Ab Initio Molecular Orbital Study of the Conformational Behavior of the Sugar−Phosphate Linkage. Toward an Understanding of the Catalytic Mechanism of Glycosyltransferases

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The conformational properties of the sugar−phosphate linkage (the phosphate functional group is linked to the anomeric carbon in hexopyranosides) have been studied with ab initio methods using the 2-*O*-methylphosphono-tetrahydropyran anion (**1**) and sodium 2-*O*-methylphosphono-tetrahydropyran (**2**) as models. The ab initio energy and geometry of the conformers around the C1−O1 and O−P bonds have been determined at various levels of theory and solvent effects evaluated. At all levels of ab initio theory, **1** prefers the trans to the gauche conformer around the C1−O1 bond. The repulsive electrostatic interactions between the ring oxygen and the PO_2^- group are assumed to be responsible for this phenomenon, which has been termed the reverse *exo*-anomeric effect. For the PO_2^- group, we estimate a 2.3 kcal/mol magnitude for the reverse *exo*-anomeric effect in a vacuum. The presence of the sodium counterion completely reverses the relative energy of the conformers, such that in the ion-pair complex **2**, the gauche conformer about the C1−O1 bond is favored in agreement with crystallographic data. Ab initio results were compared with results obtained by several quantum semiempirical and molecular mechanics methods. On the basis of structural differences between **1** and **2,** we suggest that the metal cofactor plays three roles in the catalytic mechanism of transferases. It stabilizes a suitable conformation for the sugar donor, activates the C1−O1 glycosidic linkage, and enables the protonation of the glycosidic oxygen to begin the scission of the glycosidic bond.

1. Introduction

The oligosaccharide chains of *N*- and *O*-linked glycoproteins play a crucial role in a number of biological processes.[1,2] Their biosynthesis and degradation pathways are therefore areas of significant interest for biology, medicine, and biotechnology. The assembly of the various types of oligosaccharides involves several glycosidases and glycosyltransferases. In comparison with glycosidases, the mechanisms of which have been characterized in some detail,[3−5] mechanistic investigations on glycosyltransferases have not yet undergone much scrutiny, although some kinetic studies have been reported.[6−18] Glycosyltransferases are a diverse group of enzymes that catalyze the transfer of a single monosaccharide unit from a donor to the hydroxyl group of an acceptor saccharide.[19−21] The acceptor can be either a free saccharide, glycoprotein, glycolipid, or polysaccharide. The donor can be a nucleotide−sugar, dolichol−phosphate−sugar, or dolichol−pyrophosphate−oligosaccharide. Glycosyltransferases show a precise specificity for both the sugar acceptor and donor and generally require the presence of a metal cofactor. Unfortunately, no crystal structures of glycosyltransferases have been reported so far. In the absence of experimental data, high-level ab initio calculations on the reactants might shed some light into the structural and energetic characteristic of the enzymatic reaction of glycosyltransferases.

A prerequisite for the characterization and understanding of the mechanism of glycosyltransferases is a detailed description of the conformational behavior of the key substrates for the biosynthesis of oligosaccharides. For sugar donors, the main degrees of conformational freedom are those occurring through rotations about the anomeric and phosphate torsion angles within the sugar−phosphate linkage. The conformational properties of the sugar and phosphate are influenced by the classical stereoelectronic effects termed the anomeric and *exo*-anomeric effects. Different manifestations of these effects have been extensively reviewed and discussed.[22−32] The phosphate group is an important part of many biological molecules and several ab initio theoretical studies of the dimethyl phosphate anion and similar molecules have been performed[33−35] (and references therein) to model this sequence of atoms. As well, ab initio analyses of the conformational behavior of carbohydrate model compounds have been carried out.[36−41] However, the conformational analysis of the sugar−phosphate linkage has never been performed. Owing to the above-mentioned stereoelectronic effects, the following fundamental question arises: what are the consequences of connecting the sugar and phosphate groups together on the anomeric and *exo*-anomeric effects and on the conformational behavior of the sugar−phosphate linkage? Given the general requirement for a metal cofactor to activate an enzyme, another question occurs: what role does the metal cofactor play in the mechanism of transferases? To answer these questions, and as a first step in a theoretical investigation of the catalytic mechanism of glycosyltransferases, ab initio calculations which examine the structure and stability of conformers about the sugar−phosphate linkage and the effect of the metal cofactor on these properties are presented.

2. Models and Computational Procedures

The conformational behavior of the monophosphate functional group linked to the anomeric carbon in hexopyranosides has been studied with ab initio methods using the 2-*O*-methylphosphono-tetrahydropyran anion (**1**) and the sodium 2-*O*-

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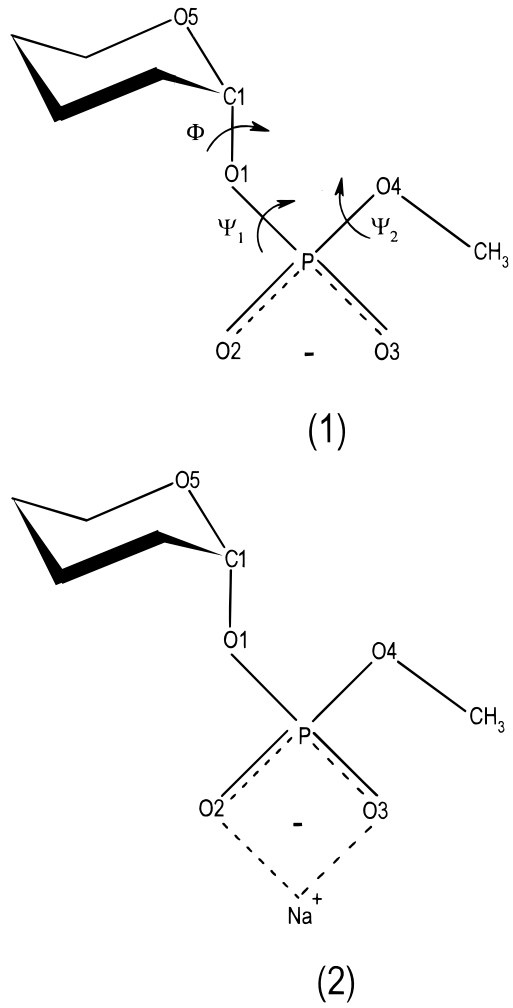

Figure 1. Schematic representation of the 2-*O*-methylphosphono-tetrahydropyran anion (**1**) and sodium 2-*O*-methylphosphono-tetrahydropyran (**2**).

methylphosphono-tetrahydropyran (**2**) as models. Structures of the compounds with axially oriented phosphate groups are illustrated in Figure 1. These two species were chosen to represent different conformational features of the possible structures of the nucleotide−sugar donors in the enzyme-binding site. For a description of atoms (Figure 1) we will use the numbering of atoms as in the carbohydrate nomenclature, where the anomeric carbon is denoted as C1, etc. The rotation about the anomeric C1−O1 linkage is described by the dihedral angle Φ [$\Phi = \Phi$(O5−C1−O1−P)], and the orientation of the phosphate group is described by dihedral angles Ψ_1 [$\Psi_1 = \Psi_1$ (C1−O1−P−O4)] and Ψ_2 [$\Psi_2 = \Psi_2$ (O1−P−O4−C)]. Conformations of compounds **1** and **2** are then described using three torsion angles Φ, Ψ_1, and Ψ_2. Three staggered orientations about the C1−O1, O1−P, and P−O4 bonds are denoted as G (*synclinal*, gauche, 60°), T (*antiperiplanar*, trans, 180°), and mG (*-synclinal*, -gauche, −60°), respectively. In this notation, the description of the torsion angle Φ is stated first, then the torsion angles Ψ_1 and Ψ_2. In this way, e.g., GTmG means that the angles Φ, Ψ_1, and Ψ_2 are approximately in *synclinal* (*sc*) or gauche (G), *antiperiplanar* (*ap*) or trans (T), and *-synclinal* (*-sc*) or -gauche (mG) conformations, respectively.

The ab initio calculations were carried out with the Turbomole 95.0 program[42] using standard basis sets. First, three possible staggered conformers around each of the C1−O1, O1−P, and P−O4 bonds were assumed, giving altogether 27 different

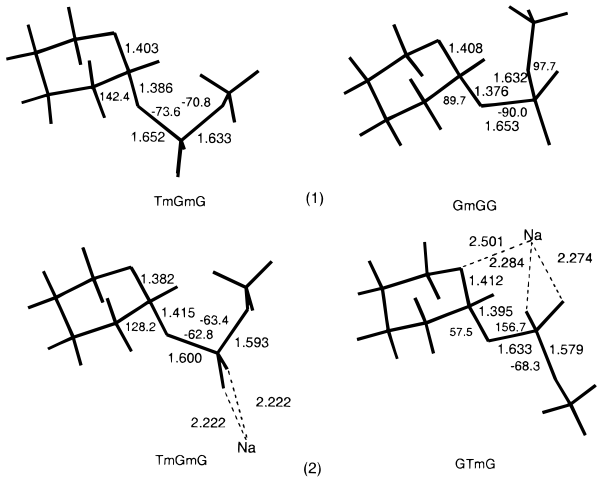

Figure 2. Comparison of the selected geometry values for the *ap* and *sc* lowest energy conformers about the C1−O1 bond calculated using the ab initio method at the 6-31G* level. Bond lengths are given in angstroms, torsion angles in degrees.

starting conformations for geometry optimization. The optimization of the geometry was performed at the SCF level with the 6-31G* basis set. The geometry was fully optimized using the gradient optimization routines of the program without any symmetry constraints. Next, single-point calculations were performed for each minimum using the triple-ζ basis set plus one set of polarization functions (tzp). It has been shown[33,37] that inclusion of electron correlation at the MP2/6-31G* level does not improve the results, therefore, electron correlation at this level was not included in the present study. Instead, a hybrid Hartree−Fock density functional scheme, the adiabatic connection method[43] (ACM) of density functional theory[44] (DFT), was used to treat electron correlation effects for some conformers with standard 6-31G* and tzp basis sets. For all minima, the vibrational frequencies were calculated at the 6-31G* level, and the zero-point energy, thermal and entropy corrections were evaluated. The effect of solvent on conformational equilibrium has been investigated using the polarized continuum (PCM) method as implemented in Gaussian94.[45] Cavity contribution was estimated within the scaled particle theory[46] and was described previously together with dispersion contributions.[47] Calculations were carried out for solvation by four solvents, namely carbon tetrachloride ($\epsilon = 2.24$), octanol ($\epsilon = 10.34$), methanol ($\epsilon = 32.70$), and water ($\epsilon = 78.30$). Semiempirical calculations were performed with the AMPAC/MOPAC module of the InsightII program[48] using the MNDO Hamiltonian. Molecular mechanics calculations were also executed with the DISCOVER program[49] using the CVFF and AMBER force fields.

3. Results and Discussion

A. 2-*O*-Methylphosphono-tetrahydropyran Anion (1). The linkage of the phosphate group to the anomeric carbon results in three internal rotational degrees of freedom, associated with the C1−O1, O1−P, and P−O4 bonds. The assumption of three staggered conformers for each bond leads to 27 (3^3) conformers to be considered. However, the balance of electrostatic, steric, and lone-pair intramolecular interactions is such that not all of these structures are minima. Indeed, from the 27 starting conformers only 16 minima were obtained. The results performed at various levels of computation are gathered in Tables 1 and 2 and in Figure 2.

TABLE 1: Ab Initio Relative Energy, Vibrational Correction, and Free Energy (kcal/mol), and the Position (torsion angles in degrees) of Conformational Minima of the 2-Methylphosphono-tetrahydropyran Anion (1)

conformer	Φ	Ψ_1	Ψ_2	Δ*E* 6-31G*	Δ*E* tzp//6-31G*	Δcorr 6-31G*	Δ(ΔG_{298}) 6-31G*
GGG	66.9	50.5	80.0	4.07	4.44	0.32	3.30
GGmG	60.6	34.0	−112.9	3.63	4.42	1.36	3.91
GmGG	89.7	−90.0	97.7	1.40	1.78	0.78	1.09
GmGT	87.4	−88.5	174.8	2.26	2.28	0.01	1.17
GTG	72.6	142.5	75.8	1.71	2.06	0.65	1.27
GTmG	69.6	149.2	−72.9	1.40	1.83	1.08	1.39
GTT	75.2	141.1	163.5	2.78	3.24	0.00[c]	1.68
mGGG	−56.8	78.1	67.0	7.31	7.33	0.86	7.07
mGGT	−59.0	85.9	172.2	9.38	9.46	0.33	8.62
mGmGmG	−100.6	−70.6	−75.0	6.66	6.97	1.34	6.91
mGmGT	−100.7	−69.0	−177.8	7.68	7.97	0.82	7.41
TGG	160.3	97.4	81.7	1.31	1.52	1.13	1.35
TGT	159.1	95.2	176.6	2.02	2.24	0.44	1.37
TmGmG	142.4	−73.6	−70.8	0.00[a]	0.00[b]	1.09	0.00[d]
TmGT	145.9	−78.8	171.4	1.77	1.76	0.37	1.05
TTG	170.3	−148.0	70.4	0.85	1.11	0.83	0.59

[a] $E = -595\,737.37$ kcal/mol. [b] $E = -595\,880.04$ kcal/mol. [c] $\Delta\text{corr} = \text{ZPE} + \Delta H_{298} - T\Delta S = 237.1$ kcal/mol. [d] $\Delta G_{298} = -595\,500.28$ kcal/mol.

TABLE 2: Comparison of the ab Initio Energies and Relative Energies (kcal/mol) of Selected Conformers of the 2-*O*-Methylphosphono-tetrahydropyran Anion (1) Calculated by Different Methods

geometry	energy	TmGmG	TTG	GmGG	GTmG
6-31G*	6-31G*	−595 737.373	0.85	1.40	1.40
	tzp	−595 839.780	1.03	1.89	1.95
	ACM/6-31G*	−595 156.359	0.66	0.95	0.94
ACM/6-31G*	ACM/6-31G*	−598 159.744	0.64	0.61	0.87
	ACM/tzp	−598 315.372	0.91	1.00	1.44
ACM/tzp	ACM/tzp	−598 315.636	0.92	1.02	1.38

The ab initio potential energy profiles for rotation about the glycosidic C1−O1 linkage have been previously investigated in detail.[37] For 2-methoxytetrahydropyran with an axially oriented methoxy group, regardless of the method, only one deep well was predicted corresponding to the *sc* (G) orientation of the methyl group with respect to the ring oxygen (Φ = 64.2°). The relative energy calculated for the *ap* (T) and -*sc* (mG) conformations at the 6-31G* level was 4.0 and 10.5 kcal/mol, respectively. Similarly, the preference for the *sc* over the *ap* conformation has also been observed for the C1−X bond in *C*-, *N*- and *S*-glycosyl compounds.[39] This feature has been attributed to the presence of the *exo*-anomeric effect.

Surprisingly, our results on the conformational properties about the C1−O1 bond for the 2-*O*-methylphosphono-tetrahydropyran anion are completely different from those found in the earlier studies discussed above. Calculations on **1** predicted three staggered conformers about the glycosidic C1−O1 bond. The order of stability of the orientations about the C1−O1 bond appears to be as follows: first the *ap* (T), then the *sc* (G), and finally the -*sc* (mG) orientation. Each of these orientations consists of a set of conformers (Table 1) that differ in the conformation of the phosphate group. The lowest energy minimum is the TmGmG conformation which corresponds to the *ap* (Φ = 142.4°) orientation about the glycosidic C1−O1 bond. The lowest energy conformer for the *sc* orientation is the GmGG (Φ = 89.7°) which has an energy at the 6-31G* level 1.4 kcal/mol above that of the TmGmG conformer. For the third orientation about the C1−O1 bond, the -*sc*, the lowest energy minimum mGmGmG (Φ = −100.6°) has a relative energy of 6.66 kcal/mol. The preference for the *ap* orientation about the C1−O1 bond is thus clear, since from five conformers having this orientation, three of them (TmGmG, TTG, and TGG) have lower energy than the GmGG conformer, which is the lowest energy conformer for the *sc* orientation. It is noteworthy that values of the torsion angle Φ adopted by the individual minima lie in the range (61°; 90°) for the *sc* (G) conformers, (−57°; −101°) for the -*sc* (mG) conformers, and (142°, 170°) for the *ap* (T) conformers, respectively. The relatively large ranges of values for the Φ torsion angle indicate that the potential energy surface about the anomeric C1−O1 is nearly flat in these regions. This suggests that the negatively charged sugar phosphates exhibit considerably larger conformational flexibility about this bond compared to glycosides.

The recent high-level ab initio calculations of the dimethyl phosphate anion[33−35] gave (Ψ_1, Ψ_2 = *sc*, *sc*) or its symmetrically equivalent conformer (-*sc*, -*sc*) as the most stable orientation. The relative energy of other conformers depended on the method of calculation but was found to be relatively small, usually within 3 kcal/mol. The (*ap*, *sc*) conformer is the next most favored, then the (*sc*, -*sc*) conformer having both methyl groups oriented on the same side of the molecule, and the (*ap*, *ap*) conformation is the least stable local minimum. These results clearly illustrate the importance of the anomeric effect for the stereochemistry of the phosphate group. In the 2-*O*-methylphosphono-tetrahydropyran anion the phosphate group exhibits a similar degree of flexibility. However, due to the presence of the hexopyranose ring, the stable conformers are not symmetrically related as in the case of the dimethyl phosphate anion. Moreover, the stability of the conformers about the O1−P and P−O4 bonds depends on the conformation about the C1−O1 bond and is influenced by interactions with the six-member ring. Despite these structural differences, the anomeric effect remains a dominant factor in the stability of the phosphate conformations in **1**. This can be seen from the data in Table 1. For the *ap* (T) orientation about the C1−O1 bond, the TmGmG minimum that corresponds to the (-*sc*, -*sc*) conformation for the dimethyl phosphate anion has the lowest energy. The next lowest energy conformers are the TTG (*ap*, *sc*) and TGG (*sc*, *sc*) conformations, with energies approximately 0.9 and 1.3 kcal/mol higher than the TmGmG conformation. For the *sc* (G) orientation, the lowest energy conformer of the phosphate group is the GmGG (-*sc*, *sc*). The GTmG (*ap*, -*sc*) has approximately the same energy and the GTG (*ap*, *sc*) is 0.3 kcal/mol higher. For this orientation about the C1−O1 bond, surprisingly, the GGG (*sc*, *sc*) is the highest energy conformer (Δ*E* = 4.1 kcal/mol). The relative energy of the GmGG conformer, with the phosphate group in the (-*sc*, *sc*) conformations is unexpectedly low. For

the dimethyl phosphate anion, the relative energy[33−35] of the (-*sc*, *sc*) conformer is approximately 2.7 kcal/mol because of a steric C···C contact. For **1**, steric interactions in the GmGG conformer are slightly relieved by a large deviation of the three torsion angles Φ, Ψ_1, and Ψ_2 (90°, −90°, 98°) from the exact gauche orientation and by opening the C1−O1−P and P−O4−C bond angles (123°, 120°). An inspection of the spatial structure of the GmGG conformer revealed that the distance between the ring oxygen atom and one of the hydrogens of the methyl group is 2.63 Å and with the methyl carbon is 3.44 Å. This suggests that a weak O5...H−C hydrogen bond interaction might be responsible for this stabilization.

To estimate the influence of the basis set on the relative energy of conformers, single-point calculations with the tzp//6-31G* basis set have been carried out. A comparison of the relative energies (Table 1) reveals that enlargement of the basis set increases the relative energy of some conformers. This change is usually less than 0.5 kcal/mol. Interestingly, the energy differences between the TmGmG and GmGG (GTmG) conformers increased from 1.4 kcal/mol to 1.78 kcal/mol (1.83 kcal/mol). A larger increase was found for the GGmG conformer, 3.63 vs 4.42 kcal/mol. In this basis set, all *ap* conformers, except the TGT, have a lower energy compared to the other orientations. Thus, the predicted preference of the *ap* over the *sc* conformers about the C1−O1 bond is slightly larger at the higher level of calculation. To evaluate further the effect of the electron correlation and basis set, we have calculated the relative energies of four relevant conformers (TmGmG, TTG, GmGG, and GTmG) of **1** using the 6-31G* and tzp basis sets with the ACM method. The results of the ACM calculations are summarized in Table 2. The energies of the TTG, GmGG, and GTmG conformers relative to the TmGmG conformer are 0.64, 0.61, and 0.87 kcal/mol at the ACM/6-31G*//ACM/6-31G* level. Thus inclusion of electron correlation within the ACM DFT method at the 6-31G* level decreased the energy difference between the TmGmG and GmGG conformers about the C1−O1 bond from 1.40 kcal/mol to 0.61 kcal/mol. It can be seen from Table 2 that the ACM relative energies are basis set dependent and a further extension of the basis set increases the energy differences between conformers. Indeed, the ACM/tzp//ACM/6-31G* relative energies of the TTG, GmGG, and GTmG conformers changed to 0.91, 1.00, and 1.44 kcal/mol, respectively. The optimization of geometry with the ACM method at the tzp level only marginally altered these values to 0.92, 1.02, and 1.38 kcal/mol. It is also noteworthy that the absolute energies of conformers at the ACM/tzp//ACM/tzp are very similar (within 0.5 kcal/mol) to those calculated at the ACM/tzp//ACM/6-31G* level. The relative energy values appear to be approaching those predicted by the 6-31G* basis set without taking into account electron correlation. The largest differences (∼0.4 kcal/mol) between the 6-31G* and ACM/tzp methods have been found for the GmGG conformer. Similar behavior has been observed for dimethyl phosphate[33,34] and carbohydrate model compounds.[40] Overall, these results support our previous suggestion[36−40] that, due to fortuitous compensation of errors, calculations with the 6-31G* basis set at the HF level provide a reliable set of conformational energies and geometries for carbohydrate molecules.

To see what effect the zero-point energy, thermal energy, and entropy might have on the calculated energy differences, we have calculated the vibrational frequencies using the 6-31G* basis set. These were used to determine the zero-point energy, thermal energy, and entropy of conformers. The results are given in Table 1. It can be seen that the conclusions regarding the stability conformers remain unchanged, supporting the preference of the *ap* over the *sc* orientation about the C1−O1 bond. The free energy differences are smaller than the energy differences, and nine conformers have their free energy within 1.5 kcal/mol. The TmGmG conformer remains the preferred species. Zero-point energies (130.95−131.09 kcal/mol) and thermal energies (138.16−138.33 kcal/mol) are very similar for all conformers. The entropy contribution (107.06−110.91 cal/mol K) is responsible for the decrease in relative free energies.

Clearly, the negatively charged phosphate group linked to anomeric carbon completely changes the conformational behavior of the anomeric C1−O1 bond in comparison to that observed in glycosides, where the gauche orientation of the aglycon carbon with respect to the ring oxygen is usually the dominant conformer due the *exo*-anomeric effect. These unexpected results suggest that the influence of the *exo*-anomeric effect on the relative energy of conformers is not a decisive factor in negatively charged sugar phosphates. In other words, joining two groups, the acetal group (C5−O5−C1−O1−C) with the negatively charged phosphate group (C1−O1−PO_2^-−O−C), that displays strong anomeric and *exo*-anomeric effects, diminishes the role of the *exo*-anomeric effect on the stability of conformers. This finding is in contrast to results on α,α-trehalose, the nonreducing disaccharide resulting from the combination of two α-D-glucopyranose molecules through a (1 → 1) glycosidic linkage. Conformational analysis of α,α-trehalose revealed[50,51] the synergistic effect of joining two acetal sequences. As a result, the "all-gauche" conformation of the C5−O5−C1−O1−C1′−O5′−C5 segment was found to be the dominant conformer due to additivity of the strong anomeric and *exo*-anomeric effects. A qualitative rationalization of the conflicting results found for **1** can be obtained considering intramolecular electrostatic interactions. According to this interpretation, the *sc* conformers about the C1−O1 bond should be destabilized by electrostatic interactions compared to the *ap* conformer. The ESP charges of the O5 atom and PO_2^- group in **1** depend on the conformation but both are always negative. For example, the ESP charge of the O5 atom is −0.626 au in the TmGmG conformer and −0.537 au in the GmGG conformer. Similarly, the effective ESP charge of the PO_2^- group in corresponding conformers is −0.324 and −0.310 au, respectively. The O5···P interatomic distance is larger in the *ap* conformation compared to that in the *sc* conformer (3.74 Å vs 3.39 Å). Therefore, the repulsive electrostatic interactions between the O5 atom and PO_2^- group are always larger in the latter and destabilize the *sc* orientation over the *ap* one. The important role played by electrostatic interactions in stabilization of the *ap* conformer of **1** supports the interpretation of the anomeric effect based on a balance of electrostatic and delocalization interactions.[52] In α,α-trehalose, the effective ESP charge of the corresponding CH_2 group is always positive. Consequently, the attractive electrostatic interactions help to stabilize the *sc* orientation.

The preference of the *ap* (T) conformer about the anomeric C1−O1 bond for the 2-*O*-methylphosphono-tetrahydropyran anion represents a unique conformational behavior that has not been previously observed. Such a preference does not occur in other compounds with the C1−X (where X = CH_2, O, NH, NH_2^+, and S) anomeric linkage.[39] In all these compounds, the *sc* orientation about the anomeric C1−X bond is preferred over the *ap* orientation. The preference of the anomeric carbon substituent for the equatorial over the axial orientation has been termed the reverse anomeric effect.[53] By analogy, we will term the unusual conformational behavior of the negatively charged

TABLE 3: Ab Initio Relative Energy, Vibrational Correction, and Free Energy (kcal/mol), and the Position (torsion angles in degrees) of Conformational Minima of the Sodium 2-Methylphosphono-tetrahydropyran (2)

conformer	Φ	Ψ_1	Ψ_2	ΔE 6-31G*	ΔE tzp//6-31G*	Δcorr 6-31G*	$\Delta(\Delta G_{298})$ 6-31G*
GGG	67.5	49.5	76.0	7.01	6.56	1.19	4.68
GGmG	60.9	32.3	−109.0	6.24	6.04	2.14	4.87
GGT	61.0	37.2	−160.1	6.60	5.94	1.52	4.61
GmGG	83.5	−82.3	94.5	5.46	5.04	1.83	3.78
GmGmG	86.7	−88.9	−76.8	5.23	5.18	3.67	5.38
GmGT	81.2	−80.9	174.6	6.08	5.19	1.22	3.79
GTG	58.1	153.2	74.9	0.39	0.36	3.22	0.09
GTmG	57.5	156.7	−68.3	0.00[a]	0.00[b]	3.51	0.00[d]
GTT	58.4	153.7	170.4	0.85	0.78	2.96	0.30
mGGG	−76.9	74.5	62.9	12.05	11.22	3.97	12.51
mGmGmG	−101.4	−64.1	−74.0	11.49	10.83	1.99	9.97
mGmGT	−100.7	−69.0	−177.8	12.15	11.23	2.01	10.64
TGT	121.0	85.3	−179.2	5.92	4.84	0.65	3.06
TmGmG	128.2	−62.8	−63.4	4.84	4.11	1.82	3.15
TmGT	134.2	−64.9	174.1	6.89	5.83	0.98	4.36
TTG	150.0	−134.9	58.9	6.44	5.73	3.80	6.72
TTmG	148.3	−151.7	−66.1	7.21	6.37	0.00[a]	3.69

[a] $E = -697\,326.84$ kcal/mol. [b] $E = -697\,467.91$ kcal/mol. [c] $\Delta\text{corr} = \text{ZPE} + \Delta H_{298} - T\Delta S = 236.0$ kcal/mol. [d] $\Delta G_{298} = -697\,087.33$ kcal/mol.

phosphate group linked to the anomeric carbon as the reverse *exo*-anomeric effect. The magnitude of the *exo*-anomeric effect can be obtained from a comparison of the *sc* − *ap* energy difference in a given compound and in 2-ethyltetrahydropyran (0.9 kcal/mol).[39] Using the 0.9 kcal *sc* − *ap* energy difference for the 2-ethyltetrahydropyran, we estimated a 2.3 kcal/mol magnitude for the reverse *exo*-anomeric effect for the negatively charged phosphate group.

The relation between the anomeric and *exo*-anomeric effects and the angular dependence of some geometrical parameters has been well established experimentally and theoretically.[22−32] As a consequence of lone-pair delocalizations, the rotation about the C1−O1 bond from the *sc* to the *ap* conformation increases the C1−O1 bond length and decreases the O5−C1−O1 bond angle in carbohydrates. Similar structural changes are observed in the bond lengths and bond angles for different conformers of **1**. These changes are illustrated in Figure 2. However, they are not so straightforward because there is a subtle interplay between the delocalization of the O1 lone pairs into the anomeric carbon region and/or phosphorus region. Nevertheless, the C1−O1 bond length is the shortest C−O bond. For the same conformation of the phosphate group, for example GG, the C1−O1 bond is shorter in the *sc* conformation compared to the *ap* conformation. Similarly, the O1−P bond is longer in the *ap* compared to the sc conformation. The O1−P bond is also longer than the P−O4 bond. The exceptions are the mGGT, mGmGT, TmGT, and TGT conformers where the conformation about the P−O4 bond is the *ap*. The key bond angles, O5−C1−O1 and O1−P−O4, are significantly larger in the *sc* conformers. The O5−C1−O1 angle is approximately 4° larger for the *sc* conformation (111.8°−114.8°) compared to the *ap* conformation (108.2°−109.1°). For the O1−P−O4 angle, the smallest angle (94.5°) was found for the GTT conformation where both P−O bonds adopted the *ap* conformation. The magnitude of the geometrical parameters was also found to be influenced by steric interactions.[37] Values of the C1−O1−P bond angle clearly illustrate their significance. This angle has larger values (133.3°−135.5°) in conformers with the *-sc* orientation about the C1−O1 bond where the phosphate group lies below the ring. Steric interactions in these conformers are relieved by a 10° increase in the C1−O1−P angle. This illustrates the complexity of the conformational space for **1** and suggests that several factors must be taken together, steric and electrostatic interactions and the anomeric and *exo*-anomeric effects, to explain the conformational properties and geometries of conformers. Inclusion of electron correlation increased the bond lengths by about 0.03 Å but did not change bond angles significantly.

TABLE 4: Comparison of the ab Initio Energies and Relative Energies (kcal/mol) of Selected Conformers of the Sodium 2-*O*-Methylphosphono-tetrahydropyran (2) Calculated by Different Methods

geometry	energy	GTmG	GTG	TmGmG	TTG
6-31G*	6-31G*	−697 326.843	0.39	4.84	6.44
	tzp	−697 467.905	0.36	4.11	5.73
	ACM/6-31G*	−699 988.868	0.51	5.80	7.15
ACM/6-31G*	ACM/6-31G*	−699 991.974	0.52	5.94	6.52
	ACM/tzp	−700 143.883	0.55	5.34	6.11
ACM/tzp	ACM/tzp	−700 144.152	0.42	5.36	6.49

B. Sodium 2-*O*-Methylphosphono-tetrahydropyran (2). The 27 different staggered conformers of C1−O1−P−O4 linkage were used as starting structures for geometry optimization of **2**. For the location of the sodium ion, only the position depicted in Figure 1 has been considered. This choice was based on the previous calculations of dimethyl phosphate and on a survey of phosphate crystal structures.[33,34] In this position, the sodium cation is coordinated directly by two partially charged phosphate oxygens so that electrostatic and charge-transfer interactions are maximized.

Among the 27 starting structures, 10 of them, namely mGGmG, mGGT, mGmGG, mGTG, mGTmG, mGTT, TGG, TGmG, TmGG, and TTT, collapsed to different optimized conformers which reduced the number of minima to 17. The relative energies of these 17 final minima are summarized in Tables 3 and 4. The calculations of dimethyl phosphate and its sodium salt showed[33−35] that for both systems, the conformations and their relative energies are very similar. The (*sc*, *sc*) was the most stable conformer and the (*ap*, *ap*) the least one. The calculations reported here predict a completely different conformational behavior for **2**. The most stable structure of **2** appears to be the GTmG completely different from the most stable conformation of **1**, TmGmG. In the case of **2**, the TmGmG conformer has a relative energy of approximately 4.8 kcal/mol at the 6-31G* level which decreases to 4.1 kcal/mol at the tzp level. Comparison of the results for **1** and **2** in Tables 1−4 revealed other significant differences between both compounds. A completely reversed order of the *ap* and *sc* conform-

ers about the C1−O1 bond can be seen from these data. From 17 conformers of **2**, the three lowest energy conformers (GTmG, GTG, and GTT) correspond to the *sc* orientation of phosphorus with respect to the ring oxygen in accordance with the *exo*-anomeric effect. The conformations adopted by the phosphate group in these three minima are the (*ap*, *-sc*) for GTmG, the (*ap*, *sc*) for GTG, and the (*ap*, *ap*) for GTT. The relative energy of the GTG and GTT conformers are 0.4 and 0.9 kcal/mol at both the 6-31G* and tzp//6-31G* levels. The least stable orientation (*-sc*) about the C1−O1 bond for **2** is the same as for **1**. The 6-31G* relative energies of the three conformers having such an orientation about the C1−O1 bond are higher than 11.5 kcal/mol.

The ACM method predicted slightly different relative energies (Table 4), namely 0.5 kcal/mol for GTG and 5.9 kcal/mol for TmGmG at the ACM/6-31G*//ACM/6-31G* level and very similar values, 0.6 and 5.3 kcal/mol, at the ACM/tzp//ACM/6-31G* level. The corresponding energy differences for the TTG conformer are 6.5 and 6.1 kcal/mol, respectively. The conformational energies for **2**, after optimization at the ACM/tzp level, remain very similar to the values obtained with the ACM/6-31G* geometry. Similarly to **1**, the changes of the zero-point and thermal energies calculated between the conformations of **2** are very small and do not influence the free energy differences between the relevant conformers. Only the entropy term showed significant dependence on the conformation (106.5−121.0 cal/mol K). After taking into account these vibrational contributions, the free energy differences of the GTG, GTT, and TmGmG conformers relative to the GTmG conformer are 0.1, 0.3, and 3.2 kcal/mol, respectively. Thus, for **2**, the *sc* orientation about the C1−O1 is dominant at all levels of computation.

These results clearly indicate that the sugar−phosphate linkage in sodium salt exhibits an *exo*-anomeric effect about the C−O glycosidic linkage. The qualitative interpretation of the *ap* preference for **1** based on electrostatic interactions may also be used to rationalize the *sc* preference for **2**. In this context, the complexation of the phosphate group with sodium in **2** compensates the negative charge on the PO_2^- group. Consequently, for the GmGG conformer, the effective ESP charge of the $PO_2^-Na^+$ group is +0.382 au in **2** compared to −0.537 au for the PO_2^- group in **1**. Therefore, the corresponding electrostatic interactions changed from repulsive in **1** to attractive in **2**. As a result, the combination of the *exo*-anomeric effect and electrostatic interactions has a synergistic effect and leads to a large preference of the *sc* over the *ap* conformer. An analysis of the geometry between the sodium and ring atoms revealed that in all conformers having Φ in *sc* and Ψ_1 in *ap* orientation the O5···Na distance is ∼2.5 Å. Such arrangement observed in the low-energy conformers GTmG, GTG, and GTT allows the sodium atom to interact with the phosphoryl and ring oxygens simultaneously. This indicates that the O5···Na interactions might contribute to the stabilization of these conformers.

The location of the sodium ion varied slightly within the 17 conformers. Since the two charged oxygen atoms are not entirely symmetrical, the sodium atom binds in a nonsymmetrical fashion. The O···Na distances range from 2.2 to 2.3 Å. The sodium atom is usually slightly displaced from the O2−P−O3 plane in a direction away from the six-member ring and the P−O−Na bond angles are between 85° and 100°. An exception is the lowest energy conformer GTmG where the sodium atom is displaced in direction to the ring oxygen. Complexation by sodium induced very interesting deformations in the geometry of the sugar−phosphate linkages. For **2**, a comparison of the torsion angles Φ, Ψ_1, and Ψ_2 with those for the same conformation of **1** shows only slight changes in most cases. An exception is the Φ angle in some conformers where shifts as large as 40° are observed (for the TGT conformer from 159° in **1** to 121° in **2**). Another important feature of the sodium−phosphate interactions emerges from a comparison of the bond lengths of **1** and **2**. The most relevant changes are associated with the variation in bond lengths connecting the sugar and phosphate groups (Figure 2). Changes for some bond lengths as large as 0.06 Å are predicted between the two compounds. In the sugar part of the sodium complex **2**, the C5−O5 and C1−O1 bonds are lengthened, while the O5−C1 bond is shortened compared to the anion **1**. For the phosphate part, the O1−P and P−O4 bonds are shortened, while the P−O2 and P−O3 are lengthened in **2**. Small variations, less than 5°, are predicted for the O5−C1−O1 and C1−O1−P bond angles. The O2−P−O3 bond angle narrows from (122°−125°) to (111°−114°) while the O1−P−O4 angle slightly widens from (94°−101°) to (100°−104°). As it was found for **1**, the inclusion of electron correlation within the ACM method increased the bond lengths by ∼0.03 Å.

To assess a reliability of calculated results a comparison with X-ray data is important. In a search of the Cambridge Structural Data Base[54] we found four examples of sugar−monophosphate structures (CSD Refcodes: CIMDUX, GLCTSM, JUGTAG, and KGLUCP02). Only structures presenting the pyranose ring in a conformation close to $^4C_1$ have been considered. All these structures are complexed either with metal counterions (sodium salt: CIMDUX and potassium salts: JUGTAG, KGLUCP02) or water molecules (hydrate: GLCTSM). Therefore, structural information they provide can only be used for comparison with our model of complex, the sodium 2-*O*-methylphosphono-tetrahydropyran (**2**). The conformation about the glycosidic bond of all the salts is in close agreement with our calculations on **2.** The conformation around the C1−O1 bond is the *sc* orientation with $\Phi = 89.1°$, 63.8°, and 87.2° for CIMDUX, JUGTAG, and KGLUCP02, respectively. The hydrate structure (GLCTSM) shows a slightly different conformation around the glycosidic bond with $\Phi = 106.2°$ deviated from the usual *sc* orientation. Specific interactions between the charged ammonium and phosphate groups that are observed in this structure could be responsible for this change. The absence of any metal cofactor in the hydrate might also contribute to this shift in the Φ dihedral angle. All crystal structures found show different packing patterns and are stabilized by numerous nonbonded interactions. For instance, the phosphate oxygens appear to be involved in one or several nonbonded interactions either with the metal, water molecules, or intermolecular hydrogen bonding. There are additional differences between these crystallographic data and theoretical calculations: the mixed nature of the counterion, the different type of sugar investigated. Therefore, these data are not sufficient for reliable statistical analysis and all comparisons should be made with caution. Nevertheless, they support calculated prediction on conformation of the sugar−phosphate linkage in **2**.

C. Solvent Effect. To investigate the relative stabilization of the *ap* and *sc* conformers about the C1−O1 anomeric bond by a dielectric continuum, calculations using the polarized continuum model (PCM) method as implemented in Gaussian94[45] were performed. This method yielded most reliable aqueous solvation energies for pyrophosphates.[55] All solvation calculations were carried out at the 6-31G* level. The PCM calculations yield the electrostatic contribution to solvation energies. The cavitation and dispersion contributions to solvation free energies were calculated using previously described pro-

TABLE 5: Ab Initio Relative Free Energy, $\Delta(\Delta G_{298})$, (kcal/mol), of Relevant Conformational Minima of the 2-Methylphosphono-tetrahydropyran Anion (1) in Carbon Tetrachloride, *n*-Octanol, Methanol, and Water

conformer	vacuum	CCl_4	octanol	methanol	water
GGG	3.30	2.82	2.68	2.07	3.21
GGmG	3.91	4.03	3.15	4.98	3.56
GmGG	1.09	1.19	5.47	6.34	5.21
GmGT	1.17	0.50	0.99	0.96	3.59
GTG	1.27	1.21	0.86	0.90	1.67
GTmG	1.39	1.73	1.69	3.05	4.23
GTT	1.68	1.51	0.95	1.21	1.10
TGG	1.35	1.84	3.19	5.16	6.39
TGT	1.37	1.76	0.86	2.71	3.00
TmGmG	0.00	0.00	0.00	0.00	0.00
TmGT	1.05	0.84	0.67	1.16	2.39
TTG	0.59	1.05	1.00	1.09	1.55

TABLE 6: Ab Initio Relative Free Energy, $\Delta(\Delta G_{298})$, (kcal/mol), of Relevant Conformational Minima of the Sodium 2-Methylphosphono-tetrahydropyran (2) in Carbon Tetrachloride, *n*-Octanol, Methanol, and Water

conformer	vacuum	CCl_4	octanol	methanol	water
GGG	4.68	4.32	3.40	4.03	2.67
GGmG	4.87	5.58	5.94	6.21	3.55
GGT	4.61	4.78	5.17	5.88	4.42
GmGG	3.78	2.20	5.28	4.89	2.97
GmGmG	6.10	6.23	6.29	7.45	6.58
GmGT	3.79	3.95	3.01	3.41	4.97
GTG	0.09	0.00	0.00	0.00	0.00
GTmG	0.00	0.24	1.53	2.51	1.05
GTT	0.30	0.62	0.91	1.20	3.14
TGT	3.06	3.47	3.23	3.79	1.78
TmGmG	3.15	2.56	3.75	3.80	3.49
TmGT	4.36	5.21	5.32	7.04	5.15
TTG	6.72	7.60	6.83	10.38	9.02
TTmG	3.69	3.49	4.57	4.54	3.50

cedures.[46,47] The calculated relative free energy difference between relevant conformers in four solvents are given in Tables 5 and 6. From solvation in four solvents at 6-31G* level, a clear trend emerges from these results. The conformational preference about the C1−O1 anomeric bond in solution is essentially the same as that found in a vacuum calculations. In all solvents, the *ap* conformation remains to be a dominant orientation about the anomeric bond for **1**, whereas for **2**, the preference shifts to the *sc* conformation. Of course, this is not to say that solvation influences all conformers by the same way. On the contrary, the effect of a dielectric continuum is different for individual conformers.

For **1**, vacuum calculations predict the TmGmG and TTG conformers as two preferred conformers. Their free energies correspond to the population of 43% and 16%, respectively. Both conformers exhibit the *ap* orientation about the anomeric bond. The TmGmG conformer is the dominant conformer in all solvents and its equilibrium fraction gradually increases to 76% in aqueous solution. On the other hand, the population of the TTG conformers decreases to 5%. Recent evaluation of solvation effects on the conformers of dimethyl phosphate anion[35] showed that the *ap* conformers are stabilized in aqueous solution compared to gas-phase. The similar effect of solvent was predicted for the 2-methoxytetrahydropyran conformers.[37] A presence of two functional groups in **1** makes a quantitative comparison impossible. Density distributions are very different in all molecules and in **1** they depend on the mutual orientation of the pyranoid ring and the phosphate group. Nevertheless it is noteworthy that among conformers with the *sc* orientation about the anomeric linkage and with the *ap* orientation about the P−O bonds, the GTT conformer is the most stabilized by solvent effects. The values given in Table 5 show a decrease in the relative free energy of the GTT conformer from 1.7 kcal/mol in a vacuum to 1.1 kcal/mol in water. Thus, the population of the GTT conformer increases from 2% in a vacuum to 12% in aqueous solution where it is the second most stable conformer. However, when comparing the *ap* and *sc* conformers about the anomeric C1−O1 bond, it appears that their ratio is not very sensitive to the solvent. For example, the *ap*:*sc* ratio is 71:29 in a vacuum, 65:35 in carbon tetrachloride, 69:31 in methanol, and 83:17 in water. The relative solvation energies of minima vary by 3.8 kcal/mol in carbon tetrachloride and by 6.4 kcal/mol in water. As expected, the dominant contribution to the solvation energy is the electrostatic term. The cavity and dispersion contribution act in opposite directions, and therefore, a sum of the cavity and dispersion contribution is less sensitive to conformation changes. For example, a sum of their contributions varied by 1.8 kcal/mol in carbon tetrachloride and 0.8 kcal/mol in water.

As can be seen from Table 6 there is no change due to solvent effects in the predicted preference for the *sc* orientation about the C1−O1 anomeric bond in **2**. However, calculations predict a shift in the ordering of the conformers. Compared to vacuum, where the GTG (35%), GTmG (40%), and GTT (24%) conformers are dominant, solvent effects gradually increase the relative energy of the GTT conformer. In particular, the solvation energy of the GTT conformer is significantly increased in water (3.1 kcal/mol) compared to vacuum (0.3 kcal/mol) This results in the dominance of the GTG (80%) and GTmG (14%) conformers. A contribution of the *ap* orientation to equilibrium in **2** is not significant, though for aqueous solution the relative free energy of the TGT conformer is 1.75 kcal/mol compared to 3.86 kcal/mol in a vacuum. As a result, the population of the TGT conformer increases to 4%. The calculated solvation energy values for **1** and **2** are somewhat uncertain and should be used with caution. In particular the contributions from specific solvation interactions are neglected in this model and there are still some uncertainties in the description of the cavity.[55] Nevertheless, the results of solvent effect calculations discussed above support the results from vacuum calculations.

D. Comparison with the Results of Semiempirical Molecular Orbital and Molecular Mechanics Calculations. The large number of heavy atoms and torsion angles in sugar donors such as nucleotide sugars, make high-level ab initio calculations of conformational properties for these molecules computationally inaccessible, necessitating the use of less sophisticated computational methods. Additional difficulties are caused by the anomeric and *exo*-anomeric effects. To model such behavior in complex molecules, it is important to determine the reliability of the predicted relative energies and geometries for the sugar−phosphate linkage as calculated by molecular mechanics programs or semiempirical molecular orbital methods. To answer this question we have examined the performance of the three commercially available and commonly used programs, namely the MNDO semiempirical molecular orbital method and the molecular mechanics method using the CVFF and AMBER force fields. The calculated relative energies for **1** and **2** are summarized in Tables 7 and 8, and the optimized geometries for the relevant MNDO conformers are illustrated in Figure 3.

The results in Tables 7 and 8 indicate that the preferred orientation about the C1−O1 bond in **1** and **2** calculated using the MNDO method is correctly predicted. The *ap* orientation is found to be preferred for the 2-*O*-methylphosphono-tetrahydropyran anion (**1**), while with the sodium counterion present (**2**), the conformational preference changes completely in favor

TABLE 7: Comparison of the Relative Energies (kcal/mol) Calculated by MNDO and Different Molecular Mechanics Methods for the 2-Methylphosphono-tetrahydropyran Anion (1)

conformer	MNDO	CVFF	AMBER
GGG	0.69	4.73	6.93
GGmG	1.10	4.51	6.39
GGT		3.00	6.14
GmGG		2.60	2.85
GmGmG		2.18	
GmGT		1.14	2.87
GTG	1.23		4.30
GTmG	1.44		4.56
GTT			4.26
mGGG	4.80	7.79	11.56
mGGT		7.59	12.36
mGmGmG	5.18	9.76	12.42
mGmGT		8.28	12.10
mGTG	6.18	8.05	
mGTmG		7.80	
mGTT		6.93	
TGG		2.67	0.51
TGT		1.65	0.66
TmGG	1.41		
TmGmG	0.00	1.91	0.45
TmGT		1.30	0.92
TTG	1.35	0.86	0.00
TTmG		1.01	0.09
TTT		0.00	0.27

TABLE 8: Comparison of the Relative Energies (kcal/mol) Calculated by MNDO and Different Molecular Mechanics Methods for the Sodium 2-Methylphosphono-tetrahydropyran (2)

conformer	MNDO	CVFF	AMBER
GGG		5.96	6.76
GGmG	3.04	5.75	6.48
GGT		6.14	8.35
GmGG	2.08	5.24	4.19
GmGmG	0.92	4.88	4.27
GmGT		5.65	6.09
GTG	0.00	0.06	0.00
GTmG	0.77	0.20	0.22
GTT		0.00	1.18
mGGG	5.65	9.18	12.53
mGGmG			17.20
mGGT	8.05		16.12
mGmGmG	5.63	10.40	13.04
mGmGT		10.73	14.95
mGTG		13.85	
mGTmG		13.61	
mGTT		15.70	
TGG		5.57	3.23
TGmG			6.51
TGT		6.47	5.44
TmGG		8.50	5.68
TmGmG		5.81	4.07
TmGT		7.33	7.18
TTG		7.01	6.50
TTmG		7.15	
TTT		8.84	10.24

of the *sc* orientation. However, in contrast to the ab initio results, conformers with the *ap* orientation have not been predicted for **2**. Another principal difference with ab initio results resides in the reduced number of minima: 10 vs 16 for **1**, and 8 vs 17 for **2**. The lowest energy conformer for **1** (TmGmG) is in agreement with the ab initio results. Nevertheless, the energy differences between the consecutive minima differ. Indeed, the next two low-energy minima GGG and GGmG correspond to the *sc* orientation about the C1−O1 bond with relative energies 0.7 and 1.1 kcal/mol, respectively (Table 7). In the case of **2**, the two lowest energy minima, GTG and GTmG, display the *sc*

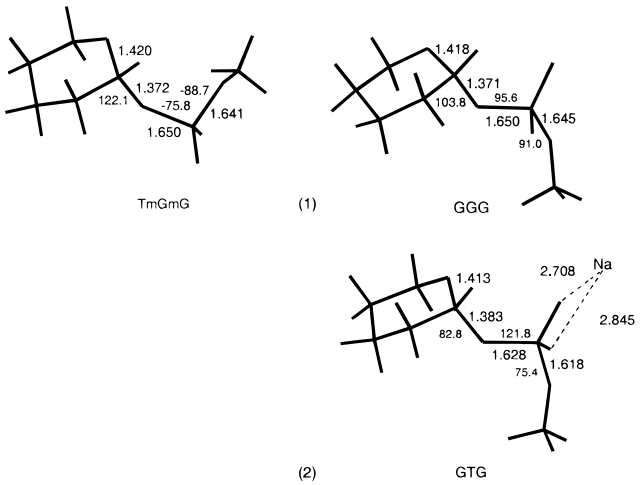

Figure 3. Comparison of the selected geometry values for the *ap* and *sc* lowest energy conformers about the C1−O1 bond calculated using the semiempirical MNDO method. Bond lengths are given in angstroms, torsion angles in degrees.

orientation with respect to the C1−O1 bond but, contrary to the ab initio results, GTG is the preferred. The GTmG conformer is 0.8 kcal/mol higher in energy than the GTG conformer using the MNDO method but it is lower by 0.4 kcal/mol at the 6-31G* level. The geometrical features observed for **1** and **2** show the same overall geometrical trends compared to those observed by ab initio calculations. The C1−O1 bond is lengthened in **2** compared to that in **1**, whereas the O1−P bond is shortened (Figure 3) and the O2−P−O3 angle narrowed. A comparison of the torsion angles reveals differences in the exact location of the minima in Φ dihedral space as predicted by the MNDO and the ab initio methods. For example, in the *sc* orientation, the MNDO values of the Φ torsion angle are close to 100°, whereas the corresponding 6-31G* values are closer to 60°. Similarly, slight differences between the magnitude of bond lengths and bond angles predicted by semiempirical and ab initio calculations are observed. The C1−O1 bonds are usually predicted approximately 0.01 Å shorter and the O1−P bonds 0.02 Å longer than the corresponding 6-31G* values. For the bond angles, the most remarkable difference was found for the C1−O1−P angle that is approximately 10° larger in MNDO structures. On the other hand, MNDO values adequately describe the structural trends observed between **1** and **2** in the ab initio calculations.

The relative energies of the conformers of **1** and **2** obtained by molecular mechanics calculations using CVFF and AMBER force fields are summarized in Tables 7 and 8. Larger numbers of minima was found for both compounds **1** and **2** by these methods compared to the ab initio results. Both molecular mechanics methods provided different results and incorrectly predicted the lowest energy conformer for **1**. As well, the relative energies of the conformers for the 2-*O*-methylphosphono-tetrahydropyran anion calculated by CVFF and AMBER methods are in poor agreement with ab initio results. Nevertheless, both force fields correctly predicted the *ap* orientation about the C1−O1 bond as the most favored for **1**. In contrast, both methods failed to describe accurately the energies of conformations about the phosphate linkages. For **2**, both force fields seem to give reasonable results. However, in most of the cases, the three lowest conformations from ab initio results (GTmG, GTG, and GTT) are predicted in a different order by molecular mechanics. The purpose of this comparison has been to estimate the reliability of semiempirical molecular orbital and molecular

mechanics methods to model the sugar−phosphate linkage. Results suggest that none of the molecular mechanics methods used reproduce in a satisfactory way the ab initio results. The obtained energy and geometry of conformers might be used as bench marks for refinements of force fields. From the comparison of the results summarized in Tables 7 and 8, it seems that the semiempirical MNDO method gives the closest results to ab initio ones, at least in a qualitative way.

E. Implications Concerning the Catalytic Mechanism of Glycosyltransferases. Kinetic studies with some transferases[6,8,11−13] indicate an ordered sequential mechanism, with the sugar donor binding first. For most transferases the presence of a metal cofactor has been shown to be required, and furthermore the metal binds to the enzyme prior to the donor nucleotide−sugar. Then, before any of the products leaves the enzyme, a sugar acceptor has to be bound. The metal cofactor is released from the enzyme in the form of a complex with the nucleotide−phosphate. It would appear that these experimental findings and the predicted changes in conformational behavior between **1** and **2** are not coincidental. It is suggested that the complexation of a positive metal cation with the negatively charged phosphate group in the sugar donor molecule represents an important step in the mechanism of glycosyltransferases. The complexation of phosphate with the metal ion would appear to fulfill three different purposes. One purpose could be to change the conformation in order to adopt the correct shape for optimal enzymatic recognition and thereby achieve maximal catalytic efficiency. A second possibility is to activate the C1−O1 bond for cleavage by the enzyme. The third possible purpose is to enable the protonation at the glycosidic phosphate oxygen to begin the scission of the C1−O1 glycosidic bond. In **1**, the protonation of O1 would be relatively unfavorable because of the competition from the partially charged phosphate oxygens.

It is generally assumed that one of the important aspects of enzymatic catalysis is the binding of the substrate and its conversion into a reactive conformation. Assuming that the *sc* conformation about the C1−O1 bond is more reactive than the *ap* one, then the enzyme has to distort **1** into a less populated and higher energy conformation. During this binding, several rotational degrees of freedom must also be lost. Both, the enthalpic and entropic internal effects are unfavorable for the reaction rate. However, as shown in previous sections, complexation with sodium reverses the conformational equilibrium. In the case of **2**, the *sc* orientation is dominant and relative energy of the *ap* orientation (4 kcal/mol) is significantly higher. Since the structure of **2** is restricted into the most reactive conformation, unfavorable entropic effects from this rotational degree of freedom (the rotation about the C1−O1 bond) should be lower in **2** compared to **1**. This might give an estimated 2−4 kcal/mol in favor of cleavage via the *sc* conformation relative to the *ap* one. In this qualitative argument we have ignored enthalpic considerations from the phosphate group. However, as shown above, the energy differences between the conformers of the phosphate group are quite modest compared to those about the C1−O1 bond.

Ab initio modeling of the sugar−phosphate linkage using **1** and **2** at the 6-31G* revealed that the C1−O1 bond length between the anomeric carbon and glycosidic oxygen elongates by 0.02 Å and that the O1−P bond between the glycosidic oxygen and phosphorus shortens by 0.02 Å upon formation of the complex. In this context, the complexation with sodium imposes a perturbation of the geometry for the sugar phosphate linkage along the reaction coordinate of the catalytic reaction toward the transition state. This is illustrated in Figure 2 where the structures and selected geometrical parameters for lowest energy conformers of **1** and **2** are presented. The C1−O1 bond is cleaved during the catalytic reaction. Therefore, its elongation activates this bond and decreases the reaction barrier. A similar effect might be expected from the shortening of the O1−P bond length since this bond is shorter in the product of this reaction, sodium methyl phosphate (1.607 Å), compared to its magnitude in **1** or **2**. For spontaneous acetal hydrolysis, the relationship between the C1−O1 bond length and the free energy of activation has been established.[56,57] It has been argued that small changes in the C1−O1 bond lengths decrease the reaction barrier as much as 10 kcal/mol per 0.05 Å. This gives an upper estimate of 3−5 kcal/mol in the decrease in the reaction barrier by complexation with sodium.

The above arguments suggest that the theoretically predicted differences in geometry and conformational equilibrium between **1** and **2** might be a prerequisite for the binding of the sugar donor to the enzyme and to begin the catalytic reaction. The difference between the reactivity of different substrates (in our case, **1** and **2**) is given by the differences between the ground and transition state free energies. Enzymatic catalysis is usually a result of the stabilization of the transition state of the reaction.[58] In the above discussion we have considered qualitatively only the likely consequences that follow from perturbations of the ground state, completely ignoring the role of intermolecular effects. We emphasize that the influence of these factors may either mask or strengthen the effects discussed above. The investigation of these effects is beyond the scope of this paper and is currently under evaluation in our laboratory.

4. Conclusions

The preference of the *ap* over the *sc* orientation about the C1−O1 bond has been predicted for the 2-*O*-methylphosphono-tetrahydropyran anion (**1**) at all levels of ab initio calculations. This unusual phenomenon has been termed, by analogy with the reverse anomeric effect, as the reverse *exo*-anomeric effect. The 2.3 kcal/mol magnitude of this effect has been estimated for the negatively charged phosphate group in a vacuum. For the sodium 2-*O*-methylphosphono-tetrahydropyran (**2**), all calculations have predicted the dominance of the *sc* over the *ap* orientation about the C1−O1 bond in accordance with the *exo*-anomeric effect. Structural variations in geometrical parameters have shown changes characteristic of the anomeric and *exo*-anomeric effects.

Differences in the structure of the 2-*O*-methylphosphono-tetrahydropyran anion (**1**) and sodium 2-*O*-methylphosphono-tetrahydropyran (**2**) suggest an important function of the metal cofactor in the catalytic mechanism of transferases. Three different roles of the cofactor have been postulated: causing a change in the conformation for optimal bonding to the enzyme, activating the C1−O1 glycosidic bond by elongating the C1−O1 bond, and changing the charge distribution to make the protonation of the O1 glycosidic oxygen more favored.

Supporting Information Available: A listing of calculated geometrical parameters for all conformers of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Varki, A. *Glycobiology* **1993**, *3*, 97−130.
(2) *Glycoproteins and Disease*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1995.
(3) Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171−1202.
(4) Legler, G. *Carbohydr. Res.* **1993**, *250*, vii−xx.

(5) Withers, S. G.; Aebersold, R. *Protein Sci*. **1995**, *4*, 361−372.
(6) Khatra, B. S.; Herries, D. G.; Brew, K. *Eur. J. Biochem*. **1974**, *44*, 537−560.
(7) Tsopaknakis, A. D.; Herries, D. G. *Eur. J. Biochem*. **1978**, *83*, 179−188.
(8) Bendiak, B.; Schachter, H. *J. Biol. Chem*. **1987**, *262*, 5784−5790.
(9) Nishikawa, Y.; Pegg, W.; Paulsen, H.; Schachter, H. *J. Biol. Chem*. **1988**, *263*, 7321−7322.
(10) Kim, S. C.; Singh, A. N.; Raushel, F. M. *J. Biol. Chem*. **1988**, *263*, 10151−10154.
(11) Kim, S. C.; Singh, A. N.; Raushel, F. M. *Arch. Biochem. Biophys.* **1988**, *267*, 54−58.
(12) Kearns, A. E.; Campbell, S. C.; Westley, J.; Schwartz, N. B. *Biochemistry* **1991**, *30*, 7477−7483.
(13) Ats, S.-C.; Lehmann, J.; Petry, S. *Carbohydr. Res*. **1992**, *233*, 125−139.
(14) Nakazawa, K.; Furukawa, K.; Narimatsu, K.; Kobata, A. *J. Biochem*. **1993**, *113*, 747−753.
(15) Yin, H.; Bennett, G.; Jones, J. P. *Chem. Biol. Interactions* **1994**, *90*, 47−58.
(16) Breuer, W.; Bause, E. *Eur. J. Biochem*. **1995**, *228*, 689−696.
(17) Strokopytov, B.; Penninga, D.; Rozeboom, H. J.; Kalk, K. H.; Dijkstra, B. W. *Biochemistry* **1995**, *34*, 2234−2240.
(18) Qiao, L.; Murray, B. W.; Shimazaki, M.; Schultz, J.; Wong, C. *J. Am. Chem. Soc*. **1996**, *118*, 7653−7662.
(19) Beyer, T. A.; Sadler, J. E.; Rearick, J. I.; Paulson, J. C.; Hill, R. L. *Adv. Enzymol*. **1981**, *52*, 23−175.
(20) *Glycoproteins*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1995.
(21) Kleene, R.; Berger, E. G. *Biochim. Biophys. Acta* **1993**, *1154*, 283−325.
(22) *The Anomeric Effect, Origin and Consequences*; Szarek, W. A. Horton, D., Eds.; ACS Symposium Series 87; American Chemical Society: Washington, DC, 1979.
(23) Kirby, A. J. In *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: Berlin, 1983.
(24) Deslongchamps, P. In *Stereoelectronic Effects in Organic Chemistry*; Pergamon: Oxford, U.K., 1983.
(25) Tvaroska, I.; Bleha, T. *Chem. Papers* **1985**, *39*, 805−847.
(26) Tvaroska, I. In *Theoretical Chemistry of Biological Systems*; Naray-Szabo, G., Ed.; Elsevier: Amsterdam, 1986; pp 283−348.
(27) Gorenstein, D. G. *Chem. Rev*. **1987**, *87*, 1047−1077.
(28) Sinnott, M. L. *Adv. Phys. Org. Chem*. **1988**, *24*, 113−204.
(29) Tvaroska, I.; Bleha, T. *Adv. Carbohydr. Chem. Biochem*. **1989**, *47*, 45−123.
(30) Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019−5087.
(31) *The Anomeric Effect and Associated Stereoelectronic Effects*; Thatcher, G. R. J., Ed.; ACS Symposium Series 539; American Chemical Society: Washington, DC, 1992.
(32) Juaristi, E.; Cuevas, G. In *The Anomeric Effect*; CRC Press: Boca Raton, FL, 1994.
(33) Schneider, B.; Kabelac, M.; Hobza, P. *J. Am. Chem. Soc*. **1996**, *118*, 12207−12217.
(34) Florian, J.; Baumruk, V.; Strajbl, M.; Bednarova, L.; Stepanek, J. *J. Phys. Chem*. **1996**, *100*, 1559−1568.
(35) Florian, J.; Strajbl, M.; Warshel, A. *J. Am. Chem. Soc.* **1998**, *120*, 7959−7966.
(36) Tvaroska, I.; Carver, J. P. *J. Phys. Chem*. **1994**, *98*, 6452−6458.
(37) Tvaroska, I.; Carver, J. P. *J. Phys. Chem*. **1994**, *98*, 9477−9485.
(38) Tvaroska, I.; Carver, J. P. *J. Phys. Chem*. **1995**, *99*, 6234−6241.
(39) Tvaroska, I.; Carver, J. P. *J. Phys. Chem*. **1996**, *100*, 11305−11313.
(40) Tvaroska, I.; Carver, J. P. *J. Phys. Chem. B* **1997**, *101*, 2992−2999.
(41) Tvaroska, I.; Carver, J. P. *J. Mol. Struct., THEOCHEM* **1997**, *395*−*396*, 1−15.
(42) *Turbomole 95.0 User Guide*; October 1995, San Diego: Biosym/MSI, 1995.
(43) Becke, A. D. *J. Chem. Phys*. **1993**, *98*, 5648−5652.
(44) Parr, R. G.; Yang, W. In *Density-Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989.
(45) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, Revision B.1; Gaussian, Inc., Pittsburgh, PA, 1995.
(46) Pierotti, R. A. *Chem. Rev.* **1976**, *76*, 717−726.
(47) Tvaroska, I.; Kozar, T. *J. Am. Chem. Soc*. **1980**, *102*, 6929−6936.
(48) *InsightII 95.0 User Guide*; October 1995, San Diego: Biosym/MSI, 1995.
(49) *Discover 95.0 User Guide*; October 1995, San Diego: Biosym/MSI, 1995.
(50) Tvaroska, I.; Vaclavik, L. *Carbohydr. Res*. **1987**, *160*, 137−149.
(51) Dowd, M. K.; Reilly, P. J.; French, A. D. *J. Comput. Chem*. **1992**, *13*, 102−114.
(52) Tvaroska, I.; Bleha, T. *Tetrahedron Lett*. **1975**, *4*, 249−252.
(53) Lemieux, R. U.; Morgan, A. R. *Can. J. Chem*. **1965**, *43*, 2205−2213.
(54) Allen, F. H.; Davies, J. E.; Galloy, J. J.; Johnson, O.; Kennard, O.; Macrae, C. F.; Mitchell, E. M.; Mitchell, G. F.; Smith, J. M.; Watson, D. G. *J. Chem. Inf. Comput. Sci.* **1991**, *31*, 187−204.
(55) Colvin, M. E.; Evleth, E.; Akacem, Y. *J. Am. Chem. Soc*. **1995**, *117*, 4357−4362.
(56) Jones, P. G.; Kirby, A. J. *J. Am. Chem. Soc*. **1984**, *1068*, 6207−6212.
(57) Bürgi, H.-B.; Dubler-Steudle, K. C. *J. Am. Chem. Soc*. **1988**, *110*, 7291−7299.
(58) Warshel, A.; Florian, J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5950−5955.