ParaHydrogen Induced Polarization via Side Arm Hydrogenation (PHIP-SAH) allows hyperpolarization of acetate and [1-13C] pyruvate.

Francesca Reineri[1], Tommaso Boi[2], and Silvio Aime[3]

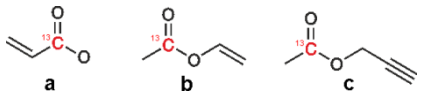
[1]*Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy,* [2]*Bracco Imaging Spa, Italy,* [3]*Molecular Biotechnology and Health Sciences, Universitty of Torino, Torino, Italy*

Introduction

Hyperpolarization methods represent a recent break-through in field of diagnostic tools by Magnetic Resonance since they allow direct *in vivo* observation of cellular metabolism. Dynamic Nuclear Polarization (DNP) is the hyperpolarization technique that has been most successfully applied in the field of Metabolic Magnetic Resonance Imaging (MMRI). The *in vivo* administration of hyperpolarized ^{13}C-labelled substrates and, in particular, of [1-^{13}C] pyruvate, can provide outstanding information for early detection of pathologic states. A limitation to the widespread use of this approach relies on the fact that it requires access to complex and expensive instrumentation. Parahydrogen Induced Polarization (PHIP) is an alternative route to hyperpolarization that has the advantage of being cheaper and easier to handle with respect to DNP. The application of PHIP to bio-medical studies has been limited by the availability of unsaturated precursors of the hyperpolarized metabolites. This study has been performed in order to bring hyperpolarization from parahydrogen to ^{13}C carboxylic signals of biologically relevant molecules such as pyruvate and acetate.

Materials and Methods

Hyperpolarization of ^{13}C carboxylate signal is usually obtained through parahydrogenation of an unsaturated group (double or triple bond) adjacent to the target ^{13}C carboxylate signal (molecule type **a**). In this work we have used a new class of esters in which parahydrogen is added to the alcoholic moiety. The vinyl ester of acetate has been considered first (type **b** compound). Then, propargyl esters (structure **c**) of acetate and pyruvate have been synthesized and parahydrogenation has been carried out in organic solvent and aqueous phase. In order to obtain polarization transfer from parahydrogen to the ^{13}C carboxylate signal, magnetic field has been cycled between earth and zero field, using a mu-metal shield. After the polarization transfer step, hydrolysis of the parahydrogenated esters has been carried out using an aqueous basic solution.

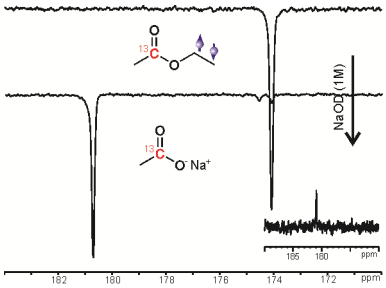


Results

Parahydrogenation of vinyl acetate and successive application of magnetic field cycling leads to high polarization level on the ^{13}C carboxylate signal of acetate. Hyperpolarization level, after the application of magnetic field cycling, is the same as that obtained on hydroxyl ethyl propionate (HEP) on which parahydrogen is added at adjacent positions to the 13C carboxylate nucleus (type **a** compound). Parahydrogenation of the propargyl ester of acetate leads to the same polarization level on the ^{13}C carboxylate signal, following to field cycling application.

Hydrolysis of the parahydrogenated ester allows to obtain ^{13}C hyperpolarized Sodium acetate.

From the parahydrogenation of propargylic ester of pyruvate, hyperpolarization is obtained on [1-^{13}C] signal of pyruvate. Then, hydrolysis is carried out by the addition of an aqueous basic solution and [1-^{13}C] hyperpolarized pyruvate is obtained in aqueous phase.



Upper spectrum: ^{13}C carboxylate signal of Papahydrogenated ethyl acetate in aqueous phase and (lower spectrum) after hydrolysis and release of the parahydrogenated alcoholic moiety.

Discussion and conclusions

The experimental results show that high polarization level can be obtained on the ^{13}C carboxylate signal of parahydrogenated esters on which parahydrogen is added to the alcoholic moiety. Theoretical study of field cycling supports the experimental observation that polarization level on the ^{13}C carboxylate signal is the same as that achievable on molecules where parahydrogen is added at adjacent positions to the target ^{13}C nucleus. After polarization transfer to the carboxylate signal, the parahydrogenated synthon can be removed by means of hydrolysis.

The herein presented method, namely ParaHydrogen Induced Polarization by means of Side Arm Hydrogenation (PHIP-SAH), relies on the use of an unsaturated alcohol (vinyl or propargyl alcohol) as a removable synthon to bring polarization to molecules containing a carboxylic group. It markedly widens the applicability of the PHIP approach and opens a very interesting perspective for the use of parahydrogen-based procedures for the generation of hyperpolarized, biologically relevant, molecules. The access to hyperpolarized molecules by means of the easy-to implement PHIP-SAH procedure is expected to promote renewed interests in the field of MRS-MRI hyperpolarization that have been precluded by the high cost and complexity of the DNP methodology.