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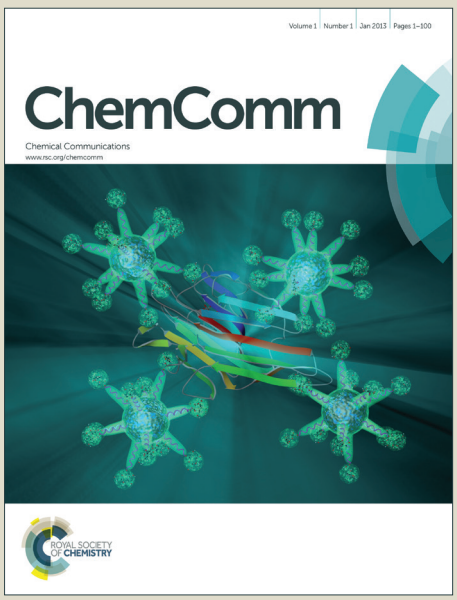

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Deactivation of signal amplification by reversible exchange catalysis, progress towards *in vivo* application

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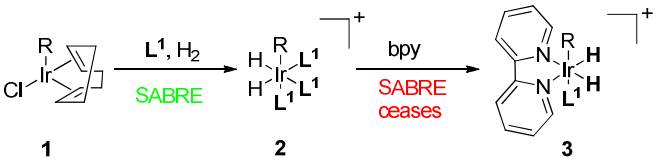
The catalyst which is used in the signal amplification by reversible exchange (SABRE) process facilities substrate hyperpolarisation while acting to speed up the rate of relaxation. Consequently, the lifetime over which the hyperpolarised contrast agent is visible is drastically reduced. We show that the addition of a chelating ligand, such as bipyridine, rapidly deactivates the SABRE catalyst thereby lengthening the agent's relaxation times and improving the potential of SABRE for diagnostic MRI.

Hyperpolarization techniques are finding widespread use in overcoming the inherent sensitivity issue associated with NMR and MRI. Recently, various groups have turned their attention to developing these techniques clinical context where improved signal-contrast facilitates studies of the fate of biologically relevant species. While long-lived ^{13}C-signals of pyruvate,[1-3] created by Dynamic Nuclear Polarization (DNP), have entered clinical trials for diagnosis of colon cancer, optically pumped noble gases are finding use in the study of lung disease.[4-7]

A related technique, known as Signal Amplification By Reversible Exchange (SABRE),[8] exploits the singlet state of *para*hydrogen (p-H_2) to polarize substrates without changing their chemical identity. This approach builds on the earlier PHIP (*para*hydrogen induced polarisation) effect that was pioneered by Weitekamp[9, 10] and Eisenberg[11] by transferring polarisation from p-H_2 at low magnetic field into a bound substrate via a transient J-coupling framework. This framework is created at a metal where the resulting complexes hydride ligands and substrate spin ½ nuclei readily share their magnetisation.[12-18] SABRE has the potential to provide a low-cost route to the production of hyperpolarised and biologically relevant molecules that could be applied as contrast agents in MRI. The approach benefits from the opportunity to deploy continuous polarization to create a permanently available source for bolus injection or slow infusion.[19]

Currently, homogeneous methods are most successfully employed for polarisation transfer catalysis (PTC) under SABRE[8, 13, 15, 17, 17, 20, 20] and while examples of heterogeneous catalysis have been reported their efficiency is lower.[21] If SABRE is to be used clinically, however, it is highly likely that the catalyst must be deactivated prior to injection, or ideally attached to a chemically robust scaffold. This communication demonstrates that catalyst deactivation is achievable whilst still delivering high levels of ^{1}H polarisation into the analyte.

Scheme 1 Formation of SABRE active **2** and deactivated **3** from **1** (R = IMes).



We achieve this by demonstrating that catalysis can be effectively stopped after polarisation transfer by targeting the hyperpolarisation of nicotinamide (**L^1**) in the presence and absence of 2,2'-bipyridine (bpy). **L^1** has been examined previously under SABRE conditions and can be polarised in methanol as well as more biologically relevant solutions.[8, 20] The addition of this substrate to [Ir(IMes)(cod)Cl],[22] **1**, under an atmosphere of p-H_2 and in deuterated methanolic solution, results in the formation of [Ir(H)$_2$(IMes)(L^1)$_3$]Cl, **2** according to Scheme 1. Samples containing SABRE catalyst **2** were then hyperpolarised in low magnetic field prior to being transferred into a 9.4 T NMR spectrometer where ^{1}H NMR spectra were recorded.[12, 20] When methanol-d_4 samples consisting of **2** and **L^1** in the ratio of 1 : 5 were hyperpolarised by SABRE in a magnetic field of 65 G, ^{1}H NMR signal enhancements were observed for the proton resonances of **L^1** that range from −450 fold (H_A) to −73 fold (H_C). The corresponding relaxation times

of the magnetic states that give rise to signals H_A-H_D were also determined at 9.4 T. These data are shown in Table 1.

Table 1. ^{1}H NMR signal enhancements (fold) following SABRE at 65 G by **2**, and T_1 data in the absence of air but under H_2 for the indicated proton spins of $\mathbf{L^1}$, with and without **2** or **3** at 9.4 T.

Proton label	^{1}H enhancement / (fold, %)	T_1 (free form) /s	T_1 /s (with **2**)	T_1 /s (with **3**)
H_A	−450 (1.5)	43.1	7.0	38.0
H_B	−334 (1.0)	11.3	6.1	11.5
H_C	−73 (0.2)	13.5	3.7	14.0
H_D	−355 (1.1)	6.6	4.3	6.8

The process of SABRE[16, 22] must catalyse magnetisation transfer in both directions, so in conditions where *p*-H_2 is no longer present it should lead to the quenching of the resulting hyperpolarization by polarising hydrogen. This effect can be viewed by probing the T_1 values of the associated resonances which should then correspond to their exchange weighted average.[23, 24] We analysed the data for H_a, and while the observed T_1 value of H_a for free and equatorially bound nicotinamide was *ca*. 7.0 s, deconvolution of the exchange effect suggested their real values were 1.13 s when bound and 39.9 s when free which validate this hypothesis.

We then tested the effect of adding an equimolar amount of 2,2'-bipyridine (bpy), relative to $\mathbf{L^1}$, to the hyperpolarisation sample. We observed that the light yellow solution of **2** turned bright yellow and that the corresponding ^{1}H NMR spectra showed evidence for a number of effects. The first of these involved the detection of a new iridium complex that yields a dominant hydride signal at δ −20.12. The species responsible for this signal is $[Ir(H)_2(IMes)(bpy)(L^1)]Cl$, **3**, where the bpy ligand lies *trans* to both hydride ligands, and $\mathbf{L^1}$ is *trans* to IMes. This complex was fully characterised by NMR and X-ray diffraction (see SI) and exists with a minor isomer where the bpy ligand is *trans* to hydride and IMes at 4% of the major isomer level. Under these conditions, the ^{1}H NMR signals for $\mathbf{L^1}$ remain hyperpolarised in the trace recorded immediately after bpy addition. Subsequent re-polarisation of the sample, now containing **2** and **3**, results in a drop in the level of visible hyperpolarisation in $\mathbf{L^1}$ as SABRE catalysis is reduced in efficiency. At 298 K, the nicotinamide dissociation rate in **2** is 6.7 ± 0.1 s^{-1} and hence the minimum time for 97 % conversion to **3** is *ca*. 1.2 s and so rapid quenching is possible. Figure 1 portrays the time-dependent drop in hyperpolarisation level after byp addition when the concentrations of **2** and bpy were 6.25 and 32 mmol dm^{-3} respectively. The rate of fall in hyperpolarisation for resonance H_a is 0.022 s^{-1} under these conditions, which reflect concentrations that facilitate the NMR observation of the quenching process.

The formation of **3** also results in an increase in the $T_{1(\text{effective})}$ values of the ^{1}H nuclei of $\mathbf{L^1}$ as shown in Table 1 relative to those that are measured when an equivalent concentration of **2** is present. This means that the period for which the hyperpolarisation target retains a detectable level of hyperpolarisation is dramatically extended. In the former case, after 3 T_1's or, 19.5 seconds just 5 % of the hyperpolarised H_a signal remains, but after bpy addition the time taken to reach this point increases to 136 seconds. While we recognise that methanol is not suitable for in vivo study, the polarisation of nicotinamide in ethanol has been reported and dilution for biocompatibility is feasible.[20, 25]

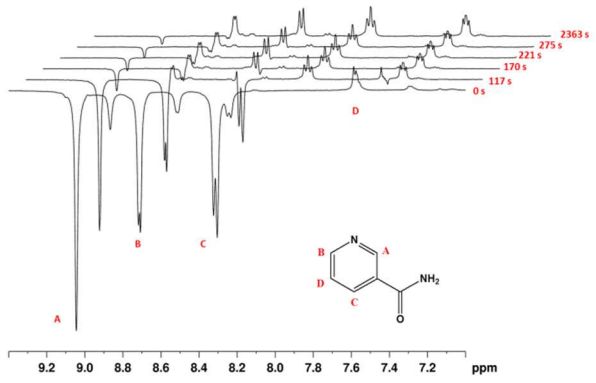


Figure 1. Organic region of a series of ^{1}H NMR traces following polarisation transfer from *p*-H_2 at 65 G of a sample that initially contained just $\mathbf{L^1}$ and **1** (ratio 7 : 1), after the addition of 5.1 equivalents of bpy relative to $\mathbf{L^1}$. These trace reveal the quenching of SABRE by bpy.

A series of selective NOESY spectra were recorded over the temperature range 273–313 K to rationalise this behaviour. These data established that the hydride ligands of the major isomer of **3** do not exchange on the NMR timescale with the protons of free dihydrogen. As this process follows substrate dissociation,[12, 13] these data confirm its inertness to SABRE and rationalise the dramatic extension in T_1 values seen for $\mathbf{L^1}$.

In a separate study, bpy was replaced by 1,10-phenanthroline. In this case, **2** reacts to form $[Ir(H)_2(IMes)(1,10\text{-phenanthroline})(L^1)]Cl$, **4**, a similar product to **3**, with hydride signal at δ −19.95. Furthermore, the rate of fall in the level of hyperpolarised magnetisation is 33% faster, and the corresponding minor isomer is seen at a *ca*. 2%. Hence 1,10-phenanthroline reflects the better trapping agent. A methanol-d_4 solution of **2** and $\mathbf{L^1}$ in the ratio of 1 : 7 was hyperpolarised by SABRE and then treated with 1,10-phenanthroline (8 fold relative to **2**) by injection through air and the hyperpolarised signal probed by a series of low-tip angle pulses in order to monitor the hyperpolarised signal decay rate. It took *ca*. 170 seconds for the proton NMR signal of $\mathbf{L^1}$ to return to that expected for magnetisation at thermal equilibrium.

Our studies with 1,10-phenanthroline were then extended to include the substrate pyridine ($\mathbf{L^2}$). This time, however, a sample consisting of a four-fold excess of $\mathbf{L^2}$ relative to **1** was hyperpolarised under SABRE with the result that the three proton sites of pyridine yielded -868.2 (*ortho*), -166.1 (*meta*) and -470.1 (*para*) fold enhancements. This sample was then repolarised and rapidly treated with 1,10-phenanthroline (two-fold excess relative to $\mathbf{L^2}$) by injection while exposed to air. The resulting signal enhancement now proved to be 338-fold across the five ^{1}H protons of $\mathbf{L^2}$. When this hyperpolarised signal was probed by a series of low-tip angle pulses, it took *ca*.

94 seconds for the hyperpolarisation effect to disappear. In this case, the quenched catalyst $[Ir(H)_2(IMes)(1,10\text{-phenanthroline})(py)]Cl$, **5** provides a hydride signal at δ −20.01.

We also tested whether light could be used to re-activate the SABRE catalyst as we hoped to produce a light controllable system. However, when a solution containing **3** and $\mathbf{L^1}$ was placed in a quartz cell and irradiated by UV-light no polarisation transfer to $\mathbf{L^1}$ occurred. We conclude that UV-light is unable to promote the necessary bpy loss.

Despite this lack of photochemical activity, we have shown that the addition of 2,2'-bipyridine or 1,10-phenanthroline to solutions of $[Ir(H)_2(IMes)(L)_3]Cl$ leads to the partial displacement of **L** and the formation of $[Ir(H)_2(IMes)(bpy)(L^1)]Cl$ and $[Ir(H)_2(IMes)(1,10\text{-phenanthroline})(L^2)]Cl$ respectively. These complexes fail to actively catalyse the SABRE process with the result that the experimentally observed relaxation times of the proton spins in the free substrate **L** are dramatically increased. Experimentally this means that the visible lifetime of the hyperpolarised signals are also extended such that the potential of SABRE for clinical MRI measurement is increased. We seek to use this approach to aid in the removal of the catalyst from solution in the future and note that DNP uses a functionalised polyethylene frit[10] to remove the electron paramagnetic agent (EPA) used for hyperpolarisation transfer and hence there is precedent for successful catalyst sequestration prior to in vivo injection.[9]

Notes and references

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