

Determination of Sugar Structures in Solution from Residual Dipolar Coupling Constants: Methodology and Application to Methyl β -D-Xylopyranoside

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Abstract: We have developed methodology for the determination of solution structures of small molecules from residual dipolar coupling constants measured in dilute liquid crystals. The power of the new technique is demonstrated by the determination of the structure of methyl β -D-xylopyranoside (**1**) in solution. An oriented sample of **1** was prepared using a mixture of C₁₂E₅ and hexanol in D₂O. Thirty residual dipolar coupling constants, ranging from -6.44 to 4.99 Hz, were measured using intensity-based *J*-modulated NMR techniques. These include 15 *D*_{HH}, 4 ¹*D*_{CH}, and 11 ⁿ*D*_{CH} coupling constants. The accuracy of the dipolar coupling constants is estimated to be $\leq \pm 0.02$ Hz. New constant-time HMBC NMR experiments were developed for the measurement of ⁿ*D*_{CH} coupling constants, the use of which was crucial for the successful structure determination of **1**, as they allowed us to increase the number of fitted parameters. The structure of **1** was refined using a model in which the directly bonded interatom distances were fixed at their ab initio values, while 16 geometrical and 5 order parameters were optimized. These included 2 CCC and 6 CCH angles, and 2 CCCC and 6 CCCH dihedral angles. Vibrationally averaged dipolar coupling constants were used during the refinement. The refined solution structure of **1** is very similar to that obtained by ab initio calculations, with 11 bond and dihedral angles differing by 0.8° or less and the remaining 5 parameters differing by up to 3.3°. Comparison with the neutron diffraction structure showed larger differences attributable to crystal packing effects. Reducing the degree of order by using dilute liquid crystalline media in combination with precise measurement of small residual dipolar coupling constants, as shown here, is a way of overcoming the limitation of strongly orienting liquid crystals associated with the complexity of ¹H NMR spectra for molecules with more than 12 protons.

Introduction

Liquid crystal NMR spectroscopy is a well-established method for obtaining accurate geometries of small, reasonably rigid molecules.¹ It is through vibrationally averaged dipolar couplings of oriented solutes that this information can be retrieved. Although this method has been applied during the past three decades to numerous molecules, the route from dipolar couplings to molecular structures is not an easy one. The main complication is that the solutes in liquid crystals normally exhibit complex, second-order spectra. Beyond 10 interacting spins, such spectra usually become too complicated to be analyzed properly.² Various approaches have been developed to simplify

the studied systems, including selective³ or random⁴ deuteration, multiple-quantum filtration,⁵ separated local field (SLF) spectroscopy in combination with variable-angle sample spinning (VASS),⁶ and proton-detected local field (PDLF) spectroscopy.⁷ Reducing the dipolar couplings to an extent that makes spectral

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interpretation possible was explored by designing special pulse sequences⁸ and by application of VASS⁹ or the switched-angle spinning technique (SAS).¹⁰

The recent introduction of *dilute liquid crystalline media*¹¹ brought about the possibility of imposing very low order on the solute molecules, resulting in significant reduction of dipolar coupling constants, referred to as *residual* dipolar couplings (RDCs). Preserving the first-order character of spectra in such media greatly facilitates the extraction of RDCs, which have become a rich source of structural information for large biomolecules.¹² Their application to smaller molecules has been mostly limited to carbohydrates, for which the RDCs are used to obtain information about the orientation of individual monosaccharide rings of oligosaccharides.¹³ RDCs of small organic molecules have been interpreted in a qualitative manner,¹⁴ providing useful stereochemical information. For example, conformational analysis of an alanine dipeptide in water-based liquid crystals using 13 relatively small (2–130 Hz) dipolar couplings has been reported recently.¹⁵ In this report the dipolar coupling constants were obtained from the best-fit simulated spectra.

The precision of molecular structures emerging from liquid crystal NMR data arises directly from the precision with which dipolar couplings can be measured. As dipolar couplings of up to several thousand hertz are observed in strongly oriented systems, as opposed to only several hertz (or tens of hertz) in dilute liquid crystalline media, it is more difficult to achieve the same relative precision in the latter case. We have recently developed intensity-based methods for the measurement of proton–proton¹⁶ and one-bond proton–carbon¹⁷ residual dipolar couplings of small molecules, which provide coupling constants with the precision of a *few hundredths of a hertz*. These techniques were designed for the measurement of dipolar coupling constants from unresolved proton multiplets containing numerous proton–proton dipolar coupling constants. Here we extend our methodology (a) to the measurement of long-range proton–carbon coupling constants, (b) by using a vibrational force field, calculated *ab initio*, to derive vibrational corrections to the dipolar coupling constants, and (c) by using all three types of coupling constants (D_{HH} , ${}^1D_{CH}$, ${}^nD_{CH}$) to determine, for the

first time, the structure of a small molecule dissolved in a dilute liquid crystalline medium. On the basis of 30 residual dipolar couplings ranging from -6.44 to $+4.99$ Hz and with an average value of $|D| = 1.56$ Hz, we have determined the structure of a simple monosaccharide, methyl β -D-xylopyranoside (compound **I**, $C_6H_{12}O_5$), the first almost-complete determination of the structure of a sugar in solution. We compare the solution-phase structure with the lowest energy *ab initio* and the neutron diffraction¹⁸ structures of **I** and discuss the strengths and limitations of this approach to the liquid-state structure determination of small molecules.

Materials and Methods

Spectra were recorded on an 800 MHz AVANCE spectrometer using a triaxial gradient triple-resonance 5 mm probe. The temperature was set to 25 °C. The air flow was increased to 1200 L/h in order to eliminate any temperature gradients across the sample volume. No decoupling was used during the experiments in order to eliminate any external sample heating. The temperature stability during the pulse sequences was checked by using a sample of ethylene glycol, a widely used NMR temperature standard. Spectra recorded before and immediately after 10 min of pulsing using the pulse sequences employed throughout this work did not register any temperature variations. The sample was prepared by dissolving 50 mg of methyl β -D-xylopyranoside (**I**), purchased from Sigma-Aldrich, in 99.99% D_2O . An oriented sample was prepared¹⁹ by dissolving 50 mg of **I** in 13.8% (w/w) $C_{12}E_5$ + hexanol in D_2O . The molar ratio of $C_{12}E_5$:*n*-hexanol was 0.99:1. The residual quadrupolar coupling constant of D_2O was 64 Hz. All spectra were acquired during the course of one week, during which the alignment did not change, judging from the splitting of the D_2O signal.

Parameters of NMR Experiments. The proton–proton coupling constants were measured using the 1D directed-COSY experiments (pulse sequences of Figure 1a,b). The optional refocusing interval was used for measurement of small scalar coupling constants and all dipolar coupling constants. Values of 50 and 200 ms were used for this interval, the latter for coupling constants less than 3 Hz. All selective pulses were 30 ms Gaussian-shaped pulses. Sixteen data sets were acquired by varying delay T from 65 ms to 1.1 s in variable-time experiments and from 65 ms to 3.5 s in the constant-time experiments. In one experiment, 480 ($= 16 \times 15 \times 2$) 1D spectra were acquired for 15 pairs of coupled protons and 16 evolution intervals. Eight scans were accumulated using an acquisition time of 2 s and relaxation delay of 1.5 s for isotropic samples, while 1 and 2.5 s, respectively, were used for aligned samples. This resulted in an overall acquisition time of approximately 5 h per one experiment.

The one-bond proton–carbon coupling constants were obtained by analysis of 12 2D *jch_h* spectra acquired using the pulse sequence of Figure 1c by incrementing the evolution interval, T , in 1 ms steps. To minimize the effects of evolution of geminal proton–proton coupling constants in CH_2 groups (i.e., C_5 in **I**), this interval was centered around $n^2J_{H_{5eq},H_{5ax}}$ in the isotropic ($n = 4$, $T_6 = 343$ ms) sample or $n^2J_{H_{5eq},H_{5ax}} + {}^2D_{H_{5eq},H_{5ax}}$ in the aligned ($n = 4$, $T_6 = 310$ ms) sample. In addition, a spectrum with $T \approx 0.5/J_{CH}$ was acquired. This was added to the set of fitted spectra as this improved the parametrization of the effective spin–spin relaxation time, T_2^{eff} , used in transfer functions. The spectral width in the F_1 dimension was 12 ppm, and 8 scans were accumulated in each of 32 complex increments. The acquisition times in the F_1 and F_2 dimensions were 10.6 ms and 1 s, respectively. Together with a relaxation delay of 1.2 s, this resulted in the total experimental time for one measurement of 4.25 h.

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increase the goodness of the fit compared to a three-parameter fitting procedure required for a different experiment.

Theoretical Methods. All calculations were performed on a Linux cluster using the Gaussian 98 program.²¹ All MP2 calculations were frozen core [MP2(fc)]. The molecular structure of methyl β -D-xylopyranoside was determined at the HF level using the 3-21G**²² basis set, and at HF and MP2 levels using the 6-31G**²³ basis set. The B3LYP functional²⁴ was also used with the 6-31G*, 6-311G*,²⁵ and 6-311+G* basis sets. The molecular structure of methyl β -D-xylopyranoside was also determined by self-consistent reaction field calculations at the B3LYP/6-311+G* level using the Onsager model.²⁶ The solvent system of water was investigated, with the solute set to occupy a fixed spherical cavity of radius $a_0 = 4.52 \text{ \AA}$, with a dielectric constant of $\epsilon = 78.3$ used for the solvent field. As this molecule is sitting in water, realistically there are many hydrogen-bond interactions that can take place with the molecule. As these would be difficult to anticipate and calculate, this has not been done. Analytic force fields calculated at the HF and B3LYP levels with all basis sets confirmed that the optimized structures were minima on the potential energy surface. Second derivatives of the energy with respect to the nuclear coordinates calculated at the B3LYP/6-311+G* level gave the force field, which was then used to correct the dipolar couplings for vibrational motion.

Vibrational Correction. An in-house program, bmgv, was used to correct the experimental couplings (D°) for vibrational effects to yield D^α couplings. These were then used to provide vibrationally corrected structural information about the molecule. The covariance matrices and internuclear vectors were obtained from the force field (B3LYP/6-311+G*) using the program ASYM40.²⁷ The five order-matrix elements, S_{xx-yy} , S_{zz} , S_{xy} , S_{xz} , and S_{yz} , were refined using the experimental dipolar couplings.²⁸ These were then read into bmgv along with the D° couplings to obtain the vibrationally corrected couplings D^α . These were then used to generate a new order matrix, and the second generation of vibrationally corrected dipolar couplings was then obtained. The second generation corrections (D^α) were then used in the structural analysis.

Structure Model and Refinement Method. The molecular structure of methyl β -D-xylopyranoside was defined in terms of 27 parameters, describing only the part of the structure affected by the dipolar couplings. As only proton-proton and proton-carbon dipolar coupling constants of the ring atoms of methyl β -D-xylopyranoside were measured, parameters relating to the hydroxyl groups, methoxy group,

and ring oxygen atom were not included in the refinements and were fixed at the B3LYP/6-311+G* values. A model representative of compound **I** containing atoms $C_1(H_1)C_2(H_2)C_3(H_3)C_4(H_4)C_5(H_{5ex}, H_{5ax})$ was therefore created. The 27 active parameters used to define the structure included 4 ring C-C distances, 6 $C_{ring}-H$ distances, 3 ring C-C-C angles, 6 $C_{ring}-C_{ring}-H$ angles, 2 ring C-C-C torsion angles, and 6 $C_{ring}-C_{ring}-C_{ring}-H$ torsion angles. Finally, five order parameters, S_{xx-yy} , S_{zz} , S_{xy} , S_{xz} , and S_{yz} , were defined so that they may be refined using the D^α coupling constants. The total number of parameters that could, in principle, be varied is therefore 32.

Vibrational corrections to the experimental dipolar couplings were calculated on the basis of the force field (B3LYP/6-311+G*), covariance matrix, internuclear vectors, and the five order-matrix elements using the program bmgv. These vibrationally corrected dipolar coupling constants, D^α , were then included (in lieu of experimental diffraction data) in the Edinburgh structure refinement program ED96²⁹ as additional (i.e., nondiffraction) spectroscopic data. The active structure parameters and order parameters were then refined against D^α in stages to gauge the response of the structure to the couplings (see Results). The number of structural parameters that can be refined is completely dependent on the number of dipolar couplings used and the type of structure under study. There is no predefined formula to follow in the refinement of the structure. However, as there are five order-matrix parameters being refined on this occasion, at least five structural parameters must remain fixed at the ab initio values (see Results section for further discussion of this). In this particular case, the C-C and C-H distance parameters were not refined. One CCC angle also remained fixed, as otherwise the ring began to open, giving a completely unrealistic structure. Inclusion of 11 $^nD_{CH}$ coupling constants was therefore crucial; without them, the structure could not have been refined using 16 parameters defined above. Therefore, we are confident that, in this case, the maximum number of parameters have been refined, giving as good a structure as is possible for the number of dipolar coupling constants used.

Results

Methyl β -D-xylopyranoside adopts a 4C_1 chair conformation that is, according to MM3 calculations, 2.62 kcal/mol below the nearest low-energy form.³⁰ These minima are separated by high energy barriers that would lead to observation of separate signals for both forms, which is not the case. Methyl β -D-xylopyranoside therefore exists in solution exclusively as a 4C_1 chair and represents a suitably rigid model system for testing our methodology without a complicating factor of conformational averaging. 1H NMR spectra of methyl β -D-xylopyranoside in the isotropic and oriented media are shown in Figure 2. Chemical shift differences of less than 2.0 Hz between corresponding signals of nine nonexchangeable protons in the two spectra indicate that the solute molecules are effectively in identical environments, surrounded by molecules of D_2O in both instances. Proton multiplets in the spectrum of the oriented sample are broadened by numerous homonuclear RDCs. The two-dimensional DQF-COSY spectrum of the aligned sample (data not shown) contained cross-peaks between all non-exchangeable protons of **I**, reflecting the presence of numerous dipolar interactions.

Measurement of Dipolar Coupling Constants. Residual dipolar coupling constants are determined from the differences between the splittings observed for the aligned and unaligned

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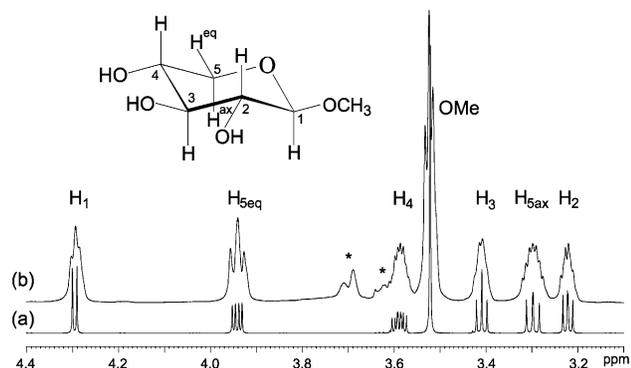


Figure 2. 800 MHz ^1H NMR spectra of methyl β -D-xylopyranoside in (a) the isotropic and (b) the aligned state. The asterisks indicate the residual signals of the aligning medium. Its intensity is much reduced compared to that of sugar resonances due to the 50 ms CPMG pulse train that was used to acquire spectrum b. The inset shows the structure of methyl β -D-xylopyranoside together with the atom numbering.

samples. This proposition assumes that the differences between the contributions to the observed splitting from the dynamic frequency shifts^{31a} and the magnetic orientations due to the anisotropic magnetic susceptibility^{31b} for the two samples are negligible. These are valid assumptions as long as all spectra are acquired at the same magnetic field. Small chemical shift anisotropy of ^1H and sp^3 ^{13}C nuclei and low anisotropy of magnetic susceptibility of methyl β -D-xylopyranoside mean that contributions to the observed resonance frequencies of individual spectral lines due to both mechanisms are very small. We therefore refer to the splitting measured for the unaligned and aligned samples as J coupling constants and $J + D$ splittings, respectively. Measurement of splittings from unresolved proton multiplets, which are typically obtained in weakly aligned media, requires the employment of intensity-based methods. Such methods were developed originally for the measurement of scalar coupling constants in biomolecules, where the proton signals are broadened by the fast spin–spin relaxation. In small molecules dissolved in dilute liquid crystalline media, the spin–spin relaxation times are long and practically identical to those observed for isotropic samples. The lack of resolution in weakly aligned samples originates from numerous D_{HH} coupling constants. Long evolution intervals can therefore be employed in the measurements of small coupling constants in small molecules—a vital prerequisite for precise determination of small dipolar coupling constants. The intensity-based methods can be implemented in two different ways. In quantitative J spectroscopy,³² each coupling constant is calculated from the intensity ratio of two peaks obtained in one or a maximum of two experiments. In J -modulated techniques,³³ peak intensities measured in several spectra are fitted to known transfer functions. The latter techniques, although requiring more spectrometer time, are more precise due to the acquisition of multiple experimental points. It is important that the intensity-based experiments are designed in such a way that corresponding transfer functions are as simple as possible, i.e., *only one coupling constant* modulates the signal intensity. When variable-

time evolution intervals are employed, each transfer function also contains a relaxation term.

Although frequency-based methods could be used for the determination of coupling constants from well-resolved proton multiplets observed in isotropic samples of small molecules, we have used identical J -modulated techniques for both isotropic and aligned samples. By doing so, we have minimized possible systematic errors in determination of RDCs, as these are calculated as differences between the values obtained from oriented ($J + D$) and isotropic (J) samples. Providing $J \gg D$ and the same method is used for isotropic and aligned samples, it is likely that accurate values of D will be obtained, even though determined J and $J + D$ values may, for some reason, deviate from their true values. The evidence of a potential problem in this way is demonstrated by the use of our J -modulated technique for the measurement of $^1D_{\text{CH}}$ coupling constants, where deviations from true J and $J + D$ values are caused by strong coupling effects.¹⁷ Nevertheless, we were able to show that when $(\Delta\delta - 0.5 \ ^1K_{\text{CH}})/K_{\text{HH}} > 5$, this technique provides accurate (± 0.02 Hz) values of $^1D_{\text{CH}}$, even though the J and $J + D$ values determined using the first-order approximation were precise but not accurate ($\Delta\delta$ is the chemical shift difference; $^1K_{\text{CH}}$ and K_{HH} are the hetero- and homonuclear constants, with values of $|J|$ or $|J + D|$ in isotropic or aligned samples, respectively). We observe a similar phenomenon for the techniques developed during the course of this work for the measurement of the $^nD_{\text{CH}}$ coupling constant. In the following, the methods for the measurement of D_{HH} and $^1D_{\text{CH}}$ are only briefly mentioned, as these have been described in detail elsewhere,^{16,17} while the new techniques for measurement of $^nD_{\text{CH}}$ are presented in full.

Proton–Proton Residual Dipolar Coupling Constants. All 15 residual dipolar proton–proton coupling constants (Table 1) between the ring protons of **I** were measured using 1D directed-COSY experiments¹⁶ (Figure 1a,b). No attempt was made to measure the coupling constants of methoxy protons, and this flexible group was not included in the structure determination. Nevertheless, many of the ring proton multiplets were broadened by dipolar interactions with the OMe protons. The 1D directed-COSY experiment relies on the selective inversion of two protons in order to achieve polarization transfer only between these protons in each experiment. In this way, the coupling constants are determined with high precision by fitting the signal intensities to a simple transfer function (see Materials and Methods). Theoretical simulations show that the condition of chemical shift difference greater than $5J$ [or $5(J + D)$] must be satisfied for this method to produce accurate values of coupling constants. This was always the case for compound **I** in both isotropic and aligned samples at 800 MHz. Typical results of 1D directed-COSY experiments, together with fitted peak intensities, are shown in Figure 3.

Coupling constants with absolute values of ≤ 0.3 Hz were measured using constant-time 1D directed-COSY (Figure 1b), as the reproducibility of measurements of smaller coupling constants using this method was higher than that with variable-time experiments. Inspection of Table 1 shows that coupling constants below 0.1 Hz are not determined accurately by either of these methods. Only absolute values ($|J|$ or $|J + D|$) are provided by these experiments, and the signs of the coupling constants must be determined by other means. For many two-

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Table 1. Scalar and Dipolar Coupling Constants (in Hz) for Methyl β -D-Xylopyranoside^a

no.	spin pair	J	$J + D$	D^{obs}	D^{α}	D^{calc}
Proton–Proton Coupling Constants ^b						
1	H ₁ –H ₂	7.87 ± 0.00	7.90 ± 0.01	0.03 ± 0.01	0.02	0.03
2	H ₁ –H ₃	−0.08 ± 0.06 ^c	1.95 ± 0.00	2.03 ± 0.06	1.97	2.00
3	H ₁ –H ₄	0.0 ^d	−0.88 ± 0.02	−0.88 ± 0.02	−0.89	−0.88
4	H ₁ –H _{5ax}	−0.04 ± 0.06 ^c	−6.57 ± 0.01	−6.53 ± 0.06	−6.44	−6.42
5	H ₁ –H _{5eq}	−0.31 ± 0.04 ^c	−1.71 ± 0.01	−1.40 ± 0.04	−1.41	−1.42
6	H ₂ –H ₃	9.33 ± 0.01	8.73 ± 0.01	−0.60 ± 0.01	−0.61	−0.63
7	H ₂ –H ₄	0.0 ^d	−4.70 ± 0.02	−4.70 ± 0.02	−4.63	−4.63
8	H ₂ –H _{5ax}	0.0 ^d	−0.33 ± 0.03	−0.33 ± 0.04	−0.33	−0.27
9	H ₂ –H _{5eq}	0.15 ± 0.03 ^c	0.0 ^b	−0.15 ± 0.03	−0.15	−0.15
10	H ₃ –H ₄	9.11 ± 0.00	8.82 ± 0.00	−0.29 ± 0.00	−0.30	−0.32
11	H ₃ –H _{5ax}	−0.20 ± 0.01 ^e	4.84 ± 0.03	−5.04 ± 0.03	4.99	4.99
12	H ₃ –H _{5eq}	−0.36 ± 0.04 ^e	0.78 ± 0.03	1.14 ± 0.04	1.15	1.21
13	H ₄ –H _{5ax}	10.55 ± 0.00	10.51 ± 0.01	−0.04 ± 0.01	−0.04	−0.11
14	H ₄ –H _{5eq}	5.49 ± 0.01	9.69 ± 0.01	4.20 ± 0.01	4.24	4.23
15	H _{5ax} –H _{5eq}	−11.65 ± 0.01	−12.88 ± 0.01	−1.23 ± 0.01	−1.32	−1.32
One-Bond Proton–Carbon Coupling Constants						
16	H ₁ –C ₁	161.77 ± 0.04	158.46 ± 0.01	−3.31 ± 0.04	−3.43	−3.42
17	H ₂ –C ₂	144.95 ± 0.02	142.03 ± 0.01	−2.92 ± 0.02	−3.07	−3.06
18	H ₄ –C ₄	145.51 ± 0.00	142.24 ± 0.01	−3.27 ± 0.01	−3.54	−3.55
19	H _{5eq} –C ₅	151.38 ± 0.03	151.72 ± 0.01	0.34 ± 0.03	0.39	0.39
Long-Range Proton–Carbon Coupling Constants						
20	H ₂ –C ₃	−4.54 ± 0.00 ^f	−5.34 ± 0.00	−0.80 ± 0.00	−0.81	−0.80
21	H ₂ –C ₁	−6.17 ± 0.01 ^f	−5.53 ± 0.01	0.64 ± 0.01	0.66	0.67
22	H ₃ –C ₂	−4.69 ± 0.00 ^f	−5.29 ± 0.00	−0.62 ± 0.00	−0.62	−0.66
23	H ₃ –C ₄	−4.44 ± 0.00 ^f	−4.47 ± 0.00	−0.03 ± 0.00	−0.03	0.00
24	H ₄ –C ₃	−3.94 ± 0.01 ^f	−4.22 ± 0.01	−0.28 ± 0.01	−0.29	−0.29
25	H ₄ –C ₅	−3.26 ± 0.00 ^f	−2.77 ± 0.00	0.49 ± 0.00	0.51	0.51
26	H _{5ax} –C ₄	−3.08 ± 0.00 ^f	−2.54 ± 0.00	0.54 ± 0.00	0.56	0.56
27	H _{5eq} –C ₄	−3.88 ± 0.01 ^f	−1.48 ± 0.01	2.40 ± 0.01	2.47	2.44
28	H _{5ax} –C ₁	2.76 ± 0.01	1.88 ± 0.01	−0.88 ± 0.01	−0.89	−0.94
29	H _{5eq} –C ₃	9.43 ± 0.00	9.85 ± 0.00	0.42 ± 0.00	0.43	0.42
30	H _{5eq} –C ₁	10.26 ± 0.01	9.78 ± 0.01	−0.48 ± 0.01	−0.49	−0.47

^a D^{obs} , D^{α} , and D^{calc} are the experimental, vibrationally corrected, and calculated dipolar coupling constants; standard deviations of experimental J and $J + D$ coupling constants were determined using three consecutive measurements; standard deviations of dipolar coupling constants were calculated as $[\delta J^2 + \delta(J + D)^2]^{1/2}$. ^b Homonuclear coupling constants were calculated using six values per coupling constant, as these were measured by starting the polarization transfer on each of the two coupled protons in separate experiments. ^c Signs determined during structure optimization. ^d No transfer of polarization observed. ^e Signs determined from E.COSY experiment. ^f Signs determined from ω_1 ¹³C-filtered DQF–COSY experiment (see Supporting Information).

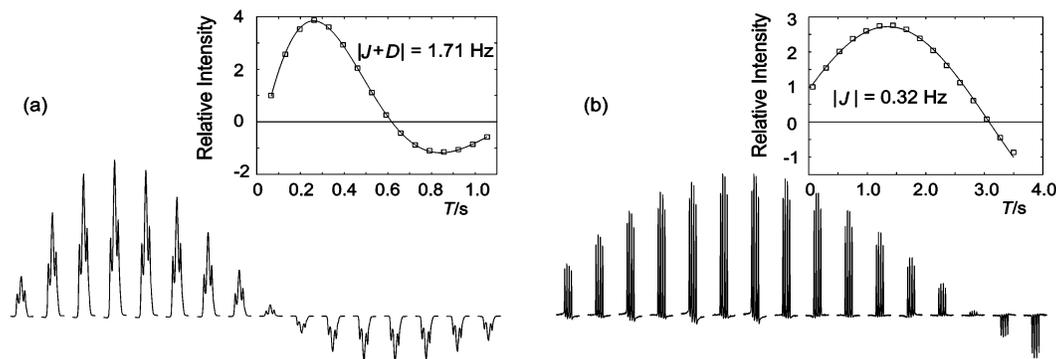


Figure 3. Illustration of the 1D directed-COSY methods. Signal intensities of proton H_{5eq} as a function of the evolution time T in (a) variable-time H₁ → H_{5eq} directed-COSY acquired using the aligned sample and (b) constant-time H₁ → H_{5eq} directed-COSY using the isotropic sample. The four-bond H₁,H_{5eq} coupling constants were determined by fitting the signal intensities to appropriate transfer functions (see Materials and Methods), as shown in the insets.

and three-bond coupling constants in weakly aligned samples, ${}^{2,3}J_{\text{HH}} \gg {}^{2,3}D_{\text{HH}}$ and the signs of the geminal and vicinal dipolar coupling constants are immediately obvious. The signs of ${}^{4,5}D_{\text{HH}}$ coupling constants are determined one at a time during the early stages of the structure refinement, by considering both positive and negative values while optimizing the order-matrix elements. Signs that yield higher sums of the squares of differences between the experimental and calculated dipolar coupling constants are rejected. The absolute values of these coupling constants depend also on the signs of ${}^{4,5}J_{\text{HH}}$ coupling constants that are usually very small. The signs of these long-range

coupling constants can, in some instances, be obtained experimentally from the analysis of the E.COSY multiplets or, as with the sign determination of ${}^{4,5}D_{\text{HH}}$ coupling constants, derived during the course of the structure calculations, typically in their later stages.

One-Bond Proton–Carbon Residual Dipolar Coupling Constants. The ${}^1D_{\text{CH}}$ coupling constants (Table 1) were measured using a modification of the *jch_h* experiment.¹⁷ A non-refocused version without the ¹³C decoupling (Figure 1c) was used to eliminate possible sample heating that could change the alignment slightly. The BIRD pulse applied in the middle

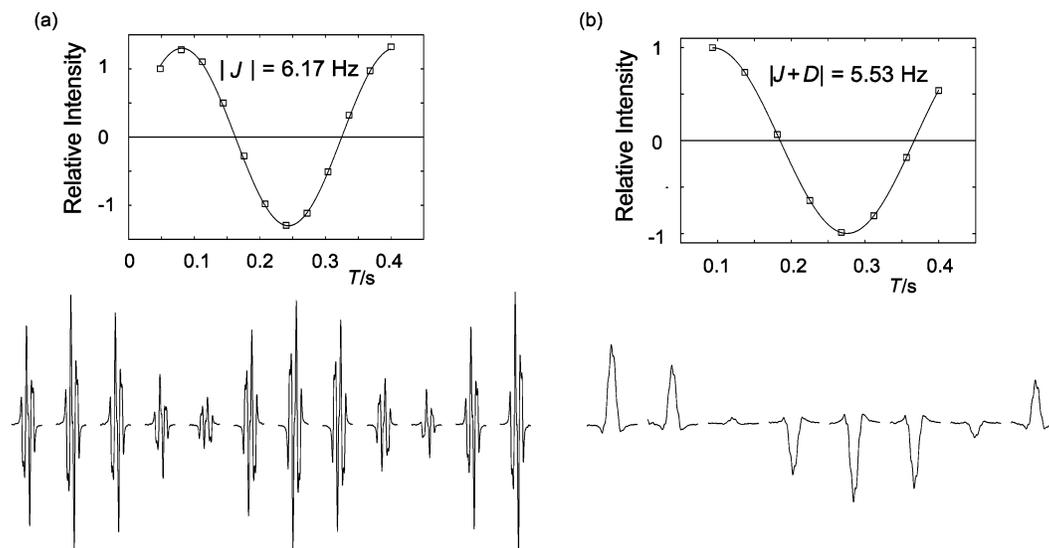


Figure 4. Illustration of the J -modulated constant-time HMBC methods. The intensities of the H_2C_1 cross-peak as a function of the evolution time T in (a) the nonselective HMBC experiment for the isotropic sample and (b) the selective HMBC experiment acquired using the aligned sample. The two-bond H_2C_1 coupling constants were determined by fitting the signal intensities to appropriate transfer functions (see Material and Methods), as shown in the insets.

of the evolution interval removes the evolution of proton–proton coupling constants, while allowing the evolution of one-bond heteronuclear coupling constants. Cross-peak intensities obtained in a series of 2D spectra as a function of the evolution interval, T , were fitted to a simple transfer function (see Material and Methods), yielding precise values of coupling constants. As stated above, only when there is sufficient chemical shift separation in the ^{13}C satellite spectra are $^1D_{\text{CH}}$ coupling constants accurately determined by this procedure. This was not the case for H_3 and $\text{H}_{5\text{ax}}$ protons in the aligned sample due to a large $^4D_{\text{H}_3, \text{H}_{5\text{ax}}}$ coupling constant. The two heteronuclear coupling constants involving protons H_3 and $\text{H}_{5\text{ax}}$ were therefore excluded from the analysis, and only four $^1D_{\text{CH}}$ coupling constants were used in the structure calculation. Since $|^1D_{\text{CH}}| \ll ^1J_{\text{CH}} > 0$, the signs of $^1D_{\text{CH}}$ coupling constants were determined automatically.

Long-Range Proton–Carbon Residual Dipolar Coupling Constants. Two new J -modulated constant-time HMBC experiments (Figure 1d,e) were designed for precise measurement of $^nD_{\text{CH}}$ coupling constants. An overall constant-time interval used in these pulse sequences ensured that equal evolution of proton–proton coupling constants took place while the evolution of heteronuclear coupling constants could be varied. The latter was achieved by changing the position of a 180° ^{13}C pulse applied within the first half of the Δ_1 interval. The ^{13}C chemical shift labeling took place during the constant-time interval Δ_2 and was achieved by increasing the t_1 interval, while simultaneously reducing the τ intervals. Sign discrimination in F_1 was done by pulsed field gradients,³⁴ and proton chemical shifts were refocused by 180° ^1H pulses applied amid Δ_i ($i = 1, 2, 3$) intervals. In the resulting spectra, the cross-peak intensities are modulated by a simple transfer function $I_k = I_k^0 \sin(\pi^n K_{\text{CH}} T)$. An example of series of multiplets extracted from 1D traces of J -modulated HMBC spectra and the analysis of a H_2C_1 cross-peak is shown in Figure 4a. These multiplets show a typical mixed-phase pattern with the heteronuclear coupling constant in anti-phase and a mixture of in-phase and anti-phase compo-

nents due to the evolution of proton–proton scalar coupling constants. While it is adequate for isotropic samples, severe signal attenuation due to line cancellation of anti-phase components makes this experiment unsuitable for aligned samples containing unresolved proton multiplets.

Simple modifications of the basic J -modulated HMBC experiment solve this problem. In the modified experiment, the evolution of proton–proton coupling constants during the Δ_i ($i = 1, 2, 3$) intervals is removed by applying selective 180° ^1H pulses instead of the nonselective ones. The in-phase rather than anti-phase multiplets with respect to the heteronuclear long-range coupling constants are obtained by setting $\Delta_3 \approx 0.5^n J_{\text{CH}}$ and applying an extra ^{13}C 180° pulse in the middle of this interval. The cumulative effect of these changes brings about a significant increase in the cross-peak intensities. The price that has to be paid for this improvement is a longer overall experimental time. A series of 2D experiments needs to be acquired for each proton independently, yielding coupling constants for all long-range coupled carbons from one set of data. An example of series of multiplets extracted from 1D traces of the selective, constant-time J -modulated HMBC experiment is given in Figure 4b. Since all carbon resonances of **I** were resolved by using a short t_1 evolution time, the Δ_2 delay was kept short ($= 16$ ms), and a nonselective 180° ^1H pulse was applied in the middle of the t_1 period.

To assess the influence of higher-order effects on the fitted values of coupling constants, a series of simulations using the NMRSIM module of the XWINNMR Bruker software was performed. The following spin system was considered: $^{13}\text{C}_a\text{H}_a - ^{12}\text{CH}_b$ with coupling constants $^1J_{\text{C}_a\text{H}_a} = 138.7$ Hz, $^3J_{\text{H}_a\text{H}_b} = 9.3$ Hz, and $^2J_{\text{C}_a\text{H}_b} = 4.3$ Hz. The proton chemical shifts were set so that the difference between the low-frequency ^{13}C satellite of proton H_a and the chemical shift of proton H_b could be expressed as a multiple of the proton–proton coupling constant [$F = (\Delta\delta - 0.5 ^1J_{\text{C}_a\text{H}_a}) / ^3J_{\text{H}_a\text{H}_b}$, where $\Delta\delta = \delta_a - \delta_b$]. A series of 2D J -modulated constant-time HMBC spectra was simulated using the pulse sequences of Figure 1d,e for F ranging from 3 to 30. 1D traces were extracted from the spectra, and multiplets

(34) Boyd, J.; Soffe, N.; John, B.; Plant, D.; Hurd, R. *J. Magn. Reson.* **1992**, *98*, 660.

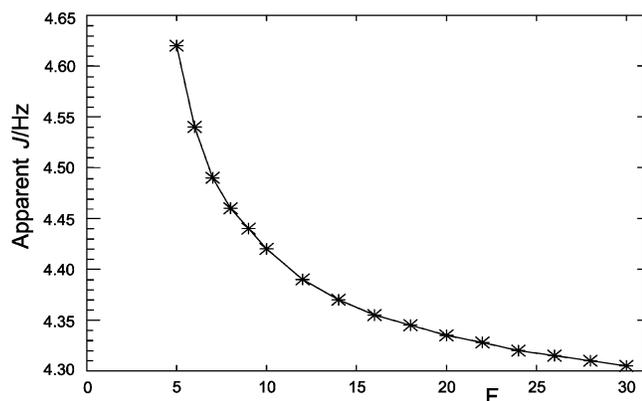


Figure 5. Relationship between the true (${}^2J_{\text{C}_5\text{H}_b} = 4.30$ Hz) and apparent ${}^2J_{\text{CH}}$ coupling constants determined from simulated HMBC experiments as a function of $F = (\Delta\delta - 0.5 {}^1J_{\text{C}_5\text{H}_b})/{}^3J_{\text{H}_b\text{H}_c}$. See text for details.

of H_b were fitted using the appropriate transfer functions (see Materials and Methods). The results of the simulation experiments can be summarized as follows. (i) Large differences were observed between the actual and calculated coupling constants, and these values converged very slowly as the factor F increased (Figure 5). (ii) Below $F = 5$, the variations in the shape of the H_b multiplets were too large for reliable determination of the relative intensities of the multiplets in a series and therefore of the apparent coupling constant. Below this threshold, variations of >0.01 Hz were observed for the apparent coupling, depending on which of the multiplets was chosen as a reference multiplet for the determination of their relative intensities. (iii) Values obtained using the selective and nonselective HMBC experiments were identical. This analysis showed that the intensity-based methods are very sensitive to higher order effects and the values they provide should be characterized only as apparent coupling constants. When aiming for precision in the determination ${}^2D_{\text{CH}}$ better than ± 0.02 Hz, weak alignments must be used so that the contribution of proton–proton dipolar coupling constants alters the F factor between the isotropic and aligned samples only marginally. If this is the case, accurate values of ${}^nD_{\text{CH}}$ are obtained from the difference $({}^nJ_{\text{CH}} + {}^nD_{\text{CH}}) - {}^nJ_{\text{CH}}$. Strong coupling affects the accuracy of determined ${}^nJ_{\text{CH}}$ coupling constants also in the frequency-based methods.³⁵ Overall, the results of this analysis suggest that a full density-matrix treatment of the J -modulated experiments is preferable, and more research in this direction is needed.

In compound **I**, factor $F > 7.7$ was observed for all protons, except for H_3 and $\text{H}_{5\text{ax}}$ in the aligned sample, where $F = 3.2$. Consequently, the ${}^3D_{\text{CH}}$ coupling constants of H_3C_5 and $\text{H}_{5\text{ax}}\text{C}_3$ pairs were excluded from structure calculations, and eight ${}^2D_{\text{CH}}$ and three ${}^3D_{\text{CH}}$ (Table 1) were eventually used. This set included all possible two-bond heteronuclear coupling constants with the exception of ${}^2D_{\text{H}1\text{C}2}$. The corresponding ${}^2J_{\text{H}1\text{C}2}$ coupling constant was too small (~ 1 Hz) to be measured accurately. The ${}^3J_{\text{CH}}$ coupling constants of axial protons in pyranose rings are usually very small because of the small dihedral angle between the interacting atoms. Given the possibility that these can be further decreased by negative dipolar coupling constants, the measurement of ${}^3D_{\text{CH}}$ coupling constants is rather difficult. Only three ${}^3D_{\text{CH}}$ coupling constants, all including protons of the CH_2 group, were therefore used in the structure calculation.

Unlike ${}^1J_{\text{CH}}$ and ${}^3J_{\text{CH}}$, which are always positive, ${}^2J_{\text{CH}}$ coupling constants can be either positive or negative.³⁶ A convenient method of sign determination of ${}^2J_{\text{CH}}$ coupling constants is provided by the ω_1 ${}^{13}\text{C}$ -filtered 2D DQF–COSY experiment (see Supporting Information). In this experiment, the tilt of the E.COSY-type cross-peaks mediated by ${}^3J_{\text{HH}}$ coupling constants is compared with the tilt of the diagonal peak, which reflects the positive sign of ${}^1J_{\text{CH}}$ coupling constants. Using this experiment, the signs of all measured ${}^2J_{\text{CH}}$ of **I** were determined as negative. Since all $|{}^nD_{\text{CH}}| < |{}^nJ_{\text{CH}}|$ in our case, the signs of ${}^nD_{\text{CH}}$ coupling constants were determined automatically.

Structure Refinement. The structure of methyl β -D-xylopyranoside in solution was refined using 30 dipolar coupling constants determined by the J -modulated methods. Standard deviations given for J and $J + D$ values in Table 1 were obtained as a result of three repetitive measurements. The average standard deviation of dipolar coupling constants was calculated to be 0.018 Hz. For the purpose of structure calculation, this value was increased to 0.05 Hz to allow for uncertainties in vibrational corrections and for any systematic errors in the bonded interatomic distances that were fixed at their ab initio values. The minimum energy ab initio (B3LYP/6-311+G*) structure of **I** calculated for a single free molecule served as a starting point for the structure refinements.

During the first step of structure calculation, all parameters of the model were fixed at their ab initio values, and five order-matrix elements were refined using the experimental dipolar coupling constants, D° . Vibrational corrections to the experimental dipolar coupling constants were then calculated using the refined order matrix and the ab initio structure of **I**. A set of vibrationally corrected coupling constants was then used to calculate a new order matrix and subsequently the second generation of vibrationally corrected coupling constants, D^α . These were then used throughout the subsequent geometry optimization. When the vibrational corrections were later calculated using the final structure, they were found to be practically identical to those used to calculate the second generation of vibrationally corrected coupling constants. Given the small difference between the ab initio and the optimized structure, this was to be expected and confirms that it was justified to calculate the vibrationally corrected coupling constants only at the beginning of the structure optimization. The largest vibrational correction observed for ${}^1D_{\text{CH}}$ coupling constants was -0.27 Hz for ${}^1D_{\text{C}4\text{H}4}$ (-3.27 Hz), while the maximum for D_{HH} coupling constants was $+0.09$ Hz for ${}^1D_{\text{H}1\text{H}5\text{ax}}$ (-6.53 Hz). These values are larger than the experimental uncertainties of dipolar coupling constants, which makes the use of vibrational corrections mandatory.

Four further rounds of structure refinement were performed, in which the order-matrix elements were always optimized together with an increasing number of molecular parameters. First two CCC dihedral angles were refined, followed by six CCCH dihedral angles, three CCH angles, and finally two out of the three CCC angles. The $\text{C}_1\text{C}_2\text{C}_3$ angle diverged when included, causing the six-membered ring to open, and it was subsequently removed from the list of refining parameters. This demonstrates the earlier comment regarding the choice of parameters to be refined being based on observation of the

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Table 2. Geometrical Parameters (Angles, in Deg) of the Solution Structure of **I**

parameter (angle \angle , or dihedral angle ϕ)	solution structure ^a	solution minus ab initio ^b	solution minus neutron structure ^c
$\angle C_1C_2C_3$	110.3 ^d		2.0
$\angle C_2C_3C_4$	111.1(8)	-0.7	-0.7
$\angle C_3C_4C_5$	111.2(6)	2.6 (4)	0.2
$\angle C_2C_1H_1$	113.0(15)	3.1 (2)	2.2
$\angle C_3C_2H_2$	109.7(8)	0.4	0.1
$\angle C_2C_3H_3$	108.7(8)	0.2	0.5
$\angle C_3C_4H_4$	108.8(8)	-0.5	0.5
$\angle C_4C_5H_{5eq}$	110.7(6)	-0.8	0.2
$\angle C_4C_5H_{5ax}$	109.5(5)	-0.1	-0.4
$\phi C_1C_2C_3C_4$	-51.2(8)	0.4	0.8
$\phi C_2C_3C_4C_5$	52.4(6)	-1.1 (2)	3.4
$\phi C_3C_2C_1H_1$	-63.7(14)	3.3 (2)	-3.8
$\phi C_4C_3C_2H_2$	66.7(9)	0.1	0.9
$\phi C_1C_2C_3H_3$	68.0(10)	0.7	0.8
$\phi C_2C_3C_4H_4$	-68.8(7)	-2.6 (3)	1.6
$\phi C_3C_4C_5H_{5eq}$	-175.2(7)	0.3	-3.4
$\phi C_3C_4C_5H_{5ax}$	64.6(7)	-0.2	-4.6

^a Estimated standard deviations obtained during the structure refinement are given in parentheses. ^b Numbers in bold indicate where the differences in angles were more than one estimated standard deviation; the deviation as a multiple of the standard deviation is given in parentheses. The distribution of deviations is statistically appropriate. ^c Differences larger than 1° are in bold. ^d This angle was fixed at its ab initio value.

behavior of the refining data, rather than being predetermined before structure refinement. The total variance of the data decreased steadily from 131.2 when only the orientation parameters were refined to 99.4, 59.1, 17.7, and finally 9.9. Similarly, the root-mean-square deviation of the dipolar coupling constants decreased steadily (0.105, 0.086, 0.062, 0.029, and 0.021 Hz). The correlation matrix for the refining parameters showed consistently small values for off-diagonal elements, indicating the absence of any major correlation between the parameters (see Supporting Information). The values of 17 parameters that define the solution-state structure of **I** are given in Table 2. As expected from the shape of the pentopyranose ring, when aligned the molecule of **I** shows large anisotropy, characterized by the following elements of the diagonalized order matrix: $S_{zz} = -46.6 \times 10^{-5}$, $S_{yy} = 39.2 \times 10^{-5}$, and $S_{xx} = 7.4 \times 10^{-5}$ (Figure 6a).

Analysis of the Structures. The agreement between the observed, vibrationally corrected dipolar coupling constants (D^{α}) of **I** and the calculated dipolar couplings (D^{calc}) based on the solution structure is very good. The rms deviation of 0.021 Hz is comparable to the average uncertainty (0.018 Hz) of the experimentally determined coupling constants (Table 1). For some pairs, the differences are larger than the errors bars on the experimental dipolar coupling constants. This is to be expected, as the calculated dipolar coupling constants are a result of an optimization procedure, and therefore their deviations follow the normal distribution. A detailed comparison of the differences between the experimental, vibrationally corrected dipolar coupling constants and those calculated on the basis of (i) the optimized solution structure, (ii) the ab initio structure, and (iii) the neutron diffraction structures is shown in Figure 7. Inspection of this figure indicates that the ab initio structure approximates the solution structure of **I** much better than does the neutron diffraction structure. We chose to use the ab initio C–H distances rather than those from neutron diffraction, as this type of parameter is likely to be reliable from the calculations, especially for the ratios of the calculated distances.

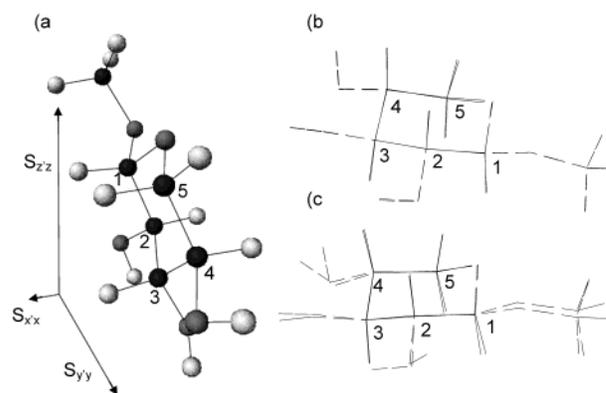


Figure 6. Comparisons of methyl β -D-xylopyranoside structures. (a) Calculated solution-phase structure of **I** in the coordinate system of the inertia tensor. The eigenvectors of the principal axis coordinate system of the alignment tensor are given with their relative lengths, showing their corresponding eigenvalues. (b) Overlay of the solution-phase and the ab initio structures. (c) The corresponding overlay of the solution-phase and the neutron diffraction structures of **I**. Dashed lines show those parts of the molecule that have not been refined against experimental data.

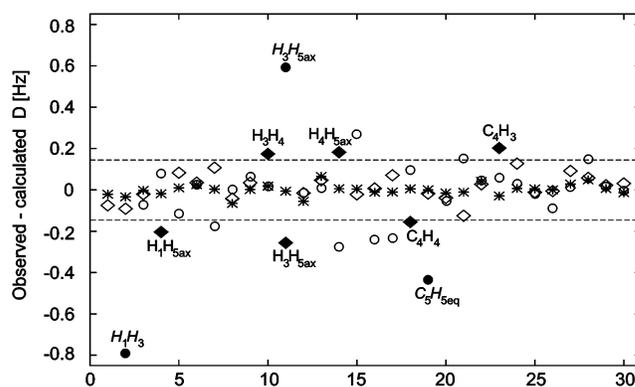


Figure 7. Differences between 30 observed, vibrationally corrected residual dipolar coupling constants and those calculated on the basis of the (*) refined solution state, (\diamond) ab initio, and (\circ) neutron diffraction structures of methyl β -D-xylopyranoside. The coupling constants are plotted in order of Table 1, and those showing the largest differences are labeled and plotted using filled symbols. The dashed lines are plotted at ± 0.15 Hz.

The closer agreement with the calculated free molecule structure suggests that the crystal environment causes significant distortion of the molecules, and that structures in solution should not be presumed to be identical to those determined crystallographically.

Several dipolar coupling constants calculated on the basis of the neutron diffraction structure showed large differences, e.g., D_{H_1,H_3} ($\Delta = -0.79$ Hz), $D_{H_3,H_{5ax}}$ ($\Delta = 0.59$ Hz), or $D_{C_5,H_{5eq}}$ ($\Delta = -0.44$ Hz). Altogether, nine coupling constants differed by more than 0.15 Hz. The largest differences involved dipolar coupling constants between H_1H_3 and H_3H_{5ax} protons, suggesting that these interproton distances are significantly different in the solid and solution-phase structures. Indeed, when the distances between the 1,3 diaxial protons of the six-membered ring were calculated, they were found to differ in the various structures (Table 3). The degree to which these differ correlates with the differences in the calculated dipolar coupling constants. Nevertheless, this is not a straightforward r^{-3} dependence, as slight differences in alignment tensors calculated for different structures contribute significantly to the calculated dipolar coupling constants.

Table 3. Distances (in Å) between the 1,3 Diaxial Protons of Methyl β -D-Xylopyranoside

structure	H ₁ –H ₃	H ₁ –H _{5ax}	H ₃ –H _{5ax}	H ₂ –H ₄
solution structure	2.67	2.40	2.63	2.66
ab initio	2.64	2.44	2.54	2.66
neutron diffraction	2.53	2.32	2.69	2.67

The largest difference in dipolar coupling constants between the refined solution-phase and ab initio gas-phase structures is -0.26 Hz ($D_{H_3, H_{5ax}}$), and only six coupling constants, labeled in Figure 7, show deviations between 0.15 and 0.26 Hz. Four of these involve either H₄ or C₄, indicating that the differences between the two structures may be localized in this part of the molecule. This observation can be correlated with the observed differences in the C₃C₄C₅ and C₂C₃C₄H₄ angles between the two structures. Overall, the ab initio and experimental solution structures are quite similar. Only 5 out of 16 bond and dihedral angles that were optimized during the calculation of the liquid-state structure differ by more than one standard deviation, and the largest deviation is less than 3.3° (Table 2). This proportion is statistically reasonable. The remaining 11 angles are identical to within 1°. A similar comparison of the solution and the neutron diffraction structures shows slightly larger ($\leq 4.6^\circ$) and more frequent (seven angles differing by $> 1^\circ$) differences in bond and dihedral angles. It is intriguing that four out of five parameters that differed significantly between the solution-state and the ab initio structure show also large differences when the solution-phase and the neutron diffraction structures are compared. Comparison of these deviations suggests that the solution structure lies between the solid-state and the gas-phase ab initio structures and is closer to the latter. Figure 6b shows an overlay of the calculated solution and ab initio structures, and the same comparison for the solution and neutron diffraction structures is shown in Figure 6c. The different orientation of the OH groups in the neutron diffraction structure is caused by the network of hydrogen bonding observed in the solid state.¹⁸ These diagrams show clearly the differences in the 1,3 diaxial proton distances.

From Table 2, it can be seen that one of the larger differences between the solution structure and the ab initio structure is $\phi_{C_2C_3C_4C_5}$, which dictates the overall conformation of the ring and therefore the position of the protons relative to each other. Long-range coupling constants therefore have an important effect on the structure refinement. Other torsions with significant differences include $\phi_{C_3C_2C_1H_1}$ and $\phi_{C_2C_3C_4H_4}$, the proton positions of which are determined from couplings including the long-range D_{H_1, H_4} coupling constant. The C₂C₁H₁ angle also deviates from the ab initio value, but the ${}^2D_{H_1, C_2}$ coupling constant was not measured. Therefore, the position of H₁ is dictated by other long-range couplings within the molecule, and this has an effect on the local parameters.

There is a fine balance between the number of parameters that can be refined and the number of data observations present. Effectively, the parameter limit is $N_D - N_S$ (where N_D is the number of dipolar coupling constants and N_S is the number of order-matrix elements). As five order-matrix elements were being refined, we were obliged to fix five potentially refinable parameters to compensate for this, and there is therefore a theoretical maximum of 25 parameters that can be refined at any one time. In practice, the maximum number of refinable parameters to obtain a reliable result is smaller by one, i.e., 24.

This number is further reduced by correlations between observations (which may effectively give the same, or similar, structural information). The distance parameters were chosen to be fixed at the ab initio values, because the ratios of these relative to one another are very well determined by theory. One angle parameter could not be refined, as it was effectively holding the sugar ring together and there was not enough residual information to refine its value once the other angle and torsion parameters were refining. At the time of the final refinement, the five order-matrix elements and 16 of the possible 24 parameters were refining. As a test, it was attempted to refine the C–C distances as a fixed ratio to one C–C distance, but this led to an unrealistic C–C distance and unrealistic estimated standard deviations. Therefore, we believe that we have extracted all possible structural information for this molecule from the vibrationally corrected dipolar coupling constants, to give the first structure of a sugar in solution.

Conclusions

We have demonstrated that it is possible to use very small residual dipolar coupling constants, measured in dilute liquid crystalline media, for the determination of solution structures of small molecules. We have measured three types of residual dipolar coupling constants, D_{HH} , ${}^1D_{CH}$, and ${}^nD_{CH}$, in order to generate sufficient experimental data to determine the molecular parameters that define the molecular structures. The incorporation of ${}^nD_{CH}$ coupling constants was crucial in the case of methyl β -D-xylopyranoside, but these coupling constants are likely to be required for any small molecule, because the proton–proton and one-bond heteronuclear interactions alone will usually be too few to allow optimization of numerous molecular parameters. To this end, we have developed new NMR experiments for precise measurement of long-range proton–carbon dipolar coupling constants. This technique extends the family of J -modulated experiments designed for accurate measurement of small dipolar coupling constants. We emphasize that these techniques provide accurate dipolar coupling constants only for first-order spin systems that satisfy the following conditions: $\Delta\delta/K_{HH} > 5$ or $(\Delta\delta - 0.5 {}^1K_{CH})/K_{HH} > 5$ for 1H or ${}^1H, {}^{13}C$ satellite spectra, respectively, where $\Delta\delta$ is the chemical shift difference between coupled protons and K is either J or $J + D$. If these conditions are met, the dipolar coupling constants can be determined by fitting the signal intensities to simple transfer functions. This was the case for all the coupling constants used in our analysis, and in order to achieve this we have resorted to using an 800 MHz NMR spectrometer. Otherwise, a full density-matrix treatment must be invoked, and further work is needed in this direction to make these techniques applicable to more strongly coupled spin systems and/or more widely used lower field NMR instruments. The use of selective pulses required for the measurement of the D_{HH} and ${}^nD_{CH}$ coupling constants may seem to be a further limitation of the proposed approach, but it is likely to be covered by the above condition of sufficient chemical shift separation. Overlap of proton resonances between spins from different spin systems should not necessarily be an obstacle to successful application of these techniques. The experimental uncertainties of the signs and sizes of very small scalar long-range proton–proton coupling constants is the limiting factor affecting the accuracy of determined dipolar coupling constants. Further method development in this area is required.

The use of ab initio methods has allowed the calculation of a force field for methyl β -D-xylopyranoside. From this, it has been possible to correct the experimental dipolar coupling constants for vibrational effects. The ab initio results have also provided an excellent starting point for the determination of the solution structure of methyl β -D-xylopyranoside. Using the corrected values of D^{α} as extra pieces of experimental observations, it was possible to use the Edinburgh programs,²⁹ usually better known for determining gaseous molecular structures, to determine the solution structure of methyl β -D-xylopyranoside. Although very small dipolar coupling constants were measured in this work, their calculated vibrational corrections often exceeded the experimental uncertainties in the values of the constants. Vibrationally corrected dipolar coupling constants must therefore be used in such structure refinements.

Reducing the orientation of solutes thus provides a general method for the determination of solution-phase structures of small molecules. This approach requires fine-tuning of the alignment, so that various dipolar coupling constants, including those of long-range coupled protons and carbons, can be measured with sufficient accuracy. With the availability of very high field NMR instruments, it is foreseeable that structures of molecules containing more than 12 protons, a limit imposed by the complexity of ^1H spectra in strongly aligning liquid

crystals, can be determined using this approach. Indeed, as the number of dipolar couplings increases with molecular size, but the number of orientation parameters is constant, the potential value of the method is greatest for large molecules.

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Supporting Information Available: Pulse sequence of the ω_1 ^{13}C -filtered COSY experiment used for the sign determination of $^2J_{\text{CH}}$ coupling constants, ω_1 ^{13}C -filtered COSY spectrum of methyl β -D-xylopyranoside, least-squares correlation matrix for the final refined structure of methyl β -D-xylopyranoside, coordinates of the solution-phase structure of methyl β -D-xylopyranoside, and ab initio coordinates from the B3LYP/6-311+G* calculation of methyl β -D-xylopyranoside. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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