

## Conformational Distribution Function of a Disaccharide in a Liquid Crystalline Phase Determined Using NMR Spectroscopy

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Proteins, nucleic acids, and carbohydrates all show conformational flexibility, but the extent is dependent on the structure and their environment. The motion in saccharides, in particular at the glycosidic linkage, defined by torsion angles  $\phi$  and  $\psi$  is of importance for molecular properties and biological function. Thus, the approximation of a single molecular structure is certainly an oversimplified picture. To obtain complete information about the molecular structure we desire to determine the conformational distribution function,  $P(\phi, \psi)$ .

Analysis of molecular conformations has for a considerable time relied on either the nuclear Overhauser effect (NOE), spin–spin ( $J$ ) couplings, or a combination of these two. Recently, the application of dilute liquid crystalline phases (bicelles) as solvents enabled a slight net orientation of nonspherical “solute” molecules and therefore determination of through-space magnetic dipole–dipole (DD) interactions. These provide a powerful tool for molecular structure analysis of saccharides in ordered phases.<sup>1–4</sup> The DD interactions depend on the spin–spin distances and on the orientations of the internuclear vectors with respect to the external magnetic field. This means that the dipolar coupling is a valuable probe of long-range order and molecular structure.

To extract useful information from the experimental DD couplings in a flexible molecule, we need a theoretical tool to be used in the analyses. Several such approaches have been considered for the interpretation of dipolar couplings. The simplest possible model assumes that only a small set of minimum-energy structures is populated. More realistic models allow for continuous bond rotations. Two approaches that have been frequently used for interpretations of dipolar couplings in bulk liquid crystals are: (i) the additive potential model (AP)<sup>5</sup> and (ii) the maximum entropy method (ME).<sup>6</sup> Both methods have been successfully applied in experimental and computational studies of the orientational order and molecular conformations in mesogenic molecules.<sup>7–9</sup> The models suffer, however, from serious limitations: the AP method requires an a priori knowledge of the functional form of the torsional potential, relevant for the investigated molecular fragment. The ME approach though, gives the flattest possible distribution consistent with the experimental data set, which results in an incorrect description of systems with low orientational order.<sup>6,9</sup> These two limitations have an obvious relevance for investigations of saccharides in dilute liquid crystals: we do not have the torsional potential function for the glycosidic linkage, and the orientational order is indeed very low.

Here we present a novel approach for construction of the conformational distribution function  $P(\phi, \psi)$  from the NMR parameters. The procedure, which is valid in the low-order limit, was

constructed as a combination of the AP and ME approaches, subsequently referred to as the APME method. In particular, the intraresidue dipolar couplings were used to determine the orientational order, while the conformational distribution function  $P(\phi, \psi)$  was constructed from the interresidue DD- and  $J$  couplings, together with NOEs. We apply our analysis to  $\alpha$ -L-Rhap-(1 $\rightarrow$ 2)- $\alpha$ -L-Rhap-OMe, shown in Figure 1, which is a model for part of the O-antigen repeating unit of the lipopolysaccharide from pathogenic *Shigella flexneri* bacteria.

The saccharide exhibits motion over the glycosidic linkage as established from a preliminary analysis of intraring dipolar couplings using the generalized degree of order (GDO) approach.<sup>1,10</sup> The GDOs in the two rigid rings differ by a factor of 1.2 ( $\vartheta_R = 0.0059$  and  $\vartheta_{R'} = 0.0072$ ), whereas identical values are expected for rigidly connected fragments.

The general expression for conformation dependent dipolar couplings,  $D_{ij}(\phi, \psi)$  can be written (in Hz) as:<sup>5</sup>

$$D_{ij}(\phi, \psi) = \frac{\mu_0}{16\pi^2} \frac{\gamma_i \gamma_j \hbar}{r_{ij}^3(\phi, \psi)} \sum_{a,b} \cos \theta_{ij}^a \cos \theta_{ij}^b S_{ab}^l(\phi, \psi) \quad (1)$$

where  $a, b = (x, y, z)$  refers to an arbitrary coordinate frame fixed in one of the two rigid units,  $l = R, R'$ , and  $\theta_{ij}^a$  is the conformation-dependent angle between the internuclear vector and the  $a$ -axis. Similarly, the conformation-dependent elements of the order matrix are denoted  $S_{ab}^l(\phi, \psi)$ . The analysis of intraring couplings is in principle simple, because we do not need to explicitly consider the  $\phi, \psi$  dependence. The interpretation of interresidue couplings, however, requires an expression for a molecular ordering matrix where both fragments contribute. To obtain  $S_{ab}^l(\phi, \psi)$ , we consider the AP<sup>5</sup> model where the singlet orientational distribution function (ODF),  $P(\beta, \gamma, \phi, \psi)$ , is related to the potential of mean torque. Note that the angles  $\beta$  and  $\gamma$  define molecular orientation in the director frame. This potential is determined by the expansion coefficients,  $\epsilon_{ab}^l$ , which depend on the orientational order and the segmental ( $R, R'$ ) anisotropic interactions. In the limit of low-molecular order, the ODF can be Taylor-expanded and truncated after the second term. This results in the following expression for the order parameter:

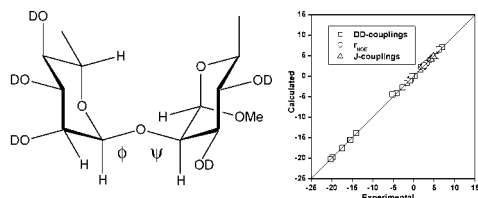
$$S_{ab}^R(\phi, \psi) = \frac{1}{5RT} (\epsilon_{ab}^R + \sum_{\mu, \nu} T_{a\mu}(\phi, \psi) \epsilon_{\mu\nu}^{R'} T_{b\nu}(\phi, \psi)) \quad (2)$$

where  $\mu, \nu = (x, y, z)$  in the frame of  $R'$ ,  $\epsilon_{ab}^l$  is defined in units of  $RT$ , and  $T_{a\mu}$  is an element of the  $\phi, \psi$ -dependent transformation matrix that relates coordinate systems fixed in rings  $R$  and  $R'$ . An equivalent relationship is obtained for  $S_{ab}^{R'}(\phi, \psi)$  by interchanging the indices  $R$  and  $R'$ . Note that two important assumptions have

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**Figure 1.** Schematic of  $\alpha$ -L-Rhap-(1 $\rightarrow$ 2)- $\alpha$ -L-Rhap-OMe. Comparison of observed and calculated NMR parameters for the disaccharide (DD- and  $J$  couplings in Hz; proton–proton distances  $r_{\text{NOE}}$  in Å). Anisotropic medium: 5% DMPC/DHPC ( $q = 3$ ) in  $\text{D}_2\text{O}$  at 37 °C. NMR experiments performed at 14.1 T: 2D  $^1J_{\text{C,H}}$  modulated  $^{13}\text{C}, ^1\text{H}$  CT-HSQC,<sup>14</sup> 2D  $^1\text{H}, ^1\text{H}$  COSY,<sup>4,15</sup> 1D DPFGE  $^1\text{H}, ^1\text{H}$  T-ROESY,<sup>16</sup> and 1D Hadamard  $^3J_{\text{C,H}}$  spectroscopy.<sup>17</sup>

been made:<sup>5</sup> (i) each rigid segment (ring) makes its own contribution to the ordering potential, and (ii)  $\epsilon_{ab}^l$  parameters are conformation-independent. Thus, the conformation dependence of the ordering tensor is achieved by transformation to a common frame ( $T_{\text{ord}}(\phi, \psi)$  in eq 2). In practice, the low-order approximation leads to an expression where the angles  $\beta$  and  $\gamma$  can be readily integrated out and the ODF becomes dependent on the  $\phi, \psi$  angles only, that is,  $P(\beta, \gamma, \phi, \psi) \rightarrow P(\phi, \psi)$ .

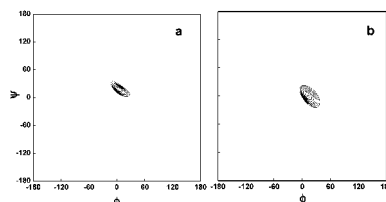
We are now in a position to construct a probability distribution function for the conformational variables  $\phi$  and  $\psi$ . Of course, there are several options for the functional form of such a distribution. In the APME approach we decided on the distribution function which is derived from the maximum-entropy principle.<sup>6</sup> The ME method provides the least biased and flattest distribution that is consistent with an experimental set of data. Thus, the conformational distribution function takes the form:

$$P(\phi, \psi) = \frac{1}{Z} \exp \left\{ - \sum_{ij} \lambda_{ij} D_{ij}(\phi, \psi) - \sum_{kl} \lambda_{kl} \frac{1}{r_{kl}^6(\phi, \psi)} - \sum_{mn} \lambda_{mn} {}^3J_{mn}(\phi, \psi) \right\} \quad (3)$$

where  $Z$  is a normalization factor and the  $\lambda$ 's are adjustable parameters that can be determined by bringing calculated NMR parameters into agreement with those experimentally determined. Here  $\lambda_{ij}$ ,  $\lambda_{kl}$ , and  $\lambda_{mn}$  are related to the motionally averaged interresidue DD couplings, NOEs, and  $^3J_{\text{C,H}}$  couplings, respectively. The recently developed Karplus-type relationship was used for interpretation of the trans-glycosidic  $^3J_{\text{C,H}}$  couplings.<sup>11</sup>

Determination of a unique conformation-dependent order tensor requires at least five independent dipolar couplings in each ring. The intraresidue DD couplings were used to obtain the  $\epsilon_{ab}^l$  parameters by combining eqs 1–3 and using  $\langle D_{ij}^{\text{intra}} \rangle = \int D_{ij}^{\text{intra}}(\phi, \psi) P(\phi, \psi) d\phi d\psi$ . The interring DD couplings (five), together with the NOEs (four) and the  $^3J_{\text{C,H}}$  couplings (two) were used to determine the  $\lambda$  parameters in eq 3. In the analysis 27 experimental points and 21 parameters (11  $\lambda$  and 10  $\epsilon$  values) were employed in determination of  $P(\phi, \psi)$ . Note that the fitting procedure must be carried out simultaneously for all the parameters ( $\lambda$  and  $\epsilon$ ). It is so, because of the conformation dependence of the ordering tensor used for calculation of all the DD couplings. Numerical fitting was performed using an in-house written computer code based on the MATLAB subroutine *fminu*.

A complication that needs to be mentioned is the sign of the five interring H–H couplings. The  $J$  contribution to these spin–spin interactions<sup>12</sup> is essentially zero, and we were therefore not able to infer the sign of the dipolar interaction. Thus, in the analysis we considered 32 possible combinations. The fitting error for several



**Figure 2.** Contour map derived for the disaccharide, where  $\phi = \text{H}1'-\text{C}1'-\text{O}2-\text{C}2$ , and  $\psi = \text{C}1'-\text{O}2-\text{C}2-\text{H}2$ . The distribution functions,  $P(\phi, \psi)$ , were determined using: (a) the APME approach (eq 3) and (b)  $^3J_{\text{C,H}}$  couplings and NOEs (only the two last terms of eq 3).

combinations was large, and these were therefore excluded from further considerations. In the final analysis we were able to identify a few sets of dipolar couplings that resulted in similar fitting errors.

We performed a test of the APME approach by calculating the ordering tensors in each ring using the intraresidue DD couplings only, and by superimposing these tensors. This attempt to analyze all the experimental data using a single molecular conformation resulted in a much larger rmsd than for the APME procedure. The large error originates from poor agreement for the interring parameters. We conclude therefore, that a proper analysis of the molecular structure requires a distribution of conformations. In addition, we determined  $P(\phi, \psi)$  using the  $^3J_{\text{C,H}}$  couplings and NOEs, but excluding the DD couplings. The conformational distribution functions  $P(\phi, \psi)$  are shown in Figure 2. The highly populated regions are in fact consistent with previously reported results derived from computer simulations of the same molecule.<sup>13</sup> The distribution functions are very similar, indicating that the APME approach is consistent with the classical analysis of molecular conformations based on  $^3J_{\text{C,H}}$  couplings and NOEs. Figure 1 shows the correlation between measured and calculated NMR parameters using the APME distribution. The agreement is indeed very good. We believe that the APME method will be applicable as an important tool in structure determination of flexible molecules in dilute liquid crystals.

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