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SABRE-Relay: A Versatile Route to Hyperpolarization

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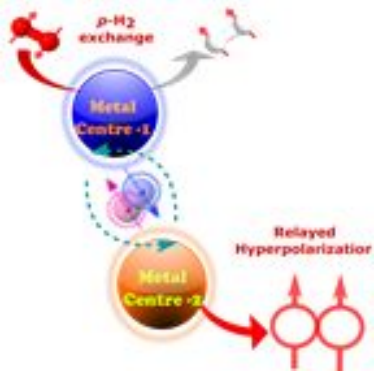
Supporting Information Placeholder

ABSTRACT: Signal Amplification by Reversible Exchange (SABRE) is used to switch on the latent *singlet* spin order of *para*-hydrogen (p-H_2) so that it can hyperpolarize a substrate (sub = nicotinamide, nicotinate, niacin, pyrimidine and pyrazine). The substrate then reacts reversibly with $[Pt(OTf)_2(bis\text{-}diphenylphosphinopropane)]$ by displacing OTf to form [Pt(OTf)(sub)(bis-diphenylphosphinopropane)]OTf. The ^{31}P NMR signals of these metal complexes prove to be enhanced when the substrate possesses an accessible singlet state or long-lived Zeeman polarization. In the case of pyrazine, the corresponding ^{31}P signal was 105 ± 8 times larger than expected, which equated to an 8-hour reduction in total scan time for an equivalent signal to noise ratio under normal acquisition conditions. Hence p-H_2 derived spin order is successfully relayed into a second metal complex via a suitable polarization carrier (sub). When fully developed we expect this route involving a second catalyst to successfully hyperpolarize many classes of substrate that are not amenable to normal SABRE.



The huge sensitivity improvement that is provided by hyperpolarization has significantly extended the scope of magnetic resonance based *in vivo* study.[1-3] While several hyperpolarization methods are available to achieve this result, the *para*-hydrogen induced hyperpolarization (PHIP) route is popular because it is fast, simple and relatively low-cost.[4-5] Classically PHIP, however, needs to chemically modify the target substrate and this makes it unsuitable for some agents.[6] This limitation has partially been overcome through the variant of PHIP known as Signal Amplification by Reversible Exchange, termed as SABRE, which no longer relies on active hydrogenation to deliver hyperpolarized material in seconds.[7] Since its inception, SABRE has become highly successful in delivering huge sensitivity enhancements to a wide range of molecular systems that are clinically relevant.[8-11] It has been shown to work for NMR active nuclei such as ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P that feature in substances such as nicotinamide, nicotinate, pyridazine, diazirine and imidazole and achieves net polarization levels as high as 50% for ^{1}H and 20% for ^{15}N.[10, 12-15] Recently, SABRE has been combined with long-lived states such that the hyperpolarized signals it creates remain visible for up to 30 minutes.[16-21] This exciting development reflects one route to overcome the normal relaxation time scale of NMR which limits many methods. Such advancements are beginning to feature in human metabolomics where the creation of tools for the diagnosis of disease is possible.[1-3]

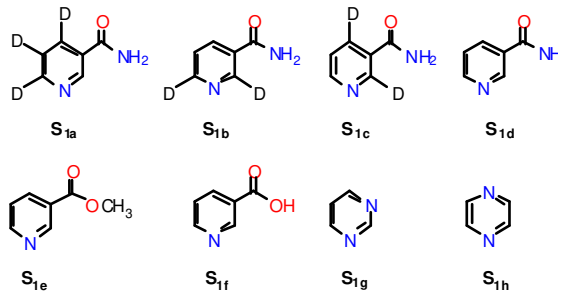
Despite the general success of the SABRE hyperpolarization technique, the signal enhancements achieved by this process are currently limited to resonances that originate in ligands which were previously bound to the polarization transfer catalyst ($\mathbf{M_1}$). Although, several types of spin system have been shown to perform well with SABRE there is a need to make this approach both robust and more generally applicable. In this study, we show how it is possible to sensitize a second metal complex ($\mathbf{M_2\text{-}S_1}$) via the *relay* of hyperpolarization from substrate ($\mathbf{S_1}$) in a process we term SABRE-*Relay*. Given that $\mathbf{M_2\text{-}S_1}$ no longer needs to react with H_2, and that with development it could contain labile ligands, we expect this approach to widen the range of substrates which can be hyperpolarized by this type of approach. Scheme 1 illustrates the basis of this effect.

Scheme 1: Schematic depiction of the SABRE-Relay; substrate S_1 binds reversibly alongside p-H_2 to metal complex M_1 and becomes hyperpolarized. S_1 then binds reversibly to M_2-S_1 and polarization is relayed into its ^{31}P response.

The singlet state concept is central to the SABRE process. In a pair of coupled spin-1/2 nuclei (e.g. H_2), the term singlet state relates to their antisymmetric spin eigenstate.[22] The PHIP concept[5] then harnesses this singlet state to facilitate enhanced NMR detection. Remarkably in 2004, it was shown by Levitt and co-workers that such singlet spin order can be created by suitable radio frequency (*r.f.*) pulses in many ordinary molecules and the resulting nuclear spin lifetime can extend beyond the more usual T_1 boundary.[23] The long-lived nature of these singlet states can be traced back to the fact that they are immune to one of the major relaxation causing mechanisms, the intra-pair dipole-dipole pro-

cess.[22] Long-lived singlet states (LLS) are now being used for hyperpolarization storage, to obtain molecular structure information, and to follow slow-molecular processes.[24-28] Warren and co-workers developed a related technique to access LLS in chemically equivalent spin systems by exploiting magnetic inequivalence.[29-30] In previous work, it has been shown that SABRE derived hyperpolarized LLS can be formed and accessed for several minutes after storing either in high or low-magnetic fields.[18-21]

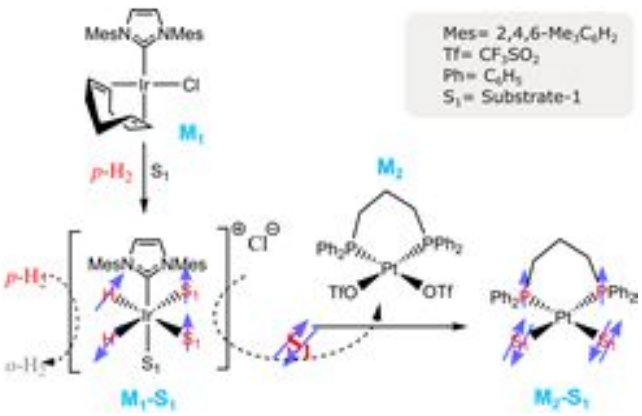
Scheme 2: Identities of S_1 used in this SABRE-Relay study.



In these experiments, SABRE is first used to hyperpolarize the exchangeable substrate $\mathbf{S_1}$ of Scheme 2 via its scalar-coupling framework. When these are two-spin substrates, the hyperpolarization is associated with both Zeeman and singlet spin order as detailed in Scheme 3. Alternatively, the resulting Zeeman polarization can be turned into singlet spin order via *r.f.* excitation as detailed.[16-21]

$\mathbf{S_1}$ is binding reversibly to $\mathbf{M_2}$-$\mathbf{S_1}$ throughout this process according to Scheme 3. If $\mathbf{S_1}$ were to have singlet spin order and its symmetry were to be broken through the *J*-coupling network of M_2-S_1, its latent polarization should be unlocked and transferred further, in this case into ^{31}P.[31] This strategy reflects the relayed transfer of polarization from *p*-H_2 into a second agent that never actually comes into contact with H_2 and hence the key requirement is that hyperpolarized $\mathbf{S_1}$ has a long lifetime. This process is depicted in Scheme 3 for [Pt(S_1)$_2$(dppm)](OTf)$_2$ ($\mathbf{M_2}$-$\mathbf{S_1}$). Here, $\mathbf{M_2}$–$\mathbf{S_1}$ rapidly forms from [Pt(OTf)$_2$(dppm)], where the bidentate phosphine controls the lability of the triflate (OTf) and the potential for any substrate ($\mathbf{S_1}$) to bind (see supporting information section S3 and S4 for more details).

Scheme 3: Precatalyst [IrCl(IMes)(COD)] ($\mathbf{M_1}$) is transformed into [Ir(H)$_2$(IMes)($\mathbf{S_1}$)$_3$]Cl ($\mathbf{M_1}$-$\mathbf{S_1}$) by adding *para*-hydrogen (*p*-H_2) gas and substrate $\mathbf{S_1}$. $\mathbf{S_1}$ then gains hyperpolarized Zeeman and singlet spin order via polarization transfer from *p*-H_2 depending on its identity. In a second step, the ^{31}P response of $\mathbf{M_2}$-$\mathbf{S_1}$ becomes hyperpolarized.



For context, it has been shown experimentally that the addition of *p*-H_2 leads to sensitization of the ^{31}P signature of Ru(H)$_2$(dppp)(PPh$_3$)(CO). This effect was explained theoretically on the basis of the coherent evolution of the zero quantum (ZQ) coherence associated with the *p*-H_2 singlet state under ^{1}H-^{31}P spin-spin coupling.[32-34] Furthermore, the complex Ir(H)$_2$Cl(PPh$_3$)$_3$ has been shown to hyperpolarize its bound ^{31}P responses in addition to that of PPh$_3$ via SABRE.[35-36] In contrast, the transfer of single spin based ^{129}Xe hyperpolarization into a second agent has been shown to proceed via the incoherent spin polarization-induced nuclear Overhauser effect (SPINOE).[37-38] There is also the possibility of coherent Zeeman order transfer.[39-40] Hence there are several well defined pathways for hyperpolarization transfer between diamagnetic materials that might operate here.

In this section, we demonstrate the experimental viability of SABRE-Relay. We employ the efficient SABRE precursor [IrCl(IMes)(COD)] ($\mathbf{M_1}$) in all cases. Standard SABRE methods are used to polarize the carrier substrates ($\mathbf{S_1}$) of Scheme 3 in the presence of $\mathbf{M_1}$. Platinum based $\mathbf{M_2}$ contains bis-diphenylphosphinopropane (dppp), bis-diphenylphosphinomethane (dppm) and bis-diphenylphospinoethane (dppe) and a pair of weakly bound triflate ligands. Synthetic details for the formation of $\mathbf{M_2}$, and its reactions to form $\mathbf{M_2}$-$\mathbf{S_1}$ can be found in the supporting information (Section S3 and S4). Samples were prepared in deuterated-methanol and contained a 1:1 ratio of $\mathbf{M_1}$ and $\mathbf{M_2}$, with each having a concentration of 5 mM and substrate loading ($\mathbf{S_1}$) of 50 mM. These solutions were then degassed prior to activation with *p*-H_2. This led to the formation of [Ir(H)$_2$(IMes)($\mathbf{S_1}$)$_3$]Cl. Subsequently, SABRE transfer was undertaken at a range of magnetic mixing fields and a series of ^{1}H and ^{31}P NMR measurements made according to the process detailed in Figure 1. After bubbling with *p*-H_2 at two different magnetic fields, the solution was then transferred into the magnet for NMR measurement which took place after the application of simultaneous 90° pulses to both channels. The magnetic field cycling illustrated is needed to optimally polarize the corresponding ^{1}H and ^{31}P responses of $\mathbf{S_1}$ and $\mathbf{M_2}$-$\mathbf{S_1}$ respectively and relates to ^{1}H-^{15}N transfer as exemplified by Theis *et al.*[16] The formation of a singlet state within the carrier substrate ($\mathbf{S_1}$) was realized either naturally (protocol-1: low-field hyperpolarization) or by a suitable *r.f.* based magnetization-to-singlet (M2S) pulse sequence (protocol-2).[23] We note that hyperpolarized Zeeman derived spin order remains present when the sample enters the high field magnet for observation because the SABRE process is on-going.

In the first measurement, $\mathbf{S_1}$ was H_1-nicotinamide $\mathbf{S_{1a}}$ of Scheme 3. The resulting ^{1}H NMR signals for $\mathbf{S_{1a}}$ showed a 370 ± 20-fold Zeeman based signal enhancement after SABRE (see Table 1) at 6 mT but no ^{31}P NMR signal was detected for $\mathbf{M_2}$-$\mathbf{S_{1a}}$ after rapid transfer into the high-field spectrometer for observation according to protocol 1. In this case, $\mathbf{S_{1a}}$ contains a single proton and hence there is no possibility to create singlet order in $\mathbf{S_{1a}}$ alone. A further control experiment was performed without *p*-H_2 and again no ^{31}P NMR signal was seen for $\mathbf{M_2}$-$\mathbf{S_{1a}}$ in a single scan measurement, although its formation was confirmed after appropriate signal averaging. Hence, we conclude that the presence of $\mathbf{M_2}$ and $\mathbf{M_2}$-$\mathbf{S_{1a}}$ does not stop SABRE from operating with $\mathbf{M_1}$, and that substrate $\mathbf{S_{1a}}$ is unable to relay polarization into $\mathbf{M_2}$-$\mathbf{S_{1a}}$. Furthermore, we note that $\mathbf{M_2}$-$\mathbf{S_1}$ is not hyperpolarized as a consequence of the *p*-H_2 that is in solution. We interpret these results to suggest that any single spin Zeeman derived hyperpolarization or SPINOE transfer is at best weak and note that the short ^{1}H-relaxation times might account for this.[41]

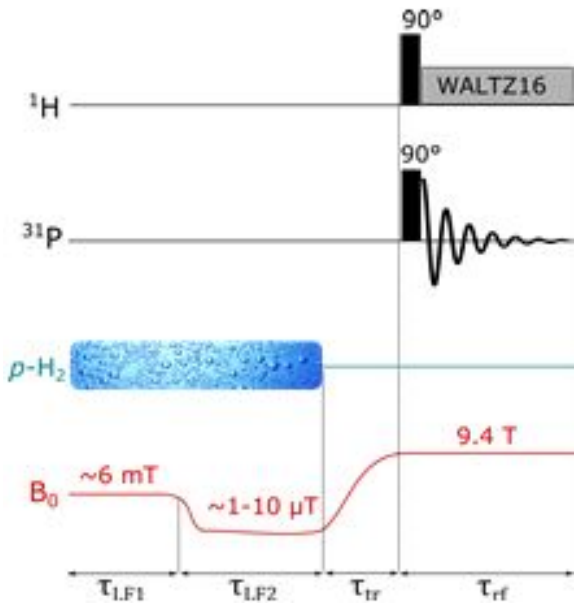


Figure 1. Experimental scheme for SABRE-Relay, showing timings, magnetic field variance and *r.f.* sequence. First the sample is mixed with enriched *p*-H_2 at low magnetic field (~6 mT and ~1-10 μT) for the durations of τ_{LF1} and τ_{LF2} before moving to high field (τ_{tr}) for NMR observation. A simultaneous 90° pulse is applied to ^{1}H and ^{31}P prior to acquiring the ^{31}P signal with ^{1}H decoupling (protocol 1). In a second variant, protocol 2, an M2S sequence[23] is applied between τ_{LF1} and τ_{LF2}.

We then examined the three variants of nicotinamide, $\mathbf{S_{1b}}$, $\mathbf{S_{1c}}$ and $\mathbf{S_{1d}}$ where we have previously demonstrated that both Zeeman and singlet order can be created in their aromatic protons via SABRE and *r.f.* driven transfer.[19] All three of these substrates now yield SABRE hyperpolarized ^{1}H NMR signals with signal gains lying between 200 and 250-fold per proton for their Zeeman polarizations after transfer at 6 mT when observed at 9.4 T. A ^{31}P NMR measurement was then made on each sample according to protocol 1 of Figure 1. This resulted in the detection of a ^{31}P signal for both of the inequivalent phosphorus centers in the corresponding $\mathbf{M_2}$-$\mathbf{S_1}$ complexes. These signals were enhanced over their thermally polarized levels by 65, 88 and 32-fold respectively. The corresponding enhancement values were determined by comparison with signal averaged ^{31}P NMR spectra of $\mathbf{M_2}$-$\mathbf{S_1}$. When protocol 2 for $\mathbf{S_{1b}}$ and $\mathbf{S_{1c}}$ was applied, the resulting ^{31}P signal gains were 42-fold and 50-fold respectively. It is likely that the weaker ^{31}P signal gains result from the longer experiment time. We note that the similarity in the single proton relaxation times of $\mathbf{S_{1a}}$ and $\mathbf{S_{1b}}$ suggests again that any SPINOE or Zeeman derived contribution would be weak.

These observations can be explained if the singlet symmetry of $\mathbf{S_1}$ is broken upon binding and polarization transfer into the ^{31}P nuclei of $\mathbf{M_2}$.$\mathbf{S_1}$ occurs. In the case of $\mathbf{S_{1b}}$ a 5-bond ^{1}H-^{31}P coupling would be involved in this step whilst for $\mathbf{S_{1c}}$ and $\mathbf{S_{1d}}$ larger four bond couplings would operate. The reduced enhancement level seen with $\mathbf{S_{1d}}$ is predicted to reflect the rapid relaxation of its singlet state in the reaction mixture (Table 1). The corresponding values for $\mathbf{S_{1b}}$ and $\mathbf{S_{1c}}$ are longer but still smaller than their 34 s and 39 s values in the absence of the catalyst. Hence in accordance with this hypothesis, the presence of $\mathbf{M_1}$ and $\mathbf{M_2}$-$\mathbf{S_1}$ is seen to impact directly on the T_{LLS} lifetimes. Figure 2 shows the corresponding ^{31}P NMR traces after the 1-shot SABRE-Relay process with $\mathbf{S_{1a}}$, $\mathbf{S_{1b}}$, $\mathbf{S_{1c}}$, and $\mathbf{S_{1d}}$ to illustrate this behavior.

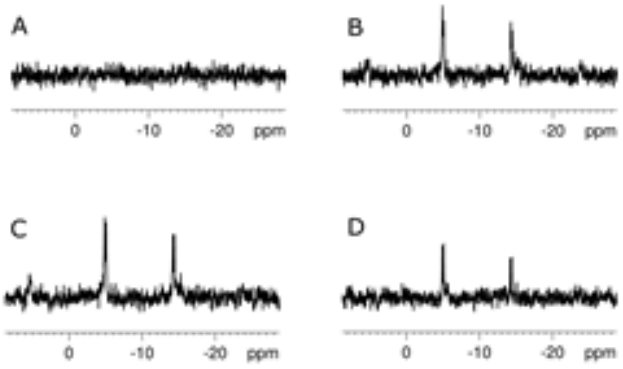


Figure 2. Single scan ^{31}P{^{1}H} NMR spectra associated with (A) $\mathbf{M_2}$-$\mathbf{S_{1a}}$; (B) $\mathbf{M_2}$-$\mathbf{S_{1b}}$; (C) $\mathbf{M_2}$-$\mathbf{S_{1c}}$; (D) $\mathbf{M_2}$-$\mathbf{S_{1d}}$ using SABRE-Relay protocol 1 of Fig. 1.

Table 1. ^{1}H and ^{31}P signal enhancements and lifetimes determined for free $\mathbf{S_1}$ and the two ^{31}P signals of $\mathbf{M_2}$-$\mathbf{S_1}$ achieved with a 50 mM loading of $\mathbf{S_1}$ at 9.4 T recorded using protocol 1.

Substrate ($\mathbf{S_1}$)	^{1}H NMR signal enhancement of $\mathbf{S_1}$	T_1 (sec) of specified free substrate protons	T_{LLS} (sec) of the free proton pair	^{31}P signal enhancement determined for $\mathbf{M_2}$ containing $\mathbf{S_1}$ and $\mathbf{S_2}$
4,5,6-d_3-nicotinamide ($\mathbf{S_{1a}}$)	370 ± 20	H2: 6.5 ± 0.4	-	None
2,6-d_2-nicotinamide ($\mathbf{S_{1b}}$)	242 ± 18	H4: 6.0 ± 0.3 H5: 7.2 ± 0.3	25.5 ± 4.4	65 ± 5
2,4-d_2-nicotinamide ($\mathbf{S_{1c}}$)	233 ± 12	H5: 13.3 ± 0.4 H6: 9.5 ± 0.4	29.0 ± 3.5	88 ± 4
Nicotinamide ($\mathbf{S_{1d}}$)	204 ± 15	H2: 11.5 ± 0.6 H4: 5.6 ± 0.3 H5: 4.8 ± 0.3 H6: 7.0 ± 0.5	H_{4-5}: 12.3 ± 3.3	56 ± 5*
Methyl Nicotinate ($\mathbf{S_{1e}}$)	638 ± 50	H2: 4.7 ± 0.5 H4: 7.4 ± 0.5 H5: 2.0 ± 0.3 H6: 3.5 ± 0.3	H_{4-5}: 10.2 ± 3.6	35 ± 5
Niacin ($\mathbf{S_{1f}}$)	120 ± 8	H2: 13.1 ± 0.8 H4: 8.2 ± 0.5 H5: 4.2 ± 0.3 H6: 8.9 ± 0.7	H_{4-5}: 13.5 ± 4.0	25 ± 5
Pyrimidine ($\mathbf{S_{1g}}$)	571 ± 65	H2: 15.5 ± 0.6 H4/H6: 10.4 ± 0.6 H5: 8.4 ± 0.3	$H_{4/6-5}$: 18.4 ± 4.2	102 ± 12
Pyrazine ($\mathbf{S_{1h}}$)	352 ± 18	3.8 ± 0.2	-	105 ± 8

*protocol 2

An improvement in the level of ^{31}P NMR signal gain, from 32-fold to 56-fold is observed with $\mathbf{M_2\text{-}S_{1d}}$ when protocol 2 is used. This again suggests the involvement of a coherent spin order transfer mechanism leading to the hyperpolarized ^{31}P NMR signal. Similar experimental strategies were then employed to examine related $\mathbf{S_{1e}}$ and $\mathbf{S_{1f}}$. In the case of $\mathbf{S_{1e}}$, while very strong ^{1}H SABRE results the relayed ^{31}P signal gains are lower. This is again likely to be a consequence of their relatively short magnetic state lifetimes.

When substrates $\mathbf{S_{1g}}$ (pyrimidine) and $\mathbf{S_{1h}}$ (pyrazine) were examined, the observed ^{31}P NMR signal enhancements increased to over 100-fold (Table 1). Figure 3 shows the corresponding ^{31}P NMR spectra of $\mathbf{M_2\text{-}S_{1g}}$ and $\mathbf{M_2\text{-}S_{1h}}$ when a 50 mM substrate concentration was employed with $\mathbf{M_2}$ at the 5 mM level. This improvement confirms that the identity and properties of $\mathbf{S_1}$ are important in controlling the visibility of $\mathbf{M_2\text{-}S_1}$. In these two cases, slow exchange is predicted which confirms that catalyst lifetime plays a role in this process.

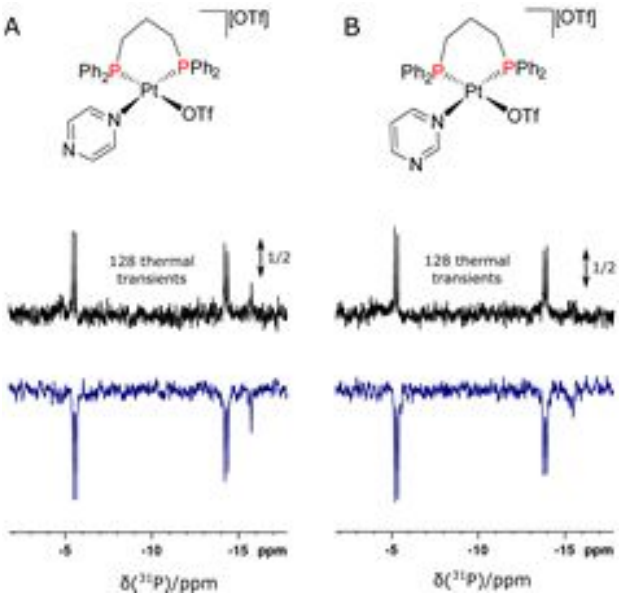


Figure 3. ^{31}P{^{1}H} NMR spectra associated with $\mathbf{M_2\text{-}S_{1g}}$ and $\mathbf{M_2\text{-}S_{1h}}$ (structures above NMR spectrum) that form from $\mathbf{M_2}$ and $\mathbf{S_{1g}}$ or $\mathbf{S_{1h}}$ respectively. In both cases, the upper ^{31}P NMR spectrum is the control which involved 128 transients, whilst lower NMR spectrum was acquired by the SABRE-Relay through process 1 and associated with a single detection pulse according to Figure 2.

In a final refinement, we studied two other platinum metal complexes - [Pt(OTf)$_2$(dppm)] and [Pt(OTf)$_2$(dppe)], and similar SABRE-Relay experiments were performed. While ^{31}P-signal enhancements were again observed, they were significantly lower than those seen for the dppp complex. The decrease in enhancement factor reflects a combination of residence time and relaxation effects. We are currently studying these effects in greater detail. Nonetheless, the associated signal enhancements confirm that several metal complexes are active for SABRE-Relay. The experimental details, and results for [Pt(OTf)$_2$(dppm)] and [Pt(OTf)$_2$(dppe)] are presented in supporting information sections S3-S5.

In summary, we have demonstrated how SABRE can be cascaded into a second metal complex via a coherent transfer pathway involving a series of hyperpolarized substrates. This involved the detection of enhanced ^{31}P NMR responses in a metal complex which does not interact directly with H_2. Hence, we have presented a route to overcome one of the key SABRE limitations, associated with *p*-H_2 being the singlet carrier. N-heterocycles can bind to many metal complexes which in turn may contain other labile ligands. We expect to be able to use SABRE-Relay to enhance new classes of agents that are not amenable to the traditional SABRE hyperpolarization route. The mechanism of transfer is likely to be based on a coherent spin order route such as that involved in singlet state evolution via S_1 which is directly analogous to the original SABRE concept. However, we note that this process simply involves propagation of the low-field created ZQ coherence but other routes involving coherent polarization transfer from Zeeman order under these low field conditions may contribute and we are now seeking to differentiate their contributions.[40, 42] Regardless of the pathway, we take advantage here of what would be expected to be relatively large ^{31}P couplings to propagate these effects and therefore expect an efficient second step. Given the interest in hyperpolarized MRI the potential of this approach to improve magnetic resonance sensitivity may be significant and we are currently working on the optimization of this technique.

ASSOCIATED CONTENT

Supporting Information

Synthetic routes; experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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