ELSEVIER

Carbohydrate Research 337 (2002) 1851–1859

CARBOHYDRATE RESEARCH

www.elsevier.com/locate/carres

A DFT/ab initio study of hydrogen bonding and conformational preference in model cellobiose analogs using B3LYP/6-311++G**☆

Gina L. Strati, Julious L. Willett, Frank A. Momany*

Plant Polymer Research, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, 1815 N. University St., Peoria, IL 61604, USA

Received 3 May 2002; accepted 30 July 2002

Abstract

A series of β-cellobiose analogs were studied at the B3LYP/6-311++G** level of theory to isolate and understand how the various electronic components of the β-(1→4)-linked disaccharide, cellobiose, contribute to the energetic stability of the molecule in vacuo. Previous studies on β-cellobiose (see accompanying paper) showed that the most energetically stable conformation was that in which the dihedral angle phi (ϕ_H) was 'flipped' by ~180° relative to the 'normal' form. From our examination of eight sets of structures in which different combinations of functional hydroxyl and hydroxymethyl groups were removed, it was determined that only β-cellobiose and one other analog (analog 7, β-xylobioside), an analog in which both hydroxymethyl groups were removed but the exocyclic hydroxyl groups retained, can form a 'cooperative' hydrogen-bonding network. Only in these two molecules did we find continuous synergistic 'communication' through hydrogen bonding from one sugar moiety to the other. This 'cooperative' hydrogen bonding energetically stabilizes the 'flipped' conformation of β-cellobiose and β-xylobioside, while the other analogs studied were unable to form a 'cooperative' grouping of hydrogen bonds and thus were more stable in their 'normal' conformational state.

Keywords: Density functional; ab initio; β-Cellobiose; Analogs; B3LYP/6-311++G**

1. Introduction

Disaccharides are important molecules in the fields of biochemistry and plant structural science. They have not been well-characterized through the years by high level electronic methods because of their complexity: disaccharides contain many weak inter- and intramolecular interactions. However, these are important molecules, and it is imperative to more fully understand the electronic energetics, conformational preferences, and overall structures of these compounds. When considering the solid-state or solution environment in which carbohydrates exist, hydrogen bonding immediately becomes an important element to investigate. In order to understand the role these weak interactions play, we have examined the vacuum conformational preferences of β-cellobiose in detail using very high-level density functional/ab initio methods[1] and a series of specifically designed analogs based on cellobiose.

In the first paper of this series,[1] 27 β-cellobiose conformers were studied at a high level of theory (B3LYP/6-311++G**). These results confirmed that in vacuo the most energetically stable form of cellobiose is a structure in which the conformational angle ϕ_H was close to 180°. These results also suggested that previous calculations on cellobiose[2–12] did not completely explore, at a significant level of theory, the complex interactions in the cellobiose molecule. French and co-workers[2,3] performed Hartree–Fock level ab initio calculations on a cellobiose analog that was completely stripped of functional groups, i.e., no exocyclic hydroxyl groups and no hydroxymethyl side chains, and

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

* Corresponding author. Fax: +1-309-681-6362

E-mail address: momanyfa@ncaur.usda.gov (F.A. Momany).

0008-6215/02/$ - see front matter
PII: S0008-6215(02)00269-0

suggested that a 'normal' form of the molecule was most energetically stable. MM3 interactions were then included to simulate the hydrogen bonding and added to the ab initio energies from the stripped-down analog.[2,3] This method led those authors to conclude that cellobiose retains the 'normal' conformational preference in vacuo.[2,3] Calculations reported here on the identical analog, but at a higher level of theory including density functionals, i.e., B3LYP/6-311++G** instead of HF/6-31G*, support their findings for that particular analog. Nonetheless, when full DFT/ab initio methods were applied to many different cellobiose conformations, the results were very different with the 'normal' conformations being several kcal/mol higher in energy than the 'flipped' forms. So, the question to be answered must be: why is the lowest energy form of β-cellobiose calculated in vacuo at our high level of theory one that is 'flipped' in conformational angle ϕ_H?

This paper attempts to answer this question by DFT/ab initio study of eight sets of cellobiose analogs. The analogs were carefully constructed to explore specific inter- and intraresidue hydrogen bonding interactions, interactions that the authors felt were crucial to the energetic stability of β-cellobiose. The results clearly show that one cannot ignore the conformational stability arising from the functional groups on cellobiose and its analogs; in particular, one cannot ignore the 'cooperative' hydrogen-bonding effects available to specific 'flipped' conformations and which are not available in any of the 'normal' forms.

Results include an analysis of the geometry effects arising from various hydrogen-bonding donor–acceptor combinations, as well as the energetic differences arising from sets of ring and side-chain hydroxyl groups oriented in different directions. Clearly, the analogs selected do not cover all possible combinations of the exocyclic and hydroxymethyl groups, but can be considered a representative set for the specific interactions under study. The results obtained with these analogs confirm our previously stated reason, i.e., cooperative hydrogen bonding, as the cause of the energetic stabilization of the 'flipped' forms.

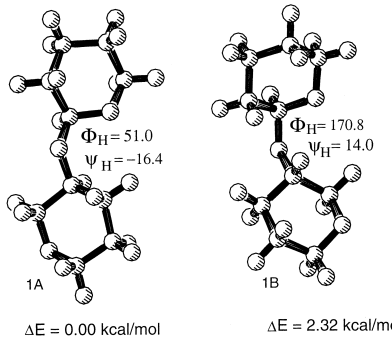

Fig. 1. Cellobiose analogs completely stripped of side groups, numbered **1A** and **2A** for the 'normal' and 'flipped' conformations.

2. Methodology

The eight analogs described here were constructed by selectively removing specific functional groups from β-cellobiose using MSI Insight software on a Silicon Graphics (SGI) computer. The structures were then geometry optimized with MSI Discover[13] software using an in-house force field, AMB01C.[14] The eight sets of analogs chosen for this study contain at least one structure with (ϕ_H, Ψ_H) ~ (40°, −20°) (labeled A in all figures) and one in which the conformational angles are (ϕ_H, Ψ_H) ~ (180°, 0°) (labeled B in all figures). In structure set **7**, four conformers were studied and in structure set **8**, three conformers were studied, to explore the importance of the orientation of the exocyclic hydroxyl groups of the reducing ring, either clockwise (c′) or anticlockwise (r′). Conformers 'flipped' in ϕ_H were not included in this study due to the high relative energy of these conformations.[1] The series was designed only to explore specific interactions, particularly those involving intraresidue communication via hydrogen bonding.

All analogs were gradient optimized using density functionals and the triple valence basis set, with two diffuse and two polarization functions, denoted B3LYP/6-311++G** on structures first optimized at the split valence B3LYP/6-31+G* level as discussed previously.[1] It should be noted that upon optimization in this manner, the conformer from set **8B** with exocyclic groups in the c′ orientation optimized to an identical structure as that with r′ orientation.

Completely stripped down versions of the cellobiose analog, i.e., structures **1A** and **1B** of Fig. 1, devoid of exocyclic hydroxyl groups and hydroxymethyl side chains were also examined using a numerical Hessian (NUMHESS) algorithm available from Parallel Quantum Solutions (PQS)[15]). For all ab initio calculations, PQS software on the PQS-600, PQS-800 and PQS-1000 machines was used. Structures were considered completely optimized when the gradient between optimization cycles was less than 0.0001 and the energy change between cycles was less than 0.000001.

3. Results and discussion

Entropy, enthalpy, zero point vibrational energy, and free energy.—We will describe the entropy, enthalpy, and resulting Gibbs free energy in terms of the different analogs studied. These energy terms are obtained from the calculation of the harmonic vibrational frequencies. Comparisons between different analogs are not possible as they are structurally different, but one can compare free energy values between conformations of the same analog.

In general, the 'flipped' or B forms have smaller entropy (S) values than the 'normal' A forms due to

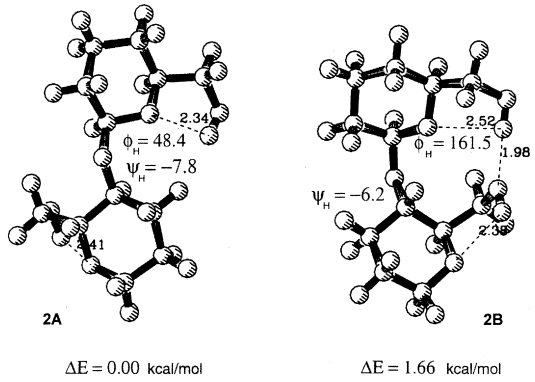

Fig. 2. Cellobiose analogs with only a hydroxymethyl side chain present, numbered **2A** and **2B**.

stabilization through cross-ring interactions only available in the 'flipped' forms. This difference is large in structure **2A** and **2B** where the 'flipped' form can engage in intraresidue communication via hydrogen bonding of the hydroxymethyl side chains, O-6···O-6′ (see Fig. 2). The A structure does not have any means with which to form intraresidue hydrogen bonds, and so the molecule has more flexibility and a larger *S* value, 129.32 cal/mol-K instead of 124.21 cal/mol-K. This 5.11 cal/mol-K difference reflects a free energy difference of $\sim$1.5 kcal/mol at 300 K, which is a significant stabilization resulting from the intraresidue interactions. In other structures, this intraresidue communication is not as crucial and the *S* values differ only by a few tenths of a calorie per mol-K, for example in structures **3A** and **3B** the *S* value is only 0.10 cal/mol-K larger in A.

Structures **1A** *and* **1B***: completely stripped, no hydroxyl or hydroxymethyl groups.*—The energy differences and dihedral angles between the **1A** and **1B** conformations are presented in Table 1 and Fig. 1. The 'normal' form is $\sim$2.3 kcal/mol more favorable in ZPVE corrected electronic energy than the 'flipped' conformation. The free energy is $\sim$2.5 kcal/mol more favorable, indicating a slight entropic preference for the 'normal' form. This result is in agreement with previous studies carried out at the HF/6-31G* level of theory.[2,3]

Structures **2A** *and* **2B***: analogs with one CH_2OH group on each sugar ring.*—Structures **2A** and **2B** focus our attention on the interactions of the hydroxymethyl side chains present in cellobiose. Energies and dihedral angles for these structures are summarized in Table 1 and Fig. 2. The A structure is 1.66 kcal/mol more stable than **2B** by ZPVE corrected electronic energy and 2.87 kcal/mol more stable in relative free energy. The entropy value for the **2B** structure is about 5 cal/mol-K less than the **2A** structure. In structures of type **2B**, where $(\phi_H, \Psi_H) \sim (180°, 0°)$, the hydroxymethyl side chains are brought into close proximity where they can interact. These interactions can and do affect certain bond lengths within the structures as evidenced by the results in Table 2 and Table 3.

In structure **2A**, there is hydrogen bonding between O-5–HO-6 as well between O-5′–HO-6′, with H–O distances of 2.34 and 2.41 Å, respectively (see Fig. 2). A corresponding hydrogen-bonding pattern is present in the **2B** structure, but O-5–HO-6 is 0.18 Å longer in the nonreducing ring and O-5′–HO-6′ is only slightly shorter in the reducing ring at a distance of 2.38 Å. Structure **2B** has additional intraresidue hydrogen bonding via HO-6–O-6′ at a length of 1.98 Å. The strong hydrogen bond between the residues does increase the C-6′–O-6′ bond length in structure **2B** by 0.004 Å over **2A** and correspondingly C-6–O-6 is 0.009 Å shorter in **2B**. The C-5–C-6 and O-6–HO-6 bonds are 0.007 and 0.008 Å longer in **2B** than **2A**, but the corresponding bond lengths in the reducing ring do not change significantly.

Structures **3A** *and* **3B***: analogs with –OH groups at the 2,3′-positions.*—Structures **3A** and **3B** explore the stabilizing effect of an exocyclic hydroxyl group placed at positions 2 and 3′ (see Fig. 3). These functional groups were placed at crucial positions to provide intraresidue hydrogen bonding. The hydroxyl groups in these analogs did not electronically stabilize structure **3B** as much as did the hydroxyl groups on the hydroxymethyl in analog **2B**. Structure **3B** is 3.60 kcal/mol higher in ZPVE corrected electronic energy than **3A** and 4.02 kcal/mol higher in free energy even though the entropy term is only 1 cal/mol-K less.

There is very little opportunity for hydrogen bonding in analog **3**. In structure **3A**, there is a hydrogen bond between the exocyclic hydroxyl group at the 2-position with the bridging oxygen (O-2–O-1 $\sim$ 2.47 Å) and a very strong intraresidue hydrogen bond, 1.89 Å, between O-5 and HO-3′. Structure **3B** only has one intraresidue hydrogen bond, HO-2–O-3′ $\sim$ 2.00 Å.

The strong hydrogen bond in **3A** between O-5–HO-3′ seems to shorten the C-3′–O-3′ bond while lengthening the O-3′–HO-3′ bond. The C-3′–O-3′ bond is 0.021 Å shorter in **3A** relative to **3B**, and the O-3′–HO-3′ bond is 0.008 Å longer than in structure **3B**. This is a result of the HO-1–O-3′ hydrogen bond in **3B**, which although a fairly short hydrogen bond, it is not sufficient to bring the energy of **3B** down to that of **3A**. This suggests that the O-5–HO-3′ interaction (i.e., the ether ring oxygen to hydroxyl hydrogen) is more favored energetically than the HO-1–O-3′ interaction (i.e., hydroxyl to hydroxyl). One must still contend with the electrostatic repulsion between the two ether oxygen atoms (O-5 and O-5′) at the shorter distances in all the B conformers relative to the A forms.

Structures **4A** *and* **4B***: analogs with a $-CH_2OH$ group on each sugar ring and –OH groups at the 2,3′-positions.*—This particular structure contains functional groups that are oriented for intraresidue hydrogen

Table 1
Energies (kcal/mol), entropy (cal/mol-K), and dihedral angles (°) of cellobiose analogs

	1A	**1B**	**2A**	**2B**	**3A**	**3B**
Φ_H	51.0	170.84	48.4	161.5	38.9	177.9
Ψ_H	−16.4	13.97	−7.8	−6.2	−23.4	9.4
E	−387635.54	−387633.10	−531423.21	−531422.14	−482071.74	−482068.09
ZPVE	174.82	174.698	226.31	226.9	188.78	188.72
E_{corr}	−387460.73	−387458.40	−531196.90	−531195.24	−481882.96	−481879.37
ΔE	0	2.32	0	1.66	0	3.60
H	182.95	182.814	237.02	237.30	197.69	197.59
S	109.70	109.187	129.32	124.21	114.54	113.00
G	−387485.28	−387482.82	−531224.73	−531221.86	−481908.19	−481904.17
ΔG	0	2.46	0	2.87	0	4.02

	4A	**4B**	**5A**	**5B**	**6A**	**6B**
Φ_H	48.8	167.6	38.6	178.1	39.2	177.8
Ψ_H	−8.9	−1.8	−27.0	5.6	−24.2	8.6
E	−625858.47	−625855.58	−576504.41	−576502.04	−576506.29	−576503.76
ZPVE	232.29	232.62	194.54	137.48	194.73	195.14
E_{corr}	−625626.18	−625622.96	−576309.87	−576307.56	−576311.56	−576308.62
ΔE	0	3.21	0	2.31	0	2.93
H	244.16	244.32	204.78	204.76	205.16	205.29
S	136.37	132.61	124.24	123.14	124.60	122.43
G	−625654.94	−625650.78	−576336.66	−576333.98	−576338.26	−576334.96
ΔG	0	4.17	0	2.68	0	3.30

	7A (c′)	**7A** (r′)	**7B** (r′)	**7B** (c′)	**8A** (c′)	**8A** (r′)
Φ_H	51.4	51.3	170.5	169.8	39.4	28.9
Ψ_H	−18.9	−18.4	13.8	15.8	−27.0	−67.5
E	−576501.20	−576504.79	−576502.84	−576498.52	−670938.73	−670936.35
ZPVE	194.59	194.56	194.21	194.61	200.35	200.12
E_{corr}	−576306.61	−576310.22	−576308.64	−576303.91	−670738.38	−670736.24
ΔE	3.62	0	1.58	6.31	1.45	3.60
H	204.94	204.96	204.64	204.93	212.05	211.91
S	125.76	126.00	126.56	125.75	134.45	134.93
G	−576333.73	−576337.37	−576335.92	−576331.07	−670766.75	−670764.65
ΔG	3.64	0	1.45	6.30	1.00	3.11

	8B (r′)
Φ_H	178.3
Ψ_H	4.5
E	−670940.54
ZPVE	200.71
E_{corr}	−670739.84
ΔE	0
H	212.27
S	132.48
G	−670767.75
ΔG	0

Table 2
Selected bond lengths of cellobiose analogs in angstroms (Å)

	2A	**2B**	**3A**	**3B**	**4A**	**4B**
C-2–O-2			1.421	1.415	1.422	1.416
O-2–HO-2			0.964	0.970	0.964	0.968
C-3–O-3						
O-3–HO-3						
C-4–O-4						
O-4–HO-4						
C-5–C-6	1.522	1.529			1.522	1.529
C-6–O-6	1.420	1.411			1.418	1.412
O-6–HO-6	0.964	0.972			0.965	0.971
C-1′–O-1′						
O-1′–HO-1′						
C-2′–O-2′						
O-2′–HO-2′						
C-3′–O-3′			1.417	1.438	1.422	1.436
O-3′–HO-3′			0.971	0.963	0.970	0.963
C-5′–C-6′	1.524	1.524			1.526	1.523
C-6′–O-6′	1.423	1.427			1.421	1.425
O-6′–HO-6′	0.965	0.964			0.965	0.964

	5A	**5B**	**6A**	**6B**	**7A** (c′)	**7A** (r′)
C-2–O-2	1.424	1.417	1.420	1.414		
O-2–HO-2	0.963	0.971	0.964	0.970		
C-3–O-3	1.420	1.422			1.432	1.432
O-3–HO-3	0.965	0.966			0.962	0.962
C-4–O-4			1.426	1.423	1.420	1.420
O-4–HO-4			0.962	0.962	0.964	0.964
C-5–C-6						
C-6–O-6						
O-6–HO-6						
C-1′–O-1′			1.393	1.395	1.398	1.398
O-1′–HO-1′			0.965	0.964	0.965	0.964
C-2′–O-2′	1.421	1.433			1.432	1.422
O-2′–HO-2′	0.965	0.962			0.962	0.964
C-3′–O-3′	1.421	1.431	1.417	1.437		
O-3′–HO-3′	0.972	0.965	0.971	0.963		
C-5′–C-6′						
C-6′–O-6′						
O-6′–HO-6′						

	7B(r′)	**7B** (c′)	**8A** (c′)	**8A** (r′)	**8B** (r′)
C-2–O-2			1.422	1.426	1.416
O-2–HO-2			0.964	0.963	0.971
C-3–O-3	1.433	1.433	1.423	1.426	1.424
O-3–HO-3	0.962	0.962	0.964	0.964	0.965
C-4–O-4	1.420	1.419	1.418	1.421	1.421
O-4–HO-4	0.964	0.964	0.965	0.964	0.964
C-5–C-6					
C-6–O-6					
O-6–HO-6					
C-1′–O-1′	1.399	1.399	1.396	1.396	1.396
O-1′–HO-1′	0.964	0.965	0.966	0.964	0.964
C-2′–O-2′	1.422	1.433	1.423	1.424	1.424
O-2′–HO-2′	0.964	0.962	0.965	0.964	0.964
C-3′–O-3′			1.419	1.425	1.431
O-3′–HO-3′			0.972	0.965	0.966
C-5′–C-6′					
C-6′–O-6′					
O-6′–HO-6′					

bonding in both structures **4A** and **4B** (see Fig. 4). Structure **4B** does engage in strong hydrogen bonding between HO-6–O-6′ (1.98 Å), HO-6–O-5 (2.54 Å) and HO-2–O-3′ (2.09 Å), whereas in **4A** there is weak hydrogen bonding at HO-6–O-3′ (2.66 Å), HO-2–O-1 (2.54 Å), HO-3′–O-1 (2.53 Å), HO-6–O-5 (2.53 Å), a strong hydrogen bond between HO-3′–O-5 (1.94 Å), and essentially no hydrogen bonding between HO-2–O-

Table 3
Inter and intraresidue hydrogen bond distances of cellobiose analogs (Å)

	2A	**2B**	**3A**	**3B**	**4A**	**4B**
HO-2–O-1			2.47		2.54	
O-2–O-3						
HO-2–O-3′				2.00		2.09
HO-2–O-6′					4.56	
O-3–O-4						
O-5–HO-6	2.34	2.52			2.53	2.54
O-5–HO-3′			1.89		1.94	
HO-6–O-3′					2.66	
HO-6–O-6′		1.98				1.98
O-1′–HO-2′						
HO-1′–O-5′						
O-2′–HO-3′						
HO-3′–O-1					2.53	
O-5′–HO-6′	2.41	2.38			2.41	2.43
	5A	**5B**	**6A**	**6B**	**7A** (c′)	**7A** (r′)
HO-2–O-1	2.54		2.50			
O-2–O-3	2.42	2.37				
HO-2–O-3′		1.96		2.00		
HO-2–O-6′						
O-3–O-4					2.44	2.44
O-5–HO-6						
O-5–HO-3′	1.88		1.89			
HO-6–O-3′						
HO-6–O-6′						
O-1′–HO-2′					2.34	2.48
HO-1′–O-5′				2.40		
O-2′–HO-3′	2.38	2.39				
HO-3′–O-1						
O-5′–HO-6′						
	7B (r′)	**7B** (c′)	**8A** (c′)	**8A** (r′)	**8B** (r′)	
HO-2–O-1			2.57	2.55		
O-2–O-3			2.48	2.48	2.42	
HO-2–O-3′					1.96	
HO-2–O-6′						
O-3–O-4	2.43	2.44	2.46	2.46	2.45	
O-5–HO-6						
O-5–HO-3′			1.88			
HO-6–O-3′						
HO-6–O-6′						
O-1′–HO-2′	2.47	2.34	2.34	2.53	2.53	
HO-1′–O-5′	2.46			2.51	2.48	
O-2′–HO-3′			2.42	2.43	2.38	
HO-3′–O-1						
O-5′–HO-6′						

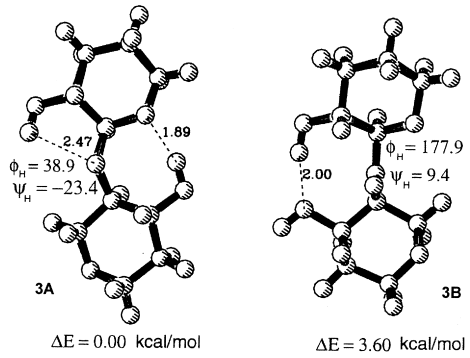

Fig. 3. Cellobiose analogs with only two exocyclic hydroxyl groups at the 2,3′ positions, numbered **3A** and **3B**.

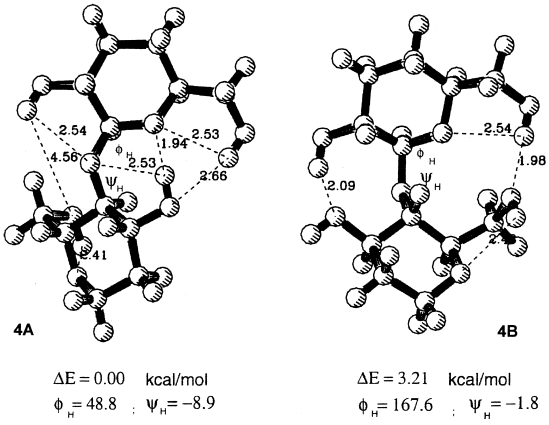

Fig. 4. Cellobiose analogs with one hydroxymethyl side-chain on each sugar ring and one exocyclic hydroxyl group at the 2- and 3′-positions, numbered **4A** and **4B**.

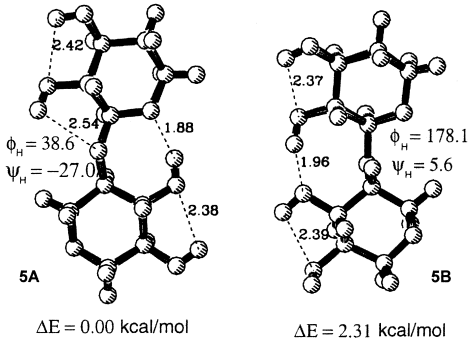

Fig. 5. Cellobiose analogs with only four exocyclic hydroxyl groups at the 2,3- and 2′,3′-positions, numbered **5A** and **5B**.

6′ (4.56 Å). Despite the intraresidue hydrogen bonding in **4B**, this structure is still 3.21 kcal/mol higher in ZPVE corrected electronic energy than **4A** and 4.17 kcal/mol higher in free energy. The entropy term in **4B** is about 3.75 cal/mol-K smaller than **4A**, which contributes to the free energy difference.

Structure **4B** has fewer hydrogen bonding opportunities than structure **4A** (see Table 3). The hydrogen bond HO-3′–O-5, which is 1.94 Å, long appears responsible for the 0.014 Å shortening of the C-3′–O-3′ bond relative to **4B**, and is also responsible for the slight shortening of the O-3′–HO-3′ bond in **4B**.

Structures **5A** *and* **5B***: analogs with –OH groups at 2,3 and 2′,3′.*—Structures **5A** and **5B** provide the opportunity for consecutive hydrogen bonding, i.e., a polarization of electron clouds, through the hydrogen bonding interactions of HO-3–O-2, HO-2–O-3′ and O-2′–HO-3′ (see Fig. 5). In structure **5A**, the O-2–HO-3 hydrogen bond is slightly longer than in **5B**, 2.42 instead of 2.37 Å, and there is a weak hydrogen bond between HO-2 and the bridging oxygen, O-1 (2.54 Å). The hydrogen bond HO-3–O-2 is slightly stronger in B, and HO-2 can intraresidue hydrogen bond with O-3′ at a hydrogen bond distance of 1.96 Å. The hydrogen bonding flow continues in **5B** with a hydrogen bond distance for O-2′–HO-3′ of 2.39 Å. This extensive hydrogen-bonding communication in **5B** is insufficient to overcome the preference for the normal form of this conformer, but the difference in ZPVE corrected electronic energy, 2.31 kcal/mol for **5B**, is not very large indicating the start of a cooperative effect in **5B**. Structure **5A** is apparently stabilized due to the intraresidue hydrogen bond, O-3′–O-5 not available in **5B**. It is a relatively short hydrogen bond at 1.88 Å.

Structures **6A** *and* **6B***: analogs with –OH groups at 2,4–1′,3′.*—This set of structures explores the placement of exocyclic hydroxyl groups in a discontinuous arrangement, i.e., the hydroxyl groups on a sugar ring cannot hydrogen bond to each other but in **6B** type structures intraresidue hydrogen bonding may be possible (see Fig. 6). This set of structures compares directly to the previously described set **5A** and **5B** in the sense that both contain the same number of exocyclic hydroxyl groups, but in set **6** the groups are noncontiguous. The placement of these exocyclic hydroxyl groups can influence the energetic stability of these cellobiose analogs. In analog set **6**, the 'normal' form or type **6A** structure is 2.93 kcal/mol lower in energy than the **6B** structure. However, in analog set **5A** and **5B** the energy

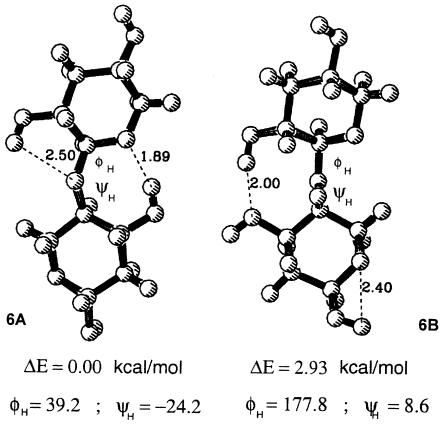

Fig. 6. Cellobiose analogs with only four exocyclic hydroxyl groups at the 2,4- and 1′,3′-positions, numbered **6A** and **6B**.

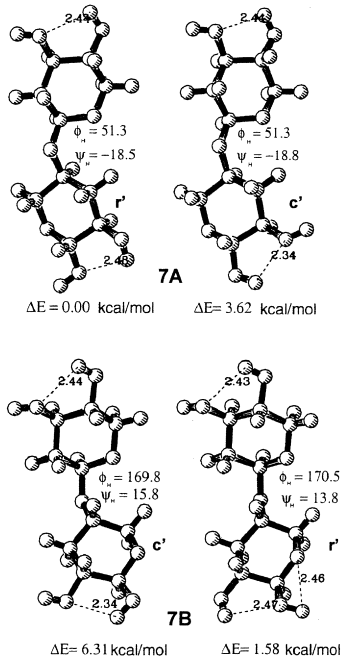

Fig. 7. Cellobiose analogs with only four exocyclic hydroxyl groups at the 3,4- and 1′,2′-positions, numbered **7A** and **7B**. Each structure in A and B was studied with different orientations of the exocyclic hydroxyl groups, c′ and r′.

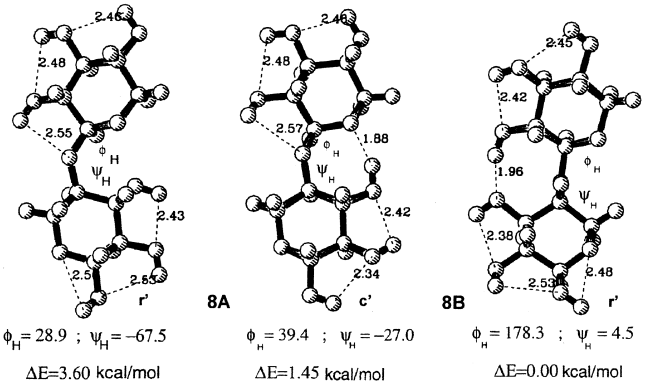

Fig. 8. Cellobiose analog (β-xylobioside) with all six exocyclic hydroxyl groups at the 2,3,4- and 1′,2′,3′-positions, numbered **8A** and **8B**. Each structure in A and B was studied with different orientations of the exocyclic hydroxyl groups, c′ and r′. Structure B is a minimum with orientation r′ regardless of starting geometry.

difference was 2.31 kcal/mol in favor of structure **5A**. Thus, the hydrogen bonding between the exocyclic hydroxyl groups in set **5** stabilized **5B** relative to **5A** by almost 0.60 kcal/mol when compared to set **6**. The free-energy difference between **6A** and **6B** is large at 3.30 kcal/mol, favoring **6A** which, when comparing set **6** with set **5** makes the free energy difference in structure set **6** again 0.60 kcal/mol larger.

Some hydrogen bonding is possible in **6A** and **6B** (see Fig. 6). Structure **6B** can form two hydrogen bonds, O-2–O-1 and O-3′–O-5, essentially one strong intraresidue hydrogen bond (1.89 Å) and a hydrogen bond of 2.50 Å with the bridging oxygen. In structure **6B** there is only one intraresidue hydrogen bond between O-2–O-3′ (2.00 Å) and a hydrogen bond on the reducing ring involving the ring oxygen between O-1′–O-5′ (2.40 Å). Correspondingly, the C–O bonds are slightly longer when the oxygen is involved in back-donation. For example, in structure **6B**, the hydrogen bond between O-2 and O-3′ shortens C-2–O-2 to 1.414 Å. The corresponding O-2–HO-2 bond length is 0.970 Å. However, the C-3′–O-3′ bond length is 1.437 Å, and it is the acceptor part of the hydrogen bond. The O-3′–HO-3′ bond length is correspondingly shorter at 0.963 Å

Structures **7A** *and* **7B***: analogs with –OH groups at 3,4 and 1′,2′.*—In analog set **7**, two exocyclic hydroxyl groups were put on the cellobiose analog in positions where they could hydrogen bond to each other, but without an opportunity for interring communication in either structure **7A** or **7B** (see Fig. 7). There is, however, opportunity for hydrogen bonding with the ring oxygen and so two forms of each structure type, **7A** and **7B**, were explored: one form with the exocyclic hydroxyl groups of the reducing ring in a clockwise orientation (c′) and one in which the exocyclic hydroxyl groups of the reducing ring are in a counter-clockwise orientation (r′). Exploring the orientation of the exocyclic groups required four structures for this set. The nonreducing ring remained in the r orientation.

Comparing like orientations of the exocyclic hydroxyl groups, the type A structures remained energetically favored. Structure **7B** (r′) was only 1.58 kcal/mol higher in energy than **7A** (r′) while **7B** (c′) was 2.69 kcal/mol higher in energy than **7A** (c′). The smaller difference in the former probably is due to the ability of **7B** (r′) to form a weak hydrogen bond between HO-1′ and O-5′ (2.46 Å), whereas no such bond is possible in **7B** (c′) since the hydroxyl group is pointing away from the ring oxygen. The two type **7A** structures are very similar in backbone dihedral angles and hydrogen bonding patterns yet the r′ orientation seems to energetically stabilize the molecule, something that is true in the **7B** type structures as well. Structure **7B** (c′) is energetically high in this set at + 6.31 kcal/mol over **7A** (r′) the lowest energy structure. The free-energy values mirror these trends as well.

The bond lengths in this set of analogs are very similar with the exception of C-2′–O-2′ and O-2′–HO-2′. In **7A** (r′) and **7B** (r′), these bond lengths are 1.422 and 0.964 Å, respectively, while in **7A** (c′) and **7B** (c′) the bond lengths are 1.432(3) and 0.962 Å. The difference occurs from the nature of the hydrogen bond, i.e., in the r′ orientation O-2′ is a donor. The O-1′–HO-2′ hydrogen bond lengths are ∼2.48 Å in **7A** (r′) and **7B** (r′), and it is ∼2.34 Å in **7A** (c′) and **7B** (c′).

Structures **8A** *and* **8B***: analogs with –OH groups at 2,3,4-1′, 2′,3′.*—In the final set of structures, all three exocyclic hydroxyl groups were included in the structure

such that the groups formed a 'network' of hydrogen bonding. In the **8A**-type structures (see Fig. 8), these functional groups formed two distinct networks surrounding each sugar residue. However, in the **8B**-type structure, the network extended over both sugar residues, i.e., there was enhanced communication between the reducing and the nonreducing rings of the disaccharide. This latter network provides cooperative hydrogen bonding resulting in lower energy. As with analog set **7**, four different structures were studied with r′ and c′ orientations at the exocyclic hydroxyl groups. However, structure **8B** (c′) geometry optimized to a structure identical to **8B** (r′) at the B3LYP/6-311++G** level so only the results for **8B** (c′) are presented here. This set of analog structures studied is the only one in which the **8B**-type structure, i.e., a structure with $(\phi_H, \Psi_H) \sim (180°, 0°)$, is energetically stabilized relative to **8A**. The lowest energy of type **8A** is structure (c′) which is 1.42 kcal/mol higher in energy than **8B** and the other A-type structure (r′) is 3.60 kcal/mol higher in energy than **8B**. Interestingly, in analog set **7A** and **7B**, the structures with c′ orientation were higher in energy than r′, a result opposite of the one presented for set **8A** and **8B**. As a matter of fact, the **8B** structure prefers the r′ orientation. Apparently, the ability to form a 'cooperative' hydrogen-bond network is more important energetically than the orientation of the exocyclic hydroxyl groups. Since this 'cooperative' hydrogen bonding stabilizes the energetics of this analog set, it stands to reason that it will also stabilize the parent β-cellobiose. In β-cellobiose not only is there a network of exocyclic groups, but the hydroxymethyl side chain at position C-5(C-5′) can also participate in the 'cooperative' hydrogen bonding. In structures where $(\phi_H, \Psi_H) \sim (180°, 0°)$, the proximity of the side chains on the reducing and nonreducing ring can extend the hydrogen-bonding network completely around the molecule. This 'cooperativity' is the reason that structures with $\phi \sim 180°$ are stabilized over the 'normal' form. The collapse of this 'cooperative' hydrogen-bonding network occurs upon addition of water, crystal packing hydrogen bonds, or other polar solvents, which explains why the 'normal' form is overwhelmingly observed experimentally both in the crystal form and in solution from experimental measurements by NMR, IR and X-ray diffraction methods.[2] Any interactions in which the hydrogen bonds around the rings are distorted or interfered with will break the cooperativity effect. However, in the far-IR spectrum at helium temperatures, it is possible to observe vibrational spectra of cellobiose,[16,17] which strongly suggests that both the normal and some 'flipped' forms are present, their being many frequencies that can be fit to the 'flipped' conformations and which are not found from our calculations to be from the 'normal' forms (data not shown) or from the crystal lattice modes.

4. Conclusions

This study provides evidence to support and explain our previous results on cellobiose.[1] Clearly, one must be careful when using analogs in which the possible 'cooperative' hydrogen bonding is removed and then extrapolate the results to β-cellobiose. The 'cooperative' hydrogen bonding in β-cellobiose is crucial to understanding the conformational energetics of the molecule. Also, because of the importance of the hydrogen bonding to the conformational stability, it is imperative to use a basis set that has polarization functions and diffuse functions as in the 6-311++G** basis set to obtain good hydrogen-bonding interactions. These weak interactions are difficult to predict empirically or with small basis sets, and most empirical force fields do not take the 'cooperative' effect into account as much as they should for carbohydrates.

Also, the effectiveness of the 'cooperative' hydrogen bonding on energetically stabilizing these β-cellobiose analogs, suggests that carbohydrate results in the laboratory will be greatly affected by the environment. A polar solvent could easily disrupt the 'network' of hydrogen bonding that favors the 'flipped' form of β-cellobiose. With this disruption, the 'normal' structure becomes energy stabilized relative to the 'flipped' form.

References

1. Strati, G. L.; Willett, J. L.; Momany, F. A. *Carbohydr. Res.*, previous paper.
2. French, A. D.; Kelterer, A.-M.; Johnson, G. P.; Dowd, M.; Cramer, C. J. *J. Comput. Chem.* **2001**, *22*, 65–78.
3. French, A. D.; Johnson, G. P.; Kelterer, A.-M.; Dowd, M.; Cramer, C. J. *J. Quantum Chem.* **2001**, *84*, 416–425.
4. Dowd, M. K.; French, A. D.; Reilly, P. J. *Carbohydr. Res.* **1992**, *233*, 15–34.
5. French, A. D. *Carbohydr. Res.* **1989**, *188*, 206–211.
6. French, A. D. *Biopolymers* **1988**, *27*, 1519–1525.
7. Tvaroska, I.; Pérez, S. *Carbohydr. Res.* **1986**, *149*, 389–410.
8. Lipkind, G. M.; Shashkov, A. S.; Kochetkov, N. K. *Carbohydr. Res.* **1985**, *141*, 191–197.
9. Lipkind, G. M.; Verovsky, V. E.; Kochetkov, N. K. *Carbohydr. Res.* **1984**, *133*, 1–13.
10. Melberg, S.; Rasmussen, K. *Carbohydr. Res.* **1979**, *71*, 25–34.
11. Rees, D. A.; Smith, P. J. C. *J. Chem. Soc., Perkin Trans. 2* **1975**, 836–840.
12. Rees, D. A.; Skerrett, R. J. *Carbohydr. Res.* **1968**, *7*, 334–348.
13. InsightII 4.0 and Discover 4.0 programs, Molecular Simulations, San Diego, CA.
14. Momany F. A.; Strati, G.; Willett, J. L., in preparation.
15. Parallel Quantum solutions, 2013 Green Acres, Suite A, Fayetteville, AR, 72703.
16. Hineno, M.; Yoshinaga, H. *Spectrochim. Acta* **1972**, *28A*, 2263–2268.
17. Hineno, M.; Yoshinaga, H. *Spectrochim. Acta* **1973**, *29A*, 1575–1578.