

Motional properties of a pentasaccharide containing a 2,6-branched mannose residue as studied by ^{13}C nuclear spin relaxation

Lena Mäler^{a,*}, Göran Widmalm^b and Jozef Kowalewski^a

^aDivision of Physical Chemistry and ^bDepartment of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-10691 Stockholm, Sweden

Received 19 October 1995

Accepted 11 December 1995

Keywords: ^{13}C relaxation; Dynamics; Model-free approach; Pentasaccharide

Summary

^{13}C relaxation data obtained at three different magnetic fields, 9.4, 11.8 and 14.1 T, and at two temperatures, 303 and 318 K, are reported for the pentasaccharide *p*-trifluoroacetamidophenyl 2,6-di-*O*-[β -D-galactopyranosyl-(1 \rightarrow 4)-*O*-2-acetamido-2-deoxy- β -D-glucopyranosyl] α -D-mannopyranoside. The pentasaccharide consists of two disaccharide units, attached at positions 2 and 6 to the central mannopyranoside residue. The relaxation data were interpreted with the Lipari–Szabo model-free approach. For the central mannose residue in the molecule a high order parameter ($S^2=0.91$) was found and the relaxation data could be interpreted with the truncated form of the Lipari–Szabo model. The motional behavior of the two 2-acetamido-2-deoxy-glucopyranoside residues was found to differ. The one attached at the primary hydroxylic position displayed more extensive local motion ($S^2=0.75$ – 0.77) than the one attached at the secondary hydroxylic position ($S^2=0.83$ – 0.85). More extensive local motion for the two outer galactopyranoside residues was found ($S^2=0.56$ – 0.59), but no significant difference in motional behavior between the two residues could be observed. Analysis of the relaxation data for the exocyclic carbons confirmed the results for the rings. For the mannose C6, the same motional parameters as obtained for the substituting 2-acetamido-2-deoxy-glucopyranoside residue were found. The two exocyclic carbons on the 2-acetamido-2-deoxy-glucopyranoside residues showed more extensive local motion, with lower order parameters ($S^2=0.59$ – 0.66).

Introduction

In the field of carbohydrate research, nuclear magnetic relaxation is frequently used to establish the motional behavior of different sugars (McCain and Markley, 1986, 1987; Adams and Lerner, 1992; Hricovini et al., 1992; Poppe and Van Halbeek, 1992; Braccini et al., 1993; Hajduk et al., 1993; Roy et al., 1993; Dais, 1994; Poppe et al., 1994; Hricovini and Torri, 1995). Motional features of carbohydrates are important aspects in the understanding of the chemistry behind reactions with oligosaccharides. A very common approach to the investigation of dynamical properties is by means of heteronuclear relaxation measurements (McCain and Markley, 1986, 1987; Braccini et al., 1993; Hajduk et al., 1993; Roy et al., 1993; Dais, 1994; Poppe et al., 1994; Hricovini and Torri, 1995). The relaxation parameters indirectly contain dy-

namical information through the spectral density functions. Information on the motional parameters can be obtained by using a motional model, and the ‘model-free’ approach developed by Lipari and Szabo (1982a,b) has become a widely used method for the evaluation of relaxation data. Typically, the heteronuclear relaxation parameters T_1 , T_2 and NOE are measured for CH vectors in the molecule and the relaxation rates are interpreted with this model to obtain a global correlation time for the overall motion as well as the parameters describing the local motion, a short local correlation time and an order parameter indicating the restriction of the local motion. Previously, we have reported several studies of the dynamical properties of oligosaccharides where we have used ^{13}C relaxation as a tool for examining the motions occurring in these molecules (Kovacs et al., 1989; Bagley et al., 1992; Kowalewski and Widmalm, 1994; Mäler et al., 1995).

*To whom correspondence should be addressed.

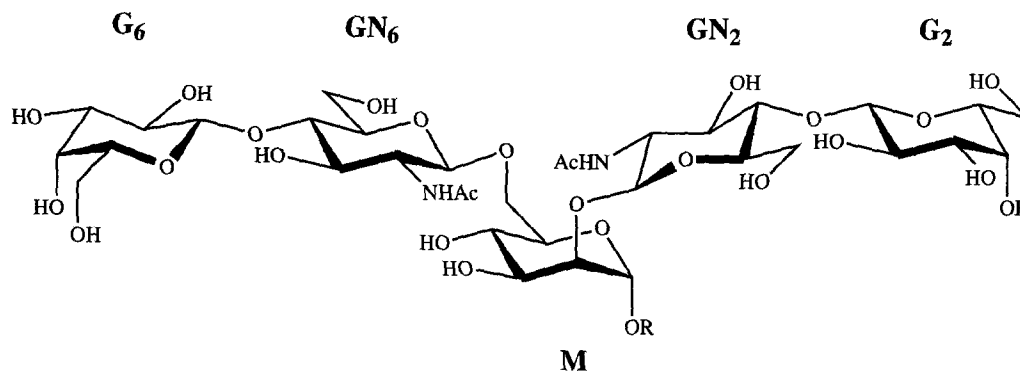


Fig. 1. Structure of the pentasaccharide. The galactose residues are labeled G, the 2-acetamido-2-deoxy-glucopyranoside residues GN and the mannose residue M. R is a *p*-trifluoroacetamidophenyl group. The subscripts 2 and 6 indicate the substitution position of the branching mannose residue.

The present investigation deals with the dynamical features of a pentasaccharide, *p*-trifluoroacetamidophenyl 2,6-di-*O*-[β -D-galactopyranosyl-(1 \rightarrow 4)-*O*-2-acetamido-2-deoxy- β -D-glucopyranosyl] α -D-mannopyranoside (Fig. 1). Carbohydrates of this type occur in glycoproteins and are involved in important biological processes (Wieland and Reutter, 1993), such as regulation of the poly-*N*-acetyl-lactoseamine biosynthesis (Do and Cummings, 1993). The molecule consists of a mannopyranoside residue to which two disaccharide units of the same kind are attached. These disaccharide units are linked to the mannopyranoside in different ways; one is linked through a primary hydroxylic position, whereas the other is linked through a secondary position (see Fig. 1). The fact that they are connected in different ways to the mannopyranoside residue suggests that their motional properties should differ. In order to investigate the dynamical features of the pentasaccharide, we have performed ^{13}C longitudinal and transverse relaxation measurements at different magnetic field strengths and at two temperatures. The relaxation data were interpreted with the model-free approach of Lipari and Szabo to obtain information on the overall reorientation correlation time as well as on the local dynamics in the rings.

Theory

For ^{13}C relaxation in systems where the carbons have directly bonded protons, the relaxation is dominated by dipole-dipole (DD) relaxation due to direct dipolar interactions between the carbon and the attached proton(s). However, the chemical shift anisotropy (CSA) cannot be neglected as a source for relaxation, as it can significantly contribute to cross-correlation effects (Shimizu, 1964). Cross-correlation effects will interfere in measurements of conventional relaxation parameters if no precautions are taken to eliminate these effects. Under the conditions of broadband decoupling, longitudinal relaxation is a single exponential process, characterized by a rate constant T_1^{-1} . In measurements of transverse relaxation rates, T_2^{-1} ,

several ways of removing cross-correlation in relaxation experiments have been proposed (Kay et al., 1992; Palmer III et al., 1992). Cross-relaxation between a carbon and the attached proton leads to an intensity increase of the carbon signal by a factor $1 + \eta$. The relaxation parameters can be expressed in terms of spectral density functions taken at different combinations of the carbon and proton Larmor frequencies. For carbons with one directly bound proton, the expressions for the relaxation parameters become:

$$T_1^{-1} = \frac{1}{4} (\text{DCC})^2 [J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad (1)$$

$$T_2^{-1} = \frac{1}{8} (\text{DCC})^2 \times [4J(0) + J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H) + 6J(\omega_H + \omega_C)] \quad (2)$$

$$\eta = \left(\frac{\gamma_H}{\gamma_C} \right) \frac{6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)}{J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)} \quad (3)$$

The factor $\text{DCC} = (\mu_0 / 4\pi) \gamma_C \gamma_H \hbar r_{\text{CH}}^{-3}$ is the dipolar coupling constant and is related to the distance between the two spins. $J(\omega)$ is the reduced spectral density function. For carbons with two protons directly attached, like the exocyclic CH_2 carbons in the carbohydrate, the expressions for T_1^{-1} and T_2^{-1} should be multiplied by two, while the expression for η remains the same, provided that cross-correlation between individual CH vectors can be neglected.

To interpret the relaxation parameters in terms of reorientational dynamics, the 'model-free' approach by Lipari and Szabo (1982a) is often used. In this model, two kinds of motion are assumed to modulate the interaction causing relaxation, i.e., a rapid, local motion and a slower, global motion. If the two motions are statistically independent and if the global molecular reorientation is isotropic, the reduced spectral density function can be written as:

$$J(\omega) = \frac{2}{5} \left(\frac{S^2 \tau_M}{1 + \omega^2 \tau_M^2} + \frac{(1 - S^2) \tau}{1 + \omega^2 \tau^2} \right) \quad (4)$$

where $\tau^{-1} = \tau_M^{-1} + \tau_c^{-1}$. τ_M is a correlation time for the global motion, common to the whole molecule, τ_c is the correlation time for fast local motion, specific for every individual axis in the molecule, and S is a generalized order parameter. S reflects the spatial restriction of the local motion. If the first term in Eq. 4 is much larger than the second, the equation can be truncated to obtain:

$$J(\omega) = \frac{2}{5} \left(\frac{S^2 \tau_M}{1 + \omega^2 \tau_M^2} \right) \quad (5)$$

which is identical to the expression for isotropical small-step diffusion with an amplitude scaling factor S^2 .

Experimental

The synthesis of the pentasaccharide *p*-trifluoroacetamidophenyl 2,6-di-*O*-[β -D-galactopyranosyl-(1 \rightarrow 4)-*O*-2-acetamido-2-deoxy- β -D-glucopyranosyl] α -D-mannopyranoside has been described previously by Arnarp et al. (1983). The pentasaccharide was dissolved in a 7:3 molar ratio of D₂O:DMSO-*d*₆ to give a 50 mM solution. The mixed solvent was chosen for its good cryogenic properties and its high viscosity, facilitating measurements outside the extreme narrowing regime. The sample was transferred to a 5 mm NMR tube and degassed by several freeze-pump-thaw cycles before being sealed under vacuum. Homo- and heteronuclear two-dimensional correlation spectra were recorded for assignment on JEOL GSX 270 and Varian U500 and U600 spectrometers operating at 6.3, 11.8 and 14.1 T, respectively. Long-range proton-carbon correlations were measured for assignment according to Nishida et al. (1995), using a pulsed field gradient-enhanced version. The ¹³C selective excitation was performed using a 90° Gaussian shaped pulse of 200 ms duration. Chemical shifts are referenced relative to residual DMSO-*d*₅ for ¹H ($\delta_H = 2.61$ ppm) and internal DMSO-*d*₆ for ¹³C ($\delta_C = 39.38$ ppm).

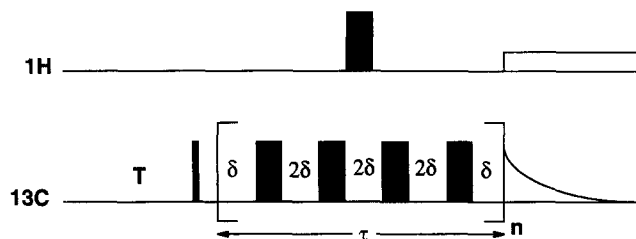


Fig. 2. The Carr-Purcell-Meiboom-Gill sequence used for the T_2 measurements. The sequence has been modified to remove cross-correlation effects according to Kay et al. (1992). The thin bar represents a $\pi/2$ -pulse and thick bars represent π -pulses. The phase of the first carbon $\pi/2$ -pulse is $x, x, -x, -x$ and the phase of the π -pulses is $y, -y$. T is the waiting period between individual scans.

¹³C relaxation measurements were performed using the following spectrometers: Jeol Alpha 400 (9.4 T), Varian U500 (11.8 T) and Varian U600 (14.1 T). T_1 and heteronuclear NOE were measured at all three fields, whereas T_2 was only measured at 9.4 T. T_1 was measured using the fast inversion-recovery (FIR; Canet et al., 1975) method with broadband proton decoupling, using 10–16 τ values between the inversion pulse and the read pulse. NOE was measured with the dynamic NOE sequence (Kowalewski et al., 1978), using one long irradiation period ($>5 \times T_1$) and one short period (1 ms). The waiting period between scans for the NOE measurements was typically 2–4 s. T_2 was measured with the Carr-Purcell-Meiboom-Gill sequence (CPMG; Carr and Purcell, 1954; Meiboom and Gill, 1958), modified to remove cross-correlation effects (Kay et al., 1992; Palmer III et al., 1992) during the relaxation period. The sequence is shown in Fig. 2. The delay, δ , between the π -pulses was set to 250 μ s. The lengths of the carbon and proton π -pulses were around 12 and 30 μ s, respectively. The δ delays bracketing the proton π -pulse were compensated for the duration of this pulse. The waiting period between scans, T , was set to 1.2 s. Series of spectra with 10–14 τ values were recorded for each experiment. The longitudinal relaxation times, T_1 , were evaluated by a nonlinear three-parameter fit of peak intensities and the transverse relaxation times, T_2 , by a nonlinear two-parameter fit of peak intensities. The NOE factors were evaluated by taking the ratio between peak intensities obtained with the long ($>5 \times T_1$) irradiation period and intensities obtained with a short delay (1 ms). The errors in the T_1 and T_2 relaxation parameters are estimated to be less than 5% and the accuracy in the NOE factor is estimated to be better than 0.1. One-dimensional proton-detected ¹³C relaxation measurements were performed at 11.8 and 14.1 T using the sequences described by Skelton et al. (1993), modified to obtain one-dimensional experiments. Proton-detected T_1 and NOE measurements were performed with series of 10–12 τ values for the T_1 and one long ($>5 \times T_1$) and one short (1 ms) delay for the NOE measurements. The evaluation of T_1 parameters and NOE parameters was done as described above. All measurements were performed twice and the average values are reported. Experiments were carried out at two temperatures, 303 and 318 K. Standard temperature control equipment provided by the manufacturers was used with all instruments. Least-squares fitting of the relaxation rates to the Lipari-Szabo model was done with the program GENLSS (DeTar, 1972), running on an IBM RISC 6000 computer.

Results and Discussion

The assignment of the carbon and proton spectra was done by means of 1D long-range proton-carbon correlation measurements and 2D homo- and heteronuclear

TABLE 1
CHEMICAL SHIFTS (ppm) OF THE SIGNALS IN THE ^1H AND ^{13}C NMR SPECTRA OF THE PENTASACCHARIDE AT 318 K IN $\text{D}_2\text{O}:\text{DMSO-}d_6$, 7:3

Sugar residue	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6
→2,6)-α-D-Manp-(1→R	5.47	4.10	3.86	3.45	3.65	3.58, 4.02
	97.1	77.4	70.0	67.9	73.6	69.7
→4)-β-D-GlcpNAc-(1→2)	4.63	3.57	3.67	3.49	3.47	~3.71, ~3.86
	100.3	55.9	72.3	80.7	75.6	60.9
→4)-β-D-GlcpNAc-(1→6)	4.45	3.59	3.59	3.44	3.41	~3.71, ~3.86
	101.7	55.6	73.0	80.9	75.5	61.1
β-D-Galp-(1→4) ₂	4.33	3.43	3.47	3.77	3.58	~3.63
	104.1	71.5	73.6	69.0	76.1	61.4
β-D-Galp-(1→4) ₆	4.32	3.43	3.47	3.77	3.58	~3.63
	104.1	71.5	73.6	69.0	76.1	61.4

correlation spectroscopy. In the two terminal galactopyranoside residues, only one signal from each residue could be individually assigned. In the ^{13}C spectrum the overlap between the signals from the two residues was complete, but in the proton spectrum the two proton signals corresponding to the C1 atoms could be separated. The terminal galactose residues, linked to O4 of the 2-acetamido-2-deoxy-glucopyranoside residues, were assigned by observing the heteronuclear long-range correlation to their anomeric proton from the corresponding C4 carbons in the 2-acetamido-2-deoxy-glucopyranoside residues. The proton and carbon chemical shift assignments are presented in Table 1.

^{13}C relaxation rates were only measured for well-resolved signals, which means that no relaxation parameters could be determined for the two galactopyranoside residues by means of conventional ^{13}C spectroscopy. Therefore, we performed proton-detected T_1 and NOE measurements at the two higher fields and at the highest temperature to determine the relaxation parameters of one carbon on each of the outer galactopyranoside residues. The T_1 and NOE values of some other carbons on the other residues were also evaluated from the proton-detected experiments to confirm that the results obtained by the different methods agreed within the experimental error limits. The results of the different methods agreed well when comparing T_1 and NOE data for the anomeric

carbons of the 2-acetamido-2-deoxy-glucopyranoside residues.

The analysis of the relaxation parameters was done for each ring individually, using the Lipari–Szabo model (Lipari and Szabo, 1982a). It was found that the relaxation parameters for the individual carbons in each ring were similar; hence it is justified to treat the ring carbons in each ring as being dynamically equivalent. Thus, in the following analysis we used the average relaxation parameters for each ring. In Table 2, relaxation data for the individual rings at 318 K are presented. These relaxation data reveal significant differences between the various residues, indicating differences in motional properties. The data sets obtained at the two temperatures were analyzed separately, following the work of Kowalewski and Widmalm (1994). A carbon–proton distance of 109.8 pm was assumed, corresponding to a dipolar coupling constant (DCC) of 143.40 kHz. In the analysis of the data at 318 K for the five rings, several different fitting procedures were adopted (Table 3). The quality of the respective fits for the three inner rings can be judged in Fig. 3, where calculated and experimental relaxation parameters are displayed. A condition for the Lipari–Szabo model to be valid is that the global motion of the whole molecule must be isotropic. In the case of the pentasaccharide, the same global correlation time should be obtained for all rings. When examining Table 3, where

TABLE 2
RELAXATION PARAMETERS, AVERAGED FOR EACH RING, FOR THE PENTASACCHARIDE OBTAINED AT DIFFERENT MAGNETIC FIELDS AT 318 K

Ring	9.4 T			11.8 T		14.1 T	
	T_1 (ms)	T_2 (ms)	1+NOE	T_1 (ms)	1+NOE	T_1 (ms)	1+NOE
M	198	108	1.29	239	1.17	315	1.15
GN ₂	216	117	1.35	264	1.29	322	1.15
GN ₆	227	140	1.31	265	1.32	332	1.31
G ₂				319	1.57	368	1.50
G ₆				333	1.59	385	1.51

TABLE 3
MOTIONAL PARAMETERS FOR THE PENTASACCHARIDE, OBTAINED AT 318 K FROM LEAST-SQUARES FITS OF RING CARBONS

Ring	Fit ^a	τ_M (ns)	S^2	τ_c (ps)	Δy^b
M	a	1.62 ± 0.08	0.91 ± 0.02		4.2
GN ₂	a	1.54 ± 0.11	0.83 ± 0.02		5.7
GN ₆	b	1.35 ± 0.10	0.75 ± 0.02	20 ± 16	4.3
G ₂	c	1.50	0.59 ± 0.02	68 ± 9	3.5
G ₆	c	1.50	0.56 ± 0.03	62 ± 10	4.7

^a Fit (a) is a two-parameter fit to the truncated Lipari-Szabo model (Eq. 5), fit (b) is a three-parameter fit to the Lipari-Szabo model (Eq. 4) and fit (c) is a two-parameter fit to the Lipari-Szabo model where the value of τ_M is fixed.

^b Standard deviation (%) of the dependent variable.

the results obtained at 318 K are collected, it can be seen that the global correlation time, τ_M , ranges between 1.4 and 1.6 ns, which is in reasonable agreement with an isotropic global motion. However, these results were obtained with different fitting procedures. Starting with the inner ring, the mannopyranoside residue, a two-parameter fit to the truncated Lipari-Szabo model was used. It has previously been shown (Bagley et al., 1992; Kowalewski and Widmalm, 1994; Mäler et al., 1995) that the truncated form of the Lipari-Szabo equation can be used when the molecule is fairly rigid. A high order parameter does not allow for the determination of a short, local correlation time, as the second term in the Lipari-Szabo model in this case becomes negligible compared to the first term. The two-parameter fit provided an S^2 value of 0.91, indicating a very rigid core in the oligosaccharide. The correlation time for the overall tumbling was found to be 1.6 ns. Two disaccharide units are attached to the inner mannopyranoside residue, one β -(1 \rightarrow 2)-linked and the other β -(1 \rightarrow 6)-linked. The fact that one is attached to a primary hydroxylic position and the other to a secondary position should be reflected in their respective motional properties. This, in fact, proved to be the case. For

the 2-acetamido-2-deoxy-glucopyranoside residue attached at the secondary position (labelled GN₂), an S^2 of 0.83 was obtained, while this value was 0.75 for the 2-acetamido-2-deoxy-glucopyranoside residue attached at the primary position (labelled GN₆). For the GN₆ residue, the best fit was obtained when a three-parameter fit to Eq. 4 was performed, resulting in values for both the order parameter and the short, local correlation time. This was not possible for the other residue, GN₂, clearly indicating more extensive local motions in the GN₆ residue as compared to the GN₂ residue.

Turning to the results obtained for the mannopyranoside residue and the two GN units at 303 K, collected in Table 4, a global correlation time in the range 2.1–2.4 ns was obtained for the three inner rings. Again, this is in reasonable agreement with isotropic global motion. The order parameters obtained for the three rings are similar to the values found at 318 K, again indicating more extensive local motion in the GN₆ residue. However, at this temperature it was not possible to obtain the short, local correlation time for the GN₆ residue. From the results obtained at the two temperatures, some conclusions can be drawn concerning the temperature dependence of the mo-

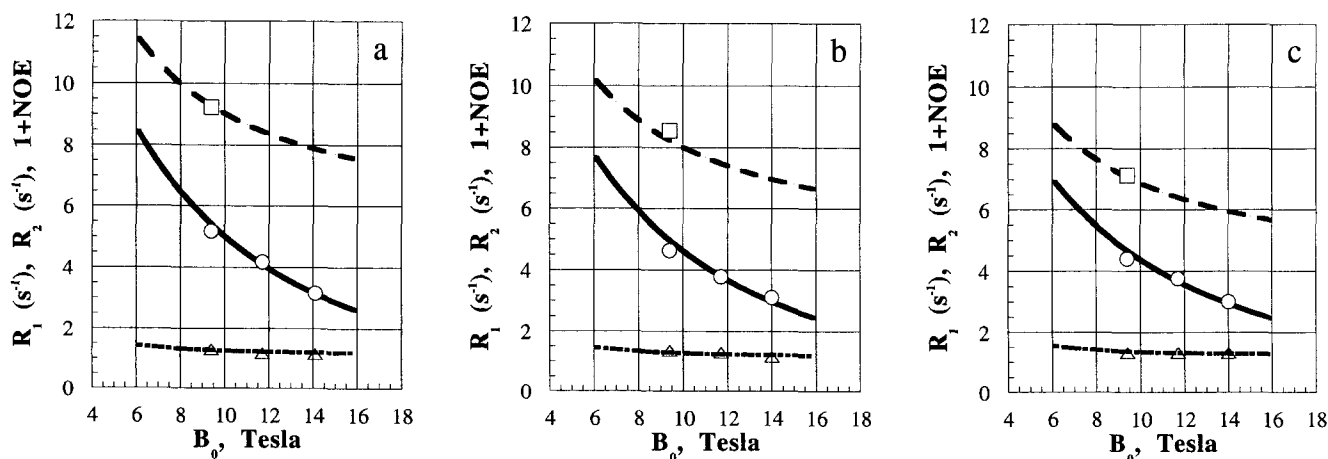


Fig. 3. Plots of calculated longitudinal relaxation rates (solid lines), transverse relaxation rates (dashed lines) and nuclear Overhauser enhancement factors (1 + NOE, dotted lines) for the ring carbons at 318 K. Shown are the relaxation parameters for (a) the M-ring; (b) the GN₂-ring; and (c) the GN₆-ring. The experimental values for T_1 are shown as circles, those for T_2 as squares and those for 1 + NOE as triangles.

TABLE 4
MOTIONAL PARAMETERS FOR THE PENTASACCHARIDE, OBTAINED AT 303 K FROM LEAST-SQUARES FITS OF THE RING CARBONS TO THE TRUNCATED LIPARI-SZABO MODEL (EQ. 5)

Ring	τ_M (ns)	S^2	Δy^a
M	2.38 ± 0.10	0.91 ± 0.03	4.6
GN ₂	2.13 ± 0.13	0.85 ± 0.03	6.0
GN ₆	2.08 ± 0.11	0.77 ± 0.02	4.4

^a Standard deviation (%) of the dependent variable.

tional parameters. The global correlation time is temperature dependent, whereas the order parameter is roughly temperature independent. This is consistent with previous findings, and if an Arrhenius temperature dependence according to $\tau = \tau_0 \exp(E_a / RT)$ is assumed, for an average global correlation time for the three rings one obtains an activation energy of 25 kJ/mol, which is also reasonable and in agreement with previous findings for oligosaccharides (Bagley et al., 1992; Kowalewski and Widmalm, 1994) of similar size and in the same solvent.

The two outer units, two galactopyranoside residues β -(1 \rightarrow 4)-linked to the 2-acetamido-2-deoxy-glucopyranoside residues, could only be characterized by proton-detected measurements on one carbon signal in each residue. The relaxation data for these two residues (labelled G₂ and G₆, respectively; see Fig. 1) are therefore not as reliable as those obtained for the inner residues. Furthermore, measurements had to be performed at only one temperature and at the two higher magnetic field strengths in order to obtain spectra with sufficient resolution for evaluating the relaxation parameters individually for the two residues. It was, however, possible to obtain the motional parameters also for these two residues at 318 K by performing a two-parameter fit to the Lipari-Szabo model, assuming a fixed global correlation time of 1.5 ns, an average value obtained from the results of the other rings. These results are presented in Table 3, together with the results for the inner three rings. Thus, under the assumption of isotropic global motion for the outer rings, S^2 values of 0.59 for G₂ and 0.56 for the G₆ ring were obtained. The local motions in these residues were further characterized by short correlation times of 68 and 62 ps, respectively. It should be pointed out that the results are obtained under the assumption of overall isotropic re-orientation, as an average global correlation time ob-

tained from the inner rings was used. However, it is clear that both outer rings display rather extensive local motion, which is reflected in the lower order parameter for these residues as compared with the inner ones. The difference in order parameter is too small to speculate on the more extensive local motion seen in the GN₆ residue as compared to what was seen in the GN₂ residue. The same behavior was found in the linear tetrasaccharide studied by Bagley et al. (1992), for which a much lower order parameter in the two outer rings was found.

We also analyzed the relaxation parameters for the exocyclic carbons at 318 K. The molecule contains five such carbons, but only three can be resolved, since the two exocyclic CH₂ carbons on the outer galactopyranoside residues are completely overlapped in both the carbon and proton spectra. The motions of the three exocyclic carbons, the two C6 carbons on the two 2-acetamido-2-deoxy-glucopyranoside residues and the mannopyranoside C6 carbon, were analyzed individually and the analysis was done by a three-parameter fit of the relaxation data to the Lipari-Szabo model. The fits produced an overall correlation time, τ_M , and the parameters describing the local motion of the CH₂ groups, S^2 and τ_c . The results are presented in Table 5. When comparing the global correlation times found for the three carbons, in the range 1.2–1.6 ns, we see that these are similar to what was found when analyzing the ring carbon data. This is satisfactory, since it is a requirement for using the Lipari-Szabo model. The order parameters for the three carbons are, however, quite different. The order parameter found for the C6 carbon situated on the mannopyranoside residue (labelled C_M-6) is almost identical to that of the substituent 2-acetamido-2-deoxy-glucopyranoside residue ring carbons. This is not unreasonable, since the CH₂ is in fact linking the mannopyranoside residue to the 2-acetamido-2-deoxy-glucopyranoside residue and it is likely that this carbon displays the same motional behavior as the substituent and shows somewhat restricted internal motion compared to what has previously been found for exocyclic carbons in oligosaccharides (Bagley et al., 1992; Kowalewski and Widmalm, 1994). The other two exocyclic carbons, situated on the two 2-acetamido-2-deoxy-glucopyranoside residues (labelled C_{GN₂}-6 and C_{GN₆}-6), display more extensive local motion, reflected in a lower order parameter for both carbons. There is, however, a significant difference between the flexibility for the two

TABLE 5
MOTIONAL PARAMETERS FOR THE CH₂ CARBONS IN THE PENTASACCHARIDE, OBTAINED AT 318 K FROM LEAST-SQUARES FITS TO THE LIPARI-SZABO MODEL (EQ. 4)

Carbon	τ_M (ns)	S^2	τ_c (ps)	Δy^a
C _M -6	1.56 ± 0.14	0.78 ± 0.03	44 ± 25	6.0
C _{GN₂} -6	1.30 ± 0.10	0.66 ± 0.02	29 ± 11	4.1
C _{GN₆} -6	1.19 ± 0.11	0.59 ± 0.02	29 ± 11	4.5

^a Standard deviation (%) of the dependent variable.

carbons. C_{GN_6-6} has a lower order parameter than what is found for C_{GN_2-6} . As was seen in the analysis of the ring carbon data, C_{GN_6-6} is situated on the more flexible 2-acetamido-2-deoxy-glucopyranoside residue and this flexibility is also displayed for the CH_2 carbon on this residue. This fact supports the finding that the enhanced mobility of the disaccharide unit attached at the primary position is substantial. The increased flexibility of certain parts of the molecule may be important in recognition processes. Selection of certain conformational families by different receptors can facilitate diversity in signalling, still using the same carbohydrate molecule.

Conclusions

For the interpretation of the ^{13}C relaxation data for the pentasaccharide studied here, we find that the model-free approach of Lipari and Szabo works satisfactorily. The relaxation for the individual carbons within each ring is seen to be similar, thus it is justified to treat the individual rings as being dynamically equivalent. However, the relaxation behavior of each individual ring is different, a finding that can be explained by differences in the contributions from internal motion. The relaxation data for the inner mannopyranoside residue were analyzed with the truncated form of the Lipari–Szabo equation, yielding an S^2 value of around 0.9, consistent with a very rigid center of the molecule. The 2-acetamido-2-deoxy-glucopyranoside residue attached at the secondary position on the mannopyranoside residue was also analyzed with the truncated form of the Lipari–Szabo model, and an order parameter of 0.83–0.85 was found in the temperature range of the study. The motional parameters for the other 2-acetamido-2-deoxy-glucopyranoside residue, attached at the primary C6 position of the mannopyranoside residue, displayed a lower S^2 value (0.75–0.77). In addition, for this ring the full Lipari–Szabo equation could be used at 318 K to obtain a local correlation time as well. This is consistent with a higher degree of mobility for the disaccharide attached at the mannopyranoside C6 position. The results for the three analyzed exocyclic carbons support this finding. The motion of the mannopyranoside CH_2 carbon, acting as the link between the mannopyranoside ring and the substituted disaccharide unit, was found to agree with the results for the substituent, the 2-acetamido-2-deoxy glucopyranoside residue. Different order parameters were found for the two exocyclic carbons on the two 2-acetamido-2-deoxy-glucopyranoside residues, supporting the finding that the disaccharide attached at the mannopyranoside C6 position is more flexible. The outer two galactopyranoside residues showed the highest mobility ($S^2 = 0.56$ – 0.59) and, at 318 K, a short local correlation time of around 60 ps was obtained for both residues.

Acknowledgements

This work has been supported by the Swedish Natural Science Research Council. Use of the U500 and U600 spectrometers at the Swedish NMR Center is gratefully acknowledged. We are grateful to Dr. Charlotta Johansson for assistance with operating these instruments.

References

- Adams, B. and Lerner, L. (1992) *J. Am. Chem. Soc.*, **114**, 4827–4829.
- Arnarp, J., Baumann, H., Lönn, H., Lönngrén, J., Nyman, H. and Ottosson, H. (1983) *Acta Chem. Scand. B*, **37**, 329–334.
- Bagley, S., Kovacs, H., Kowalewski, J. and Widmalm, G. (1992) *Magn. Reson. Chem.*, **30**, 733–739.
- Braccini, I., Michon, V., Hervé du Penhoat, C., Imberty, A. and Pérez, S. (1993) *Int. J. Biol. Macromol.*, **15**, 52–55.
- Canet, D., Levy, G.C. and Peat, I.R. (1975) *J. Magn. Reson.*, **18**, 199–204.
- Carr, H.Y. and Purcell, E.M. (1954) *Phys. Rev.*, **94**, 630–638.
- Dais, P. (1994) *Carbohydr. Res.*, **263**, 13–24.
- DeTar, D.F. (1972) *Computer Programs for Chemistry*, Vol. IV, Academic Press, New York, NY.
- Do, K.-Y. and Cummings, R.D. (1993) *J. Biol. Chem.*, **268**, 22028–22035.
- Hajduk, P.J., Horita, D.A. and Lerner, L.E. (1993) *J. Am. Chem. Soc.*, **115**, 9196–9201.
- Hricovini, M., Shah, R.N. and Carver, J.P. (1992) *Biochemistry*, **31**, 10018–10023.
- Hricovini, M. and Torri, G. (1995) *Carbohydr. Res.*, **268**, 159–175.
- Kay, L.E., Nicholson, L.K., Delaglio, F., Bax, A. and Torchia, D.A. (1992) *J. Magn. Reson.*, **97**, 359–375.
- Kovacs, H., Bagley, S. and Kowalewski, J. (1989) *J. Magn. Reson.*, **85**, 530–541.
- Kowalewski, J., Ericsson, A. and Vestin, R. (1978) *J. Magn. Reson.*, **31**, 165–169.
- Kowalewski, J. and Widmalm, G. (1994) *J. Phys. Chem.*, **98**, 28–34.
- Lipari, G. and Szabo, A. (1982a) *J. Am. Chem. Soc.*, **104**, 4546–4559.
- Lipari, G. and Szabo, A. (1982b) *J. Am. Chem. Soc.*, **104**, 4559–4570.
- Mäler, L., Lang, J., Widmalm, G. and Kowalewski, J. (1995) *Magn. Reson. Chem.*, **33**, 541–548.
- McCain, D.C. and Markley, J.L. (1986) *J. Am. Chem. Soc.*, **108**, 4259–4264.
- McCain, D.C. and Markley, J.L. (1987) *J. Magn. Reson.*, **73**, 244–251.
- Meiboom, S. and Gill, D. (1958) *Rev. Sci. Instrum.*, **29**, 688–691.
- Nishida, T., Widmalm, G. and Sandor, P. (1995) *Magn. Reson. Chem.*, **33**, 596–599.
- Palmer III, A.G., Skelton, N.J., Chazin, W.J., Wright, P.E. and Rance, M. (1992) *Mol. Phys.*, **75**, 699–711.
- Poppe, L. and Van Halbeek, H. (1992) *J. Am. Chem. Soc.*, **114**, 1092–1094.
- Poppe, L., Van Halbeek, H., Acquotti, D. and Sonnino, S. (1994) *Biophys. J.*, **66**, 1642–1652.
- Roy, R., Tropper, F.D., Williams, A.J. and Brisson, J.-R. (1993) *Can. J. Chem.*, **71**, 1995–2006.
- Shimizu, H. (1964) *J. Chem. Phys.*, **40**, 3357–3364.
- Skelton, N.J., Palmer III, A.G., Akke, M., Kördel, J., Rance, M. and Chazin, W.J. (1993) *J. Magn. Reson. Ser. B*, **102**, 253–264.
- Wieland, F. and Reutter, W. (Eds.) *Glyco- and Cellbiology*, Springer, Berlin, 1993.