Measurements of motionally averaged heteronuclear dipolar couplings in MAS NMR using R-type recoupling

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Abstract

A novel MAS NMR approach is presented for the determination of heteronuclear dipolar couplings in unoriented materials. The technique is based on the proton-detected local field (PDLF) protocol, and achieves dipolar recoupling by R-type radio-frequency irradiation. The experiment, which is called R-PDLF spectroscopy, is demonstrated on solid and liquid-crystalline systems. For mobile systems, it is shown that the R-PDLF scheme provides better dipolar resolution as compared to techniques combining conventional separated local field (SLF) spectroscopy with R-type recoupling.
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1. Introduction

A wide range of NMR techniques have been developed for measuring dipolar couplings in various systems including solids [1–3], liquid crystals [4,5], and membrane-associated peptides and proteins [6]. Since the dipolar interaction has a well-defined orientation and distance dependence, it is often straightforward to translate the experimentally determined couplings into conformational and dynamical constraints. The extraction of dipolar interactions in complex unoriented materials is, however, demanding because the NMR experiments have to be carried out under magic-angle spinning (MAS) conditions. While fast MAS provides the required sensitivity and chemical-site resolution, it also suppresses the informative through-space spin–spin couplings. Fortunately, it is possible to recouple the dipolar interactions under MAS by suitably designed radio-frequency (RF) pulse sequences [1–3].

Recently, Levitt [3] presented symmetry theorems that are useful in the design of RF schemes for selective recoupling and decoupling in MAS solid-state NMR. For example, it was found that certain R-type sequences [7] lead to reactivation of heteronuclear dipolar couplings whereas homonuclear interactions are suppressed. It has also been demonstrated that the combination of R-type RF irradiation and two-dimensional (2D) separated local field (SLF) spectroscopy is an efficient approach for measuring and assigning heteronuclear dipolar interactions in solids [7–10]. Below we refer to this experiment as R-SLF spectroscopy.

In this paper, we compare the efficiencies of various local field methods based on R-type recoupling. Our principal objective is to study their performances when applied to unoriented materials exhibiting a high degree of molecular mobility. The measurement of motionally averaged dipolar couplings in such systems, which for instance include liquid crystals and biomembranes, is of great interest since these interactions provide valuable information on molecular order, structure, and dynamics [4–6]. In addition to the original R-SLF experiment, we explore the behavior of a novel technique which incorporates R-type recoupling into the proton-detected local field (PDLF) protocol [5,11–13]. The new

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MAS method is called R-PDLF spectroscopy. We focus here on measurements of one-bond ^1H–^{13}C dipolar couplings in ^{13}CH and $^{13}CH_2$ groups. The R-SLF and R-PDLF schemes are, however, also applicable to other ^1H_n-S systems where S is a rare spin-1/2 nucleus.

2. Implementation

2.1. Local field spectroscopy using R-type recoupling

The ^1H–^{13}C local field techniques discussed in the present work are based on the rotor-synchronized sequence $R18_1^7$ [7]. It consists of a repetitive scheme of $180_{70}180_{-70}$ pulse pairs, where each 180° pulse occupies exactly 1/18 of a rotor period. This means that the $R18_1^7$ sequence requires an RF field strength of 9 times the spinning frequency. When applied to ^1H spins, $R18_1^7$ irradiation leads to recoupling of ^1H–^{13}C dipolar couplings and ^1H chemical shift anisotropies (CSA) whereas homonuclear dipolar interactions among the ^1H spins are suppressed [7]. For isolated ^1H–^{13}C spin pairs at high spinning frequencies, the recoupled dipolar powder pattern resembles the $n = 2$ rotary resonance lineshape [14] and exhibits singularities at $\pm\sqrt{3}\kappa b_{CH}/2$, where b_{CH} is the ^1H–^{13}C dipolar coupling and κ the theoretical scaling factor as defined in [7]. The effective scaling factor may, for the $R18_1^7$ sequence, be defined as $\sqrt{3}\kappa = 0.315$. For higher spinning frequencies than used in this work, alternative R-type sequences are available [7].

The original R-SLF experiment was introduced by Zhao et al. [7] and is shown in Fig. 1A. The pulse sequence starts by ramped cross-polarization (CP) enhancement of the transverse ^{13}C magnetization [15]. During the variable evolution period t_1, the CP-enhanced ^{13}C transverse magnetization evolves in the presence of $R18_1^7$ RF irradiation of the protons. Proton heteronuclear decoupling is subsequently applied for an interval $T − t_1$ with a ^{13}C 180° pulse inserted at $T/2$. The duration T is kept constant and equal to an even number of rotor periods. The signal is finally observed during the detection period t_2 as it evolves under the ^{13}C chemical shift interaction and TPPM decoupling [16].

In the new R-PDLF method schematically illustrated in Fig. 1B, ^1H polarization is allowed to evolve under ^1H $R18_1^7$ irradiation during t_1. This polarization, modulated by the ^1H–^{13}C dipolar and ^1H CSA interactions, is then transferred to ^{13}C spins for detection during t_2. Details of the polarization transfer step from ^1H to ^{13}C are discussed in Section 2.2.

In the R-SLF technique, the rare ^{13}C spins evolve under the influence of all dipolar local fields produced by the surrounding protons. In contrast, the R-PDLF spectra are governed by simple two-spin interactions because the dipolar field is probed at the abundant ^1H
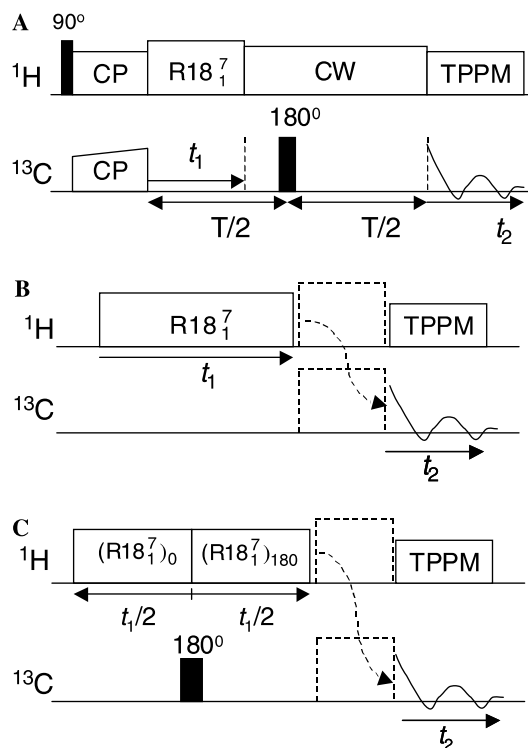

Fig. 1. Pulse sequences for: (A) 2D R-SLF spectroscopy, (B) 2D R-PDLF spectroscopy, and (C) 2D R-PDLF spectroscopy with ^1H CSA refocusing during t_1.

spins rather than at the rare ^{13}C spins. As demonstrated below, this often results in a higher dipolar resolution in R-PDLF spectroscopy as compared to that observed in R-SLF spectroscopy.

The ^1H CSA is also recoupled by the $R18_1^7$ sequence and may affect the dipolar lineshape. A modified version of the R-SLF method has been suggested to suppress this term [17]. In this experiment, two R sequences are implemented during a symmetrically placed variable evolution period t_1. If the overall phase shift between the two R blocks is set to 180° and a ^{13}C 180° pulse is applied at their junction, the ^1H CSA term is partially suppressed while the ^1H–^{13}C dipolar coupling is retained [17]. As shown in Fig. 1C, the same idea can be exploited in the R-PDLF technique.

2.2. ^1H to ^{13}C polarization transfer in R-PDLF spectroscopy

The ^1H to ^{13}C polarization transfer step in the R-PDLF experiment is important as far as spin-pair selectivity is concerned. In solids, the Hartmann–Hahn CP sequence shown in Fig. 2A is the most popular method to transfer polarization from ^1H to ^{13}C [18]. This technique is, however, susceptible to ^1H spin diffusion, which results in a redistribution of ^1H polarization during the spin-lock sequence. Shortening the CP contact time reduces the impact from spin diffusion at the
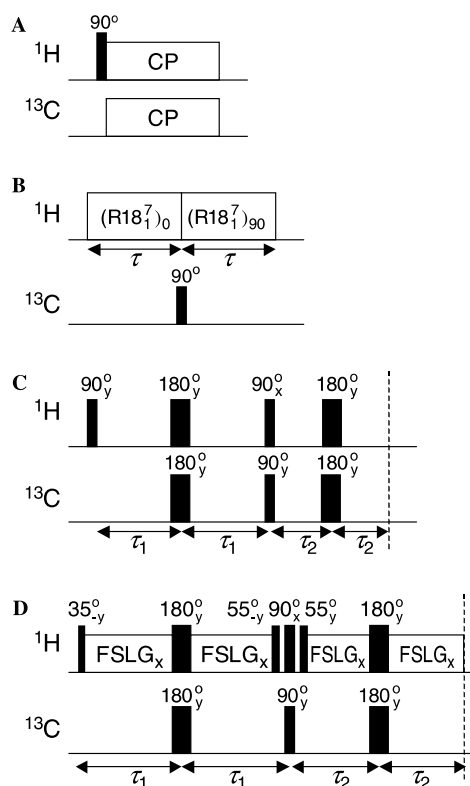
Fig. 2. Heteronuclear polarization transfer schemes used in R-PDLF spectroscopy: (A) conventional CP, (B) PRESTO, (C) refocused IN-EPT, and (D) refocused INEPT combined with FSLG decoupling.

of the rotor period [29]. These parameters are in practice optimized empirically and are, typically, in the range of a millisecond for a one-bond J_{CH} coupling of $\sim 140\,Hz$. In samples exhibiting little or no molecular mobility, strong ^1H–^1H dipolar couplings interfere unfavorable with INEPT. As indicated in Fig. 2D, this problem may be solved by combining the INEPT sequence with homonuclear decoupling by, e.g., FSLG ^1H irradiation [34,35]. With homonuclear and heteronuclear dipolar interactions suppressed by FSLG and MAS, the evolution during INEPT is solely determined by scaled J_{CH} couplings.

3. Results

3.1. R-SLF and R-PDLF spectroscopy of rigid solids

In this section, we examine the performances of the original R-SLF and the new R-PDLF experiments when applied to solid amino acids. R-SLF subspectra of the ^{13}CH spin system in [2-^{13}C,^{15}N]-L-alanine, and the $^{13}CH_2$ spin system in [2-^{13}C,^{15}N]-glycine are shown in Figs. 3A and 4A, respectively. Both dipolar spectra exhibit a three-peak structure, and the lineshapes agree with those reported in [7]. The splitting between the two outer peaks is $6.6 \pm 0.2\,kHz$, and numerical simulations show that this corresponds to a ^1H–^{13}C dipolar coupling of $22.3 \pm 0.7\,kHz$. This matches results from previous solid-state NMR investigations of rigid sp^3-hybridized ^{13}C sites [7,9,10].

The R-PDLF method is first demonstrated on [2-^{13}C,^{15}N]-L-alanine. Under moderately fast sample spinning (10 kHz) and FSLG decoupling, this sample yields a resolved J_{CH} splitting for the α carbon in the one-dimensional (1D) ^{13}C spectrum (not shown). Similar findings were reported by Lesage et al. [36]. This proves that it is possible to transfer magnetization from ^1H to ^{13}C by using a combination of INEPT and FSLG. In Fig. 3, we show R-PDLF subspectra of alanine without (Fig. 3B) and with (Fig. 3C) ^1H CSA refocusing during the evolution period. INEPT-FSLG polarization transfer with a homonuclear decoupling field strength corresponding to 90 kHz was used in both cases. Essentially the same R-PDLF dipolar lineshapes were obtained by employing CP with a short contact time (40 μs), or LG-CP with contact times up to 200 μs instead of the INEPT-FSLG sequence (spectra not shown).

The numerical simulations in Fig. 3 capture the main features of the R-SLF and R-PDLF subspectra of the ^{13}CH spin system in alanine well. Note in particular that the weak outer shoulders in the experimental R-PDLF lineshape in Fig. 3B are also visible in the calculated spectrum. These features, which are due to recoupled ^1H CSA, are clearly suppressed by using ^1H CSA refocusing during the evolution period (see Fig. 3C).

expense of lower signal intensity. Other ways to approach this problem include the use of Lee–Goldburg (LG) CP schemes which quench the dipolar-driven spin diffusion [11,19–22]. Recently, the PRESTO method (see Fig. 2B) was introduced for heteronuclear polarization transfer in MAS NMR [23]. This is a symmetry-based technique, which implements simultaneous heteronuclear recoupling and homonuclear decoupling. The delay τ is adjusted so that maximum polarization transfer is achieved between the coupled spins of interest. In this work, we employ $180_{70}180_{-70}$ pulse pairs as building blocks in PRESTO.

Both CP-based methods and PRESTO rely on polarization transfer using heteronuclear through-space dipolar couplings. A higher selectivity in the polarization transfer step can be achieved by utilizing through-bond J couplings. For example, INEPT is a well-established pulse sequence for polarization transfer in isotropic liquids which is based on this interaction [24–27]. INEPT has also been employed in NMR studies of lipid bilayers [28–32] and mesostructured materials [33], where the dipolar couplings are attenuated by molecular motions and rapid sample spinning. In general, higher spinning frequencies provide higher J coupling resolution and INEPT transfer efficiency. In the MAS analogue of the refocused INEPT method (see Fig. 2C), the delays τ_1 and τ_2 are set to integer multiples
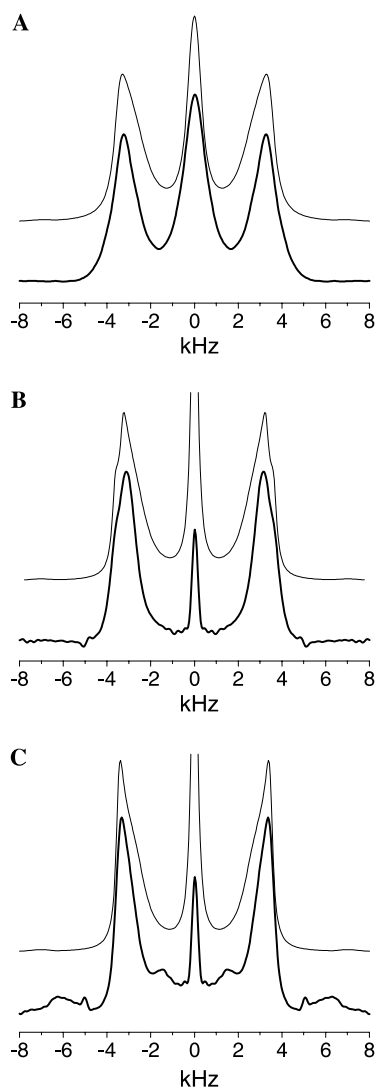
Fig. 3. Experimental (bold lines) and simulated (thin lines) dipolar spectra of [2-^{13}C,^{15}N]-L-alanine obtained at a spinning frequency of 10.0 kHz using the: (A) R-SLF sequence, (B) R-PDLF sequence, and (C) R-PDLF sequence with ^{1}H CSA refocusing. INEPT-FSLG was used in the R-PDLF experiments ($\tau_1 = \tau_2 = 1.3$ ms). Numerical simulations assumed an isolated ^{13}CH group with a ^{1}H–^{13}C dipolar coupling of 22.3 kHz, a ^{1}H CSA of −3.0 ppm and an asymmetry parameter of 0.3, and line broadenings of 600 Hz (A) and 300 Hz (B and C).

Fig. 4. Experimental (bold lines) and simulated (thin lines) dipolar spectra of [2-^{13}C,^{15}N]-glycine obtained at a spinning frequency of 8.0 kHz using the: (A) R-SLF sequence, (B) R-PDLF sequence with CP polarization transfer (contact time = 40 μs), and (C) R-PDLF sequence with PRESTO polarization transfer ($\tau = 83.33$ μs which corresponds to 6 [180$_{70}$180$_{-70}$] pulse pairs). Numerical simulations assumed an isolated ^{13}CH$_2$ group with ^{1}H–^{13}C dipolar couplings of 22.3 kHz, a ^{1}H–^{1}H interaction of 20.4 kHz, a ^{1}H CSA of −1.5 ppm and an asymmetry parameter of 0.1, an H–C–H bond angle of 109.5°, and line broadenings of 700 Hz (A) and 600 Hz (B and C).

Fig. 4 compares the results from R-SLF and R-PDLF spectroscopy of the ^{13}CH$_2$ spin system in [2-^{13}C,^{15}N]-glycine. The R-PDLF subspectrum in Fig. 4B was obtained without ^{1}H CSA refocusing, and conventional CP was used for ^{1}H–^{13}C polarization transfer (contact time = 40 μs). Similar R-PDLF lineshapes were observed by employing LG-CP with longer contact times. It was not possible to resolve the J_{CH} couplings in this sample under the experimental conditions used in this study (see Section 6 for details), which hampers the usage of INEPT-FSLG polarization transfer. Implementing ^{1}H CSA refocusing during t_1 did not affect the dipolar lineshape, which may indicate that the ^{1}H CSA of the α protons in glycine is

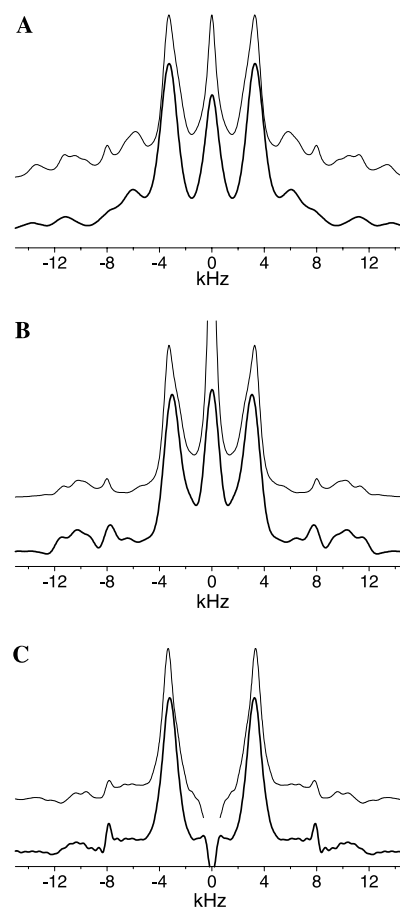relatively small. Note that the broad shoulders present in the R-SLF lineshape (in the spectral region between 5 and 7 kHz in Fig. 4A), which are characteristic of recoupled R-SLF dipolar spectra of rigid ^{13}CH$_2$ groups, are absent in the R-PDLF subspectrum in Fig. 4B. The lineshape in Fig. 4B is in fact rather similar to those of the ^{13}CH group in alanine (cf. Fig. 3). This is expected because the R-PDLF subspectrum of the ^{13}CH$_2$ group in glycine consists of a superposition of two identical ^{13}CH lineshapes. The dipolar spectrum in Fig. 4C was obtained by using PRESTO polarization transfer in the R-PDLF experiment. The central part of the subspectrum is suppressed because the PRESTO sequence was intentionally optimized so that crystallites exhibiting large ^{1}H–^{13}C couplings were selected [23]. The numerical simulations of an isolated ^{13}CH$_2$ three-spin system reproduce the main features of the experimental lineshapes in Fig. 4.

3.2. R-SLF and R-PDLF spectroscopy of liquid crystals

We tested the performances of the R-SLF and R-PDLF techniques for measuring motionally averaged dipolar interactions in a columnar mesophase formed by 1,2,3,5,6,7-hexaoctyloxy-rufigallol (RufH8O) [37], see Fig. 5. The molecule was ^{13}C enriched to 10% at the α

A C_4

B α_3

C α_3
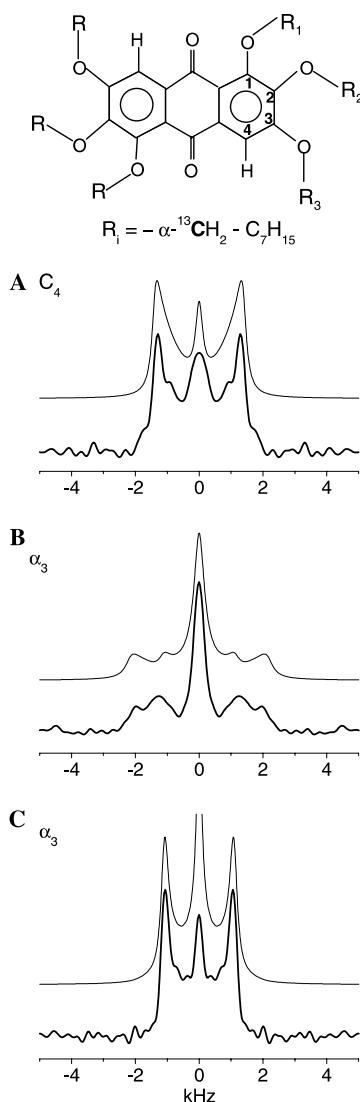
Fig. 5. Experimental (bold lines) and simulated (thin lines) dipolar spectra of unoriented RufH8O-10%-$^{13}C_\alpha$ in the liquid-crystalline phase at 80 °C (spinning frequency = 8.0 kHz). (A) C_4 cross-section through a 2D R-SLF spectrum. The numerical simulation assumed an isolated ^{13}CH group with a ^{1}H–^{13}C dipolar coupling of 8.9 kHz, a ^{1}H CSA of −1.0 ppm and an asymmetry parameter of 0, and a line broadening of 250 Hz. (B) α_3 cross-section through a 2D R-SLF spectrum. The numerical simulation assumed an isolated ^{13}CH$_2$ group with ^{1}H–^{13}C dipolar couplings of 7.0 kHz, a ^{1}H–^{1}H interaction of 15.0 kHz, zero ^{1}H CSA, an H–C–H bond angle of 0°, and a line broadening of 400 Hz. (C) α_3 cross-section through a 2D R-PDLF spectrum. CP was used for polarization transfer (contact time = 200 μs). The same simulation parameters as in (B) were employed (except that the line broadening was 250 Hz).

methylene positions. The heteronuclear ^{1}H–^{13}C couplings in RufH8O were recently estimated by means of various local field NMR experiments under both stationary and sample spinning conditions [9,38,39].

Dipolar cross-sections extracted from 2D MAS spectra of unoriented RufH8O-10%-$^{13}C_\alpha$ in the mesophase are shown in Fig. 5. The R-SLF subspectrum of the aromatic ^{13}CH two-spin system in Fig. 5A exhibits the characteristic three-peak structure. Apart from a reduction of the one-bond ^{1}H–^{13}C coupling strength due to fast molecular reorientation about the columnar axis, the lineshape is similar to the recoupled spectra of the ^{13}CH group in solid alanine (cf. Fig. 3). The splitting between the two outer peaks is 2.8 ± 0.1 kHz and corresponds to a residual dipolar coupling of 8.9 ± 0.3 kHz, which agrees with the value observed in oriented RufH8O [9,38]. A similar dipolar lineshape for C_4 was observed by using the R-PDLF experiment (not shown).

The R-SLF subspectrum of the α methylene carbon in the R_3 chain (denoted α_3) shown in Fig. 5B is qualitatively different as compared to the ones observed for the same site in the solid phase [9], or for the ^{13}CH$_2$ group in solid glycine (cf. Fig 4A). The reason for this difference is that in this liquid-crystalline phase, the motionally averaged directions of the two CH bond vectors in the methylene moiety are collinear and, furthermore, that the two residual couplings are equal. Therefore, the contribution from each ^{13}CH$_2$ spin-system to the powder spectrum is a 1:2:1 triplet with an orientation-dependent splitting. This results in an R-SLF lineshape, which is dominated by an uninformative zero-frequency peak. In contrast, the R-PDLF dipolar spectrum of the α_3 methylene group shown in Fig. 5C exhibits sharp features. This is because the spectrum is governed by simple two-spin interactions resulting in a doublet spectral structure. This spectrum was obtained using the pulse sequence in Fig. 1B, and CP was employed for polarization transfer (a combination of INEPT and FSLG decoupling could also have been used for this sample). Implementing ^{1}H CSA refocusing did not affect the dipolar lineshape. The residual $^1H_{\alpha3}$–$^{13}C_{\alpha3}$ dipolar coupling is readily estimated to 7.0 ± 0.2 kHz from Fig. 5C, which is consistent with results reported elsewhere [9,38,39].

Another example of R-PDLF spectroscopy of unoriented liquid crystals is shown in Fig. 6. This figure displays cross-sections extracted from a 2D MAS spectrum of the discotic compound 2,3,6,7,10,11-hexahexyl-thiotriphenylene (HHTT). HHTT has previously attracted much attention due to its spectacular charge transport properties [40]. The experiment reported here was carried out with the pulse sequence shown in Fig. 1C, where standard INEPT was used for ^{1}H to ^{13}C polarization transfer. This transfer technique is well suited for HHTT in the columnar mesophase since extensive molecular dynamics results in linewidths that are

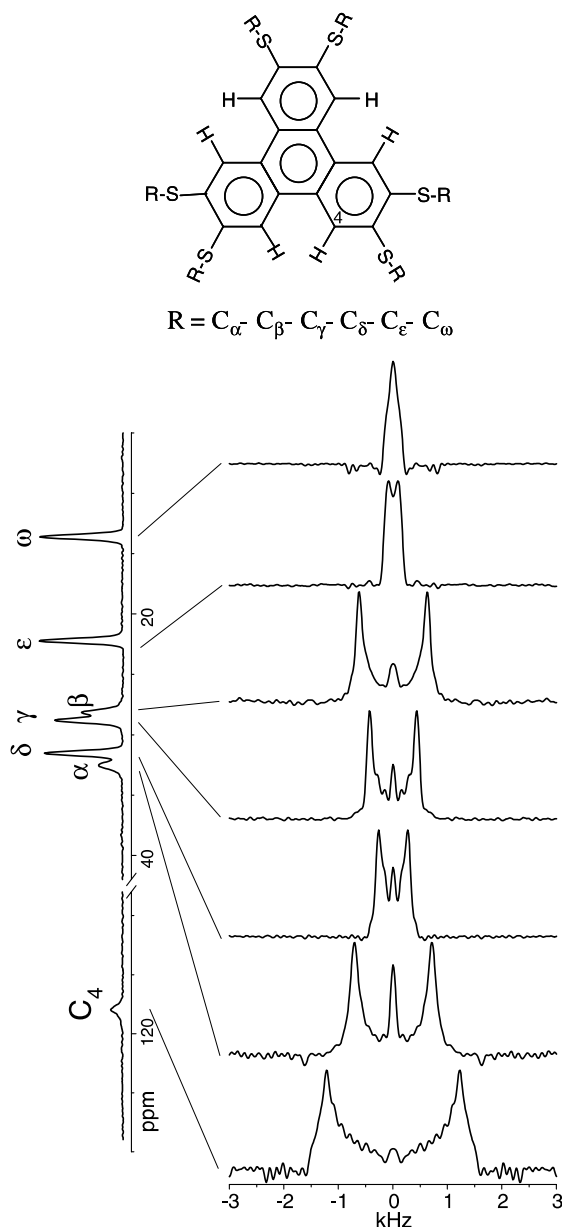R = C_α- C_β- C_γ- C_δ- C_ϵ- C_ω

Fig. 6. Dipolar cross-sections through a 2D R-PDLF spectrum of unoriented HHTT in the liquid-crystalline phase at 80 °C (spinning frequency = 8.0 kHz). INEPT was used for polarization transfer ($\tau_1 = 1.5$ ms and $\tau_2 = 1.0$ ms). The 1D ^{13}C INEPT-MAS spectrum is shown to the left. ^{13}C resonances were assigned by an INADEQUATE experiment in the isotropic phase, and a ^{1}H–^{13}C HETCOR experiment in the mesophase.

less than J_{CH} under sample spinning conditions. Fig. 6 clearly demonstrates that the R-PDLF method produces highly resolved subspectra with simple splittings for both ^{13}CH and ^{13}CH$_2$ groups. The dipolar splittings correspond to residual ^{1}H–^{13}C couplings ranging from 8.0 ± 0.2 kHz for C_4, to 0.6 ± 0.1 kHz for the ϵ methylene site. Due to the multiple-spin effects discussed above, the R-SLF experiment results also for this liquid crystal in poorly resolved ^{13}CH$_2$ dipolar spectra.

4. Discussion

The original R-SLF pulse sequence was designed for measurements of strong heteronuclear couplings in unoriented solids [7]. This experimental approach has been put to good use in NMR studies of rigid amino acids [7,8], discotic [9], and carbohydrate [10] systems. We have in this work demonstrated that the R-SLF technique is useful also for measurements of motionally averaged ^{1}H–^{13}C interactions in ^{13}CH groups. Due to the poorly resolved triplet structure shown in Fig. 5B, however, the method is less promising for extracting precise dipolar couplings in ^{13}CH$_2$ groups in systems exhibiting fast axial motions. The novel R-PDLF sequence, in contrast, produces high-resolution dipolar spectra in both solid and liquid-crystalline phases irrespective of the ^{1}H multiplicity. This spectral simplification occurs due to the fact that each ^{1}H spin in the ^{13}CH$_n$ group experiences the local dipolar field from only one ^{13}C spin.

An additional advantage of the R-PDLF technique as compared to many other 2D local field experiments is the possibility to select certain spin-pairs in the ^{1}H to ^{13}C polarization transfer step. If J_{CH} couplings are exploited, polarization transfer occurs almost exclusively between directly bonded heteronuclei. Therefore, INEPT is the method of choice for measurements of one-bond dipolar interactions by R-PDLF spectroscopy. This approach is applicable in studies of systems exhibiting resolved J_{CH} splittings in the 1D ^{13}C spectrum, either with or without homonuclear decoupling. As demonstrated both in this work and elsewhere [28–33], such systems include solids, lipid bilayers, and liquid crystals. For materials in which polarization transfer through J_{CH} couplings is difficult, CP-based schemes or PRESTO may be used. PRESTO has the advantage that it is possible to suppress the central part of the powder spectra [23], thus enhancing the dipolar resolution somewhat. Due to the sensitivity of the transfer efficiency to the strength of the dipolar interaction, however, it is problematic to employ PRESTO in systems exhibiting a broad distribution of motionally averaged ^{1}H–^{13}C couplings. For such systems, CP-based methods may offer sensitivity advantages.

One may argue that other MAS recoupling techniques with higher dipolar scaling factors would be more suitable for studies of mobile systems. For example, sideband LG-CP and PISEMA-type recoupling schemes, having a scaling factor of 0.577, were recently introduced [21,22,39,41]. These experiments, however, do not provide the opportunity to select directly bonded spin-pairs using the J_{CH} coupling. Another 2D MAS technique, which enables measurements of residual ^{1}H–^{13}C couplings in liquid crystals and fluid phase lipids, is the DROSS experiment [29]. The scaling factor of this pulse sequence is 25% higher as compared to that of

the R18$_1^7$ scheme. However, the lack of active ^{1}H–^{1}H decoupling in DROSS compromises the dipolar resolution. Methods that exploit artificial coupling amplification may be useful for estimating motionally averaged heteronuclear interactions [42–45]. In these experiments, the couplings are extracted from the dipolar sideband patterns. This is, however, not straightforward if multiple-spin interactions are active, and/or few sidebands are present.

5. Conclusions

We have in this paper compared the efficiencies of various local field schemes based on R-type heteronuclear recoupling. In particular, the performances of the original R-SLF method [7] and a novel technique called R-PDLF have been studied. Their behaviors are comparable when applied to rigid solids. In liquid-crystalline systems, however, the R-PDLF experiment results in simpler ^{13}CH$_2$ spectra with higher dipolar resolution. Furthermore, the R-PDLF method is very flexible in the sense that both through-space and through-bond interactions can be used in the polarization transfer step. One of the limitations of the MAS pulse sequences discussed in this work is the relatively small scaling factor. Still, it has been demonstrated that ^{1}H–^{13}C dipolar couplings as small as 0.6 kHz can be readily measured by employing the R-PDLF scheme. We expect, therefore, that this experiment will become a widely used method in studies of partially mobile systems such as unoriented liquid crystals and biomembranes.

6. Experimental

Unlabeled HHTT and [α-^{13}C]-labeled RufH8O were synthesized according to previously described procedures [46]. A detailed discussion of RufH8O and HHTT including their phase diagrams can be found in [37,47] and [40,48], respectively. Labeled amino acids ([2-^{13}C,^{15}N]-L-alanine and [2-^{13}C,^{15}N]-glycine) were purchased from Cambridge Isotope Laboratories and used without further purification.

All NMR experiments were performed at a magnetic field of 9.4 T on a Chemagnetics Infinity-400 spectrometer equipped with 6 mm double-resonance and 4 mm triple-resonance MAS probes. The spinning frequency varied between 8 and 10 kHz, and was stabilized to ± 2 Hz. Initial ^{13}C transverse magnetization was, for the R-SLF sequence, created by ramped ^{1}H–^{13}C CP [15] with nutation frequencies of 40–60 kHz, and contact times of 1–3 ms. Heteronuclear decoupling during the detection period in solid and liquid-crystalline phases was achieved by, respectively, 70 and 50 kHz TPPM irradiation [16]. The ^{1}H RF field strength was set to

90 kHz during FSLG decoupling. Typically, 64 points were collected in the first time domain, and t_1 consisted of an increasing number of 180$_{70}$180$_{-70}$ pulse pairs. Only cosine-modulated data sets were collected in t_1 yielding symmetric spectra in the dipolar dimension. Numerical simulations were performed using the SIMPSON programming package [49].

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