Supporting Information

The Impact of Gd^{3+} on DNP of [1-^{13}C]Pyruvate Doped with Trityl OX063, BDPA, or 4-Oxo-TEMPO

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I. Microwave DNP spectra of trityl-doped [1-^{13}C]pyruvate samples with longer irradiation times

Figure S1a shows the microwave DNP spectra (plot of NMR signal intensity as a function of the microwave irradiation frequency) of 100 µL aliquots of 1.4 M [1-^{13}C]pyruvate in 1:1 (v/v) glycerol:water doped with 15 mM trityl OX063 in the presence (4 mM) and absence of Gd^{3+}. These data were taken by plotting the NMR signal intensity of the sample after 1 hour of microwave irradiation at a particular microwave frequency at 3.35 T and 1.4 K. To ensure that both ^{13}C and ^{1}H remnant DNP-enhanced polarizations from the previous microwave frequency are destroyed, the frozen sample was taken out of the HyperSense polarizer to melt and depolarize for 5 minutes at room temperature outside the polarizer before starting polarization at the next microwave frequency. Figures S1b and S1c display the corresponding polarization buildup curves across the ^{13}C microwave spectrum of Gd-free and Gd-doped samples, respectively.
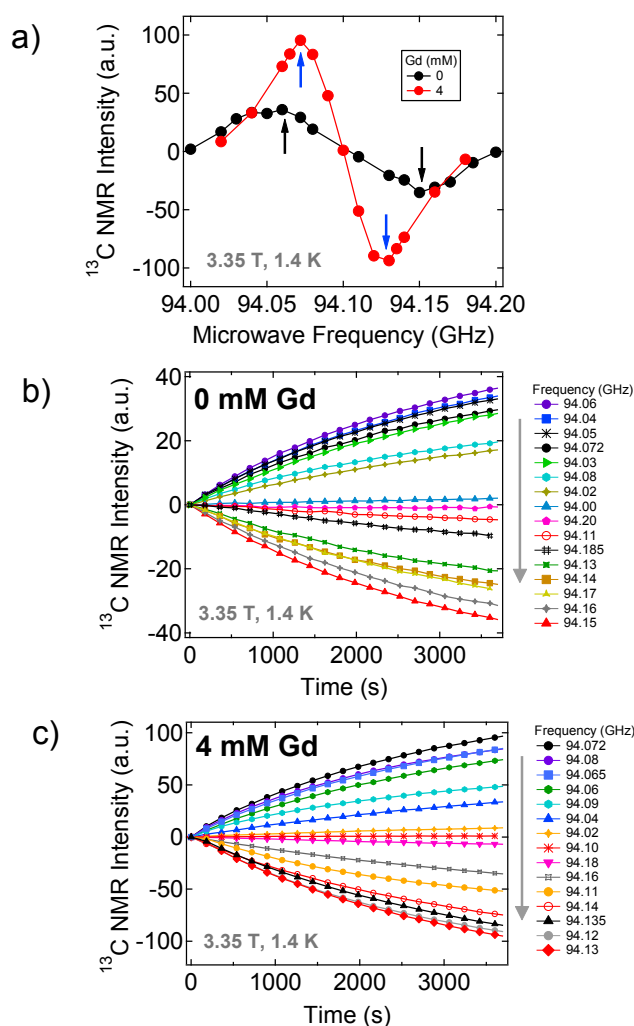

Figure S1: a) Microwave DNP spectra of 100 µL trityl-doped ^{13}C pyruvate samples (1.4 M [1-^{13}C]pyruvate in 1:1 v/v glycerol:water doped with 15 mM trityl OX063 in the presence (4 mM) and absence of Gd^{3+}. The data points were collected by irradiating the samples at different microwave frequencies for 1 hour in the HyperSense at 3.35 T and 1.4 K with a 100 mW microwave source. The corresponding buildup curves of the two samples are shown in b) 0 mM Gd^{3+} and c) 4 mM Gd^{3+}.

II. ^{13}C pulse width calibration at DNP Conditions (3.35 T and 1.4 K)

At low temperatures, the spin-lattice relaxation time T_1 of nuclei such as carboxyl ^{13}C spins in pyruvate in the HyperSense (3.35 T, 1.4 K) can be on the order of several thousand seconds, thus pulsewidth calibration at cryogenic conditions via the conventional method, i.e. incremental rf pulsewidth change every $5T_1$ is impractical. Figure S2 shows an alternative method of pulse calibration in DNP experiments. Two trityl-doped ^{13}C pyruvate samples were polarized and after the samples reached the maximum polarization in the polarizer, the microwaves were turned off. A hard pulse (in our case, pulsewidth=30 µs, transmitter power=53 dB, 200 W) was applied every TR=1 s and the decay curve was fitted to an equation which accounts for the decay of the hyperpolarized NMR signal due to rf excitation and T_1 relaxation:[1]

$$M_z(t)=M_0\sin(\theta)[\cos(\theta)]^{t/TR}\exp(-t/T_1) \qquad (1)$$

Note that due to the estimated large flip angle and the very frequent repetition time, the decay of the hyperpolarized signal will be almost exclusively due to rf pulsing; the decay of the magnetization due to T_1 (estimated to be a few thousand seconds) relaxation will be negligible. The flip angle θ can therefore be calculated from the above equation. The estimated 90 degree pulse in our HyperSense built-in probe is 155 µs for ^{13}C. Another important note is that we only use 20-degree pulse to measure both the hyperpolarized and thermal NMR signals for enhancement calculations to avoid hole-burning in the NMR spectra.
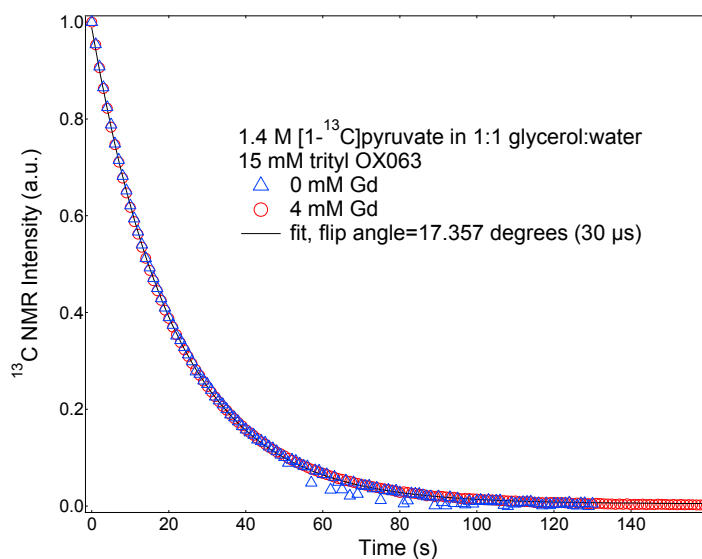

Figure S2: Decay curves of hyperpolarized ^{13}C NMR signals emanating from 1.4 M [1-^{13}C]pyruvate samples with and without Gd-HP-DO3A at 3.35 T and 1.4 K. The decay was monitored by applying 30 us pulse every 1 s. The decay curves were fitted with Equation 1.

III. Optimization of trityl OX063 concentration in [1-^{13}C] pyruvate samples

The optimum concentration of trityl OX063 free radical in ^{13}C pyruvic DNP samples is around 15 mM as reported in previous studies.[2,3] Figure S3 shows the same findings for [1-^{13}C] pyruvate samples (1.4 M in 1:1 v/v glycerol:water) at 3.35 T and 1.4 K in the HyperSense polarizer.

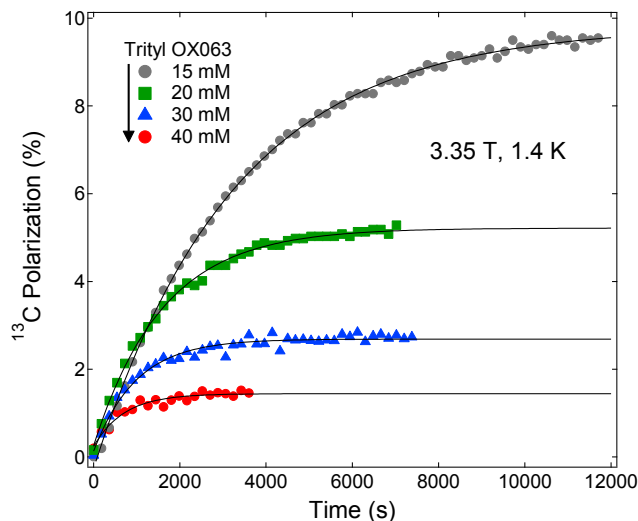Figure S3: Polarization buildup curves of 1.4 M [1-^{13}C] sodium pyruvate samples doped with different concentrations of trityl OX063 free radical. The measurements were done in the HyperSense at 3.35 T and 1.4 K.

IV. ^{13}C solid-state nuclear polarization of trityl-doped (15 mM) 1:1 [1-^{13}C]pyruvic acid:sulfolane with and without Gd-HP-DO3A

Figure S4 shows the polarization buildup curves of 1:1 [1-^{13}C]pyruvic acid:sulfolane doped with 15 mM trityl OX063 in the presence or absence of Gd^{3+}. The maximum solid-state polarization achieved for a Gd-free sample was 12 % and with the addition of 2.5 mM Gd^{3+}, the nuclear polarization doubled to 25 %.
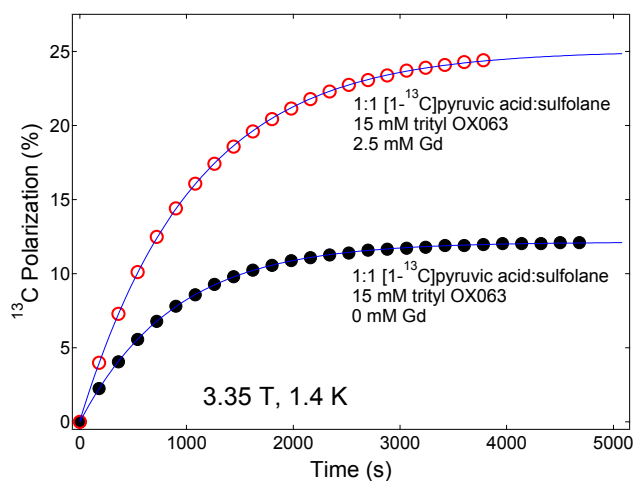

Figure S4: Polarization buildup curves of 1:1 (v/v) [1-^{13}C]pyruvic acid:sulfolane samples doped with 15 mM trityl OX063. The sample in the upper curve was mixed with 2.5 mM Gd-HP-DO3A.

V. Solid-state P_{dnp} and T_1 decay curves of BDPA-doped (20 mM) [1-^{13}C]pyruvic acid samples

Figure S5 shows the polarization buildup curves of 1:1 [1-^{13}C]pyruvic acid:sulfolane doped with a lower BDPA concentration (20 mM) and mixed with varying Gd^{3+} concentration. Without Gd^{3+}, the maximum polarization achieved was 13 % and with 2.5 mM Gd^{3+}, the polarization improved to ~17 %. Concomitant with these changes in polarization is the monotonic decrease of solid-state ^{13}C T_1 as displayed in Figure S6.
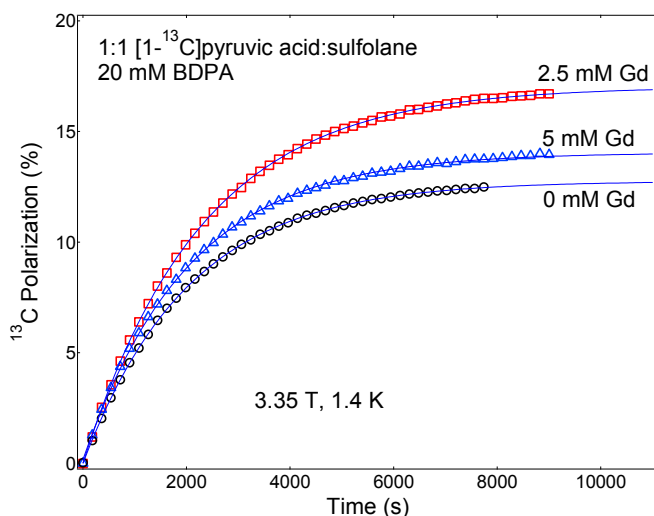

Figure S5: Polarization buildup curves 1:1 (v/v) [1-^{13}C]pyruvic acid:sulfolane doped with 20 mM BDPA and mixed with varying Gd^{3+} concentration. The samples were polarized at 3.35 T and 1.4 K with a 100 mW microwave source.
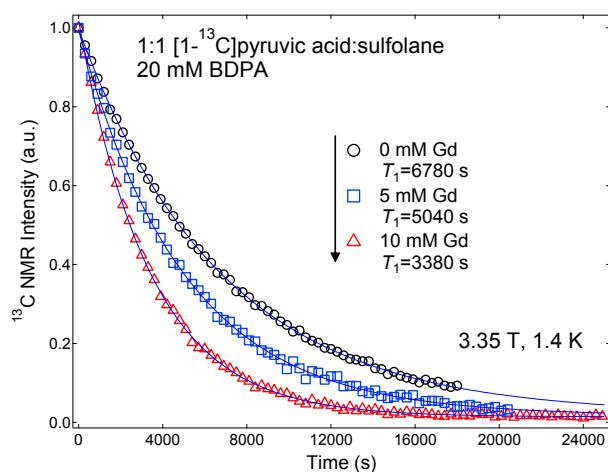

Figure S6: Decay of the hyperpolarized ^{13}C NMR signals emanating from polarized 1:1 (v/v) [1-^{13}C]pyruvic acid:sulfolane samples doped with 20 mM BDPA and mixed with varying Gd^{3+} concentration.

VI.   Solid-state polarization of 4-oxo-TEMPO-doped (20 mM) [1-^{13}C]pyruvate samples

The optimum concentration of TEMPO in DNP samples that gives the maximum nuclear polarization in a reasonable microwave irradiation time was found to be around 30-50 mM.[4] Figure S7 shows the growth of the nuclear polarization with microwave irradiation time of 1.4 M [1-^{13}C] sodium pyruvate in 1:1 (v/v) glycerol:water and doped 4-oxo-TEMPO-doped at a reduced concentration (20 mM). Without Gd^{3+}, the maximum polarization attained is ~4.5 % and with the addition of Gd^{3+}, the polarization improved to ~6 %.

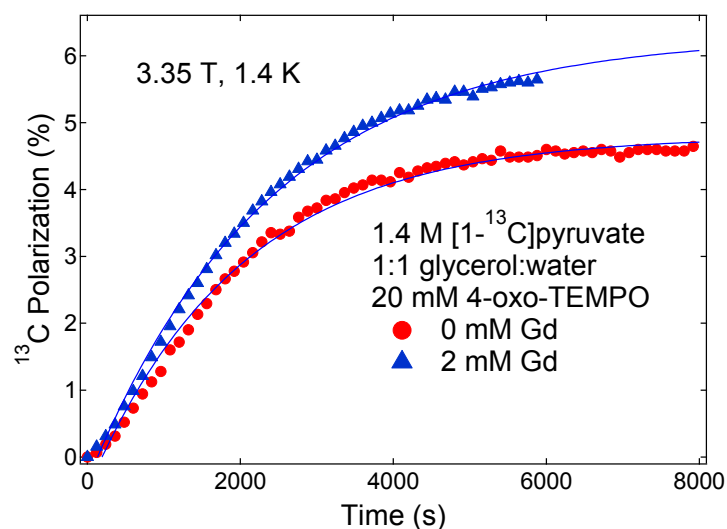Figure S7: Polarization buildup curves of 1.4 M [1-^{13}C]sodium pyruvate samples doped with 20 mM 4-oxo-TEMPO in the presence and absence of Gd^{3+}. The measurements were done in the HyperSense polarizer at 3.35 T and 1.4 K.

VII.   References

(1) Patyal, B. R.; Gao, J.-H.; Williams, R. F.; Roby, J.; Saam, B.; Rockwell, B. A.; Thomas, R. J.; Stolarski, D. J.; Fox, P. T. *J. Magn. Reson.* **1997**, *126*, 58.
(2) Ardenkjaer-Larsen, J. H.; Macholl, S.; Johannesson, H. *Appl. Magn. Reson.* **2008**, *34*, 509.
(3) Johanneson, H.; Macholl, S.; Ardenkjaer-Larsen, J. H. *J. Magn. Reson.* **2009**, *197*, 167.
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