Using $^2$H labelling to improve the NMR detectability of pyridine and its derivatives by SABRE

Philip Norcott$^1$, Michael J. Burns$^1$, Peter J. Rayner$^1$, Ryan E. Mewis$^{1,2}$, Simon B. Duckett$^1$

$^1$Department of Chemistry, University of York, York YO10 5DD, UK
$^2$Division of Chemistry and Environmental Science, Manchester Metropolitan University, Manchester, Greater Manchester, UK

Correspondence
Simon B. Duckett, Department of Chemistry, University of York, Heslington, York YO10 5DD, UK.
Email: simon.duckett@york.ac.uk

Funding information
Wellcome Trust, Grant/Award Number: 092506 and 098335

By introducing a range of $^2$H labels into pyridine and the para-substituted agents, methyl isonicotinate and isonicotinamide, we significantly improve their NMR detectability in conjunction with the signal amplification by reversible exchange process. We describe how the rates of $T_1$ relaxation for the remaining $^1$H nuclei are increased and show how this leads to a concomitant increase in the level of $^1$H and $^{13}$C hyperpolarization that can ultimately be detected.

KEYWORDS
hyperpolarisation, NMR, parahydrogen, SABRE

1 | INTRODUCTION

Hyperpolarization techniques overcome the inherent insensitivity of nuclear magnetic resonance (NMR) and magnetic resonance imaging by manipulating the spin distribution across Zeeman-split energy levels away from equilibrium where a typical population difference used to produce a magnetic resonance (MR) signal can be less than 1 in 100,000. The employment of hyperpolarized states can therefore result in very substantial MR signal gains. This process can be achieved through a number of methods, the most common of which are dynamic nuclear polarization$^{[1–3]}$ and parahydrogen induced polarization.$^{[4–6]}$ Signal amplification by reversible exchange (SABRE)$^7$ has recently emerged as a powerful alternative that can rapidly and repeatedly hyperpolarize a substrate through the interaction of parahydrogen with an iridium catalyst. In SABRE, parahydrogen ($p$-$H_2$) adds to a precatalyst, such as [IrCl(COD)(IMes)] (1, where IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene and COD = cis,cis-cycloocta-1,5-diene), in the presence of a coordinating substrate (sub) to form an iridium hydride complex such as [Ir(H)$_2$(IMes)(sub)$_3$]Cl (2)$^{[8,9]}$ (see Scheme 1). Polarization from the parahydrogen derived hydride ligands is then transferred into the bound substrate through the $J$-coupling network that exists in this complex.$^{[10]}$ Throughout the process, parahydrogen and the substrate, which are located in the bulk solution, are in reversible exchange with the corresponding ligands that are bound to the iridium complex. This results in a build-up of hyperpolarized substrate in bulk solution and hence the signal gain grows with parahydrogen exposure time, although a limit is reached because relaxation acts to destroy the hyperpolarization that has been created through SABRE.$^{[11]}$ As the rates of ligand
exchange are relatively fast, substantial hyperpolarization levels can be produced in just a few seconds. SABRE has been demonstrated for a wide range of substrates which include pyridine and its derivatives, pyrazines, imidazoles, pyrazoles, pyridazines, pyrimidines, amino acids, diazirines, and acetonitrile. It hyperpolarizes not only their 1H nuclei but the wide array of heteronuclei that they contain.

It has recently been shown that the selective, partial 2H labelling of substrates such as pyridine, nicotinamide and methyl nicotinate greatly extends the T1 relaxation times of the remaining 1H nuclei through inhibition of scalar relaxation processes. In addition, through 2H labelling of the IMes ligand of the iridium catalyst, the relaxation times of the bound substrate increase which further results in a dramatic improvement in the 1H hyperpolarization levels that can be achieved through SABRE. In fact, they exceed 50% polarization when 5 bar of parahydrogen is used in conjunction with a co-ligand. In this current work, we seek to report further developments that employ this 2H labelling strategy by applying it to the related compounds pyridine, methyl isonicotinate, and isonicotinamide. We seek to ascertain its effect on the efficacy of the resulting 1H and 13C hyperpolarization levels that can be achieved through SABRE. For synthetic procedures and characterisation data please see Supporting information.

2 | RESULTS AND DISCUSSION

Pyridine (3) was one of the first compounds to be hyperpolarized by the SABRE method. Because of its relatively simple structure, we recognized that there was an opportunity to investigate the effect of selective 2H labelling on the level of hyperpolarization. A selection of partially deuterated pyridines shown in Figure 1 were therefore synthesized. This approach gave us access to three isotopologues of pyridine, 3a, 3b, and 3c, in which 1H nuclei are retained at the ortho, meta, and para positions of the ring, respectively.

When protio-pyridine 3 (20 mM, 4 eq.) is mixed with [IrCl(COD)IMes] (5 mM) and parahydrogen in ethanol-d6 and shaken at 65 gauss for 10 s followed by immediate transport into the spectrometer for detection, the signal for Hα in Figure 2 appears with an intensity that is approximately 2,400 times larger than that observed under Boltzmann conditions in the corresponding thermally equilibrated control measurement. In contrast, the corresponding signals for Hb and Hc are 1,120 and 1,258 times larger than those of the corresponding reference signals (at 9.4 T). Figure 2 (left) illustrates a typical measurement. The new signal intensity reflects the detection of 3.9% Hα polarization, created after just 10 s of exposure to 3 bar of parahydrogen. Furthermore, we note that if 20 s were needed to repeat the control measurement which uses thermally equilibrated polarization levels, it would take 1,333 days of data averaging to reproduce the hyperpolarized signal intensity. It is for this reason that hyperpolarization reflects a method that could transform clinical diagnosis as it may facilitate the facile detection of markers that probe underlying physiology.

When the agent 3,4,5-d3-pyridine (3a) was employed under identical conditions to those described for 3, the signal enhancement seen for the remaining ortho protons increases to 4,166 (6.7% polarization), shown in Table 1. However, upon moving to 2,4,6-d3-pyridine (3b), the remaining meta protons exhibit a smaller gain associated with an enhancement of just 541 (0.9% polarization).
Finally, 2,3,5,6-d₄-pyridine (3c) results in very little signal gain with its remaining proton showing a 163-fold enhancement (0.51% polarization). These values are the average of three distinct measurements and are hence reproducible.

The lower polarization levels seen for 3b and 3c can be rationalized as the result of the fact that their protons are magnetically isolated from those of the hydride ligands in the iridium catalyst. Hence, smaller J-couplings are involved in the polarization transfer step and less effective SABRE transfer results.[37] By comparison, 3a features two ortho ¹H nuclei that couple more strongly to the hydride ligands in the SABRE-catalyst and they are therefore able to more readily receive magnetization. An analogous trend was found when methanol-d₄ was used as the solvent, and in this case, a polarization level of 5.8% could be achieved with 3a.

The reduction in hydride-substrate J-coupling is somewhat offset by relaxation changes. At 400 MHz, in degassed ethanol-d₆, prototype-pyridine (3) was found to exhibit T₁ relaxation times of between 18.2 and 26.7 s (Table 1). Its isotopologue 3a, however, has relaxation times for the two ortho protons of 72.4 s, whereas in 3b, the meta protons exhibit a 72.6 s value and in 3c, the para proton has a T₁ of 57.5 s. The values determined in the ²H-labelled isotopologues therefore reflect a dramatic improvement on those of the unlabelled 3. We note, however, that under the catalytic conditions used in SABRE, interactions with the iridium catalyst act to reduce the observed T₁ values. This is because the free and bound forms are in equilibrium and the observed value therefore reflects a weighted average of the two. For unlabelled pyridine, the new values lie between 3.2 and 5.3 s, whereas for 3a, 3b, and 3c, they are 4.2, 13.8, and 25.0 s, respectively. As percentages, the reductions caused by the presence of the catalyst in solution are therefore 94%, 81%, and 56%, respectively. This serves to demonstrate that the effect is most strongly manifest in the ortho protons, which exhibit the strongest spin-spin coupling to the hydride ligands when bound to the iridium. Although 3b exhibits a better T₁ value for the meta position, it fails to receive strong magnetization through
SABRE, a feature that is further demonstrated for the para proton of 3c. Again, analogous relaxation and polarization trends were observed in methanol-\(d_4\) solution. However, relaxation times for 3b and 3c were significantly extended in methanol-\(d_4\), compared to ethanol-\(d_6\).

Hence, for optimal SABRE activity we can conclude that it is desirable to locate a proton next to the catalyst binding site, where \(J_{HH}\) is maximized. However, we tension this need with the fact that such an arrangement will also reduce the effective lifetime of the polarization under catalytic conditions. Clearly, the interplay between the \(T_1\) of the proton site and the efficacy of polarization transfer must therefore be carefully considered when designing an optimized agent.

A series of hyperpolarized \(^{13}\)C measurements were then conducted using 3,4,5-\(d_3\)-pyridine (3a) in the presence of 1 for a 20:1 loading after SABRE at approximately 0.5 G. The resulting fully coupled single-scan \(^{13}\)C hyperpolarized NMR spectrum is shown in Figure 3 alongside its thermally equilibrated \(^{13}\)C counterpart, which is a 2,048 scan average. A significant increase in the \(^{13}\)C resonances’ signal-to-noise ratios is clearly observed in the hyperpolarized spectrum. Furthermore, the ortho peak (148 ppm) is observed as an antiphase doublet of doublets, whereas the meta (124 ppm) and para (137 ppm) peaks are split into antiphase triplets of doublets. This is consistent with the creation of \(I_SJ\) terms, where the antiphase component is associated with the small, indirect \(J_{HC}\) coupling. The meta and para signals are associated with the detection of a \(^{13}\)CD signal for the meta and para sites, which accounts for the in-phase 1:1:1 splitting.

When a similar experiment was recorded with concurrent \(^1\)H decoupling a much weaker signal was observed (Figure 4). This confirms that the dominant terms that are created under SABRE at 60 G are indeed \(I_SJ\) based rather than \(S_J\). We expect that the levels of signal gain that are observed may be improved through the incorporation of a \(^{15}\)N label, which has recently been shown to reduce \(^{13}\)C polarization transfer losses due to quadrupolar relaxation.\(^{[38]}\)

Following these observations, we turned our attention to the para-substituted pyridine derivatives, methyl isonicotinate (4) and isonicotinamide (5). A range of doubly deuterated isotopologues of these compounds were synthesized according to Scheme 2, and the results of the related NMR studies are shown in Table 2. These substrates provide a range of \(^1\)H spin systems, and we expected them to exhibit markedly different hyperpolarization characteristics. This reflects the fact that 4a and 5a possess pairs of ortho and meta \(^1\)H nuclei that exhibit a mutual three-bond coupling, in contrast, 4b and 5b contain two weakly coupled ortho and meta nuclei (5-bond coupling), whereas 4c, 5c, 4d, and 5d contain pairs of equivalent ortho and meta nuclei, respectively.

![Scheme 2](image-url)  
**Scheme 2** Synthetic route to the doubly \(^2\)H-labelled methyl isonicotinates 4 and isonicotinamides 5

![Figure 3](image-url)  
**Figure 3** Top: Thermal \(^{13}\)C NMR spectrum of 3a (100 mM) and SABRE catalyst (5 mM) in methanol-\(d_4\) after 2,048 scans. Bottom: single-scan hyperpolarized \(^{13}\)C NMR spectrum
TABLE 2 $T_1$ relaxation times (s) and polarization levels found for the methyl isonicotinates 4 and the isonicotinamides 5. Conditions: 20 mM substrate, 5 mM [IrCl(COD)IMes], activated with 3 bar $p$-$H_2$. $o$ = ortho and $m$ = meta.

| Substrate | Methanol-$d_4$ | Site $T_1$(no cat.)/s | Site $T_1$(with cat.)/s | Polarization level (%) |
|-----------|----------------|-----------------------|-------------------------|------------------------|
|           |                | $o$—14.2              | $o$—1.9                 | $o$—4.8 ± 0.1          |
| 4         |                | $m$—14.1              | $m$—3.9                 | $m$—1.0 ± 0.1          |
|           |                | $o$—9.6               | $o$—2.9                 | $o$—2.2 ± 0.1          |
| 4a        |                | $m$—9.6               | $m$—5.8                 | $m$—1.4 ± 0.1          |
|           |                | $o$—61.9              | $o$—3.5                 | $o$—5.4 ± 0.4          |
| 4b        |                | $m$—61.2              | $m$—21.4                | $m$—5.6 ± 0.3          |
|           |                | $o$—70.7              | $o$—4.1                 | $o$—7.4 ± 0.3          |
|           |                | $m$—72.8              | $m$—16.1                | $m$—7.4 ± 0.4          |
|           |                | $o$—8.2               | $o$—1.7                 | $o$—2.6 ± 0.2          |
| 5         |                | $m$—8.3               | $m$—3.5                 | $m$—0.2 ± 0.02         |
|           |                | $o$—7.4               | $o$—2.3                 | $o$—3.5 ± 0.3          |
| 5a        |                | $m$—8.3               | $m$—4.1                 | $m$—0.4 ± 0.1          |
|           |                | $o$—18.2              | $o$—4.2                 | $o$—11.0 ± 0.5         |
| 5b        |                | $m$—42.7              | $m$—16.0                | $m$—11.1 ± 0.5         |
|           |                | $o$—23.4              | $o$—2.9                 | $o$—5.8 ± 0.7          |
|           |                | $m$—47.2              | $m$—8.3                 | $m$—4.2 ± 0.1          |

**FIGURE 5** Left: single scan $^1H$ NMR spectra for hyperpolarized and fully relaxed isonicotinamide 5 at 400 MHz. Right: corresponding single scan $^1H$ NMR spectra of 5b. The normal traces are shown with a ×8 vertical expansion relative to the hyperpolarized traces.
For these ester and amide substrates, deuteration at the 2- and 3-positions (4a and 5a) resulted in limited changes in their relaxation times relative to their 1H counterpart and somewhat similar polarization levels as a result of SABRE were observed (Table 2). These results indicate that relaxation within these molecules is driven by interactions between the adjacent 1H nuclei. Analogues b–d, with more isolated 1H spin systems, might therefore be predicted to have higher T1 values. In support of this, we found that deuteration at the 2- and 5-positions (4b and 5b) does indeed result in significantly improved relaxation times and SABRE polarization levels (Figure 5). Now, the T1 values are over 60 s for methyl 2,5-\(d_2\)-isonicotinate 4b, which compare to 14 s for protio 4. In the case of isonicotinamide 5b, the SABRE polarization levels improve from the original 2.6% and 0.2% levels for the ortho and meta positions of 5 (in methanol-\(d_4\)), respectively, to 11.0% and 11.1%, respectively. In this case, the extension of the T1 values determined for the meta proton in the presence of the SABRE catalyst greatly assists in

**FIGURE 6** Series of hyperpolarized single-scan \(^{13}\)C NMR spectra of 5–5d (100 mM) and [IrCl(COD)(IMes)] (5 mM) in methanol-\(d_4\) under \(p\)-H\(_2\) after transfer at 0.5 G. Thermally polarized reference \(^{13}\)C NMR spectrum (top), acquired over 4,096 scans. \(\varepsilon\) = enhancement factor, fold gain
increasing the observed polarization level of the proton at this position.

In general, compounds with only ortho $^1$H nuclei ($4c$ and $5c$) gave slightly improved levels of polarization and $T_1$ values that compare to those of the parent, but still suffer from short relaxation times in the presence of the SABRE catalyst. This is consistent with the results outlined earlier for $3a$. Compounds $4d$ and $5d$, with only meta $^1$H nuclei now give higher $T_1$ values, but as expected, the improvement in achieved polarization level is minimal, in accordance with the weaker $J$-coupling that connects them to hydride ligands in the catalyst.

These trends confirm our earlier conclusions that substrates containing a $^1$H nucleus at the ortho position result in efficient polarization transfer and that $^1$H nuclei that are isolated from each other, and the hydride ligands of the metal catalyst, lead to increased relaxation times under SABRE. These changes contribute cooperatively to produce a strong, long-lived hyperpolarized signal.

Related hyperpolarized $^{13}$C NMR experiments on the labelled isonicotinamides ($5a$–$5d$) showed that superior signal enhancements could be achieved with these substrates (Figure 6). With a 20-fold concentration of substrate to catalyst and polarization transfer at approximately 0.5 gauss, compound $5a$ showed strong SABRE responses for the quaternary carbons, including those adjacent to the $^2$H labels. The remaining CH positions showed a much lower, but detectable signal enhancement, an effect which is predicted to be due to an increased rate of relaxation. Compound $5b$ produced a similar outcome; the higher levels of $^1$H polarization enabling increased magnetization transfer to be relayed into the most distant quaternary carbon. In the case of compound $5c$, a clear SABRE signal was observed for all carbons on the aromatic ring, notably including that of the CH at the ortho position. The carbonyl carbon showed significantly smaller polarization, as only a weak $^4J_{CH}$ coupling to the ortho proton is possible from that site for transfer. Analogue $5d$ gave very little $^{13}$C polarization, with only the carbonyl position being visible. It should be noted that in all cases, the dominant peaks appear in antiphase due to the observation of $I_zS_z$ derived states.

### 3 | CONCLUSIONS

An investigation has been made into the effect of selectively incorporating $^2$H labels into a number of pyridine-based substrates and the subsequent effect this change had on the observed hyperpolarization levels achieved through the SABRE process. We completed studies on three isotologues of pyridine (3), four isotologues of methyl isonicotinate (4), and four isotologues of isonicotinamide (5). We find that harnessing hyperpolarization sites adjacent to the nitrogen centre of pyridine, which binds to the SABRE catalyst metal centre, is crucial for efficient polarization transfer through its stronger $^3J_{HH}$ coupling to the para-hydrogen-derived metal hydride ligands. Furthermore, we confirm that the presence of spin-isolated hyperpolarization sites in these agents both increases signal lifetime through reduced relaxation and allows the detection of strongly hyperpolarized $^1$H responses. These changes also facilitate the detection of strong $^{13}$C NMR signals, most notably for the corresponding quaternary and CD positions. These signals typically appear with anti-phase character due their origin in a heteronuclear longitudinal two spin order term ($^1$H–$^{13}$C) involving an indirect coupling. Agent $5b$ produced the strongest carbonyl signal as a consequence of a $^3J_{HC}$ coupling, which must lead to limited internal peak cancelation due to its small value. The $^{13}$CH signals of $5c$ are also dramatically stronger than those of $5a$, $5b$, and $5d$ in accordance with a predicted long relaxation time. The spin isolation of the C–$^{13}$CONH$_2$ groups in $5c$ and $5d$ though acts to reduce its detectability, although the signal for C–$^{13}$CONH$_2$ is strongly visible in $5a$ and $5b$. Hence, we conclude that a $^2$H-labelling strategy can be used to control not only $^1$H signal gains but also those of $^{13}$C. We expect that this strategy will enable improvement in polarization in molecules containing other heteronuclei, such as $^{15}$N, and work is ongoing to achieve this goal. Additionally, a detailed investigation into the mechanism of polarization transfer in molecules containing $^2$H nuclei would be of interest.

### ACKNOWLEDGEMENT

We thank the Wellcome Trust (092506 and 098335) for funding.

### ORCID

Philip Norcott [http://orcid.org/0000-0003-4082-2079](http://orcid.org/0000-0003-4082-2079)
Michael J. Burns [http://orcid.org/0000-0002-5569-1270](http://orcid.org/0000-0002-5569-1270)
Peter J. Rayner [http://orcid.org/0000-0002-6577-4117](http://orcid.org/0000-0002-6577-4117)
Ryan E. Mewis [http://orcid.org/0000-0002-3756-6505](http://orcid.org/0000-0002-3756-6505)
Simon B. Duckett [http://orcid.org/0000-0002-9788-6615](http://orcid.org/0000-0002-9788-6615)

### REFERENCES

[1] J. H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning, K. Golman, Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR, Proc. Natl. Acad. Sci. U. S. A. 2003, 100(18), 10158.
[2] K. R. Keshari, D. M. Wilson, Chemistry and biochemistry of $^{13}$C hyperpolarized magnetic resonance using dynamic nuclear polarization, Chem. Soc. Rev. 2014, 43(5), 1627.

[3] J. H. Ardenkjaer-Larsen, On the present and future of dissolution-DNP, J. Magn. Reson. 2016, 264(Supplement C), 3.

[4] C. R. Bowers, D. P. Weitekamp, Transformation of symmetrization order to nuclear-spin magnetization by chemical reaction and nuclear magnetic resonance, Phys. Rev. Lett. 1986, 57(21), 2645.

[5] C. R. Bowers, D. P. Weitekamp, Parahydrogen and synthesis allow dramatically enhanced nuclear alignment, J. Am. Chem. Soc. 1987, 109(18), 5541.

[6] K. Golman, O. Axelsson, H. Jóhannesson, S. Månsson, C. Olofsson, J. S. Petersson, Parahydrogen-induced polarization in imaging: Subsecond 13C angiography, Magn. Reson. Med. 2001, 46(1), 1.

[7] R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. López-Serrano, D. C. Williamson, Reversible interactions with parahydrogen enhance NMR sensitivity by polarization transfer, Science 2009, 323(5922), 1708.

[8] M. J. Cowley, R. W. Adams, K. D. Atkinson, M. C. R. Cockett, S. B. Duckett, G. G. R. Green, J. A. B. Lohman, R. Kerssebaum, D. K. Kilgour, R. E. Mewis, Iridium N-heterocyclic carbene complexes as efficient catalysts for magnetization transfer from para-hydrogen, J. Am. Chem. Soc. 2011, 133(16), 6134.

[9] L. S. Lloyd, A. Asghar, M. J. Burns, A. Charlton, S. Coombes, M. J. Cowley, G. J. Dear, S. B. Duckett, G. G. R. Green, G. R. Green, L. A. R. Highton, A. J. J. Hooper, M. Khan, I. G. Khazal, K. J. Lewis, R. E. Mewis, A. D. Roberts, A. J. Ruddlesden, Hyperpolarisation through reversible interactions with parahydrogen, Catal. Sci. Technol. 2014, 4(10), 3544.

[10] R. W. Adams, S. B. Duckett, R. A. Green, D. C. Williamson, G. G. R. Green, A theoretical basis for spontaneous polarization transfer in non-hydrogenative parahydrogen-induced polarization, J. Chem. Phys. 2009, 131(19), 194505.

[11] D. A. Barskiy, A. N. Pravdivtsev, K. L. Ivanov, K. V. Kevtunov, I. V. Koptyug, A simple analytical model for signal amplification by reversible exchange (SABRE) process, PCCP 2016, 18(1), 89.

[12] K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, J. López-Serrano, A. C. Whitwood, Spontaneous transfer of parahydrogen derived spin order to pyridine at low magnetic field, J. Am. Chem. Soc. 2009, 131(37), 13362.

[13] J.-B. Hövener, N. Schwanderlapp, R. Borowiak, T. Lickert, S. B. Duckett, R. E. Mewis, R. W. Adams, M. J. Burns, L. A. R. Highton, G. G. R. Green, A. Olaru, J. Hennig, D. von Elverfeldt, Toward biocompatible nuclear hyperpolarization using signal amplification by reversible exchange: Quantitative in situ spectroscopy and high-field imaging, Anal. Chem. 2014, 86(3), 1767.

[14] P. J. Rayner, M. J. Burns, A. M. Olaru, P. Norcott, M. Fekete, G. G. R. Green, L. A. R. Highton, R. E. Mewis, S. B. Duckett, Delivering strong 1H nuclear hyperpolarization levels and long magnetic lifetimes through signal amplification by reversible exchange, Proc. Natl. Acad. Sci. U. S. A. 2017, 114(16), E3188.

[15] K. D. Atkinson, M. J. Cowley, S. B. Duckett, P. I. P. Elliott, G. G. R. Green, J. López-Serrano, I. G. Khazal, A. C. Whitwood, Parahydrogen induced polarization without incorporation of parahydrogen into the Analyte, Inorg. Chem. 2009, 48(2), 663.

[16] H. Zeng, J. Xu, J. Gillen, M. T. McMahon, D. Artemov, J.-M. Tyburn, J. A. B. Lohman, R. E. Mewis, K. D. Atkinson, G. G. R. Green, S. B. Duckett, P. C. M. van Zijl, Optimization of SABRE for polarization of the tuberculosis drugs pyrazinamide and isoniazid, J. Magn. Reson. 2013, 237, 73.

[17] S. S. Roy, P. J. Rayner, P. Norcott, G. G. R. Green, S. B. Duckett, Long-lived states to sustain SABRE hyperpolarised magnetisation, PCCP 2016, 18(36), 24905.

[18] M. Fekete, P. J. Rayner, G. G. R. Green, S. B. Duckett, Harnessing polarization transfer to indazole and imidazole through signal amplification by reversible exchange to improve their NMR detectability, Magn. Reson. Chem. 2017, 55(10), 944.

[19] M. L. Truong, T. Theis, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, E. Y. Chekmenev, $^{15}$N hyperpolarization by reversible exchange using SABRE-SHEATH, J. Phys. Chem. C 2015, 119(16), 8786.

[20] A. N. Pravdivtsev, A. V. Yurkovskaya, H.-M. Vieti, K. L. Ivanov, RF-SABRE: A way to continuous spin hyperpolarization at high magnetic fields, J. Phys. Chem. B 2015, 119(43), 13619.

[21] E. B. Dücker, L. T. Kuhn, K. Münne mann, C. Griesinger, Similarity of SABRE field dependence in chemically different substrates, J. Magn. Reson. 2012, 214, 159.

[22] S. S. Roy, P. Norcott, P. J. Rayner, G. G. R. Green, S. B. Duckett, A Hyperpolarizable $^1$H magnetic resonance probe for signal detection 15 minutes after spin polarization storage, Angew. Chem. Int. Ed. 2016, 55(50), 15642.

[23] K. M. Appleby, R. E. Mewis, A. M. Olaru, G. G. R. Green, I. J. S. Fairlamb, S. B. Duckett, Investigating pyridazine and phthalazine exchange in a series of iridium complexes in order to define their role in the catalytic transfer of magnetisation from para-hydrogen, Chem. Sci. 2015, 6(7), 3981.

[24] S. S. Roy, P. Norcott, P. J. Rayner, G. G. R. Green, S. B. Duckett, A simple route to strong carbon-13 NMR signals detectable for several minutes, Chem. - Eur. J. 2017, 23(44), 10496.

[25] A. M. Olaru, A. Burt, P. J. Rayner, S. J. Hart, A. C. Whitwood, G. G. R. Green, S. B. Duckett, Using signal amplification by reversible exchange (SABRE) to hyperpolarise 119Sn and 29Si NMR nuclei, Chem. Commun. 2016, 52(100), 14482.

[26] S. Gloggler, R. Muller, J. Colell, M. Emonts, M. Dabrowski, B. Blumich, S. Appelt, Para-hydrogen induced polarization of amino acids, peptides and deuterium-hydrogen gas, PCCP 2011, 13(30), 13759.

[27] K. Shen, A. W. J. Logan, J. F. P. Colell, J. Bae, G. X. Ortiz, T. Theis, W. S. Warren, S. J. Malcolmson, Q. Wang, Diazirines as potential molecular imaging tags: Probing the requirements for efficient and long-lived SABRE-induced hyperpolarization, Angew. Chem. Int. Ed. 2017, 56, 12112.

[28] R. E. Mewis, R. A. Green, M. C. R. Cockett, M. J. Cowley, S. B. Duckett, G. G. R. Green, R. O. John, P. J. Rayner, D. C. Williamson, Strategies for the hyperpolarization of acetonitrile and related ligands by SABRE, J. Phys. Chem. B 2015, 119(4), 1416.
[29] M. J. Burns, P. J. Rayner, G. G. R. Green, L. A. R. Highton, R. E. Mewis, S. B. Duckett, Improving the hyperpolarization of 31P nuclei by synthetic design, The Journal of Physical Chemistry B 2015, 119 (15), 5020.

[30] A. N. Pravdivtsev, A. V. Yurkovskaya, H. Zimmermann, H.-M. Vieth, K. L. Ivanov, Transfer of SABRE-derived hyperpolarization to spin-1/2 heteronuclei, RSC Adv. 2015, 5 (78), 63615.

[31] A. N. Pravdivtsev, A. V. Yurkovskaya, H.-M. Vieth, K. L. Ivanov, Spin mixing at level anti-crossings in the rotating frame makes high-field SABRE feasible, PCCP 2014, 16 (45), 24672.

[32] A. J. Holmes, P. J. Rayner, M. J. Cowley, G. G. R. Green, A. C. Whitwood, S. B. Duckett, The reaction of an iridium PNP complex with parahydrogen facilitates polarisation transfer without chemical change, Dalton Trans. 2015, 44 (3), 1077.

[33] P. Norcott, P. J. Rayner, G. G. R. Green, S. B. Duckett, Achieving high 1H nuclear hyperpolarization levels with long lifetimes in a range of tuberculosis drug scaffolds, Chem. - Eur. J. 2017, 23 (67), 16990.

[34] J. W. Pavlik, S. Laohhasurayotin, Synthesis and spectroscopic properties of isomeric trideuterio- and tetradeuterio pyridines, J. Heterocycl. Chem. 2007, 44 (6), 1485.

[35] J. W. Pavlik, S. Laohhasurayotin, The photochemistry of 3,4,5-trideuteriopyridine, Tetrahedron Lett. 2003, 44 (44), 8109.

[36] J. Kurhanewicz, D. B. Vigneron, K. Brindle, E. Y. Chekmenev, A. Comment, C. H. Cunningham, R. J. DeBerardinis, G. G. Green, M. O. Leach, S. S. Rajan, R. R. Rizi, B. D. Ross, W. S. Warren, C. R. Malloy, Analysis of cancer metabolism by imaging hyperpolarized nuclei: Prospects for translation to clinical research, Neoplasia 2011, 13 (2), 81.

[37] N. Eshuis, R. L. E. G. Aspers, B. J. A. van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, S. S. Wijmenga, M. Tessari, Determination of long-range scalar 1H–1H coupling constants responsible for polarization transfer in SABRE, J. Magn. Reson. 2016, 265 (Supplement C), 59.

[38] D. A. Barskiy, R. V. Shchepin, C. P. N. Tanner, J. F. P. Colell, B. M. Goodson, T. Theis, W. S. Warren, E. Y. Chekmenev, The absence of Quadrupolar nuclei facilitates efficient 13C hyperpolarization via reversible exchange with Parahydrogen, ChemPhysChem 2017, 18 (12), 1493.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Norcott P, Burns MJ, Rayner PJ, Mewis RE, Duckett SB. Using 2H labelling to improve the NMR detectability of pyridine and its derivatives by SABRE. Magn Reson Chem. 2018;56:663–671. https://doi.org/10.1002/mrc.4703