

Electronic Supplementary Information

Influence of deuteration in the glassing matrix on ^{13}C dynamic nuclear polarization

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I. Materials and Methods

Materials. The free radical trityl OX063 (tris{8-carboxyl-2,2,6,6-benzo(1,2-d:4,5-d)-bis(1,3)dithiole-4-yl}methyl sodium salt) was obtained from Oxford Instruments Molecular Biotools (Tubney Woods, UK). The other free radicals BDPA (1,3-bisdiphenylene-2-phenylallyl), galvinoxyl ((2,6-di-tert-butyl- α -(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyloxy), DPPH (2,2-diphenyl-1-picrylhydrazyl), and 4-oxo-TEMPO (4-Oxo-2,2,6,6-tetramethyl-1-piperidinyloxy) were purchased from Sigma Aldrich (St. Louis, MO). The structures of these free radicals are shown in Fig. 1 in the main text. The substrates [$1-^{13}\text{C}$]sodium pyruvate, [^{13}C]urea, [$1-^{13}\text{C}$]ethyl acetate and [$1-^{13}\text{C}$]acetate as well as the deuterated and non-deuterated glassing solvents (glycerol/ d_8 -glycerol, $\text{H}_2\text{O}/\text{D}_2\text{O}$, sulfolane/ d_8 -sulfolane, ethyl acetate/ d_8 -ethyl acetate, DMSO/ d_8 -DMSO) were also obtained from commercial sources (Sigma-Aldrich, St. Louis, MO and Thermo-Fisher Scientific, NJ) and were used without further purification.

Sample preparations. (1) **BDPA:** 4 mg BDPA (4.0 mg) was dissolved in sulfolane (100 μL) as described previously² and DMSO (100 μL) was added to the solution. [^{13}C]Urea (25.2 mg) was dissolved in the resulting mixture. The final concentration of BDPA in the solution was 40 mM. The same sample preparations were made for BDPA-doped [^{13}C]urea samples with 1:1 (v/v) d_8 -DMSO:sulfolane and d_8 -DMSO: d_8 -sulfolane glassing matrices. (2) **Trityl OX063:** Solutions of [$1-^{13}\text{C}$]sodium pyruvate (31.1 mg) were made in 1:1 (v/v) glycerol:water (200 μL), glycerol: D_2O (200 μL), or d_8 -glycerol: D_2O (200 μL). Trityl OX063 (4.3 mg) was added to each solution. The final concentration of trityl OX063 in each solution was 15 mM. (3) **Galvinoxyl:** [$1-^{13}\text{C}$]Ethyl acetate (20 μL) was added to a mixture containing unlabeled ethyl acetate (80 μL) and DMSO (100 μL). Galvinoxyl (3.4 mg) was added to the solution. The final concentration of galvinoxyl in the solution was 40 mM. The same sample preparations were made for galvinoxyl-doped [$1-^{13}\text{C}$]ethyl acetate samples with d_8 -ethyl acetate (80 μL):DMSO (100 μL) and d_8 -ethyl acetate (80 μL): d_8 -DMSO (100 μL). (4) **DPPH:** Solutions of [^{13}C]urea (24.5 mg) were prepared in 1:1 (v/v) sulfolane:DMSO (200 μL), sulfolane: d_8 -DMSO (200 μL), and d_8 -sulfolane: d_8 -DMSO (200 μL). DPPH (3.2 mg) was dissolved in each solution. The final concentration of DPPH in each solution was 20 mM. (5) **4-oxo-TEMPO:** Solutions of [$1-^{13}\text{C}$]sodium pyruvate (31.1 mg) were prepared in 1:1 (v/v) glycerol:water (200 μL), glycerol: D_2O (200 μL), and d_8 -glycerol: D_2O (200 μL). 4-Oxo-TEMPO (1.4 mg) was added to each solution. The final concentration of 4-oxo-TEMPO in each solution was 40 mM.

^{13}C microwave DNP spectra. Aliquots (100 μL) of ^{13}C -labelled samples with non-deuterated (ND) or fully deuterated (FD) glassing matrices for each free radical were inserted in the HyperSense commercial polarizer (equipped with Edwards 250 m^3/h vacuum pump and a roots blower pump) at 3.35 T and 1.1 K. The ^{13}C microwave DNP spectra for each free radical with ND and FD glassing matrices were measured by recording the ^{13}C NMR signal as a function of microwave irradiation frequency with a 10 MHz sweep increment. Each data point was obtained with a 3-minute irradiation time using a 100 mW microwave source. A series of hard rf pulses was applied to destroy the remnant magnetization prior to the measurement at the next microwave irradiation frequency.³ The optimum ^{13}C positive P(+) and negative P(-) polarization peaks for each free radical were determined from these measurements. In the case of 4-oxo-TEMPO, which has the largest ESR linewidth among the free radicals in this study, only the positive polarization peak was recorded due to the limited sweepable range of the microwave source (93.9-94.3 GHz). A full ^{13}C microwave DNP spectrum of a TEMPO-doped sample from a previous study revealed that the separation between P(+) and P(-) is about 330 MHz for this closely related free radical.¹

^{13}C polarization buildup curves. The relative ^{13}C nuclear polarization buildup curves for each sample (100 μL aliquots with non-deuterated, partially deuterated, and fully deuterated glassing matrices) was measured by applying a small flip angle rf pulse every 2 or 5 minutes as the sample is irradiated at P(+) with a 100 mW microwave source at DNP conditions 3.35 T and 1.1 K. These measurements were done in triplicate for each sample. The average and standard deviations of the maximum ^{13}C signal intensity were calculated and plotted as bar graphs as depicted in the main text.

II. W-band ESR spectra of the free radical polarizing agents at 100 K

Fig. S1 shows the field-swept ESR spectra of a) BDPA (20 mM), b) trityl OX063 (15 mM), c) galvinoxyl (40 mM), d) DPPH (20 mM), and e) 4-oxo-TEMPO (40 mM) measured in the W-band at 100 K. The details of the sample composition were discussed in the sample preparation subsection in Page S2 and Table I in the main text. These ESR measurements were performed at the National High Magnetic Field Laboratory in Tallahassee, Florida using a commercial Bruker E680 W-band ESR spectrometer with TE₀₁₁ cylindrical cavity. Capillary tubes (0.15 mm ID) were used as containers for these samples. The ESR spectra were recorded using the standard electron spin-echo method. The ESR linewidths (D) indicated in Fig. S1 correspond to the widths at 2 % height from the base of the ESR spectra.

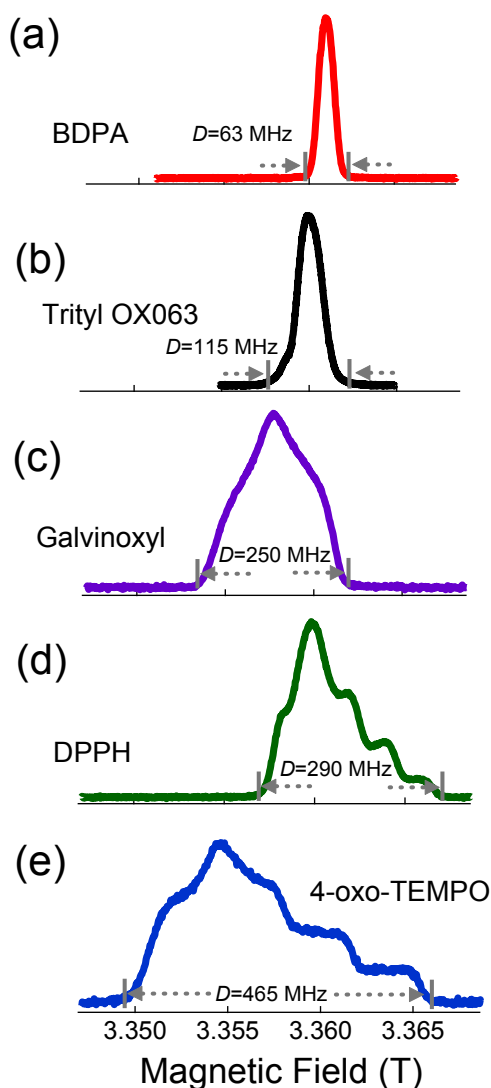


Fig. S1 W-band ESR spectra of (a) BDPA, (b) trityl OX063, (c) galvinoxyl, (d) DPPH, and (e) 4-oxo-TEMPO measured at 100 K. The ESR linewidth D shown in each spectrum is defined as the width at 2 % height from the base of the ESR spectrum.

III. Relative ^{13}C polarization buildup curves for ^{13}C DNP samples with ND, PD, or FD glassing matrices

A. ^{13}C DNP using narrow ESR D free radicals BDPA and trityl OX063 (ESR $D < ^1\text{H } \omega_n$). Fig. S2 shows the relative ^{13}C polarization buildup curves of ^{13}C DNP samples doped with a) BDPA and b) trityl OX063 taken at 3.35 T and 1.1 K irradiated at P(+) with a 100 mW microwave source. The ^{13}C substrates were prepared in non-deuterated (ND), partially deuterated (PD), or fully deuterated (FD) glassing matrices. The buildup curves were fitted with a single-exponential buildup equation $P(t) = P_0[1 - \exp(-t/\tau_{\text{bu}})]$ where t is the microwave irradiation time, P_0 is the maximum NMR intensity, and τ_{bu} is the polarization buildup time constant. The error bars shown are standard deviations of $N=3$ trials. The ESR linewidths of BDPA ($D=63$ MHz) and trityl OX063 ($D=115$ MHz), as shown in Fig. S1, are smaller compared to the ^1H Larmor frequency at 3.35 T ($^1\text{H } \omega_n=143$ MHz). This implies that the ^1H nuclear Zeeman system is essentially decoupled from the electron dipolar system (EDS).⁴ Replacement of protons by deuterons will lead to increased total heat capacity of the nuclear Zeeman system, since ^2H spins will also be in thermal contact with EDS in addition to ^{13}C spins. It can be seen for both free radicals that full deuteration of glassing matrices led to almost 50 % decrease in the maximum ^{13}C polarization compared to those obtained with protonated glassing matrices. Partial deuteration led to intermediate decreases in the relative ^{13}C maximum polarization. Thus, for free radical with ESR D less than the $^1\text{H } \omega_n$, deuteration of the glassing matrix is not recommended. In addition, the buildup time constants τ_{bu} tend to become longer with glassing solvent deuteration for both BDPA and trityl OX063-doped ^{13}C samples. This may be ascribed to having the same number of electrons “servicing” polarization transfer not only for ^{13}C spins but for ^2H spins as well, thus leading to longer buildup times.

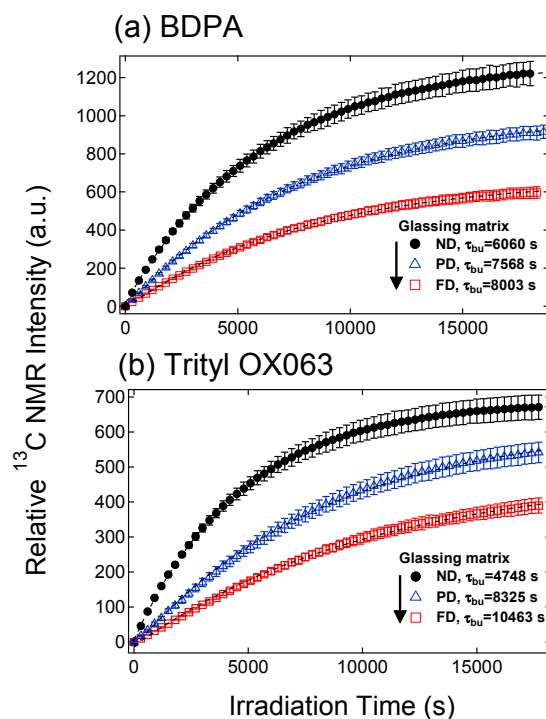


Fig. S2 Relative ^{13}C polarization buildup curves for ^{13}C samples doped with a) BDPA and b) trityl OX063 prepared with non-deuterated (ND), partially deuterated (PD), and fully deuterated (FD) glassing matrices. The data were collected in triplicate at 3.35 T and 1.1 K with a 100 mW microwave source. The buildup time constants τ_{bu} were also indicated for each curve. The error bars are standard deviations for $N=3$ trials.

B. ^{13}C DNP using large ESR D free radicals galvinoxyl, DPPH, and 4-oxo-TEMPO (ESR $D \geq ^1\text{H } \omega_n$).

Fig. S3 shows the relative ^{13}C polarization buildup curves of ^{13}C DNP samples doped with a) galvinoxyl ($D=250$ MHz), b) DPPH ($D=290$ MHz), and c) 4-oxo-TEMPO ($D=465$ MHz) taken at 3.35 T and 1.1 K. The ESR linewidths of these free radicals are greater than, or comparable to the ^1H Larmor frequency at 3.35 T ($^1\text{H } \omega_n=143$ MHz). This implies that the ^1H nuclear Zeeman system is already thermally coupled to the EDS.⁴ Replacement of protons by deuterons will decrease the total heat capacity of the nuclear Zeeman system, since ^2H spins will also be in thermal contact with EDS and with a lower γ , which is equivalent to a lesser heat load. It can be seen in Fig. S3 that full deuteration of glassing matrices led to significant improvements in the maximum ^{13}C polarization compared to those obtained with protonated glassing matrices. Partial deuteration led to intermediate increases in the relative ^{13}C maximum polarization. Thus, for a free radical with an ESR linewidth D greater than, or comparable to the proton Larmor frequency ($^1\text{H } \omega_n$), deuteration of the glassing matrix is recommended. The DPPH-doped ^{13}C samples showed the largest improvement in nuclear polarization with deuteration; this may be attributed to a better matching of the DPPH electron dipolar energy with the proton nuclear Zeeman energy, thus when protons are replaced by deuterons, significant improvements are observed. Galvinoxyl-doped ^{13}C samples showed relatively modest improvement with deuteration; this may be ascribed to a lesser contact of proton Zeeman system with EDS (considering that galvinoxyl ESR D is slightly less than $^1\text{H } 2\omega_n$), thus only slight enhancements are seen with deuteration. We also note that, similar to what were seen with BDPA and trityl OX063, the buildup time constants generally increase with deuteration.

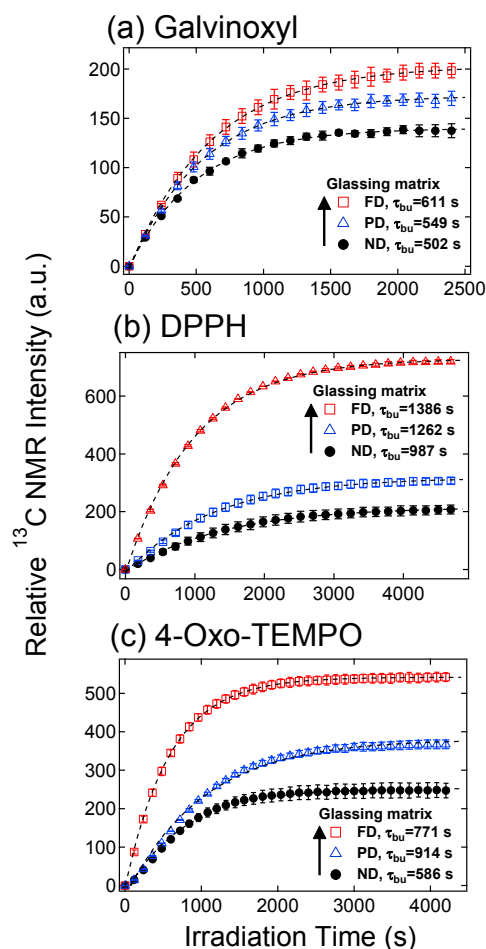


Fig. S3 Relative ^{13}C polarization buildup curves for ^{13}C samples doped with a) galvinoxyl, b) DPPH, and c) 4-oxo-TEMPO prepared with non-deuterated (ND), partially deuterated (PD), and fully deuterated (FD) glassing matrices. The data were collected in triplicate at 3.35 T and 1.1 K with a 100 mW microwave source. The buildup time constants τ_{bu} were also indicated for each curve. The error bars are standard deviations for $N=3$ trials.

References

- [1] S. Jannin, A. Comment, F. Kurdzesau, J. A. Konter, P. Hautle, B. van der Brandt and J. J. van der Klink, *J. Chem. Phys.*, 2008, **128**, 241102.
- [2] L. Lumata, S. J. Ratnakar, A. Jindal, M. Merritt, A. Comment, C. Malloy, A. D. Sherry and Z. Kovacs, *Chem. Eur. J.*, 2011, **17**, 10825-10827.
- [3] L. Lumata, M. E. Merritt, C. R. Malloy, A. D. Sherry and Z. Kovacs, *J. Phys. Chem. A*, 2012, **116**, 5129–5138.
- [4] S. T. Goertz, *Nucl. Instrum. Methods Phys. Res., Sect. A*, 2004, **526**, 28-42.