How to Predict Conformations Accessible to a Molecule in Solution: Validation of a Force Field-Based Prediction of NOE Distances by Comparison with the Experimental Data for the Series of Compounds $CH_3C[CH_2P(Bzl)R]_3Mo(CO)_3$ (R = Ph, *m*-Xyl)☆

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NMR-NOE analysis of the three compounds (*RRS*/*SSR*)-$CH_3C(CH_2PPhBz)_3Mo(CO)_3$ (**1**), (*RRR*/*SSS*)-$CH_3C(CH_2PPhBz)_3Mo(CO)_3$ (**2**), and (*RRR*/*SSS*)-$CH_3C[CH_2P$(*m*-Xyl)-$Bz]_3Mo(CO)_3$ (**3**), leads to experimental values for a number of intramolecular H···H contacts in each case. By the very nature of the NOE method, and with the type of molecules studied here, these values have to be understood as a Boltzmann weighted average over all the conformations accessible to the molecules in solution. – A Boltzmann weighted force field approach is used to predict these values on the basis of sets of force field parameters derived earlier for this class of compounds. The agreement between observed and calculated NOE distances is highly satisfactory in each case (rms = 0.2 Å to 0.3 Å). By a statistical analysis it is shown that the predictions made by applying this Boltzmann weighted force field approach are highly significant. There is only a chance of 3 in 100 000 that this quality of prediction might be obtained by chance. This is taken as a validation, albeit indirect, of the energy scale reproduced by the force field parameters as developed. The methods described give a detailed insight into the conformational flexibility of molecules of the type studied. Solid state structures of the molecules are also correctly modelled by the force field used.

Introduction

When force field methods are used to predict possible structural arrangements of coordination compounds, the parameters describing the interactions involving the metal atom are generally deduced from solid-state structures of a series of similar compounds. An optimisation method based on Genetic Algorithms has been proposed as an unbiased method to derive such parameters from a set of solid-state molecular structures.[1] The success of this novel methodology has been demonstrated by correctly predicting general trends as well as specific conformations in the chemistry of *tripod*$Mo(CO)_3$ compounds [*tripod* = $CH_3C(CH_2X)(CH_2Y)(CH_2Z)$, X, Y, Z = PRR′]. It remains an open question as to whether force field approaches developed on the basis of solid-state molecular structures might have some bearing on the behaviour of molecules in solution. Since most reactions in coordination chemistry, including those mediating homogeneous catalysis, in fact take place in solution, this is a serious question. Only if it can be shown that the conformational properties of coordination compounds in solution are appropriately modelled by these methods, can these methods be of help to the preparative chemist in the design and optimisation of ligand-metal-templates with predefined properties. In order to address this problem, information about the conformational behaviour of coordination compounds in solution is clearly necessary.

It has long been known that the Nuclear Overhauser Effect (NOE) allows the determination of H···H distances of molecules in solution.[2] The application of this type of distance measurement in solution has for decades been the preserve of the specialist. Only in recent years have NMR techniques, both methods and equipment, been developed to a state where this kind of measurement might even become a routine tool. Indeed, NMR analysis of protein structures[3] has made enormous progress in the last few years.[4]

In the chemistry of small molecules including the subclass of coordination compounds considered here, NOE measurements are routine only in their qualitative form, i.e. to find out whether two protons in a given molecule are close together or far away from each other. In a quantitative way the method is not used too often in this context. One reason for this is evident: it is rather easy to obtain a crystal structure determination once a compound has been crystallised. A second reason is more intricate: the number of H···H distances accessible to NOE measurements in small molecules is generally too low to uniquely define a structure. There is kind of a paradox: if a molecule increases in size, the number of NOE distances increases and, even

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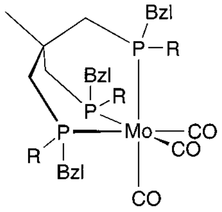
though a larger molecule will have more degrees of freedom in conformational space, the increased number of measurable distances may well counterbalance this increase so that proteins with (depending on the kind and degree of labelling) 200 or more amino acids may be fully structurally characterised by this method, while simple structures such as the ones considered in this paper still pose severe problems. Nevertheless NOE data represent the most valuable and easy way to access information about an ensemble of conformations of molecules in solution.

It is the purpose of this paper to analyse the appropriateness of the force field description[1] of *tripod*Mo(CO)$_3$ compounds[5][6] by comparing the NOE distances measured for a specific molecule with those predicted by the straightforward application of the force field model. This type of analysis is performed for the three coordination compounds **1**−**3** (Figure 1).

Figure 1. Schematic representation of compounds **1** to **3**. The configuration at the phosphorus centers of the racemates to which the paper refers is indicated by the (*R*/*S*) nomenclature



1 R=C$_6$H$_5$ (RRS/SSR)
2 R=C$_6$H$_5$ (RRR/SSS)
3 R=3,5-xylyl (RRR/SSS)

Results

Compound 1

Three compounds of the type *tripod*Mo(CO)$_3$[5][6] (**1**−**3**, Figure 1) have been selected for this analysis in the hope that the number of short and hence detectable H···H contacts might be sufficient to warrant a detailed comparison of measured and calculated H···H distances. All three compounds are chiral and the NMR analysis has been performed on the racemate in each case. In addition, compounds **1** and **2** are diastereomers. It was expected that the reduced symmetry of **1** relative to **2**, together with the bulkiness of the substituents, would strongly differentiate the conformational behaviour of these two isomers so that a maximum of information would result from their analysis.

Compound **1** was a member of the data base used to derive the force field approach and its solid state structure[5] is neatly reproduced[1] by the force field as developed.[1] A projection of the structure of **1** as determined by X-ray analysis is given in Figure 2 (the plane of projection is defined by the three P atoms). The designators of the hydrogen atoms that are involved in NOE contacts, as shown in Figure 2, refer to the contacts given in Table 1.

The NOE distances elaborated for **1** in solution in CD_2Cl_2 at 275 K (see Experimental Section) do not fully agree with the distances observed in the crystal structure. This is not an unexpected result since **1**, especially given its benzylic constituents, is expected to have a high degree of flexibility (Figure 2).

Figure 2. Projection of the structure of **1** onto the plane defined by the phosphorus atoms of $CH_3C(CH_2PPhBzl)_3Mo(CO)_3$. The numbering scheme used throughout is indicated as is the configuration at the phosphorus centers for the (*RRS*) enantiomer of the racemic pair of **1** studied

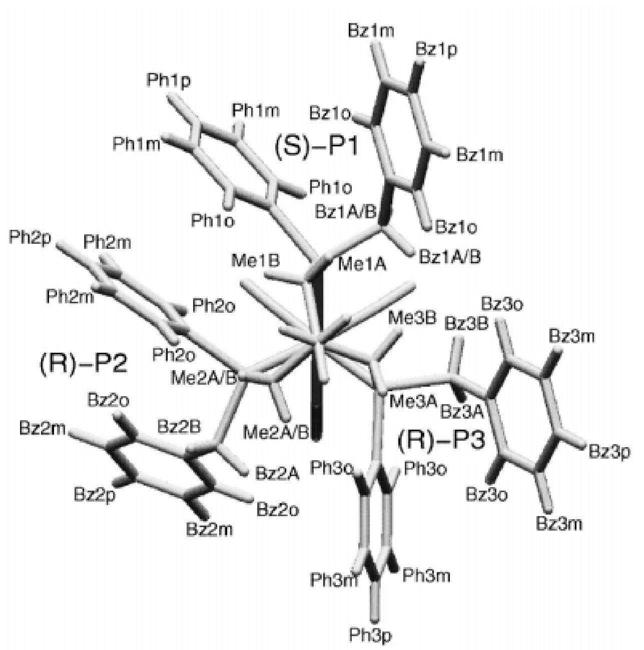


Table 1. Proton-proton distances in **1** as determined by NOE analysis (solution) and X-ray analysis (single crystal) in comparison with these distances as calculated from the force field approach

proton 1	proton 2	d_{NOE} [Å]	$d_{X\text{-}ray}$ [Å]	$d_{Boltzmann}$ [Å][a] *mm2f*	*mm2t*
Bz1A/B	Bz1o	2.30(10)	2.37(1)	2.42	2.43
Bz2A	Bz2o	2.60(15)	2.35(1)	2.45	2.44
Bz2B	Bz2o	2.50(15)	2.56(1)	2.46	2.47
Bz3A	Bz3o	2.60(10)	2.57(1)	2.46	2.46
Bz3B	Bz3o	2.40(15)	2.37(2)	2.47	2.45
Me1/3A	Bz1o	2.50(10)	2.57(2)	2.45	2.41
Me1/3B	Bz1o	2.80(10)	2.49(1)	2.91	2.76
Me2A/B	Bz2o	2.70(15)	4.57(0)	2.44	2.42
Me3A	Bz3o	2.60(15)	3.44(2)	3.17	3.16
Me1/3B	Bz3o	2.80(10)	2.46(1)	2.35	2.34
Me1A	Ph1o	2.20(10)	2.90(3)	2.75	2.74
Me3A	Ph3o	2.00(15)	2.04(2)	2.06	2.08
Me2A/B	Ph2o	2.10(15)	1.84(1)	2.05	2.09
Me3A	Bz2o	2.60(15)	6.29(2)	3.09	2.78
Bz2A	Ph2o	2.60(10)	4.40(1)	2.91	2.84
Bz3A	Ph3o	2.60(15)	2.47(1)	2.61	2.72
Bz3o	Ph3o	2.80(15)	3.19(0)	2.99	3.08
Bz2o	Ph3o	2.20(15)	3.66(0)	3.06	3.34
Bz3o	Bz1o	2.90(20)	2.78(0)	3.06	3.24
rms	[Å]	−	1.16	0.34	0.38

[a] *mm2f* and *mm2t* refer to different sets of force field parameters.[1] Column header $d_{Boltzmann}$ refers to the Boltzmann weighted average of the relevant distances in each case. − [b] rms deviation between the experimental NOE distances and the distances derived by the methods given in the respective column headers.

A flexible molecule like **1** might adopt a number of different conformations in solution and a distance extracted from NOE measurements is some kind of weighted average of all these individual distances between a specific pair of H atoms. Due to the fact that the NOE decreases with the inverse sixth power of the distance, there is an experimental limit to H···H contacts leading to NOE signals, which in the present analysis is around 3 Å. Even if only a narrow range of H···H contacts somewhere between 2 and 3 Å has been accessible by NMR for **1**, these distances are quite restrictive as far as the range of possible conformations is concerned. A number of different approaches have been used in the literature to transform the limited information available from a few NOE distances into structural models that are not excluded by the measurements.[7][8] All of these approaches use the constraints set by the measured NOE distances in one way or another to find acceptable conformations.[9]

A different approach is taken in the work described here. Be it assumed for the moment that a force field approach exists that correctly describes the energy hypersurface pertaining to a molecule, and that all the energy minima at this hypersurface are hence correctly placed in both position and in energy (relative to the energy of the global minimum). In this case the Boltzmann weighted average of the NOE-relevant geometric functions should reproduce the average of this function as observed. The function itself is, according to the physics of NOE, proportional to the inverse of the sixth power of the distance between a pair of hydrogen atoms. The NOE distances, as determined by measurement, thus present a criterion to evaluate and possibly validate a specific force field description.[10] Since molecules like **1**−**3** are still small enough for a complete and systematic search to be performed on the energy hypersurface defined by the force field they allow these lines of investigation to be followed.

To effectively scan all of the relevant conformational space[11] defined by the three rotations of phenyl groups (φ, Figure 3), the six rotational parameters describing the orientations of the benzyl groups (ψ, ω, Figure 3), and the three parameters describing the skew of the chelate cage (τ, Figure 3), the following simplifications were introduced to generate an appropriate set of starting conformations. The skew of the scaffolding was represented by just one torsion τ, thus effectively imposing C_3 symmetry on the torsional arrangement of this cage to start with. This simplification is well warranted given the observations made on 82 *tripod* metal compounds whose conformation had been statistically analysed[11] as well as by the observations made on a sample of *tripod*$Mo(CO)_3$ compounds.[1] Of course this constraint imposed on the starting conformations does not exclude the fact that during the conformational search any type of deviation from this C_3 symmetry will occur. The conformational space that has to be analysed is 10-dimensional, i.e. 3 × PPh- (φ) plus 6 × PBzl-rotations (ψ, ω) plus 1 cage twist (τ). A complete grid search was performed. The resolution for producing the starting conformations was defined by any combination of the values of the torsion angles τ, φ, ψ and ω (Figure 3) as given in Table 2. This leads to 11 664 ($= 2{\cdot}3^3{\cdot}3^3{\cdot}2^3$) starting geometries.

Figure 3. Schematic drawing corresponding to Figure 2 with the definition of the conformational variables φ, ψ, ω, and τ shown. The definition illustrated here is also adopted for **2** and **3**

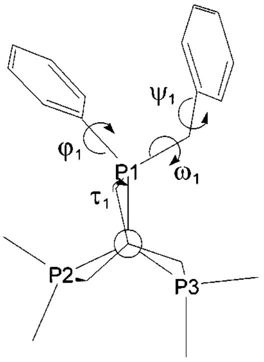


Table 2. Grid values for generating the starting geometries of **1**−**3**[a]

	τ [°]	φ [°]	ψ [°]	ω [°]	combinations	C_3 symmetric	starting geometries
1	20 −20	60 0 −60	120 0 −120	90 0	11664	−	11664
2,**3**	20 −20	60 0 −60	120 0 −120	90 0	11664	2·3·3·2 = 36	3912

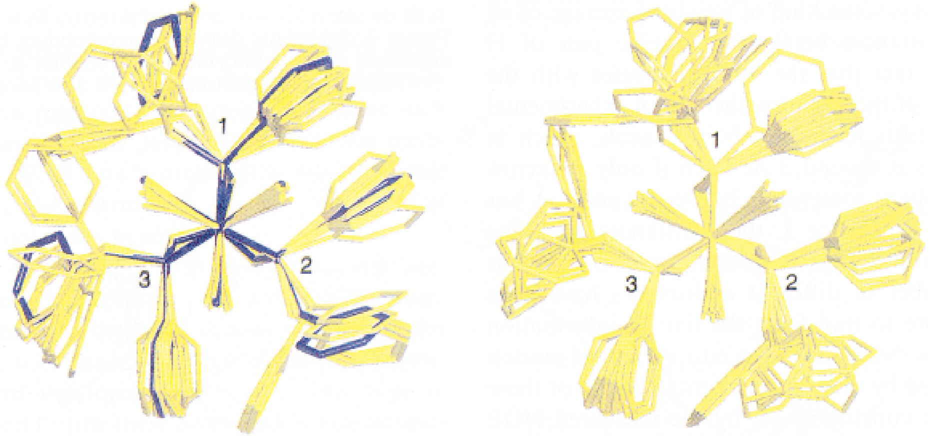
[a] There are 11 664 combinations in each case. Due to the constitutional C_3 symmetry of **2** and **3** the number of geometrically independent starting geometries is reduced to 3912 in these cases.

In a sterically crowded molecule like **1** this a priori setting of conformations may lead to unforeseeable entangling of the individual constituents of the molecule and so the *rubberband* protocol[12] was applied during refinement: the bonds radiating from the phosphorus atoms to the phenyl and benzyl groups were elongated to a distance of 4 Å initially, moving these groups away from the core of the molecule to begin with. During refinement these distances were then allowed to relax in a stepwise manner back to the normal. Entangling of the groups is elegantly[12] avoided by this procedure.

The starting conformations as defined were then allowed to adapt to the force field by an energy minimisation procedure. Depending on the force field implementation used, 191 (*mm2f*[1]) or 165 (*mm2t*[1]) conformations corresponding to local energy minima on the hypersurface were finally found. To illustrate the part of conformational space occupied by these conformations they were clustered according to the distance separating them in the 10-dimensional conformational space as defined by φ, ψ, ω, and τ. The lowest energy conformation in each cluster was used to represent its conformational characteristics. An overlay of these conformations is shown in Figure 4 arranged according to the sense of the torsion of the chelate cage.

It is evident that there is a smaller spread in the orientation of the phenyl groups as compared to that of the

Figure 4. Graphical representation of the section of conformational space occupied by the local minima on the *mm2f* hypersurface of **1**



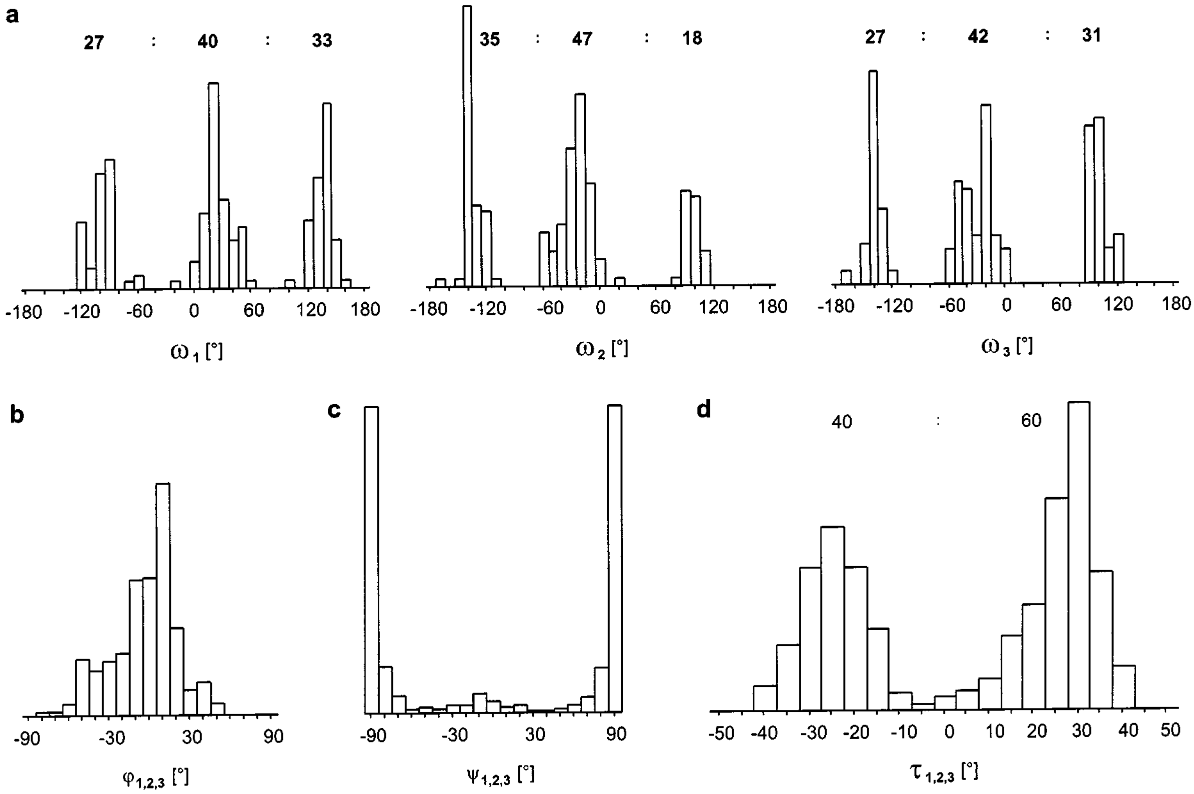
[a] The conformation adopted by **1** in the solid state is shown in blue. On the left hand side conformations with τ > = 0° are shown; the right hand side represents conformations with τ < 0°. In order to make the diagram clearer the conformations were clustered with respect to their distance in conformational space so that each conformation shown represents the properties within each cluster. Note that the sense of numbering is opposite to the one given in Figure 2 due to the opposing directions of view taken in Figures 2 and 4.

benzyl residues. The conformation as determined by X-ray analysis is also indicated in Figure 4.

An alternative view of the diversity of possible orientations of the individual groups is presented by the histographic analyses of the φ-, ω-, ψ-, and τ-values adopted by the minimum energy conformations (Figure 5).

It is seen that the torsion around the P−C-bonds of the benzylic substituents ω basically conforms to the threefold rotational potential as expected. The different type of neighbourhood of benzyl group 2 in comparison to benzyl groups 1 and 3, i.e. benzyl group 2 having two phenyl neighbours (Figure 2) and benzyl groups 1 and 3 having one phenyl and one benzyl neighbour (Figure 2), is mirrored by a difference in the appearance of the corresponding histograms (compare the percentage values given in Figure 5). The histogram referring to the phenyl rotations φ (Figure

Figure 5. Histographic representation of the variance of the conformational parameters characterising the geometry of **1**

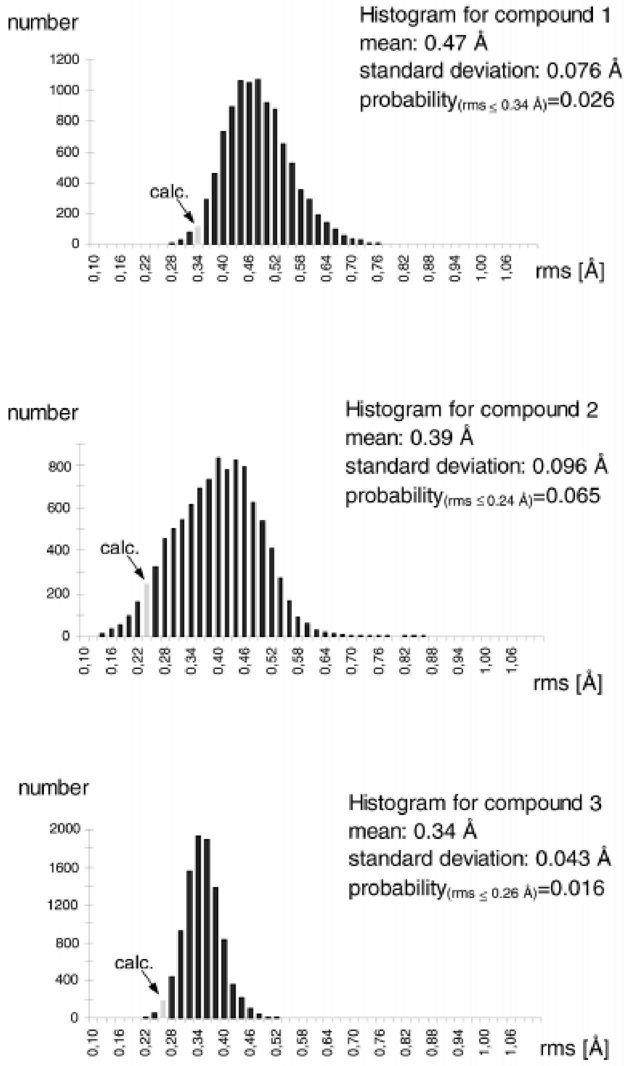


5) shows considerable spread while the histogram referring to the rotation ψ of the phenyl groups at the benzylic positions shows a marked preference at ±90°. The reason for this is that these phenyl groups are by and large outside the sterically crowded part of the molecule and can thus adopt the optimal orientation with respect to the neighbouring CH_2 groups, while the phosphorus-bound phenyl groups have to respond to the steric strain within the core of the molecule. As shown by the histogram, the space accessible to the torsion of the chelate cage (τ) is densely populated not only at the values of ±20° as set for the starting conformations but also at larger and smaller values. Since the analysis was done for the *RRS* enantiomer of the racemic pair **1**, the asymmetry in the distribution of τ values (Figure 5) is not at all unexpected (two (*R*)-configured PRR′ groups opposing only one (*S*)-configured group of this type).

The spread of the conformations, which correspond to local minima on the hypersurface as depicted in Figure 4 and Figure 5, makes no reference to their relative energy. The conformation as found in the crystal structure is at a computed energy of 5 $kJ\cdot mol^{-1}$ (*mm2f*) or 9 $kJ\cdot mol^{-1}$ (*mm2t*) above the global minimum, which differs from it by a 120° rotation around ω_2. As this amount of energy may easily be compensated for by the energy of the crystal lattice this result is not disturbing. With respect to the observation that the NOE distances are not consistent with the crystal structure, it is even a favourable observation. Indeed, none of the conformations, independent of their energy, is able to reproduce the observed NOE distances with an rms of less then 0.57 Å. If it is assumed that just two conformations would be sufficient to reproduce these distances, then all possible pairwise combinations of conformations should be analysed with their relative percentage of contribution being evaluated by a least squares procedure. By testing this hypothesis it was found that the best solution still has an rms deviation of 0.47 Å from the NOE distances. Analysing all possible combinations of three and even four conformations (combinatorial explosion does practically impede the analysis for higher multiples!) does not improve the agreement dramatically (3 conformations: rms = 0.43 Å; 4 conformations: rms = 0.43 Å). A favourable agreement between observed and calculated distances is, however, obtained when a Boltzmann weighted average (referring to d^{-6}) is taken over the complete set of minimum energy conformations. The rms deviation between observed and calculated average distances over all observable H···H pairs amounts to 0.34 Å for the *mm2f* force field and 0.38 Å for the *mm2t* approach (Table 1).

In order to develop a feeling for the relevance of this type of agreement and hence for the relevance of the Boltzmann weighting scheme employed, the following computer experiment was performed: for each conformation the relative energy was set at random in the range between 0.0 and 100 kJ/mol. This randomly generated energy was used to weight each conformation according to the Boltzmann scheme. The weighted average of the relevant distances was calculated and the calculated distances were compared with the measured ones so as to characterise each of these experiments by the corresponding rms value. This experiment was repeated with 10 000 sets of random weights generated in this way. The result of this computer experiment is shown in Figure 6 (**1**) in histographic form.

Figure 6. Computer experiments relevant to the significance of the approach used to predict NOE distances



[a] 10 000 computer experiments were performed in each case. All conformations corresponding to local minima on the *mm2f* hypersurfaces were taken into account. NOE distances were calculated as a Boltzmann type average over all these conformations with the Boltzmann weights set at random in each experiment. For each experiment the rms deviation between the observed and the calculated NOE distances was derived. The spread of these rms values is shown in the diagrams and it is seen that the agreement obtained when the weights are derived by the force field (calc) is rather good. It is highly improbable that this quality of agreement could be fortuitous. The probability of the hypothesis that the results represented by the bars designated with "calc" might be fortuitous is below 7% for each individual compound. The probability that the quality of fit obtained by the approach taken might be fortuitous for all three compounds is in fact less than 3 in 100 000.

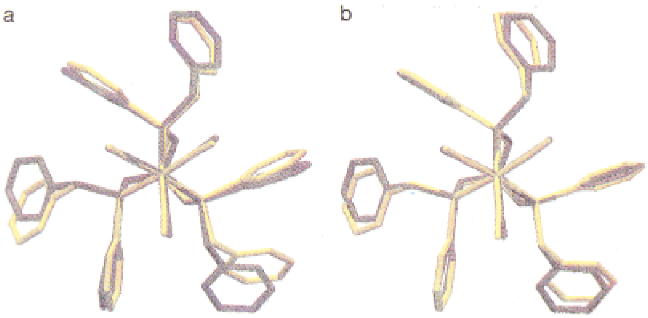
It is clear that the 10 000 rms numbers thus generated are not at all exhaustive with respect to the number of possible combinations of conformations and the weights attributed to them. In principle, an exhaustive solution would only be obtained after an infinite number of experiments of this type; even if a discrete approximation were taken in order to allow as many different weights as there are conformations, the total number of combinations between conformations and weights would be $n!$ for a given number n of conformations! The histograms obtained will nevertheless approximate the distribution of possible solutions. It is observed that an rms value as good as that obtained by the Boltzmann weighting scheme as applied is highly unlikely to occur by chance. The probability of obtaining a solution with an rms value of 0.34 Å or lower by chance is only around 3% (Figure 6). The hypothesis that the quality of fit as obtained by using the energies derived from the force field in the Boltzmann weighting approach might be fortuitous may therefore be denied ata high level of significance.

The approach taken here to reproduce the observed NOE contacts by a force field approach was equally applied to compounds **2** and **3** (see below). The histograms reproducing the distribution of rms values as obtained from 10 000 computer experiments are also given in Figure 6. It is seen that the probability of obtaining the quality of fit (Figure 6, calc) derived from using the energies calculated by the force field approach fortitiously is less than 10 (**2**) and 5 (**3**) percent for these compounds. The probability of achieving the degree of approximation as obtained by applying the Boltzmann weighting scheme on the basis of the force field energies for all three compounds fortitiously is less than 3 in 100 000 as judged from the product of the probabilities given for each compound in Figure 6.

Compound 2

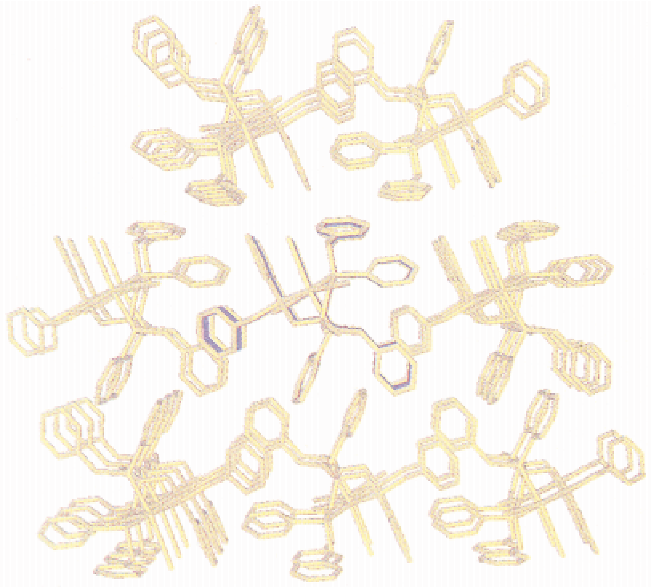
As indicated above the type of analysis as described for **1** was repeated with two additional compounds.The conformational space of **2** was defined by the same set of torsional parameters as already described for **1** (Figure 3). The grid spacing used to populate this space with starting conformations was numerically equal to the one used in the analysis of **1**. From the 11 664 starting conformations thus generated only a subset is symmetrically independent: those conformations that correspond to a C_3 symmetric arrangement occur only once each. The number of such conformations amounts to $2 \cdot 3 \cdot 3 \cdot 2 = 36$ in accordance with the number of values allowed for τ, φ, ω, and ψ. All the others will be represented at three symmetry related points in conformational space as three isoenergetic conformations. Each of these triplets corresponds to just one symmetrically independent conformation. The total number of independent conformations is thus $36 + (11664 - 36)/3 = 3912$ (Table 2). Force field refinement starting from these 3912 points in conformational space leads to 68 (*mm2f*) or 50 (*mm2t*) minimum energy conformations of which nine (*mm2f*) or eight (*mm2t*) have C_3-symmetry. Independent of the parameter set used (*mm2t, mm2f*), the conformation observed for **2** in the crystal closely corresponds to the global minimum in each case. This is a satisfactory result in that compound **2** had not been incorporated in the data base used to derive the force field approach.[1] There is some slight discrepancy between the calculated and the observed conformations as far as the precise orientation of two of the benzyl groups is concerned (Figure 7a).

Figure 7. Comparison of calculated (blue) and observed conformations of **2**; (a) free molecule; (b) molecule embedded in the crystal



Assuming that this discrepancy might be caused by the environment of **2** in the crystal, a force field refinement of the structure in its solid state environment was performed. Contributions from all the atoms that are at a maximum distance of 8 Å from any atom of the molecule to be refined were taken into account. The atoms in this crystal environment were held at fixed positions during refinement. A view of the crystal environment as generated by the nineteen neighbouring molecules that meet the above criterion is given in Figure 8.

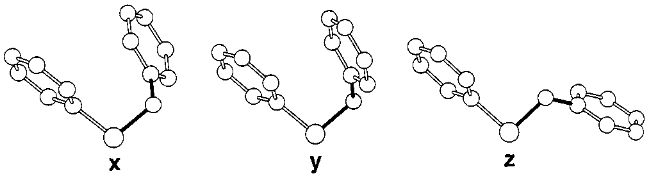
Figure 8. Crystal environment of **2** (blue: specified molecule, yellow: crystal surrounding)



The result of this refinement is shown in Figure 7b (*mm2f*). The agreement between the calculated and the observed structures is significantly increased and the rms difference between the observed and the calculated structures decreases from 0.53 Å to 0.26 Å when the crystal environment is taken into account.

If the ensemble of conformations obtained by the minimisation process is systematically analysed, it is observed that the main difference between the individual conformations may be traced back to the relative orientations that the benzyl groups occupy as characterised by ω. Three individually favourable conformations are possible for each such group, corresponding to the threefold potential governing this rotation (Figure 5). With reference to the coordination polyhedron, these three positions may be described in the following way: the benzyl group may point backward away from the carbonyl groups with its phenyl residue oriented such that it is close to the phenyl group that is directly bound to the phosphorus atom that supports both groups. Referring to Figure 9 this type of orientation is designated as **x**.

Figure 9. Relative orientation of the benzyl groups in **2** and letter codes used to specify them



[a] The plots refer to a plane of projection and direction of view as used in Figure 7.

On the other hand, the benzyl group still pointing backward may be oriented such that its phenyl residue is close to the phenyl residue sustended by the neighbouring phosphorus atom (**z**, Figure 9). A third type of orientation (designated as **y**, Figure 9) results when the benzyl group is oriented towards the side of the carbonyl ligands with its phenyl part close to the phenyl residue supported by the same phosphorus atom. It is observed that there is an energetic preference for the orientations termed **x**. The sequence of orientations **xxx** < **xxy**, **xxz** < **xyy** < **xyz**, **xzy** < **yyy**, **xzz** < **yyz** < **zzy** < **zzz** (e.g. **xxy**: ω_1 = **x**, ω_2 = **x**, ω_3 = **y**; **x**, **y**, **z**: Figure 9) is found by ranking the orientations according to the energy at which they are observed for the first time amongst the manifold obtained from the conformational search procedure. This energetic ordering is also apparent by the selection of conformations shown in Figure 10 where the relative energies of some prototypic conformations are indicated.

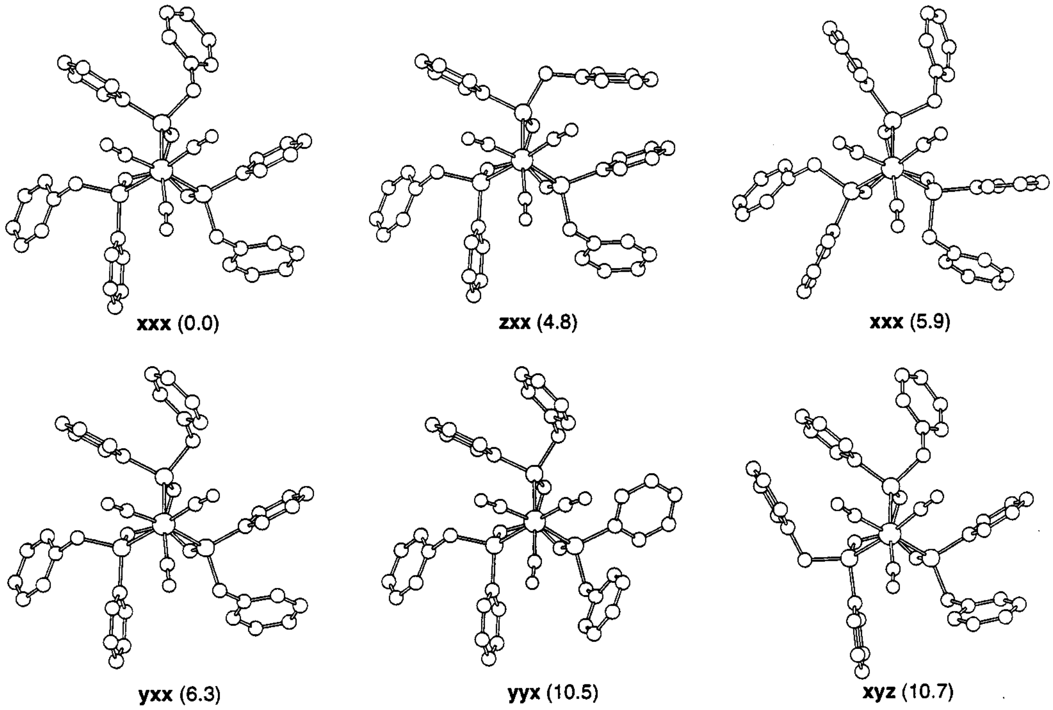
An NOE analysis of **2** resulted in a number of NOE distances (Table 3). The protons involved in these NOE contacts and the designators used for them are shown in Figure 11.

It is seen from Table 3 that the conformation as found in the crystal already reproduces the NOE distances with an rms of only 0.28 Å. It might therefore appear that the conformation adopted by **2** in solution is close to that observed in the crystal, which itself is very close to the conformation calculated as the global minimum (see above).

On the other hand, based on the assumption that the force field approach correctly reproduces the energy hypersurfaces, a Boltzmann weighted average of the relevant geometric parameters should give an unbiased estimate of the NOE distances. Application of this procedure to the 68 conformations corresponding to the 68 energy minima obtained (*mm2f*, see above) leads to an rms discrepancy between the observed and the calculated NOE distances of 0.24 Å. In summing up the individual terms, appropriate care was taken to correctly handle symmetry: while C_1 symmetric conformations will appear at three different points in conformational space, reflecting the constitutional symmetry of the problem, C_3 symmetric conformations will occur just once. For instance, if φ2, φ3, and φ5 [or ω1, ω2, and ω3 etc. (Figure 3)] each have different values, their cyclic permutation will project itself into conformational space onto three different points; if on the other hand the values for φ1, φ3, φ5 etc. are equal, any cyclic permutation will project itself onto one and the same point in this space. The weight accounting for C_3 symmetric conformations is therefore only one third of those showing C_1 symmetry. Application of the same procedure to the conformations obtained by the *mm2t* parameter set likewise gave an rms of 0.33 Å. The result obtained by considering just the minimal energy conformation (rms = 0.25 Å) and that obtained by a Boltzmann weighted average over all conformations are not significantly different (Figure 6, **2**). It is observed that other single conformations, as well as pairwise combinations of conformations, numerically give an even better agreement of 0.19 Å (*mm2f*, single conformation: E_{rel} above global minimum: 5.9 kJ·mol) and 0.13 Å [*mm2f*, 2 conformations: E_{rel} = 5.9 (64%) and 21.1 kJ·mol^{-1} (36%)] respectively. With respect to the computer experimental distribution of rms values (Figure 6) these results are not totally unexpected. There are obviously weighted combinations of conformations in each case analysed (Figure 6) that lead to an rms value lower than that obtained by using the relative energies as the weighting criterion (bars designated with "calc" in Figure 6). This Boltzmann weighted average, however, is an a priori prediction of what the NOE distances should be. These predictions are not left to chance but a definitive formula is used to make these predictions and it is seen from Figure 6 that, by using this predictor, reliable predictions are made for completely independent sets of experiments (**1**−**3**). With the predictor being based on sound physical grounds this result is especially satisfying and, by its very methodology, is far superior to any method involving a posteriori fitting of the weights of individual conformations to achieve optimal agreement.

A caveat is in place here: as shown in the histograms (Figure 6) and in the paragraph above, there are always weighted linear combinations of conformations that neatly reproduce the NOE values with rms values that are even below the ones obtained with the Boltzmann predictor (Figure 6), and there are even single conformations (see above) that meet this criterion. These conformations would, of course, be found as the appropriate solutions by applying a distance geometry approach[8] and would naturally be considered to be very important because of the excellent agreement. In the light of the Boltzmann predictor it appears that these solutions, even though numerically excellent, might well be far from reality. Only if the energy of the relevant conformations is appropriately taken into account, as in the case of the Boltzmann predicting scheme, will a somewhat more realistic view be gained. We feel that

Figure 10. Conformations of **2** ranked according to increasing energy



xxx (0.0) **zxx** (4.8) **xxx** (5.9)

yxx (6.3) **yyx** (10.5) **xyz** (10.7)

[a] From the complete set of minimum energy conformations as obtained by an exhaustive conformational search applying the parameter set *mm2f* those members are selected for which a specific arrangement (**x**, **y**, **z**, see Figure 8) of a benzyl group is first observed when scanning through these conformations in the direction of increasing energy.

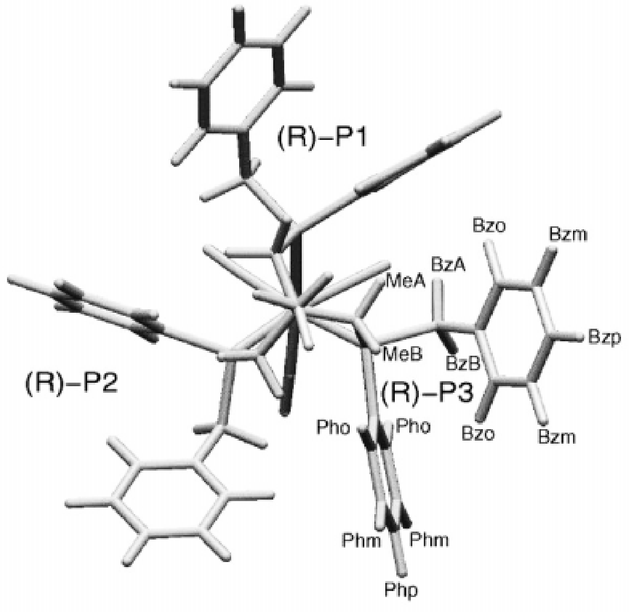
Table 3. Proton-proton distances in **2** as determined by NOE analysis (solution) and X-ray analysis (single crystal) in comparison with these distances as calculated from the force field approach. Meaning of abbreviations see Figure 11 and legend to Table 1

proton 1	**proton 2**	d_{NOE} [Å]	$d_{X\text{-ray}}$ [Å]	$d_{Boltzmann}$ [Å] *mm2f*	*mm2t*
Pho	Bzo	3.16(7)	2.91(0)	3.21	3.48
Pho	BzB	2.73(5)	2.60(1)	2.89	3.11
Pho	MeB	2.18(6)	1.95(1)	2.23	2.38
Pho	MeA	2.41(5)	2.23(1)	2.44	2.58
Bzo	BzB	2.63(5)	2.53(1)	2.73	2.84
Bzo	BzA	2.53(5)	2.23(2)	2.73	2.83
Bzo	MeB	2.60(8)	3.16(1)	3.20	3.19
Bzo	MeA	2.62(3)	2.55(1)	2.50	2.63
BzA	MeA	2.89(4)	2.67(1)	3.01	3.12
rms	[Å]	–	0.28	0.24	0.33

the approach taken here, while taking away the simple and aesthetically appealing picture of just one dominating conformation, is perhaps more relevant in a physical sense.

Force field based approaches to evaluate NOE distances of conformationally flexible coordination compounds had of course already been used. In this context C. R. Landis has described a very thorough attempt to derive the conformational flexibility of a series of catalytically relevant rhodium complexes containing chiral biphosphines[12] as well as of other coordination compounds.[13] His use of the force field approach is, however, restricted to an a posteriori fit of observed NOE distances to weighted averages of force field derived conformations. These conformations are in turn obtained by clustering individual conformations to a tractable number of average conformations. It was observed, as it is in this paper, that linear combinations of the conformations may be found that neatly reproduce the observed NOE's. In such an approach any information about the relative energies of the conformations is com-

Figure 11. Projection of the structure of **2** onto the plane defined by the three phosphorus atoms. The numbering scheme used throughout is indicated, as is the configuration at the phosphorus centers for the (*RRR*) enantiomer of the racemic pair of **2** studied



pletely neglected. No attempt to incorporate these force field derived energies is made. In contrast, in the present paper an a priori prediction of the expected NOE distances is made using all the conformations as well as their energies as given by the force field. With the above caveat in mind the physical relevance of these two approaches might be quite different.

Compound 3

Having been made curious by the results obtained so far, a third molecule (**3**) was analysed by the same type of procedure. Compound **3** is an analogue of **2**: it is a chiral compound, the racemate of which has been analysed. The configuration at each of the three chiral phosphorus centers in **3** is the same (the situation is analogous to the one in **2**). The phenyl substituents at the phosphorus centers in **2** are replaced by 3,5-$Me_2C_6H_3$ residues in **3**. A complete conformational search applying the procedures as already described (see Table 2) reduced the set of 3912 starting conformations to 158 (*mm2f*) or 104 (*mm2t*) conformations corresponding to minimum energy positions on the hypersurface. The conformation of **3** as observed in the solid state is reproduced by these calculations as the global minimum with an rms difference of only 0.46 Å (*mm2f*). The parameter set mm2t gives an equally good agreement (rms = 0.46 Å). The difference between the two estimates (*mm2f* / *mm2t*) is only 0.09 Å.

If the NOE data are compared with the appropriate H···H distances characterising the global minimum conformation, an rms agreement of 0.27 Å is observed (Figure 12, Table 4).

Figure 12. Projection of the structure of **3** onto the plane defined by the three phosphorus atoms. The numbering scheme used throughout is indicated, as is the configuration at the phosphorus centers for the (*RRR*) enantiomer of the racemic pair of **3** studied

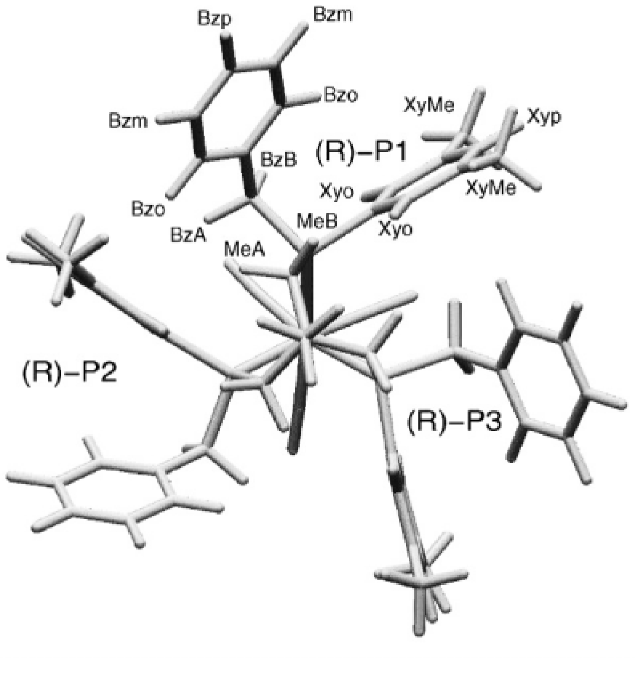


Table 4. Proton-proton distances in **3** as determined by NOE analysis (solution) and X-ray analysis (single crystal) in comparison with these distances as calculated from the force field approach. Meaning of abbreviations see Figure 12 and legend to Table 1

proton 1	**proton 2**	d_{NOE} [Å]	$d_{X\text{-ray}}$ [Å]	$d_{Boltzmann}$ [Å] *mm2f*	*mm2t*
Xyo	MeA	2.36(9)	2.51(1)	2.25	2.26
Xyo	MeB	2.00(7)	2.06(1)	2.07	2.16
Xyo	BzA	2.94(11)	3.24(1)	2.83	3.45
Xyo	BzB	2.54(9)	2.68(1)	2.68	2.74
Xyo	Bzo	2.82(12)	3.12(0)	2.95	3.12
Bzo	BzB	2.42(9)	2.43(1)	2.58	2.64
Bzo	MeA	2.63(10)	2.54(1)	2.35	2.39
Bzo	MeB	2.64(10)	2.87(2)	2.98	3.18
Bzo	BzA	2.43(9)	2.32(2)	2.56	2.58
Bzo	XyMe	2.96(11)	2.52(0)	2.60	2.72
MeA	XyMe	3.29(14)	3.83(1)	3.73	3.93
BzA	MeA	2.83(13)	2.63(4)	2.83	2.85
rms	[Å]	–	0.27	0.24	0.34

A Boltzmann-weighted average taken over the whole conformational ensemble leads to rms values of 0.24 Å (*mm2f*) and 0.34 Å (*mm2t*). The hypothesis that it is the ensemble of conformations and not just the one global minimum conformation that gives rise to the observed NOE distances is therefore acceptable. The distribution of rms values given in Figure 6 (**3**) shows that the solution obtained by applying the Boltzmann weighting scheme (calc) has a high level of significance. It is interesting to note that in the case of **3**, in a similar way to the findings made for **2**, there are pairs of conformations that, irrespective of their relative energy, are able to reproduce the experimental findings when used in appropriately weighted combinations. The best pair of such combinations leads to an rms value of only 0.14 Å (*mm2f*) even though the relative energies of the two contributing conformations are computed well above the global minimum (conformation 1: 42%, E_{rel} = 0.0 kJ/mol; conformation 2: 58%, E_{rel} = 24.8 kJ/mol). The best single conformation still gives an rms of 0.24 Å (E_{rel} = 7.5 kJ/mol). This may again be taken as a warning against the application of a merely distance based search procedure that does not appropriately account for the energetic distribution. This warning of course applies to the analysis of highly flexible molecules that are neither thermodynamically nor kinetically trapped in just one single conformation. When this happens, as is usually observed for proteins, the dimensionality of the problem is reduced and a distance based approach will generally lead to one statically correct solution as soon as there are enough NOE data points available to uniquely define the topology.[4]

For flexible small molecules the approach taken here appears to be a more reliable one as compared to mere distance geometry calculations. However, a prerequisite for this approach to work is a force field approximation that reproduces the molecular conformations on an energy hypersurface that is not distorted too far from reality.

Assessment of the Force Fields

Two sets of parameters describing the interactions involving the molybdenum heavy atom were applied in the work described in this paper. Both of these sets had been derived by an optimisation procedure involving Genetic Algorithms[1] on the basis of a large number of solid state conformations. It had already been argued[1] that the parameter set *mm2t* which, as an extension of the *mm2f* set, includes a number of torsional parameters might not be too reliable in view of the insufficient variance of the data basis with respect to these variables.[1] It is now found that in the analysis of **1**−**3** there is a tendency (Tables 1, 3, 4) for the *mm2f* set to better reproduce the experiments. While the static structures, i.e. individual conformations, are closely equivalent when calculated with the two sets of parameters, the dynamic situation analysed in this paper is better reproduced by the *mm2f* parameter set. This means that the energy scales produced by the two sets are different and the one implied by *mm2f* is the more realistic one.

The quality of fit obtained in modelling NOE distances suggests that the relative energies according to which the conformations are sorted by the force field are somehow on scale to reality. Otherwise an agreement such as that observed would not be expected. A more rigorous validation of the statement that the force field correctly assigns relative energies would of course consist in directly comparing energy differences accessible to measurement with their force field derived counterparts. The result of such a test will be described in a forthcoming paper.

Conclusion

With the force field approach used minimum energy conformations are correctly reproduced. It is found that the solid state structures of **2** and **3** correspond to conformations at globally minimal energy. The rms deviation of observed (X-ray) and calculated structures is less than 0.5 Å in each case.

It is observed that the average distances as determined for molecules **1**−**3** by NOE techniques are well reproduced by the force field model. While it is always possible to find weighted linear combinations of conformations that will reproduce the NOE distances, and while even one single conformation irrespective of its energy might give a good fit to the NOE data by an a posteriori analysis, the method reported here makes use of an a priori predictor: a Boltzmann weighted average taken over all conformations that correspond to minima on the relevant hypersurfaces will predict NOE distances with a highly satisfactory agreement. By a statistical analysis of the results obtained it is shown that the agreement achieved is far better than might be expected if it were a chance result. In fact the quality of the results obtained by applying the Boltzmann weighting scheme is estimated to have a statistical probability of below 3 in 100000, lending considerable significance to the predictor as applied. The fact that this is so is in favour of the statement that the force field approach as applied does correctly place the conformations in space as well as in energy on the molecular hypersurface. It is also evident, however, that more stringent analyses with a more direct experimental access to energy differences have to be performed in order to independently justify the use of the approach reported.

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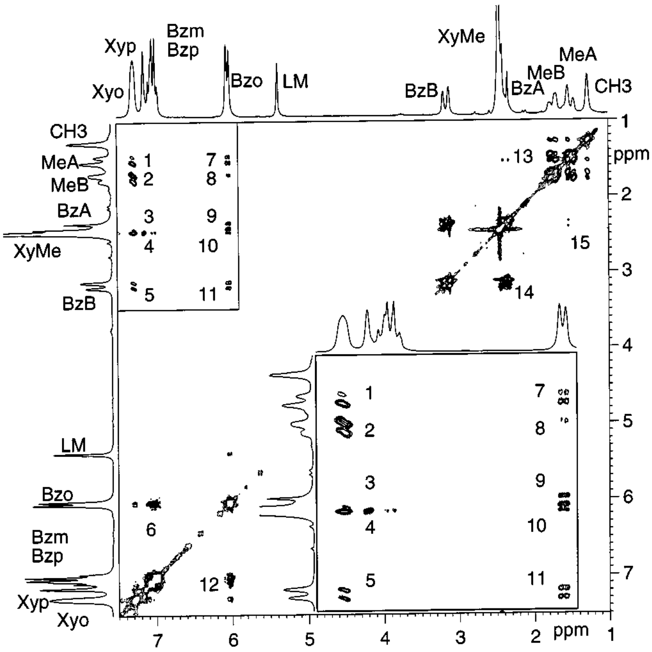
Experimental Section

NMR Analysis: Compounds **1**−**3** were obtained as previously described.[5][6] All measurements were carried out with a Bruker DPX200 (200.130 MHz, 50.323 MHz, and 81.014 MHz for ^{1}H, ^{13}C, and ^{31}P, respectively). For ^{1}H- and ^{13}C-NMR spectra CD_2Cl_2 was used as solvent ($\delta_H = 5.31$, $\delta_C = 53.8$), TMS as external standard, for ^{31}P NMR 85% H_3PO_4 ($\delta = 0$) as external standard. For NMR analysis almost completely saturated solutions of the compounds in CD_2Cl_2 sealed in nmr tubes under argon were used. Unequivocal assignment of all the ^{1}H, ^{13}C, and ^{31}P resonances was performed by a combination of 1D and 2D experiments[14] ({^{1}H-^{13}C}-HMQC,[15] {^{1}H-^{13}C}-HMBC,[16] DQF-COSY,[17] TOCSY[18]). Experimental conditions are listed in Table 5. Assignment and shifts of the ^{1}H-NMR signals of compounds **1**−**3** are given in Table 6.

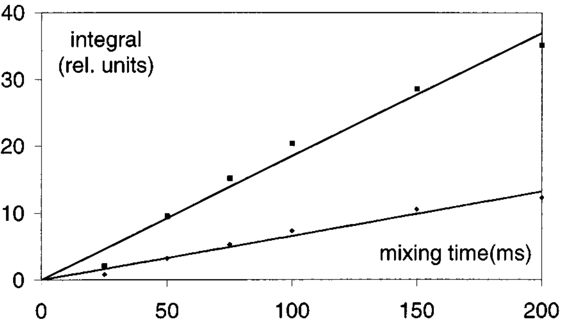
Two dimensional NOE measurements[19] were performed for each compound at a number of different mixing times. As an example the spectrum obtained for **3** with a mixing time of 200 ms is shown in Figure 13.

Figure 13. NOESY spectrum of **3** with 200 ms mixing time. The section referring to the arene protons is shown in the inlay in a magnified form. The numbers given in the diagram also refer to Table 7. Black: negative signals; grey: positive signals



In general, the two integral values[20] thus obtained for each correlation were found to be in good agreement. In some cases the value of the integral taken from below the main diagonal was found to be definitely smaller than the one taken from above it. This discrepancy is a systematic one and indicates that, in those cases where it appeared, the relaxation time was insufficient to allow for a complete relaxation. This will always lead to integrals that are smaller than the maximum obtainable by one NOE effect and it is therefore good practice to take the above diagonal value as the more appropriate data. The initial slope of integral value versus mixing time was evaluated in each case. Correlation coefficients were generally better than 95% (Figure 14) but occasionally coefficients as low as 70% were obtained.

Figure 14. Evolution of NOE cross polarisation with mixing time. The Figure refers to **3** with the pairs Xyo-MeB (upper trace) and Xyo-MeA (lower trace) as two examples



were analysed by three different approaches and the results of these are given in Table 7.

In one approach all the slopes were transformed into distances using the Bzm-Bzo reference (Table 7, aromatic reference). Transformation of the same data using the BzA-BzB distance for all data led to a slightly different set of distances (Table 7, aliphatic reference). In yet another approach the reference distance taken from the arene protons was applied to contacts between aromatic protons. The CH_2 reference distance was applied to contacts between aliphatic protons. Distances between aliphatic and aromatic protons were evaluated by applying the mean between the constants derived from the arene and from the methylene references (Table 7, individual reference). The spread of distances thus calculated (Table 7) from three different systematic approaches is a kind of a measure of the systematic error introduced by the systematically different procedures of evaluation. It is seen that for individual distances the maximum deviation between the three estimates amounts to around 0.3 Å. The average deviation between the three estimates is only $\sigma = 0.1$ Å in the mean. This is in agreement with the estimate of the reliability of the NOE distances determined for **1**−**3** as inferred from the calculus of error propagation. By whatever means an estimate for the reliability of the NOE derived distances is made, this estimate is around 0.1 Å on average. Since there is no a priori argument as to which of the three approaches applied to evaluate the data should be preferred, the mean of the three estimates[21] was considered throughout (Table 7, average) when experimental and modelled distances had to be compared. The data are thus good enough to allow for the assessment of the force field approach as described.

Table 5. Setup of NMR experiments

2D-Experiment	**{^{13}C-^{1}H}-HMQC** [15]	**{^{13}C-^{1}H}-HMBC** [16]	**DQF-COSY** [17]	**TOCSY**[18]	**NOESY**[19]
Pulse Program[a]	invbtp	inv4lplrnd	cosydftp	mlevtp	noesytp
Spectral Width F_2 [Hz]	1302	1302	1302	1302	1302
Time Domain F_2 [Word]	2 K	2 K	2 K	2 K	2 K
Size F_2 [Word]	1 K	1 K	2 K	1 K	1 K
Spectral Width F_1 [Hz]	6039	6039	1302	1302	1302
Time Domain F_1 [Word]	512	512	512	256	256
Size F_1 [Word]	1 K	1 K	1 K	512	512
Scans	44	44	32	32	32
Mixing Time [ms]	250	60	−	125	10−1400
Relaxation Delay [s]	2	2	1.5	1.0	2

[a] The acronyms in this row refer to pulse sequences supplied under these names for the BRUKER series of instruments.

As far as the distances pertinent to the slopes in each case are concerned, this uncertainty is not dramatic because the physics of the NOE process are such that the distances are proportional to the sixth root of the slopes. Application of the laws of error propagation to the data sets obtained shows that the maximum error inferred from the uncertainty in the slopes is less than 0.1 Å in every case. Reference distances are needed in order to transform the values obtained for the slopes into estimates of proton-proton distances. There are up to three possible choices for such distances for the compounds **1**−**3**: as a standard tool of the absolute assessment of distances, the distance between two vicinal methylene protons at a CH_2 group is often used. This type of reference distance is available for all three compounds (CH_2 in the chelate cage, CH_2 of the benzyl groups, standard value: 1.75 Å). Another standard tool which is often used as an absolute reference is the distance between the protons at *o*- and *m*-positions of a six membered arene ring (accepted standard value: 2.45 Å). The data for compound **1** were evaluated using: Bz2A-Bz2B, Bz3A-Bz3B, Bz1o-Bz1m, Bz2o-Bz2m, Bz3o-Bz3m. The NOE distances of compound **2** were evaluated on the basis of the methylene distance BzA-BzB (Figure 11) as well as on the basis of the Bzo-Bzm distance.

For compound **3** the same type of two different reference distances was used. In order to develop a feel for the reliability of the distances obtained by the different ways of evaluation, the data

Force Field Calculations: The commercial program set MacroModel[22] was used for the calculations throughout. Starting conformations were generated using a program, written in C, for this purpose.[1] The torsion angles used to define these conformations have the following meanings (see Figure 3):

φ: torsion angle Du−P−C_{ipso}−C_{ortho} with the position of the Dummy atom Du generated and defined as given[1]

τ: torsion angle Mo−P−$C_{methylene(cage)}$−C

ω: torsion angle Du−P−$C_{methylene(benzyl)}$−C_{ipso}

ψ: torsion angle P−$C_{methylene(benzyl)}$−C_{ipso}−C_{ortho}.

To avoid entangling of individual groups in the starting conformations as well as during refinement, a rubberband protocol was used.[12] The P−C bonds radiating to the phenyl and benzyl substituents respectively were stretched to an initial value of 4 Å with the direction of these bonds unchanged. For the purpose of refine-

Table 6. ^{1}H-NMR data for compounds **1**−**3**

Nucleus	Molecule 1 Group 1[a]	Group 2[a]	Group 3[a]	Molecule 2	Molecule 3
Me	———	1.32 (bs, 3 H, [2.1])[f]	———	1.24 (bs, 3 H)	1.23 (bs, 3 H)
MeA	1.83−2.10 (m, 4 H, $^2J_{HH}$ 16.0)[b], [e]	1.42−1.58 (m, 2 H, $^2J_{HH}$ 16.0)[b]	1.83−2.10 (m, 4 H, $^2J_{HH}$ 16.0)[b], [e]	1.57 (d, 3 H, $^2J_{HH}$ 13.0)	1.46 (d, 3 H, $^2J_{HH}$ 15.0)
MeB				1.76 (d, 3 H, $^2J_{HH}$ 13.0)	1.64 (d, 3 H, $^2J_{HH}$ 15.0)
BzA	3.46 (dd, 1 H, $^2J_{HH}$ 14.0, [3.9])[c]	2.88 (dd, 1 H, $^2J_{HH}$ 14.6, [2.9])[c]	3.58 (dd, 1 H, $^2J_{HH}$ 14.4, [3.8])[c]	2.38 (d, 3 H, $^2J_{HH}$ 14.0)	2.32 (d,3 H, $^2J_{HH}$ 14.0)
BzB	3.31 (dd, 1 H, $^2J_{HH}$ 14.0, [2.4])[c]	2.38 (d, 1 H, $^2J_{HH}$ 14.6)	3.19 (d, 1 H, $^2J_{HH}$ 14.4)	3.13 (d, 3 H, $^2J_{HH}$ 14.0)	3.11 (d, 3 H, $^2J_{HH}$ 14.0)
Pho/Xyo	7.18−7.22 (m, 2 H)[g]	6.95−7.03 (m, 8 H)[e], [g]	7.63−7.78 (m, 2 H)[g]	7.61 (bs, 6 H)[g]	7.28 (bs, 6 H) [Xyo][g]
Phm/XyMe	6.95−7.03 (m, 8 H)[d], [e], [g]	6.95−7.03 (m, 8 H)[d], [e], [g]	7.45−7.55 (m, 3 H)[d], [g]	7.41−7.58 (m, 9 H)[d], [g]	2.39 (s, 18 H) [XyMe][g]
Php/Xyp					7.27 (s, 3 H) [Xyp][g]
Bzo	6.65 (d, 2 H, $^3J_{HH}$ 4.0)	5.97 (d, 2 H, $^3J_{HH}$ 7.0)	6.46 (d, 2 H, $^3J_{HH}$ 5.4)	5.95 (d, 6 H, $^3J_{HH}$ 7.2)	5.97 (d, 6 H, $^3J_{HH}$ 7)
Bzm, Bzp	7.05−7.12 (m, 9 H)[d]	6.83−6.93 (m, 9 H)[d]	7.12−7.18 (m, 9 H)[d]	6.89−7.12 (m, 9 H)[d]	6.91−7.15 (m, 9 H)[d]

[a] The three $CH_2P(Ph)(Bzl)$ groups of **1** are chemically different. Refering to the designators given in Figure 2, data given in row "nucleus xyz" and column "group i" refer to proton xyiz, e.g. the data in the line MeA and the column "group 1" refer to the Me1A. For **2** and **3** these groups are chemically equivalent. − [b] Higher order multiplets, $^2J_{HH}$ coupling constants were determined by DQF-COSY experiments. − [c] The $^2J_{PH}$ coupling-value is given in square brackets. − [d] Higher order multiplets, see for example Bzm, Bzp, Figure 13. The NOE′s observed in this spectral range are by virtue of the geometry of the compounds (Figures 1, 11, 12) due to the dipolar interactions with the *meta*-protons in each case. − [e] Signals of individual groups overlap. − [f] The $^4J_{PH}$ coupling-value is given in square brackets. − [g] The value is given for the phenyl-protons Pho, Phm, or Php respectively; the xylyl-protons are marked by "xyz" in square brackets

Table 7. Dependence of the NOE distances of **3** on relevant reference distances[a]

crosspeak No.	interaction interaction	slope	distance estimates [Å] aromatic reference	aliphatic reference	individual reference	average
1	Xyo/MeA	0.073(2)	2.46(3)	2.25(5)	2.37(3)	2.36(9)
2	Xyo/Meb	0.193(3)	2.09(3)	1.91(5)	2.01(3)	2.00(7)
3	Xyo/BzA	0.020(1)	3.06(3)	2.80(5)	2.95(3)	2.94(11)
4	Xyo/XyMe	0.048(2)	2.64(3)	2.41(5)	2.54(3)	2.53(9)
5	Xyo/BzB	0.047(5)	2.65(3)	2.42(5)	2.55(3)	2.54(9)
6	Xyo/Bzo	0.027(2)	2.90(3)	2.65(5)	2.90(3)	2.82(12)
−	Xyp/XyMe	0.049(1)	2.63(3)	2.40(5)	2.53(3)	2.52(9)
7	Bzo/MeA	0.038(7)	2.75(4)	2.51(6)	2.64(4)	2.63(10)
8	Bzo/MeB	0.037(11)	2.76(6)	2.52(7)	2.65(5)	2.64(10)
9	Bzo/BzA	0.061(5)	2.53(3)	2.31(5)	2.44(3)	2.43(9)
10	Bzo/XyMe	0.019(3)	3.09(4)	2.83(5)	2.97(4)	2.96(11)
11	Bzo/BzB	0.063(9)	2.52(3)	2.31(5)	2.43(3)	2.42(9)
12	Bzo/Bzm	0.075(11)	2.45(4)	2.24(5)	2.45(4)	2.38(10)
13	BzA/MeA	0.022(3)	3.00(4)	2.74(5)	2.74(3)	2.83(13)
14	BzA/BzB	0.328(74)	1.91(5)	1.75(6)	1.75(5)	1.80(8)
15	MeA/XyMe	0.009(3)	3.49(6)	3.19(7)	3.19(6)	3.29(14)

[a] Crosspeak refers to the numbering scheme shown in Figure 12; the numbers refer to the numbers assigned to individual crosspeaks (Figure 13). Slope derived from measurements at different mixing times; the numbers in brackets refer to the standard deviation in terms of the least significant digit given in each case. Aromatic reference: The Bzm-Bzo distance (Figure 12) is used throughout. Aliphatic reference: The BzA-BzB distance (Figure 12) is used throughout. Individual reference: The reference distances BzA-BzB (aliphatic) and Bzm-Bzo (aromatic) are used where appropriate. For contacts between aromatic and aliphatic protons the mean between the two estimates is taken. The integrals of the crosspeaks were interpreted with reference to the mean of the two estimates. The numbers given in brackets for these three columns refer to the standard deviations inferred from the standard deviations of the slopes. Average designates the mean derived from the three values in the three columns at left; the numbers in brackets are the root mean square deviations from the mean.

ment these artificial restraints were reduced in a stepwise manner: reducing the distance after every hundred iterations to values of 3 Å, 2.5 Å, and 2.1 Å. The force constants referring to the artificially elongated bonds were set at 100.0, 175.18, 583.4, 1166.7 kJ/Å^2 mol in the sequence given. In the last step, now applying the original P−C equilibrium bond length and the original force constants using the *mm2f* / *mm2** force field (1166.7 kJ/mol = 2.91 mdyn/Å), refinement was performed without constraints (convergence criteria: maximum number of iterations = 99 999, convergence gradient: 0.01 Å/iteration).

The Polak Ribiere Conjugate Gradient algorithm was used for energy minimisation.[23]

In each case the refinement resulted in a largely reduced number of different conformations, each of which corresponding to at least a local minimum (for exact numbers: see text). Once all the conformations were known, the following procedure was applied: for each of the conformations the NOE relevant distances were extracted in the following way: whenever a pair of protons to which the NOE measurement refers could be uniquely assigned, the distances relevant to such pairs were calculated. In those cases where the nmr experiments could not differentiate between constitutionally equivalent sets of protons, as for instance in the case of the conformationally diastereotopic pair of *ortho*-protons at a phenyl residue or, in some cases, for the configurationally diastereotopic protons at a methylene position, the shortest contact amongst the set of experimentally indiscernible contacts of this type was used. Whenever configurationally equivalent, but conformationally different groups were analysed, e.g. PBzPh in **2**, all the contacts relevant to these groups were calculated and again the shortest one was taken as the representative. This means that contacts involving the methyl groups of the 3,5-xylyl groups in **3** were extracted by calculating up to 72 individual distances. This number results from the fact that three methyl protons have to be considered for each individual methyl group; there are six such groups representing a total of 18 different methyl proton positions. If these protons are involved in contacts to *ortho*-hydrogens of the benzyl groups, two positions of these protons at the left hand side as well as on the right hand side are potential candidates thus multiplying the number of possible origins of the protons which may be at three different fixed positions, to a total of $18 \times 4 = 72$.

The diastereotopic protons at the CH_2 constituents of the chelate cages, as well as of the CH_2 phenyl substituents, had been uniquely assigned in the nmr experiments with the exception of the protons Bz1A/B (Figure 2) and Me2A/B (Figure 2) for compound **1**. Independent of this assignment, which had been possible in most cases, the analysis of contacts involving these groups was done without incorporating this specification. All possible combinations corresponding to a specific assignment referring to these groups were evaluated in each case. The quality of the assignment was judged by calculating a Boltzmann weighted average over all conformations with this specific assignment and comparing the predicted distances with the observed ones. For compounds **2** and **3** the assignment as originally deduced from nmr analysis was reproduced by this procedure. With compound **1** the situation is more difficult due to its low symmetry. The best fit was obtained with the assignment shown in Figure 2. To evaluate the sums of states a temperature of $T = 300$ K was used throughout in accordance with the measuring temperature of the NOE experiments.

In order to answer the question of how well the NOE distances might be reproduced on the basis of a combination of just a few conformations, a program was written to find the best linear combination of the weights with which these conformations will contribute. The constraint that the sum of the weights has to add up to one has been incorporated in terms of a Lagrangean multiplier.[24][25]

In order to optimise the structure of compound **2** in its crystal environment, the complete set of those 19 molecules in its neighbourhood was selected for which at least one contact to the given molecule is shorter than 8 Å (Figure 8). This environment was fixed to the coordinate values found in the crystal structure and the selected molecule that is embedded by this crystal surrounding was refined. The crystal environment was generated by using the appropriate feature in the MSI program package, refinement was performed by using MacroModel. The calculations were carried out on two Silicon Graphics Indigo^2 MIPS R4400 workstations, 200 MHz, 128 MB RAM, operating under IRIX 5.3.

☆ Dedicated to Professor *Hans Bock* on the occation of his 70th birthday.

[1] J. Hunger, S. Beyreuther, G. Huttner, U. Radelof, K. Allinger, *Europ. J. Inorg. Chem.* **1998**, 693−702.

[2] F. Anet, A. Bourn, *J. Am. Chem. Soc.* **1965**, *87*, 5250−5251.

[3] K. Wüthrich, *Acta Cryst. D* **1995**, *51*, 249−270.

[4] The volumes of *nature structural biology* demonstrate the continously increasing number of protein structures which have been solved by NMR techniques.

[5] O. Walter, T. Klein, G. Huttner, L. Zsolnai, *J. Organomet. Chem.* **1993**, 63−81.

[6] M. Büchner, G. Huttner, U. Winterhalter, A. Frick, *Chem. Ber.* **1997**, *130*, 1379−1392.

[7] A. E. Torda, W. F. v. Gunsteren, in K. B. Lipkowitz, D. B. Boyd (Eds.): *Reviews in Computational Chemistry, Vol. 3,* VCH Publishers, Inc., New York **1992**, p. 143−172.

[8] J. M. Blaney, J. S. Dixon, in K. B. Lipkowitz, D. B. Boyd (Eds.): *Reviews in Computational Chemistry, Vol. 5,* VCH Publishers, Inc., New York **1994**, p. 299−335.

[9] For a recent application of distance geometry methods see H. Steinhagen, M. Reggelin, G. Helmchen, *Angew. Chem.* **1997**, *109*, 2199−2202. For molecular dynamics simulations which make use of distance constraints as well as of constraints derived from a Boltzmann weighting scheme see: J. Fennen, A. E. Torda and W. F. van Gunsteren, *Journal of Biomol. NMR*, **1995**, 6, 163−170.

[10] The underlying assumption is of course that the system is in its thermodynamic equilibrium. For the compounds studied this means that none of the rotations of either phenyl or benzyl groups is slowed down to such an extent that this assumption would not be valid. For the present case complete dynamic equilibration of the conformers is observed on the NMR time scale.

[11] S. Beyreuther, J. Hunger, G. Huttner, S. Mann, L. Zsolnai, *Chem. Ber.* **1996**, *129*, 745−757.

[12] J. S. Giovannetti, C. M. Kelly, C. R. Landis, *J. Am. Chem. Soc.* **1993**, *115*, 4040−4057.

[13] [13a] C. P. Casey, S. L. Hallenbeck, J. Monty Wright and C. R. Landis, *J. Am. Chem. Soc.* **1997**, 119, 9680−9690. − [13b] C. Landis, V. Allured, *J. Am. Chem. Soc.* **1991**, 113, 9493−9499.

[14] [14a] T. Dierks, Diplomarbeit, Heidelberg **1995**. − [14b] S. Cunskis, Diplomarbeit, Heidelberg **1996**.

[15] A. Bax, R. H. Griffey, B. L. Hawkins, *J. Magn. Reson.* **1983**, *55*, 301−315.

[16] A. Bax, M. F. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.

[17] M. Rance, O. W. Sørensen, G. Bodenhausen, G. Wagner, R. R. Ernst, K. Wüthrich, *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479−485.

[18] L. Braunschweiler, R. R. Ernst, *J. Magn. Reson.* **1983**, *53*, 521−528.

[19] [19a] J. Jeener, B. H. Meier, P. Bachmann, R. R. Ernst, *J. Chem. Phys.* **1979**, *69*, 4546−4553. − [19b] D. Neuhaus, M. Williamson, *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH, New York, **1989**.

[20] XWIN-NMR 1.3, Bruker, **1995**.

[21] "*Medio tutissimus ibis*", Ovid, *Metamorphoses*, II, 137.

[22] The *mm2* force field as implemented in Macromodel 5.0 (*mm2**) has been used throughout (see F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comp. Chem.* **1990**, *11*, 440−467).

[23] E. Polak, G. Ribiere, *Revue Francaise Informat. Recherche Operationelle* **1969**, *16*, 35.

[24] P. S. Shenkin, D. L. Yarmush, R. M. Fine, H. Wang, C. Levinthal, *Biopolymers* **1987**, *26*, 2053.

[25] R. Courant, *Vorlesungen über Differential- und Integralrechnung. Zweiter Band. Funktionen mehrerer Veränderlicher, 3rd* ed., Springer Heidelberg, **1955**.

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