

Conformational Flexibility of Carbohydrates: A Folded Conformer at the ϕ Dihedral Angle of a Glycosidic Linkage

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Abstract: The trisaccharide β -D-Glcp-(1 \rightarrow 2)- β -D-Glcp-(1 \rightarrow 3)- α -D-Glcp-OMe has been studied by Langevin dynamics (LD) simulations and NMR spectroscopy including measurement of transglycosidic $^3J_{C,H}$ coupling constants, $^3J_{H,OH}$ coupling constants, temperature dependence of the chemical shift of hydroxyl protons, and 2D NOESY and T-ROESY experiments as well as their 1D DPFGE analogues. A folded conformer at the ϕ_H dihedral angle of the (1 \rightarrow 2)-linkage observed in the LD simulation was corroborated by experimental NMR data, in particular spatial proximity of hydroxyl protons in nonadjacent sugar residues as deduced from a 2D T-ROESY experiment. The results from the present study with an “anti” ϕ_H conformer show that oligosaccharides exhibit large conformational flexibility under certain conditions and that this inherent property needs to be taken into account in the analysis of carbohydrate structure.

Introduction

An understanding of the flexibility of oligo- and polysaccharides is important for the description of the inherent properties of the molecules as well as their interactions with, in particular, proteins. In the field of glycobiology, oligosaccharides act, e.g., as receptors and regulate protein function and trafficking. The interactions of oligosaccharides render their study of great interest and call for detailed investigation of their 3-dimensional (3D) structure and dynamics.

The overall 3D shape of an oligosaccharide can readily be obtained by a simplified molecular mechanics approach such as HSEA.¹ In general, there is a “syn” relationship between sugar residues, i.e., the dihedral angles at the glycosidic linkage are around 0°. The torsional angles are denoted by ϕ_H (H1-C1-OX-CX) and ψ_H (C1-OX-CX-HX), where X is the linkage atom. The degree of an “anti” (folded) conformer ($\sim 180^\circ$) of the ψ_H dihedral angle has recently been addressed by NMR spectroscopy and molecular mechanics (MM) simulations as well as by *ab initio* calculations. Through observation of isotope effects in the 1H NMR spectrum from a $^1H/^2H$ mixture at the hydroxyl groups in a β -(1 \rightarrow 3)-linked disaccharide in dimethyl sulfoxide (DMSO), it could be shown that, in addition to the usual syn conformer, an anti ψ_H conformer was also present.² The low-energy folded conformer from the *ab initio* calculation of a β -(1 \rightarrow 4)-linked disaccharide also had the ψ_H dihedral angle in an anti state.³ Recently, conformational differences between C- and O-glycosides were investigated,⁴ addressing the above topic.

The ϕ_H dihedral angle in an oligosaccharide is governed by the exo-anomeric effect,^{1,5} which leads to the major conformer with ϕ_H close to $+60^\circ$ or -60° , depending on anomeric

configuration and absolute configuration of the sugar residue. Often, this is the exclusive conformational state from MM calculations when Boltzmann weighting is taken into account.

In the present study, we have investigated the trisaccharide β -D-Glcp-(1 \rightarrow 2)- β -D-Glcp-(1 \rightarrow 3)- α -D-Glcp-OMe (**1**) by Langevin dynamics (LD) simulations and NMR spectroscopy. Trisaccharide **1** was previously synthesized and studied with respect to glycosylation shift changes and its overall conformation.⁶ Out of four trisaccharides in that study, trisaccharide **1** showed some anomalies in ^{13}C glycosylation shifts and conformational flexibility, i.e., the β -(1 \rightarrow 3)-linkage in the trisaccharide was more rigid than in its constituent disaccharide, but the overall 3D structure was a syn conformation as deduced from HSEA calculations. We here report the combined use of stochastic dynamics simulations and NMR spectroscopy in the identification of a folded conformer at a ϕ_H dihedral angle, a finding with implications for the flexibility of carbohydrates in general and their interactions with other molecules in biological systems.

Materials and Methods

The synthesis of the trisaccharide β -D-Glcp-(1 \rightarrow 2)- β -D-Glcp-(1 \rightarrow 3)- α -D-Glcp-OMe (**1**) has been described previously⁶ together with 1H and ^{13}C NMR assignments in D_2O . Atoms in the terminal glucose residue are denoted by a double prime, those in the middle residue are by a prime, and those in the O-methyl residue are unprimed.

NMR Spectroscopy. For NMR measurements in D_2O , compound **1** was lyophilized twice from D_2O and then dissolved in D_2O to a concentration of 100 mM (long-range $^{13}C, ^1H$ coupling constants) or 50 mM (NOESY). For NMR measurements in DMSO- d_6 , **1** was lyophilized from H_2O and from a $\sim 1:1$ $H_2O:D_2O$ mixture and then dissolved in DMSO- d_6 , both samples to a concentration of 20 mM.

NMR measurements were performed on Varian Unity 500 and Inova 600 spectrometers equipped with 5 mm PFG inverse-detected triple-resonance probes. 1H NMR chemical shifts were referenced to residual DMSO- d_5 , $\delta = 2.56$. 1H NMR assignments of **1** in DMSO- d_6 were performed at 40 °C using 2D DQF-COSY and 2D TOCSY experiments. All 2D spectra were recorded in the phase sensitive mode. The temperature dependence of the chemical shifts of the hydroxyl protons was investigated at 20–45 °C, at 5 °C increments. Long-range coupling

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constants over the glycosidic linkages were measured at 37 °C. NOESY and T-ROESY experiments were performed at 40 °C, and to lower the exchange rate of hydroxyl protons in DMSO-*d*₆, measurements were also performed at 16 °C, just above the freezing point for the sugar: DMSO-*d*₆ solution.

Measurement of long-range ¹³C, ¹H coupling constants was performed at 500 MHz using a multiple ¹³C site selective excitation experiment^{7,8} extended with PFGs for coherence selection and suppression of artefacts.⁹ A Hadamard-4 shape was generated using the program Pandora's Pulse Box (Varian). Half-Gaussian shaped pulses of 50 ms duration were used for the selective ¹³C excitations of C1'', C2', C1', and C3. A spectral width of 1200 Hz was sampled with 8192 data points and 32 000 transients. FIDs were processed using Varian VNMR software. Zero-filling 8 times and multiplication of the FID with an exponential weighting function with a line broadening of 0.3 Hz was applied prior to Fourier transformation. ³J_{C,H} values were extracted using the *J*-doubling procedure with 8 Δ functions in the frequency domain.^{10,11}

1D DPGSE NOESY and 1D DPGSE T-ROESY spectra^{12–18} were recorded at 600 MHz using selective inversion pulses (i-SNOB-2)¹⁹ of 85–243 ms duration, leading to a band width of 20–7 Hz. The spectral width was 2300 Hz sampled with 16384 data points. Typically, 2048 transients were acquired for a selective excitation and a given mixing time. Zero-filling once and an exponential line broadening of 1–3 Hz was applied prior Fourier transformation. 2D NOESY and T-ROESY spectra were also recorded at 600 MHz using a sweep width of 2300 Hz, commonly with 2048 × 256 complex data points and 32 scans/*t*₁ increment. Zero-filling to 4 K × 4 K complex data points was followed by multiplication with a Gaussian weighting function prior to Fourier transformation. The mixing times in the NOESY and T-ROESY experiments were 50–500 ms, and the relaxation delays were 2–5 s. In the T-ROESY experiments the spin lock had an RF field strength of 2.0 kHz.

Langevin Dynamics Simulations. The trisaccharide was simulated *in vacuo* using stochastic dynamics based on the Langevin eq 1, which describes the motion of a particle of mass *m* as

$$m \frac{d^2x}{dt^2} = F(t) - \zeta \frac{dx}{dt} + R(t) \quad (1)$$

where *x* is the position, *t* is the time, *F* is the systematic force, ζ is the friction constant, and *R* is the random force. The latter two terms approximate the influence of the surroundings by coupling the molecule to a heat bath. The simulation was performed with a constant dielectric of unity, a time step 1 fs, a collision frequency, γ=ζ/*m*, of 50 ps⁻¹ (chosen to simulate the effects of water) on all heavy atoms and a temperature of 300K. The system was simulated for 50 ns on a Silicon Graphics Indy workstation using Quanta/CHARMm version 4.0 with PARM22 (Molecular Simulations Inc., San Diego, CA, USA).

Results and Discussion

The trisaccharide β-D-Glcp-(1→2)-β-D-Glcp-(1→3)-α-D-Glcp-OMe (**1**) was investigated by LD simulations and NMR

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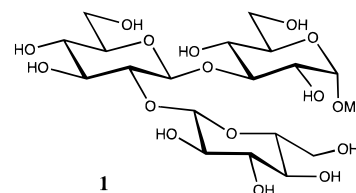


Figure 1. Schematic representation of the trisaccharide β-D-Glcp-(1→2)-β-D-Glcp-(1→3)-α-D-Glcp-OMe (**1**) in the “syn” conformation of the dihedral angles at the glycosidic linkages.

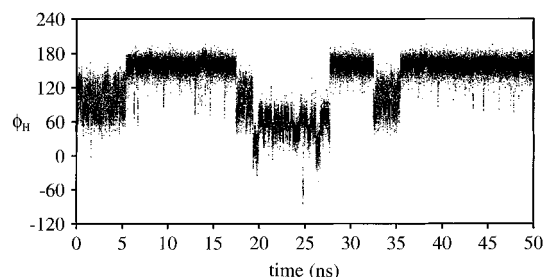


Figure 2. Langevin dynamics trajectory of the φ_H dihedral angle for the (1→2)-linkage of **1**.

spectroscopy, and a schematic drawing of it in the syn conformation is shown in Figure 1. Langevin dynamics²⁰ of oligosaccharides²¹ facilitate simulation well into the nanosecond time regime, in contrast to the use of explicit solvent in the simulation. From a 50 ns LD simulation, a trajectory for the φ_H dihedral angle of the (1→2)-linkage of **1** is shown in Figure 2. In addition to three different syn conformers being observed several times during the simulation, an anti φ_H conformer is significantly populated through a number of transitions. However, the exact population of this state will be dependent on the force field used for the molecule as well as on the model used for the treatment of solvent effects. Note that changes between major conformational states take place on a ns time scale. For the ψ_H dihedral angle of the (1→2)-linkage, an anti state was observed once, which is very reasonable in the light of the previous investigations.² Both dihedral angles of the (1→3)-linkage in **1** populated conformational regions of syn conformers.

To investigate the possible occurrence of a φ_H folded conformer, NMR spectroscopy was employed. For anti conformers present in solution, the ³J_{C,H} coupling constant across the glycosidic linkage should attain a larger value than that in the usual syn case, as deduced from a Karplus relationship.²² The magnitude of the transglycosidic ³J_{C,H} values in ²H₂O at 37 °C, measured by a Hadamard-4 selective ¹³C excitation pulsed field gradient experiment and extracted using a *J*-doubling procedure, showed that the ³J_{C,H} value for the φ_H dihedral angle of the (1→2)-linkage, 4.5 Hz, was larger than that for the φ_H dihedral angle of the (1→3)-linkage, 4.1 Hz. The difference is small, but larger than the experimental uncertainty, and the latter value is the same as observed for similar linkages of disaccharides in water.²³ Thus, the larger ³J_{C,H} value for the φ_H dihedral angle of the (1→2)-linkage suggests that a folded conformer at this linkage can be present.

By turning to a DMSO solution additional parameters can be measured through the hydroxyl protons. The ¹H NMR

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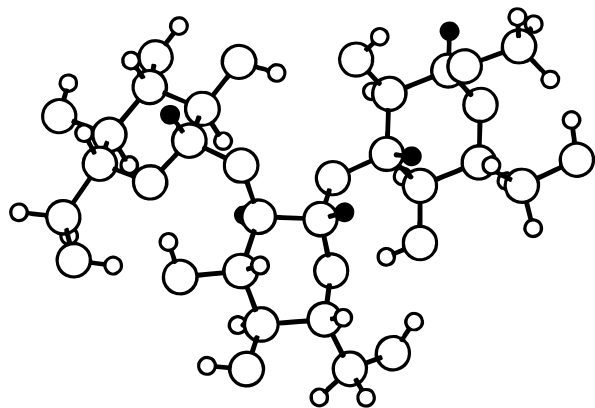
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Table 1. Chemical Shift (ppm) of the Signals in the ^1H NMR Spectra of the Trisaccharide $\beta\text{-D-Glcp-(1}\rightarrow\text{2)-}\beta\text{-D-Glcp-(1}\rightarrow\text{3)-}\alpha\text{-D-Glcp-OMe}$ at 40 °C in $\text{DMSO-}d_6^a$

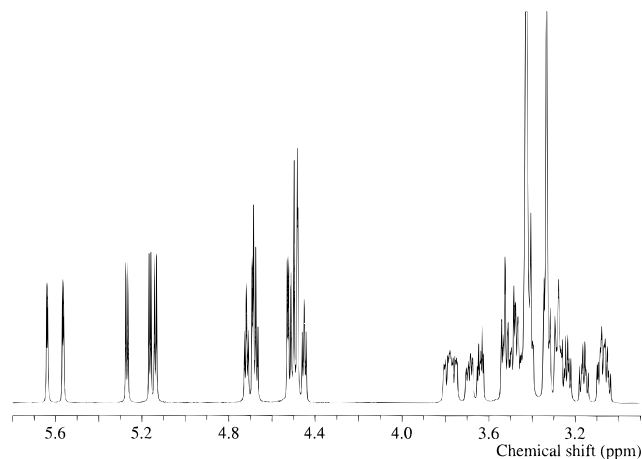
sugar residue	H1	H2	HO2	H3	HO3	H4	HO4	H5	H6	HO6	OMe
$\beta\text{-D-Glcp-(1}\rightarrow\text{)}$	4.49 (7.8)	3.09	5.48 (3.3) [-6.4]	3.25	4.99 (5.2) [-7.0]	3.08	4.98 (5.5) [-6.3]	3.28	3.50/3.79	4.32 (4.8,6.1) [-5.4]	
$\rightarrow\text{2-}\beta\text{-D-Glcp-(1}\rightarrow\text{)}$	4.50 (8.0)	3.33		3.48	5.45 (3.4) [-4.8]	3.18	5.12 (5.7) [-6.1]	3.33	3.45/3.76	4.60 (4.8,5.9) [-5.1]	
$\rightarrow\text{3-}\alpha\text{-D-Glcp-OMe}$	4.69 (3.6)	3.63	4.47 (3.8) [-2.7]	3.54		3.28	4.42 (2.2) [-2.4]	3.42	3.53/3.69	4.52 (5.9) [-6.4]	3.36

^a $J_{\text{H1,H2}}$ and $^3J_{\text{H,OH}}$ values (Hz) in parenthesis and $\Delta\delta/\Delta T$ [ppb/K] of hydroxyl protons in square brackets.

**Figure 3.** Snapshot from the LD simulation of the trisaccharide $\beta\text{-D-Glcp-(1}\rightarrow\text{2)-}\beta\text{-D-Glcp-(1}\rightarrow\text{3)-}\alpha\text{-D-Glcp-OMe}$ in the "anti" conformation of the ϕ_{H} dihedral angle for the (1 \rightarrow 2)-linkage. Protons at glycosidic linkages are denoted by filled circles.

assignments, $^3J_{\text{H,OH}}$ coupling constant and temperature dependence of the chemical shift, $\Delta\delta/\Delta T$, are shown for **1** in Table 1. Indications of hydroxyl groups being involved in hydrogen bonding may be obtained from (i) $^1\text{H}/^2\text{H}$ isotope effects,^{24–26} (ii) a $^3J_{\text{H,OH}}$ coupling constant²⁷ different from that obtained by averaging (~ 5.4 Hz), and (iii) low-temperature dependence of the chemical shift for the hydroxyl protons,²⁸ $\Delta\delta/\Delta T$ (< 3 ppb/K). Two protons, HO2'' and HO3', have their chemical shifts at low field, but a downfield chemical shift displacement of a ^1H NMR resonance is not, *per se*, an indication of the presence of a hydrogen bond.²⁹ From a $\sim 1:1$ mixture of $^1\text{H}/^2\text{H}$ on the hydroxyl groups, no large isotope effects could be observed. Lower $^3J_{\text{H,OH}}$ values, 2.2–3.8 Hz, were observed for HO2'', HO3', HO2, and HO4, and lower $\Delta\delta/\Delta T$ values were observed for HO2 and HO4, -2.7 and -2.4 ppb/K, indicating structures stabilized by hydrogen bonding. From the LD simulation the most probable hydrogen bonding could be identified for HO4–O5', as expected from the $\Delta\delta/\Delta T$ data, and in the ϕ_{H} folded conformer of the (1 \rightarrow 2)-linkage for HO3'–O5'' and HO2''–O2 (Figure 3). This leads to a structure with a cyclic network of inter-residue hydrogen bonds. Hence, intramolecular hydrogen bonding would stabilize a folded conformer. The average O2''–O2 distance is ~ 5 Å for the syn conformation during the dynamics simulation, whereas it is ~ 3 Å for the ϕ_{H} folded conformer. Thus, hydroxyl protons should in the first case be well apart, but in the second case in close proximity.

Further information was gained by a combination of data from 2D NOESY and T-ROESY experiments and their 1D DPGSE analogues which used selective pulses for excitation. 1D T-ROESY experiments in $^2\text{H}_2\text{O}$ demonstrated that syn conformations

**Figure 4.** ^1H NMR spectrum of **1** in $\text{DMSO-}d_6$ at 16 °C.

were present at the (1 \rightarrow 2)-linkage and the (1 \rightarrow 3)-linkage, as expected, by observation of transglycosidic NOEs for H1''–H2' and H1'–H3 upon selective excitation of the resonances from the anomeric protons. In the LD simulation, the H2''–H2' distance was 4.4 Å in the syn conformation and 2.3 Å in the anti conformation. 1D NOESY experiments showed an NOE between H2'' (selectively excited) and H2' indicating that a folded conformation can be present since the distance obtained using the isolated spin pair approximation (ISPA)³⁰ was < 4 Å for the proton pair.

2D NOESY experiments in $\text{DMSO-}d_6$ at 40 °C showed cross-peaks, *inter alia*, between HO2 and HO2'' as well as between HO2 and residual water. Thus, proton exchange occurs, possibly being mediated by residual water, and/or an NOE is present between HO2 and HO2''. By turning to 2D ROESY experiments, one can differentiate direct NOE cross-peaks, of opposite sign compared to the diagonal peaks, and cross-peaks that occur by chemical exchange or by indirect NOEs, which have the same sign as the diagonal peaks.³¹ In a recent study, a transient hydrogen bond was inferred to be present between O2g and O1f in sucrose, since a contribution from chemical exchange could be detected.³² When the temperature was lowered to 16 °C, just above the freezing point for the sugar: $\text{DMSO-}d_6$ solution, the chemical exchange rate could be lowered and the HO2 resonance appeared at 4.53 ppm, i.e., low-field to the anomeric protons at ~ 4.50 ppm (Figure 4). A 2D T-ROESY experiment resulted in an ROE cross-peak between the HO2'' and HO2 protons (Figure 5), which is of opposite sign to the diagonal peaks. Thus, a nuclear Overhauser effect arising from proximity in space between the HO2'' and HO2 protons is present, in agreement with a folded conformer along the ϕ_{H} dihedral angle at the (1 \rightarrow 2)-linkage as identified in the LD simulations. As expected, no cross-peak between the HO3'–HO2 protons is observed in the spectrum. Furthermore, an ROE

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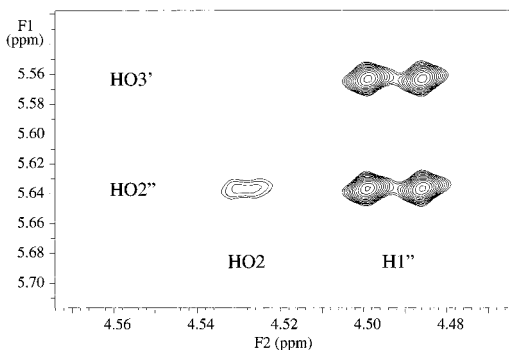


Figure 5. Part of a $^1\text{H},^1\text{H}$ T-ROESY NMR spectrum of **1** in $\text{DMSO-}d_6$ at $16\text{ }^\circ\text{C}$ using a τ_m of 300 ms. ROE cross-peaks are annotated.

cross-peak was observed, *inter alia*, between $\text{HO2}''$ and $\text{HO3}'$, which is consistent with the presence of a syn conformation at the (1 \rightarrow 2)-linkage, as shown above. The syn conformation governed by the exo-anomeric effect for the ϕ_{H} dihedral angle of this linkage places $\text{H1}''$ and $\text{HO3}'$ in close proximity. An ROE cross peak $\text{H1}''\text{--HO3}'$ for the protons across the glycosidic

linkage as well as the intraresidue cross-peak for the $\text{H1}''\text{--HO2}''$ protons could also be observed in the 2D T-ROESY spectrum.

In conclusion, the presence of a folded conformer at a ϕ_{H} dihedral angle of an oligosaccharide in solution is supported by 1D NOESY experiments, the magnitude of the $^3J_{\text{C,H}}$ coupling constant across the glycosidic linkage, possible hydrogen bonding stabilization inferred from low $^3J_{\text{H,OH}}$ values of hydroxyl protons as well as the occurrence of the anti conformer significantly populated in the LD simulation, and in particular, a 2D T-ROESY experiment. The results from the present study with an anti ϕ_{H} conformer together with those identifying an anti ψ_{H} conformer² show that oligosaccharides exhibit large conformational flexibility under certain conditions and that this inherent property needs to be taken into account in the analysis of carbohydrate structure and the interaction of carbohydrates with other types of molecules.

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