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Ab initio computational study of β-cellobiose conformers using B3LYP/6-311++G**☆

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Abstract

The molecular structure of 27 conformers of β-cellobiose were studied in vacuo through gradient geometry optimization using B3LYP density functionals and the 6-311++G** basis set. The conformationally dependent geometry changes and energies were explored as well as the hydrogen-bonding network. The lowest electronic energy structures found were not those suggested from available crystallographic and NMR solution data, where the glycosidic dihedral angles fall in the region $(\phi, \psi) \sim (40°, -20°)$. Rather, 'flipped' conformations in which the dihedral angles are in the range $(\phi, \psi) \sim (180°, 0°)$ are energetically more stable by ~2.5 kcal/mol over the 'experimentally accepted' structure. Further, when the vibrational free energy, ΔG, obtained from the calculated frequencies, is compared throughout the series, structures with (ϕ, ψ) in the experimentally observed range still have higher free energy (~2.0 kcal/mol) than 'flipped' forms. The range of bridging dihedral angles of the 'normal' conformers, resulting from the variance in the ϕ dihedral is larger than that found in the 'flipped' forms. Due to this large flat energy surface for the normal conformations, we surmise that the summation of populations of these conformations will favor the 'normal' conformations, although evidence suggests that polar solvent effects may play the dominant role in providing stability for the 'normal' forms. Even though some empirical studies previously found the 'flipped' conformations to be lowest in energy, these studies have been generally discredited because they were in disagreement with experimental results. Most of the DFT/ab initio conformations reported here have not been reported previously in the ab initio literature, in part because the use of less rigorous theoretical methods, i.e. smaller basis sets, have given results in general agreement with experimental data, that is, they energetically favored the 'normal' forms. These are the first DFT/ab initio calculations at this level of theory, apparently because of the length and difficulty of carrying out optimizations at these high levels.

Keywords: Ab initio; Density functional; Cellobiose; Geometry optimization; Conformations

1. Introduction

The disaccharide, β-cellobiose,† is commonly found as a fragment of the biopolymer cellulose and has importance as a fundamental unit of structure in the field of plant structural sugars. This disaccharide can be obtained from the partial hydrolysis of cellulose resulting in a β-(1→4) linkage between the two D-glucopyranose residues. β-Cellobiose, therefore, serves as a good model compound for exploring β-(1→4) glycosidic linkages (Fig. 1).

In this paper, the authors present in detail the structural parameters for twenty seven DFT/ab initio gradient optimized β-cellobiose conformations using B3LYP density functionals and the 6-311++G** basis set. This study provides evidence that the lowest electronic energy conformer in vacuo is not the 'accepted' experimental structure but one in which the molecular conformation is 'flipped', i.e. the dihedral angle ϕ is close to 180°. This collection of structural and energetic

☆ 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.

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† β-Cellobiose is β-D-glucopyranosyl-(1→4)-β-D-glucopyranose.

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parameters appears to be the largest set of high-level DFT/ab initio calculations obtained to date on any disaccharide, being a more complete set of structural data than that previously published by the authors on maltose.[1]

Empirical computational studies of cellobiose[2–17] and methyl α-lactoside[18,19] have been published. A detailed review of this literature is not made here, although it is of interest to point out that there appear to be only three empirical studies[8,17–19] in which the 'flipped' form was found to be of lowest energy. Previous molecular orbital calculations[15] on cellobiose in the literature are from basis sets (6-31G**) that have been shown to be inadequate for study of carbohydrates.[1] For example, from calculations at the Hartree–Fock 6-31G** level[15] three minimum-energy conformations were reported with ϕ_H and ψ_H values of (49°, −7°), (29°, −52°) and (36°, 175°). These sets of dihedral angles are in general agreement with minimum-energy positions on empirical isoenergy contour maps.[8–10] The first and third conformations are in low-energy regions found in this work, while the second conformation is not a minimum with our very large basis set. When plotted in ϕ–ψ space, experimental X-ray data on cellobiose and related β-linked carbohydrates covers a spread in dihedral angles of over 70° in ϕ and 40° in ψ (see fig. 3 of Ref. 11), and for that reason it is difficult to predict where the vacuum cellobiose structures will reside in ϕ–ψ space. Also, since the glycosidic linkage is strongly affected by the orientation of the exocyclic hydroxyl groups and the hydroxymethyl groups, it is very difficult to predict a limited ϕ–ψ space as being preferred in electronic energy. As the results presented here show, the spread in ϕ–ψ conformations in the region that experimental data are found is rather large even for in vacuo studies, the spread resulting only from optimizing conformations in which the variations occur in hydroxymethyl side-chain dihedral angles, χ, and in the cc' and rr' exocyclic hydroxyl groups.

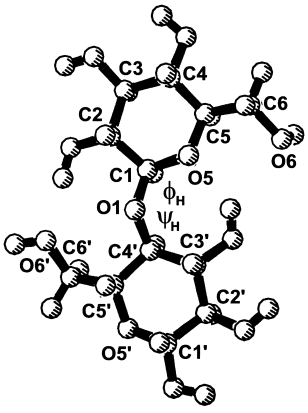

Fig. 1. β-Cellobiose (with numbering and β-(1→4) linkage indicated).

2. Methodology

Computational methods.—Initial conformations were chosen using a revision of the empirical force field AMB99C, described previously for α-(1→4)-linked disaccharides,[20] and parameterized here in part for β-(1→4)-linked disaccharides. β-Cellobiose conformations were examined for minimum-energy structures using results from vacuum dynamics simulations and search algorithms. Previous computational empirical molecular mechanics studies on cellobiose[2–17] were used to include obvious low-energy structures from different authors as starting structures. Different force fields have given remarkably different sets of minimum energy conformations for cellobiose, and not only the conformational parameters but also the energy differences vary significantly between force fields. Those conformations chosen for DFT/ab initio optimization were selected from our empirical (AMB01C) calculations using previous empirical calculations[2–17] as starting conformations.

The structures chosen were first gradient optimized at the B3LYP/6-31+G* level of theory and the same density functional, B3LYP, was used to further optimize the geometry using a large triply split valence basis set denoted 6-311++G**. Initial optimization at the smaller basis set shortened the overall time for the calculation by providing a better 'starting structure' for the higher level calculations, although we do not report results from the smaller basis set here. Other basis sets were explored both with and without density functionals, but a discussion of these studies and the resulting differences in structural parameters and energies with basis sets is best reserved for a more in-depth deliberation.[21] However, after examining the results from different basis set calculations, as well as those from previous calculations, on maltose,[1] it became clear that 6-311++G** basis set was nearly optimal for achieving high-quality internal coordinates and energies in excellent agreement with experimental structural parameters.

The DFT/ab initio calculations were performed on Parallel Quantum Solutions (PQS) machines QSA-500, QSA-600 and QSA-1000 using PQS software.[22] The β-cellobiose structures were energy and gradient optimized with a satisfactory convergence obtained when successive iterations were within 0.0001 in gradient and 0.000001 in energy of each other. Geometry changes between successive optimization cycles were generally less than one part in 10^5, meaning that changes in bond lengths between optimization cycles amount to less than 0.0001 Å and less than 0.001° and 0.01° for associated bond and torsional angles, respectively. Molecular Simulations, Inc.[23] Insight II 4.0 graphics and Discover 4.0 programs were used for visualization, structure building and empirical geometry optimization purposes.

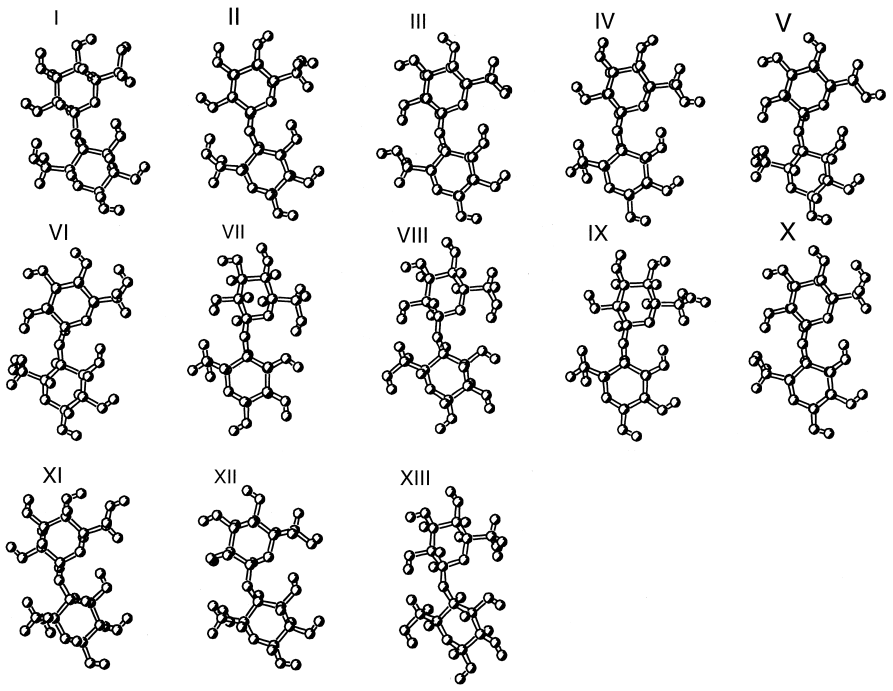

Fig. 2. Pictorial representation of the thirteen 'normal' β-cellobiose conformations.

Nomenclature and conventions.—The β-cellobiose numbering scheme used for all conformations in this paper follows convention (Fig. 1) with a prime (′) used to specify the reducing unit. The dihedral angles ϕ_H and ψ_H are defined by H-1–C-1–O–C-4′ and C-1–O–C-4′–H-4′, respectively, while ϕ and ψ are defined using the heavy atoms O-5–C-1–O–C-4′ and C-1–O–C-4′–C-5′, respectively. Conformers are denoted as 'flipped' if either ϕ_H or ψ_H lies near 180°.

The hydroxymethyl side chains are described by the orientation about the C-5–C-6 and C-5′–C-6′ bonds (χ and χ'), and the literature convention *g* (*gauche*) or *t* (*trans*). For each side chain, a complete description is obtained by specifying O-6 (O-6′) with respect to O-5 (O-5′) and O-6 (O-6′) with respect to C-4 (C-4′) to give *gg*, *gt* or *tg* (*gg′*, *gt′*, *tg′*). The orientations of the exocyclic hydroxyl groups on the sugar residues are defined in this paper as clockwise, denoted *c*, or anti-clockwise, denoted *r*, as per the literature convention.[24] Once again a prime is used to specify hydroxyl groups that are present on the reducing sugar unit.

3. Results

Conformers.—The 27 conformers in this study were chosen to give several examples of each possible conformational state (see Figs. 2 and 3), but do not constitute a complete set of structures as many thousands (531,441 if 3-fold rotation around each available torsion is assumed)[25] of conformational possibilities exist, most of which constitute high-energy conformers. The conformers studied here are classified as follows: thirteen 'normal' conformers with ϕ_H and ψ_H angles between 0° and 75° (Table 1 and Fig. 2), nine conformers with dihedral angle ϕ_H 'flipped', i.e., ϕ_H close to 180°, and five conformers with the dihedral angle ψ_H 'flipped' (Table 2 and Fig. 3). These structures can be further classified by the orientation of the exocyclic hydroxyl groups on the sugar rings, clockwise (*c*) or anti-clockwise (*r*), as well as one of the three favored rotamers of the hydroxymethyl side chain. Disaccharides are very complex structures and so the importance of each of these parameters on the overall stability of the conformers will be discussed in the following sections in terms of both the electronic and the relative free energy of the molecules.

Energetics.—The total electronic energy (E_{elec}), difference energy (ΔE), relative zero point vibrational energy ($ZPVE$) and Gibbs free energy differences (ΔG) of the cellobiose conformers studied are summarized in Tables 1 and 2. The enthalpy (H) and entropy terms (S) are also included so that one may observe the term that dominates the free-energy differences. The total electronic energy for each conformer is shown corrected for zero point vibrational energy obtained from the calculated vibrational frequencies. The calculated vibrational frequencies are not included in this paper but will be described elsewhere[21] in conjunction with vibrational frequency calculations from other carbohydrates, both alone and solvated with water molecules.

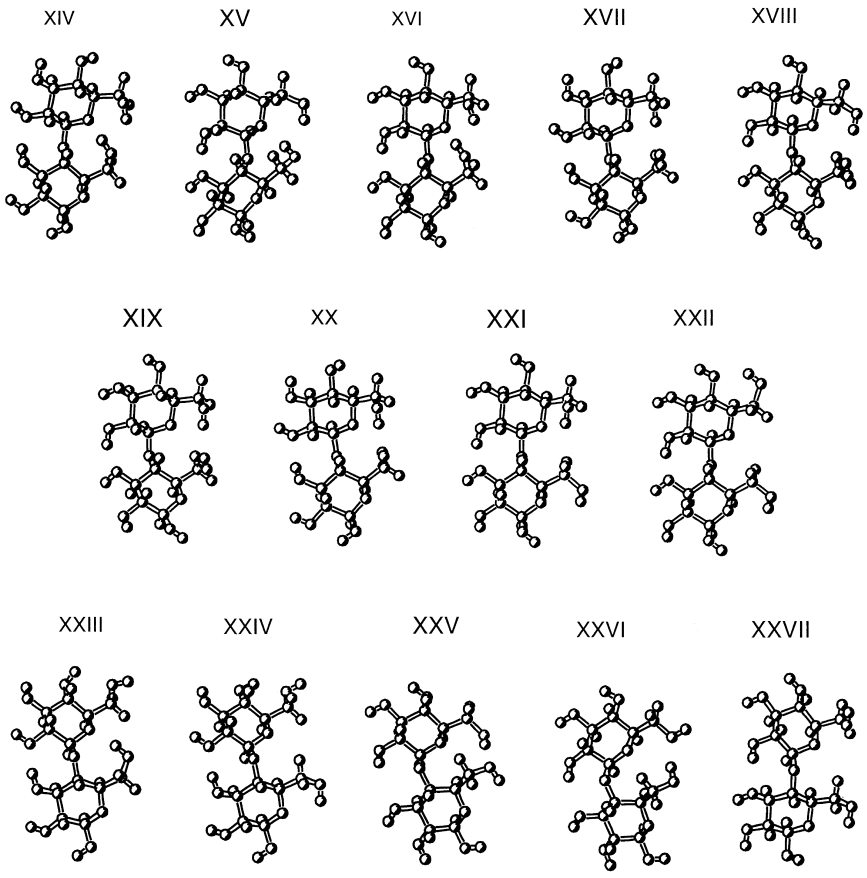

Fig. 3. Pictorial representation of nine β-cellobiose conformers 'flipped' in ϕ (top), and five conformers 'flipped' in ψ (bottom).

The Gibbs free energy of the system is calculated from Eq. (1) where T is taken as 298 K.

$$G = E_{\text{elec}} + (H - TS) \quad (1)$$

The enthalpic term, H, is fairly constant for all the conformers differing by about 1.5 kcal/mol between all conformers, while the entropy term, S, varies by about 7 cal/mol-K. The entropy term relates directly to the amount of disorder within the structure. That is, those conformers with large rotational motions of lower energy will have the greatest entropy contribution to the free energy. From this must follow that the 'normal' conformers have more freedom of motion than those conformers that are 'flipped'. In the 'normal' structures, for example, the hydroxymethyl side chains are located on opposite sides of the disaccharide and therefore do not interact or constrain each others motion. However, in the 'flipped' structures these side chains may interact forming favorable hydrogen bonds, thus inhibiting the rotation of the hydroxymethyl groups and diminishing rotations about the conformational dihedral angles ϕ and ψ. These interactions can essentially lock the disaccharide in place, raising specific vibrational frequencies and decreasing the low-frequency contributions to the entropy term.

Our calculations at the B3LYP/6-311++G** level indicate conformer **XIX**, with $(\phi_H, \psi_H) = (179.4°, -0.6°)$, is the most energetically stable structure and remains lowest in free energy. Surprisingly, this conformer is 'flipped' in the dihedral angle ϕ_H, a result which is contrary to the crystallographically determined cellobiose structures where $(\phi_H, \psi_H) \sim (40°, -20°)$ (see Ref. 16 for crystallographic references and dihedral angles). An overview of the relative electronic energy differences of the cellobiose conformers (Fig. 4), as well as free energy differences (Fig. 5), indicate that structures 'flipped' in ϕ_H are generally lower in energy than both the normal structures and those structures 'flipped' in ψ_H, with only 'flipped' conformer **XIV** (+6.64 kcal/mol) being of much higher energy than the 'normal' conformations. 'Flipped' conformer **XIX** has a zero point vibrational corrected energy (ZPVCE) that is approximately 2.6 kcal/mol lower than conformer **X**, the lowest ZPVCE 'normal' structure, where $(\phi_H, \psi_H) = (28.5°, -25.2°)$, while the Gibbs free energy difference of these two conformers is ~ 2.25 kcal/mol. A single point energy calculation for conformers **XIX** and **X** at the MP2/6-311++G** level of theory and the B3LYP/6-311++G** geometry gave $\Delta E(\text{SCF}) = 4.3$ and $\Delta E(\text{SCF}+\text{MP2}) = 6.7$ kcal/mol with the

Table 1
Energies and dihedral angles (°) of normal conformers[a]

Conformer	I	II	III	IV	V	VI	VII
ϕ_H	30.6	32.2	29.9	32.8	19.7	17.8	46.0
ψ_H	−22.9	−22.6	−30.5	−28.1	−41.1	−36.7	−7.1
E_{ele}	−814723.37	−814721.88	−814724.00	−814720.88	−814724.11	−814724.73	−814723.64
ZPVE	243.44	243.81	244.01	243.76	243.68	243.77	243.78
E_{corr}	−814479.90	−814478.07	−814479.99	−814477.12	−814480.43	−814480.96	−814479.86
ΔE	4.03	5.89	3.97	6.84	3.53	3.00	4.09
H	257.82	257.82	258.45	258.36	258.26	258.20	258.34
S	152.77	152.77	152.97	155.59	154.43	153.45	156.14
G	−814511.07	−814509.59	−814511.14	−814508.88	−814511.87	−814512.26	−814511.83
ΔG	3.48	4.97	3.42	5.68	2.69	2.29	2.730
pop (n/n_0)	0.003	0.0002	0.003	0.000	0.011	0.021	0.010
%pop	0.142	0.013	0.157	0.008	0.542	1.053	0.507

Conformer	VIII	IX	X	XI	XII	XIII
ϕ_H	45.3	39.4	28.5	36.7	28.9	62.0
ψ_H	−10.0	−13.0	−25.2	−13.3	−33.0	3.5
E	−814723.16	−814722.51	−814725.31	−814724.18	−814722.07	−814722.27
ZPVE	243.70	243.63	243.90	243.37	243.36	243.59
E_{corr}	−814479.46	−814478.88	−814481.40	−814480.82	−814478.72	−814478.70
ΔE	4.49	5.08	2.55	3.14	5.24	5.26
H	258.28	258.24	258.17	257.94	258.06	257.77
S	156.20	156.63	151.58	155.35	157.01	152.08
G	−814511.44	−814510.94	−814512.31	−814512.54	−814510.80	−814509.83
ΔG	3.12	3.62	2.25	2.01	3.76	4.72
pop (n/n_0)	0.005	0.002	0.022	0.033	0.002	0.000
%pop	0.262	0.113	1.138	1.688	0.089	0.017

[a] All energies are in kcal/mol except S, whose units are cal/mol-K. All ΔE and ΔG values are calculated with respect to **XIX**.

'flipped' conformer favored even more than similar energy values using the B3LYP density functional (see Tables 1 and 2). Conformer **XI** is the lowest Gibbs free energy 'normal' conformer but is still $\sim$2.0 kcal/mol higher in energy than **XIX**.

With the exception of structure **XXV**, those conformers 'flipped' to approximately 180° in ψ_H are higher in energy than any of the 'normal'conformers or structures 'flipped' in ϕ_H. Structure **XXV**, the most energetically stable ZPVCE conformer 'flipped' in ψ_H, (ϕ_H, ψ_H) = (35.3°, 173.4°) is approximately 3.3 kcal/mol higher than **XIX** and falls energetically below many of the 'normal' structures even when considering its Gibbs free energy.

A ϕ_H–ψ_H isoenergetic contour plot can be constructed from the ZPVCE data from the 27 conformers studied here (Fig. 6). Note that Fig. 6 is not the usual ϕ–ψ isoenergetic plot where energies are obtained at a grid point and extrapolation between points is then made. However, even though no grid has been used, the regions of ϕ_H–ψ_H space mapped out by the energies of the various geometry optimized conformers is similar in the regions of the conformers to that published previously[8–10] using empirical potentials, and the area of low energy fits nicely with the observed crystallographic data in the 'normal' region. From this result it is now evident that the orientation of the side-chain groups is extremely important on the overall minimum energy ϕ–ψ conformation of the molecule.

Conformationally dependent geometry changes.—A selection of conformers as extensive as the ones presented in this paper and at such sophisticated levels of theory, require examination of all geometric parameters such as bond lengths, bond angles and dihedral angles. These molecular parameters can change with variation in conformation and can also change with specific hydrogen bonding interactions within the structure.

Bond lengths.—When comparing the bond lengths of the various cellobiose conformers presented here, one realizes that the differences are very subtle. For this reason, the bond lengths surrounding the O-5(O-5′) ring oxygen, the O-1 bridging oxygen, the hydroxymethyl side chains and the important exocyclic hydroxyl groups at positions 2, 4, 1′ and 3′ were closely exam-

ined. The results are tabulated in Tables 3 and 4. Although the 27 conformers all vary in conformational dihedral angles, hydroxymethyl side chain orientation, and exocyclic hydroxyl orientations, some of the internal bond lengths are very consistent. The ring C–C bonds vary over all conformations by only ~ 0.01 Å. For example, in the non-reducing ring, the C-1–C-2 bond length is 1.53 Å–1.54 Å in the 'normal' and 'flipped' structures. The structures 'flipped' in ϕ_H had C-1–C-2 bond lengths at 1.54 Å, while the conformers 'flipped' in ψ_H were consistently 1.53 Å. In the reducing sugar unit, the C-1′–C2′ bond lengths are only slightly shorter at 1.52 Å–1.53 Å.

The bonds surrounding the bridging oxygen, O-1, vary slightly within groups of conformers, by about 0.02 Å. The C-1–O-1 bond varies from 1.38 Å–1.40 Å in normal structures and from 1.39 Å to 1.41 Å in 'flipped' structures. The slightly longer bond length in the 'flipped' structures is probably due in part to the twisting caused by the larger conformational dihedral angles. However, for both normal and 'flipped' structures, the bond between C-1–O-1 is much shorter than between O-1–C-4′. The O-1–C-4′ bond lengths vary from 1.43 Å to 1.44 Å in normal structures and 1.43 Å to 1.45 Å in 'flipped' structures.

The O–H bond lengths in the normal and 'flipped' structures are, in general, dependent on the amount and strength of hydrogen bonding in which they are involved. The effect of the hydrogen bonding on the O–H bond length depends, however, on whether these atoms are involved as donors or acceptors. The O–H bond is shorter in cases of back donation. The length of an O–H bond in these structures varies from 0.96 Å to 0.98 Å in the normal form and 0.96 Å–0.97 Å in 'flipped' structures. Conformer **III**, for example, has an O-2–HO-2 bond length of 0.98 Å. The O-2...O-6′ hydrogen bond is strong, which lengthens the O-2–HO-2 bond length. The O-6′–HO-6′ bond length is, of course, much shorter with a length of 0.96 Å. This hydrogen bonding influences the length of the corresponding

Table 2
Energies and dihedral angles (°) of 'flipped' conformers[a]

Conformer	**XIV**	**XV**	**XVI**	**XVII**	**XVIII**	**XIX**	**XX**
ϕ_H	−178.3	−177.8	−177.0	179.1	169.5	179.4	179.0
ψ_H	0.2	1.3	−0.8	0.1	−3.9	−0.6	0.2
E	−814721.62	−814726.98	−814726.55	−814726.60	−814727.28	−814729.15	−814726.26
ZPVE	244.30	258.60	244.03	244.33	244.23	245.19	243.89
E_{corr}	−814477.32	−814482.76	−814482.52	−814482.27	−814483.05	−814483.96	−814482.37
ΔE	6.64	1.20	1.44	1.69	0.91	0	1.59
H	258.55	258.60	258.28	258.55	258.54	259.34	258.25
S	151.60	152.06	151.01	150.40	151.20	150.18	151.23
G	−814508.24	−814513.69	−814513.27	−814512.87	−814513.80	−814514.56	−814513.08
ΔG	6.31	0.87	1.29	1.69	0.76	0	1.48
pop (n/n_0)	0.000	0.231	0.114	0.058	0.277	1.0	0.083
%pop	0.001	11.728	5.762	2.924	14.063	50.762	4.190

Conformer	**XXI**	**XXII**	**XXIII**	**XXIV**	**XXV**	**XXVI**	**XXVII**
ϕ_H	179.2	178.8	7.2	11.9	35.3	24.2	12.6
ψ_H	−3.0	−2.4	−176.8	−179.3	173.4	173.4	179.1
E	−814725.37	−814725.76	−814718.83	−814721.88	−814724.72	−814723.05	−814722.73
ZPVE	244.02	244.02	243.85	243.37	244.07	243.93	245.07
E_{corr}	−814481.34	−814481.74	−814474.98	−814478.51	−814480.65	−814479.12	−814477.66
ΔE	2.62	2.22	8.98	5.45	3.31	4.84	6.30
H	258.61	259.63	258.28	258.01	258.44	258.55	259.63
S	154.62	155.72	153.98	155.16	152.29	154.19	155.72
G	−814512.83	−814512.5	−814506.44	−814510.11	−814511.66	−814510.45	−814509.50
ΔG	1.73	2.03	8.11	4.45	2.89	4.11	5.06
pop (n/n_0)	0.054	0.032	0.000	0.001	0.008	0.001	0.000
%pop	2.737	1.649	5×10^{-5}	0.027	0.383	0.049	0.010

[a] All energies are in kcal/mol except S, whose units are cal/mol-K. All ΔE and ΔG values are calculated with respect to conformer **XIX**.

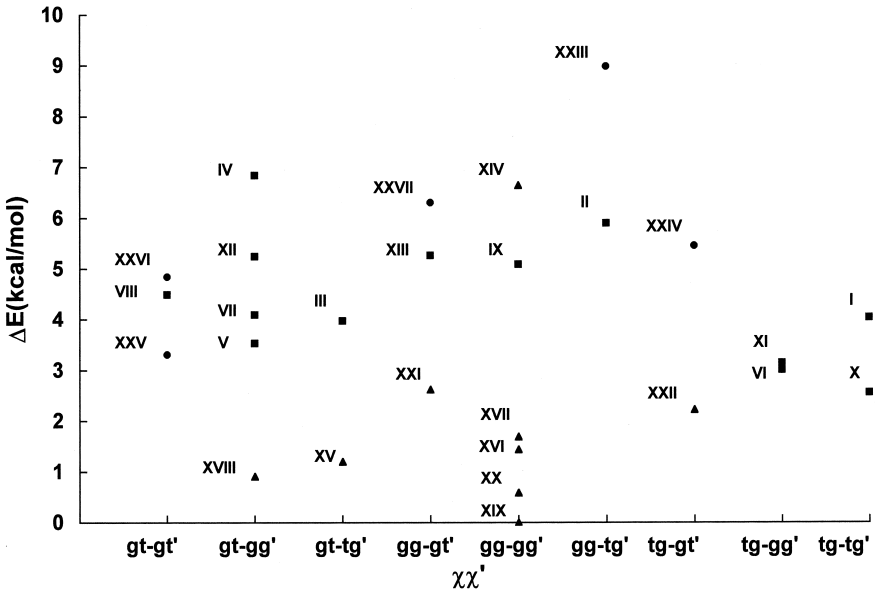

Fig. 4. Zero point corrected electronic energies of β-cellobiose conformers. ■, normal; ▲, 'flipped' in ϕ; ●, 'flipped' in ψ.

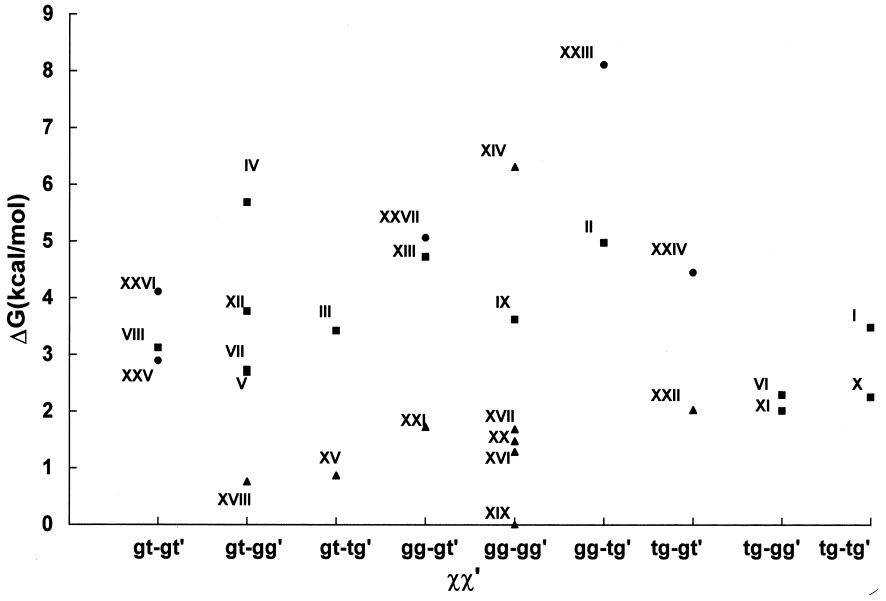

Fig. 5. Gibbs free energy of β-cellobiose conformers. Symbols are the same as in Fig. 4.

C–O bond. When the O–H bond is lengthened, the C–O bond will be shorter as evidenced in **III**, where C-2–O-2 is 1.42 Å, while C-6′–O-6′ is slightly longer at 1.44 Å. The same trend is observed in all the conformers. In the 'flipped' structures, the variance of O–H bond lengths is less. The O-2–HO-2 bond length is constant at 0.97 Å, since this oxygen seems to always be involved in hydrogen bonding to some extent, but for the other O–H bonds, the values vary from 0.96 Å to 0.97 Å. In structure **XIX**, for example, the O-2–HO-2 bond length is 0.97 due to the hydrogen-bond distance of 1.94 Å between O-2...O-3′ (see Tables 7 and 8). However, O-4–HO-4 is 0.96 Å since O-4...O-3 is a much weaker hydrogen bond, 2.44 Å. The C–O bonds vary in the same manner as the normal forms.

Bond angles and respective dihedral angles.—Hydrogen-bonding effects in disaccharides can greatly influence the geometry of the cellobiose structures including, among other parameters, the bond angles of the ring hydroxyl groups. For example, one interesting bond angle is the HO-4–O-4–C-4 angle, i.e., the angle formed by the –OH group situated next to the hydroxymethyl side chain. This angle is essentially constant at $\sim 107°$, but opens up by as much as 3° to 109° when the ring hydroxyl groups are oriented in a clockwise fashion (see, for example, structure **IX** in Table 3). This

clockwise orientation points HO-4 directly at the hydroxymethyl side-chain, but since the side chain oxygen is oriented away from the hydroxyl group in this structure, there is less hydrogen bonding capability, and the angle opens up. Therefore, the value for this angle, as well as other C–O–H angles, is dependent on whether the hydroxyl group participates in donor or acceptor hydrogen bonding. The associated dihedral angle, HO-4–O-4–C-4–C-5, also exhibits some conformationally directed structural effects. When the ring hydroxyl groups are in a clockwise orientation, this dihedral angle ranges from 87.3° to 88.9° for 'flipped' structures (Table 6) unless hydrogen bonding is possible with O-4, as it is, for example, in **XXIV**, where the dihedral angle value is much smaller at $-49.4°$.

The HO-3′–O-3′–C-3' angle, where the –OH group has the opportunity of hydrogen bonding between the sugar residues, is similarly affected by the orientation of the exocyclic hydroxyl groups. In the 'flipped' structures, where a hydrogen bond to either O-2 or the oxygen of the ether linkage between residues is possible, this angle opens up to 109° from 107° again when the exocyclic groups are clockwise (Table 4). In the normal form, it appears that only the orientation of the exocyclic groups of the reducing ring directly affects the value of this angle, probably due to the limited hydrogen-bonding interactions between residues.

Another important bond angle in disaccharides is C-5–O-5–C-1, the angle formed by the ether linkage within a glucose unit. In general for 'normal' structures, this angle is in the range of 113.5°–114.9° with the exception of structure **XIII** where the angle is 116.8°. In this structure, the hydroxymethyl side chain is *gg*, which may cause the ring to pucker a bit. In the 'flipped' structures, this angle has a somewhat larger range, 113.4°–117.5°, and the average value is also larger. Further, the *gg* conformation of the hydroxymethyl side chains appears to flatten the glucose ring. The dihedral ring angle C-4–C-5–O-5–C-1 is fairly constant in the 'normal' forms with a range of 60.0°–64.5°, a spread of less than 4° (Table 5). However, in forms 'flipped' in ϕ_H and ψ_H, the spread in the C-4–C-5–O-5–C-1 dihedral angle is much larger, being 8.2° and 6.8°, respectively.

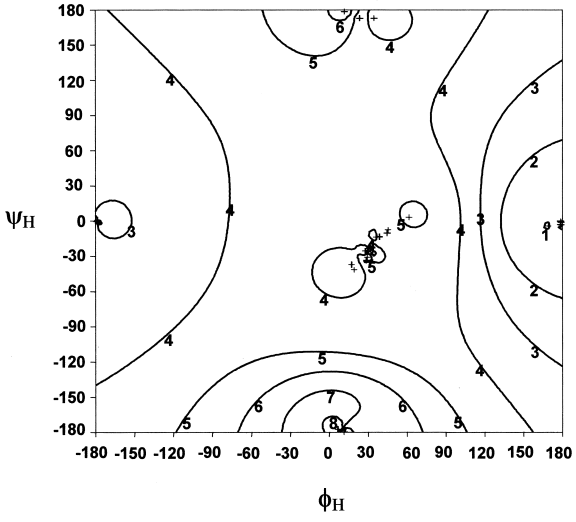

Fig. 6. ϕ_H–ψ_H isoenergetic contour plot including all twenty seven β-cellobiose conformers. Plus signs indicate ϕ_H–ψ_H positions of individual conformations. The contour lines are mathematical interpolations on the ZPVCE energy surface. Dihedral angles are in degrees.

The nearby C-6–C-5–O-5 angle is also dependent on the position of the side chain ranging from $\sim 104°$ to 109° with the larger angles corresponding to hydroxymethyl side chains in the *gg* conformation. The dihedral angle O-6–C-6–C-5–O-5 takes up energy-minimized values, $\sim 150°$, when the side chain is started in the *tg* conformation.

The angle C-1–O-1–C-4′ is directly dependent on the ϕ_H and ψ_H dihedral angles since it is the angle that defines the 'link' between the two sugar units and is attached to the anomeric carbon atom. This bond angle ranges from 116.1° to 119.0° in the 'normal' structures and 118.2°–120.7° in the 'flipped' structures. The range of angles is slightly larger in the 'normal' forms, but it is not significantly affected by backbone conformation. In the 'normal' structures with (ϕ_H, ψ_H) $\sim$ (40°, $-7°$), the value of C-1–O-1–C-4′ is smaller than when (ϕ_H, ψ_H) nears $\sim$(30°, $-30°$), where the angle opens to almost 120°. In the 'flipped' forms the differences are more subtle since the ϕ_H–ψ_H range is so small. However, it appears that those structures with ψ_H values approaching zero also have larger C-1–O-1–C-4′ angles.

The HO-6′–O-6′-C-6′ angle is very interesting in that it is influenced by hydrogen bonding with the linking O and in 'flipped' structures with the hydroxymethyl side chain of the non-reducing ring. In normal conformers this angle is either 107.1° or 109.9°. In the 'flipped' structures, this value is 105.0° or 109.3°.

The conformationally dependent geometry of the hydroxyl group at the anomeric site of the reducing ring is also of interest. In the normal structures, the C–O–H angle is either 107° or 109°. This angle opens up when the exocyclic hydroxyl groups of the reducing ring are in an anti-clockwise orientation. In the anticlockwise orientation the HO-1 hydrogen is in a position to hydrogen bonding to the ring oxygen, O-5′. This donor interaction reduces the optimized C–O–H angle. When the ring hydroxyl groups are oriented in a clockwise manner, the –OH group is positioned away from the ring oxygen and the angle opens up. In the structures 'flipped' in ϕ_H the same is true: the angle is either $\sim 107°$ or 109°, with the larger angles corresponding to structures in which the ring hydroxyl orientation is

Table 3
Selected bond lengths of regular conformers (Å)

	I	II	III	IV	V	VI	VII
C-1–C-2	1.54	1.53	1.53	1.53	1.53	1.53	1.53
C-2–O-2	1.42	1.42	1.42	1.42	1.42	1.42	1.43
O-2–HO-2	0.96	0.97	0.98	0.97	0.97	0.97	0.96
C-1–O-5	1.44	1.44	1.43	1.44	1.42	1.43	1.41
O-5–C-5	1.43	1.44	1.43	1.43	1.43	1.44	1.43
C-5–C-6	1.53	1.52	1.52	1.52	1.52	1.53	1.53
C-6–O-6	1.43	1.43	1.43	1.42	1.43	1.42	1.42
O-6–HO-6	0.96	0.96	0.96	0.96	0.96	0.97	0.97
C-4–O-4	1.42	1.43	1.42	1.43	1.42	1.43	1.42
O-4–HO-4	0.97	0.96	0.97	0.96	0.97	0.97	0.97
C-1–O-1	1.38	1.38	1.39	1.38	1.39	1.40	1.40
O-1–C-4′	1.44	1.44	1.43	1.44	1.43	1.43	1.44
C-3′–C-3′	1.42	1.42	1.42	1.42	1.42	1.42	1.42
O-3′–HO-3′	0.96	0.97	0.97	0.97	0.97	0.97	0.97
C-1′–C-2′	1.53	1.53	1.53	1.53	1.53	1.53	1.52
C-1′–O-1′	1.40	1.40	1.40	1.40	1.40	1.40	1.40
O-1′–HO-1′	0.97	0.97	0.97	0.97	0.97	0.97	0.97
C-1′–O-5′	1.41	1.41	1.42	1.41	1.42	1.42	1.42
O-5′–C-5′	1.43	1.43	1.43	1.43	1.43	1.43	1.43
C-5′–C-6′	1.54	1.54	1.53	1.53	1.53	1.53	1.53
C-6′–O-6′	1.41	1.41	1.44	1.42	1.43	1.43	1.42
O-6′–HO-6′	0.97	0.97	0.96	0.97	0.96	0.96	0.96

Conformer	VIII	IX	X	XI	XII	XIII
C-1–C-2	1.53	1.53	1.53	1.53	1.53	1.53
C-2–O-2	1.43	1.42	1.41	1.42	1.43	1.43
O-2–HO-2	0.96	0.97	0.98	0.97	0.97	0.96
C-1–O-5	1.41	1.44	1.44	1.44	1.43	1.41
O-5–C-5	1.43	1.43	1.44	1.43	1.43	1.43
C-5–C-6	1.52	1.52	1.53	1.53	1.52	1.52
C-6–O-6	1.42	1.43	1.42	1.43	1.43	1.42
O-6–HO-6	0.97	0.96	0.97	0.96	0.96	0.97
C-4–O-4	1.42	1.43	1.43	1.42	1.42	1.42
O-4–HO-4	0.97	0.96	0.97	0.97	0.97	0.96
C-1–O-1	1.40	1.38	1.39	1.38	1.38	1.40
O-1–C-4′	1.44	1.44	1.43	1.44	1.44	1.44
C-3′–O-3′	1.42	1.42	1.42	1.42	1.42	1.42
O-3′–HO-3′	0.97	0.97	0.97	0.97	0.97	0.97
C-1′–C-2′	1.52	1.52	1.53	1.53	1.53	1.52
C-1′–O-1′	1.40	1.40	1.40	1.40	1.40	1.40
O-1′–HO-1′	0.96	0.97	0.97	0.97	0.97	0.96
C-1′–O-5′	1.42	1.41	1.42	1.41	1.41	1.42
O-5′–C-5′	1.43	1.43	1.42	1.43	1.43	1.44
C-5′–C-6′	1.52	1.53	1.54	1.53	1.52	1.52
C-6′–O-6′	1.42	1.42	1.43	1.42	1.43	1.42
O-6′–HO-6′	0.96	0.97	0.96	0.97	0.96	0.96

anticlockwise and pointing at the ring oxygen. The associated dihedral angle HO-1′–O-1′–C-1′–C-2′ mirrors this trend with values from ~59° to 68° when the ring hydroxyl groups are in an anti-clockwise configuration and ~39°–45° when they are in a clockwise orientation.

4. Discussion

Disaccharides have interesting and complex structures. The presence of hydroxyl groups on the sugar residues and hydroxymethyl side chains present the possibility of sophisticated chemistry. However, these

groups also make these sugars difficult to study by quantum mechanics methods. For that reason, it was decided that density functionals coupled with the high-level triple split basis set 6-311++G** should be used throughout since this basis set includes both polarization and diffuse orbitals, both of which are necessary for modeling hydroxyl-containing compounds.[1]

At this level of theory, the most stable structure for β-cellobiose is one 'flipped' in ϕ_H. Some previous ab initio calculations[15,26] have suggested that a 'normal'

Table 4
Selected bond lengths of 'flipped' conformers (Å)

	XII	**XV**	XVI	**XVII**	**XVIII**	**XIX**	**XX**
C-1–C-2	1.54	1.54	1.54	1.54	1.54	1.54	1.54
C-2–O-2	1.43	1.41	1.43	1.42	1.42	1.42	1.43
O-2–HO-2	0.97	0.97	0.97	0.97	0.97	0.97	0.97
C-1–O-5	1.43	1.43	1.42	1.41	1.41	1.42	1.42
O-5–C-5	1.44	1.44	1.43	1.43	1.43	1.44	1.43
C-5–C-6	1.53	1.53	1.52	1.53	1.53	1.53	1.53
C-6–O-6	1.42	1.42	1.42	1.41	1.41	1.42	1.41
O-6–HO-6	0.97	0.97	0.97	0.97	0.97	0.97	0.97
C-4–O-4	1.43	1.42	1.43	1.42	1.42	1.42	1.43
O-4–HO-4	0.97	0.97	0.96	0.97	0.97	0.96	0.96
C-1–O-1	1.39	1.40	1.40	1.41	1.41	1.41	1.40
O-1–C-4′	1.45	1.44	1.44	1.43	1.43	1.43	1.44
C-3′–C-3′	1.42	1.43	1.42	1.43	1.43	1.43	1.42
O-3′–HO-3′	0.97	0.97	0.97	0.97	0.97	0.97	0.97
C-1′–C-2′	1.53	1.52	1.53	1.53	1.53	1.53	1.53
C-1′–O-1′	1.40	1.40	1.40	1.40	1.40	1.40	1.40
O-1′–HO-1′	0.97	0.96	0.97	0.96	0.96	0.96	0.97
C-1′–O-5′	1.41	1.42	1.41	1.42	1.42	1.42	1.41
O-5′–C-5′	1.43	1.44	1.43	1.44	1.44	1.44	1.43
C-5′–C-6′	1.53	1.54	1.52	1.52	1.52	1.52	1.52
C-6′–O-6′	1.42	1.42	1.43	1.42	1.42	1.43	1.43
O-6′–HO-6′	0.96	0.97	0.97	0.96	0.96	0.96	0.96

Conformer	**XXI**	**XXII**	**XXIII**	**XXIV**	**XXV**	**XXVI**	**XXVII**
C-1–C-2	1.54	1.54	1.53	1.53	1.53	1.53	1.53
C-2–O-2	1.42	1.42	1.43	1.43	1.42	1.42	1.42
O-2–HO-2	0.97	0.97	0.97	0.97	0.97	0.97	0.97
C-1–O-5	1.43	1.43	1.43	1.43	1.42	1.42	1.42
O-5–C-5	1.44	1.43	1.44	1.43	1.44	1.43	1.44
C-5–C-6	1.53	1.53	1.52	1.53	1.52	1.52	1.53
C-6–O-6	1.42	1.42	1.43	1.43	1.42	1.43	1.42
O-6–HO-6	0.97	0.97	0.96	0.96	0.97	0.96	0.96
C-4–O-4	1.42	1.43	1.43	1.42	1.42	1.42	1.42
O-4–HO-4	0.96	0.97	0.96	0.97	0.97	0.97	0.96
C-1–O-1	1.4	1.40	1.40	1.40	1.40	1.40	1.40
O-1–C-4′	1.44	1.43	1.44	1.44	1.43	1.43	1.43
C-3′–O-3′	1.43	1.43	1.42	1.42	1.42	1.42	1.43
O-3′–HO-3′	0.97	0.97	0.97	0.97	0.97	0.97	0.97
C-1′–C-2′	1.53	1.53	1.53	1.53	1.53	1.53	1.53
C-1′–O-1′	1.40	1.40	1.40	1.40	1.40	1.40	1.40
O-1′–HO-1′	0.96	0.96	0.97	0.97	0.96	0.96	0.96
C-1′–O-5′	1.42	1.42	1.41	1.41	1.42	1.42	1.42
O-5′–C-5′	1.43	1.44	1.43	1.43	1.44	1.44	1.44
C-5′–C-6′	1.53	1.52	1.54	1.52	1.52	1.53	1.52
C-6′–O-6′	1.42	1.42	1.42	1.42	1.43	1.42	1.42
O-6′–HO-6′	0.97	0.96	0.97	0.97	0.96	0.97	0.96

Table 5
Selected bond angles of regular conformers (°)

Conformer	**I** *cc tg–tg′*	**II** *cc gg–tg′*	**III** *rc gt–tg′*	**IV** *cc gt–gg′*	**V** *rc gt–gg′*	**VI** *rc tg–gg′*	**VII** *rr gt–gg′*
HO-6–O-6–C-6	109.5	109.3	109.2	109.5	109.1	107.7	107.3
O-6–C-6–C-5	111.8	109.5	112.1	107.6	107.1	112.2	110.9
C-6–C-5–O-5	105.5	109.1	106.9	106.8	107.0	107.4	104.7
C-5–O-5–C-1	114.5	113.5	113.7	114.8	113.8	114.3	114.2
O-5–C-1–C-2	108.6	108.8	108.7	107.9	107.4	107.3	108.6
HO-2–O-2–C-2	107.9	107.9	108.8	107.3	110.7	111.3	108.1
C-1–O–C-4’	118.8	118.8	118.9	118.4	117.1	117.5	116.1
HO-6′–O-6′–C-6′	109.9	109.9	108.1	107.1	109.4	109.6	107.7
O-6′–C-6′–C-5′	113.8	113.8	110.9	111.9	114.5	114.4	112.4
C-6′–C-5′–O-5′	106.4	106.5	104.5	105.8	109.3	109.3	105.6
C-5′–O-5′–C-1′	113.1	113.1	113.1	113.7	111.7	112.0	113.6
O-5′–C-1′–O-1′	106.0	106.0	105.9	106.1	105.9	105.7	108.8
C-1′–O-1′–HO-1′	107.0	107.0	107.0	107.0	107.1	107.1	109.3
HO-3′–O-3′–C-3′	108.3	108.3	110.2	110.4	110.3	108.5	106.6

Conformer	**VIII** *rr gt–gt′*	**IX** *cc gg–gg′*	**X** *rc tg–tg′*	**XI** *cc tg–gg′*	**XII** *rc gt–gg′*	**XIII** *rr gg–gt′*
HO-6–O-6–C-6	107.4	109.2	107.8	109.5	109.4	107.0
O-6–C-6–C-5	110.8	109.5	112.1	108.1	111.9	111.8
C-6–C-5–O-5	104.7	109.2	107.6	105.0	106.8	104.5
C-5–O-5–C-1	114.3	113.8	113.7	114.9	114.7	116.8
O-5–C-1–C-2	108.8	107.8	108.3	107.7	108.4	108.3
HO-2–O-2–C-2	108.5	107.2	110.0	107.1	109.9	106.4
C-1–O–C-4’	116.9	117.4	119.0	117.6	117.7	117.1
HO-6′–O-6′–C-6′	107.3	107.2	109.4	107.3	107.6	107.2
O-6′–C-6′–C-5′	111.8	112.0	114.0	112.3	112.6	111.7
C-6′–C-5′–O-5′	105.7	106.1	105.4	106.2	106.7	105.8
C-5′–O-5′–C-1′	113.0	113.7	113.2	113.7	113.0	113.0
O-5′–C-1′–O-1′	109.1	105.9	105.9	105.9	106.0	109.0
C-1′–O-1′–HO-1′	109.5	107.0	107.1	107.0	107.0	109.4
HO-3′–O-3′–C-3′	106.6	108.4	108.7	108.3	110.5	106.5

structure is of lowest energy, as is seen experimentally, but those calculations were carried out using a small basis set[15] or in another study they were carried out on analogs stripped of functional groups with empirical energy terms added to the ab initio result.[26] Clearly, this work and the next paper in this series,[27] show that analogs in which the hydroxyl groups have been removed can be misleading and may result in incorrect conformations being considered to be those of lowest energy. The cooperative stabilizing effect of the hydrogen-bonded hydroxyl groups around the rings apparently is not inherent in the empirical force field used previously.[28]

When carefully examining the cellobiose structures, both as ‘normal’ and ‘flipped’ forms, it becomes evident that interactions of the side chains and ring hydroxyl groups change with the (ϕ_H, ψ_H) angles. Changes in this conformational dihedral can bring the hydroxymethyl side chains and exocyclic groups into close proximity to each other or direct these functional groups away from each other. The exocyclic hydroxyl groups and hydroxymethyl side chains present in β-cellobiose are involved in extensive hydrogen-bonding in the molecule. The hydroxyl groups can form a type of hydrogen-bonding network that can extend around the sugar residues and can include O-6 (O-6′). In addition to this hydrogen-bonding network within each residue of the disaccharide, there arises a possibility for intraresidue hydrogen bonding via the hydroxymethyl side chains. Hydrogen bonding cooperative networks are important in these systems and can easily stabilize the in vacuo conformers in which they are prevalent.

A selection of conformers was studied to explore various combinations of clockwise and anticlockwise exocyclic group orientations, with the all the hydroxyl groups of one residue oriented in the same manner. As

Table 6
Selected bond angles of 'flipped' conformers (°)

Conformer	**XIV** *cc gg–gg'*	**XV** *rr gt–tg'*	**XVI** *rr gg–gg'*	**XVII** *cc gg–gg'*	**XVIII** *rr gt–gg'*	**XIX** *rr gg–gg'*	**XX** *cc gg–gg'*
HO-6–O-6–C-6	109.7	109.8	109.7	108.8	107.9	108.7	108.8
O-6–C-6–C-5	113.4	113.5	113.6	112.8	113.1	112.9	112.8
C-6–C-5–O-5	107.6	107.1	108.0	105.5	106.2	105.8	105.5
C-5–O-5–C-1	115.8	115.4	115.0	117.5	115.6	116.8	117.5
O-5–C-1–C-2	108.8	108.8	108.8	108.0	108.5	107.9	108.0
HO-2–O-2–C-2	107.1	106.9	107.1	106.9	106.1	106.5	106.9
C-1–O–C-4'	120.7	120.4	120.4	118.9	118.2	118.9	118.9
HO-6'–O-6'–C-6'	105.0	106.8	105.4	107.9	108.6	108.4	107.9
O-6'–C-6'–C-5'	114.1	114.0	113.9	112.4	113.4	112.5	112.4
C-6'–C-5'–O-5'	107.9	104.6	108.0	105.4	106.6	105.6	105.4
C-5'–O-5'–C-1'	112.9	114.0	113.2	113.8	113.6	114.1	113.8
O-5'–C-1'–O-1'	106.2	109.2	109.1	105.8	108.9	108.9	105.8
C-1'–O-1'–HO-1'	107.0	109.2	109.1	107.1	109.3	109.3	107.0
HO-3'–O-3'–C-3'	110.2	107.1	107.2	110.1	107.0	107.1	110.1

Conformer	**XXI** *rr gg–gt'*	**XXII** *rr tg–gt'*	**XXIII** *cc gg–tg'*	**XXIV** *cc tg–gg'*	**XXV** *rr gt–gt'*	**XXVI** *rr gt–gt'*	**XXVII** *rr gg'–gt*
HO-6–O-6–C-6	107.4	107.8	109.3	109.5	109.9	109.0	107.5
O-6–C-6–C-5	112.7	102.2	109.6	108.1	112.3	108.0	112.5
C-6–C-5–O-5	106.7	107.5	108.9	104.6	106.8	107.2	106.0
C-5–O-5–C-1	114.3	113.6	114.0	114.6	113.6	113.4	114.7
O-5–C-1–C-2	109.0	109.0	106.7	107.4	108.7	108.2	107.4
HO-2–O-2–C-2	106.5	106.5	107.7	107.6	108.2	108.7	109.6
C-1–O–C-4'	120.0	119.7	120.6	119.7	119.2	119.2	120.3
HO-6'–O-6'–C-6'	107.4	107.4	109.3	107.2	108.8	108.7	107.5
O-6'–C-6'–C-5'	111.4	111.1	113.5	111.4	112.1	112.7	111.6
C-6'–C-5'–O-5'	105.7	105.9	106.7	105.9	106.9	108.1	106.2
C-5'–O-5'–C-1'	113.5	113.4	113.4	113.7	113.4	113.6	113.8
O-5'–C-1'–O-1'	109.2	109.2	106.0	105.9	109.0	108.6	109.0
C-1'–O-1'–HO-1'	109.4	109.4	107.1	107.1	107.4	108.7	109.4
HO-3'–O-3'–C-3'	107.0	107.0	108.1	107.9	107.2	107.0	107.1

mentioned above, the hydroxyl groups in the 'flipped' conformation with *cc'* orientation of the exocylcic hydroxyl groups can achieve a type of cooperative stabilizing hydrogen-bonding network around the disaccharide unit. This stabilizing effect is important to the overall energetics of the molecule; however, there did not seem to be a clear relationship between the amount of stabilization and the orientation of the exocyclic hydroxyl groups on one or both sugar units. Superficially, it appears as though the 'flipped' structures are stabilized if the exocyclic groups are in a counterclockwise orientation, in part a result of favorable electrostatic interactions between HO-1' and O-5'. However, since the hydroxyl groups are predominately involved in a hydrogen-bonding network around one sugar residue, the hydrogen-bond network can be achieved regardless of the orientation these groups. Thus we suggest a more long-range cooperativity must be at work between sugar rings. The overall orientation of these exocyclic groups in the ring, therefore, has only a minor contribution to the overall stabilization of the structure. The cooperative hydrogen-bonding effect described here is examined in detail using model analogs in the following paper.[27]

The hydroxymethyl side chains are equally important to the overall stability of the structure. These side chains are readily involved in hydrogen bonding both as part of the hydroxyl net and across the residue to either another side chain or hydroxyl group. When the cellobiose structures are in the 'flipped'conformation, the side chains are brought in close proximity. In the proper orientation, the hydrogen-bonding interaction between side chains of the reducing and nonreducing units can extend the hydrogen-bonding network nearly completely around the disaccharide. In conformer **XIX**, the cross-residue hydrogen bond distance is 2.0 Å, while

Table 7
Selected dihedral angles of regular conformers (°)

Conformer	**I** *cc tg–tg′*	**II** *cc gg–tg′*	**III** *rc gt–tg′*	**IV** *cc gt–gg′*	**V** *rc gt–gg′*	**VI** *rc tg–gg′*	**VII** *rr gt–gg′*
O-6–C-6–C-5–O-5	−177.0	159.3	59.8	64.7	64.0	166.0	55.0
HO-6–O-6–C-6–C-5	−87.3	147.4	77.5	−173.7	179.5	52.6	−52.2
C-4–C-5–O-5–C-1	60.6	63.7	60.9	92.7	62.6	60.7	63.5
C-5–O-5–C-1–C-2	−61.1	−61.3	−62.1	−62.2	−60.2	−56.8	−66.0
HO-4–O-4–C-4–C-5(C-3)	−53.2	−86.0	49.7	−90.7	48.1	49.2	52.3
HO-3–O-3–C-3–C-4(C-2)	56.4	57.1	−54.1	61.3	−56.9	−57.8	−57.2
HO-2–O-2–C-2–C-3(C-1)	−51.7	−53.0	54.7	−50.6	46.8	47.6	70.9
H-1–C-1–O–C-4′	30.2	31.7	29.9	32.8	19.7	17.8	46.0
C-1–O–C-4′–H-4′	−23.4	−23.1	−30.6	−28.2	−41.1	−36.7	−7.2
HO-6′–O-6′–C-6′–C-5′	76.0	75.8	−156.2	58.2	17.8	72.0	59.4
O-6′–C-6′–C-5′–O-5′	157.6	157.5	164.5	−58.1	−82.4	−81.7	−61.7
C-4′–C-5′–O-5′–C-1′	63.6	63.4	61.1	58.9	65.4	65.6	59.3
C-5′–O-5′–C-1′–C-2′	−65.2	−64.9	−65.2	−64.2	−62.4	−61.9	−62.8
HO-1′–O-1′–C-1′–C-2′(O-5′)	44.2	43.9	40.1	39.9	43.3	45.3	−66.4
HO-2′–O-2′–C-2′–C-3′(C-1′)	−54.1	−53.5	−50.7	−51.6	−51.9	−53.9	63.4
HO-3′–O-3′–C-3′–C-4′(C-2′)	54.6	53.2	67.4	59.6	72.8	62.5	−51.6

Conformer	**VIII** *rr gt–gt′*	**IX** *cc gg–gg′*	**X** *rc tg–tg′*	**XI** *cc tg–gg′*	**XII** *rc gt–gg′*	**XIII** *rr gg–gt′*
O-6–C-6–C-5–O-5	55.6	−78.8	166.2	−176.5	59.1	−51.8
HO-6–O-6–C-6–C-5	−52.1	163.7	52.2	−179.9	79.4	55.2
C-4–C-5–O-5–C-1	63.7	64.5	61.6	60.9	62.3	56.3
C-5–O-5–C-1–C-2	−65.5	−61.5	−60.1	−61.8	−62.7	−57.1
HO-4–O-4–C-4–C-5(C-3)	52.5	−81.4	49.7	−50.0	51.6	55.0
HO-3–O-3–C-3–C-4(C-2)	−57.2	56.8	−55.8	56.6	−49.6	−56.1
HO-2–O-2–C-2–C-3(C-1)	71.7	−51.0	57.8	−48.6	−41.2	68.3
H-1–C-1–O–C-4′	45.3	39.4	28.5	36.7	29.0	62.0
C-1–O–C-4′–H-4′	−10.0	−13.0	−25.2	−13.3	−33.0	3.5
HO-6′–O-6′–C-6′–C-5′	−58.8	59.9	−70.0	60.3	62.6	−57.5
O-6′–C-6′–C-5′–O-5′	62.2	−59.8	164.5	−59.8	−64.9	60.62
C-4′–C-5′–O-5′–C-1′	61.0	60.0	61.8	60.1	61.9	62.2
C-5′–O-5′–C-1′–C-2′	−64.5	−63.5	−64.3	−63.6	−64.1	−63.5
HO-1′–O-1′–C-1′–C-2′(O-5′)	−68.9	42.4	40.8	42.2	41.9	−68.5
HO-2′–O-2′–C-2′–C-3′(C-1′)	63.0	−52.3	−53.4	−53.2	−50.7	62.3
HO-3′–O-3′–C-3′–C-4′(C-2′)	51.1	44.6	55.9	44.6	70.3	−51.7

in conformer **X** there is no cross-residue hydrogen bonding from the hydroxymethyl groups (Tables 9 and 10). When a strong hydrogen bond between the residues exists, particularly via these side-chain interactions, the overall electronic energy of the structure decreases significantly more than one expects just from the addition of a single hydrogen bond. This intraresidue hydrogen bonding need not occur only via the hydroxymethyl side chain, for it can also occur for example between O-2 and O-3′, i.e., via the hydroxyl groups of the reducing and nonreducing rings. Although it appears that the interactions of the hydroxymethyl side chains contribute considerably to the overall stability of the molecule, the ability to achieve a direct synergistic hydrogen-bonding network is the crucial aspect for energetic stability.

From these calculations we believe that the following conclusion can be drawn. The overall electronic energy of a disaccharide conformer is lowered proportionally with the number, strength and synergistic effect of the hydrogen bonds that are present. Conversely, structures with little or no hydrogen-bonding possibilities will be much higher in electronic energy. Although extensive hydrogen-bonding will dictate the motional freedom within a molecule and hence affect the entropy contribution to the free energy in these conformers, it is not enough to overcome the cooperative electronic energy difference of the

molecules and so mirrors the electronic energy conformational trends.

An example of the synergistic effects of the above-described intraresidue hydrogen-bonding phenomenon is found in conformations **XIV** and **XX**. These two 'flipped' conformers are identical except for a slight rotation of the hydroxymethyl side chain away from O-5′ in structure **XX**. The intraresidue hydrogen bond is therefore 1.96 Å in **XX** and 2.26 Å in **XIV**. Other exocyclic configurations are similar for these two conformers, yet the energy difference is ~ 5 kcal/mol in favor of **XX**. The hydroxymethyl side chain and the ability to extend the hydrogen-bonding network are very important and can drive the conformational preferences of cellobiose.

Those conformers that are 'flipped' in ψ_H also have the opportunity for intraresidue hydrogen bonding to occur since the hydroxymethyl side chains are on the same side of the molecule. However, these structures are more bent at the glycosidic linkage, thus making it difficult for the side chains to get close enough to hydrogen bond. In this series, the shortest hydrogen-bond distance between side chains is 2.01 Å (Table 8).

When the side chains can hydrogen bond to each other, the conformation is stabilized and the electronic energy is lowered. Hence, conformer **XXV** is the lowest energy structure 'flipped' in ψ_H. Conformer **XXIII**, on the other hand, does not have any possibility for this cross-ring communication because HO-6′ of the reducing ring is pointing towards O-5 instead of the side

Table 8
Selected dihedral angles of 'flipped' conformers (deg.).

Conformer	**XIV** *cc gg–gg′*	**XV** *rr gt–tg′*	**XVI** *rr gg–gg′*	**XVII** *cc gg–gg′*	**XVIII** *rr gt–gg′*	**XIX** *rr gg–gg′*	**XX** *cc gg–gg′*
O-6–C-6–C-5–O-5	−68.0	65.3	−62.8	−63.1	54.2	−59.1	−63.2
HO-6–O-6–C-6–C-5	88.2	−74.3	88.5	71.5	−73.1	68.9	71.6
C-4–C-5–O-5–C-1	61.5	60.0	60.0	55.5	56.1	54.0	55.4
C-5–O-5–C-1–C-2	−60.2	−62.4	−61.6	−56.0	−63.1	−57.3	−55.9
HO-4–O-4–C-4–C-5(C-3)	−88.9	49.6	54.5	−87.3	51.4	54.7	−87.4
HO-3–O-3–C-3–C-4(C-2)	56.8	−53.3	−52.7	55.8	−50.9	−51.3	55.8
HO-2–O-2–C-2–C-3(C-1)	−47.4	74.6	75.9	−44.9	78.4	78.0	−44.9
H-1–C-1–O–C-4′	−178.3	−177.8	−177.0	179.1	169.5	−179.4	179.0
C-1–O–C-4′–H-4′	0.2	1.3	−0.8	0.1	−3.9	−0.6	0.2
HO-6′–O-6′–C-6′–C-5′	−71.2	65.1	−74.4	58.5	61.5	59.2	58.5
O-6′–C-6′–C-5′–O-5′	−76.5	162.5	−74.9	−59.0	−58.5	−59.0	−59.0
C-4′–C-5′–O-5′–C-1′	63.3	61.6	62.7	61.0	61.0	59.9	61.0
C-5′–O-5′–C-1′–C-2′	65.6	−64.6	63.7	63.5	60.9	61.9	−63.4
HO-1′–O-1′–C-1′–C-2′(O-5′)	41.7	178.5	−59.9	42.1	−66.4	−66.4	42.3
HO-2′–O-2′–C-2′–C-3′(C-1′)	53.5	−178.3	62.1	−51.5	62.8	62.9	−51.3
HO-3′–O-3′–C-3′–C-4′(C-2′)	57.6	−172.2	−48.4	58.1	−47.1	−48.3	58.0

Conformer	**XXI** *rr gg–gt′*	**XXII** *rr tg–gt′*	**XXIII** *cc gg–tg′*	**XXIV** *cc tg–gt′*	**XXV** *rr gt–gt′*	**XXVI** *rr gt–gt′*	**XXVII** *rr gg–gt′*
O-6–C-6–C-5–O-5	−59.0	166.4	−80.3	−176.8	65.6	64.7	−58.9
HO-6–O-6–C-6–C-5	61.2	50.9	154.4	−179.5	73−.5	172.8	58.0
C-4–C-5–O-5–C-1	59.5	62.2	64.6	60.1	63.2	62.1	57.8
C-5–O-5–C-1–C-2	−62.1	−62.9	−61.8	−60.4	−63.3	−61.8	−58.8
HO-4–O-4–C-4–C-5(C-3)	54.1	50.2	−84.6	−49.4	50.3	49.0	54.7
HO-3–O-3–C-3–C-4(C-2)	−52.5	−53.1	55.8	55.3	−57.1	−56.0	−54.9
HO-2–O-2–C-2–C-3(C-1)	78.1	77.9	−50.6	−49.4	53.4	50.0	52.0
H-1–C-1–O–C-4′	179.2	178.8	7.3	11.9	35.3	24.2	12.6
C-1–O–C-4′–H-4′	−3.0	−2.4	−176.8	−179.3	173.4	173.4	179.1
HO-6′–O-6′–C-6′–C-5′	−58.8	−59.3	57.7	−56.0	−65.4	78.3	−60.0
O-6′–C-6′–C-5′–O-5′	61.9	61.2	148.5	62.0	79.5	87.4	61.3
C-4′–C-5′–O-5′–C-1′	62.5	62.8	62.8	61.6	60.8	62.0	61.4
C-5′–O-5′–C-1′–C-2′	63.4	63.7	64.8	−64.0	−63.3	−63.9	−63.4
HO-1′–O-1′–C-1′–C-2′(O-5′)	−68.3	−68.0	42.0	41.8	−66.9	−59.2	−67.7
HO-2′–O-2′–C-2′–C-3′(C-1′)	32.4	62.0	−52.9	−52.0	61.9	61.4	62.6
HO-3′–O-3′–C-3′–C-4′(C-2′)	−47.5	−47.6	71.1	69.9	−51.9	−49.4	−50.1

Table 9
Selected hydrogen bond lengths of regular conformers (Å)

Conformer	I	II	III	IV	V	VI	VII
O-6–O-4	2.05	2.05	–	–	–	2.06	–
O-4–O-3	2.47	2.47	2.40	2.50	2.37	2.37	2.43
O-3–O-2	2.42	2.42	2.49	2.39	2.51	2.53	2.48
O-6–O-5	–	–	–	–	–	–	2.24
O-3′–O-2′	2.46	2.45	2.41	2.38	2.42	2.47	2.35
O-1′–O-2′	2.36	2.36	2.34	2.33	2.37	2.38	2.54
O-6′–O-5′	–	–	–	2.37	–	–	2.44
O-2′–O-6′	1.99	2.00	1.95	–	2.17	2.06	–
O-3′–O-5′	1.94	1.92	2.04	2.01	2.13	2.03	–
O-3′–O-6′	–	–	2.6	2.49	2.56	–	2.36

Conformer	VIII	IX	X	XI	XII	XIII
O-6–O-4	–	2.88	2.06	1.99	–	–
O-4–O-3	2.43	2.47	2.38	2.45	2.43	2.44
O-3–O-2	2.48	2.41	2.50	2.37	2.45	2.46
O-6–O-5	2.25	–	–	–	–	2.25
O-3′–O-2′	2.34	2.43	2.45	2.44	2.39	2.36
O-1′–O-2′	2.54	2.34	2.35	2.34	2.35	2.55
O-6′–O-5′	2.42	2.42	–	2.42	–	2.39
O-2′–O-6′	–	–	1.91	–	3.41	–
O-3′–O-5′	–	1.94	1.95	1.95	2.07	–
O-3′–O-6′	2.30	–	–	–	2.45	2.68

chain. Structure **XXIV** also lacks hydrogen-bonding opportunities via hydroxymethyl side chain interactions between sugar residues, yet it is lower in energy than **XXIII** by about 3.5 kcal/mol. One reason for this may be the synergistic participation of the side chain of the nonreducing ring in the hydrogen-bonding network formed by the exocyclic hydroxyl groups. The hydrogen-bond distance from side chain to nearest hydroxyl group is 1.98 Å. In **XXIII**, the corresponding bond distance is 2.8 Å, and the side chain is not as involved in the exocyclic hydroxyl network.

Conformer **X** is the lowest energy 'normal' structure. It is 2.7 kcal/mol lower in energy than **XII**, the highest energy structure. The main reason for the difference in energy lies in the hydrogen-bonding network capabilities. In structure **X**, the exocyclic hydroxyl groups and cross-chain interactions form a very complete network. The hydroxymethyl side chain hydrogen, OH-6, points directly at O-4 to add to the synergistic network. Not only is there a continuous hydrogen bond network around each residue via the exocyclic hydroxyl groups, but also there is a strong hydrogen bond between H-5 and O-6′ of 1.91 Å. In conformer **XII**, not only is HO-6 pointing away from O-4 and hence cannot contribute to the hydrogen-bonding network, but also O-6′–HO-6′ is pointing away from the nonreducing unit and so the hydrogen bond between the residues is weak at a distance of 3.41 Å. The three lowest energy 'normal' structures are respectively, **X**, **VI** and **XI**, and all have the side chain on the non-reducing unit taking part in the exocyclic hydroxyl hydrogen-bond network.

Most of the structures studied that are 'flipped' in the conformational angle ϕ_H, can participate in strong cross-ring hydrogen-bonding interactions between the hydroxymethyl side chains on both sugar residues. The lowest electronic structure, **XIX**, has strong hydrogen bonding and communication between the hydroxymethyl side chains (2 Å) and further, both interact simultaneously with their respective ether ring oxygen at the O-5 and O-5′ positions. Conformer **XVIII** is the next lowest energy conformer with a distance of 2.03 Å between hydroxymethyl side chains. The two structures that do not have this side chain interaction, **XXII** and **XXI**, are slightly higher in energy than the others. The ability to hydrogen bond via the hydroxymethyl side chain completes the cooperative synergistic effect of the hydrogen-bond network that encompasses the disaccharide.

Table 10
Selected hydrogen bond lengths of 'flipped' conformers (Å)

Conformer	XIV	XV	XVI	XVII	XVIII	XIX	XX
O-6–O-4	–	–	–	–	–	2.64	–
O-4–O-3	2.46	2.40	2.44	2.46	2.43	2.44	2.45
O-3–O-2	2.35	2.43	2.41	2.33	2.40	2.39	2.33
O-6–O-5	2.87	2.67	2.84	2.58	2.52	2.52	2.58
O-3′–O-2′	2.43	2.34	2.33	2.41	2.32	2.32	2.41
O-1′–O-2′	2.35	2.55	2.56	2.36	2.56	2.56	2.36
O-6′–O-5′	–	–	–	2.41	2.48	2.42	2.40
O-2′–O-3′	1.97	1.89	1.87	1.99	2.04	1.94	1.99
O-6′–O-6′	2.26	2.27	2.27	1.96	2.03	2.00	1.96
O-5′–O-6′	1.83	1.85	1.83	–	–	–	–

Conformer	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII
O-6–O-4		2.06	2.80	1.98	–	–	2.62
O-4–O-3	2.45	2.40	2.47	2.45	2.40	2.39	2.44
O-3–O-2	2.41	2.44	2.40	2.39	2.52	2.52	2.48
O-6–O-5	2.45	–	–	–	2.65	–	2.4
O-3′–O-2′	2.31	2.31	2.44	2.44	2.41	2.40	2.40
O-1′–O-2′	2.56	2.55	2.35	2.35	2.53	2.54	2.55
O-1′–O-5′	–	–	–	–	2.53	2.46	2.54
O-6′–O-5′	2.41	2.40	–	2.39	2.75	–	2.43
O-2′–O-3′	1.90	1.91	1.94	2.01	2.91	2.42	2.11
O-6′–O-6′	–	–	–	–	2.05	2.01	–
O-5′–O-6′	–	–	1.94	–	–	2.83	–

5. Conclusions

This paper reports a very complete set of data at the B3LYP/6-311++G** level of theory for β-cellobiose. From these calculations, the lowest energy structure is a structure 'flipped' in the dihedral angle ϕ_H, conformer **XIX**. In general, those conformers with ϕ_H approaching 180° are lower in electronic and free energy than either the 'normal' form or the structures 'flipped' in ψ_H. One reason for this stabilization is the presence of a cooperative hydrogen-bonding network across the entire disaccharide that is facilitated by the close proximity of the hydroxymethyl side chains and ring hydroxyl groups in the structures 'flipped' in ϕ_H. This cooperative 'network' of hydrogen bonds that exists in cellobiose conformers 'flipped' in ϕ may influence many properties of cellulosic materials; in particular it may be involved in how cellulose is solvated.

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