Hyperpolarized Fumarate via Parahydrogen

Barbara Ripka,a† James Eillsb,†*, Hana Kouřilováb, Markus Leutzscht, Malcolm H. Levittb, and Kerstin Münnemannbd

Received 00th January 20xx, Accepted 00th January 20xx
DOI: 10.1039/x0xx00000x

We produce hyperpolarized [1-13C]fumarate in the proton nuclear spin singlet state by pairwise trans-addition of parahydrogen to a molecular precursor using a ruthenium-based catalyst in water. The proton singlet state is transformed into observable carbon magnetization by radiofrequency pulses to enhance the 13C signal by a factor of 1000 using 50% para-enriched hydrogen gas.

NMR methods suffer from intrinsically weak signals, which makes in vivo imaging of metabolites at physiological concentrations very challenging. To overcome this barrier, hyperpolarization of the nuclear spins is a necessity, and can lead to signal enhancements of ~10^5-10^6 in a typical MRI scanner [1-3]. For example, hyperpolarized magnetic resonance imaging may be used to study the enzymatic conversion of fumarate (trans-butenedioic acid) into malate (2-hydroxybutanedioic acid), which acts as a marker for dying or diseased cells [4-6].

Currently, dissolution-dynamic nuclear polarization (dDNP) [7,8] is the technique used to hyperpolarize fumarate. Despite significant advances in this field over the last decade, dDNP still has some limitations: expensive equipment is required, the hyperpolarized material is delivered in batch mode, and the polarization build-up time per batch is in the order of one hour. An alternative hyperpolarization method is PHIP (Para-Hydrogen Induced Polarization) [9-11], which uses the nuclear spin singlet form of hydrogen gas as a polarization source. In hydrogenative PHIP a molecule of parahydrogen is chemically reacted with a molecular precursor to produce a 1H-hyperpolarized product. The proton polarization can be observed directly, or transferred to a heteronuclear spin (i.e. 13C or 15N) which allows for the generation of hyperpolarized metabolites in a cheap, continuous manner [12-16].

Here we show that PHIP can be used to prepare hyperpolarized fumarate by pairwise hydrogenation of an acetylene dicarboxylate precursor in water (Figure 1), using a commercially available ruthenium-based catalyst to achieve trans-hydrogenation [17-21]. This is in contrast to the vast majority of parahydrogenation reactions reported in the literature, which lead to the para-H₂ protons occupying cis-positions on the product molecule. For example, maleate, the cis isomer of butenedioic acid, is readily formed after

![Diagram of the catalyst mechanism](https://example.com/catalyst Mechanism.png)

Fig 1. Acetylene [1-13C]dicarboxylate reacts with parahydrogen in the presence of [Cp*Ru(CH₃CN)₃]PF₆ to form [1-13C]fumarate. The proton nuclear spin singlet state is indicated by opposing red arrows. The expected catalyst mechanism, modified from reference [19], is shown in the box.
hydrogenation of acetylene dicarboxylate with parahydrogen using the most widely used hydrogenative PHIP catalyst, i.e. [1,4-bis(diphenylphosphino)butane][1,5-cyclooctadiene]rhodium(I) tetrafluoroborate [22-24]. Maleate itself is a toxic compound, so for in vivo application a second hydrogenative step is used to produce the metabolite succinate [24].

If 13C nuclei are absent, the protons in fumarate are chemically and magnetically equivalent, so the hyperpolarized proton singlet order remains in an NMR-silent form, and enhanced NMR signals cannot be directly observed. The hyperpolarized 1H NMR signals can be released by enzymatic conversion to maleate [25], or chemical desymmetrization [26]. In the cases of maleate and succinate, magnetic inequivalence caused by a difference in J\_HH couplings to a 13C spin has be used to convert the 1H singlet order into observable 13C magnetisation [23,24]. Here we use the same principle: 13C labelling of one of the carboxylate moieties breaks the magnetic equivalence of the protons, which have different 1H-13C couplings to the 13C label. Since the protons remain close to magnetic equivalence, the application of a simple π/2 pulse on 13C leads to relatively weak hyperpolarized 13C NMR signals, which are due to singlet-triplet mixing [27]. A specific pulse sequence such as S2hM (singlet-to-heteronuclear magnetization) [23][28], on the other hand, leads to full conversion of proton singlet order into 13C magnetization, leading to strongly enhanced 13C NMR signals.

The S2hM pulse sequence, shown in Figure 2, exploits the molecular J-couplings (and not chemical shifts) to achieve polarization transfer, and is optimised for the case of small heteronuclear coupling to the parahydrogen proton pair. The sequence is designed to work independent of magnetic field homogeneity [23], which is an improvement on similar sequences [29,30]. It can additionally be made very robust to RF field inhomogeneity by the use of composite pulses, and can therefore be readily implemented on large sample volumes at low magnetic field, as is commonly the case in a clinical setting.

It should be noted that although trans-hydrogenation has been used before in combination with parahydrogen [17-20], the product molecules formed in those cases have chemically inequivalent proton pairs, giving rise to so-called PASADENA-enhanced NMR signals. This approach is ineffective for releasing the proton singlet order as observable magnetization for the chemically equivalent protons of fumarate.

To generate hydrogen gas at 50% para enrichment, hydrogen gas (purity 99.995%) was passed through a home-built parahydrogen generator containing an iron(III) oxide catalyst cooled to 77 K. The solution prior to hydrogenation reactions was 6 mM [Cp*Ru(CH$_3$CN)$_3$]PF$_6$, 100 mM sodium acetylene [1-13C]dicarboxylate and 100 mM sodium sulphite in D$_2$O. For sample hydrogenation, parahydrogen gas was bubbled at 5 bar and 50 °C for 30 s through 1/16 inch PEEK tubing that extended to the bottom of a pressurizable 5 mm NMR tube. The bubbling was performed inside the magnet and was controlled manually by hand-operated valves. NMR experiments were performed at 11.7 T in a 5 mm BBO probe using a Bruker AVANCE III console.

To demonstrate hyperpolarization of the 13C spins, we hydrogenated the sample as described, and then applied the S2hM pulse sequence optimised for the J-couplings in fumarate on the 13C channel before 13C detection. For comparison, the hyperpolarized NMR signal was allowed to decay, and a thermal equilibrium spectrum was acquired with a π/2 pulse. The results are shown in Figure 3. A typical 13C spectrum of [1-13C]fumarate shown a triplet peak structure from J-coupling to the strongly coupled proton pair. However, the S2hM sequence enhances only the central peak of the expected triplet [23], and this observation is supported by a SpinDynamica [31] simulation of the spectrum. The out-of-phase outer peaks are given by weakly allowed proton singlet-triplet state mixing, and are not discussed further here. There is no evidence of formation of the cis-product maleate in the 13C NMR spectra, which would appear at 167.3 ppm.

To quantify the catalyst selectivity for trans-hydrogenation over cis-hydrogenation, we acquired 1H NMR spectra of the reaction mixture after sample hydrogenation (see supplementary information). There is no detectable maleate signal in a 32 transient 1H spectrum, and the ratio of trans:cis product is therefore over 500:1. If the sodium sulphite is omitted, the ratio is only 3:1. This modification to the catalyst activity by sodium sulphite is not currently understood, and is subject to ongoing research. Additionally, succinate is produced as a side-product in 9% yield compared to fumarate. Succinate is a metabolite safe for injection in vivo, so this is less concerning than the formation of maleate, which is toxic. It has previously been shown that succinate forms through a Ru-carbene intermediate in a separate catalytic pathway, and not form the over-reduction of fumarate [19,20]. Importantly, this means that once fumarate is formed, it is not over-hydrogenated to succinate or isomerised to maleate.

The signal enhancement after the S2hM sequence was measured to be over 1000 at a field of 11.7 T, corresponding to a polarization level of over 1%. We plan to improve this enhancement factor and reaction yield by optimizing the reaction conditions, i.e. parahydrogen pressure, temperature, increasing the catalyst concentration, reducing the reaction time to limit losses due to relaxation, and reducing the precursor concentration to achieve complete hydrogenation. The proton singlet order generated from parahydrogen can also be increased by a factor of 3 by equilibrating the hydrogen gas over a catalyst at 25 K, instead of the 77 K used here.

Spin relaxation times were measured on a sample of 20 mM [1-13C]fumarate in a 40 mM phosphate buffer (pH 7) in water. The data are shown in Table 1 for a sample with and without...
The proton singlet lifetime is expected to be much longer (many minutes) [25,26,35] in the non \( ^{13}\text{C} \)-labelled molecule, because the \( ^{1}\text{H} \)-\( ^{13}\text{C} \) dipolar coupling relaxation contribution is removed. This is appealing for the production of hyperpolarized fumarate, but the oxygen sensitivity should still limit the \( T_1 \) for in vivo application.

In summary, a method for producing hyperpolarized fumarate from parahydrogen using a ruthenium-based catalyst in water has been presented. With radiofrequency pulse sequences we converted the hyperpolarized singlet order into magnetization on the \( ^{13}\text{C} \) spin in \([\text{-}^{13}\text{C}]\)fumarate. Although the achieved polarization level is relatively low in this preliminary work, there is much room for optimization of the catalyst, the parahydrogen preparation, and the reaction conditions. If these important issues are addressed, this might lead to a practical route for the convenient and inexpensive preparation of hyperpolarized fumarate for magnetic resonance spectroscopy and imaging experiments.

This work was supported by the Bundesministerium für Bildung und Forschung (grant number VIP HYPER-MRI 03V0443), the Max Planck Graduate Center, Mainz, the Deutsche Forschungsgemeinschaft for the financial support within the Collaborative Research Center SFB/TRR173 Spin+X, the Engineering and Physical Sciences Research Council (grant numbers EP/N002482/1, EP/M508147/1, EP/P009980, EP/N009304/1), the Royal Society, and Bruker BioSpin. J.E. would like to thank the Royal Society of Chemistry for a Researcher Mobility Grant.

**Conflicts of interest**

Conflict of Interest: Hana Kouřilová is a co-founder of Hyperspin Scientific Ltd, U.K.

**Notes and references**

6. Nielsen, P. M.; Eildirdi, A.; Bertelsen, L. B.; Jørgensen, H. S.; Ardenkjær, Larsen, J. H.; Laustsen, C. Fumarase activity: an in vivo...