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Probing Deoxysugar Conformational Preference: A Comprehensive Computational Study Investigating the Effects of Deoxygenation

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Abstract

Deoxysugars are intrinsic components in a number of antibiotics, antimicrobials, and therapeutic agents that often dictate receptor binding, improve efficacy, and provide a diverse toolbox in modifying glycoconjugate function due to an extensive number of unique isomers and inherent conformational flexibility. Hence, this work provides a comprehensive examination of the conformational effects associated with deoxygenation of the pyranose ring. Both the location and degree of deoxygenation were evaluated by interrogating the energetic landscape for a number of mono- and dideoxyhexopyranose derivatives using DFT methods (M05–2X/cc-pVTZ(-f)). Both anomeric forms and in some cases, the alternate chair form, have been investigated in the gas phase. As was documented in a preceding study, variation of the C-6 oxidation state has been shown to affect the anomeric preference of select glucose stereoisomers. Similar results were also observed for several deoxysugar isomers in this work, wherein the alternate anomer was favored upon reduction to the 6-deoxyhexose derivative or oxidation to the hexonic acid. Additionally, comparison of relative Gibbs free energies revealed C-3 deoxygenation imparts greater instability compared to C-2 or C-4 deoxygenation, as indicated by an increase in free energy for 3-deoxysugars. A polarizable continuum solvation model was also applied to empirically validate theoretical results for several deoxysugars, wherein good agreement with both carbon ($\sigma = 1.6$ ppm) and proton ($\sigma = 0.20$ ppm) NMR shifts was observed for the majority of isomers. Solvated and gas phase anomeric ratios were also calculated and compared favorably to reported literature values, although some discrepancies are noted.

Graphical Abstract

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SUPPORTING INFORMATION

The supporting information includes an atomic numbering scheme, scheme of all deoxysugar structures investigated, extended compilation of literature references for computational studies and synthetic preparation. A corresponding Data in Brief article contains all three dimensional ball-and-stick structures, number of unique rotamers, Cartesian coordinates, number of imaginary frequencies, computed electronic and Gibbs free energies, entropies, enthalpies, and zero point energies of optimized structures.³⁰

INTRODUCTION

Deoxyhexoses have received increasing interest within recent years due to their presence in cardiac glycosides, polysaccharides, antigens, and a number of naturally derived antibiotics. Through the removal of one or more hydroxyl groups from the sugar ring accompanied by variations in stereochemical configuration, deoxysugars provide an extensive means to altering bioactivity.¹ A number of glycosylated natural products and drugs containing these types of sugars often exhibit anti-tumor, anti-microbial, and chemotherapeutic activity – thereby drawing much interest from the wider carbohydrate and biochemical communities.

The degree and location of deoxygenation uniquely affects deoxysugar activity and biological function. For example, 2-deoxysugars often play a role in the inhibition of human leucocyte and human leukemic cell glycolysis, wherein they are used to treat seizures and paroxysmal disorders.² 3- and 4-deoxysugars are less biologically relevant, although several are found in a number of *O*-polysaccharides and *O*-antigens. More specifically, the *O*-polysaccharides of *Escherichia coli* O162 contain 4-deoxy-D-*arabino*-hexose (4-deoxy-D-altrose),³ which is also found to occupy the side chain terminal position as an immunodominant sugar for other *O*-antigens such as *Yersinia frederiksenii*,⁴ *Franconibacter pulveris*,⁵ and *Citrobacter*.^{6,7}

In regard to antibiotic relevance, motifs of 2,6-dideoxysugars are found to be essential components of anti-tumor and anti-viral drugs such as olivomycin, flambamycin, chlorothricin, and jadomycin B – amongst others.^{8–11} 3,6-dideoxysugars are known to occur in the cell wall lipopolysaccharides of Gram-negative bacteria and function as the dominant serological determinants for several antigens.¹² Of these, abequose (3,6-dideoxy-D-*xylo*-hexose) has been found in the polysaccharide of *P. pseudotuberculosis* type II and tyvelose (3,6-dideoxy-D-*arabino*-hexose) was found to be related to *Salmonellae*.^{13,14} Another rare class of deoxysugars is the 4,6-dideoxyhexoses, whose 3-methyl ether derivatives can be found in the antibiotics chalcomycin, lankamycin, and neutramycin.^{15–17}

Of the more extensively deoxidized carbohydrate class, two 2,3,6-trideoxyhexoses have been isolated from bacteria. The most common, D-amicetose, is a constituent of the antibiotic amicetin, a universally recognized antiviral and antibacterial agent which functions as a peptidyl transferase inhibitor.¹⁸ The second, L-rhodinose, has been isolated from the antibiotics rhodomycin and streptolydigin, the latter of which has been found to inhibit the catalytic function of ribonucleic acid (RNA) polymerase.^{19,20} Although 2,4,6- and 3,4,6-trideoxyhexoses have not yet been found to be biologically relevant, efforts have been made to synthetically access specific isomers within this series and theoretically investigate their NMR spin-couplings in solution.^{21,22} Further evaluation of these highly deoxidized compounds could prove to be useful in future modification of antibiotic and antimicrobial function.

Due to the extensive role of deoxysugars in biochemical processes, as were previously highlighted, understanding the conformational landscape of these molecules is of high importance. A strong understanding of conformational preference is essential when determining biological structure-activity relationships, as well as predicting synthetic reactivity. Although a number of deoxysugars have been isolated from natural sources or have been synthetically prepared, the majority of mono-, di- and trideoxyhexoses have not been theoretically modeled. To date, the conformational landscapes of only four dideoxyaldohexoses have been interrogated using molecular mechanics²³ or molecular dynamics simulations.²⁴ These isomers include D-digitoxose (2,6-dideoxy-D-*ribo*-hexopyranose), abequose (3,6-dideoxy-D-*xyl*o-hexopyranose), paratose (3,6-dideoxy-D-*ribo*-hexopyranose), and tyvelose (3,6-dideoxy-D-*arab*ino-hexopyranose). In addition, very few theoretical studies have focused on the conformational preferences of monodeoxysugars. The solvation free energies for 4-deoxy- β -D-*xyl*o-hexopyranose, 3-deoxy- β -D-*xyl*o-hexopyranose, and 2-deoxy- β -D-*lyx*o-hexopyranose were calculated by Daranas and coworkers in regard to the thermodynamic binding with the L-arabinose binding protein.²⁵ The only other theoretical study was carried out by Lonardi and coworkers wherein the influence of solvent polarity on hydroxymethyl rotameric state was investigated for 4-deoxy- β -D-*xyl*o-hexopyranose.²⁶ In regard to trideoxysugars, knowledge of their conformational preference is also limited with only the *J*-spin coupling constants calculated for both anomers of 3,4,6-trideoxy-D-*erythro*hexopyranose and 3,4,6-trideoxy-D-*threo*-hexopyranose via DFT methods.²² Considering that very few deoxysugars have been theoretically studied to date, this work aims to provide further knowledge regarding carbohydrate deoxygenation and its effect on conformational preference at the monosaccharide level.

Through a systematic investigation of the conformational preferences imparted by deoxygenation at various locations around the hexopyranose ring, valuable insights can be gained regarding changes in electronic distribution, steric influences, and variations in intramolecular hydrogen-bonding patterns – all of which have potential for informing reactivity. Additionally, future comparison of these deoxysugar derivatives to their fully hydroxylated parent sugars aims to elucidate important structural features that drive overall carbohydrate conformation. Compared to conventional spectroscopic methods, which are hampered by conformational averaging and offer limited structural information, computational methods are a valuable alternative that provide access to discrete

conformational states and a broader understanding of the overall energetic landscape.²⁷ In order to fully assess the implications of deoxygenation, a large compilation of deoxysugar stereoisomers were computationally modeled in this study and a simplified scheme of these structures is provided in Figure 1. An expanded version including all discrete deoxysugar structures modeled (Figures S2 and S3), as well as a comprehensive literature compilation of all synthetic preparative routes and related computational studies (Tables S1 and S2) can be found in the SI.

Due to a large number of possible rotameric states (9–729 potential rotamers per isomer) and a high degree of ring flexibility associated with this class of molecules, studying their conformational landscape theoretically can prove to be challenging. In terms of ring preference, previous experimental and theoretical studies have shown that deoxygenation can increase the population of the alternate chair conformation, as well as result in larger contributions of the furanose form.^{23,28} However, the ⁴C₁ canonical chair conformation in the pyranose form is still commonly found to be the dominant conformer within the overall population, as evidenced by previous publications of deoxysugars.^{23,24} Accordingly, all deoxyhexoses within this study were modeled in the ⁴C₁ conformation in an effort to minimize the number of structures in the sugar set and limit the subsequent number of calculations needed. Due to our previous findings where several β -hexuronic acid isomers were found to show preference for the ¹C₄ conformation (i.e. β -alluronic acid, β -guluronic acid, β -iduronic acid), a number of beta dideoxy- and trideoxyhexonic acids in the alternate chair conformation were also investigated to elucidate any common structural features that may drive this particular ring conformational preference.²⁹

In an effort to further validate theoretical results, solvation calculations for several deoxysugar isomers were carried out using a polarized continuum solvation model. A comparison of calculated and experimental ¹H and ¹³C NMR shifts, as well as solvated anomeric ratios, is discussed in the following section.

RESULTS AND DISCUSSION

Lowest Energy Deoxysugar Conformations

The relative gas phase free Gibbs energies of the lowest energy conformers for the dideoxyhexoses in the ⁴C₁ conformation with varying oxidation state at the C-6 position were calculated relative to the lowest energy structure in each sugar series (see Table S5 in Ref. 30). All three-dimensional ball-and-stick structures are provided (see Figures S1–S3 in Ref. 30), the number of unique rotamer structures investigated (see Table S1 in Ref. 30), and calculated enthalpies, entropies, and zero point energies are tabulated (see Table S4 in Ref. 30).

In addition, the monodeoxyhexoses with varying oxidation state at the C-6 position were also theoretically investigated and their relative Gibbs free energies are provided in Table S6. Free energies for this subset were calculated in the same way as for the dideoxyhexoses, relative to the lowest energy structure in each sugar series. All three-dimensional ball-and-stick structures are provided (see Figures S4–S6 in Ref. 30), the number of unique rotamer structures evaluated (see Table S2 in Ref. 30), and calculated enthalpies, entropies, and zero

point energies are tabulated (see Table S4 in Ref. 30). Cartesian coordinates for all rotamers are also provided (see Tables S7–S24 in Ref. 30).

Changes in Deoxysugar Anomeric Preference Upon Oxidation or Reduction at the 6-Position

A previous study carried out by our group showed the inherent anomeric preference of several glucose stereoisomers was affected by changes in oxidation state at the C-6 position.²⁹ Namely, derivatives of allose, mannose, talose, and idose showed shifts in preference from one anomer to the other upon oxidation or reduction of the hydroxymethyl substituent to either the methyl or carboxylic acid group. In order to further investigate the underlying driving forces regarding anomeric preference, the corresponding deoxysugar substrates were investigated. Figures 2 and 3 highlight the difference between the relative free energy values for the α - and β -anomers [ΔG_{FAV}] of the mono- and dideoxysugar derivatives in the 4C_1 conformation *in vacuo*. A positive value corresponds to a preference for the α -anomer, whereas a negative value corresponds to favoring of the β -anomer. Surprisingly, for the dideoxysugar derivatives, the presence of an axial hydroxyl group in either the 2- or 4-position was found to affect anomeric preference upon changes in oxidation state at the C-6 position. In the presence of an axial hydroxyl at the 2-position, both the aldohexose (3,4-dideoxy-D-*threo*-hexopyranose) and hexonic acid (3,4-dideoxy-D-*threo*-hexonic acid) derivatives showed preference for the β -anomer by 0.96 and 0.11 kcal mol⁻¹, respectively. However, upon reduction at C-6 (3,4,6-trideoxy-D-*threo*-hexopyranose), a switch in preference to the α -anomer by 0.33 kcal mol⁻¹ was observed. The opposite effect was found for deoxysugar derivatives with an axial hydroxyl at the 4-position. In this case, the aldohexose (2,3-dideoxy-D-*threo*-hexopyranose) and hexonic acid (2,3-dideoxy-D-*threo*-hexonic acid) significantly favored the α -anomer by 2.48 and 2.60 kcal mol⁻¹. Reduction of the oxidation state at C-6 to give the 6-deoxysugar (2,3,6-trideoxy-D-*threo*hexopyranose) resulted in a shift in preference to the β -anomer by 0.49 kcal mol⁻¹. All other dideoxyhexose isomers within the series were shown to favor the α -anomer by more than 0.57 kcal mol⁻¹.

Investigation of the monodeoxysugar series also revealed that variation in the C-6 oxidation state affected anomeric equilibrium for three isomers (Figure 3). Specifically, the deoxy- (4,6-dideoxy-D-*arabino*-hexopyranose) and aldohexose (4-deoxy-D-*arabino*-hexopyranose) derivatives with an axial hydroxyl at the 2- and 3-positions both showed a slight preference for the α -anomer. However, oxidation to the hexonic acid (4-deoxy-D-*arabino*-hexonic acid) shifted the equilibrium to favor the β -anomer by 0.56 kcal mol⁻¹. Conversely, the aldohexose (2-deoxy-D-*lyxo*-hexopyranose) and hexonic acid (2-deoxy-D-*lyxo*hexonic acid) derivatives with an equatorial hydroxyl at the 3-position and axial hydroxyl at the 4-position showed preference for the α -anomer, while reduction at C-6 to give the dideoxysugar (2,6-dideoxy-D-*lyxo*-hexopyranose) showed very slight favoring of the β -anomer by 0.02 kcal mol⁻¹. The third isomer that displayed variation in anomeric preference upon changes in C-6 oxidation state was the derivative with an axial hydroxyl at the 2-position and equatorial hydroxyl at the 4-position. The aldohexose derivative (3-deoxy-D-*arabino*-hexopyranose) showed a 0.24 kcal mol⁻¹ preference for the α -anomer, while reduction to the dideoxysugar (3,6-dideoxy-D-*arabino*-hexopyranose) or oxidation to the hexonic acid (3-deoxy-D-

arabino-hexonic acid) slightly shifted the preference to the β -anomer by 0.02 and 0.14 kcal mol⁻¹, respectively.

Interestingly, additional evaluation of anomeric preference for this series indicates favoring of the β -anomer for two isomers regardless of C-6 oxidation state variation. Due to the anomeric effect, the α -anomer would typically be expected to be favored in the gas phase. However, this is not the case for derivatives of 4-deoxy-D-*ribo*-hexopyranose and 3-deoxy-D-*lyxo*-hexopyranose where the β -anomer is favored for all structures by 0.26–1.37 kcal mol⁻¹. Initial conformational analysis of the 4-deoxy-D-*ribo*-hexopyranose derivatives would suggest that the instability of the α -anomer could be imparted by a disfavored 1,3-diaxial interaction between the anomeric hydroxyl and axial substituent at the 3-position. However, the stereochemistry at the 2-position also plays a role in the stability of the sugar, as the isomer with an axial hydroxyl at C-2 shows α -anomer preference for the 6-deoxy- (4,6-dideoxy-D-*arabino*-hexopyranose) and aldohexose (4-deoxy-D-*arabino*-hexopyranose) derivatives, but maintains the β -anomer preference for the hexonic acid sugar (4-deoxy-D-*arabino*-hexonic acid). This suggests that the stereochemistry at the 2-position and oxidation state at C-6 affect the anomeric centre in a codependent fashion when there is a lack of a hydroxyl at the 4-position and presence of an axial hydroxyl at the 3-position.

Evaluation of the 3-deoxy-D-*lyxo*-hexopyranose series also reveals a similar positional synergy, where the presence of an axial hydroxyl at the 2-position and either an axial or equatorial hydroxyl at the 4-position shifts anomeric preference to favor the β -anomer (Figure 4). Assessment of the discrete three-dimensional structures for these isomers reveal that the unexpected anomeric preference may be due to a weak intramolecular hydrogen-bonding event between the β -anomeric hydroxyl group and the axial hydroxyl at the 2-position. As was observed in previous studies, the stability of a discrete carbohydrate conformation is often strengthened by the presence of intramolecular hydrogen bonds and reliant upon the ability of the hydroxyl groups to form hydrogen bonding networks or multiple adjacent hydrogen bonding contacts. Although it is still unclear whether the location, direction, or number of hydrogen bonds follows a particular trend in relation to overall carbohydrate stability, analysis of this system indicates that the presence and particular direction of a hydrogen bond between the anomeric hydroxyl and secondary hydroxyl at the 2-position is a stabilizing factor. It is apparent that the anomeric preference is highly dependent on the stereochemistry of the C-2 position in terms of potential hydrogen bond formation. Isomers with an equatorial 2-OH (3-deoxy-D-*ribo*- and 3-deoxy-D-*xylo*-derivatives), irrespective of 4-position orientation or C-6 oxidation state, show favoring of only the α -anomer, while isomers with an axial hydroxyl at the same position show a greater preference for the β -anomer (3-deoxy-D-*lyxo*- and 3-deoxy-D-*arabino*-derivatives) – with the exception of 3-deoxy-D-*arabino*-hexopyranose. The directionality of the hydrogen bond at the anomeric position also plays a role in the conformational stability, as a stronger β -anomer preference is observed when the C-2 hydroxyl serves as the hydrogen bond acceptor and the anomeric hydroxyl as the hydrogen bond donor. This alignment allows for an additional hydrogen-bonding contact between the C-2 hydroxyl hydrogen and C-4 hydroxyl oxygen, further extending the consecutive hydrogen-bonding network. Therefore, the presence of an axial hydroxyl at the C-4 position increases the stability of the β -anomer more than an equatorial 4-OH. Congruently, a decrease in β -

anomer preference is observed for both 3-deoxy-D-*arabino*-hexopyranose and 3,6-dideoxy-D-*arabino*-hexopyranose, where the anomeric and 2-OH hydrogen bond direction is reversed.

Instability Associated with C-3 Deoxygenation

In order to fully understand the conformational effects associated with deoxygenation, an initial comparison regarding the location of deoxygenation (removal of one or more secondary hydroxyls at the 2-, 3-, or 4-positions) was carried out in respect to isomer and anomeric stability. For the mono- and dideoxysugar series, comparison of relative energies and location of deoxygenation revealed that removal of a hydroxyl group at the 3-position resulted in a decrease in stability (higher Gibbs free energy) ranging from 0.16–5.31 kcal mol⁻¹ for the majority of isomers – when either one secondary hydroxyl at the 2-, 3- or 4-position was axial or all other hydroxyls were equatorial (Table 1).

Carbohydrate instability associated with deoxygenation at the C-3 position has been documented in previous literature accounts empirically, but never fully explored computationally. Specifically, Angyal and Pickles reported that removal of the hydroxyl group at the C-3 position of D-glucose to give 3-deoxy-D-*ribo*hexopyranose resulted in a 29% increase in the furanose form, indicating that a major interaction had been removed.²⁸ The authors suggest the instability is due to varying interaction between the C-3 hydroxyl and hydroxymethyl substituent in a pyranose versus furanose ring. It was proposed that C-3 deoxygenation alleviates the steric interaction between these substituents in the furanose form, thereby increasing the furanose population present at equilibrium. Empirical studies carried out by Pratt and Richtmyer comparing the reactivity of 3-deoxy-D-*arabino*-hexose and 3-deoxy-D-*ribo*-hexose with their parent sugars (D-mannose/D-altrose; D-glucose/D-allose) further demonstrate the reactivity differences between deoxysugars and fully hydroxylated substrates.³¹ It was observed that the formation of anhydride in aqueous acid is dependent on the orientation and presence of the C-3 hydroxyl group. Starting from the 3-deoxy-D-*arabino*-hexose substrate, 29% of the anhydride product was obtained, while only 1% was formed from D-mannose and 57% from D-altrose. Comparatively, the 3-deoxy-D-*ribo*-hexose substrate yielded 10% of the anhydride, while the fully hydroxylated epimers showed varying yields (D-Glc >2%; D-All 14%). These results demonstrate that deoxygenation at the 3-position increases reactivity when compared to fully hydroxylated substrates with equatorial substituents, but also that stereochemistry plays a role as evidenced by the cases of altrose and allose where the axial hydroxyl at C-3 increases the amount of anhydride product. These differences in yield further demonstrate the varying reactivity profiles imparted by deoxygenation and epimerization.

In an effort to provide an explanation for the destabilizing effect associated with deoxygenation at the 3-position, three-dimensional structures for the lowest energy rotamers of 2-, 3-, and 4-deoxyglucose were evaluated and indicate that the instability may be due to disruption of the cooperative hydrogen-bonding network (Figure 5). Consecutive hydrogen bonds in this context are defined as a network of hydrogen bond donors and acceptors with the same directionality, either clockwise or counter-clockwise (as indicated with blue dotted lines). Removal of a hydroxyl at the 2- or 4-positions still allows for potential hydrogen

bonding between two to three consecutive hydroxyl group partners, whereas removal of the hydroxyl group at the C-3 position decreases the potential number of hydrogen bonding interactions to only one or two – depending on the identity of the C-6 substituent (CH₂OH or CH₃).

Although this certainly is a possible explanation for the instability observed for 3-deoxysugars, other effects such as electronic or steric implications may also be contributing factors even if they are not apparent at this time. This is evidenced by the fact that oxidation at the C-6 position to the carboxylic acid results in an even greater destabilizing event for three isomers in the series (4-deoxy- β -D-*xyl*o-hexonic acid, 4-deoxy- β -D-*lyx*o-hexonic acid, 4-deoxy- α -D-*ribo*-hexonic acid) which are higher in energy than their 3-deoxysugar counterparts (Table 3).

Location and Degree of Deoxygenation

Another interesting trend related to anomeric preference was found when comparing the relative stability of the 2-, 3-, and 4- β -deoxysugars. For the 6-deoxy- and aldohexose sugar series, the relative energy order amongst isomers is consistent, with all 4-deoxysugars displaying the lowest energy, followed by the 2-deoxysugars and then the 3-deoxysugars with the highest relative energy (Table 2). This trend, however, does not hold for the α -deoxysugars or hexonic acid series. Comparison of the relative energies for the α -deoxysugars in Table 1 indicates 3-deoxygenation raises the energy for all isomers within the 6-deoxy and aldohexose series, but there is no consistency in the relative order for the 2- or 4-deoxygenated isomers. Similar results are observed for the hexonic acid derivatives, where no consistent trends in relative energy are apparent. Analysis of deoxysugars with more than one axial hydroxyl group also show similar results, wherein no correlation between deoxygenation location and relative energy was observed.

Boltzmann Population Distribution and Predicted Anomeric Ratios

The calculated percentages of each anomer for all mono- and dideoxysugar derivatives in the gas phase are reported in Table 3. All values include the total ensemble of rotamers for each given isomer and have been calculated using the Boltzmann distribution. Isomers are specified using conventional nomenclature with the stereochemistry (R or S) of each present secondary hydroxyl group(s) at the 2-, 3-, and/or 4-position(s). The presence of a hydroxyl group at the anomeric centre is indicated as (1) considering the values correspond to anomeric ratios where both orientations are included.

A comparison between the anomeric preference and stereochemistry for mono- and dideoxysugars reveals that the α -anomer is favored for sugars with all equatorial hydroxyl groups at the 2-, 3-, and/or 4-positions, regardless of degree or location of deoxygenation and C-6 oxidation state (Figure 6). Alternatively, the presence of one or more secondary axial hydroxyl groups is found to increase the β -anomer preference for several isomers. Deoxysugars with an axial hydroxyl at the 2- and 4-positions showed the greatest favoring of the β -anomer for all C-6 oxidation states ((1)2S, 4R).

In terms of the degree of deoxygenation, the majority of trideoxyhexoses favor the α -anomer, with the only exception being 2,3,6-trideoxy-D-*threo*hexopyranose, which slightly

favors the β -anomer by 11% (Table 3, entry (1)4R). In contrast, a greater number of mono- and dideoxyhexoses favor the β -anomer overall, thus indicating that an increase in the number of hydroxyls on the pyranose ring may contribute to the stability of the less thermodynamically favored β -anomer.

Evaluation of the Preference for the 1C_4 Chair Conformation Associated with β -Hexuronic Acid Parent Sugars

As reported in a previous study on the conformational preference of glucose stereoisomers in the gas phase, several β -hexuronic acid derivatives were found to favor the alternate 1C_4 chair over the commonly preferred 4C_1 canonical conformation.²⁹ Namely, the β -anomers of alluronic acid, guluronic acid, and iduronic acid were shown to favor the 1C_4 conformer by 0.04–1.38 kcal mol⁻¹. Evaluation of the discrete conformational structures of these sugars provided little insight when attempting to rationalize this switch in ring conformational preference. Therefore, in an effort to further understand any steric and/or electronic implications that may be driving this unusual conformational behavior, the mono- and dideoxyhexonic acid daughter structures were theoretically investigated in the 1C_4 conformation (see Figure S4 in the SI). The relative gas phase free Gibbs energies of the lowest energy conformers for this subset of deoxysugars are presented in Table 4, relative to the lowest energy structure in each series (i.e. 3,4-dideoxy- β -D-*threo*-hexonic acid and 4-deoxy- β -D-*arabino*-hexonic acid). The percentage of 4C_1 and 1C_4 conformers for the total rotamer ensemble is also included as calculated via the Boltzmann distribution. All three-dimensional ball-and-stick structures are provided (see Figure S8 in Ref. 30), as well as the number of unique rotamer structures investigated (see Figure Table S3 in Ref. 30) and calculated enthalpies, entropies, and zero point energies (see Table S4 in Ref. 30). Cartesian coordinates are provided in Tables S25 and S26 in Ref. 30.

Interestingly, several deoxysugar derivatives favor the 1C_4 conformation with the highest preference observed for 4-deoxy- β -D-*arabino*-hexonic acid (2:98). In regard to the dideoxyhexonic acid series, it is apparent that isomers with an axial hydroxyl group at either the 2-, 3-, or 4-position favor the 1C_4 conformer by approximately 70% while isomers with an equatorial hydroxyl at either the 2- or 4-position show 4C_1 preference. This trend, however, does not propagate within the monodeoxyhexonic acid series, where only three isomers were observed to favor the 1C_4 chair. A correlation between secondary hydroxyl stereochemistry and deoxygenation location for this series is not apparent, as isomers with both axial and equatorial perturbations and varying deoxygenation patterns do not show a related conformational preference. The effects observed within the monodeoxysugar series are therefore not additive, wherein adding a second axial hydroxyl group does not increase 1C_4 conformer population. This is especially evident in the case of 3-deoxy- β -D-*lyxo*-hexonic acid where the 4C_1 chair is highly preferred (99:1) in spite of two axial hydroxyl groups at the 2- and 4-position.

Solvation of Deoxysugars: Comparison to Experimental NMR Shifts

In an effort to further validate theoretical results, solvation calculations were carried out using a polarizable continuum model that implicitly treats solvent based on its dielectric constant. As it is well known that solvent has a significant effect on the anomeric ratio and

overall conformation of carbohydrates, these solvation calculations aim to provide a more accurate account of deoxysugar shape when in solution. Solvation calculations were carried out using the solvent for which the experimental NMR was taken, either deuterated methanol ($\epsilon = 32.7$), deuterium oxide ($\epsilon = 78.5$), chloroform-d ($\epsilon = 4.8$) or dimethyl sulfoxide ($\epsilon = 47.2$). Although several publications provided routes to synthetically access deoxysugars, NMR values were not included in some reports. In addition, many of these publications did not report coupling constants that would be indicative of ring conformation ($^3J_{H1,H2}$, $^3J_{H2,H3}$, $^3J_{H3,H4}$, $^3J_{H4,H5}$), thus necessitating the use of NMR chemical shift values for comparison. As a result, NMR shielding values were only calculated for deoxysugars for which experimental proton and/or carbon NMR shifts were explicitly stated in the literature. 32–42

The calculated NMR values correspond to the lowest energy rotamer in the 4C_1 chair out of the solvated rotamer ensemble, see Tables S26 and S27 in Ref. 30. Initial comparison of the calculated NMR predictions with empirical values showed general agreement with an average deviation of 1.6 ppm (^{13}C NMR) and 0.20 ppm (1H NMR). Although the 4C_1 chair conformation commonly predominates the conformer population, careful consideration of the contribution of other ring conformational states must be taken into account when comparing these values. Some disagreement between predicated and empirical values is expected where spectral overlap or conformational averaging from alternate conformer contributions may lead to difficult interpretation of some NMR shifts. Although incorporation of these alternate conformers would likely improve agreement with empirical values, especially inclusion of the 1C_4 chair, the number of calculations required for the extensive number of deoxysugars evaluated here is not feasible within this context and may instead be included in future solvation studies. In addition, several NMR experiments were carried out as a mixture of anomers or were known to include furanose forms and are denoted with an asterisk. For example, the spectra for 3-deoxy-D-ribo-hexopyranose (3-deoxyglucose) was obtained from a mixture of 51% β -pyranose, 26% α -pyranose, 17% β -furanose, 6% α -furanose.³⁶

Solution phase anomeric ratios are also provided for select sugars for which experimental values were reported (Table 5). Calculated gas phase anomeric ratios vary greatly compared to solution, with many isomers showing preference for the opposite anomer in the alternate phase. Although an ensemble of rotamers was used to calculate the solution phase anomeric ratios, the solvation effect was not completely captured and large disagreements between theoretical and empirical ratios were observed. This may be attributed to the lack of furanose forms or alternate ring conformations included in the theoretical population, as it is well known that deoxysugars show a higher contribution of the furanose and 1C_4 chair conformation in solution. Additionally, several experimental studies only reported anomeric ratios for mixtures of deoxysugars, without delineating between conformer or anomer form, further contributing to discrepancies. Furthermore, polarizable continuum solvation models often suffer from accurately representing bulk solvent effects and incorporating explicit hydrogen-bonding interactions, which also likely contributes to the inconsistencies observed.⁴³ As this study was limited by system size, it is recommended that future investigations aim to include these additional conformational states or use molecular dynamics simulations when calculating solvated deoxysugar structures. A recent publication by Pancyzk and

Plazinski demonstrated the use of molecular dynamics simulations in modeling carbohydrate and solvent interactions.²⁴ Calculated anomeric ratios for the dideoxyaldohexose series were not compared to experimental values within the study, however, but slightly better agreement for 2,6-dideoxy-D-*ribo*hexopyranose (digitoxose) was observed using molecular dynamics simulations (67:33) versus the polarizable continuum model (79:21). Therefore, future solvation calculations of highly deoxidized sugars may significantly benefit from the use of molecular dynamics or alternate approaches that incorporate all conformational states and explicit solvent molecules (i.e. high level QM/MD simulations).

CONCLUSIONS

The conformational space of mono- and dideoxyhexopyranose derivatives was evaluated using density functional theory methods in an effort to more thoroughly understand the correlation between the degree and location of deoxygenation and its effect on anomer and ring conformer preference. Initial investigation of the lowest energy rotamers within each deoxysugar series showed that the formation of cooperative hydrogen-bonding networks, as well as the directionality of these hydrogen-bonding contacts, could have significant stabilizing effects on deoxysugar conformation in the gas phase. In addition, it was found that deoxygenation at the C-3 position, which disrupts the intramolecular hydrogen-bonding network, has a destabilizing effect exemplified by a greater increase in Gibbs free energy for 3-deoxysugar isomers compared to deoxygenation at either the 2- or 4-position, where partial networks are still maintained.

In regard to the total rotamer ensemble, anomeric ratios were calculated via the Boltzmann population distribution for all deoxyhexose derivatives. Interestingly, trideoxyhexoses were found to mostly favor the α -anomer, with the exception of 2,3,6-trideoxy-D-*threo*-hexopyranose, while a greater number of mono- and dideoxyhexose isomers favored the less thermodynamically favorable β -anomer. In addition to anomeric preference, the conformational preference for select β -hexonic acid isomers was also investigated in order to rationalize the 1C_4 conformer preference previously observed for several β -hexuronic acid parent sugars (i.e. β -alluronic acid, β -guluronic acid, β -iduronic acid). It was found that several deoxysugars within the series also favored the 1C_4 chair conformation, with the highest preference observed for 4-deoxy- β -D-*arabino*-hexonic acid. Dideoxyhexonic acids with an axial secondary hydroxyl group were also found to highly favor the alternate chair conformation, while derivatives with equatorial substituents showed preference for the commonly favored 4C_1 conformer. This trend, however, was not found to extend to the monodeoxyhexonic acid series, where secondary hydroxyl stereochemistry and conformational preference showed minimal correlation.

Finally, validation of theoretical results was carried out through comparison of calculated proton and carbon NMR shifts with experimental values. General agreement between chemical shifts was observed with small standard deviations. A comparison between empirical and calculated anomeric ratios in solution was also carried out for select deoxysugars. Less agreement between these values was observed and is likely due to incomplete capture of bulk solvent effects and inexplicit representation of hydrogen-bonding interactions, as well as a lack of potential low energy conformers that may contribute

significantly to the overall population when in solution. It is recommended that quantum mechanical methods in combination with molecular dynamics simulations or solvation models that are explicitly parameterized for carbohydrate molecules be used in future studies involving deoxysugars.

Overall, it is apparent that the conformational landscape of deoxysugars is highly complex and influenced by a number of interrelated stereochemical factors. In this regard, theoretical methods provide valuable structural insight when interpreting structure-activity relationships and may potentially be used to predict carbohydrate reactivity in the future with more extensive interrogation of protecting group effects. We hope this work will inspire more experimental work on carbohydrate ring conformations as well as serve as the starting point for machine learning approaches⁶³ to predict electronic structure and chemical reactivity.

COMPUTATIONAL METHODS

The conformational landscape of each stereoisomer was initially investigated by performing a Monte-Carlo type conformational search to generate a series of unique monosaccharide rotamers for a given structure using the Global-MMX program (GMMX) in PC Model 9.30⁴⁴ employing the MMX force field which is derived from MM2 with π -VESCF routines taken from the MMP1 program.^{45–47} The π -VESCF routines were modified for open shell species by McKelvey, whereas Gajewski improved the calculations of heat of formation. A large number of low energy rotamers were located, but only structures that were within 4.00–4.50 kcal mol⁻¹ of the nearest low energy rotamer were included in the final set. Due to occasional unwanted relaxation of the ring geometry to skew-boat or boat-like structures during the conformational search, a substructure method was employed to freeze the ring atoms and maintain ring conformation throughout the simulation in order to avoid ring interconversion and epimerization. For all chair conformers, a substructure of the ring atoms was created and not minimized during the rotamer search.

The use of GMMX allowed a rapid generation of relevant conformers, but to get a more accurate picture of the energetics, this set of structures was additionally characterized with higher-level electronic structure calculations. Further optimization using DFT was carried out in the Jaguar 8.1 suite of ab initio quantum chemistry programs.⁴⁷ Geometry optimizations and vibrational entropies were calculated at the B3LYP/6–31G** level of theory,⁴⁹ followed by additional single point calculations using the Minnesota functional M05–2X⁵⁰ and Dunning's correlation-consistent polarized triple- ζ basis set cc-pVTZ(–f), which includes a double set of polarization functions on all atoms.⁵¹ Frequency calculations confirmed all structures were true minima. Energy components were calculated as follows:

$$\Delta G(\text{gas}) = \Delta H(\text{gas}) - T\Delta S(\text{gas})$$

(1)

$$\Delta H(\text{gas}) = \Delta E(\text{SCF}) + \Delta \text{ZPE}$$

(2)

where $G(\text{gas})$ is the change in gas phase free energy; $H(\text{gas})$ is the change in gas phase enthalpy; T is temperature (298.15 K); $S(\text{gas})$ is the change in gas phase entropy; $E(\text{SCF})$ is the self-consistent field energy, i.e., “raw” electronic energy as calculated at the triple- ζ level using M05–2X unless noted otherwise; ZPE is the change in vibrational zero point energy (ZPE).

Duplicate structures present after geometry optimization were removed by carrying out a statistical analysis, wherein the root-mean-square deviation (RMSD) was calculated for all rotamer populations. Quantitative comparison of RMSD values revealed similarity in three-dimensional (3D) structure based on atomic coordinate displacement. Duplicate rotamers were subsequently removed from the data set. Anomeric ratios are calculated using relative free-energy values for the total rotamer ensemble.

The choice of functional for obtaining final Gibbs free energies is based on previous work showing improved accuracy for monosaccharide modeling compared to the popular B3LYP exchange-correlation functional, even with dispersion corrections added.^{52,53} A functional and basis set screen was also performed confirming M05–2X had the best agreement with MP2 calculations (Fig. S1). It is important to note that to actually distinguish between small differences in anomeric ratios (i.e., 70:30 vs. 30:70) involves accurately calculating relative energetics on the order of less than 1 kcal mol^{−1}. Generally this is considered well beyond the error limit of modern DFT as shown in several benchmarking studies.^{54–56} Despite this, we are optimistic about the accuracy of the results reported herein for several reasons. Previous work by Schnupf and coworkers showed that DFT methods could be used to accurately determine anomeric ratios based on comparison with experimentally determined values.⁵⁷ We found that, despite using different methodology for our conformational searches, we obtained results similar to this work in most cases, suggesting that our computational methods likely have the same level of rigor. Exceptions to this are discussed below. We believe that the high accuracy of DFT in this case can be ascribed to the fact that we are comparing structures that are both extremely similar to each other, and in all cases relatively simple as well. This leads to massive error cancelations for our purpose of determining the conformational landscape. It is still always dangerous to over-interpret calculated data, however, and hence we emphasize that our most important results are the qualitative trends predicted by our calculations.

Although the conformational shape of these highly polarized molecules is fairly flexible in solution and interaction with solvent molecules may cause changes in conformation,^{58–60} accurately incorporating solvation effects in computer models remains a significant challenge. Molecular dynamics (MD) simulations that incorporate explicit solvent molecules have most commonly been used, as they better represent specific hydrogen-bonding

interactions and bulk solvent effects compared to implicit solvation.⁴³ While promising the most sophisticated handling of solvation effects, however, MD simulations are unfortunately time-intensive and computationally expensive. As a result, very few DFT-MD studies on sugars are found in the literature and only include one structure or a small subset of structures per publication due to the more intensive nature of these simulations.^{57,61} DFT-MD calculations require a run for each low energy rotamer which exponentially increases computational time. In addition, the interpretation of simulations can often be complicated due to conformational transitions of the pyranose ring to other conformers such as boat or skew-boat structures.

As an alternative, polarizable continuum models (PCM) have been developed that treat solvent implicitly by applying a dielectric constant to account for electrostatic interactions imparted by solvation.⁴³ They offer many advantages over MD simulations in that computational times are often shorter and are less expensive, while still offering relatively excellent predictive power. As such, solvation calculations were carried out using a polarizable continuum model (Poisson-Boltzmann) in the Jaguar 8.1 suite of ab initio quantum chemistry programs. Solution phase single point calculations were carried out at the M05-2X/cc-pVTZ(-f) level of theory, while vibrational frequency calculations and NMR shielding values were calculated at the B3LYP/6-31G** level of theory. The choice of solvent was determined by the NMR experiment for which empirical values were obtained, either deuterated methanol ($\epsilon = 32.7$), deuterium oxide ($\epsilon = 78.5$), chloroform-d ($\epsilon = 4.8$) or dimethyl sulfoxide ($\epsilon = 47.2$). Tetramethylsilane (TMS) was used as the reference molecule to calculate all NMR shifts. The TMS structure was geometry-optimized at the B3LYP/6-31G** level of theory and the corresponding solvated NMR shielding values were calculated at the same level of theory. The predicted proton and carbon isotropic shieldings were then averaged and used to calculate the deoxysugar NMR shifts ($\delta = \sigma_{\text{standard}} - \sigma_{\text{sugar}}$). The reported theoretical NMR shifts were calculated using only the lowest energy solvated rotamer in each sugar series. Jaguar's shielding values are expected to be accurate within ± 0.3 ppm for organic compounds.⁶² Although a more rigorous approach would involve a weighted average of several low energy conformers to give improved calculated shifts, we find that the NMR values calculated for only the lowest energy species is sufficient within this context.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The location and degree of deoxygenation of 108 mono-, di-, and trideoxyhexopyranose derivatives were evaluated using DFT methods (M05–2X/cc-pVTZ(-f)).
- Application of a polarizable continuum solvation model validated these theoretical results for several deoxysugars through comparison with published carbon and proton NMR data.
- Solvated and gas phase anomeric ratios were also calculated and compared favorably to previously reported experimental data.
- The modeling of the complete set of deoxysugars reveals trends to inform gas phase or synthetic chemistry experiments.

Figure 1.

The mono- and dideoxyhexopyranose stereoisomer structures investigated with varying oxidation state at C-6 in the 4C_1 chair conformation

Figure 2.

Changes in anomeric preference for dideoxyhexopyranoses (structures 1–36) with varying oxidation state at C-6 in the 4C_1 conformation. Gibbs free energy values (kcal mol $^{-1}$) are calculated as the difference between the β - and α -anomer [$\Delta(\Delta G)_{\text{FAV}} = G_{\beta} - G_{\alpha}$].

Figure 3.

Changes in anomeric preference for monodeoxyhexopyranoses (structures **37–108**) with varying oxidation state at C-6 in the 4C_1 conformation. Gibbs free energy values (kcal mol $^{-1}$) are calculated as the difference between the β - and α -anomer [$(\Delta G)_{FAV} = G_{\beta} - G_{\alpha}$].

Figure 4.

Three-dimensional structures of the preferred anomers for the 3,6-dideoxy-D-aldohexopyranoses, 3-deoxy-D-hexopyranoses, and 3-deoxy-D-hexonic acids. Gibbs free energies are reported as relative preference over the opposite anomer. (black = carbon, white = hydrogen, red/orange/blue = oxygen)

Figure 5.

Three-dimensional ball and stick structures of both anomers of 2-, 3-, and 4-deoxyglucose. Consecutive hydrogen bonding interactions are indicated with blue dotted lines while additional hydrogen bonds are shown in red; interatomic distances ($\text{\AA}$) are provided for reference (black = carbon, white = hydrogen, orange = oxygen). Gibbs free energies (kcal mol^{-1}) are calculated relative to the lowest energy isomer within the series (i.e. 2-deoxy- α -D-ribohexopyranose).

Figure 6.

Percentage of alpha anomer in relation to the number of secondary hydroxyl substituents at the 2-, 3-, and/or 4-position in the axial orientation

Table 1.

Comparison of Gibbs free energies (kcal mol⁻¹) for mono- and dideoxyhexoses with all equatorial secondary hydroxyls or one axial hydroxyl at either the 2-, 3-, or 4-position with varying oxidation state at C-6. Energies were calculated relative to the lowest energy isomer for each sugar series (i.e. 2,6-dideoxy- α -D-*ribo*-hexopyranose; 2-deoxy- α -D-*ribo*-hexopyranose; 2-deoxy- α -D-*ribo*-hexonic acid). The highest energy isomer within each set is denoted in red.

R =	Deoxy-	All Equatorial		2-OH Axial		3-OH Axial		4-OH Axial	
		α	β	α	β	α	β	α	β
CH ₃	2-Deoxy	1.44	2.93	1.44	2.93	0.00	2.48	2.32	2.30
	3-Deoxy	2.43	3.15	3.10	3.08	2.43	3.15	2.46	2.83
	4-Deoxy	0.59	1.57	1.53	1.82	1.47	0.75	0.59	1.57
CH ₂ OH	2-Deoxy	1.39	2.87	1.39	2.87	0.00	1.73	1.04	2.82
	3-Deoxy	2.59	4.30	3.31	3.55	2.59	4.30	3.26	3.45
	4-Deoxy	1.29	2.25	2.24	2.71	1.77	1.19	1.29	2.25
COOH	2-Deoxy	2.77	4.46	2.77	4.46	0.00	0.20	4.91	7.03
	3-Deoxy	5.26	5.51	5.37	5.23	5.26	5.51	5.78	7.09
	4-Deoxy	5.14	5.66	5.13	5.45	5.80	5.16	5.14	5.66

Table 2.

Comparison of relative Gibbs free energies (kcal mol⁻¹) for β-deoxysugars with all equatorial secondary hydroxyls or one axial hydroxyl at either the 2-, 3-, or 4-position with varying oxidation state at C-6. Energies were calculated relative to the lowest energy isomer for each sugar series (i.e. 2,6-dideoxy-α-ribo-hexopyranose; 2-deoxy-α-D-hexopyranose; 2-deoxy-α-D-ribohexonic acid).

R =	Deoxy-	All Eq.	2-OH Axial	3-OH Axial	4-OH Axial
CH ₃	3-Deoxy	3.15	3.08	3.15	2.83
	2-Deoxy	2.93	2.93	2.48	2.30
	4-Deoxy	1.57	1.82	0.75	1.57
CH ₂ OH	3-Deoxy	4.30	3.55	4.30	3.45
	2-Deoxy	2.87	2.87	1.73	2.82
	4-Deoxy	2.25	2.71	1.19	2.25
COOH	3-Deoxy	5.51	5.23	5.51	7.09
	2-Deoxy	4.46	4.46	0.20	7.03
	4-Deoxy	5.66	5.45	5.16	5.66

Highest Free Energy ← → Lowest Free Energy

Table 3.

Boltzmann distributions of the total ensemble of rotamers for all monoand dideoxyhexoses with varying C-6 oxidation state at the M05–2X/cc-pVTZ(-f) level of theory (*in vacuo*).

Isomer	R = CH ₃		R = CH ₂ OH		R = COOH	
	α : β	Preferred Anomer	α : β	Preferred Anomer	α : β	Preferred Anomer
(1)2S	51:49	α	26:74	β	58:42	α
(1)2R	77:23	α	87:13	α	68:32	α
(1)3S	90:10	α	87:13	α	95:5	α
(1)3R	97:3	α	95:5	α	96:4	α
(1)4S	83:17	α	80:20	α	90:10	α
(1)4R	39:61	β	97:3	α	98:2	α
(1)2S,3R	51:49	α	38:62	β	50:50	α/β
(1)2S,3S	56:44	α	67:33	α	64:36	α
(1)2R,3R	42:58	β	59:41	α	37:63	β
(1)2R,3S	85:15	α	89:11	α	75:25	α
(1)2S,4R	12:88	β	40:60	β	30:70	β
(1)2S,4S	48:52	β	60:40	α	44:56	β
(1)2R,4R	73:27	α	52:48	α	92:8	α
(1)2R,4S	79:21	α	91:9	α	72:28	α
(1)3R,4R	99:1	α	94:6	α	94:6	α
(1)3R,4S	92:8	α	96:4	α	58:42	α
(1)3S,4R	68:32	α	92:8	α	95:5	α
(1)3S,4S	91:9	α	91:9	α	91:9	α

Table 4.

Relative Gibbs free energies (kcal mol⁻¹) of the lowest energy rotamers of select mono- and dideoxyhexonic acids in the ¹C₄ conformation calculated at the M05–2X/cc-pVTZ(-f) level of theory (*in vacuo*), as well as calculated ⁴C₁:¹C₄ ratios via the Boltzmann distribution.

Compound	Systematic Name	Stereochemistry	(G) (kcal mol ⁻¹)	4C1:1C4	Preferred Conformer
28	3,4-Dideoxy-β-D- <i>threo</i> -hexonic acid	1R, 2S	0.00	29:71	1C4
36	2,3-Dideoxy-β-D- <i>threo</i> -hexonic acid	1R, 4S	0.37	36:64	1C4
26	3,4-Dideoxy-β-D- <i>erythro</i> -hexonic acid	1R, 2R	0.77	62:38	4C1
30	2,4-Dideoxy-β-D- <i>erythro</i> -hexonic acid	1R, 3R	0.80	34:66	1C4
34	2,3-Dideoxy-β-D- <i>erythro</i> -hexonic acid	1R, 4R	0.96	81:19	4C1
86	4-Deoxy-β-D- <i>arabino</i> -hexonic acid	1R, 2S, 3R	0.00	2:98	1C4
108	2-Deoxy-β-D- <i>xylo</i> -hexonic acid	1R, 3R, 4R	1.78	24:76	1C4
90	4-Deoxy-β-D- <i>ribo</i> -hexonic acid	1R, 2R, 3R	2.75	61:39	4C1
96	3-Deoxy-β-D- <i>lyxo</i> -hexonic acid	1R, 2S, 4R	2.80	99:1	4C1
106	2-Deoxy-β-D- <i>ribo</i> -hexonic acid	1R, 3R, 4S	2.99	100:0	4C1
98	3-Deoxy-β-D- <i>ribo</i> -hexonic acid	1R, 2R, 4S	3.05	45:55	1C4
100	3-Deoxy-β-D- <i>xylo</i> -hexonic acid	1R, 2R, 4R	3.85	60:40	4C1

Table 5.

Boltzmann distributions for select mono- and dideoxyhexoses calculated in the gas and solution phases at the M05–2X/cc-pVTZ(-f) level of theory. Experimental data was taken from references indicated below.

Systematic Name	$\alpha:\beta$ Ratio			Reference (Solvent)
	<i>In Vacuo</i>	Soln.	Exp.	
2,4,6-Trideoxy-D- <i>threo</i> -hexopyranose	90:10	72:28	-----	----- (CD ₃ OD)
2,3,6-Trideoxy-D- <i>erythro</i> -hexopyranose	83:17	82:18	-----	----- (CDCl ₃)
2,4-Dideoxy-D- <i>threo</i> -hexopyranose	87:13	58:42	-----	----- (CD ₃ OD)
4,6-Dideoxy-D- <i>lyxo</i> -hexose	56:44	72:28	-----	----- (D ₂ O)
2,6-Dideoxy-D- <i>ribo</i> -hexopyranose	92:8	79:21	67:33	33 (D ₂ O)
2,6-Dideoxy-D- <i>arabino</i> -hexopyranose	91:9	49:51	60:40	33 (D ₂ O)
2,6-Dideoxy-D- <i>xylo</i> -hexopyranose	99:1	35:65	80:20	33 (D ₂ O)
2,6-Dideoxy-D- <i>lyxo</i> -hexopyranose	68:32	35:65	50:50	33 (D ₂ O)
4-Deoxy-D- <i>xylo</i> -hexopyranose	89:11	65:35	33:67	35 (D ₂ O)
4-Deoxy-D- <i>lyxo</i> -hexopyranose	67:33	84:16	60:40	36 (D ₂ O)
3-Deoxy-D- <i>ribo</i> -hexopyranose	91:9	59:41	68:32	36 (D ₂ O)
3-Deoxy-D- <i>xylo</i> -hexopyranose	52:48	35:65	27:73	28 (D ₂ O)
2-Deoxy-D- <i>ribo</i> -hexopyranose	96:4	75:25	27:73	28 (D ₂ O)
2-Deoxy-D- <i>arabino</i> -hexopyranose	91:9	64:46	47:53	28 (D ₂ O)
2-Deoxy-D- <i>lyxo</i> -hexopyranose	92:8	74:26	48:52	28 (D ₂ O)
4-Deoxy-D- <i>xylo</i> -hexonic acid	75:25	70:30	-----	----- (D ₂ O)
2-Deoxy-D- <i>arabino</i> -hexonic acid	91:9	79:21	-----	----- (D ₂ O)