

Efficient preparation of hyperpolarized aqueous succinate from the para-hydrogenation and hydrolysis of maleic anhydride

F. Reineri¹, A. Viale¹, S. Ellena¹, T. Boi¹, R. Gobetto¹, and S. Aime¹
¹University of Torino, Torino, IT, Italy

Purpose

Aim of this work is the set-up of efficient methods for the preparation of aqueous solutions of para-hydrogenated molecules by a phase extraction procedure that minimizes the loss of hyperpolarization during the experimental work-up. The method has been applied to yield hyperpolarized succinate from the para-hydrogenation of maleic anhydride.

Introduction

Para-Hydrogen Induced Polarization (PHIP) is currently under intense scrutiny for the preparation of hyperpolarized ¹³C contrast agents for Magnetic Resonance Imaging (MRI) [1]. In order to attain large PHIP effects in para-hydrogenation products, the hydrogenation reaction is carried out in organic solvents and in the presence of homogeneous hydrogenation catalysts. The catalyst and the organic solvent must be removed after para-hydrogenation in order to have non-toxic formulations of the hyperpolarized agents for their *in vivo* use.

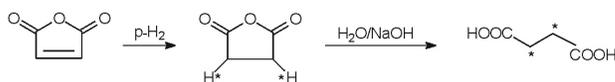
Here we report a new method based on the para-hydrogenation, in an organic solvent phase, of an unsaturated precursor that, after hydrogenation, is converted into the water-soluble substrate by an hydrolysis reaction.

Methods

Parahydrogenation reactions were carried out in a low field NMR spectrometer (built by STELAR/Invento) consisting of a 50 mT permanent magnet equipped with a ¹H/¹³C double resonance wide bore probe which holds the hydrogenation reaction chamber. The reaction takes place by spraying the solution of the catalyst [Rh(cyclooctadiene)(diphenylphosphinobutane)][BF₄] (10mM) and the unsaturated reagent (100mM) into the chamber previously pressurised with 4 atm of 50% enriched para-H₂, while CW decoupling is applied (5s). When chloroform was used as solvent, a small amount of acetone-d⁶ - about 1:5 with respect to chloroform - was necessary for the activation of the catalyst. Hydrolysis was carried out by addition of 0.4 ml of basic D₂O to the hyperpolarized product. The mixture was then let to stand for few seconds in the test-tube and the upper phase was transferred by a syringe into a NMR tube for acquisition. The amount of NaOD dissolved in D₂O was calculated in order to obtain a neutral pH for the final succinate solution (NaOD : maleic anhydride = 2.1:1). NMR (14.1 T) and ICP analysis of the obtained succinate water solution revealed no traces of other organic compounds.

Results and Discussion

The substrate chosen as model to test the proposed procedure is maleic anhydride, which affords succinic anhydride by hydrogenation. Succinic anhydride can then be converted to succinate by rapid hydrolysis under basic conditions.



Hyperpolarized ¹³C-succinate is thought to be a good candidate for metabolic imaging studies in tumour cells.

Para-hydrogenation has been carried out in chloroform solution and hydrolysis of the formed succinic anhydride has been achieved by addition of water basified with NaOH. The formed succinate results then dissolved in the aqueous phase. The ¹³C polarization has been measured by acquiring single-pulse spectra on a 14.1 T high resolution NMR spectrometer. As shown in the figure the loss of net ¹³C magnetization is minimized on passing from succinic anhydride in the organic phase to succinate in the aqueous phase.

The herein presented methodology appears to be a valid alternative to the direct hydrogenation of fumaric acid for the production of hyperpolarized succinic acid for MRI applications. More in general, in addition to hydrolysis of anhydrides or of activated esters that afford carboxylic acids, other hydrogenation reactions on lightly stabilized precursors may be considered to prepare compounds of potential biological interest that may be tested as MRI HP probes. The proposed procedure allows to obtain water solutions of the compounds of interest, which are free of the organic solvent and of the catalyst, in one step only by a simple phase extraction process, avoiding the use of high para-H₂ pressures and further manipulations of the hyperpolarized product that invariably lead to polarization loss.

References

[1] Golman, K. *et al.*, *Magn. Res. Med.* **2001**, *46*, 1 ; Svensson, J. *et al.*, *Magn. Reson. Med.* **2003**, *50*, 256; Olsson, L.E. *et al.*, *Magn. Res. Med.* **2006**, *55*, 731; Ishii, M. *et al.*, *Magn. Res. Med.* **2007**, *57*, 459; Johansson, E. *et al.*, *Magn. Reson. Med.* **2004**, *51*, 464; Johansson, E. *et al.*, *Magn. Reson. Med.* **2004**, *52*, 1043; Magnusson, P. *et al.*, *Magn. Reson. Med.* **2007**, *57*, 1140.

