^{13}C Dynamic Nuclear Polarization using SA-BDPA at 6.7 T and 1.1 K

Coexistence of Pure Thermal Mixing and Well-Resolved Solid Effect

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**^{13}C Dynamic Nuclear Polarization using SA-BDPA at 6.7 T
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^{13}C Dynamic Nuclear Polarization using SA-BDPA at 6.7 T and 1.1 K: Coexistence of Pure Thermal Mixing and Well-Resolved Solid-Effect

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ABSTRACT

SA-BDPA is a water-soluble, narrow-linewidth radical previously used for dynamic nuclear polarization (DNP) signal enhancement in solid-state magic angle spinning NMR spectroscopy. Here, we report the first study using SA-BDPA under dissolution DNP conditions (6.7 T and 1.15 K). Longitudinal-detected (LOD)-electron spin resonance (ESR) and ^{13}C DNP measurements were performed on samples containing 8.4 M ^{13}C urea dissolved in 50:50 water:glycerol (v/v) doped with either 60 mM or 120 mM SA-BDPA. Two distinct DNP mechanisms, both “pure” thermal mixing and a well-resolved solid effect could clearly be identified. The radical’s ESR linewidth (30–40 MHz), broadened predominantly by dipolar coupling, excluded any contribution from the cross

effect. Microwave frequency modulation increased the enhancement by DNP at the lower radical concentration but not at the higher radical concentration. These results are compared to data acquired with trityl radical AH111501, highlighting the unusual ^{13}C DNP properties of SA-BDPA.

TOC GRAPHICS

KEYWORDS: dissolution DNP, SA-BDPA, ^{13}C , Thermal Mixing, Cross Effect, Solid Effect, Hyperpolarization, microwave frequency modulation (mod).

Dynamic nuclear polarization (DNP) is the physical phenomenon describing the transfer of spin order from unpaired electrons to nuclei upon microwave irradiation at the appropriate frequency.^{1,2} Known since the 1950s through the seminal work of Overhauser, Carver and Slichter,^{3,4} it remained a high energy physics niche topic⁵ before

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4 its adoption by the NMR community as a way to enhance the signal arising from the nuclei
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7 in both solid-state with magic angle spinning (MAS)-DNP,^{6–10} and liquid state with
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10 Overhauser DNP¹¹ or dissolution DNP (dDNP).¹²
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15 As its name implies, dDNP produces solutions of hyperpolarized nuclei by dissolving solid
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18 samples prepared by DNP, usually at low temperature (1 – 1.4 K) and moderate magnetic
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21 field (3.35 – 7 T). Low-power microwaves (50 – 100 mW) excite unpaired electron spins,
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24 added to the sample in the form of organic free radicals, and trigger the polarization
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27 transfer. These radicals are routinely present at concentrations ranging from 10 mM to 60
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30 mM, depending on the radical species and magnetic field strength.¹³ Low- γ nuclei, such
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33 as ^{13}C , ^6Li and ^{15}N , are often the target of dDNP experiments, as they have a wide range
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36 of possible applications^{14–19} and their relatively slow longitudinal relaxation rates extend
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39 the useful time frame for exploiting the enhanced signal.
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47 Under conditions typical for dDNP, the nuclear hyperpolarization can be explained mainly
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50 *via* two mechanisms relying on the saturation of first order (or “allowed”) electron spin
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53 transitions which are on resonance with the microwave field: the cross effect (CE), which
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is based on quantum mechanics and relaxation on small spin systems; and thermal mixing (TM), which originates from the macroscopic concept of spin temperature.^{20–23} A different mechanism, the solid effect (SE),¹ can also enhance the nuclear polarization but, its contribution under dDNP conditions is generally considered minor. Indeed, SE involves the saturation of second order (or “forbidden”) electron-nucleus spin transitions (i.e. zero-quantum and double-quantum), making high microwave power a key requirement.^{8,23–25} Motivated by the need for optimized dDNP conditions and samples preparation, a substantial effort has been made during the last decades to achieve a better theoretical understanding of the underlying physical phenomena. In particular, several studies have been published concerning the circumstances that promote CE over TM and vice versa.^{26–32} Very recently, T. W. Wenckebach provided a more unified vision demonstrating that CE and TM represent two extreme cases of the same physical phenomenon: nuclear polarization enhancement via triple spin flips, i.e. the simultaneous spin transitions of two electron spins and one nuclear spin.^{33,34} A triple spin flip entails a simultaneous modification of the electron Zeeman interaction and electron dipolar interaction that, under the right conditions, can both provide the appropriate energy

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4 quanta to be exchanged with the nuclear spin system.^{33,34} More specifically, CE requires
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7 the presence of at least two electron spins with Zeeman energy difference corresponding
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10 to that of the nuclear Zeeman ($\hbar\omega_I$), whereas TM requires a sufficiently strong dipolar
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13 interaction between electron spin pairs that fulfills the cut-off frequency condition $\omega_{CO} >$
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16 ω_I . The cut-off frequency represents the maximum possible value of dipolar coupling
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19 between a pair of electron spins, thus corresponding to the minimum possible distance
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22 between them. Therefore, CE can be identified as the flow of electron Zeeman energy
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25 and TM as the flow of electron dipolar energy.^{33,34} Although in theory, the relative
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28 strengths of the two flows of energy depends on the ratio between ω_{CO} and ω_I , it is hard
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31 to distinguish them in practice. Most of the time, the two mechanisms occur
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34 simultaneously because the magnetic field values and radicals typically used in dDNP
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37 (trityls and nitroxides), lead to electron spin resonance (ESR) linewidths greater than ω_I
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40 and a minimum possible distance between electron spin pairs small enough to provide
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43 sufficient dipolar coupling. Recently, Guarin et al., studying samples with increasing
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46 radical concentration (10 – 100 mM), provided an elegant experimental demonstration of
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49 the conditions necessary to exclude TM from being the dominant mechanism when
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performing ^{13}C dDNP at 6.7 T on water:glycerol amorphous solids doped with nitroxide radicals. They concluded that TM was quenched at low radical concentration when sufficiently strong dipolar coupling was not provided.²⁷ To the best of our knowledge, no demonstration has been provided to date for the opposite case under these experimental conditions, i.e. polarization transfer to ^{13}C taking place solely via TM. This would require a water-soluble, narrow-ESR line radical broadened predominately by electron-electron dipolar coupling.

We herein report the use of the polarizing agent SA-BDPA (see Figure 1 for molecular structure) to perform dDNP on $[^{13}\text{C}]$ urea at 6.7 T and 1.1 K. SA-BDPA is a sulfonated water-soluble derivative of BDPA that, to date, has mainly been studied at MAS-DNP conditions.^{35–39}

Figure 1. Planar (left) and tridimensional (right) molecular structure of SA-BDPA. In the tridimensional representation the unpaired electron site is reported in blue.

We collected longitudinal-detected (LOD)-ESR and ^{13}C DNP measurements on a sample of 8.4 M of ^{13}C urea dissolved in a solution of water:glycerol 50:50 (v/v) and doped with either 60 or 120 mM of SA-BDPA (“SA60 sample” and “SA120 sample”, respectively). For comparison, we also investigated a sample doped with 30 mM of AH111501 trityl radical instead (“TR30 sample”).

Figure 2. ^{13}C DNP and ESR properties of 8.4 M ^{13}C urea doped with 60 mM SA-BDPA radical (SA60 sample). Panel A: normalized LOD-ESR spectrum of the radical and ^{13}C DNP frequency sweep profiles at different microwave frequency modulation amplitudes (modulation rate: 1 kHz); the LOD-ESR spectrum intensity was normalized to 1, while the DNP profiles were normalized according to best performing conditions (i.e. 25 MHz microwave frequency modulation amplitude). Panel B: representative T_{1e} experimental curve and fit. All measurements were performed at 6.7 T, 1.1 K.

Details about the experimental methods used in this study are available in Supporting Information.

Results for the SA60 sample are reported in Figure 2. Panel A shows the radical LOD-ESR spectrum along with three DNP spectra, acquired with monochromatic microwaves as well as with microwave frequency modulation (10 MHz and 25 MHz modulation

amplitude at 1 kHz modulation rate, following a sine function modulation scheme). The ESR spectrum, with ω_S centered at 187.97 GHz, revealed a symmetric appearance, as one would expect at high magnetic field for an electron spin system with negligible g-tensor anisotropy and unresolved hyperfine coupling to protons.^{35,36} The full-width-at-half-maximum (FWHM) was only 30.5 ± 1.0 MHz, less than half of the ^{13}C ω_I at 6.7 T (71.8 MHz), in good agreement with prior reports.^{35,36} Surprisingly, the DNP sweep collected using monochromatic microwaves revealed an unconventional pattern. In addition to the usual two lobes overlapping with the ESR spectrum, positive DNP enhancement was also measured at frequencies around 187.90 GHz and negative DNP enhancement around 188.04 GHz. The relative maxima with lower intensity than the central lobes were separated by 144 ± 1.0 MHz. When modulating the microwave frequency with an amplitude of 10 MHz, the four lobes grew in intensity and the central ones also shifted further away from ω_S . With a frequency modulation amplitude of 25 MHz, the two positive lobes merged, as did the two negative ones, and the overall enhancement almost doubled compared to the non-modulated spectrum. Moreover, the positive and negative maximum enhancements appeared to shift by 25 MHz away from ω_S , compared to the non-

modulated DNP sweep. The radical longitudinal relaxation time (T_{1e}) measured 230 ± 4 ms, as shown in Figure 2B.

Figure 3. ^{13}C DNP and ESR properties of 8.4 M ^{13}C urea doped with 120 mM SA-BDPA radical (SA120 sample). Panel A: normalized LOD-ESR spectrum of the radical and ^{13}C DNP frequency sweep profiles at different microwave frequency modulation amplitudes; the LOD-ESR spectrum intensity was normalized to 1, while the DNP profiles were normalized according to best performing conditions (i.e. 25 MHz microwave frequency modulation amplitude). Panel B: representative T_{1e} experimental curve and fit. All measurements were performed at 6.7 T, 1.1 K.

For the SA120 sample, T_{1e} decreased by approximately 10% compared to SA60, revealing a relatively small dependence on radical concentration (Figure 3B). The ESR linewidth, however, increased by 30%, with a FWHM of 41.5 ± 1.0 MHz. This is consistent

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3 with a stronger dipolar interaction among the electron spins due to the increased radical
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7 concentration (Figure 3A). Doubling the radical concentration also affected the DNP
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11 spectra. When applying microwaves without modulation four lobes were still discernable,
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14 but the separation between the central and side lobes was less marked, particularly for
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17 the positive enhancements (Figure 3A). Applying microwave frequency modulation, the
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21 two positive and two negative lobes were merged already applying a modulation
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24 amplitude of 10 MHz and no increase in DNP enhancement was observed, even with a
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28 modulation amplitude of 25 MHz.
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33 When comparing the non-modulated microwaves sweeps of the two SA-BDPA samples,
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36 it is clear that the DNP process takes place by two different mechanisms: the DNP pattern
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40 overlapping with the radical ESR line is related to energy transfer via triple spin flips, while
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43 the furthest peaks carry the clear signature of SE.^{25,33,34} While the latter is unusual under
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46 these experimental conditions, the two outer lobes have maxima located at microwave
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50 frequencies equal to $\omega_S - \omega_I$ and $\omega_S + \omega_I$, respectively corresponding to the double-
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54 quantum and zero-quantum spin transitions. The intensity of the outer lobes is relatively
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low compared to the central lobes because SE is a second-order process, strongly depending on microwave power.^{35,36,40} For a radical like SA-BDPA, one would expect TM to be responsible for the central lobes. Its g-anisotropy being negligible, especially at high magnetic field, it is unlikely to find two distinct electron spin packets S_1 and S_2 with a Zeeman energy difference large enough to satisfy the CE relation $\hbar\omega_{S1} - \hbar\omega_{S2} = \hbar\omega_l$. On the other hand, conditions for TM can still be met, even though the ESR spectrum FWHM of both samples is smaller than ω_l . As noted before, one important parameter for TM is the relation between ω_l and the cutoff frequency ω_{CO} . For an ensemble of randomly oriented electron spins, it can be expressed in frequency units as (see Supporting Information for a derivation of the formula):²⁵

$$\Delta_0 = \sqrt{\frac{1}{5\pi^2 4\pi}} \frac{\mu_0 \hbar \gamma_s^2}{r_0^3} \quad (1)$$

with r_0 the minimal distance between two radicals. Using molecular modeling (see Supporting Information), this value for SA-BDPA was estimated to be 8.0 Å, which corresponds to a Δ_0 value of 91 MHz, i.e. 1.3 times the ^{13}C Larmor frequency at 6.7 T. It

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4 is therefore reasonable to ascribe the center lobes of the microwave spectrum to electron
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7 dipolar energy transfer to nuclei.^{32,34} This interpretation is further supported by the
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10 behavior observed in the SA120 sample, as follows: by doubling the radical concentration,
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13 and thus increasing the dipolar coupling, TM becomes more efficient, and microwave
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16 frequency modulation does not lead to any further polarization enhancement. This is
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19 readily explained considering one of the essential elements of this DNP mechanism,
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22 namely spectral diffusion.^{25,29,41} When spectral diffusion is fast compared to the electron
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25 spin-lattice relaxation time, the hole burned by the microwave into the ESR spectrum
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28 spreads across its whole width via energy-conserving electron-electron flip-flops. In this
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31 case, all the electron spins participate in the polarization transfer to nuclei. Conversely,
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34 with slow spectral diffusion, only a fraction of electron spins will participate, making the
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37 DNP mechanism less efficient.^{25,29} The flip-flop transition probability is a function of the
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40 electron spins' average dipolar coupling and therefore increases with radical
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43 concentration.^{25,33} If the coupling is not sufficiently strong compared to the ESR line width,
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46 microwave frequency modulation can help in broadening the initial hole by engaging more
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49 spin packets.^{42,43}
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The simplest cut-off frequency model for TM does not consider the possibility of radicals clustering. To this regard, an interesting remark can be made concerning an old experiment performed in 1976 by W. de Boer for ^1H nuclei in a *m*-xylene- d_6 sample doped with BDPA at 2.5 T and 0.5 K.²³ In that case the well-resolved was the main mechanism, but some TM was still visible although protons ω_1 was 106 MHz and radical the concentration only 6×10^{18} spins/cm³ (10 mM).

It is worth noting that, in the SA60 sample, microwave frequency modulation also yielded a clear increase in the SE enhancement. Especially for a microwave frequency modulation amplitude of 10 MHz, where the distinction between the SE and TM was still clear, the SE enhancement improvement was higher than the TM enhancement improvement. This suggests that the improvement we observed on the external lobes was not an effect of superposition between TM and SE. Moreover, upon modulation the SE lobes became wider and the buildup of polarization faster (data not shown). This phenomenon is new and deserves further investigation. Herein, we propose a qualitative argument. Being the g-anisotropy negligible (see Fig 3 of ref 35), a possible explanation

might be related to SA-BDPA unresolved hyperfine interaction to protons.³⁵ It has been reported earlier that, when modulating the frequency with sufficient amplitude, all hyperfine lines within the modulated range share the power. As a consequence, a larger part of the ESR spectrum responds to the excitation rather than only the electron spins on resonance with monochromatic irradiation.⁴⁴ Therefore, several forbidden-transitions can be excited at the same time speeding up the DNP process and making it more efficient.

Figure 4. ^{13}C DNP and ESR properties of 8.4 M ^{13}C urea doped with 30 mM AH111501 radical (TR30 sample). Panel A: normalized LOD-ESR spectrum of the radical and ^{13}C DNP frequency sweep profiles. Right: representative T_{1e} experimental curve and fit. All measurements were performed at 6.7 T, 1.1 K.

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4 When considering the trityl radical sample (TR30), the ESR spectrum showed an
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7 asymmetric line-shape due to its characteristic g-tensor anisotropy, in good agreement
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10 with previous studies at high field (Figure 4A).^{45,46} The center of gravity was measured at
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13 188.015 GHz, and the FWHM was 110 ± 5 MHz. In this case, no DNP enhancement was
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16 measured outside the ESR line, and a typical two-lobed DNP pattern was observed
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19 (Figure 4A). The $T_{1\rho}$ of 610 ± 15 ms (Figure 4B) was about three times longer than that
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22 of the two SA-BDPA samples (Figure 2B and 3B). As previously reported for similar
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25 samples,^{47,48} microwave frequency modulation did not improve the DNP enhancement
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28 (data not shown).
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Figure 5. Representative $[^{13}\text{C}]$ urea polarization buildup curves of the SA-BDPA and AH11501 samples at 6.7 T, 1.1 K. While the trityl data were acquired shining monochromatic radiation, the SA-BDPA datasets were acquired with a modulation amplitude of the microwave frequency of 25 MHz. For each sample the irradiation frequency was the one providing the highest enhancement with respect to the different DNP sweeps (see Figure 2, 3 and 4). The reported polarization levels are the back-calculated values from the liquid-state measurements. The manual transfer of the hyperpolarized solution took 10 s.

To compare the three samples, we measured polarization buildups at best DNP conditions (Figure 5, see Table 1 for experimental details). All solid-state polarization values were back calculated from liquid-state polarization measurements after dissolution and transfer to a 9.4 T vertical NMR magnet (see Supporting Information). A ^{13}C polarization of $25.8 \pm 2.6\%$ was measured on the SA60 sample, and this value improved

to 42.7 ± 3.4 % for the SA120 sample. The polarization build-up time constant decreased from 16200 ± 2000 s to 3070 ± 60 s for increased SA-BDPA concentration. The TR30 sample showed the best performance in terms of both polarization level and build-up rate, with values comparable to those previously reported for $[1-^{13}\text{C}]$ pyruvic acid at similar experimental conditions.^{47–49} The polarization transfer in this latter case can be ascribed to CE. The AH11150 trityl radical molecule is too large (i.e. 10.5 Å) to fulfill the cut-off frequency condition ($\Delta_0 = 40$ MHz). Differently its g-tensor anisotropy results in electron spin packets spreading across almost 150 MHz, thus matching the CE condition. In this regard, it is worth noting that J. Wolber et al., in the context of ^1H dDNP at 3.35 T using trityl radicals, did not report any enhancement overlapping with the radical ESR line. Indeed, with a proton $\omega_1 = 144$ MHz, neither the TM condition nor the CE one could be fulfilled in that case.⁴⁰ The DNP performance difference between trityl and SA-BDPA was remarkable. The latter can be ascribed to the cut-off frequency being very close to the ^{13}C Larmor frequency for SA-BDPA samples. This means that only few radical pairs hold enough dipolar energy to participate to TM, making polarization transfer less efficient than the trityl sample, which relies a well-matched CE condition.

	<u>LOD ESR</u>		<u>Solid-State NMR</u>				<u>Liquid-state NMR</u>	
Sample	FWHM (MHz)	T _{1e} (ms)	μ w frequenc y (GHz)	Mod amplitud e (MHz)	Buildup time constant (s)	¹³ C polarizatio n (%)	Relaxatio n time (s)	¹³ C polarizatio n (%)
SA60	30.5 ± 1.0	230 ± 4	187.93	25	16200 ± 2000	30.6 ± 3.0	58 ± 4	25.8 ± 2.6
SA120	41.5 ± 0.5	204 ± 8	187.91	25	3070 ± 60	42.8 ± 3.4	58 ± 1	36.0 ± 3.0
TR30	110.0 ± 5.0	610 ± 15	187.93	0	2060 ± 160	64.5 ± 1.8	55 ± 5	53.5 ± 1.5

Table 1. Numerical summary of results and experimental parameters used for the three samples of the study.

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4 In this study we investigated the properties of the water-soluble radical SA-BDPA at high
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7 field dDNP conditions for direct [^{13}C]urea hyperpolarization. The unique properties of this
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10 polarizing agent at 6.7 T allowed us to observe two distinct DNP mechanisms, well-
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13 resolved SE and TM, in the same sample with ^{13}C . To the best of our knowledge, this is
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16 the first experimental demonstration of such a resolution of separate DNP mechanisms
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19 on a low- γ nucleus.
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26 At 3.35 T, the most common dDNP magnetic field strength, BDPA and its benzyl
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29 derivatives at concentrations between 40 mM and 60 mM have been reported to yield
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32 excellent polarization levels in a very short time,^{50–52} whereas SA-BDPA yielded high ^{13}C
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35 polarization only at very high radical concentration. Although the latter did not have any
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38 detrimental effect on the ^{13}C urea liquid state relaxation time (see Table 1), investigating
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41 whether this high amount of radical would present an issue for possible future applications
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44 remains to be determined. Finally, we note a surprising feature: the generally-used
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47 criterion for TM mechanism occurrence compares the radical ESR spectrum FWHM (or
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50 the square root of the second moment) to the Larmor frequency of the nucleus of interest
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(i.e. $\text{FWHM} > \omega_I$).^{1,25} While this criterion may be appropriate for very broad radicals such as nitroxides when investigating low- γ nuclei, we demonstrate here that this is not necessarily the case for all radicals. With the cut-off frequency being a crucial parameter, it would be more appropriate to instead consider the full ESR spectral width at its base. In our case these values measured 81 ± 5 MHz for the SA60 sample and 99 ± 5 MHz for the SA120 sample, thus matching the TM condition for the ^{13}C Larmor frequency at 6.7 T. An extreme case would be represented by radicals showing an ESR linewidth much smaller than the nuclear Larmor frequency. Here, the strong dipolar couplings offering a mechanistic basis for TM-DNP would exceed by far the spectrum FWHM and tend to broaden into the background, making them difficult to be detected experimentally. This has been recently demonstrated to be possible by Equbal et al. in the case of ^1H TM-DNP at 7 T using trityls at very high concentration.⁵³ They reported proton enhancement spreading over 300 MHz, while the ESR linewidth measured only 100 MHz. The latter represents a clear case where the ESR spectrum FWHM does not correspond to the DNP mechanism involved because radical clustering is involved. This study provided also experimental evidence of another parameter of remarkable importance for efficient

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3 thermal mixing: the asymmetry of the dipolar coupling network.³² Although we could not
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7 observe it in our experiments, this asymmetry is the key for a pure quantum mechanics
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10 treatment of TM.
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NOTES

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ASSOCIATED CONTENT

Supporting Information material is available free of charge via the Internet at

<http://pubs.acs.org>. Supporting Information includes:

- Experimental methods
 - Sample preparation
 - Radical concentration measurements
 - LOD-ESR measurements
 - DNP experiments
 - Estimation of inter-radical distance
 - Derivation of equation 1
- Additional results
 - Evaluation via ESR of SA-BDPA concentration in DNP samples
 - DNP buildup at different microwave frequency modulation amplitude
 - Molecular dimensions of SA-BDPA and trityl AH111510

REFERENCES

- (1) Abragam, A.; Goldman, M. Principles of Dynamic Nuclear-Polarization. *Reports on Progress in Physics* **1978**, *41* (3), 395–467. <https://doi.org/Doi 10.1088/0034-4885/41/3/002>.
- (2) Abragam, A.; Goldman, M. *Order and Disorder*; Clarendon Press, Oxford, 1982.
- (3) Overhauser, A. W. Polarization of Nuclei in Metals. *Physical Review* **1953**, *92* (2), 411.
- (4) Carver, T. R.; Slichter, C. P. Polarization of Nuclear Spins in Metals. *Physical Review* **1953**, *92* (1), 212.
- (5) van den Brandt, B.; Hautle, P.; Konter, J. A.; Kurdzesau, F. Dynamic Nuclear Polarization – from Polarized Targets to Metabolic Imaging. *Appl Magn Reson* **2008**, *34* (3–4), 475–481. <https://doi.org/10.1007/s00723-008-0111-y>.
- (6) Becerra, L. R.; Gerfen, G. J.; Temkin, R. J.; Singel, D. J.; Griffin, R. G. Dynamic Nuclear Polarization with a Cyclotron Resonance Maser at 5 T. *Phys. Rev. Lett.* **1993**, *71* (21), 3561–3564. <https://doi.org/10.1103/PhysRevLett.71.3561>.
- (7) Gerfen, G. J.; Becerra, L. R.; Hall, D. A.; Griffin, R. G.; Temkin, R. J.; Singel, D. J. High Frequency (140 GHz) Dynamic Nuclear Polarization: Polarization Transfer to a Solute in Frozen Aqueous Solution. *The Journal of chemical physics* **1995**, *102* (24), 9494–9497.
- (8) Wind, R. A.; Duijvestijn, M. J.; van der Lugt, C.; Manenschijn, A.; Vriend, J. Applications of Dynamic Nuclear Polarization in ^{13}C NMR in Solids. *Progress in Nuclear Magnetic Resonance Spectroscopy* **1985**, *17*, 33–67.
- (9) Singel, D. J.; Seidel, H.; Kendrick, R. D.; Yannoni, C. S. A Spectrometer for EPR, DNP, and Multinuclear High-Resolution NMR. *Journal of Magnetic Resonance (1969)* **1989**, *81* (1), 145–161. [https://doi.org/10.1016/0022-2364\(89\)90273-4](https://doi.org/10.1016/0022-2364(89)90273-4).

- (10) Afeworki, M.; McKay, R. A.; Schaefer, J. Selective Observation of the Interface of Heterogeneous Polycarbonate/Polystyrene Blends by Dynamic Nuclear Polarization Carbon-13 NMR Spectroscopy. *Macromolecules* **1992**, *25* (16), 4084–4091. <https://doi.org/10.1021/ma00042a006>.
- (11) Griesinger, C.; Bennati, M.; Vieth, H. M.; Luchinat, C.; Parigi, G.; Höfer, P.; Engelke, F.; Glaser, S. J.; Denysenkov, V.; Prisner, T. F. Dynamic Nuclear Polarization at High Magnetic Fields in Liquids. *Progress in Nuclear Magnetic Resonance Spectroscopy* **2012**, *64*, 4–28. <https://doi.org/10.1016/j.pnmrs.2011.10.002>.
- (12) Ardenkjaer-Larsen, J. H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M. H.; Servin, R.; Thaning, M.; Golman, K. Increase in Signal-to-Noise Ratio of > 10,000 Times in Liquid-State NMR. *Proceedings of the National Academy of Sciences of the United States of America* **Sep 2**, *100* (18), 10158–10163.
- (13) Ardenkjaer-Larsen, J. H. On the Present and Future of Dissolution-DNP. *J Magn Reson* **2016**, *264*, 3–12. <https://doi.org/10.1016/j.jmr.2016.01.015>.
- (14) Nelson, S. J.; Kurhanewicz, J.; Vigneron, D. B.; Larson, P. E. Z.; Harzstark, A. L.; Ferrone, M.; van Criekinge, M.; Chang, J. W.; Bok, R.; Park, I.; Reed, G.; Carvajal, L.; Small, E. J.; Munster, P.; Weinberg, V. K.; Ardenkjaer-Larsen, J. H.; Chen, A. P.; Hurd, R. E.; Odegardstuen, L. I.; Robb, F. J.; Tropp, J.; Murray, J. A. Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [$1\text{-}^{13}\text{C}$]Pyruvate. *Science Translational Medicine* **Aug 14**, *5* (198), 198ra108 1–10. <https://doi.org/ARTN 198ra108 DOI 10.1126/scitranslmed.3006070>.
- (15) van Heeswijk, R. B.; Uffmann, K.; Comment, A.; Kurdzesau, F.; Perazzolo, C.; Cudalbu, C.; Jannin, S.; Konter, J. A.; Hautle, P.; van den Brandt, B.; Navon, G.; van der Klink, J. J.; Gruetter, R. Hyperpolarized Lithium-6 as a Sensor of Nanomolar Contrast Agents. *Magn Reson Med* **2009**,

61 (6), 1489–1493. <https://doi.org/10.1002/Mrm.21952>.

(16) Bowen, S.; Hilty, C. Time-Resolved Dynamic Nuclear Polarization Enhanced NMR Spectroscopy. *Angewandte Chemie* **2008**, *120* (28), 5313–5315.

(17) Lee, Y.; Heo, G. S.; Zeng, H.; Wooley, K. L.; Hilty, C. Detection of Living Anionic Species in Polymerization Reactions Using Hyperpolarized NMR. *Journal of the American Chemical Society* **2013**, *135* (12), 4636–4639.

(18) Lerche, M. H.; Jensen, P. R.; Karlsson, M.; Meier, S. NMR Insights into the Inner Workings of Living Cells. *Analytical Chemistry* **2015**, *87* (1), 119–132.

(19) Harris, T.; Eliyahu, G.; Frydman, L.; Degani, H. Kinetics of Hyperpolarized ^{13}C -1-Pyruvate Transport and Metabolism in Living Human Breast Cancer Cells. *Proceedings of the National Academy of Sciences* **2009**, *106* (43), 18131–18136.

(20) Borghini, M. *Mechanism of Nuclear Dynamic Polarization by Electron-Nucleus Dipolar Coupling in Solids*; European Organization for Nuclear Research, Geneva (Switzerland), 1971.

(21) Borghini, M. Spin-Temperature Model of Nuclear Dynamic Polarization Using Free Radicals. *Physical Review Letters* **1968**, *20* (9), 419-. <https://doi.org/DOI10.1103/PhysRevLett.20.419>.

(22) Hwang, C. F.; Hill, D. A. Phenomenological Model for the New Effect in Dynamic Polarization. *Phys. Rev. Lett.* **1967**, *19* (18), 1011–1014. <https://doi.org/10.1103/PhysRevLett.19.1011>.

(23) de Boer, W. Dynamic Orientation of Nuclei at Low Temperatures. *J Low Temp Phys* **1976**, *22* (1–2), 185–212. <https://doi.org/10.1007/BF00655221>.

- (24) de Boer, W.; Borghini, M.; Morimoto, K.; Niinikoski, T. O.; Udo, F. Dynamic Polarization of Protons, Deuterons, and Carbon-13 Nuclei: Thermal Contact between Nuclear Spins and an Electron Spin-Spin Interaction Reservoir. *Journal of Low Temperature Physics* **1974**, *15* (3–4), 249–267.
- (25) Wenckebach, W. Th. *Essentials of Dynamic Nuclear Polarization*; Publications, S., Series Ed.; Ridderprint B.V. Ridderkerk: The Netherlands, 2016.
- (26) Serra, S. C.; Filibian, M.; Carretta, P.; Rosso, A.; Tedoldi, F. Relevance of Electron Spin Dissipative Processes to Dynamic Nuclear Polarization via Thermal Mixing. *Phys Chem Chem Phys* **2014**, *16* (2), 753–764. <https://doi.org/10.1039/c3cp52534a>.
- (27) Guarin, D.; Marhabaie, S.; Rosso, A.; Abergel, D.; Bodenhausen, G.; Ivanov, K. L.; Kurzbach, D. Characterizing Thermal Mixing Dynamic Nuclear Polarization via Cross-Talk between Spin Reservoirs. *J. Phys. Chem. Lett.* **2017**, *8* (22), 5531–5536. <https://doi.org/10.1021/acs.jpcllett.7b02233>.
- (28) Kundu, K.; Feintuch, A.; Vega, S. Theoretical Aspects of the Cross Effect Enhancement of Nuclear Polarization under Static Dynamic Nuclear Polarization Conditions. *J. Phys. Chem. Lett.* **2019**, *10* (8), 1769–1778. <https://doi.org/10.1021/acs.jpcllett.8b03615>.
- (29) Wenckebach, W. T. Spectral Diffusion and Dynamic Nuclear Polarization: Beyond the High Temperature Approximation. *J Magn Reson* **2017**, *284*, 104–114. <https://doi.org/10.1016/j.jmr.2017.10.001>.
- (30) Wenckebach, W. T. Dynamic Nuclear Polarization via Thermal Mixing: Beyond the High Temperature Approximation. *J Magn Reson* **2017**, *277*, 68–78. <https://doi.org/10.1016/j.jmr.2017.01.020>.

- (31) Hovav, Y.; Feintuch, A.; Vega, S. Theoretical Aspects of Dynamic Nuclear Polarization in the Solid State – Spin Temperature and Thermal Mixing. *Phys. Chem. Chem. Phys.* **2013**, *15* (1), 188–203. <https://doi.org/10.1039/C2CP42897K>.
- (32) Karabanov, A.; Kwiatkowski, G.; Perotto, C. U.; Wiśniewski, D.; McMaster, J.; Lesanovsky, I.; Köckenberger, W. Dynamic Nuclear Polarisation by Thermal Mixing: Quantum Theory and Macroscopic Simulations. *Phys. Chem. Chem. Phys.* **2016**, *18* (43), 30093–30104. <https://doi.org/10.1039/C6CP04345C>.
- (33) Wenckebach, W. Th. Dynamic Nuclear Polarization via the Cross Effect and Thermal Mixing: A. The Role of Triple Spin Flips. *Journal of Magnetic Resonance* **2019**, *299*, 124–134. <https://doi.org/10.1016/j.jmr.2018.12.018>.
- (34) Wenckebach, W. Th. Dynamic Nuclear Polarization via the Cross Effect and Thermal Mixing: B. Energy Transport. *Journal of Magnetic Resonance* **2019**, *299*, 151–167. <https://doi.org/10.1016/j.jmr.2018.12.020>.
- (35) Haze, O.; Corzilius, B.; Smith, A. A.; Griffin, R. G.; Swager, T. M. Water-Soluble Narrow-Line Radicals for Dynamic Nuclear Polarization. *J. Am. Chem. Soc.* **2012**, *134* (35), 14287–14290. <https://doi.org/10.1021/ja304918g>.
- (36) Michaelis, V. K.; Smith, A. A.; Corzilius, B.; Haze, O.; Swager, T. M.; Griffin, R. G. High-Field ^{13}C Dynamic Nuclear Polarization with a Radical Mixture. *J. Am. Chem. Soc.* **2013**, *135* (8), 2935–2938. <https://doi.org/10.1021/ja312265x>.
- (37) Can, T. V.; Walish, J. J.; Swager, T. M.; Griffin, R. G. Time Domain DNP with the NOVEL Sequence. *The Journal of Chemical Physics* **2015**, *143* (5), 054201. <https://doi.org/10.1063/1.4927087>.

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2020. <https://doi.org/10.1017/9781108567435>.

(45) Lumata, L.; Kovacs, Z.; Sherry, A. D.; Malloy, C.; Hill, S.; van Tol, J.; Yu, L.; Song, L.; Merritt, M. E. Electron Spin Resonance Studies of Trityl OXo63 at a Concentration Optimal for DNP. *Physical Chemistry Chemical Physics* **2013**, *15* (24), 9800–9807.

(46) Walker, S. A.; Edwards, D. T.; Siaw, T. A.; Armstrong, B. D.; Han, S. Temperature Dependence of High Field C-13 Dynamic Nuclear Polarization Processes with Trityl Radicals below 35 Kelvin. *Phys Chem Chem Phys* **2013**, *15* (36), 15106–15120. <https://doi.org/10.1039/c3cp51628h>.

(47) Capozzi, A.; Patel, S.; Wenckebach, W. T.; Karlsson, M.; Lerche, M. H.; Ardenkjær-Larsen, J. H. Gadolinium Effect at High-Magnetic-Field DNP: 70% ^{13}C Polarization of $[\text{U-}^{13}\text{C}]$ Glucose Using Trityl. *J. Phys. Chem. Lett.* **2019**, *10* (12), 3420–3425. <https://doi.org/10.1021/acs.jpcllett.9b01306>.

(48) Ardenkjær-Larsen, J. H.; Bowen, S.; Petersen, J. R.; Rybalko, O.; Vinding, M. S.; Ullisch, M.; Nielsen, N. C. Cryogen-Free Dissolution Dynamic Nuclear Polarization Polarizer Operating at 3.35 T, 6.70 T and 10.1 T. *Magnetic Resonance in Medicine* **2018**, *accepted*.

(49) Yoshihara, H. A. I.; Can, E.; Karlsson, M.; Lerche, M. H.; Schwitter, J.; Comment, A. High-Field Dissolution Dynamic Nuclear Polarization of $[\text{1-C-}^{13}\text{C}]$ Pyruvic Acid. *Phys Chem Chem Phys* **2016**, *18* (18), 12409–12413. <https://doi.org/10.1039/c6cp00589f>.

(50) Muñoz-Gómez, J. L.; Monteagudo, E.; Lloveras, V.; Parella, T.; Veciana, J.; Vidal-Gancedo, J. A Benzyl Alcohol Derivative of the BDPA Radical for Fast Dissolution Dynamic Nuclear Polarization NMR Spectroscopy. *Org. Biomol. Chem.* **2015**, *13* (9), 2689–2693. <https://doi.org/10.1039/C4OB02356K>.

- (51) Muñoz-Gómez, J. L.; Monteagudo, E.; Lloveras, V.; Parella, T.; Veciana, J.; Vidal-Gancedo, J. Optimized Polarization Build-up Times in Dissolution DNP-NMR Using a Benzyl Amino Derivative of BDPA. *RSC Adv.* **2016**, *6* (32), 27077–27082. <https://doi.org/10.1039/C6RA00635C>.
- (52) Lumata, L.; Merritt, M. E.; Malloy, C. R.; Sherry, A. D.; Kovacs, Z. Impact of Gd³⁺ on DNP of [1-C-13]Pyruvate Doped with Trityl OXo63, BDPA, or 4-Oxo-TEMPO. *J Phys Chem A* **2012**, *116* (21), 5129–5138. <https://doi.org/10.1021/Jp302399f>.
- (53) Equbal, A.; Li, Y.; Tabassum, T.; Han, S. Crossover from a Solid Effect to Thermal Mixing ¹H Dynamic Nuclear Polarization with Trityl-OXo63. *J. Phys. Chem. Lett.* **2020**, 3718–3723. <https://doi.org/10.1021/acs.jpcllett.occo830>.