

Conformational studies of polymorphic *N*-octyl-D-gluconamide with ^{15}N (labeled) ^{13}C (natural abundance) REDOR spectroscopy

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Magnetic dipolar couplings between the ^{15}N atom (labeled) and neighboring ^{13}C atoms (natural abundance) in three solid modifications of *N*-octyl-D-gluconamide are measured with rotational echo double resonance (REDOR). A unique spectral assignment of ^{13}C resonances is possible by means of their dipolar dephasing. While in the monolayer crystal and in the fiber modification the assignment is amenable to the solution spectra, in the bilayer crystallites a different assignment is found. The dipolar couplings in the range 45 to 1220 Hz are converted into CN distances. These distances are employed in conjunction with the ^{13}C chemical shieldings of the CP-MAS spectra to determine sets of possible torsion angles, which define the molecular conformation in the neighborhood of the amide group. In contrast to the monolayer crystal, for the fiber and bilayer crystallite modifications a *gauche* bend at the C2–C3 bond is found, giving the molecules the shape of a ${}_2\text{G}$ sickle.

Introduction

Open chain carbohydrate derivatives containing secondary amide groups form a large variety of fibrous assemblies in water due to hydration of chiral centers and strong linear amide hydrogen chains.^{1–4} In order to understand the mechanism of the creation of micelles in this group of compounds, it is important to know their individual molecular conformation, which is related to the supermolecular arrangement. Therefore, the correlation of (chemical) molecular structure and supermolecular structure has been studied systematically on *N*-octyl-D-hexonamides.^{3,4}

While it was feasible to directly determine the conformations of the carbohydrate chain of the class of well crystallizing compounds by X-ray diffraction, the non- or poorly crystallizing solids were analyzed by an indirect approach employing isotropic ^{13}C chemical shielding (CS) data obtained from ^{13}C CPMAS solid state NMR spectra. Structural information was obtained comparing these data to ^{13}C CS of similar hexonamides with known conformation. The latter approach is based on the availability of a large number of crystal structures of different conformers within the closely related classes of compounds, namely of open-chain glyconamides, glycosylesters and -acetals. The spectral assignment of their ^{13}C atoms was performed by virtue of the ^{13}C CS in solution.

However, as will be shown in detail below, the sensitivity of ^{13}C CS to conformational features⁵ or hydrogen bonding may cause an interchange of signal positions in CP-MAS spectra due to different secondary structures. Thus, solution CS values by no means guarantee safe assignments of the resonance frequencies in the solid modifications and the correlation of the liquid-state assigned ^{13}C NMR signals with related crystal data can only in fortunate cases yield the real conformation of the molecule.

In contrast, dipolar solid state NMR spectroscopy can give direct insight into the conformation of a molecule in the solid

state and does not depend on the availability of suitable single crystals.^{6,7} Two prerequisites are needed for a successful conformational analysis by dipolar solid state NMR: (i) a high spectral resolution to assign individual signal lines to particular atomic positions in the molecule by virtue of their isotropic CS and (ii) a network of adjacency, which contains the information about the interatomic distances.

High resolution is achieved by application of the magic angle spinning (MAS)⁸ technique, often in combination with cross polarization (CP-MAS)⁹ from protons to enhance the signal of the X-nuclei. CP-MAS spectra obtained in this way do not contain direct geometrical information. Therefore, in recent years an impressive number of experiments has been invented to achieve dipolar recoupling under MAS. These experiments allow the measurement of dipolar couplings under high resolution. A review of some of these techniques can be found in Bennett *et al.*¹⁰ The basic idea of these recoupling experiments is to periodically disturb the evolution of the spin system by rotor-synchronized RF pulses. For heteronuclear systems of two X-nuclei, the rotational echo double resonance (REDOR) experiment^{11–17} is of particular importance. This method allows the recoupling of dipolar interactions between different X-nuclei (for example ^{15}N and ^{13}C) by periodically inverting the sign of the heteronuclear dipolar interaction.

Most REDOR studies have been performed by investigating either doubly labeled systems or systems of abundant spins. However, REDOR spectroscopy allows also the combination of a single spin label, for example a ^{15}N nucleus inserted in a molecule, with low abundance spins of another species, for example ^{13}C in natural abundance.¹⁸ These ^{15}N (labeled) ^{13}C (natural abundance) REDOR experiments have several important advantages: (i) expensive and often unfeasible selective ^{13}C isotope labeling is avoided; (ii) a single REDOR experiment gives several dipolar couplings, *i.e.* distances, (iii) distortions of the evolution of the REDOR decay due to ^{13}C – ^{13}C homonuclear interactions in a multi- ^{13}C labeled system are avoided. The problem with this ^{13}C REDOR approach is the low signal to noise ratio of ^{13}C (natural abundance) spectra of large molecules, in particular

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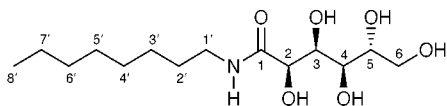


Fig. 1 ^{15}N -octyl-D-gluconamide (GA).

at low or medium B_0 fields. Therefore, a careful operation with the magnetization in the observation channel is required, in particular if small couplings, *i.e.* large distances, are desired.

In the present study the molecular conformations of three modifications of ^{15}N -octyl-D-gluconamide (GA, Fig. 1) in the vicinity of the amide ^{15}N label are studied by a combination of ^{13}C (natural abundance) ^{15}N -labeled REDOR NMR and ^{13}C CP-MAS spectroscopy. GA is a typical example of a polymorphic *N*-octylhexonamide. It forms various gels and fibers of different structures in water.¹⁹ It exhibits structural polymorphism in the solid state. Three modifications are known (Fig. 2): (a) a head-to-tail monolayer crystal²⁰ (GA I, Fig. 2a); (b) tail-to-tail bilayer crystallites² (GA II, Fig. 2b); (c) a micellar quadruple helix^{3,4} (GA III, Fig. 2d). This helix consists of stacked elliptical arrangements of 24 molecules (Fig. 2c).

The structure of GA I has been determined by X-ray spectroscopy.^{20,21} The GA II crystallites were originally found in ^{13}C CP-MAS spectra¹ after tempering a sample of GA I at 80 °C for a short time. Due to their poorly crystallizing behavior it was not possible to achieve single crystals suitable for X-ray structure determination. The micellar fibers of GA III are formed in bulk water and have been isolated in the solid state by lyophilization. By electron microscopy it was found that these fibers have a bimolecular thickness and form extended quadruple helices.³ The geometrical structure of the amide group in GA III was studied recently by dipolar chemical shift NMR spectroscopy.²² The GA III modification is not amenable to X-ray diffraction analysis, due to its non-crystalline nature.

The driving force of the polymorphism in GA is an interplay of strong hydrogen bond interactions between the sugar head group of GA with hydrophobic/hydrophilic interactions

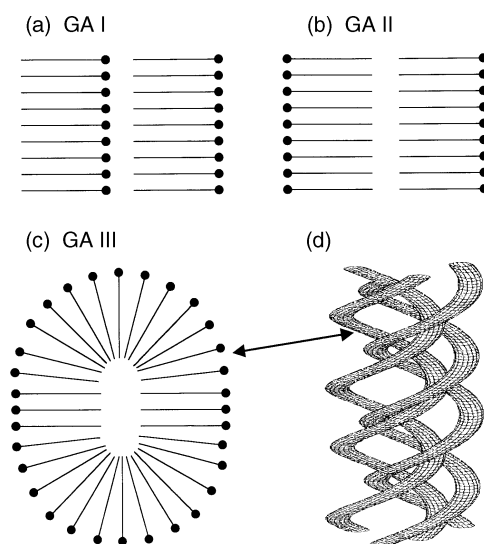


Fig. 2 Schematic superstructures of *N*-octyl-D-gluconamide in its different modifications, the black discs represent the sugar head groups and the lines the alkyl chains. (a) Simple monolayer crystal with linear head-to-tail arrangement of the molecules (GA I). (b) Bilayer crystallites with head-to-head arranged molecule pairs (GA II). (c) Micellar fiber modification (GA III) showing a tail-to-tail arrangement of 24 gluconamide molecules, which gives long fiber structures intertwining to the extended quadruple helix (d). The X-ray structure is known only for GA I.

of the head and tail chains with the solvent in the course of the preparation.

We demonstrate that it is feasible to evaluate quantitatively the dipolar dephasing without the necessity of ^{13}C isotope enrichment, even for such a relatively large molecule: From the ^{13}C (natural abundance) REDOR experiments, a faithful and unambiguous assignment of the isotropic CS in all three investigated modifications is obtained. These CS data in conjunction with the distances calculated from the REDOR dephasing curves are employed for determining the molecular conformations.

The rest of the article is organized as follows. First a brief survey of the REDOR technique is given. Then a short summary of our experimental setup, sample synthesis and preparation follows. Next the experimental results are presented, discussed and finally summarized.

The ^{13}C (natural abundance) ^{15}N (labeled) REDOR experiment

In the REDOR^{10–12} experiment the dipolar coupling between two spin-half heteronuclei is recovered under magic angle spinning by the irradiation of two π -pulses during one rotor period, the first one at time τ and the second one at the end of the rotor period (T_r). The recoupled dipolar frequency of a specific spin packet depends on the orientation of the dipolar vector with respect to the external field, defined by the polar angles α, β ,

$$\varpi_D(\alpha, \beta, \tau) = \frac{1}{T_r} \left\{ \int_0^\tau \omega_D(\alpha, \beta, \tau') dt' - \int_\tau^{T_r} \omega_D(\alpha, \beta, t') dt' \right\} \quad (1)$$

with:

$$\omega_D(\alpha, \beta, t) = \pm \frac{1}{2} D \{ \sin^2 \beta \cos 2(\alpha + \omega_r t) - \sqrt{2} \sin 2\beta \cos(\alpha + \omega_r t) \} \quad (2)$$

The heteronuclear dipolar coupling D , which depends on the distance (r) of the two nuclei I and S as

$$D = \frac{\mu_0}{4\pi} \hbar \frac{\gamma^I \gamma^S}{r^3}, \quad (3)$$

contains the structural information.

The measured REDOR signal S_R can be expressed as a function of the number N of applied REDOR cycles and the spinning frequency $1/T_r$:

$$S_R(\alpha, \beta) = \text{Tr}(\rho(\alpha, \beta) S_x) = \cos\{\varpi_D(\alpha, \beta, T_r/2) N T_r\} \quad (4)$$

For non-oriented powder samples the integral over the polar angles α, β has to be taken, yielding the REDOR signal:

$$S_R = \frac{1}{4\pi} \int_{\alpha, \beta} S_R(\alpha, \beta) \sin \beta d\beta d\alpha \quad (5)$$

The most efficient dipolar recoupling is achieved if the first pulse is irradiated at the center of the rotor cycle, *i.e.* $\tau = T_r/2$. In this case there exists an analytical expression for the REDOR signal ($J_n(x)$: cylindrical Bessel function of the first kind; n : number of rotor cycles),

$$S_R(nv_D T_r) = \frac{\pi}{2\sqrt{2}} J_{1/4}(n\sqrt{2}v_D T_r) \times J_{-1/4}(n\sqrt{2}v_D T_r) \quad (6)$$

and a direct transformation of the decay function from the time domain into the frequency domain,^{23,24} the so-called REDOR transform.

To take relaxation processes during the REDOR evolution periods (nT_r) into account, a reference experiment under the same conditions, but without recoupling pulses is performed.

With the reference signal S_0 the normalized dipolar dephasing is given as:

$$\frac{\Delta S}{S_0} = \frac{S_0 - S_R}{S_0} \quad (7)$$

Experimental

The spectrometer

A detailed discussion of our home built three channel NMR spectrometer has been given recently.^{25,26} Here only some salient features are reproduced. All experiments were performed at a magnetic field of 6.98 T, corresponding to a proton resonance frequency of 297.8 MHz on a standard Oxford wide bore magnet (89 mm) equipped with a room temperature shim unit. For the ^1H channel a Creative Electronics 1 kW class C amplifier was used. For the ^{13}C -channel a 1 kW class AB and for the ^{15}N -channel a 2 kW class AB amplifier, both from AMT, were employed. All amplifiers are equipped with an RF blanking for suppressing the noise during data acquisition. All experiments were performed using a 7 mm Bruker triple resonance NMR probe operating at room temperature. To improve the mutual RF isolation of the three channels, commercial band pass filters (Texscan) in conjunction with home built notch filters were employed. The RF of the observed channel was fed through a crossed diode duplexer, connected to the detection preamplifier and through the filters into the probe. The other two channels were fed directly through the filters into the probe. A typical 90° pulse width was 6.5 μs for all three channels, corresponding to 38 kHz B_1 -field in frequency units for the cross polarization and REDOR pulses. For the proton decoupling the ^1H - B_1 was changed to 52 kHz to avoid unwanted cross polarization by mismatching the Hartmann Hahn condition and to improve the decoupling efficiency. This was sufficient to remove ^1H -X dipolar line broadening. The rotation frequency was controlled by a Doty spin rate controller. Deviations in the rotation frequency were below 2 Hz. The spectra were measured by first cross polarizing the observed nucleus from the protons and then recording the signal of the observed X nuclei under proton decoupling. Repetition time of the experiments was 3 s for the GA. The referencing of the ^{13}C spectra was accomplished by using adamantane as a secondary reference. All experiments were performed at 5 kHz rotation speed, which is fast enough to suppress all rotational side bands with the exception of the amide carbon C(1).

In the case of the ^{13}C (natural abundance) ^{15}N (labeled) GA, relatively large distances are studied in a diluted ^{13}C spin system. Therefore, a variant of the REDOR experiment is applied, where only a single echo pulse is given in the ^{13}C (observing) channel and all other recoupling pulses are applied in the ^{15}N (dephasing) channel. A mirror-symmetric pulse scheme, where N is stepwise increased in units of two rotor cycles, was found to yield the most reliable dipolar dephasing (Fig. 3). The compensation of pulse imperfections is ensured by XY-4 phase cycling.^{27,28} The known distances of GA I are employed as external standards for controlling the REDOR NMR distances determined in the other two modifications.

The decay of the echo spectra is evaluated by adjusting the dipolar coupling constant in eqn. (5) and eqn. (6), respectively, to match the observed REDOR curve. The errors of the determined dipolar couplings have been estimated by determining upper and lower limits of the dipolar coupling strengths in such a way that 70% of the experimental data are enclosed within the limiting curves.

Samples and preparation

The synthesis of selectively ^{15}N labeled GA samples is described in detail in ref. 29. The isotope enrichment was

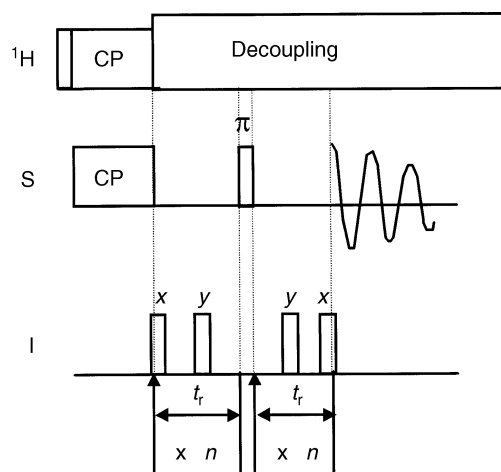


Fig. 3 Pulse Sequence of the REDOR experiment, which provides the least distorted observation of the ^{13}C spins in natural abundance. The recoupling pulses in the ^{15}N dephasing channel were phase cycled in a mirror symmetric XY-4 scheme.

better than 99% using ^{15}N -ammonia produced from ^{15}N -ammonium chloride. The GA I modification was prepared by crystallizing the solution from ethanol. The GA II sample was prepared by packing of the rotor with GA I and then heating above 90° . The GA III fibers were produced by rapid cooling of a 5% (w/v) aqueous gel in liquid nitrogen followed by freeze-drying. The material was placed into a standard 7 mm Bruker rotor closed with Kelf caps, which sealed the sample sufficiently tight to prevent structural modifications by moist air. Due to the size of the molecule, no isotope dilutions were necessary for the suppression of intermolecular ^{15}N - ^{15}N dipolar interactions.

Results and discussion

Experimental results

In a first step the ^{13}C CP-MAS spectra of the three samples have been recorded. Fig. 4 shows the conventional ^{13}C (natural abundance) CPMAS spectra of the investigated GA I, GA II and GA III samples. The spectral regions corresponding to the chemically different types of carbons in the molecule

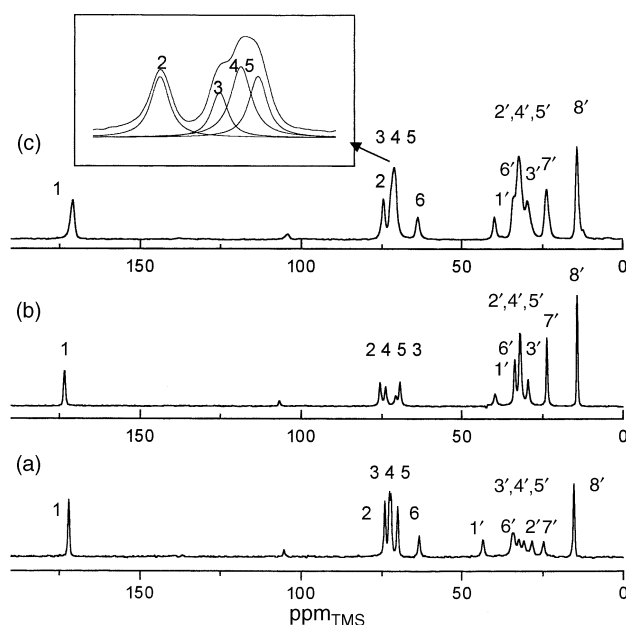


Fig. 4 ^{13}C (natural abundance) CP-MAS spectra of GA. GA I (a); GA II (b); GA III (c).

are clearly resolved. All carbon positions are visible in the spectra with the exception of the terminating carbon C6 in the GA II. This carbon has an unfortunate T_2 relaxation time at room temperature due to molecular motions.¹ The assignment of the spectral line positions to individual carbons was performed by means of the REDOR dephasing (see below). The ^{13}C CP-MAS spectra display significant CS differences between the investigated modifications, both in the range of the sugar head group and in the range of the alkyl tail group carbons.

In the CP-MAS spectrum of GA II, all carbon signals are well resolved, except for the C2', C4' and C5' signals of the alkyl tail group. In particular the neighboring carbons of the ^{15}N (C1, C2, C3, C4, C1', C3') are well separated and allow spectrally resolved REDOR experiments.

In the GA III sample the individual lines exhibit a strongly increased line width, as compared to the GA II sample, resulting in a stronger line overlap in both sugar and alkyl chain. Differences in the individual CS of the ^{13}C resonances in the alkyl and sugar region are observed. The assignment of the signal lines to individual resonances has been performed by means of the REDOR experiment (see below). The spectral deconvolution of the overlapping signals at 70 ppm resolves the signals of C3, C4 and C5, as shown in the insert in Fig. 4.

The spectrum of the GA I sample exhibits a narrow line width. In the sugar region the spectrum is similar in appearance to that of the GA III sample. In the alkyl region the largest spread of CS values is observed.

Comparing the three ^{13}C CP-MAS spectra, an important difference in the isotropic CS of the sugar carbons is evident: In GA I and GA III the CS values of the sugar carbons are ordered as C2, C3, C4, C5. In GA II the CS values are ordered as C2, C5, C4, C3, *i.e.* the relative positions of C3 and C5 are interchanged.

In the next step the three modifications are studied by ^{15}N (labeled) ^{13}C (natural abundance) REDOR spectroscopy. As an example of the REDOR experiments Fig. 5 displays the 2D REDOR spectrum of the GA II sample. The horizontal axis corresponds to the normal ^{13}C CS frequency and the vertical axis to the dipolar ^{15}N - ^{13}C frequency. In this 2D spectrum the signal reduction due to dipolar dephasing is visible in the width of the spectral lines in the dipolar frequency dimension. The dipolar interaction is clearly visible for the resolved signals of C1, C2, C3, C4, C1', C3', in particular for the two

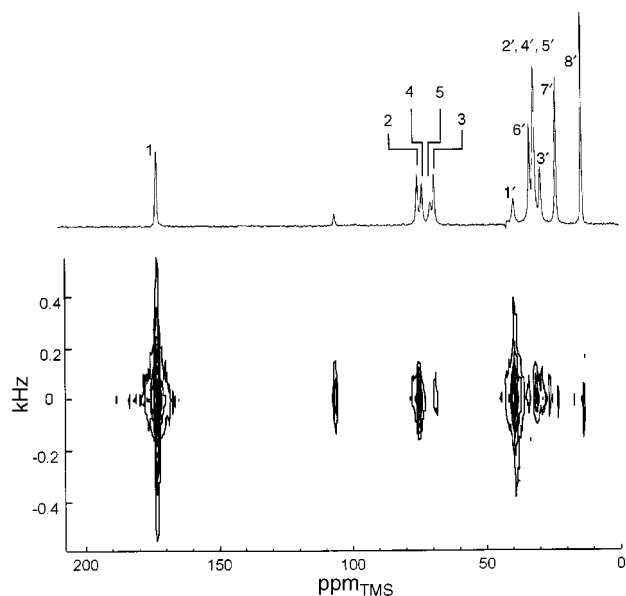


Fig. 5 2D dipolar ^{13}C (natural abundance) ^{15}N (labeled) spectrum of GA II. The ^{13}C - ^{15}N dipolar couplings are visible in the width of the peaks in the dipolar dimension.

carbons C1 and C1', which are directly bound to the ^{15}N . Moreover, the dipolar dephasing is visible also for the unresolved carbons C2' and C4' in the dipolar dimension.

In principle it would be possible to obtain a more quantitative representation of the 2D spectrum by performing a REDOR instead of a Fourier transformation in the dipolar dimension. However the increasing oscillatory behavior of the kernel of the inverse REDOR transformation resulted in strong wiggles in the REDOR spectrum, which prevented a quantitative evaluation of the signals in this way. Therefore for the quantitative evaluation it is advantageous to evaluate the REDOR echo decay curves instead of the REDOR spectra. This is especially true for small dipolar couplings, which give only a very small contribution to the line width, respectively only a weak decay of the echo, observable in the time window of the T_2 relaxation time.

In the GA I sample the distances calculated from the dipolar dephasing (not shown) of the C1, C2 and C3 signal lines agree well with the corresponding distances found in the X-ray crystal structure,^{20,21} showing the reliability of the REDOR distances. The decay of the C4 signal line is too weak to allow for a quantitative evaluation of the REDOR dephasing within the 15 ms REDOR evolution time. A longer REDOR evolution time than 15 ms suitable for an observation of extremely weak couplings could not be achieved, due to the T_2 signal relaxation.

In the GA II and GA III sample a dephasing of the C1, C2, C3 and C4 signals is observed and quantitatively evaluated. This is already an indication of a conformational difference in the vicinity of the amide group between GA II and GA III on the one side and GA I on the other side. Fig. 6 displays the experimental and fitted REDOR echo decay curves of GA II and GA III for C1, C2, C3 and C4 of the head group and C1' and C3' of the tail group. The partly overlapping lines C2, C3, C4, C5 in the ^{13}C (natural abundance) spectrum of the GA III sample at 70 ppm have been evaluated by spectral deconvolution into individual Lorentz lines, as shown in the insert in Fig. 4.

The carbons C1 and C1' directly bound to ^{15}N exhibit very strong dephasing already after 0.8 ms evolution time, then the typical oscillations and finally total dephasing after 4 ms follow. The line of the C2 carbon exhibits strong dephasing after 5 ms and the typical overshoot at 8 ms, which corresponds to the dipolar oscillation. Due to T_2 relaxation, the full leveling to the final value is not visible in the decay curve. For the more weakly coupled carbons C3, C3' and C4, only the initial part of the decay curves is visible, due to T_2 relaxation. The unresolved C2', C4' and C5' alkyl carbon lines in the spectra of both GA II and GA III could not be deconvoluted by line shape analysis, due to the following reasons: (i) Their chemical shielding differences are too small to allow a direct deconvolution of the lines. (ii) The relative intensities of the signals of these three carbons are not known, due to possibly different cross polarization efficiencies or differences in their T_2 relaxation times. (iii) The expected differences in their dipolar couplings are not large enough to allow an exact separation of the carbons by REDOR.

Discussion

From the REDOR assignment of the individual carbon resonances, differences in the spectral line positions in the sugar head group are evident. For the GA III the REDOR assignment of the ^{13}C head group signals corresponds to the assignment which has been accomplished by solution NMR.¹ The data show an increasing strength of the dipolar coupling correlated with a lower field signal position, *i.e.* the carbons more close to the amide nitrogen exhibit a low field shift. Comparing this to the GA II sample, an interchange of the signal lines C3, C4 and C5 is evident. For the isotropic CS of the tail

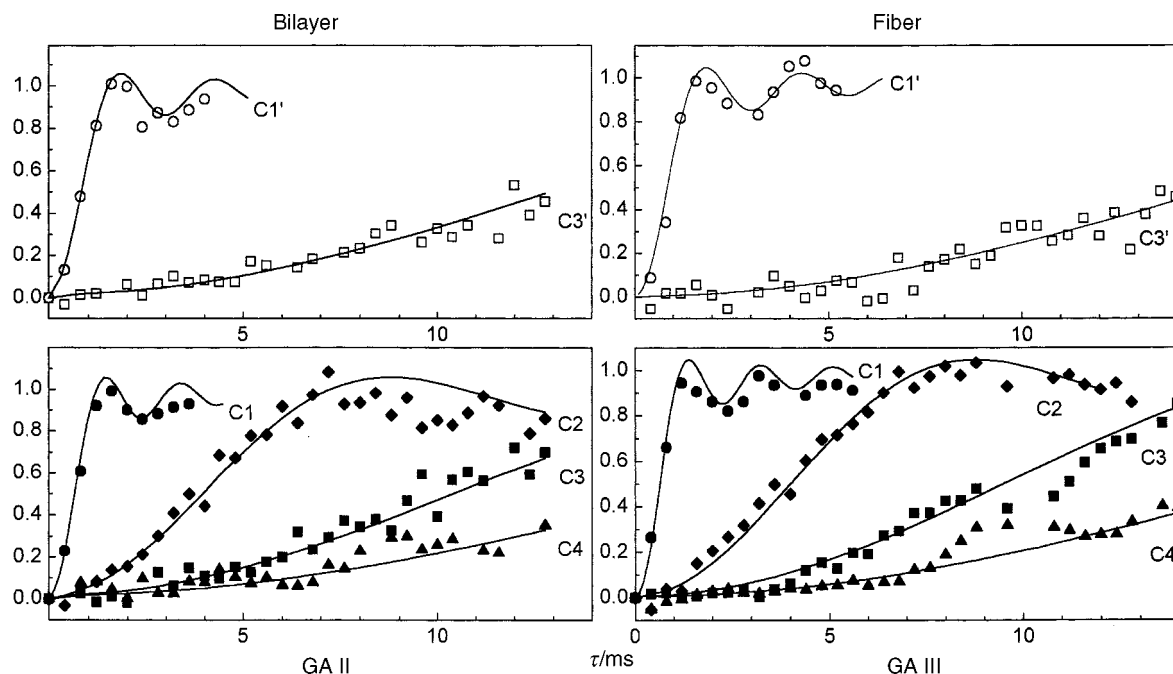


Fig. 6 ^{13}C (natural abundance) ^{15}N (labeled) REDOR of spectrally resolved carbons in GA II and GA III. Upper panel: alkyl chain, lower panel: sugar head group. The symbols mark the experimental points and the solid lines the fit of these data.

group signals, no significant differences are found between the GA II and GA III sample.

The dipolar couplings determined with the REDOR experiments and the corresponding distances are summarized in Table 1. The CN distances of carbons C1, C2 and C3 of the GA I sample are in good agreement with the X-ray data. The very weak dephasing of C4 is compatible with the CN distance of 4.83 Å found by X-ray analysis. This distance corresponds to a dipolar ^{13}C - ^{15}N coupling of 28 Hz, which is not observable within the dipolar evolution period of our REDOR experiments.

For the sugar head group of GA II and GA III the couplings to C1, C2, C3 and C4 have been elucidated. The observed couplings range from 45 to 1120 Hz. They correspond to CN distances in the range from 4.1 down to 1.40 Å. For the alkyl chain, only the couplings to the α -carbon C1' and to the γ -carbon C3' have been determined. Due to line overlap, the coupling to the β -carbon C2' and the δ -carbon C4' could not be determined. Comparing the distances from the REDOR experiment to the values obtained from X-ray structure analysis of GA I, the following results are evident.

For the alkyl chain, the distances to the resolved carbons C1 (bilayer: 1.49 ± 0.01 Å; fiber: 1.50 ± 0.02 Å) and C3 (bilayer: 3.74 ± 0.06 Å; fiber: 3.93 ± 0.15 Å) of both investigated modifications are very close to the X-ray values of the GA I (1.47 Å, respectively 3.82 Å, *i.e.* a nearly all-*trans* conformation). The all-*trans* conformation corresponds to the longest possible distance between the amide nitrogen and the carbon C3. From this it can be concluded, that the alkyl chain has a similar structure in GA II and GA III as in GA I, *i.e.* the chain is close to an all-*trans* conformation.

For the sugar head group differences between the X-ray and REDOR distances of GA I on the one hand and the REDOR data of GA II and GA III on the other hand are found: The distances from the amide nitrogen to carbons C1, C2 and C3 of GA II and GA III are close to the corresponding distances found for GA I. However, the C4 signal of both GA II and GA III reveal a dipolar coupling of 45 Hz, corresponding to N-C4 distance of 4.07 Å. This distance is *ca.* 0.8 Å shorter than the N-C4 distance of 4.83 Å in the GA I.

In principle there are two different explanations for this difference in the N-C4 distance: (i) Strong motional effects

Table 1 Dipolar interactions and corresponding distance vectors from ^{13}C (natural abundance) ^{15}N (labeled) REDOR of GA II (A) and GA III (B), compared to the X-ray data of GA I (C)^{20,21}

		C1	C2	C3	C4	C1'	C2	C3'	C4'
(A)	D/Hz	1120 (50)	190 (10)	72 (3)	45 (5)	920 (20)	—	58 (3)	—
	$r/\text{Å}$	1.40 (0.02)	2.47 (0.04)	3.48 (0.04)	4.07 (0.15)	1.49 (0.01)	—	3.74 (0.06)	—
(B)	D/Hz	1120 (50)	190 (10)	80 (7)	45 (5)	900 (30)	—	50 (5)	—
	$r/\text{Å}$	1.40 (0.02)	2.47 (0.04)	3.36 (0.10)	4.07 (0.15)	1.50 (0.02)	—	3.93 (0.15)	—
(C) X-ray REDOR	$r/\text{Å}$	1.32	2.45	3.4	4.83	1.47	2.48	3.82	5.01
	D/Hz	1200 (50)	210 (10)	80 (10)	—	900 (30)	—	—	—
	$r/\text{Å}$	1.36 (0.02)	2.44 (0.04)	3.45 (0.15)	>4.2	1.50 (0.02)	—	—	—

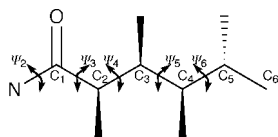


Fig. 7 Definition of the torsion angles $\Psi_2 \dots \Psi_6$ which determine the conformations of the head groups.

change the value of the effective dipolar coupling constant. (ii) A change of the torsion angles Ψ_3 or Ψ_4 has occurred, *i.e.* a rotation around the C1–C2 or C2–C3 bond direction (see Fig. 7 for the definition of the torsion angles). To solve this question, the following arguments can be employed:

(1) From the studies of similar compounds,³⁰ it is known that strong intermolecular hydrogen bonds between the sugar head groups of adjacent molecules exist, which fix the supermolecular structure and immobilize the inner sugar carbons.

(2) The distances between the amide nitrogen and the carbons C1, C2 and C3 agree with the distances from X-ray structure analysis. It follows that only the outer carbons C4, C5 and C6 can be influenced by this molecular vibration. To achieve an increase of the effective dipolar coupling, it is necessary that the vibration causes an oscillation of the N–C4 distance, because the $\langle 1/r^3 \rangle$ averaging of the dipolar interaction favors the shorter distances.

Assuming a harmonic stretching vibration of the N–C4 distance, a simple estimation of the amplitude necessary to explain the observed difference of 0.8 Å, results in a value of *ca.* 1.7 Å. A vibration with such an amplitude would destroy the molecule and can be safely excluded. It follows that the differences between X-ray crystal structure and REDOR data are caused by structural reasons, *i.e.* changes of the torsion angles. Such torsional rotations produce sickle shaped sugar groups for GA II and GA III compared to the nearly linear shape of GA I.

The number of available REDOR distances is not sufficient to directly determine possible molecular conformations. Therefore, the following assumptions are made for the interpretation of the distance data: (i) Bond lengths and bond angles are taken from a geometry optimization using a molecular modeling program (GAUSSIAN94).³¹ The calculated bond lengths and bond angles are in good agreement with those of the monolayer crystal, determined by X-ray diffraction.^{20,21} (ii) The structure of the amide group is taken from ref.²²

Using these assumptions there is one degree of freedom left for the position of C3, the N–C3 torsion angle (Ψ_3), and two degrees of freedom are left for the position of C4, the N–C3 and C1–C4 torsion angles (Ψ_3 and Ψ_4). Fig. 8 shows a Ramachandran-type plot of the calculated CN distances as a function of the torsion angles Ψ_3 and Ψ_4 for the sugar group of GA II and GA III. Four different pairs of angles are found corresponding to two pairs of symmetry related molecular conformations. A similar result is found for the sugar group of GA III. The resulting pairs of torsion angles are summarized in Table 2.

Further insight into the head group conformations of GA II and GA III can be gained by inspecting their CS values. Assuming that mainly the γ -*gauche* effect⁵ of the γ -neighborhood

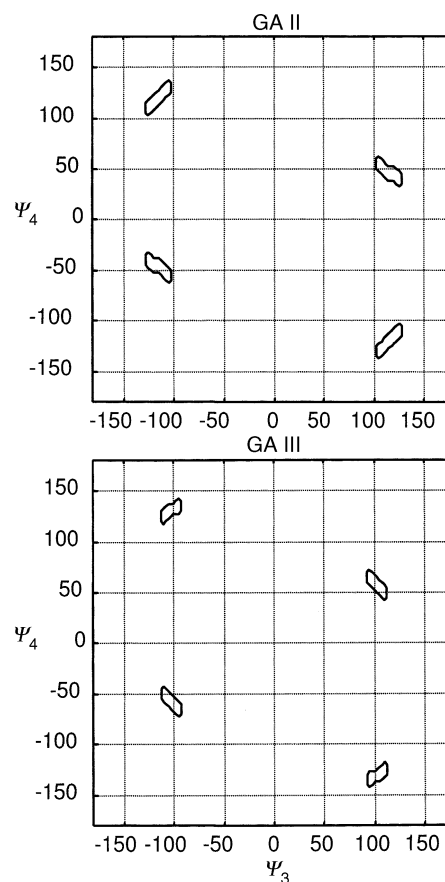


Fig. 8 Ramachandran-type plots of the possible torsion angle pairs Ψ_3 and Ψ_4 (in degree) for the GA II and GA III sugar group conformation.

heavy atoms in the sugar chain is responsible for different CS values in the ^{13}C CP-MAS spectra, we can correlate changes of the ^{13}C CS to variations in the torsion angles Ψ_3 and Ψ_4 . (Note: We use the convention of ref. 32, *i.e.*: *cis* = 0°, *anti* = 60°, *gauche* = 120° and *trans* = 180°, and not the definition of ref. 5, which is shifted by 180° with respect to the other convention, *i.e.*: *trans* = 0°, *etc.*)

Table 3 lists the ^{13}C CS values and their differences for C1, \dots C5 and those torsion angles which influence the CS *via* the γ -*gauche* effect. The torsion angles Ψ_1 and Ψ_2 are known from the structure of the amide group and the nearly all-*trans* conformation of the alkyl chain. The largest CS difference between GA II and GA III is found for carbon C3, a high field shift of -2.9 ppm. The CS of C3 is influenced by Ψ_3 and Ψ_6 .

Table 2 Possible pairs of torsion angles for the gluconamide modifications

Modification	Ψ_3	Ψ_4
GA II	$+113^\circ \pm 15^\circ$	$+43^\circ \pm 15^\circ$; $-113^\circ \pm 15^\circ$
	$-113^\circ \pm 15^\circ$	$-43^\circ \pm 15^\circ$; $+113^\circ \pm 15^\circ$
GA III	$+100^\circ \pm 15^\circ$	$+53^\circ \pm 15^\circ$; $-123^\circ \pm 15^\circ$
	$-100^\circ \pm 15^\circ$	$-53^\circ \pm 15^\circ$; $+123^\circ \pm 15^\circ$

Table 3 Resolved ^{13}C chemical shifts of the sugar group carbons of GA II and GA III, CS difference between these modifications and torsion angles which influence these shifts *via* the γ -*gauche* effect

	C1	C2	C3	C4	C5
GA II/ppm	173.6	75.7	69.4	74.0	70.7
GA III/ppm	171.5	74.5	72.3	71.5	70.9
$\Delta\sigma$ /ppm	+2.1	+1.2	-2.9	+2.5	-0.2
γ - <i>gauche</i> torsion angles	Ψ_1, Ψ_4	Ψ_2, Ψ_5	Ψ_3, Ψ_6	Ψ_4	Ψ_5

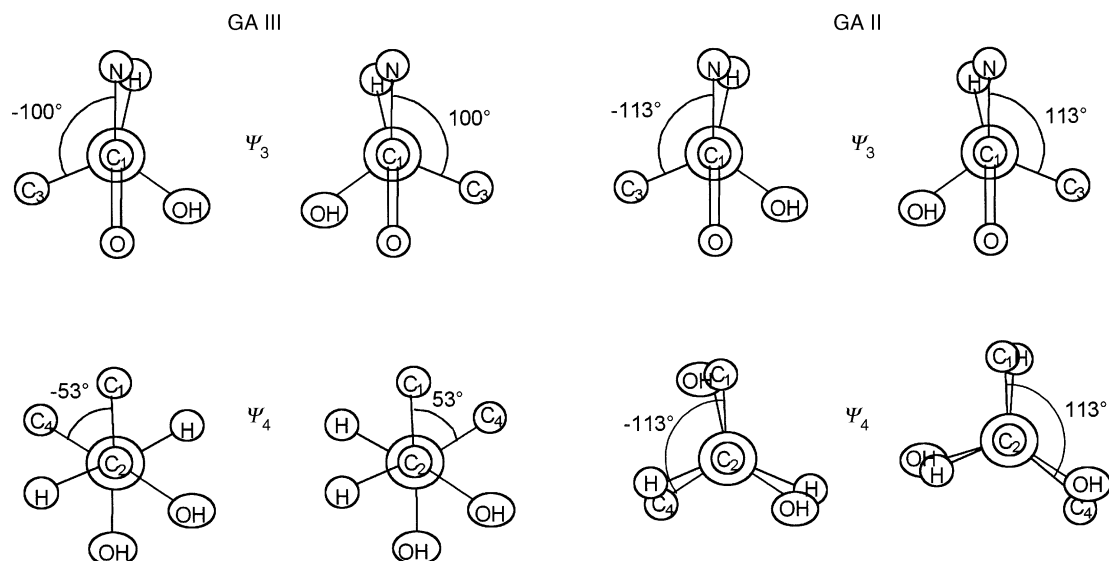


Fig. 9 Newman projections of the possible molecular conformations of the sugar group of GA for the possible pairs of torsion angles Ψ_3 and Ψ_4 determined by REDOR under the assumption that the ^{13}C γ -*gauche* effect is responsible for the changes of the ^{13}C chemical shieldings.

From the REDOR data it is known, that Ψ_3 equals $\pm 113^\circ$ for the GA II and Ψ_3 equals $\pm 100^\circ$ for GA III, *i.e.* N–C1–C2–C3 is close to an *anti* conformation in both modifications. Thus one can assume that Ψ_6 is responsible for the difference, *i.e.* that Ψ_6 is close to *gauche* in GA II and close to *trans* or *anti* in GA III.

The second large difference is found for C4 with a shift of +2.5 ppm in GA II, relative to GA III. In this case it follows that Ψ_4 is *gauche* in GA III and *trans* or *anti* in GA II. This result for Ψ_4 is corroborated by the CS of C1, which exhibits a shift of +2.1 ppm, similar to C4. Comparing this with the REDOR data of Ψ_4 we can attribute the torsion angle pairs $\Psi_4 = \pm 53^\circ$ to GA III and $\Psi_4 = \pm 113^\circ$ to GA II. Ψ_5 is influencing C2 and C5. The CS difference is +1.2 ppm for C2 and –0.2 ppm for C5. It follows that Ψ_5 is identical in both GA II and GA III. Fig. 9 summarizes the possible conformations of the sugar groups as Newman projections.

In the previous solution NMR and ^{13}C CP-MAS NMR investigations^{3,4} an all-*trans* conformation was proposed for the alkyl chain of both GA II and GA III, similar to the GA I structure. For the sugar head group of GA III a *gauche* bend (G) at C2 (${}_2\text{G}$) and for GA II at C3 (${}_3\text{G}$) was proposed.

The REDOR-NMR results of GA III fully corroborate this structure proposal of a ${}_2\text{G}$ sickle. However, for GA II the deduced *gauche* bend is one bond closer to the amide nitrogen than it was proposed originally, *i.e.* also a ${}_2\text{G}$ sickle. Due to

the inherent ambiguity of the sign of rotation in the torsion angles, it is not possible to decide by REDOR distance constraints, whether the rotation is positive or negative, *i.e.* whether the bend is ${}_2\text{G}^+$ or ${}_2\text{G}^-$. However, by virtue of the REDOR dephasing a reliable and unambiguous assignment of the ^{13}C signal lines exists and it is now safe to employ the comparison to data of the similar compounds with a known structure, as mentioned in the introduction. The result of this comparison is that the ${}_2\text{G}^+$ sickle has to be attributed to GA II and that the ${}_2\text{G}^-$ sickle has to be attributed to GA III. A sketch of the resulting molecular structures is displayed in Fig. 10. Now we can conclude that the different superstructures are at least partially the result of the different molecular conformations and not only caused by different arrangements of an identical molecular structure.

Our interpretation of the ^{13}C CS data excludes all effects of intra- and intermolecular hydrogen bonding. The sugar chain is effectively treated as a simple alkyl chain, neglecting the influence of the –OH groups on the chemical CS. This simplification seems reasonable due to the fact that the sugar head groups of both GA II and GA III are very similar to each other and should be influenced by these effects in the same manner. Nevertheless this reasoning is no proof of the structure and a final determination of the sign of the ${}_2\text{G}$ sickles can only be obtained by a quantum mechanical *ab initio* calculation of the chemical shifts. In these calculations not only one single molecule, but several of them have to be included to model the effects of the intermolecular hydrogen bonding on the CS. Such advanced calculations on a higher theoretical level are within the scope of future work.

Summary and conclusion

In summary it has been shown that the combination of ^{15}N ^{13}C REDOR NMR spectroscopy and ^{13}C CP-MAS spectroscopy is a very sensitive technique for conformational studies of organic solids. The ^{15}N (labeled) ^{13}C (natural abundance) REDOR experiment permits the measurement of several dipolar couplings in a single experiment. These dipolar couplings not only allow the determination of internuclear distances but also a reliable and unambiguous assignment of the ^{13}C chemical shifts.

This technique has been applied to three modifications of ^{15}N -octyl-D-gluconamide, namely GA I, GA II and GA III. ^{13}C - ^{15}N dipolar couplings in the range 40 to 1.2 kHz were detected and converted into distances of 1.4 to 4.07 Å. The

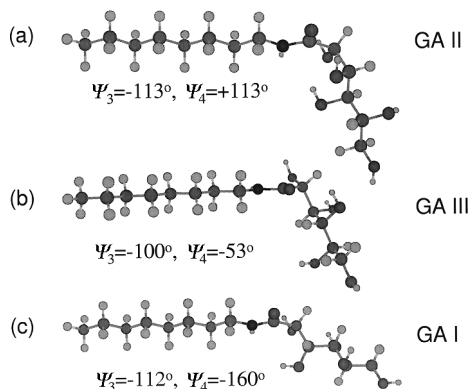


Fig. 10 Steric view of the conformations of GA; (a): GA II; $\Psi_3 = -113^\circ$, $\Psi_4 = +113^\circ$; (b): GA III; $\Psi_3 = -100^\circ$, $\Psi_4 = -53^\circ$; (c): GA I; $\Psi_3 = -112^\circ$, $\Psi_4 = -160^\circ$. For the alkyl chains the all-*trans* conformation of the monolayer crystal is used. Only one of the symmetry equivalent forms of Fig. 9 is shown for each modification.

distances found in the GA I modification are in very good agreement with the X-ray distances, showing the reliability of the method. The range of distances measured in the GA II and GA III is large enough to determine possible sets of torsion angles, which describe the conformations of the molecules in the vicinity of the ^{15}N label. The combination of the REDOR results with ^{13}C CS data allows a further reduction of the number of possible conformations, showing that a ${}_2\text{G}$ bend has occurred in both sugar head groups of GA II and GA III. A comparison of the CS values to other hexonamides with known structure finally reveals that the ${}_2\text{G}^+$ sickle has to be attributed to GA II and that the ${}_2\text{G}^-$ sickle has to be attributed to GA III.

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