SABRE Hyperpolarization of Sodium [1-^{13}C]Pyruvate Using Our Low-cost High-Pressure Clinical-Scale Liquid Nitrogen-Based Parahydrogen Generator

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Parahydrogen Induced Polarization (PHIP) is a simple and fast hyperpolarization approach, which holds the key to revolutionizing clinical production of HP contrast agents. Parahydrogen (p-H_2) is employed as a source of polarization by both hydrogenative PHIP and its non-hydrogenative variant (SABRE) allowing hyperpolarization of a wide range of biologically relevant compounds. In those cases, p-H_2-derived polarization is often transferred to other spin-1/2 nuclei including ^{13}C, ^{15}N, ^{1}H, ^{31}P, ^{19}F, and others reaching nuclear spin polarization (*P*) of >50% in some cases. Therefore, we report on robust and inexpensive design of liquid N_2-based p-H_2 generator (o-p catalyst-filled copper tubing spiral, Fig. 1a) for operation at up to 35 atm (1). The produced exiting p-H_2 gas is quantified by 'real-time' NMR spectroscopy using bench-top 1.4 T NMR spectrometer. The design reproducibility has been evaluated with N=3 devices. Moreover, we investigated ortho-para catalyst activation using exposure to high temperature to achieve production rate of 1,000 sccm with ~48% p-H_2 fraction (Fig. 1b, 1). We anticipate the reported design can be employed for p-H_2 production at higher flow rates of up to 4,000 sccm (2). The utility of the reported device was further evaluated for SABRE-SHEATH hyperpolarization of concentrated sodium [1-^{13}C]pyruvate, a metabolic contrast agent under investigation in numerous clinical trials. The study yielded ^{13}C signal enhancement of over 14,000-fold (Fig. 1c) at clinical relevant magnetic field of 1 T corresponding to approximately 1.2% ^{13}C polarization – if near 100% parahydrogen would have been employed, the reported value would be tripled to ^{13}C polarization of 3.5% (1).

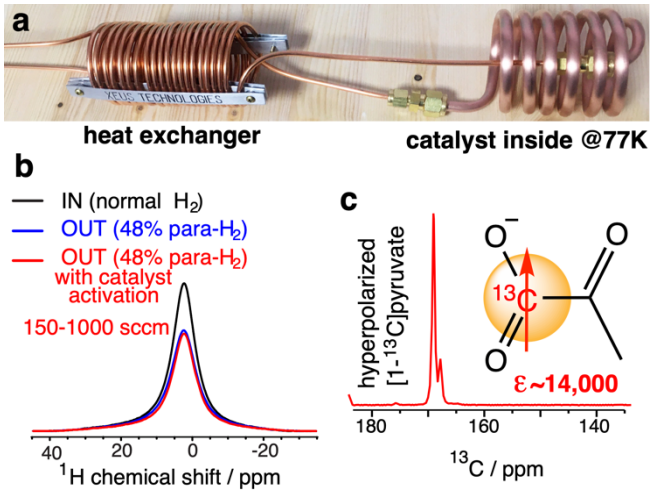

Figure 1. a) Annotated photo of p-H_2 generator for operation in liquid N_2 bath (77 K); b) Parahydrogen quantification using 1.4 T bench-top NMR spectrometer using 8 atm gas samples: 1024 scans, SW=5 kHz, t_{acq}=52 ms, ~102 s experimental time; c) NMR spectrum of SABRE-SHEATH hyperpolarized sodium [1-^{13}C]pyruvate yielding ^{13}C signal enhancement >14,000-fold at 1 T corresponding to approximately 1.2% ^{13}C polarization.

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ACKNOWLEDGEMENTS: Molecular Products Inc. for providing Ionex – Type O-P Catalyst, Hydrous Ferric Oxide, NSF CHE-1416268, CHE-1416432, CHE-1905341, and CHE-1904780, DOD CDMRP W81XWH-15-1-0271, W81XWH-15-1-0272, W81XWH-20-10576, and W81XWH-20-10578, NCI 1R21CA220137, NIBIB 1R01EB029829, NHLBI 1R21HL154032. HHSN261200800001E.

Toward clinical-scale heterogeneous hyperpolarization of propane gas at 1 atm

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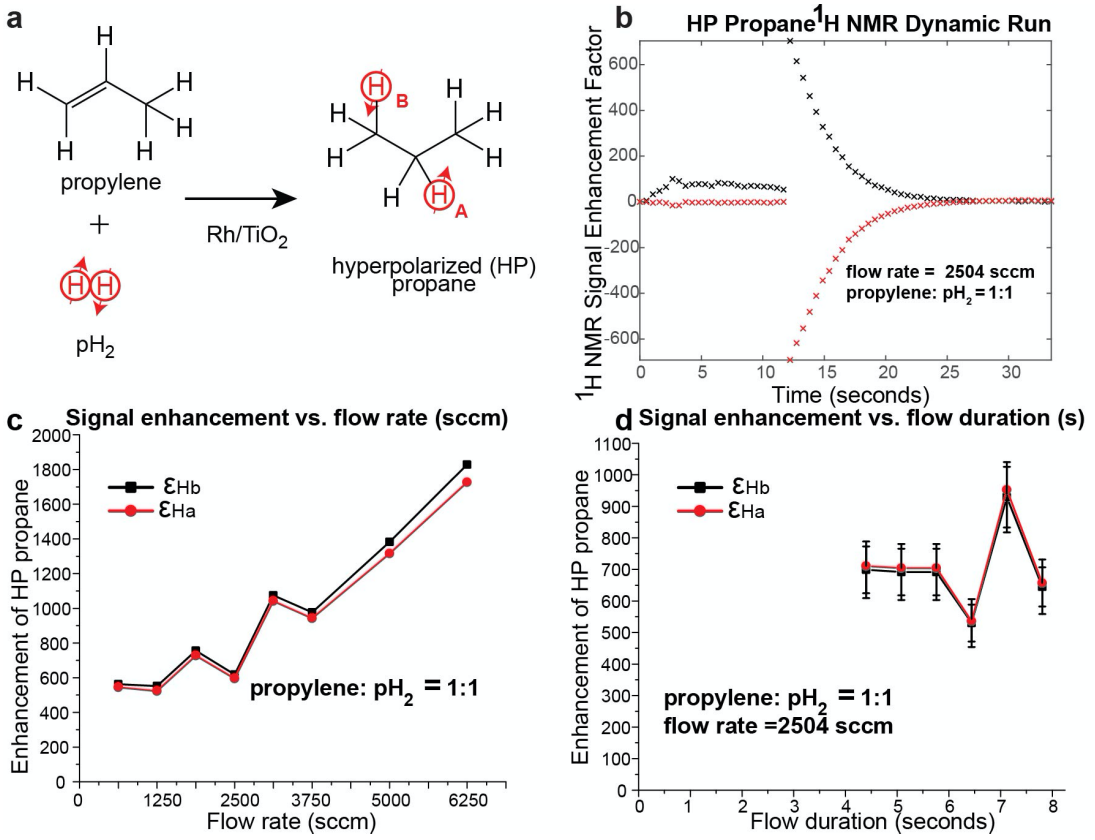
Our long-term goal is to develop *proton*-hyperpolarized (HP) propane as inhalable contrast agent for ultrafast pulmonary imaging. In this work, we study the feasibility of HP propane production at physiologically relevant condition of 1 atm total pressure via heterogeneous parahydrogen addition to propylene substrate (Figure 1a) using fast pseudo 2D signal acquisition method and 1.4 T bench-top NMR spectrometer (Nanalysis NMR Pro60), Figure 1b, and 87% parahydrogen generator.



The effect of the gas flow of the mixture of propylene and parahydrogen was studied over a wide range of flow rates from 620 standard cubic centimeters (sccm) – 8800 sccm using a gas phase heterogeneous hydrogenation reaction carried out at outer reactor temperature of 100 °C. We observe substantial dependence of proton signal enhancement (SE) of Ha and Hb protons on the gas flow rate, Figure 1c, with SE increasing at higher flow rates.

Figure 1. a) PHIP reaction to produce HP propane via heterogeneous hydrogenation. b) Pseudo 2D acquisition of HP propane signal. c) and d) HP propane signal enhancement dependence on gas flow rate and flow duration respectively.

HP propane SE dependence on total reactor pressure was studied with respect to different reactor pressure values for a 1:1 gas mixture of propylene and parahydrogen. However, reactor pressure does not seem to have a significant effect in the range of the pressure values studied in this work, Figure 1d. A detailed study of pressure dependence for variable gas compositions will be presented. These findings bode well for developing disposable clinical-scale hyperpolarizer operating at 1 atm (physiological condition).

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Acknowledgments: NSF CHE-1904780, NHLBI R21 HL154032, DOD W81XWH-15-1-0271 / W81XWH-20-1-0576. We thank Dr. Garett Leskowitz for implementation of custom data acquisition sequence.

Observation of Anti-Phase Hyperpolarized Orthohydrogen Signals with ~1000-Fold Enhancement Using a Heterogeneous MOF-Based SABRE Catalyst

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In PHIP and SABRE, parahydrogen (p-H_2) is used as the source of spin order to achieve hyperpolarization (1). A frequent byproduct is the spin-isomer *ortho*hydrogen (o-H_2). In SABRE (2), where substrate hyperpolarization is achieved under conditions of reversible exchange, the transfer of spin order from p-H_2 can give rise to hyperpolarized o-H_2 (3). The increased ^{1}H T_1 from the gas-phase millisecond regime to 2-3 s in solution allows significant polarization—particularly with p-H_2 bubbling and high-field acquisition (e.g. ~100-fold enhancement at 9.4 T) (3). In such cases, the hyperpolarized o-H_2 signal is usually absorptive (compared to the typically emissive phase of ^{1}H SABRE). However, when PHIP is manifested by irreversible hydrogenation, the p-H_2 is consumed in the reaction—often preventing HP o-H_2 formation. Exceptions generally require some process whereby molecular H_2 is regenerated (e.g. PHIP insertion/elimination (4)). Of particular interest are cases where the the signals from HP o-H_2 are antiphase (5,6); such an observation is paradoxical, because the two transitions within the triplet manifold should cancel. This "partial negative line" effect was recently explained in the context of PHIP by the late Konstantin Ivanov (6), who noted that binding of H_2 with the Rh catalyst causes the two H spins to become non-equivalent, leading them to precess at different frequencies and giving rise to free o-H_2 enriched in the T_0 state; exchange gives rise to residual shifts for the two tranitions, yielding a significant antiphase signal.

Here we describe a novel manifestation of this effect using a heterogeneous SABRE catalyst constructed in a metal-organic framework (MOF). Large antiphase o-H_2 signals are observed, with ^{1}H enhancements exceeding 1000-fold (P_H>3%). Moreover, the lifetime of the HP o-H_2 state is extended by ~4-5-fold compared to the homogeneous case (3). Finally, we report on efforts to perform HET-SABRE-SHEATH (7) with this novel MOF catalyst.

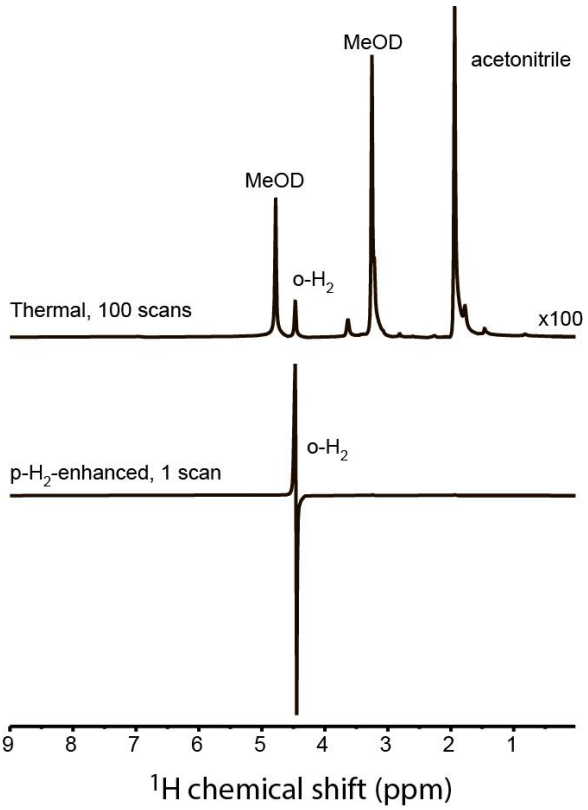

Figure 1: Parahydrogen-enhanced spectrum (1 scan, bottom) compared to a corresponding thermal spectrum (100 scans, scaled 100 times, top). Bottom spectrum was obtained after 30 s pH2 bubbling at ~65 gauss and trapid manual transport of the sample to high field (9.4 T).

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Ab initio calculations of ^{15}N chemical shifts of antibiotic drugs for hypoxia metabolic sensing

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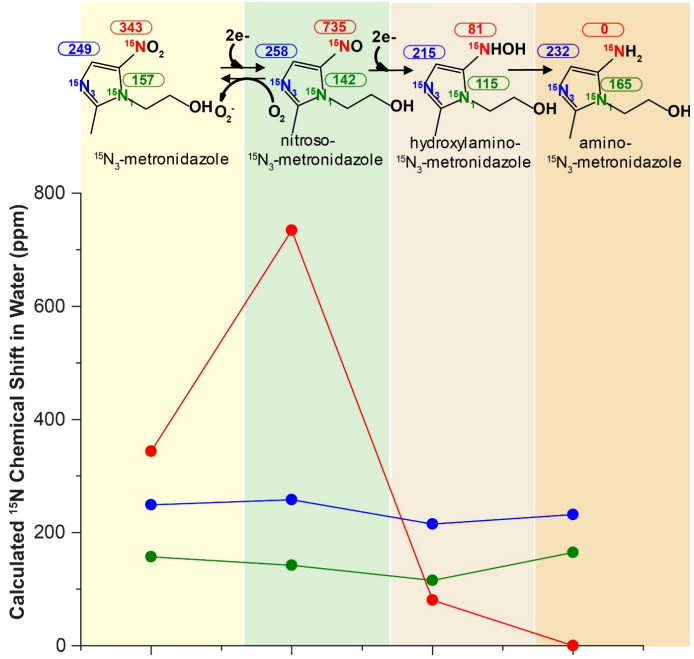

Figure 1. Results summary of *ab initio* calculations of ^{15}N NMR chemical shifts of [$^{15}N_3$]metronidazole and its metabolites from Gaussian'09.

The nuclear magnetic resonance (NMR) chemical shift is a great property of molecular structure. NMR has been employed for the characterization of molecules, and it ultimately helps to create magnetic resonance images. Moreover, it enables spectroscopic imaging via monitoring the metabolic transformation of hyperpolarized contrast agents. The NMR chemical shift also can be determined by *ab initio* calculation. We have used Density-Functional Theory (DFT) and Gaussian'09 software for our calculation of ^{15}N NMR chemical shifts in aqueous media. Ground-state DFT calculations have been performed to determine the on a series of metabolites (nitroso-, hydroxylamino- and amino-) of [$^{15}N_3$]metronidazole, [$^{15}N_3$]nimorazole, [$^{15}N_3$]ornidazole, [$^{15}N_3$]secnidazole, [$^{15}N_3$]benznidazole, and [$^{15}N_3$]evofosfamide for screening of the sensitivity of ^{15}N chemical shifts sites to metabolic reduction process, *e.g.*, due to hypoxia. ^{15}N chemical shifts of the drugs and their metabolites were obtained after the three-level geometry optimization with STO-3G, 3-21G, and 6-311++g(d,p) basis sets. The NMR chemical shift tensors of optimized structures were calculated by a single-point GIAO method using the correction consistent aug-cc-pVDZ Dunning basis set. While all compounds exhibited a clear sensitivity trend to the reduction process for all three ^{15}N sites, *i.e.*, their ^{15}N chemical shifts can clearly provide sensitive mechanism for ^{15}N hypoxia sensing, [$^{15}N_3$]metronidazole was deemed the optimum choice in the context of hypoxia sensing.

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Hyperpolarization of common cancer drugs with SABRE

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NMR and MRI are indispensable tools that work without the use of ionizing radiation and have an unparalleled ability to elucidate morphological and chemical structure. These techniques have untapped potential as the absolute sensitivity is very small due to low thermal polarization of spin states. Hyperpolarization methods are employed to increase sensitivity. Signal amplification by reversible exchange (SABRE)[1] is a parahydrogen based method that generates high levels of polarization[2][3] on substrates without chemical modifications directly in room temperature liquids. These features make SABRE a promising technique for future biological studies, including hyperpolarized MR contrast agents for in vivo sensing at physiological concentrations. Nitrogen-15 contrast agents are of growing interest because they do not compete with strong proton background and because they tend to have longer hyperpolarization lifetimes.[4][5]

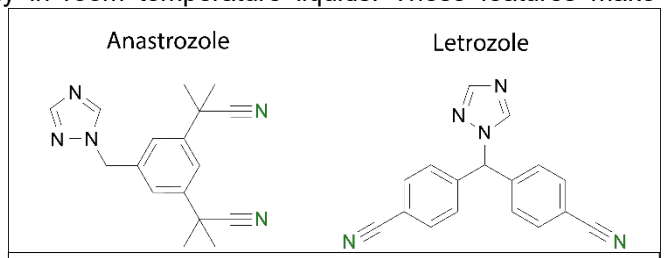

Figure 1. Chemical structure of the target substrates, anastrozole and letrozole. Nitrogen atoms highlighted green are the hyperpolarized nuclei in Figure 2.

In this study, we extend the biologically relevant SABRE substrate scope by hyperpolarizing both ^{1}H and ^{15}N nuclei on two common cancer drugs, anastrozole and letrozole illustrated in **Fig. 1**. *Specifically, we conduct polarization transfer field sweeps* (**Fig. 2** for ^{15}N field sweeps)*, temperature sweeps and hyperpolarization lifetime studies for both drugs*.

For ^{15}N nuclei on the nitrile substituents, we found that polarization transfer efficiency increases in a linear fashion with an increase in temperature from 25°C to 50°C and that the optimum polarization transfer field is ~0.3μT. We plan on extending the high field (9.4T) hyperpolarization lifetime on ^{15}N nuclei (~20 seconds for both drugs) by storing the sample at lower field strengths, e.g., 1T and EMF. ^{1}H polarization transfer efficiency is maximized at lower temperatures (~40°C) compared to ^{15}N nuclei and the optimum polarization transfer field is ~6mT.

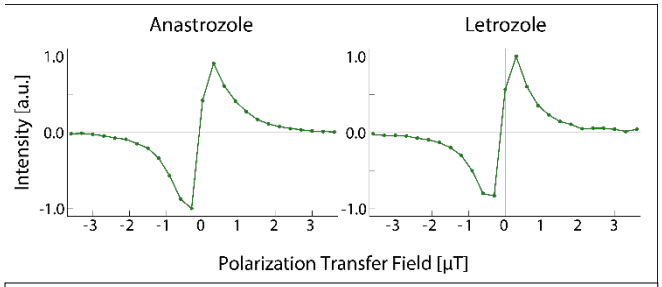

Figure 2. ^{15}N polarization transfer field sweep of anastrozole and letrozole. Hyperpolarized nulcei are highlighted green in Figure 1.

These optimization studies shed light on complex SABRE dynamics and bring mechanistic insights into the hyperpolarization process. In conclusion, this study broadens the SABRE substrate scope and furthers the fields direction towards highly sensitive MR contrast agents.

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New Reactor Design for Bulk Hyperpolarization of Metabolites from Parahydrogen

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Hyperpolarized metabolites (HMs) can facilitate kinetic studies of metabolic processes as biomarkers for disease detection and monitoring via PHIP Side Arm Hydrogenation (PHIP-SAH).[1,2] Typically, liquid phase PHIP experiments are performed by bubbling parahydrogen through a solution containing a dissolved rhodium catalyst and the side-arm precursor. The reaction rate, hyperpolarization levels, and polarization yields achieved by the conventional bubbling method are limited by the molecular transport and mixing process as well as spin-lattice relaxation losses. Spray injection systems have been shown to be more efficient than bubbling for the fast production of bulk hyperpolarized substrates that is well-suited to in-vivo applications.[3] Spray injection can deliver the required quantities of highly hyperpolarized metabolites. We will present details on the instrumentation, operating conditions, and PHIP performance for a novel mixing process that can provide controlled and repeatable HM production. Exemplary PHIP spectra for the hydrogenation of propargyl acetate with parahydrogen using this new reactor system are presented in Figure 1.

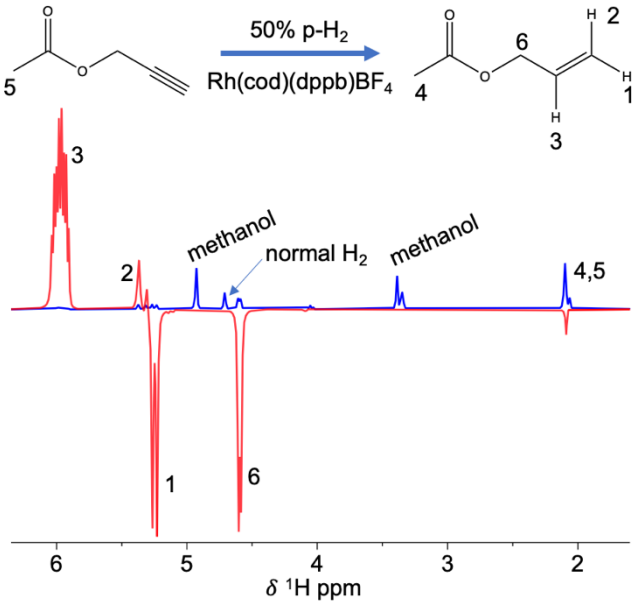

Figure 1. ^{1}H ALTADENA spectrum (red, 1 scan) and thermally polarized spectrum (blue, 4 scans) of propargyl acetate hydrogenation acquired from the novel reactor system.

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NMR Signal Enhancement Over Mesoporous Silica-Encapsulated Pt-Sn Nanoparticles

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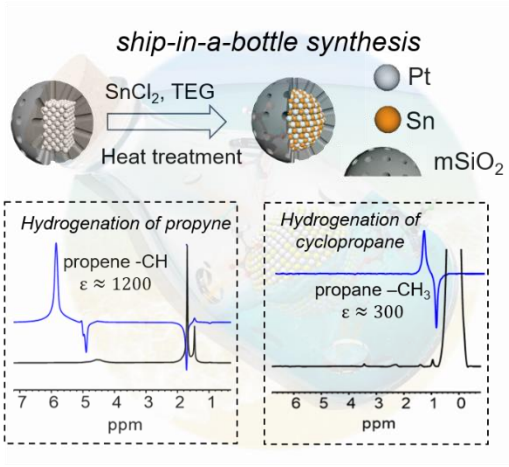

Figure 1: Signal enhancement of propene and propane by parahydrogen enhanced NMR over Pt-Sn@$mSiO_2$ nanoparticles

Supported Pt nanoparticles (NPs) are widely used in parahydrogen enhanced NMR. However, the stepwise transfer and fast diffusion of H atoms on the Pt surface significantly limit the pairwise addition in the hydrogenation reaction. Significant improvements can be made on pairwise selectivity through the rational design of heterogeneous catalysts. In 2016, a facile approach was developed to synthesize mesoporous silica ($mSiO_2$) encapsulated Pt-Sn intermetallic nanoparticles (iNPs), where the silica shell provides the catalyst with high-temperature stability up to 750 °C.[1] In the present study, parahydrogen enhanced NMR in the hydrogenation of propyne, propene, and cyclopropane will be presented using these three Pt-Sn@$mSiO_2$ iNPs. A significant difference in pairwise selectivity was observed among three catalysts and PtSn@$mSiO_2$ iNPs delivered >1000-fold NMR signal enhancements in the hydrogenation product: propene and propane.[2,3] The difference of performance was attributed to the elimination of 3-fold Pt hollow sites on the catalyst surface, thereby restricting the dissociative H_2 chemisorption and H diffusion across the surface sites. The hyperpolarized gases achieved by parahydrogen enhanced NMR over PtSn@$mSiO_2$ iNPs have many potential applications, ranging from mechanistic probing of chemical processes to real-time pulmonary imaging.

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Raising the Bar of ^{13}C SABRE-SHEATH Hyperpolarization of [1-^{13}C]Pyruvate

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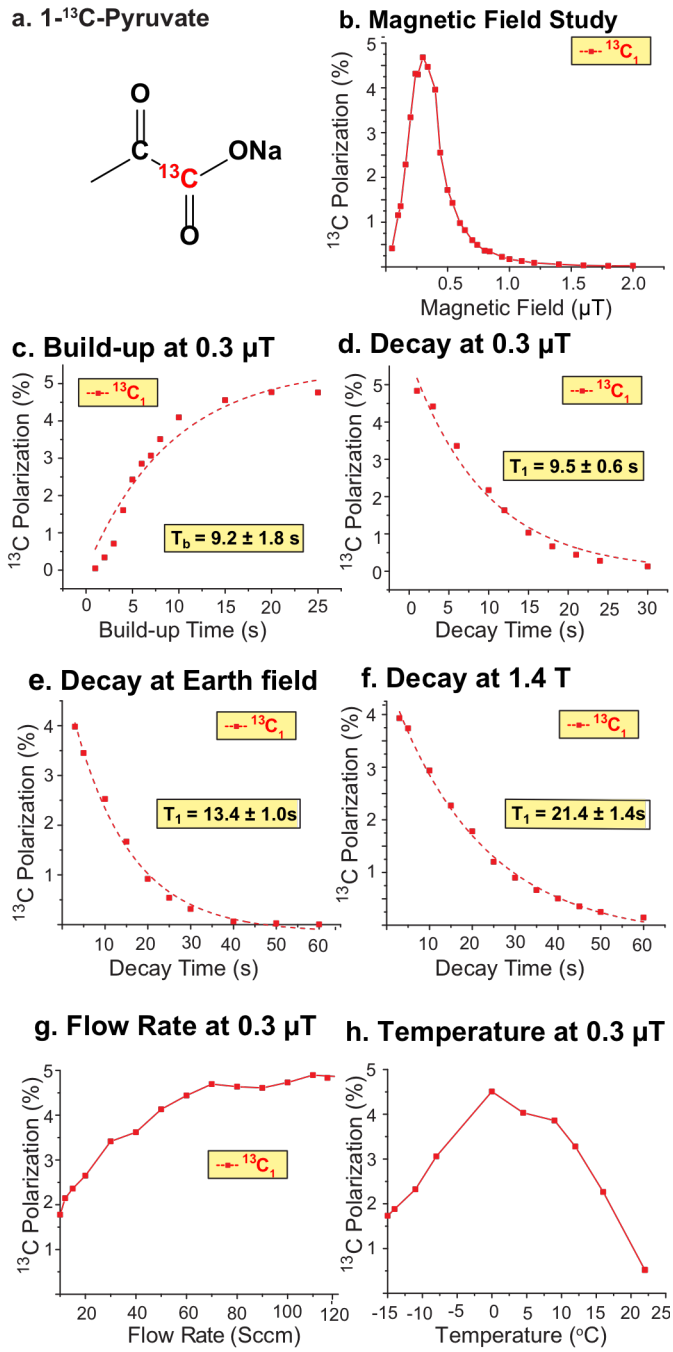

NMR signal enhancement through hyperpolarization improves the diagnosis and treatment of medical conditions such as cancer. [1-^{13}C]pyruvate is the leading hyperpolarized contrast agent, which in under investigation in many clinical trials and studies. Signal Amplification by Reversible Exchange (SABRE) in shield enables alignment transfer to heteronuclei (SABRE-SHEATH) including ^{13}C. This approach can give rise to strong ^{13}C signals through simultaneous chemical exchange of parahydrogen and to-be-hyperpolarized substrate (*e.g.*, to-be hyperpolarized contrast agent). In 2020, Duckett and co-workers have demonstrated that [1-^{13}C]pyruvate can be hyperpolarized via SABRE-SHEATH. Here, we demonstrate how ^{13}C signal enhancement of [1-^{13}C]pyruvate, an important metabolic compound, can be optimized using SABRE-SHEATH technique via signal readout using a benchtop 1.4 T ^{13}C NMR spectrometer. By hyperpolarizing [1-^{13}C]pyruvate via an Iridium based transfer catalyst [Ir(H)$_2$(η^2-pyruvate)(DMSO)(IMes)] and pilot optimization of experimental parameters (**Figure 1**), P_{13C} of ~5% was achieved corresponding to ^{13}C signal enhancement by ~40,000-fold. We expect that further optimization of experimental parameters space will allow improving the level of polarization beyond 20%. When combined with other recent advances including catalyst purification, these findings bode well for future *in vivo* and clinical translation.

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Figure 1. a) Structure of sodium [1-^{13}C]pyruvate employed for the studies of the following SABRE-SHEATH composition: 20 mM DMSO, 30 mM [1-^{13}C]pyruvate, 6 mM IrIMes pre-catalyst in 0.6 mL CD_3OD, 7.7 atm pH_2 pressure. b) Magnetic field sweep. c) ^{13}C polarization build-up at 0.3 μT. d) ^{13}C T_1 decay at 0.3 μT. e) ^{13}C T_1 decay at the Earth's magnetic field. f) ^{13}C T_1 decay at 1.4 T. g) P_{13C} dependence on flow rate. h) P_{13C} dependence on temperature.

Optimizing Hyperpolarization and Catalyst Separation in PHIP-SAH Agents with Natural ^{13}C Abundance Using High-Field and Benchtop NMR Spectrometers

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PHIP and SABRE are attractive hyperpolarization methods because they are fast, cheap, scalable, and have relatively modest instrumentation/infrastructure requirements (1). The reversibility of SABRE (2) also enables systematic experimental repetition over considerable periods of time, facilitating optimization--a feature that is particularly important when using labeled compounds or exploring multi-dimensional parameter spaces. On the other hand, PHIP-SAH (3,4) has successfully demonstrated high polarizations in ^{13}C spins in biologically relevant molecules, but the irreversibility of substrate hydrogenation presents a challenge towards technique development, particulary given the significant expense of ^{13}C-labeled compounds (relative to ^{15}N).

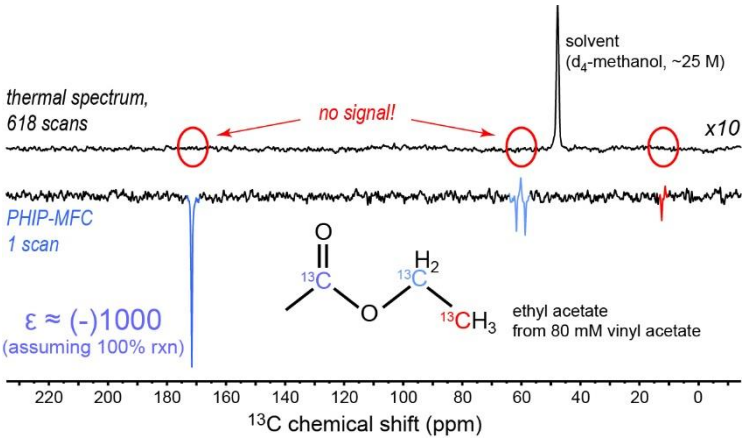

Figure 1: (bottom) Natural abundance (~1%) ^{13}C spectrum of HP ethyl-acetate, achieved after 15 s of p-H_2 bubbling at earth's field and 45 °C. After bubbling, the sample was place in a magnetic shield and removed (~3 s); then, subsequent transfer to 9.4 T (Vinyl-acetate: 80 mM; Rhodium catalyst, 5 mM).

Here we are investigating the use of unlabeled (naturally abundant ^{13}C) molecules and both high-field and benchtop NMR spectrometers for optimizing protocols for hyperpolarization and catalyst removal for PHIP-SAH agents. In one set of experiments, a commercial Rh (Wilkinson's) catalyst and PHIP with magnetic field cycling (MFC) was used to obtain ~1000-fold ^{13}C signal enhancement at 9.4 T when converting vinyl acetate to ethyl acetate; given a 25% reaction yield, this corresponds to a ~4000-fold enhancement in polarization for the product (or ~3.2%). In another set of experiments, a benchtop NMR setup was used, and P_{13C}=10% was achieved in the same system; in both cases the naturally abundant signal is sufficently strong for relaxation studies to be performed, obviating the need for ^{13}C enrichment. In other experiments, commercial surface-functionalized silica particles (5) were used to rapidly sequester the catalyst; doing so rapidly quenched any observable ^{1}H PHIP in both the original solution and the supernatant. SEM-EDX shows successful uptake of the Rh catalyst by the functionalized silica particles. The approach should be amenable to systematic mapping of parameter space and comparison of different approaches for PHIP catalyst removal (5-9).

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