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Title: Using Para-hydrogen to Hyperpolarize Amines, Amides, Carboxylic Acids, Alcohols, Phosphates and Carbonates.

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One Sentence Summary: Para-hydrogen is used to give efficient NMR detection of array of amines, amides, alcohols, carboxylates, carbonates and phosphates.

Abstract:
Hyperpolarization turns weak NMR and MRI responses into strong signals so normally impractical measurements are possible. We use para-hydrogen here to rapidly hyperpolarize appropriate $^1$H, $^{13}$C, $^{15}$N and $^{31}$P responses of analytes such as NH$_3$ and important amines such as phenylethylamine, amides such as acetamide, urea and methacrylamide, alcohols spanning methanol through octanol and glucose, the sodium salts of carboxylic acids such as acetic acid and pyruvic acid, sodium phosphate, disodium adenosine 5’ triphosphate and sodium hydrogen carbonate. The associated signal gains are used to demonstrate it is possible to collect informative single-shot NMR spectra of these analytes in seconds at the micromole level in a 9.4 T observation field. To achieve these wide ranging signal gains, we first employ the Signal Amplification By Reversible Exchange (SABRE) process to hyperpolarize an amine or ammonia and then employ their exchangeable NH protons to relay polarization into the analyte without changing its identity. We found the $^1$H signal gains reach as high as 650-fold per proton, while for $^{13}$C, the corresponding signal gains achieved in a $^1$H-$^{13}$C refocused INEPT experiment exceed 570-fold and those in a direct detected $^{13}$C measurement 400-fold. Thirty one examples are described to demonstrate the applicability of this technique.

Introduction
Nuclear magnetic resonance (NMR) is one of the most powerful methods for the study of materials and magnetic resonance imaging (MRI) plays a vital role in clinical diagnosis. However, the low sensitivity of these techniques limits their applicability. Remarkably, the hyperpolarization method, dynamic nuclear polarization (DNP) improves the detectability of analytes such as pyruvate to the level that the MRI based diagnosis of disease is now possible.\(^{(1)}\) Para-hydrogen ($^p$-$H_2$), which is cheap to prepare and exists as a pure nuclear spin state, was shown to enhance the strength of an NMR signal in 1987\(^{(2)}\), although such methods have not yet been used clinically. This may reflect the fact that $^p$-$H_2$ was originally used to sensitize chemically modified hydrogenation products\(^{(3, 4)}\), and only recently has a method been developed where the original identity of the sensitized analyte is retained.\(^{(5)}\) This approach, Signal Amplification By Reversible Exchange (SABRE), harnesses $^p$-$H_2$ in the form of metal bound hydride ligands and transfers hyperpolarization into a weakly bound substrate\(^{(6-8)}\) via the small $J$-couplings that connect them.\(^{(9)}\) Ligand exchange then builds up a pool of hyperpolarized substrate according to Scheme 1a.\(^{(10)}\) SABRE is successful for analytes with multiple bonds to nitrogen such as nicotinamide\(^{(11)}\), isoniazid\(^{(12)}\), pyrazole\(^{(13)}\) and acetonitrile\(^{(14)}\) with $^1$H polarizations of 50%\(^{(11)}\) and $^{15}$N values of 20%\(^{(15)}\) being achieved. Furthermore, while it works for other nuclei\(^{(11, 16-20)}\) there are many classes of analyte it fails to sensitize.

Here we describe a method where $^p$-$H_2$ hyperpolarizes a range of amines, amides, carboxylic acids, alcohols, phosphates and carbonates without changing their chemical identity. Our method starts with the hyperpolarization of ammonia (the hyperpolarization transfer agent). Subsequently, polarization is relayed into the specified analyte through proton exchange as outlined in Scheme 1b. Spontaneous low-field transfer then creates the hyperpolarized analyte which we detect. We called this approach SABRE-RELAY, and predict that when it is fully optimized it will have a major impact on NMR and MRI in accordance with the fact that we exemplify it for thirty one analytes.
Scheme 1: a) Hyperpolarization via SABRE, and b) hyperpolarization via SABRE-RELAY. SABRE is used to hyperpolarize the transfer agent $\text{NH}_2\text{R}$, where $R = H$ or CH$_2$Ph or CH$_2$CH$_2$Ph (etc) which relays polarization to the analyte (HR'', Route A) where R'' = amide, carboxyl, phosphate or alkoxide (etc). This process involves both proton exchange and spin-spin interactions and may be mediated by an intermediary HOR', where R' = H, or suitable scaffold (Route B). Centre, reaction scheme shows the formation of SABRE active 2-NH$_3$ which leads to NH$_3$.

Results

We achieve SABRE-RELAY by reacting ammonia with the most versatile of the current SABRE catalysts, [IrCl(COD)(IMes)]$_2$ [21, 22] (1) [where IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene, COD = cycloocta-1,5-diene] and H$_2$ to form [Ir(H)$_2$(IMes)(NH$_3$)$_3$]Cl (2-NH$_3$) according to Scheme 1. When this reaction is competed in dichloromethane-$d_2$, 2-NH$_3$ exhibits equatorial and axial NH$_3$ ligand signals at $\delta$ 2.19 and 2.88 in the corresponding $^1$H NMR spectrum, alongside a broad NH$_3$ response at $\delta$ 0.47, as detailed in Fig. 1a. When this sample is examined after exposure to a 2 bar pressure of $p$-H$_2$ gas at 60 G, the resulting $^1$H NMR signal for free NH$_3$ now shows an ~10-fold signal enhancement per proton with the bound NH$_3$ ligand signal at $\delta$ 2.19 showing a 3-fold enhanced response. These observations confirm that 2-NH$_3$ undergoes SABRE to produce hyperpolarized ammonia. When the same process is repeated in methanol-$d_4$, 2-NH$_3$ exhibits a hydride resonance at $\delta$ ~23.2 that rapidly separates into several components as H-D exchange proceeds to form an array of isotopologues. However, when $p$-H$_2$ is used a hyperpolarized NMR signal is readily seen at $\delta$ 5.06 for the exchangeable proton of CD$_3$OH which exhibits a 32-fold intensity gain over its thermally equilibrated signal. We therefore added a 5% loading of H$_2$O, relative to iridium, to the CD$_2$Cl$_2$ sample and re-examined it. Under these conditions, the free NH$_3$ signal gain resulting from SABRE...
proved to increase to 40-fold per proton, whilst the corresponding equatorial ligand signal now showed an 85-fold per proton gain (Fig. 1b). Additionally, the free H$_2$O signal was enhanced by 75-fold per proton, a result which compares well with other solvent signal enhancements.\textsuperscript{[23-25]}

Exchange spectroscopy measurements were then used to confirm that free NH$_3$ and the equatorially bound NH$_3$ ligand of 2-NH$_3$ are in chemical exchange, with the observation of further exchange peaks between free NH$_3$ and H$_2$O demonstrating the rapid transfer of protons between them. Based on this selectivity, we conclude that when the ammonia is bound, proton exchange between NH$_3$ and H$_2$O is suppressed as the nitrogen lone pair is involved in bonding to the metal center. Consequently it now becomes hyperpolarized by SABRE. Proton exchange proceeds though after NH$_3$ dissociation and this leads to observation of hyperpolarization in the chemical-exchange averaged response of H$_2$O (or HOCD$_3$) according to Scheme 1b. We show now how it is possible to harness this proton exchange process to hyperpolarize the NMR signals of a series of added analytes.

First we consider whether the SABRE hyperpolarization of NH$_3$ can be relayed into the $^1$H and $^{13}$C responses of a series of alcohols CH$_3$(CH$_2$)$_n$OH (where n = 0-7). In order to do this we prepared a range of dichloromethane-$d_2$ solutions that contained [Ir(H)$_2$(IMes)(NH$_3$)$_3$]Cl (2-NH$_3$), NH$_3$ and one microliter of each alcohol (typical concentration 20 mM). After hyperpolarization transfer from p-H$_2$ transfer process was undertaken and a fully coupled $^{13}$C NMR measurement made instead of a $^1$H NMR measurement, molecule-diagnostic $^{13}$C and $^1$H-$^{13}$C refocused INEPT based responses could also be recorded in one scan at 9.4 T for all of the alcohols, as illustrated in Fig. 2b for 1-pentanol with the associated signal gains reaching 570-fold for the C$_\alpha$ signal of 1-hexanol. The SABRE-RELAY effect results in the detection of hyperpolarized NMR signals for all of the spin 1/2 nuclei in these molecules. Additionally, as with SABRE, the hyperpolarized NMR terms reflect a mixture of longitudinal single spin, and higher order states, whose relative amplitudes depends on the magnetic field the sample experiences during the polarization transfer step.\textsuperscript{[16, 26]} Furthermore, by reducing the concentrations of these analytes below the concentration

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*Fig. 1: (a) Thermally polarized control $^1$H NMR spectrum showing peaks for 2-NH$_3$, NH$_3$ and H$_2$ at 298 K in dichloromethane-$d_2$, x128 vertical expansion relative to (b). (b) Corresponding single scan $^1$H NMR spectrum in the presence of p-H$_2$ with the hyperpolarized responses for H$_2$O, NH$_3$(free), Ir-NH$_3$(equatorial) and Ir-NH$_3$(axial) of 2-NH$_3$ indicated.*
of NH$_3$, it is possible to improve on SABRE-RELAY efficiency. This is beneficial when studying low concentration analytes, as when propanol was studied, the $^1$H NMR signal gains seen for its OH resonance increased by 100% on moving from a 15 mM to 1.5 mM concentration (Fig S4), while its CH resonances showed a ca. 50% improvement in enhancement level; a $^1$H-$^{13}$C refocused INEPT response was still clearly visible in one scan where the three signals, from the OH end, were 639, 538 and 603 times larger respectively than those in the corresponding $^{13}$C response. This polarization transfer method is also applicable to complex branched alcohols, and when a sample of $^{13}$C labelled glucose was analyzed, a single scan $^{13}$C response could be seen for all of the expected $\alpha$ and $\beta$ forms signals which serves to illustrate the wider significance of this effect (Fig. S15e). Furthermore, our studies show that when SABRE-RELAY is carried out under anhydrous conditions with the straight chain alcohols superior results are obtained.

Our next goal was to expand on the range of materials that can be sensitized by this method. We started with pyruvic acid but found its addition to a solution of 2-NH$_3$ and NH$_3$ resulted in ammonium salt precipitation which acted to limit hyperpolarization efficacy. This can be overcome by the addition of a pH modifier such as Cs$_2$CO$_3$ but working with the corresponding sodium salt proved optimal. Now when $^{13}$C-labelled sodium pyruvate, acetate or propanoic acid samples were studied in the presence of p-H$_2$ strong $^1$H and $^{13}$C signals were seen; the $^{13}$C signal gain for propionic acid was 109 fold. Furthermore, sodium dihydrogen phosphate, adenosine 5’triphosphate disodium, and $^{13}$C-labelled sodium hydrogen carbonate, provided strong $^{31}$P and $^{13}$C responses (Fig. 3a and 3b) while the amides acetamide, urea and methacrylamide show substantial $^1$H, $^{13}$C and $^{15}$N signal gains; for urea a $^{13}$C signal gain of 408-fold was observed. These studies could be completed with NH$_3$/H$_2$O or NH$_3$/CH$_3$OH as detailed in the supplementary material to promote the necessary proton exchange and the observations establish that analytes containing the four common functional groups OH, NH$_2$, CO, PO, and COOH can be employed. In some cases, we see evidence for Schiff base condensation at long reaction times but could suppress this process by adding water.
Fig. 3: Single scan SABRE-RELAY NMR spectra recorded in dichloromethane-$d_2$ with NH$_3$ and 2-NH$_3$ in the presence of $p$-H$_2$ for: (a) sodium adenosine 5'-triphosphate, $^1$H-$^{31}$P refocused INEPT spectrum (OH transfer) and (b) sodium $^{13}$C-labelled pyruvate, $^{13}$C NMR spectrum. Single scan SABRE-RELAY NMR spectra recorded with PEA and 2-PEA in the presence of $p$-H$_2$ for: (c) $^{15}$N-$^{13}$C-labelled urea, $^{13}$C NMR spectrum, 25 mM concentration and (d) $^{15}$N-$^{13}$C-labelled urea, $^{15}$N NMR spectrum, 25 mM concentration. The corresponding thermally polarized spectra are detailed in Fig. S29a, S18a, S23a and S23c and yield no signal.

In order to examine the role of the hyperpolarization transfer agent, we replaced NH$_3$ with benzylamine (BnNH$_2$) or phenethylamine (PEA). Both react with $I$ and H$_2$, forming [Ir(H)$_2$(IMes)(NH$_2$Bn)$_3$]Cl (2-BnNH$_2$) and [Ir(H)$_2$(IMes)(PEA)$_3$]Cl (2-PEA) respectively. For the corresponding 2-BnNH$_2$ sample, signal gains for free BnNH$_2$ of 72- (NH), 53- (CH) and 170-fold (aromatic) respectively per proton are observed (Fig. 4) and these measurements can be repeated if the same sample is probed with $p$-H$_2$ several days after the first observation was made. Phenethylamine (PEA) proved to perform better than BnNH$_2$, with the corresponding NH$_2$ signal gain being 108-fold per proton for a 10-fold loading of $I$ with signal gains of 50- (NCH$_2$), 45- (CH$_2$), 92- (ortho), 50- (meta) and 20- (para) resulting for the other groups. These observations show how polarization transfer through the aliphatic carbon chain into the aromatic protons is possible. BnNH$_2$ and PEA also proved suitable for SABRE-RELAY. In the case of PEA, the efficiency of urea hyperpolarization was found to improve (Fig. 3c and 3d) over that achieved with NH$_3$, although the measured response of $^{13}$C-labelled glucose was found to reduce. Furthermore, replacing BnNH$_2$ with its $d_7$-form C$_6$D$_5$CD$_2$NH$_2$ led to further improvements in observed analytes response level because the initially created SABRE hyperpolarization is now optimally focused into just the NH$_2$ protons.

Given the wide range of amine pK$_b$ values it may be possible to remove the need for an auxiliary base when dealing with acidic analytes through a process of amine variation. We therefore conclude that studies on the role of the amine will be important for the optimization of SABRE-RELAY, and may even allow the introduction of selectivity into the hyperpolarization process. Furthermore as improvements in analyte detectability with SABRE can be easily achieved by varying the polarization transfer field, reducing
relaxation within the analyte, and optimizing the catalyst’s lifetime whilst minimizing its relaxivity we expect the signal gains reported here to be similarly improved upon in the future.\(^{(5)}\)

Discussion

In summary, we have shown that SABRE-RELAY can be used to hyperpolarize a wide range of biologically relevant materials. In the initial step, SABRE, is used to enhance the NH proton response of the selected hyperpolarized transfer agent (the free amine) by between 10- and 120 fold per proton. When this is achieved in the presence of propanol, proton exchange results in its OH signal being amplified by between 250 and 500-fold. The non-equilibrium magnetic state of the OH proton is then successfully relayed into its aliphatic \(^1\)H resonances such that the corresponding signals are amplified by between 650-790 fold per proton. We used this \(^1\)H signal gain, to record a single scan \(^1\)H-\(^{13}\)C refocused INEPT NMR spectrum using just \(1 \times 10^{-7}\) moles of material, although direct transfer to \(^{13}\)C means that a weaker fully coupled \(^{13}\)C response can also be seen. On the basis of these signal gains we hope that this route can be developed to allow the phenotyping of urine via lower-field \(^{13}\)C detection in the future as an alternative to the current high-field \(^1\)H detection methods.\(^{(28)}\) However, as exchangeable protons feature heavily in biochemical NMR we expect harnessing this effect to be of significant interest to biochemists, especially if it is augmented with high field transfer via the “Low-Irradiation Generation of High Tesla-SABRE”\(^{(29)}\) approach. Additionally, as hyperpolarized urea, glucose and pyruvate reflect successful MRI probes of disease\(^{(30, 31)}\), when SABRE-RELAY is coupled with catalyst removal and biocompatibility, we expect this route to become clinically important as it can theoretically deliver a continuously hyperpolarized bolus.\(^{(32)}\) Moreover, as studies of catalysis with \(p\)-\(\text{H}_2\) have made significant contributions to process optimization\(^{(33-37)}\) we expect this
approach to provide insight into important reactions such as transfer hydrogenation,(38) hydroamination(39) and N₂ fixation in the future.(40)

Materials and Methods

Experimental Design
The measurements undertaken in this work were completed on a 400 MHz Avance (III) spectrometer and involved ¹H, ¹³C, ¹⁵N and ³¹P detection as detailed in the supplementary material. Enhancement values were determined according to the methods defined here and sample details will allow the repetition of these measurements which involve the following procedures.

SABRE-RELAY polarization transfer method with NH₃
The polarization transfer experiments that are reported in this study were conducted in 5 mm NMR tubes that were equipped with a J. Young’s Tap. Samples for these polarization transfer experiments were based on a 5 mM solution of [IrCl(COD)(IMes)] and the indicated substrate and NH₃ loadings in methanol-d₄ or dichloromethane-d₂ (0.6 mL). The samples were degassed prior to the introduction of NH₃. Subsequently, parahydrogen at a pressure of ca. 3 bar was added. Samples were then shaken for 10 s in the specified fringe field of an NMR spectrometer before being rapidly transported into the magnet for subsequent interrogation by NMR spectroscopy. This whole process takes ca. 15 seconds to achieve.

SABRE-RELAY polarization transfer method with BnNH₂ or PEA
The polarization transfer experiments that are reported were conducted in 5 mm NMR tubes that were equipped with a J. Young’s Tap. Samples for these polarization transfer experiments were based on a 5 mM solution of [IrCl(COD)(IMes)], the indicated BnNH₂ or PEA loading and the indicated additional substrate at the specified loading in dichloromethane-d₂ (0.6 mL). The samples were degassed prior to the introduction of parahydrogen at a pressure of ca. 3 bar. Samples were then shaken for 10 s in the specified fringe field of an NMR spectrometer before being rapidly transported into the magnet for subsequent interrogation by NMR spectroscopy.

Supplementary Materials
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content
SABRE-RELAY polarization transfer method with NH₃
SABRE-RELAY polarization transfer method with BnNH₂ or PEA
Polarization enhancement quantification procedures
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Fig. S1 Inept pulse sequence.
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Fig. S3 SABRE-RELAY NMR spectra methanol.
Fig. S4 SABRE-RELAY NMR spectra ethanol.
Fig. S5 SABRE-RELAY NMR spectra propanol.
Fig. S6 SABRE-RELAY NMR spectra propanol, low concentration.
Fig. S7 SABRE-RELAY NMR spectra butanol.
Fig. S8 SABRE-RELAY NMR spectra pentanol.
Fig. S9 SABRE-RELAY NMR spectra hexanol.
Fig. S10 SABRE-RELAY NMR spectra heptanol.
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Fig. S14 SABRE-RELAY NMR spectra D-glucose.
Fig. S15 SABRE-RELAY NMR spectra D-glucose-¹³C.
Fig. S16 SABRE-RELAY NMR spectra Glycerol.
Fig. S17 SABRE-RELAY NMR spectra sodium acetate-¹³C.
Fig. S18 SABRE-RELAY NMR spectra sodium pyruvate-¹³C.
Fig. S19 SABRE-RELAY NMR spectra sodium acetate-1,2 ¹³C₂.
Fig. S20 SABRE-RELAY NMR spectra propionic acid-¹³C.
Fig. S21 SABRE-RELAY NMR spectra Sodium hydrogen carbonate-¹³C.
Fig. S22 SABRE-RELAY NMR spectra Urea-¹³C.
Fig. S23 SABRE-RELAY NMR spectra Urea-¹³C-¹⁵N₂.
Fig. S24 SABRE-RELAY NMR spectra Urea$^{13}$C-$^{15}$N$_2$.
Fig. S25 SABRE-RELAY NMR spectra acetamide.
Fig. S26 SABRE-RELAY NMR spectra methacrylamide.
Fig. S27 SABRE-RELAY NMR spectra cyclohexyl methacrylamide.
Fig. S28 SABRE-RELAY NMR spectra mono sodium dihydrogen orthophosphate.
Fig. S29 SABRE-RELAY NMR spectra adenosine 5-triphosphate disodium salt.
Fig. S30 SABRE-RELAY NMR spectra ammonia in methanol.
Fig. S31 SABRE-RELAY NMR spectra ammonia in dichloromethane.
Fig. S32 SABRE-RELAY NMR spectra benzylamine.
Fig. S33 SABRE-RELAY NMR spectra benzylamine-$^{15}$N.
Fig. S34 SABRE-RELAY NMR spectra, mixture of urea, propanol and PEA.

Table S1: Alcohol $^1$H SABRE-RELAY signal enhancement values
Table S2: Alcohol $^{13}$C SABRE-RELAY signal enhancement values
Table S3: NMR data for $^{2}$NH$_3$
Table S4: NMR data for $^{2}$BnNH$_2$

References


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Competing interests: The authors declare no competing interests. The University of York as filed a patent application based on the results presented in this paper. Data and materials availability: Raw data can be found via: DOI: 10.15124/5e44a9c2-c968-42e9-b8f0-885a552bafa0.