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Influence of ^{13}C Isotopic Labeling Location on Dynamic Nuclear Polarization of Acetate

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Abstract

Dynamic nuclear polarization (DNP) via the dissolution method has alleviated the insensitivity problem in liquid-state nuclear magnetic resonance (NMR) spectroscopy by amplifying the signals by several thousand-fold. This NMR signal amplification process emanates from the microwave-mediated transfer of high electron spin alignment to the nuclear spins at high magnetic field and cryogenic temperature. Since the interplay between the electrons and nuclei is crucial, the chemical composition of a DNP sample such as the type of free radical used, glassing solvents, or the nature of the target nuclei can significantly affect the NMR signal enhancement levels that can be attained with DNP. Herein, we have investigated the influence of ^{13}C isotopic labeling location on the DNP of a model ^{13}C compound, sodium acetate, at 3.35 T and 1.4 K using the narrow electron spin resonance (ESR) linewidth free radical trityl OX063. Our results show that the carboxyl ^{13}C spins yielded about twice the polarization produced in methyl ^{13}C spins. Deuteration of the methyl ^{13}C group, while proven beneficial in the liquid-state, did not produce an improvement in the ^{13}C polarization level at cryogenic conditions. In fact, a slight reduction of the solid-state ^{13}C polarization was observed when ^2H spins are present in the methyl group. Furthermore, our data reveal that there is a close correlation between the solid-state ^{13}C T_1 relaxation times of these samples and the relative ^{13}C polarization levels. The overall results suggest the achievable solid-state polarization of ^{13}C acetate is directly affected by the location of the ^{13}C isotopic labeling via the possible interplay of nuclear relaxation leakage factor and cross-talks between nuclear Zeeman reservoirs in DNP.

TOC Graphic

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1. Introduction

In vivo and *in vitro* nuclear magnetic resonance (NMR) spectroscopy and imaging (MRI) of nuclei with relatively low gyromagnetic ratio γ such as ^{13}C is quite difficult and can be prohibitively time-consuming due to the inherently low Boltzmann thermal polarization of these nuclei at ambient conditions. One way to alleviate this NMR insensitivity issue is by employing a technique known as dynamic nuclear polarization (DNP)—a standard method used in preparing highly polarized protons and deuterons in the nuclear and particle physics community since 1960s. In DNP, the high electron spin alignment is transferred to the nuclear spins via microwave irradiation at cryogenic temperatures and high magnetic field, thus increasing the nuclear polarization and hence amplifying the NMR signal.¹ The NMR signal-enhancing capabilities of DNP was previously used primarily in nuclear scattering experiments at cryogenic temperatures until the invention of the dissolution DNP method in 2003.² This major breakthrough, pioneered by Ardenkjaer-Larsen and co-workers,² has extended the NMR signal amplification capability of DNP to the liquid-state in which the signals of low- γ nuclei such as ^{13}C , ^{15}N , ^{89}Y , etc.^{2–6} are enhanced by several thousand-fold. Since its inception, dissolution DNP has become an emerging biomedical technique for *in vitro* and *in vivo* metabolic research using hyperpolarized ^{13}C -enriched biomolecules. In particular, it has found practical applications in real-time metabolic assessment of healthy and diseased tissues with excellent sensitivity and high specificity afforded by hyperpolarized ^{13}C NMR and MRI.^{7–13}

Since DNP involves the microwave-mediated interaction between electrons and nuclear spins, the choice of the sources of free electrons, typically provided by stable organic free radicals, can significantly affect the maximum achievable polarization level.^{14–19} Furthermore, certain sample preparation methods such as the addition of trace amounts of lanthanides in the DNP sample can significantly boost the DNP-enhanced NMR signals.^{5,20–23} Previous studies have shown that ^{13}C enrichment²⁴ and deuteration^{17,21,25} of the glassing matrix can expedite and also improve the DNP enhancement levels, respectively. All of these DNP sample optimization practices so far were additives or certain isotopic enrichment in the glassing matrix. In this work, we have investigated the effect of ^{13}C isotopic enrichment location on the DNP of ^{13}C substrate, in this case, ^{13}C sodium acetate using the free radical trityl OX063 (see structure in Fig. 1) as the polarizing agent. Acetate is chosen for this work due to its availability, biochemical importance, and the usefulness of the carboxyl location as a model for DNP optimization of other important DNP substrates

such as [1- ^{13}C] pyruvate. In particular, hyperpolarized ^{13}C acetate has been used to probe myocardial,^{26,27} hepatic,²⁸ and cerebral²⁹ metabolism *in vivo*.

In this study, we have recorded and compared the relative ^{13}C polarization levels achieved for carboxyl and methyl ^{13}C spins of acetate samples with the following isotopic enrichments (see structures in Fig. 2): the carboxyl [1- ^{13}C], deuterated carboxyl [1- ^{13}C ,d₃], methyl [2- ^{13}C], deuterated methyl [2- ^{13}C ,d₃], doubly-labeled [1,2- $^{13}\text{C}_2$], and doubly-labeled and deuterated [1,2- $^{13}\text{C}_2$, d₃] sodium acetate. While it is well-known in the liquid-state that carboxyl ^{13}C spins have relatively longer T_1 relaxation time because of its relative isolation from the protons and that deuteration of the methyl ^{13}C group would lead to longer ^{13}C T_1 and higher liquid-state enhancement,^{30–32} there is a dearth of comparative data of these ^{13}C isotopomers in terms of solid-state ^{13}C DNP polarization levels and T_1 relaxation times at cryogenic temperatures. The main goal of this study is to investigate the effect on DNP of these different ^{13}C isotopic labeling locations of acetate in which intra-molecular forces between magnetically-active nuclei may play a significant role on the polarization transfer process at cryogenic temperature. In addition, this study also aims to provide details as to the effect of ^2H enrichment of the methyl group, a practice which is proven to be beneficial in preserving the ^{13}C polarization in the liquid-state, on the ^{13}C DNP at cryogenic temperature from which the NMR signal amplification originates. Furthermore, the corresponding solid-state ^{13}C T_1 relaxation times of the different ^{13}C isotopic locations will also be measured and discussed in conjunction with the enhanced ^{13}C polarization levels. With the plethora of ^{13}C -labeled carboxylate compounds used as hyperpolarized ^{13}C NMR and MRI agents,^{7–9,30} the results of this study may provide insights with regards to the DNP efficiency of these ^{13}C isotopomers of acetate or a carboxylate in general prior to the dissolution process.

2. Experimental Section

Sample preparation:

The ^{13}C -labeled acetate compounds (99% ^{13}C enrichment), glassing solvents and free radical used in this study were obtained commercially and were used without further purification. The following amounts of ^{13}C -enriched acetate compounds (Sigma-Aldrich, St. Louis, MO) were weighed out and prepared using Discovery semi-micro analytical balance (Ohaus, Parsippany, New Jersey): 24.9 mg [1- ^{13}C]sodium acetate (mw=83.03 g/mol), 24.9 mg [2- ^{13}C]sodium acetate (mw=83.03 g/mol), 25.8 mg [1- ^{13}C , d₃]sodium acetate (mw=86.04 g/mol), 25.8 mg [2- ^{13}C , d₃]sodium acetate (mw=86.04 g/mol), 25.2 mg [1,2- ^{13}C]sodium acetate, and 26.1 mg [1,2- ^{13}C ,d₃]sodium acetate (mw=87.04 g/mol). 100 μL solutions were prepared in 1 mL microcentrifuge (Scientific USA, Ocala, FL) by mixing these compounds with 1:1 glycerol:water. 2.14 mg of trityl OX063 free radical (Oxford Instrument Biotools, MA) was added to each solution, then rapidly mixed and prepared using vortex mixer and microcentrifuge (ThermoFisher Scientific, WI). The final concentrations of ^{13}C acetate and trityl OX063 in the solutions were 3 M and 15 mM, respectively. Fresh sample for each trial was prepared 10 hours prior to experiment and stored at -80°C in an ultra-low temperature freezer (Thermo Scientific, WI) to prevent degradation. All the procedures of sample preparation, storage, insertion, and acquisition were done in triplicate for each ^{13}C acetate isotopomer.

Microwave frequency sweep.

The ^{13}C microwave DNP spectrum, which determines the optimum microwave frequencies to polarize the samples, was done using a built-in NMR program in the HyperSense polarizer (Oxford Instruments, UK). 100 μL ^{13}C acetate sample was placed in a PEEK cup and was quickly inserted into the polarizer which operates at 3.35 T and 1.4 K base temperature. The microwave power was set at 100 mW and the frequency was swept in the range 94.00–94.20 GHz with step interval of 5 MHz. The sample was irradiated for 3 minutes at each frequency step and the ^{13}C NMR signal was automatically recorded for each frequency.

^{13}C polarization buildups.

The ^{13}C acetate samples were all irradiated at the positive polarization peak P(+) determined from the microwave frequency sweep. 100 μL aliquots of ^{13}C acetate samples were placed in the polarizer at 3.35 T and 1.4 K. A ^{13}C NMR spectrum was recorded every 5 minutes and plotted as a function of time. The ^{13}C polarization buildup curves for each sample were done in triplicate. Since the same 100- μL volume was used for the doubly-labeled ^{13}C samples ([1,2- ^{13}C]sodium acetate and [1,2- ^{13}C , d_3]sodium acetate), the polarization buildup intensities of these 2 samples were multiplied by $\frac{1}{2}$ to be able to compare with the relative ^{13}C DNP intensities of singly ^{13}C -labeled samples ([1- ^{13}C]sodium acetate, [1- ^{13}C , d_3]sodium acetate, [2- ^{13}C]sodium acetate, and [2- ^{13}C , d_3] sodium acetate) with the sample ^{13}C spin count. The relative ^{13}C maximum DNP signals were determined (mean $\pm$ standard deviation) for each sample and compared in bar graph representations.

^{13}C T_1 decay curves.

Each ^{13}C acetate sample was polarized to its maximum ^{13}C DNP intensity at 3.35 T and 1.4 K. Once the samples reached their maximum polarization, the microwave source was then turned off and the decay of hyperpolarized ^{13}C NMR signal of the frozen sample at 3.35 T and 1.4 K was recorded every 20 minutes using a 2-degree RF pulse. To do this, an RG-58U RF cable from a Varian VNMR 400 MHz console (Agilent Technologies, Santa Clara, CA) was connected to the top-tuned NMR circuit box of the HyperSense polarizer. Depending on the ^{13}C T_1 , each T_1 decay curve from a ^{13}C acetate sample can take 5–10 hours of NMR signal recording. The T_1 decay curves were then fitted using an equation that accounts ^{13}C NMR signal decay due to T_1 relaxation and RF pulsing.⁵ The ^{13}C T_1 values of these samples were extracted using the aforementioned equation.

3. Results and Discussion

The free radical trityl OX063 is very commonly used in dissolution DNP, and its electron spin resonance (ESR) linewidth is among the narrowest of all free radicals used in DNP.^{18,34,35} This narrow linewidth allows for the DNP process to occur either via the solid effect and thermal mixing, depending on the nucleus being polarized.^{24,34,36,37} The solid effect is dominant when the nuclear Larmor frequency is much larger than the linewidth of the free radical as is the case for the polarization of ^1H . In this mechanism, polarization is achieved through direct activation of zero or double quantum transitions by microwave irradiation at a

frequency $\nu = \nu_s \pm \nu_I$ where ν_s and ν_I are the electron and nuclear Larmor frequencies, respectively. Thermal mixing, on the other hand, is dominant when the nuclear Larmor frequency is comparable to or less than the free radical ESR linewidth.³⁸ Even for a narrow linewidth radical such as trityl OX063, low γ nuclei meet this condition at the field considered in this work, so it is expected that ^{13}C and ^2H polarization will proceed by way of thermal mixing.^{18,39,40} In this mechanism, spin systems are treated as thermodynamic heat reservoirs with an associated spin temperature.^{38,41,42} In particular, reservoirs include the nuclear and electron Zeeman systems (NZS and EZS) and the electron dipolar system (EDS). Thermodynamically, polarization transfer proceeds when microwave irradiation near the electron resonance brings the three reservoirs into thermal contact and “cools” the NZS to a spin temperature value below thermal equilibrium.^{1,43,44}

The P(+) and P(−) polarization peaks in the microwave frequency sweep data in Fig. 1 denote the optimum microwave frequencies in which the trityl OX063-doped ^{13}C acetate samples can be irradiated to achieve the maximum polarization enhancement. Conventionally, DNP at P(+) will result in more nuclear spins populating the lower Zeeman energy level and conversely, microwave irradiation at P(−) will cause more spins to populate the upper Zeeman energy state. This polarization gradient in the microwave frequency sweep can be qualitatively described by the Borghini or spin temperature model of DNP.⁴⁰ The ^{13}C DNP samples in this study were irradiated at the P(+) microwave frequency. During DNP, all NMR-active nuclei in the DNP sample whose Larmor frequencies are comparable to the trityl ESR linewidth such as ^{13}C , ^{23}Na , and ^2H spins are expected to be in thermal contact with the trityl OX063 EDS. Protons, on the other hand, have a Larmor frequency that is much larger than the trityl OX063 ESR linewidth at 3.35 T and therefore ^1H spins are not in thermal contact with EDS as experimentally confirmed in a previous report.⁴⁵ We will revisit the pertinent discussion of this phenomenon later in the ^{13}C DNP efficiency when ^1H spins are replaced with ^2H spins in the methyl group of acetate.

Representative hyperpolarized solid-state ^{13}C NMR spectra of the ^{13}C isotopomers of sodium acetate at 3.35 T and 1.4 K are shown in Fig. 2. Since these frozen samples are under static or non-magic angle spinning (MAS) conditions, dipolar broadening is the most prominent feature visible in these ^{13}C NMR spectra at cryogenic conditions. For instance, the ^{13}C NMR spectrum of $[1-^{13}\text{C}]$ sodium acetate in Fig. 2a has a full-width at half-height value of around 9 kHz. Deuteration of the methyl group appears to have no significant effect on the solid-state ^{13}C NMR spectrum of the carboxyl ^{13}C spins of acetate as shown in Fig. 2b. Despite the large broadening, some of the NMR spectral features of these acetate samples with different ^{13}C isotopic labeling locations are distinguishable. In particular, there is a slight difference in NMR resonance locations between the carboxyl ^{13}C spins in Fig. 2a and methyl ^{13}C spins in Fig. 2c. Furthermore, the NMR spectrum of methyl ^{13}C spins in Fig. 2c has a slightly broadened shoulder ascribed to the coupling of methyl ^{13}C spins with methyl protons. When protons are replaced with deuterons in the methyl group, the broadened shoulders seen previously in Fig. 2c disappear and the resulting NMR spectrum of deuterated methyl ^{13}C spins in Fig. 2d resembles a symmetric single Gaussian or Lorentzian shape. On the other hand, the ^{13}C NMR spectrum of the doubly-labeled $[1,2-^{13}\text{C}_2]$ acetate in Fig. 2e appears to be a superposition of the ^{13}C NMR spectra of the

carboxyl ^{13}C spins in Fig. 2a and the methyl ^{13}C spins in Fig. 2c. In a similar fashion, the NMR spectrum of doubly-labeled and deuterated $[1,2-^{13}\text{C}_2, \text{d}_3]$ acetate displayed Fig. 2f is reminiscent of a combination of the ^{13}C NMR spectra of $[1-^{13}\text{C}]$ acetate in Fig. 2a and $[2-^{13}\text{C}, \text{d}_3]$ acetate in Fig. 2d.

Next, we have investigated the effect of ^{13}C isotopic labeling location in acetate on the efficiency of ^{13}C DNP. Inspection of Fig. 3 reveals that not only do they strongly affect the NMR spectra, but the different ^{13}C isotopic labels can significantly affect the achievable maximum ^{13}C DNP levels. Relative ^{13}C polarization buildup curves in Fig. 3a show that ^{13}C labeling in the carboxyl location yielded about twice the ^{13}C polarization obtained for the methyl or deuterated methyl ^{13}C spins in acetate. Deuteration of the methyl group resulted in a slight but not statistically significant decrease in the solid-state polarization of the carboxyl ^{13}C spins. In addition, the doubly-labeled $[1,2-^{13}\text{C}_2]$ and $[1,2-^{13}\text{C}_2, \text{d}_3]$ acetate samples yielded ^{13}C polarization levels that are comparable with the methyl or deuterated methyl ^{13}C spins in acetate. It should be noted that the ^{13}C polarization buildup curves displayed in Fig. 3a were normalized to the same number of ^{13}C spins for direct comparisons of relative solid-state ^{13}C polarization levels. A bar graph of the relative maximum ^{13}C polarizations that can be achieved from each isotopomer is shown in Fig. 3b. While it is expected that the ^{13}C labeling in the carboxyl location can be polarized most efficiently due to its relative isolation from protons, it is quite surprising that the ^{13}C polarization levels of the other samples are nearly equal. Based on dipolar relaxation arguments, it is also anticipated that methyl ^{13}C spins would yield lower ^{13}C polarization enhancement compared to ^{13}C DNP of carboxyl ^{13}C spins because of the direct coupling or proximity of methyl ^{13}C to methyl protons which are a dominant source of fluctuating magnetic fields. As noted before, deuteration of the methyl ^{13}C group is beneficial in the liquid-state because it increases ^{13}C T_1 and thus higher liquid-state ^{13}C NMR enhancements due to longer ^{13}C polarization preservation time.^{30–32} However, our solid-state ^{13}C DNP results indicate that there is no improvement in the solid-state ^{13}C polarization levels when the methyl group is deuterated for both the singly- and doubly- ^{13}C labeled acetate samples. Although not statistically significant as shown in Fig. 3b, deuteration in the methyl location, in fact, causes a slight reduction in the average ^{13}C polarization enhancement.^H

To explain these behavior regarding the effect of deuteration, we revisit the previous finding that the use of trityl OX063 as the polarizing agent, at least at 3.35 T and temperature close to 1 K, allows for the exclusion of ^1H spins from the polarization process via thermal mixing.⁴⁶ When ^1H is replaced with ^2H , the NZS of ^2H spins in the methyl groups is now brought into thermal contact with the EDS, thus increasing the total heat load for the EDS to cool, and thereby reducing the polarization enhancement.^{21,25,38} A similar effect has been observed when ^2H -enrichment is used in the glassing solvents where the ^{13}C polarization is reduced significantly by 30–50%.^{21,25} In the case of isotopic labeling of the substrate itself as in this work, ^2H labeling in the methyl group resulted in similar or slightly lower ^{13}C polarization compared to ^{13}C DNP of non-deuterated methyl ^{13}C spins, suggesting the extra ^2H NZS heat load of the deuterons in the methyl group is not as significant as the DNP effect of ^2H enrichment in the glassing matrix.^{21,25} This is borne out by considering the relative number of hydrogen atoms in the two systems. In the glassing matrix, water and glycerol are approximately 55 M and 13 M respectively, far exceeding the 3 M concentration

of acetate. As such, the reduction of ^{13}C polarization induced by ^2H labeling in the methyl group is small compared to that witnessed in deuteration of the glassing matrix. This is of great interest as it suggests that ^2H labeling may be utilized to lengthen liquid-state ^{13}C T_1 with only minor reductions in the solid-state ^{13}C polarization enhancement.

Based on the high polarization of singly-labeled carboxyl ^{13}C and the low polarization for other labels, it is suggested that polarization is strongly affected by relaxation interactions between nuclear spins which can be encompassed in a “leakage factor” f .^{34,38} It should be noted that f varies with γ^2 of the nucleus in question, suggesting that ^{13}C bonding to ^1H would lead to large f , while bonding to ^2H or ^{13}C would lead to comparatively small f .^{1,46} From this, one possible scenario is that the ^{13}C polarization of the methyl ^{13}C spins is governed primarily by the leakage factor given the proximity with protons. When methyl ^{13}C samples are deuterated, the leakage factor is reduced because of the weaker magnetic moment of ^2H , but the heat load of the NZS is increased, ultimately resulting in slightly weaker polarization enhancement. In addition, our suggested explanation for the lower ^{13}C polarization values of the doubly labeled $[1,2-^{13}\text{C}_2]$ and $[1,2-^{13}\text{C}_2, \text{d}_3]$ acetate samples is that the presence of both carboxyl and methyl ^{13}C labels in acetate contribute to mutual sources of fluctuating magnetic fields via dipolar interaction, leading to shorter solid-state ^{13}C T_1 values and thus lower ^{13}C polarization levels.

In light of the suggested DNP explanations related to nuclear relaxation, we have performed ^{13}C T_1 relaxation time measurements of these different ^{13}C isotopomers of acetate at 3.35 T and 1.4 K. The ^{13}C hyperpolarization decay curves of these different acetate samples along with the fits to the equation⁵ accounting for ^{13}C T_1 relaxation and RF pulsing are displayed in Fig. 4a. We should note that for doubly labeled samples, the displayed T_1 is calculated by fitting a decay curve to the total signal obtained from both ^{13}C peaks. In the supporting information, it is shown that the relaxation of the individual peaks mirror that of the convolution of the two spectra. The calculated average solid-state ^{13}C T_1 values of these trityl OX063-doped ^{13}C acetate isotopomers are displayed as a bar graph in Fig. 4b. It is apparent that the solid-state ^{13}C T_1 values of the different acetate isotopomers shown in Fig. 4 closely correlate with their corresponding relative ^{13}C polarization levels shown in Fig. 3. One prominent observation from Fig. 4 is that the carboxyl ^{13}C T_1 relaxation value is about twice the ^{13}C T_1 of the methyl ^{13}C spins and the other isotopomers of acetate. These solid-state ^{13}C T_1 results roughly scale with their solid-state ^{13}C polarization values. Another important observation from Fig. 4 is that there seems to be a significant reduction of ^{13}C T_1 when the methyl group is deuterated. This ^{13}C T_1 reduction effect by deuteration is, as noted before, mirrored on a weaker scale by ^{13}C polarization reduction for the deuterated versions of the acetate isotopomer shown in Fig. 3. The shortening of methyl ^{13}C T_1 in the solid-state when ^1H ($\gamma=42.6$ MHz/T) is exchanged for ^2H ($\gamma=6.54$ MHz/T) in acetate is somewhat unexpected considering that deuterons have weaker magnetic moments than protons, and therefore weaker sources of fluctuating magnetic fields for relaxation. As noted previously, the exact opposite occurs in the liquid-state in which ^2H enrichment of the molecule leads to longer ^{13}C T_1 .⁴⁷ Though the cause of this counterintuitive result is not immediately clear, one possible explanation is that of heteronuclear “cross-talk” between hyperpolarized ^{13}C and ^2H spins. It has been found that there may be polarization exchange between ^1H and ^2H in the presence of electrons, which is termed the heteronuclear cross effect, suggesting the

possibility of a similar exchange between ^{13}C and ^2H .⁴⁸ This exchange causes the equalization of polarization enhancements of the two different spin species, suggesting its application to the present case. The solid-state relaxation time of deuterium is much shorter than that of ^{13}C , so as ^2H relaxes, these spins act as a “sink” for ^{13}C polarization. That is, as ^2H spins relax, polarization is pulled from the ^{13}C spins, leading to slower ^2H and faster ^{13}C relaxation. Further studies are needed to confirm this or in general, pinpoint the exact cause of this effect on solid-state ^{13}C T_1 by deuteration. Testing of the effect would require monitoring of the hyperpolarized ^2H NMR signal during relaxation which is currently outside the capability of our hyperpolarizer. Nevertheless, our results here have indicated that the location of ^{13}C isotopic labeling has a significant effect on the maximum achievable ^{13}C polarization levels.

4. Conclusion

It has been shown that the ^{13}C isotopic labeling of acetate has a significant effect on ^{13}C DNP efficiency when using the trityl OX063 free radical. When considering both the polarization enhancement and T_1 , $[1\text{-}^{13}\text{C}]$ acetate has significant advantages over the other labelings studied due to its isolation from other nuclear magnetic spins. ^{13}C labeling in the methyl group greatly reduced the efficiency of DNP, leading to lesser polarization enhancement and short T_1 due to the coupling to adjacent nuclear spins which increases the nuclear relaxation leakage factor. Contrary to liquid-state results, deuteration of the methyl group leads to smaller solid-state ^{13}C polarization gain and faster relaxation due to possible increased NZS heat load and heteronuclear cross-talk between hyperpolarized ^{13}C and ^2H spins. Our results suggest that there is a close correlation between the maximum achievable solid-state ^{13}C polarization values and their corresponding solid-state ^{13}C T_1 relaxation times.

5. Supporting Information

In the supporting information, we show the deconvolution of ^{13}C NMR spectra for the doubly labelled isotopomers of acetate. This allows for the determination of solid-state T_1 for each ^{13}C location as well as the overall ^{13}C signal of the molecule. For $[1,2\text{-}^{13}\text{C}_2]$ sodium acetate, the methyl ^{13}C signal is dwarfed by the carboxyl, making an accurate assessment of the methyl T_1 difficult. In the case of $[1,2\text{-}^{13}\text{C}_2, \text{d}_3]$ sodium acetate, the peaks corresponding to the two locations are approximately equal in intensity, making the deconvolution more effective. This sample shows that there is “cross-talk” between the ^{13}C spins in different locations, resulting in all ^{13}C spins, regardless of location, to relax at approximately the same rate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The free radical polarizing used in this work (left) and a representative ^{13}C microwave DNP spectrum (right) of trityl OX063-doped $[1-^{13}\text{C}]$ acetate sample showing the optimum microwave irradiation frequencies P(+) and P(-) for DNP at 3.35 T and 1.4 K. All the other ^{13}C isotopomers of acetate have the same locations of P(+) and P(-).

Figure 2.

Structures and representative normalized hyperpolarized ^{13}C NMR spectra of frozen solutions of sodium acetate with different isotopic labeling locations: (a) $[\text{1-}^{13}\text{C}]$, (b) $[\text{1-}^{13}\text{C}, \text{d}_3]$, (c) $[\text{2-}^{13}\text{C}]$, (d) $[\text{2-}^{13}\text{C}, \text{d}_3]$, (e) $[\text{1}, \text{2-}^{13}\text{C}_2]$, and (f) $[\text{1}, \text{2-}^{13}\text{C}_2, \text{d}_3]$. These ^{13}C NMR spectra were taken at 3.35 T and 1.4 K from 100 μL aliquots of 3 M ^{13}C acetate samples in 1:1 v/v glycerol:water doped with 15 mM trityl OX063.

Figure 3.

Relative solid-state ^{13}C DNP signals of various isotopic labeling locations of sodium acetate taken at 3.35 T and 1.4 K: (a) Buildup curves of the relative ^{13}C polarization as a function of microwave irradiation time. The error bars are standard deviations for trials done in triplicate. The down arrow indicates the direction of decreasing polarization level for the different isotopic labeling locations. (b) Maximum ^{13}C DNP signals for the different isotopomers of sodium acetate taken from data in (a). The data for the doubly-labeled $[1,2-^{13}\text{C}_2]$ acetate and $[1,2-^{13}\text{C}_2, \text{d}_3]$ acetate samples were normalized according to the same

^{13}C spin count for direct comparison with the maximum ^{13}C DNP signals of the singly-labeled ^{13}C acetate samples.

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Figure 4.

Lifetimes of the solid-state ^{13}C polarizations of hyperpolarized ^{13}C isotopomers of sodium acetate at 3.35 T and 1.4 K: (a) Decay of the hyperpolarized ^{13}C NMR intensity of each sample monitored by applying a 2-degree RF pulse every 20 minutes after the microwave source was turned off. The down arrow indicates the direction of decreasing relaxation times for the various ^{13}C acetate isotopomers. The ^{13}C DNP decay data were fitted to a single-exponential equation accounting for the DNP signal loss due T_1 decay and RF pulsing. (b) Comparative solid-state ^{13}C T_1 values of the ^{13}C -enriched acetate samples calculated from

data in (a). The error bars are standard deviations for trials done in triplicate. The ^{13}C T_1 values reported for the doubly-labeled $[1,2-^{13}\text{C}_2]$ sodium acetate and $[1,2-^{13}\text{C}_2, \text{d}_3]$ sodium acetate were done by integrating the areas of both the carbonyl and methyl ^{13}C spins. See Supporting Information for separate T_1 analysis of the deconvoluted ^{13}C NMR spectra of doubly-labeled ^{13}C acetate samples.