



PAPQMD parametrization of molecular systems with cyclopropyl rings: Conformational study of homopeptides constituted by 1-aminocyclopropane-1-carboxylic acid

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Summary

The suitability of ab initio, semiempirical and density functional methods as sources of stretching and bending parameters has been explored using the PAPQMD (Program for Approximate Parametrization from Quantum Mechanical Data) strategy. Results show that semiempirical methods provide parameters comparable to those compiled on empirical force fields. In this respect the AM1 method seems to be a good method to obtain parameters at a minimum computational cost. On the other hand, harmonic force fields initially developed for proteins and DNA have been extended to include compounds containing highly strained three-membered rings, like 1-aminocyclopropane-1-carboxylic acid. For this purpose the cyclopropyl ring has been explicitly parametrized at the AM1 level considering different chemical environments. Finally, the new set of parameters has been used to investigate the conformational preferences of homopeptides constituted by 1-aminocyclopropane-1-carboxylic acid. Results indicate that such compounds tend to adopt a helical conformation stabilized by intramolecular hydrogen bonds between residues i and $i + 3$. This conformation allows the arrangement of the cyclic side chains without steric clashes.

Molecular mechanical (MM) and dynamical (MD) methods have become useful tools for the study of the interactions and conformational properties of biological macromolecules [1–3]. All MM and MD methods are based on the assumption that the energy of a molecular system can be described as the addition of two contributions: (i) the bonded term, which includes the energy of stretching, bending, proper and improper torsions, and (ii) the non-bonded term, where the electrostatic, hydrogen bonding and van der Waals interactions are considered. The set of analytical expressions used to represent these interactions is known as the force field. To date, several force fields have

been developed and implemented in different computational programs [4–14], which are currently used in a wide range of chemical and biochemical problems.

All the analytical expressions used to compute both the bonded and non-bonded terms include several parameters, whose values are selected so that results derived from force field methods reproduce experimental data. Therefore, the study of any molecular system by MM and MD techniques implies the previous knowledge of all the parameters describing such a system. This leads to one of the most serious shortcomings of MM and MD methods, since even though MM and MD programs include large parameter data

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sets, several parameters describing a molecular system are often not available.

Experimental parametrization is the best alternative, but in most cases this is not possible due to the lack of experimental data. Quantum mechanical based parametrization appears to be a suitable alternative [15]. In this respect, some strategies have been reported to compute the non-bonded force field parameters [16–26]. Moreover, other strategies have been developed to calculate the bonded force field parameters, like those upon the decomposition of the quantum mechanical Hessian of the equilibrium geometry [27–32], or those based upon the fitting of molecular and quantum mechanical energy surfaces corresponding to the perturbation of selected parameters [33–35].

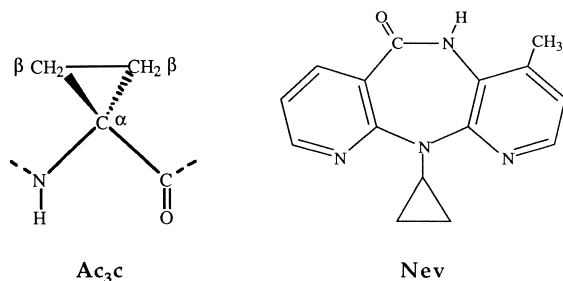
We are interested in the parametrization of highly strained compounds for simple force fields like AMBER [6,7] and TRIPOS [10]. These force fields use a simple harmonic approximation to represent the stretching and bending terms:

$$E_{\text{stretching}} = k_s(d - d_0)^2 \quad (1)$$

$$E_{\text{bending}} = k_b(\theta - \theta_0)^2 \quad (2)$$

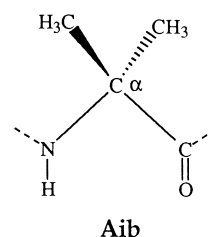
k_s and k_b are the stretching and bending force constant; d_0 and θ_0 are the equilibrium bond length and bond angle. It is well known that highly strained compounds are reasonably described by force fields with anharmonic and cross terms [14,36], i.e. named complex force fields, whereas harmonic force fields are much weaker in the representation of such systems. However, harmonic force fields have been explicitly parametrized for the simulation of large biological systems like proteins and DNA, and are much less computer time demanding than complex force fields. In contrast, complex force fields are usually employed in structural and spectroscopic studies of small organic systems.

It is interesting to note the recent appearance of some important biological compounds which contain a highly strained three-membered ring, i.e., 1-aminocyclopropane-1-carboxylic acid (Ac₃c) and nevirapine (Nev).



Nev contains a cyclopropyl ring attached to a seven-membered ring. Nev is one of the most promising non-competitive inhibitors of HIV-1 reverse transcriptase, which is of central importance for the therapeutic treatment of AIDS [37,38]. The structure of NEV complexed with enzymes was determined by X-ray crystallography [39]. However, the lack of suitable parameters hampers the design of a pharmacophoric model for non-competitive inhibitors of HIV-1 reverse transcriptase using MM or MD simulations.

On the other hand, Ac₃c is a C^{α,α}-disubstituted α-amino acid residue with a cyclic side chain. X-ray diffraction studies on a variety of peptides of this residue up to the tetramer level have indicated the marked structural preferences of Ac₃c for type-I β-turns [40,42]. Furthermore, ab initio quantum mechanical calculations on the conformational preferences of the Ac₃c dipeptide have been recently reported [43]. Computational studies [44–48] and X-ray diffraction analyses [49–51] of a variety of α-aminoisobutyric acid (Aib) based peptides have concluded that C^α-methylation induces a significant propensity for β-turns, providing 3₁₀-helices, i.e. consecutive β-turns, in the resulting homopeptides.



It should be noted that both Aib and Ac₃c are α, α-dimethylated compounds, the difference between them being determined by the bond between the two C^β-atoms in the latter. However, no study about the 3₁₀-helix forming tendency of Ac₃c in large homopeptides has been reported so far. Force field calculations of systems containing one or several Ac₃c residues would be useful to improve our understanding about the conformational preferences of such a residue. However, such calculations are not possible at the present time due to the lack of suitable force field parameters for the three-membered ring.

Parametrization of harmonic stretchings and bendings from quantum mechanical data can be difficult if accurate parameters are needed. The difficulty arises from the inadequacy of RHF-SCF calculations to reproduce molecular properties of perturbed geometries, where electron correlation effects must be considered

to obtain a proper description [52–56]. This is especially noticeable when stretching parameters which will represent elongated bonds are to be determined. In these cases, the quality of the calculations is increased up to multiconfigurational level wavefunctions [16,52–56].

Force constants obtained at this level of sophistication are completely comparable to stretching parameters compiled on empirical force fields, although high level ab initio multiconfigurational calculations are often computationally prohibitive. The last few years we have successfully used AM1 semiempirical calculations to obtain stretching and bending parameters for a wide range of compounds [34,35,57–59]. Thus, electron correlation is assumed to be partially included in semiempirical Hamiltonians by means of the parametrization of such methods. However, to our knowledge no rigorous comparison between harmonic force constants obtained from semiempirical and ab initio methods has been reported so far.

In this work a parametrization study of highly strained systems is presented. Furthermore, the derived parameters have been used to investigate the conformational preferences of Ac₃c homopeptides. The work has been divided in four different parts. First, we have compared the suitability of ab initio and semiempirical quantum mechanical methods to provide stretching and bending parameters. For this purpose equilibrium and force constants of 10 stretchings and 8 bendings were estimated at the HF/6-31G(d,p), MP2/6-31G(d,p), MP4/6-31G(d,p), B3-LYP/6-31G(d,p) and AM1 levels of theory. Second, we have explored the suitability of harmonic force fields to describe highly strained systems. Thus, we have computed all the stretching and bending parameters of cyclopropane (Cpr) using AM1 calculations. Such parameters were validated by comparison of equilibrium geometry and vibrational frequencies with available experimental data. Third, we have computed all the stretching and bending parameters for the three-membered rings of Ac₃c and Nev. Finally, force field calculations of homopeptides constituted by Ac₃c residues have been performed using the explicitly developed parameters.

Computational procedure

Quantum Mechanical Calculations

Geometrical parameters and force constants of 10 bonds and 8 angles corresponding to 7 different mole-

cules were computed at the ab initio SCF, MP2 and MP4 levels using the 6-31G(d,p) [60] basis set and at the semiempirical level using the AM1 [61] Hamiltonian. On the other hand, density functional theory (DFT) offers a promising tool that may be applied to large systems due to its computational efficiency. We have compared the performance of DFT calculations to provide stretching and bending parameters with that of ab initio and semiempirical methods. Becke's [62] tree-parameter hybrid functional with gradient corrections provided by the Lee, Yang and Parr [63] functional (B3-LYP) was used in this study. B3-LYP calculations were performed using the 6-31G(d,p) basis set. Stretching and bending parameters of Cpr and the three-membered rings of Ac₃c and Nev were obtained at the AM1 level of theory.

Parametrization Procedure

The optimized geometries were used to determine the equilibrium bond distances (d_o) and angles (θ_o). The stretching (k_s) and bending (k_b) force parameters were derived using the PAPQMD (Program for Approximate Parametrization from Quantum Mechanical Data) program [34]. Accordingly, each bond and angle type was perturbed from its equilibrium geometry. The quantum mechanical energy (ΔE^{QM}) of these perturbed structures was computed and then used in Equation 3, in which all the parameters are changed until the difference between force field (ΔE^{FF}) and quantum mechanical energy is minimal.

$$(\Delta E^{QM} - \Delta E^{FF})^2 = \text{minimum} \quad (3)$$

ΔE^{FF} is expressed (Equation 4) as the addition of the energy directly related to the variation of the perturbed geometric variable (ΔE^j) and the energy related to all the other variations in the molecular geometry (ΔE^{per}).

$$\Delta E^{FF} = \Delta E^j + \Delta E^{per} \quad (4)$$

Considering Equation 4, Equation 3 can be rewritten as:

$$(\Delta E^{QM} - \Delta E^j - \Delta E^{per})^2 = \text{minimum} \quad (5)$$

The strategy computes the corresponding perturbation term according to Equation 1 and 2, and subtracts it from the quantum mechanical energy. The corrected energy is then defined by:

$$\Delta E^{QM,cor} = \Delta E^{QM} - \Delta E^{per} \quad (6)$$

Consequently, the determination of the force parameters is carried out by considering all the perturbations that the modification of one geometrical variable

causes in all the others. For each bond and angle six perturbed geometries were considered. The large perturbations were 0.20 Å (single bonds), 0.15 Å (double bonds) and 12° (angles).

Force Field Frequency Calculations

Torsional and non-bonded parameters, which were used in the force field geometry optimization and frequency calculations of some small model molecules, were taken from the AMBER all atom force field (see Ref. 6). All the geometry optimizations were performed in vacuo ($\epsilon = 1$) using the conjugate gradient method, with a convergence test of 10^{-7} kcal/mol. Optimized geometries were used to compute the Hessian matrix by using a Langevin–Newton–Rampson method [64]. Such calculations allowed us to confirm the minima properties of the optimized geometries, as well as to compute the normal mode frequencies.

Conformational Analysis of Ac₃c Homopeptides

The conformational analysis of Ac₃c homopolypeptide has been carried out using MM by minimization of the conformational energy with respect to all the atomic coordinates. The conformational preferences of oligopeptides (Ac₃c)_m (the number of residues *m* varying from 5 to 13) were investigated. The peptides were blocked with an acetyl group at the N-terminal end and with an N-methylamide at the C-terminal end. In order to avoid end effects on the conformation, only the *m*–4 middle residues were considered when analyzing the results for a peptide with *m* residues [44,65].

Force field parameters for Ac₃c were derived in the present work, with the only exception of the torsional parameters that were taken from AMBER libraries [6]. Similarly, the parameters for the terminal groups were taken from AMBER libraries with the exception of the charges that were re-evaluated to make them coherent with those of Ac₃c. Charges for the Ac₃c residue as well as the acetyl and N-methylamide terminal groups were determined by fitting the AM1 molecular electrostatic potential (MEP) of the Ac₃c dipeptide to a set of atomic centered charges [66,67].

The energy was computed using a factor of 0.5 for the 1–4 interactions. Geometry optimizations were performed using a conjugate gradient algorithm. All the structures were minimized until the difference in energy between two successive iterations was less than 10^{-7} kcal/mol or the norm of the gradient for two

successive steps in the minimization was less than 0.1 kcal/mol Å.

Starting conformations for energy minimization were generated using the minima characterized at the HF/6-31G(d) level on the fully relaxed energy potential surface of Ac₃c dipeptide [43]: C₅ ($\phi, \psi = 180^\circ, 180^\circ$), C₇ ($\phi, \psi = -78.9^\circ, 38.7^\circ$) and P_{II} ($\phi, \psi = -71.2^\circ, 145.6^\circ$). For similarity with homopeptides constituted by Aib, a ₃₁₀-helix structure ($\phi, \psi = -40^\circ, -30^\circ$) was also considered as starting conformation, although it is not a minimum in the potential energy surface of Ac₃c dipeptide. Due to the achiral nature of the Ac₃c residue, these conformations are twofold degenerated, $\phi, \psi = -\phi, -\psi$. For each Ac₃c oligopeptide the four structures were built by repeating forms of these conformations, i.e., assigning to each residue of the chain the same values to the dihedral angles ϕ, ψ .

Ab initio computations were performed using the Gaussian-94 program [68]. AM1 calculations were carried out using a modified version of the MOPAC program [69,70]. The fitting of the energy profiles was carried out using the PAPQMD program [34, 35]. Force field geometry optimizations and frequency calculations were performed with the MIN and NMODE modules of the AMBER 3.0a set of programs [71]. Computations were carried out on the CRAY-YMP of the ‘Centre de Supercomputacio de Catalunya’ (CESCA).

Results and Discussion

Effect of the Theoretical Level in the Equilibrium Parameters

Equilibrium bond lengths and angles determined at the HF, MP2, MP4, AM1 and B3-LYP levels are compared with experimental values [72] in Table 1. The results reveal a notable similarity between the different methods. In general, the agreement between the experimental equilibrium parameters and the theoretical ones is quite reasonable. Thus, considering both the precision of the force field calculations and the computational efficiency of the AM1 method, semiempirical calculations are enough to provide equilibrium stretching and bending parameters.

Effect of the Theoretical Level in the Force Parameters

Stretching and bending force parameters computed from HF, MP2, MP4, AM1 and B3-LYP data are com-

Table 1. Equilibrium bond lengths (in Å) and bond angles (in degrees) determined from HF/6-31G(d,p), MP2/6-31G(d,p), MP4/6-31G(d,p), B3-LYP/6-31G(d,p) and AM1 computations. Experimental values are also included

#	Molecule	HF	MP2	MP4	B3-LYP	AM1	EXP ^a
Bond							
O-H	H ₂ O	0.943	0.974	0.972	0.969	0.961	0.957
C-C	CH ₃ CH ₃	1.526	1.531	1.533	1.530	1.500	1.536
C-H	CH ₃ CH ₃	1.086	1.098	1.099	1.096	1.117	1.091
C-O	CH ₃ OH	1.398	1.427	1.425	1.418	1.410	1.425
O-H	CH ₃ OH	0.942	0.973	0.971	0.969	0.964	0.945
H-S	H ₂ S	1.324	1.333	1.327	1.350	1.317	1.328
C=O	H ₂ CO	1.185	1.232	1.223	1.220	1.227	1.208
C-H	H ₂ CO	1.093	1.114	1.112	1.099	1.110	1.116
C=C	CH ₂ CH ₂	1.316	1.344	1.342	1.331	1.326	1.339
C-H	CH ₂ CH ₂	1.076	1.090	1.090	1.087	1.098	1.086
Angle							
H-O-H	H ₂ O	105.9	105.1	105.4	103.6	103.5	104.5
H-C-C	CH ₃ CH ₃	111.2	111.2	111.2	111.4	110.7	110.9
H-C-O	CH ₃ OH	112.1	112.9	112.8	110.7	110.8	108.5
C-O-H	CH ₃ OH	109.5	108.7	109.0	107.7	107.2	107.0
H-S-H	H ₂ S	94.5	93.1	92.8	92.8	98.1	92.2
H-C=C	CH ₂ CH ₂	121.7	121.8	122.0	121.9	122.7	121.2
H-C-H	CH ₂ CH ₂	116.6	116.8	116.6	116.3	114.9	119.4

^aFrom reference 59.

pared in Tables 2 and 3, respectively. These results show some clear trends about the importance of the level of theory used as reference. Thus, it can be noted that MP calculations lead to an important reduction in the ab initio HF force parameters. Similar effects were observed by other authors using sophisticated MCSCF and CI wavefunctions [27,52,54–56]. The results indicate that MP2 and MP4 calculations provide very similar force parameters, while the former is fairly less expensive than the latter. On the other hand, it should be noted that, in general terms, B3-LYP calculations also lead to a reduction of the HF force constants, which is even larger than that provided by MP calculations. These results suggest that the B3-LYP functional is better able to collect the effects of electron correlation in the force constants than the MP methods. Our computations also suggest that the electron correlation effects are about 10% more relevant for double bonds than for single ones, which stems from the large electron density between the atoms [60]. On the other hand, the suitability of Dewar's semiempirical methods [61,73,74] as a source of stretching parameters was recently tested by comparing the AM1, MNDO and MINDO/3 derived parameters with those reported in empirical force fields

[35]. This study suggested that if approximate values of stretching force constants are necessary, inexpensive and fast parametrization from the AM1 method seems a good choice. Results displayed in Tables 2 and 3 permit a comparison between AM1 derived force parameters and those obtained by both ab initio and DFT calculations.

The effects that the introduction of electron correlation has on equilibrium parameters and vibrational frequencies have been explored in the last years [27,52,74,75]. Different conclusions appear from such studies, depending on the parametrization procedure. Thus, some authors [27,75,76] claim that the effect of electron correlation is important for the determination of equilibrium parameters, since it produces a notable enlargement with respect to SCF results, but it is not relevant for the force parameters if they are obtained from a Hessian matrix computed at an accurate geometry. However, other authors using numerical methods like that of the present work, indicate that the effect of electron correlation is important, since it implies a reduction in the force constants of around 10–20% [52]. Our results agree with the latter, according to which correlation effects should be taken into account for

Table 2. Stretching force parameters (in kcal/mol Å²) for several bonds determined from HF/6-31G(d,p), MP2/6-31G(d,p), MP4/6-31G(d,p), AM1 and B3-LYP/6-31G(d,p) computations

Bond	Molecule	HF	MP2	MP4	AM1	B3-LYP	AMBER	TRIPOS
O-H	H ₂ O	747	622	621	640	508	553	504
C-C	CH ₃ CH ₃	369	344	346	390	320	310	317
C-H	CH ₃ CH ₃	428	402	400	374	331	350	346
C-O	CH ₃ OH	497	426	429	484	376	320	341
O-H	CH ₃ OH	747	622	621	505	549	553	331
H-S	H ₂ S	347	313	298	181	309	–	–
C=O	H ₂ CO	1273	997	1023	1219	1001	–	778
C-H	H ₂ CO	412	361	361	364	353	350	346
C=C	CH ₂ CH ₂	863	721	732	805	748	–	670
C-H	CH ₂ CH ₂	460	445	443	383	404	–	346

Experimental values compiled in the most used force fields for these bonds are listed in the last two columns, sequentially including the Kollman's AMBER and TRIPOS parameters (see text).

Table 3. Bending force parameters (in kcal/mol rad²) for several bonds determined from HF/6-31G(d,p), MP2/6-31G(d,p), MP4/6-31G(d,p), AM1 and B3-LYP/6-31G(d,p) computations.

Bond	Molecule	HF	MP2	MP4	AM1	B3-LYP	AMBER	TRIPOS
H-O-H	H ₂ O	56	52	53	64	59	35	32.8
H-C-C	CH ₃ CH ₃	80	74	74	48	45	35	26.3
H-C-H	CH ₃ CH ₃	74	58	59	36	48	35	26.3
H-C-O	CH ₃ OH	95	87	88	57	39	35	26.3
C-O-H	CH ₃ OH	62	58	59	69	64	55	32.8
H-S-H	H ₂ S	65	58	59	53	56	–	32.8
H-C=C	CH ₂ CH ₂	87	76	76	35	45	–	39.4
H-C-H	CH ₂ CH ₂	59	55	55	28	45	–	19.7

Experimental values compiled in the most used force fields for these bonds are listed in the last two columns, sequentially including the Kollman's AMBER and TRIPOS parameters (see text).

the parametrization of force constants from quantum mechanical data.

Results reveal qualitative similarity between the parameters obtained from the AM1 semiempirical method and those derived at different levels of sophistication. The change from AM1 to ab initio MP implies a reduction in the stretching parameters of 12% (MP2) and 9% (MP4), whereas the change to ab initio HF represents an increase in the stretching parameters of 11%. On the other hand, the B3-LYP parameters are about 6% smaller than the MP4 ones. Regarding the bendings, a poor intercorrelation was obtained between the different methods. However, in general terms semiempirical force constants are underestimated with respect to the ab initio ones, particularly with respect to the HF. Thus, the behaviour of the AM1 method seems to be better than that of

ab initio HF. Indeed, electron correlation is assumed to be partially included in semiempirical Hamiltonians by means of the parametrization of these methods.

Comparison of force parameters with those obtained experimentally is very difficult due to the discrepancy existing between the different force field parameters [34,35,52]. This discrepancy between empirically derived parameters is surprising at first glance, since all the force fields were determined by accurate parametrization procedures from experimental data. Therefore, this disagreement should be attributed to the fact that force field parameters are not fully transferable, since they strongly depend on the set of molecules which was considered during the parametrization procedure. This is clearly shown in Tables 2 and 3 where the force parameters reported in the

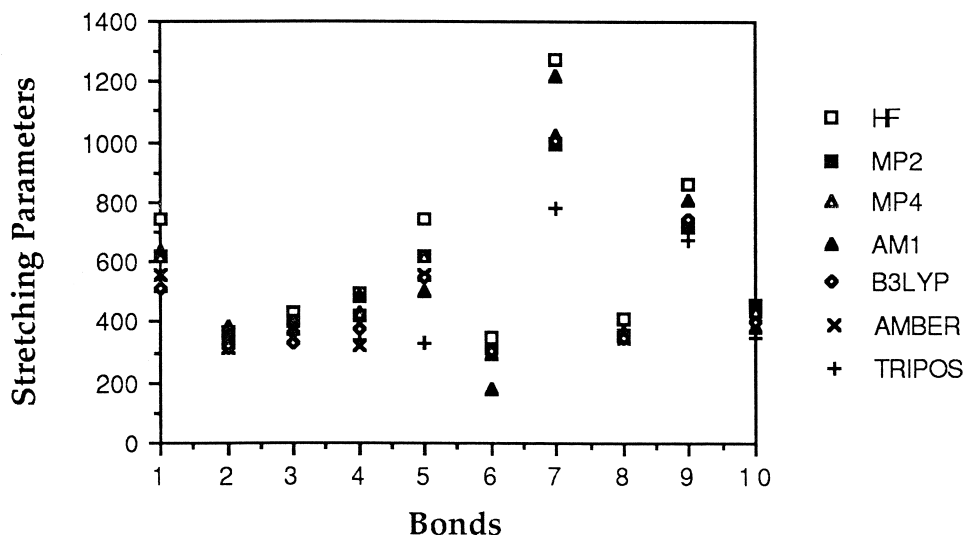


Figure 1. Graphical representation of the variation between stretching force constants obtained from quantum mechanical calculations and empirically derived ones: AMBER and TRIPOS.

AMBER [6,7] and TRIPOS [10] force fields, for the explored stretchings and bendings, are compared.

Figures 1 and 2 show graphically the variation between the empirical and quantum mechanical derived stretchings and bendings, respectively. As a general rule, ab initio HF considerably overestimates the rigidity of bonds and angles with respect to the empirical force fields, especially with respect to TRIPOS. The differences obtained between both B3LYP and AM1 derived force constants and those reported in the AMBER force field are small, whereas greater discrepancies appear when TRIPOS is considered as reference. On the other hand, our results state that the deviations between MP and empirically derived parameters, especially AMBER ones, are similar to those existing between empirical force fields, which suggests that a significant improvement is obtained when electron correlation is introduced in the computations.

The strong discrepancies between TRIPOS and the remainder parameters considered in this work, i.e. AMBER and quantum mechanical derived ones, must be attributed to the universal character of this force field. Thus, the so-called 'all purpose' force fields include parameters developed to represent any molecule. Consequently, this disagreement should mainly be related to the wide and different sets of molecules considered in the parametrization of the TRIPOS force field. On the other hand, the AMBER force field was developed for the study of nucleic acids and proteins. The good agreement found between quantum mechan-

Table 4. Experimental (see reference 76) and theoretical frequencies (in cm^{-1}) for methanol

Assignment	$\nu(\text{EXP})$	$\nu(\text{HF})$	$\nu(\text{MP4})$	$\nu(\text{AM1})$	$\nu(\text{B3-LYP})$
OH (str)	3682	4312	3932	3546	3696
CH ₃ (asym str)	2977	3347	3235	3125	2942
CH ₃ (asym str)	2977	3346	3234	3124	2941
CH ₃ (sym str)	2844	3229	3116	3012	2830
CH ₃ (def)	1477	2138	1947	1614	1605
CH ₃ (def)	1455	2018	1803	1439	1604
CH ₃ (def)	1430	2017	1802	1394	1538
COH (bend)	1340	1512	1425	1392	1374
CO (str)	1034	1143	1064	1052	915
CH ₃ -O (tor)	270	304	295	294	294

ical and AMBER parameters strongly suggests that parametrization in the latter was performed in molecules with prototypical bonds similar to those studied in this work, which contain the main functional groups found in both nucleic acids and proteins.

Finally, in order to have a better description of the quality of the theoretically derived force fields, we have calculated the frequencies of vibration for ethane, methanol and ethene using the parameters displayed in Tables 1, 2 and 3. Atomic charges for the three compounds were taken from previous work [78]. Results for methanol obtained using the parameters derived from HF, MP4, AM1 and B3-LYP calculations are displayed in Table 4, where the experimental val-

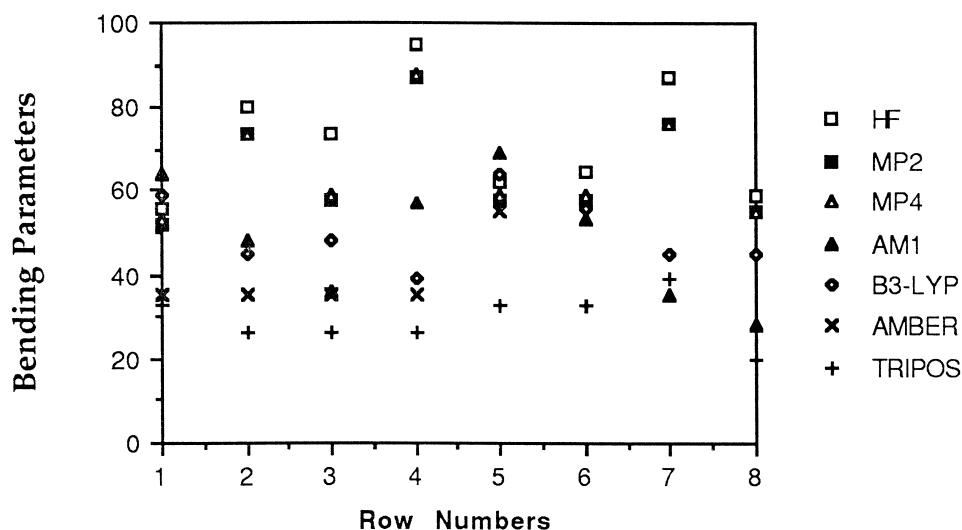


Figure 2. Graphical representation of the variation between bending force constants obtained from quantum mechanical calculations and empirically derived ones: AMBER and TRIPOS.

ues [77] are also listed. Results clearly point out the general quality of the PAPQMD derived parameters since the correlations between force field and experimental frequencies are almost perfect ($r > 0.99$) in all cases. However, the value of the scaling coefficients arising from such correlations indicates a strong dependence with the level of theory used to derive the force field parameters. Thus, the global deviations between the theoretical and experimental frequencies are 18% (HF), 10% (MP4), 2% (AM1) and 1% (B3-LYP). The poor behavior of HF parameters is consistent with the results previously found by other authors [27,52,54–56]. It is worth to note the excellent results provided by both AM1 and B3-LYP parameters, giving support to the general quality of the force field parameters derived from such quantum mechanical methods. Results for both ethane and ethene (data not shown) were similar to those of methanol, the best theoretical vibrational spectra being also provided by AM1 and B3-LYP parameters.

Parametrization of Cyclopropane

First, we have checked that AM1 and B3-LYP are the most suitable methods for the parametrization of strained rings, since these would be more demanding than simple molecules. Thus, we have computed the vibrational frequencies for the minimized structures of Cpr at the HF, MP2, AM1 and B3-LYP levels. Results are shown in Table 5, where experimental data [79] are also included. In all cases an excellent correlation ($r > 0.99$) was obtained between theoret-

ical and experimental frequencies. Experimental data are overestimated by 10% (HF), 7% (MP2) and 4% (AM1 and B3-LYP). These results suggest that semi-empirical and DFT are suitable quantum mechanical methods for the force field parametrization. Thus, we computed all the force field parameters for Cpr using the AM1 method due to its computational efficiency.

The equilibrium bonds and angles, and the stretching and bending force parameters of Cpr are displayed in Table 6. A qualitative comparison between the parameters derived for Cpr and those provided in the AMBER force field for acyclic compounds, which are also included in Table 6, reveals a reasonable agreement in the stretching parameters. Thus, the largest deviations are 0.025 Å (2%) in equilibrium bonds and 32 kcal/mol Å² (10%) in force constants. However, important differences appear in the bending parameters, being especially dramatic for the C-C-C angle. The force parameter derived for the C-C-C bending is 192 kcal/mol rad² (480%) larger than provided in the AMBER force field. This large value points out the highly strained nature of Cpr.

In order to get insight into the reliability of harmonic force fields to describe compounds containing three-membered rings, we compared both the optimized geometry and frequencies of vibration for Cpr computed from the AMBER force field using the AM1 parameters (AMBER/AM1) with those obtained using the complex force field (CFF) developed by Hagler and co-workers [14]. In Table 7 we present the molecular geometry of Cpr derived by different method-

Table 5. Experimental (see reference 79) and theoretical frequencies (in cm^{-1}) computed at the HF, MP2, AM1 and B3-LYP levels for cyclopropane

Assignment	$\nu(\text{EXP})$	$\nu(\text{HF})$	$\nu(\text{MP2})$	$\nu(\text{AM1})$	$\nu(\text{B3-LYP})$
CH (str)	3102	3399	3313	3208	3239
CH (str)	3083	3377	3296	3208	3218
CH (str)	3083	3377	3296	3208	3218
CH (str)	3038	3317	3218	3169	3153
CH (str)	3024	3303	3210	3140	3145
CH ₂ (bend)	1482	1680	1587	1615	1547
CH ₂ (bend)	1438	1616	1532	1435	1499
CH ₂ (bend)	1438	1616	1532	1435	1498
CH ₂ (rock)	1187	1331	1267	1403	1227
CH ₂ (rock)	1187	1331	1267	1213	1226
Ring breathing	1188	1303	1254	1167	1225
CH ₂ (twist)	1126	1269	1200	1156	1162
CH ₂ (wag)	1070	1225	1119	1154	1090
CH ₂ (wag)	1028	1193	1111	1025	1069
CH ₂ (wag)	1028	1193	1111	1025	1067
Ring defm	869	961	934	1025	894
Ring defm	869	961	934	1025	840
CH ₂ (rck)	854	925	889	860	867
CH ₂ (twist)	738	808	770	785	742
CH ₂ (twist)	738	807	770	785	742

ologies: (i) X-ray [80]; (ii) ab initio HF/6-31G(d,p) and MP4/6-31G(d,p); (iii) DFT B3-LYP/6-31G(d,p); (iv) semiempirical AM1; (v) AMBER/AM1; (vi) AMBER force field using the standard parameters [6] for acyclic compounds (AMBER/AC); and [vii] CFF developed by Hagler and co-workers [14].

The comparison of calculated and X-ray geometries points out the ability of force fields to reproduce experimental geometries. This is not surprising for the CFF since anharmonicity and cross terms have been included in the functional form of the force field. However, it is remarkable that the AMBER/AM1 optimized geometry, which has been derived using a diagonal quadratic force field, deviates on average only 0.007 Å and 1.7° from experimental data. These values are lower than the deviations of 0.011 Å and 3.9° obtained when using the standard AMBER/AC. On the other hand, average deviations of 0.001 Å and 0.4° are obtained between the CFF and AMBER/AM1 force fields.

The frequencies of vibration of Cpr obtained from AMBER/AM1, AMBER/AC and CFF of Hagler and co-workers (taken from reference 14) are displayed in Table 8, which can be compared with the experimental

Table 6. Equilibrium values and force field constants for stretching and bending of cyclopropane, computed from AM1 data and experimental parameters for acyclic compounds included in the AMBER force field

Stretching	AM1		AMBER	
	d_o	k_s	d_o	k_s
C-C	1.501	342	1.526	310
C-H	1.104	324	1.090	340
Bending	AM1		AMBER	
	θ_o	k_b	θ_o	k_b
C-C-C	60.0	232	109.5	40
H-C-C	119.1	36	109.5	50
H-C-H	111.7	24	109.5	35

Distances are given in Å and angles in degrees. The force parameters of stretching and bending are given in kcal/mol Å² and kcal/mol rad², respectively.

values displayed in Table 5. The graphical representation of theoretical versus experimental frequencies is shown in Figure 3. Results clearly point out the general suitability of the AMBER/AM1 force field. Thus, not only is the correlation between AMBER/AM1 and experimental frequencies almost perfect ($r > 0.98$), but the value of the scaling coefficient arising from such a correlation suggests a global deviation between AMBER/AM1 and experimental frequencies of 4%. The comparison of AMBER/AC and experimental frequencies gives a deviation of 6%. These results suggest that harmonic force fields are able to give a reasonable representation of the equilibrium structure of highly strained compounds like Cpr, but an explicit and rigorous parametrization is required. Finally, CFF frequencies present a deviation with respect to the experimental ones of 1%. As expected, this complex force field is able to fit experimental data better than the diagonal quadratic one.

Parametrization of

1-Aminocyclopropane-1-carboxylic Acid and Nevirapine

Tables 9 and 10 report the stretching and bending parameters for the cyclopropyl rings of Ac₃c and Nev, respectively. There is a general agreement between the parameters of Cpr (see Table 6) and those of Ac₃c and Nev. However, force constants for Ac₃c are slightly larger than those of Cpr and Nev since the former has the three-membered ring more strained than the latter. The worst agreement is found for the C^β-C^α-C^β angle,

Table 7. Comparison of experimental and calculated geometries for cyclopropane (distances and angles in Å and degrees respectively). Values in parentheses are deviations from the experiment.

	X-ray	HF	MP4	B3-LYP	AM1	AMBER/AM
C-C	1.514	1.497 (−0.017)	1.509 (−0.005)	1.509 (−0.005)	1.501 (−0.013)	1.501 (−0.013)
C-H	1.099	1.076 (−0.023)	1.089 (−0.010)	1.087 (−0.012)	1.104 (+0.005)	1.104 (−0.005)
C-C-H	117.9	118.1 (+0.2)	118.1 (+0.2)	118.1 (+0.2)	119.1 (+1.2)	118.9 (+1.0)
H-C-H	114.5	114.0 (−0.5)	114.0 (−0.5)	114.0 (−0.5)	111.7 (−2.8)	112.1 (−2.4)

Table 8. Force field frequencies (in cm^{-1}) for cyclopropane

Assignment	$\nu(\text{AMBER/AM1})$	$\nu(\text{AMBER/AC})$	$\nu(\text{CFF})$
CH (str)	2910	2956	3052
CH (str)	2921	2965	3049
CH (str)	2921	2965	3049
CH (str)	2854	2864	3009
CH (str)	2838	2847	3001
CH ₂ (bend)	1604	1725	1451
CH ₂ (bend)	1501	1604	1456
CH ₂ (bend)	1501	1604	1456
CH ₂ (rock)	1293	1170	1232
CH ₂ (rock)	1293	1170	1232
Ring breathing	1168	1171	1314
CH ₂ (twist)	998	1005	1017
CH ₂ (wag)	751	613	875
CH ₂ (wag)	1187	1000	1048
CH ₂ (wag)	1187	1000	1048
Ring defm	674	682	831
Ring defm	674	682	831
CH ₂ (rck)	607	352	650
CH ₂ (twist)	649	374	711
CH ₂ (twist)	649	374	711

Table 9. Equilibrium values and force field constants for stretching and bending of 1-aminocyclopropane-1-carboxylic acid, computed from AM1 data

Stretching	d_0	k_s
C ^α -C ^β	1.526	398
C ^β -H	1.106	350
C ^β -C ^β	1.497	376
Bending	θ_0	k_b
C ^α -C ^β -C ^β	60.6	248
H-C ^β -C ^α	118.8	40
H-C ^β -H	111.7	28
C ^β -C ^α -C ^β	58.7	288
H-C ^β -C ^β	117.8	36

Distances are given in Å and angles in degrees. The force parameters of stretching and bending are given in kcal/mol Å² and kcal/mol rad², respectively.

Table 10. Backbone dihedral angles (ϕ, ψ ; in degrees) of (Ac₃c)_m, where the number of residues m ranges from 5 to 13, obtained after energy minimization

m	C ₅	C ₇	P _{II}	3 ₁₀ -Helix
3	158, −170	−76, 20	−37, 111	−42, −36
5	150, −155	−77, 27	−46, 117	−43, −34
7	163, −168	−77, 26	−50, 120	−43, −32
9	161, −166	−77, 27	−52, 122	−43, −32
11	166, −171	−77, 28	−58, 127	−43, −30
13	162, −163	−77, 28	−62, 131	−43, −30

which is about 25% stronger for Ac₃c than for Cpr and Nev. These results point out the important role of the chemical environment on the parametrization procedure. Thus, the parametrization should be performed in a molecular model which largely resembles the molecule of interest.

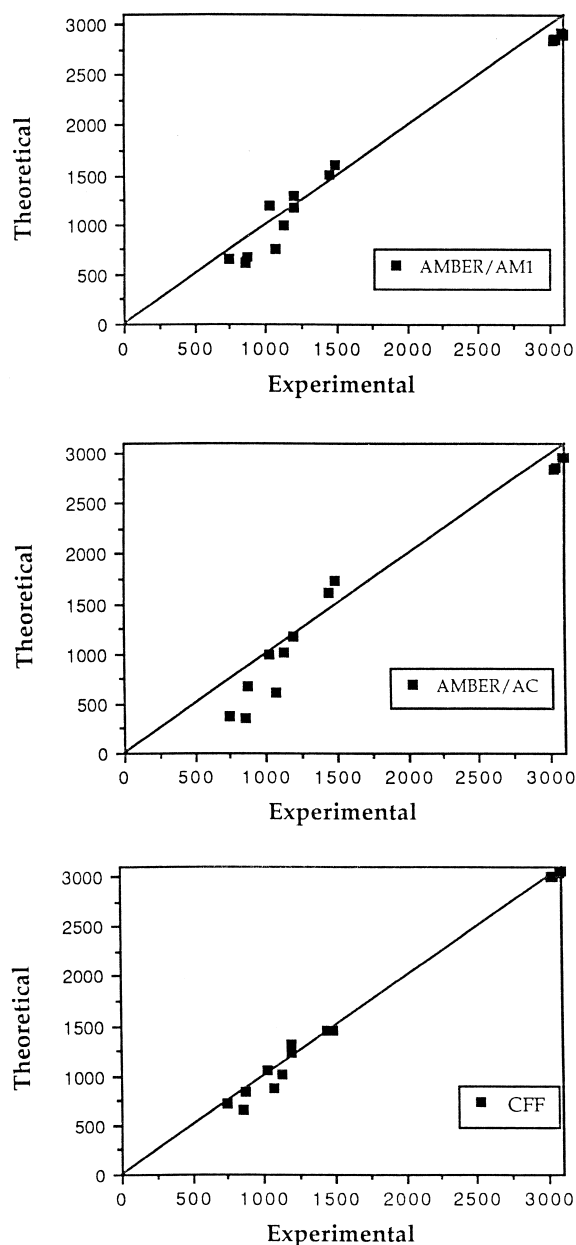


Figure 3. Graphical representation of the AMBER/AM1, AMBER/AC and CFF frequencies versus the experimental values (all in cm^{-1}) for cyclopropane.

Force Field Calculations of Homopeptides Constituted by 1-Aminocyclopropane-1-carboxylic Acid

The effects of atomic point charges are of dramatic importance in the reliability of force field calculations. Accordingly, the charges were obtained for the Ac_3c residue by fitting the Coulombic monopole-monopole

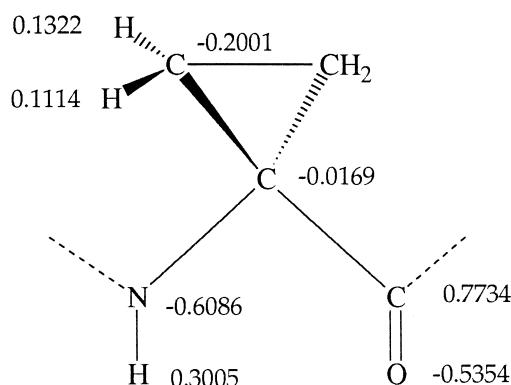


Figure 4. Electrostatic charges for the Ac_3c residue.

electrostatic potential to the AM1 electrostatic potential. Electrostatic charges derived from AM1 wavefunctions have been proven to be of similar quality to those derived at the HF/6-31G(d) level [26,81]. On the other hand, different authors have pointed out a dependence of the atomic charges on the conformation, which is more remarkable for the buried backbone atoms [58,82–84]. Accordingly, atomic charges were computed for the three minimum energy conformations characterized at the HF/6-31G(d) level for Ac_3c dipeptide [43]: C_7 , C_5 and P_{II} .

Results indicate that only those atoms buried in the Ac_3c residue, i.e. the carbon and nitrogen atoms of the amide group and the central α -carbon atom, are sensitive to the environment, showing a range of variation greater than 0.1 eu. The nitrogen atom shows the largest variation, from -0.431 to -0.700 . Thus, following the approach used in previous work [58,82,83] the charges evaluated from different conformations were weighted according to the Boltzmann populations. The weights are given by the standard Boltzmann formula at room temperature ($T = 298.15 \text{ K}$) using the MP2/6-31G(d) energies of the geometries optimized at the HF/6-31G(d) level [43]. The multiple conformations electrostatic charges computed for the Ac_3c residue are shown in Figure 4.

As stated in the Methods section, starting geometries for $(\text{Ac}_3\text{c})_m$ with $m = 5\text{--}13$ were generated considering C_5 , C_7 , P_{II} , and 3_{10} -helix regular conformations, i.e. identical dihedral angles for each residue. The dihedral angles (φ, ψ) obtained after energy minimizations are listed in Table 10. The variations of the angles (φ, ψ) with the peptide length were almost negligible, indicating that the present results could be extended to an infinite chain of poly- (Ac_3c) . Furthermore, the optimized angles for the C_7 and 3_{10} -helix

are very similar to the starting ones (see the Computational Procedure section), whereas the angles of C_5 and P_{II} , present deviations of about 20° and 10° respectively with respect to the initial values.

The change in the relative energy for the four different structures on the number of residues is shown in Figure 5. For a given number of residues the energy differences are determined per residue, i.e. in kcal/mol residue, and relative to the energy of the 3_{10} -helix.

It is worth to note that the 3_{10} -helix is the most favoured structure, irrespective of the number of residues. This structure contains an intramolecular hydrogen bond set between the $C=O$ group of residue i and the NH group of residue $i + 3$ (C_{10} form or type III β bend). Inspection of the optimized conformation (Figure 6a) reveals that there are no steric clashes between the cyclic side chains, but they pack within the structure in a very suitable way. The stability of the C_7 structure decreases slowly from $m = 5$ to $m = 13$, being about 4 kcal/mol residue less stable than the 3_{10} -helix. This structure consists of consecutive γ -turns, i.e. C_7 conformations, involving the carbonyl group of residue i and the NH group of residue $i + 2$, which give place to a 2_7 -ribbon (Figure 6b). The P_{II} structure is not stabilized by intramolecular hydrogen bonds, being about 6 kcal/mol residue less stable than the 3_{10} -helix (Figure 6c). Finally, the C_5 -conformation, which is an extended structure (Figure 6d), is about 8 kcal/mol residue less favoured than the 3_{10} -helix. This structure seems to be very constrained due to the cyclic side chains.

Unfortunately, there is no experimental information about the preferred conformation of homopeptides constituted by Ac_3c residues. However, recent X-ray studies have indicated that, when inserted in a peptidic sequence, Ac_3c is accommodated in β -bends which can be related to the 3_{10} -helix [40,42]. This trend is in good agreement with our predictions for $(Ac_3c)_m$ homopeptides. On the other hand, comparison with previous ab initio calculations on Ac_3c dipeptide also reveals a good agreement [43]. Thus, the relative energy order found at both HF/6-31G(d) and MP2/6-31G(d) levels of theory for the Ac_3c dipeptide was: $C_7 < P_{II} < C_5$, which agrees with that found for the $(Ac_3c)_m$ homopeptides using force field calculations. Obviously, the 3_{10} -helix conformation was not considered for the Ac_3c dipeptide since there were not enough residues to form such intramolecular hydrogen bonds.

Conclusions

The results presented in this paper allow us to obtain several general conclusions about the level of sophistication for the quantum mechanical calculations used as reference in the force field parametrization procedure. MP2 and MP4 calculations lead to an important reduction in the HF stretching and bending force parameters. On the other hand, B3-LYP calculations also lead to a reduction of the HF force constants, which is even larger than that provided by MP calculations. These results suggest that the B3-LYP functional is able to collect the effects of the electron correlation in the force constants more than the MP methods. The range of error from semiempirical AM1 calculations is smaller than that found from more sophisticated HF calculations, especially when the empirical AMBER force field data base is used as reference. Comparison between theoretical and experimental frequencies for a set of small molecules reveals the excellent results provided by both AM1 and B3-LYP parameters. Thus, semiempirical force constants, which can be obtained at a low computational cost, seem to be adequate to represent the minima and the closely related conformations of molecular systems.

We have used the AM1 method to derive the stretching and bending parameters of cyclopropyl rings for different compounds. The predicted parameters have been tested in Cpr by computing both the molecular geometry and frequencies. Furthermore, such test calculations have been used to investigate the goodness of diagonal quadratic force fields in highly strained systems. Results are satisfactory. Thus, although complex force fields provide better results than simple force field, the latter give a reasonable representation of the cyclopropyl ring.

Finally, the developed parameters have been used to investigate the conformational preferences of homopeptides constituted by Ac_3c residues. Results have indicated that a helical conformation stabilized by intramolecular hydrogen bonds set between residues i and $i + 3$ is the most favoured arrangement, in good agreement with experimental data.

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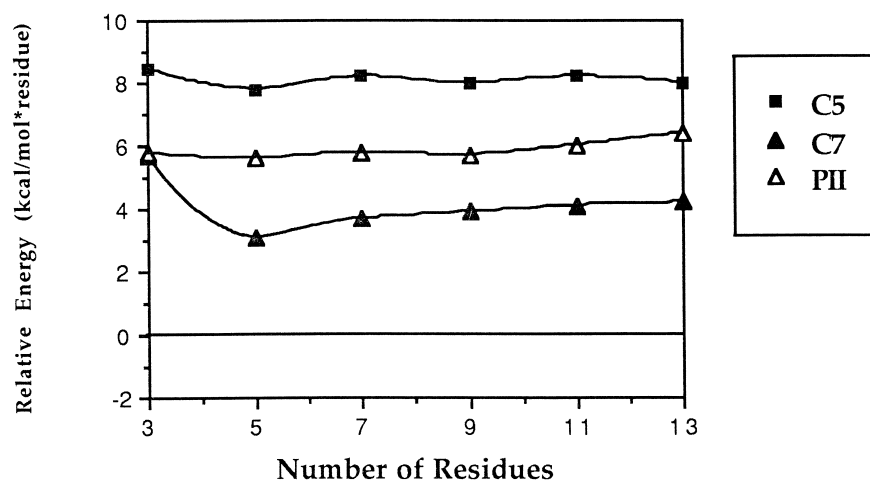


Figure 5. Relative energies (kcal/mol residue) of the C₅, C₇ and P_{II} structures relative to the 3₁₀-helix for homopeptides of Ac₃c.

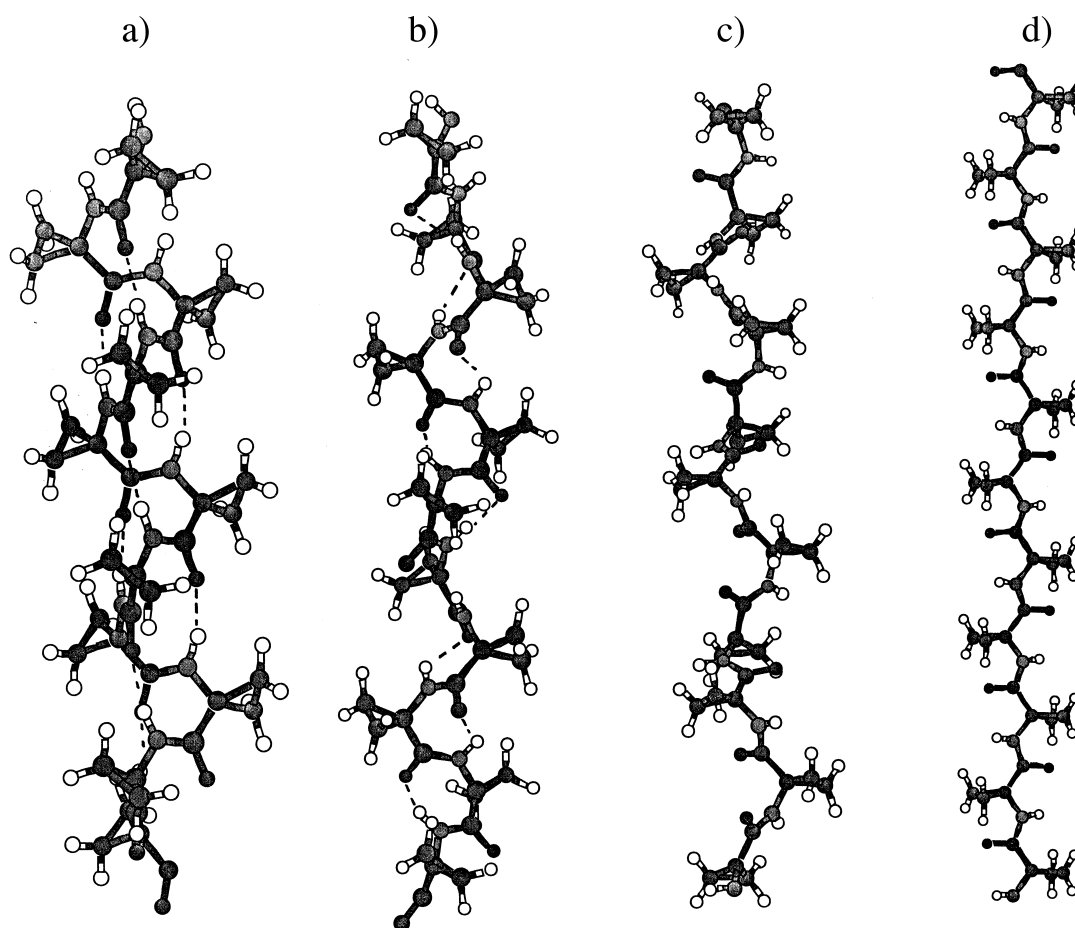


Figure 6. Energy minimized structures for (Ac₃c)₁₃: (a) 3₁₀-helix; (b) C₇ structure; (c) P_{II} structure; and (d) C₅ structure.

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