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¹⁹F Hyperpolarization of ¹⁵N-3-¹⁹F-Pyridine Via Signal Amplification by Reversible Exchange

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Abstract

We report synthesis of ¹⁵N-3-¹⁹F-pyridine via Zincke salt formation with the overall 35% yield and 84% ¹⁵N isotopic purity. Hyperpolarization studies of Signal Amplification by Reversible Exchange (SABRE) and SABRE in SHield Enables Alignment Transfer to Heteronuclei (SABRE-SHEATH) were performed to investigate the mechanism of polarization transfer from parahydrogen-derived hydride protons to ¹⁹F nucleus in milli-Tesla and micro-Tesla magnetic field regimes in ¹⁵N-3-¹⁹F-pyridine and ¹⁴N-3-¹⁹F-pyridine. We found the mismatch between ¹⁵N and ¹⁹F magnetic field hyperpolarization profiles in the micro-Tesla regime indicating that the spontaneous hyperpolarization process likely happens directly from parahydrogen-derived hydride protons to ¹⁹F nucleus without spin-relaying via ¹⁵N site. In case of SABRE magnetic field regime (milli-Tesla magnetic field range), we found that magnetic field profiles for ¹H and ¹⁹F hyperpolarization are very similar, and ¹⁹F polarization levels are significantly lower than ¹H SABRE polarization levels and lower than ¹⁹F SABRE-SHEATH (i.e. obtained at micro-Tesla magnetic field) polarization levels. Our findings support the hypothesis that in milli-Tesla magnetic field regime, the process of ¹⁹F nuclei hyperpolarization is relayed via protons of substrate, and therefore is very inefficient. These findings are important in the context of improvement of the hyperpolarization hardware and rational design of the hyperpolarized molecular probes.

Graphical Abstract

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INTRODUCTION

Nuclear spin polarization P can be enhanced by several orders of magnitude by NMR hyperpolarization techniques.^{1–10} This significant P increase results in the corresponding gains of NMR and MRI signals, which is very attractive for a wide range of bioimaging applications.^{6, 11–19}

One of the most recent hyperpolarization techniques Signal Amplification by Reversible Exchange (SABRE) was pioneered by Duckett and co-workers in 2009.^{20–24} It is relatively inexpensive and can produce hyperpolarized compounds in a minute or less^{19–23, 25–26} for imaging applications.^{27–33} SABRE technique employs simultaneous chemical exchange processes of parahydrogen ($p\text{-H}_2$) and an appropriate to-be-hyperpolarized substrate on an Iridium metal complex, Scheme 1a. The $p\text{-H}_2$ is the source of hyperpolarization in SABRE technique.^{20–21} While many flavors of SABRE have been recently developed,^{34–35} the approach relying on spontaneous polarization transfer from $p\text{-H}_2$ -derived hydride protons (Scheme 1a) has been demonstrated the most efficient to date. In this approach, the static magnetic field (B_{evo}) is matched to the combination of spin-spin couplings between $p\text{-H}_2$ and the nucleus of interest in the to-be-hyperpolarized substrate.^{20–21, 23, 28, 36–38} In case of protons, i.e. homonuclear ($^1\text{H}_{\text{hydride}} \rightarrow ^1\text{H}_{\text{substrate}}$) polarization transfer, B_{evo} in the range of a few milli-Tesla can lead to P_{H} of up to 50%.³³ In heteronuclear case (e.g. $^1\text{H}_{\text{hydride}} \rightarrow ^{15}\text{N}_{\text{substrate}}$), the optimum B_{evo} is significantly lower than for protons and in micro-Tesla range $P_{^{15}\text{N}}$ can reach more than 40%^{25, 39} and for $P_{^{13}\text{C}}$ can reach up to 15%⁴⁰, and high polarizations for other nuclei have been demonstrated.^{27, 41–43} Matching static magnetic field B_{evo} also enables spin-relayed polarization transfer within substrate molecule.^{20–21, 40, 44–46} For example, homonuclear ($^1\text{H}_{\text{substrate}} \rightarrow ^1\text{H}_{\text{substrate}}$ and $^{15}\text{N}_{\text{substrate}} \rightarrow ^{15}\text{N}_{\text{substrate}}$), and heteronuclear ($^{15}\text{N}_{\text{substrate}} \rightarrow ^{13}\text{C}_{\text{substrate}}$) polarization transfer have been demonstrated in the milli-Tesla and micro-Tesla regimes respectively.^{20–21, 40, 44–45} This intramolecular polarization transfer is accomplished via spin relays of the spin-spin coupled spin-1/2 nuclei. As a result, long-range nuclear sites can be hyperpolarized in the substrate molecule even if the direct spin-spin couplings between hydride protons and the nucleus of interest are too weak.⁴⁴

In the work presented here, we study the effect of spin-relayed polarization transfer in ^{15}N -3- ^{19}F -pyridine by comparison with compound at natural abundance of ^{15}N isotope ($\sim 0.3\%$), i.e. ^{14}N -3- ^{19}F -pyridine (Scheme 1b, 1c, 1d, 1e). ^{19}F is present in approximately 20% of FDA-approved drugs,⁴⁷ and therefore, efficient SABRE hyperpolarization of ^{19}F sites would be of interest for bioimaging and theranostic applications. Moreover, ^{19}F is spin-1/2 nucleus with nearly 100% natural abundance, i.e. additional isotopic labeling is not required. Furthermore, ^{19}F NMR detection sensitivity is similar to that of proton nuclei, and

the ^{19}F resonance frequency is similar to that of ^1H : so, in principle, ^{19}F imaging can be accomplished on conventional proton MRI scanners with specialized RF coil.^{48–50}

METHODS

General Considerations.

Unless otherwise stated, all reagents and solvents were purchased from commercial suppliers and used without further purification. ^1H NMR spectra were recorded on a Bruker 400 UltraShield® (9.4 T, Avance III) spectrometer in deuterated solvent and chemical shifts were reported in ppm (δ) relative to tetramethylsilane with the solvent resonance employed as the internal standard (CD_3OD , δ 3.31 ppm). ^{13}C NMR spectra were recorded on a Bruker 400 UltraShield® (9.4 T, Avance III) spectrometer (at 101 MHz) in deuterated solvent with ^1H decoupling, and ^{13}C chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (CD_3OD , δ 49.0 ppm). ^{19}F and ^{15}N NMR spectra were recorded on Bruker 9.4 T and 7.1 T NMR spectrometers. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz) and integration.

High-resolution mass spectrometry (HR-MS) was performed by direct liquid infusion using an Orbitrap mass spectrometer (Thermo-Finnigan, San Jose, CA) equipped an Ion-Max source housing and an atmospheric pressure chemical ionization (APCI) probe in positive ion mode at a resolving power of 60,000 (at m/z 400).

^{15}N -3- ^{19}F -pyridine (84% ^{15}N enrichment) synthesis.

3- ^{19}F -pyridine (43 g, 443 mmol) and 2,4-dinitrochlorobenzene (65 g, 150 mmol) were placed in a pre-weighted oven-dried round bottom flask (1 L) supplied with a magnetic stir bar. The solids were dissolved and left to stir at 60 °C for 3 days under the inert atmosphere (Argon). After that the flask was placed on a rotational evaporator to remove excess of unreacted 3- ^{19}F -pyridine. Then anhydrous Et_2O (200 mL) and dry acetone (60 mL) were added to the reaction mixture. White amorphous residue was formed on the bottom of the flask. Solvent was decanted carefully, leaving the residue on the bottom of the flask. Another portion of anhydrous Et_2O (100 mL) and dry acetone (30 mL) was added rapidly, and the flask was rotated several times and left for several minutes before solvent was carefully decanted from the residue. Washing of residue was repeated in the following manner: 2×30 mL dry acetone, and 4×100 mL dry THF. The flask was placed on a rotational evaporator to remove the remaining traces of THF from 3- ^{19}F -pyridine-Zincke salt (ZS). 27.4 g (91 mmol) of ZS was obtained (60%). Then anhydrous methanol (150 mL) was added, the flask was flashed with Argon and put aside. To an oven-dried round bottom flask (5 L) supplied with a magnetic stir bar, ammonium- ^{15}N chloride (16.6 g, 305 mmol) and anhydrous methanol (3.5 L) were added. The solution was cooled to 0 °C and sodium methoxide methanolic solution (25 wt%, 275 mmol, 63 mL) was added dropwise. After that methanolic solution of 3- ^{19}F -pyridine-Zincke salt (described above) was added dropwise to the flask in 3 h. The resulting solution was left to stir under the inert atmosphere (Argon) for 2 days. The supernatant solution was distilled, and 2 M solution of hydrochloric acid (850 mL) in Et_2O was added to distillate (pH ~ 1), and after that the solution was evaporated to dryness under vacuum. The

resulting crude ^{15}N -3- ^{19}F -pyridine hydrochloride was placed in 100 mL flask. After that the excess of solid sodium hydroxide (10 g) was added and mixture was distilled. The excess of CaH_2 was added carefully to the obtained ^{15}N -3- ^{19}F -pyridine. The second distillation over CaH_2 allowed preparing 2.5 g of ^{15}N -3- ^{19}F -pyridine with the overall yield of 35% from ZS. ^{15}N isotopic purity is 84% as confirmed by HRMS, Figure S1. NMR spectra can be found in the Supporting Information (SI): Figures S2–S6.

Characterization of ^{15}N -3- ^{19}F -pyridine.

^1H NMR (400 MHz, CD_3OD): δ 7.47–7.51 (m, 1H), 7.62–7.67 (m, 1H), 8.38–8.42 (m, 1H), 8.45–8.48 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 123.3 (dm, $J = 18.3$ Hz, C-4), 124.9 (m, C-5), 137.1 (d, $J = 24.2$ Hz, C-2), 145.0 (d, $J = 4.2$ Hz, C-6), 159.8 (dm, $J = 254.6$ Hz, C-3); ^{19}F NMR (375 MHz, CD_3OD): δ –125.6; $\text{C}_5\text{H}_4\text{F}^{15}\text{N}$ ($\text{M}+\text{H}^+$): 99.03709; found 99.03695 (–1.41 ppm).

NMR hyperpolarization.

SABRE and SABRE-SHEATH experiments were performed as described recently,^{44–45} and they employed 81–86%-enriched p-H_2 . If not specified, the samples were maintained at room temperature during p-H_2 bubbling. On average, the sample transfer procedure (from p-H_2 cessation to NMR signal detection) took 4–7 seconds for SABRE-SHEATH experiments (in micro-Tesla shield) and 2–4 seconds for SABRE (in the fringe field of NMR spectrometer). All samples were polarized for at least 1 minute to achieve steady-state condition of hyperpolarization.^{27, 45} For example, Figure S7 shows the ^{19}F build-up curve in SABRE-SHEATH regime indication that a steady-state condition is certainly achieved in less than a minute.

Calculation of NMR Signal Enhancement and Nuclear Spin Polarization.

In case of ^1H and ^{19}F , the HP signal intensities were compared directly to intensities of thermally polarized resonances, and the enhancement factor ϵ was computed as the ratio of HP and thermal signal intensities. In case of ^{15}N experiments, the thermally polarized signals had insufficient signal intensities due to low signal-to-noise ratio (SNR). Therefore, external ^{15}N signal reference was employed,⁴⁴ and $\epsilon_{^{15}\text{N}}$ was computed as:

$$\epsilon = \frac{I_{\text{HP}}}{I_{\text{REF}}} \times \frac{C_{\text{REF}}}{C_{\text{HP}}} \times \frac{A_{\text{REF}}}{A_{\text{HP}}} \times \frac{N_{\text{REF}}}{N_{\text{HP}}}$$

where I_{HP} and I_{REF} are NMR peak integrals of HP and thermally polarized states respectively, C_{HP} and C_{REF} are the effective ^{15}N concentrations of HP and thermally polarized samples respectively, A_{REF} and A_{HP} is the cross-section of the NMR tubes containing reference and HP samples respectively: $A_{\text{REF}}/A_{\text{HP}}$ was ~ 1.85 as noted in the previously described setup.^{28, 44} N_{REF} and N_{HP} are the number of NMR equivalent ^{15}N sites per molecule in thermally polarized and HP samples respectively.

Nuclear spin polarization $P_{^1\text{H}}$, $P_{^{19}\text{F}}$ and $P_{^{15}\text{N}}$ were calculated as the product of the corresponding ϵ and the equilibrium nuclear spin polarization of ^1H , ^{19}F and ^{15}N respectively at the field of interest and 298 K. For example, the following values of nuclear

spin polarization were employed in our calculation: $P_{1H} \sim 3.2 \times 10^{-3}\%$, $P_{19F} \sim 3.0 \times 10^{-3}\%$, and $P_{15N} \sim 3.3 \times 10^{-4}\%$.

RESULTS AND DISCUSSION

Synthesis of ^{15}N -3- ^{19}F -pyridine.

Isotopically ^{15}N -labeled compounds are of considerable interest for SABRE hyperpolarization technique, because they increase ^{15}N natural abundance ($\sim 0.4\%$) by nearly 250-fold resulting in corresponding gains in NMR signal. Moreover, ^{15}N sites (compared to proton and ^{13}C sites) typically have much longer relaxation time constants,^{45, 52–55} and therefore can retain their HP state much longer than ^1H and ^{13}C sites – a clear advantage in the context of in vivo applications.⁵⁶ Synthesis of ^{15}N -enriched pyridine and imidazole derivatives is often laborious and expensive.^{57–61} A limited number of methods for the pyridine ring formation exist, especially for those unsubstituted in positions 2 and 6. ^{15}N -labeled pyridine can be synthesized successfully by the reaction of 2-ethoxy-3,4-dihydro-2*H*-pyran with $^{15}\text{NH}_4\text{Cl}$ in the presence of methylene blue (scheme 1, $\text{R} = \text{H}$).⁵⁹ Synthesis of substituted pyridines according this scheme is complicated due to the absence of substituted pyran derivatives. This is the reason why only one substituted ^{15}N -pyridine was synthesized by this approach (Scheme 2, $\text{R} = \text{Me}$) to date.⁶²

An alternative synthetic approach for ^{15}N -enriched pyridine derivatives is reaction of Zincke salts⁶³ with $^{15}\text{NH}_4\text{Cl}$. ^{15}N -nicotinamide was synthesized by this approach, Scheme 3, $\text{R} = \text{CONH}_2$.^{58, 61, 64} Based on the limited previous observations, this approach is the most rational for the synthesis of pyridines substituted in position 2 or 3, while it should be noted, that the major disadvantage of this approach is relatively low percentage of ^{15}N enrichment. Here, ^{15}N -3- ^{19}F -pyridine (Scheme 3, $\text{R} = \text{F}$) was synthesized by a two-step method through the Zincke salt intermediate.

We found that 3- ^{19}F -pyridine-Zincke salt formation proceeds relatively slowly than in the case of unsubstituted pyridine most likely due to the electron-withdrawing effect of fluorine atom. The best yield of a 3- ^{19}F -pyridine-Zincke salt of about 60% was achieved by keeping reaction mixture at 60 °C for 3 days. However, the amount of impurities dramatically increased with temperature, and the presence of impurities was noticeably even at room temperature (RT), but the yield of 3- ^{19}F -pyridine-Zincke salt was only 45% after 20 days of reaction at RT. It should be noted that during the second stage reaction of ammonium chloride with 3- ^{19}F -pyridine-Zincke salt, the ^{15}N enrichment depends on several factors including the reagent concentrations. The best obtained ^{15}N -enrichment of 84% was observed at 0.025 M concentration of 3- ^{19}F -pyridine-Zincke salt. Therefore, all experimental data presented below was obtained using ^{15}N -3- ^{19}F -pyridine with 84% ^{15}N enrichment.

General Considerations of SABRE and SABRE-SHEATH Hyperpolarization.

The reader is reminded that SABRE polarization condition is typically referred to the process of spontaneous polarization transfer from p- H_2 -derived hydride protons to substrate protons due to homonuclear ($^1\text{H}_{\text{hydride}} \rightarrow ^1\text{H}_{\text{substrate}}$) matching static magnetic field (B_{evo})

conditions of a few milli-Tesla. SABRE-SHEATH polarization condition is typically referred to the process of spontaneous polarization transfer from p-H₂-derived hydride protons to substrate heteronuclei due to heteronuclear (e.g. $^1\text{H}_{\text{hydride}} \rightarrow ^{15}\text{N}_{\text{substrate}}$) matching static magnetic field B_{evo} conditions on the order of a micro-Tesla.^{20–21, 23, 28, 36–37}

¹⁹F SABRE and SABRE-SHEATH Hyperpolarization.

SABRE and SABRE-SHEATH experiments were carried out with solutions of ^{15}N -3- ^{19}F -pyridine (Scheme 1c) or natural abundance ^{19}F -pyridine (i.e. with predominant form of ^{14}N -3- ^{19}F -pyridine) (Scheme 1b) and [Ir(IMes)(COD)Cl]^{38, 51, 65} SABRE pre-catalyst (5 mM) in CD₃OD. Parahydrogen pressure was 70 psig; p-H₂ gas flow rate was 60 or 70 standard cubic centimeters per minute (sccm); p-H₂ was produced at 42–47 K corresponding to 81–86% p-H₂ enrichment. In SABRE-SHEATH^{27–28} experiments, p-H₂ gas was bubbled through the sample solution placed in magnetic shield. The corresponding ^{19}F NMR spectra with hyperpolarization are presented in Figure 1a and Figure 1c, with reference spectra of thermally polarized samples presented in Figure 1b and Figure 1d. The maximum ^{19}F NMR signal enhancements achieved for ^{15}N -3- ^{19}F -pyridine and ^{14}N -3- ^{19}F -pyridine molecules are similar (~195, corresponding to ~0.59% ^{19}F polarization). The ^{19}F signal enhancement values are in line with those reported earlier at the same concentration.²⁷ The field dependence of ^{19}F SABRE-SHEATH polarization was investigated. Figure 2 demonstrates that the optimum magnetic field for the transfer of polarization from p-H₂-derived hydride protons to ^{19}F via SABRE-SHEATH technique with 3- ^{19}F -pyridine is ~3–4 μT for both ^{15}N -labeled and non-labeled forms, although the overall profile for these two forms is somewhat different. Taken together, the results presented in Figure 1 and Figure 2 indicate that the overall contribution of the spin relayed polarization transfer for this spin system is relatively small, and the polarization is predominantly transferred via direct polarization transfer route (Scheme 1d and Scheme 1e). Based on the similarity of the results for the ^{19}F signal enhancement values ($\epsilon \sim 195$ and ($\epsilon \sim 194$), Figure 1, and the similarity of the magnetic field profiles, Figure 2), we estimate that the contribution of the spin relayed polarization transfer is less than 10% (determined by the reproducibility of the signal enhancement values (which is significant due to relatively short ^{19}F T_1) and by the signal-to-noise ratio of the thermal spectra, Figure 1b and Figure 1d). This explanation is also supported by the presence of small (but not negligible) spin-spin coupling between p-H₂-derived hydride protons and 3- ^{19}F nucleus, which we estimate as ~0.34 Hz based on the pyridine proton data presented by Eshuis and co-workers.⁶⁶ An alternative explanation for such similar magnetic field profiles and signal enhancement levels for ^{15}N -labeled and unlabeled 3- ^{19}F -pyridine forms is relayed polarization transfer via substrate proton spin network. However, proton polarization efficiency at SABRE-SHEATH conditions has been shown to be relatively low.⁴⁴

It should also be noted that ^{19}F hyperpolarization was performed under magnetic field conditions of canonical (i.e. homonuclear) SABRE effect in the milli-Tesla range in a manner similar to that described recently by Olaru and co-workers,⁴³ and by Buckenmaier and co-workers.⁶⁷ These experiments were performed by p-H₂ bubbling through solutions in fringe field of the high-field magnet. The achieved ^{19}F polarization levels were significantly lower compared to those of SABRE-SHEATH approach described above despite

optimization of the magnetic field, Figure 3. The best results were as follows: $\epsilon_{19F} \sim 15$ for ^{15}N -3- ^{19}F -pyridine, corresponding to $P_{19F} \sim 0.046\%$, and $\epsilon_{19F} \sim 32$ for ^{14}N -3- ^{19}F -pyridine, corresponding to $P_{19F} \sim 0.098\%$. These results indicate that SABRE-SHEATH is a better strategy for hyperpolarization of ^{19}F nuclei.

^{15}N SABRE-SHEATH hyperpolarization.

^{15}N SABRE-SHEATH experiments with ^{15}N -3- ^{19}F -pyridine were carried out in the magnetic shield. The signal enhancement was calculated using 4.4 M solution of $^{15}N_2$ -imidazole (prepared as described previously⁵⁷) in D_2O as a signal reference. The SABRE-SHEATH polarization transfer was found to be relatively efficient, and ^{15}N HP resonance of ^{15}N -3- ^{19}F -pyridine was enhanced by ~ 997 -fold, Figure 4a versus Figure 4b. The observed ^{15}N signal enhancements and the appearance of ^{15}N spectra were similar to those reported for ^{15}N -pyridine earlier.^{28, 30, 68} We note that ^{15}N signal enhancement values for ^{15}N -3- ^{19}F -pyridine reported here are significantly lower than the record ^{15}N values for other substrates reported in literature,^{25, 37, 39} which can be explained by three primary factors: difference of the actual substrates, the relatively high concentration (~ 100 mM) employed here, and non-optimized polarization conditions (e.g. lower p- H_2 pressure and flow rate).

The magnetic field profile of ^{15}N SABRE-SHEATH of ^{15}N -3- ^{19}F -pyridine shown in Figure 5 reveals the maximum at ~ 1 μT . While this result is in line with previous studies of similar ^{15}N -heterocycles (we additionally note that more careful and precise measurement of ^{15}N magnetic field profile for ^{15}N -pyridine were performed in Ref. # 69),^{25, 45, 69} there is a clear mismatch with the ^{19}F magnetic field profile shown in Figure 2. This magnetic field mismatch is informative, because it reveals that while ^{15}N site is most efficiently hyperpolarized at ~ 1 μT , this magnetic field range is a poor choice for relayed polarization transfer ($^{15}N \rightarrow ^{19}F$, Scheme 1e). On the other hand, this mismatch also explains why the dominant mechanism for polarization transfer at > 1 μT magnetic field is dominated by the direct $^1H_{hydride} \rightarrow ^{19}F$ polarization transfer, because ^{15}N sites are poorly hyperpolarized at this magnetic field regime. We also note that the maximum obtained ^{15}N and ^{19}F polarization levels were relatively similar for ^{15}N - ^{19}F -pyridine: $P_{15N} \sim 0.33\%$ versus $P_{19F} \sim 0.59\%$ in the SABRE-SHEATH field regime indicating the overall efficiency of each process is comparable for direct polarization transfer from p- H_2 -derived hydride protons to heteronucleus: ^{15}N or ^{19}F . These findings are important, because they not only explain the challenges for spin-relayed SABRE-SHEATH in the context of polarization of ^{19}F sites, but also point to how these issues can be potentially remedied in the future. For example, the more advanced and automated SABRE-SHEATH setup would benefit a great deal from a functionality allowing to switch the magnetic field in the micro-Tesla field regime from the optimum for efficient ^{15}N polarization from p- H_2 -derived hydride protons (i.e. ~ 1 μT) to that enabling the best relay transfer from HP ^{15}N nucleus to ^{19}F nuclei (note this field regime was not determined in this work). Such setup should have a very fast switch delays and sample transfer to minimize the polarization losses, because ^{15}N T_1 ⁴⁵ and ^{19}F T_1 (Figure S8) are relatively short in micro-Tesla field regime. Unfortunately, such automated setup was not available to us during these studies, but is subject to future development in our laboratories.

¹H SABRE experiments.

¹H SABRE experiments were carried out in the fringe field of the 9.4 T NMR spectrometer. ¹H SABRE NMR spectra acquired after p-H₂ bubbling through the solution of ¹⁵N-3-¹⁹F-pyridine and ¹⁴N-3-¹⁹F-pyridine and SABRE catalyst in CD₃OD are shown in Figure 6. The signal reference ¹H NMR spectrum of SABRE solution after relaxation of polarization was used for the estimation of the signal enhancement. We found $\epsilon_{1H} \sim 105$ for ¹⁵N-3-¹⁹F-pyridine, corresponding to $P_{1H} \sim 0.339\%$, and $\epsilon_{1H} \sim 199$ for ¹⁴N-3-¹⁹F-pyridine, corresponding to $P_{1H} \sim 0.643\%$.

We also note that the maximum obtained ¹H and ¹⁹F polarization levels were distinctly different for ¹⁴N-¹⁹F-pyridine: $P_{1H} \sim 0.643\%$ versus $P_{19F} \sim 0.098\%$ (a ~6.5-fold difference) in the SABRE field regime indicating that the overall efficiency of polarization transfer process from p-H₂-derived hydride protons to ¹⁹F is greatly diminished compared to that for ¹H_{hydride} → ¹H_{substrate}. Moreover, the comparison of the corresponding magnetic field profiles for ¹⁹F (Figure 3) and ¹H (Figure 7) clearly indicates that these magnetic field profiles are very similar with a maximum around 5.5–6 milli-Tesla (We note that a similar observation on the magnetic field profiles in 3-¹⁹F-pyridine was also reported by Buckenmaier and co-workers,⁶⁷ but they did not provide the explanation due to experimental limitations of their study: i.e. their report stated “However, while the transfer to ¹H nuclei of pyridine derivatives was strongly field-dependent, the field dependence for ¹⁹F hyperpolarization is much less pronounced”). These observations are important, because they support the following overall concept: ¹H_{hydride} → ¹H_{substrate} polarization transfer occurs first, and then a small fraction of ¹H_{substrate} polarization is leaked to ¹⁹F nuclei. Unlike in SABRE-SHEATH, where magnetic field profiles are mismatched completely for ¹⁵N and ¹⁹F nuclei, and the ¹H_{hydride} → ¹⁹F_{substrate} transfer occurs directly (Scheme 1d and Scheme 1e), in SABRE conditions (B_{evo} in milli-Tesla regime), the polarization transfer to ¹⁹F nuclei is likely inefficiently spin-relayed over the protons of the substrate.

Temperature dependence.

SABRE and SABRE-SHEATH experiments were optimized with respect to temperature as one of the key factors influencing the rate of ligands exchange and the overall process efficiency. Overall, all data obtained indicated the polarization maximum at 12–15 °C, Figure S9 and Figure S10.

¹⁵N High-Field SABRE.

Significant ¹⁵N NMR signal enhancement of ¹⁵N-3-¹⁹F-pyridine (Figure 4) that is similar to ¹⁵N NMR signal of SABRE hyperpolarized ¹⁵N-pyridine reported recently^{30, 68} allows to use ¹⁵N-3-¹⁹F-pyridine in High Field (HF) SABRE approach.^{65, 70} It was successfully shown that ¹⁵N NMR resonances can be detected during p-H₂ bubbling through the sample of conventional SABRE pre-catalyst ([Ir(IMes)(COD)Cl]) in CD₃OD, Figure S11. Obtained results show that presence of ¹⁹F atom decreases the efficiency of spontaneous polarization transfer from ¹H to ¹⁵N by approximately 3 times. The causes of lower spontaneous high-field SABRE hyperpolarization efficiency due to ¹⁹F presence are not exactly known, but steric hindrance effect may be responsible for these observations.³⁶

CONCLUSION

The synthesis of ^{15}N -3- ^{19}F -pyridine via preparation of Zincke salt is reported with overall 35% yield and 84% ^{15}N isotopic purity. SABRE and SABRE-SHEATH hyperpolarization studies were performed to investigate the mechanism of polarization transfer from p- H_2 -derived hydride protons to ^{19}F nucleus in milli-Tesla and micro-Tesla static magnetic field regimes in ^{15}N -3- ^{19}F -pyridine and ^{14}N -3- ^{19}F -pyridine. We found the mismatch between ^{15}N and ^{19}F magnetic field profiles in the micro-Tesla regime indicating that the polarization process likely happens directly from p- H_2 -derived hydride protons to ^{19}F nucleus without spin-relaying via ^{15}N site. Future developments to improve SABRE-SHEATH instrumentation may help to relay the polarization from p- H_2 -derived hydride protons to ^{19}F sites via ^{15}N with the overarching goal of significantly improving the overall polarization transfer efficiency. We note that while ^{19}F site on 3- ^{19}F -pyridine can be hyperpolarized directly via SABRE-SHEATH (to a similar level as that of ^{15}N), other N-heterocyclic targets (e.g. fluorine-containing drugs) may not necessarily have sufficient J -coupling between p- H_2 -derived hydride protons and ^{19}F to establish efficient direct polarization transfer. In case of SABRE with B_{evo} in milli-Tesla magnetic field range, we found that magnetic field profiles for ^1H and ^{19}F hyperpolarization are very similar, and ^{19}F polarization levels are significantly lower than ^1H SABRE polarization levels. Moreover, ^{19}F polarization levels with B_{evo} in milli-Tesla regime are also lower than ^{19}F polarization levels with B_{evo} in micro-Tesla regime. Our findings support the hypothesis that in SABRE with B_{evo} in milli-Tesla regime (i.e. optimal for homonuclear SABRE hyperpolarization), the process of ^{19}F nuclei hyperpolarization is inefficiently spin-relayed via protons of 3- ^{19}F -pyridine substrate. These findings are important in the context of future potential improvement of the hyperpolarization hardware and rational design of the HP molecular probes.

Supplementary Material

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Figure 1.

(a) ^{19}F SABRE-SHEATH NMR spectrum acquired after bubbling of $p\text{-H}_2$ through the solution of ^{15}N -3- ^{19}F -pyridine and SABRE catalyst in CD_3OD at $\sim 3.8\ \mu\text{T}$ magnetic field; $\epsilon_{^{19}\text{F}} \sim 195$ corresponding to $P_{^{19}\text{F}} \sim 0.593\%$. (b) Thermal ^{19}F NMR spectrum of the same ^{15}N -3- ^{19}F -pyridine solution used as a signal reference. (c) ^{19}F SABRE-SHEATH NMR spectrum acquired after bubbling of $p\text{-H}_2$ through the solution of ^{14}N -3- ^{19}F -pyridine and SABRE catalyst in CD_3OD at $\sim 3.8\ \mu\text{T}$ magnetic field; $\epsilon_{^{19}\text{F}} \sim 194$ corresponding to $P_{^{19}\text{F}} \sim 0.588\%$. (d) Thermal ^{19}F NMR spectrum of the same ^{14}N -3- ^{19}F -pyridine solution used as a signal reference. Note that spectra (b) and (d) are multiplied by a factor of 32.

Figure 2. Magnetic field dependence of ^{19}F SABRE-SHEATH polarization for ^{15}N -3- ^{19}F -pyridine (black squares) and ^{14}N -3- ^{19}F -pyridine (red circles).

Figure 3.
Magnetic field dependence of ^{19}F SABRE polarization for ^{14}N -3- ^{19}F -pyridine.

Figure 4.

(a) ^{15}N SABRE-SHEATH NMR spectrum acquired after bubbling of p- H_2 through the solution of ^{15}N -3- ^{19}F -pyridine and SABRE catalyst in CD_3OD at 0.1 μT magnetic field. $\epsilon_{^{15}\text{N}} \sim 997$, corresponding to $P_{^{15}\text{N}} \sim 0.326\%$. (b) ^{15}N NMR spectrum of 4.4 M solution of thermally polarized $^{15}\text{N}_2$ -imidazole in D_2O used as a signal reference.

Figure 5. Magnetic field dependence of ^{15}N SABRE-SHEATH polarization for ^{15}N -3- ^{19}F -pyridine.

Figure 6.

(a) ^1H SABRE NMR spectrum acquired after bubbling of p-H_2 through the solution of ^{15}N -3- ^{19}F -pyridine and SABRE catalyst in CD_3OD at ~ 9.4 mT magnetic field. (b) Thermal ^1H NMR spectrum of the same ^{15}N -3- ^{19}F -pyridine solution used as a signal reference. (c) ^1H SABRE NMR spectrum acquired after bubbling of p-H_2 through the solution of ^{14}N -3- ^{19}F -pyridine and SABRE catalyst in CD_3OD at ~ 9.4 mT magnetic field. (d) Thermal ^1H NMR spectrum of the same ^{14}N -3- ^{19}F -pyridine solution used as a signal reference. Note that spectra (b) and (d) are multiplied by a factor of 32.

Figure 7.
Magnetic field dependence of ^1H SABRE polarization for ^{14}N -3- ^{19}F -pyridine.

a Signal Amplification By Reversible Exchange (SABRE)

Scheme 1.

(a) Schematic of the SABRE hyperpolarization process using transfer of spin order from parahydrogen ($p\text{-H}_2$) on an Ir–IMes hexacoordinate complex.^{38, 51} (b) The schematic of SABRE hyperpolarization process with 3- ^{19}F -pyridine at natural abundance of spin isotopes: the dominant species is shown. (c) The schematic of SABRE hyperpolarization process with ^{15}N -3- ^{19}F -pyridine. SABRE is accomplished via spin–spin couplings between $p\text{-H}_2$ -derived hydrides and spin-1/2 nuclei of the exchangeable ligands in equatorial positions of the complex. (d) Relevant spin-spin interactions for direct polarization transfer

from p-H₂-derived hydrides to ¹⁹F in 3-¹⁹F-pyridine in SABRE process. (e) Relevant spin-spin interactions for direct polarization transfer from p-H₂-derived hydrides to ¹⁹F in ¹⁵N-3-¹⁹F-pyridine in direct and spin-relayed (via ¹⁵N) SABRE process.

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Scheme 2.

Reaction scheme of R-substituted ¹⁵N-pyridine synthesis from 2-ethoxy-3,4-dihydro-2H-pyrans.

Scheme 3.

Reaction scheme formation of ^{15}N -pyridine derivatives via Zincke salt.