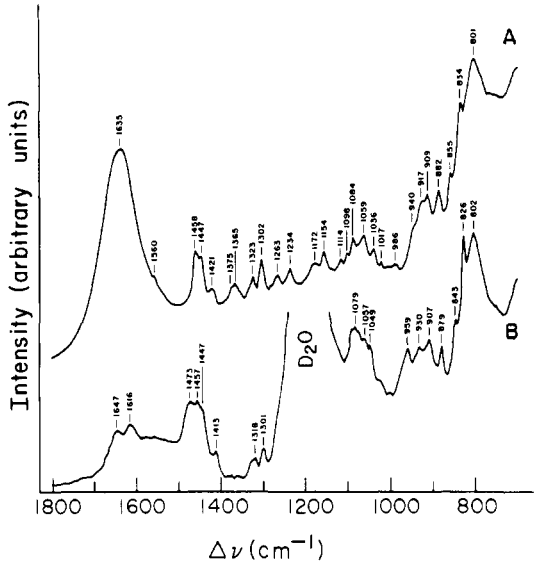


**Figure 1.** Raman spectra of 0.03 M solutions of *cyclo*(L-Ala-Gly-Aca) in $H_2O$ (A) and in $D_2O$ (B).

peptide groups which are not restricted to be planar. They are listed in Table I in order of increasing computed energy. The table also shows the bend type[24,25] corresponding to each conformation.

The computed coordinates of these structures were used as input for the normal vibration calculations. The force field was that used in the earlier $\beta$-bend calculations,[10,11] and transition dipole coupling between peptide groups was included.[15,16] The force constants for the $\epsilon$-aminocaproyl residue were transferred from hydrocarbons.[26] Computations were carried out for structures with external hydrogen bonds of constant strength, as in earlier calculations.[10,11,27] Internal hydrogen bonds were incorporated where required by the O···H separation. This was particularly important for conformations 1 and 3, in which the H···O distance is 2.62 and 2.31 Å, respectively, for the Gly-N-H···O=C-Aca hydrogen bond, and for conformations 7 and 10, in which the H···O distance is 2.51 and 2.40 Å, respectively, for the Aca-N-H···O=C-Aca hydrogen bond. In such cases, the force constant was related to the hydrogen bond length by a relationship described previously.[11] Effective transition moments were taken to be 0.45 D for amide I and 0.40 D for amide II. These values were suggested by earlier calculations.[8,11,12]

## III. Results

Infrared and Raman spectra of *cyclo*(L-Ala-Gly-Aca) are presented in Figures 1–4. Figure 1 shows the Raman spectra in $H_2O$ and $D_2O$ in the 1800–800-$cm^{-1}$ region. Spectra of the normal and deuterated species in the solid state are given in Figure 2 for the IR from 1800 to 500 $cm^{-1}$

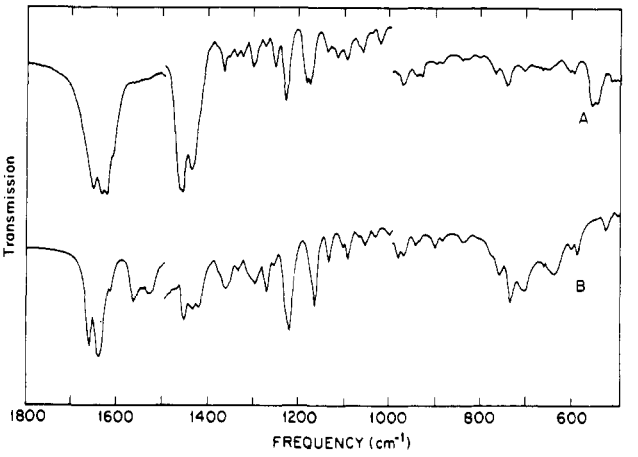


**Figure 2.** Infrared spectra of solid (A) N-deuterated *cyclo*(L-Ala-Gly-Aca) and (B) *cyclo*(L-Ala-Gly-Aca).

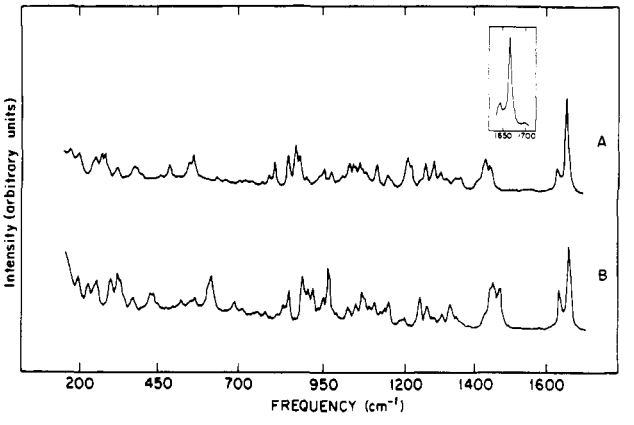


**Figure 3.** Raman spectra of solid (A) *cyclo*(L-Ala-Gly-Aca) and (B) N-deuterated *cyclo*(L-Ala-Gly-Aca) at room temperature. The inset shows the 1650–1700-$cm^{-1}$ region of the Raman spectrum of the nondeuterated compound, taken at liquid nitrogen temperature.

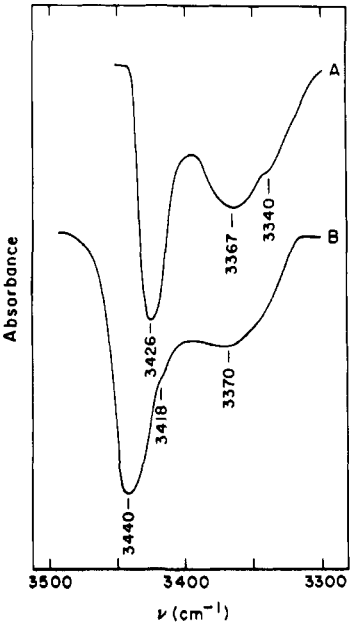


**Figure 4.** Infrared spectra of $10^{-4}$ M solutions in chloroform of (A) *cyclo*(L-Ala-Gly-Aca) and (B) Ac-L-Ala-Gly-NHMe.

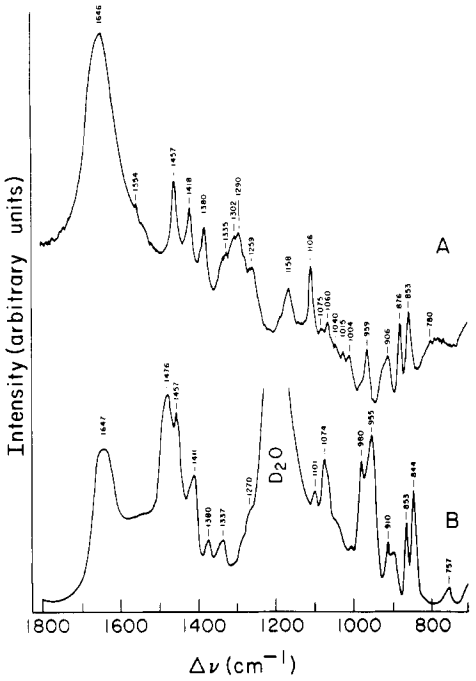


**Figure 5.** Raman spectra of 0.12 M solutions of Ac-L-Ala-Gly-NHMe in $H_2O$ (A) and in $D_2O$ (B).

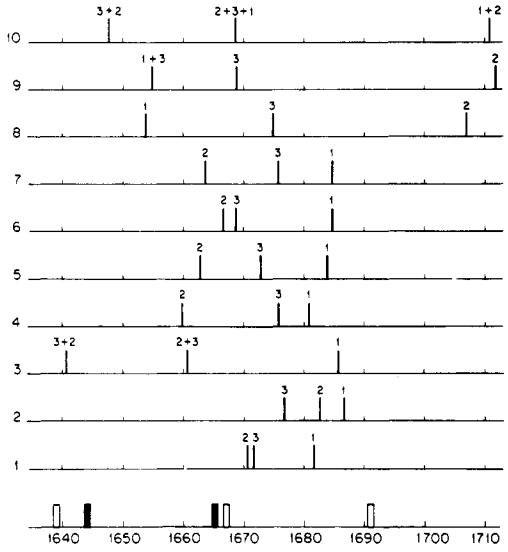


**Figure 6.** Calculated frequencies in the amide I region for the ten computed conformations of Table I. The assignable[28] observed infrared (solid bar) and Raman (open bar) bands are shown on the bottom line. Numbers above the calculated frequencies represent the groups involved in the vibration: 1 refers to the peptide group between Aca and Ala, 2 to the peptide group between Ala and Gly, and 3 to the peptide group between Gly and Aca.

and Figure 3 for the Raman from 1800 to 200 $cm^{-1}$. In Figure 4, we present IR spectra in chloroform in the 3500–3300-$cm^{-1}$ region. Raman spectra of Ac-L-Ala-Gly-NHMe in $H_2O$ and $D_2O$ are shown in Figure 5. The IR spectrum of this compound in chloroform in the 3500–3300-$cm^{-1}$ region is given in Figure 4. The peaks and shoulders did not change in the concentration range from $5 \times 10^{-5}$ to $5 \times 10^{-4}$ M, suggesting that none were unique to intermolecularly associated species.

The results of the normal mode vibration calculations are presented in terms of the frequencies of the characteristic amide modes. A detailed analysis of the assignments for the preferred structure of the cyclic compound

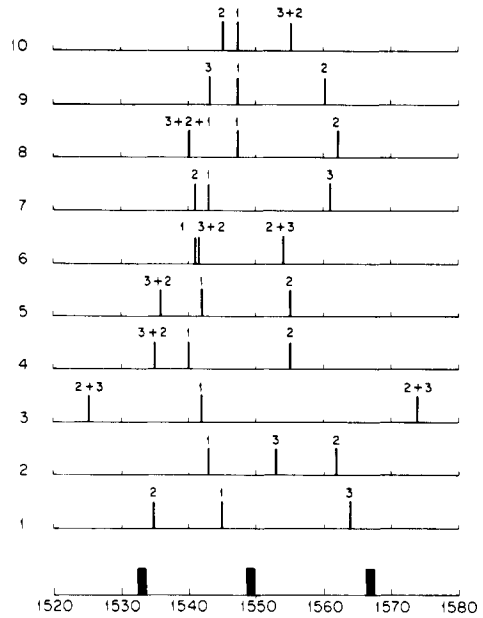


**Figure 7.** Calculated frequencies in the amide II region for the ten computed conformations of Table I. The assignable[28] observed infrared bands are shown on the bottom line. Numbers above the calculated frequencies represent the groups involved in the vibration (see legend of Figure 6).

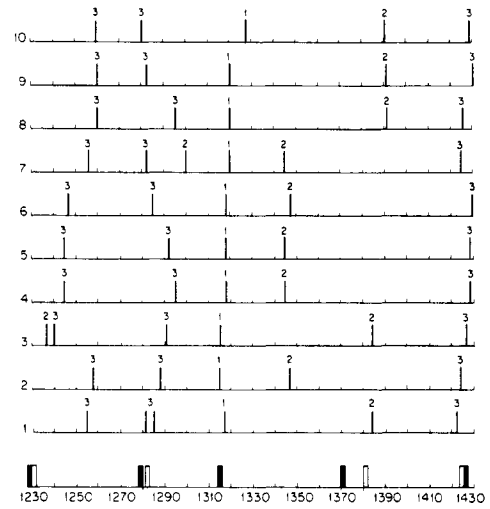


**Figure 8.** Calculated frequencies in the amide III region for the ten computed conformations of Table I. The assignable[28] observed infrared (solid bar) and Raman (open bar) bands are shown on the bottom line. Numbers above the calculated frequencies represent the groups involved in the vibration (see legend of Figure 6).

will be given elsewhere.[28] In Figures 6–9, we show the calculated amide I, II, III, and V frequencies for the ten conformations of Table I. The observed IR and Raman bands, assignable[28] to these modes, are shown by bars at the bottom of these figures (only frequencies, not relative intensities, are given). The numbers above the calculated frequencies represent the peptide group(s) involved in the vibration (1 + 2 means that the mode contains significant contributions from peptide groups 1 and 2, with the eigenvector contribution of 1 being greater than that of 2). In Figures 8 and 9, the only frequencies included are those whose NH in-plane bend contribution to the potential energy distribution is 9% or larger.

## IV. Discussion

**A. *cyclo*(L-Ala-Gly-Aca) in the Solid State. Amide I Region.** Analysis of the amide I mode can provide de-

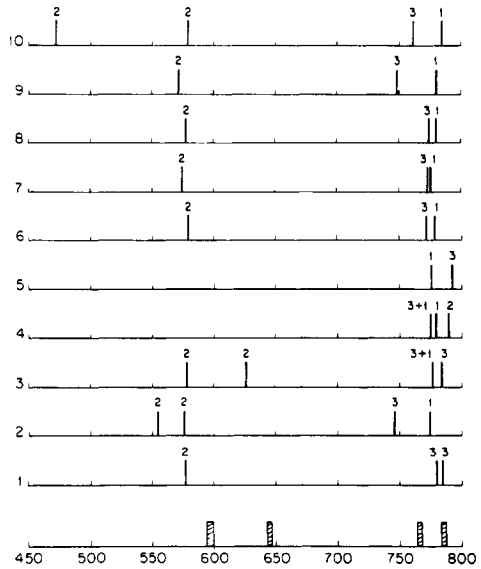


**Figure 9.** Calculated frequencies in the amide V region for the ten computed conformations of Table I. The assignable[28] observed infrared and Raman bands occur at the same frequencies and are indicated by the shaded bars on the bottom line. Numbers above the calculated frequencies represent the groups involved in the vibration (see legend of Figure 6).

tailed information about the conformation of the molecule. This is a consequence of the effect of transition dipole coupling[15,16] on the splittings of the C=O stretching frequencies. The maximum splitting of amide I modes, calculated for the ten computed conformations in the absence of transition dipole coupling, ranges from 7 to 18 $cm^{-1}$, but it ranges from 10 to 63 $cm^{-1}$ in the presence of such coupling (Table I). This sensitivity is, of course, a result of the dependence of the transition dipole interaction energy on the inverse cube of the separation between, and on the relative orientation of, the dipole moments.

Figure 6 shows that significantly different patterns of amide I frequencies are predicted for the various conformations. The 1692-$cm^{-1}$ Raman band is not seen in the room-temperature spectrum, but it shows up as a weak band in spectra of low-temperature samples (Figure 3, inset). The calculated modes of conformation 3, a type II bend, appear to agree best with the observed IR and Raman bands. This conclusion is based on the following reasoning: (1) none of the type I or III bends (conformations 2 and 4–7) predicts a band near 1640 $cm^{-1}$, the lowest prediction being 1660 $cm^{-1}$; (2) conformation 1, another type II bend, cannot account for the large observed amide I splittings of about 50 $cm^{-1}$ (see range of amide I frequencies on bottom line of Figure 6) because its predicted frequencies span a range of only 11 $cm^{-1}$; and (3) the type I′ and III′ bends (conformations 8–10), while having large amide I mode splittings, do not agree as well with the observed bands as does conformation 3.

**Amide II Region.** This mode is a combination of NH in-plane bend and CN stretch. The three bands in the IR spectrum at 1567 (ms), 1549 (vw), and 1533 (m) $cm^{-1}$ are readily assigned[28] to the amide II mode, particularly since they weaken on N-deuteration (Figure 2). A comparison of these frequencies with the calculated amide II frequencies for the ten computed low-energy conformations of the cyclic molecule, shown in Figure 7, indicates that the two type II bends (conformations 1 and 3) agree somewhat better with the observations than any of the others. They are the only ones which predict the large observed spread in frequencies. It should be noted that the exact values of the frequencies depend on the forms of the vibrations, which in turn are sensitive to the detailed nature of the hydrogen bonding to the NH group. The Gly NH group bonds to the Aca C=O, rather than to an "external" O atom, in conformations 1 and 3, but not in any of the others.[5,29] This changes the potential energy distribution in the amide II modes quite significantly. Thus, if the dihedral angles of the actual conformation differ somewhat from those computed for conformation 1 or 3, the effect on amide II would be large even though the other modes would be influenced very little. Although the amide II bands are not conclusive by themselves, the observed spectra are most consistent with conformation 3. In particular, it should be noted that conformation 10, which was a possibility on the basis of its amide I modes, is definitely excluded because its predicted amide II splittings are too small.

**Amide III Region.** The amide III mode is usually considered to be another combination of NH in-plane bend and CN stretch. In fact, it is usually much more complex, involving other internal coordinates and being dependent on side-chain composition. For this reason, caution is necessary in using it to characterize peptide conformations.[30] In the case of $\beta$ bends, the NH in-plane bend contribution covers a large spectral region, with characteristic frequencies[10,11] between 1230 and 1430 $cm^{-1}$, and it varies significantly with the type of turn.[11]

For *cyclo*(L-Ala-Gly-Aca) in the solid state, it is possible to assign five bands in the 1230–1430-$cm^{-1}$ region that, on the basis of their behavior on N-deuteration, contain an NH in-plane bend contribution.[28] These are shown in Figure 8, together with the calculated frequencies of modes having such a contribution. Again we find that the type II bends (conformations 1 and 3) give better agreement with observation than any of the other conformations. They reproduce the observed 1370–1380-$cm^{-1}$ bands, in contrast to the type I and III bends (conformations 2 and 4–7). These bands are also reproduced by conformations 8–10, but these were excluded already on the basis of the amide I and II regions. Of the two type II bends, conformation 3 is more satisfactory in accounting for the lowest observed frequency, near 1230 $cm^{-1}$. While not definitive, the amide III region nevertheless adds support to the conclusions drawn from the amide I annd II regions.

**Amide V Region.** The observed amide V bands, containing NH out-of-plane bend contributions, are characteristic of conformations in $\beta$ bends.[11] Bands that can be assigned[28] to such modes, together with calculated frequencies for the ten structures, are shown in Figure 9. In this case, the evidence convincingly favors conformation 3 over the others: it is the only one that has a predicted mode in the 600–700-$cm^{-1}$ region, and a band is observed, at 645 $cm^{-1}$, that is significantly influenced by N-deuteration.

The comparison of observed and calculated frequencies in all four amide regions definitely indicates the presence of a type II bend conformation in the solid state. Of the two computed type II conformations, conformations 1 and 3, the latter is favored by the results because its computed spectra are most compatible with the observed bands in all four amide regions. The computed spectrum of conformation 1 is compatible in the amide II and III regions, but not in the amide I and V regions.

**B. *cyclo*(L-Ala-Gly-Aca) in Solution. N–H Stretching Region.** The most definitive information about the conformation of *cyclo*(L-Ala-Gly-Aca) in solution comes from an analysis of the N–H stretching frequencies in $CHCl_3$. These vibrations can contain contributions from Fermi resonance with overtones and combinations,[31] and

Table II
NH Stretching Frequencies ($cm^{-1}$) for Several Compounds in $CHCl_3$ (IR)

| molecule | conformational assignment of $\nu_{NH}$: unperturbed NH | with nearby $C^\beta$ ($\phi \approx -60°$) | $C_5$ interaction | intermolecularly H bonded | intramolecularly H bonded |
|---|---|---|---|---|---|
| Ac-Gly-NHMe[a] | 3450 | no $C^\beta$ | 3416 | 3300-3370[b] | n.o.[c] |
| Ac-L-Ala-NHMe[a] | 3450 | 3437 | 3420<br>3410 | 3300-3370[b] | n.o.[c] |
| cyclo(L-Ala-Gly-Aca) | n.o.[c] | 3426 | n.o.[c] | | 3367<br>3340 |
| Ac-L-Ala-Gly-NHMe | broad band at 3340 | | 3418 | | 3370 |

[a] These assignments are discussed in detail, along with other model compounds, in ref 17. [b] These concentration-dependent bands were observed above $10^{-3}$ M. No bands could be detected in this region below $5 \times 10^{-4}$ M. [c] n.o. = none observed.

a detailed normal mode analysis of the effects of conformation on these frequencies is not available. Extensive experimental studies with model compounds have been carried out, however, and much is known about the effects of various interactions on the N–H stretching frequencies.[17-19] Some of the results obtained with model compounds in $CHCl_3$ are summarized in Table II, as an aid to the interpretation of the spectrum of *cyclo*(L-Ala-Gly-Aca). Three points should be noted. First, the unperturbed N–H stretch of the amide occurs at 3450 $cm^{-1}$ for both *N*-acetyl-*N'*-methylglycinamide and *N*-acetyl-*N'*-methyl-L-alaninamide.[17] Second, the presence of a nearby $\beta$ carbon lowers the N–H stretching frequency. Thus, the 3437-$cm^{-1}$ band in *N*-acetyl-*N'*-methyl-L-alaninamide is assigned to the alanyl NH conformations where the NH is close to the $CH_3$ group of the alanyl side chain (i.e., $\phi_{Ala} \approx -60°$).[17,32] Studies with substituted amides have shown[33] that nearby methyl groups can perturb the amide NH stretching frequency as much as 31 $cm^{-1}$. Third, intramolecular hydrogen bonds (e.g., in the $C_7^{eq}$ conformation) or intermolecular hydrogen bonds shift the N–H stretching frequency below 3400 $cm^{-1}$. For *N*-acetyl-*N'*-methylsarcosinamide, in which the $C^7$ conformation is energetically strongly favored, an absorption at 3368 $cm^{-1}$ was observed in $CHCl_3$ solution.[17] For *N*-acetyl-*N'*-methyl-L-alaninamide and *N*-acetyl-*N'*-methylglycinamide, no absorptions between 3400 and 3300 $cm^{-1}$ were observed in $10^{-4}$ M $CHCl_3$ solutions,[17] although such absorptions occur at higher concentrations, due to intermolecular hydrogen bonding.

In the infrared spectrum of *cyclo*(L-Ala-Gly-Aca) in $CHCl_3$ (Figure 4A), there are no absorptions above 3440 $cm^{-1}$ which would correspond to the unperturbed N–H stretching frequency. Thus, all three NH groups in the cyclic peptide must either be involved in hydrogen bonds or be near other groups which would affect the N–H stretching frequency. This severely limits the number of possible conformations; only type II bends similar to conformation 1 are consistent with this condition. (See Figure 4 of ref 5 for an illustration of conformation 1.) (a) The Ala NH is close to the $CH_3$ group of the Ala side chain in all conformations, with $\phi_{Ala}$ near −80°, i.e., in all type I, II, and III bends (conformations 1–7). As discussed above, this interaction can reduce the N–H stretching frequency by as much as 31 $cm^{-1}$, thus accounting for the 3426-$cm^{-1}$ band. (b) In conformations 1 and 3, but in none of the others, the Gly NH group is involved in a hydrogen bond with the Aca CO group, corresponding to the $C_7^{eq}$ conformation of the Ala residue, so that an N–H stretching frequency near 3370 $cm^{-1}$ can be expected.[17] (c) The Aca NH group could be hydrogen bonded to the Aca CO group in type I bends, or in a type II bend which can be obtained by a slight readjustment[5] of conformation 1, but not in conformation 3 or in a conformation similar to the latter. This hydrogen bond (which has to be highly nonlinear, but not more so than the hydrogen bond in a $C_7^{eq}$ conformation) produces a large frequency shift. Thus, the NH groups of the Gly and Aca residues in a type II bend conformation which is similar to conformation 1 could account for the broad absorption at 3367 $cm^{-1}$ and the 3340-$cm^{-1}$ shoulder of the cyclic peptide in $CHCl_3$.

The infrared spectrum of *cyclo*(L-Ala-Gly-Aca) in $CHCl_3$ is not compatible with the presence of type I (or III) bends as major components since the Gly NH points away from the center of the ring in conformations 2 and 4–7. This NH would have no significant perturbations in these conformations, and an N–H stretching frequency near 3450 $cm^{-1}$ would be expected. Type I' and III' bends can be excluded since their Ala NH would be unperturbed, also producing a 3450-$cm^{-1}$ band. The absence of such a band excludes the presence of type I (and III) and I' (and III') bends in amounts exceeding 10%. Thus, the N–H stretching region of the infrared spectrum in chloroform is consistent only with the type II $\beta$ bend of conformation 1.

**Amide I Region.** A broad band at 1680 $cm^{-1}$ is observed in the infrared spectrum of the cyclic peptide in chloroform (not shown here), with a pronounced shoulder at 1654 $cm^{-1}$ and, possibly, a weak shoulder near 1695 $cm^{-1}$. The observed splitting of the amide I band indicates the presence of interactions between the amide groups. This observation is consistent with the presence of a type II bend, but does not exclude other possible bends. A specific conformation cannot be assigned on the basis of comparison of these amide I bands with the normal mode calculations (Figure 6) since the latter include external hydrogen bonds which are not present in the dilute chloroform solutions.

In aqueous ($D_2O$) solution, amide I' Raman bands[34] are observed at 1647 and 1616 $cm^{-1}$ (Figure 1B). The splitting of these bands is similar to that observed in the infrared spectrum of the $CHCl_3$ solution (see above), but the frequencies are lowered by about 35 $cm^{-1}$. We have found that the amide I' frequencies of *N*-acetyl-*N'*-methylglycinamide and *N*-acetyl-*N'*-methyl-L-alaninamide in $D_2O$ are also about 35 $cm^{-1}$ lower than the corresponding amide I frequencies in the infrared spectra of chloroform solutions. The similar splitting in the chloroform and aqueous solutions of *cyclo*(L-Ala-Gly-Aca) suggests that the molecule may have similar preferred conformations in both solvents (i.e., a type II bend).

**Amide III Region.** In aqueous solutions, bands that weaken or shift on N-deuteration are observed at 1375, 1365, 1302, and 1234 $cm^{-1}$ (Figure 1). The 1234-$cm^{-1}$ region, overlapping with the $D_2O$ absorption, was observed by subtracting out the $D_2O$ Raman spectrum with a computer. These bands are in the same general region as the

bands observed in the solid state. In particular, the bands near 1370 $cm^{-1}$ are consistent with type II bends (conformations 1 and 3) as the dominant species, but they would not be consistent with *large* amounts of type I or III bends (Figure 8).

The Raman spectra in aqueous solution are most consistent with a type II bend as the predominant conformation, similar to the computed structures 1 or 3. The presence of small amounts of type I (+III) bends in aqueous solution cannot be excluded on the basis of the observed spectra.

**C. Ac-L-Ala-Gly-NHMe in Solution.** This molecule is an open-chain analogue of the peptide part of *cyclo*(L-Ala-Gly-Aca). Comparison of the spectra of the two compounds illustrates the effects of cyclization in restricting conformational flexibility.

**N–H Stretching Region.** The IR spectra of *cyclo*(L-Ala-Gly-Aca) and of the terminally blocked single residues are helpful for interpreting the spectrum of Ac-L-Ala-Gly-NHMe (Figure 4B). The open-chain dipeptide has a broad band, attributable to NH groups which are not hydrogen bonded, near 3440 $cm^{-1}$, and a broad band, centered near 3370 $cm^{-1}$, which is probably due to a variety of hydrogen-bonded conformations. There are several indications of conformational flexibility in this molecule. There is a small shoulder at 3418 $cm^{-1}$, which indicates that a small percentage of these molecules have one of the residues in a $C_5$ (extended) conformation.[17,19] The band near 3440 $cm^{-1}$ in Ac-L-Ala-Gly-NHMe is considerably broader than the 3426-$cm^{-1}$ band in *cyclo*(L-Ala-Gly-Aca) or the 3450-$cm^{-1}$ band in Ac-Gly-NHMe.[17] The bands attributed to the free N–H stretch in Ac-L-Ala-NHMe showed clear splitting into several components.[17] In Ac-L-Ala-Gly-NHMe, the separate components are not resolved, and a broad band at 3440 $cm^{-1}$ appears. The broadness and lack of clearly resolved components in this band are probably due to an ensemble of several conformations.

The 3370-$cm^{-1}$ band seen for Ac-L-Ala-Gly-NHMe is of interest because no N–H stretching band below 3400 $cm^{-1}$ was observed[17] in dilute chloroform solutions of the blocked single residues of Gly and Ala. A possible interpretation is that this band is due to the formation of a hydrogen bond between the terminal NH and CO groups of the dipeptide. This hydrogen bond occurs in some bends,[24,25,29] but it cannot be formed in the blocked single residues. It might also be postulated that the presence of a second residue increases the formation of seven-membered hydrogen-bonded rings. Conformational energy calculations indicate, however, that the percentage of residues in the $C_7^{eq}$ conformation is not greater in dipeptides than in the blocked single residues.[35] The broadness of the 3370-$cm^{-1}$ band indicates that there are several hydrogen-bonded conformations in equilibrium, possibly including a mixture of $\beta$-bend and $C_7^{eq}$ conformations. The intensity of this band indicates that a large fraction of the molecules in solution have a hydrogen bond. The fact that this band is centered near 3370 $cm^{-1}$ indicates that the hydrogen bonds are not particularly strong on the average, since this frequency is typical of the relatively weak hydrogen bond observed in the $C_7^{eq}$ conformation. A similar band was reported for tetrapeptides forming $\beta$ bends in deuteriochloroform solutions.[20]

**Amide I and III Regions.** In the amide I region of Ac-L-Ala-Gly-NHMe in $CHCl_3$, a broad band centered near 1674 $cm^{-1}$ was observed (not shown here). In contrast to the cyclic peptide, shoulders could not be resolved clearly. Simiarly, in $D_2O$, a single broad amide I′ band was observed at 1647 $cm^{-1}$ (Figure 5B). In contrast to the cyclic peptide, for which intramolecular interactions clearly split the amide I′ band, no splitting could be observed in the amide I′ region of the open-chain dipeptide. These amide I bands indicate that the conformation of the open-chain dipeptide differs from that of the cyclic peptide. The position of this band is similar to that observed by us in *N*-acetyl-*N*′-methylalaninamide or *N*-acetyl-*N*′-methylglycinamide in $D_2O$ (unpublished data). This indicates that, on the average, the amide I mode of the open-chain analogue is not perturbed by neighboring groups.

Amide III bands occur at 1259, 1290, 1302, and 1380 $cm^{-1}$. These amide III bands cannot be compared directly with the vibrations of the cyclic peptide since cyclization can result in different mixing of internal vibrations.[28] A complete analysis of this region is not possible without normal mode calculations on the open-chain peptide.

## V. Conclusion from the Infrared and Raman Studies

Infrared and Raman spectra indicate that the predominant conformation of *cyclo*(L-Ala-Gly-Aca) both in chloroform and aqueous solutions and the only conformation in the solid state is a type II $\beta$ bend. The conformation of the molecule may differ somewhat in the solid state and in solution. In the solid state, the computed conformation 3 best fits the observed data. In solution, the spectra favor a conformation similar to the lowest energy computed conformation 1. Both computed conformations are type II bends. They differ from each other only in their different orientation of the third peptide group, i.e., the one between the Gly and Aca residues.[5,29] A minor component with type I(+III) bend conformations may be present in aqueous solution but not in chloroform solution. This is consistent with the CD measurements,[6] which suggest that the fraction of type I (+III) bends is much smaller in methanol and trifluoroethanol than in water. It is reasonable to assume that the equilibrium would be shifted even further toward the type II bend in the less polar chloroform solution.

The open-chain analogue Ac-L-Ala-Gly-NHMe has greater conformational flexibility than the cyclic peptide. In chloroform solution, however, a significant fraction of the molecules form intramolecular hydrogen bonds.

## VI. Overall Conclusions

This section summarizes the conclusions reached in this series of studies, based on the experimental results reported here and in the preceding paper[6] and on the theoretical results in the first paper.[5]

**Conformation of *cyclo*(L-Ala-Gly-Aca).** Consistent conclusions have been reached from the NMR, CD, IR, and Raman spectroscopic measurements in solution and from the IR and Raman measurements in the solid. *cyclo*(L-Ala-Gly-Aca) has limited internal flexibility. It exists in solution predominantly in a type II $\beta$-bend conformation, with two weak (bent) hydrogen bonds between the Gly NH and Aca NH groups, respectively, and the Aca C=O group. These results confirm the prediction made by theoretical conformational energy calculations,[5] which indicated that the lowest energy conformation of the molecule is a type II $\beta$ bend. The lowest energy computed conformation contains only a bent Gly-N–H···O=C-Aca hydrogen bond, but a slight readjustment of the dihedral angles would permit the formation of the bent Aca-N–H···O=C-Aca hydrogen bond as well. The computed dihedral angles also agree closely with those deduced from the NMR studies. The conformational energy computations did not include interactions with the solvent and entropy effects. Solvent

effects may contribute up to 1–2 kcal/mol to the energy differences listed in Table I, and hence the fraction of various bend components may change somewhat in different solutions.

Normal mode analysis of the IR and Raman spectra indicates that another type II bend might be prevalent in the solid state. This bend differs from the first bend only by the reversal of the orientation of the peptide bond between the Gly and Aca residues, corresponding to a decrease[5] of 136° in the dihedral angle $\psi_2$. As a result, this conformation does not contain the Aca-N-H···O=C-Aca hydrogen bond. The computations show that this conformation is 0.93 kcal/mol higher in energy than the other type II bend conformation. The energy barrier between the two conformations is low (estimated[5] to be of the order of 3–4 kcal/mol), so that it is possible that there is interconversion between the two conformations when the environment is changed from solution to the solid form.

Different solvents had to be used with the various experimental methods because of experimental limitations. Nevertheless, the results obtained by different techniques are consistent with each other. This indicates that the same conformations are prevalent in various environments. Changes of the solvent may alter the equilibrium distribution between various types of bend conformations, and they may cause small changes in the dihedral angles. Examples are seen in the temperature and solvent dependence of the ellipticities[6] and the change from one preferred type II bend in solution to another in the solid form, as suggested by the IR and Raman spectra.

**Use of Various Spectroscopic Techniques as Diagnostic Tools for the Presence of $\beta$ Bends.** The presence of bends in peptides can be inferred, making joint use of several methods, as exemplified in the analysis presented here. Some of the techniques may differentiate between various types of bends.

(1) Alterations of N-H proton chemical shifts and of their temperature dependence indicate hydrogen bonding and/or inaccessibility to solvent. The lowering of N-H stretching frequencies also can be attributed to hydrogen bonding or to other perturbations (e.g., by a neighboring $CH_3$ group). Such altered NMR and IR spectra may indicate the presence of bends, if one can reasonably exclude the possibility of hydrogen bonding to other parts of the peptide. A smaller lowering of the N-H stretching frequency, as seen here for the Ala NH group, not accompanied by modification of the NMR signals of this group, indicates that the IR shift is not due to hydrogen bonding or inaccessibility. The attribution of the perturbation to a neighboring $CH_3$ group served to specify $\phi_{Ala}$. This information narrows the choice of values of $\phi$, based on coupling constant measurements.

(2) The normal mode analysis of several amide bands, combined with observed spectra, demonstrated that this analysis is capable of distinguishing between various types of bends.

(3) Nuclear Overhauser effects can also distinguish between type I and II bends,[36,37] because of the different value of $\psi$ for the first residue in the bends (Ala in this case). The usefulness of this effect was confirmed by the present study.[6]

(4) Finally, CD spectroscopic measurements[6] can distinguish between various types of bends, and provide information about the relative amounts of bend conformations when more than one is present.

## References and Notes


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(2) (a) Baker Laboratory of Chemistry, Cornell University. (b) Biophysics Research Division and Department of Physics, The University of Michigan. (c) Russell Grimwade School of Biochemistry, University of Melbourne.

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