CONFORMATIONAL PREFERENCES OF ETHYL 2,3-DIDEOXY-3-[(α-D-GLUCOPYRANOSYL)METHYL]-β-L- AND -D-*arabino*-HEXOPYRANOSIDES

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Received April 1, 2005
Accepted July 19, 2005

Conformational behavior of two *C*-disaccharides, containing D-glucopyranose moiety at the non-reducing end and L- or D-2-deoxy-*arabino*-hexopyranose moiety at the reducing end, has been studied using MM3 calculations and NMR experiments. The obtained results show that the conformational preference around the *C*-glycosidic bond is the same in both compounds and corresponds with the *exo*-anomeric effect. On the other hand, both compounds differ markedly in the conformational arrangement around the *C*-aglycone bond where the population of conformers is controlled by 1,3-diaxial-like interactions.

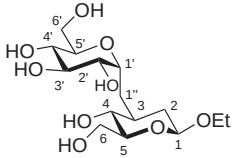
Keywords: Carbohydrates; *C*-Glycosides; *C*-Disaccharides; Conformational analysis; NMR spectroscopy; MM3 calculations.

Cell-surface oligosaccharides, in the form of glycoproteins or glycolipids, play a crucial role in processes by which cells communicate with each other or with e.g. pathogenic microorganisms[1]. In recent years great effort has been devoted to the synthesis of compounds in which the glycosidic oxygen atom in an oligosaccharide molecule is replaced with methylene group. Unlike natural oligosaccharides, such modified compounds (called *C*-oligosaccharides) are resistant against enzymic hydrolysis. It is assumed that these compounds, as non-hydrolyzable carbohydrate mimics, could disturb glycoprotein biosynthesis and/or intercell communication, which in favorable cases could lead to their therapeutic use[2].

However, the replacement of the glycosidic oxygen atom by a methylene group results in a change of the size and electronic properties of the glycosidic linkage, which in natural oligosaccharides and corresponding

C-oligosaccharides may manifest itself in a different population of preferred conformers around the glycosidic and aglycone bonds. Therefore a question arises how closely e.g. *C*-disaccharides simulate the conformational behavior of natural disaccharides. The problem was intensively studied mainly by groups of Kishi[3] and Jimenéz-Barbero[4]. Studies published so far have shown that the *C*-glycosyl analogues are more flexible than corresponding natural glycosides. Nevertheless, both the *C*-glycosidic and *O*-glycosidic bonds in disaccharides qualitatively prefer the same conformer which corresponds with the *exo*-anomeric effect.

In this paper we present results obtained by study of conformational preferences in α-(1→3)-*C*-disaccharides **1** and **2** in which D-glucopyranose is linked with L- or D-2-deoxy-*arabino*-hexopyranose moiety. The studied compounds **1** and **2** were prepared by Zemplén deacetylation of the corresponding peracetyl derivatives as described by us recently[5].



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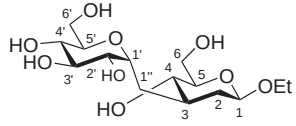

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Conformational preferences in several other α-(1→3)-*C*-disaccharides have been estimated. The Kishi[6] and Vogel[7,8] groups have studied the conformational behavior of α-(1→3)-*C*-disaccharides containing D-galactopyranose or D-mannopyranose at the non-reducing end. It has been found that configuration on C-2 of a non-reducing monosaccharide (galactose vs mannose) has no significant influence on conformation around the *C*-glycosidic bond, and it was confirmed that in all cases the *C*-glycosidic bond prefers the *+sc* conformer (see Appendix) that corresponds with the *exo*-anomeric effect. On the other hand, the conformation around the *C*-aglycone bond strongly depends mainly on the position of hydroxy group at C-4 of the reducing saccharide. When this group is axial, like in α-D-Gal*p*-(1→3)-*C*-D-Gal*p* [3,6] or in α-D-Man*p*-(1→3)-*C*-D-GalNAc or α-D-Man*p*-(1→3)-*C*-D-TalNAc [8], then the *C*-disaccharide markedly prefers the *ap* conformer **A** shown in Fig. 1. In the case of equatorial hydroxy group the very unfavorable destabilizing 1,3-diaxial-like interactions between C_4-OH and *C*-glycosidic bond lead to preferred conformers **B** (*–sc*) in α-D-Gal*p*-(1→3)-*C*-D-Man*p* [3,7] and **C** (*+sc*) in α-D-Gal*p*-(1→3)-*C*-D-Glc*p* [3,6] (Fig. 1).

Having in hands the compounds **1** and **2** containing 2-deoxyhexopyranose (moreover also in the L-configuration) at the reducing end we decided to find out whether their conformational population will be in accord with the same rules that have been hitherto observed for other α-(1→3)-*C*-disaccharides[3,6–8] (i.e. the preference of such conformation around the C-glycosidic bond that corresponds with the *exo*-anomeric effect, the destabilizing influence of 1,3-diaxial-like interactions, etc.).

Appendix

Herein we would like to remark on considerable differences in the description of conformations in *C*-disaccharides. The relevant papers make use of analogy with the structure of natural disaccharides and in accord with Recommendations of IUPAC-IUB Joint Commission on Biochemical Nomenclature[9] designate the torsion angle around the *C*-glycosidic bond as ϕ whereas that around the *C*-aglycone bond as ψ. For a qualitative description of conformers arising by rotation around the *C*-glycosidic bond in (1→*n*)-*C*-disaccharides a notation describing the three possible staggered conformations as *exo*/*anti*, *nonexo*/*gauche* and *exo*/*gauche* is often used[3,4]. The three possible staggered conformations around the *C*-aglycone bond are then denoted e.g. as *C*-*anti*-C_{n-1}, *C*-*anti*-C_{n+1} and *C*-*anti*-*H* [3]. Other authors describe the conformers by exact values of torsion angles ϕ and ψ; unfortunately, different authors specify these torsion angles using different reference atoms and this inconsistency thus complicates comparison of results from different authors and laboratories.

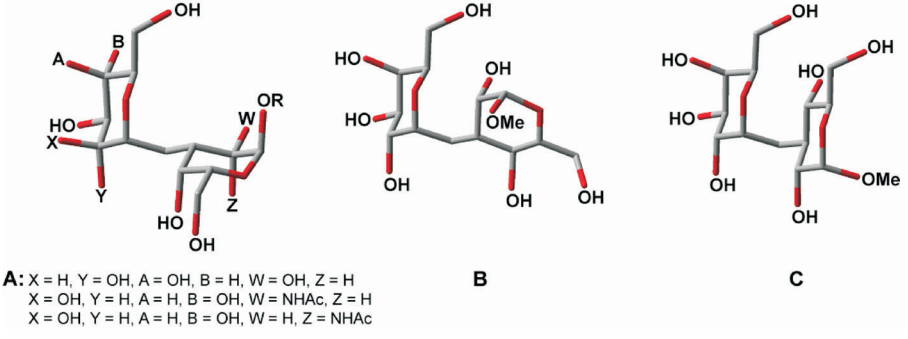

FIG. 1
Preferred conformers of known α-(1→3)-*C*-disaccharides

In the present paper we shall adhere to the Recommendations of IUPAC-IUB Joint Commission on Biochemical Nomenclature on symbols for specifying the conformation of polysaccharide chains[9], according to which "two torsion angles, ϕ and ψ, are required to describe the glycosidic bond from the (*i*)th unit to a carbon atom located in the ring of the (*i*–1)th unit. The angle ϕ about the bond from the anomeric carbon to the oxygen that joins the two residues is specified using the ring oxygen as a reference atom. The torsion angle ψ about the bond from the glycosylated oxygen of the (*i*–1)th residue to a carbon of this residue uses the carbon atom one lower in numbering as a reference atom". In accord with these recommendations, in the case of our (1→3)-*C*-disaccharides **1** and **2** the conformation around the *C*-glycosidic bond is defined by torsion angle ϕ (O5′–C1′–C1″–C3) and the conformation around the *C*-aglycone bond by torsion angle ψ (C1′–C1″–C3–C2). For a qualitative description of staggered conformers by torsion angles ϕ and ψ we shall use the usual recommended symbols, i.e. *+sc*, *–sc* and *ap*.

EXPERIMENTAL

Materials

Ethyl 2,3-dideoxy-3-[(α-D-glucopyranosyl)methyl]-β-L-arabino-hexopyranoside (**1**). Ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)methyl]-β-L-*arabino*-hexopyranoside[5] (150 mg, 0.25 mmol) was dissolved in methanol (11 ml) and then a 1 M solution of MeONa (1.3 ml) was added. After 40 min the reaction mixture was neutralized with methanolic suspension of Dowex (H^+ form). After filtration and evaporation, the residue was chromatographed (ethyl acetate–methanol 2:1), affording 84 mg (96%) of product, R_F 0.20 (chloroform–methanol 3:1). $[\alpha]_D$ +83.4 (*c* 1, CH_3OH). ^{1}H NMR (CD_3OD): 4.58 dd, 1 H, *J*(1,2eq) = 1.2, *J*(1,2ax) = 9.3 (H-1); 4.05 ddd, 1 H, *J*(1′,2′) = 2.5, *J*(1′,1″*proR*) = 2.8, *J*(1′,1″*proS*) = 12.8 (H-1′); 3.97 dq, 1 H, *J* = 7.1, *J* = 9.5 (-O-C**H**$_2$-CH_3); 3.82–3.94 m, 2 H (H-6a, H-6′a); 3.72 dd, 1 H, *J*(5,6b) = 5.9, *J*(6a,6b) = 11.7 (H-6b); 3.55–3.68 m, 4 H (H-6′b, H-2′, H-5′, -O-C**H**$_2$-CH_3); 3.52 m, 1 H (H-3′); 3.31 m, 1 H (H-5); 3.26 dd, 1 H, *J*(3′,4′) = 9.4, *J*(4′,5′) = 9.4 (H-4′); 3.07 dd, 1 H, *J*(3,4) = 9.7, *J*(4,5) = 9.7 (H-4); 2.34 ddd, 1 H, *J*(3,1″*proS*) = 2.4, *J*(1′,1″*proS*) = 12.8, *J*(1″*proR*,1″*proS*) = 12.8 (H-1″*proS*); 2.08 brd, 1 H, *J*(2ax,2eq) = 12.8, (H-2eq); 1.77 m, 1 H (H-3); 1.32 ddd, 1 H, *J*(1′,1″*proR*) = 2.8, *J*(3,1″*proR*) = 12.7, *J*(1″*proS*,1″*proR*) = 12.8 (H-1″*proR*); 1.21 t, 3 H, *J* = 7.1 (C**H**$_3$$CH_2$); 1.16 ddd, 1 H, *J*(1,2ax) = 9.3, *J*(2,3) = 9.8, *J*(2ax,2eq) = 12.8 (H-2ax). ^{13}C NMR (125 MHz, $CDCl_3$): 102.49 (C-1); 80.92 (C-5); 75.25, 72.92 (C-2′, C-5′); 74.14 (C-3′); 73.35 (C-1′); 72.48 (C-4′); 71.38 (C-4); 65.32 (-O-CH_2-CH_3); 63.40, 63.28 (C-6, C-6′); 37.68 (C-3); 36.58 (C-2); 27.67 (C-1″); 15.48 (-O-CH_2-CH_3). MS (FAB): 375.1 (M + Na)$^+$. HRMS (FAB) calculated for $C_{15}H_{28}NaO_9$ (M + Na)$^+$: 375.163103, found: 375.162435.

Ethyl 2,3-dideoxy-3-[(α-D-glucopyranosyl)methyl]-β-D-arabino-hexopyranoside (**2**). In the same manner as described in the preceding experiment, 150 mg of ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)methyl]-β-D-*arabino*-hexopyrano-

side[5] was converted into 85 mg (96%) of the title compound, R_F 0.20 (chloroform–methanol 3:1). $[\alpha]_D$ +15.4 (*c* 1, CH_3OH). ^1H NMR (CD_3OD): 4.57 dd, 1 H, *J*(1,2eq) = 1.1, *J*(1,2ax) = 9.6 (H-1); 4.18 ddd, 1 H, *J*(1′,1″*proR*) = 4.5, *J*(1′,2′) = 5.4, *J*(1′,1″*proS*) = 10.1 (H-1′); 3.96 dq, 1 H, *J* = 7.2, *J* = 9.5 (-O-C**H**$_2$-CH_3); 3.78–3.90 m, 2 H (H-6a, H-6′a); 3.63–3.71 m, 2 H (H-6b, H-6′b); 3.49–3.61 m, 4 H (-O-C**H**$_2$-CH_3, H-2′, H-3′, H-5′); 3.18–3.33 m, 3 H (H-4, H-5, H-4′); 1.98–2.13 m, 2 H (H-2eq, H-1″*proR*); 1.81 m, 1 H (H-3); 1.69 ddd, 1 H, *J*(3,1″*proS*) = 6.2, *J*(2′,1″*proS*) = 10.1, *J*(1″*proS*,1″*proR*) = 12.8 (H-1″*proS*); 1.35 ddd, 1 H, *J*(1,2ax) = 9.6, *J*(3,2ax) = 9.6, *J*(2eq,2ax) = 12.9 (H-2ax); 1.21 t, 3 H, *J* = 7.1 (C**H**$_3$$CH_2$). ^{13}C NMR (125 MHz, $CDCl_3$): 102.44 (C-1); 81.02 (C-4); 76.53 (C-1′); 74.95, 74.76 (C-3, C-5′); 73.08 (C-2); 72.44 (C-5); 71.68 (C-4′); 65.28 (-O-C**H**$_2$-CH_3); 63.34, 63.12 (C-6, C-6′); 40.33 (C-3); 38.25 (C-2); 28.30 (C-1″); 15.47 (-O-CH_2-C**H**$_3$). MS (FAB): 375.1 $(M + Na)^+$. HRMS (FAB) calculated for $C_{15}H_{28}NaO_9$ $(M + Na)^+$: 375.163103, found: 375.162253.

MM3 Calculations

MM3(1996)[10] was implemented on a Linux-based PC. A single minimization took about 0.5 s. In the systematic mapping of conformational space, we considered three possible starting orientations (*–sc*, *+sc*, *ap*) for each hydroxymethyl group and two orientations (*c* = clockwise or *r* = anticlockwise) for each secondary hydroxy group. For the glycosidic as well as aglycone bonds three starting orientations for the anomeric ethoxy group were considered. All combinations of these starting orientations were taken into account. Adiabatic maps for torsions ϕ and ψ were constructed in 20° steps, which gives 104 976 starting geometries for each structure. In all calculations the dielectric permittivity (ε) was set at 4.0. The convergent criterion was based on energy differences between two subsequent optimization steps and the optimization was terminated when ΔE was smaller than $0.00008N$ kcal/mol, where *N* is the number of atoms in the molecule.

NMR Experiments

The spectra were taken on a Bruker DRX 500 Avance spectrometer at 500.1 MHz for ^1H and 125.8 MHz for ^{13}C. The measurements were performed at 298 K in methanol-d_4. Chemical shifts in ppm are referenced to Me_4Si and *J* values are given in Hz. ^1H NMR spectra were measured with a spectral width of 7500 Hz, data size 32 K, recycle time 3.1 s, and 16 scans. ^{13}C NMR spectra were measured with a spectral width of 26.5 kHz, data size 32 K, recycle time 2.6 s, and 3000 scans. The spin systems were identified by 2D COSY (128 t_1-increments of 1024 data points, 16 scans, spectral width 3000 Hz) and by ^1H-^{13}C HMQC (128 t_1-increments, spectral widths 3000 Hz in ^1H and 23.7 kHz in ^{13}C dimensions, 16 scans, polarisation transfer delay 3.5 ms). 1D ^1H DPFGSE-NOE experiment was performed using selective q3-Gaussian-cascade of 79.2 ms, the mixing time was 1 s. Typical $\pi/2$-pulses were 9.5 μs for ^1H and 12 μs for ^{13}C.

RESULTS AND DISCUSSION

Conformational behavior of compounds **1** and **2** was studied using MM3[11] molecular mechanics calculations. The MM3 force field is very suitable for saccharide modelling[12], because it contains inter alia also explicite terms for the hydrogen bond and an updated parametrization for the anomeric

grouping. However, there are certain problems with the hydroxymethyl group[13] and, for example, with saccharose.

The validity of the calculations has been then tested by NMR experiments. The assignment of the resonances was made by combination of ^{1}H, ^{13}C, COSY and HMQC experiments. In a few cases where the resonances were overlapped by other protons, proton homodecoupling was used for determination of the key coupling constants.

*Conformational Analysis of **1***

Combination of three staggered conformations around the *C*-glycoside bond (ϕ = +*sc*, –*sc* and *ap*) and three staggered conformations around the *C*-aglycone bond (ψ = +*sc*, –*sc* and *ap*) affords for the compound **1** nine ideal conformers. From the molecular models it is clear already at the first sight that combinations with ϕ = –*sc* lead to crowded conformers and consequently their population must be negligible.

The results of MM3 calculation for compound **1** are summarized in the adiabatic contour map given in Fig. 2.

The contour map shows that up to the 2 kcal/mol level, two main local minimum regions exist. The first, **1A** corresponding to torsion angles ϕ = +56° (+*sc*) and ψ = +61° (+*sc*), represents the global minimum (Figs 2 and 3). The second minimum corresponds to the conformer **1B** with torsion angles ϕ = +78° (+*sc*) and ψ = +166° (*ap*). The geometry of this conformer allows

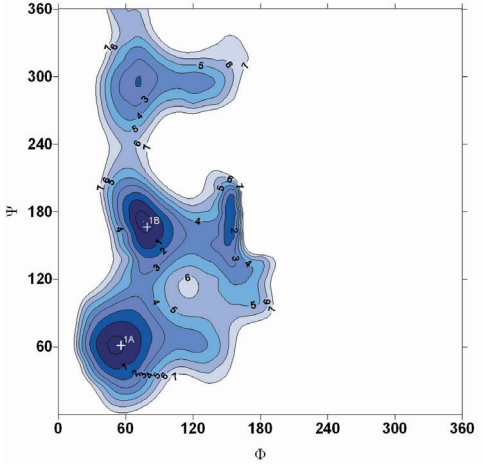

FIG. 2
Adiabatic contour map for compound **1**

the formation of intramolecular hydrogen bond C4–OH···O–C6′. In both the local minima there is the same +*sc* arrangement of hydroxymethyl groups around bonds C5–C6 and C5′–C6′, and also the +*sc* orientation of the anomeric ethoxy group is practically identical (+77 and +78°, respectively).

Assuming negligible entropy differences between different conformers, the population of the (*i*)th conformer P_i depends on its relative energy E_i according to the relation

$$P_i = \exp(-E_i/RT)/\Sigma[\exp(-E_i/RT)] .$$

Using the above equation, MM3 calculations afforded the values 53 and 42% for the relative proportions of the energy region around the conformer **1A** and **1B**, respectively, and thus compound **1** shoud exist as a mixture of conformers **1A** and **1B** in the ratio about 5:4.

The ^{1}H NMR spectrum of compound **1** exhibits well-separated and discerned signals of protons in positions 1, 1′, 1″, 2, 3 and 4. Combination of coupling constants and NOE enabled us to obtain sufficient amount of data for conformational analysis. For estimation of the preferred conformation, the coupling constants of protons H-1′ and H-3 with two diastereotopic protons H -1a″ (2.34 ppm) and H-1b″ (1.32 ppm) are important. Proton H-1′ exhibits strong coupling (J = 12.8) with proton H-1a″ and weak coupling (J = 2.8) with proton H-1b″. On the other hand, proton H-3 shows weak coupling (J = 2.4) with proton H-1a″ and strong coupling (J = 12.7) with proton H-1b″. The mentioned coupling constants are very close to the theoretical values for ideal *ap* and *sc* arrangements of vicinal protons and suggest that in methanolic solution the compound **1** exists almost exclusively in a conformation where proton H-1′ is antiperiplanar to proton H-1a″ and synclinal to proton H-1b″, and proton H-3 is antiperiplanar to

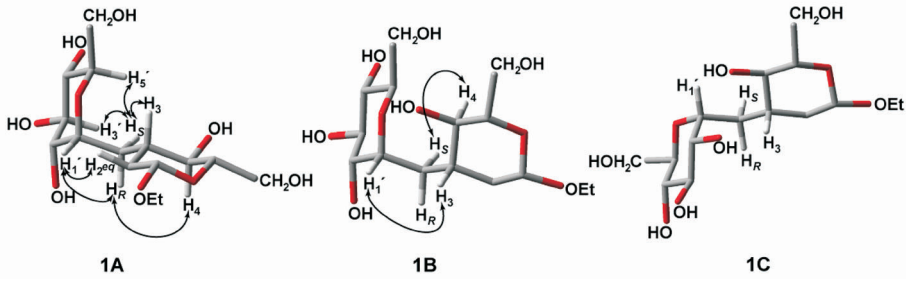

FIG. 3
Selected conformers and observed NOEs for compound **1**

proton H-1b″ and synclinal to proton H-1a″. Of the nine possible staggered conformers of **1**, only two satisfy this requirement: one of them is the conformer **1A**, in which proton H-1′ is antiperiplanar to proton H-1″*proS* and synclinal to proton H-1″*proR* and, at the same time, proton H-3 is antiperiplanar to proton H-1″*proR* and synclinal to proton H-1″*proS*. The second possible one is the conformer **1C**, in which proton H-1′ is antiperiplanar to proton H-1″*proR* and synclinal to proton H-1″*proS* and, at the same time, proton H-3 is antiperiplanar to proton H-1″*proS* and synclinal to proton H-1″*proR*.

These two conformers were distinguished by using NOE experiments. The selected experimental NOE contacts are listed in Table I.

As seen from the data, the compound **1** exhibits strong NOE for the pair of protons H-1′/H-2eq, which unequivocally excludes the conformer **1C**. (According to the MM3 model, the distance between the mentioned protons in the ideal conformer **1A** is about 1.9 Å whereas in the conformer **1C** it is 4.8 Å.) All strong NOEs in Table I comply perfectly with the conformer **1A**, and thus we can assume with great probability that the compound **1** markedly prefers this structure (see Fig. 3; the observed NOEs are denoted by arrows). The experimental values of NOE enable us to assign also chemical shifts to prochiral protons H-1″*proS* (2.34) and H-1″*proR* (1.32). The data further show that the compound **1** also exhibits very weak NOEs between proton pairs H-1′/H-3 and H-4/H-1″*proS*. These weak NOEs indicate a very small proportion of the conformer **1B**.

TABLE I
Selected NOEs for compound **1**

Proton pair	NOE intensity[a]	Proton pair	NOE intensity[a]
H-1/H-2eq	s	H-4/H-2ax	s
H-1/H-3	s	H-4/H-1″*proR*	s
H-1/H-5	s	H-4/H-1″*proS*	w
H-1′/H-2′	s	H-1″*proS*/H-3	s
H-1′/H-2eq	s	H-1″*proS*/H-3′	s
H-1′/H-3	w	H-1″*proS*/H-5′	s
H-1′/H-1″*proR*	m	H-1″*proS*/H-1″*proR*	s

[a] Strong, medium, and weak NOEs are denoted by s, m, and w, respectively.

The observed NOEs thus confirm that the compound **1** exists in two conformers **1A** and **1B** corresponding to the calculated local minima. However, the experimental values of vicinal coupling constants of protons at the *C*-aglycone bonds in methanolic solution definitely do not correspond to the calculated population (about 5:4). The values of vicinal coupling constants can be theoretically calculated using the Karplus equation[14] with Haasnot–Altona's parametrization[15] that takes into account the dependence of *J* on the dihedral angle of the H–C–C–H fragment, on electronegativities and on orientation of α- and β-substituents. To this end, we used the Karplus equation in the form

$$^3J_\theta = A \cos(2\theta) + B \cos(\theta) + C \sin(2\theta) + D$$

where θ corresponds to various dihedral angles H1′–C1′–C1″–H1″*proR*, H1′–C1′–C1″–H1″*proS*, H3–C3–C1″–H1″*proR*, H3–C3–C1″–H1″*proS*. The values of parameters *A*, *B*, *C* and *D*, used for the compound **1**, are the same as were published for *C*-nigerose[16].

The coupling constant, as a macroscopic quantity, reflects the contributions of all individual elements (i.e. conformers) in the set; therefore, for each vicinal spin-spin coupling of the given protons, we calculated weighted averages according to the equation

$$^3J = \Sigma P_i \, J_\theta \, .$$

In this manner we calculated the given coupling constant values for the individual conformers **1A** and **1B**, corresponding to the local minima, and then for the set of all conformations of **1** as found by the MM3 calculations. The obtained values were compared with the experimentally observed couplings in the compound **1** in methanolic solution. The data are given in Table II.

As seen from Table II, for the *C*-glycosidic bond (vicinal couplings of proton H-1′) there is a relatively good accord of calculated and experimental values. However, for the *C*-aglycone bond (vicinal couplings of proton H-3) the values agree very poorly. On the other hand, experimental values for the compound **1** agree very well with those calculated for conformer **1A**. This means that the MM3 calculations strongly overestimate the population of the conformer **1B** and that in methanolic solution the conformer **1A** is very pronouncedly preferred whereas the population of the second most stable conformer **1B** is very low.

Conformational Analysis of ***2***

An adiabatic contour map for the compound **2** (Fig. 4) closely resembles that for the structurally similar α-D-Glc*p*-(1→3)-*C*-β-D-Glc*p*-OMe (*C*-nigeroside) published by Mikros and coworkers[16]. In this case, it is apparent that up to the 2 kcal/mol level there are three significant local minimum regions. The first one, with torsion angles ϕ = +77° (+*sc*) and ψ = +67° (+*sc*), represents the global minimum and corresponds to the conformer **2C** in Fig. 5. The second low energy region is located around the local minimum with torsion angles ϕ = +51° (+*sc*) and ψ = –175° (*ap*) and corresponds to the conformer **2A**. The third region centred around ϕ = +78° (+*sc*) and ψ = –60° (–*sc*), corresponds to the conformer **2B**. All the local minima have a +*sc*

TABLE II
Experimental and calculated coupling constants for compound **1**

Structure	$J_{H1',H1''proR}$	$J_{H1',H1''proS}$	$J_{H3,H1''proR}$	$J_{H3,H1''proS}$
1 exp.	2.8	12.8	12.7	2.4
1 calc.[a]	3.3	11.1	7.7	6.6
1A calc.[b]	3.6	11.6	12.6	2.4
1B calc.[c]	1.6	11.5	1.2	12.3

[a] Values for the ensemble average. [b] Values for the global minimum. [c] Values for the local minimum.

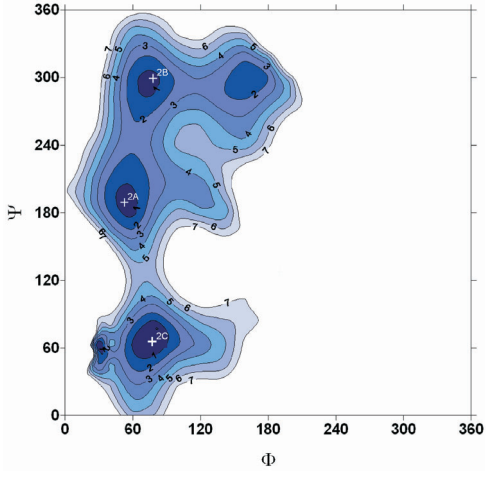

FIG. 4
Adiabatic contour map for compound **2**

(66°) oriented hydroxymethyl group and a *–sc* (–76°) oriented ethoxy group.

Using the same procedure as described for the compound **1**, we calculated the proportion of regions around the conformers **2C**, **2A** and **2B** as 47, 25 and 21%, respectively. Thus, the compound **2** should exist as a mixture of the conformers **2C**, **2A** and **2B** in about 2:1:1 ratio (Fig. 5).

In contrast to the compound **1**, the ^{1}H NMR spectrum of the compound **2** exhibited a greater signal overlap, which complicated the interpretation; however, also in this case it was possible to obtain sufficient data for conformational analysis. Proton H-1′ showed strong coupling (J = 10.1) with proton H-1a″ (1.69) and a weaker coupling (J = 4.5) with proton H-1b″ (2.02). On the other hand, proton H-3 exhibited medium coupling (J = 6.2) with proton H-1a″ and a little weaker coupling (J = 4.7) with proton H-1b″. The observed coupling constants of protons H-1′ and H-3 with two diastereotopic protons H-1a″ and H-1b″ indicate that the conformational population in the compound **2** is significantly different from that in the compound **1** and that the former apparently does not exist in only one pre-

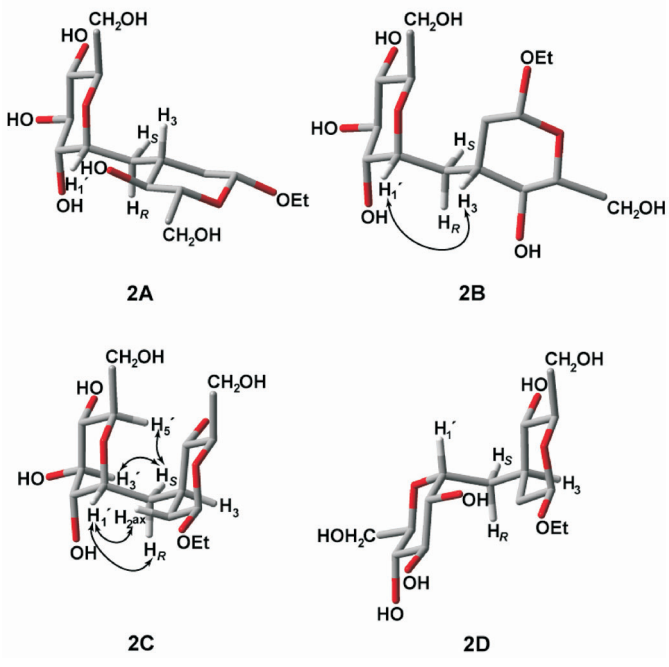

FIG. 5
Selected conformers and observed NOEs for compound **2**

dominant conformer, as the latter does. The coupling constants indicate that in the most populated conformer of the compound **2** the proton H-1′ is antiperiplanar to proton H-1a″ and synclinal to proton H-1b″, similarly as found for the compound **1**. An entirely different situation, however, is in the case of proton H-3 which, in a first approximation, may be assumed to have the same (synclinal) relation to proton H-1a″ as well as to proton H-1b″. Of the nine possible staggered conformers of the compound **2** again only two conform with this situation: either conformer **2C** or conformer **2D**. In the conformer **2C** the proton H-1′ is antiperiplanar relative to proton H-1″*proS* and synclinal to prochiral proton H-1″*proR*, whereas the proton H-3 is synclinal relative to both diastereotopic protons H-1″*proR* and H-1″*proS*. In the conformer **2D**, the proton H-1′ is antiperiplanar relative to proton H-1″*proR* and synclinal to proton H-1″*proS*, whereas the proton H-3 is synclinal relative to both diastereotopic protons H-1″*proR* and H-1″*proS*. Also in this case, distinction between the conformers was made using NOE experiments. Selected experimental connectivities of NOEs are given in Table III.

The presence of NOE between protons H-1′/H-2ax (Table III) indicates that, in accord with the MM3 calculations, the compound **2** exists predominantly in the conformer **2C**, because in this conformer the distance H-1′/H-2ax is significantly shorter than in the conformer **2D**. From this it follows that the most populated conformations of the compound **2** have a geometry close to the structure **2C** (see Fig. 5; selected experimental NOEs

TABLE III
Selected NOEs for compound **2**

Proton pair	NOE intensity[a]	Proton pair	NOE intensity[a]
H-1/H-2eq	s	H-3/H-5	s
H-1/H-3	s	H-3/H-2eq	m
H-1/H-5	s	H-1″*proS*/H-3′	s
H-1′/H-2′	s	H-1″*proS*/H-5′	s
H-1′/H-2ax	m	H-1″*proS*/H-1″*proR*	s
H-1′/H-3	m	H-2ax/H-4	s
H-1′/H-1″*proR*	m	H-2ax/H-2eq	s

[a] Strong, medium, and weak NOEs are denoted by s, and m, respectively.

are designated by arrows). On the basis of this structure, the 2.02 ppm signal can be ascribed to prochiral proton H-1″*proR* and the 1.69 ppm one to prochiral proton H-1″*proS*. The compound **2** also exhibits NOE of medium intensity between protons H-1′/H-3, which indicates the presence of a non-negligible amount of the conformer **2B** (the corresponding NOE is designated by arrow in Fig. 5). Although we were not able to confirm the conformer **2A** by the NOE experiments, this does not exclude its presence in the conformation mixture.

Vicinal coupling constants for the most stable conformers of the compound **2** as well as for the set of all conformations found for the compound **2**, were calculated using the modified Karplus equation as described for the compound **1**. These values, together with the values experimentally observed for the compound **2** in methanolic solution, are given in Table IV.

In the case of the compound **2** there is a very good accord between the calculated and observed values and therefore we may assume that the calculated population of the conformers **2A**, **2B** and **2C** in the ratio of about 1:1:2 corresponds to the actual situation.

In conclusion both the NMR experiments and molecular mechanics calculations demonstrate that the two studied *C*-disaccharides **1** and **2**, containing D-glucopyranose moiety at the non-reducing end and L- or D-2-deoxy-*arabino*-hexopyranose moiety at the reducing end, definitely prefer the +*sc* conformer around the *C*-glycosidic bond. This conformer corresponds with the *exo*-anomeric effect and its preference has been confirmed also in other α-(1→3)-*C*-disaccharides[6–8]. However, the compounds significantly differ in the conformational arrangement around the *C*-aglycone bond where the conformational preference can be explained by the presence and character of 1,3-diaxial-like interactions as recently for-

TABLE IV
Experimental and calculated coupling constants for compound **2**

Structure	$J_{H1',H1''proR}$	$J_{H1',H1''proS}$	$J_{H3,H1''proR}$	$J_{H3,H1''proS}$
2 exp.	4.5	10.1	4.7	6.2
2 calc.[a]	3.0	9.5	4.9	6.0
2B calc.[b]	3.7	9.8	12.6	2.6
2C calc.[b]	1.5	9.5	3.2	12.4
2A calc.[c]	1.5	9.8	2.9	4.3

mulated by Kishi[3]. The compound **1** with L-2-deoxy-*arabino*-hexopyranose moiety markedly prefers the conformer **1A**, as there is no 1,3-diaxial-like interaction. On the other hand, all three staggered conformers of *C*-aglycone bond in the compound **2** (**2A**, **2B** and **2C**) possess a 1,3-diaxial-like interaction, although of different type. Like in other studied α-(1→3)-*C*-disaccharides containing equatorial hydroxy group at C-4 of the reducing D-saccharide[6,7], in the compound **2** the conformer **2A** does not dominate because it contains the most destabilizing 1,3-diaxial-like interaction between bonds C4–OH and C1″–C1′. Therefore the most populated is the conformer **2C** having only a less destabilizing 1,3-diaxial-like interaction between bonds C1′–O5′ and C3–C4. In addition to **2C** and **2A** a non-negligible amount of the conformer **2B**, which also contains only a less destabilizing 1,3-diaxial-like interaction between bonds C1′–O5′ and C3–C2, is present in the conformational equilibrium.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (grant No. 6046137305).

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