Dynamic nuclear polarization with vanadium(IV) metal centers

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SUMMARY

Dynamic nuclear polarization (DNP) harnesses the large polarization of electron spins to dramatically increase nuclear magnetic resonance (NMR) sensitivity. This study expands the scope of DNP beyond its traditional focus on hyper-polarizing the solvent network using exogenous polarizing agents (PAs). We introduce ^{1}H DNP with endogenous V^{4+} centers positioned in a set of vanadyl complexes with tunable V^{4+}-^{1}H distances. We traced the polarization transfer from V^{4+} to ^{1}H spins, specifically differentiating between direct V^{4+}-^{1}Hs polarization transfer and the ^{1}H spin-diffusion mediated bulk solvent ^{1}H polarization buildup, and illuminated the effect of the V^{4+}-^{1}H distance on these processes. These results deepen our understanding of polarization pathways and expand the catalogue of PAs to broad-line transition metals. This study establishes crucial first steps towards employing strategically positioned endogenous paramagnetic metal centers for DNP, and the conceptual framework of hyperfine DNP spectroscopy that merges both spatial and chemical diagnosis of target nuclear spins.

Keywords: spin diffusion barrier, spin dynamics, endogenous DNP, vanadium DNP, direct DNP, coordination chemistry, transition metal EPR, spin decoherence

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy, a widely used tool to elucidate fundamental chemical, structural, and dynamical information in molecules and materials, is inherently limited by the poor polarization of nuclear spins. Dynamic nuclear polarization (DNP) is the most broadly applicable hyperpolarization method to enhance the nuclear magnetic resonance (NMR) signal by orders of magnitudes, relying on polarization transfer from highly polarized electron spins (*e*) to the surrounding nuclear spins (*n*). In a typical DNP experiment, a source of unpaired electron spins known as a polarizing agent (PA) is mixed with the sample in a ^{1}H-rich glassing matrix. Microwave (μw) irradiation near the electron paramagnetic resonance (EPR) frequency of the PA can drive polarization transfer from the electron to the surrounding ^{1}H nuclear spins. Current state-of-the-art DNP methodologies have already transformed the scope of NMR in fields from structural biology to materials science.[1] To date, nitrogen-centered nitroxide or carbon-centered trityl radicals are used nearly exclusively as PAs owing to their stability, solubility, molecular geometry, relatively long electron spin relaxation time and an electron spin *g* factor near 2.0, matching that of a free electron.[1] However, these PAs are exogenously introduced and do not serve as a polarization source to report on specific locales around paramagnetic active sites in molecules and functional materials.

A huge opportunity exists in the use of paramagnetic transition metal centers intrinsic to the system of interest as PAs. To date, highly electronically symmetric paramagnetic metals such as Gd^{3+}, Mn^{2+}, and Cr^{3+} with narrow central EPR transition bands have been used as exogenous PAs for high-field (>5 T) DNP.[2-5] Corzillius and coworkers employed endogenous paramagnetic metal centers, such as Mn^{2+} of a hammerhead ribozyme complex to enhance the ^{13}C NMR signal, and Leskes and coworkers used Mn^{2+} and Fe^{3+} ions to hyperpolarize $^{7/6}$Li and ^{17}O in

battery materials.[6-8] However, many transition metal ions, such as Ni^{+}, Cu^{2+}, Ni^{3+} and V^{4+}, that are widely present and central to the function of battery materials, catalytic compounds and metalloenzymes are considered inaccessible to DNP due to their wide EPR lines and g-values significantly shifted from 2.0. Expanding DNP capabilities to utilize these metal centers as PAs would provide a significant step towards DNP-enhanced NMR studies with endogenous paramagnetic metal centers.

Attaining local chemical and structural information with DNP-enhanced NMR studies using endogenous paramagnetic transition metals necessitates a rigorous understanding of their polarization pathways. Fundamentally, bulk polarization of nuclear spins by DNP comprises two stages. First, polarization transfer from the PAs to discrete nuclear spins by PA-nuclear spin hyperfine interactions. Second, polarization transfer by nuclear spin diffusion, in which polarized nuclear spins exchange energy with nearby, unpolarized spins to propagate polarization to bulk nuclei. These processes, and the detected NMR spectrum, are influenced by paramagnetic effects such as paramagnetic relaxation enhancement (PRE)[9-11] and contact and pseudo-contact shifts (CS and PCS)[12-14], all of which are determined by the proximity of the nuclear spins to the paramagnetic center. These paramagnetic effects give rise to the "spin diffusion barrier"[15] that defines how effectively the nuclear spins can transfer the polarization from near the paramagnetic center outwards to other nuclear spins through nuclear spin diffusion after getting hyperpolarized. A number of studies have explored the concept of the spin-diffusion barrier around lanthanides, transition metal centers, and organic radicals.[16-18] The exact size of the spin diffusion barrier is a critical parameter in determining the DNP polarization pathway and buildup rate, as it determines the location of the nearest nuclear spins that serve as a conduit for nuclear spin diffusion to remote nuclei. Hence, the rate of nuclear spin diffusion depends on the closest paramagnetic metal-nuclear spin distance for nuclei located beyond the spin-diffusion barrier that should give rise to a gradient of nuclear spin diffusion rates. Many DNP models rely on knowledge of the spin diffusion barrier and the polarization transfer rate,[19-21] that can be aided by experimentally validated knowledge of the effect of the electron-nuclear spin distance on the DNP buildup rates. These rates determine the spatial propagation of nuclear hyperpolarization and modulate the sensitivity enhancement by DNP.

In this study, we demonstrate the viability of V^{4+} ions as PAs to enhance the ^{1}H NMR signal of localized protons around the transition metal center at 6.9 T. We designed a series of vanadyl complexes with deliberately installed ^{1}H-containing trimethylene groups at varying distances from the V^{4+} center on an otherwise nuclear spin-free ligand backbone.[22] These transition metal-nuclear spin rulers allow a systematic study of the effectiveness of the ^{1}H's to conduct the spin-diffusion process as a function of their distance to the V^{4+} centers. We present the first demonstration of DNP using a wide-line transition metal by broad-band irradiation of the V^{4+} EPR transitions. These experiments were enabled by a versatile and unique (to date) DNP NMR instrument powered by a frequency tunable (193 – 201 GHz) solid-state microwave source with arbitrary waveform generation (AWG). DNP-enhanced ^{1}H NMR spectra and polarization buildup curves quantified the radius of the spin diffusion barrier to be between 4.0–6.6 Å, and revealed spin-diffusion mediated bulk ^{1}H polarization to directly depend on the position of the trimethylene ^{1}H nuclei relative to the barrier. Crucially, we demonstrate direct polarization transfer to and NMR detection of ^{1}H nuclei located 12.6 Å away from the V^{4+} center via V^{4+}-^{1}H hyperfine interaction. This work comprises the first systematic study of the effect of the spin diffusion barrier around a paramagnetic metal center on polarization transfer and DNP buildup rates, and paves the way towards elucidating structural and chemical information around paramagnetic active sites and cofactors. To distinguish DNP of local, select nuclear spins from DNP of bulk nuclei to achieve global NMR sensitivity enhancement, we dub this novel category of experiments hyperfine DNP spectroscopy.

RESULTS

Electron Paramagnetic Resonance

The V^{4+}-^{1}H rulers, i.e. the vanadyl complexes with controlled average V^{4+}-^{1}H distances ($R_{V\text{-}H}$), are shown in Figure 1a, with $R_{V\text{-}H}$ = 4.0 Å, 6.6 Å, 9.3 Å and 12.6 Å for complexes **1**–**4**, respectively. The complexes with chemical formulas $(Ph_4P)_2[VO(C_3H_6S_2)_2]$ (**1**), $(Ph_4P)_2[VO(C_5H_6S_4)_2]$ (**2**), $(Ph_4P)_2[VO(C_7H_6S_6)_2]$ (**3**), and $(Ph_4P)_2[VO(C_9H_6S_8)_2]$ (**4**) have tetraphenylphosphonium cations and were dissolved in 99.5% deuterated

dimethylformamide (DMF). The field-swept echo detected EPR spectra of the vanadyl complexes were recorded at a μw frequency ($\omega_{\mu w}$) of 240 GHz by sweeping the field from 8.4 T to 9 T at 5 K (see Figure S1). The principle components of the *g* factor and hyperfine coupling (*A*) between paramagnetic V^{4+} (*[Ar]3d1, S* = $^1/_2$) and 100% abundant ^{51}V isotope (*I* = $^7/_2$) were extracted by fitting these echo detected EPR spectra using EasySpin.[23] Figure 1b depicts the EPR line-shapes simulated at 6.9 T based on the fitted EPR parameters. The *g* and *A* tensor values found for all vanadyl complexes are well resolved at the high field and frequency employed in this study (Table S1, S2), and in agreement with the previously reported values for the same complexes determined by X-band CW EPR analysis.[22] The EPR lines of the vanadyl complexes span more than 3 GHz, which are significantly broader compared to the nitroxide-based radicals that span 0.6–1 GHz at 6.9 T and 4 K. The EPR spectrum is inhomogenously broadened as a result of the g-anisotropy of V^{4+}, as corroborated by data fitting in EasySpin (See Figure S1 and Table S1). While transition metal centers can have EPR line broadening spanning 100s of GHz,[24] only narrow-line radicals (line-width < 800 MHz) have been utilized in the current state-of-the-art DNP experiments due to the limited μw frequency range in commercial instruments. In this context, paramagnetic metals with EPR linewidth exceeding 1 GHz are categorized as wide-line PA for DNP. In the previous EPR study of these complexes, the size of the spin-diffusion barrier was reported to be between 4.0 and 6.6 Å. Here we explore the viability of V^{4+} centers as PA for DNP, determine the size of spin-diffusion barrier under DNP conditions (at high field of 6.9 T) using NMR detection and study its influence on the DNP process.

DNP Frequency Profiles and DNP-Enhanced ^{1}H NMR Spectra

To determine whether the V^{4+} ions are viable polarization agents, we investigated the DNP frequency profiles for **1–4**. The DNP frequency profiles of these broad line V^{4+} centers were recorded using the EPR-NMR pulse sequence, in which the μw irradiation frequency was varied over a 3 GHz span (Figure 2a). The NMR signal enhancement factors were determined by calculating the ratio $\varepsilon = (S_{ON}\text{-}S_{OFF})/S_{OFF}$, where S_{ON} and S_{OFF} are NMR signal intensities under μw-on and μw-off conditions at equal buildup times, respectively.

Figure 2b shows the DNP frequency profiles recorded with chirped μw pulse trains. The DNP frequency profiles across all complexes were broad and asymmetric, with maximum positive and negative enhancement positions separated by ~1 GHz. These complexes exhibited larger negative enhancements at around 192.4 GHz compared to positive enhancements at around 191.5 GHz. The same general features for the DNP profiles were observed with monochromatic CW irradiation with the exception of lower overall enhancement values ($\varepsilon_{CW\text{-}DNP}$) (Figure S2). The DNP frequency profiles of the complexes provide key insight into the mechanism of polarization transfer in these systems. The DNP profiles for all complexes display a sharper intensity slope at the higher frequency end and a longer tail towards low frequencies, reflecting the broad and asymmetric dispersion of the EPR spectrum caused by inhomogeneous broadening (Figure 1b). This indicate that the underlying mechanism is the differential solid-effect (SE).[25] In conventional SE, electron-nuclear dipolar interactions permit the forbidden electron-nuclear double quantum and zero quantum excitations that lead to the characteristic positive and negative enhancements separated by twice the nuclear Larmor frequency. Differential SE gives rise to the superposition of such SE profiles whose center frequencies span the inhomogeneously broadened EPR line. In such cases, the shape (width and symmetry) of DNP frequency profile in dominated by the EPR line shape, which in current case result into an asymmetric and broad DNP frequency profile. The basic feature of such DNP profiles could be replicated by numerical simulations, in which the difference of the positive and negative enhancements according to SE DNP for each frequency point was calculated from the respective EPR signal intensity to compute the net DNP enhancement, and the DNP profile reconstructed for each complex (see Figure S3).

DNP-enhanced ^{1}H NMR spectra measured at the maximum positive enhancement frequency ($\omega_{\mu w}$ = 191.30, 191.50, 191.35, 191.40 GHz for **1**–**4**, respectively) yielded $\varepsilon_{Chirp\text{-}DNP}$ ~ 1,19,12 and 9 for **1–4** using chirped μw pulses (Figure 3, solid line). CW μw irradiation yielded enhancements of $\varepsilon_{CW\text{-}DNP}$ ~ 0.5, 1.7, 1.7 and 1.8 for **1**–**4**, respectively (Figure 3, dashed-lines). Corresponding ^{1}H NMR spectra collected at the maximum negative enhancement with chirped μw pulses revealed similar enhancement values (Figure S4). The higher enhancement observed in sample **2** is attributed to the high solubility of the complex in the DMF solvent (77.2 mM),[26] resulting in a relatively large number of V^{4+} metal centers in the sample.

Crucially, these results demonstrate, for the first time, ^{1}H polarization enhancement with a wide-line EPR transition in V^{4+} paramagnetic ions using broad-band microwave pulses. The significant DNP performance boost obtained by transitioning from CW monochromatic to broad-band chirp train μw irradiation has been reported recently in organic biradicals.[27] Broad-band pulse trains are crucial for DNP to access a greater population of wide-line transition metal centers that constructively participate in polarization enhancement. The inhomogeneous broadened EPR lines due to large *g* anisotropy in these metals spreads out the electron spin density across a wide frequency range. As a result, monochromatic CW microwave irradiation would only engage a small population of the V^{4+} centers in DNP that hence results in small NMR signal enhancements. Thus, by using shaped microwave pulses to fully saturate the broad EPR transition, we could successfully access V^{4+} spin centers that generate significant polarization enhancement. The gain factor was $\varepsilon_{Chirp\text{-}DNP}/\varepsilon_{CW\text{-}DNP} > 4$ for all samples **1–4**, and reaching up to ~10 for **2** (with $\varepsilon_{Chirp\text{-}DNP} = -33$ and $\varepsilon_{CW\text{-}DNP} = -3.5$ in the region of negative enhancement). By implementing broad-band chirp μw pulses, we demonstrate ^{1}H polarization with V^{4+} paramagnetic metal centers traditionally believed to be inaccessible for DNP.

Proton NMR line-shape and polarization buildups

To realize DNP with endogenous paramagnetic metal centers, we need to understand the polarization transfer mechanism and pathways. Specifically, we need to know the polarization characteristics of protons relative to their proximity to the polarization agent. Because differential SE DNP is fundamentally based on electron-nuclear dipolar interactions, complexes **1**–**4** presents a unique opportunity to systematically investigate polarization pathways with modular V^{4+}-^{1}H dipolar interactions. Essential to this investigation is identifying the distinct nuclear spins participating in the polarization process. Based on our sample preparation, the enhanced ^{1}H NMR signal observed can originate from three different sources: the trimethylene moieties on the vanadyl complex, the tetraphenylphosphonium (PPh_4+) counterion, and the 0.5% protons in the DMF-d_7 solvent. To begin our investigation, we turned to the DNP-enhanced NMR spectra of **1**–**4** (Figure 3), which show a common narrow signal across the complexes. However, the ^{1}H NMR spectral line-shapes of **4** clearly indicate the presence of two spectral components with different line widths. In order to distinguish between the two signals in **4** and identify the common narrow signal across **1**–**3**, the inter-pulse delay (τ) was varied between 30–200 μs (see Figure S5) to observe whether the broad signal in **4** can be selectively suppressed with increasing τ. The ^{1}H spectra of **4** recorded with τ = 50 μs (cyan, solid-line) and τ = 200 μs (magenta, dashed-line) are shown in Figure 4a. With a 200 μs inter-pulse delay, we observe only a single narrow spectral component. This indicates that the ^{1}H nuclear spins constituting the broad spectral component have significantly shorter transverse relaxation times (T_{2n}) relative to those of the narrow spectral component. A deconvolution of the ^{1}H NMR acquired with τ = 50 μs revealed that the narrow peak is centered at -5 ppm and the broad peak upfield shifted to -14 ppm (Figure S7). The combination of a shorter T_{2n}, broad line-shape, and an upfield-shifted peak position suggests that these nuclear spins experience greater paramagnetic effects (PRE and PCS) than those of the narrow signal. This is further reinforced by solution-state ^{1}H NMR spectra of **4**, wherein the peaks of the complex protons (centered at 2.22 ppm) were broader compared to that of the solvent ^{1}H (counterion protons at 7.77 ppm and the DMF protons at 7.91, 2.80, and 2.64 ppm) as shown in Figure S6. The upfield shift at low temperatures has also been reported in previous work on $S = ½$ vanadium complexes.[28] Thus, we assigned the broad signal component with shorter T_{2n} to the protons covalently attached to the complex, referred to as "complex protons", and the narrow signal with longer T_{2n} to "solvent protons" that include protons on the counter ions and the DMF solvent. To discard the possibility of the counter-ion protons being too close to the V^{4+} centers leading to the broad signal, we performed additional pulsed hyperfine EPR experiments (known as Electron Spin Echo Envelope Modulation – ESEEM) to detect V^{4+}-^{31}P hyperfine couplings (ESEEM is sensitive to V^{4+}-^{31}P distances in the 3-7 Å range) between V^{4+} of the complexes and ^{31}P of the PPh_4 ions (data not shown). However, no modulations were observed, indicating that the counter-ions are not in close proximity to V^{4+}. This observation is consistent with the size of the solvation shell being sufficiently large for the tetraphenylphosphonium ion, such that the ions are separated far enough from the vanadyl complex. Moreover, the broad signal was absent in complexes **1-3**, which further confirm that only the protons on the complex give rise to this signal. The absence of the broad components in samples **1–3** is a result of paramagnetic quenching due to strong PRE at

shorter $R_{V\text{-}H}$ compared to **4**. The complex ^{1}Hs of sample **4**, on the other hand, are clearly visible as a broad spectral component.

With the chemical identity of the protons that constitute the observed NMR signals confirmed, we then proceeded to investigate the ^{1}H polarization buildup times of the different nuclei. The DNP buildup times of the ^{1}H NMR signal of complexes **2–4** were recorded as shown in Figure 4b, including those of the two ^{1}H signals of **4**. Note that the DNP buildup of complex **1** is not shown, as there was no DNP enhancement observed with this sample (Figure 3). To test whether the total sample concentration affected the buildup curve, two concentrations of sample **2**, 77.2 mM (at saturation) and 13 mM (comparable with the other three complexes) were measured. All DNP buildup curves were fitted to a stretched exponential:

$$I = I_0\left[1 - e^{-\left(\frac{t}{T_{DNP}}\right)^n}\right]$$

Where I_0 is the NMR signal intensity at DNP saturation, T_{DNP} is the time constant for polarization buildup, and n is the stretch factor. The value of n (≤ 1) provides key information about the nature of the polarization process. We expect that polarization buildup dominated by ^{1}H nuclear spin diffusion to be a mono-exponential process, resulting in $n \sim 1$. Should other processes, such as V^{4+}-^{1}H hyperfine coupling interactions, contribute to polarization buildup, we expect a multi-exponential process that lowers n towards a value of 0.5.[29] The T_{DNP}, n and I_0 values obtained from the fits are given in Table 1.

Table 1. Fitted parameters for the proton build up curves in 2-4 for chirped DNP experiments.

Complex#	T_{DNP} (s)	n	I_0
4(complex)	18.2±1.5	0.60±0.03	1.003±0.020
4(solvent)	47.7±6.5	0.79±0.06	1.021±0.049
3	88.0±4.0	0.78±0.01	1.176±0.018
2	142.7±28.3	0.80±0.09	1.053±0.072
2 (77 mM)	120.4±9.0	0.93±0.02	1.253±0.048

Between the two types of ^{1}H nuclei, the complex protons on **4** have the shortest T_{DNP} and smallest n values relative to the solvent protons in **2**–**4**. These nuclei exhibit a stretching parameter n = 0.6 that is very close to 0.5, which we therefore attribute to polarization by V^{4+}-^{1}H hyperfine interactions and not nuclear spin diffusion. Polarization via hyperfine interactions involves the direct transfer of polarization through the double and zero quantum transitions. This single-step process is expected to be faster than polarization by the stochastic, multi-step nuclear spin diffusion processes. Therefore the combination of the short T_{DNP} time and near n = 0.5 stretch parameter lead us to attribute polarization of complex protons via V^{4+}-^{1}H hyperfine interactions. The solvent protons in **2**–**4** exhibit longer T_{DNP} values, in addition to larger n values approaching 1. The increase of both parameters indicates a more homogenous buildup process facilitated by the influence of nuclear spin diffusion, which is relatively slower. However, the value of n (0.8) is still less than 1.0, implying that the buildup is still a multiexponential process in the polarization of solvent protons. This may be due to the contribution of both direct V^{4+}-^{1}H transfer and ^{1}H nuclear spin diffusion processes, or due to a multiexponential spin-diffusion process among the solvent protons. The distinct polarization buildup of different types of ^{1}H nuclei may provide a powerful tool to elucidate additional structural and chemical information unseen in conventional ^{1}H NMR spectra. This concept is routinely used in solid-state NMR techniques such as double cross-polarization[30] and Transferred-echo double-resonance (TEDOR),[31] in which the buildup curve of polarization transferred from one to another nuclear spin provide information on their spatial proximity and relative orientation.

We next turned our attention to the solvent protons observed across **2**–**4**, which do not exhibit equal polarization buildup rates. The buildup time constant increases from 47.7 s in **4** to 88.0 s in **3** and 142.7 s in **2** while n remains relatively constant, suggesting that the polarization buildup mechanism is consistent amongst the complexes. The only difference between the complexes is the distance between the V^{4+} ion and the ^{1}H nuclei on the ligand scaffold. Since the electron-nuclear hyperfine coupling is dependent on the distance between

the spins, we expect V^{4+}-^{1}H coupling strength to increase as V^{4+}-^{1}H distance decreases from **4** to **2**. If hyperfine coupling is the mechanism of polarization transfer, we expect a stronger hyperfine interaction to increase state mixing that enhances differential SE and produce a faster buildup rate. However, we observe a decrease in the buildup rate as the coupling strength increases. We hypothesize that the complex protons in **2**–**4** serve as a conduit to transfer polarization to the solvent protons via spin-diffusion, the rate of which determines the solvent proton polarization buildup rates. Nuclear spin diffusion from the complex protons to the solvent protons is heavily dependent on the V^{4+}-^{1}H hyperfine coupling strength. Strong V^{4+}-^{1}H coupling gives rise to strong paramagnetic effects (PRE and PCS) that significantly alter the frequency of the complex ^{1}H spins. If the complex protons are key conduits for nuclear spin diffusion, large frequency (energy) changes would reduce the tendency of the complex ^{1}Hs to participate in nuclear spin diffusion process, leading to long polarization buildup times. Indeed, the trend in T_{DNP} mirror the trend in V^{4+}-^{1}H hyperfine interaction strength, which increases from **4** to **2**. In **2**, we observe that the buildup rate increases (T_{DNP} = 120.4 s) on increasing the concentration from 13 mM to 77.2 mM and the *n* value gets closer to 1 (0.93). We attribute this to the formation of a stronger proton spin network due to the larger total number of protons from the complex and counterion in solution at higher complex concentrations, leading to a relatively faster and more uniform nuclear spin-diffusion process. The general trend of increasing T_{DNP} and *n* from complex **4** to **2** was recapitulated by CW DNP (see Figure S9a and Table S3) as well as by the spin-lattice relaxation time (T_{1n}) of the solvent protons (see Figure S9b and Table S3). Note that for nuclear spins other than ^{1}H such as ^{13}C, ^{29}Si, etc, the rate of nuclear spin diffusion is far slower due to their lower natural abundances and gyromagnetic ratios. In such cases, the rate of bulk polarization buildup will be significantly slower, such that DNP will selectively enhance the signal of the nuclear spins surrounding the paramagnetic center.

We modelled the aforementioned experimental observations on the proton polarization builudp curves with quantum mechanical simulations. A three spin model consisting of one electron and two proton spins was simulated in the SpinEvolution software, wherein one proton spin was strongly coupled to the electron spin (complex protons) and another weakly coupled to the electron spin (solvent protons).[32] The simulations showed that increasing the hyperfine interaction strength between the electron spin and the complex proton spin slows the buildup rate of the solvent proton spin polarization (Figure S10), supporting the aforementioned hypothesis and results. Taken together, these detailed explorations into the buildup rates of distinct nuclear spins provide foundational understanding of DNP using transition metal-based PAs, paving the way towards DNP with endogenous metal centers.

The strong dependence of polarization rate on the V^{4+}-^{1}H hyperfine interaction strength in **1**–**4** can be explained with the concept of the spin diffusion barrier. The spin diffusion barrier, governed by electron-nuclear hyperfine interaction strength, determines the nearest nuclei that can facilitate polarization transfer via nuclear spin diffusion. Nuclei within the barrier are too strongly interacting with the electronic spin and hence do not participate in nuclear spin diffusion. With these concepts, we can approximate the spin diffusion barrier using the polarization behavior across **1**–**4**. Complex **1** showed negligible DNP enhancement, and **2**–**4** showed increasing polarization rates for the solvent ^{1}H's. We ascribe the lack of polarization in complex **1** to the suppression of nuclear spin diffusion when the nearest complex protons are located inside the spin-diffusion barrier. In complex **2**, the complex protons are near or outside the barrier, enabling polarization via nuclear spin diffusion to occur. The progressive increase in polarization rate across **2**–**4** is the result of weaker electron-nuclear coupling that enables better energy matching between complex and solvent protons that facilitate spin diffusion. Taken together, these results suggest that the spin-diffusion barrier lies between 4.0 Å and 6.6 Å from the paramagnetic center.

Phase memory relaxation times

We further corroborated the size of the spin diffusion barrier by measuring the phase memory relaxation time, which is equivalent to the electron spin decoherence time (T_m) of **1**–**4** at comparable concentrations at 8.63 T and at 5 K, as shown in Figure 5. The T_m values published previously at 0.35 T and at 40 K are also shown for comparison. The characteristic timescale for electron spin decoherence can be modulated by multiple factors such as spin-spin relaxation (T_{2e}), spectral, spin and instantaneous diffusion processes, as well as nuclear spin flip-flop processes.[33] Here, the limiting mechanism for electron spin decoherence of V^{4+} are

nuclear spin flip-flop processes, which generates magnetic noise that induces transverse electron spin relaxation, hence shortening T_m. These energy-conserving nuclear flip-flop processes are analogous to those involved in nuclear spin diffusion. Thus, T_m can serve as a probe for the efficacy of complex protons to facilitate nuclear spin diffusion that is dominated by solvent and cation protons from PPh_4^+ and DMF-d_7, as demonstrated in a computational study of the same complexes.[34]. At 8.63 T, the T_m values dropped significantly from 10.59 µs for **1** to 4.53 µs for complex **2**. Interestingly, T_m increased from 4.53 µs in complex **2** to 4.80 µs in **3** and 6.43 µs in **4**. For both fields, T_m was found to be longest for complex **1.** The longer T_m of **1** compared to **2**–**4** suggests that the nearest nuclear spins (complex protons) are well inside the spin diffusion barrier, and hence do not participate in facilitating the electron spin decoherence. The minimum in T_m for **2** can be rationalized by the complex protons positioned just outside the spin diffusion barrier, where they are maximally detrimental. The subsequent increase in T_m in **3** and **4** is explained by less efficient state mixing caused by the weaker hyperfine interactions at higher fields where the Zeeman levels are well separated. These results lend support to complex protons being the dominant contributor dictating the electron spin coherence of V^{4+}, reinforcing the proposed 4.0–6.6 Å spin diffusion barrier radius. Note that the EPR signals for complexes **2**–**4** do not indicate any sign of aggregation, as no broad EPR line component or signal quenching was observed. In complex **1**, we observe signal quenching by reduction of the EPR signal amplitude, but not the width of the visible EPR spectrum compared to that of complexes **2**–**4** (see Figure S1). This indicates that a population of complex **1** has clustered in solution and exhibits relaxation rates too fast to be detected by EPR. Our measurements on **1** are performed on the non-aggregated population of complex **1**, as supported by the absence of paramagnetically shifted features in the ^{1}H NMR spectra (Figure 3). Thus, we can exclude aggregation as a cause for the absence of DNP enhancement in complex **1,** and ascribe the observation to the lack of spin-diffusion from complex protons to the solvent protons.

Conclusions

The sum of this work demonstrates, significant NMR signal enhancements up to ~33 fold in this first study of V^{4+} transition metal complexes as DNP PAs. The use of AWG shaped microwave pulses allowed broad-band saturation of an inhomogeneously broadened EPR line, which is essential for efficient DNP. DNP-enhanced NMR spectroscopy and polarization buildup studies on synthetically modular V^{4+}-^{1}H nuclear spin rulers uncovered distinct polarization pathways for different types of polarizable ^{1}H. We identify the key role of the complex protons as conduits to transfer polarization to bulk nuclei, and that their position relative to the V^{4+} centre influences the nuclear spin-diffusion rates. The polarization buildup curves and the phase memory relaxation time measurements were used to determine the spin diffusion barrier to be between 4.0–6.6 Å from the V^{4+} centre. In contrast to the previous study on these complexes by EPR T_m measurements at low field, in this study the spin-diffusion barrier is determined by detecting its effect on the DNP amplification of the ^{1}H NMR signal. This is the first demonstration of a gradient effect of the nuclear spins (^{1}H) to participate in the spin-diffusion process as a function of their proximity to the paramagnetic center. Moreover, we report on a direct V^{4+}-^{1}H DNP transfer up to a distance of 12.6 Å. The observation that DNP enhancement diminished when the nearest protons were located inside the spin-diffusion barrier demonstrates that the nuclear spins inside the spin diffusion barrier can trap polarization to propagating outwards. This study provides critical insights for endogenous DNP using transition metal centers.

Expanding the catalogue of polarizing agents to wide-line transition metal ions such as V^{4+} offers furthermore the potential to enable endogenous polarization within molecules and materials of interest in the future. Specifically, vanadium acts as catalytically active species in both heterogeneous catalyst systems, such as vanadium oxides, and metalloenzymes, including vanadium haloperoxidase.[35; 36] This proof-of-concept study demonstrates a pathway to harness paramagnetic vanadium centers within catalyst systems to selectively enhance and illuminate the NMR signal sensitivity of target nuclei approximately 6 to 12 Å (or perhaps farther) from chemically-active V^{4+} sites. Immense future potential remains in exploiting the anisotropic electronic structure of these metals to engender orientation-selective DNP-enhanced NMR spectroscopy, wherein orientation and distance information can be extracted from the polarization of target nuclei. In fact, the established technique of hyperfine EPR spectroscopy[37] relies on similar principles to extract orientation and/or distance information between a paramagnetic metal and nearby nuclei, except EPR detection offers less detailed chemical information on the nuclear spins compared to NMR. This work provides a critical

first step towards DNP-enhanced hyperfine NMR spectroscopy with endogenous polarization agents that couple high sensitivity with local structural information.

EXPERIMENTAL PROCEDURES

Resource Availability

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Songi Han (songihan@ucsb.edu).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The NMR experimental data including the buildup curves, DNP profiles, Proton spectra, processed text files, Matlab script for figure generation and the figure files generated during this study are available at figshare: https://figshare.com/s/17782f99048db974f94e

Materials and Methods

Unless otherwise noted, all solvents and reagents were purchased from commercial vendors and used without further purification. All solvents were dried and degassed according to literature procedures prior to use.[38] All synthetic manipulations were performed under an N_2 atmosphere in an MBraun Unilab Pro glovebox. Complexes **1**–**4** were synthesized and dried according to literature procedures.[22] (d_{20}-Ph_4P)Br was synthesized following the preparation of Marcoux and Charette starting with C_6D_5Br and $P(C_6D_5)_3$.[39] Complex **4'** was synthesized following the same procedure as **4** using (d_{20}-Ph_4P)Br.

Concentrations of **1**–**4** in dimethylformamide were determined through UV-visible spectroscopy, which were collected on a Varian Cary 5000 spectrometer. Concentration series for complexes **1**–**4** were prepared in dimethylformamide (DMF) in an N_2 atmosphere within the glovebox. Serial dilution of a stock solution of **1**–**4** was performed to generate four samples of incrementally decreasing concentrations per series. All UV-visible absorption spectra were baseline corrected with a blank containing DMF. Absorbances at select wavelengths for each complex were fitted with a linear regression to generate a calibration curve (Tables S5–S8, Figures S8 and S9) for concentration determination of the saturated solutions of **1**–**4**. Saturated solutions of **1**–**4** were prepared in DMF, then diluted 20-fold with DMF for **1** and 100-fold for **2**–**4** to generate samples with absorbances within the range of the concentration curves (Table S10, Figures S8). The same aforementioned procedures were repeated for complex **4'**.

The solution of the four vanadyl complexes (see Figure 1a) in 99.5% deuterated dimethylformamide (DMF) solvent were prepared as described in a previous publication by Graham et al.[22] The V^{4+} ion concentrations for the complexes in saturated solutions are 10.5 mM for **1**, 77.2 mM for **2**, 14 mM for **3** and 13.2 mM for **4** (see section 6 of the SI). The saturated solutions were flame sealed in EPR quartz tubes of length ~2 cm, with inner and outer diameters of 2.2 mm and 3.0 mm, respectively, to avoid any air exposure. In case of complex **2**, a sample with 13 mM V^{4+} concentration was also prepared to confirm the observed trends in buildup rate at comparable concentrations as the other complexes.

The field-swept echo-detected EPR spectra as well as phase memory relaxation time (T_m) measurements were performed using a 55 mW microwave source tuned to 240 GHz. The fields were swept in a range from 8.4 T to 9.0 T, while EPR spin-echoes were recorded using the microwave pulse sequence (p1-t-p2-t-echo), where p1 = 800 ns and p2 = 1 us. These measurements were performed at temperature 5K. Phase memory time measurements were performed with a two-pulse Hahn echo pulse sequence (p1-t-p2-t-echo) where p1 = 800 ns and p2 = 1 us. T_m was found by fitting the echo decay E(2t) as a function of t to $E(2t) = A*\exp(-2t/T_m) + C$.

The DNP NMR experiments were performed with a broad-band quasi-optics (QO) based dual DNP-EPR instrument, operating at 6.9 T and at 4–10 K. Details of the home-built DNP instrumentation have been described previously.[40-42] All static ^{1}H NMR experiments were recorded using the solid echo (90x-t-90y) pulse sequence shown in Figure 2a. To perform DNP

experiments, the samples were freeze-quenched in liquid nitrogen by dipping the sample loaded probe into the cryostat filled with liquid nitrogen for glass formation. After evaporating the nitrogen, the cryostat was cooled to 4 K using liquid He for the experiments. The microwave power and buildup time of 120 mW and 60 s were used in the frequency profiles for both the chirp and CW DNP case. The chirp pulse sweep width was tested in a range from 0 (CW) to 600 MHz, and the sweep width that optimizes the DNP effect found to be 200 MHz. Hence, a train of chirp pulses with sweep width ($\Delta\omega_{ch}$) = 200 MHz and pulse length (t_{ch}) = 300 µs was used repetitively over the buildup time (t_{DNP} = 60s). The static proton NMR spectra were recorded using solid-echo pulse sequence with a delay of 50 µs between the pulses except for complex 4 in which 200 µs was used to obtain the T_2-filtered signal from solvent protons. For the buildup curves the microwave power was 120 mW and the frequencies were set to the positive maxima positions, i.e. $\omega_{\mu w}$ = 191.30, 191.50, 191.35, 191.40 GHz for **1**-**4**, respectively.

SUPPLEMENTAL INFORMATION

Document S1 is the main supplemental PDF that includes: The EPR spectra of the vanadyl complexes, DNP frequency profiles, proton NMR of complex **4**, the polarization buildup curves and fittings, T_m measurement data, and sample concentration measurements.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.K.J., C.-J.Y., D.E.F. and S.H.; Methodology, S.K.J., C.-J.Y., B.W., and T.T.; Writing-Original Draft S.K.J., C.J.Y., D.E.F. and S.H.; Visualization S.K.J. and C.-J.Y; Supervision D.E.F. and S.H.; Funding Acquisition D.E.F. and S.H.

DECLARATION OF INTERESTS

The authors declare no competing interests

Figure 1 Molecular Structures of the Complexes and Their EPR Spectra (a) Structures of the four V^{4+}-^{1}H rulers presented in Graham et al. that were used in this study.[22] (b) The EPR line-shapes of the complexes **1**–**4** shown in magenta, orange, gray and cyan respectively. The spectra were simulated for 6.9 T field using the experimental data from 240 GHz CW EPR spectra at 5 K.

Figure 2 DNP Pulse Sequence and Frequency Profiles

(a) Solid-echo pulse sequence used to collect ^{1}H NMR signals. Blue and magneta squares represent microwave and radio wave pulses, respectively. Microwaves are applied for DNP experiments and turned off for normal NMR experiments. (b) DNP frequency profiles for **1**–**4** are shown in magenta, orange, gray and cyan respectively. The experiments were performed at 4 K on a 6.9 T magnet with using chirped µw pulses with the parameter $\Delta\omega_{ch}$ = 200 MHz, t_{ch} = 300 µs, t_{DNP} = 60 s, $P_{\mu w}$ = 120 mW, and the interpulse delay τ = 50 µs. Sample concentrations are given in the legend.

Figure 3 ^{1}H NMR Spectra of the Four Complexes

^{1}H NMR spectra acquired under μw off (dotted-lines), and with CW (dashed-lines) and chirp DNP (solid-lines) for **1**–**4** shown in magenta, orange, gray and cyan colors, respectively. $\omega_{\mu w}$ was set to positive maximum signal enhancement for each complex and the remaining experimental parameters are identical to those reported In Figure 2.

Figure 4 Complex vs Solvent Proton NMR and DNP Builpup Curves

(a) Chirp DNP-enhanced ^{1}H NMR spectra from complex **4** with inter-pulse delays of 50 μs (cyan, solid-line) and 200 μs (magenta, dashed-line), recorded on a 6.9 T magnet at 4 K temperature. The spectrum with τ = 200 μs was scaled up to match the peak heights of the two spectra to highlight the suppression of the broad component. (b) The bulk polarization buildup using chirp DNP experiments (plot markers) and fitted curves (lines) for **2** at 77 mM (orange-circles) and 13 mM (orange-square), **3** at 14 mM (gray), and **4** at 13.2 mM from the solvent protons (cyan-circles) and complex protons (cyan-square). The fitted parameters are given in Table 1.

Figure 5 Phase Memory Relaxation Time Constants and Echo Decay Curves

(a) Phase memory relaxation time constants (T_m) for **1**–**4** in magenta, orange, gray and cyan colors respectively, measured at 8.63 T field and 5 K temperature (triangles) in saturated solutions. The squares in the same color coding are data points recorded at 0.35 T and 40 K published in Graham et al.[22] (b) The experimental data (plot markers) and the fitted exponential decay curves (cyan lines) to determine T_m for complexes **1**–**4** at 8.63 T field and 5 K temperature.

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26. We hypothesize that the solubility of complex 2 in DMF is particularly high due to the optimal balance between the non-polar nature of the organic ligands and the overall charge of the complex. With the longer linkers in complex 3 and 4, the nonpolar nature of those ligands are dominant and inhibit solubility in DMF. With complex 1, the ligand is more ionic in nature, and combined with the 2– charge of the complex may render the complex highly ionic, hence reducing its solubility in DMF. Taken together, we propose that the nonpolar nature of the ligands on complex 2 balance the ionic nature of the complex, thereby enhancing its solubility in DMF.
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