Molecular Mechanics Calculations of the Conformers of the Dipeptide Boc–Gly–Leu–NMe and Tripeptide Boc–Gly–Leu–Gly–NMe. Searching the Conformational Space by the Build-up Method

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The sequences of amino acids Gly–Leu and Gly–Leu–Gly frequently recur in elastin, the protein responsible for the elasticity of the tissues of many vertebrates. In this paper we have carried out a theoretical conformational study by molecular mechanics, using a modified version of the build-up method for searching the conformational space, to determine the set of the low-energy conformers for the dipeptide Boc–Gly–Leu–NMe and the tripeptide Boc–Gly–Leu–Gly–NMe. The conformational energy, torsional and H-bond distributions of the conformers, determined by the algorithm, have been calculated. The outcome is successfully compared with experimental and theoretical results.

The sequences of amino acids Gly–Leu and Gly–Leu–Gly frequently recur in elastin (*e.g.* in human tropoelastin 25 and 15 times, respectively), the protein responsible for the elasticity of the tissues of many vertebrates,[1] and may belong to those repetitive sequences responsible for the elasticity. The synthetic peptides comprising the sequences Gly–X–Gly (X = Val or Leu) have been the object of detailed and extensive circular dichroism (CD) and NMR conformational studies.[2,3] In this paper we describe our theoretical conformational study by molecular mechanics, using a modified version of the build-up method[4,5] to determine the set of low-energy conformers for the dipeptide Boc–Gly–Leu–NMe and the tripeptide Boc–Gly–Leu–Gly–NMe (Fig. 1). The conformational energy, torsional and H-bond distributions of the conformers have been calculated.

The properties of real systems usually represent averages resulting from a multitude of populated low-energy structures. The set of all populated minima, or at least a representative sampling, are required if quantitative molecular modelling is to be achieved. Nevertheless, conformational-searching methods are far from providing a general solution to the multiple-minima problem.[6,7]

The multiple-minima problem for peptides constitutes the main difficulty of locating the populated conformers (low-energy local minima)[8] in the conformational space of the molecule, directly from the primary structure.[6] The simplest means to solve the problem is to minimize from a very large number of starting points, chosen randomly or uniformly in the conformational space, but this procedure does not focus the search efficiently enough. In particular, a grid search procedure[9] to explore systematically the conformational subspaces defined by φ_1, ψ_1, φ_2, χ_2^1, χ_2^2, ψ_2, for the dipeptide and φ_1, ψ_1, φ_2, χ_2^1, χ_2^2, ψ_2, φ_3, ψ_3, for the tripeptide, would be hardly feasible because of the number of the torsion angles and of the grid spacing to be taken into account. In fact, by varying the torsions φ and ψ from 0° to 360° even by using steps of 40° and by assuming for the torsions χ only the values of ±60° and 180°, sets of $9^4 \times 3^2$ and $9^6 \times 3^2$ starting conformations to be minimized would be selected for the di- and tri-peptide, respectively.

Instead, in a recently developed version of the build-up method[10] the conformers of amino acid residues (*ca.* 5 kcal mol^{-1} above the global minimum) are taken into account to focus the search on the most probable regions of the conformational space. The search starts at the N-terminus, and adds one residue at a time. At some point in the build-up search

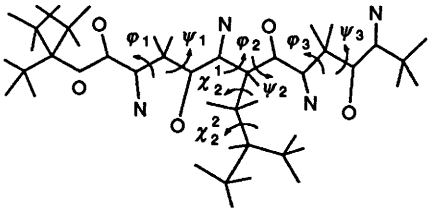

Fig. 1 Molecular structure of the tripeptide Boc–Gly–Leu–Gly–NMe with the notation of φ_i, ψ_i and χ_i torsional angles. Labels are reported for non-hydrogen and carbon atoms.

a set of conformations is selected for a *n*-residue growing chain by the combination of torsional sets of the *n*-th residue and of the *n* − 1-residue growing chain. These conformations are subjected to energy minimization at a rigid valence geometry, then are selected by a sorting procedure, comparing the backbone conformation. Firstly, they are ordered by increasing energy, and equivalent conformations are discriminated; secondly, they are compared with an energy cut-off and high-energy conformations are excluded from further consideration. The remaining low-energy conformations are used in extending the chain. The new residue is added at the C-terminus of the growing chain. The procedure is repeated until it reaches the final C-terminus of the chain. At that point the set of all populated conformers of the peptide are determined.

In our version of the build-up method, four improvements to the procedure of ref. 10, have been made: (1) all the resulting conformational energy minima of amino acid residues are taken into account (no energy cut-off of 5 kcal mol^{-1} is used); (2) the Cartesian coordinate energy minimization, in which complete geometry optimization of all atomic degrees of freedom is carried out, is used (no energy minimization at a rigid valence geometry is used); (3) all the *n*-residue growing chains are taken into account including the high energy ones (no energy cut-off is used), in order not to neglect those structures whose stability depends on long-range interactions;[10] (4) the minimum energy structures with any difference in either backbone or side-chain conformation are selected (not only backbone conformations are considered).

Theoretical Computations

The Model Force Field.—The AMBER program[11–14] was used for molecular mechanics calculations. The force field equation is of the form given in eqn. (1).

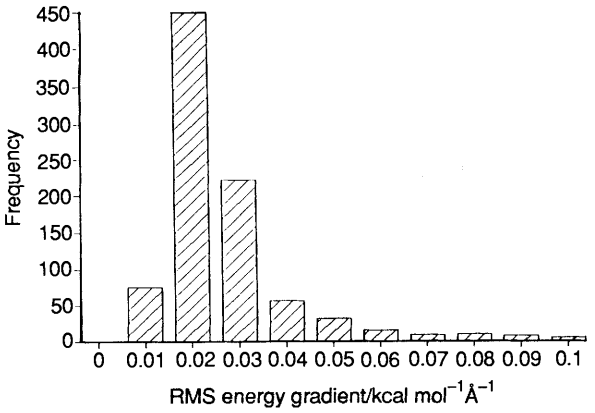

Fig. 2 RMS energy gradient distribution for the 1015 conformers of the tripeptide Boc–Gly–Leu–Gly–NMe

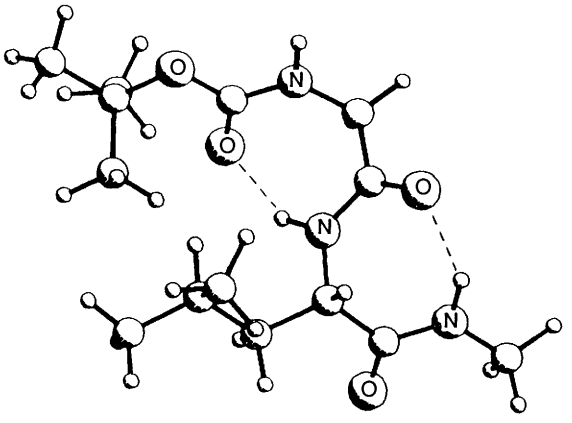

Fig. 3 The lowest-energy conformer of the dipeptide Boc–Gly–Leu–NMe. H-Bonds are shown (dashed lines). Labels are reported for non-hydrogen and carbon atoms.

$$E_{tot} = \Sigma_{bonds}\, k_r(r - r_{eq})^2 + \Sigma_{angles}\, k_\tau(\tau - \tau_{eq})^2 + \Sigma_{dihedrals}\, U_n/2\,[1 + \cos(n\varphi - \delta)] + \Sigma_{i<j}\, w(A_{ij}/R_{ij}^{12} - B_{ij}/R_{ij}^{6} + 332.2\, q_i q_j/\varepsilon R_{ij}) + \Sigma_{H\text{-}bonds}\,(C_{ij}/R_{ij}^{12} - D_{ij}/R_{ij}^{10}) \quad (1)$$

The calculations were carried out on a VAX 8530 computer under the VMS 5.4 operating system. Throughout all calculations the all-atoms force field was used, *i.e.* all atoms were explicitly represented by the force field. The force field parameters are those of ref. 12. The 1–4 non-bond and electrostatic interactions are weighted by a scale factor $w = 0.5$ and no cut-off for the non-bonded interactions was used. All energies are expressed as $\Delta E = E - E_o$, where E_o is the energy corresponding to the most stable conformer.

For the coulombic term the value of the relative permittivity, ε, has been taken as 1.0. Net atomic charges, q_k, for the amino acid residue atoms (Gly and Leu) and for the NMe CO–terminal group were those of ref. 12, those for the Boc NH–terminal group were derived by us in ref. 15. In all cases the charges were calculated by fitting to a point charge model the quantum mechanically derived electrostatic potential.[16]

Multiple-minima Search.—The multiple-minima search for the dipeptide Boc–Gly–Leu–NMe and the tripeptide Boc–Gly–Leu–Gly–NMe was performed on the basis of the build-up method. In Fig. 1 the molecular structure with the notation of φ_i, ψ_i and χ_i for the torsional angles is presented for the tripeptide. We started from all minima, without any energy cut-off, for the *N*-acetyl-*N'*-methylamide of Gly and Leu (5 and 49 minima, respectively) as derived by us, in the Kollman Force

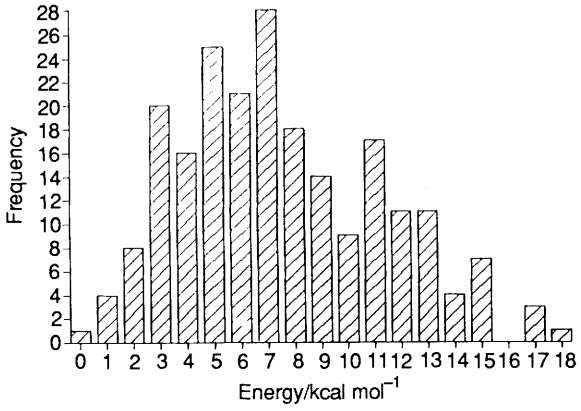

Fig. 4 Histogram of the conformational energy distribution for the 215 conformers of the dipeptide Boc–Gly–Leu–NMe

Field with a Newton–Raphson minimization method, from starting conformations selected as described by Zimmermann *et al.*[17] The eigenvalues of the second-derivative energy matrix (non-negative) at the minimum were checked.

By combination of the torsional sets φ, ψ, and eventually, χ of the conformers of Gly and Leu, assuming a *trans* conformation for the peptide bonds and the *tert*-Boc group, and staggered for the methyl groups, $5 \times 49 = 245$ starting conformations for the dipeptide Boc–Gly–Leu–NMe were identified. They were subjected to conjugate gradient energy minimization in Cartesian coordinates. The convergence criterion of $\Delta E = 10^{-6}$ kcal mol^{-1} between two successive iterations was adopted, obtaining values of the RMS energy gradient lower than 10^{-3} kcal mol^{-1} Å^{-1} for the minimized structures. A maximum number of iterations was set at a value of 10 000 in order to avoid an 'infinite loop' in those cases where convergence could not be satisfied. Therefore, the high number of iterations cannot interfere with the convergence which generally occurs after 300–500 steps. The resulting energy-minimized structures were selected by the following sorting procedure: they were ordered by increasing energy, then, duplicate structures were identified and only the one with the lowest energy was stored. Structures are defined as duplicate when all torsional angles φ_1, ψ_1, φ_2, χ_2^1, χ_2^2 and ψ_2, are equal within a tolerance (absolute value of the angular difference) of 15°. By doing so 215 non-equivalent conformers were determined.

In the same way, by the combination of the torsional sets of the dipeptide with those of Gly, $215 \times 5 = 1075$ starting conformations for the tripeptide Boc–Gly–Leu–Gly–NMe were identified and subjected to energy minimization (assuming a maximum number of 3000 iterations), the resulting energy minimized structures were selected. This determined 1015 non-equivalent conformers. The high number of final non-equivalent conformers as compared to the initial conformations for both di- and tri-peptide, should mean good efficiency of the method used in the sampling of the conformational space. In Fig. 2 the RMS energy gradient distribution for the conformers of the dipeptide is reported: the shape is sharp, the values are contained in the range 0.01–0.1 kcal mol^{-1} Å^{-1} and the highest frequency occurs at 0.02 kcal mol^{-1} Å^{-1}.*

Results and Discussion

Boc–Gly–Leu–NMe.—The outcome produced by the algorithm for searching the conformational space consists of 215

* Cartesian coordinates in pdb format for all the conformers of the dipeptide Boc–Gly–Leu–NMe and the tripeptide Boc–Gly–Leu–Gly–NMe on magnetic tape, are available from the authors.

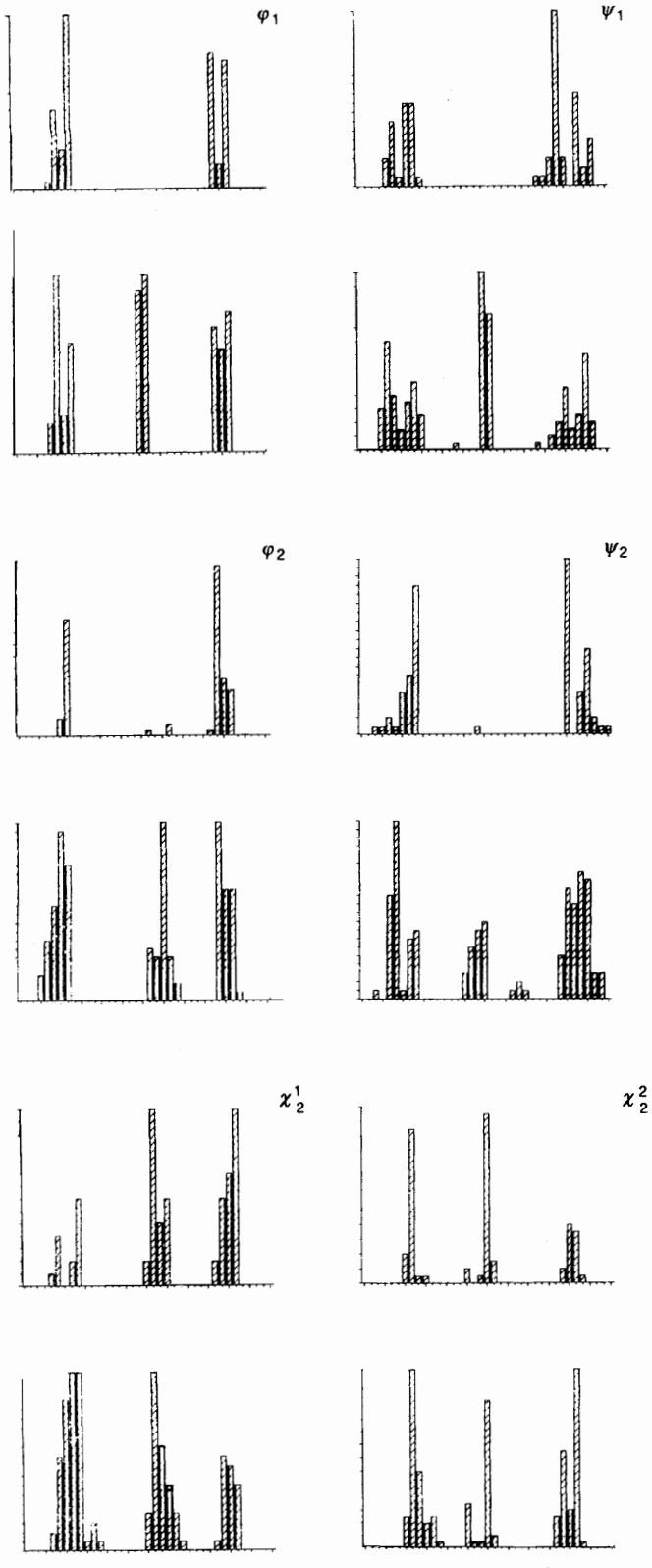

Fig. 5 Torsional distributions of φ_1, ψ_1, φ_2, χ^1_2, χ^2_2, and ψ_2 (degrees) of the dipeptide Boc–Gly–Leu–NMe. Upper picture: $\Delta E \leqslant 5$ kcal mol^{-1}, lower picture: $\Delta E > 5$ kcal mol^{-1}.

conformers spread over an energy range of 18 kcal mol^{-1} above the most stable. In Fig. 3 the lowest-energy conformer is reported; it is stabilized by consecutive seven-membered hydrogen-bonded rings, C_7, [Boc]C=O···HN[Leu$_2$] and [Gly$_1$]C=O···HNMe, in accordance with the preferred conformations of oligopeptides.[18–20]

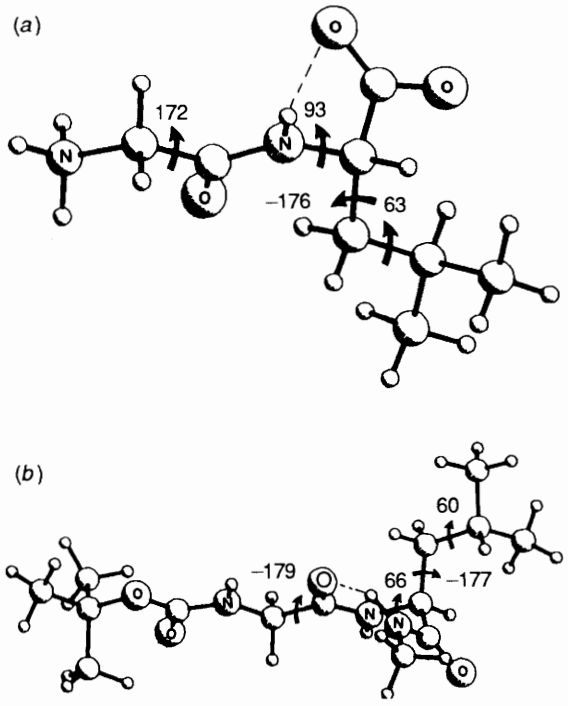

Fig. 6 Molecular conformation observed in crystal structure of Gly–Leu as a zwitterion[19] (*a*) and the most similar conformer of Boc–Gly–Leu–NMe among the calculated ones (*b*). The experimental and the calculated values of ψ_1, φ_2, χ^1_2, and χ^2_2 (degrees) are reported. H-Bonds are shown (dashed lines). Labels are reported for non-hydrogen and carbon atoms.

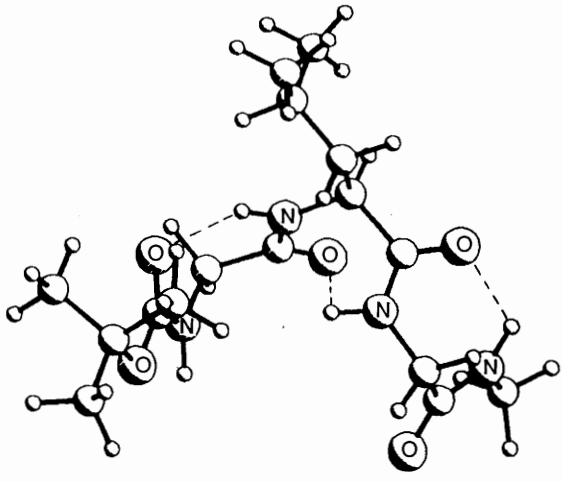

Fig. 7 The lowest-energy conformer of the tripeptide Boc–Gly–Leu–Gly–NMe. H-Bonds are shown (dashed lines). Labels are reported for non-hydrogen and carbon atoms.

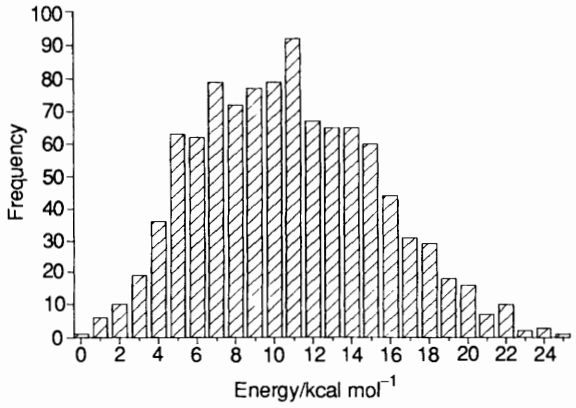

Fig. 8 Histogram of the conformational energy distribution for the 1015 conformers of the tripeptide Boc–Gly–Leu–Gly–NMe

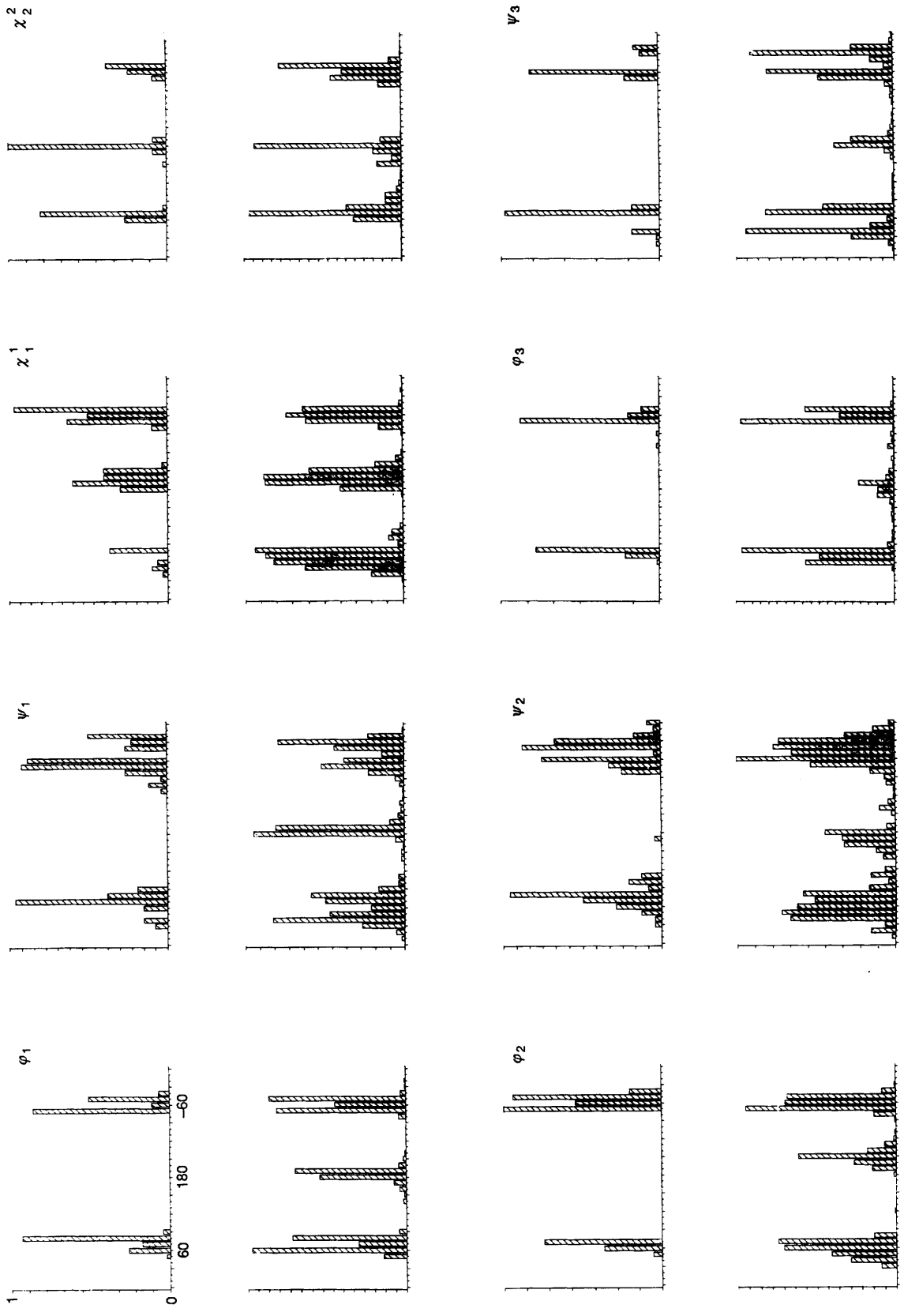

Fig. 9 Torsional distributions of φ_1, ψ_1, φ_2, χ^1_2, χ^2_2, ψ_2, φ_3, and ψ_3 (degrees) of the tripeptide Boc–Gly–Leu–Gly–NMe. Upper picture: $\Delta E \leqslant 5$ kcal mol^{-1}, lower picture: $\Delta E > 5$ kcal mol^{-1}.

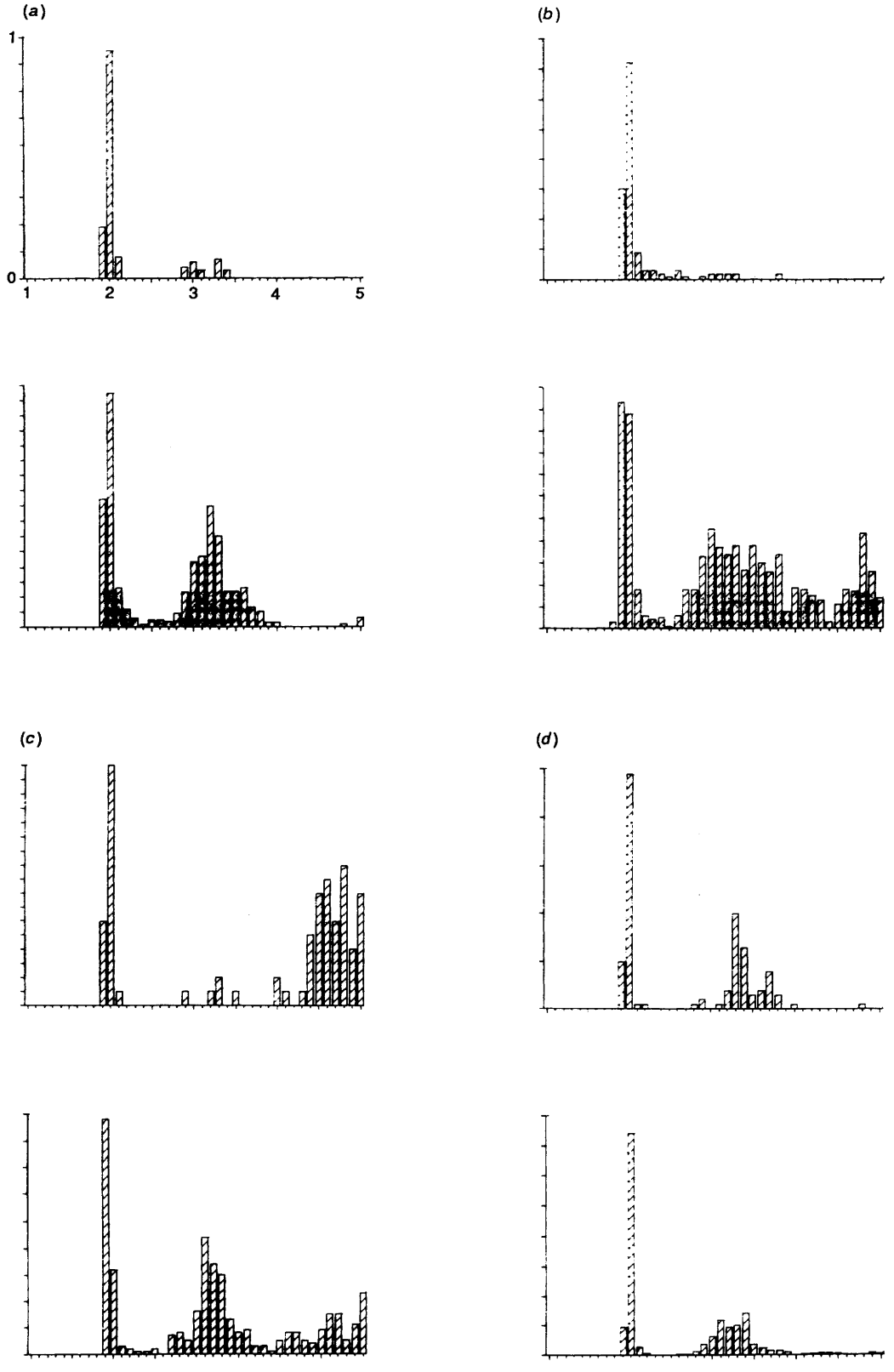

Fig. 10 Interaction distances O···H (Å) distributions for [Boc]C=O···HN[Leu_2] (*a*), [Gly_1]C=O···HN[Gly_3] (*b*), [Gly_1]C=O···HNMe (*c*), and [Leu_2]C=O···HNMe (*d*) of the tripeptide Boc–Gly–Leu–Gly–NMe. Upper picture: $\Delta E \leqslant 5$ kcal mol^{-1}, lower picture: $\Delta E > 5$ kcal mol^{-1}.

A histogram of the conformational energy distribution is reported in Fig. 4. The plot of frequency *vs.* energy states reveals that there is a continuous asymmetric distribution (skewness > 0, Maxwell-like) of energy states; the highest frequency occurs in the range 5–7 kcal mol^{-1} above the global minimum.

In Fig. 5 the statistical analysis of the torsional distribution of φ_1, ψ_1, φ_2, χ_2^1, χ_2^2 and ψ_2 is reported for the 74 states with $\Delta E \leqslant 5$ kcal mol^{-1} and for the 141 states with $\Delta E > 5$ kcal mol^{-1}. The distributions that result are essentially bi- or tri-modal with the frequency maxima near the *t*, $g^{\pm}$; states (180, $\pm 60°$). The angular region *t* is unlikely for the low-energy ($\Delta E \leqslant 5$ kcal mol^{-1}) distributions φ_1, ψ_1, φ_2 and ψ_2, however it

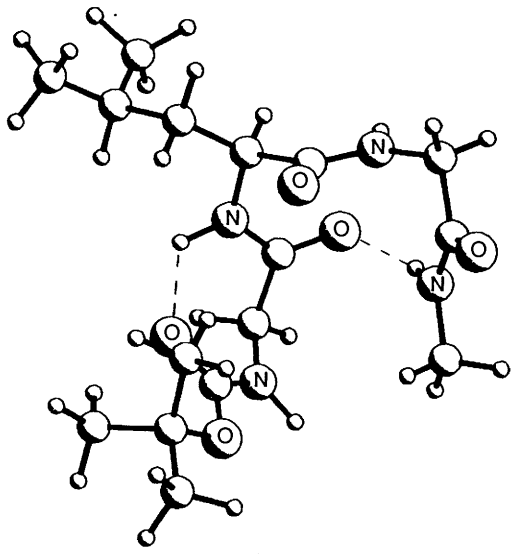

Fig. 11 The most stable conformer among those showing the type II β-turn [Gly_1]C=O···HNMe of the tripeptide Boc–Gly–Leu–Gly–NMe. H-bonds are shown (dashed lines). Labels are reported for non-hydrogen and carbon atoms.

is populated for $\Delta E > 5$ kcal mol^{-1}. Modifications of the relative amount of the $g^{\pm}$ and t populations take place for the distributions χ_2^1 and χ_2^2 passing from low to high energies.

In Fig. 6(*a*) and 6(*b*) the molecular conformation observed in the crystal structure of Gly–Leu as zwitterion[21] and the most similar conformer of Boc–Gly–Leu–NMe (at $\Delta E = 8.0$ kcal mol^{-1}) among the calculated ones are shown. The experimental and calculated values of the torsion angles ψ_1, φ_2, χ_2^1 and χ_2^2 are reported. The agreement is particularly satisfying, taking into account the differences in the chemical structures, and the constraints imposed by the crystal packing and by the particular hydrogen-bonding of the experimental structure. In fact, only the difference of the torsion φ_2 is appreciable, $\approx 25°$, owing to the presence in the zwitterion of the C_5 COO^- ···HN[Leu_2] H-bond, while in the dipeptide the C_7 [Gly_1]-C=O···HNMe H-bond occurs.

Boc–Gly–Leu–Gly–NMe.—The outcome of the search consists of 1015 conformers, spread over an energy range of 25 kcal mol^{-1} above the most stable. In Fig. 7 the lowest-energy conformer is reported; it is stabilized by consecutive C_7 [Boc]C=O···HN[Leu_2], [Gly_1]C=O···HN[Gly_3], and [Leu_2]C=O···HNMe H-bonds. The conformational energy distribution is reported in Fig. 8. The plot reveals that there is a continuous distribution of energy states, which is more symmetric than that of the dipeptide (skewness ≈ 0, Gauss-like). The highest frequency occurs in the range 7–11 kcal mol^{-1} above the lowest-energy minimum. This range is larger, and centred at higher energies, than for the dipeptide.

A priori, nothing can be said about the minima distribution on the potential energy surface for a complex molecule. We found continuous distributions of energy states with bell-like functional shape. We think that the actual distributions are well approximated taking into account the efficiency of our version of the build-up method for searching the conformational space and detecting minimum energy structures.

In Fig. 9 the torsional distribution of φ_1, ψ_1, φ_2, χ_2^1, χ_2^2, ψ_2, φ_3 and ψ_3 are reported for the 135 conformers with $\Delta E \leqslant 5$ kcal mol^{-1} and for the 880 conformers with $\Delta E > 5$ kcal mol^{-1}, respectively. Except for small variations, for example peak amplitude and relative populations of the most probable states, the corresponding torsional distributions are invariant

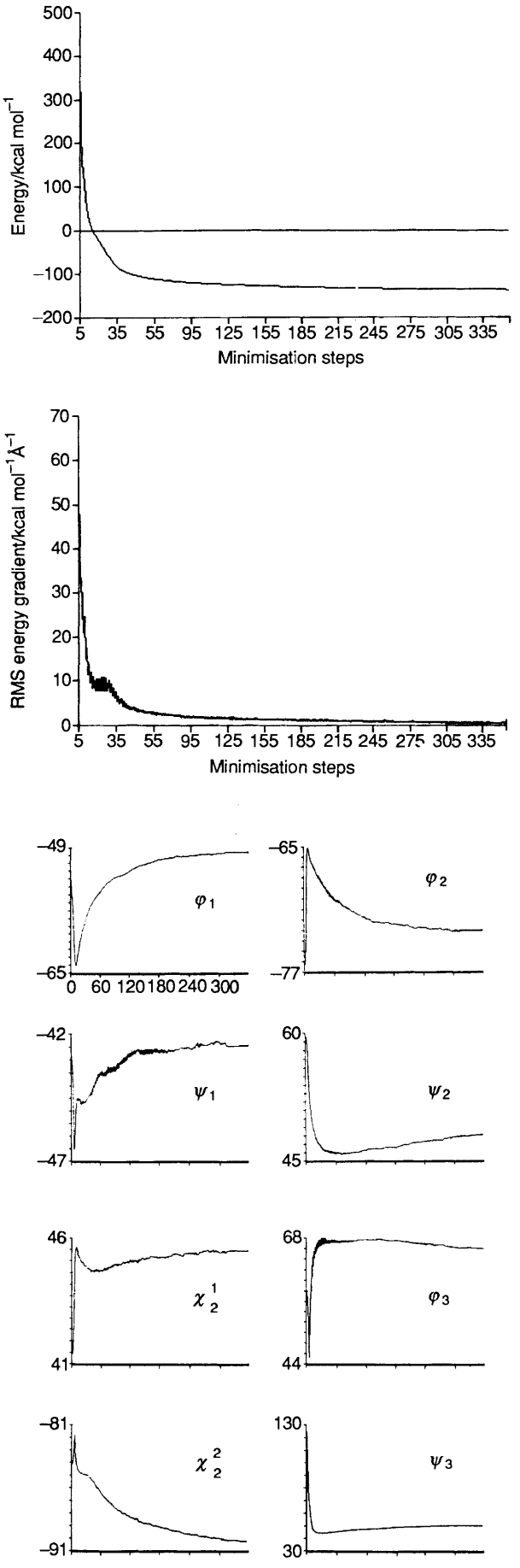

Fig. 12 History of the energy, RMS energy gradient, and torsions as functions of the minimization iteration steps for case C (see text)

on passing from the dipeptide to the tripeptide. The possible 16 distributions of the interaction distances O··· H, for the >C=O and HN< groups have been calculated. In Fig. 10 the distributions which present a significant peak in correspondence of the H-bond distance (≈ 2 Å) for the conformers with either $\Delta E \leqslant 5$ or $\Delta E > 5$ kcal mol^{-1} are reported. Only four H-bonds are populated: [Boc]C=O···HN[Leu_2] (C_7), [Gly_1]C=O···HN[Gly_3] (C_7), [Gly_1]C=O···HNMe (C_{10}) and [Leu_2]C=O···HNMe (C_7). These are the same H-bonds found to be present in the lowest-energy conformer. Therefore, all the C_7 hydrogen-bonded cycles which can in principle occur in the tripeptide are apparently stabilized, according to the literature.[18–20] Moreover, the presence of the β-turn [Gly_1]-C=O···HNMe agrees with the experimental results[2,3] and, in particular, with those obtained *via* CD for the tripeptide Boc–Gly–Val–Gly–OMe, which is similar to our one, apart from the Val→Leu substitution.[3] A systematic examination of the torsion angles φ_2, ψ_2, and φ_3, ψ_3, which define the type of secondary structure[22] involving [Gly_1]C=O···HNMe H-bond, allowed us to find the type II β-turn in 41 conformers. In Fig. 11 the most stable conformer among those showing the type II β-turn (at $\Delta E = 3.0$ kcal mol^{-1} above the global minimum) is reported. The comparison with the lowest-energy conformer (Fig. 7) shows that the C_7 [Boc]C=O···HN[Leu_2] is present in both structures, while the C_7 [Gly_1]C=O···HN[Gly_3] and [Leu_2]C=O···HNMe are replaced by the type II β-turn [Gly_1]C=O···HNMe.

The sequences Boc–Gly–Leu–Gly of the tripeptide Boc–Gly–Leu–Gly–NMe and Boc–Gly–Val–Gly in the pentapeptide Boc–Gly–Val–Gly–Gly–Leu–OMe, previously studied by us,[15] differ only in the substitution Val→Leu. So we have compared in an exhaustive way the torsional sets φ_i, ψ_i ($i = 1, 2, 3$) of the conformers of the tripeptide with the 14 low-energy structures of the pentapeptide determined by molecular mechanics and dynamics calculations.[15] Two conformations are defined as duplicate when all torsion angles are equal within a determined tolerance. The tolerance value has been varied from 15° to 60° in steps of 5°. Most of the conformations found for the pentapeptide[15] are also present in the tripeptide, as far as φ_i and ψ_i are concerned, within a tolerance range of 40°. As a general trend, the substitution Val→Leu does not significantly affect the identified conformations.

We have verified that the absence of some conformations in the tripeptide, which are present in the pentapeptide, is due to the possibility of the latter having structures stabilized by many H-bonds involving also Gly_4 and Leu_5, which are absent in the tripeptide. For example, conformer B[15] of the pentapeptide shows the maximum number of such H-bonds, in particular [Val_2]C=O···HN[Leu_5], [Gly_3]C=O···HN[Leu_5], and [Leu_5]C=O···HN[Val_2]. However, the agreement between the torsional patterns of most conformers shows that many structural features of the pentapeptide, and most importantly the torsion angles φ_2, ψ_2 and φ_3, ψ_3 which determine the β-turn involving the residues Gly_1 and Gly_4, can also be found in the tripeptide.

Starting Conditions and Convergence in the Energy Minimization of the Tripeptide.—From the examination of the 1075 minimizations carried out for the tripeptide at least three important representative behaviours emerge: favourable starting conformation (attractive energy and low RMS energy gradient) with fast convergence (A); favourable starting conformation without convergence (B); unfavourable starting conformation (strongly repulsive energy and high RMS energy gradient) with fast convergence (C).

The values of energy, RMS energy gradient and torsions have been analysed as a function of the minimization iteration steps for cases A, B and C. In Fig. 12 the plots for a representative example of case C are reported. In all cases the trends quickly (after *ca.* 50 iterations) assume an asymptotic behaviour, with small torsional variations in A and B ($\leqslant 15°$) and large in C (up to 80 °C). In case A, as we would expect, the convergence criterion is easily satisfied (after *ca.* 300 iterations); similarly for case C, although the energy and the gradient of the starting conformation are particularly high. In case B (15 cases) the convergence criterion is not satisfied and the calculation would be indefinitely iterated. This could derive either from conformations close to a turning point on the surface or from conformations on the way to other fully converged conformers which are unable to reach convergence because of some inadequacy in the optimiser. This will require further investigation in future work. In any case, the minimiser becomes unstable, varying between two fixed values, after the convergence has been substantially reached ($\Delta E \approx 10^{-5}$ kcal mol^{-1} between two successive iterations and RMS energy gradient about 10^{-2} kcal mol^{-1} Å^{-1} before the instability arises). We think that these conformations must be considered among the minima.

From the behaviour of the minimizations we conclude that the unfavourable conformations, even those with very high energy, cannot be *a priori* ignored, at least in the build-up procedure. The choice of a correct maximum number of iterations is important to avoid wasted CPU time, when the convergence criterion cannot be satisfied (*e.g.* in case B). This could be matched by using an iterative procedure in which the conformations are subjected to partial minimization with the exclusion of the duplicate conformations. The procedure is repeated until equivalent conformations are no longer selected. At that point a final full minimization follows.

Conclusions

The set of low-energy conformers for the dipeptide Boc–Gly–Leu–NMe and the tripeptide Boc–Gly–Leu–Gly–NMe has been determined using a modified version of the build-up method for searching the conformational space. The outcomes produced by the algorithm consist of 215 and 1015 conformers for the dipeptide and the tripeptide, respectively. We think that the set of populated low-energy minima is carefully described taking into account the effectiveness of our version of the build-up method for searching the conformational space and detecting minimum energy structures. Nevertheless, we cannot exclude the possibility that there may be more minima on the potential surface than those actually found.

The torsional and H-bond distributions as a function of energy have been determined and analysed. The most stable conformer for both peptides is stabilized by repeating C_7 secondary structures, according to the literature. The torsional distributions are essentially bi- or tri-modal with the frequency maxima near the t, $g^{\pm}$ states and are invariant on passing from the di- to the tri-peptide. From the tripeptide H-bond distributions it is clear that all the possible C_7 structures and the C_{10} β-turn [Gly_1]C=O···HNMe are populated. By comparing our results on the tripeptide Boc–Gly–Leu–Gly–NMe and the previous ones found for the pentapeptide Boc–Gly–Val–Gly–Gly–Leu–OMe, it can be said that many similarities in the structural features appear in their quasi-common sequence Boc–Gly–Leu(Val)–Gly–, despite the Leu→Val substitution.

The emerging general picture, indicating that the peptides are characterized by a combination of flexibility and conformational preferences, is in agreement with previous experimental findings and adds new aspects to the description of the

microscopic behaviour of these molecules suggesting a more detailed interpretation of previous data.

From the examination of starting conditions and convergence in the energy minimizations of the tripeptide we conclude that the unfavourable starting points cannot be ignored *a priori* and the maximum number of iterations is critical. These precautions would be satisfied using an iterative algorithm in which the partial minimization and sorting procedure are carried out until equivalent conformations are no longer detected, followed by a final full minimization.

Acknowledgements

We would like to acknowledge the CISIT of *Universita' della Basilicata* for a generous gift of computer time. This work has been partially supported by MURST and CNR (*Progetto Finalizzato Chimica Fine e Secondaria*) grants.

References

1 M. Bashir, Z. Indik, H. Yeh, W. Abrams, N. Ornstein-Goldstein, J. C. Rosenbloom, M. Fazio, J. Uitto, R. Mecham, W. Parks and J. Rosenbloom in *Elastin: Chemical and Biological Aspects*, eds., A. M. Tamburro and J. M. Davidson, Congedo, Italy, 1990, p. 45.
2 A. M. Tamburro, V. Guantieri, L. Pandolfo and A. Scopa, *Biopolymers*, 1990, **29**, 855.
3 A. M. Tamburro in *Elastin: Chemical and Biological Aspects*, eds., A. M. Tamburro and J. M. Davidson, Congedo, Italy, 1990, p. 125.
4 M. Vasquez and H. A. Scheraga, *Biopolymers*, 1985, **24**, 1437.
5 K. D. Gibson and H. A. Scheraga, *J. Comput. Chem.*, 1987, **8** 826.
6 K. D. Gibson and H. A. Scheraga in *Structure and Expression*, eds., M. H. Sarma and R. H. Sarma, Adenine Press, Guilderland, New York, 1988, vol. I, p. 67.
7 G. Chang, W. C. Guida and W. Clark Still, *J. Am. Chem. Soc.*, 1989, **111**, 4379.
8 J. Dale, *Top. Stereochem.*, 1976, **9**, 199.
9 H. A. Scheraga, *Chem. Rev.*, 1971, **71**, 195.
10 M. H. Lambert and H. A. Scheraga, *J. Comput. Chem.*, 1989, **10**, 798.
11 U. C. Singh, P. K. Weiner, J. Caldwell and P. A. Kollman, AMBER 3.0, University of California, San Francisco, 1987.
12 S. J. Weiner, P. A. Kollman, D. T. Nguyen and D. A. Case, *J. Comput. Chem.*, 1986, **7**, 230.
13 P. K. Weiner and P. A. Kollman, *J. Comput. Chem.*, 1981, **2**, 287.
14 S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta jnr. and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765.
15 F. Lelj, A. M. Tamburro, V. Villani, P. Grimaldi and V. Guantieri, *Biopolymers*, 1992, **32**, 161.
16 U. C. Singh and P. Kollman, *J. Comput. Chem.*, 1984, **5**, 129.
17 S. S. Zimmermann, M. S. Pottle, G. Nemethy and H. A. Scheraga, *Macromolecules*, 1977, **10**, 1.
18 P. N. Lewis, F. A. Momany and H. A. Scheraga, *Biochim. Biophys. Acta*, 1973, **303**, 211.
19 E. Ralston and L. de Coen, *J. Mol. Biol.*, 1974, **83**, 393.
20 A. T. Hagler, P. S. Stern, R. Sharon, J. M. Becker and F. Naider, *J. Am. Chem. Soc.*, 1979, **101**, 6842.
21 V. Pattabhi, K. Venkatesan and S. R. Hall, *J. Chem. Soc., Perkin Trans. 2*, 1974, **3**, 1722.
22 G. D. Rose, L. M. Gierasch and J. A. Smith, *Adv. Protein Chem.*, 1985, **37**, 1.

Paper 2/01449A
Received 18*th March* 1992
Accepted 30*th July* 1992