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Conformational Analysis of Benzyloxycarbonylglycyl-L-proline

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The conformational preferences of the protected dipeptide derivative benzyloxycarbonylglycyl-L-proline (L-ZGP), in neutral and anionic forms, have been investigated by molecular mechanics calculations using the MMX force field. A sequential build-up procedure of L-ZGP from *N*-acetylproline was used to span efficiently the conformational space. This procedure resulted in the identification of 243 conformations of neutral L-ZGP and 41 conformations of the anionic form with relative steric energies below 3 kcal mol^{-1}. The anionic form of L-ZGP predominantly adopts conformations with a *cis*-arrangement around the glycylproline amide bond. Such conformations have ψ_{gly} and $\phi_{gly} \approx -60°$. According to the calculations, the *cis*- and the *trans*-carbamates are equally favoured. The neutral form of L-ZGP shows a high structural flexibility with a strong preference for a *trans*-glycylproline amide bond. MMX calculations show that the *cis*-oriented carbamates are more stable than the *trans*-carbamates. ^1H and ^{13}C NMR spectroscopic data support the results from the calculations.

The commercially available dipeptide derivative benzyloxycarbonylglycyl-L-proline (L-ZGP) acts as a chiral selector in liquid ion-pair chromatography of various racemic amino alcohols when added to the organic mobile phase.[1] The enantiomers of several pharmacologically interesting compounds have been separated by this method.[1–3] The factors determining the degree of chromatographic resolution have not previously been identified. However, computational studies of potentially important interactions between L-ZGP and the chiral amino alcohols have now helped in identifying critical factors for enantiomer separation.[4]

In this investigation, which forms the basis of the aforementioned study, we have explored the conformational space of neutral and ionized L-ZGP by means of unrestricted MMX calculations. The results are compared with NMR spectral data obtained under conditions similar to those during the chromatographic separations and with data from the literature. MMX calculations as well as NMR spectroscopic experiments demonstrate that ionized L-ZGP prefers to adopt conformations with a *cis*-arrangement around the glycylproline amide bond, whereas the neutral form shows a preference for a *trans*-arrangement. This difference is due to electrostatic repulsions between the carboxylate and the amide oxygen which appear in *trans*- but not in *cis*-conformations of deprotonated L-ZGP. According to the calculations, conformations with a *cis*-oriented carbamate group show unexpectedly high stability both in the carboxylic acid and in the carboxylate.

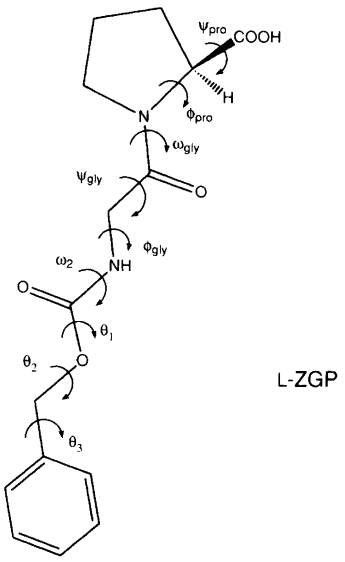
Methods

General comments. The structural modelling of L-ZGP was done with the PCMODEL program (version PI 3.1).[5] Energy-minimized geometries were obtained by use of the MMX force field without restrictions in the minimization process. MMX uses the block diagonal Newton–Raphson minimization method and the convergence criterion is 10^{-5} eV. Point charges are used to calculate the electrostatic interactions. The H-BND command was activated during each minimization in order to take into account potential hydrogen bonds. Calculations were done on an IBM PS/2 A21 computer equipped with a math-coprocessor.

Commercial samples of L-ZGP and *N*-acetyl-DL-proline were used in the NMR spectroscopic studies.

^{13}C NMR spectroscopy was performed on a JEOL FX90Q spectrometer, whereas ^1H NMR spectra were recorded on a Varian VXR 300 or a Varian VXR 400 instrument. *N*-Acetylproline and L-ZGP were dissolved in $CDCl_3$ and tetramethylsilane was added as an internal reference. Triethylamine (1 equiv.) was added to the sample solutions. L-ZGP was also dissolved in CH_3OH containing about 1 % of D_2O (the methanol resonance at $\delta = 49.3$ was used as the reference).

Definition of conformational descriptors of L-*ZGP.* The backbone of a peptide chain is normally described by two dihedral angles per amino acid residue (ϕ and ψ) in addition to ω which describes the geometry of the amide bond (Fig. 1). To describe the geometry of the peptide backbone of L-ZGP, four parameters are needed: ψ_{pro} [N–C^{α}–C′–O(H)] defines the orientation of the carboxylic acid group, ω_{gly} defines the glycylproline amide bond arrangement, and ψ_{gly} and ϕ_{gly} define the orientation of the glycyl moiety. In proline, ϕ_{pro} is restricted to angles of $-60° \pm 20°$ dependent on the pyrrolidine ring puckering. Therefore, it is more relevant to define the



L-ZGP

Fig. 1. Structure and conformational descriptors of benzyloxycarbonylglycyl-L-proline (L-ZGP).

geometry of the proline moiety by substituting ϕ_{pro} with parameters that describe the ring puckering in pyrrolidine. The ring puckering can be described by five different dihedral angles but it is more convenient to use the abbreviated nomenclature introduced by Chacko *et al.*[6] which describes the pyrrolidine ring using the concept of pseudorotation. The carbamate functionality is planar and adopts only two conformations, the *trans/trans* ($\omega_2 = 180°$, $\theta_1 = 180°$) and the *cis/trans* ($\omega_2 = 0°$, $\theta_1 = 180°$) conformations.[7] Two additional parameters (θ_2 and θ_3; Fig. 1) are required to describe fully the geometry of the benzyloxycarbonyl moiety.

Results and discussion

MMX calculations. The molecular-mechanics calculations on L-ZGP were performed on the carboxylate ion and on the carboxylic acid. To span the conformational space of L-ZGP we used a strategy involving a stepwise build-up, starting from *N*-acetylproline. In the starting geometries of *N*-acetylproline, the following conformational parameters were varied: the pyrrolidine ring puckering (ten envelope conformations were employed), the amide bond geometry (two different amide bond rotamers, $\omega_{gly} = 0°$ and 180°, were used) and the orientation of the carboxylic acid (carboxylate ion) functionality ($\psi_{pro} = 0°$ and 180° were used). Thus, in all, 40 starting geometries were constructed. Fully relaxed energy minimizations afforded eight conformations of the neutral form of *N*-acetylproline and four of the carboxylate ion with relative steric energies below 3 kcal mol^{-1}.

Only two pyrrolidine ring puckerings were observed in *N*-acetylproline, the twist conformers ${}_{\gamma}T^{\beta}$ and ${}_{\beta}T^{\gamma}$ having phase angles of pseudorotation (*P*) of about 25° and 160°, respectively. The same result has been obtained from molecular-mechanics calculations on proline.[8] In the solid state, the pyrrolidine ring of L-ZGP adopts the twist conformation ${}_{\beta}T^{\gamma}$,[9] and it has been shown, by NMR spectroscopy, that the pyrrolidine ring of glycylproline (in D_2O) interconverts rapidly between two half-chair formers puckered at C_β and C_γ,[10] thus corroborating the MMX calculations.

The calculations produced two stable amide bond rotamers having $\omega = 0°$ ($\pm 10°$) and $\omega = 180°$ ($\pm 10°$) (the *trans/cis* interconversion in acylated proline derivatives has been studied extensively[11–19]) and the carboxylic acid functionality adopts two orientations $\psi_{pro} = -30°$ ($\pm 15°$) and 135° ($\pm 15°$).

A Boltzmann distribution of identified conformations of *N*-acetylproline (at 25°C) indicates that the neutral carboxylic acid predominantly adopts *trans*-amide conformations, whereas the carboxylate ion exhibits a pronounced preference for a *cis*-arrangement around the amide bond. This observation may be rationalized in terms of unfavourable electrostatic interactions between the amide carbonyl group and the carboxylate ion in the *trans*-conformation.[16] We observed an excellent correlation ($r^2 = 0.98$) between the electrostatic energy component (E_{qq}) and the total steric MMX energy of the *N*-acetylproline conformations. Thus, the electrostatic interactions appear strongly to influence the relative steric energies of these conformations. These results are supported by results from NMR experiments (see below).

The acetyl hydrogens of the eight low-energy *N*-acetylproline conformers were replaced with amino groups thus forming 72 starting geometries of glycyl-L-proline having ϕ_{gly} (lone pair–N–C^{α}_{gly}–C'_{gly}) = +60°, 180° and −60° which were energy minimized.

As the next stage in the stepwise construction of L-ZGP, we added a *trans*-acetyl group to the nitrogen atom of all conformers of glycyl-L-proline. The *cis*-amide starting geometries were generated by amide bond rotation of the energy minimized *trans*-conformations to $\omega_2 = 0°$. It is noteworthy that the MMX calculations predicted an unexpectedly high stability for the *cis*-acetyl conformations.

To construct the carbamate functionality of L-ZGP, we replaced the methyl group of the acetamide function in the energy-minimized *N*-acetylglycylproline conformers with a methoxy group so that $\theta_1 = 180°$ (according to Benedetti *et al.*,[7] only such *trans*-conformations are stable). The resulting starting geometries were energy minimized and finally, to complete the structure of L-ZGP, a phenyl group was added at each methoxy-hydrogen position in each of the conformations of methoxycarbonylglycyl-L-proline ($\theta_3 = 90°$).

Starting from the eight energy-minimized conformers of *N*-acetylproline, a total of 432 staggered conformations of L-ZGP are plausible [$8(N\text{-acetylproline}) \times 3(\psi_{gly}) \times 3(\phi_{gly}) \times 2(\omega_2) \times 1(\theta_1) \times 3(\theta_2) \times 1(\theta_3) = 432$]. However, during each step in the build-up procedure, pairs of starting geometries converge into identical local minima, thus decreasing the total number of conformations of

L-ZGP. Therefore, a total of only 369 starting geometries of the neutral form and 318 of the carboxylate ion of L-ZGP were energy minimized. The MMX calculations produced 243 conformations of neutral L-ZGP and 41 conformations of the carboxylate ion with relative steric energies below 3 kcal mol^{-1}. These conformations were divided into 22 different groups defined by ω_{gly}, ψ_{gly}, ϕ_{gly} and ω_2 (Table 1). The parameters related to the orientation of the carboxylic acid functionality (ψ_{pro}) and the pyrrolidine ring puckering were not considered in the classification since these conformational changes do not influence the overall shape of L-ZGP. In addition, changes in ψ_{pro} and in ring puckering normally induced energetic effects smaller than 1 kcal mol^{-1}. Similarly, θ_2 and θ_3 were not used in the classification since, in general, the rotamers have similar steric energies.

A Boltzmann distribution (at 25°C) indicates that the 22 groups contain more than 97% of the conformations available for both the carboxylic acid and the carboxylate ion. Each group contains at least 1% of the total number of conformations (Fig. 2 and Table 1). The carboxylic acid predominantly adopts conformations belonging to groups 1–16 which contain conformers having a *trans*-glycylproline amide bond. However, the carboxylate ion populates only groups 17–22 which are characterized by a *cis*-amide bond arrangement.

Preferentially, ionic L-ZGP adopts conformations in groups 19 and 22, which represent more than 85% of the conformational distribution. In addition to a *cis*-amide bond, these conformations have ψ_{gly} and $\phi_{gly} \approx -60°$. The difference between the two groups is the *trans/cis*-arrangement of the carbamate group. The neutral L-ZGP, on the other hand, shows a high degree of structural flexibility in that several low-energy conformations are available. The conformer of lowest energy, which belongs to group 11, amounts to only 6% of the total conformational distribution. Surprisingly, the majority of low-energy conformers of the carboxylic acid shows a *cis*-oriented carbamate group with a *trans/cis* ratio of 1 : 3, whereas this ratio is 1 : 1 for the carboxylate ion.

The X-ray geometry of L-*ZGP.* The conformation of L-ZGP in the solid state has been determined by X-ray crystallography by Tanaka *et al.*[9] The X-ray structure belongs to group 5 (Table 1). When the X-ray conformation was energy minimized in the MMX force field, a conformer close to the X-ray conformation was obtained. This local minimum conformation had a relative steric energy of 2.2 kcal mol^{-1}. The main difference between the X-ray geometry and the MMX generated conformer is described by ϕ_{gly} and θ_2. A comparison between the two geometries is shown in Fig. 3. When all atoms were fitted the average distance between fitted atoms was 0.21 Å. In contrast, when only the atoms in the carbamate group were superimposed a much better fit was obtained in which the top halves of the molecules also appeared to fit well (Fig. 3). A comparison including the pyrrolidine ring, the amide and the carboxylic groups gave an average distance between fitted atoms of 0.09 Å.

L-ZGP crystallizes with the molecules hydrogen bonded to each other in an antiparallel arrangement.[9] This crystal structure has also been verified by solid-

Table 1. Parameters defining groups of conformations of L-ZGP found in the MMX-calculations. Mean values of dihedral angles are given with standard deviations in parentheses. Also included is the Boltzmann distribution calculated at 25°C for the carboxylic acid and the carboxylate ion of L-ZGP.

	Conformational descriptor				Boltzmann distribution (%)	
Group	ω_{gly}	ψ_{gly}	ϕ_{gly}	ω_2	Neutral ZGP	Deprotonated ZGP
1	179(3)	75(7)	−166(5)	−178(1)	2	
2	179(1)	119(2)	−61(5)	179(2)	1	
3	177(3)	−160(9)	76(6)	−176(3)	2	
4	179(2)	−179(6)	−178(3)	180(1)	10	
5	179(2)	168(7)	−81(4)	176(2)	2	
6	178(3)	−71(7)	161(8)	178(2)	4	
7	175(2)	−55(6)	−55(2)	175(4)	3	
8	178(3)	62(8)	60(4)	2(1)	6	
9	180(2)	73(7)	−172(3)	0(2)	2	
10	175(2)	130(2)	−67(1)	0(1)	9	
11	178(2)	−165(13)	70(3)	0(2)	18	
12	179(2)	−178(9)	−179(8)	0(1)	6	
13	179(3)	172(10)	−74(4)	−1(2)	4	
14	178(5)	−124(2)	74(7)	3(1)	9	
15	176(3)	−69(6)	171(4)	0(1)	3	
16	178(4)	−60(7)	−60(6)	−1(2)	10	
17	4(4)	82(6)	82(1)	177(1)		4
18	−1(4)	79(5)	170(5)	178(2)		6
19	5(4)	−52(3)	−71(4)	177(4)		37
20	6(4)	64(5)	58(3)	2(1)	1	1
21	−1(4)	159(5)	−70(3)	0(1)	1	
22	5(5)	−55(2)	−67(5)	−2(2)		50

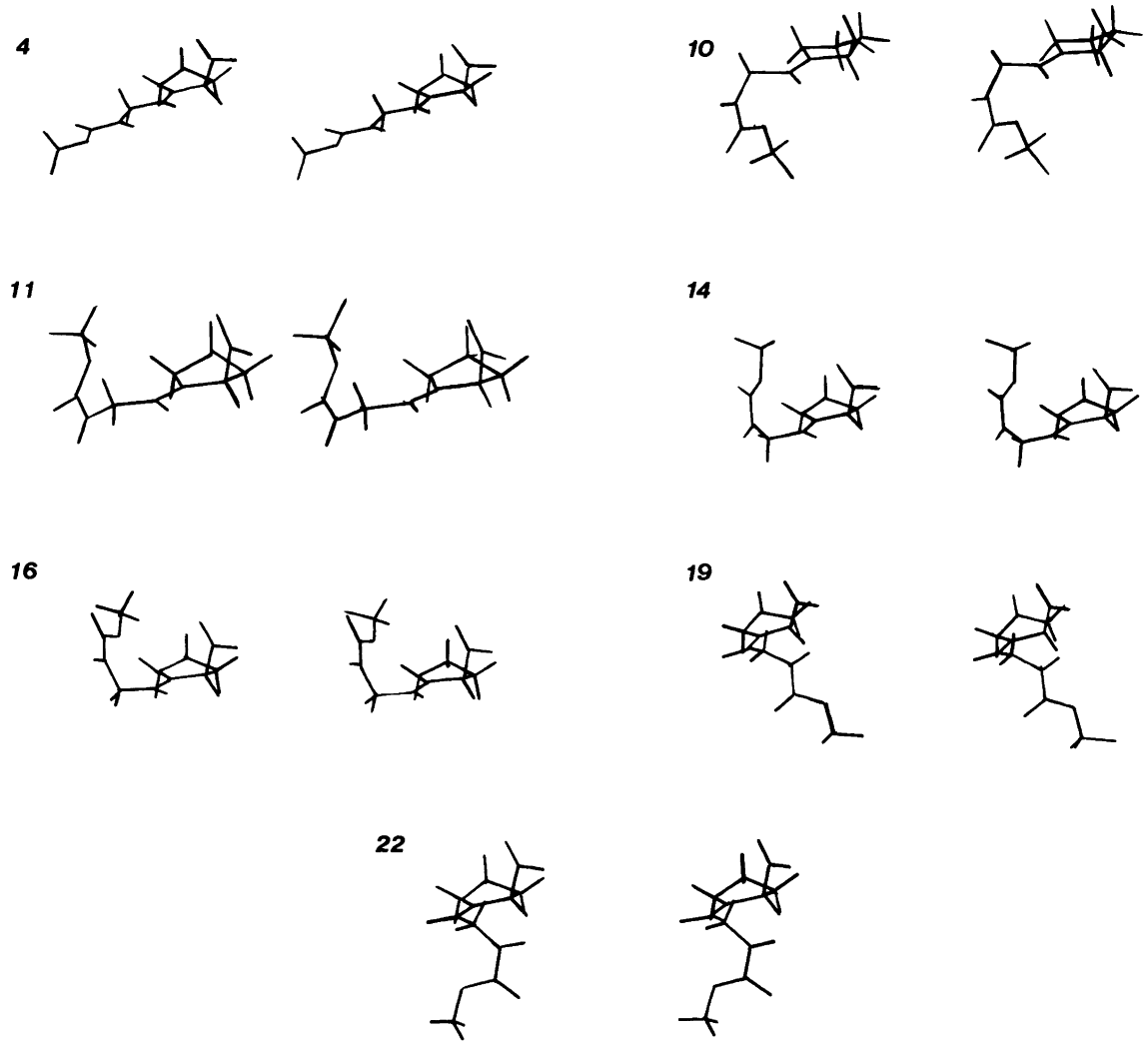


Fig. 2. Stereoscopic views of conformations from selected groups (see Table 1) representing the predominant conformations of L-ZGP found in the MMX calculations. Parameters defining the conformations included in each group are given in Table 1. For convenience in the stick models, the phenyl group in L-ZGP is omitted.

state MAS-NMR spectroscopy.[20,21] The intermolecular hydrogen bonds as well as crystal packing forces probably stabilize the observed conformation of L-ZGP in the crystalline state.

NMR spectroscopy. To check the reliability of the MMX calculations the conformational distribution of *N*-acetylproline and L-ZGP were studied by NMR spectroscopy.

^{13}C NMR spectroscopy provided information on the orientation around the glycylproline amide bond. The pyrrolidine resonances, especially those of the β- and γ-carbons, appeared as well separated signals for each rotamer.[12,22–26] Doubling of signals, which is probably due to *cis/trans* isomerism in the benzyloxycarbonyl group, was also observed in the ^{13}C NMR spectra.[12]

^{1}H NMR spectroscopy was also informative,[27] but potentially useful proton resonances were often partially obscured by complex multiplets due to the proline ring protons, preventing accurate measurements. However, it was possible to obtain an indication of the *trans/cis* ratio of the amide bond by comparing the H_α-signals. The NH_{gly} proton appeared as two resonances in spectra of L-ZGP, possibly reflecting the *trans/cis* isomerism around the benzyloxycarbonyl group.

Spectra were run with and without triethylamine (1 equiv.) present to allow studies of differences in the conformational distribution of *N*-acetylproline and L-ZGP in their ionized and protonated forms, respectively.

NMR spectroscopy of N-*acetylproline.*[8,22,23,28] ^{1}H NMR spectra of *N*-acetylproline in $CDCl_3$ show a strong predominance of the *trans*-amide isomer. The *trans/cis* ratio was estimated to be 9 : 1. However, in the presence of triethylamine, conformations with a *cis*-amide bond appear to be favoured, the *trans/cis* ratio being 2 : 3. The

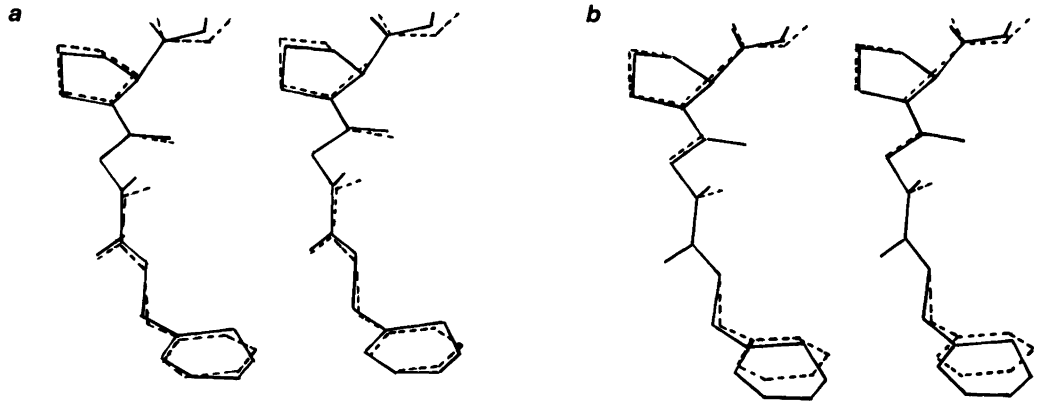


Fig. 3. Stereoscopic representation of a comparison between the conformation of L-ZGP in the solid state found by X-ray crystallography[9] (solid lines) and a similar conformation found by MMX minimization (dashed lines). The MMX generated conformation has a relative steric energy of 2.2 kcal mol^{-1}. (a) Fit of all atoms except the hydrogens located on the carboxylic acid and in the carbamate groups, respectively. The average distance between fitted atoms was 0.21 Å. (b) The atoms in the carbamate group were superimposed to give an average distance between fitted atoms of 0.04 Å.

^{13}C NMR spectral data show the same trend with *trans/cis* ratios of 4 : 1 and 2 : 3 for *N*-acetylproline in neutral and ionized forms, respectively. The discrepancy between ^{1}H and ^{13}C NMR data might arise from uncertainties in the signal area measurements in ^{13}C NMR spectra and/or differences in relaxation times of the nuclei in the *trans*- and *cis*-amide isomers. The data obtained in the MMX calculations of the acids show similarities with the NMR data. According to a Boltzmann distribution at 25°C, the *trans/cis* ratio of the neutral form is 9 : 1. However, calculations on the carboxylate ion predict that almost all the conformations present at 25°C had a *cis*-amide bond. In contrast, the NMR spectroscopic data show only a slight preference for conformations having a *cis*-amide versus a *trans*-amide bond.

In more polar solvents like CD_3OD and DMSO-d_6 the preference for the *trans*-amide bond was less pronounced, showing a *trans/cis* amide ratio of 5 : 2 in both solvents. After addition of one equivalent of triethylamine the ratio decreased to 3 : 2 in CD_3OD and to 1 : 1 in DMSO-d_6.

NMR spectroscopy of L-*ZGP.* ^{13}C NMR spectra of L-ZGP in $CDCl_3$ show the same trend as that observed for the other compounds. The *cis*-glycylproline amide conformers become stabilized when the amount of triethylamine is increased, i.e., when the amount of neutral dipeptide decreases. The *trans/cis* ratio changes from 4 : 1 to 2 : 3 upon addition of one equivalent of triethylamine (Fig. 4). Further addition of amine does not influence the ratio. However, when methanol is used as the solvent, the *trans/cis* ratio becomes 1 : 1 when one equivalent of triethylamine is added. This is not surprising since a polar, protic solvent, such as methanol, is likely to neutralize unfavourable electrostatic interactions and will therefore tend to stabilize the *trans*-amide conformers. As expected, addition of HCl increases the preference for the *trans*-amide conformation. The ^{13}C NMR spectra did not provide clear-cut information regarding the conformation of the carbamate group. Doubling of the carbamate carbonyl and the benzyl methylene carbon signals indicates the presence of both *cis*- and *trans*-carbamate conformers in solution since the relative peak ratio within each doublet, which was 2 : 1, did not change on addition of triethylamine. We have not been able unambiguously to assign the doubled signals to the *cis*- or *trans*-carbamate conformers.

Calculations on ion pairs. The strong electrostatic repulsion observed in the calculations of the *trans*-amide conformers of the carboxylate ions of *N*-acetyl-L-proline and L-ZGP could, in part, be an effect of performing the calculations on the free anions without involving a counter ion. Such naked ions are unlikely to exist in solution. Inclusion of a positive counter ion in the calculations will partly neutralize an otherwise exaggerated negative charge. A small counter ion would not lead to major steric interactions which might impose conformational changes in the anions. However, the MMX force field is not parametrized for ions such as Li^+ or Na^+. In addition, the program does not allow for manual reduction of the charges on the carboxylate oxygen atoms. To investigate whether the results from the calculations on the anions were accurate in a qualitative sense we therefore reminimized each conformer identified of *N*-acetyl-L-proline and L-ZGP using the hydronium ion (H_3O^+) as the counter ion. One serious drawback with the use of a hydronium counter ion is related to its ability to participate in hydrogen bonding. Consequently, this counter ion may induce conformational changes and additional stabilization of otherwise less stable conformations of the anions. Our results show that some ion pairs of L-ZGP and the hydronium ion were stabilized by additional intermolecular hydrogen bonding due to the hydronium ion. However, the majority of conformers did not change their geometry appreciably when the counter ion was included in the calculations.

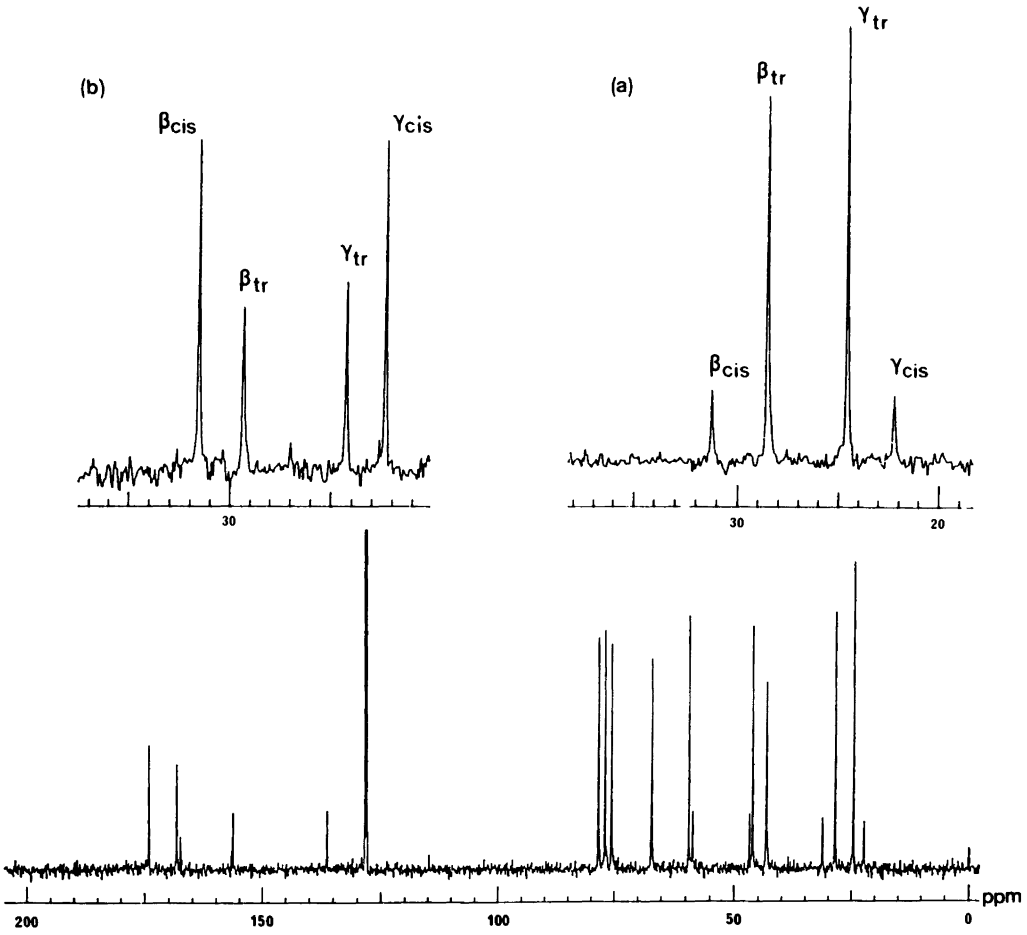


Fig. 4. ^{13}C NMR spectrum of L-ZGP in $CDCl_3$ (bottom). Top: (a) Expanded proton-decoupled ^{13}C NMR spectrum showing the resonances from C_β and C_γ of the pyrrolidine ring. (b) The C_β and C_γ region after addition of one equivalent of triethylamine to the sample solution.

Eight different conformers with similar relative energies were found for the ion pair of the carboxylate ion of *N*-acetyl-L-proline and the hydronium ion. A Boltzmann distribution at 25°C gave a *trans/cis* amide ratio of 1 : 1 which compares better with the ^1H and ^{13}C NMR spectroscopic data (*trans/cis* ratio 2 : 3) than did the results from the calculations on the free carboxylate ion (only *cis*-amide conformations were of low energy).

The conformers of L-ZGP identified were also reminimized as ion pairs. The ion-pair conformations which were stabilized by intermolecular hydrogen bonding were excluded when calculating the Boltzmann distribution. The *trans/cis* ratio was found to be 1 : 3 which may be compared with the NMR data which showed a *trans/cis* ratio of 2 : 3.

A statistical treatment of the results. In order to establish how the MMX program treated the three different species of L-ZGP (carboxylic acid, carboxylate ion, and ion pair), the relative energies of the conformers included in groups 1–22 (Table 1) were compared by regression analysis. The results were analysed by dividing groups 1–22 into four subgroups: *trans*-amide/*trans*-carbamate (groups 1–7), *trans*-amide/*cis*-carbamate (groups 8–16), *cis*-amide/*trans*-carbamate (groups 17–19) and *cis*-amide/*cis*-carbamate (groups 20–22) (Table 2).

There is no correlation between the relative steric energies of conformers of the carboxylate ion and the carboxylic acid, or between those of the carboxylic acid and ion-pair conformers since the regression coefficients are low ($r^2 = 0.36$ and 0.12, respectively) and the slopes of the regression lines are negative (slope = -1.98 and -0.48, respectively). This might indicate that the strong repulsive electrostatic interactions observed in conformers with *trans*-amide bond arrangements in the free carboxylate ion are not eliminated in the ion pairs although they are considerably weaker. A correlation was found only between energies of conformers of the carboxylate ion and those of the ion pair. The highest correlation coefficient ($r^2 = 0.83$) was observed in the *trans*-amide/*trans*-carbamate group, and the lowest in the *cis*-amide/*trans*-carbamate group ($r^2 = 0.18$). The slopes of the regression lines are about $+0.5$ in the four subgroups. These results are hard to rationalize and demonstrate some of the

Table 2. Results from the statistical treatment of all conformers of L-ZGP included in Table 1 divided into four subgroups. Data from correlation diagrams between the relative energies (kcal mol^{-1}) of different species of L-ZGP. The correlation coefficient (r^2) and the slope of the regression lines are given.

	Deprotonated ZGP/ neutral ZGP		Neutral ZGP/ ion pair		Deprotonated ZGP/ ion pair	
Group	r^2	Slope	r^2	Slope	r^2	Slope
trans-Amide/*trans*-carbamate	0	−0.15	0	+0.55	0.83	+0.51
trans-Amide/*cis*-carbamate	0	−0.24	0	+0.28	0.56	+0.57
cis-Amide/*trans*-carbamate	0	−0.06	0	−0.18	0.18	+0.47
cis-Amide/*trans*-carbamate	0.18	−1.12	0	−0.25	0.58	+0.58

problems associated with molecular-mechanics calculations of charged species.

Concluding remarks

Conformational analysis of small peptides pose interesting problems due to the large number of energetically accessible conformers. These problems are complicated further by potential intramolecular hydrogen bonds, which may stabilize otherwise disfavoured conformations, and interactions between charged or highly polarized functional groups, which may have a quite different impact on the conformational preferences of a peptide *in vacuo* compared with, e.g., in aqueous solution.

In the present study we have used a sequential 'build-up strategy' to span the conformational space of neutral and ionized L-ZGP by use of MMX calculations. The computational results are in fair agreement with experiment and indicate that this molecular-mechanics program handles charged as well as non-charged peptides with reasonable accuracy.

Recently, the MM2 force field was extended to allow predictions of amide geometries and rotational barriers to be made within experimental error.[29] Since the MMX force field and the extended version of MM2 give approximately the same bond lengths, bond angles and rotational barriers in formamide, acetamide and their *N*-methyl derivatives, it is not surprising that a peptide derivative such as L-ZGP may be handled by MMX. In addition, the MMX force field and the ECEPP force field developed by Scheraga *et al.*[30,31] produce the same minimum-energy conformer of *N*-acetylprolinamide.[32]

Initially, we were surprised by the MMX-derived result that the *cis*-oriented carbamates are more stable than the *trans*-carbamates. However, a comparison between MMX and ECEPP computations[7] on Z–Ala–*N*–Me and Z–Pro–*N*–Me reveals that the MMX-force field predicts a larger percentage of *cis*-carbamate conformers of the former and a larger amount of *trans*-carbamate conformers of the latter. Similarly, no experimental evidence seems to contradict the MMX-derived conformational preferences of the carbamate functionality. In conclusion, we believe that the computational results presented herein provide a good description of the conformational preferences of L-ZGP when dissolved in chloroform.

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