Published in final edited form as:

J Phys Chem B. 2013 June 27; 117(25): 7546–7553. doi:10.1021/jp403719g.

Bifurcated Hydrogen Bonding and Asymmetric Fluctuations in a Carbohydrate Crystal Studied via X-ray Crystallography and Computational analysis

Xibing He¹, Elizabeth Hatcher¹, Lars Eriksson², Göran Widmalm³, and Alexander D. MacKerell Jr.¹

¹Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, 20 Penn Street, Baltimore, MD 21201

²Department of Materials and Environmental Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

³Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

Abstract

The structure of the *O*-methyl glycoside of the naturally occurring 6-*O*-[(*R*)-1-carboxyethyl]- β -D-galactopyranose, C₁₀H₁₈O₈, has been determined by X-ray crystallography at 100 K, supplementing the previously determined structure obtained at 293 K (Acta Cryst., 1996, C52, 2285-2287). Molecular dynamics simulations of this glycoside were performed in the crystal environment with different numbers of units cells included in the primary simulation system at both 100 K and 293 K. The calculated unit cell parameters and the intra-molecular geometries (bonds, angles, and dihedrals) agree well with experimental results. Atomic fluctuations, including B-factors and anisotropies, are in good agreement with respect to the relative values on an atom-by-atom basis. In addition, the fluctuations increase with increasing simulation system size, with the simulated values converging to values lower than those observed experimentally indicating that the simulation model is not accounting for all possible contributions to the experimentally observed B-factors which may be related to either the simulation time scale or size. In the simulations the hydroxyl group of O7 is found to form bifurcated hydrogen bonds with O6 and O8 of an adjacent molecule, with the interactions dominated by the HO7-O6 interaction. Quantum mechanical calculations support this observation.

Keywords

CHARMM force field; carbohydrates; molecular dynamics simulation; molecular modeling; monosaccharides

Correspondence to: A. D. MacKerell, Jr., alex@outerbanks.umaryland.edu, phone: (+1)410-706-7442; G. Widmalm, gw@organ.su.se, phone: (+46)8-163742.

Supporting Information: CCDC 802592 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Two tables comparing the calculated and experimental bond lengths and valence angles as well as two figures are included in the Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Introduction

Knowledge of atomic position and fluctuations of molecules allow for an understanding of their properties that can have chemical or biological consequences. However, obtaining such information is still not trivial and requires a combination of methods to fully access the properties being analyzed. X-ray crystallography is a powerful approach that allows for identification of the positions of atoms in crystals along with information on their fluctuations to be obtained.¹⁻³ This is especially true in high-resolution small molecule crystals, such as those found in the Cambridge Structural Database (CSD),⁴ in which the positions of hydrogen atoms can often be inferred with a high level of confidence. In addition, information on the nature of the motion of individual atoms, including the asymmetry of those motions can often be obtained.

While X-ray approaches are quite powerful, they are limited even in high resolution small molecule crystals. Usually the hydrogen positions are derived from the positions of heavy atoms and the use of standard covalent geometry information. The source of atomic fluctuations is also ambiguous. The nature of those motions as a function of time cannot be accessed and the magnitude of the atomic fluctuations themselves include multiple contributions.^{5,6} The most obvious contribution is the atomic motions themselves, but these are confounded by lattice disorder as well as other possible imperfections in crystals. To both elucidate the positions of hydrogens and better understand the contributions of different phenomena to the measured fluctuations computational methods may be useful. Computational methods, either quantum or molecular mechanics, can identify the locations of hydrogens. By using molecular dynamics (MD) simulations the time dependent nature of atomic motions can be monitored and it is possible, in principle, to separate the contribution of lattice disorder from atomic fluctuations themselves to the experimentally observed fluctuations.

Previously, we reported the crystal structure of the glycoside, methyl 6-O-[(*R*)-1-carboxyethyl]- β -D-galactopyranoside.⁷ The structure was determined with $R = 0.0562$ at a resolution of $0.83 \text{ \AA}$ at 293 K . While the structure was well resolved and the asymmetric temperature factors deduced, the proton on one hydroxyl moiety, O7-HO7, which could potentially hydrogen bond with two different acceptor oxygens, O6 and O8 (Figure 1), could not be unambiguously located. To better understand the nature of this hydrogen bond a second structure of the crystal was determined at 100 K as part of the present study. In addition, computations were undertaken to facilitate interpretation of the X-ray data with respect to the O7 hydrogen bond and to understand the underlying contributions to and the nature of the atomic motions in the system.

Methods

Experimental

Single crystal X-ray diffraction patterns were recorded with a STOE IPDS on a Mo-radiation source ($\lambda = 0.71073 \text{ \AA}$) with ω -scans. The sample-detector distance was 70 mm . Indexing, cell refinements, and integration of reflection intensities were performed using the diffractometer software.⁸ Absorption correction was neglected as the small size of the crystal and the low absorption coefficient made an absorption correction of minor importance. The structure was solved by direct methods using SHELXS97 (Ref. 1) giving electron density maps where most of the non-hydrogen atoms could be resolved. The rest of the non-hydrogen atoms were located from difference electron density maps and the structure model was refined with full matrix least square calculations on F^2 using the program SHELXL97.¹ All non-hydrogen atoms were refined with anisotropic displacement parameters. The hydrogen atoms were placed at geometrically calculated positions and were

allowed to ride on the atoms they were bonded to. Rigid bond restraints were applied according to Hirshfeld⁹ as implemented with the DELU command in the program SHELXL97.¹ The effects of large anisotropic displacement parameters show up as apparent bond-shortening¹⁰ due to libration, particularly for the 293 K data.

Molecular Dynamics Simulations

Two series of MD simulations of the *O*-methyl glycoside in its crystal environment were performed at 100 K and 293 K. Each series includes 4 systems, which contains 1 complete crystal unit cell having two molecules, 2×2×2 unit cells (16 molecules), 4×4×4 unit cells (128 molecules) and 8×8×8 unit cells (1024 molecules), respectively. Initial geometries were generated from the crystal structures of the glycoside. All MD simulations were performed with the CHARMM program¹¹ along with the CHARMM36 additive carbohydrate force field.¹²⁻¹⁶ Periodic boundary conditions were applied in accordance with the length and angle parameters of the respective crystals. The particle mesh Ewald approach was used to calculate the long-range electrostatic interactions with a real-space cutoff of 12 Å, a kappa value of 0.36 Å⁻¹, and a six-order spline.¹⁷ Nonbonded interactions were updated heuristically out to 14 Å with a force switch smoothing function from 10 to 12 Å used for the Lennard-Jones interactions.¹⁸ All simulations were carried out in the constant pressure-constant temperature (NPT) ensemble with the Leapfrog integrator employing an integration time step of 1 fs. The temperature was maintained at 100 K and 293 K, respectively, by a Nosé-Hoover thermostat.¹⁹ A constant pressure of 1 atmospheric pressure (101325 Pa) was controlled using the Langevin piston barostat.²⁰ Covalent bonds between hydrogen atoms and heavy atoms were constrained to their equilibrium values by means of the SHAKE/RATTLE algorithm.²¹ After equilibration, each system was simulated for 50 ns. Coordinates were saved every 1 ps from the MD simulations for all subsequent analyses.

The calculated positional anisotropies of atom *i* was obtained by:

$$ANI_{i,x} = \langle u_{i,x}^2 \rangle = \langle (x_i - \bar{x}_i)^2 \rangle \quad (1)$$

$$ANI_{i,y} = \langle u_{i,y}^2 \rangle = \langle (y_i - \bar{y}_i)^2 \rangle \quad (2)$$

$$ANI_{i,z} = \langle u_{i,z}^2 \rangle = \langle (z_i - \bar{z}_i)^2 \rangle \quad (3)$$

where (*x_i*, *y_i*, *z_i*) are the coordinates of atom *i* and (*u_{i,x}*, *u_{i,y}*, *u_{i,z}*) are the displacements of atom *i* from its average position. The angle brackets denote the ensemble average over the whole trajectory. The corresponding experimental anisotropies in the X, Y and Z directions for each non-hydrogen atoms were taken directly from the columns “_atom_site_aniso_U_1_1”, “_atom_site_aniso_U_22” and “_atom_site_aniso_U_33”, respectively, from the crystal data files.

The calculated B factor of atom *i* was obtained from

$$B_{cal,i} = 8\pi^2 \langle u_{i,r}^2 \rangle / 3 = 8\pi^2 \langle u_{i,x}^2 + u_{i,y}^2 + u_{i,z}^2 \rangle / 3 \quad (4)$$

and the experimental B factor was obtained by:

$$B_{exp}=8\pi^2(U_{11}+U_{22}+U_{33})/3 \quad (5)$$

where U_{11} , U_{22} , and U_{33} were obtained from the columns “_atom_site_aniso_U_11”, “atom_site_aniso_U_22” and “atom_site_aniso_U_33”, respectively, in the crystal data files.

Quantum Mechanical Calculations

A single complete unit cell was subjected to quantum mechanical (QM) calculations performed using the program QCHEM version 4.0.²² The positions of all heavy (non-hydrogen) atoms were fixed at their 100 K crystallographic locations, and the positions of all hydrogen atoms were optimized at the RIMP2 theory with 6-31G* basis and RIMP2-VDZ auxiliary basis to default tolerances.^{23,24}

Results and Discussion

Experiments

Selected crystal data are given in Table 1, selected bond lengths in Table S1 and selected bond angles in Table S2. The average bond length elongation¹⁰ for the 100 K structure could be described as an effect due to decreased librational motion. This is also clearly shown by the smaller thermal ellipsoids of the 100 K structure as shown in Figure S2.

Crystal MD Simulations

Crystal MD simulations were carried out on the glycoside at both 100 and 293 K, as in the experimental studies. In addition, simulations were performed on different representations of the crystal. These increased in size from a single unit cell (i.e. $1\times1\times1$ system), which contains two glycoside molecules (Figure 1) to a $2\times2\times2$, a $4\times4\times4$ and an $8\times8\times8$ systems where the integers indicate the number of unit cells in the 3 dimensions. Studying differently sized systems allowed for the impact of simulation size on the atomic fluctuations to be determined, including the contribution of lattice disorder on the dynamics. The latter was evaluated by determining the impact of aligning the individual molecules versus the full simulation systems when calculating the atomic fluctuations, as described below.

Initial analysis involved the ability of the force field to maintain the unit cell parameters and the intra-molecular geometries. Table 2 shows the averaged unit cell parameters from the simulations compared to the experimental ones. The α and β angles, not shown in Table 2, were restrained to 90 degrees to maintain the lattice type observed in the experimental data. Overall, unit cell parameters from the MD simulations tabulated by averaging over snapshots from the trajectories were consistent with the experimental values, with the relative errors less than 4% for most of the cases. The relative errors for the systems at 293 K are slightly higher than the systems at 100 K, and the relative errors for the largest systems ($8\times8\times8$) are higher than for smaller systems.

Figures 2 & 3 show the calculated bond lengths and valence angles averaged over the 50 ns trajectories of the $1\times1\times1$ unit cell systems. Detailed information on the bonds and angles is given in Tables S1 & S2 in the supplementary material. All calculated values are close to the corresponding experimental values from the crystallographic analysis. The calculated bond lengths are within 0.02 Å of the experimental values (Table S1), except for C7-C8 & C8-C9 at 293 K, which differ by approximately 0.04 Å. The differences between the calculated and experimental angle values are mostly less than 2 degrees (Table S2). The root mean squared (RMS) fluctuations are smaller at 100 K than that at 293 K as expected. Figure 4 shows the distributions of 5 dihedral angles from simulations of the $1\times1\times1$ systems. All distributions

show a single Gaussian peak, and the peak positions are close to the experimental values. Again, as expected, the distributions are narrower at 100 K than that at 293 K. Figures 2, 3 and 4 demonstrate that the CHARMM36 carbohydrate force field used in this study maintains the intramolecular geometries of the crystals of the target glycoside.

The anisotropic fluctuations of all non-hydrogen atoms were calculated using Eq. (1) ~ (3) and are shown in Figure 5. Two different strategies were used to re-orient the systems and calculate the average positions and fluctuations. Note that the $1\times 1\times 1$ single unit cell contains 2 target glycoside molecules, the $2\times 2\times 2$ system contains 8 unit cells and 16 molecules, the $4\times 4\times 4$ system contains 128 molecules, and the $8\times 8\times 8$ system contains 1024 molecules. The first strategy is that for each snapshot, each molecule was least-squared reoriented with respect to its own initial coordinates, and then the fluctuations calculated. The second strategy is that all molecules in all unit cells of each system were least-squares re-oriented together to the crystal coordinates of the whole system, and then the fluctuations were calculated. The first strategy considers only the contributions of the individual molecules to the atomic fluctuations. The second strategy includes shifts of the molecules relative to each other in the crystal lattice, which may be considered the contributions from lattice disorder. The anisotropic fluctuations calculated by the first strategy are denoted by dotted lines in Figure 5 and are almost identical for the systems of different size. This is expected since the environment of each molecule is almost identical in the crystal simulations for all the different system sizes. However, if lattice disorder is considered when calculating the anisotropic fluctuations using the second strategy, the impact of system size is significant. The fluctuations in the $1\times 1\times 1$ system (red dot dash line) system are systematically larger than for the monomer alone, indicating that fluctuations of the two molecules with respect to each other in the individual unit cells make a significant contribution to the experimentally observed fluctuations. It should be noted that this contribution does not formally conform to lattice disorder as the fluctuations occur within the same unit cell. However, upon going to the $2\times 2\times 2$ system (blue dashed line) a further increase in the anisotropic fluctuations is observed, representing the contribution of lattice disorder to atom motions. Although small, further increases occur upon going to the $4\times 4\times 4$ system and then to the $8\times 8\times 8$ system, with the latter approaching the experimental anisotropic fluctuations, though still lower than the experiment.

Figure 6 shows the B-factors calculated by Eq. (4) that are compared to the experimental values obtained from Eq. (5). Just like anisotropies, the B-factors calculated following of re-orientation of each individual molecule are indistinguishable for differently sized systems, and the B-factor following of re-orientation of the whole simulation box increase as the box size increases. In addition, the higher temperature results in larger B-factor values.

The quality of the simulations in reproducing the experimental fluctuations on an atom-by-atom basis is good. Lower fluctuations are observed in atoms up to C6, which correspond to the galactopyranose ring. Significantly higher fluctuations are present in the carboxyethyl moiety of the molecule, with the highest fluctuations occurring in O8 followed by O7, a trend reproduced in the calculations. In general, the relative fluctuations on an atom-by-atom basis observed experimentally are reproduced in the MD simulations.

The magnitude of the fluctuations on an atom-by-atom basis suggests the source of difference between the experimental and simulated results. At 100 K the magnitude of the fluctuations in the galactopyranose ring are in near quantitative agreement, with the simulated results being only slightly underestimated compared to experimental data. However, the underestimation is significantly larger in the carboxyethyl atoms, suggesting that additional conformational sampling is occurring that is not sampled in the MD simulations. This may be associated with the length of the simulations as studies of proteins

suggest that conformational transitions occur on longer simulation time scales,²⁵ although the possibility that longer range disorder in the crystal, not accessible in our 8×8×8 system, may contribute. The 293 K simulation results are consistent with this scenario indicating that additional conformational excursions not sampled in the simulation occurs throughout the glycoside at the higher temperature. This trend is consistent with a number of previous computational and experimental studies on protein and nucleic acids that show the presence of a transition in the nature of the atomic fluctuations in the vicinity of 200 K. The transition involves the atomic motions changing from a primarily isotropic nature at low temperatures to an anisotropic nature, in which the anisotropy is indicated to be associated with the atoms sampling distinct conformational substates.^{26,27}

A primary motivation for the present study was the nature of the proton, HO7, on the O7 hydroxyl moiety. As is evident in Figure 1, the hydrogen can donate to either the O6 or O8 atoms in the adjacent molecule in the crystal. To analyze the behavior of the hydrogen bonding the HO7 to O6 and HO7 to O8 distances were analyzed as a function of simulation time over a selected region of the simulation (Figure 7), with 2D probability distributions obtained from the entire 50 ns time series presented in Figure 8. At 100 K there are two ranges of distances, with the HO7—O6 sampling systematically shorter distances than HO7---O8 (Figure 7A). This leads to a well-defined local distribution in the corresponding 2D distribution (Figure 8A), with the maxima in the distances occurring at 1.97 and 2.58 Å for HO7—O6 and HO7—O8, respectively. At the higher temperature additional thermal fluctuations lead to more overlap of the HO7—O6 and HO7—O8 time series (Figure 7B). This manifests a broader 2D distribution, with a tail in the distribution associated with shorter HO7---O8 and longer HO7—O6 distances evident. In the 293 K time series, excursions of the distances to significantly longer and shorter values for the HO7—O8 and HO7—O6 distances, respectively, indicate the nature of anisotropic motions occurring at the higher temperature, similar to the previously reported transitions of atomic motions in proteins and nucleic acids.^{26,27} These results suggest that while hydrogen bonding is primarily occurring with the O6 atom (distance maximum at 1.97 Å), rapid, short-lived excursions of the hydrogen leading to hydrogen bonding with the O8 atom do occur (distance maximum at 2.65 Å).

One of most striking aspects of the non-hydrogen atom anisotropies (Figure 5) is the large anisotropy of O8 in the Y and Z directions, but not in the X direction. Figure S1 in the supplementary material shows a snapshot of three neighboring glycoside molecules in the crystal. Visual analysis shows that a hypothetical hydrogen bond between adjacent HO7 and O8 atoms is located almost entirely in the Y-Z plane. When the hydrogen bond between HO7 and O8 forms and breaks, the carbonyl O8 atom is expected to fluctuate in the Y-Z plane with a higher magnitude than other non-hydrogen atoms. The X direction for the O8 atom is almost perpendicular to the HO7...O8 hydrogen bond and parallel to the C=O bond; therefore, the fluctuation of O8 in X direction is much less than in Y and Z directions. Due to the constraints of two covalent bonds (C6-O6, O6-C8), the fluctuation of O6 atom is smaller than O8 which is a terminal atom.

QM Calculations

To verify the MD simulation results indicating hydrogen bonding to occur primarily with the O6 atom a quantum mechanical calculation was performed. The calculation involved constraining the non-hydrogen atoms to their 100 K crystallographic locations and allowing the hydrogen atoms to relax to favorable positions. From the QM geometry optimization calculation an HO7---O6 distance of 1.93 Å was obtained as compared to a HO7---O8 distance of 2.46 Å. The QM distances are in good agreement with the most probable distances from MD simulations, verifying the results from those simulations.

Conclusions

In this study, the structure of methyl 6-O-[(*R*)-1-carboxyethyl]- β -D-galactopyranose, was determined by X-ray crystallography at 100 K, which can be compared to the previously reported structure at 293 K.⁷ Molecular dynamics simulations were also performed on crystals of different sizes at both 100 K and 293 K. The CHARMM36 carbohydrate force field used in simulations was found to reproduce the experimental unit cell parameters and the intra-molecular geometries (bonds, angles, and dihedrals). The calculated atomic fluctuations (B-factors and anisotropies) were also consistent with the experimental results, though the magnitude of the fluctuations in the largest simulation system underestimates the experimentally determined values, indicating that the simulations are not identifying long range conformational sampling in terms of either simulation length or size. The hydroxyl group of O7 is found to form hydrogen bonds primarily with O6 of an adjacent molecule, but it also can form hydrogen bonds with O8 of the adjacent molecule, especially at the higher temperature.

Bifurcated hydrogen bonding (HB) is a common phenomenon in nature. It is found to be important in the rearrangements and reorientation of water,^{28,29} self-association of organic molecules in solvent,³⁰ formation of crystal structure of inorganic molecules,³¹ stabilization of conformations of proteins,^{32,33} nucleosides,^{34,35} and DNA-protein complexes.³⁶ In the Cambridge Structural Database there are to date (accessed April 8, 2013) 6398 carbohydrate structures out of which 2490 have C-O-H...O atomic arrangements with an oxygen-oxygen distance of < 3.4 Å. By limiting the O-H...O angle to be between 135° and 180° and analyzing for bifurcated HBs one finds 141 structures fulfilling the geometrical criteria. Thus, bifurcated HBs do occur in carbohydrate crystal structures, e.g., in methyl-3,4-*O*-ethylidene- α -L-arabinopyranoside³⁷ and in the tetrahydrate of methyl 3-*O*- β -D-mannopyranosyl- β -D-glucopyranoside.³⁸ In this work, we were able to elucidate the intermolecular bifurcated HB in the glycoside methyl 6-O-[(*R*)-1-carboxyethyl]- β -D-galactopyranoside, thereby describing the dual nature of the HB interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

A.D.M. would like to thank the National Institutes of Health (NIH) for financial support (GM051501, GM070855) and GW. would like to thank the Swedish Research Council for financial support.

References

1. Sheldrick GM. Acta Crystallogr. 2008; A64:112–122.
2. Thorn A, Dittrich B, Sheldrick GM. Acta Crystallogr. 2012; A68:448–451.
3. Eriksson L, Widmalm G. Acta Crystallogr. 2012; E68:o2221–o2222.
4. Allen FH. Acta Crystallogr. 2002; B58:380–388.
5. Kuriyan J, Petsko GA, Levy RM, Karplus M. J Mol Biol. 1986; 190:227–254. [PubMed: 3795269]
6. Kundu S, Melton JS, Sorensen DC, Phillips GN Jr. Biophys J. 2002; 83:723–732. [PubMed: 12124259]
7. Eriksson L, Pilotti Å, Stenutz R, Widmalm G. Acta Crystallogr. 1996; C52:2285–2287.
8. Stoe IPDS software, version 2.87. Stoe & CIE GmbH; Darmstadt, Germany: 1997.
9. Hirshfeld FL. Acta Crystallogr. 1976; A32:239–244.
10. Busing WR, Levy HA. Acta Crystallogr. 1964; 17:142–146.

11. Brooks BR, Brooks CL III, MacKerell AD Jr, Nilsson L, Petrella RJ, Roux B, Won Y, Archontis G, Bartels C, Boresch S, Caflisch A, Caves L, Cui Q, Dinner AR, Feig M, Fischer S, Gao J, Hodoseck M, Im W, Kuczera K, Lazaridis T, Ma J, Ovchinnikov V, Paci E, Pastor RW, Post CB, Pu JZ, Schaefer M, Tidor B, Venable RM, Woodcock HL, Wu X, Yang W, York DM, Karplus M. *J Comput Chem*. 2009; 30:1545–1614. [PubMed: 19444816]
12. Guvench O, Greene SN, Kamath G, Brady JW, Venable RM, Pastor RW, MacKerell AD Jr. *J Comput Chem*. 2008; 29:2543–2564. [PubMed: 18470966]
13. Guvench O, Hatcher ER, Venable RM, Pastor RW, MacKerell AD Jr. *J Chem Theory Comput*. 2009; 5:2353–2370. [PubMed: 20161005]
14. Hatcher E, Guvench O, MacKerell AD Jr. *J Chem Theory Comput*. 2009; 5:1315–1327. [PubMed: 20160980]
15. Hatcher E, Guvench O, MacKerell AD Jr. *J Phys Chem B*. 2009; 113:12466–12476. [PubMed: 19694450]
16. Raman EP, Guvench O, MacKerell AD Jr. *J Phys Chem B*. 2010; 114:12981–12994. [PubMed: 20845956]
17. Darden T, York D, Pedersen L. *J Chem Phys*. 1993; 98:10089–10092.
18. Steinbach PJ, Brooks BR. *J Comput Chem*. 1994; 15:667–683.
19. Nosé S. *J Chem Phys*. 1984; 81:511–519.
20. Feller SE, Zhang Y, Pastor RW, Brooks BR. *J Chem Phys*. 1995; 103:4613–4621.
21. Ryckaert JP, Ciccotti G, Berendsen HJC. *J Comput Phys*. 1977; 23:327–341.
22. Shao Y, Molnar LF, Jung Y, Kussmann J, Ochsenfeld C, Brown ST, Gilbert AT, Slipchenko LV, Levchenko SV, O'Neill DP, Distasio RA, Lochan RC, Wang T, Beran GJ, Besley NA, Herbert JM, Lin CY, Van Voorhis T, Chien SH, Sodt A, Steele RP, Rassolov VA, Maslen PE, Korambath PP, Adamson RD, Austin B, Baker J, Byrd EF, Dachsel H, Doerksen RJ, Dreuw A, Dunietz BD, Dutoi AD, Furlani TR, Gwaltney SR, Heyden A, Hirata S, Hsu CP, Kedziora G, Khalliulin RZ, Klunzinger P, Lee AM, Lee MS, Liang W, Lotan I, Nair N, Peters B, Proynov EI, Pieniazek PA, Rhee YM, Ritchie J, Rosta E, Sherrill CD, Simmonett AC, Subotnik JE, Woodcock HL, Zhang W, Bell AT, Chakraborty AK, Chipman DM, Keil FJ, Warshel A, Hehre WJ, Schaefer HF, Kong J, Krylov AI, Gill PM, Head-Gordon M. *Phys Chem Chem Phys*. 2006; 8:3172–3191. [PubMed: 16902710]
23. Feyereisen M, Fitzgerald G, Komornicki A. *Chem Phys Lett*. 1993; 208:359–363.
24. Weigend F, Haser M. *Theor Chem Acc*. 1997; 97:331–340.
25. Cerutti DS, Freddolino PL, Duke RE, Case DA. *J Phys Chem B*. 2010; 114:12811–12824. [PubMed: 20860388]
26. Norberg J, Nilsson L. *Proc Natl Acad Sci U S A*. 1996; 93:10173–10176. [PubMed: 8816771]
27. Steinbach PJ, Brooks BR. *Proc Natl Acad Sci U S A*. 1996; 93:55–59. [PubMed: 8552674]
28. Keutsch FN, Saykally RJ. *Proc Natl Acad Sci U S A*. 2001; 98:10533–10540. [PubMed: 11535820]
29. Laage D, Hynes JT. *Science*. 2006; 311:832–835. [PubMed: 16439623]
30. Chipanina NN, Aksamentova TN, Sherstyannikova LV, Sterkhova IV, Shainyan BA, Turchaninov VK. *Russ J Org Chem*. 2005; 41:1415–1420.
31. Muniz-Miranda F, Pagliai M, Cardini G, Righini R. *J Phys Chem A*. 2012; 116:2147–2153. [PubMed: 22309150]
32. Liu AZ, Lu ZW, Wang JF, Yao LS, Li Y, Yan HG. *J Am Chem Soc*. 2008; 130:2428–2429. [PubMed: 18247612]
33. Torshin IY, Weber IT, Harrison RW. *Protein Eng*. 2002; 15:359–363. [PubMed: 12034855]
34. Jiang DW, He Y, Budow S, Kazimierczuk Z, Eickmeier H, Reuter H, Seela F. *Acta Crystallogr*. 2010; C66:o561–o564.
35. Šponer J, Leszczynski J, Hobza P. *Biopolymers*. 2002; 61:3–31. [PubMed: 11891626]
36. Kondo J, Westhof E. *Nucleic Acids Res*. 2011; 39:8628–8637. [PubMed: 21737431]
37. Fridey SM, Srikrishnan T. *J Chem Crystallogr*. 2003; 33:695–700.
38. Eriksson L, Widmalm G. *Acta Crystallogr*. 2008; E64:o1639–o1640.

Figure 1.
The structure of the crystal unit cell of methyl 6-O-[(*R*)-1-carboxyethyl]- β -D-galactopyranoside.

Figure 2.
The experimental (black X) and averaged bond lengths from MD simulations (red circle) of the systems of single unit cell at temperatures of 100 K (A) and 293 K (B).

Figure 3.

The experimental (black X) and averaged valence angles from MD simulations (red circle) of the systems of single unit cell at temperatures of 100 K (A) and 293 K (B).

Figure 4. The distributions of five dihedral angles from MD simulations of the systems of a single unit cell at temperatures of 100 K (A) and 293 K (B). Corresponding

Figure 5.
The anisotropies of non-hydrogen atoms at 100 K (A) and 293 K (B).

Figure 6.
The B-factors of non-hydrogen atoms at 100 K (A) and 293 K (B).

Figure 7. The HO7--O6 (red X) and HO7--O8 (green plus) distances from a 600 ps time window (total simulation time 50 ns) at 100 K (A) and 293 K (B). The 600 ps windows are presented to allow for visualization of the time series. Analyses of the full 50 ns simulations yielded similar results.

Figure 8.

The distributions of the distances between HO7 and O6 or O8 in the adjacent molecule at 100 K (A) and 293 K (B).

Table 1

Crystal data and data collection parameters.

CSD reference code/CCDC	TEJHOF	802592
Diffractometer	Stoe AED2	Stoe IPDS
Wavelength	Cu K (= 1.54184 Å)	Mo K (= 0.71073 Å)
μ/mm^{-1}	1.122	0.131
Temperature/K	293(2)	100(1)
$a/\text{\AA}$	5.840(3)	5.835(2)
$b/\text{\AA}$	8.063(6)	8.067(2)
$c/\text{\AA}$	12.923(7)	12.855(3)
$\beta/^\circ$	101.74(6)	101.99(3)
$V/\text{\AA}^3$	595.8(6)	591.8(4)
Density/ g cm^{-3}	1.484	1.494
2θ (min, max) $^\circ$	6.98, 136.72	3.3, 52.1
Resolution (min, max)/ $\text{\AA}$	12.7 - 0.83	12.3 - 0.81
Number of measured reflections	4008	4584
Number of unique-reflections	2045(all); 1516 ($I > 2\sigma(I)$)	2278(all), 2030 ($I > 2\sigma(I)$)
Rint	0.0673	0.0305
Coverage	0.958	0.984
wR2	0.1411	0.1050
R1	0.0562	0.0390
$\rho_{\text{min}}, \rho_{\text{max}}/\text{e \AA}^{-3}$	-0.307, 0.236	-0.398, 0.418

Table 2

The averaged unit cell parameters from crystal MD simulations at 100 and 293 K.

100K	expt	1×1×1		2×2×2		4×4×4		8×8×8	
		calc	% err	calc	% err	calc	% err	calc	% err
a (Å)	5.835	5.96	2.1	6.02	3.2	5.96	2.1	6.16	5.6
b (Å)	8.067	8.08	0.2	8.02	-0.6	8.08	0.2	8.15	1.0
c (Å)	12.855	12.66	-1.5	12.75	-0.8	12.44	-3.2	12.39	-3.6
	101.99	101.88	0.1	99.85	-2.1	95.16	-6.7	100.98	-1.0
V (Å ³)	591.8	596.6	0.8	606.5	2.5	596.6	0.8	610.6	3.2
293K	expt	1×1×1		2×2×2		4×4×4		8×8×8	
		calc	% err	calc	% err	calc	% err	calc	% err
a (Å)	5.840	6.04	3.4	6.03	3.3	6.03	3.3	6.46	10.6
b (Å)	8.063	8.17	1.4	8.16	1.2	8.16	1.2	8.17	1.4
c (Å)	12.923	12.77	-1.2	12.79	-1.0	12.58	-2.6	12.54	-2.9
	101.74	101.39	-0.3	101.36	-0.4	94.66	-7.0	103.76	2.0
V (Å ³)	595.8	617.7	3.7	617.0	3.6	616.9	3.5	642.8	7.9