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Conformational analysis of six- and twelve-membered ring compounds by molecular dynamics

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Summary

A molecular dynamics (MD)-based conformational analysis has been performed on a number of cycloalkanes in order to demonstrate the reliability and generality of MD as a tool for conformational analysis. MD simulations on cyclohexane and a series of methyl-substituted cyclohexanes were performed at temperatures between 400 and 1200 K. Depending on the simulation temperature, different types of interconversions (twist-boat–twist-boat, twist-boat–chair and chair–chair) could be observed, and the MD simulations demonstrated the expected correlation between simulation temperature and ring inversion barriers. A series of methyl-substituted 1,3-dioxanes were investigated at 1000 K, and the number of chair–chair interconversions could be quantitatively correlated to the experimentally determined ring inversion barrier. Similarly, the distribution of sampled minimum-energy conformations correlated with the energy-derived Boltzmann distribution. The macrocyclic ring system cyclododecane was subjected to an MD simulation at 1000 K and 71 different conformations could be sampled. These conformations were compared with the results of previously reported conformational analyses using stochastic search methods, and the MD method provided 19 out of the 20 most stable conformations found in the MM2 force field. Finally, the general performance of the MD method for conformational analysis is discussed.

Introduction

The majority of biologically interesting compounds are flexible, or contain some flexible parts, and a detailed knowledge of the conformational characteristics is a prerequisite for understanding the relationship between molecular structure and a variety of properties, especially biological activity. Numerous methods for studying the conformational behavior of flexible compounds have been developed [1].

For molecules with a limited number of degrees of freedom, it is desirable to map the complete potential energy surface by systematically generating all possible conformations followed by energy minimization with respect to all except certain structural variables. This approach not only allows a determination of all minima on the potential energy surface, but also the estimation of barrier heights associated with interconversion between the low-energy conformations. Unfortunately, for very flexible compounds, this approach requires considerable computer resources, although a number of improvements have been developed to speed up the systematic search approach [2].

However, for many purposes, it is sufficient to identify minima on the potential energy surface. One way of reducing the combinatorial problem of searching the conformational space is to incorporate different kinds of constraints. In heuristic methods conformational rules are used to combine templates with precalculated conformational characteristics [1,3,4], whereas distance-geometry-based methods use intramolecular distances as constraints [1,5].

Nonsystematic search methods include stochastic (Monte Carlo) and time-dependent (molecular dynamics, MD) based methods [6]. In a Monte Carlo search the conformational space is explored by imposing small ran-

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dom or pseudorandom variations to the initial structure. If allowed to run for sufficient time, the stochastic search will cover all regions of the conformational space. In an MD simulation the motion of a molecule can be simulated by a combination of the molecular mechanics principle for calculation of energy and Newton's equations of motion. The trajectory contains information about the structural and energetic changes of the molecule as a function of time. Since the energy fluctuates during an MD simulation, energy barriers may be crossed and new conformations generated.

MD simulations have been widely used in protein modelling for years, and it has become an integral part of experimental structure determination as well [7,8]. The potential of MD simulations in the conformational analysis of low-molecular-weight compounds is, however, still relatively unexplored. A number of research groups have applied MD for the investigation of specific conformational problems of different type and complexity (e.g. neurotransmitters [9], insecticides [10], cryptands [11] and saccharides [12]). Only a few groups have focused on the application of the method in general. Itai and co-workers [13] used high-temperature MD simulations for mapping the pathways for the conformational interconversion of cyclohexane. Saunders et al. [14] investigated a number of stochastic search methods for the conformational analysis of cycloheptadecane and they included MD simulations and distance geometry for comparison. Although none of the methods were able to identify all low-energy conformations in a single run, some methods performed better than others. The MD simulations turned out rather poorly, but as pointed out in the paper a referee had considered MD simulations to be more efficient as a global search method than originally suggested by the authors. In the work by Böhm et al. [15], a nine-membered lactam was used for comparing the efficiency of various search procedures and the authors concluded that the stochastic search and MD simulations were comparable in completeness and efficiency of searching the conformational space. Both these papers deal with the efficiency of finding low-energy conformations, whereas Zhang and Mattice [16] focused more on the ability to correlate with experimental data. They showed that MD simulations on cyclohexadecane provided results that were in good agreement with experimentally determined conformer populations. It was also demonstrated that very long (ns) simulations were required for obtaining equilibrium conditions.

A general problem with an MD-based conformational analysis is to explore the conformational space properly during a simulation at normal temperature and within a practical simulation time, because the simulation time normally is too short compared to the actual time scale of conformational interconversions [17]. Furthermore, the barrier height between low-energy conformations may also prevent or diminish the interconversion between low-energy conformations. In principle, this problem can be solved by increasing the simulation temperature, since the probability of passing high-energy barriers increases with the temperature, but simulations at very high temperature may also be problematic because more distorted conformations are generated [18].

Here, we report the results of conformational analyses of six- and 12-membered ring compounds by MD. The aim of the study is to verify MD simulations as a valuable tool in the conformational analysis of small molecules in terms of simplicity, generality and reliability.

We have chosen to study cyclohexane and a number of methyl-substituted cyclohexanes, because this class of compounds probably represents one of the most intensively studied classes of compounds [19] and thus enables a critical reference frame for our MD simulations. 1,3-Dioxane and a number of methyl-substituted dioxanes have previously been investigated by NMR [20]. As these compounds show a remarkable variation in inversion barriers, they have been used to demonstrate the correlation between experimentally determined ring inversion barriers and the number of transitions in an MD simulation. Furthermore, we have studied the conformational flexibility of a macrocyclic ring system, cyclododecane, by MD. This cycloalkane was selected because it has previously been studied in great detail using different search methods [21–23], and this allows a direct comparison between different approaches for conformational analysis.

Methods

The SYBYL molecular modelling system (versions 6.03 and 6.1) [24] was used for manipulation and comparison of the different structures. Energy minimizations were performed using the Tripos force field [25] and the MAXIMIN2 minimizer [26] with a convergence criterion of 0.005 kcal/(mol Å). The dielectric constant was set to $\varepsilon = 20$ in order to provide a more reasonable balance between steric and electrostatic interactions (I.T. Christensen and F.S. Jørgensen, submitted). Partial point charges were calculated by the PM3 method (MOPAC 5.0) [27].

MD simulations were performed at constant temperature by coupling the system to a thermal bath. The temperature coupling factor was reduced to 10 fs in order to ensure a satisfactory number of molecular transitions. The integration time step was set to 1 fs and nonbonded interactions were updated every 25 fs. Initial velocities were obtained from a Boltzmann distribution and bonds to hydrogen atoms were constrained by the SHAKE algorithm. The system was heated to the simulation temperature during 10 ps. After the heating period, each simulation was continued for an additional 1000 ps. A simulation temperature of 1000 K was applied if nothing else is reported. Coordinates were saved every 1 ps during

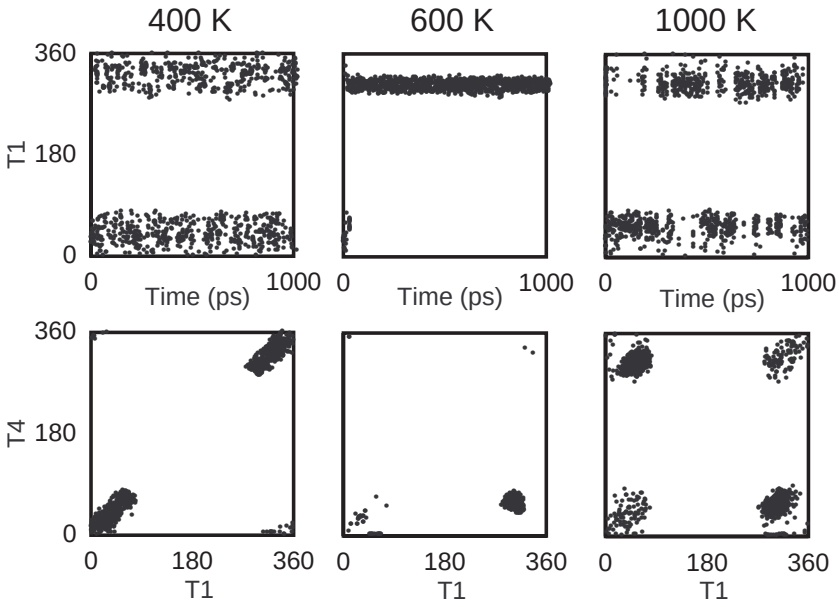


Fig. 1. MD simulation of cyclohexane at 400, 600 and 1000 K with a twist-boat conformation as the starting geometry. In the upper row, a ring torsional angle (T1) is shown as a function of simulation time, illustrating the frequency of conformational transitions. In the bottom row, two oppositely positioned ring torsional angles (T1 and T4) are shown, illustrating the distribution of conformations. At 400 K cyclohexane readily interconverted between various twist-boat conformations. At 600 K the molecule was converted to a chair conformation during the heating period, but no chair–chair interconversions were seen. At 1000 K several ring inversions were seen.

the simulation period. In order to be able to observe low-energy transitions, coordinates from the heating period were included in the analysis. Analyses of MD trajectories were performed by a number of SPL (SYBYL Programming Language) macros*.

The programs MM2(91) [28] and MM3(92) [29] were accessed through the MacMimic [30] molecular modelling system.

Results and Discussion

Cyclohexane and methyl-substituted cyclohexanes

Saturated six-membered rings are capable of adapting a maximum of eight different minimum-energy conformations: two chair conformations and six twist-boat conformations. Depending on the symmetry of the molecule, some of these minima are either enantiomeric or degenerate conformations. The eight conformations can be unambiguously classified by measuring four consecutive ring torsional angles.

Initially, a series of MD simulations on cyclohexane using different start conformations and different simulation temperatures were performed. At 400 K the twist-boat conformation of cyclohexane readily interconverted between other twist-boat forms without possessing sufficient energy to convert to any of the two chair conformations. By increasing the temperature to 600 K the twist-boat was converted to one of the chair conformations during the heating period, but no chair–chair interconversions were observed. A further increase of the temperature to 1000 K yielded chair as well as twist-boat conformations and several chair–chair interconversions were observed (cf. Fig. 1).

The energy barriers for the ring inversion of cyclohexane, 1,1-dimethylcyclohexane, 1,1,3,3-tetramethylcyclohexane and 1,1,4,4-tetramethylcyclohexane have been determined by NMR spectroscopy [31] (cf. Table 1). The

TABLE 1
EXPERIMENTALLY DETERMINED RING INVERSION BARRIERS FOR METHYL-SUBSTITUTED CYCLOHEXANES AND 1,3-DIOXANES

Compound	Inversion barrier[a]	Reference
Cyclohexane	10.5	31
1,1-Dimethylcyclohexane	10.6	31
1,1,3,3-Tetramethylcyclohexane	9.6	31
1,1,4,4-Tetramethylcyclohexane	11.6	31
1,3-Dioxane	9.9	20
2,2-Dimethyl-1,3-dioxane	7.8	20
4,4-Dimethyl-1,3-dioxane	8.6	20
5,5-Dimethyl-1,3-dioxane	11.2	20
2,2,4,4-Tetramethyl-1,3-dioxane	<5.5	20
2,2,5,5-Tetramethyl-1,3-dioxane	8.9	20
4,4,5,5-Tetramethyl-1,3-dioxane	10.1	20
4,4,6,6-Tetramethyl-1,3-dioxane	5.9	20
2,2,4,4,5,5-Hexamethyl-1,3-dioxane	6.5	20

[a] Energies are given in kcal/mol.

*The macros are available, on request, from the authors (e-mail: inge@medchem.dfh.dk).

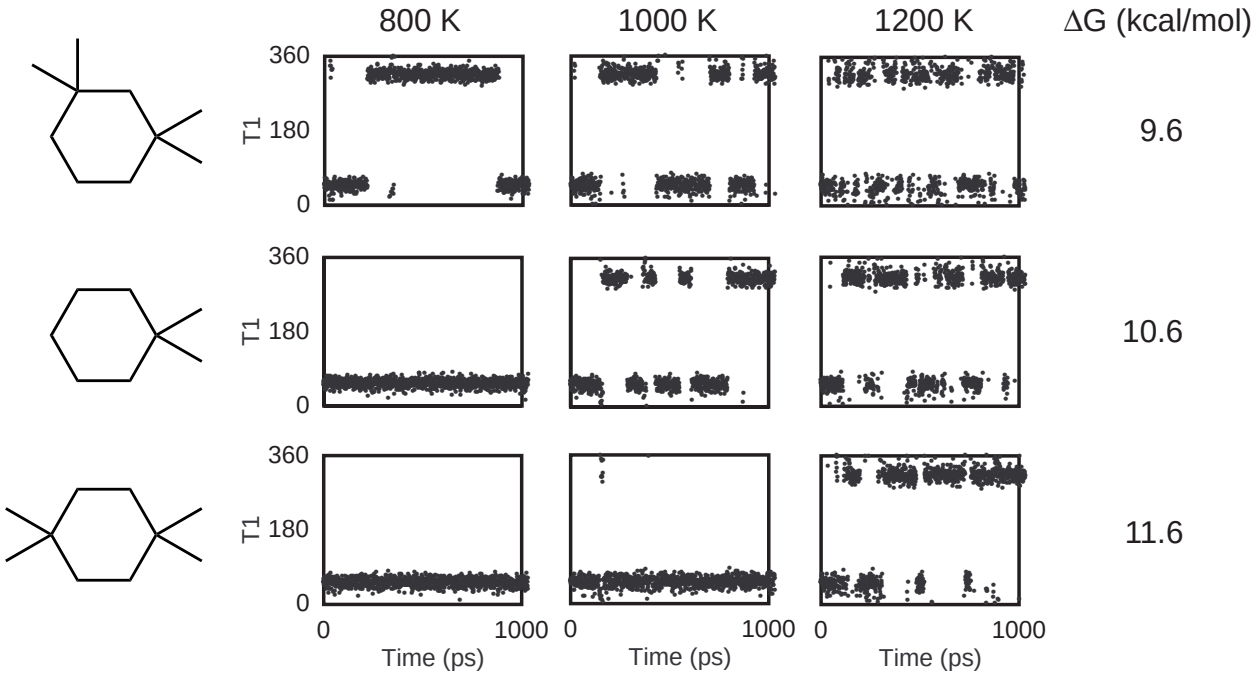


Fig. 2. MD simulations on 1,1-dimethylcyclohexane, 1,1,3,3-tetramethylcyclohexane and 1,1,4,4-tetramethylcyclohexane at 800, 1000 and 1200 K. A ring torsional angle (T1) is shown as a function of simulation time, illustrating the number of chair–chair interconversions at different temperatures. To the right, the experimentally determined ring inversion barriers [31] are listed.

ring inversion barrier of the disubstituted analogue is similar to that of the parent compound, 10.6 and 10.5 kcal/mol for 1,1-dimethylcyclohexane and cyclohexane, respectively. By introducing two additional methyl groups in the ring system, the inversion barrier either becomes lower (1,1,3,3-tetramethylcyclohexane) or higher (1,1,4,4-tetramethylcyclohexane). The reduced barrier height of the former is due to unfavorable 1,3-diaxial interactions in the chair conformations giving rise to a destabilization of the ground state, whereas the increased barrier height of the latter is probably due to destabilization of the transition state.

TABLE 2
COUNTS AND RELATIVE ENERGIES OF SAMPLED LOW-ENERGY CONFORMATIONS OF METHYL-SUBSTITUTED 1,3-DIOXANES[a]

1,3-Dioxane	Unsubstituted	2,2-	4,4-	5,5-	2,2,4,4-	2,2,5,5-	4,4,5,5-	4,4,6,6-	2,2,4,4,5,5-
Chair	**924**	**772**	**346**	**941**	**212**	**861**	**460**	**340**	**431**
	0	0	0	0	0	0	0	0	0
Chair'			**476**		**182**		**449**		**287**
			0		0		0		0
Twist-boat 1	**21**	**121**	**33**	**39**		**54**	**26**	**214**	**129**
	6.5	5.2	5.8	6.8		5.7	6.4	0.8	3.0
Twist-boat 1'	**26**	**118**	**36**	**31**		**96**	**32**	**197**	**131**
	6.5	5.2	5.8	6.8		5.7	6.4	0.8	3.0
Twist-boat 2	**19**		**37**		**223**		**18**	**133**	
	7.4		6.3		1.6		7.6	2.5	
Twist-boat 2'			**36**		**241**		**6**		
			6.3		1.6		7.6		
Twist-boat 3			**27**		**79**		**13**	**3**[b]	**17**
			6.4		3.9		8.5	4.8	7.0
Twist-boat 3'	**21**		**20**		**74**		**7**	**124**	**16**
	7.4		6.4		3.9		8.5	2.5	7.0
Transitions	**47**	**112**	**77**	**27**	**173**	**59**	**52**	**105**	**93**
Inversion barrier	9.9	7.8	8.6	11.2	<5.5	8.9	10.1	5.9	6.5

[a] Bold numbers refer to counts and plain numbers to energies (kcal/mol).
[b] Distorted twist-boat 2 conformation.

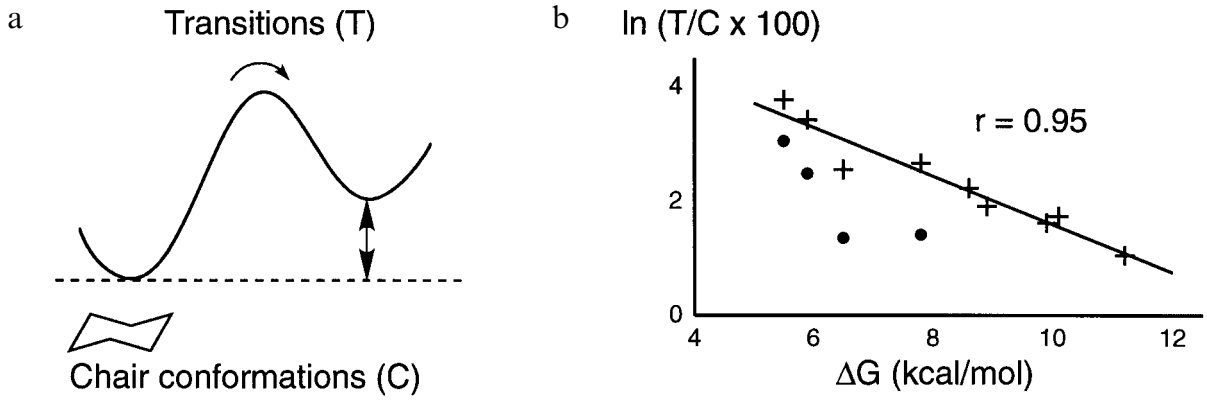


Fig. 3. Ring inversion frequencies of methyl-substituted 1,3-dioxanes as obtained by MD simulations compared to the experimentally determined ring inversion barriers [20]. (a) Ring inversion frequencies were calculated as the number of times a molecule leaves a chair conformation (T) relative to the total number of chair conformations (C). (b) Ring inversion frequencies expressed as $\ln(T/C \times 100)$ as a function of the experimentally determined ring inversion barriers (+: 1000 K; ●: 800 K). The line represents the best line through the data obtained at 1000 K (correlation coefficient 0.95).

The three methyl-substituted cyclohexanes were subjected to MD simulations at 600, 700, 800, 900, 1000, 1100 and 1200 K. The variations of one of the ring torsional angles during representative MD simulations are displayed in Fig. 2. For 1,1-dimethylcyclohexane a limited number of chair–chair interconversions were observed at 1000 K, whereas the ring inversion frequency increased considerably at 1100 and 1200 K. Simulations on 1,1,3,3-tetramethylcyclohexane and 1,1,4,4-tetramethylcyclohexane nicely reflected the relative magnitude of experimentally determined ring inversion barriers. Thus, 1,1,3,3-tetramethylcyclohexane interconverted at 800 K, whereas 1,1,4,4-tetramethylcyclohexane did not interconvert below 1200 K.

Simulations on cyclohexane and the methyl-substituted analogues illustrate what should be expected from theory: kinetic energy (simulation temperature) determines the ability to cross energy barriers. A comparison of the methyl-substituted cyclohexanes also indicates that, within closely related molecules, it is possible to deduce the ranking of energy barrier heights from the trajectory. Thus, MD simulations on simple molecules can, in favorable cases, be analyzed directly from the high-energy conformations sampled during the simulation without subsequent energy minimization.

1,3-Dioxane and methyl-substituted 1,3-dioxanes

1,3-Dioxane and a series of methyl-substituted analogues have previously been investigated by dynamic NMR [20]. From Table 1 it is obvious that the energy for ring inversion depends heavily on the substitution pattern. As for the cyclohexane series, 1,3-substitution lowers the inversion barrier considerably. Furthermore, it can be concluded that substitution in the 5-position increases the barrier, whereas substitution in the 2-position lowers the barrier. These effects are most pronounced for the four iso-

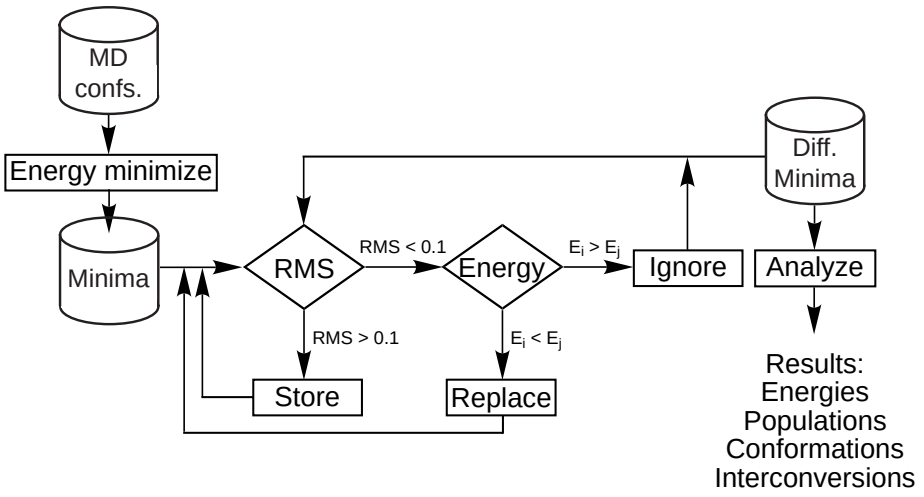


Fig. 4. Flow chart illustrating the method for the classification of low-energy conformations.

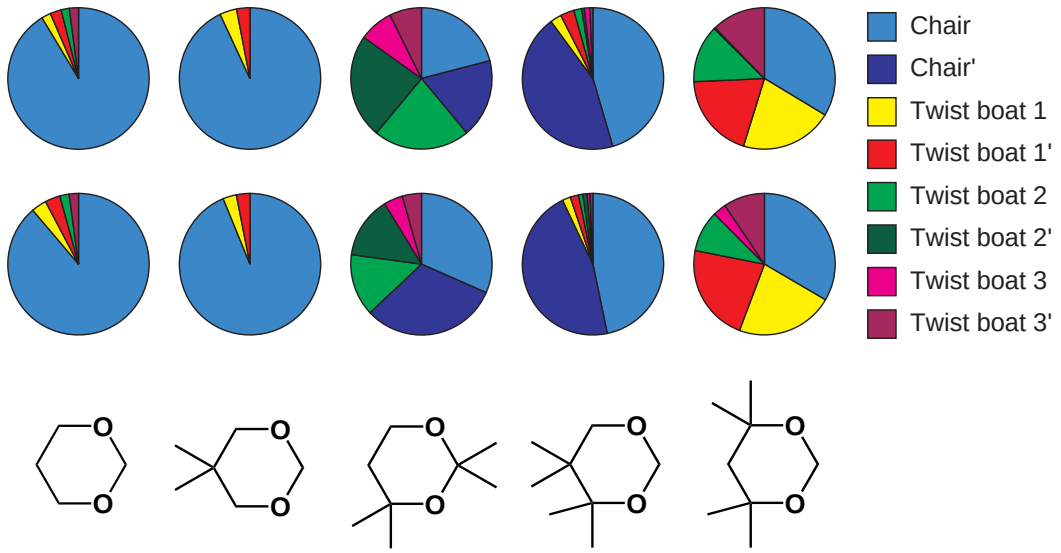


Fig. 5. Conformer populations of selected methyl-substituted 1,3-dioxanes as obtained by MD simulations compared to the calculated Boltzmann distributions. The Boltzmann distributions (top row) were calculated using the steric energy and a temperature of 1000 K. The MD-derived conformations (bottom row) were classified and counted according to Fig. 4.

meric tetrasubstituted analogues 4,4,5,5-, 2,2,5,5-, 4,4,6,6- and 2,2,4,4-tetramethyl-1,3-dioxane, displaying inversion barriers ranging from 10.1 to <5.5 kcal/mol.

Initially, the nine dioxane analogues were subjected to MD simulations at 1000 K in 1000 ps. For each simulation all sampled conformations were energy minimized and classified according to four consecutive ring torsional angles. Then the number of chair–chair interconversions were counted as the number of times the molecule leaves a chair conformation. As the relative energies of the twist-boat conformations were strongly dependent on the substitution pattern, there was a considerable variation in the total number of chair conformations within the series of analogues (cf. Table 2). Therefore, the number of interconversions (T) were taken relative to the total number of chair conformations (C) in order to reflect the frequency of ring inversion (cf. Fig. 3a). In Fig. 3b the ring inversion frequencies obtained by MD simulations are compared with the experimentally determined inversion barriers.

Figure 3 illustrates that there was a strong correlation between the number of transitions observed in the MD simulations and the barrier heights measured by NMR. Only the heavily substituted analogue 2,2,4,4,5,5-hexamethyl-1,3-dioxane seemed to be an outlier. For the four low-barrier compounds, 2,2,4,4-tetramethyl-1,3-dioxane, 4,4,6,6-tetramethyl-1,3-dioxane, 2,2,4,4,5,5-hexamethyl-1,3-dioxane and 2,2-dimethyl-1,3-dioxane, the simulations were repeated at 800 K. The results of these calculations were consistent with the 1000 K data; thus, the hexasubstituted analogue was still an outlier, whereas the other three compounds displayed the same correlation between calculated and experimental values. A similar analysis of the cyclohexanes revealed that the two series of molecules did not fit to the same curves (data not shown). Qualitatively, the cyclohexanes displayed a similar correlation between calculated and experimental values, but due to the small number of compounds and the narrow range of experimental data a correlation coefficient was not calcu-

TABLE 3
COUNTS AND TRANSITIONS FROM FIVE INDIVIDUAL MD SIMULATIONS OF 1,3-DIOXANE AT 1000 K

Conformation	MD run 1	MD run 2	MD run 3	MD run 4	MD run 5	Mean
Chair + chair'	912	892	916	911	926	911
Chair	446	580	453	371	334	437
Chair'	466	312	463	540	592	475
Twist-boat 1	23	29	25	19	25	24
Twist-boat 1'	26	25	25	22	20	24
Twist-boat 2	13	17	13	13	12	14
Twist-boat 2'	18	20	11	14	7	14
Twist-boat 3	12	15	13	14	11	13
Twist-boat 3'	7	13	8	18	10	11
Transitions	50	54	53	57	42	51

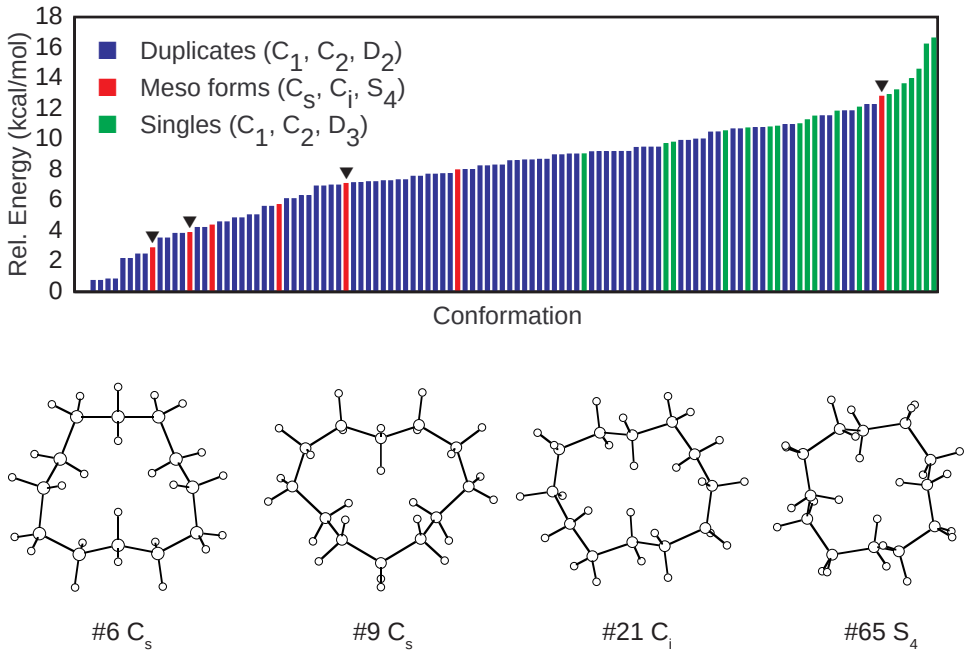


Fig. 6. Relative energies of minimum-energy conformations of cyclododecane obtained by MD simulation at 1000 K in 1000 ps. The conformations are sorted according to increasing energy and color coded according to symmetry. Chiral conformations are expected to be found in duplo (blue), but some high-energy conformations are only represented by one of the enantiomers (green). Achiral conformations are shown in red. The three-dimensional representations of four selected meso forms marked by triangles are shown at the bottom.

lated. Thus, it can be concluded that the correlation is only valid within a series of closely related molecules and it should not be extrapolated to other series of compounds. This finding may explain why the single hexasubstituted dioxane apparently did not fit to the dioxane curve.

Cyclohexanes and 1,3-dioxanes represent a very simple group of compounds as all low-energy conformations can easily be described by four consecutive ring torsional angles. In order to extend the generality of the method, it was necessary to develop another classification method. The principle of the classification method is illustrated in Fig. 4. All sampled structures were subjected to an energy minimization and the minimum-energy conformations were stored in a database. Then, all conformations were compared two by two in terms of an rms fit for all heavy atoms, considering conformations with an rms distance below 0.1 Å (0.2 Å for cyclododecane) as being identical. In the case of identical conformations, the conformation possessing the lowest energy was kept. Finally, the number of representatives of each low-energy conformation was counted. The resulting counts for the MD simulations on the dioxanes are listed in Table 2.

For 1,3-dioxane five individual MD simulations at 1000 K were performed in order to establish whether the simulations were reproducible. These calculations showed that the number of transitions varied within approximately 20%. From Table 3 it can be seen that there also was a considerable variation in the absolute number of representatives of the different conformations. This is most

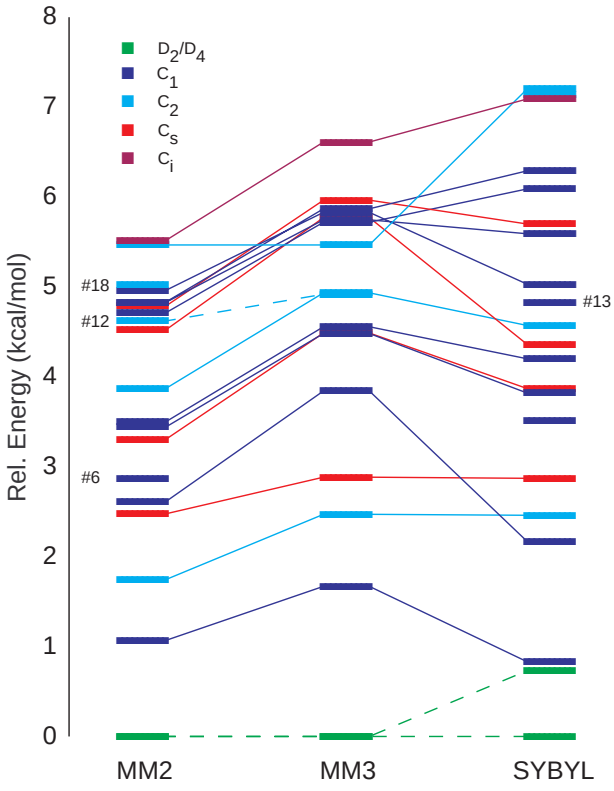


Fig. 7. Energy diagram showing the 20 most stable conformations of cyclododecane as found by stochastic search [23] and by MD simulation. Identical conformations are connected by full lines, whereas similar conformations are connected by broken lines. Conformations are colored according to symmetry. For details, see text.

TABLE 4
CONFORMER POPULATION FROM MD SIMULATION AND CALCULATED BOLTZMANN DISTRIBUTION FOR 1,1,3,3-TETRAMETHYLCYCLOHEXANE

Conformation	ΔE (kcal/mol)	800 K		1000 K		1200 K	
		Count	Population (%)	Count	Population (%)	Count	Population (%)
Chair + Chair'	0	95.0	92.0	90.8	84.3	74.4	76.4
Twist-boat 1	5.6	1.0	2.6	3.2	4.9	7.0	7.2
Twist-boat 1'	5.6	1.4	2.6	1.9	4.9	8.1	7.2
Twist-boat 2' + 3'	6.7	1.3	1.4	2.1	2.9	4.4	4.6
Twist-boat 2 + 3	6.7	1.2	1.4	2.1	2.9	6.1	4.6

clearly seen by comparing the counts of two enantiomeric conformations, e.g. chair and chair' or twist-boat 1 and twist-boat 1'. The reason for this uncertainty is doubtless that the systems have not reached equilibrium during the MD simulation.

As the aim of this study was to verify the usefulness of MD simulations as a tool for conformational analysis, it was crucial to demonstrate that the method was reliable in terms of identifying all low-energy conformations. Therefore, it was tested whether the obtained counts were in accordance with the ideal energy-derived Boltzmann distribution. In Table 4 the Boltzmann-derived populations are compared with the obtained counts from three simulations on 1,1,3,3-tetramethylcyclohexane. The results clearly show the temperature dependence of the conformer distribution. At low temperatures the global minimum is favored, but increased temperatures secure the population of local minima of higher energies. Similarly, the Boltzmann distribution was calculated for the whole series of dioxanes. In Fig. 5 the results from five selected compounds are illustrated. The upper row corresponds to the Boltzmann distribution, whereas the bottom row corresponds to the counts obtained by the MD simulations. For some of the compounds, not all the eight possible low-energy conformations are minima on the potential energy surface. For the symmetrically substituted compounds, the two chair conformations chair and chair' are identical. Except for one compound, the counts and the population according to the Boltzmann distribution were very similar. 2,2,4,4-Tetramethyl-1,3-dioxane was clearly an outlier, apparently giving too high counts for the two chair conformations during the MD simulation. This discrepancy is, however, more likely to be caused by the calculated Boltzmann distribution as these populations were calculated from the enthalpy. Generally, the chair conformations of saturated six-membered rings are favored by enthalpy, whereas the twist-boat conformations are favored by entropy due to the lack of symmetry and the possibility of pseudorotation [32]. Thus, it seems likely that by calculating the Boltzmann distribution solely from the enthalpy, the stabilities of the most stable twist-boat forms of 2,2,4,4-tetramethyl-1,3-dioxane are underestimated.

Cyclododecane

As previously mentioned, various macrocyclic compounds have been subjected to comparative conformational analyses using different search methods [14,15]. Here we report the results of an MD simulation on cyclododecane. Although cyclic, the molecule is extremely flexible; thus, a 1000 ps simulation was not expected to give an exhaustive exploration of the conformational space. Nevertheless, the simulation provided important information on the reliability of MD simulation as a tool for conformational analysis.

The trajectory was analyzed as described above following the principles of Fig. 4. The resulting 116 low-energy conformations were sorted according to energy as shown in Fig. 6. The majority of conformations were found as pairs of enantiomeric conformations, but even among the conformations of low relative energy some were found only as one stereoisomer. All these conformations were analyzed individually, and out of 26 single conformations seven possessed internal mirror planes (C_s, C_i or S_4 symmetry) and thereby could be classified as meso forms. Most important, all single conformations of low energy were meso forms. These results indicate that within 9 kcal/mol above the global minimum, all low-energy conformations were found, provided no low-energy meso forms were overlooked. Thus, even in a relatively short simulation time the MD method provided 45 pairs of enantiomeric conformations, seven meso forms and 19 conformations only represented by one of the enantiomers, giving a total of 71 different conformations. Most often, enantiomeric conformations are pooled in conformational analysis, but in this case they served as a measure of reliability, as all conformations ideally should be generated as enantiomeric pairs.

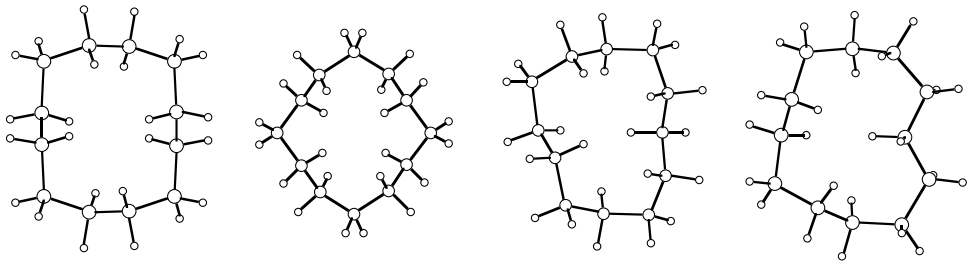
Cyclododecane has previously been subjected to conformational analysis [21–23]. By far, the most comprehensive study was done by Kolossváry and Guida [21], who identified 121 energy minima at the MM2 potential energy surface and, in addition, a complete set of interconversion pathways. Pertsin et al. [22] used cyclic alkanes as a test system in a Monte Carlo search, but only the five most stable conformations of cyclododecane were listed. Among these conformations, MM2_4 (conformation #4

in the MM2 force field) was not found. Using a stochastic search procedure, Saunders [23] found 102 and 87 conformations in MM2 and MM3, respectively. By combining the results of these two searches, a total of 111 MM2 conformations and 90 MM3 conformations were identified. Thus, none of the methods provided all the possible conformations in one run. Compared to MM2 and MM3, the MD search provided a considerably smaller number of conformations, but it should be emphasized that only 1000 conformations were sampled in the MD search, whereas as many as 3500 conformations were sampled in the stochastic searches by Saunders [23].

In Fig. 7 the energies of the listed conformations from Saunders' work [23] are compared with the MD-generated conformations. Out of the 20 most stable MM2 conformations, 18 matches could be identified in the MM3 search. No counterparts to the conformations MM2_6 and MM2_18 were found by MM3, but as the tabulated conformations are the combined results of both MM2 and MM3 searches it must be concluded that MM2_6 is not a minimum at the MM3 potential energy surface, whereas MM2_18 probably corresponds to an MM3 conformation outside the range of the 20 tabulated conformations. Similarly, the results obtained by Saunders were compared with the MD search. Enantiomeric conformations were deleted from the list of MD-generated conformations and all conformations were classified in terms of symmetry. All three methods provided a highly symmetrical conformation as the global minimum. In MM3 this conformation had D_4 symmetry, whereas the MM2 counterpart was slightly distorted as pointed out by Saunders. The two most stable conformations found in the MD search were very similar to MM3_1, but they both had slightly lower symmetry (D_2) like in MM2. It was found that out of the 20 most stable MM2 conformations, 19 matches could be identified in the MD search. The MD search did not provide a conformation corresponding to MM2_12. The reason for this discrepancy could either be that the actual conformation was not a minimum at the potential energy surface or that the search procedure failed to find it. Consequently, MM2_12 was constructed and energy minimized within SYBYL. In MM2 the conformation had C_2 symmetry and yielded upon energy minimization in the Tripos force field a very similar conformation. The corresponding MM3 conformation (MM3_9) possessed two additional mirror planes having C_{2v} symmetry. By energy minimization the achiral conformation converged to the mirror image of the C_2 symmetric conformation found by MM2 (MM2_12). Thus, a pair of enantiomeric C_2 symmetrical conformations similar to the one found in MM2 were found to be stable in the Tripos force field. The relative steric energy of the missing conformation was determined to be 6.6 kcal/mol, which is significantly higher than the energies of the corresponding conformations in MM2 and MM3 (4.6 and 4.9 kcal/mol, respectively). This might provide an explanation as to why the MD search failed to find it. Furthermore, the conformation was symmetrical and was therefore expected to be disfavored by entropy as well as by enthalpy.

The MD search provided a conformation (MD_13) that was not found among the 20 listed MM2 and MM3 conformations. Therefore, the conformation was constructed in MM2 as well as in MM3 and geometry optimized. The conformation did not correspond to a minimum-energy conformation at the MM3 potential energy surface as the final geometry and energy corresponded perfectly to conformation MM3_2 (equal to MM2_2 and MD_3). Energy minimization in MM2 did, however, provide a new energy minimum at the potential energy surface. Although somehow related to MM2_2, this conformation was unique (rms 0.39 Å between MM2_2 and the new conformation), and with a relative steric energy at 3.6 kcal/mol it was expected to be in the range of the 20 tabulated conformations.

Although the system was not expected to be in equilibrium, the obtained counts were compared to the relative



Conformation	#1 D_2	#2 D_2	#3 C_1	#4 C_1
Rel. Energy	0.0	0.7	0.8	2.2
Count (+)	30	6	46	28
Count (-)	33	7	64	58

Fig. 8. Three-dimensional representations of four selected low-energy conformations of cyclododecane. The relative energies and the obtained counts of the two corresponding enantiomeric conformations (count (+) and count (–)) are shown at the bottom.

energies. In Fig. 8 four low-energy conformations are shown together with the relative steric energies and the obtained counts. From these numbers it is clear that the counts not only reflect the enthalpy but also include differences in entropy. Thus, the D_2 symmetrical conformations MD_1 and MD_2 were disfavored by entropy and, thereby, the counts were lower than expected from the enthalpy. Similarly, all the other symmetrical conformations were found in relatively low counts.

Conclusions

The conformational analysis of small, flexible molecules is a very essential operation in molecular modelling. The major problem is to find all low-energy conformations with a high degree of certainty, irrespective of the different types and degrees of conformational flexibility. Thus, the most important features of a conformational analysis method are that it should be reliable, general and simple.

In the previous section it has been demonstrated that the MD method is a powerful tool for exploring the conformational space of even very flexible compounds. The method was reliable in terms of finding all low-energy minima, and the obtained counts were generally in accordance with the Boltzmann distribution. For cyclododecane, the entropic terms greatly influenced the correlation between obtained counts and expected conformer population. However, as this compound is very unusual in terms of symmetry a more direct correlation between enthalpy-derived Boltzmann population and obtained counts should be expected for almost any compound of biological interest. Similarly, it was demonstrated that there was a nice correlation between simulation temperature and number of conformational transitions on the one hand and experimentally determined energy barriers on the other hand.

By combining MD simulations with a series of macros, a simple and generally applicable procedure for conformational analysis has been developed. The procedure comprises a 1000 ps simulation at 1000 K, followed by an energy minimization of all sampled structures. Then, all minimum-energy conformations are sorted and counted based on an rms fit and, finally, a database containing all low-energy conformations is obtained.

By performing a number of MD simulations on three groups of model compounds, we have shown that the MD method is a reliable, simple and general tool for conformational searching. Thus, the method represents a very useful alternative to other methods for the conformational analysis of small and flexible compounds.

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