Remarkable Levels of $^{15}$N Polarization Delivered through SABRE into Unlabeled Pyridine, Pyrazine, or Metronidazole Enable Single Scan NMR Quantification at the mM Level

Marianna Fekete, Fadi Ahwal, and Simon B. Duckett*

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ABSTRACT: While many drugs and metabolites contain nitrogen, harnessing their diagnostic $^{15}$N NMR signature for their characterization is underutilized because of inherent detection difficulties. Here, we demonstrate how precise ultralow field signal amplification by reversible exchange (±0.2 mG) in conjunction parahydrogen and an iridium precatalyst of the form IrCl(COD)-(NHC) with the coligand $d_5$-benzylamine allows the naturally abundant $^{15}$N NMR signatures of pyridine, pyrazine, metronidazole, and acetonitrile to be readily detected at 9.4 T in single NMR observations through >50% $^{15}$N polarization levels. These signals allow for rapid and precise reagent quantification via a response that varies linearly over the 2−70 mM concentration range.

INTRODUCTION

Hyperpolarization methods have been shown to dramatically improve the sensitivity of nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) in a process that involves increasing the purity of the magnetic states they detect. Signal amplification by reversible exchange (SABRE) reflects one such method. It harnesses the nuclear spin order of parahydrogen ($p$-H$_2$)$^{3,5}$ and is a consequence of the pioneering work of Weitekamp$^6$ and Eisenberg.$^7$ For SABRE to operate, the symmetry of $p$-H$_2$ is first broken by temporarily placing it in a metal complex so that the new hydride ligands couple distinctly to NMR active spins within the ligand sphere of the product. A process of reversible binding then allows a suitable substrate to become hyperpolarized through a catalytic process that transfers nuclear spin order within the complex rather than achieving a change in the chemical identity.$^{5,6}$ Typically, this process takes place in a specified magnetic field that is often called the polarization transfer field (PTF) and can be selected to optimize efficiency.$^{5,9}$ The selection of this field is made according to the chemical shift difference that exists between the interacting nuclear spins and their spin−spin couplings$^{10,11}$ in a process that has been accurately modeled.$^{1,2}$ As the active SABRE catalyst may break the symmetry of the two protons that were initially located in $p$-H$_2$ through chemical or magnetic inequivalence effects, the process of catalysis can be complex.$^{5,13}$ This is because for the spin order transfer from the $p$-H$_2$ derived hydride ligands to take place, the receiving ligand nuclei must exhibit different spin−spin couplings to these two protons.

Knowledge of this behavior has influenced the SABRE catalyst design,$^{1,4}$ and the resulting sensitization process has enabled the easy NMR detection of low-abundance inorganic species.$^{13}$ Other studies have used deuterated coligands to improve the spin-order yields in SABRE by reducing waste through the focusing of polarization transfer into fewer receptor sites.$^{15}$ When this is achieved in conjunction with $^3$H labeling, the associated extension of the nuclear spin-order lifetime has proven to be particularly beneficial as decoherence within the SABRE catalyst reflects one route to reduce the overall processes efficiency.$^{16}$ These two effects combine to extend the duration, over which signals remain visible to NMR. As in classical terms, one $T_1$ period is associated with 63% destruction of the hard-won polarization level. Not surprisingly, the extended lifetimes associated with molecular singlet states$^{17−21}$ and their derivatives feature extensively in hyperpolarization research as one goal is often to study in vivo reactivity.$^{22}$ In further developments, Tessari et al. have shown how $^3$H-SABRE can achieve precise analyte quantification at low substrate loadings by the involvement of a slow exchanging coligand.$^{23,24}$ Furthermore, Iali et al. extended SABRE to the hyperpolarization of primary amines through catalysts of the...
form $[\text{Ir}(\text{H})_2(\text{Imes})(\text{amine})]_2\text{Cl}$, and it was noted that sterically hindered amines, which failed to bind efficiently, benefited by the addition of smaller NCMe, which enables the formation of $[\text{Ir}(\text{H})_2(\text{Imes})(\text{aniline})(\text{NCMe})]_2\text{Cl}$. The successful use of amines reflects an important boost to SABRE because the hyperpolarized NH response can be used to sensitize other molecules through proton exchange. More recently developments of this ligand design route have enabled the hyperpolarization of pyruvate and acetate.

Recent developments of this ligand design route have enabled formation of $[\text{Ir}(\text{H})_2(\text{IMes})(\text{aniline})(\text{NCMe})]_2\text{Cl}$. The benefits involved examining an NMR tube containing methanol-$d_4$ solutions of these reagents under 3 bar (absolute) pressure of $p$-H$_2$ at 99% purity. $p$-H$_2$ gas is first dissolved by shaking the NMR tube while it is located in a preset magnetic field that lies between $\pm 1$ mG and $\pm 70$ G for $\sim 10$ s (relative to the main NMR magnetic field orientation). Subsequently, the sample is placed in a 9.4 T magnet where the final NMR signal detection step occurs.

Under these conditions, the SABRE catalyst $[\text{Ir}(\text{H})_2(h_{22-1})(\text{py})_2]_2\text{Cl}$ forms and a $^1$H NMR signal gain of 1452-fold can be seen for the ortho proton resonance of free pyridine that is present in the solution after being transferred from a 60 G field. This polarization transfer step takes 10 s to complete and the resulting polarization level ($P_1$) is 4.65% ($P_r$ reflects the percentage polarization associated with nuclei $x$). In this case, the catalyst breaks the symmetry of the two $p$-H$_2$-derived protons through magnetic inequivalence effects, and hence, the spin order transfer flows optimally within the equatorial plane that contains the hydride ligands into bound pyridine. For $^{15}$N, however, the large trans two bond $^1$H -- $^{15}$N coupling of $\sim 19$ Hz that connects these hydride ligands to nitrogen in $[\text{Ir}(\text{H})_2(h_{22-1})(\text{py})_2]_2\text{Cl}$ enables the efficient transfer of polarization at an approximate $\sim 1$ mG field that is of the same sense to the main 9.4 T observation field. The consequence of this process is a 39200-fold ($\pm 2\%$) $^{15}$N NMR signal gain, which means the corresponding $P_1$/$P_r$ value is 12.9% ($\pm 2\%)$. Hence, this unlabeled 35 mM sample of pyridine can be detected by $^{15}$N NMR spectroscopy in a single scan NMR measurement at a magnetic field of 9.4 T with a signal to noise ratio of 11 using a routine inverse detection probe.

Establishment of Coligand Benzylamine As Beneficial to the Hyperpolarization of the $^{15}$N NMR Signal of Pyridine. When the coligand $d_2$-benzylamine ($d_2$-BnNH$_2$) was added to such a sample, at an initial concentration of 17.5 mM, it proved to rapidly convert into its $d_2$-benzylamine isotopologue. Consequently, we refer to $d_2$-BnND$_2$ throughout this article even though $d_2$-BnNH$_2$ is actually added to the samples. The resulting $^1$H NMR spectra reveal that in addition to this labeling change, two new inorganic species are formed, which yield pairs of hydride ligand signals at $\delta = -22.14$ and $-22.58$, and $\delta = -23.34$ and $-23.73$, respectively. These hydride ligand signals arise from $[\text{Ir}(\text{H})(h_{22-1})(d_2$-BnND$_2$)(py)$_2]_2\text{Cl}$ and $[\text{Ir}(\text{H})(h_{22-1})(d_2$-BnND$_2$)$_2]$Cl, respectively, that are present in the solution in the ratio 2.6:1. The two complexes contain inequivalent hydride ligands that differ from one another according to the identity of the axial ligands in the complex, as detailed in Scheme 1 and the Supporting Information. Furthermore, as their proportions match the value seen when a similar sample is created by the initial addition of benzylamine and H$_2$ to $[\text{IrCl(COD)}(h_{22-1})]$, but before pyridine addition takes place, it can be concluded that these two complexes are in equilibrium. Hence, the separation of their roles in the underlying SABRE process is impractical, but we note it would be expected that both will contribute to this process. In addition, it is important to recognize that both of these complexes contain chemically and magnetically distinct hydride ligands. The result of this change is that spin-order transfer can now proceed into ligands that lie trans and cis to hydride, which means that spin dilution, associated with polarization of the axial ligands, is expected and this will reduce the SABRE signal gains that are seen for the free substrate. Hence, the involvement of polarization transfer protecting $d_2$-BnND$_2$, which limits spin-order wastage should be of significant benefit to the SABRE outcome.

When the resulting $d_2$-BnND$_2$ solutions were examined for SABRE, the $^1$H NMR response resulting from this mixture of catalysts proved to contain a free pyridine ortho proton...
Hydride ligands. The resulting $^1$H NMR form in these experiments, in a 2:1 ratio. They both possess when compared to that achieved by $[Ir(H)_{2}((dp-BnND_{2})_{2})(NCMe)_{3}]Cl$. This reflects a 27% improvement in the SABRE efficiency when compared to that achieved by $[Ir(H)_{2}((dp-BnND_{2})_{2})(py)]Cl$ and confirms that there is a benefit to using the coligand $d_{6}$-benzylamine when seeking $^{15}$N polarization.

Upon changing to $[IrCl(d_{22}-1)(COD)]$, and completing a similar series of $d_{6}$-BnND$_{2}$ promoted measurements, the levels of signal gain seen in the pyridine ortho proton $^1$H NMR signal rises to 1324-fold, although the $^{15}$N polarization level proved to be unaffected. Hence, while catalyst deuteration is not successful at improving SABRE $^{15}$N activity, it is able to improve the level of $^1$H signal gain because of reduced spin order wastage and improved $^1$H relaxation. This suggests that low-field $^{15}$N-relaxation within the catalyst is not improved.

While it is well known that the optimum SABRE catalyst changes with the identity of the substrate, it has been clearly demonstrated here that there is also a further dependence on the efficiency of SABRE transfer within a given substrate according to whether $^1$H or $^{15}$N is the target. The optimum rate of ligand exchange for $^1$H transfer has been proposed by Barskiy to be 4.5 s$^{-1}$ in complexes of the type $[Ir(H)_{2}((h_{22}-1)(py))]Cl$. Consequently, the rate of pyridine substrate dissociation in $[Ir(H)_{2}((h_{22}-1)(py))_{2}(dp-BnND_{2})]Cl$ in methanol-$d_{4}$ solution was determined using the EXSY method and found to be 0.06 s$^{-1}$ at 268 K. This value increases to 1.04 s$^{-1}$ upon warming at 298 K, and 2.1 s$^{-1}$ at 308 K. Our associated SABRE measurements reveal that the corresponding $^1$H NMR signal gains change from 600-fold, through 4530-fold to 3550-fold at the 308 K setting. Hence, it appears that a rate closer to 1.04 s$^{-1}$ is optimal for $^1$H transfer into pyridine using this catalyst. Our experiments also reveal that there is a 30% growth in efficiency of $^{15}$N polarization for pyridine on moving from 268 to 298 K, and a further 22% improvement on moving to 308 from 298 K. Consequently, we can confirm that the two different nuclei are best served with different rates of ligand exchange.

Hyperpolarization of the $^{15}$N NMR Signal of Acetonitrile. In order to develop this method further, acetonitrile was tested at a similar 35 mM concentration in conjunction with the SABRE catalyst $[Ir(H)_{2}((h_{22}-1)(NCMe)_{3})Cl].$ This catalyst also relies on magnetic inequivalence to break the symmetry of the hydride ligands and it yields a $^1$H NMR signal gain of just 83-fold per methyl proton in the unbound acetonitrile present in the solution after transfer at 298 K from a 70 G field. The SABRE-derived $^{15}$N NMR signal gain for CH$_3$CN was found to be far more substantial, at 41,800 ± 6000-fold (14% polarization) after transfer from an approximate −1 mG field.

Acetonitrile hyperpolarization was then studied in conjunction with 3.6 equivalents of the coligand $d_{6}$-benzylamine relative to 5.2 mM iridium concentration. Both $[Ir(H)_{2}(dp-BnND_{2})_{2}(NCMe)((h_{22}-1))Cl]$ and $[Ir(H)_{2}(dp-BnND_{2})((h_{22}-1))]Cl$ form in these experiments, in a 2:1 ratio. They both possess chemically distinct hydride ligands. The resulting $^1$H NMR response after SABRE showed an improved $^1$H NMR signal gain of 160-fold per proton for CH$_3$CN while its $^{15}$N polarization level rose to 19% (Figure 1b).

For the corresponding $^2$H labeled precatalyst $[IrCl(d_{22}-1)(COD)]$, the $^1$H NMR signal again improves further to 367-fold per proton in accordance with reduced spin dilution that arises as a consequence of hydride ligand chemical

Figure 1. Polarized $^{15}$N NMR signals of (a) pyridine, (b) acetonitrile, (c, d) pyrazine, and (e) metronidazole. Levels indicted in figure alongside agent. In (d), the series of $^{15}$N NMR signals for pyrazine vary in intensity according to the magnitude of the PTF. (f) Shake time dependence of the $P_{15N}$ level in metronidazole with $[IrCl(d_{22}-4)(COD)]$. (g) $P_{15N}$ level for metronidazole (black) and pyrazine (red) in a 10 mm sample tube as a function of PTF magnitude.
Table 1. Absolute Value of $^1$H (Total Proton) and $^{15}$N NMR (per Site) Signal Enhancement Levels for Pyrazine and Metronidazole at the Specified PTF for Samples with $d_{9}$-Benzylamine as a Coligand

| nucleus (PTF) | $^{1}$H (Total Proton) | $^{15}$N (per Site) |
|--------------|-----------------------|---------------------|
|              | 1 $d_{22}$-1          | 2 $d_{22}$-2        | 3 $d_{22}$-3        | 4 $d_{22}$-4        | error, % ±  
| pyrazine     | 1372 ± 3151           | 2220 ± 6028         | 2533 ± 558          | 556 ± 673          | 4 ±  
|              | $^{15}$N (% of indicated PTF mG) | 37 ± (−19) | 37 ± (−5) | 26 ± (−2) | 31 ± (−4) | 32 ± (−5) | 28 ± (−3) |
| metronidazole-$N_{1}$ | 326 ± 474 | 560 ± 446 | 814 ± 1038 | 676 ± 856 | 5 ±  
|              | $^{15}$N (% of PTF of $d_{22}$-2) | 22 ± 27 | 24 ± 27 | 23 ± 23 | 51 ± 51 | 3 ±  

The rate of pyrazine dissociation from $[\text{Ir}(H)_{2}(pz)]_{2}([d_{9}-\text{BnND}_{2}](h_{22}-2))\text{Cl}$ was determined using the EXSY method to be 0.33 s$^{-1}$ at 268 K when the $^1$H NMR signal gain is 660-fold. This rate increases to 1.8 s$^{-1}$ at 298 K where the $^1$H signal gain is 2200-fold. Our experiments reveal that the 20% growth in the efficiency of $^{15}$N polarization on moving from 268 to 298 K for pyrazine is a consequence of this rate increase, which is faster than that of pyridine loss in the related complex $[\text{Ir}(H)(h_{22}-1)(py)_{2}([d_{9}-\text{BnND}_{2}])\text{Cl}]$. This kinetic difference is consistent with the relative $^{15}$N polarization efficiencies of 44.2% and 18%, respectively.

**Hyperpolarization of the $^{15}$N NMR Signal of Metronidazole.** Biologically significant metronidazole has been well-studied by Chekmenev et al.$^{32-35}$ We conducted control measurements for 5.2 mM methanol-$d_{4}$ solutions of $[\text{IrCl(COD)}(h_{22}-1)]$ and $[\text{IrCl(COD)}(h_{22}-2)]$ with a sevenfold excess of metronidazole relative to iridium and a 3 bar pressure of $p$-$H_{2}$ but failed to see significant polarization in either samples. However, once a 3.6-fold excess of $d_{9}$-benzylamine was added, polarization transfer to proton and $^{15}$N was readily seen with both precursors. For $[\text{IrCl(COD)}(h_{22}-1)]$, the $P_{15}$ value was 22% while for $[\text{IrCl(COD)}(h_{22}-2)]$, it was 24% (transfer at $−2$ mG and 2% $P_{15}$ seen for $d_{9}$-benzylamine itself). When the $^1$H labeled versions of these catalysts, $[\text{IrCl(COD)}(d_{22}-1)]$ or $[\text{IrCl(COD)}(d_{22}-2)]$, were used, these $P_{15}$ values rose to 27%. In all cases, the reaction with $d_{9}$-benzylamine and metronidazole formed $[\text{Ir}(H)_{2}(mtz)_{2}([d_{9}-\text{BnND}_{2}])\text{NHC}]\text{Cl}$ and $[\text{Ir}(H)_{2}([d_{9}-\text{BnND}_{2}])_{2}(mtz)]\text{(NHC)}\text{Cl}$ with the ratio being 1:4:1 for $d_{22}-2$.

Data were now collected on the $d_{22}-2$ system to demonstrate that the PTF value can be used to control which of the two substrates present in solution receives polarization. This serves to illustrate how selectivity can be introduced into the analysis of mixtures if peak overlap is an issue (see the Supporting Information). Furthermore, a catalyst change to $[\text{IrCl(COD)}(d_{22}-4)]$ increased the $N_{1}$ value to 51% for metronidazole with 4% polarization being achieved on $N_{2}$ and 1% on $d_{9}$-benzylamine (Table 1).

The rates of metronidazole dissociation from the resulting complex $[\text{Ir}(H)_{2}(mtz)_{2}([d_{9}-\text{BnND}_{2}])_{2}(d_{22}-4)]\text{Cl}$ were determined in methanol-$d_{4}$ solution at 268, 298, and 308 K by the EXSY method as being 0.80, 2.37, and 5.5 s$^{-1}$, respectively. For the $^1$H signal gain, 298 K proved to be the best, yielding an enhancement of 856-fold. We now see an 80% growth in the efficiency of $^{15}$N polarization on moving from 268 to 298 K, but the $P_{15}$ value falls to just 18% at 308 K. Hence, increasing the ligand exchange rate beyond 2.4 s$^{-1}$ seems detrimental.

**Usage of Higher Proportions of $p$-$H_{2}$ to Improve the NMR Signal Gain.** A series of measurements were then completed on metronidazole using a 10 mm NMR tube to deploy a larger excess of $p$-$H_{2}$ in conjunction with $[\text{IrCl} (\text{COD})(d_{22}-4)]$ and $d_{9}$-benzylamine. A slight increase in the
$^{15}$N polarization level to 54% results alongside a reduction in response variability to 2%. Consequently, as shown in Figure 1g, a $\sim 3.6$ mg PTF can be deduced as being optimal. Similar 10 mm measurements were then made for pyridine with [IrCl(COD)/(h$_{22}$)2], acetonitrile with [IrCl(COD)/(h$_{22}$)2], and pyrazine with [IrCl(COD)/(h$_{22}$)2] in the presence of $d_3$-benzylamine. These studies saw the $P_{\text{N}}$ level for pyridine increase to 48% at 4 bar $p$-H$_2$ pressure. When acetonitrile was examined, a 30.7% $P_{\text{N}}$ level was reached, but for pyrazine it became 59.4% per nitrogen. Further increases in the pyrazine percentage $P_{\text{N}}$ level can be achieved through reagent dilution such that when an initial 5 mM solution of [IrCl(COD)/(h$_{22}$)2] with a 3.6-fold excess of $d_3$-benzylamine and sevenfold excess of pyrazine based on iridium is diluted 10 fold, the $P_{\text{N}}$ value increases to 79%; the S/N ratio in this case is 11.3. In this case, the effect is directly analogous to increasing the volume of $p$-H$_2$ available.

Quantification of Reagent Concentrations at the mM Level through a SABRE-Enhanced $^{15}$N Signal. Once we had ascertained how to achieve these polarization levels, we tested how the magnitude of the pyridine, pyrazine, and metronidazole response varied as a function of substrate concentrations between 2.2 and 70 mM. These solutions were made up by simply diluting a stock solution with an initial concentration of $d_3$-benzylamine and the square of the sample correlation coefficient—$R^2$—confirms linear behavior.

In the second series of studies, we maintained a constant iridium and coligand concentration while changing the pyrazine concentration. A linear change in the $^{15}$N signal intensity was again observed (Figure 3) despite, in this case, observing some changes in the catalyst form. The PTF was optimized for each substrate. The stock solution of the sample ([Ir] = 6.5 mM, substrate $= 70$ mM, and 22.7 mM $d_3$-BnND$_2$) was diluted during these measurements, from 70 mM substrate to 2.2 mM substrate concentration. The straight lines result from linear regression analysis and the square of the sample correlation coefficient—$R^2$—confirms linear behavior.

![Figure 3](https://doi.org/10.1021/acs.jpcb.9b02583)

**Figure 3.** Raw signal intensity resulting from a series of hyperpolarized $^{15}$N NMR spectra of pyrazine as a function of its concentration. The PTF used was $\sim 1.9$ mG. The concentration of the [Ir]-precatalyst ([IrCl(COD)/(h$_{22}$)2]) was kept constant at 6.5 mM. 3.6 equivalents of $d_3$-BnND$_2$ were added relative to the metal. Subsequently, the concentration of added pyrazine was varied from 8.2 to 70 mM. Straight line behavior results thereby confirmed that the absolute concentration of pyrazine can be estimated from such data.

SABRE—$^{15}$N catalyst is the type B complex. We are currently exploring this behavior in more detail. These data, therefore, confirm that substrate detection and quantification is feasible via a $^{15}$N SABRE signal (see the Supporting Information).

**CONCLUSIONS**

We have described here how the addition of the coligand $d_3$-benzylamine to a precatalyst based on [IrCl(NHC)(COD)] under $p$-H$_2$ results in very high levels of $^{15}$N polarization in a range of substrates. The high field measurements were made in conjunction with the simple shake and drop approach, and it takes approximately 17 s to complete a measurement. In the case of the substrates pyridine and acetonitrile, [IrCl(h$_{22}$-1)(COD)] led to $P_{\text{N}}$ values of 48 and 30.9%, respectively, after being transferred from an appropriate mG field. In contrast, a 59.4 $P_{\text{N}}$ value for pyrazine was achieved using the precatalyst [IrCl(h$_{22}$-2)(COD)]. These reactions involve the formation of a range of SABRE catalysts of the form [Ir(H)$_2$(sub)$_3$(d$_3$-BnND$_2$)(NHC)]Cl and [Ir(H)$_2$(sub)$_3$(d$_3$-BnND$_2$)(NHC)]Cl, which are in equilibrium.

Previous studies have established that using deuterated NHC ligands ($d_{22}$-1 and $d_{22}$-2) improve SABRE hyperpolarization transfer efficiency into methyl nicotinate. This improvement is based on an extension of the hydride ligand relaxation times. Studies here confirm that higher $P_{\text{N}}$ values result in all cases in support of this benefit. However, deuteration is not beneficial for the $^{15}$N transfer in pyridine, pyrazine, and acetonitrile. Barskii’s observations that in microtesla transfer fields, scaler relaxation of the second kind associated with the quadrupolar $^{14}$N—$^{13}$C interaction limits the level of $^{13}$C polarization under SABRE offer a route to explain this view.

For metironidazole, however, an improved value of 54% on N$_1$ results with $d_3$-benzylamine and [IrCl(COD)/(d$_{34}$-4)], compared to that seen with precatalyst [IrCl(COD)/(h$_{22}$-4)]. Hence, $^3$H labeling of the catalyst can also be of significant benefit to $P_{\text{N}}$.

The rates of ligand exchange were also assessed alongside the collection of variable temperature SABRE data. It was found that the rate of optimum ligand exchange could slower than that found for $^1$H transfer, despite the larger $^1$H—$^{15}$N
transfer coupling. We are currently exploring this behavior in more detail.

Data were also presented that was collected from larger 10 mm NMR tubes using a 4 bar pressure of $p$-$\text{H}_2$. This acted to increase the relative excess of the hyperpolarization fuel $p$-$\text{H}_2$ relative to the substrate and proved to result in greatly improved response reproducibility. Consequently, results demonstrated that a PTF precision of $\pm 0.2$ mG is needed for optimal $^{15}\text{N}$ transfer. In addition, $\sim 50\%$ $^{15}\text{N}$ polarization levels could now be achieved in pyrazine, pyridine, or metronidazole, which makes them all highly detectable even at low concentrations.

In order to demonstrate an analytical use for these $^{15}\text{N}$ signals, results were presented to demonstrate that the magnitude of the resulting NMR response scales linearly with concentration over the range 2.2$−70$ mM. This means that such SABRE-derived data can be used to quantify their amount in the solution when set against a suitable reference trace. Tessari have completed a growing range of studies, which demonstrate that $^{1}\text{H}$ detection levels can be linked to both speciation and quantity, while we have described how $^{13}\text{C}$ signals in glucose can be linked to amount. These studies employed a methylated triazol coligand to simplify the exchange kinetics in order to produce the necessary linear response. We were unable to benchmark our data with that of the triazol coligand as it is not commercially available. We did, however, test $d_6$-DMSO, which is finding widespread use as a coligand for the sensitization of weakly binding substrates as an alternative. As detailed in the Supporting Information, the corresponding SABRE performance was degraded.

It is therefore clear that SABRE offers a simple and yet efficient route to analyte quantification by $^{15}\text{N}$ NMR spectroscopy. Not surprisingly, we predict these results will, therefore, be of benefit if you wish to use $^{15}\text{N}$ NMR as a characterization tool, or simply to quantify precise, and yet low, levels of nitrogen-containing drugs that are present in solution or to collect $^{15}\text{N}$-MRI data.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.0c02583.

Experimental details, NMR data, and hyperpolarization details (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

Simon B. Duckett — Department of Chemistry, University of York, York YO10 5DD, U.K.; orcid.org/0000-0002-9788-6615; Email: simon.duckett@york.ac.uk

**Authors**

Marianna Fekete — Department of Chemistry, University of York, York YO10 5DD, U.K.

Fadi Ahwal — Department of Chemistry, University of York, York YO10 5DD, U.K.

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jpcb.0c02583

**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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The authors declare no competing financial interest.

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