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Verdazyl-ribose: A new radical for solid-state dynamic nuclear polarization at high magnetic field

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ABSTRACT

Solid-state dynamic nuclear polarization (DNP) using the cross-effect relies on radical pairs whose electron spin resonance (ESR) frequencies differ by the nuclear magnetic resonance (NMR) frequency. We measure the DNP provided by a new water-soluble verdazyl radical, verdazyl-ribose, under both magic-angle spinning (MAS) and static sample conditions at 9.4 T, and compare it to a nitroxide radical, 4-hydroxy-TEMPO. We find that verdazyl-ribose is an effective radical for cross-effect DNP, with the best relative results for a non-spinning sample. Under non-spinning conditions, verdazyl-ribose provides roughly 2× larger ^{13}C cross-polarized (CP) NMR signal than the nitroxide, with similar polarization buildup times, at both 29 K and 76 K. With MAS at 7 kHz and 1.5 W microwave power, the verdazyl-ribose does not provide as much DNP as the nitroxide, with the verdazyl providing less NMR signal and a longer polarization buildup time. When the microwave power is decreased to 30 mW with 5 kHz MAS, the two types of radical are comparable, with the verdazyl-doped sample having a larger NMR signal which compensates for its longer polarization buildup time. We also present electron spin relaxation measurements at Q-band (1.2 T) and ESR lineshapes at 1.2 and 9.4 T. Most notably, the verdazyl radical has a longer T_{1e} than the nitroxide (9.9 ms and 1.3 ms, respectively, at 50 K and 1.2 T). The verdazyl electron spin lineshape is significantly affected by the hyperfine coupling to four ^{14}N nuclei, even at 9.4 T. We also describe 3000-spin calculations to illustrate the DNP potential of possible radical pairs: verdazyl-verdazyl, verdazyl-nitroxide, or nitroxide-nitroxide pairs. These calculations suggest that the verdazyl radical at 9.4 T has a narrower linewidth than optimal for cross-effect DNP using verdazyl-verdazyl pairs. Because of the hyperfine coupling contribution to the electron spin linewidth, this implies that DNP using the verdazyl radical would improve at lower magnetic field. Another conclusion from the calculations is that a verdazyl-nitroxide bi-radical would be expected to be slightly better for cross-effect DNP than the nitroxide-nitroxide bi-radicals commonly used now, assuming the same spin-spin coupling constants.

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1. Introduction

Dynamic nuclear polarization (DNP) transfers spin polarization from unpaired electron spins to nuclear spins. This can increase the nuclear spin polarization, which improves the sensitivity of nuclear magnetic resonance (NMR) measurements. Crucial to DNP are radical molecules which can be doped into the sample to provide the unpaired electron spins. The radicals used for DNP need to satisfy several different criteria. Most importantly, the radicals need to be efficient at polarizing the nuclear spins. There are several different mechanisms for DNP including the cross-effect [1,2], the solid-effect [3,4], thermal mixing [5–7], and the Overhauser effect [8,9], which have different requirements for the radicals, especially for the electron spin resonance (ESR) linewidth. In our case of DNP of ^{1}H nuclei at high magnetic field (9.4 T) with moderate strength microwave irradiation of the solid-state sample, the cross-effect is the dominant mechanism. Cross-effect DNP needs two electron spins whose ESR frequencies differ by the NMR frequency [2,10]. Currently, nitroxide radicals are primarily used for cross-effect DNP of ^{1}H [11,12], because the large g-anisotropy of the nitroxides allows two nitroxides with different radical orientations to have a frequency difference equal to the ^{1}H frequency. Although nitroxide pairs are effective for cross-effect DNP of ^{1}H, however they are not as efficient as they could be because only a fraction of the possible nitroxide pair orientations will have a frequency difference match-

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ing the NMR frequency [10]. Thus, it is useful to investigate the DNP properties of other radicals.

In addition to efficient nuclear polarization, for our application of DNP to biomolecules [13,14], we generally want the radicals to be stable and soluble in water/glycerol mixtures, so the radicals can be easily mixed into a solution with the sample of interest. Then, the water/glycerol mixture is frozen, providing a glassy matrix for the radicals and biomolecules during DNP measurements at low temperatures.

This paper reports high field solid-state DNP experiments testing a recently synthesized water-soluble verdazyl radical molecule, verdazyl-ribose (Fig. 1) [15]. Verdazyl radicals were discovered in 1963 [16,17], and can be very stable in the solid-state and in solution. Under some conditions, verdazyl-ribose is more stable than nitroxide radicals. For example, ascorbic acid rapidly reacts with nitroxide radicals, but does not react significantly with the verdazyl [15]. Verdazyl radicals have been used successfully for liquid-state DNP at relatively low magnetic field (up to 1.4 T) [18,19], but to our knowledge have not been previously used for high magnetic field DNP. In this article, we measure solid-state DNP using the verdazyl-ribose radical at 9.4 T, both with and without MAS, and compare it to the nitroxide radical, 4-hydroxy-TEMPO. The verdazyl-ribose radical does provide significant cross-effect DNP. With a non-spinning sample, the sample with verdazyl gives roughly 2× larger ^{13}C CP NMR signal than the sample with nitroxide, and similar polarization buildup times. With 7 kHz MAS and 1.5 W microwave power at 30 K, the sample with verdazyl provides only 60% of the signal of the nitroxide sample, and also has a longer polarization buildup time. When the microwave power is reduced to 30 mW at 5.2 kHz MAS, the DNP from the two radicals is comparable, with the larger NMR signal from the verdazyl sample compensating for its longer polarization buildup time.

We also present solid-state ESR spectra of the verdazyl radical at 1.2 T (Q-band) and 9.4 T at 50 K, and the temperature dependence of ESR T_{1e} and T_m relaxation measurements at 1.2 T. The ESR spectra show that the electron spin has a nearly axial g-tensor and strong anisotropic hyperfine couplings to all four ^{14}N nuclei in the ring of the radical molecule. Even at 9.4 T, these hyperfine couplings have a significant effect on the ESR lineshape. Quantum chemistry calculations suggest that the principal axis perpendicular to the plane of the verdazyl ring has the smallest g-value and the largest ^{14}N hyperfine coupling. From the relaxation measurements, the most notable result is that the verdazyl has a significantly longer T_{1e} than the nitroxide at 1.2 T and 50 K.

Also presented here are calculations of spin systems to estimate the cross-effect DNP efficiency of hypothetical bi-radicals, either verdazyl-verdazyl, nitroxide-nitroxide, or verdazyl-nitroxide pairs. The polarization dynamics of 1000 three-spin systems (two electron and one nuclear spins) is simulated using the Landau-Zener equation [20,21]. These calculations show that the ESR spectrum of this verdazyl radical is only barely wide enough for pairs of verdazyl radicals to meet the cross-effect frequency matching condition for ^{1}H nuclei at 9.4 T. This suggests that the verdazyl radical would be more efficient for DNP at lower magnetic field, because the field-independent hyperfine couplings would give the radical a larger ESR linewidth relative to the NMR frequency. The calculations also suggest that a hypothetical verdazyl-nitroxide bi-radical should be slightly better than a nitroxide-nitroxide bi-radical, if both bi-radicals have the same spin-spin couplings.

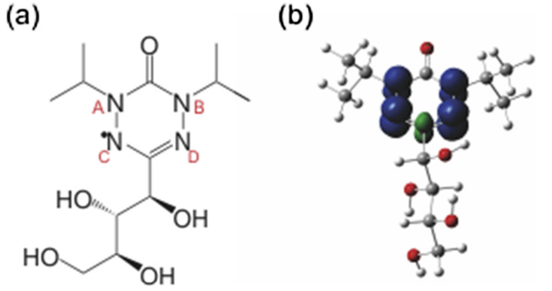

Fig. 1. (a) Structure of verdazyl-ribose. The four nitrogen atoms are labelled for reference in Table 3 and the text. (b) Electron spin density surface shown in blue (+) and green (−) on top of the molecular geometry from a quantum chemistry calculation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Experimental methods

The NMR experiments used the home-built ultra-low-temperature DNP-MAS NMR probe described previously [22] and were performed at 9.39 T (400.9 MHz and 100.8 MHz ^{1}H and ^{13}C NMR frequencies) using a Bruker Avance III NMR spectrometer console. For experiments with 30 mW of microwaves, the microwave source was a diode source from Virginia Diodes, Inc. mounted with the quasi-optical interferometer system previously described [23]. For experiments with 1.5 W of microwaves, the microwave source was an extended interaction oscillator (EIO) from Communications & Power Industries, which transmits its microwaves through a corrugated waveguide to the quasi-optical interferometer system [24]. The 45 μl NMR samples contained 40 mM of radicals, either verdazyl-ribose or 4-hydroxy-TEMPO, and 150 mM ^{15}N,$^{13}C_3$-L-alanine in partially protonated glycerol/water (60/30/10 volume% d_8-glycerol/D_2O/H_2O). The radical concentration is determined by measuring the optical absorption [15,25]. For the verdazyl radical, the optical absorption at the 382 nm peak in the glycerol/water solvent was 1100 cm^{-1} M^{-1}. Sample temperatures were determined from measurements of the spin-lattice relaxation of ^{79}Br in KBr contained in a glass capsule placed in the MAS rotor along with the sample [26].

For CP ^{13}C measurements, an 800 μs CP contact time and 40 kHz ^{13}C radio-frequency field strength were used, with one dummy scan to establish a steady state before NMR signal acquisition. In all ^{13}C NMR experiments, 75 kHz proton decoupling fields with two-pulse phase modulation [27] were applied during detection of free-induction decay (FID) signals. The ^{1}H nuclear polarization buildup times, τ_{DNP}, were measured by recovery from saturation of the ^{13}C CP signals, with the microwaves on. The recovery time from saturation for ^{13}C CP NMR was also measured with the microwaves off (^{1}H T_{1n}) at 31 K with MAS for the verdazyl dopant sample, and the time was not significantly different from the polarization buildup time with the microwaves on.

^{13}C CP NMR signals and DNP enhancements in Table 1 are calculated from the integrals of all alanine ^{13}C lines (CO, Cα, and Cβ signals) for measurements with MAS. Without MAS, the entire aliphatic region was integrated, thus including the alanine Cα and Cβ signals, and glycerol natural abundance ^{13}C signals. The NMR signal measurements were taken with repetition delays equal to twice the polarization buildup times. Measurement uncertainties are listed in Table 1, dominated by the uncertainty in absolute signal size, estimated at ~20% from multiple measurements on some samples.

1.2 T (Q-band) ESR measurements used a Bruker E-580 spectrometer and a model ER5107D2 probe. The Q-band ESR samples were 0.1 mM concentration of verdazyl or nitroxide in 60/40 volume% d_8-glycerol/D_2O. The 1.2 T ESR spectrum of Fig. 4 was taken by integrating the full echo from a $\pi/2$-τ_m-π-τ_m-echo pulse sequence, with a 512 ns π pulse and 5 μs τ_m. The microwave frequency was 33.94465 GHz for the verdazyl Q-band spectrum at