Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 340 (2005) 841–852
Carbohydrate RESEARCH

The two-conformer hypothesis: 2,3,4,6-tetra-*O*-methyl-mannopyranosyl and -glucopyranosyl oxacarbenium ions

Tomoo Nukada,[a] Attila Bérces,[b] LiJie Wang,[b] Marek Z. Zgierski[b] and Dennis M. Whitfield[c,*]

[a]*The Institute for Physical and Chemical Research (RIKEN), Wako-shi, 351-01 Saitama, Japan*
[b]*Steacie Institute for Molecular Science, National Research Council Canada, 100 Sussex Dr., Ottawa, Ontario, Canada K1A 0R6*
[c]*Institute for Biological Sciences, National Research Council Canada, 100 Sussex Dr., Ottawa, Ontario, Canada K1A 0R6*

Received 13 September 2004; accepted 10 December 2004
Available online 2 February 2005

This paper is in honour of the contributions to carbohydrate chemistry that have been made by Professor David A. Brant

Abstract—Computational chemistry can give information about the probable conformations of reactive intermediates that are difficult to determine experimentally. Based on density functional theory (DFT) calculations of tetra-*O*-methyl-D-mannopyranosyl and -glucopyranosyl oxacarbenium ions, two families of conformations, which we call **B0** and **B1**, were found. For the *manno* configuration, a $^4H_3$ and ^3E almost isoenergetic pair were found, whereas for the *gluco*-configuration a $^4H_3$ and $^5S_1$ pair favouring $^4H_3$ were calculated. These results corroborate earlier results and suggest that this two or more conformer hypothesis is general. Nucleophilic attack on these pairs of cations was modelled with methanol and led to four cases to consider namely α- or β-attack on **B0** or **B1**. The resulting complexes (**G0**, **G1** and **F0**, **F1**) demonstrate facial selectivity. The relative energies of these complexes are dominated by intramolecular hydrogen bonding and the conformational consequences to the pyranose ring of changes in the C-5–O-5–C-1–C-2 torsion angle. Constrained variation of the nucleophilic oxygen (methanol) to C-1 distance shows that these ion dipole complexes are the only minima with this constraint.

Keywords: Glycosylation; Oxacarbenium ions; Density functional theory; Conformational analysis

1. Introduction

Most sugars are found in Nature either as glycosides of other natural products, or joined together to form oligosaccharides and polysaccharides. In order to synthesize such compounds one must solve the complexities of making the R^1**COC**–**O**CR^2 linkage. A large body of empirical observations have been made on glycosylation reactions but still only a few generalizations can be made.[1] Of particular importance is the control of the stereochemistry at C-1 and avoiding side reactions. Experimentally it is very difficult to study glycosylation reactions due in part to the short lifetimes of the presumed oxacarbenium ion intermediates.[2] Of most relevance to the present discussion is the unequivocal demonstration of the formation of dioxolenium ions under glycosylation reaction conditions.[3] One of the emerging strategies to study this stereochemical problem is to perform mechanistic studies using modern theoretical chemistry methods. The recent advances in computer hardware and software have now made molecules of the size of protected monosaccharides amenable to quantum mechanical calculations.[4] Of particular interest is to determine the probable conformations of the calculated species, since the conformation is expected to be an important determinant of the stereochemical outcome of glycosylation reactions.

Several years ago we initiated a program to develop an understanding of the glycosylation reaction mechanism through density functional theory (DFT) calculations of plausible intermediates and transition states

* Corresponding author. Tel.: +1 613 993 5265; fax: +1 613 952 9092; e-mail: dennis.whitfield@nrc-cnrc.ca

0008-6215/$ - see front matter
doi:10.1016/j.carres.2004.12.021

(TS) where the initial structures were derived from hypotheses developed from the body of empirical experimental observations.[5] Our goal is to assist experimentalists in developing stereospecific glycosylation reactions that are free of side reactions. As we developed our studies it has become increasingly clear to us that our studies also have implications for the mechanistic study of glycosyltransferases and glycosidases.

Our first studies were of the relatively rigid 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-D-galactopyranosyl cations, **1**. These bicyclic ions were anticipated to show less flexibility than monocyclic ions and therefore be less prone to conformational ambiguities during calculations. The relative energetics and conformational preferences of these glycosyl oxacarbenium ion test the S_N1 (D_N+A_N) model of reactive intermediates generated from glycosyl donors during a glycosylation reaction. The earliest kinetic studies of glycosylation reactions showed that only with very reactive nucleophiles that true S_N2 (A_ND_N) kinetics could be observed.[6] Since most glycosylation reactions involve weak nucleophiles it follows that most reactions have considerable S_N1 character. Prompted by detailed analysis of enzymatic glycosylation reactions, more refined mechanistic studies also consider the degree of association of the nucleophile at the TS as well as the degree of disassociation of the leaving group.[7] More recent kinetic studies find a dependence on both the donor and acceptor, suggesting a more complex mechanism than simple S_N1.[8] However, all of these mechanisms share a key feature, which is that C-1 has appreciable sp^2 character at the TS. This progression from sp^3 in the reactants to sp^2 in the TS and back to sp^3 in the product must entail changes in the pyranosyl ring conformation. For simplicity we only consider six-membered rings. This hybridization change sequence is fully operational with fully ionized glycosyl oxacarbenium ions and is an important feature of our model. Initial studies are gas-phase models with a continuum solvent correction. We anticipate that as computational resources and programs improve solvent and counterions can be included in the models.

Classical intuitive conformational analyses of glucopyranosyl oxacarbenium ions have considered $^4H_3$ and $^3H_4$ half chair conformations for D and L sugars, respectively, since these conformations allow the 3,4-substituents to be pseudo-equatorial instead of pseudo-axial.[9] The adjacent in conformational space (see spherical representation in Fig. 1) to $^4H_3$ conformers ^4E and E_3 have been considered for tetrahydropyranyl oxacarbenium ions.[10] Evidence for 'flattened chair' conformations have

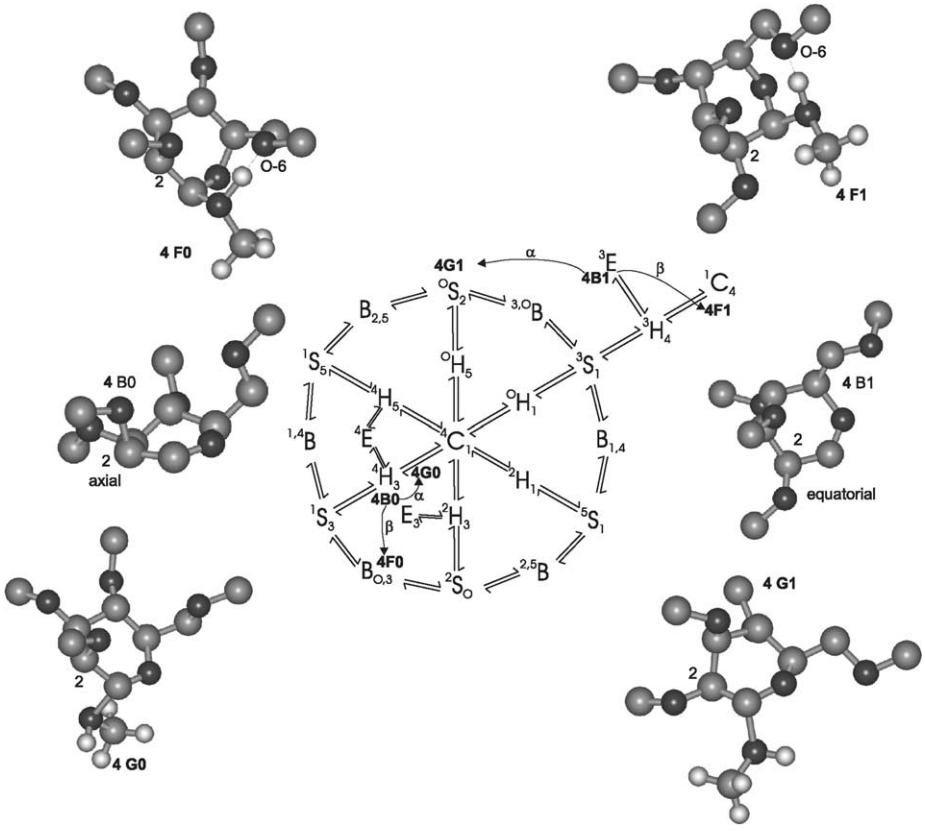


Figure 1. Ball and stick representation of **4** and **4** plus MeOH in its minimum conformations (grey = C, dark grey = O and light grey = H). Planar representation of the spherical representation of the conformations of six-membered rings showing the $^4C_1$ hemisphere. The arrows indicate the conformational change from α-attack and β-attack for **4**. The extension on the right shows a portion of the $^1C_4$ hemisphere. The C-2 atom is labelled in all structures for orientation and sugar hydrogens have been removed for clarity.

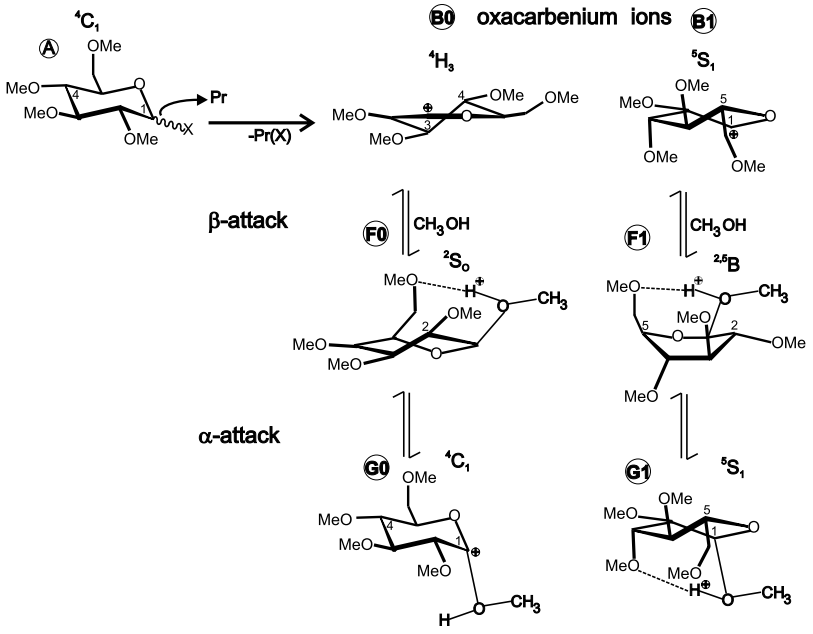
been found for reactions of diequatorially substituted cyclohexane derivatives.[11] Following extensive review of the glycosylation literature we believe that most people only consider one conformation of glycopyranosyl oxacarbenium ions. In contrast, our studies of **1** found two families of conformers, one in the $^{2}S_{O}$ skew boat and the other in the $B_{2,5}$ boat, ring conformations. We named the conformation closest to the starting chair conformation in conformational space **B0** and the second conformation **B1**. The barrier to interconversion for **1** was found to be 34 kJ mol^{-1} with a $^{O}S_{2}$ TS.[12] The $B_{2,5}$ ring conformation allowed for dioxolenium ion formation from the 2-*O*-acetyl group. This discrepancy from $^{4}H_{3}$ and $^{3}H_{4}$ conformations is almost undoubtedly related to the fused isopropylidene ring. Such rings are known to torsionally deactivate glycosylation reactions.[13] Increasing the flexibility by studying the 2,6-di-*O*-acetyl-3,4-di-*O*-methyl-D-galactopyranosyl cations (**2**) also found two families of conformers in the E_{3} and $^{4}H_{5}$ conformations. The latter allowing for dioxolenium ion formation. The discrepancy in this case could be the result of the difference between *gluco* and *galacto* configured sugars. More recent studies of the 2,3,4-tri-*O*-methyl-D-lyxopyranosyl cations (**3**) did indeed find the $^{4}H_{3}$ and $^{3}H_{4}$ ring conformations but surprisingly the inverted $^{3}H_{4}$ conformation was the most stable by 12.6 kJ mol^{-1} suggesting the importance of the C-2–O-2 conformation. Note that C-2–O-2 is pseudo-equatorial in the $^{3}H_{4}$ conformation of **3**. The electron donating and electron withdrawing characteristics of O-2 substituents are well known to influence the reactivity of both chemical and enzyme catalyzed glycosylation reactions.[14] The influence of C-2 substituents on ring conformations other than the possibilities of neighbouring group participation are much less studied, see for examples Ref. 15.

Based on the results for **1** to **3**, we developed the two conformer hypothesis of glycosyl oxacarbenium ions, which is that glycopyranosyl oxacarbenium ions can exist in at least two families of conformers. Furthermore, when assessing the reactivity of donors in glycosylation reactions that could go through such ions at least four cases need to be considered, which are α- or β-attack on each of the two families (see Scheme 1). Detailed studies may indicate the population of more than two conformations but for arguments sake we limit the discussion to two. We wanted to assess this hypothesis with a more fundamental test. Thus, we have now studied the 2,3,4,6-tetra-*O*-methyl-mannopyranosyl (**4**) and 2,3,4,6-tetra-*O*-methyl-glucopyranosyl (**5**) oxacarbenium ions. This pair only differ configurationally at C-2 and should form a basis for all other glycopyranosyl oxacarbenium ions (see Chart 1).

2. Results and discussion

2.1. Development of the model

We choose *O*-methyl substituents for two reasons. Firstly, the absence of hydroxyl groups prevents intramolecular hydrogen bonding. Conformations with intramolecular hydrogen bonding are found as the lowest energy ones by gas-phase calculations of neutral monosaccharides, and this factor may obscure other factors influencing reactivity.[16] Secondly, methyl is the smallest possible



Scheme 1. Activation of donor **A** leads to oxacarbenium ions **B0** and **B1**, which can be attacked by methanol from the α face or the β face leading to four possible cases, **F0**, **F1** and **G0** and **G1**. The example shown is for glucose derivative **5**. The **A**–**G** nomenclature is from Ref. 5, Pr = promoter.

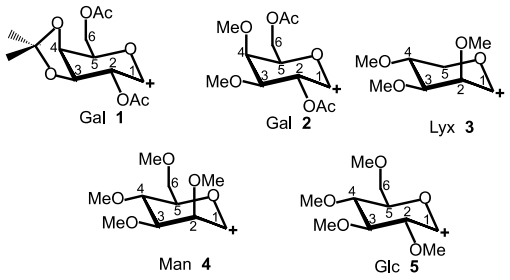


Chart 1. Structures of oxacarbenium ions **1**–**5**.

protecting group and begins to address the important issue of the effect of protecting groups on reactivity.

For simplicities sake the only nucleophile considered so far is methanol. Thus, all issues of mismatched TS's and the anticipated stereoelectronic effects of the protecting groups of the nucleophile are not considered but are expected to modify the trends observed in our calculations.[17] We have previously calculated the relative energetics of α- versus β-attack for **1**–**3** and some closely related species with methanol. In all cases ion–dipole complexes with varying degrees of hydronium ion character were found as minima implying a preassociation of nucleophile model ($D_N * A_N$) for glycosylation reactions.[18] Recent DFT dynamic calculations of the acid catalyzed glycosidic bond formation reaction find a $D_N * A_N$ mechanism even in water.[19] Our observation of a marked stereoselectivity for the α- versus β-attack on glycopyranosyl oxacarbenium ions is in sharp contrast to most of the mechanistic discussions in the literature that assume little or no stereoselectivity, for example Ref. 20. From our results two important factors determining stereoselective attack on glycopyranosyl oxacarbenium ions have been found.[21] One factor is intramolecular hydrogen bonding between the nucleophilic hydroxyl and the electronegative atoms of the oxacarbenium ions especially in cases where the species have appreciable hydronium ion character. The relative energetics of this effect are over estimated because intermolecular hydrogen bonding is not considered.[21] Secondly, the change in pyranosyl ring conformation from the isolated cation to the ion–dipole complex can be favourable or unfavourable. In all the isolated cations the C-5–O-5–C-1–C-2 (τ_5) torsion angle is nearly planar, as expected. For the D sugars considered so far this torsion angle increases for β-attack and decreases for α-attack. Depending on the starting conformation this change may be favourable or unfavourable. It was previously noted that some rotamers about C-5–C-6 (ω_H = H-5–C-5–C-6–O-6) allowed for hydrogen bonding between the nucleophile and O-6 or its substituents but otherwise the effect of ω_H conformation was not considered in detail. Based on these observations and the effect of the absence of C-6 in **3**, the influence of the ω_H conformation is considered in more detail in this work. For numbering see Chart 2.

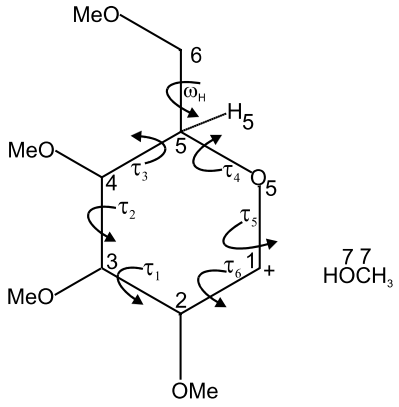


Chart 2. Planar representation showing atom numbering and dihedral angles.

In the study of **1** above, it was found that the pyranose ring conformational change was the dominant factor determining the barrier to dioxolenium ion formation between the ^2S_O and the $B_{2,5}$ conformations. In order to model these transitions we have developed a modification of Hendrickson's spherical representation of six-membered rings[22] that allows the conformations of six-membered rings to be described by three vectors, which we have named chair (*C*), boat (*B*) and skew boat (*S*).[23] With suitable normalization, linear combinations of these three vectors describe any six-membered ring conformation.[24] Throughout this work this description is used.

For this study we used DFT calculations as implemented in the Amsterdam Density Functional, ADF, program (see Computational methods). To start we optimized **4** and **5** in each of the two families of conformers. Input structures were either generated from idealized $^4H_3$ and $^3H_4$ ring conformations[24] or by appropriate in silica modifications of the **B0** and **B1** structures found for **2**. Both procedures led to the same minima. We assume that donors **A** ionize irreversibly to oxacarbenium ions and thus ignore ion-pairing or more implicitly assume that its effect is equivalent for all species considered. All our calculations do include a continuum solvent correction calibrated to dichloromethane as solvent. For each case all three staggered rotamers of C-5–C-6 were optimized; for numbering see Chart 2. The lowest energy conformers were then checked to be true minima by frequency calculations. Subsequently all minima were reoptimized within the continuum solvent model (see Table 1).

2.2. Isolated 4 and 5

For both the mannose (**4**) and glucose (**5**) configured glycopyranosyl oxacarbenium ions studied here two conformers that differ by ring inversion **B0** and **B1** were found. Figure 1 compare ball and stick representations of **B0** and **B1** for **4** and Figure 2 compare **B0** and **B1**

Table 1. Relative energies (kJ mol^{-1}) of **4** and **5** and their methanol complexes **F0**, **F1** and **G0**, **G1** with selected geometric parameters (optimized with solvation and corrected for ZPE)

Species	ω_H (°)	C-1–O-7 (Å)	∠O-5–C-1–O-7 (°)	∠O-5–C-1–O-7–C-7 (°)	Energy	–H-Bond
4(B0)	−178.7	—	—	—	0.0	
4(B1)	−50.8	—	—	—	1.9	
4(G0)	178.0	1.860	107.9	14.1	−21.2	
4(F0)	179.3	1.498	110.5	−68.2	−51.3	−23.3
4(G1)	−50.1	1.672	106.6	140.6	−30.9	
4(F1)	178.4	1.510	105.9	−176.8	−49.5	−21.5
5(B0)	−179.9	—	—	—	−4.5	
5(B1)	−168.1	—	—	—	0.3	
5(G0)	176.8	1.620	109.3	77.7	−47.9	
5(F0)	−175.0	1.518	110.6	−65.6	−60.7	−32.7
5(G1)	50.8	1.539	110.3	66.3	−59.8	−31.8
5(F1)	−86.1	1.519	108.3	−80.9	−47.5	−19.5

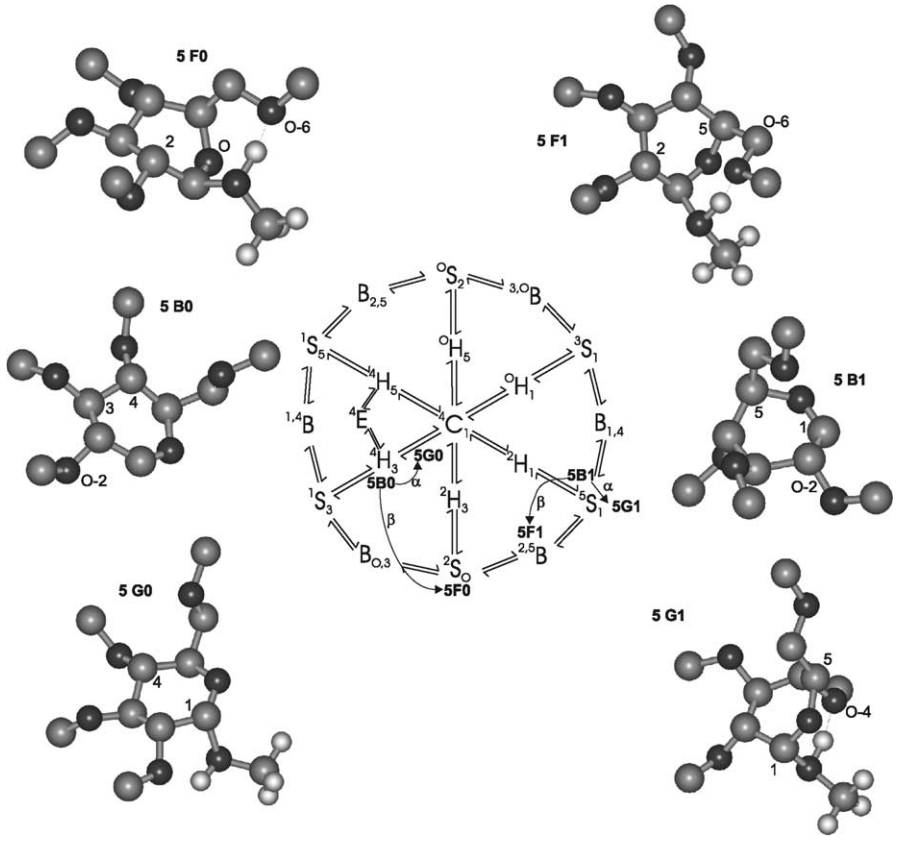


Figure 2. Ball and stick representation of **5** and **5** plus MeOH in its minimum conformations (grey = C, dark grey = O and light grey = H). Planar representation of the spherical representation of the conformations of six-membered rings showing the $^4C_1$ hemisphere. The arrows indicate the conformational change from α-attack and β-attack for **5**. The ring atoms that are used for the IUPAC descriptors are indicated for orientation. Sugar hydrogens have been removed for clarity.

for **5**. As discussed above we focus on the conformations of these species in particular the conformation of the pyranose rings. For both oxacarbenium ions the **B0**'s are found to be in the $^4H_3$ ring conformation long postulated to be a probable conformation for these ions. The *gluco* configured cation is an almost perfect half chair with a coefficient of 1.013 $^4H_3$ derived from 0.507 $^4C_1$ and 0.509 $^3S_1$ whereas the *manno* cation (0.566 $^4C_1$,) has a smaller contribution from the skew boat 0.397 $^3S_1$ resulting in a distorted 0.793 $^4H_3$ conformation (see Table 2). The **B1** conformations are ^3E for **4** (ΔE 1.9 kJ mol^{-1}) and $^5S_1$ for **5** (ΔE 4.7 kJ mol^{-1}) (see Table 2). The ^3E conformation is near in configurational space to $^3H_4$ but $^5S_1$ is relatively far (see Figs. 1 and 2). Examination of Figure 2 shows that this conformation allows O-2 to be pseudo-equatorial while O-3, O-4, and C-6 are pseudo-axial.

As expected τ_5 is almost planar in all isolated oxacarbenium ions (see Table 2). The exocyclic C-5–C-6 conformations follow the trends for neutral pyranosides where the

Table 2. Ring conformations of **4** and **5** and their methanol complexes **F0**, **F1** and **G0**, **G1**

Species	τ_1 (°)	τ_2 (°)	τ_3 (°)	τ_4 (°)	τ_5 (°)	τ_6 (°)	Chair	Boat	Skew-boat	Half-chair/envelope
4(B0)	−42.9	57.9	−48.5	24.5	−10.3	19.8	$^4C_1$, 0.566	$B_{2,5}$ 0.043	$^1S_3$ 0.397	$^4H_3$ 0.793
4(B1)	54.8	−58.7	35.0	−6.2	3.7	−29.5	$^1C_4$ 0.522	$^{0,3}B$ 0.432	$^5S_1$ 0.048	^3E 0.864
4(G0)	−53.5	59.4	−47.8	32.5	−28.7	39.4	$^4C_1$ 0.726	$^{2,5}B$ 0.052	$^1S_3$ 0.252	—
4(F0)	−61.4	59.3	−7.3	−46.5	44.3	10.7	$^4C_1$ 0.133	$B_{0,3}$ 0.881	$^5S_1$ 0.031	—
4(G1)	66.1	−34.6	−23.2	56.9	−24.0	−37.7	$^1C_4$ 0.095	$^{1,4}B$ 0.010	$^OS_2$ 1.015	—
4(F1)	51.9	−45.1	41.0	−44.9	52.1	−56.6	$^1C_4$ 0.810	$^{0,3}B$ 0.000	$^1S_5$ 0.126	—
5(B0)	−44.2	61.1	−46.8	15.9	0.3	14.8	$^4C_1$ 0.507	$B_{2,5}$ 0.015	$^1S_3$ 0.509	$^4H_3$ 1.013
5(B1)	−17.6	−27.2	57.4	−42.2	−4.6	37.0	$^1C_4$ 0.188	$B_{0,3}$ 0.117	$^5S_1$ 0.779	—
5(G0)	−48.3	57.3	−54.9	46.2	−39.5	40.5	$^4C_1$ 0.796	$^{1,4}B$ 0.025	^2S_o 0.021	—
5(F0)	−63.4	44.2	11.5	−54.8	35.3	24.1	$^4C_1$ 0.083	$^{1,4}B$ 0.183	^2S_o 0.976	—
5(G1)	−21.9	−30.4	52.2	−17.3	−38.1	59.5	$^4C_1$ 0.052	$^{0,3}B$ 0.122	$^5S_1$ 0.925	—
5(F1)	−24.4	−12.9	54.7	−62.6	23.3	21.2	$^1C_4$ 0.299	$^{2,5}B$ 0.679	$^1S_3$ 0.088	—

Table 3. Effect of C-5–C-6 torsion angle on relative energies (calculated with solvent correction only)

Species	ω_H/° (H-5–C-5–C-6–O-6)	C-1–O-7/Å	Energy/kJ mol^{-1}
4(B0)	−178.73	—	0.0
	−43.86	—	3.8
	58.84	—	17.4
4(B1)	−50.81	—	0.0
	170.15	—	9.4
	57.06	—	8.1
4(G0)	178.89	2.184	0.0
	−43.18	2.167	1.6
	54.28	2.164	14.7
4(F0)	−178.86	1.537	0.0
	−57.65	1.615	23.2
	61.24	1.618	36.5
4(G1)	−49.19	1.969	0.0
	178.55	2.027	1.8
	63.39	1.995	11.1
4(F1)	178.79	1.553	0.0
	−54.71	1.675	3.5
	56.92	1.647	1.9
5(B0)	−179.86	—	0.0
	−43.51	—	5.9
	58.85	—	18.0
5(B1)	−168.09	—	0.0
	−55.39	—	3.3
	53.93	—	8.9
5(G0)	175.67	1.769	0.0
	−48.25	1.721	0.2
	54.55	1.722	13.8
5(F0)	−173.06	1.575	10.1
	−85.46	1.578	0.0
	59.38	2.067	62.4
5(G1)	51.42	1.575	0.0
	−53.86	1.605	1.4
	−175.49	1.615	8.2
5(F1)	−85.78	1.582	0.0
	49.59	2.034	58.0
	166.43	2.472	75.5

$\omega_H = -60°$ (*gt*) and 180° (*gg*) conformers are near in energy and the +60° (*tg*) conformer is less stable (see Table 3).[25] The only exception is the **4(B1)** 180° conformation, which is destabilized, perhaps sterically. It should be noted that a rotation about C-5–C-6 is predicted for interconversion between **B0** and **B1** for **4** but not for **5**. The small **B0** versus **B1** energy differences, especially for **4**, mean that different protecting groups, solvents, counterions, etc. could well stabilize **B1** over **B0**.

In all cases examined so far the preferred CH–OCH_3 rotamers have a lone pair *anti* to the sugar methine and the methyl carbon *syn* to the methine (Figs. 1 and 2). This conformational preference has been observed before in calculated structures of permethylated disaccharides.[26] This *syn* preference has been found for 2,6-disubstituted-1-methoxycyclohexanes and was ascribed to steric effects.[27] Calculated conformers with C-2–O-2(CH_3) *anti* were found to be minima but are typically 10 kJ mol^{-1} less stable then the *syn* conformers. The origin of this effect is currently under investigation and the results will be reported elsewhere.

2.3. Static 4 and 5 plus methanol

In order to assess the possible origins of stereoselectivity in glycosylation reactions the inherent preference of the α and β faces was investigated using the model nucleophile methanol. We well recognize that other factors should contribute to stereoselectivity but it is our goal to determine the inherent selectivities under idealized S_N1-like conditions. We anticipate that this knowledge could be useful for the development of stereoselective glycosylation methods. A similar optimization process to that for isolated **4** and **5** was taken for the methanol ion–dipole complexes with one additional consideration. Each initial minima was subjected to a constrained; optimization procedure (Linear Transit, LT) where the O-7–C-1 (O-7 from MeOH) bond length was varied in approximately 0.1 Å increments forwards and backwards, allowing all other degrees of freedom to minimize. Once a minimum O-7–C-1 bond length was found the other two staggered ω_H conformers were optimized. That these overall conformations were indeed minima was then checked by frequency calculations. Reoptimization with-

in the continuum solvent model was then done as a preliminary for more detailed LT studies, see below. This procedure in no way ensures that the conformational space is completely searched but it does encompass a number of the plausible conformers.

Ball and Stick representations of the resulting conformations are shown schematically in Figure 1 for **4** + MeOH and in Figure 2 for **5** + MeOH. The relative energies, ω_H angles and the C- 1–O-7 bond lengths are shown in Table 1. Table 2 gives the ring dihedrals τ_1(C-1–C-2–C-3–C-4), τ_2(C-2–C-3–C-4–C-5), to τ_6(O–C-1–C-2–C-3) and the conformational descriptors in terms of the chair, boat and skew-boat canonical vector representations of six-membered rings. Table 3 gives the values for the ω_H search. All complexes have considerable hydronium ion character.

As above, we focus on the conformations of the six-membered rings. Surprisingly for **4** the β-face complexes (**F0** and **F1**) are calculated to be more stable than those for α-face complexes (**G0** and **G1**). Most of this extra stabilization comes from hydrogen bonding to O-6 even though this forces the ring into boat $B_{0,3}$ (**F0**) or inverted chair $^1C_4$ (**F1**) conformations, respectively. For **4(F0)** O-7–O-6 = 2.464 Å, O-7–OH-7 = 1.120 Å and ∠O-7–OH-7–O-6 = 167.8°. For **4(F1)** O-7–O-6 = 2.456 Å, O-7–OH-7 = 1.108 Å and ∠O-7–OH-7–O-6 = 166.9°. The second consequence of hydrogen bonding is a shorter O-7–C-1 bond length. Previously, we had estimated the energetic contribution of hydrogen bonding to be 28 kJ mol^{-1} by calculating for a deoxy analogue in the same conformation as the parent oxygen containing hydrogen bond accepting compound.[21] Table 1 also shows relative energy values corrected for neglect of intramolecular hydrogen bonding. These 'corrected' values show the expected trend of α more favourable than β for mannopyranosyl donors. That this correction value is reasonable is suggested by the relative energies at different ω_H values for **4(F0)** where the 180° conformer allows hydrogen bonding but the others do not (ΔE = 23.2 and 36.5 kJ mol^{-1}). Note that for **4(F1)** all conformers allow for hydrogen bonding. In both these cases the C-1–O-7 bond lengths are comparable. For **5(F1)** the C-1–O-7 bond length is much shorter at the hydrogen-bonded minimum, which results in a large effect for ω_H variation.

For **5** a similar picture emerges with β-face complexes (**F0** and **F1**) more stable than α-face complexes (**G0** and **G1**) but in this case the differences are smaller. In fact these differences are small enough that many combinations of different protecting groups, specific solvation or ion-pairing could change the order. Also, once corrected for neglect of hydrogen bonding the energetics show a clear preference for α-glycosides. For **5(F0)** O-7–O-6 = 2.474 Å, O-7–OH-7 = 1.090 Å and ∠O-7–OH-7–O-6 = 162.7°. For **5(F1)** O-7–O-6 = 2.497 Å, O-7–OH-7 = 1.102 Å and ∠O-7–OH-7–O-6 = 167.9°.

In accordance for the results with **1** to **3** the changes in ring conformation with the changes in τ_5 is a dominant factor. Figures 1 and 2 show projections of the spherical representation of six-membered ring conformation and the relative positioning of these changes can be discerned. For both **B0**'s in the $^4H_3$ conformation α-attack lowers this angle and is favourable since this pushes the conformation back towards a chair whereas β-attack increases this value and pushes the ring towards unfavourable conformations $B_{0,3}$ for **4** and ^2S_O for **5**. For **4(B1)** β-attack pushes the ring towards the inverted $^1C_4$ chair whereas α-attack leads to a $^OS_2$ skew boat conformation. For **5(B1)** β-attack pushes the ring towards the unfavourable $^{2,5}B$ boat whereas α-attack maintains the $^5S_1$ skew boat conformation that allows for a hydrogen bond to O-4. For **5(G1)** O-7–O-4 = 2.457 Å, O-7–OH-7 = 1.106 Å and ∠O-7–OH-7–O-4 = 163.1°.

It is known that in some cases large protecting groups at O-6 favour the formation of α products perhaps by disfavouring hydrogen bonding.[28] Hammett studies of *p*-substituted benzoyl esters at O-6 of *gluco* configured donors support a role for hydrogen bonding to O-6 as a determinant of β products.[29] Other, as yet unidentified factors could also play roles.

2.4. C-1–O-7 bond length

For each of these eight minima the C-1–O-7 bond length was varied forwards and backwards from the minima in small increments as above, that is, LTs. The preliminary gas-phase LTs with relatively large increments had indicated methodological problems. Notably, oscillations in energy and the inability to dissociate to the expected free **B0** or **B1** conformations at long C-1–O-7 bond lengths. These problems were addressed in two ways first by including the continuum solvent effect in the constrained optimizations (not as a correction factor as above) and by using very small increments in the constraint. Both of these procedures increase computational resources and not all cases led to satisfactory results. Representative plots of relative energy versus C-1–O-7 bond length are shown in Figure 3a–c for **4** and **5**.

Also shown on the plots are the leading conformational descriptors for selected points. The same two methodological problems are apparent in these plots. Some plots show small discontinuities even with small step sizes. We interpret this methodological short coming to a need to couple the nucleophilic attack, C-1–O-7 bond length, to the pyranose ring conformation by using a more complex constraint in the calculations.[30] Secondly, the complexes with hydrogen bonds in the minima did not always dissociate the hydrogen bond even at C-1–O-7 bond lengths >2 Å. In these cases the pyranose ring conformations at 3 Å separation are not necessarily those of the isolated cation. This creates a timing dilemma on the importance of such hydrogen

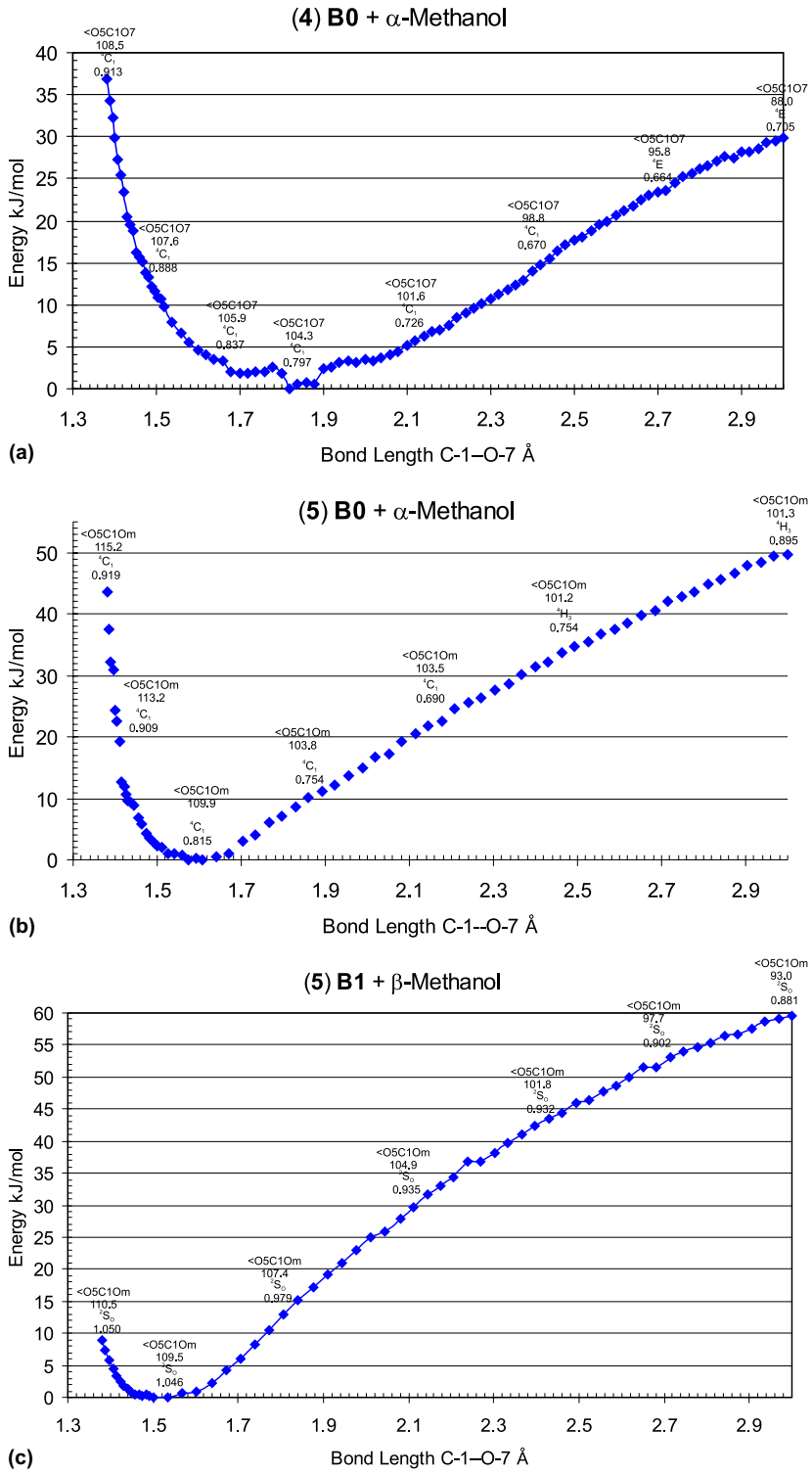


Figure 3. Plots of C-1–O-7 bond length (O-7 from MeOH) versus energy with the minimum set to 0.0 kJ mol^{-1}. At selected points the leading conformational descriptors and the ∠O-5–C-1–O-7 bond angle are shown, (a) **4** (**B0**)+βMeOH, (b) **5** (**B0**)+βMeOH, (c) **5** (**B1**)+αMeOH. The minima are **4** (**G0**), **5** (**G0**) and **5** (**F1**), respectively. All calculations done with a continuum dielectric parameterized to CH_2Cl_2 solution.

bonds and the relative timing of hydrogen bond formation and ring conformational change. Case 1, which our calculations model, has the nucleophile selecting a ring conformation favourable for hydrogen bonding. However, if the oxacarbenium ions do indeed exist for sufficient time to equilibrate before nucleophilic attack then they should exist in their optimum (**B0**, **B1**, etc.) conformations and a case 2, not modelled by our calculations,

should occur where ring conformational changes follow after nucleophile association. A more sophisticated computational methodology is necessary to distinguish these possibilities.

For **4(G0)** (Fig. 3a) the minimum is very shallow with a region from C-1–O-7 between 1.6 and 2.1 Å lying within 5 kJ mol^{-1} of the minimum. The conformation at C-1–O-7 of 3.0 Å is the ^4E and not the $^4H_3$ conformation expected. However the energy is also 20–35 kJ mol^{-1} less than all other cases studies at this separation. The O-5–C-1–O-7 bond angle changes from close to 90° to 108.5° see below for discussion. The data for **5** is more clear than for **4** in Figure 3b the **5(G0)** goes forward from the minimum to a true chair conformation and the proton transfers to O-2. Whereas on lengthening C-1–O-7 the ring conformation transforms as expected to a $^4H_3$ conformation. In Figure 3c for the **5(F1)** case the hydrogen bonding dilemma prevents the ring from changing from the ^2S_O conformation. As well, the hydroxylic proton transfers to O-6 at short C-1–O-7 bond lengths.

In spite of these methodological shortcomings several important features can be identified. One, the minimum about the intermediate is very shallow in all cases. Examination of the C-1–O-7 bond lengths in Table 3 versus Table 1 shows that in all cases the C-1–O-7 bond length shortens by up to 0.3 Å for α-attack on **4** (**4(G0)**) when solvation is included in the optimization. Consequently, the minima are best described as longer than a normal *exo*-glycosidic bond length of about 1.42 Å and shorter than about 2 Å with appreciable hydronium ion character. Two, on going to shorter C-1–O-7 bond lengths from the intermediates stable structures can be formed by proton transfer. In cases with hydrogen bonding at the shortest C-1–O-7 bond lengths the hydroxylic proton does indeed transfer to the oxygen that was the hydrogen acceptor. In chemical glycosylation reactions there is often no added base or only very sterically hindered bases such as 2,6-di-*tert*-butylpyridines or molecular sieves in the reaction mixture, in these cases such transient proton transfers may actually occur. The timing of proton transfer in chemical glycosylations is not known. From our studies it seems unlikely that proton transfer would occur before the hydroxylic proton of the complex assumes sufficient hydronium character so that it will easily transfer. Three, plotting the ∠C-7–O-7–C-1 as a function of the C-1–O-7 bond length reveals that at long distances this angle approaches 90° whereas at short distances it is close to the tetrahedral angle of 109°. In classical organic chemistry terms this angle change is consistent with a transition from an initial π-complex to a σ-complex, mirroring the sp^2 to sp^3 hybridization change. The changeover is at close to 2 Å and in some cases a TS could be found by optimization and frequency calculation at near this separation. A similar TS has been found by semi-empirical calculations for glycoside hydrolysis, C-1–O-7 = 1.89 Å.[31] However, the smooth nature of the curves in Figure 3a–c suggests that this is not the overall TS for the reaction. A second TS should also be associated with the proton transfer step at short distances but in our still too simple model this could not be separated from nucleophilic attack and ring conformational changes. Four, the pyranose ring conformation changes relatively slowly with C-1–O-7 bond length and does not show any abrupt changes. In other words these two motions appear to be only loosely coupled and reiterates the need for a more sophisticated computational model.

3. Conclusions

Our hypothesis is that for any reaction that proceeds through oxacarbenium ion intermediates at least two conformers of glycopyranosyl oxacarbenium ions need to be considered. For glycosylation reactions that proceed through oxacarbenium ion intermediates this means that at least four-cases need to be considered to evaluate the inherent diastereoselectivity of such reactions, that is, α- or β-attack on these two conformers. The easiest to calculate are the four ion–dipole complexes of the nucleophile and the oxacarbenium ions since these are relatively easily found minima on the PE surface. The corresponding TS's that lead to glycosides are much more difficult to find since these involve at least three separate motions to occur. The C-1–O$_{nuc}$ where O$_{nuc}$ is O-7 in our examples must shorten, the pyranose ring must revert to a chair concomitant with sp^2 to sp^3 rehybridization at C-1 and the hydroxylic proton must be transferred.

Our calculations are only aimed at identifying important factors for glycosylation and not the much bigger task of modelling the stereoselectivity of glycosylation. Perhaps fortuitously, the results for **1** to **3** were that the lowest energy ion–dipole complex corresponded to the experimentally observed α- or β-selectivity. For 2,3,4,6-tetra-*O*-methyl-mannopyranosyl donors reacting with excess methanol near 50:50 α- and β-glycosidic mixtures have been reported. For 2,3,4,6-tetra-*O*-methyl-glucopyranosyl donors reacting with excess methanol under similar conditions β-glycosides predominate, typically 93:7.[6] Our calculations for **5** agree with these results but those for **4** do not unless H-bonding is neglected. However, if H-bonding is neglected for **5** than α-glycosides are predicted. Clearly a method for determining the exact effect of H-bonding is required. It should be noted that the experimental results refer to conditions of excess methanol, which probably affects H-bonding.

Our results do show the thermodynamic feasibility of oxacarbenium ions accessing two or more families of

conformations and that at least four cases need to be considered when considering α/β-selectivities. In a non-sugar example, it has been suggested that depending on the reaction conditions a carbocation is formed in one of two possible ring conformations leading to different stereochemical outcomes of the reaction.[32] This observation is analogous to our **B0 B1** hypothesis.

Although suggested for many years[33] it is only in the last few years that experimental evidence for 'distorted' conformations for the TS of glycosidases have been shown.[34] Attempts to rationalize the specificity of certain glycosidase inhibitors and their conformational similarity to $^4H_3$ conformations have been made[35] although the role of shape versus charge of inhibitors is controversial.[36] Recently it has been shown that one enzyme appears to be pick a low population conformer of an inhibitor from among the family of accessible conformers lending support for the hypothesis that there is a need for particular conformations for inhibition.[37] In order to test the relevance of 'distorted' conformations one synthetic group has prepared glycosides with fixed $^{2,5}B$ conformations and this does not inhibit hydrolysis, which it would if a markedly different conformation was necessary for hydrolysis.[38] Thus, our considerations may have important implications for the design of glycosyltransferase and glycosidase TS inhibitors.[39] Although, a full discussion is outside the scope of this article it seems highly possible that some enzymes may stabilize **B0** and others **B1** conformations in their TSs.[40] For example, this may be one explanation for the specificity of inhibitors, that is, a TS state inhibitor that mimics a **B0** conformation is not likely to inhibit an enzyme that stabilizes **B1** conformers and vice versa even though the two enzymes have similar substrates.[41] A recent example provides another twist where the inhibitor 1-deoxymannojirimycin is found in a $^1C_4$ conformation when bound to a class I α-(1→2)-mannosidase[42] but in a $^4C_1$ conformation when bound to a class II α-(1→2)-mannosidase.[43] These results suggest that class I enzymes use **B1** and class II enzymes use **B0** conformers.

How does our two conformer hypothesis assist the development of stereoselective glycosylation reactions? Experimentally β-mannopyranosides and α-glucopyranosides are the most difficult to obtain and hence methods for their formation the most desirable. Our results suggest two plausible strategies. A first one is to use conformationally constrained glycopyranosyl donors that can only access one oxacarbenium conformation not two or more. The best studied example of this strategy is 4,6-*O*-alkyl(aryl)idene protected gluco- or mannopyranosyl donors. Experimentally 4,6-*O*-alkyl(aryl)idene-mannopyranosyl donors give preferentially, sometimes even exclusively β-glycosides, whereas 4,6-*O*-alkyl(aryl) idene-glucopyranosyl donors give mainly α-glycosides.[44] Other similarly protected donors of different configurations are known to be selective in some cases.[45] We have previously shown that distortions of the τ_5 torsion angle from its isolated oxacarbenium ion values to new angles in the ion–dipole complexes adequately models this observed selectivity.[21] The second strategy that follow for **4** and analogues is that in order to obtain β-glycosides H-bonding to O-6 should be assisted by increasing the electron density at O-6 and minimizing the steric hindrance about both the nucleophile and O-6, that is, use small electron donating protecting groups at O-6. For **5** and analogues in order to obtain α-glycosides large electron withdrawing protecting groups should be used at O-6. Thus, we have developed a prototype of a method to assess the diastereoselectivty of a particular glycosyl donor before a glycosylation reaction.

4. Computational methods

The DFT calculations were carried out with the Amsterdam Density Functional (ADF) program system, ADF2000.[46] The atomic orbitals were described as an uncontracted double-ζ Slater function basis set with a single-ζ polarization function on all atoms, which were taken from the ADF library. The 1s electrons on carbon and oxygen were assigned to the core and treated by the frozen core approximation. A set of s, p, d, f and g Slater functions centred on all nuclei were used to fit the electron density, and to evaluate the Coulomb and exchange potentials accurately in each SCF cycle. The local part of the V_{xc} potential (LDA) was described using the VWN parameterization,[47] in combination with the gradient corrected (CGA) Becke's functional[48] for the exchange and Perdew's function for correlation (BP86).[49] The CGA approach was applied self-consistently in geometry optimizations. Second derivatives were evaluated numerically by a two point formula. The solvation parameters were dielectric constant $\varepsilon = 9.03$, ball radius = 2.4 Å, with atomic radii of C = 1.7, O = 1.4 and H = 1.2 Å.

Acknowledgements

The authors gratefully acknowledge the use of the VPP770 Fujitsu parallel computer situated in the RIKEN Computer Center. This work was partly supported by the iHPC multiscale modelling initiative of the NRC. This is NRC paper #42496.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2004.12.021.

References

1. (a) Whitfield, D. M.; Douglas, S. P. *Glycoconjugate J.* **1996**, *13*, 5; (b) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155; (c) Ogawa, T. *Chem. Soc. Rev.* **1994**, 397; (d) Demchenko, A. V. *Curr. Org. Chem.* **2003**, *7*, 35.
2. (a) Banait, N. S.; Jencks, W. P. *J. Am. Chem. Soc.* **1991**, *113*, 7951; (b) Bennet, A. J. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1207; (c) Chiappe, C.; Lo Moro, G.; Munforte, P. *Tetrahedron* **1997**, *53*, 10471.
3. (a) Wallace, J. E.; Schroeder, L. R. *J. Chem. Soc., Perkin Trans. 2* **1977**, 795; (b) Paulsen, H.; Herold, C. P. *Chem. Ber.* **1970**, *103*, 2450; (c) Crich, D.; Dai, Z.; Gastaldo, S. J. *Org. Chem.* **1999**, *64*, 5224.
4. Schleyer, P. V. R.; Maerker, C. *Pure Appl. Chem.* **1995**, *67*, 755.
5. Nukada, T.; Bérces, A.; Zgierski, M. Z.; Whitfield, D. M. *J. Am. Chem. Soc.* **1998**, *120*, 13291.
6. Rhind-Tutt, A. J.; Vemon, C. A. *J. Chem. Soc. Part 2* **1960**, 4637.
7. Schramm, V. L.; Shi, W. *Curr. Opin. Struct. Biol.* **2002**, *11*, 657.
8. Bowden, T.; Garegg, P. J.; Maloisel, J. L.; Konradsson, P. *Isr. J. Chem.* **2000**, *40*, 271.
9. Lemieux, R. U. *Chem. Canada* **1964**, *16*, 14.
10. Andrews, C. W.; Fraser-Reid, B.; Bowen, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 8293.
11. Tichý, M.; Pánková, M. *Coll. Czech. Chem. Commun.* **1975**, *40*, 647.
12. Bérces, A.; Nukada, T.; Whitfield, D. M. *J. Am. Chem. Soc.* **2001**, *123*, 5460.
13. (a) Fraser-Reid, B.; Wu, Z.; Andrews, C. W.; Skowronski, E.; Bowen, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 1434; (b) Dean, K. E. S.; Kirby, A. J.; Komariv, I. V. *J. Chem. Soc., Perkin Trans. 2* **2002**, 337.
14. (a) Fraser-Reid, B.; Merritt, J. R.; Handlon, A. L.; Andrews, C. W. *Pure Appl. Chem.* **1993**, *65*, 779; (b) Zhang, Z.; Ollman, I. R.; Ye, X.-S.; Wishnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734; (c) Douglas, N. L.; Ley, S. V.; Lucking, U.; Warriner, S. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51.
15. (a) Jones, O. K.; Liotta, D. C. *Tetrahedron Lett.* **1993**, *34*, 7209; (b) Durham, T. B.; Roush, W. R. *Org. Lett.* **2003**, *5*, 1871; (c) Chong, P. Y.; Roush, W. R. *Org. Lett.* **2002**, *4*, 4523; (d) Bravo, F.; Viso, A.; Alcázar, E.; Molas, P.; Bo, C.; Castillón, S. *J. Org. Chem.* **2003**, *68*, 686.
16. (a) Barrows, S. E.; Dulles, F. J.; Cramer, C. J.; French, A. D.; Truhlar, D. G. *Carbohydr. Res.* **1995**, *276*, 219; (b) Whitfield, D. M. *J. Mol. Struct. (Theochem)* **1997**, *395*, 53; (c) Polavarapu, P. L.; Ewig, C. S. *J. Comput. Chem.* **1992**, *13*, 1255; (d) Jeffrey, G. A. *J. Mol. Struct.* **1990**, *237*, 75.
17. (a) Spijker, N. M.; Basten, J. E. M.; van Boeckel, C. A. A. *Reel. Trav. Chim. Pays-Bas* **1993**, *112*, 611; (b) Spijker, N. M.; van Boeckel, C. A. K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 180; (c) Green, L. G.; Ley, S. V. In *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, 2000; Vol. 1, 427pp.
18. Mayr, E. L.; Minegishi, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 4493.
19. Stubbs, J. M.; Marx, D. *J. Am. Chem. Soc.* **2003**, *125*, 10960.
20. Chiba, H.; Funasaka, S.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 1629.
21. Nukada, T.; Bérces, A.; Whitfield, D. M. *Carbohydr. Res.* **2002**, *337*, 765.
22. Hendrickson, J. B. *J. Am. Chem. Soc.* **1967**, *89*, 7047.
23. Stoddart, J. F. *Stereochemistry of Carbohydrates*; Wiley-Interscience: Toronto, 1971.
24. Nukada, T.; Bérces, A.; Whitfield, D. M. *Tetrahedron* **2001**, *57*, 477.
25. (a) Wladkowski, B. D.; Chenoweth, S. A.; Jones, K. E.; Brown, J. W. *J. Phys. Chem. A* **1998**, *102*, 5086; (b) Tvaroška, I.; Taravel, F. R.; Utille, J. P.; Carver, J. P. *Carbohydr. Res.* **2002**, *337*, 353.
26. Mendonca, A.; Johnson, G. P.; French, A. D.; Laine, R. A. *J. Phys. Chem. A* **2002**, *106*, 4115.
27. Anderson, J. E.; Ijeh, A. I. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1965.
28. (a) Maroušek, V.; Lucas, T. J.; Wheat, P. E.; Schuerch, C. *Carbohydr. Res.* **1978**, *60*, 85; (b) Houdier, S.; Vottero, P. J. A. *Carbohydr. Res.* **1992**, *232*, 349.
29. Fréchet, J. M.; Schuerch, C. *J. Am. Chem. Soc.* **1972**, *94*, 604.
30. Bérces, A.; Whitfield, D. M.; Nukada, T.; Santos, Z. I.; Obuchowska, A.; Krepinsky, J. J. *Can. J. Chem.* **2004**, *82*, 1157.
31. Blériot, Y.; Genre-Grandpierre, A.; Imberty, A.; Tellier, C. *J. Carbohydr. Chem.* **1996**, *15*, 985.
32. Doan, L.; Yagi, H.; Jerina, D. M.; Whalen, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 14382.
33. Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed.* **1999**, *38*, 751; Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515.
34. (a) $^{2,5}B$ Glc: Guérin, D. M. A.; Lascombe, M.-B.; Costabel, M.; Souchon, H.; Lamzin, V.; Béguin, P.; Alzari, P. M. *J. Mol. Biol.* **2002**, *316*, 1061; (b) $^{2,5}B$ Xyl: Sidhu, G.; Withers, S.; Nguyen, N. T.; Mclntosh, L. P.; Ziser, L.; Brayer, G. D. *Biochemistry* **1999**, *38*, 5346; (c) 'sofa' GlcNAc probably $^{4}H_{3}$: Strynadka, N. C. J.; James, M. N. G. *J. Mol. Biol.* **1991**, *220*, 401; (d) distorted Glc:Sulzenbacher, G.; Driguez, H.; Henrissat, B.; Schülein, M.; Davies, G. J. *Biochemistry* **1996**, *35*, 15280; (e) $^{1}S_{3}$ Glc: Davies, G. D.; Mackenzie, L.; Varrot, A.; Dauter, M.; Brzozowski, A. M.; Schülein, M.; Withers, S. G. *Biochemistry* **1998**, *37*, 11707; (f) $^{2,5}B$ Xyl/Glc: Notenboom, V.; Williams, S. J.; Hoos, R.; Withers, S. G.; Rose, D. R. *Biochemistry* **2000**, *39*, 11553; (g) $^{4}H_{3}$ or $^{2,5}B$ Glc: Williams, S. J.; Hoos, R.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 2223.
35. Winkler, D. A. *J. Med. Chem.* **1996**, *39*, 4332.
36. (a) Ganem, B.; Papandreou, G. *J. Am. Chem. Soc.* **1991**, *113*, 8984; (b) Hansen, S. U.; Bols, M. *J. Chem. Soc., Perkin Trans. 2* **2000**, 665; (c) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340.
37. Johnson, M. A.; Jensen, M. T.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2003**, *125*, 5663.
38. (a) Blériot, Y.; Vadivel, S. K.; Greig, I. R.; Kirby, A. J.; Sinaÿ, P. *Tetrahedron Lett.* **2003**, *44*, 6687; (b) Blériot, Y.; Vadivel, S. K.; Herrera, A. J.; Greig, I. R.; Kirby, A. J.; Sinaÿ, P. *Tetrahedron* **2004**, *60*, 6813.
39. (a) Lorthiois, E.; Meyyappan, M.; Vasella, A. *J. Chem. Soc., Chem. Commun.* **2000**, 1829; (b) Remen, L.; Vasella, A. *Helv. Chim. Acta* **2002**, *85*, 1118; (c) Bülow, H. S.; Plesner, I. W.; Bols, M. *J. Am. Chem. Soc.* **2000**, *122*, 8567.
40. Davies, G. J.; Ducros, V. M.-A.; Varrot, A.; Zechel, D. L. *Biochem. Soc. Trans.* **2003**, *31*, 523.
41. A possible example: Søhoel, H.; Liang, X.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1584.
42. Vallée, F.; Karaveg, K.; Herscovics, A.; Moremen, K. W.; Howell, P. L. *J. Biol. Chem.* **2000**, *275*, 41287.

43. Shah, N.; Kuntz, D. A.; Rose, D. R. *Biochemistry* **2003**, *42*, 13812.
44. (a) Abdel-Rahman, A. A.-H.; Jonke, S.; El Ashry, E. S. H.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 2972; (b) Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64*, 4926; (c) Crich, D.; Li, H. *J. Org. Chem.* **2000**, *65*, 801; (d) Weingart, R.; Schmidt, R. R. *Tetrahedron Lett.* **2000**, *41*, 8753; (e) Yun, M.; Shin, Y.; Chun, K. H.; Nam Shin, I. E. *Bull. Korean Chem. Soc.* **2000**, *21*, 562; (f) Crich, D.; Dudkin, V. *Org. Lett.* **2000**, *2*, 3941; (g) Ito, Y.; Ohnishi, Y.; Ogawa, T.; Nakahara, Y. *Synlett* **1998**, 1102.
45. (a) Tamura, S.; Abe, H.; Matsuda, A.; Shuto, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1021; (b) Yule, J. E.; Wong, T. C.; Gandhi, S. S.; Qiu, D.; Riopel, M. A.; Koganty, R. R. *Tetrahedron Lett.* **1995**, *36*, 6839; (c) Toshima, K.; Nozaki, Y.; Tatsuta, K. *Tetrahedron Lett.* **1991**, *32*, 6887.
46. (a) Baerends, E. J.; Ellis, D. E.; Ros, P. *Chem. Phys.* **1973**, *2*, 41; (b) te Velde, G.; Baerends, E. J. *J. Comput. Phys.* **1992**, *99*, 84; (c) Fonseca Guerra, C.; Snijders, J. G.; te Velde, G.; Baerends, E. J. *Theor. Chim. Acta* **1998**, *99*, 391; (d) Versiuis, L.; Ziegler, T. *J. Chem. Phys.* **1988**, *88*, 322; (e) Fan, L.; Ziegler, T. *J. Chem. Phys.* **1992**, *96*, 9005.
47. Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200.
48. Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.
49. Perdew, J. P. *Phys. Rev. B* **1986**, *34*, 7506.