Ac-L-2-MePro-NHMe[14] has backbone dihedral angles $\phi = -60.5°$ and $\psi = -24.9°$, similar to those of Ac-L-pro-NHMe[39] ($\phi = -76.3°$, $\psi = -15.9°$), while in Ac-2,4-MePro-NHMe, $\phi = \pm 29°$. For this reason, 2-methylproline[13,14] may be a better proline analogue than 2,4-methanoproline for selectively stabilizing X-pro peptide bonds while simultaneously maintaining a ϕ,ψ conformational space similar to that of trans L-Pro. On the other hand, replacement of L-Pro with 2,4-MePro selectively stabilizes the trans peptide bond and also contrains $\phi = \pm 29°$. Hence, both 2-MePro and 2,4-MePro should be useful proline analogues for polypeptide molecular design.

An attempt was made to compare the 2,4-methanopyrrolidine structure with structures which have been determined for similar bicyclic rings. To our knowledge, there is no atomic resolution molecular structure available for the corresponding nitrogen-containing 2-azabicyclo[2.1.1]hexane. For bicyclo[2.1.1]hexane, in which the nitrogen atom is replaced by carbon, the molecular structure based on gas-phase electron diffraction[40] has been criticized by subsequent ab initio calculations[41] and by comparisons of the calculated photoelectron spectrum with that observed experimentally.[42] For both the electron diffraction[40] and ab initio calculations, a symmetric bicyclic structure was assumed a priori, while the X-ray diffraction data presented in this paper clearly indicate an asymmetric methanopyrrolidine structure for Ac-2,4-MePro-NHMe.

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The experimental and theoretical results presented in this paper indicate that proper modeling of the conformational properties of 2,4-MePro in peptides requires that its backbone (i.e., ϕ) and side-chain conformational chirality be taken into account. Although the introduction of the 2,4-methylene bridge into L-Pro results in selective stabilization of the trans peptide bond conformation,[15] the constraints of $\phi = \pm 29°$ restrict 2,4-MePro to a conformational space different from that of L-Pro. In particular, $\phi = \pm 29°$ prevents 2,4-MePro from adopting some extended conformations which are accessible to L-Pro. The effects of these contraints on the conformational properties of 2,4-MePro compared with L-Pro in polypeptides are addressed with conformational energy calculations in the accompanying paper.[19]

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Supplementary Material Available: Table 1S, fractional coordinates and thermal parameters for Ac-2,4-MePro-NHMe; Table 2S, comparison of bond lengths in Ac-2,4-MePro-NHMe, Ac-Pro-NHMe, and 2,4-MePro; Table 3S, comparison of bond angles in Ac-2,4-MePro-NHMe, Ac-Pro-NHMe, and 2,4-MePro; Table 4S, hydrogen bonds and other short intermolecular distances in the Ac-2,4-MePro-NHMe crystal (5 pages). Ordering information is given on any current masthead page.

Conformational Properties of 2,4-Methanoproline (2-Carboxy-2,4-methanopyrrolidine) in Peptides: Theoretical Conformational Energy Analysis of Restrictions of the Polypeptide Chain Conformation

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Abstract: The α-amino acid 2,4-methanoproline (2-carboxy-2,4-methanopyrrolidine, 2,4-MePro) can serve as an analogue of proline in studies of the folding of globular proteins and of collagen structure and function. Conformational energy computations have been carried out on the terminally blocked residue Ac-2,4-MePro-NHMe and on the dipeptides Ac-2,4-MePro-X-NHMe and Ac-X-2,4-MePro-NHMe, where X = L-Ala or L-Tyr. The trans form of the peptide bond preceding the 2,4-MePro residue is strongly stabilized over the cis form, by at least 5.9 kcal/mol, in agreement with experimental NMR studies of this residue, and in contrast to the cis/trans equilibrium in prolyl peptides (where the trans form is preferred by only about 2 kcal/mol). The value of the dihedral angle ψ also is more strongly constrained than in prolyl peptides. The conformational constraints exerted by the 2,4-MePro and Pro residues on the residue *following* them in a dipeptide are similar, except that preferences for partially folded (such as A and D) conformations, compared with more extended (such as E and F) conformations, are stronger in the case of 2,4-MePro. On the other hand, Pro strongly constrains the conformational freedom of the residue *preceding* it in a dipeptide, while such constraints are much less severe in the case of a residue preceding 2,4-MePro. The probability of bend formation in both the Ala-Pro and Pro-Ala dipeptides is strongly enhanced by substitution of 2,4-MePro for Pro. Therefore, the 2,4-MePro residue can serve as a model for Pro where bulkiness and rigidity of the polypeptide chain is required, with selective stabilization of the trans peptide bond, if some change of the conformational preferences of the neighboring residues can be allowed.

1. Introduction

The rare achiral amino acid[2] 2,4-methanoproline (2-carboxy-2,4-methanopyrrolidine) occurs naturally as a free amino acid in some plants.[3,4] It is not one of the naturally occurring amino acid components of proteins. It is of potential importance, however, in conformational studies of proteins and peptides as an analogue of proline, especially in the study of the cis–trans isomerization about the peptide bond preceding proline. It has

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(2) Abbreviations used: Ac, *N*-acetyl terminal group; 2,4-MePro, 2,4-methanoprolyl residue; NMR, nuclear magnetic resonance.
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been suggested that the rate-limiting step in protein refolding[5-8] and in the assembly of the triple-stranded collagen molecule[9] is the cis–trans isomerization about the peptide bond preceding prolyl residues. For this peptide bond, the cis and trans forms have comparable energies in small peptides, with a computed energy difference[10,11] $\Delta E_{t\rightarrow c}$ = 2.0 kcal/mol and observed energy differences of a similar magnitude.[12-15] Thus, both forms may coexist in solutions of Pro-containing peptides, unlike other amino acids for which the trans form is highly preferred.[10,11] This conformational heterogeneity may result in complex refolding kinetics when Pro residues are present. The cis–trans equilibrium can be modified by substitutions or alterations of the prolyl ring, as indicated by investigations of proline analogues.[16-19] The analysis of the role of cis–trans isomerization in protein folding would be aided if one could use an analogue of proline which behaves sufficiently similarly to proline but exists in only one of the forms, i.e., either as a cis or a trans peptide. If such an analogue is incorporated into a polypeptide and the folding kinetics of the original and modified polypeptides are compared, this may shed light on the mechanism of the refolding process. Proline analogues are also of interest in the study of collagen structure and function.[20]

2,4-MePro was chosen as a possible structural analogue of Pro, because it has a constrained geometry, differing somewhat from that of Pro. Solution NMR data indicate[21] that the cis–trans equilibria for the two amino acid residues are different. This paper is part of a series of studies[21,22] (referred to hereafter as parts 1 and 2). In the first part,[21] NMR spectroscopy was used to show that replacement of Pro by 2,4-MePro in the peptides Ac-L-Pro-NHMe and Ac-L-Tyr-L-Pro-NHMe results in selective stabilization of the trans conformation of the X-Pro peptide bond.

In the second part,[22] the structure of the Ac-2,4-MePro-NHMe molecule was determined by X-ray crystallography. Although, on the basis of its covalent structure, the bridged pyrrolidine ring might have been expected to possess a plane of symmetry (with the C^{α}, N, C^{δ}, and C^{γ} atoms lying in one plane and with the $C^{\beta 1}$ and $C^{\beta 2}$ atoms positioned symmetrically with respect to this plane), the observed structure exhibits considerable asymmetry.[23,24] Two enantiomeric molecules, with opposite distortions, exist in the crystal.[22] The asymmetry is manifested in differences of corresponding bond lengths (i.e., C^{α}–$C^{\beta 1}$ and C^{α}–$C^{\beta 2}$, as well as $C^{\beta 1}$–C^{γ} and $C^{\beta 2}$–C^{γ}) and of bond angles around the C^{α}, $C^{\beta 1}$, $C^{\beta 2}$, and C^{γ} atoms, as well as in the observed values of the dihedral angles: the C′–N–C^{α}–C'_0 dihedral angle, ϕ, is either −28.9 or +28.9° in the two enantiomeric molecules, and the C^{α}–N–C^{δ}–C^{γ} dihedral angle is 2.0 or −2.0°, respectively, while both would be 0° in a symmetrical structure. Possible reasons for these deviations are discussed in the accompanying paper.[22] As a result of the asymmetry, the N atom and its three substituents (viz., C^{α}, C^{δ}, and C'_0) are not coplanar in the observed structure; i.e., the substituents on the N atom are arranged in a pyramid, in contrast

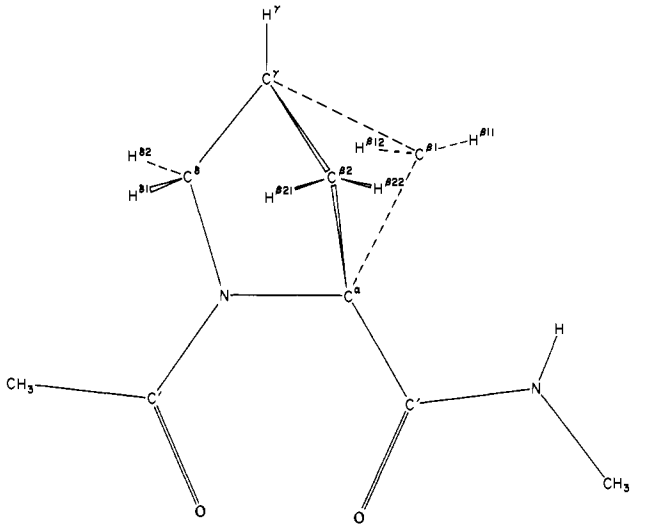

Figure 1. Diagram of the Ac-2,4-MePro-NHMe molecule, indicating the nomenclature for atoms.[25,27]

Table I. Bond Lengths, Bond Angles, and Fixed Dihedral Angles Used for Ac-2,4-MePro-NHMe in the Computations[a]

	experimental geometry[b]	symmetrized geometry
	Bond Lengths (Å)	
N–C^{α}	1.48	1.48
C^{α}–$C^{\beta 1}$	1.55[c]	1.55
C^{α}–$C^{\beta 2}$	1.55[c]	1.55
$C^{\beta 1}$–C^{γ}	1.55[d]	1.55
$C^{\beta 2}$–C^{γ}	1.54[d]	1.55
C^{γ}–C^{δ}	1.53	1.53
C^{δ}–N	1.49	1.49
C^{α}–C′	1.51	1.51
	Bond Angles (deg)	
C'_0–N–C^{α}	126.9	128.1
C'_0–N–C^{δ}	125.8	128.1
C^{α}–N–C^{δ}	103.9	103.9
N–C^{δ}–C^{γ}	97.0	97.0
N–C^{α}–C′	116.6	116.6
N–C^{α}–$C^{\beta 1}$	100.6[e]	100.1
N–C^{α}–$C^{\beta 2}$	99.7[e]	100.1
$C^{\beta 1}$–C^{γ}–C^{δ}	103.1[f]	101.7
$C^{\beta 2}$–C^{γ}–C^{δ}	100.5[f]	101.7
C^{α}–$C^{\beta 1}$–C^{γ}	81.9[g]	82.1
C^{α}–$C^{\beta 2}$–C^{γ}	82.4[g]	82.1
	Dihedral Angles (deg)[i]	
C^{α}_0–C'_0–N_1–C^{α}_1 (ω_0)	175.9	180
O'_0–C'_0–N–C^{α}	4.1	0
O'_0–C'_0–N–C^{δ}	159.6	180
C'_0–N–C^{α}–C′ (ϕ)	−29.0	0
C^{α}–N–C^{δ}–C^{γ}	2.0	0
C'_0–N–C^{δ}–C^{γ}	−158.0	180
C'_0–N–C^{α}–$C^{\beta 1}$	114.1[h]	+135.8
C'_0–N–C^{α}–$C^{\beta 2}$	−157.9[h]	−135.8

[a] Bond lengths and bond angles not listed are those used in the standard ECEPP residue and end-group geometry.[28,29] [b] Observed in the crystal structure.[22] [c–h] Numerical values of each pair labeled by the same superscript must be interchanged for the enantiomeric molecule (with opposite distortion of the geometry). [i] The sign of all dihedral angles must be interchanged for the enantiomeric molecule.

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to Pro and other amino acid residues in which these substituents and the N atom are coplanar.

In this paper, the conformational restrictions of the polypeptide chain, caused by the presence of the 2,4-MePro residue, are studied by means of theoretical conformational energy computations. The goal of this study was to predict the conformational preferences and restrictions introduced when Pro is replaced by 2,4-MePro.

2. Methods

The standard convention for the nomenclature of polypeptide conformations has been followed.[25,26] The numbering of the atoms in the 2,4-MePro residue is shown in Figure 1.[27] Backbone and side-chain conformational states of the Ala and Tyr residues are indicated with the letter code according to the nomenclature of Zimmerman et al.[10] Subscript numbers denote the residues,[25] with 0 indicating the acetyl end group, 1 the first amino acid residue, etc. Additional notation is introduced here to describe the conformation of the 2,4-MePro residue as follows. The letters t and c refer to the trans and cis conformational states, respectively, about each peptide bond. The letters n and p refer to the deformation of the bridged pyrrolidine ring in the 2,4-MePro residue,[22] as described in section 1, where n indicates the geometry as given in the second column of Table I, corresponding to the negative value of $\phi = -28.9°$, and p indicates the geometry of the enantiomeric molecule (with opposite distortion of the geometry), with $\phi = 28.9°$. Superscripts + and − on n and p indicate the sign of ψ.

The normalized Boltzmann probability P_J for conformational state J is given[11] as

$$P_J = Q^{-1} \sum_{j \in J} \exp(-\Delta E_j / RT) \quad (1)$$

with

$$Q = \sum_{i=1}^{m} \exp(-\Delta E_i / RT) \quad (2)$$

where j indicates all conformations belonging to conformational state J, m is the total number of conformations, and J = p or n, or J = + or − for the conformations of the 2,4-MePro residue, or J = A, C, D, E, F, G, or A* for the backbone conformations of the other residues.

Conformational energy computations have been carried out using the updated version[28] (ECEPP/2) of the ECEPP algorithm.[29] The conjugate direction Powell algorithm[30] was used for energy minimization. The computations were carried out on a Prime 550 minicomputer with an attached Floating Point Systems AP-120B array processor.[31]

2.1. Asymmetrical Geometry. Some changes had to be introduced in the ECEPP method for generation of the polypeptide chain, in order to account for the special structural features of the 2,4-MePro residue (Figure 1). The bond lengths and bond angles adopted for the heavy atoms of the bridged pyrrolidine ring were those determined by means of X-ray crystallography for Ac-2,4-MePro-NHMe with trans peptide bonds[22] (column 2 of Table I). They reflect the asymmetry of the molecule, observed in the crystal structure[22] and described in section 1.

The same geometry was used throughout the computations for both the trans and the cis forms of the peptide bond between the Ac- end group and the 2,4-MePro residue, because no experimental information is available for the cis form. The bond angles around the N atom differ from those in the Pro residue, because of the change of the ring geometry. The observed intra-ring bond angle C^{α}–N–C^{δ} is 103.9° (as compared to 113.0° in Pro). The two observed bond angles exterior to the ring, viz. C'_0–N–C^{α} and C'_0–N–C^{δ} are 126.9 and 125.8°, respectively. These angles were used in the computations for both the trans and the cis form.[32,33]

The bond lengths and bond angles used for heavy atoms outside the bridged pyrrolidine ring were the standard values in ECEPP for proline-like residues,[29] for the sake of consistency in the ECEPP computations (Table I). Hydrogen atoms were placed at positions corresponding to standard ECEPP bond geometry[28,29] (with C–H and N–H bond lengths of 1.09 and 1.00 Å, respectively, and hydrogen-atom bond angles that reflect deviations from tetrahedral symmetry[34]), instead of the experimentally determined positions, because of the relatively larger uncertainty of hydrogen positions in the X-ray diffraction study. All bond lengths and bond angles were kept constant in the calculations, as is usual for ECEPP computations.[29] This implies, among others, that the dihedral angle ϕ was fixed at a value $\phi = +28.9$ or $-28.9°$. The partial charges on the atoms were determined from the Mulliken population analysis carried out in a CNDO/2 calculation[35] on several conformers of the Ac-2,4-MePro-NHMe molecule.

2.2. Symmetrized Geometry. We also investigated whether the deviation of the 2,4-MePro residue from symmetry plays a significant role in the conformational properties. Therefore, a second set of computations was carried out for a hypothetical Ac-2,4-MePro-NHMe molecule with adjusted bond lengths and bond angles that correspond to a symmetrical structure of the bridged pyrrolidine ring (last column of Table I). The symmetrized structure was obtained by making the following changes in the observed[22] geometry. The C^{α}, N, C^{δ}, and C^{γ} atoms were made coplanar, without any change of bond lengths and bond angles, by fixing the C^{α}–N–C^{δ}–C^{γ} dihedral angle at 0°. The four C^{α}–C^{β} and C^{β}–C^{γ} bond lengths were set equal to the average value of the observed C^{α}–$C^{\beta 1}$, C^{α}–$C^{\beta 2}$, $C^{\beta 1}$–C^{γ}, and $C^{\beta 2}$–C^{γ} bond lengths. The two N–C^{α}–C^{β} bond angles were set equal to the average of the corresponding observed bond angles. The C'_0–N–C^{α} and C'–N–C^{δ} bond angles were set equal to each other. Their value was chosen as 128.1°, in order to make the N atom and its substituents coplanar. The dihedral angle ϕ was fixed at 0°. In addition, the C' atom of the 2,4-MePro residue and its three substituents were made coplanar, by adding equal increments to the observed bond angles around the C' atom, so that their sum became 360°. The two peptide groups were fixed to be planar ($\omega_0 = \omega_1 = 180°$).

2.3. Selection of Starting Points and Energy Minimization. For the terminally blocked single residue, Ac-2,4-MePro-NHMe, the energy was computed as a function of ψ_1, for the four combinations of trans and cis conformational states of the two peptide bonds, i.e., with ω_0 and ω_1 fixed at either 180 or 0°, with all other variable angles fixed at 180°. The computed minimum-energy values of ψ_1 were then used as starting points in the complete energy minimization in which all backbone dihedral angles (θ_0, ω_0, ψ_1, ω_1, θ_2) were allowed to vary.[26] These computations were carried out for both the asymmetrical and the symmetrized geometry.

In the computations of the terminally blocked 2,4-MePro-Ala and Ala-2,4-MePro dipeptides, the starting points for conformational energy minimization were all combinations of the minimum-energy conformations of Ac-2,4-MePro-NHMe with *experimental* geometry, computed in this study, with the seven low-energy conformations of Ac-L-Ala-NHMe, computed earlier,[11] and denoted C, E, A, D, F, G, and A*. Energy minimization was carried out with respect to all nine variable dihedral angles (including methyl group rotations in Ala and in the end groups). No computations were carried out with symmetrized geometry.

For the two terminally blocked dipeptides of Tyr and 2,4-MePro, the starting points for conformational energy minimization were all combinations of only the four trans–trans minimum-energy conformations of Ac-2,4-MePro-NHMe with experimental geometry with the 30 low-energy conformations of Ac–Tyr–NHMe, computed earlier.[11] Energy minimization was carried out with respect to all backbone and side-chain dihedral angles.

3. Results and Discussion

3.1. Ac-2,4-MePro-NHMe. Both the trans and the cis conformation were considered for each peptide bond next to the 2,4-MePro residue, corresponding to four possible conformational states of the molecule: trans–trans (tt), cis–trans (ct), trans–cis

(25) IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **1970**, *9*, 3471–3479.

(26) θ_0 and θ_2 (or θ_3) denote the dihedral angles for the rotation of the CH_3 groups in the *N*-acetyl (Ac–) and *N'*-methylamide (–NHMe) end groups, respectively.

(27) In the other papers of this series[21,22] an alternative notation is used, based on prochiral assignments, in order to aid the visualization of spatial relationships of H and C atoms with the bicyclic ring. The relationship of that notation to the standard nomenclature of polypeptides[25] is as follows: $C^{\beta 1}$ corresponds to $C^{\beta,R}$ in the alternative notation, $C^{\beta 2}$ to $C^{\beta,S}$, $H^{\beta 11}$ to $H^{\beta 1,endo}$, $H^{\beta 12}$ to $H^{\beta 1,exo}$, $H^{\beta 21}$ to $H^{\beta 2,exo}$, $H^{\beta 22}$ to $H^{\beta 2,endo}$, $H^{\delta 1}$ to $H^{\delta,S}$, and $H^{\delta 2}$ to $H^{\delta,R}$.

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Table II. Minimum-Energy Conformations of Ac-2,4-MePro-NHMe with Two Choices of Covalent Geometry

peptide bond conformations	conformational letter code for the ring geometry[a]	experimental geometry					symmetrized geometry				
		energy,[b] ΔE (kcal/mol)	dihedral angles[c] (deg)				energy,[d] ΔE (kcal/mol)	dihedral angles[c] (deg)			
			ω_0	ϕ_1[e]	ψ_1	ω_1		ω_0	ϕ_1[e]	ψ_1	ω_1
trans–trans	n^-	0.00[f]	176	(−29)	−50	180	0.00	180	(0)	−76	180
	p^+	0.00	−1.76	(29)	50	180	0.00	180	(0)	76	180
	n^+	0.24[f]	175	(−29)	93	180					
	p^-	0.24	−175	(29)	−93	180					
cis–trans	n^-	5.90[f]	−24	(−29)	−56	−179	10.65	−42	(0)	−73	−179
	p^+	5.90	24	(29)	56	179	10.65	42	(0)	73	179
	n^+	7.18[f]	−23	(−29)	123	180					
	p^-	7.18	23	(29)	−123	180					
trans–cis[g]	n^-	14.57[f]	174	(−29)	−40	−45	14.77	170	(0)	−57	−36
cis–cis[g]	n^-	21.39[f]	−29	(−29)	−42	−42	24.99	−48	(0)	−57	−34

[a] Indication of the choice of asymmetric (distorted) geometry of the ring and of the sign of the backbone dihedral angles, as defined in the text: n and p correspond to $\phi_1 = -29°$ and $\phi_1 = 29°$, respectively, while the superscript + or − refers to the sign of ψ_1. [b] $\Delta E = E - E_0$, where $E_0 = -19.15$ kcal/mol for the lowest energy nonsymmetrical trans–trans conformation. [c] All dihedral angles were allowed to vary. The values of θ_1 and θ_2, describing the terminal methyl group rotations,[26] are not listed. They fall into the range 180 ± 15°. [d] $\Delta E = E - E_0$, where $E_0 = -18.53$ kcal/mol for the lowest energy symmetrized trans–trans conformation. The values of E_0 given in footnotes *b* and *d* cannot be compared with each other because they do not include the energy of the distortion of the geometry. [e] Rigidly fixed by the choice of the ring geometry (distortion). [f] Dihedral angles for the experimental geometry in this line correspond to the geometry described by the data in column 2 of Table I. Dihedral angles in the other lines correspond to the enantiomeric geometry. [g] Only the lowest energy minimum is listed for this conformational state of the peptide bonds, and only one of the two equivalent enantiomeric conformations is shown.

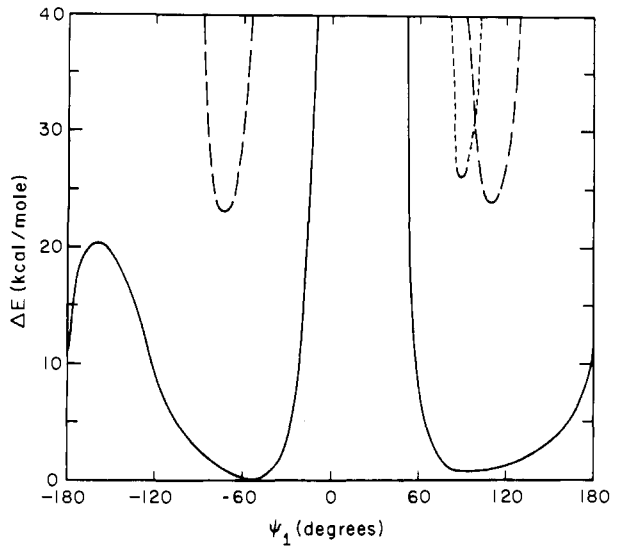

Figure 2. Curve of ΔE vs. ψ for Ac-2,4-MePro-NHMe with asymmetriic geometry, with the two peptide bonds fixed in the trans–trans (full line), cis–trans (long-dashed line), or trans–cis (short-dashed line) conformations. The energy of the cis–cis conformation is too high on the scale of this drawing. The conformation of the bicyclic ring was kept fixed, with $\phi_1 = -28.9°$, $\theta_0 = \theta_2 = 180°$.

(tc), and cis–cis (cc). The energy was computed as a function of ψ_1, with all other variable dihedral angles fixed at 180°, for each of these four conformational states. Computations were carried out for both the asymmetrical geometry (i.e., for both enantiomeric asymmetries) and symmetrized geometry. In each case, the ΔE vs. ψ_1 curve has two minima, one at a negative and one at a positive value of ψ_1. For the symmetrized geometry, the position and depth of the two minima is symmetrical with respect to $\psi_1 = 0°$. For the experimental geometry, the two minima differ in position and depth (Figure 2). The energy of the conformation with the smaller absolute value of ψ_1 is always lower than that of the other minimum.

Next, a complete energy minimization was carried out, in which all backbone dihedral angles were allowed to vary. The results are shown in Table II for both choices of geometries. One of the two lowest energy conformations is shown in Figure 3.

The trans–trans (tt) state has the lowest energy (for both the asymmetric and the symmetric geometries), followed by ct, tc, and cc.

The energy is always very high ($\Delta E > 14$ kcal/mol) when the peptide group *following* the 2,4-MePro residue is cis. The behavior

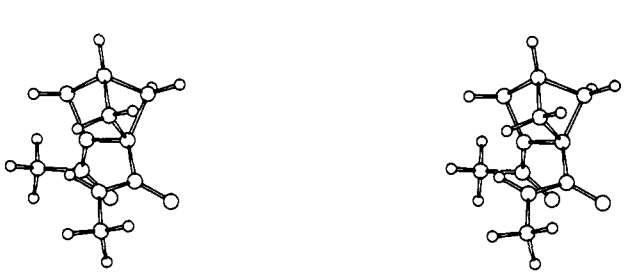

Figure 3. Stereoscopic picture of the Ac-2,4-MePro-NHMe molecule in one of its two lowest energy computed conformations, with $(\omega_0, \phi_1, \psi_1) = (176°, -29°, -50°)$. This choice of ϕ_1 corresponds to the experimental geometry given in column 2 of Table I.

is analogous to that of all amino acid residues.[36,37] On the other hand, the energy difference between the trans and cis forms for the peptide group *preceding* 2,4-MePro is 5.90 kcal/mol.[38] This value is lower than that for nonprolyl residues,[36,37] but considerably higher than the 2.0 kcal/mol computed[11] for Pro. The result indicates that Ac-2,4-MePro-NHMe exists practically entirely in the trans–trans form. This conclusion agrees with solution NMR observations.[21] The minima are narrow in all states except tt (Figure 2). This implies that the tt state is also favored entropically.

The values of ΔE listed in Table II show that the energy of the trans–cis isomerization for each of the two peptide groups is nearly independent of the state of the other peptide group, because the values of ΔE are nearly additive (within 1 kcal/mol). On the other hand, the large deviation of all cis peptide bonds from planarity (i.e., from the value of $\omega = 0°$) suggests strong steric hindrance in the cis forms of both peptide units. The trans–cis and cis–cis forms are very high in energy. Therefore, they will not be considered further, and we shall henceforth use the terms trans and cis to refer to the state of the peptide bond *preceding* the 2,4-MePro residue.

A symmetrical geometry is energetically unfavorable because of several repulsive close atomic contacts. In such a geometry, either the O atom or the CH_3 group of the acetyl end group (for

(36) Zimmerman, S. S.; Scheraga, H. A. *Macromolecules* **1976**, *9*, 408–416.

(37) Némethy, G.; McQuie, J. R.; Pottle, M. S.; Scheraga, H. A. *Macromolecules* **1981**, *14*, 975–985.

(38) The value of $\Delta E = 5.90$ kcal/mol represents an upper limit for the trans–cis energy difference. The actual value could be somewhat lower if the geometry of the cis form were significantly different from that of the trans form. In the present calculation, the same geometry was used for both forms (as described in section 2.1) because no experimental information is available for a cis form, so that any possible geometrical relaxation on going to the cis form could not be accounted for.

trans or cis forms, respectively) is coplanar with the N, C^{α}, and C′ atoms of the 2,4-MePro residue, and is near them. This results in repulsive overlaps of the O atom or the terminal CH_3 group with the C^{α} and C′ atoms. In addition, there are, in the trans form, repulsive interactions between the acetyl CH_3 group and the $C^{\delta}H_2$ group of the ring. Despite the latter, the energy of the cis conformation is 10.65 kcal/mol higher than that of the trans conformation in the symmetrized molecule. All of these repulsive interactions are lessened, however, by the distortion of the molecule into the (observed) nonsymmetrical form. This accounts for the computed difference of E_0 for the two geometries (footnotes *b* and *d* of Table II). The difference between the values of E_0 listed may not be equal to the actual energy difference between the two geometrical forms of the molecule, because it does not include any energies of possible distortions of bond lengths and bond angles. Nevertheless, it indicates that the nonbonded and electrostatic interactions, taken by themselves, already help to stabilize the nonsymmetrical form of the molecule. A similar conclusion was drawn from CNDO/2 conformational energy calculations described in the accompanying paper.[22]

The observed distortion of the symmetry of the bridged pyrrolidine ring is sufficient to move the 2,4-MePro C′ atom out of the C'_0–N–C^{α} plane by 0.65 Å, resulting in a decrease of the repulsion between the acetyl O atom and the 2,4-MePro C′ atom (in the trans form). The only additional significant repulsive interactions in the nonsymmetrical trans form occur between the acetyl CH_3 group and the $C^{\delta}H_2$ group. The two computed values of ψ_1 at the minima (near −50 and 90°) correspond to nearly opposite orientations of the C-terminal peptide group and end group, relative to the bridged ring. The two peptide groups are roughly perpendicular to each other in both conformations. Close inspection shows that repulsions between the O_1, N_2, and H_2 atoms of the C-terminal peptide group, on the one hand, and the acetyl C'_0 and O_0 atoms and the nearest $C^{\beta}H_2$ group of the ring, on the other hand, are minimized with these values of ψ_1.

In the cis form, the acetyl O_0 atom (instead of the acetyl CH_3 group) is near the ring $C^{\delta}H_2$ group, but no significant repulsion occurs. On the other hand, there is a close approach of the acetyl CH_3 group to the C'_1, O_1, and N_2 atoms of the second peptide group. Repulsions between these atoms are lessened somewhat by the strong nonplanarity of the first peptide group ($\omega_0 = \pm 24°$ instead of 0° for a planar cis peptide, Table II). This nonplanar conformation is the resultant of the opposing effects of the torsional energy for the peptide bond (which would favor planarity) and of the repulsive interactions between the atoms mentioned (which would favor even larger nonplanarity). As a result, the total energy of the cis form relative to the trans form is raised by 5.9 kcal/mol.[39] It should be noted that the three successive dihedral angles ω_0, ϕ_1, and ψ_1 are either all negative or they are all positive in the most stable cis form (Table II). This is the choice of signs which maximizes the distance between the acetyl CH_3 group and the N atom of the C-terminal end group (thereby reducing the repulsion between them). With this choice of signs, the C_0, C'_0, N_1, C^{α}_1, C'_1, and N_2 atoms are located so that they form an arrangement resembling an irregular helix, thereby increasing the distance between the C_0 and N_2 atoms.

For the ring deformation corresponding to the experimental geometry in Table I (i.e., with $\phi_1 = -28.9°$), the computation indicates that a negative value of $\psi_1 = -50°$ corresponds to a minimum with a slightly lower energy than the positive value of $\psi = 93°$, with $\Delta E = 0.24$ kcal/mol. (The corresponding signs of ψ_1 are reversed for the enantiomeric ring deformation.) On the other hand, the observed value of ψ_1 in the crystal structure is positive, viz. 114.5°. This dihedral angle permits hydrogen bonding of the N–H group at the C terminus of one molecule with the acetyl C=O group of a neighboring molecule of the crystal.

(39) This can be contrasted with the interaction in the cis form of Ac-Pro-NHMe. There, the C'_1 atom of Pro lies far outside the plane defined by the C'_0, N_1, and C^{α}_1 atoms (because $\phi = -75°$). Therefore, the distance between the acetyl CH_3 group and the C'_1, O_1, and N_2 atoms is larger, thereby reducing the repulsion. As a result, the nonplanarity is smaller ($\omega_0 \approx -6°$) and the trans-cis energy difference is much less ($\Delta E \approx 2.0$ kcal).[11]

Table III. Minimum-Energy Conformations of Ac-2,4-MePro-Ala-NHMe[a]

conformational letter code[b]	ΔE[c] (kcal/mol)	dihedral angles (deg)[d]				
		ω_0	ψ_1	ω_1	ϕ_2	ψ_2
tn^-D	0.00	176	−44	177	−143	45
tp^-A	0.10	−173	−85	−173	−70	−37
tp^-D	0.18	−175	−89	178	−147	41
tn^-A	0.38	175	−45	−173	−69	−35
tn^-C	0.76	176	−53	179	−82	72
tp^-C	1.10	−176	−98	180	−82	72
tp^+C	1.38	−176	48	−178	−81	76
tn^+A^*	1.53	174	88	177	55	43
tn^+C	1.68	175	92	−179	−80	76
tp^+A^*	1.68	−175	45	176	55	42
tp^+E	1.85	−176	50	−179	−155	159
tn^-E	1.98	176	−48	178	−154	155
tn^+E	2.23	176	96	180	−154	160
tp^+A	2.23	−176	48	180	−75	−34
tp^+F	2.29	−176	48	−179	−76	145
tp^+D	2.44	−176	49	180	−151	44
tp^-E	2.47	−176	−94	179	−155	153
tn^+A	2.48	175	90	179	−74	−35
tp^+G	2.56	−176	47	−179	−160	−57
tn^+F	2.57	175	92	180	−75	145
tn^+D	2.73	175	92	179	−151	43
tn^+G	2.73	175	88	179	−160	−57
tn^-G	3.36	176	−47	180	−157	−57
tp^-G	3.60	−175	−88	−179	−157	−57
tn^-A^*	3.79	176	−50	180	54	45
tp^-A^*	4.03	−175	−93	−179	54	46

[a] All minimum-energy conformations with $\Delta E < 5.0$ kcal/mol are listed. The experimental geometry was used for the 2,4-MePro. [b] The first lower-case letter denotes the conformational state of the peptide bond preceding the 2,4-MePro residue. The second lower-case letter and its superscript refer to the 2,4-MePro residue (see footnote *a* of Table II). The capital letter indicates the conformation of the Ala residue, according to the letter code by Zimmerman et al.[10] [c] For each minimum, $\Delta E = E - E_0$, where $E_0 = -18.72$ kcal/mol, the computed energy of the lowest energy minimum. [d] The dihedral angles θ_0, θ_2, and χ^1_2, corresponding to the rotation of methyl groups in the end groups and in the Ala side chain, and ω_2 are not listed. $\theta_0 = -165 \pm 1°$ for n conformations and $\theta_0 = 165 \pm 1°$ for p conformations; θ_2 and χ^1_2 are $180 \pm 2°$, and $\omega_2 = 180 \pm 2°$.

The computed energy of the isolated molecule at the crystallographic value $\psi_1 = 114.5°$, with respect to the energy at $\psi_1 = 93°$, is $\Delta E = 0.45$ kcal/mol (after minimization with respect to all other dihedral angles). This slight increase in the intramolecular energy apparently is compensated for by the favorable intermolecular interactions in the crystal, among others, an intermolecular hydrogen bond between the N_2–H_2 group of a molecule and the C_0=O_0 group of its neighbor.

3.2. Dipeptides of Ala and 2,4-MePro. The possible constraining effects of the 2,4-MePro residue on the backbone conformation of the neighboring residue can be seen from the conformational analysis of the terminally blocked 2,4-MePro-Ala and Ala-2,4-MePro dipeptides.

3.2.1. Ac-2,4-MePro-Ala-NHMe. The computed minimum-energy conformations are listed in Table III. Low-energy conformations are obtained only when both peptide units are in the trans form. The cis form for the peptide unit preceding the 2,4-MePro residue is destabilized even more than in Ac-2,4-MePro-NHMe. The energy difference between the lowest energy trans and cis conformations is 7.28 kcal/mol, compared with 5.90 kcal/mol for Ac-2,4-MePro-NHMe.

In contrast to the single 2,4-MePro residue, the energies of corresponding conformations with enantiomeric ring geometries (n^- and p^+ or n^+ and p^-, respectively) are not equal to each other in the dipeptide, because of the presence of the chiral L-Ala residue. Nevertheless, the overall Boltzmann statistical weights of the ensembles of conformations having n and p ring geometries are equal in this dipeptide (Table IV). On the other hand, the presence of the Ala residue results in a strong preference for negative values of ψ_1, i.e., for the n^- and p^- conformations of the 2,4-MePro residue; the sum of their normalized Boltzmann sta-

Table IV. Computed Frequency of Occurrence of the Two Enantiomeric Ring Deformations and of the Two Orientations around the C^{α}–C′ Bond of the 2,4-MePro Residue

compound	Boltzmann probability: ring deformation[a] P_n	ring deformation[a] P_p	C^{α}–C′ bond rotation[b] P_-	C^{α}–C′ bond rotation[b] P_+
Ac-2,4-MePro-NHMe	0.50	0.50	0.50	0.50
Ac-2,4-MePro-L-Ala-NHMe	0.50	0.50	0.88	0.12
Ac-2,4-MePro-L-Tyr-NHMe	0.49	0.51	0.84	0.16
Ac-L-Ala-2,4-MePro-NHMe	0.15	0.85	0.15	0.85
Ac-L-Tyr-2,4-MePro-NHMe	0.73	0.27	0.27	0.73

[a] The probabilities are obtained as sums over all conformations with n and p ring deformation, respectively. [b] The probabilities are obtained as sums over all conformations with negative and positive values of ψ, respectively.

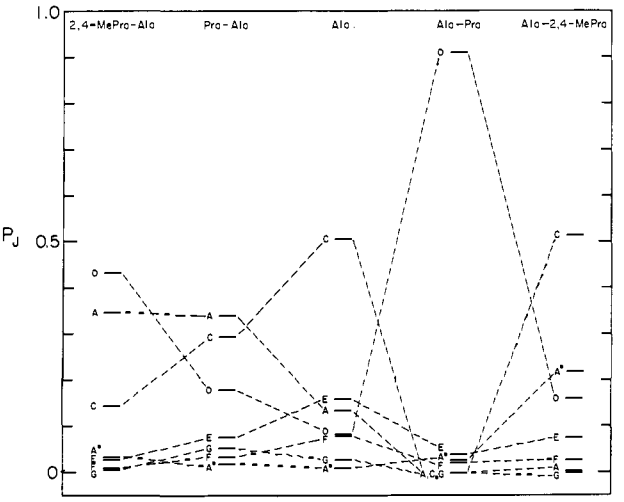

Figure 4. Comparison of the Boltzmann probabilities, P_J, for various conformations J of the Ala residue in (a) Ac-2,4-MePro-Ala-NHMe, (b) Ac-Pro-Ala-NHMe, (c) Ac-Ala-NHMe, (d) Ac-Ala-Pro-NHMe, and (e) Ac-Ala-2,4-MePro-NHMe. The conformations J = A, C, D, E, F, G, A* (ref 10) are indicated in the figure. Dashed lines are used only for ease of reading in comparing P_J values of various columns.

tistical probabilities is $P_- = 0.88$.

The effect of the 2,4-MePro residue on the conformations of the Ala residue following it is comparable to the corresponding effect of a Pro residue in the Ac-Pro-Ala-NHMe dipeptide.[40] The Boltzmann probabilities for various conformational states of the Ala residue in the terminally blocked single residue, Ac-Ala-$NHCH_3$, and in the two dipeptides are compared in the left-hand half of Figure 4. The effects of Pro and of 2,4-MePro on the conformational preferences of Ala are generally similar, but the presence of 2,4-MePro results in an enhanced stabilization or destabilization of some Ala conformational states, as compared with the presence of Pro. Thus, the C conformation, strongly preferred for the blocked single Ala residue, is destabilized, while the D and A conformations are stabilized, etc.

3.2.2. Ac-Ala-2,4-MePro-NHMe. The computed minimum-energy conformations are listed in Table V. The trans–cis energy difference is 8.79 kcal/mol; i.e., it is even higher than in the blocked single residue or in the dipeptide discussed above. There is a strong preference for the p ring conformation (its Boltzmann probability is $P_p = 0.85$, as shown in Table IV) and for positive values of ψ_2 ($P_+ = 0.85$). Thus, the interactions of the 2,4-MePro residue with the preceding residue exert a stronger constraining effect on the conformation of the bicyclic ring than the interactions with the residue following.

Comparison of the Boltzmann probabilities of the Ala residue in this dipeptide with those in Ac-Ala-NHMe and Ac-Ala-Pro-NHMe, shown in the right-hand half of Figure 4, indicates that the 2,4-MePro residue exerts less constraint on the preceding residue than does Pro. In the case of the Ac-Ala-Pro-NHMe dipeptide, the A, C, and G conformations of the Ala residue are very strongly destabilized, while the D conformation becomes most stable. Such changes, relative to the blocked single Ala residue, are much smaller in the Ac-Ala-2,4-MePro-NHMe dipeptide. The C conformation (with a high value of $\psi_1 = 127°$, near the boundary of the C and F conformational regions) remains strongly preferred. There is some destabilization of the A and E conformations and some stabilization of the A* conformation.

(40) Gibson, K. D.; Chin, S.; Némethy, G.; Clementi, E.; Scheraga, H. A., manuscript in preparation.

Table V. Minimum-Energy Conformations of Ac-Ala-2,4-MePro-NHMe[a]

conformational letter code[b]	ΔE[c] (kcal/mol)	dihedral angles (deg)[d] ϕ_1	ψ_1	ω_1	ψ_2
Ctp^+	0.00	−60	127	−164	43
A^*tp^+	0.52	51	49	−169	43
Dtn^-	1.36	−149	88	174	−50
Dtp^+	1.51	−151	55	−173	50
Dtp^-	1.56	−151	55	−173	−94
Dtn^+	1.66	−149	88	173	94
Etn^-	1.74	−156	166	170	−49
Etn^+	1.92	−155	166	170	94
Etp^+	1.99	−152	134	−172	49
Ftn^+	2.26	−78	163	171	93
Etp^-	2.34	−153	134	−172	−94
Ftn^-	2.39	−79	163	171	−49
Ftp^-	2.46	−75	131	−171	−92
A^*tp^-	3.06	53	53	−171	−90
Atn^-	3.30	−64	−31	158	−45
A^*tn^+	3.98	54	80	174	92
A^*tn^-	4.25	54	80	174	−49

[a] All minimum-energy conformations with $\Delta E < 5.0$ kcal/mol are listed. The experimental geometry was used for the 2,4-MePro. [b] The capital letter indicates the conformation of the Ala residue.[10] The first lower-case letter denotes the conformational state of the peptide bond preceding the 2,4-MePro residue. The second lower-case letter and its superscript refer to the conformation of the 2,4-MePro residue (see footnote a of Table II). [c] For each minimum, $\Delta E = E - E_0$, where $E_0 = -18.24$ kcal/mol, the computed energy of the lowest energy minimum. [d] The dihedral angles θ_0, θ_2, and χ_1^1, corresponding to the rotation of methyl groups, and ω_2 are not listed. θ_0 and θ_2 are 180 ± 1°, $\chi_1^1 = 180 \pm 9°$, and $\omega_2 = 180 \pm 1°$.

3.3. Dipeptides of Tyr and 2,4-MePro. These dipeptides indicate the constraining effect of the 2,4-MePro ring on the large neighboring tyrosine side chain.[21,22]

3.3.1. Ac-2,4-MePro-Tyr-NHMe. The computed minimum-energy conformations are listed in Table VI. The distribution of backbone conformations is similar to that for Ac-2,4-MePro-Ala-NHMe (Table IV). The trans–cis energy difference is 7.06 kcal/mol, i.e., about the same as in the corresponding Ala dipeptide. The computed distribution of Tyr side-chain conformations is strongly affected in the dipeptide. While the three rotameric states g^-, t, and g^+ for the C^{α}–C^{β} bond (χ_2^1) are nearly uniformly populated in Ac-Tyr-NHMe,[11] the g^- conformation is strongly preferred in both Ac-Pro-Tyr-NHMe and Ac-2,4-MePro-Tyr-NHMe, with $P_g = 0.82$ for the latter (Table VII).

3.3.2. Ac-Tyr-2,4-MePro-NHMe. The computed minimum-energy conformations are listed in Table VIII. The backbone conformations differ considerably from those in Ac-Ala-2,4-MePro-NHMe. The nearly extended E and F, as well as the D conformations are strongly favored over the others in the Tyr-containing dipeptide. The n ring geometry is strongly preferred ($P_n = 0.73$), in contrast to Ac-Ala-2,4-MePro-NHMe (Table IV). On the other hand, the preference for P_+ is similar in the Ala-2,4-MePro and Tyr-2,4-MePro dipeptides. The trans–cis energy difference is 7.75 kcal/mol; i.e., it is somewhat less than in the corresponding Ala dipeptide, but it is higher than in Ac-2,4-MePro-NHMe.

The distribution of Tyr side-chain conformations is affected in this dipeptide as well, but in a manner different from the dipeptide discussed above. The t rotamer is preferred for the C^{α}–C^{β} bond, with $P_t = 0.64$ (in contrast to Ac-Tyr-Pro-NHMe

Table VI. Minimum-Energy Conformations of Ac-2,4-MePro-Tyr-NHMe[a]

conformational letter code[b]	ΔE[c] (kcal/mol)	dihedral angles (deg)[d]						
		ω_0	ψ_1	ω_1	ϕ_2	ψ_2	χ_2^1	χ_2^2
tn⁻Dg⁻	0.00	176	−44	180	−140	38	−56	−78
tn⁻Dg⁻	0.14	176	−44	180	−140	38	−56	102
tp⁻Dg⁻	0.14	−176	−89	−179	−142	34	−55	−80
tp⁻Dg⁻	0.28	−175	−89	−179	−142	34	−55	100
tp⁺Cg⁻	1.04	−177	52	−177	−83	73	−59	−68
tp⁺Cg⁻	1.16	−178	52	−177	−83	73	−59	112
tp⁺Ag⁻	1.34	−177	52	179	−83	−24	−57	−67
tp⁻Ag⁻	1.47	−173	−88	−170	−83	−25	−58	−71
tp⁺Fg⁻	1.47	−178	54	−179	−85	152	−57	−68
tp⁻At	1.48	−173	−86	−174	−69	−34	180	79
tp⁻At	1.48	−173	−86	−174	−69	−34	180	−99
tn⁻Ag⁻	1.50	176	−48	−169	−89	−15	−57	−73
tp⁺Ag⁻	1.51	−178	52	−179	−83	−24	−58	112
tp⁻Ag⁻	1.54	−173	−87	−170	−82	−26	−58	109
tp⁺Fg⁻	1.55	−178	54	−179	−85	152	−58	112
tn⁻Ag⁻	1.59	175	−48	−169	−87	−17	−57	108
tp⁻Dg⁺	1.60	−175	−88	178	−146	28	53	93
tn⁻At	1.66	175	−45	−174	−68	−33	180	79
tn⁻At	1.66	175	−45	−173	−68	−33	180	−99
tn⁻Dg⁺	1.67	177	−42	180	−143	29	53	93
tp⁻Dg⁺	1.69	−175	−87	178	−146	28	52	−87
tn⁻Ag⁺	1.73	173	−42	−173	−64	−27	68	84
tn⁻Ag⁺	1.76	173	−42	−173	−64	−27	68	−96
tn⁻Dg⁺	1.76	176	−42	179	−143	29	52	−87
tn⁻Eg⁺	1.86	175	−47	178	−156	160	57	88
tn⁻Eg⁺	1.89	175	−47	178	−156	161	58	−92
tn⁻Eg⁻	1.97	175	−48	179	−146	154	−60	−78
tn⁻Eg⁻	2.07	175	−48	179	−146	154	−60	102
tn⁺Cg⁻	2.12	176	94	−176	−84	72	−59	−71
tn⁺Cg⁻	2.19	176	94	−177	−84	73	−60	109
tp⁻Ag⁺	2.19	−172	−82	−173	−67	−26	69	−96
tp⁻Ag⁺	2.20	−172	−82	−173	−68	−25	69	84
tp⁻Eg⁺	2.30	−176	−96	180	−159	160	56	88
tp⁻Eg⁺	2.34	−176	−96	180	−159	160	56	−91
tn⁺Ag⁻	2.39	176	93	−179	−84	−24	−58	−70
tp⁺Et	2.40	−175	50	180	−156	153	180	78
tp⁺Et	2.42	−175	50	180	−156	153	179	−103
tp⁻Eg⁻	2.45	−176	−96	−178	−147	152	−59	−79
tp⁻Ag⁻	2.51	176	93	−179	−84	−24	−58	110
tp⁻Eg⁻	2.55	−176	−96	−178	−147	−152	−60	100
tn⁻Cg⁻	2.57	176	−53	−177	−90	65	−58	−74
tn⁻Et	2.59	176	−48	178	−155	151	180	78
tn⁻Cg⁻	2.62	176	−53	−178	−89	66	−58	106
tn⁻Et	2.62	176	−48	178	−155	151	179	−102
tn⁺Et	2.80	176	96	180	155	153	180	78
tn⁺Et	2.81	176	96	180	155	153	180	−103
tp⁺A*g⁻	2.86	−176	45	178	50	41	−48	112
tp⁺A*g⁻	2.87	−176	45	178	50	42	−48	−69
tp⁺Eg⁺	2.88	−176	72	178	−146	163	73	−86
tp⁺Eg⁺	2.93	−175	74	178	−147	165	72	85

[a] All minimum-energy conformations with $\Delta E < 3.0$ kcal/mol are listed. The experimental geometry was used for the 2,4-MePro. [b] The first lower-case letter denotes the conformational state of the peptide bond preceding the 2,4-MePro residue. The second lower-case letter and its superscript refer to the 2,4-MePro residue (see footnote *a* of Table II). The capital letter followed by a lower-case letter (referring to χ^1) indicates the conformation of the Tyr residue, according to the letter code by Zimmerman et al.[10] [c] For each minimum, $\Delta E = E - E_0$, where $E_0 = -23.20$ kcal/mol, the computed energy of the lowest energy minimum. [d] The dihedral angles θ_0 and θ_3, corresponding to the rotation of methyl groups in the end groups, χ_2^6 and ω_2 are not listed. θ_0 is always near −165° or 165° for n and p conformations, respectively. $\theta_3 = 180 \pm 1°$, $\omega_2 = 180 \pm 3°$, and $\chi_2^6 = 0 \pm 3°$.

Table VII. Computed Frequency of Occurrence of Side-Chain Rotamers around the C^α–C^β Bond in Tyrosyl Residues

compound	g⁻	t	g⁺	ref
Ac-Tyr-NHMe	0.31	0.33	0.36	11
Ac-Pro-Tyr-NHMe	0.79	0.04	0.17	39
Ac-2,4-MePro-Tyr-NHMe	0.82	0.08	0.10	this work
Ac-Tyr-Pro-NHMe	0.48	0.32	0.20	39
Ac-Tyr-2,4-MePro-NHMe	0.12	0.64	0.24	this work

in which g⁻ is preferred, with $P_{g^-} = 0.48$, Table VII).

As discussed above, there are large differences in the backbone conformational preferences of Tyr in this dipeptide and those of the Ala residue in Ac-Ala-2,4-MePro-NHMe and of the Tyr side chain in the other Tyr peptides. Similarly, the preferred direction of deformation of the ring is changed drastically in this dipeptide, as compared with Ac-Ala-2,4-MePro-NHMe. These changes suggest that there is a strong favorable interaction between the Tyr side chain and the bicyclic ring in Ac-Tyr-2,4-MePro-NHMe. This conclusion is consistent with solution NMR data which indicate that the Tyr side chain is near one face of the 2,4-MePro ring, in the ensemble average.[21]

The average value of ψ_{Tyr} can be determined from Table VIII by computing a Boltzmann-weighted average[11] over all conformations, resulting in a value of $\langle\psi\rangle = 134°$. This result agrees with the conclusions reached from ensemble-averaged measurements[21] of the nuclear Overhauser effect that indicate that the preferred Tyr conformation lies in the range $70° < \psi < 170°$.

4. Conclusions

Steric crowding of the 2,4-MePro residue causes strong geometric distortions of the bridged ring system, because of repulsive

Table VIII. Minimum-Energy Conformations of Ac-Tyr-2,4-MePro-NHMe[a]

conformational letter code[b]	ΔE[c] (kcal/mol)	dihedral angles (deg)[d]					
		ϕ_1	ψ_1	ω_1	χ_1^1	χ_1^2	ψ_2
Ettn$^+$	0.00	−155	152	167	176	−99	91
Eg$^+$tn$^+$	0.12	−157	170	169	67	94	91
Ettn$^+$	0.18	−155	152	167	177	80	92
Eg$^+$tn$^+$	0.23	−157	170	169	66	−86	91
Dttn$^-$	0.23	−151	98	171	174	−116	−50
Dttn$^-$	0.26	−151	98	171	175	65	−50
Fttp$^+$	0.30	−57	122	−164	180	−94	44
Fttp$^+$	0.38	−57	122	−164	−179	86	44
Fttn$^+$	0.66	−74	151	167	179	−100	91
Fttn$^+$	0.85	−75	151	167	179	80	92
Eg$^+$tn$^-$	1.39	−158	168	178	70	−78	−73
Eg$^-$tn$^-$	1.50	−147	169	171	−63	−77	−49
A*g$^-$tp$^+$	1.51	46	48	−170	−52	105	44
Eg$^-$tn$^-$	1.54	−147	169	171	−64	102	−49
A*g$^-$tp$^+$	1.54	46	48	−170	−53	−75	44
Eg$^+$tn$^-$	1.57	−157	168	178	70	102	−73
Dg$^-$tn$^-$	1.59	−144	89	174	−64	−78	−50
Dg$^-$tn$^-$	1.62	−144	89	174	−64	102	−50
Eg$^-$tp$^+$	1.65	−146	138	−173	−65	−77	50
Eg$^-$tp$^+$	1.67	−146	138	−173	−65	102	50
Eg$^-$tn$^+$	1.71	−147	169	171	−63	−77	94
Eg$^-$tn$^+$	1.75	−147	169	171	−64	102	94
Dg$^-$tp$^+$	1.80	−144	55	−173	−59	−78	50
Dttn$^+$	1.81	−151	101	169	175	69	93
Fttn$^-$	1.81	−78	139	171	180	62	−62
Eg$^+$tp$^+$	1.82	−155	140	−174	60	93	50
Fttn$^-$	1.87	−78	139	171	180	−119	−61
Eg$^+$tp$^+$	1.88	−155	140	−173	61	−86	50
Dg$^-$tp$^+$	1.89	−144	55	−173	−60	102	50
Dg$^-$tp$^-$	1.89	−144	54	−173	−59	−78	−94
Dg$^-$tn$^+$	1.90	−144	90	174	−64	−78	94
Dg$^-$tp$^-$	1.95	−144	54	−173	−60	102	−94
Ettp$^+$	1.96	−152	124	−172	−178	−92	49
Dg$^-$tn$^+$	1.96	−144	90	174	−64	102	94
Ettp$^+$	1.99	−152	124	−172	−178	88	49
Eg$^-$tp$^-$	2.03	−146	138	−173	−65	−77	−94
Eg$^+$tp$^-$	2.04	−156	140	−174	61	−86	−93
Eg$^-$tp$^-$	2.06	−147	138	−173	−66	102	−94
Eg$^+$tp$^-$	2.09	−156	140	−174	61	93	−93
Dg$^+$tp$^+$	2.18	−154	40	−167	60	102	48
Ettp$^-$	2.20	−153	124	−172	−178	88	−93
Dg$^+$tp$^+$	2.22	−154	40	−167	59	−79	48
Ettp$^-$	2.25	−153	124	−172	−178	−91	−93
A*ttp$^+$	2.38	46	49	−171	−168	−116	44
A*ttp$^+$	2.50	46	49	−170	−168	64	44
Dg$^+$tp$^+$	2.57	−157	42	−165	65	104	52
Cttp$^-$	2.74	−78	123	−171	−177	88	−92
Cttp$^-$	2.76	−78	123	−171	−177	−93	−92
Dttp$^+$	2.94	−151	56	−174	−173	−118	50
Dttp$^-$	2.96	−152	56	−174	−173	−118	−94
Dttp$^-$	2.97	−151	56	−174	−173	62	−93
Dttp$^+$	2.98	−151	56	−174	−172	62	50

[a] All minimum-energy conformations with $\Delta E < 3.0$ kcal/mol are listed. The experimental geometry was used for 2,4-MePro. [b] The first capital letter, followed by a lower-case letter (referring to χ^1), indicate the conformation of the Tyr residue according to the letter code of Zimmerman et al[10]. The third letter denotes the conformational state of the peptide bond preceding the 2,4-MePro residue. The fourth letter and its superscript refer to the 2,4-MePro residue (see footnote a of Table II). [c] For each minimum, $\Delta E = E - E_0$, where $E_0 = -22.07$ kcal/mol, the computed energy of the lowest energy minimum. [d] The dihedral angles θ_0 and θ_3, corresponding to the rotation of methyl groups in the end groups, ω_0, ω_2, and χ_2^6 are not listed. θ_0 and θ_3 are 180 ± 1°, ω_0 and ω_2 are 180 ± 2, and $\chi_1^6 = 0 \pm 3°$.

nonbonded interactions with neighboring residues in an oligopeptide and/or with the Ac– or –NHMe terminal blocking groups. As a result, an asymmetric geometry is preferred over the symmetric one. The Ac-2,4-MePro-NHMe molecule exists, therefore, in two enantiomeric forms with equal energy. The peptide group following the 2,4-MePro residue can take two nearly opposite orientations, with ψ near −50 or 90° (and, for the other enantiomeric form, 50 or −90°). This constraint on ψ is stronger than on the corresponding dihedral angle in Pro peptides. In X-2,4-MePro and 2,4-MePro-X dipeptides, where X is a chiral amino acid, the two enantiomeric forms of the residue have different energies.

As a result of unfavorable steric interactions involving the two peptide groups and their substituents, the cis–trans equilibrium for the peptide bond preceding the 2,4-MePro residue is shifted strongly toward the trans form. The energy difference between the two forms, ranging from 5.9 to 8.9 kcal/mol for the peptides considered here, is lower than that for nonprolyl peptides (where it is >10 kcal/mol except for glycyl peptides[36,37]), but it is considerably higher than the 2.0-kcal/mol value computed for the prolyl residue[11] (Table IX). Therefore, 2,4-MePro can serve as an analogue of Pro in which the conformation of the peptide bond is restricted to the trans form, with the exclusion of the cis form. A similar conclusion has been reached from solution NMR studies.[21]

On the other hand, several differences exist between the Pro and the 2,4-MePro residues in terms of details of their conformations. The C′–N–C$^\alpha$–C′ dihedral angle ϕ is constrained in both residues, with $\phi = -29°$ in 2,4-MePro and $\phi = -68$ to −75° in Pro.[41,42] There is a difference of about 40° between the two values

Table IX. Computed Energy Difference for the Trans–Cis Equilibrium of the Peptide Bond Preceding Various Amino Acid Residues

peptide bond	$\Delta E_{t\rightarrow c}$	ref
Ac-Gly-NHMe	7.6	36
Ac-Ala-NHMe	11.7	36
Ac-Pro-NHMe[a]	2.0	11
Ac-Ala-Pro-NHMe[a]	3.0	39
Ac-Tyr-Pro-NHMe[a]	2.4	39
Ac-2,4-MePro-NHMe[b]	5.9	this work
Ac-2,4-MePro-Ala-NHMe[b]	7.3	this work
Ac-2,4-MePro-Tyr-NHMe[b]	7.1	this work
Ac-Ala-2,4-MePro-NHMe[b]	8.8	this work
Ac-Tyr-2,4-MePro-NHMe[b]	7.8	this work

[a] Peptide group preceding the Pro residue. [b] Peptide group preceding the 2,4-MePro residue.

Table X. Computed Bend Probabilities[a] in Dipeptides of Pro and 2,4-MePro

peptide	P_{bend}
Ac-Ala-Pro-NHMe	0.01[b]
Ac-Ala-2,4-MePro-NHMe	0.75
Ac-Pro-Ala-NHMe	0.61[b]
Ac-2,4-MePro-Ala-NHMe	0.87

[a] A bend is defined[44,45] as a conformation in which the distance between the methyl carbon atoms of the Ac– and –NHMe end groups is $R \leq 7$ Å. The bend probability P_{bend} is computed using eq 1, summing over all conformations that satisfy this inequality. [b] The numerical value of this quantity differs from that reported earlier[45] because it was computed using the updated version[28] ECEPP/2, while the earlier version[29] was used in ref 45.

of ϕ. Furthermore, the preferred values of ψ for 2,4-MePro ($\psi \approx -50$ and 90°) differ significantly from those preferred for Pro ($\psi \approx -19$, 75, and 160°).[11]

The presence of the bulky bridged pyrrolidine ring in 2,4-MePro also influences the conformational behavior of the preceding and following residues in a manner that differs from the influence of the Pro ring on neighboring residues. The relative preferences for various backbone conformations change. In general, the more tightly folded conformations (such as A and A*) become more favorable in both positions, before and after the 2,4-MePro residue. Most notably, the presence of an A conformation for the residue preceding 2,4-MePro is not quite as unfavorable as in the case of Pro. In spite of this, 2,4-MePro has a disruptive influence on an α-helix, like Pro,[43] because of its bulky side chain and its inability to contribute an amide proton for hydrogen bonding; also its preferred (ϕ, ψ) dihedral angles differ considerably from those of α-helical conformations.

The differences between the conformations of the Pro and 2,4-MePro residues and between their effects on the conformational preferences of neighboring residues are seen clearly in the bend probabilities[44,45] of their respective dipeptides (Table X). The probability of bend formation is strongly enhanced by the substitution of 2,4-MePro for Pro. This substitution favors compact conformations of the dipeptide, relative to more extended ones. As a consequence, the incorporation of 2,4-MePro in the place of Pro in a polypeptide results in local changes of the conformation of the polypeptide backbone.

In conclusion, the 2,4-MePro residue can serve as a model analogue for Pro in which the peptide bond preceding the residue is constrained to the trans form and the residue exhibits limited flexibility, but it does not reproduce exactly *all* of the conformational properties of the Pro residue. It is a useful substituent whenever bulkiness and rigidity are required at a particular position in a polypeptide, together with a preference for a trans peptide bond, without the requirement of analogy of the backbone conformation with that of other residues.

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(41) The small variation of ϕ in Pro arises from the possibility of pseudorotation which can alter the puckering of the ring.[29,42] No such flexibility of the ring exists in 2,4-MePro because of the covalent bridge within the bicyclic ring.

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