New conformational constraints in isotopically (13C) enriched oligosaccharides

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Multidimensional heteronuclear NMR studies have been applied to the resonance assignment and conformational analysis of 13C-enriched Neu5Acα2–3Galβ1–4Glc. It is demonstrated that three-dimensional ROESY-HSQC experiments provide through-space distance restraints which cannot be observed with conventional homonuclear 1H techniques due to resonance overlap. In particular, connectivities demonstrating the existence of the “anti” conformation about the Galβ1–4Glc glycosidic linkage are unambiguously observed. It is shown that 13C isotopic enrichment of the trisaccharide at a level >95% enables straightforward measurement of trans-glycosidic 1H–13C and 13C–13C coupling constants and a Karplus-type relation is derived for the latter. In total 15 conformational restraints were obtained for the trisaccharide in aqueous solution, all of which were in excellent agreement with theoretical parameters computed from a 5 ns molecular dynamics simulation of the glycan.

Key words: NMR/oligosaccharide/structure/resonance/conformational analysis

Introduction

Structural studies on oligosaccharides using high-resolution 1H NMR are plagued by the very severe overlap of the majority of proton resonances (Vliegenthart et al., 1983). Apart from rendering complete resonance assignments difficult if not impossible, there remains the problem of accurate quantitation of crosspeak intensities in experiments such as NOESY or ROESY for use as structural restraints. Furthermore, the number of such restraints is usually small, thus frustrating efforts to obtain a detailed picture of oligosaccharide structure and dynamics.

By analogy with structural studies on proteins (Marion et al., 1989), the resonance overlap problem can in principle be overcome by application of heteronuclear three-dimensional NMR methods. These offer improved spectral dispersion in comparison with their homonuclear counterparts in view of the fact that one or more dimensions in the 3D spectrum can represent the chemical shift of the heteronucleus (Xu et al., 1996). Here, we show that 13C enrichment at a high level (>95%) offers a high-sensitivity approach to the resonance assignment and conformational analysis of oligosaccharides, revealing additional conformational restraints that are not observed in conventional homonuclear NMR spectra. In addition we demonstrate that isotopic enrichment offers significant advantages for the measurement of trans-glycosidic scalar coupling constants. We illustrate these approaches in application to 13C enriched Neu5Acα2–3Galβ1–4Glc.

Results and discussion

Chemoenzymatic synthesis of Neu5Acα2–3Galβ1–4Glc

13C enriched Neu5Acα2–3Galβ1–4Glc was prepared by enzymatic sialylation of 13C-enriched Galβ1–4Glc acceptor using Trypanosoma cruzi trans-sialidase with p-nitrophenyl-[U-13C]-Neu5Ac as donor. Full details of this chemoenzymatic strategy will be presented elsewhere (Probert et al., 1997).

Resonance assignments in Neu5Acα2–3Galβ1–4Glc

Complete 1H and 13C resonance assignments in the trisaccharide have been determined by Lerner and Bax (1987), by application of three heteronuclear two-dimensional NMR techniques at natural abundance 13C. As anticipated, in 13C enriched form the trisaccharide can be assigned fully using a single two-dimensional (2D) HCCH-COSY experiment (Bax et al., 1990; Yu et al., 1993), (data not shown). These data confirm the assignments proposed by Lerner and Bax (1987).

Solution structure of Neu5Acα2–3Galβ1–4Glc

The solution structure of Neu5Acα2–3Galβ1–4Glc was initially investigated by use of 1H–1H rotating frame Overhauser effect (ROESY) measurements in the conventional manner (Botherby et al., 1984; Bax and Davis, 1985). Although Neu5Acα2–3Galβ1–4Glc is only a trisaccharide, it has a very complex 1H NMR spectrum, with most resonances concentrated within a 0.4 ppm shift range. Despite the availability of complete 1H resonance assignments (Lerner and Bax, 1987), the interpretation of the conventional two-dimensional 1H–1H ROESY spectrum is not straightforward. To illustrate this point, Figure 1a shows a 1D slice parallel to F2 at the frequency of the Gal H-1 resonance (4.56 ppm) from the 2D ROESY spectrum of unlabeled Neu5Acα2–3Galβ1–4Glc. A few peaks can be identified unambiguously, but severe overlap prevents both unambiguous identification and quantitation of certain resonances. By contrast, the observed crosspeaks were readily separated by their 13C chemical shift in a two-dimensional F2/F3 (13C/1H) slice from the 3D-ROESY-HSQC spectrum at the F1 (1H) resonance frequency of Gal H-1 (Figure 1b), and all ROE connectivities from Gal H-1 can be assigned unambiguously. In particular, the connectivity between Gal H-1 and Neu5Ac H-8 proposed in earlier work (Siebert et al., 1992), and found to be of similar intensity to the connectivity between Gal H-1 and Glcα H-6s, is absent in the present study. The reason for this discrepancy is unclear. The ROE between Gal H-3 and Neu5Ac H-8 is observed however (not shown), which is consistent with the orientation of the glycerol sidechain of Neu5Ac (θ = -60°, θ2 = 180°) suggested in previous work (Poppe and van Halbeek, 1991a; Siebert et al., 1992), and is similarly supported by 1H–1H...
coupling constants in the present study (data not shown). An intriguing aspect of the data shown in Figure 1b is the presence of weak crosspeaks from Gal H-1 to Glcαβ H-3 and H-5. These connectivities were not observable in the two-dimensional $^1$H-$^1$H ROESY spectrum due to essentially complete overlap with other resonances, and indeed to our knowledge have not been observed previously for this oligosaccharide in aqueous solution. In this regard, caution must be exercised in interpreting weak crosspeaks in ROE spectra, since these can arise from a strong ROE connectivity (in this case Gal H-1 to Glc H-4) followed by relayed coherence transfer (to Glc H-3 and Glc H-5) to other coupled protons via a HOHAHA mechanism (Neuhaus and Keeler, 1986). However, the intensities of these crosspeaks were found to be independent of the position of the spin-lock carrier, confirming that they correspond to direct connectivities.

In total seven trans-glycosidic ROEs were observed (Table I), and these were utilized in restrained dynamical simulated annealing calculations, using as input ten pseudo-random geometries generated by a dynamical quenching procedure (Homans and Forster, 1992). This resulted in three families of structures termed “A,” “B,” and “C” (Figure 2a) that differ principally in the conformation about the Neu5Acα2–3Gal glycosidic linkage; 3 of the 10 starting structures annealed to conformer “A” ($\phi, \psi = -70^\circ, +5^\circ$), five annealed to conformer “B” ($\phi, \psi = -165^\circ, -20^\circ$), and one annealed to conformer “C” ($\phi, \psi = -95^\circ, -45^\circ$). The final structure had large restraint violations and was discarded. Similar conformers about the Neu5Acα2–3Gal linkage were predicted in the original study of Neu5Acα2–3Galβ1–4GlcNAcβ−Asn by Breg et al. (1989). In order to probe the extent of torsional fluctuation about each of these conformers, the lowest energy structure of each family was arbitrarily chosen as input for a 5 ns restrained molecular dynamics simulation in vacuo. Since all MD simulations gave similar results (data not shown), only the MD simulation with the global minimum energy structure as input is considered further.

The instantaneous values of relevant torsion angles are shown in Figure 2b. In order to determine whether this dynamic behavior is a plausible model for the solution dynamics of Neu5Acα2–3Galβ1–4Glc, theoretical ROEs were back calculated from the molecular dynamics simulations using a full relaxation matrix approach (Forster, 1991) from which it is apparent that there is very good agreement between theoretical predictions and experimental values (Table I). In particular, the intensities of the relatively weak ROEs between Gal H-1 and Glc H-3 and Gal H-1 and Glc H-5 are reproduced in the theoretical simulations. These ROEs derive from the relatively small population of conformers that adopt the “anti” conformation (Figure 3) about the Galβ1–4Glc glycosidic linkage during the molecular dynamics simulation ($\psi = 180^\circ$), a result that lends strong support to indirect evidence that a small population of such conformations exists for various oligosaccharides in solution at physiological temperature (Lipkind et al., 1985, 1987; Poppe et al., 1990; Bock et al., 1994; Dabrowski et al., 1995).

### Table I. Experimental ROE intensities for $^{13}$C Neu5Acα2–3Galβ1–4Glc versus theoretical values computed from the 5 ns MD simulation of the trisaccharide

<table>
<thead>
<tr>
<th>ROE connectivity</th>
<th>Restraint</th>
<th>Experimental ROE intensity (%)a</th>
<th>Theoretical ROE intensity (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neu5Ac H-3ax - Gal H-3</td>
<td>W</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Neu5Ac H-8 - Gal H-3</td>
<td>W</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Gal H-1 - Glc H-4</td>
<td>S</td>
<td>4.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Gal H-1 - Glc H-6</td>
<td>W</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Gal H-1 - Glc H-6′</td>
<td>W</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Gal H-1 - Glc H-3</td>
<td>W</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Gal H-1 - Glc H-5</td>
<td>W</td>
<td>0.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Experimental and theoretical intensities shown are for the Glcβ anomer only, after correction for the mole fraction of this anomer.

bCalculated with a rotational correlation time of 0.13 ns.

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Fig. 1. (A) Trace parallel to F2 derived from the $^1$H-$^1$H ROESY spectrum of Neu5Acα2–3Galβ1–4Glc at the resonance frequency of Gal H-1 (4.56 ppm). (B) Two-dimensional F2/F3 slice (F1 = 4.56 ppm) derived from the three-dimensional ROESY-HSQC spectrum of $^{13}$C-enriched Neu5Acα2–3Galβ1–4Glc.
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Fig. 2. (A) Superposition of nine geometries derived from restrained dynamical simulated annealing calculations on Neu5Acα2–3Galβ1–4Glc. (B) Values of \( \phi \) vs. \( \psi \) for (i) Neu5Acα2–3Gal linkage and (ii) Galβ1–4Glc linkage, and of torsion angles \( \theta_1 \) (iii) and \( \theta_2 \) (iv) for the glycerol sidechain of Neu5Ac derived from 5 ns restrained dynamics simulation of Neu5Acα2–3Galβ1–4Glc. The restraints used in these simulations are listed in Table I.

It could be argued that the excellent agreement between experimental and theoretical ROEs in Table I would be anticipated from a molecular dynamics simulation which includes ROE restraints. However, as discussed at length previously (Rutherford and Homans, 1994), the computed ROE can in principle vary over a very wide range as a result of the choice of restraint distance bounds. Moreover, the availability of 13C enriched material enables the measurement of trans-glycosidic coupling constants with which to validate further the predicted motional behavior.

Long-range 13C–13C coupling constants

Conventionally, trans-glycosidic \(^1\)H–13C coupling constants have been utilized for the conformational analysis of oligosaccharides (Poppe and van Halbeek 1991b,c; Rutherford et al., 1993, 1994). In the present study carbon-carbon coupling constants are particu-

larly valuable since only one 13C–H coupling is available across the Neu5Acα2–3Gal glycosidic linkage. However, although experiments have been described for the measurement of long-range carbon-carbon couplings in 13C enriched material (Bax et al., 1992, 1994), and while 13C–13C coupling constants have been reported in carbohydrates (Wu and Serianni, 1992; Wu et al., 1992; Duke and Serianni, 1993; Church et al., 1996), a suitable parametrization of the Karplus relationship has not been reported for the C-C-O-C fragment. We have therefore undertaken such a parametrization, by use of model compounds in 13C enriched form containing C-C-O-C fragments with known, fixed dihedral angles.

Karplus parametrization

Parametrization of the Karplus relation ideally requires experimental \( J_{CC} \) values for C-C-O-C fragments with known dihedral angles distributed over a wide range. On grounds of sensitivity such coupling constants must be determined in compounds with a high level of enrichment with 13C, and in this regard the information that can be obtained from readily available compounds is limited. For example, the only usable coupling constant for a C-C-O-C fragment in uniformly 13C-enriched glucose is that for the C1-O-C5-C6 fragment, where the dihedral angle is 180°. The coupling constant for the C1-O-C5-C4 fragment (+60°) cannot be utilized since it occurs simultaneously with the coupling via C1-C2-C3-C4, and the two contributions cannot readily be separated. However, dihedral angle values of +60°, -60°, and -120° in C-C-O-C fragments can be obtained in two simple sugar derivatives, namely, methyl 4,6-O-(1-methylbenzylidene) \( \alpha \)-D-glucopyranoside and 2,3-O-isopropylidene 1,6-anhydro-\( \beta \)-mannopyranose (Table II). The \( J_{CC} \) values listed in Table II were fitted to a Karplus relation of the form \( J_{CC} = A \cos 2\theta + B \cos \theta + C \), giving rise to the curve shown in Figure 4. It is appreciated that with the limited experimental data used in fitting and neglect of electronegativity effects, this parametrization is semiquantitative at best, and is probably unreliable in the region 0° < \( \theta \) < 60° (not shown). However, the experimental datapoints lie within the region of the curve that is likely to be populated by trans-glycosidic \( J_{CC} \) values, and thus should predict these values with reasonable accuracy.

Table II. Long range 13C–13C coupling constants measured in 13C enriched model compounds and utilized for the parametrization of a Karplus relation for the C-C-O-C fragment

<table>
<thead>
<tr>
<th>Compound</th>
<th>Coupling</th>
<th>Angle (deg.)</th>
<th>( J_{CC} ) (Hz)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )-D-Glucose</td>
<td>( J_{C1-O-C5-C6} )</td>
<td>180</td>
<td>3.8</td>
</tr>
<tr>
<td>( J_{Me-C-O4-C4} )</td>
<td>60</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>( J_{Me-C-O6-C6} )</td>
<td>60</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>( J_{C1-C2-O-C7} )</td>
<td>109(^b)</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Error in these measurements estimated as ±0.2 Hz.  
\(^b\)Value determined from crystal structure (Lightfoot 1997, unpublished observations).

Three-bond scalar carbon–carbon couplings can be measured using 2D long-range carbon–carbon correlation spectroscopy.
Fig. 3. Diagrammatic illustration of the observed NOEs in Neu5Acα2–3Galβ1–4Glc with conformer “B” about the Neu5Acα2–3 linkage (see text) and dihedral angle ψ about the Galβ1–4Glc glycosidic linkage in the “anti” conformation (ψ∼–180°).

Fig. 4. Karplus curve for $^{3}J_{CC}$ in the C-C-O-C fragment, parametrized using the data of Table II. Experimental datapoints (○) were fitted to the function $^{3}J_{CC} = A\cos 2\phi + B\cos \phi + C$, giving rise to the constants $A = 4.4$, $B = 1.1$, and $C = 0.5$.

(LRCC, Bax et al., 1992). However, Neu5Acα2–3Galβ1–4Glc suffers from poor spectral dispersion in critical regions of the $^{13}$C spectrum. For example, Figure 5 (left) shows an extract from the 2D LRCC spectrum of the trisaccharide, illustrating severe overlap between Gal C-3,C-5 and Glcβ C-3,C-5. The three bond coupling between Gal C-1 and Glcβ C-3/C-5 clearly cannot be determined from this spectrum. It is therefore necessary to resort to the three-dimensional analog of this experiment (Bax et al., 1994), where an F2/F3 plane (Figure 5, right) at an F1 frequency of ~75 ppm (Gal C-3,C-5, Glcβ C-3,C-5) allows $^{3}J_{CC}$ between Gal C-1 and Glcβ C-5 to be measured. In total, four long-range carbon–carbon coupling constants could be determined in the trisaccharide and two others could be inferred as being < 1 Hz, and these values could be compared with those predicted theoretically from the MD simulation of the trisaccharide using the above Karplus relation. It can be seen from Table III that the theoretical values are in very good agreement with those measured experimentally, despite the fact that no angular restraints were applied during the MD simulation. These data therefore support the validity of the MD simulations as a plausible model for the solution dynamics of the oligosaccharide.

Table III. Experimental trans-glycosidic scalar coupling constants in $^{13}$C Neu5Acα2–3Galβ1–4Glc vs. theoretical values computed from the 5 ns MD simulation of the trisaccharide

<table>
<thead>
<tr>
<th>Coupling</th>
<th>Experimental (Hz)</th>
<th>Theoretical (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{3}J_{CC}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gal C-3 - Neu5Ac C-3</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Gal C-2 - Neu5Ac C-2</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Gal C-4 - Neu5Ac C-2</td>
<td>&lt;1</td>
<td>1.1</td>
</tr>
<tr>
<td>Gal C-1 - Glcβ C-5</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Gal C-2 - Glcα/β C-4</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Gal C-1 - Glcα/β C-3</td>
<td>&lt;1</td>
<td>1.1</td>
</tr>
</tbody>
</table>


$^{3}J_{HC}$

|                  |             |             |
| Gal H-1 - Glc C-4 | 3.5         | 3.7         |
| Gal C-1 - Glc H-4 | n.d.        | 4.9         |
| Neu5Ac C-2 - Gal H-3 | 4.7       | 4.5         |

*Error in these measurements estimated as ±0.5 Hz.

Long-range $^{1}H-^{13}C$ coupling constants

Trans-glycosidic $^{13}$C–$^{1}$H coupling constants can conveniently be measured in $^{13}$C enriched oligosaccharides (Gitti et al., 1994; Lippens et al., 1996; Xu and Bush 1996), and in the present work we have utilized a heteronuclear constant-time COSY sequence (Ionides et al., 1995) and iterative fitting strategy described by Titman and Keefer (1990). Two trans-glycosidic $^{13}$C–$^{1}$H coupling constants were obtained with this approach (data not shown), and were again compared with theoretical predictions based on the relevant Karplus relationship for coupling pathways of this type (Mulloy et al., 1989; Tvaroska et al., 1989). The $^{13}$C–$^{1}$H coupling constant between Gal C-1 and Glc H-4 could unfortunately not be obtained due to resonance overlap and strong coupling. As with trans-glycosidic $^{13}$C–$^{13}$C coupling constants, simulated $^{13}$C–$^{1}$H
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Fig. 5. Left, Typical trace from 2D LRCC spectrum (positive contours only) of $^{13}$C-enriched Neu5Acα2–3Galβ1–4Glc, illustrating the overlap between Gal C-3, C-5 and Glcβ C-3, C-5. (right) F2/F3 plane derived from 3D LRCC spectrum at F1~75 ppm (Gal C-3, C-5, Glcβ C-3, C-5).

coupling constants were in excellent agreement with these experimental values.

Conclusions

Isotopic enrichment of oligosaccharides at high levels (>95%) $^{13}$C offers considerable advantages for structural and conformational analysis. A major advantage is the additional spectral dispersion offered in heteronuclear experiments by virtue of an additional $^{13}$C dimension. Of course, heteronuclear experiments are possible at natural abundance provided sufficient material is available, but it is unlikely that sensitivity would be sufficient to observe some of the weaker connectivities detected in the present study. For example, weak ROE connectivities are observed which prove the existence of an “anti” conformation about the Galβ1–4Glc linkage, which to our knowledge have not been observed previously for this disaccharide in aqueous solution but have, however, been observed very recently in GlcNAcβ1–4GlcNAc (Espinosa et al., 1996). Moreover, trans-glycosidic $^{13}$C–$^{1}$H and $^{13}$C–$^{13}$C coupling constants, which are measurable with difficulty or not at all at natural abundance, have been shown to provide a valuable semiquantitative assessment of the quality of dynamic models derived from restrained molecular dynamics simulations. Given that $^{13}$C-enriched oligosaccharides can be synthesized at high yield in milligram amounts using chemoenzymatic strategies, we anticipate that further developments in the structural and conformational analysis of oligosaccharides will be apparent in the future.

Materials and methods

Sample preparation

$N$-Acetyl [$^{13}$C] Neu5Acα2–3Galβ1–4Glc. NH$_4^+$ (~7.5 mg) was dissolved and lyophilized into 99.96% D$_2$O three times followed by dissolution into 700 µl D$_2$O.

NMR experiments

NMR spectra were obtained at 300K with a $^1$H reference frequency of 500 MHz on a Varian Unity+ spectrometer equipped with a self shielded z gradient triple resonance probe. All spectra were recorded in the phase-sensitive mode with use of the States (States et al., 1982) method for quadrature detection. 2D HCCH-COSY experiments were recorded using the three-dimensional (3D) pulse scheme of Bax et al. (1990). A total of 1K complex and 2K complex points were acquired in the $t_1$ and $t_2$ dimensions, respectively, with spectral widths of 2 kHz and 10 kHz. Two- (Bax et al., 1992) and three-dimensional (Bax et al., 1994) gradient-enhanced long range carbon–carbon correlation (LRCC) experiments were recorded with a proton sweep.
width of 2 kHz, consisting of 1024 complex points and a $^{13}$C sweep-width of 11 kHz in $t_1$ (128 complex points) and a 6.5 kHz (32 complex points) in $t_2$ for the 3D experiment. A total of 64 and 8 scans were acquired per increment for the 2D and 3D experiments, respectively. $^{13}$C-$^{13}$C couplings in the compounds methyl 4,6-O-(1-methylbenzyli-

Dynamical simulated annealing calculations and restrained molecular modeling were zero filled to 256 and 64 complex points, respectively. Total acquisition times for the 2D and 3D experiments were $\sim$12 h and $\sim$48 h, respectively. The values of the long range coupling constants are derived from the ratios of cross-peaks obtained in the spectrum in the manner described by Bax et al. (1992). Long-range $^{13}$C-$^{13}$C couplings in the compounds methyl 4,6-O-(1-methylbenzylidene) $\alpha$-D-glucopyranoside and 2,3-O-isopropylidene 1,6-anhydro- $\delta$-mannopyranoside were obtained by conventional homonuclear $^{13}$C spin-decoupling experiments.

Trans-glycosidic carbon-proton coupling constants (CT-LRCH) were measured using the 2D constant-time pulse sequence of Ionides et al., (1995). Proton and carbon sweep-widths were identical to those used in the 2D LRCC spectrum with 4096 complex points in $t_2$ and 256 $t_1$ increments consisting of 16 scans per increment.

Three-dimensional $^{13}$C-edited ROESY (Bothner-by et al., 1984; Bax and Davis, 1985) experiments were acquired using a conventional ROESY-HSQC pulse sequence with offset compensation (Griesinger and Ernst, 1987) and with spectral widths of 2 kHz, 6.5 kHz, and 2 kHz and 128, 32, and 1024 complex points in $t_1$, $t_2$, and $t_3$, respectively. The proton transmitter offset was placed 500 Hz downfield of the lowest field resonance during the spin-lock period to minimize coherence transfer effects. To optimize digital resolution the Neu5Ac C5 and C3 resonances ($\sim$54 ppm and $\sim$40 ppm, respectively) were fold-in once. The effective field for spin-locking was 2 kHz and was applied for a mixing time of 250 ms. Prior to Fourier transformation, data were apodized with cosine-bell functions, and the $t_1$ and $t_2$ dimensions were zero filled to 256 and 64 complex points, respectively. Total acquisition time for the 3D spectrum was $\sim$55 h.

Molecular modeling

Dynamical simulated annealing calculations and restrained molecular dynamics simulations were computed in vacuo using described procedures (Homans and Forster, 1992; Rutherford and Homans, 1994). Theoretical ROE intensities were computed from molecular dynamics simulations using the in-house written software package MDNOE. This package incorporates a heteronuclear full relaxation matrix approach including a formalism appropriate for the computation of NOE and ROE data due to fluctuating internuclear distances arising from internal motions which are fast with respect to the rate of molecular tumbling (Tropp, 1980; Homans and Forster, 1982). A single overall isotropic correlation time for the molecule was assumed, and was obtained by fitting the ratio of the theoretical diagonal peak to crosspeak (intraresidue ROE) intensities of the Neu5Ac H-3 axial and H-3 equatorial protons to the experimentally measured values. The experimental ROE intensities (i.e., crosspeak volumes) were then normalized to the theoretical ROE intensities to generate the absolute magnitudes of the experimental ROEs. The torsion angles $\varphi$ and $\psi$ are defined as H-1 - C-1 - O-1 - C-X (C-1 - C-2 - O-2 - C-X in the case of Neu5Ac) and C-1 - O-1 - C-X - H-X, respectively, where C-X and H-X refer to the aglyconic atoms. The torsion angles $\theta_1$ and $\theta_2$ for the glycerol sidechain of Neu5Ac are defined as H-6 - C-6 - C-7 - H-7 and H-7 - C-7 - C-8 - H-8, respectively.

Acknowledgments

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Abbreviations

COSY, correlated spectroscopy; HCCH-COSY, $^1$H-$^{13}$C-$^{13}$C-$^1$H correlation via $^{13}$C couplings; ROESY-HSQC, three-dimensional rotating-frame Overhauser effect heteronuclear single-quantum correlation spectroscopy; NOESY, nuclear Overhauser effect spectroscopy; ROE, rotating frame Overhauser effect; ROESY, rotating frame Overhauser effect spectroscopy; 2D, two-dimensional; 3D, three-dimensional.

References


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