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The conformational analysis of methyl β-xylobioside: effect of choice of potential functions

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ABSTRACT

In order to determine the effect of the choice of potential function used in the conformational analysis of a carbohydrate, the NMR spectrum of methyl β-xylobioside [β-D-Xyl-(1 → 4)-β-D-Xyl-(1 → O)-Me] was interpreted using calculated $J_{^{13}C-H}$ coupling constants and nuclear Overhauser effects for protons across the anomeric linkage. Conformational flexibility was described by calculating average ϕ and ψ angles, and estimating their standard deviations. ECEPP2, ECEPP83, and HSEA potentials were used in the first series of calculations. The calculated coupling constants and nuclear Overhauser effects were averaged over the Boltzmann distribution of conformations of the disaccharide in which the entire ϕ,ψ space was scanned in ten-degree steps while retaining fixed bond distances and angles in the remainder of the molecule. In the second series of calculations, MM2, MM2CARB, and PCILO parameters were used to calculate conformational energies. Conformational optimization was done. The effect of temperature and solvent on the calculated coupling constants was negligible. Calculated properties from conformations whose energies were based on the ECEPP parameters gave the best agreement with experiment. Exploration of the conformational space in breadth rather than on a detailed level of full optimization appears to be a preferable course of action.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is used to determine the configurations and conformations of organic molecules because atoms can be distinguished according to their chemical and geometrical environments. Interpretation of spectra involves comparing experimental quantities against their counterparts calculated from candidate geometries for the compound. Coupling constants

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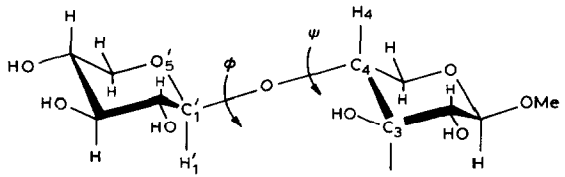

Fig. 1. The methyl β-xylobioside molecule, β-D-Xyl-(1 → 4)-β-D-Xyl-(1 → O)-Me.

(J) and nuclear Overhauser effects (NOEs) are two properties that are used frequently for analyzing geometry. The values of the properties depend indirectly on the choice of potential function selected for optimization of the geometry or for the generation of conformations and their statistical weights.

The compound studied was methyl β-xylobioside [β-D-Xyl-(1 → 4)-β-D-Xyl-(1 → O)-Me]. This compound is similar to cellobioside with the exception of the missing $-CH_2OH$ groups on C-5 of both sugars. The lack of this group was expected to yield somewhat simpler spectra and somewhat greater flexibility around the anomeric linkage. The sugar rings assumed the $^4C_1$ conformation (Fig. 1). The NMR spectrum of the methyl β-D-xylobioside had been reported in refs 1 and 2.

Although the methyl β-xylobioside is of interest in itself, the study also examined the impact of common assumptions and techniques that can be expected to be used when larger oligosaccharides and polysaccharides are studied. The tools that are often employed in the modeling of peptides and proteins can be anticipated to be applicable to sugars. For small compounds, starting geometries may be allowed to relax. However, for larger compounds, fixed geometries of the constituents (i.e., bond lengths and three-atom angles) are maintained, and only dihedral angles are allowed to change. This assumption is made because the number of internal variables becomes unmanageable as the molecule becomes larger. More effort is expended on the dihedral angles since the effects of changing dihedral angles are presumed to be much more important than the effects that may be obtained by optimizing every bond length and bond angle in the molecule. Exclusive study of dihedral angles permits the use of high speed conformational searching algorithms as well as stochastic techniques to look for the single conformation that best fits the experimental data or to collect a conformational ensemble that best represents the statistics of the system. A number of averages and standard deviations (NOEs, J values, and angles) are calculated as well. Since the disaccharide is small enough to allow exhaustive enumeration of conformations, statistical sampling *per se* may not mean much for the molecule in question. However, the investigation of the averaging procedure and its implications in the study of larger molecules by molecular dynamics or Monte Carlo techniques, in which genuine statistical sampling is done, is made easier by using a simpler model compound. Details of the manner in which the calculations were done will be presented in the Experimental section of this report.

TABLE I

Summary of calculated and experimental nuclear Overhauser effects and coupling constants (J) for methyl β-xylobioside

	Parameter sets						
	ECEPP2	ECEPP83	HSEA	MM2	MM2CARB	PCILO	Exptl
Lowest energy conformation							
ϕ	−60	−60	−70	−84	−69	−80	
ψ	110	110	150	152	152	84	
$J(\phi)$	1.6	1.6	2.5	3.0	2.4	3.3	
$J(\psi)$	5.4	5.4	4.3	4.1	4.1	3.7	
NOE: H-4 [satd H-1′]	12.3	12.3	8.2	7.9	10.8	17.0	
NOE: H-1′ [satd H-4]	11.4	11.4	5.7	5.7	8.6	16.0	
Averaged results (25°C)							
$J_{^{13}C-H}$							
$\langle J(\phi)\rangle$	2.1±1.0	1.9±0.9	2.3±0.8	4.1	2.5	3.3	4.7
$\langle J(\psi)\rangle$	5.3±0.3	5.4±0.4	4.2±1.1	3.3	4.1	3.7	5.1
NOE (% error)							
H-4 [satd H-1′]	13.6 (53%)	12.2 (48%)	12.2 (51%)	21.8	10.8	17.1	15
H-1′ [satd H-4]	12.4 (52%)	10.9 (48%)	5.9 (62%)	20.6	8.6	16.0	12
Dihedral angles							
$\langle\phi\rangle$	−65±12	−62±12	−67±10	−93	−71	−79	
$\langle\psi\rangle$	112±12	118±13	149±15	106	144	85	

RESULTS AND DISCUSSION

Table I summarizes the primary data obtained from simulation using the six methods for calculating energies and the experimental NOEs and J values across the anomeric linkage. Results are recorded for both the lowest energy conformation and the statistically weighted set of conformations using Boltzmann weights. The average dihedral angles correspond to estimates that could be made in stochastic calculations. Estimates of standard deviations were made in the course of doing the calculation using ECEPP2[3], ECEPP83[4], and HSEA[5] parameters. Fixed geometries were used in the ECEPP2, ECEPP83, and HSEA calculations. Only dihedral angles were allowed to change in ten-degree steps. The MM2[6], MM2CARB[7], and PCILO[8] calculations were done after conformational optimization which included bond lengths and bond angles in addition to the anomeric torsions. Although only results at 25°C were reported, additional calculations were done at 65°C. There were no differences in the calculated results for the two temperatures. Furthermore, temperature had little effect on the experimental results. Corrections for various solvents were applied in the MM2, MM2CARB, and PCILO calculations[9]. The calculated results were insensitive to solvent. The experimental results recorded in Table I are for the compound in water at 25°C. Nearly identical experimental values were found in methanol at the same temperature.

The two revised versions of ECEPP produced the expected anomeric angle ϕ

without requiring any special torsional potential functions. The standard C–O torsion used in both schemes was applied to all of the C–O bonds including the sugar linkages. The major differences between the results of the ECEPP variants and HSEA may be attributable to the form of the potential function. The ECEPP schemes use Lennard–Jones 6–12 functions to calculate the nonbonded interactions and 10–12 functions for hydrogen bonds. HSEA uses 6-exp functions that are "softer", and HSEA does not include hydrogen bond functions. In the ECEPP variants, C-1′ and H-4 were predicted to be nearly eclipsed as indicated by the large predicted $J(\psi)$ value. Since the C-1′–H-4 angle opened by an additional 30° in the HSEA calculation, the predicted value of $J(\psi)$ was lower.

The close resemblance between the ground-state conformation, the average conformation, and the results from averaging properties over conformations suggests that the ground state may represent satisfactorily the overall distribution of conformations for this compound. Even though many conformations can appear in the ϕ,ψ map, only the handful of conformations that were tightly clustered around the minimum energy conformation contributed to the bulk of the conformational probability. For example, in the ECEPP2 potential, 77% of the conformations were within the narrow range of ϕ: $-50°$ to $-90°$ and ψ: 100 to 120°. Meaningful probabilities were found for no other conformations. The same was true for the ECEPP83 and HSEA function sets with the center of the well in the HSEA calculation shifted to larger ψ values.

The nuclear Overhauser effect characterizes conformations by probing H–H distances in the molecule. Since the proton–proton distances are present in the formula to the 6th power, only protons that are close will affect the response. The two principal NOEs associated with ϕ and ψ are that of H-4 when H-1′ is saturated, and that of H-1′ when H-4 is saturated. The NOEs were insensitive to temperature. The differences among the NOEs reflected differences in the potential functions used. The ECEPP variants placed the H-1′ and H-4 protons closer to each other than did HSEA.

The differences in NOEs between ECEPP2 and ECEPP83 demonstrate the sensitivity of calculated NOEs to the choice of potential function. The average ψ angle changes by 6° and the average ϕ angle by only 2° when ECEPP2 and ECEPP83 results are compared. The change in average conformation was sufficient to change the values of the averaged field used to calculate NOEs by an amount that was large enough to change the NOE by a little over 1% (or approximately 10% with respect to the value of the response itself). The source of the difference is the sensitivity of the σ(H-1′,H-4) and ρ(H-4) coefficients (off-diagonal and diagonal elements in the relaxation matrix, respectively) in the NOE equations. The value of σ was 15% smaller in ECEPP83 than in ECEPP2 although the same minimum point in ϕ,ψ space was found for both ECEPP83 and ECEPP2. The slight change in average r(H-1′,H-4) caused by small changes in the distribution of angles around the same minimum point was magnified because the distance is taken to the 6th power. The ρ value is also sensitive to the surroundings

of a proton since it is the sum of r_{ij}^{-6} terms over all $j \neq i$. H-4 is an axial proton and is relatively far from the protons in its own ring (except H-2). On the other hand H-1′ is also axial, but it is on the same side of the ring as H-3′ and H-$5'_{ax}$. Its ρ value should be less sensitive to the presence of protons on the other ring.

Coupling constants were calculated by averaging over J values (tabulated as $\langle J(\text{angle})\rangle$). When the angles in question are in a quasi-linear part of the curve, for example, for local angles between 20 and 70°, the averaged quantity appears as if it were a nearly linear monotonic function. The ϕ angle in the disaccharide is an example of this situation. The local C-4–H-1′ angle is 60°, corresponding to a standard exoanomeric angle of −60° and is, therefore, in this monotonic portion of the curve.

The local angle between C-1′–H-4 corresponding to a standard ψ angle of 120° is zero. Variations around a ψ angle of 120° will give local angles that are small and close to zero. The corresponding J values will peak at 0° locally and decrease on both sides of the angle. Averaging J values will tend to give a reduced coupling constant. If a coupling constant calculated by averaging J values were used to assign a conformation by back-calculating the angle from the averaged J, the local angle found would be larger than the actual angle. In addition to misrepresenting the angle itself, averaging J values yields statistical error limits that are not very useful. The standard deviations associated with $\langle J(\psi)\rangle$ in the ECEPP potentials imply nothing about the mobility around the ψ angle in these potentials. The small standard deviations for $\langle J(\psi)\rangle$ in ECEPP2 and ECEPP3 are computational artifacts caused by the fact that the J values turn around in the region where most of the averaging is taking place.

In order to measure the flexibility of the dihedral angles, the average angles and their standard deviations were calculated. The standard deviation is a direct measure of flexibility. Since methyl β-xylobioside exhibits only one energy well, taking averages yields meaningful values. If the distribution of conformations were bimodal or trimodal, the averages would not be meaningful, and their poor quality would be flagged by very large standard deviations. The primary utility of evaluating average angles would be in inspecting portions of molecular dynamics trajectories or Markov chains from Monte Carlo calculations. Numerical estimates can be more informative than, for instance, the circle plots or conformational dials commonly used to represent changes in angles in the course of a calculation. The direct evaluation of statistics on the angles themselves can disclose more about the flexibility of a torsion angle than averaging J values, which are derived quantities. The ranges on the standard deviation limits on ψ itself indicate that the large majority of the ψ angles will be within 12 to 15° of the mean value regardless of which potential function selected.

The interactions between H-1′ and H-4 had the largest percentage errors. The errors reflect the flexibility of the ϕ,ψ angle pair. Even though the conformational densities in the ϕ,ψ angle space point to a single, rather narrow concentration of conformations, there is enough play in the angle pair to cause changes in the value

of $r(H,H')^{-6}$ that affect the σ and ρ values from which the NOEs are calculated. If there were additional conformations existing in their own potential wells with significant probabilities, the calculated NOEs would have much larger errors. The assignment of a unique conformation by finding a correspondence between a set of experimental NOEs and a set of calculated NOEs is a procedure that could easily ignore the Boltzmann distribution of conformations in which the molecule exists and the possible errors can be estimated from the averaging process.

In a second series of calculations using MM2, MM2CARB, and PCILO parameters, geometries were optimized after an initial screening for local minima. Six were found. The optimized conformations in each energy method were used to calculate coupling constants of the ϕ and ψ angles for the molecule both as an isolated species and in solvent using a previously described computational methodology[9]. The energies associated with each conformation were used to calculate average angles, average J values, and NOEs.

In the MM2 and MM2CARB calculations, the lowest energy conformation resembles that found in the ECEPP2, ECEPP83, and HSEA calculations. The other higher-energy conformations are much closer together by using MM2 instead of MM2CARB. The higher energy conformations are furthest from the lowest using PCILO and have little effect on the final results. The five higher energy conformations would have had energies in either ECEPP2 or ECEPP83 so high that they would not appear in the set of acceptable conformations. Two of the six conformations were present in the HSEA set of acceptable conformations. The other four were of very high energy in HSEA. The type of energy method one uses has a significant effect on the set of conformations that are finally included in a study of ϕ,ψ space.

All three sets of conformations optimized in a "vacuum" condition were placed in solvent, and the energies of the conformations were recalculated. Comparison of these results with the calculations on the isolated molecule showed negligible effects due to solvation. Solvation was not considered further. Since the solvation results were nearly the same as for the isolated molecule, the same observations and comments made concerning the isolated molecule are applicable to the solvated molecule.

The relevance of the theoretical calculations can be determined only by comparison with experiments. Both J values and NOEs have been determined for methyl β-xylobioside in a number of solvents and at different temperatures[1,2]. If average J values are compared, none of the three — MM2, MM2CARB, and PCILO — agree with experimental results. The ECEPP2, ECEPP83, and HSEA calculations were performed only on the isolated molecule. In all three cases, the usual $-60°$ exoanomeric ϕ angle is not open enough. The coupling constant across ϕ is too small by a factor of 2. In order to obtain coupling constants for ϕ closer to experimental results, ϕ would need to open up to ca. $-100°$. The ECEPP2 and ECEPP83 calculations of $\langle J(\psi)\rangle$ were in agreement with experimental measurements.

TABLE II

Ratios of NOEs of movable protons to NOEs of intraring protons at 25°C

Proton saturated	H-1′	H-4
Ratio of NOEs	H-4/H-3′	H-1′/H-2
Source of NOE values		
Exptl, water	1.1	0.6
ECEPP2	1.2	0.8
ECEPP83	1.0	0.7
HSEA	0.7	0.4
MM2	1.9	1.3
MM2CARB	0.9	0.5
PCILO	1.4	1.0

Measurement of the nuclear Overhauser effect should provide corroborating evidence in the analysis of the conformations of xylobiose. Although extraction of NOEs can often provide only fragmentary information, it is usually possible to extract the major effects. In order to compare the theoretical and experimental calculations, one can compare the ratio of the NOE of the movable proton to the NOEs of protons in the same ring as the saturated proton. The response of protons in the same ring as the saturated proton provides an internal standard. These ratios are recorded in Table II. Examination of this table indicates that either the ECEPP2 or the ECEPP83 potential functions provide NOE ratios in acceptable agreement with experiment. This agreement is obtained *in spite of* the fact that the ϕ angle predicted is in poor agreement with that expected from the experimental $J(\phi)$ values. On the contrary, although the MM2 potential yields averaged angles from which acceptable J values might be calculated, the calculated NOE ratios are not even close. There is a precedent for finding differences between the "NOE structure" and the "J structure" of a molecule[10]. Emphasis on one or the other property implies an emphasis on different physical behaviors, which are handled differently in the averaging process.

There are two separate optimization goals — finding the best angles and finding the best internuclear distance between the protons across the anomeric linkage. They are coupled through the interatomic potential functions used and the degree of flexibility allowed to the molecule. A set of protons may achieve satisfactory interatomic distances with many combinations of dihedral and three-atom angles and bond distances. Likewise a set of satisfactory dihedral angles may correspond to a large number of combinations of three-atom angles and bond distances. The details of the interatomic potentials will determine the resulting conformation. Relaxing one or more constraints (e.g., allowing a three-atom angle to vary) could either worsen agreement of an otherwise acceptable calculation or allow an unsatisfactory calculation achieve good agreement.

The study of the sensitivity of a parameter set to geometry is an integral part of the creation and testing of the parameter set. Examination of the references on the

parameter sets and related reports will show that extensive testing had been done. However, the testing focused on the use of a parameter set to describe the behavior of a single molecule as it may change from one conformation to another, often with the emphasis on finding the best single conformation. The development and the testing of empirical parameter sets did not consider the different kind of problems presented in this report in which not only the "best" conformation has meaning, but also other conformations of higher energy are useful in generating an averaged distance for NOE calculation or angular "field" to be used in calculating a coupling constant, respectively. The higher energy conformations became more than merely temporary geometries on the way to the "best". The results reported here indicate that commonly used methods for calculating conformational energies have shortcomings that are easily seen when tested on a small molecule like methyl β-xylobioside. Calculations on larger sugars can be expected to be affected similarly.

The difference between the "NOE structure" and the "*J* structure" of a molecule has implications in performing constrained stochastic optimization of the structure. Often, the experimental NOEs or *J* values, or both, are used in the form of a Hooke's law potential that keeps the conformation close to the geometry that would reproduce the NOEs or coupling constants. If the structure leading to good NOEs may easily differ significantly from the structure leading to good coupling constants, using constraints based on either or both quantities may be prejudicial to the final result or be inherently at cross purposes. If problems of this sort can arise with peptides, the conformations of which are somewhat better behaved than saccharides, difficulties can certainly be anticipated in the study of sugars by constrained stochastic methods.

The general conclusions of this study are as follows: the agreement of NOEs calculated from a single conformation with a set of experimental NOEs is insufficient to assign that geometry. The agreement of a set of calculated *J* values with a set of experimental *J* values is insufficient to assign an "average" conformation. Since an experimental response is obtained as the response of a Boltzmann distribution of conformations, the prediction of this experimental response ought to be obtained by Boltzmann averaging over the energies generated by the application of the model potential. The somewhat better performance of the ECEPP-based parameter sets suggests that their use might be extended (no doubt, with modifications) from proteins for which they were originally developed to sugars. Since the better results used averaged distances calculated over the Boltzmann distribution of states, it may be preferable to look for the ensemble of contributing conformations instead of searching for a best single structure.

EXPERIMENTAL

Two ways of changing the conformation were used. In the first method, the change in conformation was attributed solely to changes in the values of the two

dihedral angles of the anomeric linkage between the xylose units. The ring geometries were kept rigid. The values of the bond distances and bond angles were adapted from the Arnott and Scott[11] geometry for D-glucose. Although the conformations were not optimized, a complete set of angles can be scanned in order to represent the density of states associated with each potential energy well. The other approach was to optimize the geometries of a small set of candidate conformations and then to calculate the average properties over the set of optimized conformations.

In the first approach, conformational energy calculations over the entire ϕ,ψ space in ten-degree steps were performed on xylobiose. Angles ϕ and ψ are standard right-handed dihedral angles. Angle ϕ is the angle O-5′–C-1′–O–C-4. Angle ψ is the angle C-1′–O–C-4–C-3. The primes identify atoms on the nonreducing sugar. Three different empirical energy function sets were used: (1) ECEPP2, the ECEPP potential as revised by Scheraga and co-workers[3], (2) ECEPP83, the ECEPP potential as revised by Momany et al.[4], and (3) the HSEA potential functions of Lemieux and co-workers.[5] The disaccharide was treated as an isolated, unsolvated molecule. Only the ϕ and ψ angles changed in the calculation. The other geometrical parameters remained fixed. Using the conformational energies calculated in each potential energy function scheme, average angle values for ϕ and ψ, average coupling constants, and nuclear Overhauser effect responses across the bonds corresponding to ϕ and ψ were calculated.

In the second approach, three sets of calculations were performed in which geometries were optimized after an initial screening for local minima. The methods by which conformational zenergies were calculated were MM2[6], MM2CARB[7], and PCILO[8]. In these three methods, it was possible to treat the molecule either as an isolated species or in solvent using a previously described computational methodology[9]. Only a handful of conformations were obtained. Calculated solvent corrections and temperature dependence were found to have been negligible.

The calculation of the relative populations of conformers was based on Boltzman probabilities, $\exp(-E_i/kT)/\Sigma_j \exp(-E_j/kT)$. Each property for an individual conformation is weighted by this factor when ensemble properties are calculated. Since this problem has only two degrees of freedom, and evaluation of conformational energies was done at each point in the ten-degree grid into which the dihedral angles had been divided, the averages reported are population averages, rather than sample averages.

Nuclear Overhauser effects were calculated in approximation II of Noggle and Schirmer[12] in which the NOEs were calculated by the solution of the set of linear equations

$$\rho_i f_i + \sum_{j \neq i,\, k} \sigma_{ij} f_j = \sigma_{ik} \qquad (1)$$

in which ρ_i and σ_{ij} were formed by Boltzmann averaging over the ρ_i and σ_{ij} values calculated for individual conformations. The saturated proton is identified

by k. Methyl corrections were calculated by using Woessner's formulae[13]. The value of τ_c was chosen to be 2×10^{-10} s; τ_{methyl}, 1×10^{-13} s; and external relaxation was set at 0.03 s. The spectrometer frequency was 300 MHz.

The predicted NOEs can also be assigned error limits. Since the NOE of proton i upon saturation of proton k can be approximated by

$$f_i \approx \sigma_{ik}/\rho_i \tag{2}$$

The percentage error associated with f_i can be estimated by adding the percentage errors of σ_{ik} and ρ_i. The NOEs were calculated by solving the appropriate system of linear equations in which σ_{ik} and ρ_i were averaged over the Boltzmann distribution. The standard deviations of σ_{ik} and ρ_i were calculated in the procedure.

$J_{^{13}C-H}$ coupling constants for C-1′–H-4 and C-4–H-1′ pairs were calculated using the recently determined formula[14]

$$J_{^{13}C-H} = 5.7 \cos^2\theta - 0.6 \cos\theta + 0.5 \tag{3}$$

in which θ is the local dihedral angle between the proton and the carbon.

Coupling constants for the ensemble were calculated by summing the $J_{^{13}C-H}$ values of the individual conformations weighted by the proper Boltzmann factor.

Averaged angles were calculated using a phase angle averaging method[15]. In this method, the dihedral angle θ is represented as if it were a vector of unit length in the complex plane

$$\exp(i\theta) = \cos\theta + i \sin\theta \tag{4}$$

The average sines and cosines are given by the weighted contributions of the sines and cosines of each conformation

$$\langle \cos\theta \rangle = \sum_i f_i \cos\theta_i \tag{5a}$$

$$\langle \sin\theta \rangle = \sum_i f_i \sin\theta_i \tag{5a}$$

The "average angle" (θ_{ave}) is calculated from

$$\theta_{ave} = \arctan(\langle \sin\theta \rangle / \langle \cos\theta \rangle) \tag{6}$$

The standard deviations of the sines and cosines likewise define vectors of the form $\sigma(\cos\theta) + i\sigma(\sin\theta)$:

$$\sigma(\cos\theta) = \left(\sum_i f_i [\cos\theta_i - \langle \cos\theta \rangle]^2 \right)^{1/2} \tag{7a}$$

$$\sigma(\sin\theta) = \left(\sum_i f_i [\sin\theta_i - \langle \sin\theta \rangle]^2 \right)^{1/2} \tag{7b}$$

The combination of standard deviations of the trigonometric functions that yields the largest error range (θ_s) will be used as a conservative estimate of the angular error:

$$\theta_s = \arctan[\sigma(\sin\theta)/\sigma(\cos\theta)] \quad (8)$$

The average angle calculated by this method is rotationally invariant. Averaged angles should be treated with caution. They are used only to represent the flexibility of the angles. Like average distances from NOE calculations, average angles may not represent any genuine conformation.

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REFERENCES

1 M. Hricovini, I. Tvaroška, D. Uhrin, and Gy. Batta, *J. Carbohydr. Chem.*, 8 (1989) 389–394.
2 M. Hricovini, I. Tvaroška, J. Hirsch, and A.J. Duben, *Carbohydr. Res.*, 210 (1991) 13–20.
3 G. Nemethy, M.S. Pottle, and H.A. Scheraga, *J. Phys. Chem.*, 87 (1983) 1883–1887.
4 H. Chuman, F.A. Momany, and L. Schaefer, *Int. J. Peptide Protein Res.*, 24 (1984) 233–248.
5 H. Thogersen, R.U. Lemiex, K. Bock, and B. Meyer, *Can. J. Chem.*, 60 (1982) 44–57.
6 U. Burkert and N.L. Allinger, *Molecular Mechanics*, ACS Monograph, Vol. 177, American Chemical Society, Washington DC, 1982.
7 G.A. Jeffrey and R. Taylor, *J. Comput. Chem.*, 1 (1980) 99–109.
8 S. Diner, J.P. Malrieu, F. Jordan, and M. Gilbert, *Theor. Chim. Acta*, 15 (1969) 100–110.
9 I. Tvaroška, S. Perez, O. Noble, and F. Taravel, *Biopolymers*, 26 (1987) 1499–1508.
10 H. Kessler, Ch. Griesinger, J. Lautz, A. Muller, W.F. van Gunsteren, and H.J.C. Berendsen, *J. Am. Chem. Soc.*, 110 (1988) 3393–3396.
11 S. Arnott and W.E. Scott, *J. Chem. Soc., Perkin Trans. 2*, (1972) 324–335.
12 J.H. Noggle and R.E. Schirmer, *The Nuclear Overhauser Effect: Chemical Applications*, Academic Press, New York, 1971, pp 90–93.
13 D.E. Woessner, B.S. Snowden, and G.H. Meyer, *J. Chem. Phys.*, 50 (1969) 719–721.
14 I. Tvaroška, M. Hricovini, and E. Petrakova, *Carbohydr. Res.*, 189 (1989) 359–362.
15 A.J. Duben, *Proceedings of the Summer Computer Simulation Conference, 1989*, Austin, TX, July 24–27, 1989, Society for Computer Simulation, San Diego, CA, pp 310–314.