A more controllable way of producing ^{13}C contrast and metabolite agents in PHIP-SAH

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Abstract: Hyperpolarized metabolites and contrast agents open great opportunities for diagnostic medicine. Easy to handle and non-expensive Parahydrogen Induced Polarization (PHIP) technique is capable of production of hyperpolarized molecules with short polarization time. To get pure hyperpolarized target molecules with long relaxation time the polarization transfer from parahydrogen to heteronuclei is an essential supplement to PHIP, which we address herein.

Motivation

Magnetic resonance (MR) is an indispensable tool for medical diagnostic. Due to its intrinsically low sensitivity hydrogen is the only nucleus used in clinical routine while application of heteronuclear MR is still hindered. Hyperpolarization methods like Dynamic Nuclear Polarization (DNP) opened the possibility to obtain ^{13}C hyperpolarized metabolites and contrast agents and thus to investigate their transformation in vivo, as their T_1 times are usually longer than those of hydrogen. That would widen the diagnostic applications of MR not only in vitro but also in vivo, enhancing the signal by several orders of magnitude. Moreover, the heteronuclei can be observed without significant background.

On the other hand, DNP polarizers are expensive, which limits their rapid development in medicine. Another way of hyperpolarization is the Parahydrogen Induced Polarization (PHIP) method. In contrast to DNP it is a chemistry-based technique; it is easier to handle, cost effective, and allows much shorter polarization times. Proton polarization can reach an order of unity by simple hydrogenation reaction on homogeneous catalyst just because the protons stem from highly polarized parahydrogen. The polarization of heteronuclei is achieved by polarization transfer techniques which can be done in below earth field or in high field directly in the cryomagnet of an MRI machine (1). However, a drawback of this technique is the small number of molecule for which a deprotonated precursor is available. Thus, only few biomolecules were polarized by parahydrogenation. One option to overcome this is functionalization of biomolecules by moieties that can be hydrogenated.

PHIP-SAH (PHIP by means of side arm hydrogenation) goes further (2). It can be seen as functionalization of the molecule combined with polarization transfer from protons stemming from parahydrogen to the metabolite's ^{13}C and subsequent detaching of the hydrogenated moiety, for example by hydrolysis. The obtained target molecule is pure not only from functionalization but also from organometallic catalyst (the hydrogenation reaction can be done in organic solvent while the hydrolysis product is extracted to aqueous phase). The efficiency of the polarization transfer is crucial for the whole technique. It is governed by scalar couplings and can be achieved either by going to near zero field where proton and carbon states are mixed or by applying pulse sequences inside an MR machine. Both ways have been shown to yield an optimal polarization transfer when the added parahydrogen protons are two and three bonds away from the heteronucleus, though high polarization has been seen even for four and five bonds (like in Fig. 1) in case of PHIP-SAH with field cycling (2). However, at such a long distance, the polarization transfer goes not directly from protons originated from parahydrogen to carbon, but first to protons adjacent to carbon and then further to carbon itself. Replacing these protons by deuterons breaks the polarization transfer chain. For this "two-step" process the only parameters that can be optimized in this setting are field strength and its variation rate.

Herein we aim to do PHIP-SAH in a more controllable way inducing the two-step polarization transfer by pulse sequences inside an MR machine.

Material and methods

Measurements were performed using a 7 Tesla Bruker Spectrometer (Avance 300 MHz) equipped with a standard double resonance inverse probehead. The hydrogenation reaction was performed in a 5 mm NMR tube

while it was in the probehead in the cryomagnet at a field of 7 Tesla. Gaseous parahydrogen was delivered through a capillary and bubbled through the solution of the precursor and commercially available catalyst in organic solvent at elevated temperature (~320K). Parahydrogen was obtained using a Bruker ParaHydrogen Generator (BPHG 90). Pressure of the bubbled parahydrogen was kept at 6 bar for high concentration of the dissolved gas to increase the rate of hydrogenation reaction. Following the bubbling period (~20s) an NMR pulse sequence on proton and carbon channels was applied to transfer proton polarization to adjacent carbon and then to carboxylate. Immediately afterwards, the FID of enhanced ^{13}C signal was recorded. In the end the hydrogen gas was released and a normal NMR spectrum was recorded with great number of scans to obtain the signal of the hydrogenated product. Comparison to hyperpolarized signal allowed determining the level of polarization.

Results

Parahydrogenation reaction of triple-bonded precursors leads to fast formation of hyperpolarized double-bonded product. Although the used catalyst is optimized to hydrogenate only triple bonds, the reaction slowly continues to form a completely saturated molecule. Thus, it is important to optimize the hydrogenation time, catalyst concentration and parahydrogen pressure to increase yield of the double-bonded product on the one hand and to omit further hydrogenation on the other. 20 s hydrogenation time, 5 mM catalyst concentration and 6 bar pressure was used resulting in 20% proton polarization of 1 mM product.

The polarization transfer to ^{13}C carboxylate occurs during a pulse sequence in two steps. PH-INEPT sequence (3) transfers polarization from protons to the adjacent methylene carbon. Immediately afterwards, proton decoupling is applied while a pulsed experiment transfers carbon polarization to the desired metabolite carboxylate.

Discussion and conclusions

Despite the simplicity of the apparatus, we could already achieve 20% proton polarization and after polarization transfer 3% ^{13}C in the carboxylate. This corresponds to 5000 fold increase in signal intensity compared to the Boltzmann polarization. The pulse sequence can be further modified to result in longitudinal ^{13}C carboxylate magnetization with longer than transversal relaxation time. That would allow enough time for subsequent hydrolysis of the hyperpolarized product to obtain catalyst-free carboxylates in water solution.

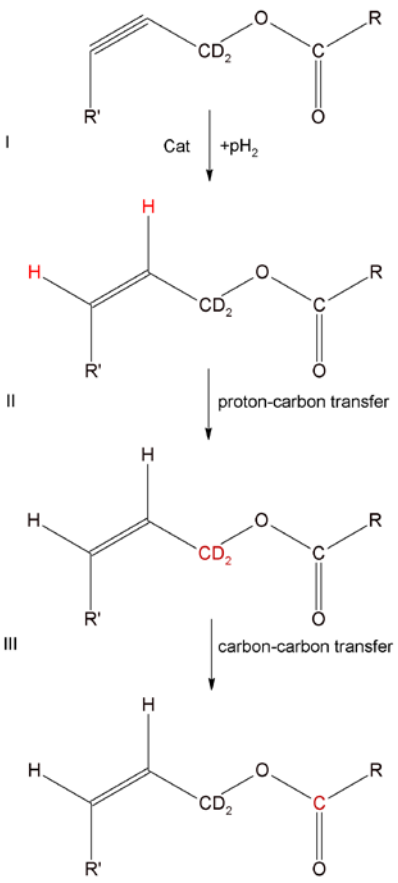

Fig. 1: Reaction scheme of parahydrogenation of triple-bonded precursor (I) and subsequent polarization transfer from the protons to the adjacent carbon (II) and then to the carboxylate (III).

References

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