Hyperpolarization

Hyperpolarizing Concentrated Metronidazole $^{15}$NO$_2$ Group over Six Chemical Bonds with More than 15 % Polarization and a 20 Minute Lifetime

Roman V. Shcheperin$^{[a]}$, Jonathan R. Birchall$^{[b]}$, Nikita V. Chukanov$^{[c, d]}$, Kirill V. Koptunov$^{[c, d]}$, Igor V. Koptyug$^{[c, d]}$, Thomas Theis$^{[e]}$, Warren S. Warren$^{[f]}$, Jurij G. Gelovani$^{[b]}$, Boyd M. Goodson$^{[g]}$, Sepideh Shokouhi$^{[a]}$, Matthew S. Rosen$^{[h]}$, Yi-Fen Yen$^{[h]}$, Wellington Pham$^{[i]}$, and Eduard Y. Chekmenev$^{[i, j]}$

Abstract: The NMR hyperpolarization of uniformly $^{15}$N-labeled [($^{15}$N)$_2$]metronidazole is demonstrated by using SABRE-SHEATH. In this antibiotic, the $^{15}$NO$_2$ group is hyperpolarized through spin relays created by $^{15}$N spins in [($^{15}$N)$_2$]metronidazole, and the polarization is transferred from parahydrogen-derived hydrides over six chemical bonds. In less than a minute of parahydrogen bubbling at approximately 0.4 μT, a high level of nuclear spin polarization ($P_{15N}$) of around 16% is achieved on all three $^{15}$N sites. This product of $^{15}$N polarization and concentration of $^{15}$N spins is around six-fold better than any previous value determined for $^{15}$N SABRE-derived hyperpolarization. At 1.4 T, the hyperpolarized state persists for tens of minutes (relaxation time, $T_1$ ≈ 10 min). A novel synthesis of uniformly $^{15}$N-enriched metronidazole is reported with a yield of 15%. This approach can potentially be used for synthesis of a wide variety of in vivo metabolic probes with potential uses ranging from hypoxia sensing to theranostic imaging.

Introduction

NMR hyperpolarization techniques transiently increase nuclear spin polarization (P) by several orders of magnitude.$^{[1-3]}$ This significant increase in P enables metabolic magnetic resonance spectroscopy (MRS) and MRS imaging (MRSI) after a bolus injection of a hyperpolarized (HP) compound to detect abnormal metabolism in cancer and other diseases.$^{[4-10]}$ The $^{13}$C isotope has been widely employed in a number of biomolecules, most notably in [1-13C]pyruvate,$^{[16,18]}$ which is typically hyperpolarized by dissolution dynamic nuclear polarization (d-DNP)$^{[11,12]}$. However, this approach has a number of shortcomings. First, the lifetime of the HP state (governed by the spin–lattice constant of exponential decay) is relatively short [e.g., compared with those of $^{19}$F and $^{13}$C positron emission tomography (PET) tracers], with the relaxation time ($T_1$) on the order of 1 min.$^{[13]}$ As a result, only a limited number of metabolic pathways are amenable to MRSI, because sufficient levels of nuclear spin polarization must persist until detection.$^{[11,14]}$ Secondly, d-DNP generally requires several tens of minutes (or longer) of hyperpolarization buildup time as well as sophisticated, costly equipment.$^{[15,16]}$

The hyperpolarization of $^{15}$N sites in biomolecules is a viable alternative approach,$^{[17-19]}$ because $^{15}$N sites can generally retain nuclear spin polarization for significantly longer periods of time, with $T_1$ values of more than 10 minutes having been observed in quaternary nitrogen sites in several model compounds.$^{[20,21]}$ However, HP $^{15}$N-labeled choline (along with its derivatives) is the only quaternary amine with biomedical rele-
vance in the context of HP MRS. [22] Unfortunately, the small difference in chemical shift observed for [15N]choline and its metabolites (ca. 0.2 ppm) would make them difficult to distinguish in vivo. [5,22] On the other hand, nitro groups occur in a wide range of drugs approved by the US Food and Drug Administration (FDA), including antibiotics, cancer radio-sensitizing agents, [23] hypoxia sensors, [24] and chemotherapeutics, [25] and can therefore potentially serve as convenient biocompatible moieties to retain the HP state for tens of minutes.

Signal amplification by reversible exchange (SABRE) is a hyperpolarization technique that employs parahydrogen as the source of hyperpolarization. [26–33] In this work we employed the SABRE in the shield enables alignment transfer to heteronuclei (SABRE-SHEA) [34,35] variant of SABRE, which relies on simultaneous chemical exchange between parahydrogen (pH2) and the to-be-hyperpolarized biomolecules (without altering their structures) within the ligand sphere of a catalyst in microtresa (μT) magnetic fields (Figure 1a). SABRE-SHEA has been shown to produce 15N HP states with P values of up to 30% in under 1 minute (although these high polarization levels have previously been obtained at a relatively low concentration of 15N spins). [38–39] Unlike d-DNP, [12] SABRE-SHEA requires relatively simple and inexpensive hardware. [34,35]

Results and Discussion

Although to date 15N hyperpolarization of several biomolecules has been demonstrated by SABRE-SHEA, which opens up a range of potential contrast agents, [38] nearly all previous reports have focused on the hyperpolarization of 15N sites that directly interact with the iridium catalytic center of the Ir-IMes (IMes = 1,3-dimesitylimidazol-2-ylidene) polarization transfer catalyst, which is the catalyst typically used in most SABRE experiments. [39] The direct polarization transfer from pH2-derived hydrides to these 15N sites is efficient (i.e., fast and yielding high values of P15N) because the two-bond 1H–15N spin–spin couplings are relatively strong (e.g., 10–20 Hz) and sufficiently different from each other as to render the exchangeable catalyst binding sites magnetically inequivalent from one another. [34,35]

Spin-relayed polarization transfer

Although in general direct SABRE-SHEA of remote spin I = 1/2 sites over three or more chemical bonds is inefficient, [40] we have recently demonstrated the concept of spin-relayed polarization transfer from SABRE-SHEA-hyperpolarized 15N sites to other 1H and 15N sites within the same molecule. [41–43] In this approach, a network of J-coupled spin I = 1/2 nuclei can transmit polarization several chemical bonds away from the pH2-derived hydrides. [41,42]

In this paper we show that uniformly 15N-labeled [15N3]miconizolone (MNZ-15N) can be efficiently hyperpolarized by the SABRE-SHEA approach to P15N > 16% for each of the three 15N sites by using 87% pH2. Figure 1c shows the spectrum of HP MNZ-15N3, in which all three 15N peaks are dramatically enhanced, with 15N polarization increased by over 300 000-fold compared with thermal 15N polarization (for an NMR spectrum of a thermally polarized signal reference compound at 1.4 T, see Figure 1b). The signal enhancements are field-dependent, because thermal polarization varies linearly with the applied magnetic field. We note that five- and six-bond spin–spin couplings between pH2-derived hydrides and 15N1 and 15N2 sites, respectively, are negligible, and therefore direct polarization transfer is highly inefficient, as described previously. [37] Analysis of the 15N–15N spin–spin couplings in MNZ-15N3 (see Figure S2 in the Supporting Information) revealed J15N–15N values of around 1.65 Hz between the 15N3 and 15N1 sites and around 1.45 Hz between the 15N1 and 15NO2 sites. No spin–spin coupling between the 15NO2 and 15N3 sites was detected. Therefore, we conclude that the 15N3 site is likely hyperpolarized first by SABRE-SHEA from pH2-derived hydrides, the 15N1 site is hyperpolarized next by spin-relayed polarization transfer from the HP 15N3 site, and the 15NO2 site is hyperpolarized last by spin-relayed transfer from the 15N1 site. We note that the 15NO2 site is not significantly spin–spin cou-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** a) Schematic of the simultaneous chemical exchange of pH2 and [15N3]miconizolone (MNZ-15N3) on the Ir-IMes polarization transfer catalyst. [26] Note the dashed red lines indicate the likely path of spin-relayed polarization transfer from pH2-derived hydrides to the 15NO2 group via the 15N3 and 15N1 sites. b) Single-scan 15N NMR signal reference spectrum of thermally polarized neat (no solvent) [15N]pyridine (12.4 μM). c) Single-scan 15N NMR spectrum of HP [15N3]miconizolone in [D6]MeOH. All NMR spectra were recorded at 1.4 T. The inset displays (in red) the path of chemical bonds over which the network of I = 1/2 spin relays is established. The following experimental conditions were employed for SABRE-SHEA hyperpolarization: Room temperature, 6.7 atm overpressure of the medium-walled NMR tube, and a flow rate of 70 sccm (standard cubic centimeters per minute) of pH2 controlled by a mass-flow controller (MFC).
plied to any other nucleus besides $^{15}$N1, further supporting our conclusion.

**$^{15}$N polarization dynamics**

Studies of the polarization field dependence, dynamics, and decay for [15N]metronidazole are summarized in Figure 2. In part because the [15N]NO$_2$ site is effectively isolated from protons in the [15N]metronidazole molecule, we found that it has a significantly longer $T_1$ (9.7 ± 0.1 min) than the [15N]3 (3.1 ± 0.4 min) and [15N]1 (3.8 ± 0.3 min) sites, respectively (Figure 2f). The relayed (versus direct) nature of the polarization transfer mechanism is also supported by the magnetic field dependence (Figure 2a) of $P_{1SN}$ for the three [15N] sites, which reveals that the maxima of $P_{1SN}$ for the [15N]NO$_2$ and [15N]1 sites coincide with that for the [15N]3 site, which is consistent with the notion that the [15N]3 site acts as the local source of polarization. Moreover, the kinetics of [15N] polarization buildup at the optimal magnetic field (ca. 0.4 μT) clearly indicates that, during the buildup process, $P_{1SN} > P_{1SN1} > P_{1SN2}$ (Figure 2b). It is worth noting that the three $P_{1SN}$ values tend towards similar values when the buildup is nearly complete (Figure 2a). On the other hand, during the [15N] polarization decay process in a field of around 0.4 μT, this order of $P_{1SN}$ values is reversed, that is, $P_{1SN1} < P_{1SN} < P_{1SN2}$ (Figure 2e). It is also worth noting that when the magnetic field is below the optimal value (left-hand slope in Figure 2a), the [15N]3 site is hyperpolarized better than the [15N]1 and [15N]NO$_2$ sites. The lower levels of polarization observed below 0.4 μT and the fact that the polarization of [15N]1 is lower than that of [15N]3 are likely explained by the “strong coupling” of protons and heteronuclei in magnetic fields in the range 100 nT–1 μT observed by Kryutin et al., which may lead to more efficient depolarization of the [15N]1 site, which is coupled more efficiently to the proton spin network (see the Supporting information for details) compared with the [15N]3 site. Because [15N] polarization of the [15N]NO$_2$ site is dependent on that of [15N]1 (because the strongest spin–spin coupling of the [15N]NO$_2$ site is with the [15N]1 site), the lower [15N] polarization of the [15N]NO$_2$ site (compared with that of the [15N]3 site) is also expected. We have employed the nonequal HP spin-state condition, that is, $P_{1SN1} > P_{1SN} > P_{1SN2}$ to study the dynamics of polarization evolution in the Earth’s magnetic field (ca. 30 μT), in which pH$_3$ can no longer efficiently hyperpolarize any heteronuclei and therefore the polarization can only decay. Figure 2c shows that $P_{1SN}$ is redistributed relatively quickly, on the timescale of seconds, to the [15N]1 and [15N]NO$_2$ sites (it is worth noting that $P_{1SN1}$ and $P_{1SN2}$ grow for the first approx. 15 s, whereas $P_{1SN}$ decreases). Although this redistribution of polarization occurs in the Earth’s magnetic field, which is approximately 30 μT (as measured in our laboratory) and significantly exceeds 0.4 μT, this observation provides additional support for the spin-relayed mechanism of SABRE-SHEATH polarization transfer in [15N]metronidazole in microtesla magnetic fields.

**[15N] polarization levels**

It should be noted that over the MNZ concentration range studied (23–41 mM), $P_{1SN}$ was 15.6 ± 0.7% on all three [15N] sites, as detected by 1.4 T NMR spectroscopy (Figure 2d; notably, the ratio of MNZ and the catalyst was maintained constant at 20:1). This polarization level is lower (by around two-fold) than the best $P_{1SN}$ values (ca. 34%) obtained for metronidazole with a natural abundance of [15N] (< 0.3%) under similar conditions (Figure 3a). This observation can likely be explained by two effects: First, the labeled compound produces an active complex with two [15N] sites in the equatorial plane and the polarization is transferred in the AA′BB′ four-spin system, whereas in the case of metronidazole with a natural abundance of [15N] polarizer transfer from the pH$_3$–derived hydrides to [15N] occurs in an AA′BB′ three-spin system. It has been shown previously that polarization transfer in such three-spin systems may be more efficient than that in four-spin systems. The second contributing factor is access to fresh pH$_3$, which can be
more limited under conditions of high $^{15}\text{N}$ density (i.e., local pH, can be depleted more quickly if there are more $^{15}\text{N}$ sites present);$^{245}$ the labeled sample studied here contains more than 600-fold more $^{15}\text{N}$ sites than the natural abundance compound studied previously.$^{246}$

$^{15}\text{N}$ polarization payload and $^{15}\text{N}$ magnetization

Comparison of the polarization payload (defined as the product of the concentration of $^{15}\text{N}$ spins, $^{15}\text{N}$ polarization, and the volume of the HP contrast agent) is more relevant in the context of biomedical applications, because in vivo experiments greatly benefit from a higher HP payload to enhance the signal-to-noise ratio and improve spatial and temporal resolution.$^{47}$ Because SABRE is a highly scalable hyperpolarization technique (i.e., varying the volume of HP liquids is relatively straightforward), it is important to highlight the $^{15}\text{N}$ magnetization achieved, that is, the product of $^{15}\text{N}$ spin concentration and $^{15}\text{N}$ polarization. The $^{15}\text{N}$ polarization levels of several biomolecules hyperpolarized by SABRE are compared in Figure 3a, and the $^{15}\text{N}$ magnetization determined for the same five compounds hyperpolarized by SABRE are presented in Figure 3b. The $^{15}\text{N}$ magnetization for $[^{15}\text{N}]{\text{MNZ}}$ is improved by around 25-fold or more compared with the previously studied molecules (i.e., compared with MNZ(n.a.) and MNZ$^{15}\text{N}_p^{13}\text{C}_2$).$^{236}$ Moreover, the $^{15}\text{N}$ magnetization calculated here for $[^{15}\text{N}]{\text{MNZ}}$ is approximately six-fold greater than that of mono-$^{15}\text{N}$-labeled $[^{15}\text{N}]{\text{nicotinamide}}$.$^{24}$

**Synthesis of $^{15}\text{N}$-enriched metronidazole**

Importantly, the above NMR results were enabled by $^{15}\text{N}$ enrichment of all three nitrogen sites of the substrate, achieved by a novel but straightforward spin-labeling synthetic procedure (Scheme 1). First, 2-methyl$[^{15}\text{N}]{\text{imidazole}}$ was prepared by using a modification of the procedure previously developed for the synthesis of $[^{15}\text{N}]{\text{imidazole}}$, in which inexpensive $^{15}\text{NH}_4\text{Cl}$ was employed as the $^{15}\text{N}$ source. In the second step, the $^{15}\text{NO}_2$ group was added by a nitration reaction using Na$^{15}\text{NO}_3$ as the second inexpensive $^{15}\text{N}$ source. Ethylene oxide was employed in the last step to introduce the hydroxyethyl moiety. The overall yield over three steps (Scheme 1) was around 15% (see Section 9 of the Supporting Information). This synthetic approach for introducing $^{15}\text{N}$ spin into the nitromidazole moiety can be potentially applied to a wide range of nitromidazole-based drugs, for example, azomycin, benzimidazole, secnidazole, ornidazole, nimorazole, TH-4000, and evofosfamide, used as hypoxia sensors, radio-sensitizing therapeutics, and theranostic imaging agents.$^{24}$

**Biomedical outlook**

Metronidazole is an FDA-approved antibiotic that can be safely administered orally and intravenously in large (multigram) doses.$^{299}$ Moreover, we have recently reported the preparation of pure (i.e., catalyst-free) HP metronidazole solutions$^{30}$ as well as heterogeneous $^{15}\text{N}$ SABRE-SHEATH$^{36}$ and $^{15}\text{N}$ SABRE-SHEATH HP metronidazole in aqueous media,$^{25}$ thereby potentially enabling the preparation of biologically compatible HP MNZ$^{15}\text{N}_1$ injectable formulation(s). We envision this contrast agent to have potential in hypoxia sensing (the focus of our future studies) in a manner similar to that of $[^{15}\text{F}]$ fluoroisonidazole (FMISO) and other nitro-containing radiotracers employed in positron emission tomography (PET)$^{32,53}$ The nitromidazole moiety is chemically reduced in the hypoxic environment by upregulated nitroreductases (i.e., in tumors or ischemic tissues),$^{24,54}$ which will likely result in large (tens of ppm) changes in the $^{15}\text{N}$ chemical shifts of the three $^{15}\text{N}$ sites. Although future studies are required to investigate the utility of HP MNZ$^{15}\text{N}_1$, for hypoxia sensing and other applications, the results presented here ($P_{1\text{NH}} \approx 16\%$ for $\geq 98\%$ $^{15}\text{N}$ enrichment, $T_1 \approx 10\,$ms, fast polarization, and straightforward isotopic enrichment) bode well for such envisioned cellular and in vivo experiments. Moreover, an in vivo study of $^{15}\text{N}$ relaxation with HP choline revealed an approximate 1.5-fold decrease in $T_1$ (from $172 \pm 16$ to $126 \pm 15\,$s) compared with that in vitro at 9.4 $T_1$.$^{22}$ The effective reduction of the $^{15}\text{N}$ $T_1$
value for HP choline in vivo was attributed to choline uptake and metabolism versus depolarization,[24] which indicates that the $^{15}$N $T_1$ value is reduced marginally in vivo compared with in vitro, largely because of the very low gyromagnetic ratio of $^{15}$N spin (a tenth of that of proton spins). Therefore, we anticipate a relatively small (30% or less) reduction in the $T_1$ values presented here for $[^{15}N]_{nitroimida}$zole in future in vivo studies.

Although the level of $^{15}$N polarization obtained here is lower than the $^{13}$C polarization obtained by using d-DNP hyperpolarizers ($^{13}$C polarization, ca. 40%[67]), the amount of actual polarization at the time of MR image acquisition will likely exceed that of $^{13}$C, because the $^{15}$NO$_2$ group of $[^{15}N]_{nitroimida}$zole has a significantly longer $T_1$ (by approximately an order of magnitude) and therefore will enjoy disproportionately lower depolarization during injection, circulation, and imaging. For example, the current time for injection of HP contrast agents in humans is around 55 s,[67] which causes an approximate three-fold decay of the $^{13}$C polarization level, whereas the $^{15}$NO$_2$ polarization of HP $[^{15}N]_{ nitroimida}$zole will decay only by roughly 1.1-fold in that time, that is, it will experience a negligible loss.

However, the low gyromagnetic ratio of $^{15}$N spins, which in part endows the long $T_1$ values demonstrated here, is a double-edged sword, because the NMR detection of low-$\gamma$ nuclei is generally significantly less sensitive even at the same nominal polarization level,[55,56] The detected signal is directly proportional to the nuclear magnetic moment (which varies linearly with gyromagnetic ratio), and the NMR signal is also directly proportional to the resonance frequency (which also varies linearly with gyromagnetic ratio). Therefore, it follows that proton detection is around 100 times more sensitive than $^{15}$N detection, $\gamma_{^{15}N}/\gamma_{^1H} \approx 100$, and $^{13}$C detection is approximately six times more sensitive than $^{15}$N detection, $\gamma_{^{13}C}/\gamma_{^{15}N} \approx 6$. Moreover, in the case of MRI and MRSI applications, spatial imaging encoding of low-$\gamma$ nuclei carries two additional disadvantages. First, the gradient strength required to achieve the same spatial resolution is directly proportional to the gyromagnetic ratio (and therefore $^{15}$N imaging requires ca. 2.5-fold greater gradient strength than $^{13}$C imaging and ca. 10-fold greater gradient strength than $^1$H imaging). Secondly, the gradient ramp rate is also directly proportional to the gyromagnetic ratio, thereby making gradient rise/fall durations of $^{15}$N imaging around 2.5-fold slower than those of $^{13}$C imaging and around 10-fold slower than those of $^1$H imaging at a fixed gradient strength (i.e., of maximum gradient power).

To summarize, $^{15}$N MRI and MRSI face significant fundamental challenges. However, we envision proton detection of $^{15}$N HP contrast agents in general and HP $[^{15}N]_{nitroimida}$zole in particular, similarly to the concept of indirect proton detection recently introduced for $^{13}$C HP contrast agents,[17,57–59] which means that the detection sensitivity of $^{15}$N HP agents will be similar to that of $^{13}$C HP agents when indirect ($^1$H) proton detection is employed. $[^{15}N]_{nitroimida}$zole has protons in its molecular structure that are weakly (2–9 Hz) coupled to the $^{15}$N sites through spin–spin interactions. As a result, it is possible to use these spin–spin couplings for hyperpolarization transfer from $^{15}$N to $^1$H[57,80,61] by using conventional polarization transfer techniques such as insensitive nuclei enhanced by polarization transfer (INEPT).[62] This hyperpolarization transfer approach leads to a large increase in sensitivity of approximately 100-fold, as described above and elsewhere,[58,57,63] and the speed of imaging readout in the case of MRI and MRSI applications is also enhanced, as described above.

In this work $[^{15}N]_{nitroimida}$zole was hyperpolarized in alcoholic solution. Although SABRE hyperpolarization in aqueous media has been demonstrated,[51,64–67] so far, the SABRE polarization levels in aqueous solutions are more than 10-fold lower than those in alcoholic solutions,[68] for example, in methanol here, largely due to significantly lower $H_2$ solubility in water.[69,70] Two potential work-arounds that can be envisioned in the context of biomedical applications are 1) the use of high-pressure hyperpolarizers[71] to increase the concentration of $\text{PH}_2$ in aqueous solutions or 2) the dilution of alcoholic solutions (e.g., based on ethanol) with a biocompatible buffer followed by in vivo injection, because the $^{15}$N $T_1$ value is nearly 10 minutes in vitro and therefore the slow injection of larger volumes over several minutes may be feasible in the context of future in-human applications.

Conclusion

We have demonstrated in this report the $^{15}$N SABRE-SHEATH hyperpolarization of $[^{15}N]_{nitroimida}$zole. Efficient hyperpolarization ($P_{15N} \approx 16\%$) of the $^{15}$NO$_2$ group is accomplished by spin relays created by the network of $^{15}$N spins in the molecular structure of $[^{15}N]_{nitroimida}$zole. In this process, the parahydrogen-derived hyperpolarization of iridium hydrides is transferred over up to six chemical bonds. The high level of nuclear spin polarization $P_{15N}$ of around 16% is achieved in about 1 minute on all three $^{15}$N sites of $[^{15}N]_{nitroimida}$zole at concentrations up to approximately 41 mM. The HP state of the $^{15}$NO$_2$ group has a long lifetime in vitro with an exponential decay constant $T_1$ of 9.7 ± 1.0 minutes at the clinically relevant magnetic field of 1.4 T. Such a long lifetime of the HP state is possible as a result of the low gyromagnetic ratio of the $^{15}$N nucleus, and in part because the $^{15}$N spin of the $^{15}$NO$_2$ group in metronidazole has no detectable spin–spin couplings with any of the protons in the molecule. We have also reported herein on the novel robust synthesis of $[^{15}N]_{nitroimida}$zole (see the Experimental Section) and $[^{15}N]_{nitroimida}$zole compounds in a yield of 15% over three steps. This synthetic approach employs inexpensive sources of $^{15}$N label ($^{15}$NH$_4$Cl and Na$^{15}$NO$_3$) and can be potentially tailored for the $^{15}$N enrichment of other nitroimidazole derivatives, for example, FDA-approved nimorazole.

Experimental Section

General: The details of the experimental setup (Scheme 2) employed for the studies reported herein have been described previously.[62,66] All synthetic and spectral characterization details relating to the preparation of $[^{15}N]_{nitroimida}$zole and $[^{15}N]_{nitroimida}$zole are provided in the Supporting Information.

SABRE-SHEATH hyperpolarization: The solutions for SABRE hyperpolarization were prepared with a 1:20 catalyst/substrate ratio. A
known mass of \(^{15}\text{N}\)metronidazole was placed in a plastic Eppendorf tube and a 1 mm solution of the Ir-Mes catalyst in \([D_2]_2\text{MeOH}\) (0.6 mL) was added to yield a 20 mm concentration of \([^{15}\text{N}]\text{metronidazole}\). The mixture was flushed with ultrapure argon gas immediately after preparation. Each solution prepared in this manner was used for SABRE activation and SABRE-SHEATH hyperpolarization experiments typically within 2 h of its initial formulation.

The Ir-Mes catalyst precursor\(^{[20]}\) was synthesized according to our previously published protocol\(^{[21]}\). The catalyst was activated by \(\text{PH}_2\) bubbling for approximately 1 h to achieve maximum performance.

Each sample was placed in a medium-walled 5 mm NMR tube (0.6 mL aliquot) and connected to the SABRE hyperpolarization setup, which has been described in detail previously\(^{[24,35,42]}\). NMR spectra were recorded by using a 1.4 T benchtop NMR spectrometer (NMR Pro 60, Nanalys, Canada) with a flow rate of 50 sccm (standard cubic centimeters per minute; the flow rate was controlled by a mass flow controller) and a 6.4 atm overpressure of \(\text{PH}_2\) gas in the NMR tube at room temperature (ca. 20–22 °C; Scheme 2). The thermally polarized reference spectrum of neat \([^{15}\text{N}]\text{pyridine}\) was recorded by \(^{15}\text{N}\)NMR spectroscopy with proton decoupling, whereas all \(^{15}\text{N}\)NMR spectra of HP \([^{15}\text{N}]\text{metronidazole}\) were recorded under conditions without proton decoupling.

The duration of \(\text{PH}_2\) bubbling in the shield was about 1 min (unless otherwise noted), and the durations of \(\tau\text{(decay)}\) (< 1 s), \(\tau\text{(evolution)}\) (ca. 1–2 s), and sample depolarization in the 1.4 T NMR spectrometer, \(\tau\text{(decay)}\) (ca. 1–2 s) were kept to a minimum to reduce \(^{15}\text{N}\) polarization losses (Figure 1c). Varying the durations of \(\text{PH}_2\) bubbling \(\tau\text{(build-up)}\), the polarization decay in the shield after cessation of \(\text{PH}_2\) bubbling \(\tau\text{(decay)}\), the evolution of polarization in the Earth’s magnetic field \(\tau\text{(evolution)}\), and sample depolarization in the 1.4 T NMR spectrometer \(\tau\text{(decay)}\) shown in Scheme 2 allowed for systematic mapping of the SABRE-SHEATH process and the spin dynamics of \(^{15}\text{N}\) polarization shown in Figure 2b,c,e,f, respectively. Systematic mapping of the static magnetic field inside the shield (using a variable resistor bank and dc power supply [GW INSTEK, GPR5 series], Scheme 2) is shown in Figure 2a.

Some samples were heated to approximately 55 °C to evaporate \([D_2]_2\text{MeOH}\) to increase the concentration of \([^{15}\text{N}]\text{metronidazole}\) for the experiments summarized in Figure 2d.

**Preparation of parahydrogen:** Parahydrogen (\(\text{PH}_2\)) enrichment was performed by using a home-made parahydrogen generator equipped with a SunPower cryocooler. The \(\text{PH}_2\) fraction was determined by \(^1\text{H}\) NMR spectroscopy as discussed previously\(^{[40]}\). Briefly, the signal of orthohydrogen in “normal” hydrogen gas (consisting of 75% ortho and 25% para states) was employed as a signal reference. The orthohydrogen fraction/percentage signal from the \(\text{PH}_2\)-enriched mixture was determined next (by using a signal reference obtained from “normal” hydrogen gas) and the fraction/percentage of \(\text{PH}_2\) was determined as the difference between 100% and the percentage of detected orthohydrogen. The NMR spectra of normal (25 % \(\text{PH}_2\)) and \(\text{PH}_2\)-enriched mixtures are shown in Figure S1 in the Supporting Information. The NMR spectra of several samples were recorded (for reproducibility) and a variability of less than 2% was achieved.

**Acknowledgements**

This work was supported by the NSF under grants CHE-1836308 (E.Y.C.) and CHE-1416432 (B.M.G.), NIH R21CA220537 (E.Y.C., B.M.G.), DOD CDMPR BRP W81XWH-12-1-0159/BC112431 (E.Y.C., B.M.G., W.P), DOD PRMRP awards W81XWH-15-1-0271 (E.Y.C.) and W81XWH-15-1-0272 (B.M.G.), and RO1CA16700 (W.P). N.V.C. and K.V.K. thank the Russian Science Foundation (Grant #17-73-20030) for their support in the synthesis of \(^{15}\text{N}\)-labeled compounds. I.V.K. thanks the Russian Ministry of Science and Higher Education (Project AAAA-A16-116121510087-5).

**Conflict of interest**

The authors declare no conflict of interest.

**Keywords:** hyperpolarization — isotopic labeling — nitrogen heterocycles — NMR spectroscopy — SABRE — spin relays

---


