Pure & Appl. Chem., Vol.55, No.4, pp. 605-622, 1983.
Printed in Great Britain.

0033-4545/83/040605-18$03.00/0
Pergamon Press Ltd.

THE PREFERRED CONFORMATION OF OLIGOSACCHARIDES IN SOLUTION INFERRED FROM HIGH RESOLUTION NMR DATA AND HARD SPHERE EXO-ANOMERIC CALCULATIONS

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Abstract - A complete understanding of the interaction between carbohydrates (oligosaccharides) and proteins (enzymes, antibodies and lectins) is to a large extent dependent on the information available about the preferred conformation of the carbohydrate molecules in solution.

Simple hard sphere (HSEA) calculations (including the exo-anomeric effect) have been shown to yield a preferred conformation of the oligosaccharide which is in excellent accord with experimental data obtained from high resolution NMR data (^{1}H and ^{13}C) of the oligosaccharide in aqueous solution. The conformations thus calculated can be evaluated with respect to hydrophobic surfaces, distances between ifferent receptor sites etc., in order to obtain a detailed information about the nature of the interaction between the carbohydrates and the proteins.

The conformational analysis will discussed in detail both with respect to the HSEA calcualtions but also with regard to the nuclear magnetic resonance parameters (^{1}H-chemical shifts and coupling constants, T_1-values, and nuclear Overhauser enhancement results, together with ^{13}C-chemical shifts, T_1-values, and ^{13}C-^{1}H-long range coupling constants), which yield information about the preferred conformation of the oligosaccharides in solution.

Examples will be given to illustrate how this approach can be used to estimate the preferred conformation of oligosaccharides having structures related to the complex glycoproteins and to the O-specific chains of lipopolysaccharides from Salmonella strains.

INTRODUCTION

Our understanding of the significance of carbohydrates in biological systems has increased during the last twenty years (1). Analytical techniques have improved tremendously during that period and Lindberg (2) has recently reviewed how structural analysis of complex oligo- and polysaccharides can be carried out on milligram amounts of compound using modern spectroscopic and chemical tools. Through synthetic work, as demonstracted by Lemieux (3), Paulsen (4) and others it is possible to prepare in relatively large amount

complex oligosaccharides. With these compounds it is possible to gain further insight into the understanding of the interaction between carbohydrates and proteins (like enzymes, antibodies or lectins) (5,6). However, in order to obtain a better picture of these interactions it is necessary to have information about the preferred conformation of the oligosaccharides in solution. The present paper primilarily discusses how a conformational analysis can be carried out using modern instrumentation and computers. Examples of the interpretation of the results in relation to the recognition of oligosaccharides will be discussed in the last part of the paper.

CONFORMATIONAL ANALYSIS OF OLIGOSACCHARIDES

Conformational analysis of oligosaccharides can be based on data from the following experiments,

1. X-Ray (or neutron)diffraction studies.
2. Chiroptical methods.
3. Nuclear magnetic resonance spectroscopic data.

The different diffraction studies require the carbohydrate molecule to be crystalline, which is a limitation because many oligosaccharides do not crystallize very easily. Furthermore, lattice forces may cause deviation from the conformation which is predominating in solution.

Chiroptical methods on the other hand yield information about the molecules in solution and have been used extensively in the study of polysaccharides (7). The results are, however, most easily interpreted when the compounds are simple repeating oligo- or polysaccharides (e.g. amylose).

A detailed conformational analysis of complex carbohydrate structures in solution is at present only possible using nuclear magnetic resonance (n.m.r.) data. High resolution n.m.r. spectrometers operating at 300 MHz or higher have made it possible to obtain experimental n.m.r. parameters, which contain detailed conformational information about the oligo- and polysaccharides in aqueous solutions (8-12). This point will be discussed in further details below. However, in order to make a complete interpretation of the n.m.r. data and in order to be able to draw relevant conclusions from the results, it is necessary to support these experimentally acquired data with a model which allows a simple theoretical evaluation of the preferred conformation of the oligosaccharides in solution.

Several theoretical approaches have been used in the study of carbohydrate conformations and the most frequently applied are the following:

1. Ab initio calculations
2. Force fields calculations
3. Hard spheres calculations

Ab initio calculations have not yet been used to calculate the preferred conformation of an oligosaccharide even though one ab initio calculation has been carried out on a fixed conformation of maltose (13). The reason is that these calculations are very expensive and in general not practically possible on large molecules. Most ab initio calculations have therefore been performed on model compounds, which simulate the atoms involved in the

glycosidic linkages (14-17). The molecular orbital method in the PCILO approximation has also been used in the study of the conformation of oligosaccharide linkages (18,19).

The simpler force fields calculations, which include both bond and angle deformations, torsional terms, non-bonded interactions, coloumbic terms, and hydrogen bonding have been carried out on several mono- and some disaccharides (20-24). These calculations are, however, still rather expensive and time consuming to carry out on molecules larger than disaccharides.

It has therefore been important to develop a simple and inexpensive method, which gives reliable results even on larger oligosaccharides, despite the fact that simple functions are used to describe the interaction between the component monosaccharides (25). Hard-spere calculations which only take into account the non-bonded interaction between the monosaccharide units of an oligosaccharide have proven to give results, which are in excellent accord with evidence from n.m.r. spectra provided the importance of the exo -anomeric effect is recognized (9-11). This method, the hard sphere exo -anomeric effect (HSEA) calculations, which has its major force in simplicity and inexpensiveness, will be described in further detail below.

HARD SPHERES CALCULATIONS

The problem in a conformational analysis of a disaccharide is relatively simple if the monosaccharide units can be considered as rigid bodies, i.e. that they exist in their regular unstrained chair conformations as indicated in Fig. 1 for maltose. Sheldrick and Akrigg (26) have published an investigation of 161 X-ray structures of compounds containing pyranose rings and discussed the validity of a rigid-body assumption. Their results indicated that it is possible to establish coordinates for an average pyranose ring. Based on these results and keeping in mind that the molecules investigated are dissolved in aqueous solutions it appears to be a reasonable assumption, as also discussed by Arnott and Scott (27).

However, in order to obtain as accurate results as possible, coordinates for the individual monosaccharides units are taken from good neutron- or X-ray diffraction experiments. Because the preferred conformation of oligosaccharides to a large extent is determined by the atoms, particularly the protons located around the glycosidic linkage, only proton coordinates from neutron diffraction studies can be used. If X-ray diffraction data are available only the experimentally determined coordinates from the heavy atoms are used and the proton coordinates are generated using a computer program. This program positions the protons at a 1.10 Å distance from the carbon atom along a vector defined by the remaining carbon-carbon and carbon-oxygen bond vectors. Hydrogen atoms of hydroxyl groups are not included in the calculations.

All these coordinates are stored in a library, which can be used directly in the conformational analysis of a specific oligosaccharide. If coordinates for a monosaccharide unit are not determined by either X-ray- or neutron-diffraction experiments, it is possible to perform bond modifications on related structures and thus calculate the appropriate coordinates.

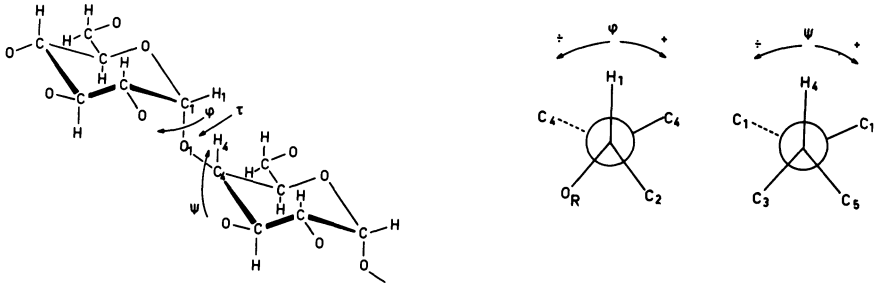

Fig. 1. The definition of the torsion angles ϕ and ψ which define the conformation of the glycosidic linkage.

The three degrees of freedom, which remain to be investigated are thus the rotations around the C_1-O_1 bond (ϕ_H rotation for the H_1-C_1-O_1-C_4-fragment) defined positive according to IUPAC recommendations (28) and rotation around the O_1-C_4 (ψ_H rotation for the C_1-O_1-C_4-H_4 fragment) and the size of the glycosidic bond angle (τ). Experimental data from X-ray or neutron diffraction studies suggest the τ-angle in most oligosacchatides can be considered constant ($\simeq 117^{o}$) (9), but the value can of course be included as a variable in the calculations.

Hard-sphere calculations primarily take into account the non-bonded interactions between the atoms. The literature (29) gives different potential functions for the interaction between the atoms, but in the present work the values published by Kitaygorodski have been chosen (30). The data have been used to estimate the non-bonded interaction between two atoms following the equation

$$V_{pot} = 3.5(-0.04/z^{6} + 8.5 \cdot 10^{3}\, e^{(-13z)}) \text{ kcal/mole} \qquad (1)$$

in which $z = r_{ij}/r_0$. The r_0 value is the equilibrium distance between the atoms and r_{ij} equal to the actual distance between the two interacting atoms. The r_{ij} value is arbitrarily set (29) at $1.11(r_i + r_j)$, where r_i and r_j are the van der Waal radii for the specific atoms investigated in the calculations (see Table I).

TABLE I.

van der Waal radii for atoms used in HSEA calculations

r_H	= 1.20 A	$r_{O,H-H}$	= 2.66 A
r_N	= 1.55 -	$r_{O,H-O}$	= 3.00 -
r_O	= 1.50 -	$r_{O,H-C}$	= 3.22 -
r_C	= 1.70 -	$r_{O,C-C}$	= 3.78 -
r_{CH_3}	= 1.85		

The potential curves for the H-H and H-O interactions are shown in Fig. 2A and B.

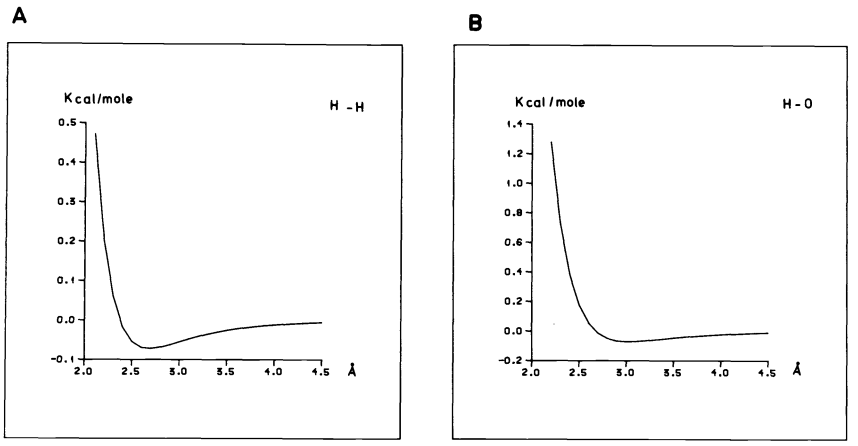

Fig. 2. Potential energy curve for H-H (A) and H-O (B) interactions calculated using equation 1.

The interaction energies for a given set of values for ϕ_H,ψ_H and τ are then summed for all interactions between two monosaccharide units and the sum represents the pure non-bonded interaction energy for that conformation. ϕ_H,ψ_H is then varied through 360^o in given intervals (e.g. 5^o) and this leads to a series of interaction energies as a function of ϕ_H and ψ_H, which can be inspected in different ways. It is most illustrative to use these numbers to describe the energy surface in a three-dimensional picture as a function of ϕ_H and ψ_H as shown in Fig. 3A and B, but a plot of isoenergy contours around the minimum is also frequently published to visualise this problem (14,17,20). Fig. 3C.

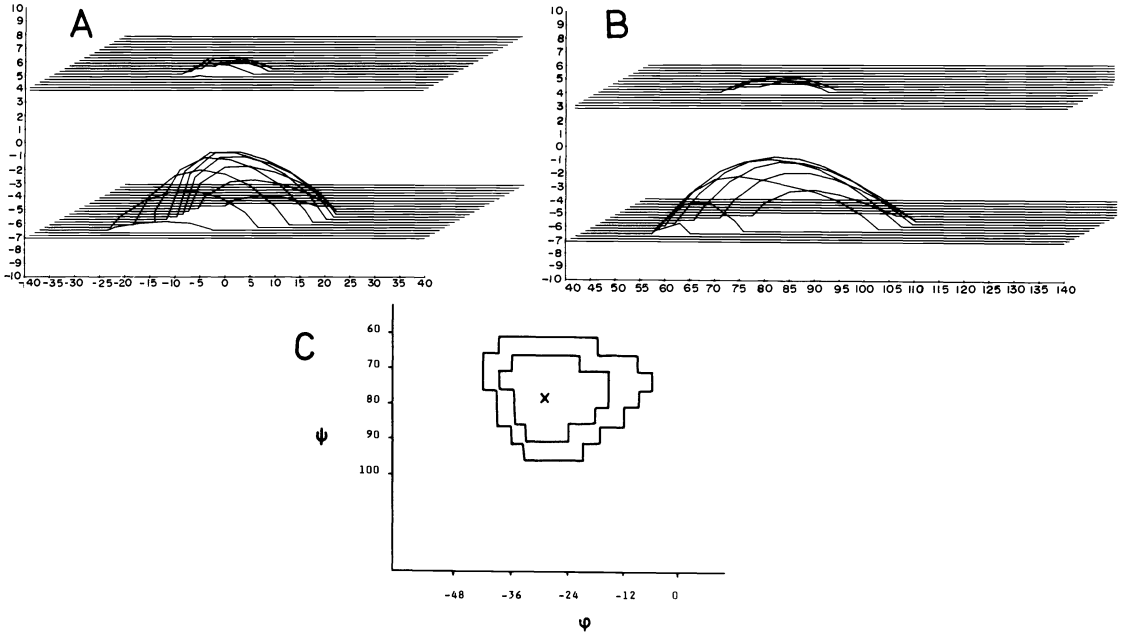

Fig. 3. Energy surface for the glycosidic bond of sucrose with cut through the surface at 0.5 and 2.5 kcal/mole, respectively. (A) as a function of ϕ and ψ. (B) As a function of ψ and ϕ. (C) Isocontour diagram for 1.0 and 2.0 kcal/mole, respectively.

However, experimental (9,10,31) and theoretical (14,17) evidence has proven that the exo-anomeric effect makes an important contribution to the preferred conformation of the glycosidic linkage. The consequence of the exo--anomeric effect is that the aglyconic carbon prefers an orientation in which the ϕ_H-angle is ~60° in β-D-glycosides and ~ -60° in α-D-glycosides, respectively, as shown in Fig. 4.

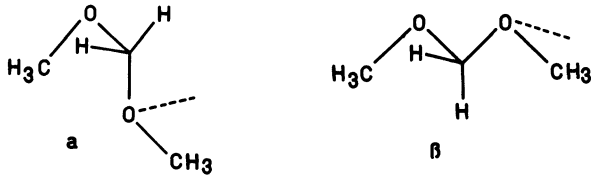

Fig. 4. The consequence of the exo-anomeric effect illustrated using dimethoxymethane as a model for an α- or β-glycoside. The most stable conformer is indicated with the dotted line.

Lemieux and coworkers (10) have discussed the quantitative aspects of the exo-anomeric effect and based on ab-initio calculations of dimethoxymethane (14) proposed a torsional potential describing the exo-anomeric effect for α- and β-glycosides respectively.

$$V_{exo-\alpha} = 1.58\ (1-\cos\phi) \quad -0.74\ (1-\cos 2\phi) \quad -0.70\ (1-\cos 3\phi) + 1.72 \text{ kcal/mole} \quad (2)$$

$$V_{exo-\beta} = 2.61\ (1-\cos\phi) \quad -1.21\ (1-\cos 2\phi) \quad -1.18\ (1-\cos 3\phi) + 2.86 \text{ kcal/mole} \quad (3)$$

This energy contribution is calculated for each φ-value and added to the non-bonded interaction energies according to equation 1 mentioned above giving the total energy encountered in these calculations, which are called hard-sphere, exo-anomeric (HSEA) calculations (9,10).

The minimum energy conformation can now be inspected with respect to short proton-proton or proton-oxygen distances or hard interactions which can be related to the experimentally determined n.m.r. parameters, which thus can support the calculated results. The coordinates determined for this minimum energy conformation can furthermore be used in standard molecular plot programs. These programs allow one to plot the molecules either as ball and stick models or as CPK models as shown in Fig. 5, which can be inspected from different angles to identify interactions between the individual monosaccharides (5). The last point is even better realised using color plot programs (32).

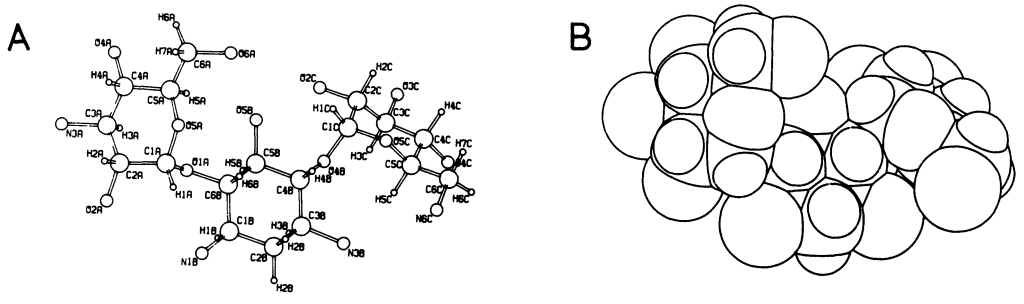

Fig. 5. Stick and ball model (A) and CPK model (B) of Kanamycin in its minimum energy conformation.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

^{1}H and ^{13}C-n.m.r. spectroscopy is the most direct method by which information about the preferred conformation of oligosaccharides in solution is obtained.

^{1}H-n.m.r. Parameters

The ^{1}H-n.m.r. parameters which yield conformational information are as follows:

1. Chemical shifts
2. Coupling constants
3. Spin lattice relaxation rates
4. Nuclear Overhauser enhancements

Prior to a discussion of the application of these parameters it is appropriate to mention briefly the assignment techniques used in the analysis of complex spectra of oligosaccharides in aqueous solutions (33).

1. Comparison with model compounds
2. Isotopic substitution (e.g. ^{2}H, ^{13}C)
3. Double-resonance experiments
4. Relaxation experiments
5. 2-Dimensional spectroscopy
6. Paramagnetic shift reagents
7. Protonation shifts
8. Solvent induced shifts

Of these methods the instrumental techniques 3,4 and 5 , which do not require chemical modifications of the oligosaccharides will be illustrated below. The major problem in the analysis of spectra of oligosaccharides in aqueous solutions is the assignment of the signals from the ring protons which resonate between δ 3.2 and 4.3.

Difference homo-decoupling experiments (33,34) are useful in assigning the chemical shifts of protons which are spin-spin coupled to protons outside the region between δ 3.2 and 4.3. Partially relaxed spectra (9,11) allow identification of the signals from the fast relaxing protons of the hydroxymethyl groups and from slowly relaxing protons (33). This experiment can be combined with a double-resonance experiment (35), which increase the possibilities for assigning "hidden resonances" (36).

The most powerful technique for the assignment of complex spectra are 2-dimensional n.m.r. experiments. 2-D-J-resolved spectroscopy (37) results in series of spectra in which the chemical shift information and the coupling constants are separated along two axes as shown in Fig. 6.

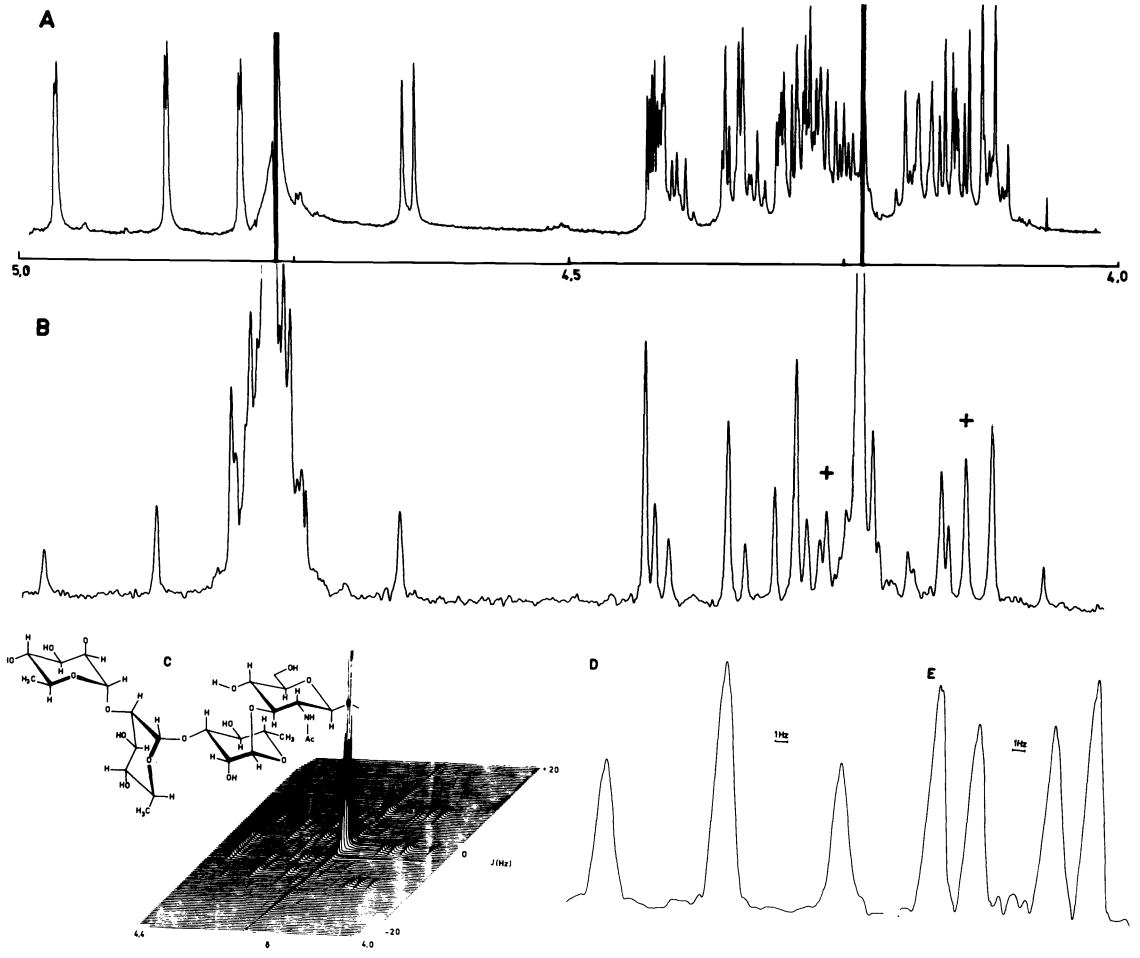

Fig. 6. 400 MHz ^{1}H-NMR spectrum of a tetrasaccharide α-L-Rham (1→2) α-L-Rham (1→3) α-L-Rham (1-3) β-D-glcNAc-OR. A Normal spectrum. B. 2-Dimensional-J resolved δ-spectrum. C. 2-D-J--resolved contour diagram. D. and E. J-spectra of lines marked with a + in B.

The spectrum obtained by projecting the data along the x-axis (the δ-spectrum, Fig. 6B) gives a ^{1}H-n.m.r. spectrum, in which the spin-spin couplings are removed. The spectrum thus shows each proton as a singlet in the same manner as a proton-decoupled natural-abundance ^{13}C-n.m.r. spectrum. Projection along the y-axis for a given proton provides the coupling pattern for that particular proton as shown in Fig. 6D. This experiment facilitates the analysis very much, but does not resolve very close or overlapping signals or protons which are strongly coupled.

Normally homo-decoupling experiments are necessary in order to establish the connectivities between the spin-spin coupled protons. This could be combined with the 2-D-J resolved experiment, but is not easily accomplished, mainly because of the time required for each 2-D-J resolved experiment. Fortunately, another 2-dimensional experiment has been proposed by Ernst and coworkers (38). This 2-D-scalar coupled n.m.r. experiment allows an identification of the connectivities between all spin-spin coupled protons in one experiment and an example is shown in Fig. 7.

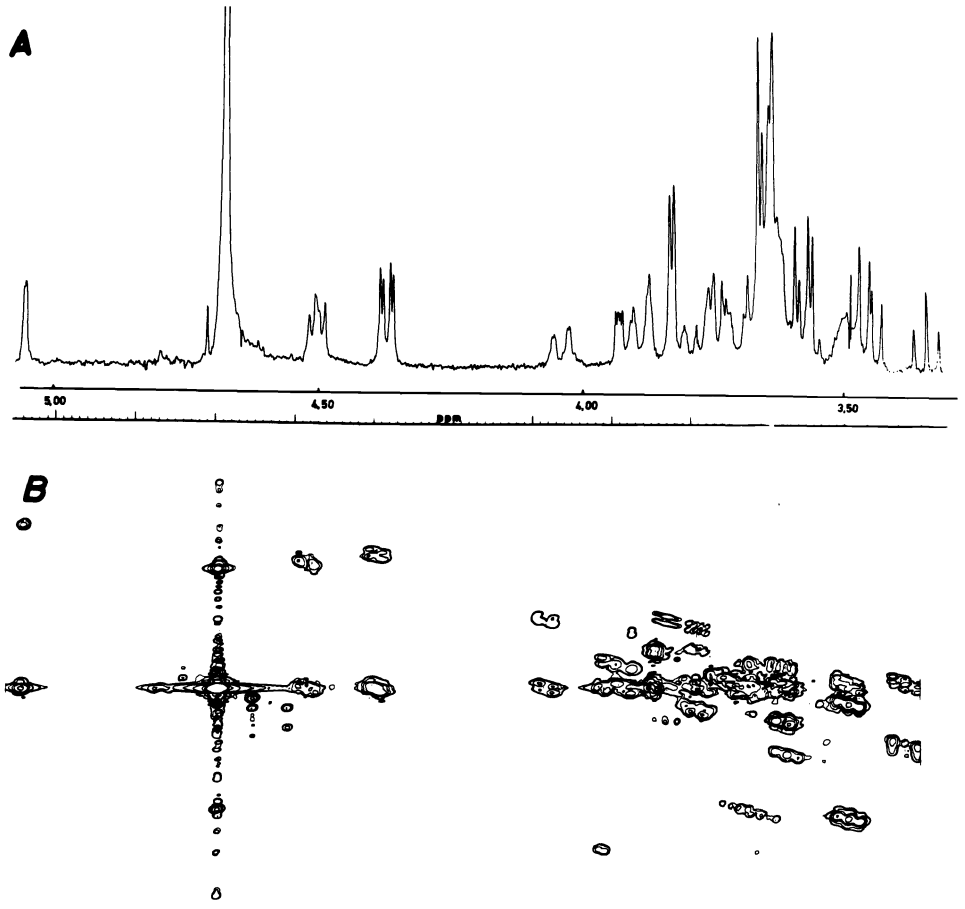

Fig. 7. 400 MHz-NMR spectrum of a pentasaccharide β-D-gal (1-4) -β-D-glcNAc (1→6) {β-D-gal (1-4)-β-D-glcNAc (1→2)}-α-D-man. in D_2O. A. Normal spectrum. B. 2-Dimensional-scalar coupled spectrum.

A combination of the above two mentioned experiments allows complete analyses of the proton n.m.r. spectra of even very complex carbohydrate structures. Thus it has been possible to perform a full analysis of the ^{1}H-n.m.r. spectra of penta- and heptasaccharides with structures related to the complex glycoproteins (39).

After a complete assignment of the ^{1}H-n.m.r, spectrum has been done it is relevant to discuss how these data can be used in a conformational analysis of an oligosaccharide.

The proton spin-spin coupling constants can be used to confirm that the chair conformations of the individual units are similar to those found in the parent monosaccharides. The proton chemical shifts can be used in the analysis of the interglycosidic conformation because protons will be downfield shifted if they are close in space to oxygen atoms (< 2.70 Å) from neighbouring units (9,10,11) as indicated in Fig. 8. This downfield shifting is similar to that observed for the H-3 and H-5 protons in pyranoses, when the anomeric configuration is changed from β- to α-, Fig. 8. Similarly, upfield shifts may be observed if the molecules contain functional groups, which exhibit strong anisotropy, like C=O groups in N-acetyl derivatives.

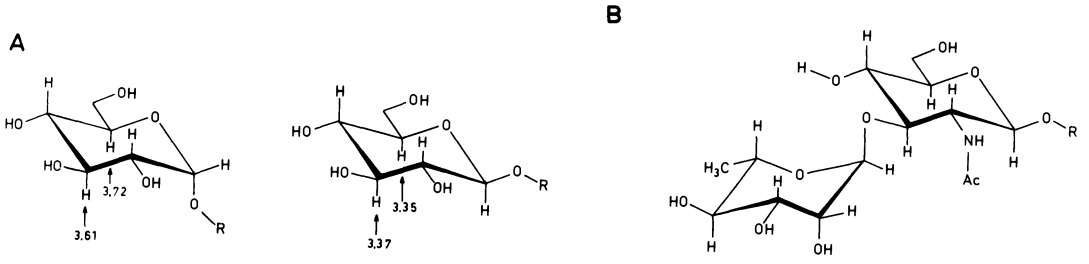

Fig. 8. A ^{1}H-NMR chemical shifts of H-3 and H-5 protons in α- and β-D-glucopyranose in D_2O. B. Downfield shifting of H-5 in α-L-Rhamose unit is expected if the molecule preferrently adopts the conformation indicated.

The application of proton spin-lattice relaxation rates for structural assignments of carbohydrates has been pionered by Hall and coworkers (40,41). Its application in the study of interglycosidic conformations rely on the strong dependence of the relaxation rates with intramolecular proton-proton distances as indicated in equation 4.

$$\frac{1}{T_1} = R_1 = \frac{3}{2} \cdot \hbar^2 \cdot \gamma_H^4 \cdot \tau c / \sum_{i \neq j} r_{ij}^{-6} = c / \sum_{i \neq j} r_{ij}^{-6} \qquad (4)$$

Generally, relaxation contributions from another monosaccharide unit can be observed if the proton-proton distances are below 3.00 Å. The problem with the application of proton-relaxation rates in a conformational analysis is that it is generally difficult to determine the individual relaxation contributions and thus obtain a quantitative measure for the proton-proton interactions in neighbouring pyranose rings.

This problem can be solved by measurement of nuclear Overhauser enhancements (42). In this experiment the individual proton-proton relaxation contributions can be determined between the proton saturated and the protons receiving relaxation contribution as indicated in equation 5.

$$NOE(d)^s = r_{ds}^{-6}/c \cdot 2 \cdot \sum_{d \neq j} r_{dj}^{-6} \qquad (5)$$

Performed in the difference mode as shown in Fig. 9,this experiment gives very reliable results and it is possible to measure enhancements as small as 2 % with confidence (9,10,11). This experiment is the most powerful tool in the conformational analysis of oligosaccharides. Furthermore,if qualitative data only can be accepted,the experiment can be performed as a 2-dimensional experiment (10,43).

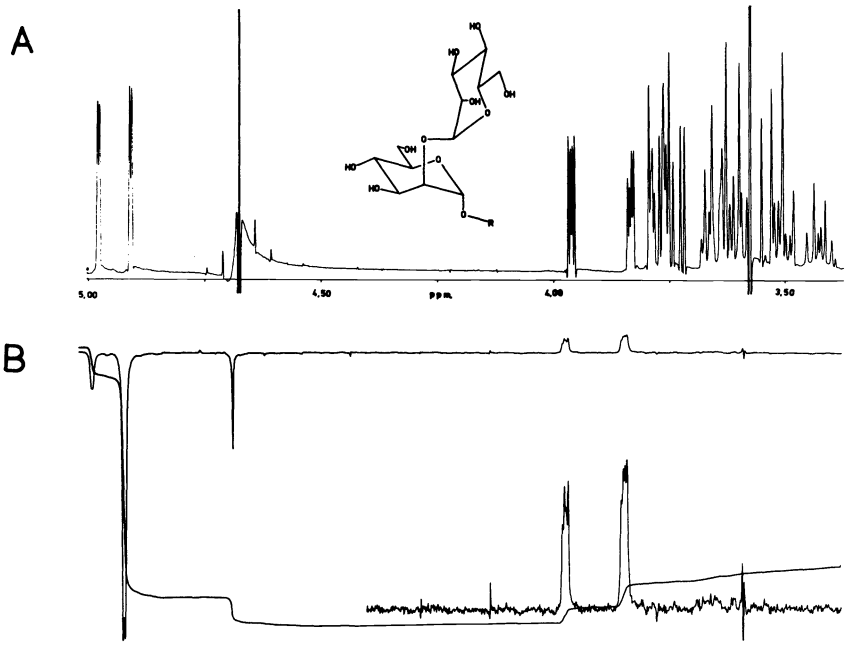

Fig. 9. 400MHz spectrum of α-D-man(1→2)α-D-man-OR in D_2O. A. Normal spectrum. B. Difference n.O.e. experiment with saturation of H-1 of the glycosidically linked mannose unit.

^{13}C-n.m.r. Parameters

Even though the carbon atoms are not located at the surface of the molecules the ^{13}C-n.m.r. data contain some conformational information. The parameters which are of importance in a conformational analysis are as follows:

1. Chemical shifts
2. Long-range coupling constants
3. Spin-lattice relaxation rates

As mentioned above it is important to assign the carbon resonance before the data can be used in a conformational analysis. Several assignment techniques can be used as discussed in reviews (44,45,46).

1. Correlation with model compounds
2. Isotopic substitution
3. Correlation with proton spectra
4. Relaxation experiments
5. Paramagnetic reagents
6. Protonation shifts
7. ^{13}C-^{13}C Satellite spectra

The most frequently used techniques are 1 and 3, but the ^{13}C-^{13}C satellite spectra either in the 1- (47) or the 2-dimensional (48) form are also used more and more. Generally,correlation with the proton spectra, either through a selective proton-decoupling experiment (44) or through hetero-nuclear 2-D correlated spectroscopy (49), is the most reliable method to assign the carbon signals in a given spectrum.

As mentioned above, the ^{13}C-chemical shifts do not yield as much information about the glycosidic conformation as the corresponding ^{1}H-n.m.r. chemical shifts. But in cases where chrowding occurs as for example in branched trisaccharides small valence and/or bond-angle deformations may take place, which will result in a different hybridisation of the ^{13}C-nucleus and thus in a different ^{13}C-chemical shift. Normally, these effects cause upfield shifts in the resultant spectra as observed for a di- and trisaccharide related to the H-blood group determinant (9.33).

Due to the angular dependence of the three bond C-O-C-H long range coupling constants (44,46,50,51) it is possible to obtain information about the interglycosidic torsion angles from high resolution proton coupled spectra of oligosaccharides (9,52) or from proton spectra of isotopically labelled molecules (12,53,54). These values have in several cases been used successfully in the conformational analysis of oligosaccharides (9,11,12,52,53,54). The only limitation to the use of these long-range coupling constants is that the line width of the resonances is to some extent dependent of the molecular size, which imply that it is generally not possible to use this approach for larger oligosaccharides.

Finally, relaxation rates can be used in the conformational analysis of oligosaccharides, but due to the dominating dipole-dipole relaxation mechanism from the protons directly bonded to the carbon atoms, these values are mainly used in the study of the molecular motion of the individual monosaccharide units in an oligo- or polysaccharide as discussed by several authors (55-59).

APPLICATIONS

The combination of experimental n.m.r. data and theoretical HSEA calculations has in several examples given convincing results concerning the preferred conformation of the oligosaccharides in aqueous solutions with blood determinants (8,9,10) and oligosaccharides related to the O-antigen of the lipopolysaccharides of Shigella Flexneri (11). Also a conformational analysis of simpler oligosaccharides like sucrose (60), trehalose (61), gentiobiose-octa acetate (62) and acetylated Forsman antigen (disaccharide) (63) has yielded results which are in very good accord with other experimental and theoretical considerations.

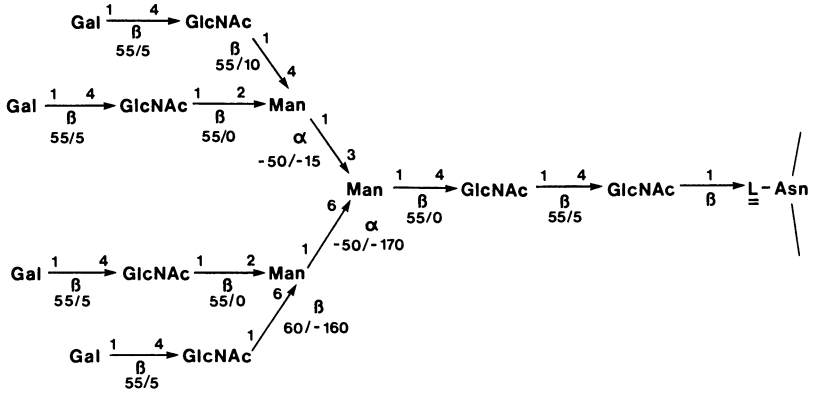

Fig. 10. Structure of tetraantennary asialo-glycan of oligosaccharides related to the complex type of glycoproteins. Numbers below the glycosidic bonds indicate the $\phi.\psi$ angles for the calculated minimum energy conformation.

This approach has also been used in a conformational analysis of oligosaccharides related to the complex type glycoproteins (39). The oligosaccharide part of this type of molecules is shown in Fig. 10 and structures with 2, 3 or 4 N-acetyl-lactosamine units have been isolated from biological systems (64). In several cases the terminal β-D-galactopyranose unit may be substituted with sialic acid residues in the 3-position. A series of two tri-, two penta- and one heptasaccharide were available through chemical synthesis (65). N.m.r. data (both ^{1}H and ^{13}C) have been determined for these 5 compounds using the methods described above (39). A 400 MHz ^{1}H-n.m.r. spectrum together with a 2-D-scalar coupled n.m.r. experiment of the pentasaccharide is shown in Fig.7, which clearly shows the potential of these advanced methods for the assignment of complex spectra.

HSEA calculations on the different components of the structure shown in Fig. 10 have been carried out (39) and the results presented in Fig. 10. The minimum energy molecule either as a stick and ball model or as a CPK model is shown in Fig. 11 for the heptasaccharide attached to the N-acetyl-chitobiose linking arm. The molecules are shown in the two conformations (gg and gt) predominating around the C5-C6 bond of the β-D-mannopyranose unit. It is clearly seen from Fig. 11 that there is no interaction between the two N-acetyl-lactosamine units and note that the distance between the C4 atoms of the terminal β-D-galactopyranose units are 25.2 Å and 24.1 Å, respectively.

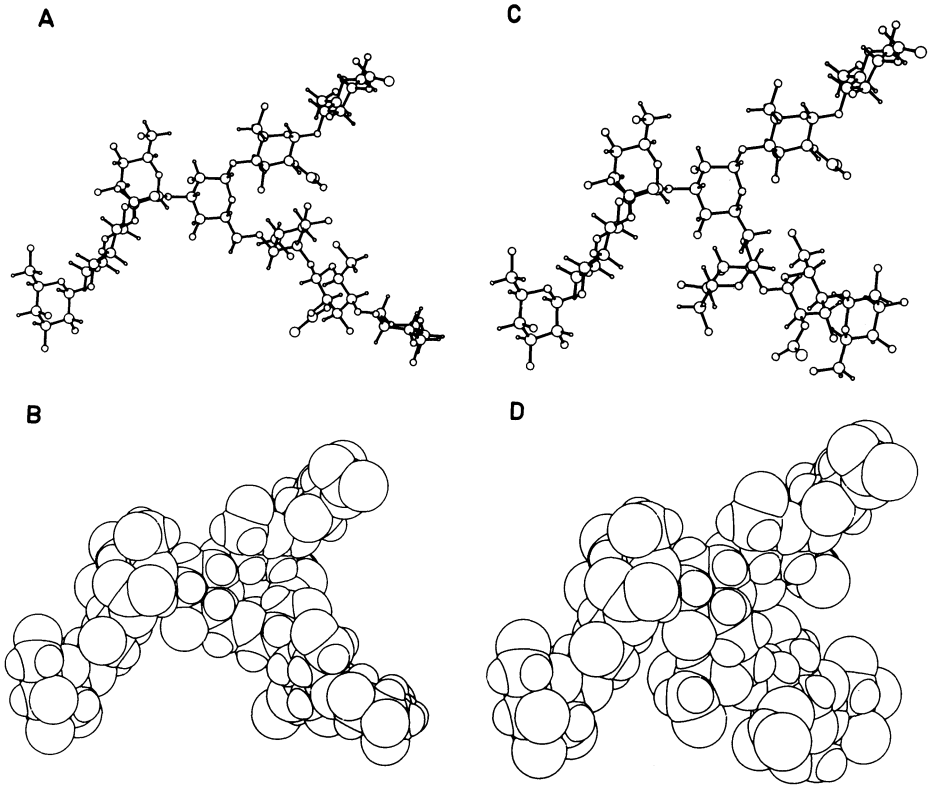

Fig. 11. Minimum energy conformation of central monosaccharide part of the oligosaccharide shown in Fig. 10. (A) and (B) with the conformation of the hydroxymethyl group of the β-D-man unit in the "gt" conformer. (C) and (D) with the same hydroxymethyl group in the "gg" conformer.

The calculated minimum energy conformation of a undecasaccharide indicated in Fig. 10 is shown in Fig. 12. This molecule has not been synthesized in total, but the component two penta- and one heptasaccharide have been available for an experimental investigation. The result of the conformational analysis shows that the N-acetyl-lactosamine units are pointing out in space like antennae. In order to get a proper view of this molecule it is necessary to look from two directions either along the C4-C1 vector of the central β-D-mannopyranose unit as shown in Fig. 12 (A and C) or perpendicular to that direction along the H4-C4 bond of the same sugar unit Fig. 12 (B and D). The NN-di-acetylchitobiose linking arm is not shown in this picture, because it would be even more difficult to obtain a proper view of the molecule.

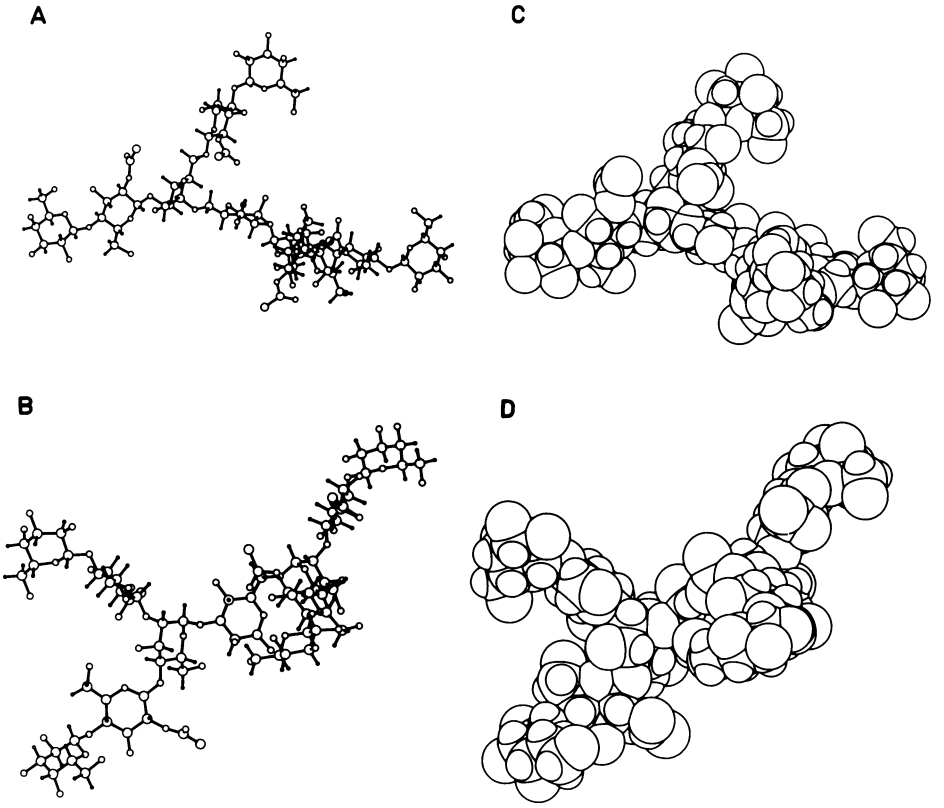

Fig. 12. Minimum energy conformation of the tetraantennary undecasaccharide shown in Fig. 10 without the N-acetyl-chitobiose part. (A) and (C) shown along the C4 to C1 vector of the central β-D-man unit. (B) and (D) shown along the H4 to C4 vector of the same unit.

It should be noted that the distance between the C4 atoms of the terminal β-D-galactopyranose units in the pentasaccharide in which the N-acetyl-lactosamine units are linked in the 2 and 4 postition of the α-D-mannopyranose unit is 15.4 Å (Fig. 12B). However, the distance between the C4 atoms of the same units in the pentasaccharide linked in the 2 and 6 position is much larger (22.5 Å (Fig. 12A). Inhibition results with these two pentasaccharides and the heptasaccharide mentioned above toward rabbit hepatic lectin, which is known to recognize terminal β-D-galactopyranose

units, has shown (66) that the pentasaccharide with N-acetyllactosamine linked in the 2 and 4 position with the short distance between the galactose units, is a much better inhibitor than the other two structures.

A similar analysis has been carried out on oligosaccharides related to the O-specific chains of the lipopolysaccharides of Salmonella strains. The basic structure of which is shown in Fig. 13. The synthesis of all the possible, di-, tri- and tetrasaccharides has been possible through a collaboration between research group in Ottawa, Stockholm and Copenhagen (67).

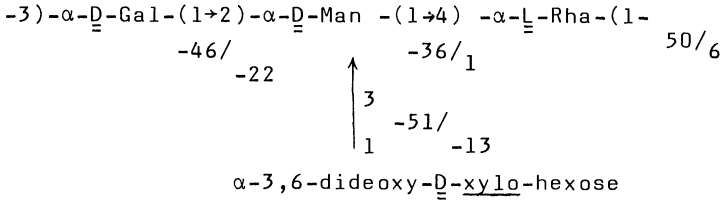

Fig. 13. Structure of the repeating unit of the O-specific chain of Salmonella strains serogroup B. The numbers below the glycosidic bonds refer to the ϕ/ψ values of the minimum energy conformation.

A complete analysis of the n.m.r. data of all these compounds together with HSEA calculations has given results (67) which indicate that the preferred conformation of the repeating tetrasaccharide structure is as indicated in Fig. 13. Extension of the calculation to the oligosaccharide chain is shown in Fig. 14, and indicates that the molecules adopt a helical structure, with three-tetrasaccharide units per turn. If this molecule is viewed from the end along the axis of the helix as shown in Fig. 14B and C it is clearly seen that a hydrophobic surface is seen from one end and a hydrophilic surface from the other. It is interesting to note that the hydrophilic region is pointing away from the surface of the bacterium, whereas the hydrophobic areas are pointing towards the bacterium. This might offer a protection for the bacteria towards antibodies which may look for binding to hydrophobic surfaces (5).

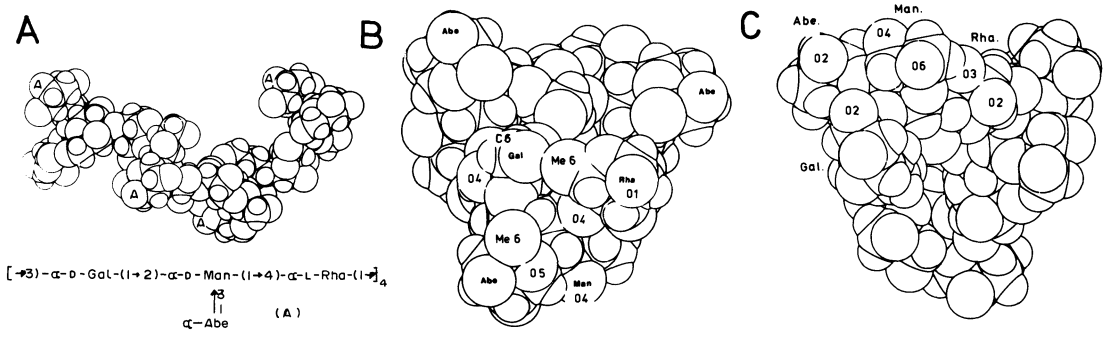

Fig. 14. Minimum energy conformation of the O-specific chain of the Salmonella strain serogroup B shown in Fig. 13. (A). Viewed perpendicular to the axis of the helix with the reducing end pointing to the left. (B). Viewed from the reducing end along the axis of the helix showing the hydrophobic part. (C). Viewed opposite to that in (B) showing the hydrophilic part of the molecule.

CONCLUSION

The results presented above indicate strongly that the simple HSEA calculations give valuable information about the preferred conformation of oligosaccharides in solution, which in oligosaccharide structures related to blood group determinants, glycoproteins and lipopolysaccharides have been supported with n.m.r. data. It is thus possible to calculate the preferred conformation of the oligosaccharide structures, which do not allow the same thorough n.m.r. analysis as discussed above. Nevertheless qualified interpretation of the biological behaviour of these molecules offers futher insight into the interactions between carbohydrates and proteins.

ACKNOWLEDGEMENTS

This work has only been possible through the stimulation and inspiring collaboration with professor R. U. Lemieux to whom the author wishes to dedicate this paper. Drs. B. Meyer, H. Thøgersen and D. Bundle have also contributed to the development of the HSEA methods. The Danish National Science Research Council supported the project financially.

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