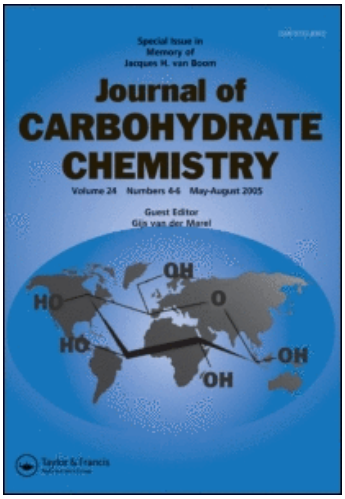


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

A Conformational Study of Hydroxymethyl Groups in Carbohydrates Investigated by ^{1}H NMR Spectroscopy

Klaus Bock[a]; Jens Ø Duus[b]
[a] Department of Chemistry, Carlsberg Laboratory, Valby, Copenhagen, Denmark [b] Beckman Institute, California Institute of Technology, Pasadena, CA

To cite this Article Bock, Klaus and Duus, Jens Ø(1994) 'A Conformational Study of Hydroxymethyl Groups in Carbohydrates Investigated by ^{1}H NMR Spectroscopy', Journal of Carbohydrate Chemistry, 13: 4, 513 — 543
To link to this Article: DOI: 10.1080/07328309408011662
URL: http://dx.doi.org/10.1080/07328309408011662

J. CARBOHYDRATE CHEMISTRY, 13(4), 513-543 (1994)

REVIEW

A CONFORMATIONAL STUDY OF HYDROXYMETHYL GROUPS IN CARBOHYDRATES INVESTIGATED BY ^{1}H NMR SPECTROSCOPY

Klaus Bock* and Jens Ø. Duus[$]

Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg Vej 10
DK-2500 Valby (Copenhagen), Denmark

[$]Current Address: Beckman Institute, California Institute of Technology,
Pasadena, CA 91125

TABLE OF CONTENTS

1. INTRODUCTION

It is generally acknowledged that information about carbohydrate conformation is important for the understanding of interaction between carbohydrates and other biomolecules, such as proteins.[1-5] The major part of oligosaccharides involved in the interactions are made up of hexopyranoses, and the overall conformation can be described by the rotation of the glycosidic linkages and the bonds to the exocyclic groups,[6,7] *e.g.*, the hydroxymethyl groups and *N*-Acetyl groups. The conformation of the hydroxymethyl group is of interest for interactions involving the hydroxyl group at this position.[2,8,9] Furthermore, the conformation of the C5-C6 linkage

determines the overall shape of oligosaccharides with glycosidic linkages to O6.[10-27] The conformational preferences of the hydroxymethyl group in mono and oligosaccharides have therefore been the subject of several investigations including both experimental and theoretical studies, as will be discussed in the following.

This report is focussed on the application of ^1H Nuclear Magnetic Resonance (NMR) spectroscopic studies to probe the hydroxymethyl group conformations in a series of hexopyranosides. This is of interest not only for the conformational description of the monosaccharides or oligosaccharides that contain hydroxymethyl functions, but furthermore the data can provide a good test for theoretical methods developed in the field of carbohydrate conformational analysis. Additionally, the structural differences between the monosaccharides investigated permits an evaluation of the forces determining the conformation of the hydroxymethyl group in carbohydrate derivatives in general. The compounds discussed are predominantly unprotected or partly protected hexopyranoside derivatives measured in D_2O, because these are of main interest for biological studies.

2. Methods used in conformational analysis of hydroxymethyl groups.

2.1 Nomenclature:

The conformation of the hydroxymethyl groups in hexopyranoses is predominantly determined by the rotation around the C5-C6 linkage,[7] as described by the dihedral angle ω (O5-C5-C6-O6) for the normal carbohydrates. In principle the ω angle can have any value in the 0 to 360° range, but generally the conformation is described by the three staggered conformations. These are denoted by the position of the O6 relative to the atoms at the C5-C6 bond (by the heavy atoms according to IUPAC rules, **Fig. 1**). It should be noted that other notations have been used in earlier literature.[28-30] One earlier frequently used notation is : g-, g+ and t corresponding to gg, gt and tg, respectively in the present notation.

The large collection of experimental data in the literature obtained from X-ray,[7,11,15,31] optical[32-39] and particularly NMR[10,18,19,22,23,29,30,40-58] studies indicates that a major effect responsible for the differences in the populations of the three conformations in the monosaccharides[31,40] is the relative orientation of the hydroxymethyl group and the substituent at the adjacent position C-4 (*cis* or *trans*, **Fig. 2**). For the normal D-sugars[31,40] this can be seen by the populations reported for *gluco* and *galacto* monosaccharides with approximate populations of gg:gt:tg ~ 6:4:0 for *gluco* (*trans*) and gg:gt:tg ~ 2:6:2 for *galacto* (*cis*). Furthermore, the populations are dependent on the configuration at C1 and the substitution pattern,[54] as will be demonstrated in the series of compounds investigated in the present study.

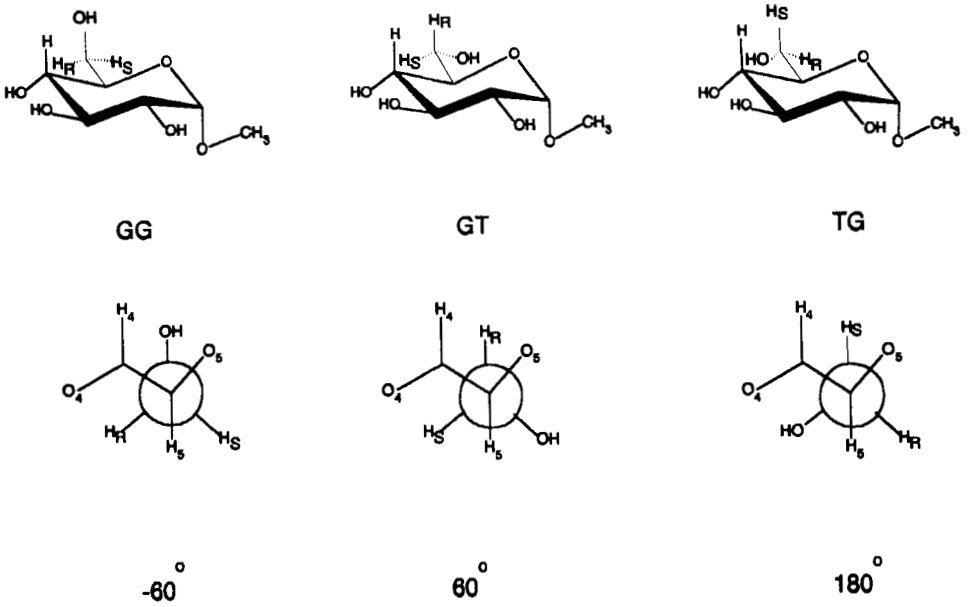


Fig. 1. Definition of the three staggered conformations for the hydroxymethyl group of D-hexopyranoses.

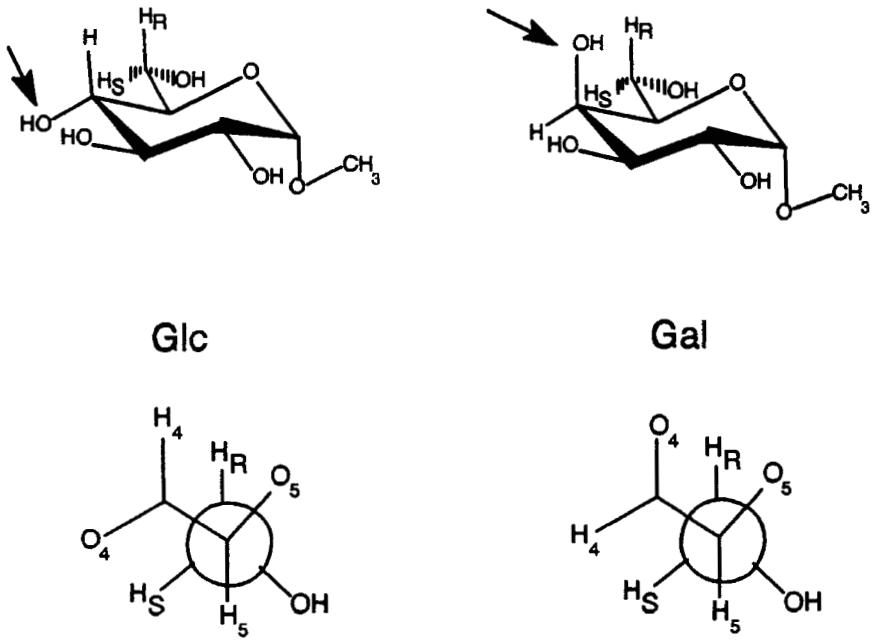


Fig. 2. Me α-D-Glucopyranoside and Me α-D-Galactopyranoside

The experimental evidence for the conformational preferences of the hydroxymethyl group in mono- and oligosaccharides mainly stems from X-ray crystallography and NMR studies. Thus a search of published X-ray data reported by Marchessault and Perez[31] gave a statistical distribution of gg:gt:tg 60:40:0 from 101 glucopyranose structures. Likewise a distribution of gg:gt:tg 8:58:34 for the fewer available (24) galactopyranose structures was observed.[31]

In the literature a few investigations of the preferred conformation of the hydroxymethyl group by optical methods have appeared.[32-39] The early studies by Lemieux and Martin[32,33] based on optical rotations gave good indications of the preferred conformation in glucose and galactose derivatives, but the methods have not been generally applied. More recent studies have applied CD techniques of, *e.g.*, benzoylated monosaccharides confirming the observations obtained by other techniques, *i.e.*, a preference for gg and gt conformations for glucose derivatives.[34,35]

Another recent extention to the conformational analysis of oligosaccharides is the application of time resolved fluorescence energy transfer, which have been utilized by Rice *et al.*[3,37-39] to obtain information about the relative populations of branched oligosaccharide structures having 1-6 linkages. The technique offers the possibility to derive information about long distances of different conformers if these are exchanging slower than about 10^{-9} s. The results are derived from the analysis of the fluorescent quenching between a fluorescent probe and a corresponding quencher which is covalently attatched at two sites in the oligosaccharide structure. The investigation of the branched structures resulted in a distance distributions in agreement with two different conformations of the 1-6 arm in good accordance with data obtained from NMR experiments.[38]

2.2 NMR spectroscopy.

The major technique used for the investigation of the hydroxymethyl group conformation in solution is NMR spectroscopy. Information about the conformational preferences of the hydroxymethyl groups can be obtained from NMR spectroscopy using the three-bond coupling constants between H5 and the two H6 protons, provided the stereospecific assignment is known or assumed. The two H6 protons are magnetically nonequivalent, and in the following the pro-R proton is denoted H6R and the pro-S proton H6S. The three-bond coupling constants of the coupled protons show a dependence on the angular orientation, with the largest coupling for an antiperiplanar orientation. This can be described by empirical equations modelling the observed relationship between couplings and angles. The type of equation is denoted "Karplus-equations" according to the first application by Karplus.[59,60] The observation that the coupling constants additionally depend on the substituent at the corresponding carbon atoms and their orientation[60-63] has led to the parameterization of the dependence of the $^3J_{H,H}$

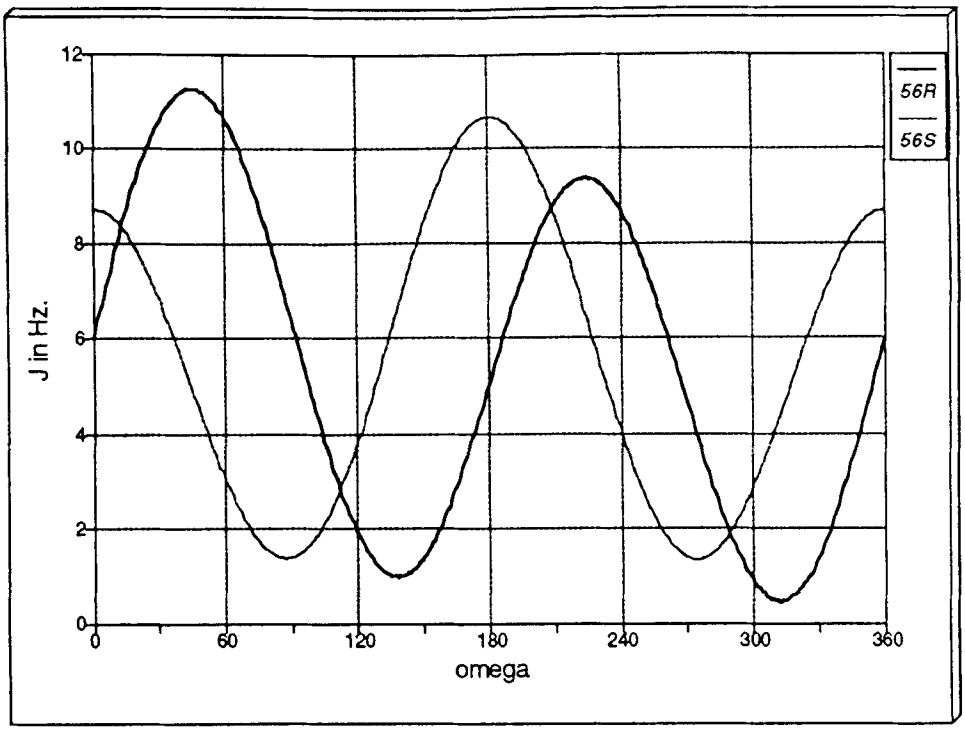


Fig. 3. Dependence of $^3J_{5,6R}$ and $^3J_{5,6S}$ on the ω angle as determined by the Haasnoot-Altona equation.[65]

on the dihedral angle and substituent electronegativity for example by Haasnoot and Altona[64,65] using electronegativies from Huggins.[66] For the three substituent case a series of 100 experimental coupling constants from compounds having restricted internal rotation were used to calculate six parameters giving the best fit between calculated and measured coupling constants with a rms-deviation of 0.49 Hz.[65] The H6R and H6S protons have different coupling constants for each of the staggered conformations (**Fig. 3**), as calculated by the above formula.[65]

The analysis of the curves shown in **Fig. 3** shows as expected that the gg rotamer (ω=-60°) with |60°| angles between both H5-C5-C6-H6R and H5-C5-C6-H6S gives rise to two small coupling constants. Both rotamers, gt and tg, have a large and a small coupling constant resulting from a *trans* and a *gauche* orientation, respectively, between H5 and the H6 protons. This means that for discriminating between gt and tg conformations the stereospecific assignment of the H6R and H6S is crucial. On the time scale of the NMR experiment the observed coupling constants will be time averaged values.[67-69] Assuming that the conformation

of the hydroxymethyl group can be described predominantly by the three staggered conformations their population can be determined from the two assigned coupling constants $J_{5,6R}$ and $J_{5,6S}$ and the fact that the sum of the population of the three conformations is one.

The limiting values for the three staggered conformations for "normal" hexo pyranosides using the Haasnoot-Altona formula[65] are as follows:

	gg	gt	tg
$J_{5,6R}$	0.9	10.7	5.0
$J_{5,6S}$	2.8	3.1	10.7

It can be seen that for the same proton-proton dihedral angles, *e.g.*, tg for H6R and gg for H6S, the coupling constants are different due to the different orientations of the electronegative substituents. Several other limiting values have been used for the determination of rotamer populations, as will be discussed later.

The equations can be solved mathematically or as shown by Bock and Pedersen[10] graphically by plotting the limiting values for the three staggered conformations in a XY-graph with $J_{5,6R}$ and $J_{5,6S}$ on the two axes. This results in a triangle in which any measured coupling constant should fit and the populations can be estimated by the "level rule". The fact that not all measured coupling constants fall within this triangle indicates that the limiting values from the Haasnoot-Altona formula might not be sufficiently accurate, as discussed in detail below, because the points outside the triangle correspond to negative populations. A recent extension of the Karplus equation by Imai and Osawa[70,71] includes more parameters and takes into account additional effects, *e.g*, through space effects. Unfortunately, these effects are not easily determined in flexible systems. Another recent extension of the Karplus-equations have been suggested by Altona and coworkers[72-74] including some higher order terms. These authors concluded that this extension of the data set have improved the performance of the equation on a set of test data, but have unfortunately not parameterized this approach generally.

Similarly to the ^1H-^1H coupling constants also the ^1H-^{13}C coupling constants provide information about the conformational preferences of the hydroxymethyl group, *e.g.* using the C-4 - H6R and C-4 - H6S coupling constants.[55,75,76] This method utilizes the dependence of the four bond coupling constants on the dihedral angle[77,78] like the corresponding ^1H-^1H coupling constants using, *e.g.*, the Karplus equation proposed by Spoormaker and de Bie[79] and gives results in accordance with data from ^1H-^1H coupling constants.[75,76] However, the accuracy using the ^{13}C-^1H long range coupling constants are less than that obtained from the corresponding ^1H-^1H values due to the smaller absolute value of the former (max. ~ 6Hz, compared to 10.7 Hz for the latter).

The combination of homo- and heteronuclear coupling constants can furthermore be used to stereospecifically assign the H6 signals.[55,76] A potential source of additional information

could be the one and two bond ^{13}C-^{13}C or ^{1}H-^{13}C coupling constants,[80,81] which have been shown to be sensitive to conformation. [80]

Recent measurements of ^{13}C relaxation times have given insight into the rotational freedom of the hydroxymethyl group of monosaccharides and oligosaccharides.[82-86] Hajduk *et al.* [82] concluded based on measurements and dynamic simulations of a series of monosaccharides that the exchange between the different conformations of the hydroxymethyl groups takes place on a time scale between 10^{-9} to 10^{-3} s. They furthermore concluded that there is no significant difference between the flexibility of glucose and galactose. Poppe[76] concludes on the basis of ^{1}H relaxation and NOE data that the exchange is on the order of 10^{-10} to 10^{-9} s for the hydroxymethyl group in gentiobiose. In a study of the disaccharide lactose, Ejchart and Dabrowski[83] concluded that the hydroxymethyl group of the glucose is more restricted than the hydroxymethyl group in the galactose unit. In amylose, Dais and Marchessault[84] have furthermore used the analysis of relaxation data to estimate the population of gg and gt to be 60 to 40 % using a two state jump model.

Recently Dzakula *et al.*[87,88] described an alternative method (CUPID) for the general determination of the rotamer distribution without any assumptions about fixed staggered conformers. These authors calculated the continuous rotamer population distribution from experimental NMR data (coupling constants and NOEs), but unfortunately six conformationally sensitive parameters are necessary to determine a Fourier expansion of the order three. Such a number of NMR parameters would generally not be accessible for a hydroxymethyl group in a hexopyranose system.

A similar approach have recently been reported by Poppe[76] for the investigation of the C5-C6 bond in gentiobiose, but using a maximum entropy method to derive the rotameric distribution from experimental constraints.

The unambiguous assignment of the resonances of the pro-R and pro-S H6 resonances has been a problem in the conformational analysis of the hydroxymethyl group. In early studies the assignment was based on the interpretation of the chemical shifts with varying success. The studies by Lemieux and Stevens[89] and by Hall and coworkers[51] initiated the investigation of many monosaccharides by ^{1}H NMR spectroscopy.[21,29,30,40,42,45,48-50,52-54,56-58] The assignment of the $^{3}J_{5,6R}$ and $^{3}J_{5,6S}$ were, however, not always correct, as the assignments were generally based on the prediction of the relative chemical shifts of H6R and H6S by empirical rules, as the syn-up rule[90] or assumptions that certain conformations were not populated, especially the assignment in galactose derivatives have been a problem. [29,30,50]

More reliable assignments have been made by the use of partly deuterated monosaccharides. The first example of this approach was the semiselective introduction of deuterium in glucose by Gagnaire *et al.*[48] However, a more selective method is widely used today as developed by Ohrui and coworkers.[42,91,92] The deuterium is introduced in 1,6-anhydro

derivatives, and monosaccharides specifically deuterated either in R or S configuration can be synthesized. This technique has been used in the investigation of the rotameric distributions in hexopyranosides[40-45,92] and unambiguously determines the assignment of the two proton resonances and furthermore, allows for measurement of coupling constants in cases where almost identical chemical shifts would prevent this. Additionally, the method has been applied to disaccharides both by the group of Ohrui[18,19,44] and by Bock *et al.*[10,47] and also to branched trisaccharides.[10,19,22,46] This has, for example, given information about the conformation for 1-6 linked disaccharides. Similar studies of the C4 hydroxymethyl group of pentofuranosides have been carried out both by the group of Ohrui[93,94] and by Serianni and coworkers.[95-97]

A major dispute in the area has been the assignment of the H6 protons in galactose derivatives and thereby the determination of the populations of the staggered conformations.[29,30,32,51,56,57] The interchange of the assignment leads to either a high population of tg or gt. This was finally settled by the elegant work by Ohrui *et al.*[42] making both the S and R isomer of methyl β-D-(6-^{2}H)-galactopyranoside, whereby they could clearly determine that the H6R is downfield from the H6S and also could determine the coupling constants more accurately than previously.

An alternative stereospecific assignment can be achieved using NOE measurements with careful analysis of measured values,[17,76,98,99] *e.g.*, between H4 and H6R or H6S. However, this technique is not universally applicable due to the close chemical shifts and the effective dipole-dipole coupling between the two geminal H6 protons.

The studies in general have shown that the rotameric distribution around the C5-C6 bond[40-42,45] for glucopyranosides is about 6:4:0 and for galactopyranosides around 2:6:2 (gg:gt:tg), but with variations, depending on the solvent[21,54] and different protecting groups.[100]

2.3 Theoretical investigations.

It is not attempted to give a full description of all the material published on the modelling of hydroxymethyl group conformation, but rather to introduce some of the concepts and problems for general background and to try to separate the individual contributions.

The effects generally described affecting the conformational preferences for systems having oxygens in close proximity, are the 1,3-diaxial interaction and the gauche effect.[7] The 1,3-diaxial interaction or Hassel-Ottar effect[101] is the electronic and steric repulsion between two electronegative substituents arranged in a diaxial fashion[101,102] like O6-O4 in the tg conformation for glucose or in the gg conformation for galactose. It has been estimated that conformations where a 1,3-diaxial interaction is present are destabilized by more than 1 kcal/mol.[102] The gauche effect is the effect stabilizing the gauche orientation of two vicinal electronegative substituents as discussed by Wolfe *et al.*,[103,104] Zefirov *et al.*,[105,106] Abe *et al.*[107]

and later by others.[108-117] Furthermore solvation effects have been shown both experimentally[21,29,33,52,54,118] and theoretically[119-125] to be important for the preferred conformation of the hydroxymethyl group.

The theoretical treatment of the hydroxymethyl group orientation has in general not been successful in reproducing the experimental results. The force-fields applied have in several cases overestimated the influence of hydrogen bonding, which have resulted in prediction of the tg conformation of glucose to be the most populated conformation.[122-125] However, recent calculations have given better agreement with the experimental distribution by inclusion of solvent effects,[119,120] but these calculations still do not reproduce the experimentally determined populations very well. However, the recent paper by Cramer and Truhlar[121] demonstrates that quantum chemical calculations modelling solvent (water) by a continuum with dielectric polarization and including a surface accessible surface area model gives the correct relative energies for the three staggered conformations in glucose. They conclude that the unfavorable solvation of the tg conformation is important in the destabilization relative to gt and gg.

3. RESULTS AND DISCUSSION

3.1 Evaluation of the limiting value coupling constants.

For the interpretation of the coupling constants it is necessary to choose a set of limiting values for the three staggered conformations and these values have generally been derived from different sources. A general problem with the values is that they necessarily are derived from measurements of coupling constants in ring systems in order to have a well defined geometry. This could lead to wrong estimates for some coupling constants as, *e.g.*, the oxygen lone pairs will be fixed relative to the coupling pathway in these model compounds. There is, however, not a simple solution to this problem and we have chosen to use the values from the Haasnoot-Altona formula.[65] One of the problems with the limiting values used has been that negative populations sometimes are the results of the values used, which is of course of no physical meaning. This is seen, *e.g.*, for Me α-D-Glc (4α) from the coupling constants of $^3J_{5,6R}$= 5.49 Hz and $^3J_{5,6S}$= 2.39 Hz (Table 1). The use of the limiting values from the Haasnoot-Altona formula gives a negative population of the tg rotamer. This is most likely caused by the too large coupling constants predicted by the formula for the 60° ^{1}H-^{1}H coupling constants like the $^3J_{5,6S}$ for gt (2.8 Hz). Observation of couplings below 2.4 Hz for other glucose derivatives indicates that this value might be too large.

One explanation for this observation could be that the staggered conformations populated are not exactly at 60°, but are offset slightly from this value. Based on reported X-ray

Table 1. Coupling constants for calculation of populations for the hydroxymethyl group

Method	$^3J_{5,6R}$			$^3J_{5,6S}$			Population[a] for Me α-D-Glc (**4α**)		
	gg	gt	tg	gg	gt	tg	gg	gt	tg
Haasnoot [65]	0.9	10.7	5.0	2.8	3.1	10.7	57(53)	50(47)	-7(0)
Nishida [40]	1.7	10.8	4.1	2.2	2.4	11.1	58	41	1
Manor [126]	1.3	11.5	5.8	1.3	2.7	11.7	56	39	5
Average 3 [b]	1.4	10.2	5.0	3.1	3.3	10.2	60(54)	51(46)	-11(0)
Average 2 [b]	1.7	9.9	5.0	3.3	3.5	9.9	63(54)	52(46)	-15(0)
Average 1 [b]	2.4	9.2	5.1	3.6	3.8	9.2	69(55)	55(45)	-24(0)
Experimental [c]	0.9	10.7	5.5	2.2	2.5	10.7	53	47	1

a. The population for the three conformations based on $^3J_{5,6R}$= 5.49 Hz and $^3J_{5,6S}$=0 2.39 Hz, values in parenthesis are values based on a tg population of zero.

b. Values from average ±60° around the staggered conformations using a three-fold potential with V_3 values of 3, 2 and 1 kcal/mol (see text).

c. Values of coupling constants based on measured values in the model compounds discussed in the text.

crystallographic data, Nishida *et al.*[40] proposed several sets of angles for the conformation of glucose derivatives, *e.g.*, ω=-67° for gg, ω=65° for gt and ω=175° for tg. Using these angles and the Haasnoot-Altona formula the limiting values shown in Table 1 were obtained.[40] According to the study the application of these values in the calculation of populations from experimental coupling constants did not change the proportion of gg to gt for glucose, but gave more reasonable populations for tg.

The coupling constants reported by Manor *et al.*[126] (Table 1) have been applied[18,40,42,43] in the calculations of the hydroxymethyl group populations. However, these values might not be appropriate as they are based on couplings in strained 5 membered rings.

The following approach was therefore tested here to obtain more realistic limiting values for the three staggered conformations. The couplings can be regarded to originate from a three state jump model, in which the three states have vibrations around the perfectly staggered conformations. The coupling constants for the three situations should then be the average values of the coupling constants with vibration around the 60°, 180° and 300°. We chose to model the flexibility around the individual minima by a three fold potential with the simplest

potential without imposing assumption about other forces. The weighted average coupling constants around the three minima were calculated as follows. The energies from the three-fold potential were calculated by a grid search of the ω angle over a range around the perfect staggered conformation of ±60° (1° steps). For each of the three staggered conformations the average coupling constants were calculated by the weighted averages over these ranges by the Boltzmann distribution and coupling constants from the Haasnoot-Altona formula.[65] The result is shown in Table 1 with three different barrier heights (V_3) to probe the influence of the flexibility on the coupling values.

It can be seen that the introduction of flexibility in the model does not solve the problem about the too high values predicted for the 60° ^{1}H-^{1}H angles. The averaging gives higher values of the small coupling constants than obtained from the ideal staggered conformation. The coupling constants resulting from averaging around ^{1}H-^{1}H angles of 180° are lower than in the ideal staggered conformations, as the maximum in the Haasnoot-Altona curve is at 180° and any deviation from this will lower the coupling constants. Interestingly, the use of the different coupling constants does not alter the proportion of gg to gt significantly, when the calculation of populations of gg and gt is done assuming a tg population of 0. This is due to the fact that when the averaging results in a smaller $^3J_{5,6R}$ in the gt conformation it also results in a larger coupling in the gg conformation.

Alternatively, the limiting values for the calculation of rotamer populations can be derived from measurement of the coupling constants in the following model compounds: 1,5-anhydro-mannitol (**1**), 1,5-anhydro-glucitol (**2**) and 1,5-anhydro-galactitol (**3**). The 1,5-anhydro-mannitol (**1**) (**Fig. 4**) can be regarded as a rigid model for the gg conformation with two 60° ^{1}H-^{1}H angles and with the oxygen in the right orientation. The couplings were measured to be $^3J_{1eq,2}$= 2.2 Hz and $^3J_{1ax,2}$= 0.9 Hz which correspond to the $^3J_{5,6S}$ and $^3J_{5,6R}$, respectively for the gg conformation. The $^3J_{1ax,2}$ is in good agreement with the value predicted, but the $^3J_{1eq,2}$ is predicted to be too high (0.6 Hz). The 1,5-anhydro-glucitol (**2**) and 1,5-anhydro-galactitol (**3**) are both rigid models for the tg conformation and the $^3J_{1eq,2}$ measured were 5.5 Hz and 5.6 Hz, respectively and for $^3J_{1ax,2}$, 10.8 Hz and ≈10 Hz respectively. The calculated values of the large coupling are in good agreement with those measured, but the value for the 60° coupling is 0.6 Hz larger than predicted. Without a good model for the gt orientation, it was estimated that the 60° coupling should be lowered by 0.6 Hz from 3.1 Hz to 2.5 Hz following the observation for the $^3J_{5,6S}$ of gg. This analysis gave the last set of values for the three conformations of the hydroxymethyl group shown in Table 1.

The coupling constants derived by different methods shown in Table 1 have fairly large variations, and it is not obvious which values should be chosen for the investigations of the hydroxymethyl group conformation. The values reported by Nishida *et al.*[40] or by Manor *et al.*[126]

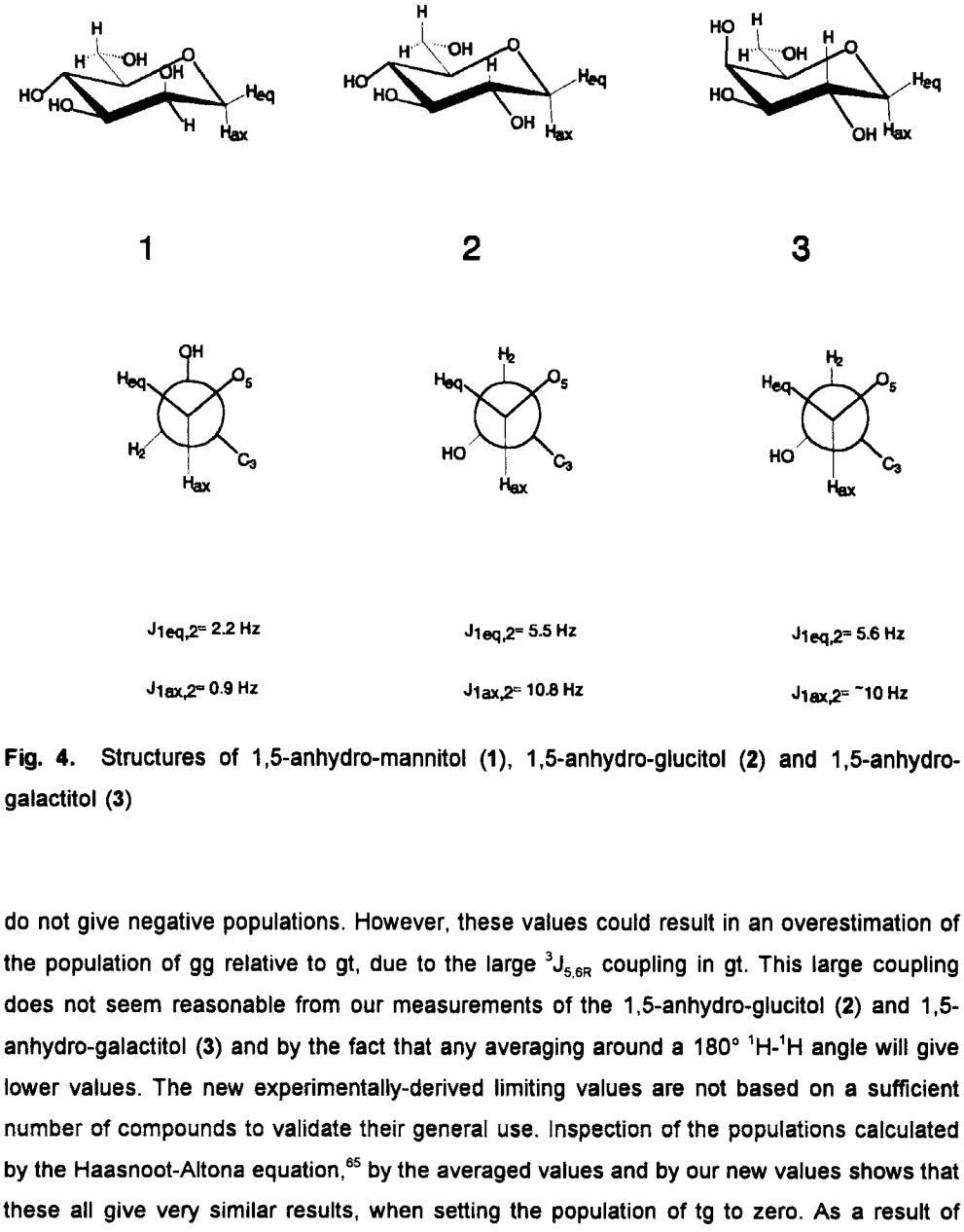


Fig. 4. Structures of 1,5-anhydro-mannitol (**1**), 1,5-anhydro-glucitol (**2**) and 1,5-anhydro-galactitol (**3**)

do not give negative populations. However, these values could result in an overestimation of the population of gg relative to gt, due to the large $^3J_{5,6R}$ coupling in gt. This large coupling does not seem reasonable from our measurements of the 1,5-anhydro-glucitol (**2**) and 1,5-anhydro-galactitol (**3**) and by the fact that any averaging around a 180° ^{1}H-^{1}H angle will give lower values. The new experimentally-derived limiting values are not based on a sufficient number of compounds to validate their general use. Inspection of the populations calculated by the Haasnoot-Altona equation,[65] by the averaged values and by our new values shows that these all give very similar results, when setting the population of tg to zero. As a result of these considerations the Haasnoot-Altona treatment for the interpretation of experimental coupling constants will be used in the following and the population of the tg rotamer will be set to zero in cases where negative tg populations are encountered.

3.2 NMR spectroscopic data for the hydroxymethyl groups of hexopyranoses.

The coupling constants given in Tables 2 and 3 are based on measurements at 500 and 600 MHz and the data reported are for several examples derived from spin system simulation.

Table 2. ^{1}H-NMR parameters obtained in D_2O at 300 K and populations of the three staggered conformations for hydroxymethyl groups of 4,5-trans-hexopyranoid structures.

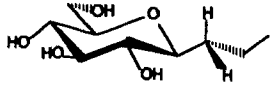
Comp.	Compounds	Chemical shift(ppm)				Coupling (Hz)			Population[a]			Source
		H4	H5	H6R	H6S	5,6R	5,6S	6R,6S	gg	gt	tg	
(4α)	Me α-D-Glc [f]	3.426	3.678	3.790	3.898	5.49	2.39	-12.35	57 (53)	50 (47)	-7	[b] + [c]
(4α)	Me α-D-Glc in CD_3OD	3.280	3.525	3.670	3.810	5.64	2.40	-11.8	56 (52)	51 (48)	-7	[c]
(4β)	Me β-D-Ic [f]	3.400	3.481	3.743	3.945	6.11	2.47	-12.31	50 (47)	56 (53)	-6	[b] + [c]
(5α)	α-D-Ic in DMSO [f]	3.041	3.551	3.441	3.595	5.0	2.3	11.2	63 (58)	45 (42)	-8	[e] 127
(5β)	β-D-Ic [f]	3.413	3.470	3.735	3.909	5.78	2.29	-12.33	55 (50)	54 (50)	-9	[b]
(5β)	β-D-Ic in DMSO [f]	3.022	3.066	3.412	3.661	5.9	2.2	11.8	55 (49)	55 (51)	-10	[e] 127
(6)	Me α-D-an [f]	3.662	3.630	3.779	3.918	6.40	2.44	-12.20	48 (44)	59 (56)	-7	[b]
(7)	Me β-D-IcNAc [f]	3.458	3.484	3.773	3.958	5.38	2.35	-12.20	59 (54)	50 (46)	-9	[b]
(8α)	6-*O*-Me-α-D-Ic	3.406	3.953	3.679	3.715	5.73	2.37	-11.04	55 (51)	63 (49)	-8	[b]
(8β)	6-*O*-Me-β-D-Ic	3.401	3.543	3.676	3.747	6.10	2.16	-11.20	53 (47)	57 (53)	-10	[b]
(9)	4,6-di-*O*-Me-β-D-Ic	3.18	3.54	3.638	3.719	5.64	1.96	-11.14	59 (52)	54 (48)	-13	[b]
(10)	Me 6-*O*-Acetate-β-D-Glc	3.53	3.69	4.32	4.44	4.9	2.3	-12.3	64 (59)	(44) 41	-8	[d] + [e] 29
(11)	Me 4,6-di-*O*-Acetate-β-D-Glc			4.36	4.18	3.8	2.1	-12.6	76 (70)	34 (30)	-10	[e] 29
(12)	6-deoxy-6-Cl-β-D-Ic	3.52	3.680	3.855	3.965	4.79	2.26	-12.49	72 (65)	39 (35)	-12	[b]
(13)	Me 6-deoxy-6-F-α-D-Ic	3.502	3.789	4.739	4.688	3.62	1.86	-10.79	71 (67)	37 (33)	-8	[c] + [e] 52
(14)	Me 6-deoxy-6-F-(6-R)-^{2}H-α-D-Glc	3.507	3.790		4.680		1.81					[c]
(15)	Me 6-deoxy-6-NH_2-β-D-Ic pH <1	3.304	3.643	3.115	3.455	9.15	3.09	-13.44	17	79	4	[b]
(15)	pH 4.75	3.351	3.675	3.160	3.498	9.20	3.10	-13.40	16	80	4	[b]
(15)	pH > 12	3.168	3.258	2.636	2.983	8.15	2.65	-13.80	32 (30)	71 (70)	-3	[b]
(16)	Me 6-*O*-PO_3-α-D-Glc pH 1.3	3.475	3.758	4.123	4.155	4.6	2.4	-11.6	66 (62) 72	41 (38) 6	-7 22	[c] [i]
(16)	pH 9.3	3.62	3.68	4.066	3.927	3.53	1.91	-12.13	81 (73) 85	32 (27) 7	-13 4	[c] [i]
(17α)	Me 4-Deoxy-α-D-Ic	1.436 1.977	3.927	3.596	3.678	6.20	3.03	-12.06	45	54	1	[b]

(continued)

TABLE 2. Continued

(17β)	Me 4-Deoxy-β-D-Ic	1.422 1.996	3.749	3.644	3.711	7.42	3.34	-12.04	31	65	4	[b]
(18)	Me 4-*O*-Acetate-β-D-Glc			3.69	3.89	5.9	2.2	-12.3	55 (49)	55 (51)	-10	[e 29]
(19)	Me 4-keto(hydrate)-α-D-Ic		3.72	3.78	3.98	8.4	2.4	-12.0	28 (23)	80 (77)	-8	[d]
(20)	Me 4-deoxy-4-S-α-D-Ic	2.70	3.68	3.88	3.93	5.0	2.0	12.5	65 (58)	47 (42)	-12	[d]
(21)	Me 4-deoxy-4-NH_2-α-D-Ic pH 2.0	3.23	3.934	3.790	3.852	4.64	3.7	-12.44	56	34	10	[d]
(21)	pH 9	2.67	3.57	3.726	3.832	5.40	2.46	-12.42	58 (54)	49 (46)	-6	[d]
(22)	Me 5-deoxy-5-S-β-D-Ic	3.626	2.949	3.86	3.956	6.16	3.29	-11.89	53	43	4	[c]
(23α)	5-deoxy-5-S-α-D-Ic	3.617	3.232	3.920	3.882	5.60	3.30	-11.91	57	37	6	[c]
(23β)	5-deoxy-5-S-β-D-Ic	3.575	3.001	3.834	3.927	6.17	3.32	-11.89	52	43	5	[c]
(24)	1-deoxynojirimycin pH 0.9[f]	3.53	3.13	3.80	3.87	5.3	3.2	-12.9	58 60	39 7	4 33	[d] [i]
(24)	pH 9.2[f]	3.19	2.50	3.59	3.79	6.4	3.0	-11.8	49 (49) 53	52 (51) 0	-1 47	[d] [i]
(25)	C-Glycoside analogue [f]			1.39	1.78	8.5	2.4		36 (25)	80 (75)	-10	[e 128]
(26α)	Pseudo-α-D-Glc [h]	3.29	1.88	3.68	3.73	5.5	3.5	-11.5	56	35	8	[e 129]
(26β)	Pseudo-β-D-Glc [h]	3.32	1.64	3.63	3.77	6.0	3.5	-11.5	52	41	7	[e 129]
(27α)	Pseudo-α-D-Man [h]	3.55	1.83	3.67	3.73	6.2	3.5	-11.8	50	43	7	[e 129]
(27β)	Pseudo-β-D-Man [h]	3.52	1.57	3.63	3.80	6.0	3.0	-11.5	56	42	2	[e 129]
(28α)	Pseudo-α-D-GlcNAc [h]	3.37	1.89	3.69	3.74	5.6	3.8	-11.0	53	36	11	[e 130]
(28β)	Pseudo-β-D-GlcNAc [h]	3.38	1.62	3.66	3.78	6.2	3.6	-11.0	50	43	8	[e 130]
(29)	Pseudo-β-D-ManNAc [h]	3.46	1.61	3.70	3.78	5.9	3.5	-11.2	53	40	7	[e 130]

[a] Populations from Haasnoot-Altona equation[65] (see text).
[b] Measured at 500 MHz and fitted with standard Bruker software (PANIC), coupling constants ±0.05 Hz
[c] Measured at 600 MHz, couplings constants measured as first order ±0.1 Hz
[d] Measured at 500 MHz, couplings constants measured as first order ±0.3 Hz
[e] Data from literature with reference as indicated.
[f] See adjacent figure for structure.
[g] Assignment of pro-R and pro-S from Ohrui et al.[40,41,45]
[h] The pseudo-monosaccharides are the 5a-carba analogues of the corresponding monosaccharides.
[i] Populations based on interchanging the assignment of H6R and H6S.



25

Table 3. ^{1}H-NMR parameters obtained in D_2O at 300 K and populations of the three staggered conformations hydroxymethyl groups of 4,5-cis-hexopyranoid structures.

Comp.	Compounds	Chemical shift (ppm)				Coupling (Hz)			Population[a]			Source
		H4	H5	H6R	H6S	5,6R	5,6S	6R,6S	gg	gt	tg	
(30α)	Me α-D-al [e]	3.996	3.922	3.779	3.767	8.6	3.7	-11.7	16	75	9	[b]
(30β)	Me β-D-al	3.943	3.716	3.814	3.776	7.50	4.83	-11.76	18	57	26	[b]
(31)	β-D-al [e]	3.924	3.699	3.762	3.737	7.92	4.28	-11.50	19	65	17	[b]
(32)	Me (6-S)-^{2}H-β-D-al	3.924	3.690	3.777		7.76						[b]
(33)	6-deoxy-6-Cl-β-D-Gal	3.98	3.82	3.65	3.69	7.9	4.0	-10.9	22	64	14	[c]
(34)	6-deoxy-6-F-α-D-Gal			4.62	4.67	7.78	3.49		25	70	5	[d] 52
(35)	6-deoxy-6-NH_2-α-D-al pH 1.2		4.117	3.315	3.271	9.72	3.10	-13.12	10	85	5	[b]
(17α)	Me 4-Deoxy-α-D-al	1.436 1.977	3.927	3.596	3.678	6.20	3.03	-12.06	45	54	1	[b]
(17β)	Me 4-Deoxy-β-D-al	1.422 1.996	3.749	3.644	3.711	7.42	3.34	-12.04	31	65	5	[b]
(19)	Me 4-keto(hydrate)-α-D-al		3.72	3.78	3.98	8.4	2.4	-12.0	28	80	-8	[b]
(36)	Me 5-deoxy-5-*S*-β-*L*-Gal [f]	4.166	3.252	3.667 [f]	3.826 [f]	7.68	6.62	-11.44	11	52	37	[c]
(37)	Pseudo-α-D-Gal [g]	4.09	2.03	3.64	3.52	8.0	6.2	-11.0	12	56	32	[d] 129
(38α)	Pseudo-α-D-GalNAc [g]	4.12	2.04	3.67	3.54	7.9	6.3	-11.0	12	55	33	[d] 130
(38β)	Pseudo-β-D-GalNAc [g]	4.06	1.78	3.69	3.57	7.7	6.5	-11.0	12	52	36	[d] 130

[a] Populations from Haasnoot-Altona[66] (see text).
[b] Measured at 500 MHz and fitted with standard Bruker software (PANIC), coupling constants ±0.05 Hz
[c] Measured at 600 MHz, couplings constants measured as first order ±0.1 Hz
[d] Data from literature with reference as indicated.
[e] Assignment of pro-R and pro-S from Ohrui et al.[42]
[f] For ease in comparison the H6R and H6S of the L monosaccharide are interchanged.
[g] The pseudo-monosaccharides are the 5a-carba analogues of the corresponding monosaccharides.

The assignment of the pro-R and pro-S H6 protons of the normal monosaccharides follows the assignment by stereospecific deuteration as reported by Ohrui *et al.*[40-42,45] as indicated with footnotes in the tables. For some compounds the data obtained for the specific deuterated compounds are included in the Tables. The assignment for all other compounds is based on a comparison with the deuterated compounds[40,41,43,45] and the observed coupling constants.

For a few compounds the populations are calculated based on an inversion of the assignment of H6R and H6S as well. For these compounds the assignment of H6R and H6S might have to be interchanged, as no convincing evidence for the stereospecific assignment has been reported.

The values in parentheses are the populations of gg and gt calculated by setting the population of tg to zero and using the $^3J_{5,6R}$. The accuracy using the Haasnoot-Altona values for the calculation of population is fairly good within the limitations discussed earlier. Especially the comparison of different compounds using the same equation can be done with high confidence. The limiting values of ^3J for the compounds having other substituents at C5 or C6 than normal have been calculated with the Haasnoot-Altona equation[65] and electronegativities reported by Huggins.[66] More accurate values for the electronegativities including group electronegativities have been published recently.[131-135] However, as the parameterization by Haasnoot-Altona was done using the electronegativities from Huggins[66] these values were used here. In fact, the difference between the electronegativities published by Huggins and the values determined recently[131-133] are marginal. In principle the electronegativity difference between the simple 6OH and the substituted compounds as 6-methoxy, 6-O-acetates could affect the coupling constants. However, the effect will be very small as the group electronegativities of OH, OCH_3 and $OCOCH_3$ from Boyd and Boyd[133] are 3.55, 3.53 and 3.57, respectively.

The calculated values reflect the effects of electronegative substituents on the coupling constants, as for example the small $^3J_{5,6R}$ of the tg rotamer for a 6-fluoro derivative having a very electronegative substituent antiperiplanar to H5. A symmetric relation is seen for the 5-CH_2 and the 6-CH_3 substituted compounds for $^3J_{5,6R}$, but not for $^3J_{5,6S}$.

3.3 Evaluation of the NMR spectroscopic data.

Information about the rotameric population is furthermore contained in the chemical shifts. However, the information in the chemical shifts will often be disguised by other effects, like the direct effect of substituents on the chemical shifts. The chemical shift of H4 for the *gluco* configurated compounds has a clear relation to the population of the gg conformation as shown in **Fig. 5.** In this Figure H4 chemical shifts minus the chemical shift of H4 for Me α-D-Glc (**4α**) are plotted against the population of the gg rotamer for compounds only differing in the substituent at the 6 position and the pH of the solution.

Table 4. $^3J_{5,6R}$ and $^3J_{5,6S}$ by Haasnoot-Altona[85]

Compound	$\Delta\chi$ [a]			$^3J_{5,6R}$			$^3J_{5,6S}$		
	4	5	6	gg	gt	tg	gg	gt	tg
Normal hexose	0.4	1.3	1.3	0.90	10.67	5.01	2.84	3.07	10.67
5-CH_2- (pseudo)	0.4	0.4	1.3	1.93	11.52	4.11	1.93	4.11	11.52
5-S-	0.4	0.4	1.3	1.93	11.52	4.11	1.93	4.11	11.52
5-NH-	0.4	0.85	1.3	1.58	10.96	4.53	2.36	3.76	10.96
6-CH_2-	0.4	1.3	0.4	1.93	11.52	4.11	3.87	2.17	11.52
6-NH_2	0.4	1.3	0.85	1.58	10.96	4.53	3.52	2.59	10.96
6-Cl	0.4	1.3	0.95	1.46	10.87	4.64	3.40	2.70	10.87
6-F	0.4	1.3	1.7	0.07	10.73	5.36	2.01	3.42	10.73

a. The electronegativities from Huggins[66] at the 4, 5 and 6 position.

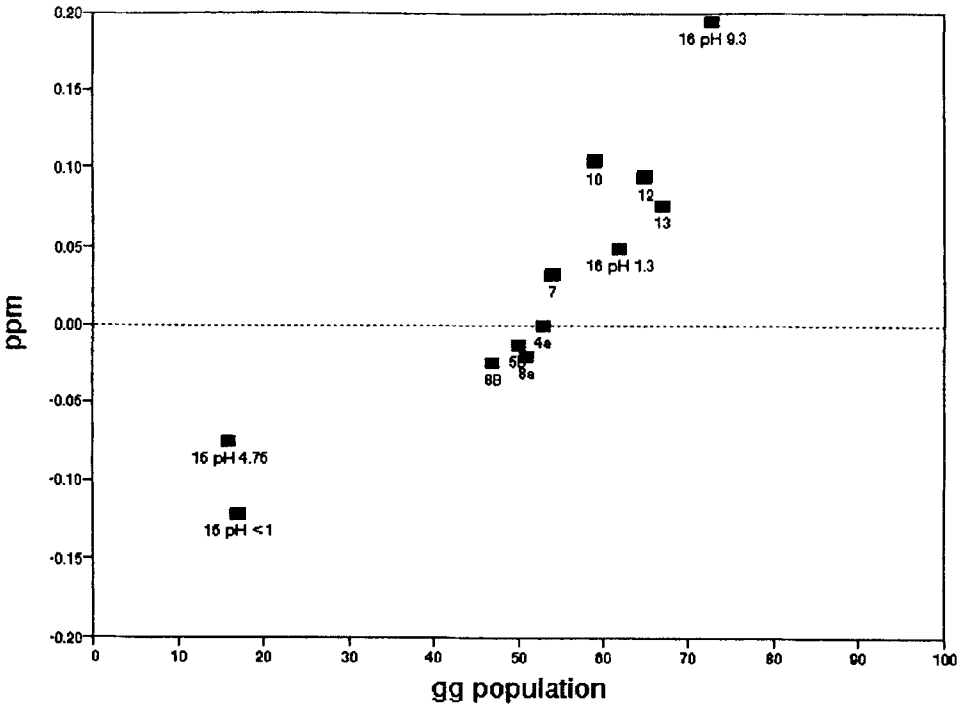


Fig.5. The chemical shifts of H4s versus the populations of the gg rotamer. (H4 chemical shifts taken relative the H4 of Me α-D-Glc (4α)).

A larger population of gg gives a downfield shift of H4, which can be explained by a 1,3-diaxial orientation of H4 and O6 in the gg conformation. In general the close contact between a proton and an oxygen has been observed to give downfield.[58,89,134,135] Extending the correlation to 0 and 100 % gg gives a difference in chemical shift of H4 of approximately 0.5 ppm between the two extremes. This value is in good agreement with the chemical shift difference, observed for bicyclic analogues of gg and gt compounds.[16] The correlation of the chemical shift of H4 with the rotameric population might be an alternative way to obtain information about the conformation of the hydroxymethyl group in cases where the coupling constants cannot be measured accurately. Similar effects will cause shifting of the H6 protons and therefore the chemical shift differences of these could also be used in assessing the conformational preferences of the hydroxymethyl groups.

In general, H6S resonates downfield from H6R for the *gluco* configurated compounds with some exceptions. The few exceptions are the Me 4,6-di-O-acetate-β-D-Glc (**11**), Me 6-deoxy-6-F-α-D-Glc (**13**) and Me 6-O-phosphate-α-D-Glc (**16**) at pH 9.3 where the H6Rs are downfield to H6S. It should be noted that the assignments of H6R and H6S for the latter compound might be inverted. The explanation for the exceptions is the larger population of gg relative to gt for these compounds, as a close contact between H6R and O4 is present in the gg rotamer, while the contact is between H6S and O4 for the gt rotamer. Thus the larger gg conformation brings H6R to lower field and H6S to higher field.

Furthermore, the chemical shifts of Me 4,6-di-O-acetate-β-D-Glc(**11**) is affected by the anisotropy of the acetate group.[29] An interesting observation is the interchange in the order of chemical shifts of H6R and H6S for the α- and β- 5-deoxy-5-S-D-Glc (**23**α) and (**23**β). One explanation might be the change in the rotameric population with the α compound having more gg and less gt, giving a larger downfield shift of H6R (H6R close to O4) and less downfield shifted H6S. In 1-6 linked disaccharides a reversal of this order is generally observed.[10,18,19,44,47]

For Me 6-O-PO_3-α-D-Glc (**16**) a set of proton-phosphorus coupling constants were measured at pH 1.3 $^3J_{H6S,P}$= 5.6 Hz and $^3J_{H6R,P}$= 6.9 Hz and at pH 9.3: $^3J_{H6S,P}$= 5.1 Hz and $^3J_{H6R,P}$= 7.2 Hz. These values indicate a fairly freely rotating C6-O6 bond in the C5-C6-O6-P fragment from the Karplus equation for this system reported by Lankhorst *et al.*,[138] but with some conformational preference.

Additionally, information can be obtained for the 6-fluoro compounds from the couplings between ^{1}H and F and between ^{13}C and F. Recently Abraham *et al.*[52] reported the measurement of the ^{1}H-^{1}H coupling constants of the 6-deoxy-6-F-D-Glc and 6-deoxy-6-F-D-Gal for the conformational assignment of these compounds and later also the fluorine-proton coupling constants in different solvents for 6-deoxy-6-F-D-Glc.[53] They concluded that the 6-deoxy-6-F-α-D-Glc has approximately the same rotamer population as glucose with only slightly higher population of the gg rotamer. The calculation of the populations based on the

coupling constants seems very dependent on the method for calculating the limiting values. Using the Haasnoot-Altona formula[65] it is indicated that the population of gg relative to gt is larger for Me 6-deoxy-6-F-α-D-Glc (**13**) than for Me α-D-Glc (**4α**). An even larger population of the gg conformer is expected from the $^3J_{H5,F}$= 28.6 Hz in accordance with the value reported by Evelyn and Hall[137] and by Abraham *et al.*[53] This indicates a very high population of the gg conformation by the Karplus curves for ^{1}H-F couplings from Wray,[138] as was also concluded earlier.[53,137,139] The observation of the $^3J_{C4,F}$= 6.8 Hz on the other hand indicates that the gt rotamer is populated to some extent. The $^3J_{C4,F}$ coupling can be compared to the $^3J_{C2,F}$ of 4-deoxy-4-F-D-Glc and 4-deoxy-4-F-D-Gal which are 8.8 Hz and 1.1 Hz respectively,[138] where the glucose configuration corresponds to a gt orientation and the galactose to a gg orientation of the ^{13}C and the F. Finally, $^2J_{C5,F}$= 17.3 Hz for Me 6-deoxy-6-F-α-D-Glc (**13**) gives information on conformational preferences, as Wray[138] showed that the 2-bond couplings are dependent on the orientation of oxygen substituents at the C in the coupling pathway. The couplings constants for rigid ring systems having F and O substituents in *gauche* orientation is 17.5±0.3 HZ and for *a trans* orientation 24.2±0.4 Hz. This indicates that the population of tg is very low, in accordance with the results from ^{1}H-^{1}H coupling constants.

The chemical shifts of *galacto* configurated compounds cannot be interpreted as easily as the chemical shifts of the *gluco* configurated compounds. The chemical shift differences between H6R and H6S are small and as all three staggered rotamers are populated, the effects determining the chemical shifts are more complicated.

3.4 Effects determining the conformation of the hydroxymethyl groups.

The description so far shows that NMR data are a good source of information about the conformational preferences of the hydroxymethyl group for monosaccharides. Additionally, the set of compounds might give information about the effects determining the conformation of the molecules, and it seems to be valid to derive some general trends.

The major effects assumed to determine the rotamer population of the hydroxymethyl group are the solvation effect, 1,3-diaxial interactions, *gauche* effect and hydrogen bonding. The effect of hydrogen bonding can be estimated by a comparison of protected and unprotected compounds or comparing the same compounds in different solvents. The effect of methylation of O6, going from β-D-Glc (**5α**) to 6-*O*-Me-β-D-Glc (**8α**), results in an increase of the $^3J_{5,6R}$ from 5.78 to 6.10 Hz, which indicates a somewhat lower population of gg relative to gt. This effect would not be expected to arise from hydrogen bonding as the possibility of hydrogen bonding is the same in gg and gt conformers. The addition of a methoxy group might lower the population of tg, as the $^3J_{5,6S}$ is decreased slightly. Compound 4,6-di-*O*-Me-β-D-Glc (**9**) has an even lower $^3J_{5,6S}$ which can be due to the lack of stabilization by hydrogen

Table 5. Effect of a 50° temperature change on the ^{1}H NMR parameters for some methyl hexopyranosides in D_2O.

Compound	δ_{350K}-δ_{300K}				J_{350K}-J_{300K}	
	H4	H5	H6R	H6S	H5,H6R	H5,H6S
Me α-D-Glc	0.02	0.00	0.01	0.00	0.02	0.10
Me β-D-Glc	0.03	0.00	0.03	0.00	-0.20	0.10
Me 4-deoxy-α-D-Glc	0.02[a] 0.02[a]	0.04	0.07	0.05	-0.07	0.30
Me α-D-Gal	0.02	-0.01	0.01	0.02	-0.33	0.09
Me β-D-Gal	0.01	-0.03	0.00	0.00	0.08	0.08

a. Chemical shift changes given both the axial and equatorial H4

bonding of the tg conformation, but might be a direct steric effect as well. The effect of changing the solvent from water to DMSO seems to be small and no conclusion about hydrogen bonding can be made from this experiment.

Investigation of the temperature dependence of the coupling constants and chemical shifts for the monosaccharides shown in Table 5 could also indicate that the hydrogen bonding is of minor importance in water, but a general solvation can be important.[121] The change in coupling constants for Me α-D-al are still small taken into consideration that the H6 signals give a higher order spin system and the uncertainty of the coupling constants might be larger than for the other monosaccahrides. A recent study by Beeson *et al.*[118] concluded that the free energy gained in a hydrogen bonding in water is small compared to other solvents and they showed that the property that determines the strength of the hydrogen bond in different solvents are the "solvent hydrogen bond basicity".

An estimation of the energy of the 1,3-diaxial interaction can be calculated from the populations of Me α-D-Glc (**4**α) and methyl α-D-*xylo*-hexopyranosid-4-ulose(hydrate) (Me 4-keto(hydrate)-α-D-Glc which is the same as Me 4-keto(hydrate)-α-D-Gal) (**19**). The relative populations gg:gt:tg for Me α-D-Glc (**4**α) of 53:47:0 is changed to 23:77:0 for Me 4-keto(hydrate)-α-D-Glc (**19**). The change of population in the gg conformation can be converted to the energy destabilization by the 1,3-diaxial interaction using the Boltzmann equation. This gives an estimate of the energy contribution for the 1,3-diaxial interaction of about 0.8 kcal/mol in good agreement with earlier proposals.

The estimate of the energy for the 1,3-diaxial interaction cannot be confirmed based on the populations for Me 4-deoxy-α-D-Glc (**17α**) or Me 4-deoxy-β-D-Glc (**17β**) relative to corresponding Me D-Glc (**4**) or Me D-Gal (**30**), as the determination of the low population of the tg rotamers is too inaccurate. Qualitatively, the effects are seen in the higher population of the tg rotamer for Me 4-deoxy-D-Glc (**17**) compared to Me D-Glc (**4**) and of the gg rotamer for Me 4-deoxy-D-Glc (**17**) compared to Me D-Gal (**30**).

The Me α- and β-4-deoxy-D-Glc (**17**) are good indicators for the importance of the *gauche* effect in having mainly the gg and gt rotamers, even when no 1,3-diaxial interaction destabilizes the tg rotamer. Estimates of the energy of the *gauche* effect cannot be calculated as no compounds completely lack a *gauche* interaction. The best indication is the higher population of tg for the pseudo monosaccharides having the *gluco* configuration, but even here a *gauche* interaction can stabilize the gg rotamer, as will be described below. The higher population of the tg rotamer indicate that the *gauche* effect for the 5-S compounds (**22**), (**23**) and (**36**) might be lower than for the oxygen analogues or it might even be zero. However, the steric effect of the larger sulphur atom might destabilize the gg and gt rotamers too, and it is not possible to separate the steric and electronic effects. It has been reported that the *gauche* effect in O-C-C-S fragments in organic solvents is repulsive.[108,113] The fairly high population of gg and gt for the 5-S compounds indicates that if this is the case, the repulsion is weak. However, the *gauche* effect can be expected to be affected by the solvent, as generally observed for electronic effects.[140-143]

The change in pH affects the population as observed for 1-deoxynojirimycin (**24**) where the $^3J_{5,6R}$ and $^3J_{5,6S}$ are changed from 5.3 Hz and 3.2 Hz at pH 0.9 to 6.4 Hz and 3.0 Hz at pH 9.2 . This can be due to both a change of the *gauche* effect or to different steric interactions with the proton of nitrogen by protonation. Presumably the hydration is also changed significantly by the change in pH. The populations indicated for (**24**) (Table 2) are calculated under the assumption that the H6R and H6S chemical shifts do not change relative position by the change in pH.

An interesting trend observed from the data in Tables 2 and 3 is the higher populations of gg rotamers relative to gt for α-anomers compared to β-anomers. This is seen *e.g.*, Me D-Glc (**4**), D-Glc (**5**) in DMSO, 6-O-Me-D-Glc (**8**), Me 4-deoxy-D-Glc (**17**), 5-deoxy-5-S-α-Glc (**23**), pseudo-D-Glc (**26**) and pseudo-D-GlcNAc (**28**). The estimation of higher populations of gg rotamers is based on the $^3J_{5,6R}$ which generally is larger for β anomers. The higher coupling constant could be an artifact of the change in environment giving a change in coupling constants for the same conformation and not originate from a change in conformation. Imai and Osawa[70] have published an extended Karplus equation taking into account (among other effects) the effect of through space interaction with the involved protons. The effect in this case

should be from O1 being close to H5 for the α anomer, but this interaction between an oxygen and a proton is reported to increase the coupling constant and not as observed to decrease it. Furthermore, the effect is reported to be very small, normally being less than 0.1 Hz. It therefore appears that the stabilization of the gg rotamer by going from the β- to the α-anomer is genuine. Studies on a series of galactose derivatives by de Vries and Buck[21,54] have shown a similar very interesting trend in the population of the tg conformation being not only dependent on the solvent but also on the configuration at C-1 and the pK_a of the aglycon. They furthermore showed that this could not be explained by changes in the chair conformation of the pyranose ring and also that a simple coulombic repulsion between O5 and O6 could not explain the difference between the α- and β-compounds. This indicates based on the results presented that the *gauche* effect may be dependent on the configuration at C-1. It is more difficult to evaluate a difference in the total solvation of the α- and β-compounds and the theoretical treatment of this problem is only now approaching the necessary accuracy in the calculation of the contribution of solvation, where the above observed fact was reproduced in the calculations.[121]

The stabilization of gg over gt can be explained by the nature of the *gauche* effect. A report by Juaristi and Antunez[114] on the *gauche* effect in 5-substituted 1,3-dioxanes describes how the *gauche* effect is influenced by the electronic environment of the protons at the O-C-C-O fragment. These authors described the *gauche* effect by the hyperconjugative mechanism as proposed by Dionne and St-Jacques[109] and as also indicated in **Fig. 6**. The illustration of the *gauche* effect by these resonance forms might not be the most correct picture,[144] but it is convenient for the interpretation of the *gauche* effect in different environments. The hyperconjugation mechanism can work in two directions for the gg rotamer, but only in one direction for the gt rotamer **(Fig. 6)**. This explains first of all the extra stabilization of the gg over the gt rotamer, which seems general for the monosaccharides. Furthermore, the stabilizing interaction between the electronegative O6 and H5 can explain some of the differences observed for monosaccharides. The difference observed between α- and β-anomers can be explained from the fact that the α-anomer has the electron rich O1 close to H5 and thus contributing to a stabilization of the hyperconjugation form by the charge of the oxygen.[114] The larger *gauche* effect in gg for the α-anomer is in agreement with the increasing charge around H5, which is generally known as the deshielding of H5 in α-anomers relative to β-anomers (downfield shift of H5 for α anomers). The energy of the extra stabilization of the gg conformer for α- over β-anomers can be estimated from its population in the α- and β-anomers. A value of 0.14 kcal/mol is obtained from the difference in population gg:gt:tg α 53:47:0 and β 47:53:0 for Me D-Glc (**4**). The other examples where both α and β anomeric compounds were measured gives values in the range from 0.1 to 0.3 kcal/mol.

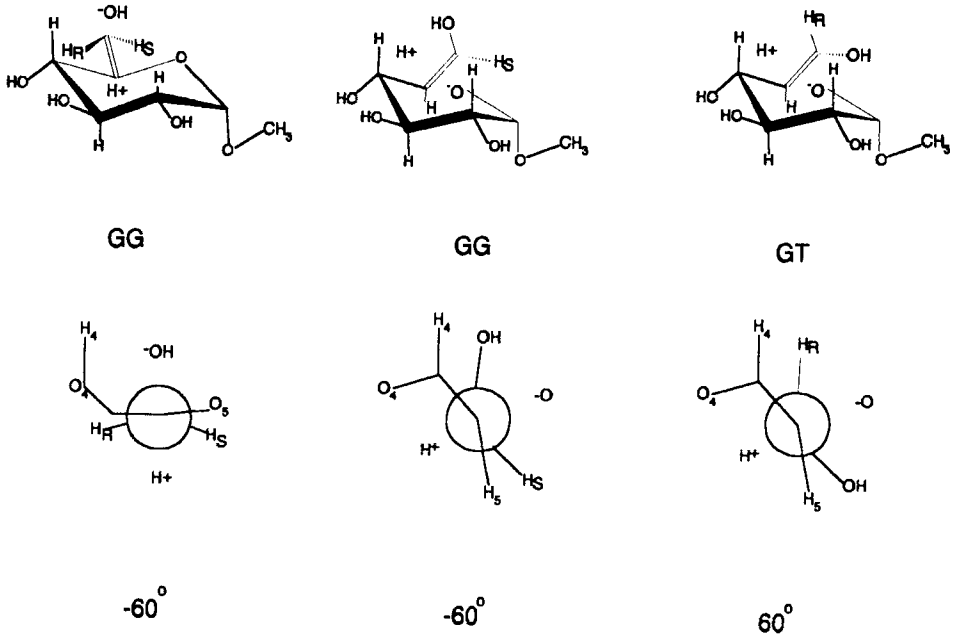


Fig. 6. The *gauche* effect for the gg and gt conformations.

The stabilization of gg over gt will be dependent on the nature of the substituent at C6, *i.e.*, OH, F etc., but interestingly the larger stabilization of gg over gt going from β to α is seen even for the pseudo monosaccharides. The fact that the effect is observed in these compounds supports the model for the *gauche* effect, as the hyperconjugation can still be important in the gg rotamer for compounds having the ring oxygen replaced by CH_2. The general stabilization of both gg and gt over tg by hyperconjugation between O5 and H6S or H6R will however not be important when O5 is replaced by CH_2. This is in accordance with the larger populations of the tg rotamer for the pseudo-monosaccharides.

The model of the *gauche* effect (**Fig. 6**) can be used to explain the larger population of the gg anomer for glucose derivatives having more electronegative substituents at C6, as the 6-*O*-acetate (**10**) and 6-deoxy-6-fluoro (**13**). The electronegative nature of the substituent is not the best measure of the size of stabilization by the hyperconjugation, but more correctly the tendency to form the negatively charged form as, e.g., OH^-, $OCOCH_3^-$. This has been correlated by Juaristi and Antunez[114] with both the nucleofugicity and the energy of the lowest unoccupied orbital (LUMO). Following this proposal it seems fair that the 6-*O*-acetate can stabilize the ionic form by resonance.

In order to determine the effect of changing the pH for *e.g.*, Me 6-deoxy-6-NH_2-β-D-Glc (**15**) or Me 6-*O*-PO_3-α-D-Glc (**16**) it would be necessary to synthesize the specifically

deuterated compound. The assignment of the H6R and H6R in these cases is not certain which is crucial for the correct interpretation of the NMR spectroscopic data.

Investigation of the *gauche* effect for the galactose derivatives is not straightforward. The extra stabilization of the gg conformation by hyperconjugation is competing with the destabilization by the 1,3-diaxial interaction. It is clear though that a stabilization of gg by a *gauche* effect is necessary to explain the fairly large population of gg for the galactose derivatives. The comparison of the population of the Me α- and β-D-Gal (**30**) indicates that the relative population of gg α:β is opposite to the relation observed for Me D-Glc (**4**), with a larger population of gg for the β-anomer. This is probably an artifact of the coupling constant assigned to Me α-D-Gal (**30α**), where the close chemical shifts of H6R and H6S gives problems in determining the accurate coupling constants. The two galactose derivatives substituted with a halogen at C6; 6-deoxy-6-Cl-β-D-Gal (**33**) and 6-deoxy-6-F-α-D-Gal (**34**), have relative large populations of the gg rotamer indicating the extra stabilization discussed above.

4. CONCLUSION

The NMR spectroscopic investigations of monosaccharides have indicated that the conformational preferences of the hydroxymethyl groups are influenced by many structural features of the compounds. The explanations for these observations are complex with elements of at least the following effects; solvent effects, steric interactions, electronic effects and hydrogen bonding.

A main limitation for an improved description of the conformational preference of hydroxymethyl groups in hexopyranoses is the limited accuracy of the empirical correlations between NMR spectroscopic data (coupling constants, chemical shifts) and the rotameric population. However, we hope that the large experimental data set presented here will stimulate theoretical investigations, which could contribute to a better understanding of the competing factors involved in the conformational preferences for hydroxymethyl groups in carbohydrates.

5. EXPERIMENTAL

The NMR data presented were obtained from 500 MHz or 600 MHz 1D spectra in D_2O at 300 K. For a few compounds literature data are presented. For a large part the data in Tables 2 and 3 are determined by simulation of the spin systems (H4, H5, H6R and H6S) using the standard Bruker software program PANIC in order to obtain accurate chemical shift and

coupling constants. For some compounds the spectra were collected at 600 MHz as well and the coupling constants measured on a first order basis are in good agreement with values from simulations. Therefore compounds measured at 600 MHz are presented without simulations. In a few cases the spectra from earlier studies have been used and therefore only 500 MHz data without simulation was available. It seems that when no chemical shift overlap is observed coupling constants from first order analysis are in good agreement with coupling constants from the more laborious simulations.

The Me 6-deoxy-6-F-(6-R)-^{2}H-α-D-Glc (**14**), Me 4-keto(hydrate)-α-D-Glc (**19**), Me 4-S-α-D-Glc (**20**) and Me 4-NH_2-α-D-Glc (**21**) compounds were synthesized by S. Refn, The Technical University of Denmark, Dept. of Organic Chemistry, Lyngby, Denmark.[145] The compound Me 6-deoxy-6-F-(6-R)-^{2}H-α-D-Glc (**14**) was synthesized from Me (6-S)-^{2}H-D-Glc by treatment with (Diethylamino)sulfur Trifluoride (DAST) which according to Card[146] gives introduction of fluorine in carbohydrates by inversion of configuration.

The compound Me 6-PO_3-α-D-Glc (**16**)[147] was a gift from Mette K. Christensen, Carlsberg Laboratory, Dept. Chemistry, Valby, Denmark. The Me 5-S-β-L-Gal (**36**) was a gift from Ole Hindsgaul, University of Alberta, Edmonton, Canada.

6. ACKNOWLEDGEMENT

We would like to thank Dr. Susanne Refn (Lyngby, Denmark), Mette K. Christensen (Copenhagen, Denmark) and Professor Ole Hindsgaul (Edmonton, Canada) for compounds used in this investigation. Dr. B. Mario Pinto (Vancouver, Canada) is thanked for valuable discussions during the preparation of this manuscript and Bent O. Petersen for careful measurements of many of the NMR data presented in the Tables.

7. REFERENCES

1. D.R. Bundle and N.M. Young, *Curr. Opinion Struct. Bio.*, **2**, 666 (1992).

2. Y. Bourne, H. van Tilbeurgh, and C. Cambillau, *Curr. Opinion Struct. Bio.*, **3**, 681 (1993).

3. K.G. Rice, P. Wu, L. Brand, and Y.C Lee, *Curr. Opinion Struct.Bio.*, **3**, 669 (1993).

4. S.W. Homans, *Prog. NMR Spectrosc.*, **22**, 55 (1990).

5. B. Meyer, *Top. Curr. Chem.*, **154**, 141 (1990).

6. K. Bock, *Pure Appl. Chem.*, **55**, 605 (1983).

7. G.A. Jeffrey, *Acta Cryst.*, **B46**, 89 (1990).

8. K. Bock and H. Pedersen, *Acta Chem. Scand.*, **B42**, 75 (1988).

9. K. Adelhorst and K. Bock, *Acta Chem. Scand.*, **46**, 1114 (1992).

10. K. Bock and H. Pedersen, *Acta Chem. Scand.*, **B 42**, 190 (1988).

11. G.A. Jeffrey and D.-B. Huang, *Carbohydr. Res.*, **222**, 47 (1991).

12. A. Imberty and S. Pérez, *Int .J. Biol. Macromol.*, **11**, 177 (1989).

13. I. Tvaroska, A. Imberty and S. Pérez, *Biopolymers*, **30**, 369 (1990).

14. I. Tvaroska, S. Pérez and R.H. Marchessault, *Carbohydr. Res.*, **61**, 97 (1978).

15. A. Imberty and S. Pérez, *Carbohydr. Res.*, **181**, 41 (1988).

16. K. Bock, J.Ø. Duus, O. Hindsgaul and I. Lindh, *Carbohydr. Res.*, **228**, 1 (1992).

17. S. Sabesan, J.Ø. Duus, S. Neira, P. Domaille, S. Kelm, J.C. Paulson and K. Bock, *J. Am. Chem. Soc.*, **114**, 8363 (1992).

18. H. Ohrui, Y. Nishida, H. Itoh and H. Meguro, *J. Org. Chem.*, **56**, 1726 (1991).

19. Y. Nishida, H. Hori, H. Ohrui, H. Meguro, S. Zushi, J. Uzawa and T. Ogawa, *Agric. Biol. Chem.*, **52**, 1003 (1988).

20. D.A. Cumming, R.N. Shah, J.J. Krepinsky, A.A. Grey and J.P. Carver, *Biochemistry*, **26**, 6655 (1987).

21. N.K. de Vries and H.M. Buck, *Recl. Trav. Chim. Pays-Bas*, **106**, 453 (1987).

22. Y. Nishida, H. Hori, H. Ohrui, H. Meguro, J. Uzawa, D. Reimer, V. Sinnwell and H. Paulsen, *Tetrahedron Lett.*, **29**, 4461 (1988).

23. H. Hori, Y. Nishida, H. Ohrui, H. Meguro and J. Uzawa, *Tetrahedron Lett.*, **29**, 4457 (1988).

24. S. Sabesan, K. Bock and J.C. Paulson, *Carbohydr. Res.*, **218**, 27 (1991).

25. D.A. Cumming and J.P. Carver, *Biochemistry*, **26**, 6676 (1987).

26. J.-R. Brisson and J.P. Carver, *Biochemistry*, **22**, 1362 (1983).

27. J. Breg, L.M.J. Kroon-Batenburg, G. Strecker, J. Montreuil and J.F.G. Vliegenthart, *Eur. J. Biochem.*, **178**, 727 (1989).

28. P.G. Goekjian, T.-C. Wu, H.-Y. Kang and Y. Kishi, *J. Org. Chem.*, **52**, 4823 (1987).

29. V.S. Rao and A.S. Perlin, *Can. J. Chem.*, **61**, 2688 (1983).

30. A. De Bruyn and M. Anteunis, *Carbohydr. Res.*, **47**, 311 (1976).

31. R.H. Marchessault and S. Pérez, *Biopolymers*, **18**, 2369 (1979).

32. R.U. Lemieux and J.C. Martin, *Carbohydr. Res.*, **13**, 139 (1970).

33. R.U. Lemieux and J.T Brewer, *Conformational Preferences for Solvated Hydroxymethyl Groups in Hexopyranose Structures*, in Advances in Chemistry Series, Number 117, pp 121-146, ACS 1973.

34. W.T. Wiesler, J.T. Vazquez and K. Nakanishi, *J. Am. Chem. Soc.*, **109**, 5586 (1987).

35. H.-W. Liu and K. Nakanishi, *J. Am. Chem. Soc.*, **103**, 5591 (1981).

36. C. Bertucci, P. Salvadori, G. Zullino, D. Pini and W.C. Johnson, Jr., *Carbohydr. Res.*, **149**, 299 (1986).

37. K.G. Rice, P. Wu, L. Brand, and Y.C. Lee, *Biochemistry*, **30**, 6646 (1991).

38. P.Wu, K.G. Rice, L. Brand, and Y.C. Lee, *Proc. Natl. Acad. Sci. USA*, **88**, 9355 (1991).

39. K.G. Rice, P. Wu, L. Brand, and Y.C. Lee, *Biochemistry*, **32**, 7264 (1993).

40. Y. Nishida, H. Hori, H. Ohrui and H. Meguro, *J. Carbohydr. Chem.*, **7**, 239 (1988).

41. H. Hori, Y. Nishida, H. Ohrui and H. Meguro, *J. Carbohydr. Chem.*, **9**, 601 (1990).

42. H. Ohrui, Y. Nishida, H. Higuchi, H. Hori and H. Meguro, *Can. J. Chem.*, **65**, 1145 (1987).

43. Y. Nishida, H. Hori, H. Ohrui and H. Meguro, *Carbohydr. Res.*, **170**, 106 (1987).

44. H. Ohrui, Y. Nishida, M. Watanabe, H. Hori and H. Meguro, *Tetrahedron Lett.*, **26**, 3251 (1985).

45. Y. Nishida, H. Ohrui and H. Meguro, *Tetrahedron Lett.*, **25**, 1575 (1984).

46. Y. Nishida, H. Hori, H. Ohrui and H. Meguro, *Agric. Biol. Chem.*, **52**, 887 (1988).

47. K. Bock and S. Refn, *Acta Chem. Scand.*, **B41**, 469 (1987).

48. D. Gagnaire, D. Horton and F. Taravel, *Carbohydr. Res.*, **27**, 363 (1973).

49. S.J. Perkins, L.N. Johnson, D.C. Phillips and R.A. Dwek, *Carbohydr. Res.*, **59**, 19 (1977).

50. J. Boyd, R. Porteous, N. Soffe and M. Delepierre, *Carbohydr. Res.*, **139**, 35 (1985).

51. L.D. Hall, J.F. Manville and N.S. Bhacca, *Can. J. Chem.*, **47**, 1 (1969).

52. R.J. Abraham, E.J. Chambers and W.A. Thomas, *Carbohydr. Res.*, **226**, C1 (1992).

53. R.J. Abraham, E.J. Chambers and W.A. Thomas, *Magn. Reson. Chem.*, **30**, S60 (1992).

54. N.K. De Vries and H.M. Buck, *Carbohydr. Res.*, **165**, 1 (1987).

55. M.L. Hayes, A.S. Serianni, and R. Barker, *Carbohydr. Res.*, **100**, 87 (1982).

56. H. Thøgersen, R.U. Lemieux, K. Bock and B. Meyer, *Can. J. Chem.*, **60**, 44 (1982).

57. A. Maradufu, G.M. Cree and A.S. Perlin, *Can. J. Chem.*, **49**, 3429 (1971).

58. G. Descotes, F. Chizat and J.C. Martin, *Bull. Soc. Chim. France*, 2304 (1970).

59. M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

60. M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).

61. D.H. Williams and N.S. Bhacca, *J. Am. Chem. Soc.*, **86**, 2742 (1964).

62. H. Booth, *Tetrahedron Lett.*, 411 (1965).

63. B. Coxon, *Tetrahedron*, 2281 (1966).

64. C. Altona and C.A.G. Haasnoot, *Org. Magn. Reson.*, **13**, 417 (1980).

65. C.A.G. Haasnoot, F.A.A.M. de Leeuw and C. Altona, *Tetrahedron*, **36**, 2783 (1980).

66. M.L. Huggins, *J. Am. Chem. Soc.*, **75**, 4123 (1953).

67. A.J. Duben, *Proc. Summer Comput. Simul. Conf.*, **21st.**, 310 (1989).

68. O. Jardetzky and G.C.K. Roberts, *NMR in Molecular Biology*, Academic Press: New York, 1981.

69. O. Jardetzky, *Biochim. Biophys. Acta*, **621**, 227 (1980).

70. K. Imai and E. Osawa, *Magn. Reson. Chem.*, **28**, 668 (1990).

71. E. Osawa, N. Ouchi, N. Saito, M. Yamato, O.S. Lee and M.-K. Seo, *Magn. Reson. Chem.*, **30**, 1104 (1992).

72. E. Diez, J. San-Fabian, J. Guilleme, C. Altona and L.A. Donders, *Mol. Phys.*, **68**, 49 (1989).

73. L.A. Donders, F.A.A.M. de Leeuw and C. Altona, *Magn. Reson. Chem.*, **27**, 556 (1989).

74. C. Altona, J.H. Ippel, A.J.A.W. Hoekzema, C. Erkelens, M. Groesbeek and L.A. Donders, *Magn. Reson. Chem.*, **27**, 564 (1989).

75. C. Morat, F.R. Taravel, and M.R. Vignon, *Magn. Reson. Chem.*, **26**, 264 (1988)

76. L. Poppe, *J. Am. Chem. Soc.*, **115**, 8421 (1993) .

77. R.U. Lemieux, T.L. Nagabhushan, and B. Paul, *Can. J. Chem.*, **50**, 773 (1972).

78. R.U. Lemieux, *Ann. New York Acad. Sci.*, **222**, 915 (1973).

79. T. Spoormaker and M.J.A. de Bie, *Rec. Trav. Chim. Pays-Bas*, **97**, 85 (1978).

80. J. Wu, P.B. Bondo, T. Vuorinen, and A.S. Serianni, *J. Am. Chem. Soc.*, **114**, 3499 (1992).

81. P.E. Hansen, *Prog. NMR Spectrosc.*,**14**, 175 (1981).

82. P.J. Hajduk, D.A. Horita, and L.E. Lerner, *J. Am. Chem. Soc.*, **115**, 9196 (1993).

83. A. Ejchart and J. Dabrowski, *Magn. Reson. Chem.*, **30**, S115 (1992).

84. P.Dais and R.H. Marchessault, *Macromolecules*, **24**, 4611 (1991).

85. D. Girlich and H.-D. Lüdemann, *Z. Naturforsch.*, **48c**, 407 (1993).

86. I. Braccini, V. Michon, C.H. du Penhoat, A. Imberty, and S. Peréz, *Int. J. Biol. Macromol.*, **15**, 52 (1993).

87. Z. Dzakula, W.M. Westler, A.S. Edison and J.L. Markley, *J. Am. Chem. Soc.*, **114**, 6195 (1992).

88. Z. Dzakula, A.S. Edison, W.M. Westler and J.L. Markley, *J. Am. Chem. Soc.*, **114**, 6200 (1992).

89. R.U. Lemieux and J.D. Stevens, *Can. J. Chem.*, **43**, 2059 (1965).

90. D. Danneels and M. Anteunis, *Org. Magn. Reson.*, **6**, 617 (1974).

91. H. Ohrui, Y. Nishida and H. Meguro, *Agric. Biol. Chem.*, **48**, 1049 (1984).

92. H. Hori, T. Nakajima, Y. Nishida, H. Ohrui and H. Meguro, *J. Carbohydr. Chem.*, **5**, 585 (1986).

93. H. Ohrui, T. Misawa and H. Meguro, *Agric. Biol. Chem.*, **49**, 239 (1985).

94. H. Ohrui, T. Misawa, H. Hori, Y. Nishida and H. Meguro, *Agric. Biol. Chem.*, **51**, 81 (1987).

95. G.D. Wu, A.S. Serianni and R. Barker, *J. Org. Chem.*, **48**, 1750 (1983).

96. P.C. Kline and A.S. Serianni, *Magn. Reson. Chem.*, **26**, 120 (1988).

97. P.C. Kline and A.S. Serianni, *Magn. Reson. Chem.*, **28**, 324 (1990).

98. L. Poppe, R. Stuike-Prill, B. Meyer and H. van Halbeek, *J. Biomol. NMR*, **2**, 109 (1992).

99. H. van Halbeek and L. Poppe, *Magn. Reson. Chem.*, **30**, S74 (1992).

100. D.M. Mackie, A. Maradufu and A.S. Perlin, *Carbohydr. Res.*, **150**, 23 (1986).

101. O. Hassel and B. Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).

102. O.A. Subbotin and N.M. Sergeyev, *Anal. Chem.*, **48**, 545 (1976).

103. S. Wolfe, *Acc. Chem. Res.*, **5**, 102 (1972).

104. S. Wolfe, L.M. Tel, W.J. Haines, M.A. Robb and I.G. Csizmadia, *J. Am. Chem. Soc.*, **95**, 4863 (1973).

105. N.S. Zefirov, L.G. Gurvich, A.S. Shashkov, M.Z. Krimer and E.A. Vorob'eva, *Tetrahedron*, **32**, 1211 (1976).

106. N.S. Zefirov, V.V. Samoshin, O.A. Subbotin, V.I. Baranenkov and S. Wolfe, *Tetrahedron*, **34**, 2953 (1978).

107. A. Abe and J.E. Mark, *J. Am. Chem. Soc.*, **98**, 6468 (1976).

108. E.L. Eliel and E. Juaristi, *J. Am. Chem. Soc.*, **100**, 6114 (1978).

109. P. Dionne and M. St-Jacques, *J. Am. Chem. Soc.*, **109**, 2616 (1987).

110. J. Huang and K. Hedberg, *J. Am. Chem. Soc.*, **111**, 6909 (1989).

111. P.I. Nagy, W.J. Dunn III, G. Alagona and C. Ghio, *J. Am. Chem. Soc.*, **113**, 6719 (1991).

112. B. Bernet, U. Piantini and A. Vasella, *Carbohydr. Res.*, **204**, 11 (1990).

113. M.C. Carreño, J.C. Carretero, J.L.G. Ruano and J.H. Rodriquez, *Tetrahedron*, **46**, 5649 (1990).

114. E. Juaristi and S. Antunez, *Tetrahedron*, **48**, 5941 (1992).

115. P.I. Nagy, W.J. Dunn, G. Alagona and C. Ghio, *J. Am. Chem. Soc.*, **114**, 4752 (1992).

116. K. Inomata and A. Abe, *J. Phys. Chem.*, **96**, 7934 (1992).

117. E. Juaristi and G. Cuevas, *J. Am. Chem. Soc.*, **115**, 1313 (1993).

118. C. Beeson, N. Pham, G. Shipps, Jr. and T.A. Diz, *J. Am. Chem. Soc.*, **115**, 6803 (1993).

119. L.M.J. Kroon-Batenburg and J. Kroon, *Biopolymers*, **29**, 1243 (1990).

120. I. Tvaroska and T. Kozar, *Theor. Chim. Acta*, **70**, 99 (1986).

121. C.J. Cramer and D.G. Truhlar, *J. Am. Chem. Soc.*, **115**, 5745 (1993)

122. J.W. Brady, *J. Am. Chem. Soc.*, **108**, 8153 (1986).

123. J.W. Brady, *J. Am. Chem. Soc.*, **111**, 5155 (1989).

124. S. Ha, J. Gao, B. Tidor, J.W. Brady and M. Karplus, *J. Am. Chem. Soc.*, **113**, 1553 (1991).

125. S.N. Ha, L.J. Madsen and J.W. Brady, *Biopolymers*, **27**, 1927 (1988).

126. P.C. Manor, W. Saenger, D.B. Davies, K. Jankowski and A. Rabczenko, *Biochim. Biophys. Acta*, **340**, 472 (1974).

127. B. Coxon, *Anal. Chem.*, **55**, 2361 (1983).

128. P.G. Goekjian, T.-C. Wu and Y. Kishi, *J. Org. Chem.*, **56**, 6412 (1991).

129. K. Bock, J.F.B. Guzman and S. Ogawa, *Carbohydr. Res.*, **174**, 354 (1988).

130. K. Bock, S. Ogawa and M. Orihara, *Carbohydr. Res.*, **191**, 357 (1989).

131. N. Inamoto and S. Masuda, *Chem. Lett.*, 1003 (1982).

132. J. Mullay, *J. Am. Chem. Soc.*, **107**, 7271 (1985).

133. R.J. Boyd and S.L. Boyd, *J. Am. Chem. Soc.*, **114**, 1652 (1992).

134. R.J. Abraham and J.S.E. Holker, *J. Chem. Soc.*, 806 (1963).

135. S. Winstein, P. Carter, F.A.L. Anet, and A.J.R. Bourn, *J. Am. Chem. Soc.*, **87**, 5247 (1965).

136. P.P. Lankhorts, C.A.G. Haasnoot, C. Erkelens and C. Altona, *J. Biomol. Struc. Dyn.*, **1**, 1387 (1984).

137. L. Evelyn and L.D. Hall, *Carbohydr. Res.*, **47**, 285 (1976).

138. V. Wray, *J. Chem. Soc., Perkin Trans. 2*, 1598 (1976).

139. A. De Bruyn, M. Anteunis, G. Aerts, and C.K. De Bruyne, *Acta Cienc. Ind.*, **4**, 103 (1978).

140. I. Tvaroska and T. Bleha, *Adv. Carbohydr. Chem. Biochem.*, **47**, 45 (1989).

141. B.M. Pinto, B.D. Johnston and R. Nagelkerke, *J. Org. Chem.*, **53**, 5668 (1988).

142. R.U. Lemieux, *Pure Appl. Chem.*, **25**, 527 (1971).

143. J.-P. Praly and R.U. Lemieux, *Can. J. Chem.*, **65**, 213 (1987).

144. N.D. Epiotis, *J. Mol. Struct. (Theochem)*, **229**, 205 (1991).

145. K. Bock and S. Refn, *unpublished work*, (1993).

146. P.J. Card, *J. Org. Chem.*, **48**, 393 (1983).

147. M. Meldal, M.K. Christensen and K. Bock, *Carbohydr. Res.*, **235**, 115 (1992).