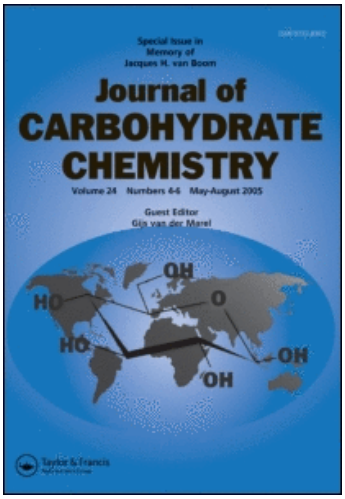

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β-LACTOSE IN THE VIEW OF A CFF-OPTIMIZED FORCE FIELD

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ABSTRACT

A recently developed force field PEF95SAC, based on Consistent Force Field (CFF) optimized potential energy parameters for alcohols and most of the naturally occurring carbohydrates, is applied to and tested on β-lactose. The properties of the potential energy surface of this disaccharide are compared to X-ray structures, NMR coupling constants and optical rotation data. The overall performance indicates good extrapolative power for the modeling of oligo- and polysaccharide structures. A new glycosidic linkage geometry region is proposed for β-lactose as being important in both solid state and water solutions. This finding is supported by calculated $J_{H,C}$ coupling constants and calculated optical rotation values.

In relation to the spectral calculations on β-lactose, the error of the use of relative energies (ΔE) in place of the Gibbs free energy (ΔG) as the basis for calculating Boltzmann distributed properties is demonstrated. In the β-lactose case it is shown that the conformational entropy is neither negligible nor uniformly distributed over the potential energy surface.

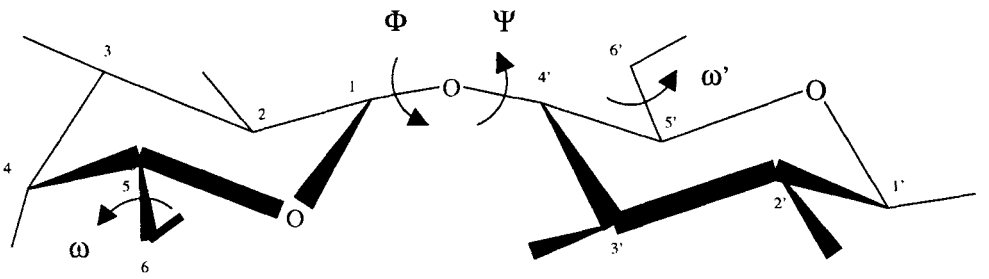

Figure 1. β-Lactose. Important torsional angles and atomic numbering.

INTRODUCTION

In order to validate a new CFF-optimized force field, PEF95SAC,[1] and to demonstrate some of the possibilities, limitations and pitfalls of molecular mechanics methods, we have applied it to β-lactose. Lactose (mother's disaccharide) which is found exclusively in the milk of mammals (approximately 5% of cow's milk) is of great importance to the dairy industry. Because of the wealth of theoretical and experimental data available and because the β(1-4) glycosidic linkage is found in a variety of natural polysaccharides, we found that β-lactose is well-suited for a test application. This molecule (in the form of ethyl β-lactoside) has recently been thoroughly investigated using three different molecular mechanics (MM) force fields (FF) and the results compared to spectral experimental data such as X-ray structures, NMR coupling constants, NOESY volumes and optical rotation data. It was found that the potential energy surfaces depend on the force field used, differing in the number and location of low energy conformers as well as in the shallowness of the dominant primary region.[2]

As previously described,[1] the PEF95SAC force field has improved the reproducibility of rotational barriers, most notably that of the *exo*-cyclic methoxy group in 2-methoxytetrahydropyrans[3] which can be considered as model compounds for the *exo*-anomeric effect.[4] For this reason the PEF95SAC promised good predictive power with respect to the glycosidic linkages when applied to standard carbohydrate molecules.

A schematic representation of β-lactose (4-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose) along with the atom numbering and the torsional angles of interest is

shown in Fig. **1**. The lactose structure involves five main conformational features assuming that the $^4C_1$ chair forms are the predominant ring conformations, a fact which is supported by results from both solid state X-ray diffraction and solution NMR experiments.[2] The primary conformational features are the orientation of the glycosidic linkage bonds as described by the torsional angles ϕ = O5-C1-O1-C4' and ψ = C1-O1-C4'-C5'. Secondary conformational features are the orientations of the hydroxymethyl groups as described by the torsional angles ω = O5-C5-C6-O6 and ω' = O5'-C5'-C6'-O6' and the intercyclic hydrogen bond HO3'...O5 which stabilizes the lactose structure in solid state as well as in water solution. The orientations of the primary hydroxyl groups are referred to as either *gauche-gauche* (GG), *gauche-trans* (GT) or *trans-gauche* (TG) depending on whether the values of the above torsion angles are closer to 300°, 60° or 180° (torsional angles are given between 0° and 360°).

THE ADIABATIC MAP

The potential energy surface of β-lactose was calculated as described by Engelsen *et al.*[2] The construction of the adiabatic map involved the computation of 36 individual relaxed maps. The relaxed maps were computed using rigid rotation followed by harmonic constraint minimization in 10° increments for ϕ and ψ spanning the whole angular range and convergence was accepted when the rms gradient was less than 0.001 kcal·mol^{-1}Å^{-2}. The optimized coordinates of each point on all 36 grids were stored for further calculations. This approach results in 46,656 possible conformations of which 24,925 were accepted for further calculations on the basis of criteria of relative energy (20 kcal·mol^{-1}) and values of ω and ω' which were not constrained in the relaxed map calculations.

The resulting potential energy surface (PES) is shown in Fig. **2**. Within 8 kcal·mol^{-1} the occupied area in (ϕ,ψ)-space is 32% and the calculated 3D-volume of the adiabatic map is 191×10^3 deg^2·kcal·mol^{-1}. These overall values indicate a relatively restricted force field which in this respect places the PEF95SAC in between the MM3(92)[5] and HGFB[6] FF's (see ref. 2).

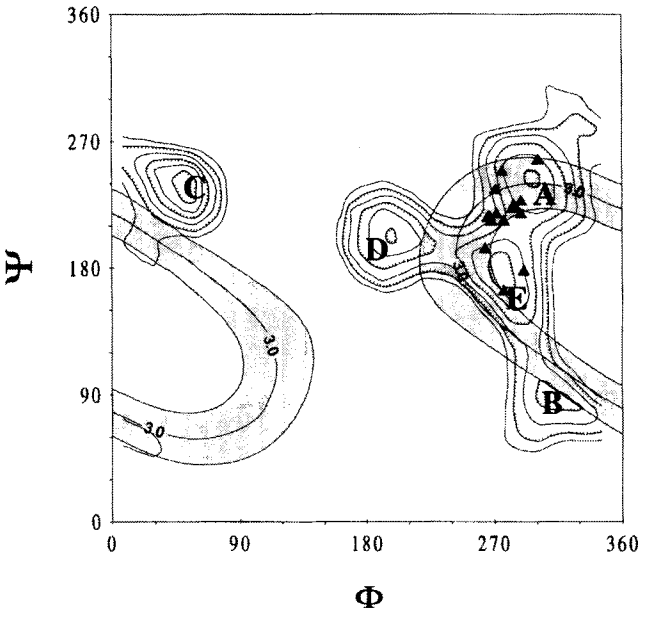

Figure 2. Adiabatic (ϕ,ψ)-map of β-lactose. Iso-energy contour lines are drawn in 1 kcal·mol^{-1} intervals within a 6 kcal·mol^{-1} range above the global minimum. Lactose conformations found in pure single crystals and lactose conformations co-complexed with proteins are marked with a black triangle (▲). Distance contours (2.5, 3.0 and 3.5 Å) of the important O3'...O5 hydrogen bond are superimposed.

Upon visual inspection, the PES of β-lactose appears as a hybrid between the PES of the PEF91L[7] and the HGFB FF's. This is reasonable, since the main difference between PEF91L and PEF95SAC is the "non-optimized" use of *ab initio* charges which has resulted in considerable increased partial charges not unlike those of the HGFB FF. The PEF95SAC FF has also in common with HGFB that it is developed as a true vacuum type force field. One main feature is unique to PEF95SAC: the lactose PES displays a new local minimum well, labeled **E**, centered about (ϕ = 280; ψ = 170) only 0.12 kcal·mol^{-1} above the global minimum in the **C**-well. It is perhaps remarkable that three of the crystal conformations of lactose co-crystallized with proteins are found in this well (within 2 kcal·mol^{-1}). As is apparent from Fig. **2**, the **E**-well can also accommodate the O5...O3' hydrogen bond for which reason it does not contradict X-ray crystallography (it is observed in all structures obtained to date[2]) and recent NMR results in water solution

which strongly suggest a high prevalence of this bond.[2,8] The important **A**-well in PEF94SAC is found to be 0.69 kcal·mol^{-1} above the **C**-minimum. It accomodates most crystal structures and combines steric-free conformational space with O5...HO3' inter-residue hydrogen bonding and optimal *exo*-anomeric configuration. The global minimum in PEF94SAC is found in the **C**-well for which we have no experimental evidence. The **C**-well accommodates conflict-free conformations with the possibility of favorable electrostatic interactions between the HO6' and O5 (bifurcated with O1), between O3' and HO2 and between HO6 and O6'. As is the case with the HBFG FF, the large numerical values of the partial charges in PEF95SAC favor these predominantly electrostatic interactions when calculations are done in vacuum.

ENTROPY CALCULATIONS

In order to investigate the discrepancy between the lack of experimental evidence and the very favorable energetics of the **C**-well in the PEF95SAC force field (and HBFG) we calculated the entropy contributions to the local and global minima of β-lactose according to the formula:[9]

$$S = S_{trans} + S_{rot} + S_{vib}$$

$$S_{trans} = R\ln\left(\frac{(2\pi mkT)^{\frac{3}{2}}kT}{h^3 p}\right) + \frac{5}{2}R$$

$$S_{rot} = R\ln\frac{8\pi^2(2\pi kT)^{\frac{3}{2}}(I_A I_B I_C)^{\frac{1}{2}}}{h^3\sigma} + \frac{3}{2}R$$

$$S_{vib} = R\sum_{i=1}^{3n-6}\frac{h\nu_i / kT}{\exp(h\nu_i / kT)-1} - R\sum_{i=1}^{3n-6}\ln(1-\exp(-h\nu_i / kT))$$

where R is the gas constant, T is the temperature, k is the Boltzmann constant, m is the molecular mass, h is the Planck constant, p is the pressure, I_A, I_B and I_C are the principal moments of inertia, σ is the symmetry number (1 for all carbohydrates), ν_i is the i'th

Table 1. Optimized grid points of the global and local energy minima. Angles are in degrees, distances are in Å, energies are in kcal·mol^{-1} and molar rotations are in deg·cm^2·dmol^{-1}

Conformer	ϕ	ψ	ω	ω'	ΔE	ΔG	p	Rg	[M]
A	295	242	TG	GG	0.69	0.30	0.27	3.56	417
B	308	90	TG	GG	2.42	2.17	0.01	3.44	-48
C	52	238	TG	GT	0.00	0.50	0.19	3.44	236
D	198	205	TG	GG	0.59	1.00	0.08	3.63	347
E	278	174	TG	GG	0.12	0.00	0.45	3.57	115

normal mode frequency and ***n*** the number of atoms in the molecule. The most important entropy contribution is the vibrational entropy, which in turn needs full evaluation of the normal mode frequencies. This computational bottleneck is the reason why Boltzmann distributed entities are often calculated on the basis of relative potential energies ΔE, assuming that the conformational entropy was either negligible or uniformly distributed, in place of the Gibbs free energy ΔG.

The results of the entropy calculations are shown in Table **1**. The Gibbs free energies at 300 K for the four local and the global minima are listed along with the free enthalpies. The table clearly indicates that the conformational entropy is neither negligible nor uniformly distributed for a molecule such as β-lactose. It has previously been indicated that the **C**-well has a more restricted conformational entropy than the **A**-well, from the point of view of a diminished conformational diversity.[10] Our point calculations of the free energy support this indication. Using the ΔG calculations, the **E**-minimum becomes the most populated followed by the **A**-minimum; in third place comes the **C**-minimum with a relative free energy of 0.5 kcal·mol^{-1} corresponding to approximately 20% population at 300 K. These ΔG point calculations reflect the local shape of the potential energy surface, a flatter minimum region resulting in increased entropy.

Among the three most important minimum conformations **A**, **C** and **E**, the **E**-conformation has the largest radius of gyration and the **C**-conformation the lowest. The

low value for the Rg and thus the more folded structure may explain why the **C**-conformation cannot easily propagate into a crystal lattice for which reason such a solid state conformation has not yet been detected. In water solution the attractive non-bonded forces which favor the **C**-conformation will be more or less neutralized by the strong competition from the water molecules. For this reason it is not likely to be significantly populated in water. The reason for the large Rg value of the **E**-minimum is an almost planar "three ring" structure where the center ring is a seven-membered pseudo ring structure, including the O5...HO3' hydrogen bond.

OPTICAL ROTATION

According to Bates,[11] the optical rotation $[\alpha]_D^{20}$ of β-lactose in water is between 34.9 and 55.4 deg, of which the first number can be converted into a molar rotation [M] of approximately 120 deg·cm^2·dmol^{-1}.

The optical rotation of β-lactose in water was calculated using a semi-empirical model based on interacting oscillators, as described by Stevens and Sathyanarayana.[12] This approach has been claimed to be useful for probing carbohydrate force fields[13] and it has previously been applied to the study of β-lactose using statistical weights from NMR studies[14] and to the study of ethyl β-lactoside using a complete Boltzmann distributed spectral calculation.[2] In practice the optical rotation model has proven difficult to interpret due to the interference from the primary hydroxyl group orientations which are rarely well reproduced by MM FFs. However, as apparent from Table **1**, primary hydroxyl group orientations are far from being the only parameters affecting the calculated molar rotation.

In this study we have restricted the optical calculations to the local and global minima (Table **1**). As apparent from Table **1**, only the **E**-minimum (115 deg·cm^2·dmol^{-1}) in the β-lactose case is able to explain the experimental [M] value corresponding to a fairly restrained system. Significant populations of **A**- and **C**-minima will have to be counterbalanced by an unrealistically large population of **B**-minima which is the only uniformly negative well. In order to further investigate this surprising result we calculated

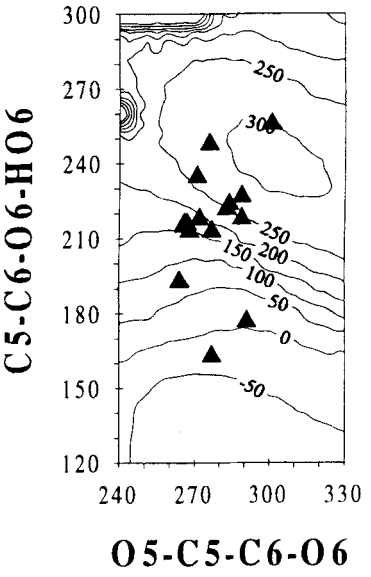

Figure 3. Molar rotation contours of the most important region of the GT-GT map. Crystal conformations of β-lactose are marked with a black triangle (▲).

an optical rotation map where the primary hydroxyl groups are held in the predominant GT-GT conformations (see under coupling constants). In this map (Fig. **3**) the **E**-well results in molar rotations between -50 and 100 deg·cm^2·dmol^{-1} with the center at about 25 deg·cm^2·dmol^{-1}, whereas both the **A**-well and the **C**-well result in molar rotations higher than 250 deg·cm^2·dmol^{-1}. The only relatively highly populated region able to explain the experimental molar rotation alone is a thin belt just below the center of gravity of the crystal conformations (in between the **A**- and **E**-wells) almost identical to the region labeled A2 by Engelsen *et al.*[10] on the MM3[5] adiabatic map.

COUPLING CONSTANTS

The dependence of molecular dihedral structure upon NMR determined vicinal coupling constants is well established. The coupling constants reflect a time-averaged structure and can be expressed by empirical Karplus-type relationships which are

Boltzmann distributed over all microstates of conformational space. In this study we have performed spectral calculations of coupling constants using relative energies as the basis for these Boltzmann distributed spectral calculations. For comparison we also calculated the Boltzmann distributed coupling constants based on the Gibbs free energy ΔG of four local minima and the global minimum.

Homonuclear coupling constants $^3J_{HH}$ for vicinal hydrogen atoms of a H-C-C-H segment were calculated using the Haasnoot-Altona equation[15] and the heteronuclear coupling constant $^3J_{H,C}$ across the glycosidic linkage was calculated using the Karplus-type equation for the C-O-C-H segment parametrized by Tvaroska *et al.*[16] (further details can be found in ref. 2).

Table **2** compares the calculated coupling constants with the experimental literature values of methyl β-lactoside[17,18] and ethyl β-lactoside. Note that the coupling constants derived from ΔG calculations (last column) of only five conformations is remarkably similar to the coupling constants derived from the spectral ΔH calculation (preceding column). Only the heteronuclear couplings and the pro*R* and pro*S* couplings related to the glucose residue are different. Especially the pro*S* coupling has improved, whereas the glycosidic $J_{H4'C1}$ has deteriorated. These differences are partly due to the differences in entropy of the five minima, but perhaps also due to the differences in conformational diversity of the five energy wells.

The calculations of the heteronuclear coupling constants (Table **2**) are quite accurately reproduced, especially the $J_{H1,C4'}$ which has improved significantly from the PEF91L force field. These calculations indicate a very good performance of the PEF95SAC force field with respect to the glycosidic linkage and provide further support for the **E**-well. Hayes *et al.*[17] argued that the glycosidic conformation in methyl β-lactoside is fairly rigid, especially the rotation about ψ based on an observed $^3J_{C1,C3'}$ coupling close to 0 Hz which will demand that the average dihedral of the contributing conformers is close to 90 degrees. Instead of being rigid around ψ, this investigation strongly suggests a conformational equilibrium between the **E**-well and the **A**-well. The mentioned average value of 90 degrees is in fact almost exactly inbetween the **E**-

Table 2. Calculated ^3J coupling constants (Hz) for β-lactose compared with the observed ^3J coupling constants for methyl β-lactoside and ethyl β-lactoside. NMR-determined population distributions are calculated from the Haasnoot-Altona equation assuming only idealized staggered conformations.

	Observed			Calculated		
^3J	Ref. 17	Ref. 18	Ref. 2	MM3[a]	PEF95[b]	PEF95G[c]
Gal*p*						
H1, H2	7.8	7.9	7.8	7.9	8.0	8.0
H2, H3	10.0	9.8	9.8	9.5	10.1	10.1
H3, H4	3.5	3.5	3.4	5.0	4.4	4.5
H4, H5	1.0	0.4	0.5	1.1	1.4	1.4
H5, H6s	3.8	4.0	3.2	2.6	10.8	10.8
H5, H6r	8.2	8.2	8.6	7.8	4.7	4.7
GG:GT:TG	20:70:10	18:69:13	20:78:2	21:76:3	0:0:100	0:0:100
Glc*p*						
H1', H2'	8.0	7.8	8.0	7.7	7.9	7.9
H2', H3'	9.5	9.8	9.8	9.2	9.8	9.8
H3', H4'	-	9.3	9.3	9.2	9.6	9.6
H4', H5'	9.9	9.3	9.3	10.0	9.9	9.9
H5', H6s'	2.2	2.3	2.1	3.4	5.3	2.6
H5', H6r'	5.1	5.2	5.0	6.8	4.8	3.3
GG:GT:TG	63:47:-9	61:47:-8	64:46:-11	33:57:10	49:19:32	81:19:0
β (1→4)						
H1, C4'	3.8	-	3.7	3.5	3.8	3.6
H4', C1	4.9	-	-	4.2	4.1	3.4

a. From Engelsen *et al.*[2] based on a Boltzmann distribution at 300 K from ΔE, using the complete MM3(92)[5] theoretical ensemble (34,443 conformations).

b. Calculated from a Boltzmann distribution at 300 K from ΔE, using the complete PEF95SAC theoretical ensemble (24,925 conformations).

c. Calculated from a Boltzmann distribution at 300 K from ΔG, using the the four local minima and the global minimum in PEF95SAC.

minimum (52) and the A-minimum (122) for the C1-O1-C4'-C3' dihedral and, in addition, the compiled crystal structures seem to be more restricted in ϕ than in ψ.

As found for all previously investigated force fields, the *axial-axial* intra-ring couplings are modeled quite exactly, indicating stable average $^4C_1$ conformations of the *gluco* and *galacto* rings. The exception is the coupling to the only equatorial hydrogen (H4) which is calculated too high. In contrast, the calculated pro*R* and pro*S* coupling constants of the primary hydroxyl groups are strongly erroneous. Despite the fact that

none of the previously investigated force fields have been able to accurately model the conformational behavior of the C5-C6 bonds (as measured by the coupling involving the prochiral hydrogens), we had some expectations to PEF95SAC due to its changed electrostatic profile and good performance on rotational barriers. Assuming only idealized staggered conformations, the population distribution can be established from the experimental pro*R* and pro*S* couplings through the Haasnoot-Altona equation.[15] For the *galacto* hydroxymethyl group this approach results in a GG:GT:TG ratio of about 20:70:10 and for the *gluco* hydroxymethyl group a GG:GT:TG ratio of about 60:40:0. These NMR results are supported by the statistics on the crystal conformations which result in an absolute GG:GT:TG ratio of 1:13:0 for the *galacto* hydroxymethyl group and 4:8:2 for the *gluco* hydroxymethyl group.[2] Using the complete calculated ensemble of β-lactose in PEF95SAC and a Boltzmann distribution based on ΔH we obtained GG:GT:TG ratios of 0:0:100 and 49:19:32 for the *galacto* and *gluco* hydroxymethyl groups, respectively.

In order to investigate this problem we calculated relaxed maps of β-D-glucose and β-D-galactose by rotating the two torsional angles O5-C5-C6-O6 and C5-C6-O6-HO6 (see Fig. **4**). The figure reveals a more restricted PES for β-D-galactose than for β-D-glucose. The absence of GG conformations on the PES for galactose can be understood in terms of the unfavorable conformation generated with the C4-O4 and the C6-O6 bond dipoles in axial and parallel arrangement (the O4...O6 distance is only about 2.5 Å). This is also called the *Hassel-Ottar* effect.[19] The strong experimental discrimination between the GT and TG conformations is less obvious. They both have the C4-O4 bond parallel with one of the C6-H6 bonds. In the TG conformation the C6-O6 bond is equatorial parallel with the C4-H4 bond, whereas the GT conformation has the C6-H6 bond parallel to the C4-H4 bond. In the GT conformation the C6-O6 bond is turned towards the O5 ring oxygen and has the possibility of a favorable intramolecular interaction (O6...O5 is approximately 2.8 Å) which is absent in the TG conformation. In the case of glucose the *Hassel-Ottar* effect predicts absence of TG conformations due to the parallel and equatorial arrangement of the C4-O4 and the C6-O6 bond dipoles. This is not accurately reflected by PEF95SAC; although the GG conformer is the global minimum (Fig. **4**) the TG conformations are highly populated. The fine details of the internal structure and the

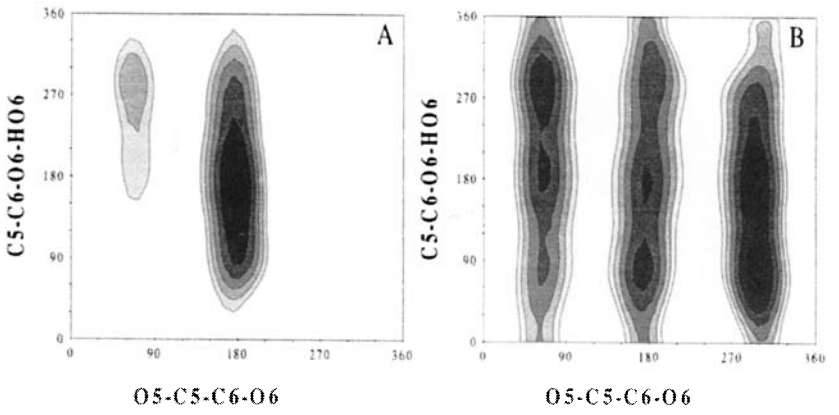

Fig. 4. Relaxed potential energy maps as a function of the primary hydroxyl group torsions O5-C5-C6-O6 and C5-C6-O6-HO6 for (A) β-galactose and (B) β-glucose. Iso-energy contour lines are drawn in 1 kcal·mol^{-1} intervals within a 6 kcal·mol^{-1} range above the global minimum.

size of non-bonded van der Waals parameters of hydrogen are considered essential in the modeling of the primary hydroxyl group orientations. The short C5-C6 bonds (1.51 Å) invariably found in crystals are modeled too long (1.52-1.53 Å) in PEF95SAC and bound to affect the orientational preference of the primary hydroxyl group.

COMPARISON WITH *AB INITIO* RESULTS

Although the results of the primary hydroxyl rotamer distributions from the X-ray structure and the NMR coupling constant data seem to point in the same direction, it has to be emphasized that they both are determined in condensed phases and that the modeling is done *in vacuo*. Since experimental data *in vacuo* on this subject are impossible, *ab initio* calculations remain the only source for validation.

Two *ab initio* studies have recently been published on this subject.[20,21] Table **3** lists some of these results and compares them to point calculations of global and local minima on the PES in PEF95SAC. The *ab initio* results of α-D-glucose indicate that a converging model has not yet been obtained and great caution should be taken when comparing with

Table 3. Relative energies of β-D-galactose, β-D-glucose and α-D-glucose conformations with different orientations of the primary hydroxyl groups. All conformations are in the counter-clockwise conformation (HO4 is pointing toward HO3, HO3 toward HO2, etc.) where the HO4 hydrogen is pointing away from the rotated primary hydroxyl group. Energies are in kcal·mol^{-1}.

Structure	6-31G*[a]	6-31G(d) MP2/ZPVE[b]	AMBER/g[a]	PEF95SAC ΔH (ΔG^{300K})[c]	NMR in D_2O[d]
β-D-gal*p* (GT)				3.80 (4.03)	
β-D-gal*p* (TG)				0.0	
β-D-gal*p* (GG)				7.35 (7.37)	
GG:GT:TG				0:0:100	19:65:17
β-D-glc*p* (GT)	2.0		2.3	0.89 (0.95)	
β-D-glc*p* (TG)	1.0		0.0	1.18 (1.30)	
β-D-glc*p* (GG)	0.0		4.4	0.00	
GG:GT:TG	82:15:3		0:2:98	74:16:10	55:54:-9
α-D-glc*p* (GT)	0.2	0.50	1.0	1.38 (1.47)	
α-D-glc*p* (TG)	0.0	0.12	0.0	1.71 (1.87)	
α-D-glc*p* (GG)	0.1	0.0	0.4	0.0	
GG:GT:TG	33:28:39	45:19:36	30:11:59	87:8:5	65:60:-25

a. From Glennon *et al.*[20] Population distributions calculated using a Boltzmann distribution considering only the three minimum conformations.

b. From Brown *et al.*[21] Population distribution calculated using a Boltzmann distribution considering only the three minimum conformations.

c. Global and local energy minima taken from the potential energy surfaces. Population distributions calculated using a Boltzmann distribution considering only the three minimum conformations.

d. Population distributions are calculated from the Haasnoot-Altona equation using the prochiral coupling constants. Coupling constants for α-D-glucose are taken from Nishida *et al.*[22]; the others are from Bock and Duus.[23]

these results. However, the table indicates that the molecular mechanics models (AMBER/g[20] and PEF95SAC) result in too high energy differences between the rotamers. The reason for this in the case of PEF95SAC could be that the points are taken from different cross-sections of the map which will increase the differences. Furthermore Table **3** shows that PEF95SAC provides quite different results from those of the other molecular mechanics force field AMBER/g. However, the validity of these very different predictions cannot be assessed before more elaborate *ab initio* studies have been carried out. MM3(92) predicts quite good results for the β-D-lactose primary hydroxyl groups in condensed phase (see Table **2**), indicating that MM3 is to be considered as a potential of mean force having the effects of the environment implicit in the parametrization.

It is obvious from the results listed in Table **3** that the influence of solvent plays a crucial role in establishing the structures and the conformational equilibria of the solute. This role must explicitly be taken explicitly into account in order to be able to further validate the force field[2] using the only experimental data present, namely the coupling constants. For the time being we cannot foresee a development where empirical tuning of the force fields[24] can be performed on these time-averaged data. This is primarily because molecular dynamics simulations in condensed phase are extremely resource-demanding and thus can hardly be done on an automated basis within, for instance, the CFF cycle.

CONCLUSION

We have applied a recently developed force field for carbohydrates, PEF95SAC, to β-lactose. Although our calculations have been done in vacuum and most of the available data are obtained in water solution, we have found a good overall agreement. These results indicate that the population distribution of glycosidic linkage geometries which is well reproduced by PEF95SAC is only little influenced by intermolecular interactions. In analogy, it seem as though the population distribution for the primary hydroxyl group rotamers which remain poorly reproduced in PEF95SAC are strongly influenced by intermolecular interactions. For this reason we believe that future

investigations of lactose should concentrate on how aqueous environment will influence the primary hydroxyl rotamer populations.

Perhaps most importantly, we have demonstrated that new results and/or new ideas to pursue in further investigations can arise from the development of a carefully optimized force field to a given problem. For this reason we hope to encourage the development of new force fields and multiple application of several force fields rather than resorting to mainstream applications of molecular mechanics. The many approximations made in molecular mechanics and the profound differences in, for example, AMBER, MM3 and PEF95SAC force fields are bound to provide differences in their predictions which may or may not be relevant to a particular application.

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