Ab Initio SCF and Force-field Calculations on Low-energy Conformers of 2-Acetylamino-2,*N*-dimethylpropanamide

Carlos Alemán[*,a] **and Jordi Casanovas**[b]
[a] *Departament d'Enginyeria Química, Escola Tecnica Superior d'Enginyers Industrials de Barcelona, Universitat Politecnica de Catalunya, Av. Diagonal 647, E-08028 Barcelona, Spain*
[b] *Departament de Química Física, Facultat de Química, Universitat de Barcelona, C/Martí i Franqués 1, E-08028 Barcelona, Spain*

The understanding of the conformational properties of α,α-disubstituted amino acids is related to the design of structural mimics for proteins. More specifically, 2-amino-2-methylpropanoic acid has been widely used in constructing helical peptide modules. In this paper, we report the results of *ab initio* SCF calculations with 3-21G, 6-31G* and 6-31G** basis sets on four low-energy conformations of 2-acetylamino-2,*N*-dimethylpropanamide. The results are used to assess the reliability of empirical potentials used in force-field methodologies. For this purpose, averaged multiple conformations' atomic point charges were determined with the 6-31G* basis set. The results point to some important deficiencies in current force-fields. Thus, the relative energies of dipeptide conformations with excessive repulsive or excessive attractive interactions are not well described by some empirical potentials.

Force-field methodologies, *i.e.* molecular mechanics (MM), molecular dynamics (MD) and Monte Carlo (MC) calculations, are of great importance in the study of the structure and dynamics of biomolecules. All these techniques are based on the implicit assumption that the energy of a system can be represented by an addition of classical terms. During the last few years, several groups have devoted their research efforts to the development of suitable force-fields[1-8] as well as to their validation.[9-16] The reliability of the calculations based on the force-field techniques depends not only on the formalism used to describe the different contributions to the energy, but also on the quality of the parameters incorporated within the force-field.[17-19] Accordingly, a large number of parameterization strategies from both quantum mechanical calculations[20-26] and experimental data[27-29] have been also developed for this crucial step.

One method that has been widely used for the validation of force-field parameters has been the comparison of calculated energies and geometries of model molecules with high quality quantum mechanical *ab initio* calculations. For example, *N*-acetyl-*N'*-methylalanilamide and *N*-acetyl-*N'*-methylglycilamide have been considered as test cases for force-field parameters derived for naturally occurring amino acids.[30-34] However, when the system under study involves residues which are not usually found in natural peptides and proteins, the determination and subsequent verification of force-field parameters is a difficult task. This is fundamentally due to the lack of high level quantum mechanical data for this set of compounds, which are sometimes of enormous importance in *de novo* protein design.[35]

2-Amino-2-methylpropanoic acid (**1**) is an α,α-dialkyl amino acid in which the replacement of hydrogen at α-C produces severe restrictions on the conformation.[36-38] Reviews on the crystal structure of peptides containing residues of **1** have confirmed a strong preference for the helical conformation in the solid state.[39,40] On the other hand, NMR experiments in non-polar solvents demonstrated that the C_5 and the C_7 conformations are favoured with respect to the helix, while in polar media the helix conformation becomes significantly more stable.[41,42]

$H_2NCMe_2CO_2H$
1

The prediction of the helical conformations of oligomers of **1** has been the subject of several theoretical studies within the MM framework.[37,43-46] Thus, they agree upon the existence of a 3_{10}-helix or an α-helix, depending on three factors: (*i*) the dielectric constant of the environment; (*ii*) the length of the polypeptidic chain; and (*iii*) the packing interactions with neighbouring chains. However, some differences were observed in the results depending on the force-field parameterization. This was explicitly pointed out in two recent works.[47,48]

On the other hand, Barone and co-workers[47] examined the potential energy surface of 2-acetylamino-2,*N*-dimethylpropanamide (**2**) at the Hartree–Fock level with an STO-3G basis set.[49] In their calculations, bond lengths were fixed and only bond and dihedral angles were optimized. The authors found four low-energy conformers: C_7, C_5, helix and P_{II}, the latter being the least favoured with respect the others (relative energies: 0.0, 0.8, 1.7 and 13.6 kcal mol^{-1}, respectively). Nevertheless, STO-3G computations are not sufficiently accurate for both a quantitative analysis and a definitive calibration of force-field parameters.

In this work, we present a study of the structural and energetic properties of the four low energy conformers, *i.e.* C_5, C_7, helix and P_{II}, of amide **2** (see Fig. 1) using *ab initio* quantum mechanical calculations. Geometry optimizations were performed at the Hartree–Fock level employing a 3-21G basis set.[50] In order to assess the basis set dependence, single point calculations with 6-31G*[51] and 6-31G**[52] basis sets

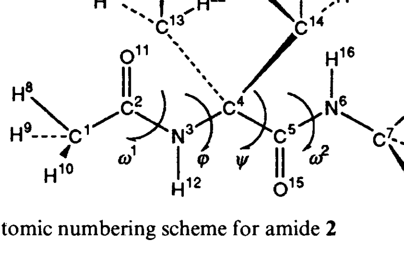

Fig. 1 Atomic numbering scheme for amide **2**

Table 1 *Ab initio* SCF relative energies (kcal mol^{-1}) of the four low-energy conformers of **2**

	C_5	C_7	Helix	P_{II}
STO-3G[a]	0.0	0.0	12.6	20.1
3-21G	0.0[b]	0.6	4.1	4.8
6-31G*	0.0[c]	0.8	1.8	3.1
6-31G**	0.0[d]	0.9	2.0	4.0

[a] From ref. 47. [b] The 3-21G zero of energy is −528.941 303 1 hartrees. [c] The 3-31G zero of energy is −531.888 210 9 hartrees. [d] The 6-31G** zero of energy is −531.916 373 3 hartrees. 1 hartree ≈4.36 × 10^{-18} J.

were performed. Subsequently, in order to provide a reliable set of parameters for representing the electrostatic effects of the residue of **1** in force-field calculations, atomic point charges for the four conformers were determined using a 6-31G* basis set. Finally, the reliability of some empirical potentials was tested. The results permit a critical evaluation of force-field techniques.

Computational Methods

We performed all *ab initio* calculations with the HONDO 7.0[53] molecular orbital package run on an IBM/RISK-6000 and an IBM/3090. Full geometry optimizations of the four low-energy conformers of **2** were performed at the Hartree–Fock level with the minimal split valence 3-21G basis.[50] The optimized 3-21G geometries were used for single point calculations with 6-31G*[51] and 6-31G**[52] basis sets. The calculations comprised a total of 127, 193 and 235 basis functions at 3-21G, 6-31G* and 6-31G** levels, respectively.

The geometry optimizations were carried out as follows. Initially, semiempirical energy optimizations of the four low-energy conformers were carried out with the AM1 Hamiltonian,[54] using a locally modified version[55] of the MOPAC program.[56] The AM1-minimized structures were used as input for the *ab initio* SCF calculations. A full optimization of all the degrees of freedom was carried out with HONDO 7.0.[53] The minimizations were continued until the maximum energy gradient dropped below 0.0008 kcal mol^{-1} Å^{-1} and the energy change over the last ten iterations was less than 0.01 kcal mol^{-1}.†

The 6-31G* wave functions calculated for the 3-21G fully minimized structures were used to compute electrostatic charges.[57,58] Accordingly, the charges were obtained by fitting the Coulombic monopole–monopole electrostatic potential to the quantum mechanical electrostatic potential by means of the Levenberg–Marquardt nonlinear optimization procedure.[59] To perform this fitting, the electrostatic potential was evaluated at a point located at four Connolly layers.[60] The inner layer was placed at 1.4 times the van der Waals radii of the atoms and a separation of 0.2 Å between layers was considered,[26] a density of 1.5 points Å^{-1} in each layer being defined. The electrostatic potential was calculated at 1570 points for the C_5 conformer, 1557 points for the C_7 conformer, 1548 for the helix conformer and at 1572 points for the P_{II} conformer. Electrostatic potential and atomic point charges were determined by using the MOPETE and MOTODO programs,[61] respectively.

Force-field geometry optimizations were performed with the MIN module of the AMBER 3.0A set of programs.[62] Bonded and van der Waals parameters were taken from the AMBER all-atoms force-field.[4] The geometry optimizations were performed *in vacuo* ($\varepsilon = 1$) using the conjugated gradient method, with a convergence test of 10^{-7} kcal mol^{-1} in the energy and 0.1 kcal mol^{-1} in the gradient of the energy. Optimized geometries were used to compute the Hessian matrix by the Langevin–Newton–Rampson method. Such calculations allowed us to confirm the minima properties of the optimized geometries.

Results and Discussion

The relative energies calculated with various basis sets are listed in Table 1. The effect of the basis size does not appear to be dramatic. Thus, the same relative order between the different conformers was provided by the different basis sets considered. The C_5 conformer was predicted to be most stable. At the 3-21G level, the separation between C_5 and C_7 is 0.6 kcal mol^{-1}, while at 6-31G* and 6-31G**, it increases to 0.8 and 0.9 kcal mol^{-1}, respectively. The C_5 intramolecular hydrogen bond geometry is characterized by r[H(12)–O(15)] = 2.064 Å and ∠N(3)–H(12)–O(15) = 110.8°. The C_7 conformation is characterized by r[O(11)–H(16)] = 1.930 Å and ∠C(2)–O(11)–H(16) = 146.8°. Comparison with STO-3G results reported in recent work by Barone and coworkers[47] reveals a poor agreement, since they found the same energy for C_5 and C_7 conformers. This must be attributed to the deficiencies of the minimal basis STO-3G.

Regarding the helix and P_{II} conformers, examination of the energy separations reveals a stabilization when the basis set increases from 3-21G to 6-31G*. Thus, the energy difference between C_5 and helix is 4.1 kcal mol^{-1} at 3-21G, and 1.8 kcal mol^{-1} at 6-31G*, remaining relatively constant at 6-31G** (2.0 kcal mol^{-1}). The energy difference between C_5 and P_{II} is 4.8, 3.1 and 4.0 kcal mol^{-1} at the 3-21G, 6-31G* and 6-31G** levels, respectively. Indeed, these results suggest that the true surface may be more flat than the 3-21G surface. Inspection of the relative energies obtained from the different wavefunctions reveals the similarity between 6-31G* and 6-31G** results. Accordingly, although atoms other than hydrogen need a basis set of at least split-valence plus polarization, hydrogen atoms can be described with a split-valence. Comparison with STO-3G[47] computations indicates a considerable overestimation of the relative energy for the P_{II} conformer.

Conformational parameters of the conformers of AAibN calculated at the 3-21G level are given in Table 2. The dependence of bond lengths and bond angles on the conformation is small. The largest change in a bond length occurs for r[N(3)–C(4)] which varies from 1.461 (C_5) to 1.480 Å (C_7). The largest change in a bond angle is found for ∠N(3)–C(4)–C(5) which varies from 105.4° (C_5) to 111.1° (helix). Conformational angles φ and ψ were found around the typical values for each conformation. Previous systematic studies seem to suggest some general rules relating the ∠N(3)–C(4)–C(5) angle and the residue conformation.[47,63–65] Specifically, small values of ∠N(3)–C(4)–C(5) favour the C_5 and helix conformations. The results displayed in Table 2 support this conformational behaviour for conformation C_5. In contrast, the helix conformation exhibits the largest ∠N(3)–C(4)–C(5) value. The same disagreement was observed in a recent study, in which the helical region of the potential energy surface of the AAibN was explored using *ab initio* and semiempirical quantum mechanical methods, as well as force-field derived methods.

An interesting feature found in the helix conformation is the markedly non-planar distortion of the peptide bond ω_1. This distortion is overestimated by SCF calculations with respect to the X-ray determined structures.[43,63–68] Thus, the average non-planar distortion found from crystallographic data analysis is around 3–6°.

The atomic point charges' effects are of dramatic importance in the reliability of force field calculations. Quantum mechanics

---

† 1 cal = 4.18 J.

Table 2 *Ab initio* 3-21G conformational parameters of **2**

Parameter	C_5	C_7	Helix	P_{II}
Bond lengths *r*/Å				
C(1)–C(2)	1.517	1.517	1.515	1.519
C(2)–N(3)	1.351	1.350	1.366	1.362
N(3)–C(4)	1.461	1.480	1.468	1.476
C(4)–C(5)	1.533	1.544	1.532	1.532
C(5)–N(6)	1.346	1.343	1.347	1.356
N(6)–C(7)	1.464	1.461	1.461	1.462
C(2)–O(11)	1.225	1.228	1.216	1.219
N(3)–H(12)	1.000	0.998	0.998	0.998
C(3)–C(13)	1.542	1.540	1.541	1.545
C(3)–C(14)	1.542	1.533	1.533	1.531
C(5)–O(15)	1.225	1.223	1.219	1.218
N(6)–H(16)	0.994	1.001	0.994	0.996
Bond angles ∠/°				
C(1)–C(2)–C(3)	114.7	114.4	114.6	114.3
C(2)–N(3)–C(4)	123.5	127.1	121.6	123.1
N(3)–C(4)–C(5)	105.4	110.3	111.1	107.4
C(4)–C(5)–N(6)	115.1	115.5	115.6	115.0
C(5)–N(6)–C(7)	122.3	121.3	120.2	121.0
O(11)–C(2)–N(3)	123.2	123.8	122.1	123.3
H(12)–N(3)–C(4)	114.5	115.1	117.0	116.7
C(13)–C(4)–C(5)	109.4	110.1	106.6	107.3
C(14)–C(4)–C(5)	109.3	108.2	109.2	109.4
O(15)–C(5)–N(6)	123.1	123.7	123.0	122.8
H(16)–N(6)–C(7)	119.6	117.8	119.3	119.0
Torsion angles/°				
ω_1	180.0	−177.9	−167.6	−170.9
φ	180.0	−74.6	−60.9	49.0
ψ	180.0	63.2	−38.5	−177.4
ω_2	180.0	178.4	−178.7	172.7

Table 3 Electrostatic charges computed from the 6-31G* wavefunctions for the four low-energy conformers of **2**

Atom	C_5	C_7	Helix	P_{II}	MCC[a]	AMCC[b]
C(1)	−0.517 682	−0.501 297	−0.503 923	−0.526 317	−0.511 568	−0.511 568
C(2)	0.893 325	0.872 063	0.825 914	0.924 201	0.878 951	0.878 951
N(3)	−0.735 262	−0.857 169	−0.782 137	−0.869 519	−0.789 013	−0.789 013
C(4)	0.420 917	0.555 330	0.410 412	0.571 370	0.470 086	0.470 086
C(5)	0.707 322	0.601 522	0.705 591	0.646 872	0.671 883	0.671 883
N(6)	−0.602 860	−0.585 168	−0.499 679	−0.530 262	−0.575 829	−0.575 829
C(7)	−0.037 073	0.010 915	−0.205 946	−0.104 544	−0.055 454	−0.055 454
H(8)	0.141 961	0.133 358	0.125 576	0.136 243	0.136 431	−0.141 668
H(9)	0.141 029	0.148 165	0.148 111	0.144 779	0.144 621	−0.141 668
H(10)	0.142 062	0.143 746	0.145 640	0.151 978	0.143 952	−0.141 668
O(11)	−0.674 316	−0.660 726	−0.609 807	−0.650 100	−0.658 417	−0.658 417
H(12)	0.327 455	0.385 746	0.317 674	0.329 795	0.343 291	0.343 291
C(13)	−0.553 470	−0.479 020	−0.439 722	−0.588 586	−0.516 150	−0.520 060
C(14)	−0.557 586	−0.492 606	−0.486 473	−0.512 941	−0.523 970	−0.520 060
O(15)	−0.600 981	−0.602 566	−0.605 981	−0.596 534	−0.602 233	−0.602 233
H(16)	0.369 176	0.385 746	0.317 674	0.326 588	0.362 684	0.362 684
H(17)	0.062 254	0.042 447	0.117 802	0.072 921	0.066 284	0.078 058
H(18)	0.061 993	0.043 148	0.099 592	0.072 921	0.063 416	0.078 058
H(19)	0.105 798	0.089 688	0.120 582	0.118 422	0.104 475	0.078 058
H(20)	0.217 086	0.122 879	0.085 861	0.118 037	0.160 914	0.139 513
H(21)	0.110 220	0.140 986	0.147 110	0.122 496	0.126 187	0.139 513
H(22)	0.127 503	0.119 068	0.150 447	0.164 363	0.131 579	0.139 513
H(23)	0.127 092	0.135 946	0.104 979	0.213 434	0.132 641	0.139 513
H(24)	0.108 007	0.106 828	0.111 755	0.140 750	0.110 779	0.139 513
H(25)	0.216 029	0.137 701	0.149 211	0.117 445	0.174 979	0.139 513
r.m.s.[c]	0.071	0.074	0.052	0.060	—	—

[a] Multiple conformations charges (see text). [b] Averaged multiple conformations' charges for equivalent methyl carbons and hydrogens (see text). [c] R.m.s. is the root mean square deviation of the fitting of the Coulombic potential generated by the electrostatic charges to the quantum mechanical molecular electrostatic potential (kcal mol^{-1}).

is the most adequate method for determining atomic point charges when the availability of experimental data is limited. Table 3 reports the electrostatic charges from SCF 6-31G* calculations for the four conformers of **2**. The atomic point charges provide an excellent representation of the SCF electrostatic potential. Thus, the r.m.s. values corresponding to the

Table 4 Dihedral angles (°) of AAibN obtained from different empirical potentials

	C_5		C_7		Helix		P_{II}	
Method	φ	ψ	φ	ψ	φ	ψ	φ	ψ
AMBER[a]	−179.3	180.0	−60.7	67.0	−47.9	−42.5	56.2	−177.5
ECEPP[b]	173	172	−76	59	−53	−40	60	169
Amodeo *et al.*[c]	180.0	180.0	−64.9	67.3	—	—	—	—
Amodeo *et al.*[d]	180.0	180.0	−76.7	61.7	−49.2	−30.5	58.8	−155.4
SCF/3-21G[a]	180.0	180.0	−74.6	63.2	−60.9	−38.5	49.0	−177.4

[a] Present work. [b] From ref. 47. [c] From ref. 8, flexible rotor model. [d] From ref. 8, rigid motor model.

Table 5 Relative energies (kcal mol^{-1}) of **2** obtained from different empirical potentials

Method	C_5	C_7	Helix	P_{II}
AMBER[a]	7.6	0.0	5.8	12.2
ECEPP[b]	15.5	0.0	−27.2	10.4
Amodeo *et al.*[c]	0.0	0.7	—	—
Amodeo *et al.*[d]	0.0	0.3	1.2	2.4
SCF/6-31G**[a]	0.0	0.9	2.0	4.0

[a] From present work. [b] From ref. 47. [c] From ref. 8, flexible rotor model. [d] From ref. 8, rigid rotor model.

fitting of the point charge electrostatic potentials to the rigorous quantum mechanical for all the conformers are in the range 0.05–0.07 kcal mol^{-1}.

Results indicate a dependence of the atomic charges on the conformation, especially on the dipeptide backbone atoms. The changes in atomic point monopole charges with the geometry could restrict the ability of force-field methodologies to sample correctly some conformational regions. In order to generate a reliable set of atomic charges valid over all the conformations considered, we followed a clever strategy developed by Richards and co-workers.[69] These authors average the charges evaluated for different conformations, weighting them according to the Boltzmann populations. The weights are given by the standard Boltzmann formula using the 6-31G* energies of the 3-21G optimized geometries at room temperature ($T = 298.15$ K). Table 3 shows calculated multiple conformations' electrostatic charges for **2**.

In force-field treatments of conformational flexible species, it is necessary to have identical charges on nuclei equivalents in terms of the simulation. Thus, all the hydrogens on a methyl group must bear the same charge, because otherwise, the three degenerate rotamers of the methyl would give rise to different energies. Forcing symmetry in methyl hydrogens have been carried out by averaging the three methyl hydrogen charges. The same procedure was followed in the case of the two equivalent β-carbon nuclei. Averaged multiple conformations charges are shown in Table 3.

The averaged multiple conformations charges were used in force-field calculations with AMBER 3.0A. Computations were performed following the procedure described in the Computational Methods section. Table 4 presents the dihedral angles found for the different conformers of **2**, and these are compared with the SCF 3-21G data. Geometries obtained with other empirical potentials, ECEPP[37] and a new force-field developed by Amodeo and Barone,[8] were also included in the Table for comparison. It is observed, in general, that force-field optimized structures are closest to the 3-21G ones. An interesting feature is that dihedral angles found with a rigid rotor model optimization, *i.e.* varying only the dihedral angles, are very similar to those found with a flexible rotor model, *i.e.* minimizing the energy with respect to all the geometrical variables.

In Table 5, we report the relative energies of our optimizations with the AMBER force-field. Results from other empirical potentials were also included for comparison. Large discrepancies occur in the relative order of the conformers of **2**. Thus, the relative ordering of the conformers provided by AMBER is $C_7 <$ helix $< C_5 < P_{II}$ as opposed to $C_5 < C_7 <$ helix $< P_{II}$ as found at all levels of *ab initio* calculations. On the other hand, ECEPP force-field provides the worst agreement with the present *ab initio* results.

Similar results were obtained recently by Böhm and Brode for the glycine dipeptide.[30] The authors found that the energy difference between the C_5 conformation and the C_7 conformation is overestimated in AMBER and other force-fields. Thus, *ab initio* SCF calculations with a triple ζ-plus polarization (TZP) basis set indicated that C_5 is 0.4 kcal mol^{-1} favoured with respect to C_7, whereas the AMBER force-field found for the C_5 conformation is 3.3 kcal mol^{-1} higher in energy than C_7.

Böhm and Brode point out some possible origins for the discrepancies between *ab initio* data and force-fields. Thus, they suspect that the hydrogen bond term in some empirical force-fields does not describe accurately the difference between C_5 and C_7 conformations. The results displayed in Table 5 give support to this idea. The use of sophisticated atomic point charges for the computation of electrostatic interactions seems not to be sufficient for obtaining reliable force-field results. On the other hand, Table 5 also shows the relative energies provided by the force-field developed by Amodeo and Barone.[8] This new generated force-field provides an excellent estimation of the relative energies for low-energy conformers of Aib dipeptide. This must be attributed to the improved description of the formalism used to describe the short-range repulsions between non bonded atoms.

In summary, force-field methodologies have proved their utility in the study of the helical region of the potential energy surface of oligomers of **1**.[37,43–48] However, the results of the present work clearly indicate that due to excessive repulsive or excessive attractive interactions, it may become necessary to recalibrate some force-fields when all the potential energy surface of the residue of **1** is considered. On the other hand, the force-field developed by Amodeo and Barone[8] seems to provide the best description of the short-range interactions between non-bonded atoms.

Conclusions

In the present study, we have investigated the relative energies for the four low-energy conformers of **2**. *Ab initio* calculations with 6-31G* and 6-31G** basis sets at 3-21G optimized geometries have pointed out a number of important conformational trends. In particular, the *ab initio* calculations indicate that the C_5 conformation is about 0.9 kcal mol^{-1} lower in energy than the C_7. The helix and P_{II} conformations are about 2.0 and 4.0 kcal mol^{-1} less stable than the C_5, respectively. In judging the relative energies shown in Table 1, it must be taken into account that correlation effects are not included at the Hartree–Fock level. Thus, it is clear that more accurate calculations including correlation effects are needed to provide a more quantitative estimation of the relative energies.

An *ab initio* SCF electrostatic force-field parameterization has been performed for **2**. Luque *et al.*[70] recently pointed out that the molecular electrostatic potential computed in regions outside the molecular van der Waals sphere remains largely unaffected by electron correlation. Consequently, the wavefunction determined at the SCF level with a 6-31G* basis set is able to reflect, with reasonable accuracy, the features of the MEP in the outer regions, including derived electrostatic atomic charges. Furthermore, multiple conformational charges were developed using the weighting of the charges for all the conformers according to the Boltzmann population.

Geometries obtained by force-field calculations are in excellent agreement with *ab initio* results. However, the relative energies found with the averaged multiple conformations charges are in poor agreement with *ab initio* results. Thus, *ab initio* calculations point to important deficiencies in some force fields with respect to the relative energies of the C_7 and C_5 conformations of **2**.

Acknowledgements

The authors are indebted to Professor F. Illas, Dr. J. Rubio, Dr. F. J. Luque and Dr. M. Orozco for computational facilities. We also thank Dr. F. J. Luque and Dr. M. Orozco for a copy of MOPETE and MOTODO programs, and for helpful discussions.

References

1 (*a*) F. A. Momany, R. F. McGuire, A. W. Burgess and H. A. Scheraga, *J. Phys. Chem.*, 1975, **79**, 2361; (*b*) S. S. Zimmerman, M. S. Pottler, G. Nemethyl and H. A. Scheraga, *Macromolecules*, 1977, **10**, 1.
2 (*a*) N. L. Allinger, *J. Am. Chem. Soc.*, 1977, **99**, 8127; (*b*) N. L. Allinger, Y. H. Yuh and J. -H. Lii, *J. Am. Chem. Soc.*, 1989, **111**, 8551.
3 P. Dauber-Osguthorpe, U. A. Roberts, D. J. Osguthorpe, J. Wolf, M. Genest and A. T. Hagler, *Proteins*, 1988, **4**, 31.
4 (*a*) S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765. (*b*) S. J. Weiner, P. A. Kollman, D. T. Nguyen and D. A. Case, *J. Comput. Chem.*, 1986, **71**, 230.
5 L. Nilsson and M. Karplus, *J. Comput. Chem.*, 1986, **7**, 591.
6 W. F. Gunsteren and H. J. C. Berendsen, *GROningen MOlecular Simulation* (*GROMOS*), ETH, Zurich, 1992.
7 W. L. Jorgensen and C. J. Swenson, *J. Am. Chem. Soc.*, 1985, **107**, 1489.
8 P. Amodeo and V. Barone, *J. Am. Chem. Soc.*, 1992, **114**, 9085.
9 K. C. Chou, G. Némethy, M. Pottle and H. A. Scheraga, *J. Mol. Biol.*, 1989, **205**, 241.
10 K. C. Chou, G. M. Maggiora, G. Némethy and H. A. Scheraga, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 4295.
11 A. T. Hagler and S. J. Lifson *J. Am. Chem. Soc.*, 1974, **96**, 5317.
12 A. T. Hagler, P. S. Stern, R. Sharon, J. M. Becker and F. Naider, *J. Am. Chem. Soc.*, 1979, **101**, 6842.
13 W. L. Jorgensen and J. Tirado-Rivers, *J. Am. Chem. Soc.*, 1988, **110**, 1657.
14 A. Y. Meyer and A. Y. Forrest, *J. Comput. Chem.*, 1985, **6**, 1.
15 D. M. Ferguson and P. A. Kollman, *J. Comput. Chem.*, 1991, **12**, 620.
16 J. E. H. Koehler, W. Saenger and W. F. Gunsteren, *J. Biomol. Struct. Dyn.*, 1988, **6**, 181.
17 I. K. Roterman, K. D. Gibson and H. A. Scheraga, *J. Biomol. Struct. Dyn.*, 1989, **7**, 391.
18 J. Mauri, F. Avbelj and D. Hadzi, *J. Mol. Struct.* (*THEOCHEM*), 1989, **187**, 307.
19 C. Alemán and J. J. Perez, submitted for publication.
20 (*a*) C. Alemán, E. I. Canela, R. Franco and M. Orozco, *J. Comput. Chem.*, 1991, **12**, 664; (*b*) C. Alemán and M. Orozco, *J. Comput.-Aided Mol. Design*, 1992, **6**, 331.
21 (*a*) J. R. Maple, U. Dinur and A. T. Hagler, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 5350; (*b*) U. Dinur and A. T. Hagler, *J. Comput. Chem.*, 1990, **11**, 1234.
22 G. Tasi, I. Kiricsi and H. Föster, *J. Comput. Chem.*, 1992, **13**, 371.
23 F. A. Momany, *J. Phys. Chem.*, 1978, **82**, 592.
24 U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1984, **5**, 129.
25 B. H. Besler, K. M. Merz and P. A. Kollman, *J. Comput. Chem.*, 1990, **11**, 431.
26 (*a*) M. Orozco and F. J. Luque, *J. Comput. Chem.*, 1990, **11**, 909; (*b*) F. J. Luque and M. Orozco, *Chem. Phys. Lett.*, 1990, **168**, 269; (*c*) M. Orozco and F. J. Luque, *J. Comput.-Aided Mol. Design*, 1990, **4**, 411. (*d*) C. Alemán, F. J. Luque and M. Orozco, *J. Comput. Chem.*, 1993, **14**, 799.
27 P. Lagant, P. Derreumaux, G. Vergoten and W. Perticolas, *J. Comput. Chem.*, 1991, **12**, 731.
28 J. L. M. Dillen, *J. Comput. Chem.*, 1992, **13**, 257.
29 K. B. Wiberg, *J. Am. Chem. Soc.*, 1979, **101**, 1718.
30 H. -J. Böhm and S. Brode, *J. Am. Chem. Soc.*, 1991, **113**, 7129.
31 T. Head-Gordon, M. Head-Gordon, M. J. Frisch, C. L. Brooks III and J. A. Pople, *J. Am. Chem. Soc.*, 1991, **113**, 5989.
32 J. H. Jensen and M. S. Gordon, *J. Am. Chem. Soc.*, 1991, **113**, 7917.
33 A. Perczel, J. Angyán, M. Kajtár, W. Viviani, J. L. Rivail, J.-F. Marcoccia and I. G. Csizmadia, *J. Am. Chem. Soc.*, 1991, **113**, 6256.
34 I. R. Gould and P. A. Kollman, *J. Phys. Chem.*, 1992, **96**, 9255.
35 K. Uma, I. L. Karle and P. Balaram, in *Proteins: Structure, Dynamics and Design*, eds. V. Renugopalaksishran, P. C. Carey, C. P. Smila, S. G. Huang and C. Storer, ESCOM, Leiden, 1991, pp. 295–300.
36 A. W. Burguess and S. J. Leach, *Biopolymers*, 1973, **12**, 2599.
37 Y. Paterson, S. M. Rumsey, E. Benedetti, G. Nemethy and H. A. Scheraga, *J. Am. Chem. Soc.*, 1981, **103**, 2947.
38 I. L. Karle, J. L. Flippen-Anderson, K. Uma and P. Balaram, *Curr. Sci.*, 1990, **59**, 1990.
39 R. Bosch, G. Jung, H. Schmitt, G. M. Sheldrick and W. Winter, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 450.
40 I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747.
41 C. Toniolo, G. M. Bonora, V. Barone, A. Bavoso, E. Benedetti, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone and C. Pedone, *Macromolecules*, 1986, **19**, 492.
42 A. Aubry, J. Protas, G. Boussard, M. Marraud and J. Neel, *Biopolymers*, 1978, **17**, 1693.
43 B. V. V. Prasad and V. Sasisekharan, *Macromolecules*, 1979, **12**, 1107.
44 G. Valle, M. Crisma, C. Toniolo, R. Beisswenger, A. Rieker and G. Jung, *J. Am. Chem. Soc.*, 1989, **111**, 6828.
45 E. E. Hodkin, J. B. Clark, K. R. Miller, G. R. Marshall, *Biopolymers*, 1990, **30**, 533.
46 C. Alemán, J. A. Subirana and J. J. Perez, *Biopolymers*, 1992, **32**, 621.
47 V. Barone, F. Fraternali and P. L. Cristinziano, *Macromolecules*, 1990, **23**, 2038.
48 C. Alemán and J. J. Perez, *Int. J. Quantum. Chem.*, 1993, **47**, 231.
49 W. J. Hehre, R. F. Stewart and J. A. Pople, *J. Chem. Phys.*, 1969, **51**, 2657.
50 J. S. Binkley, J. A. Pople and W. J. Hehre, *J. Am. Chem. Soc.*, 1980, **102**, 939.
51 P. C. Hariharan and J. A. Pople *Theor. Chim. Acta*, 1973, **28**, 213.
52 (*a*) W J. Hehre, R. Ditchfield and J. A. Pople, *J. Chem. Phys.*, 1972, **56**, 2257; (*b*) P. C. Hariharan and J. A. Pople, *Mol. Phys.*, 1974, **27** 209.
53 M. Dupuis, J. D. Watts, H. O. Villar and G. J. B. Hurst, *HONDO 7.0*, 1987.
54 M. J. S. Dewar, E. G. Zoebisch, E. H. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
55 S. Olivella, *QCPE Bull.*, 1984, **4**, 10.
56 J. J. P. Stewart, *QCPE Bull.*, 1983, **3**, 431.
57 F. A. Momany, *J. Phys. Chem.*, 1978, **82**, 592.
58 S. R. Cox and D. E. Williams, *J. Comput. Chem.*, 1981, **2**, 304.
59 W. H. Press, B. P. Flannery, S. A. Teukolsky and W. T. Vettering, *Numerical Recipes*, Cambridge University Press, Cambridge, 1986.
60 M. Conolly, *QCPE Bull.*, 1981, **1** 75.
61 F. J. Luque and M. Orozco, *MOPETE and MOTODO* programs, unpublished results.
62 U. C. Singh, P. K. Weiner, J. Caldwell and P. A. Kollman, *AMBER 3.0*, revision A by G. Seibel
63 V. Barone, F. Lelj, A. Bavoso, B. Di Blasio, P. Grimaldi, V. Pavone and C. Pedone, *Biopolymers*, 1985, **24**, 1759.
64 E. Benedetti, C. Toniolo, P. Hardy, V. Barone, A. Bavoso, B. Di

Blasio, P. Grimaldi, F. Lelj, V. Pavone, G. M. Bonora and I. J. Lingham, *J. Am. Chem. Soc.*, 1984, **106**, 8146.
65 E. Benedetti, V. Barone, A. Bavoso, B. Di Blasio, F. Lelj, V. Pavone, C. Pedone, G. M. Bonora, C. Toniolo, M. T. Laplawy, K. Kaczmarek and A. Redlinsky, *Biopolymers*, 1988, **27**, 357.
66 C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone and C. Pedone, *J. Biomol. Struct. Dyn.*, 1985, **3**, 585.
67 V. Pavone, E. Benedetti, E. Di Blasio, C. Pedone, A. Santini, A. Bavoso, C. Toniolo, M. Crisma and L. Sartre *J. Biomol. Struct. Dyn.*, 1990, **7**, 1321.
68 E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo and M. Crisma, *Biopolymers*, 1992, **32**, 453.
69 C. A. Reynolds, J. W. Essex and W. G. Richards, *J. Am. Chem. Soc.*, 1992, **114**, 9075.
70 F. J. Luque, M. Orozco, F. Illas and J. Rubio, *J. Am. Chem. Soc.*, 1991, **113**, 5203.

Paper 3/04793H
Received 9*th August* 1993
Accepted 28*th October* 1993