

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## ParaHydrogen Induced Polarization of <sup>13</sup>C carboxylate resonance in acetate and pyruvate

### This is the author's manuscript

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1526124> since 2015-10-06T14:44:15Z

*Published version:*

DOI:10.1038/ncomms6858

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

**ParaHydrogen Induced Polarization of  $^{13}\text{C}$  carbonyl resonance in acetate and pyruvate**

Francesca Reineri†, Tommaso Boi‡, Silvio Aime†\*

†University of Torino, Dept. Molecular Biotechnologies and Health Sciences

Via Nizza 52 Torino, Italy

‡ Bracco Imaging Spa, CRB, Colletterto Giacosa, Torino, Italy

\*e-mail: [silvio.aime@unito.it](mailto:silvio.aime@unito.it)

Tel. +39 670 6451

Recent years have witnessed a renewed interest in routes to hyperpolarization as a means of overcoming sensitivity issues associated with Magnetic Resonance Spectroscopy (MRS) and Magnetic Resonance Imaging (MRI). In this context, most attention has been devoted to the application of the dissolution DNP (Dynamic Nuclear Polarization) methodologies.<sup>1-3</sup> Applications of DNP have led to very important results in the field of Metabolic Magnetic Resonance Imaging (MMRI). It has been shown that the administration of hyperpolarized <sup>13</sup>C-labelled substrates such as pyruvate,<sup>2,3</sup> lactate,<sup>4,5</sup> fumarate,<sup>6</sup> acetate,<sup>7,8</sup> bicarbonate<sup>9,10</sup> can report on their intracellular transformations, providing outstanding information for early detection of pathologic states and for their response to therapy.<sup>11</sup> A limitation to the widespread use of this approach relies on the fact that it requires access to complex and expensive instrumentation, available only in a limited number of laboratories. Moreover the DNP methodology requires relatively long times for the polarization step with consequent large consumption of cryogenic liquids.

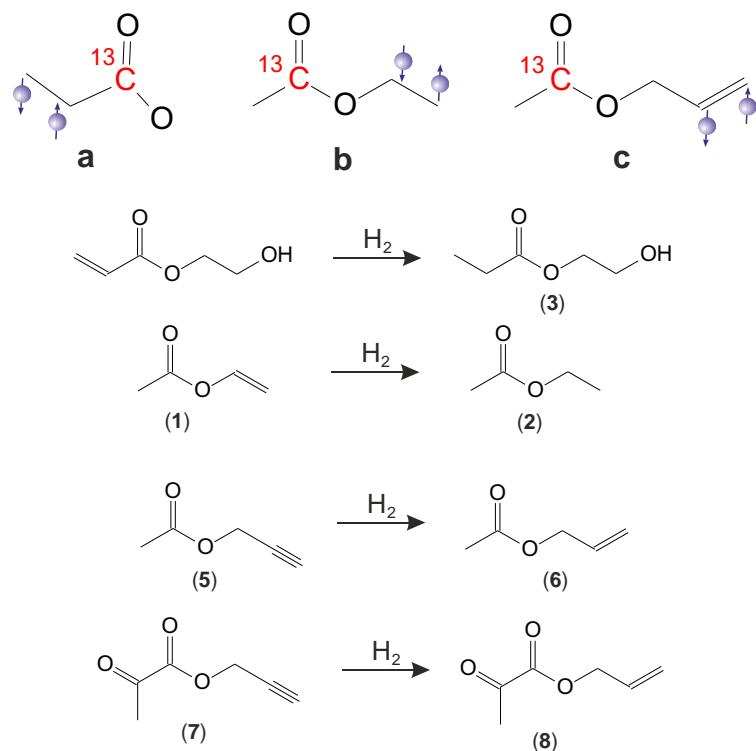
An alternative route to hyperpolarized molecules is represented by Parahydrogen Induced Polarization (PHIP)<sup>12,13</sup> that has the advantage of being cheaper and easier to handle with respect to DNP. As far as bio-medical applications are concerned, few molecules have been hyperpolarized by means of this method. Among them, succinate<sup>14,15</sup> and phospho-lactate<sup>16,17</sup> are the only metabolites for which good hyperpolarization level has been achieved until now. Alternative routes have been proposed to achieve PHIP on biologically relevant molecules by functionalizing the substrates of interest with moieties capable of hydrogenation.<sup>18,19,20</sup> However the structural modifications introduced by the unsaturated group may significantly alter their biological behavior with respect to the native substrates.

An attempt to tackle the major limitations of PHIP has been pursued with the recently introduced Signal Amplification By Reversible Exchange method.<sup>21-23</sup> With this approach parahydrogen protons are not added to the substrate but the hyperpolarization transfer takes place on a transient adduct formed by the substrate, parahydrogen and the organometallic complex. This method has been successfully tested on several

biologically relevant molecules such as pyrimidines, purines, amino acids and drugs.<sup>24,25</sup> However *in vivo* application still suffers from low polarization levels and the need for suitable organometallic complex to efficiently renew hyperpolarization.<sup>26</sup>

As far as PHIP is concerned, efficient polarization transfer from parahydrogen spin order to <sup>13</sup>C longitudinal magnetization of carbonyl groups can be achieved, by the addition of the para-H<sub>2</sub> molecule to a double or a triple bond adjacent to the carbonyl group.<sup>23</sup> The amount of polarization transferred from parahydrogen protons (A and A') to heteronuclear resonances (X) (i.e. <sup>13</sup>C) depends on the coupling asymmetry of the two parahydrogen protons with the X nucleus ( $J_{AX} - J_{A'X} / 2 * J_{AA'}$ ).<sup>28,29</sup> Therefore heteronuclear polarization cannot be achieved if the target X nucleus is symmetrically coupled with the two protons. Also, polarization is expected to be low if the difference between J<sub>AX</sub> and J<sub>A'X</sub> is small or when the moiety to be hydrogenated is far away from the heteroatom. Optimal polarization transfer has been observed when the added parahydrogen protons are two and three bonds away from the "target" heteronucleus (figure 1, structure **a**).

Herein we report PHIP results showing that high polarization levels can also be achieved from the reaction of para-H<sub>2</sub> with substrates having general formula **b** and **c** (figure 1). Moreover a successive hydrolysis step yields hyperpolarized carboxylic acids, such as acetate and pyruvate whose formation has been till now precluded by the lack of suitable de-hydrogenated precursors.

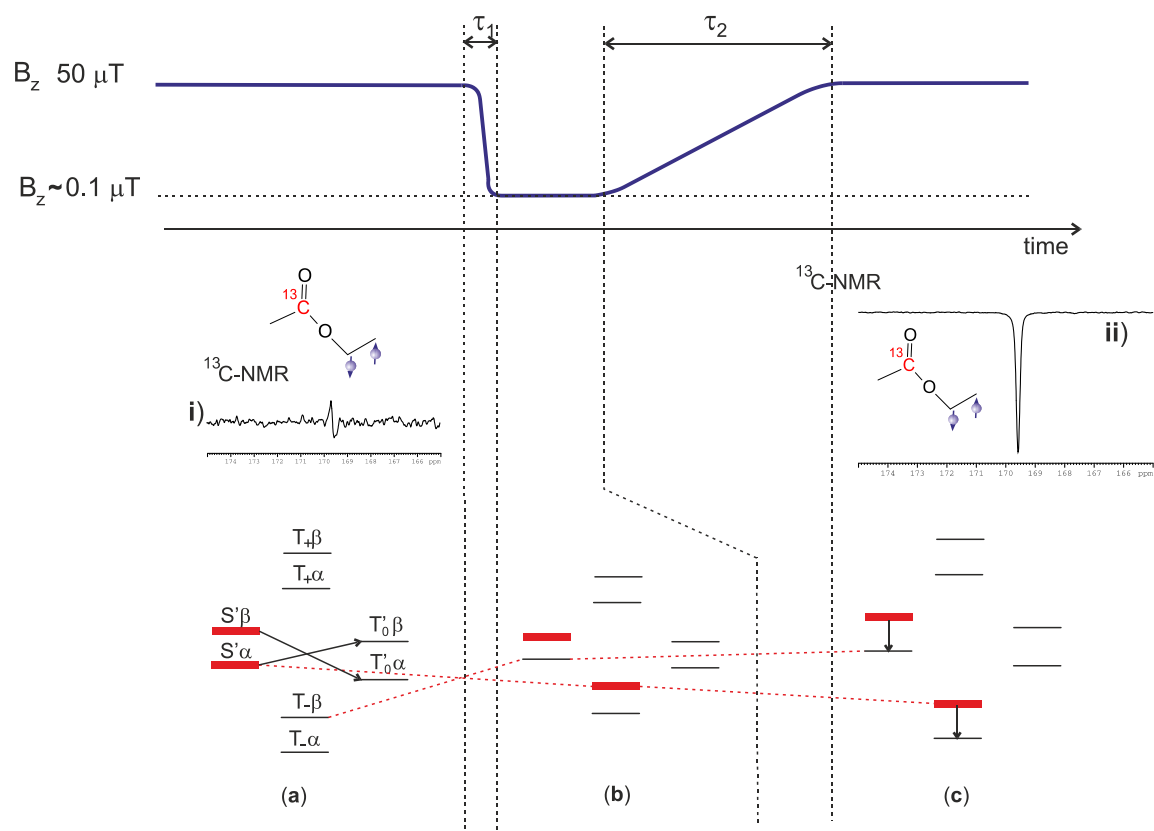


**Figure 1.**

## Results

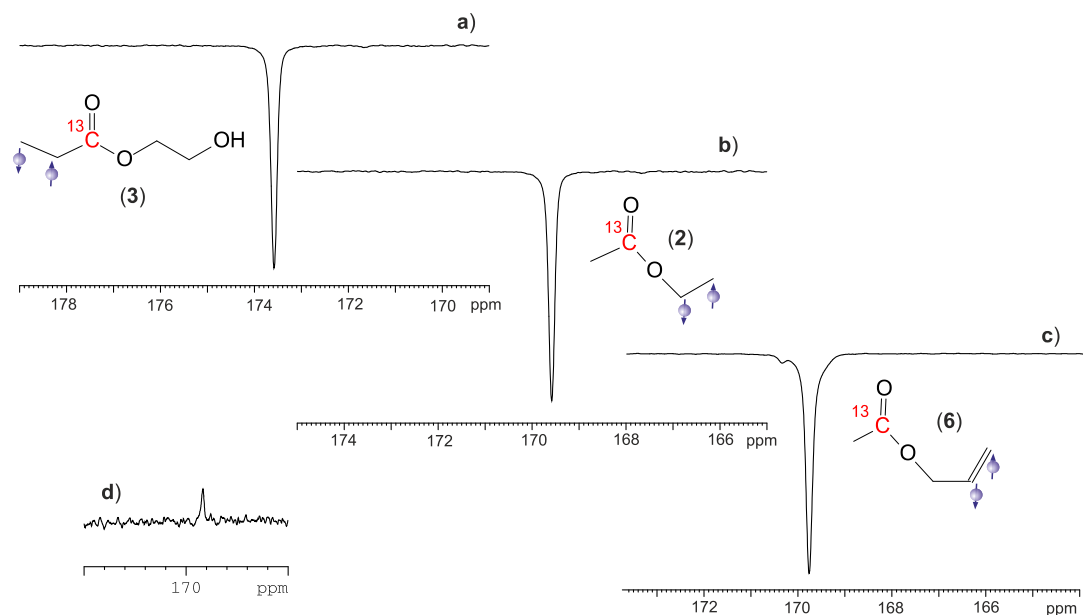
### ParaHydrogen Induced Polarization of acetate

The  $^{13}\text{C}$ -NMR spectrum acquired immediately after the parahydrogenation of vinyl acetate (**1**) (1.1 % C-13), carried out at earth's magnetic field, according to the ALTADENA method,<sup>30</sup> showed hyperpolarized signals for the aliphatic carbons (**2**) whereas no polarization was observed at the  $^{13}\text{C}$  carbonyl carbon (figure 2, the whole spectra is reported in Supplementary Fig. 2). Conversely, the application of low magnetic field cycling<sup>27,31</sup> immediately after the parahydrogenation reaction yielded an efficient polarization transfer to the  $^{13}\text{C}$  longitudinal magnetization of the carboxylate group (Figure 2).



**Figure 2.**

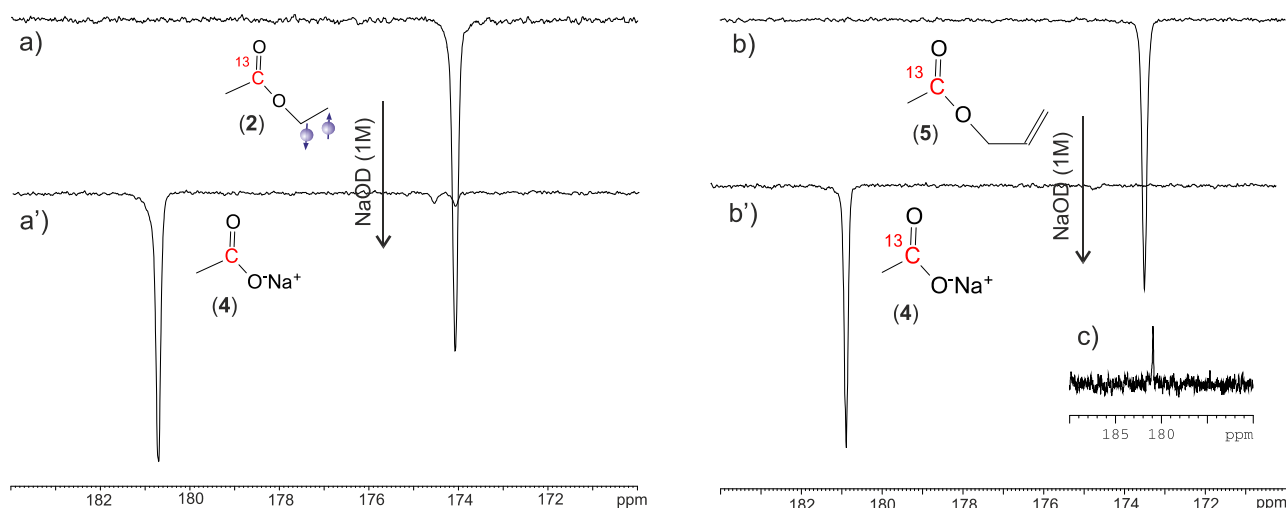
Support for the view that the polarization level attained for the  $^{13}\text{C}$ -carboxylate group of ethyl acetate is good was obtained by acquiring the  $^{13}\text{C}$ -NMR spectrum of parahydrogenated hydroxyethyl propionate (**3**, HEP). The PHIP effect on the  $^{13}\text{C}$  carboxylate signal of HEP had been previously estimated to yield polarization level as high as 20%.<sup>31</sup> As shown in figure 3, the polarization level observed for the  $^{13}\text{C}$ -carboxylate resonances of ethyl acetate and HEP are quite similar.



**Figure 3.**

After the polarization transfer step, the ethyl group was removed by hydrolysis, while the hyperpolarization is kept on the  $^{13}\text{C}$  carbonyl signal of acetate. Hydrolysis was carried out by adding sodium hydroxide (1M solution) to the reaction mixture (parahydrogenation in water) and hyperpolarized  $^{13}\text{C}$ -acetate (**4**) is instantaneously formed (figure 4).

Since the synthesis of the vinyl esters is not straightforward due to keto-enol tautomerism of ethenol and ethanal species, propargylic ester was considered –Acetic acid prop-2-ynyl ester (**5**) was synthesized, reacted with para- $\text{H}_2$  and the obtained acetic acid allyl ester (**6**) was subjected to the field cycling procedure. The hyperpolarization level obtained on the  $^{13}\text{C}$ -carboxylate signal of **5**, was similar to that observed for the corresponding vinyl ester. Again, hydrolysis yielded the free acid by maintaining a good polarization level of the parent ester (figure 4). When the deuterated propargylic alcohol was used (2- $\text{d}_2$  propargylic alcohol)<sup>19</sup> polarization transfer to  $^{13}\text{C}$  carboxylic signal was not obtained (spectrum in Supplementary Fig. 4).



**Figure 4.**

#### ParaHydrogen Induced Polarization of Pyruvate

On the basis of the results obtained on propargylic ester of acetate, propargyl alcohol was used to prepare the unsaturated ester of pyruvic acid 2-propynyl-2-oxopropanoate (**7**)<sup>32</sup> (naturally abundance  $[1-^{13}\text{C}]$  pyruvate).

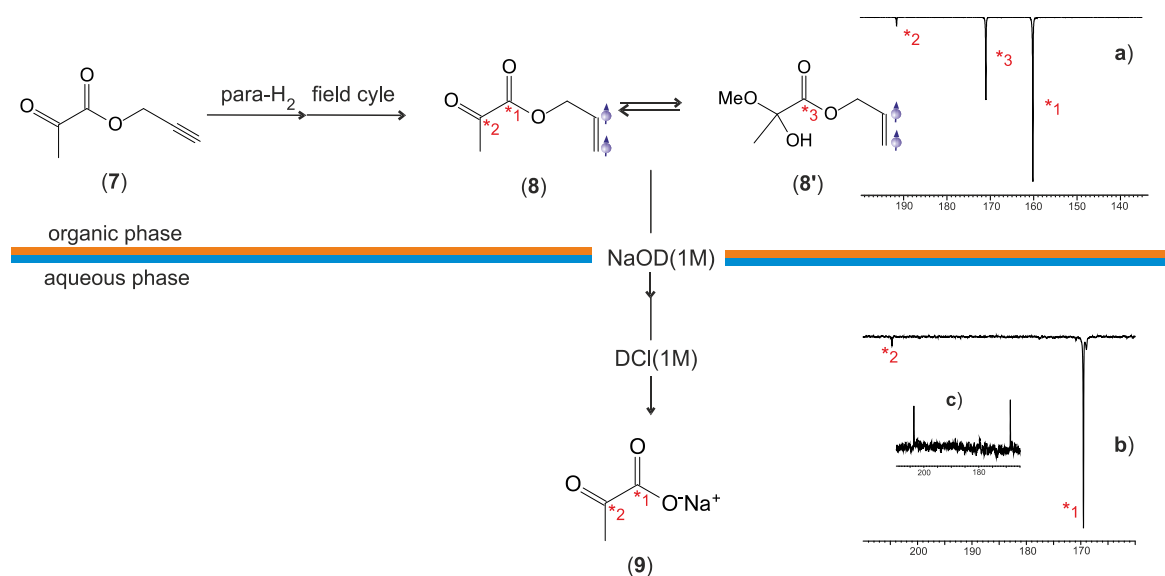
According to the reported phase transfer procedure<sup>33</sup>, hydrogenation of the ester was carried out in a chloroform (90%) methanol (10%) mixture and was followed by the addition of an aqueous basic solution (NaOD 1M). Two phases quickly separated out, the catalyst being retained in the organic phase while the hyperpolarized sodium salt was extracted in the aqueous phase. This method results in an improved hydrogenation efficiency with respect to hydrogenation in water and yielded an aqueous solution of the polarized metabolite free from the organometallic catalyst.

As shown in figure 5, a good level of polarization was observed for the C-13 carbonyl signals of the allyl ester of pyruvate (**8**) upon the application of the magnetic field cycling (thermal equilibrium  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra of the natural abundance C-13 carbonyl and acetal tautomer are reported in Supplementary Fig. 6 and 7).

Next, hydrolysis and phase separation were quickly carried out by adding NaOD (1M) and the pH of the solution was finally set to about 5 by the addition of an equimolar amount of DCl. Overall, this procedure took



about 10 seconds. The  $^{13}\text{C}$  spectrum of the aqueous phase (figure 5) shows that a good polarization level is maintained on pyruvate (9) after hydrolysis and pH equilibration. The relaxation times ( $T_1$ ) of the  $^{13}\text{C}$  carbonyl signals of allyl-pyruvate and pyruvate were measured, at 14.1T, to be  $T_1=33.5\pm 5''$  and  $T_1=40\pm 6''$  respectively. The occurrence of such a long  $T_1$ s ensured that polarization loss during the experimental work-up is limited.



**Figure 5.**

## Discussion

The results reported herein show that parahydrogen induced polarization can be obtained on molecules, such as acetate and pyruvate (and, in principle, can be extended to other carboxylic acids), by using precursors containing a side-arm capable of hydrogenation that can be hydrolyzed to yield the hyperpolarized target products. The reported method, named PHIP-SAH (PHIP by means of Side Arm Hydrogenation) relies on the following steps: i) functionalization of the target acidic molecule with an unsaturated alcoholic group (vinyl/propargyl alcohol); ii) parahydrogenation of the unsaturated ester; iii) polarization transfer to the carboxylic C-13 signal by applying magnetic field cycling; iv) release of the alcohol moiety by hydrolysis to obtain the polarized  $^{13}\text{C}$ -carboxylate containing product.

Parahydrogenation of vinyl acetate and hydrolysis have been previously reported<sup>34</sup> for obtaining hyperpolarized ethanol. Here it is shown that polarization transfer to the <sup>13</sup>C carbonyl signal is possible by means of magnetic field cycling. It was reported<sup>31</sup> that magnetic field cycling yields about 20% polarization on HEP and it is shown in this work that the same polarization level is obtained in parahydrogenated ethyl acetate, in spite of less favorable J coupling pattern.

Polarization transfer from the two parahydrogen protons (indicated with A and A') to the <sup>13</sup>C carbonyl signal (X) of ethyl acetate may be accounted for on the basis of spin state populations. Spin states are expressed as product of ortho ( $\alpha\alpha=T_+$ ,  $(\alpha\beta+\beta\alpha)=T_0$ ,  $\beta\beta=T_-$ )-para ( $(\alpha\beta-\beta\alpha)=S$ ) states for the protons and  $\alpha$  and  $\beta$  states for <sup>13</sup>C.<sup>28</sup>

When the parahydrogen molecule is added to the <sup>13</sup>C-containing substrate, at the earth's magnetic field, the singlet (S) and the triplet (T<sub>0</sub>) states are mixed thanks to asymmetric coupling of the protons with the heteroatom. The states  $S'\alpha - T_0'\alpha$  (for <sup>13</sup>C in the  $\alpha$  state) and  $S'\beta - T_0'\beta$  (for <sup>13</sup>C in the  $\beta$  state) are formed, where the prime indicates that these states are a linear combination of S and T<sub>0</sub> states with a dominating singlet (S') or triplet (T<sub>0</sub>') character (figure 2, lower part, a).<sup>28</sup> Singlet-triplet mixing allows transitions between levels  $S'\alpha - T_0'\beta$  (polarized absorption) and  $S'\beta - T_0'\alpha$  (polarized emission) and the transition probability can be calculated from the relevant J coupling values. For ethyl acetate ( $J_{AX}= 3.2$  Hz,  $J_{A'X}= 1.7$  Hz and  $J_{AA'} = 7.5$ Hz) the resulting transition probability is  $|\langle S'\alpha | I_+^X | T_0'\beta \rangle|^2 = 0.02$  while for hydroxyl ethyl propiolate ( $J_{AX}=7.2$  Hz and  $J_{A'X}= -5.6$  Hz  $J_{AA'} = 7.5$  Hz) the transition probability is 0.37 (detailed calculations are reported in Supplementary Methods). Since polarization intensity is also related to the population difference between levels, which is 0.51 for HEP and 0.98 for ethyl acetate, it can be derived that polarization on the <sup>13</sup>C carbonyl signal of parahydrogenated ethyl acetate is expected to be only about 10% of that on HEP, in agreement with what was anticipated from the J coupling ratio  $(J_{AX} - J_{A'X}) / 2 * J_{AA'}$ .

When magnetic field cycling is applied, isotropic mixing between heteronuclei takes place at nearly zero field and all the states with the same total spin  $T_{+\beta}-T_0\alpha-S\alpha$  ( $I=-1/2$ ) and  $T_{-\alpha}-T_0\beta-S\beta$  ( $I=+1/2$ ) are mixed. Due to non-adiabatic passage from earth's field to low field, the most stable state (state  $T_{+\beta}$  at earth's field) becomes the most populated state (figure 1 2, **a b**). If J couplings of ethyl acetate are considered, almost all the singlet state population is transferred to the state  $T_{+\beta}$  while, on HEP the singlet state population is shared between the three states (Supplementary Information). Then, when the sample is adiabatically transferred back to high field, the spin states populations are maintained and the hyperpolarized  $^{13}\text{C}$  transitions take place between the states  $T_{+\beta} - T_{+\alpha}$  and  $S'\beta - S'\alpha$  (figure 1 2, **c**). Note that, in this case, the transition probability is  $|\langle T_{+\alpha} | I_{+}^X | T_{+\beta} \rangle|^2 = 1$  and polarization intensity is only due to population differences. The intensity of hyperpolarized longitudinal magnetization of the  $^{13}\text{C}$  carbonyl signal of ethyl acetate is expected to be even higher than that for HEP (for more detail, see the Supplementary Information, methods). Therefore it may be expected that high polarization levels can be obtained on  $^{13}\text{C}$  carbonyl signals of the esters precursors using a finely controlled field cycling procedure.<sup>31</sup>

When propargyl esters are used, polarization transferred to the  $^{13}\text{C}$ -carbonyl signal is the same as that obtained for the vinyl ester albeit the parahydrogen protons are further from the  $^{13}\text{C}$  carbonyl atom. We surmise that, in this case, parahydrogen spin order is transferred to the methylenic protons and, from these protons, to the  $^{13}\text{C}$  carbonyl atom. The lack of polarization on the  $^{13}\text{C}$ -carbonyl signal obtained when the deuterated compound is used supports this hypothesis.

In summary, these findings open a very interesting perspective for the use of parahydrogen-based procedures for the generation of hyperpolarized, biologically relevant, molecules. The possibility of using propargyl alcohol as a removable synthon to generate parahydrogen induced polarization on  $^{13}\text{C}$  resonances markedly widens the applicability of the PHIP approach.

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.

## Methods

### Chemicals

Vinyl acetate (**1**), hydroxyl ethyl propiolate (**2**) and the catalyst

[Rh(diphenylphosphinobutane)(cyclooctadiene)][BF<sub>4</sub>] were purchased from Sigma-Aldrich and used without purification.

Propargyl esters of acetic acid and pyruvic acid (**3** and **4**) were synthesized as reported in reference 36 33 and used without further purification.

The water soluble catalyst [Rh(norbornadiene)1,4-bis-[(phenyl-3-propane sulfonate) phosphine][BF<sub>4</sub>] (3mM) was prepared according to the method described by Hövener et al.<sup>32</sup>

### Parahydrogenation experiments

Parahydrogenation of HEP, vinyl acetate and propargyl acetate were carried out in acetone-d<sub>6</sub> using the commercial catalyst [Rh(diphenylphosphinobutane)(cyclooctadiene)][BF<sub>4</sub>]. 5mm NMR tubes equipped with Young valve were charged with the catalyst ( $4 \cdot 10^{-3}$  mmol), 400  $\mu$ l of acetone-d<sub>6</sub> and the catalyst was activated by hydrogenation of the coordinated diene. The substrate was added ( $35 \cdot 10^{-3}$  mmol) and the tube pressurized with 2 bar of parahydrogen while keeping it in liquid Nitrogen in order to achieve an higher parahydrogen pressure in the NMR tube and to freeze the hydrogenation reaction. The NMR tube was then thawed to room temperature and vigorously shaken for about 10 ". Immediately after shaking, the sample was a) placed in the NMR spectrometer (Bruker Avance 600 MHz <sup>1</sup>H-NMR) and a single scan <sup>13</sup>C spectrum was acquired or,

alternatively, b) quickly dropped in the  $\mu$ -metal shield, slowly taken out of it (about 5") and immediately placed in the NMR spectrometer for  $^{13}\text{C}$  acquisition (1 scan).

For the parahydrogenation in aqueous medium, 400  $\mu\text{l}$  of the catalyst  $[\text{Rh}(\text{norbornadiene})1,4\text{-bis-}[(\text{phenyl-3-propane sulfonate})\text{phosphine}][\text{BF}_4]$  (3mM) prepared according to the reported procedure<sup>35</sup> were placed into the 5mm NMR tube equipped with Young valve, the substrate was added ( $35 \cdot 10^{-3}$  mmol) and the tube pressurized with 2 bar of parahydrogen while keeping it in liquid Nitrogen. After thawing, the NMR tube was warmed up using a water bath (90°C) for 5". Then the parahydrogenation reaction was initiated by shaking the tube and field cycle was applied as described for the reaction in organic medium.

For phase extraction experiments, 10 mm NMR tubes equipped with Young valve were used. The catalyst  $[\text{RhCOD}(\text{dppb})][\text{BF}_4]$  ( $4 \cdot 10^{-3}$  mmol) was activated in 50  $\mu\text{l}$  of methanol- $\text{d}_4$ , 500  $\mu\text{l}$  of  $\text{CDCl}_3$  and the substrate 2-propynyl-2-oxopropanoate ( $50 \cdot 10^{-3}$  mmol) were added. The NMR tube was pressurized with 2 bar of parahydrogen while keeping it in liquid Nitrogen, then it was thawed and warmed up by means of a water bath (90°C). Parahydrogenation and field cycle were applied as described above, then the NMR tube was opened and 300  $\mu\text{l}$  of NaOD solution (1M) were added to the organic solvent followed few seconds later by the addition of 300  $\mu\text{l}$  of DCl (1M). The aqueous phase is then transferred into a 5 mm NMR tube and placed into the NMR spectrometer (Bruker Avance 600 MHz) for the acquisition of the  $^{13}\text{C}$  spectrum (1 scan).

**Field cycling.** Two concentric  $\mu$ -metal cylinders (200 mm height, 30 and 50 mm diameter) (Meca-Magnetic, Amilly, France) were used to carry out field cycling experiments. NMR tubes were dropped into the centre of the shield and then slowly pulled up out of the cylinders in about 5"s.

**Parahydrogen.** Hydrogen gas was enriched to 92% parahydrogen by catalytic conversion at low temperature (36K) and collected directly into the NMR tubes for parahydrogenation reactions.

## References

- <sup>1</sup> Ardenkjær-Larsen, J.H. et al. Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR, *PNAS* **100**, 10158–10163 (2003).
- <sup>2</sup> Golman, K., in 't Zandt, R. & Thaning, M. Real-time metabolic imaging, *PNAS* **103**, 11270–11275 (2006)
- <sup>3</sup> Kurhanewicz, J. et al. Analysis of cancer metabolism by imaging hyperpolarized nuclei: Prospects for translation to clinical research *Neoplasia* **13**, 81-97 (2011).
- <sup>4</sup> Witney, T.H., Kettunen, M.I. & Brindle, K.M. Kinetic modeling of hyperpolarized <sup>13</sup>C label exchange between pyruvate and lactate in tumor cells *J. Biol. Chem.* **286**, 24572-24580 (2011).
- <sup>5</sup> Kennedy, B.W.C., Kettunen, M.I., Hu, D.-E. & Brindle, K.M. Probing lactate dehydrogenase activity in tumors by measuring hydrogen/deuterium exchange in hyperpolarized L-[1-<sup>13</sup>C,U-<sup>2</sup>H]lactate *J. Am. Chem. Soc.* **134**, 4969-4977 (2012).
- <sup>6</sup> Gallagher, F.A. et al. Production of hyperpolarized [1,4-<sup>13</sup>C<sub>2</sub>]malate from [1,4-<sup>13</sup>C<sub>2</sub>] fumarate is a marker of cell necrosis and treatment response in tumors *PNAS* **106**, 19801-19806 (2009).
- <sup>7</sup> Jensen, P.R. et al. Acetyl-CoA and Acetyl carnitine show organ specific distribution in mice after injection of DNP Hyperpolarized Acetate *Proc. Intl. Soc. Magn. Reson. Med.*, 172 (2008).
- <sup>8</sup> Comment, A. et al. In vivo DNP-enhanced <sup>13</sup>C-labeled acetate brain studies in a 9.4T animal scanner *Proc. Int. Soc. Magn. Reson. Med.* 369 (2007).
- <sup>9</sup> Gallagher, F.A., Kettunen, M.I. & Brindle, K.M. Imaging pH with hyperpolarized <sup>13</sup>C *NMR Biomed.* **24**, 1006-1015 (2011).
- <sup>10</sup> Gallagher, F.A. et al. Magnetic resonance imaging of pH in vivo using hyperpolarized <sup>13</sup>C-labelled bicarbonate *Nature* **453**, 940-943 (2008).
- <sup>11</sup> Brindle, K. New approaches for imaging tumour responses to treatment *Nature Reviews Cancer* **8**, 94-107 (2008).
- <sup>12</sup> Natterer, J. & Bargon, J. Parahydrogen Induced Polarization *Prog. Nucl. Magn. Reson. Spectrosc.* **31**, 293-315 (1997).
- <sup>13</sup> Duckett, S.B. & Mewis, R.E. Application of Parahydrogen Induced Polarization Techniques in NMR spectroscopy and imaging *Acc Chem. Res.* **45**, 1247-1257 (2012).
- <sup>14</sup> Bhattacharya, P. et al. Towards hyperpolarized (<sup>13</sup>C)-succinate imaging of brain cancer. *J Magn. Reson.* **186**, 150-155 (2007).
- <sup>15</sup> Chekmenev, E.Y. et al. PASADENA hyperpolarization of succinic acid for MRI and NMR spectroscopy *J. Am. Chem. Soc.* **130**, 4212–13 (2008).

- <sup>16</sup> Shchepin, R.V., Coffey, A.M., Waddell, K.W. & Chekmenev, E.Y. PASADENA Hyperpolarized <sup>13</sup>C Phospholactate *J. Am. Chem. Soc.* **134**, 3957–3960 (2012).
- <sup>17</sup> Shchepin, V. Coffey, A.M, Waddell, K.W. & Chekmenev, E.Y. Parahydrogen Induced Polarization of 1-<sup>13</sup>C-Phospholactate-d<sub>2</sub> for Biomedical Imaging with >30.000.000-fold NMR signal Enhancement *Anal. Chem.* **86**, 5601-5605 (2014).
- <sup>18</sup> Reineri, F. et al. Para-hydrogenated Glucose Derivatives as Potential <sup>13</sup>C-Hyperpolarized Probes for Magnetic Resonance Imaging *J. Am. Chem. Soc.* **132**, 7186–7193 (2010).
- <sup>19</sup> Reineri, F. et al. N-15 MR hyperpolarization via the reaction of parahydrogen with <sup>15</sup>N-propargylcholine *J. Am. Chem. Soc.* **134**, 11146-11152 (2012).
- <sup>20</sup> Roth, M. et al. <sup>13</sup>C hyperpolarization of a barbituric acid derivative via parahydrogen induced polarization *J. Magn. Reson.* **204**, 50-55 (2010).
- <sup>21</sup> Adams, R.W. et al. Reversible Interactions with para-hydrogen enhance NMR sensitivity by polarization transfer *Science* **323**, 1708-1711 (2009).
- <sup>22</sup> Atkinson, K.D. et al. Spontaneous Transfer of Parahydrogen Derived Spin Order to Pyridine at Low Magnetic Field *J. Am. Chem. Soc.* **131**, 13362–13368 (2009).
- <sup>23</sup> Hovener, J.B. et al. A hyperpolarized equilibrium for magnetic resonance *Nature Commun.* **4**, 2496 (2013).
- <sup>24</sup> Glöggler, S. et al. Para-hydrogen induced polarization of amino acids, peptides and deuterium–hydrogen gas *Phys. Chem. Chem. Phys.* **13**, 13759–13764 (2011).
- <sup>25</sup> Zeng, H. et al. Optimization of SABRE for polarization of the tuberculosis drugs pyrazinamide and isoniazid *J. Magn. Reson.* **237**, 73-78 (2013).
- <sup>26</sup> Cowley, B. et al. Iridium N-heterocyclic carbene complexes as efficient catalysts for magnetization transfer from para-hydrogen *J. Am. Chem. Soc.* **133**, 6134–6137 (2011).
- <sup>27</sup> Golman, K. et al. Parahydrogen-induced polarization in imaging: Subsecond <sup>13</sup>C angiography *Magn. Reson. Med.* **46**, 1-5 (2001).
- <sup>28</sup> Barkemeyer, J., Haake, M. & Bargon J. Hetero-NMR enhancement via Parahydrogen Labeling *J. Am. Chem. Soc.* **117**, 2927-2928 (1995).
- <sup>29</sup> Natterer, J., Schedletzky, O., Barkemeyer, J. & Bargon, J. Investigating catalytic processes with parahydrogen: evolution of zero-quantum coherence in AA<sub>0</sub>X spin systems *J. Magn. Reson.* **133** 92–97 (1998).
- <sup>30</sup> Pravica, M.G., Weitekamp, D.P. Net NMR alignment by adiabatic transport of parahydrogen addition products to high magnetic field *Chem. Phys. Lett.* **145**, 255–258 (1988).
- <sup>31</sup> Jóhannesson, H., Axelsson, O. & Karlsson M. Transfer of para-hydrogen spin order into polarization by diabatic field cycling *C. R. Physique* **5**, 315–324 (2004).

<sup>32</sup> Heaney, F., Fenlon, J., O Mahony, C., McArdle, P. & Cunningham, D. Oximono-esters as precursors to heterocycles – generation of oxazinone *N*-oxides and cycloaddition to alkene dipolarophiles *Org. Biomol. Chem.* **1**, 4302-4316 (2003).

<sup>33</sup> Reineri, F. et al. Use of Labile Precursors for the Generation of Hyperpolarized Molecules from Hydrogenation with Parahydrogen and Aqueous-Phase Extraction *Angew. Chem. Int. Ed.* **50**, 1-5 (2011).

<sup>34</sup> Trantschel, T. et al. Parahydrogen induced polarization in face of keto–enol tautomerism: proof of concept with hyperpolarized ethanol *Phys. Chem. Chem. Phys.* **14**, 5601–5604 (2012)

<sup>35</sup> Hövener, J.B. et al. · Quality assurance of PASADENA hyperpolarization for <sup>13</sup>C biomolecules *Magn. Reson. Mat. Phys. Biol. Med.* **22**, 123-34 (2009).

**Acknowledgements.** We gratefully acknowledge Regione Piemonte for financial support.

**Author contributions.** The experiments were designed and performed by F.R and T.B., synthesis of the organic substrates was performed by T.B., all the authors contributed to the analysis and interpretation of the results. The manuscript was written by S.A. and F.R.

**Figure 1 Unsaturated substrates and hydrogenation reactions.** In the generic formula (upper part of the figura) it is evidenced that compounds **a**, **b** and **c** differ for the number of bonds between the <sup>13</sup>C-carbonyl moiety and the site of para-H<sub>2</sub> addition. Most of PHIP experiments have been till now reported for type-a compounds as they show the most favorable J –coupling pattern. The specific reactions are listed in the lower part of the figure, the numbers are those reported in the text.

**Figure 2: Field cycling yield high polarization on <sup>13</sup>C carbonyl of ethyl acetate.** Upper part of the figure: magnetic field profile during the field cycle. The magnetic field is cycled between earth magnetic field, where the parahydrogenation reaction is carried out, and nearly zero field by using concentric cylinders made of μ-metal. The first passage from earth to zero field is fast (non-adiabatic) (τ<sub>1</sub>), the second passage is slow (adiabatic) and takes about 5 seconds (τ<sub>2</sub>). Central part of the figure: <sup>13</sup>C spectrum (one scan, carbonyl region only) of ethyl acetate obtained from parahydrogenation of vinyl acetate (85 mM), acquired at 14.1 T and 298K, before the application of field cycle (**i**) and after application of field cycling (**ii**). In **i**) the <sup>13</sup>C carbonyl signal is hardly detectable from the spectral noise, in **ii**) the same carbonyl resonance shows high polarization. Lower



part of the figure: spin states of the AA'X system and their populations immediately after parahydrogen addition (a), after non-adiabatic transport to low field (b) and successive adiabatic transport to earth field (c).

**Figure 3: C-13 carbonyl hyperpolarization on type A, B, and C molecules.** Similar level of hyperpolarization is obtained upon field cycle application, irrespective of the position where parahydrogens are added, i.e. a) at the adjacent position to the carbonyl (Hydroxy ethyl propionate, **3**), b) at three-four (ethyl acetate, **2**), c) or at four-five (acetic acid allyl ester **6**) bonds far away from the carbonyl  $^{13}\text{C}$  atom. d) Thermal equilibrium spectrum of acetic acid allyl ester, 10100 scans (30° pulse, 15h acquisition time). The concentration of the three precursors was the same, i.e. 85 mM. The parahydrogenation reaction was carried out in acetone- $\text{d}_6$ .

**Figure 4: Formation of  $^{13}\text{C}$ -hyperpolarized acetate.** Free  $^{13}\text{C}$ -hyperpolarized acetate ( $^{13}\text{C}$  natural abundance) is obtained from hydrolysis of parahydrogenated ethyl (**2**) and allyl (**5**) esters (reactions carried out in aqueous medium). Addition of Sodium hydroxide (1M) to the aqueous solutions of ethyl acetate and acetic acid allyl ester allows efficient hydrolysis with the release of the alcoholic moiety whereas polarization is fully maintained on the carbonyl moiety (A' and B'). C)  $^{13}\text{C}$  spectrum of the acetate product at thermal equilibrium, 1000 scans (90° pulse), acquisition time 56h.

**Figure 5: Formation of  $^{13}\text{C}$ -hyperpolarized pyruvate.** Organic phase: parahydrogenation of 2-propynyl-2-oxopropanoate (**7**) carried out in Chloroform/Methanol (10:1) and field cycling allow hyperpolarization of the  $^{13}\text{C}$  carbonyl of the allyl ester of pyruvate (a:  $^{13}\text{C}$  spectrum, one scan). The presence of methanol in the reaction mixture causes an equilibrium between carbonylic (**8**) and emi-acetalic (**8'**) forms. All the carbonyl signals (1, 2 and 3) are hyperpolarized. Hydrolysis was carried out by means of the addition of NaOD (1M) then DCI (1M solution) was added to reach pH 5. The aqueous phase was collected in a NMR tube, the  $^{13}\text{C}$ -NMR single scan spectrum (b) showed polarized signals of Sodium pyruvate (**9**) (about 50mM). The (c) spectrum is the corresponding thermal equilibrium spectrum acquired with 10100 scans (30° pulse, Ernst angle, acquisition time 15h 20').

