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Conformational Flexibility of C-Glycosides: Experimental Evidence of the Existence of a Gauche-Gauche Conformation Around the Glycosidic Linkage for a Lactose Analogue

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Abstract: The solution conformation of C-glycosides 3 and 4, presenting a hydroxyl group at the bridging position, has been determined by NMR and MM3* calculations. The conformation of 3 is similar to that of C-lactose, while that of 4 is rather different and shows a significant contribution of a conformer with a gauche disposition between C1'-O5' and C1'-C2' bonds.

The search for new glycosidase inhibitors has led to a group of disaccharide analogues with the glycosidic oxygen substituted by carbon.[1,2] A deeper understanding of the interaction of carbohydrates with proteins requires a detailed information on the conformational preferences of both species. Thus, the comparison of the conformational behaviour of C-glycosides with the naturally occurring O-glycosides is a topic of interest. We have recently demonstrated[3] that, in contrast with common belief,[2] conformational studies on C-disaccharides may not be directly applicable to O-glycosides. In particular, C-lactose (**1**) and its O-analogue, methyl α-lactoside (**2**) display different conformations around the aglyconic bond. Glycosidic torsion angles are defined as Φ H1'-C1'-Cα-C4 and Ψ C1'-Cα-C4-H4. In **1**, the *anti* conformation[4] (minimum A, Ψ *ca.* 180°) is more populated[3] than the *syn* one(s) (minima B/C, Ψ *ca.* 0°), which is(are) the *usual* conformation(s)[5] for **2** and for β(1→4) disaccharides.[6] In addition, **1** exhibits[3] much higher flexibility than **2**. In order to verify whether these findings could be extended to other C-glycosides, as well as to obtain information about which factors determine the relative orientation of the glycosidic linkages, we now report on the conformational analysis of C-glycosides **3** and **4** (R and S diasteromers, Fig. 1). The presence of the hydroxyl groups may alter differently the steric and electronic interactions within the molecule, and, in addition, since C-glycosides are thought to affect the activity of glycosidases *via* competitive inhibition, these derivatives may display different biological activities.

1: X=Y=H
3: X=H, Y=OH
4: X=OH, Y=H

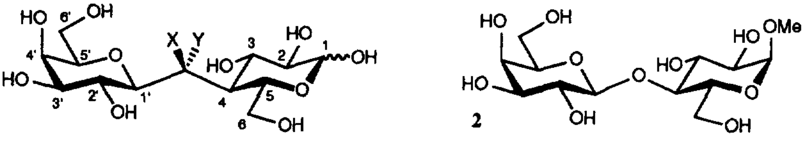
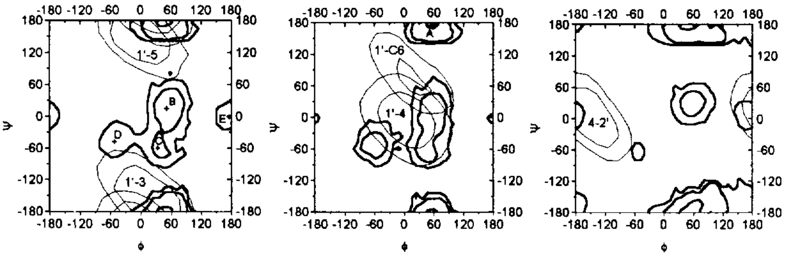


Fig. 1

Twenty-four relaxed steric energy maps, and from these, the corresponding adiabatic surfaces were calculated by MM3*[7] for **3** and **4**, using the methodology described in detail for **1**.[8,3] Probability maps are shown in Fig. 2, along with that calculated for **1**. The torsion angle values of the predicted minima for both compounds in comparison with those of **1** are shown in Table 1, which also gathers the relative populations of the different low energy regions.

Table 1. Torsion angle values (Φ,Ψ) of the predicted minima and relative MM3* populations of the different low energy regions of compounds **1**, **3**, and **4**.

	Compound	Conformer (Φ,Ψ)				
		A (36/180)	B (54/18)	C (54/-72)	D (-54/-54)	E (180/0)
Pop (%)	**1**	54.0	31.6	9.3	3.3	1.7
	3	36.5	29.6	13.8	19.8	0.3
	4	82.6	15.0	0.0	0.8	1.6



1 **3** **4**

Fig. 2. Population distribution maps (contours are given at 10%, 1%, and 0.1% of population) and short relevant interresidue proton-proton distances (contours are given at 2.5 and 3.0 Å) for compounds **1**, **3**, and **4**.

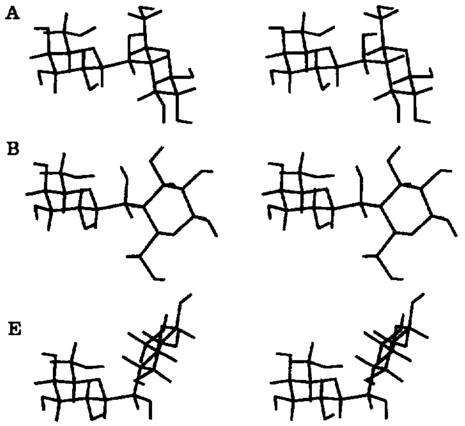


Fig. 3. Stereoviews of the A (anti) and B (syn) low energy minima of compound **3** and minimum E (gauche-gauche) of compound **4**. The corresponding A and B minima of **4** are similar to those presented for **3**. Minimum E of compound **3** is not detected.

The MM3*-based probability distribution of **3** is fairly similar to that of **1**. Nevertheless, in order to get reliable conformational information, the validity of the calculations has to be tested using experimental measurements of $^3J_{H,H}$ and NOEs. Fig. 2 shows the representation of the relevant interresidue proton-proton distances within the available conformational space. The NOEs that unequivocally characterize these regions have been dubbed *exclusive NOEs*.[4] The obtained data for **3** indicate that the NOE ratio between those exclusive of the *anti* conformation (H1'-H3 and H1'-H5) and those exclusive of the *syn* one (H1'-H4) increases in comparison with that of **1**. Besides, the *syn* exclusive H1'-H6 NOE that was present in **1** is no longer observed in **3**. These results would indicate a slight increment of the population of the *anti* region of **3** in comparison to that of **1**. In addition, since the H4-H2' NOE is not detected, the presence of minimum E can be neglected (5% for **1**). Table 2 shows the NOE values obtained from a relaxation matrix approach[9] in comparison with the experimental data. The agreement is satisfactory, and since the observed H1'-H3, H1'-H5 and H1'-Hα NOE values are larger than those expected, it seems that the population of the *anti* conformer is higher than that predicted by MM3* (>37%). Additional information can be obtained through the analysis of the observed $^3J_{HH}$ (Table 3) in comparison with the values calculated from the distribution using Altona's equation.[10] The $^3J_{H1'-H\alpha}$ value of 0.7 Hz indicates that the presence of D is smaller than that predicted by MM3* since this conformer has these protons in an *anti* disposition. In addition a $^3J_{H4-H\alpha}$ value of 2.2 Hz (Table 3) indicate that of the two *syn* conformations (B or C) is the region around minimum C the most populated, what is in agreement with the absence of the H1'-H6 NOE (Table 2). In conclusion, all the measured data indicate a *ca.* 60:40 equilibrium among minima A:B+C, in contrast with the natural O-glycoside, **2**, which only shows a 10% population of the *anti* conformer.

Table 2. Experimental and Calculated Steady State NOEs Intensities for **3** and **4** at 30° C in D_2O at 500 MHz.

Proton	Intensity (%)										
	Compound 3 (R)						Compound 4 (S)				
		Calculated						Calculated			
Pair	Exp.	Distrib.	Min. A	Min. B	Min. C	Min. E	Exp.	Distrib.	Min. A	Min. B	Min. E
H1'/H3	8.3	6.5	12.7	-	-	-	2.2	10.7	12.9	-	-
H1'/H4	2.4	3.0	-	5.3	4.2	-	2.6	0.9	-	3.4	-
H1'/H5	2.4	0.5	1.8	-	-	-	-	1.0	1.0	-	-
H1'/H6s	-	1.1	-	5.5	-	-	2.2	1.1	-	6.6	-
H1'/H3'+H5'	11.5	13.7	13.5	13.8	13.5	14.0	13.0	13.3	13.0	13.6	14.0
H1'/Hα	6.3	4.4	7.6	4.6	3.3	5.3	4.3	1.3	1.1	1.9	7.5
H4/H2	12.1	11.5	11.7	11.5	10.3	11.1	12.6	11.9	12.3	11.7	10.9
H4/H2'	-	1.0	-	-	-	17.6	5.5	0.1	-	-	16.3

In all cases τ_c = 0.17 ns and external relaxation of 0.1 s^{-1}. The experimental NOEs are obtained by adding the contribution of both anomers.

With regard to **4**, the calculations predict an increase of the *anti* population (83 %), while minima C, D, and E basically disappear. Therefore, a strong H1'-H3 NOE should be expected. However, in this case, the experimental data show that MM3* does not reproduce the solution conformational behaviour of **4**: The H1'-H3 NOE is weak (*ca.* 2.2%). In contrast, the H4-H2' NOE value is *ca.* 5.5% (minimum E). On the other hand, a value of 2.6 and 2.2% for the H1'-H4 and H1'-H6 NOEs, respectively, also confirms the presence of the *syn* conformation. The disagreement between the theoretical and the experimental data is also evident from the relaxation matrix approach data presented in Table 2. Moreover, a $J_{H1'-H\alpha}$ value of 6.9 Hz (Table 3) can not be explained if only minima *syn* and *anti* are present, since these two conformers display a pure *trans* relationship for H1'/Hα protons. Thus, this coupling value and the strong exclusive H4-H2' NOE unequivocally demonstrate that region E of **4** is also populated. The presence of the other exclusive H1'-H3 (*anti*) and H1'-H4 (*syn*) NOEs confirm that there are three regions populated in water. Since the ratio between H1'-H3/H1'-H4 NOEs is rather small, it seems that the *anti* conformation is more destabilized in **4** than in **3** and **1**. All the experimental data may be explained assuming a *ca* 10:65:25 population distribution among regions A:B:E.

Table 3. Vicinal Coupling Constants ($^3J_{HH}$) across the C4-Cα-C1' Bridge of Compounds **3** and **4** (500 MHz).

	Compound 3 (R)							Compound 4 (S)				
		Calculated							Calculated			
$^3J_{HH}$	Exp.	Distrib.	Min. A	Min. B	Min. C	Min. D	Min. E	Exp.	Distrib.	Min. A	Min. B	Min. E
H1'/Hα	0.7	2.2	0.3	0.4	0.3	8.3	4.2	6.9	7.6	8.5	8.5	1.6
H4/Hα	2.2	3.3	2.5	7.0	2.3	1.1	3.9	1.5	2.5	2.5	0.8	2.9

In conclusion, according to our results, **3** presents an important percentage of the *anti* conformer, as shown for C-lactose and for other N- and S-disaccharides.[11] In addition, **3** presents flexibility around the aglyconic bond. In contrast, 4 exhibits flexibility not only around the aglyconic bond, but also around the glycosidic linkage. To the best of our knowledge, this is the first experimental evidence for an important contribution of a *gauche-gauche* conformation (min. E shows the Cα-C4 linkage *gauche* with respect to both C1'-O5' and C1'-C2') for a free disaccharide analogue. Nevertheless, the presence of this arrangement had been detected for conformationally restricted 3,2'-O-isopropylidene acetals of lactose and cellobiose.[12] The presence of this conformation in 4 could be due to a rotation around the glycosidic bond to avoid the 1,3-*syn* Oα/O2' interaction which is present for $\Phi \approx 60°$ (conformers *syn* and *anti*).

The lack of agreement between the MM3* calculations and the experimental NMR results are probabily due to the role ot the electrostatic interactions, which are overemphasized in force fields calculations performed in vacuo.[5a] Although the use of a bulk dielectric constant (ε= 80) indeed decreases the role of the coulombic term and that of the intra-molecular hydrogen bonding, conformer A of 4 still presents the posibility of the existence of a HO2'-Oα hydrogen bond with the corresponding lowering of its energy. Therefore, although MM3* may indeed produce excellent agreement between theoretical and NMR results (for instance, compounds 1 and 3) care should be taken when performing molecular mechanics calculations for sugars without explicit water molecules. Nevertheless, MM3* still produces good low energy minimum geometries which may serve to interpret the experimental results as shown here for 4.

The present results, as well as those previously obtained for C-lactose[3], indicate that β-linked C-glycosides are fairly flexible compounds and that subtle variations of their structural and/or electronic characteristics may heavily affect their conformational behaviour which we have shown to be different to that of the natural parent compounds. The conformational changes observed within this series also reflect the small energy barriers between the different energy regions and, therefore, conformations different of the major one existing in solution may be bound by the binding sites of lectins, antibodies or enzymes. Moreover, distinct conformations of different similar compounds may well be recognized by an unique protein[13].

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