Carbohydrates: United Atom AMBER* Parameterization of Pyranoses and Simulations Yielding Anomeric Free Energies

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Abstract: The success of molecular modeling using classical potential functions (*i.e* force field calculations) rests heavily on the availability of specific, high-quality parameters that accurately describe the gas phase potential surface of the molecular system under study, on solvent models that reliably reproduce the effect of the medium, and on simulation methods that sample all significantly populated conformations of the entire system with the correct statistical weights. In this paper we present a set of molecular mechanics parameters that were developed using *ab initio* molecular orbital calculations to model pyranoses in the context of the AMBER* force field in the molecular modeling package MacroModel 5.0. These parameters were tailored to reproduce the quantum mechanical conformational energies of certain small molecules that were taken as models for common substructures in monosaccharides. Solvent was included as the GB/SA continuum model for water. The sampling problem was solved for these systems using the recently described MC(JBW)/SD simulation method that facilitates interconversion of predetermined conformational minima by alternating between smart Monte Carlo and stochastic dynamics steps. A series of such MC(JBW)/SD simulations using the new carbohydrate parameters was used to calculate anomeric α,β ratios (and thus anomeric free energy differences) for tetrahydropyran derivatives and the pyranose monosaccharides glucose, methyl glucoside, mannose, methyl mannoside, galactose, 2-deoxyglucose, and *N*-acetylglucosamine. In all cases, the simulations converged within 1 ns to yield anomeric free energies that are within 0.4 kcal/mol of the experimentally determined anomeric free energies in water.

Introduction

Carbohydrates form an exceptionally important class of organic and biological molecules, and the development of computational methods for modeling their structures and properties has been receiving significant attention in recent years. The need for special molecular mechanics treatment of carbohydrates follows from their densely packed, highly polar functionality and the dependence of their conformational behavior on stereoelectronic effects (*e.g.* anomeric, *exo*-anomeric, and *gauche* effects). These issues have long been recognized and resulted in a number of parameterization schemes. Thus Rasmussen *et al.*[1] developed potential functions to model carbohydrates based on atomic partial charges obtained from Mulliken population analysis of *ab initio* wave functions and on experimental structures for a set of appropriate model compounds. Jeffrey and Taylor[2] modified the MMI force field for carbohydrates and based their parameterization on neutron diffraction data for pyranose and methyl pyranose structures. More recently, Ha *et al.*[3] developed a CHARMM-like molecular mechanics potential for carbohydrates based on the X-ray structure and vibrational spectrum of α-D-glucose along with *ab initio*-derived atomic partial charges. These parameters were later ported to the AMBER force field by Homans[4] and expanded to include the glycosidic linkage based on crystallographic data of pyranose systems and *ab initio* calculations on dimethoxymethane. A more extensive use of *ab initio* calculations was made by Marsden *et al.*[5] in their development of carbohydrate parameters for the LUCIFER suite of programs and by Woods[6a] and by Merz[6b,c] for the AMBER force field. Finally, Grootenhuis and Haasnoot[7] developed the CHEAT approach to carbohydrate modeling by adding a new atom type, the carbohydrate hydroxyl group, to the CHARMM force field. Parameterization was performed by comparing CHEAT energetic results with a generalized steric interaction energy scheme proposed by Angyal.[8]

While most of these force fields have been used to calculate known geometries of simple carbohydrates with reasonable success, how accurately these force fields reproduce the relative energies of stereoisomeric carbohydrate structures is less clear. The main problem is that few such energy differences are well established experimentally, and without them, it is difficult to test a force field's ability to reproduce actual conformational energies. There is, however, one group of stereoisomers whose relative energies are easily measured in carbohydrates: α,β anomers. While a few calculations of anomeric energy differences have been made by simple energy minimization[1] and by separate simulations of each anomer,[9] one really wants *anomeric free energies* for comparison with experiment. Several such studies have been described recently in which free energy

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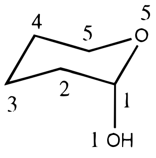
simulations were used to compute the α,β free energy difference of glucose in water.[6c,10a,b]

In this work, we use what are now standard methods of empirical force field parameterization to develop a molecular mechanics parameter set for modeling carbohydrates in the context of the united atom AMBER force field.[11] To carry out this parameterization, we derive new atomic partial charges and torsional parameters to reproduce electrostatic potentials and conformational energies of relevant model compounds as given by *ab initio* molecular orbital calculations. We then apply this new carbohydrate force field and the GB/SA continuum model[12] for solvent to compute the equilibrium anomeric ratios of a range of simple monosaccharides or other sugar-like molecules (**1**−**9**)for comparison with experiment. Our anomeric ratio calculations are carried out using the recently described MC(JBW)/SD simulation method[13] that generates a Boltzmann-weighted ensemble of states in part by jumping between different energy wells. In previous work describing the MC(JBW) method, those energy wells corresponded to different conformations—here they also include anomeric (α,β) stereoisomers. Such an interconversion between diastereomers (here anomers) in a single simulation makes it possible to compute diastereomeric free energy differences directly, a feat not readily accomplished with traditional simulation methods. Thus we use MC(JBW)/SD simulations to jump back and forth between both conformational and anomeric states and thereby to generate an equilibrium ratio of states based on their relative free energies. Determination of the computed anomeric ratio is then simply a matter of counting the number of structures that are α and the number that are β in the ensemble generated.



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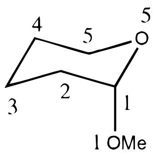

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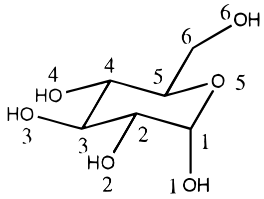

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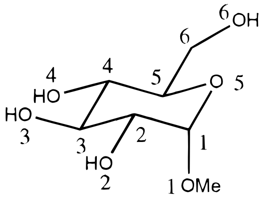

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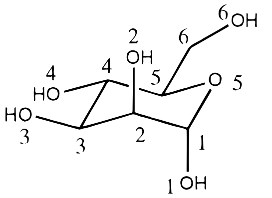

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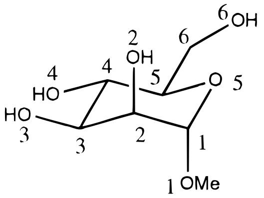

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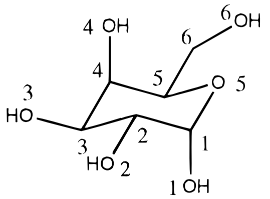

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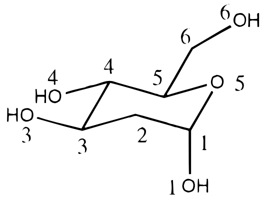

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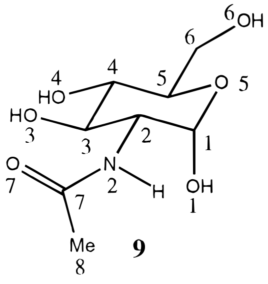

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AMBER* Parameterization of Pyranoses

Carefully parameterized molecular force fields are necessary for reproducing the properties of molecules accurately, especially with polar, densely functionalized systems such as those found in sugars. Of particular concern are the 1,1- and 1,2-diol substructures that characterize carbohydrates. These systems exhibit unusual conformational preferences that are dictated in part by stereoelectronic effects rather than by simple nonbonded forces.[14] Such stereoelectronic effects (*e.g.* the anomeric effect) are not implicitly treated in traditional molecular mechanics force fields. Carbohydrates have the additional complication that their highly polar hydroxyl and alkoxyl groups interact very strongly in molecular mechanics by simple Coulombic interactions, an approximation that appears simplistic when the functional groups are closely bound as they are in 1,1- and 1,2-diol derivatives. To model the structures and energies of carbohydrates realistically, we felt that special molecular mechanics parameters needed to be developed that accurately reproduced what was known about the conformational properties of such structures. This approach has been used in previous carbohydrate parameterizations that used quantum mechanical data on dimethoxymethane;[4,5] however, carbohydrates contain other substructures (*e.g.* 1,2-hydroxy acetals, 1,2-hydroxy ethers) that have received less scrutiny.[6b]

We began our work by developing molecular mechanics parameters for simulations of carbohydrates in the context of the united atom AMBER* force field[11] as implemented in MacroModel 5.0.[15] We based our new parameters on *ab initio* molecular orbital calculations of model compounds that represented fragments of common carbohydrates, and these parameters were ultimately combined to give a complete parameter set for intact pyranose monosaccharides. Since we were concerned with the energetic and conformational properties of carbohydrates in both vacuum and solution, we focused our efforts on obtaining an accurate atomic partial charge set and relevant torsional parameters. Both are important for determining relative conformational energies, and the former is essential for obtaining accurate solvation energies. Except for O−C−O bond length and angle parameters (O−C: $r_{eq} = 1.425$ Å, $K_r = 350$ kcal/mol Å; O−C−O: $\theta_{eq} = 111.5°$, $K_\theta = 63$ kcal/mol rad), all stretch and bend parameters were taken from the native united atom AMBER parameters for the sugar portion of uracil.[11a]

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Our parameter development basically followed the procedure outlined by Hopfinger and Pearlstein[16] and employed suitable model compounds as noted below: (1) Atomic partial charges were derived for model compounds from the *ab initio* wave functions through electrostatic potential fitting using the CHELPG procedure.[17] (2) *Ab initio* torsional energy profiles were obtained through dihedral angle driving in which a torsional angle of interest is set to specific values and all other degrees of freedom are allowed to relax. (3) Molecular mechanics torsional energy profiles were obtained in an analogous manner (constant dielectric electrostatics, $\epsilon = 1.0$) using the atomic partial charges from step 1 and the torsional parameters to be optimized set to zero. (4) A difference function between the *ab initio* and molecular mechanics energy profiles was constructed, and the torsional parameters to be optimized were adjusted to give the best possible fit (a minimized difference function) between the *ab initio* and molecular mechanics conformational energies for the model compound. (5) The resulting charge and torsional parameters were incorporated in the AMBER* force field in such a way that they would be used for the corresponding substructures of pyranoses and pyranosides. A similar approach was used to reparameterize AMBER by Merz and co-workers.[6b,c]

Ab initio calculations were carried out with GAUSSIAN92[18] and PS-GVB 2.01[19] using standard basis sets.[20] Geometry optimization was performed at the Hartree−Fock level[21] and at the GVB-PP level with GVB pair correlation between heteroatoms.[22a] In this study we chose HF/6-31G* *ab initio* calculations as a minimum theoretical treatment. This level of theory is reported to provide reliable conformational energies and reasonably accurate rotational barriers.[22] As described below, higher levels of theory were applied whenever they were practical.

We chose 2-hydroxytetrahydropyran (**1**) as an appropriate model system for parameterization of the hemiacetal fragment of sugars since the six-membered pyran ring provides geometrical constraints similar to those found in pyranoses and exhibits both anomeric and *exo*-anomeric effects. *Ab initio* calculations at various levels of theory were applied to this molecule as we considered it to be a test case for determining the most appropriate treatment for other model systems in our parameterization work.

We began by computing atomic partial charges for axially and equatorially substituted **1** using the CHELPG electrostatic fitting procedure at various levels of theory. The results are shown in Table 1 and some general trends are readily apparent. The inclusion of polarization functions on hydrogen atoms decreases the amount of charge on most heavy atoms by redistributing charge onto the hydrogens, and further expansion of the basis set to include diffuse functions increases charge on the heavy atoms, as well as on hydrogens. Correlation effects decrease the amount of charge on most of the atoms. Thus there seems to be a competing effect between basis set expansion and inclusion of correlation effects. Table 1 also lists the corresponding united atom charges. These were obtained by summing the alkyl hydrogen charges into those of the attached carbons. Interestingly, the united atom representation of charge is less sensitive to the choice of theoretical treatment. Also evident from the data in Table 1 is the fact that charges are conformationally dependent at all levels of theory. On the basis of our experience with the GB/SA water model,[12b] we chose PS-GVB/6-31G**//HF/6-31G** for computing atomic partial charges. At this level of theory (and indeed in all other calculations; see below), the energy difference between the global axial and global equatorial minima is rather small, and we obtained a single, united atom set of charges for the hemiacetal functionality of **1** by adding the charges on alkyl hydrogens to the charges of attached carbons and then simply averaging the resulting united atom charges of both minima. Thus the atomic partial charges used in our carbohydrate force field for the hemiacetals (1,1-hydroxy ethers) of sugars are the average of charges from the two chairlike conformers. We used such charge averaging whenever a model compound had multiple low-energy forms (see below); otherwise, we took charges from the global minimum.

Ab initio energy differences between the most stable axial and most stable equatorial conformers of **1** at various levels of theory are given in Table 2, some of the data being taken from Salzner and Schleyer.[23c] In all calculations, the axial form is favored. On the basis of these results we decided to use HF/6-311++G**//HF/6-31G** calculations to obtain the necessary torsional parameters for **1**. We therefore used this level of theory to calculate the 60° resolution rotational profile for the C1−O1 torsion in both axial and equatorial forms of **1**. Analogous calculations at HF/6-31G**//HF/6-31G** gave very similar conformational energies. The molecular mechanics C5−O5−C1−O1 and (C2,O5)−C1−O1−H torsional parameters were then adjusted to reproduce the quantum mechanical energy difference between the most stable axial and equatorial minima and the *ab initio* rotational profiles in the axial and equatorial forms. A comparison of the final energetic results and rotational profiles between *ab initio* and force field calculations for the axial and equatorial conformers of **1** is provided in Table 3 and Figure 1. This work shows that the rotational profile does not change significantly in either conformer upon expansion of the basis set. More importantly, the reparameterized force field calculations *in vacuo* reproduce well the *ab initio* relative energies and rotational profiles. Though one of the *ab initio* minima (axial *trans*, Table 3) is not a minimum on the AMBER* potential surface, that conformer corresponds to a high-energy region (3.6−4.0 kcal/mol) in both calculations, and we therefore view this difference between *ab initio* and AMBER* as unlikely to have a significant effect on free energies computed with the new force field.

2-Methoxytetrahydropyran (**2**) was chosen to model the acetal linkage of glycosides. The relative energies of the six rotational isomers (torsional minima and maxima) around the C1−O1 torsion in both axial and equatorial forms were available at the HF/6-311++G**//HF/6-31G* level from Tvaroska and Carver.[24]

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Table 1. All-Atom and United-Atom Partial Charges for Axial and Equatorial **1** as Determined by the CHELPG Electrostatic Fitting Procedure at Various Levels of Theory

	HF/6-31G*//HF/6-31G*		HF/6-31G**//HF/6-31G**		HF/6-311++G**//HF/6-31G*		PS-GVB/6-31G*//HF/6-31G*		PS-GVB/6-31G**//PS-GVB/6-31G**	
	all-atom	united-atom	all-atom	united-atom	all-atom	united-atom	all-atom	united-atom	all-atom	united-atom
					Axial					
O1	−0.720	−0.720	−0.710	−0.710	−0.737	−0.737	−0.644	−0.644	−0.648	−0.648
C1	0.573	0.559	0.559	0.549	0.599	0.585	0.475	0.487	0.440	0.463
C2	−0.135	−0.097	−0.135	−0.067	−0.132	−0.072	−0.150	−0.059	−0.135	−0.047
C3	0.153	0.124	0.143	0.122	0.180	0.139	0.121	0.121	0.177	0.118
C4	−0.128	−0.078	−0.131	−0.077	−0.139	−0.086	−0.149	−0.074	−0.138	−0.063
C5	0.302	0.306	0.293	0.304	0.313	0.321	0.244	0.280	0.216	0.270
O5	−0.565	−0.565	−0.561	−0.561	−0.593	−0.593	−0.515	−0.515	−0.505	−0.505
H1-OH	0.444	0.444	0.441	0.441	0.445	0.445	0.424	0.424	0.412	0.412
H1	−0.014		−0.010		−0.014		0.012		0.023	
H2a	0.028		0.030		0.025		0.047		0.046	
H2b	0.038		0.039		0.034		0.044		0.042	
H3a	0.002		0.006		−0.003		0.019		0.021	
H3b	−0.031		−0.028		−0.038		−0.019		−0.020	
H4a	0.045		0.048		0.051		0.059		0.059	
H4b	0.004		0.006		0.002		0.016		0.016	
H5a	0.011		0.014		0.011		0.027		0.035	
H5b	−0.007		−0.003		−0.003		0.009		0.019	
					Equatorial					
O1	−0.738	−0.738	−0.729	−0.729	−0.754	−0.754	−0.681	−0.681	−0.660	−0.660
C1	0.664	0.602	0.654	0.596	0.684	0.635	0.551	0.518	0.499	0.484
C2	−0.113	−0.018	−0.119	−0.018	−0.140	−0.026	−0.111	0.003	−0.101	0.023
C3	0.020	0.021	0.016	0.021	0.038	0.034	−0.057	0.005	−0.068	−0.011
C4	−0.037	−0.018	−0.044	−0.019	−0.055	−0.026	−0.031	0.010	−0.019	0.022
C5	0.253	0.258	0.248	0.258	0.268	0.273	0.162	0.212	0.136	0.204
O5	−0.553	−0.553	−0.550	−0.550	−0.577	−0.577	−0.486	−0.486	−0.475	−0.475
H1-OH	0.445	0.445	0.442	0.442	0.443	0.443	0.425	0.425	0.413	0.413
H1	−0.062		−0.058		−0.049		−0.033		−0.015	
H2a	0.068		0.071		0.080		0.037		0.040	
H2b	0.027		0.030		0.034		0.080		0.084	
H3a	−0.003		0.006		0.004		0.025		0.027	
H3b	0.004		−0.001		−0.008		0.027		0.030	
H4a	−0.007		0.029		0.033		0.004		0.003	
H4b	0.026		−0.004		−0.004		0.037		0.038	
H5a	−0.020		0.027		0.024		0.004		0.014	
H5b	0.025		−0.017		−0.019		0.046		0.054	

Table 2. *Ab Initio* Relative Energies of the Axial (C5−O5−C1−O1 = 60°; O5−C1−O1−H = 60°) and Equatorial (C5−O5−C1−O1 = 180°; O5−C1−O1−H = −60°) Conformers of 2-Hydroxytetrahydropyran (**1**) at Different Levels of Theory

theoretical level	energy (kcal/mol) equatorial − axial
HF/6-31G*//HF/6-31G*	1.32[23c]
HF/6-31G**//HF/6-31G**	1.24
HF/O;Huz, C,H:6-31G//HF/6-31G**	0.67[23c]
HF/6-311++G**//HF/6-31G**	0.69

We repeated their calculations of the two most stable conformers at the PS-GVB/6-31G**//HF/6-31G** level (with correlation of all heavy atoms) in order to derive atomic partial charges via CHELPG. As before, the charges were found to be conformationally dependent, and the energy difference between the two most stable conformers was small (0.9 kcal/mol in favor of the axial form from Tvaroska and Carver). We therefore produced a single united atom charge set by summing the charges on alkyl hydrogens into the attached carbons and then averaging of the charges of the two conformations. These charges were used in all subsequent torsional parameterizations and simulations of pyranosides.

Force field torsional parameters for the C5−O5−C1−O1 and (C2,O5)−C1−O1−C torsions were obtained by fitting the aforementioned *ab initio* axial−equatorial energy difference and the C1−O1 rotational profiles in both conformational forms of **2**. The results, presented in Table 4 and Figure 2 reveal a good reproduction of *ab initio* results by the force field calculations.

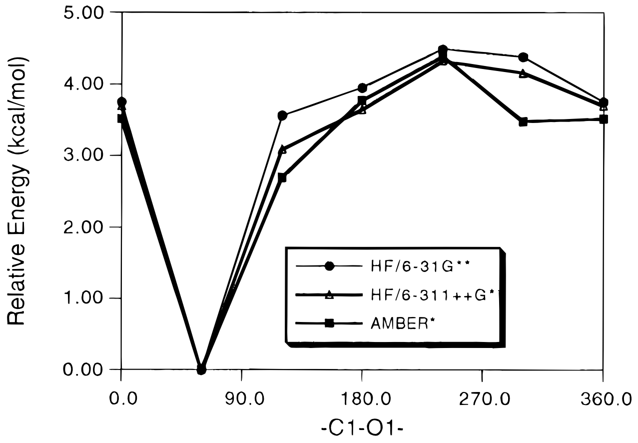
Table 3. Relative Energies (kcal/mol) of 2-Hydroxytetrahydropyran (**1**) Conformations Calculated by *ab Initio* Methods and the New Force Field

conformer	HF/6-311++G**//HF/6-31G**	HF/6-31G**//HF/6-31G**	AMBER*
axial			
trans[a]	3.63	3.98	(3.80)[d]
gauche$^-$ [b]	3.85	4.08	3.42
gauche$^+$ [c]	0.00	0.00	0.00
equatorial			
trans[a]	5.36	5.95	5.20
gauche$^-$ [b]	0.69	1.24	0.68
gauche$^+$ [c]	1.46	1.90	1.33

[a] Trans: O5−C1−O1−H = 180°. [b] Gauche$^+$: O5−C1−O1−H = 60°. [c] Gauche$^-$: O5−C1−O1−H = −60°. [d] O5−C1−O1−H constrained at 180° to prevent collapse to the global minimum.

Next we considered glucose (**3**) and methyl glucoside (**4**). Conceptually, such sugars can be considered to be built up from simpler model systems such as **1** and **2**, and indeed we used this technique to obtain hemiacetal and acetal charge and torsional parameters that we could apply to more complex pyranoses and pyranosides, respectively. Sugars, however, contain other substructures including variously substituted primary and secondary alcohol functionalities, and these were reparameterized as well. Because the hydroxyls in simple sugars are closely related to those of nucleic acids, we used the same stretch, bend, and atomic partial charge parameters for nonanomeric sugar hydroxyls as united atom AMBER uses for uracil.[11a] For hydroxyl conformational energies and barriers,

1 (OH axial)



1 (OH equatorial)

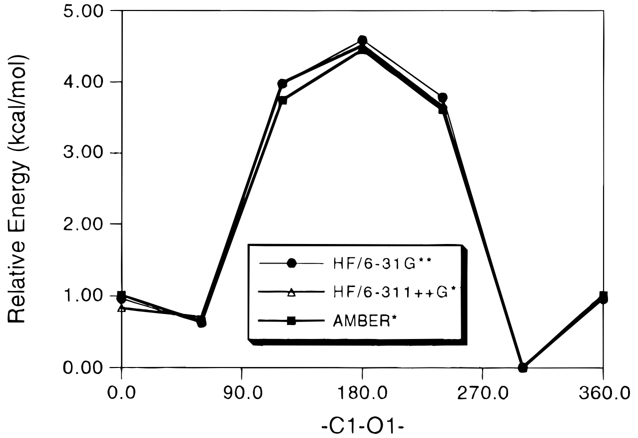


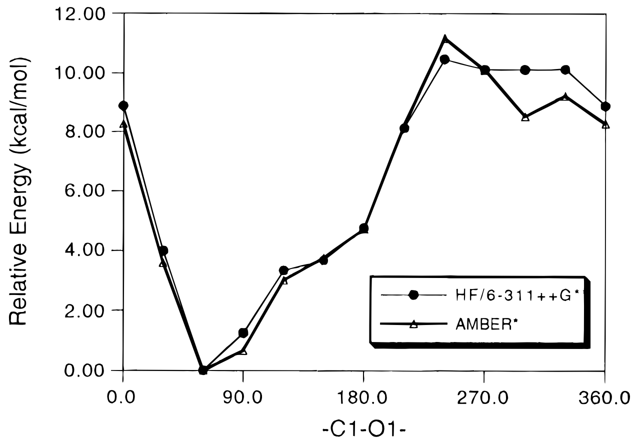
Figure 1. Rotational profiles around the C1−O1 torsion in **1** as calculated *ab initio* (HF/6-31G**//HF/6-31G** and HF/6-311++G**//HF/6-31G**) and with the reparameterized AMBER* force field.

Table 4. Relative Energies (kcal/mol) of 2-Methoxytetrahydropyran (**2**) Conformations Calculated by *ab Initio* Methods and the New Force Field[a]

conformer	HF/311++G**// HF/6-31G*[b]	AMBER*
axial		
trans	3.68	(5.53)[c]
gauche$^-$	10.11	8.73
gauche$^+$	0.00	0.00
equatorial		
trans	5.48	(6.26)[c]
gauche$^-$	0.94	0.95
gauche$^+$	3.93	4.11

[a] See footnotes of Table 3. [b] From ref 24. [c] O5−C1−O1−C constrained at 180° to prevent collapse to the corresponding global minimum.

2 (OMe axial)



2 (OMe equatorial)

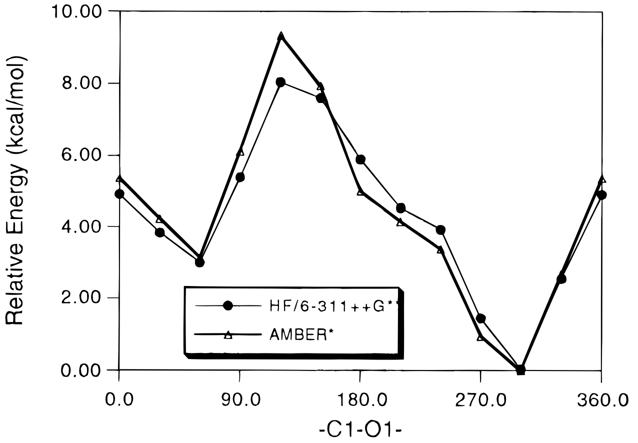


Figure 2. Rotational profiles around the C1−O1 torsion in **2** as calculated *ab initio* (HF/6-311++G**//HF/6-31G*) and with the new AMBER* force field.

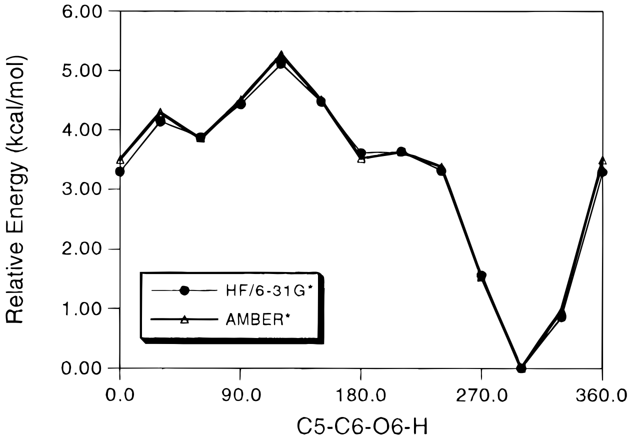


Figure 3. Rotational profiles around the C5−C6−O6−H (carbohydrate numbering) torsion in 2-methoxyethanol as calculated *ab initio* (HF/6-31G*//HF/6-31G*) and with the new AMBER* force field. In both calculations the O5−C5−C6−O6 torsion was initially set to 60°.

however, we turned to HF/6-31G**//HF/6-31G* *ab initio* calculations on model compounds 2-methoxyethanol (for the C6 primary alcohol) and 2-propanol (for the C2−C4 secondary alcohols). The *ab initio* energy profiles thus calculated for C−C−O−H torsional rotations along with the corresponding AMBER* results after parameterization are shown in Figures 3 and 4.

For parameterizing the endocyclic C3−C2−C1−O1 torsion, we used 2,4-dihydroxytetrahydropyran as the model compound. We calculated the energy difference between its *cis* and *trans* diastereomers (C4 hydroxyl equatorial) at the HF/6-311++G**//HF/6-31G** level of theory. After C3−C2−C1−O1 torsional parameterization, AMBER* exactly reproduced the *ab initio* energy difference of 1.3 kcal/mol favoring the *trans* diastereomer. For torsional parameters associated with the C2 hydroxyl, we fit the O2−C2−C1−O1 torsional parameters to HF/6-31G*//HF/6-31G* conformational energies of hydroxy hemiacetal **10**. Finally, the torsional parameters for rotation around the

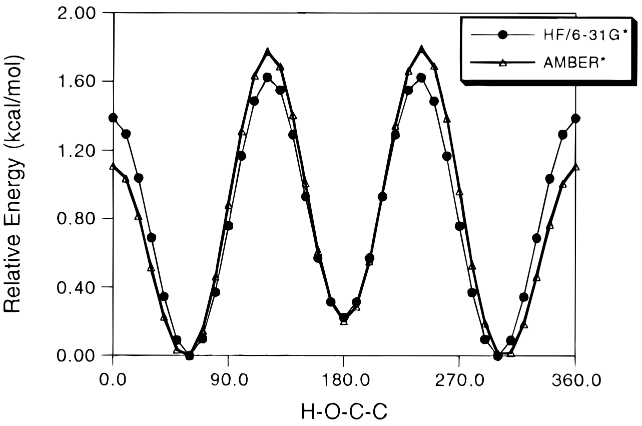
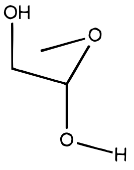
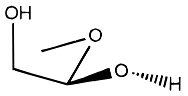


Figure 4. Rotational profiles around the C−C−O−H torsion in 2-propanol as calculated *ab initio* (HF/6-31G*//HF/6-31G*) and with the new AMBER* force field.

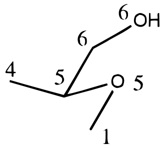
carbohydrate C5−C6 exocyclic bond were obtained by fitting HF/6-31G*//HF/6-31G* *ab initio* rotational profiles for 2-methoxypropan-1-ol (**11**) where the equivalent C4−C5−O5−C1 (sugar nomenclature) torsion was constrained at 60° to maintain a pyranose-like geometry and the hydroxyl H was *anti*. The *ab initio* and AMBER* results are show in Figure 5. With all of these model compounds, the agreement between quantum mechanics and molecular mechanics was very good.



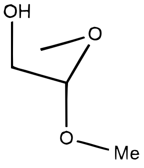
10a



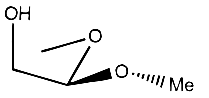
10b



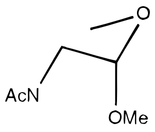
11



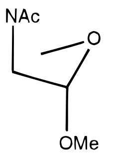
12a



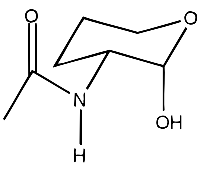
12b



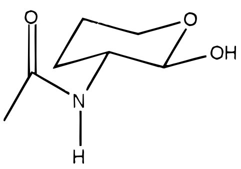
13a



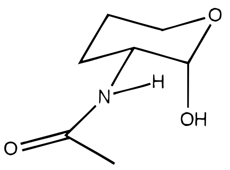
13b



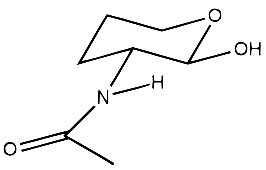
14a



14b



14c



14d

Methyl glucoside (**4**) differs from glucose (**3**) only by the replacement of the anomeric (C1) hydroxyl with a methoxyl group. Here we employed the same molecular parameters for **4** as we used for **3** except that the hemiacetal charges and torsional parameters from **1** were replaced by the acetal parameters developed for **2**, and we optimized the O2−C2−C1−O1 torsional parameters to reproduce HF/6-31G*//HF/6-31G* data for hydroxy acetal **12**.

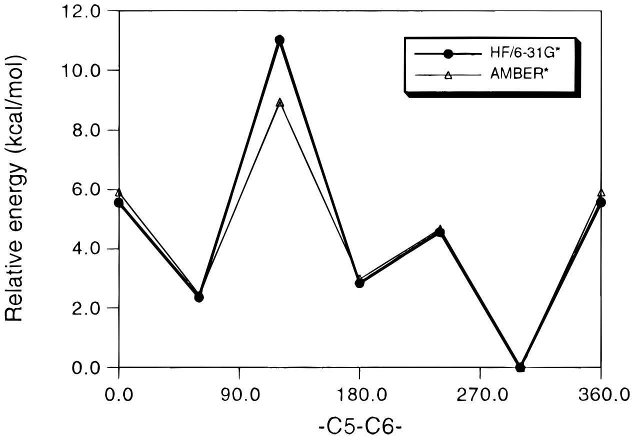


Figure 5. Rotational profiles around the C5−C6 (carbohydrate numbering) torsion in 2-methoxypropan-1-ol (**11**) as calculated *ab initio* (HF/6-31G*//HF/6-31G*) and with the new AMBER* force field.

These pyranose and pyranoside parameter sets were used for the following calculations on all oxygen-substituted monosaccharides including **3**, **4**, mannose, methyl mannoside, galactose, and 2-deoxyglucose. For *N*-acetylglucosamine, however, the acetamido substituent required further parameterization. We started by using the same charge and bonded parameters for the *N*-acetyl part that are used in united atom AMBER for peptidic acetamides. The O1−C1−C2−N2 torsional parameters were then adjusted to reproduce the HF/6-31G*//HF/6-31G* energy difference between the two diagrammed conformers of acetamido acetal **13** (*ab initio* and AMBER* calculations gave 0.19 and 0.20 kcal/mol in favor of **13a**), and the O1−C1−C2−C3, C3−C2−N2−C7, and C1−C2−N2−C7 torsional parameters were adjusted to reproduce HF/6-31G*//HF/6-31G* *ab initio* calculations of *cis*- and *trans*-3$_{eq}$-acetamido--2-hydroxytetrahydropyran (**14**) (*ab initio*: **14a**, 0.0; **14b**, 3.4; **14c**, 7.6; **14d**, 3.8 kcal/mol. AMBER*: **14a**, 0.0; **14b**, 3.5; **14c**, 9.5; **14d**, 3.7 kcal/mol).

The final AMBER* carbohydrate parameter set was used to calculate the energy difference between particular conformers of α- and *β*-glucose for comparison with *ab initio* results. Both calculations favored α-glucose *in vacuo* with *β*-glucose being higher in energy by 1.12 kcal/mol with HF/6-31G*//HF/6-31G* and by 0.80 kcal/mol with AMBER*. A complete list of all new AMBER* parameters and charges for the pyranoses and pyranosides described above are included in the supporting information.

Anomeric Free Energies of Pyranoses

The free energy difference between isomers (*e.g.* conformers or conformational families) of a molecular system is given by

$$\Delta G = -RT \ln K \tag{1}$$

where *K* is the population ratio of the isomers at equilibrium. *K* can be obtained directly from a single molecular simulation provided that (1) the simulation rapidly interconverts the isomers available to the molecular system to give a Boltzmann-weighted ensemble and (2) it is possible at each point in the simulation to determine to which isomer the currently simulated structure corresponds. In the work below, the isomers correspond to diastereoisomers at the anomeric center, *i.e.* α,*β* anomers. The first requirement is met by the rapid stereoisomeric state interconversion characteristics of the MC(JBW)/SD method that we will use here to interconvert anomers. The second requirement can be met for stereoisomeric states by measuring one or more appropriate internal coordinates (*e.g.* torsional angles).

We describe below the direct calculation of α,β anomeric free energy differences in several common tetrahydropyran derivatives and monosaccharides using MC(JBW)/SD simulations with the above described AMBER* parameters. The free energy calculation we used consists of the following steps:

(1) Perform conformational searches of both anomers of the solute (in the case of diastereomeric α,β monosaccharides) to find all conformational energy minima.

(2) Use the low-energy minima from the searches above as input to a 10 ns MC(JBW)/SD simulation at 300 K.

(3) Determine the anomeric (α/β) ratio by monitoring an appropriate torsion angle (*e.g.* an improper torsion around the anomeric carbon with sugars) during the simulation.

(4) Calculate the anomeric free energy from the cumulative α/β ratio (K) using eq 1 above.

(5) Monitor convergence by continuously calculating the cumulative α/β ratio during the simulation and observing its stability.

All the computational work described below was performed with the united atom AMBER* force field[11] as implemented in MacroModel 5.0[15] and augmented with the new carbohydrate parameters described above. For solution phase calculations, the GB/SA continuum solvent model was used.[12] Conformational searches used the internal coordinate SUMM method[25] and employed a total of 5000 search/minimization steps for tetrahydropyran derivatives and 30 000 steps for monosaccharides. For the tetrahydropyran derivatives, all conformations within ~10 kcal/mol of the global minimum (typically between 10 and 15 structures) were used as input to the MC(JBW)/SD algorithm. For the more conformationally rich monosaccharides, only the lowest 100 structures (out of 1500−2800 unique minima within 10 kcal/mol of the global minimum) were used. Such structures typically spanned an energetic range as high as 2−3.5 kcal/mol above the global minimum. The MC(JBW) part of the simulation utilized a ring closure bond within the pyranose ring that was broken and re-formed by the simulation algorithm to interconvert both ring conformers and anomers. The MC(JBW) procedure also varied the conformations of ring appendages (*e.g* hydroxyls). MC(JBW)/SD simulations were each run for 10 ns in vacuum, GB/SA chloroform, or GB/SA water as appropriate for the system under study. The acceptance rate of the MC(JBW) part of the algorithm ranged from 7 to 10% for the tetrahydropyran derivatives and from 15 to 35% for the monosaccharides. In all cases, interconversion between different ring conformers or anomers occurred at least once every 0.3 ps on average. With the monosaccharides studied here, the relatively high energy of the alternate chair forms implies that most interconversions occurred between anomers. Each 10 ns simulation therefore interconverts anomers on the order of 30 000 times and thus assures good convergence of the anomeric ratios. Indeed, the statistical uncertainties of our final free energies as measured by the method of block averages ranged from 0.01 to 0.03 kcal/mol (1σ).

Table 5. Calculated and Experimental Anomeric Free Energies (kcal/mol) for the α, β Equilibrium of the Tetrahydropyran Derivatives and Monosaccharides (Positive Values Favor the α Anomer, and Negative, the β Anomer)

	ΔG (kcal/mol)		
	AMBER* calculated		
system	Boltzmann[f]	MC(JBW)/ SD[g]	experiment/ *ab initio*
1 (vacuum)	0.87	0.28(0.02)	−0.07,[a] 0.69[b]
1 (H_2O)	−0.76	−1.02(0.02)	−0.95[26c]
2 (vacuum)	0.83	0.65(0.01)	0.90,[c] 0.94[d]
2 ($CHCl_3$)	0.54	0.36(0.01)	0.64[14]
2 (H_2O)	−0.22	−0.41(0.03)	0.1 to −0.7[26a,d]
glucose (H_2O)	0.36	−0.22(0.02)	−0.34[27]
methyl glucoside (H_2O)	0.71	0.53(0.02)	0.42 (MeOH)[e]
mannose (H_2O)	0.05	0.21(0.02)	0.34−0.45[27]
methyl mannoside (H_2O)	1.09	1.34(0.04)	1.70 (MeOH)[e]
galactose (H_2O)	0.56	−0.03(0.03)	−0.37[27]
2-deoxyglucose (H_2O)	−0.11	−0.45(0.01)	−0.05[32]
N-acetylglucosamine (H_2O)	1.32	0.50(0.03)	0.51[33]

[a] In CCl_4.[26c] [b] The energy difference between the lowest axial (C5−O5−C1−O1 = 60°; O5−C1−O1−H = 60°) and lowest equatorial (C5−O5−C1−O1 = 180°; O5−C1−O1−H = −60°) conformations from HF/6-311++G**//HF/6-31G** *ab initio* calculations in this work. [c] In CCl_4.[14] [d] The energy difference between the lowest axial (C5−O5−C1−O1 = 60°; O5−C1−O1−Me = 60°) and lowest equatorial (C5−O5−C1−O1 = 180°; O5−C1−O1−Me = −60°) conformations from HF/6-311++G**//HF/6-31G* *ab initio* calculations in ref 24. [e] The experimental value for equilibration in 1% methanolic HCl at 35 °C.[31] [f] Result based on Boltzmann-weighted average of minimum energy conformers and anomers using the new AMBER* force field. [g] Result based on 10 ns MC(JBW)/SD free energy simulation at 300 K using the new AMBER* force field, values in parentheses are statistical uncertainty (1σ) in the result and were computed from five block averages.

2-Hydroxytetrahydropyran (**1**) and 2-methoxytetrahydropyran (**2**) have often been considered as model systems for carbohydrates and consequently have been studied extensively, both by experiment[26] and by theory.[23,24] The conformational preferences of these molecules are governed, *inter alia*, by anomeric and *exo*-anomeric effects. Thus in the gas phase and in nonpolar solvents, the axial anomer is reported to be favored for both **1** and **2**. However, as the solvent polarity increases, the axial preferences decrease until, in water, both systems prefer the equatorial conformation. We carried out our first anomeric free energy calculations on these well-studied systems to test our quantum mechanically derived force field parameters and our MC(JBW)/SD methodology for interconverting anomers. In particular, we wanted to see that our methods could both reproduce the experimental trends in going from nonpolar to polar solvents and obtain anomeric free energies that are in good agreement with experiment.

Conformational searches performed as described above were run for **1** in vacuum, **1** in GB/SA water, **2** in vacuum, **2** in GB/SA chloroform, and **2** in GB/SA water, and the resulting minima were used as input for 10 ns MC(JBW)/SD simulations. With **1** and **2**, the anomers are conformers and not diastereomers, and only single conformational searches needed to be carried out for each compound in each medium. The anomeric ratios were calculated using eq 1 from the axial/equatorial conformational ratio (K) that was determined by monitoring the C3−C2−C1−O1 torsion angle (−60 ± 30° and 180 ± 30° for the axial and equatorial conformers, respectively). The anomeric free energy results are summarized in the first five entries of Table 5. They show very good agreement with available experimental data. As expected, the preference for the axial conformer decreases on going from vacuum to chloroform to water in accord with the known behavior of tetrahydropyranoid anomeric systems.[14] A notable feature of the MC(JBW)/SD algorithm is its rapid convergence with these systems. In all cases, the free energy results after 1 and 10 ns of simulation time differ by less than 0.05 kcal/mol. Furthermore, the final

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(25) Goodman, J. M.; Still, W. C. *J. Comput. Chem.* **1991**, *12*, 1110.

(26) (a) Lemieux, R. U.; Pavia, A. A.; Martin, J. C.; Watanabe, K. A. *Can. J. Chem.* **1969**, *47*, 4427. (b) de Hoog, A. J.; Buys, H. R.; Altona, C.; Havinga, E. *Tetrahedron* **1969**, *25*, 3365. (c) El-Kafrawy, A.; Perraud, R. *C. R. Acad. Sc. Paris. Ser. C* **1975**, *280*, 1219. (d) Praly, J.-P.; Lemieux, R. U. *Can. J. Chem.* **1987**, *65*, 213. (e) Wiberg, K. B.; Marquez, M. *J. Am. Chem. Soc.* **1994**, *116*, 2197.

anomeric free energy differences for **1** and **2** had standard deviations of only 0.02 kcal/mol by the method of block averages.

Several calculations of the anomeric free energies of simple sugars including glucose have been reported previously.[6c,10] Karplus *et al.* carried out free energy perturbations of glucose anomers in TIP3P water with the CHARMM force field to obtain a value of 0.31 ± 0.43 kcal/mol favoring the α (axial) anomer.[10a] After evaluating a range of different free energy simulation methods, van Eijck *et al.* used the GROMOS force field and SPC/E water to find the β (equatorial) anomer being favored by 0.86 ± 0.43 kcal/mol.[10b] Both results are in reasonable agreement with the experimental observation that α- and β-glucose in water are rather close in energy.

The actual anomeric free energy difference of gluocse in water has been obtained from a variety of measurements including NMR and optical rotation studies and corresponds to 0.34 kcal/mol in favor of the β (equatorial) anomer.[27] We began our glucose simulations with conformational searches of the α and β anomers and identified ~2800 minima within the lowest 10 kcal/mol. The lowest 100 of these structures were used as input for a 10 ns MC(JBW)/SD simulation in GB/SA water. The α/β ratios were obtained by monitoring the C2−C1−O5−O1 improper torsion ($-120 \pm 30° = \alpha$ anomer, $120 \pm 30° = \beta$ anomer; see **3**), and the corresponding free energy difference was calculated with eq 1. The result (Table 5, entry 6) favors the β conformer by 0.22 kcal/mol, a finding in good agreement with experiment.

Of some concern to us was the fact that the 100 lowest energy structures used as input for the glucose MC(JBW)/SD simulation covered energies up to only 2.7 kcal/mol above the global minimum. We therefore conducted a test to see whether or not inclusion of additional minma in the MC(JBW)/SD input list would alter the final results. Thus we repeated the simulation with 200 input structures (relative energies as high as 3.5 kcal/mol). The results obtained from a 10 ns MC(JBW)/SD simulation gave 0.25 kcal/mol, favoring β for the anomeric free energy difference, in excellent agreement with the previous value. Thus the additional structures provided no significant alteration in the simulation results, presumably because such higher energy structures contribute little to the overall free energy difference and/or because the simulation algorithm was already exploring them.

The population ratio of the rotational conformers around the C5−C6 torsion in glucose is a matter of some controversy. The three possible conformers are defined in *anti* (*a*) *gauche* (*g*) terms for two torsional angles: O5−C5−C6−O6 and C4−C5−C6−O6. Thus *ga* means that O6 is *gauche* to O5 and *anti* to C4. All the experimental data in solution[28,29] and in the solid phase[30] are consistent with the predominance of the *gg* form. However, NMR measurements of glucose found considerable amounts of the *ga* form as well (*gg:ga:ag* ratios of 56:44:0 and 53:45:2 for the α and β anomers, respectively).[28] Similar measurements of *N*-acetylglucosamine[29] were interpreted in terms of predominance of *ag* over *ga*, the latter being found only in negligible amounts. We should note, however, that a number of approximations were made in the analysis of the NMR data, and it is not clear how precise the reported ratios actually are. Several molecular simulations have also addressed this issue. Brady[9] found the *ag* conformer to predominate in both vacuum and explicit water simulations of glucose, in disagreement with the experimental data, while Grootenhuis and Haasnoot[7] obtained *gg:ga:ag* ratios of 25:65:10. Our own simulation of glucose in GB/SA water yielded *gg:ga:ag* ratios of 27:63:9, similar to those obtained by Grootenhuis and Haasnoot, but only in fair agreement with experiment.

The experimental anomeric ratio of methyl glucoside (**4**) was determined by equilibrating the system in methanol using catalytic acid at 35 °C and corresponds to a preference of the α anomer by 0.42 kcal/mol.[31] Our results for **4** obtained from a 10 ns MC(JBW)/SD simulation of the system in GB/SA water at 27 °C gave a free energy difference of 0.53 kcal/mol in favor of α (Table 5, entry 7). These anomeric free energies are similar but not strictly comparable because of the differing solvents.

The anomeric ratios in pyranoses are well-known to vary with the substituent type and the substitution pattern at C2 of the pyranose ring. Thus, the $\alpha - \beta$ free energy differences for glucose (equatorial OH at C2),[27] mannose (axial OH at C2),[27] 2-deoxyglucose (no substituent at C2),[32] and *N*-acetylglucosamine (equatorial acetamide at C2)[33] have been experimentally measured and range from −0.3 to +0.5 kcal/mol in water. Such sugars are also common components of important biological macromolecules. We therefore applied our new carbohydrate parameter set and MC(JBW)/SD simulation methodology to the anomeric free energies of these systems.

According to NMR measurements in water, the α anomer of mannose (**5**) is preferred over β by 0.34−0.45 kcal/mol.[27] Our results, obtained from a 10 ns MC(JBW)/SD simulation in GB/SA water indicate a preference for the α anomer by 0.21 kcal/mol and are therefore in good agreement with experiment (Table 5, entry 8). The anomeric free energy difference for methyl mannoside (**6**) (Table 5, entry 9) is also calculated here to be in good accord with experiment, though the latter was again evaluated in methanol instead of water. The anomeric energy for galactose (**7**) is small and in reasonable accord with experiment[27] (0.03 and 0.37 kcal/mol favoring β by calculation and experiment, respectively).

The experimental value for 2-deoxyglucose (**8**) in water suggests almost equal populations of the α and β anomers (0.05 kcal/mol favoring β).[32] Our 10 ns MC(JBW)/SD simulation of **8** in GB/SA water gave a somewhat larger energy difference of 0.45 kcal/mol in favor of the β anomer. Finally, the anomeric energy difference in *N*-acetylglucosamine (**9**) by experiment in water (0.51 kcal/mol favoring the α anomer)[33] is in good agreement with our free energy simulations that gave 0.50 kcal/mol also favoring α.

Conclusions

This work not only provides a useful molecular mechanics force field for pyranose sugar derivatives, but also shows that new force field parameters based exclusively on *in vacuo*, *ab initio* molecular orbital calculations can do a good job of reproducing experimental free energy results with highly functionalized molecules in water. While the agreement between calculation and experiment is good, it is not perfect and there is room for improvement. The two largest sources of error in such parameterization works are (1) inadequate level of theoretical treatment in the *ab initio* calculations and (2)

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inadequate choices of model compounds to represent fragments of intact carbohydrates. In this study we addressed the first issue by using *ab initio* calculations at levels of theory which are generally believed to provide good atomic partial charges and reliable conformational energies and rotational barriers. However, with these levels, our conformational potentials could easily be off by 0.2−0.5 kcal/mol according to basis set tests we ran. The second issue, choice of appropriate model compounds, may be more problematic with such densely functionalized structures as those found in carbohydrates. Thus the assumption of parameter transferability is likely to be worse here than in simpler systems, and the best model compounds for studies such as these may be the full monosaccharides. Finally, the model described here is a so-called "united atom" model because it unifies carbons and attached hydrogens into single superatoms. Calculations with such a model are by nature faster than with the corresponding all-atom model, but they do have more limited accuracy, especially when systems incorporating close van der Waals contacts are being studied.

In spite of the inexact nature of our model and force field, the free energy data obtained in this work and found experimentally reveals some interesting trends: (1) the dependence of the anomeric ratio on solvent polarity for **1** and **2** (Table 5, entries 1−5), (2) the dependence of the anomeric ratio in water on the anomeric hydroxyl or methoxyl substituent (entries 6−9), and (3) the dependence of the anomeric ratio in water on the C2 substituent and stereochemistry of the pyranose ring (entries 6−12). For each such dependence, the calculated results generally reproduce the experimental trends both in sign and approximate magnitude. Moreover, for all the molecular systems studied in this work, the calculated and experimental anomeric ratios are in semiquantitative agreement with an average unsigned error for all solvated systems (excluding aqueous **2** for which the experimental value is not well defined) of 0.22 kcal/mol and a maximum error of 0.40 kcal/mol. Also given in Table 5 are corresponding α,β energy differences calculated by a standard molecular mechanics treatment using the same force field and solvent model. Here a Boltzmann-weighted ensemble of minimum energy conformers was assumed and based on all minimum energy conformers within 50 kJ/mol of the global minimum. The average unsigned error for the solvated systems based on those molecular mechanics results is seen to be larger at 0.45 kcal/mol with a maximum error of 0.93 kcal/mol. Thus the simple Boltzmann-averaged energy minimization calculations deviate from experiment to a significantly greater extent than do the free energies obtained from our simulations, suggesting the significant role of entropy in determining the conformational energies of these systems.

In conclusion, we have employed *ab initio* molecular orbital calculations to develop a united atom molecular mechanics parameter set for pyranose derivatives and used it to compute anomeric free energies for a number of simple monosaccharides in water. The MC(JBW)/SD simulations we used converged smoothly on the nanosecond time scale to give free energy results that are in good agreement with available experimental data. We believe that the parameters and methodology described here will find useful applications in carbohydrate chemistry. We are currently developing an analogous all-atom parameter set for free energy calculations with sterically hindered carbohydrate systems (*e.g.* oligosaccharides).

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Supporting Information Available: Atomic partial charges and new torsional parameters for **1**−**9** (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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