



## Effect of low and zero magnetic field on the hyperpolarization lifetime in parahydrogenated perdeuterated molecules

Francesca Reineri, Daniela Santelia, Roberto Gobetto, Silvio Aime \*

Università degli Studi di Torino, Dep. Chemistry I.F.M., Via P. Giuria 7, 10123 Torino, Italy

### ARTICLE INFO

#### Article history:

Received 13 February 2009

Revised 15 May 2009

Available online 22 May 2009

#### Keywords:

Parahydrogen  
Hyperpolarization  
Relaxation rate  
Singlet state

### ABSTRACT

Hyperpolarization can be kept for times longer than  $T_1$  if it is maintained in a singlet state, from which transitions are not allowed. Another, more direct, way to slow the relaxation process consists in the use of perdeuterated molecules. Here both methods have been applied and the hyperpolarization (induced by para- $H_2$ ) decay rate has been measured at two different magnetic fields: earth field and zero field. While relaxation is very slow at earth field, it becomes faster at zero field: this rather unexpected finding has been explained on the basis of isotropic mixing between  $^1H$  and  $^2H$ .

© 2009 Elsevier Inc. All rights reserved.

### 1. Introduction

Since the early days of NMR spectroscopy, the search for hyperpolarization procedures has been seen as a key to overcome its intrinsic low sensitivity. In recent years there has been a renewed interest towards hyperpolarized molecules thanks to the possibility of their use in MRI investigations [1–4]. Currently, for these applications, much attention has been devoted to hyperpolarization of  $^{13}C$  and  $^{15}N$  resonances using Dynamic Nuclear Polarization (DNP) or parahydrogenation of unsaturated substrates [5,6]. The main limit to the application of both DNP and parahydrogen methods is due to the fact that equilibrium population of spin levels is quickly restored by relaxation processes. In particular, concerning the small organic molecules used in ParaHydrogen Induced Polarization (PHIP), polarization decay is mainly driven by dipolar interactions among protons [7]. Therefore substrate deuteration is commonly applied to decrease the relaxation rate [8].

Recently Carravetta et al. tackled the general problem of storing  $^1H$ -polarization for times longer than  $T_1$  on the basis of the fact that, as known from theory, transitions from singlet states are not allowed [9–11]. This is the reason why, in principle, a para-enriched  $H_2$  mixture can be kept unaltered indefinitely. When the  $H_2$  molecule is added to a substrate the two protons are usually located at chemically (or magnetically) different sites. However, even if the two protons become chemically different, the singlet state may be retained if the difference between their resonance frequencies is negligible, i.e. if they are placed in a weak magnetic field. Therefore the non-equilibrium population of the singlet state

(of parahydrogen) can be maintained on the hydrogenation product because they form an  $A_2$  spin system. In this case the symmetry is broken only when the parahydrogenated molecule is transferred into a high-field NMR spectrometer. This behavior has been experimentally assessed in parahydrogenated molecules and hyperpolarization storage was observed also when the two parahydrogen protons are coupled to other protons [12,13]. In the latter case it has been shown that three singlet states are formed in proportion of a function of  $J$  coupling constants.

In this publication we report our observations about the effect of the magnetic field strength on the polarization loss of the  $^1H$  resonances of a perdeuterated parahydrogenated molecule. We used the perdeuterated [14] methyl butynoate (**1**) (Fig. 1) as parahydrogenation substrate and investigated the polarization decay rate at two different low magnetic field, namely earth magnetic field (50  $\mu T$ ) and zero field (0.1  $\mu T$ ).

### 2. Methods and results

In order to assess the effect of the magnetic field strength on  $^1H$  polarization decay rate of (**2**) the following experimental work-up was applied:

- (1) Upon addition of para- $H_2$  to an acetone solution of **1** in a 5 mm NMR tube, the hydrogenation reaction was allowed to proceed (under vigorous shaking) for 10 s in the spectrometer fringe field, at 0.2 mT [15].
- (2) The NMR tube was quickly opened to release the non-reacted hydrogen, then it was placed (a) at 50  $\mu T$ , i.e. at earth magnetic field or (b) at 0.1  $\mu T$ , in a  $\mu$ -metal cylinder that allows to shield the external earth magnetic field.

\* Corresponding author. Fax: +39 011 670 7855.

E-mail address: [silvio.aime@unito.it](mailto:silvio.aime@unito.it) (S. Aime).

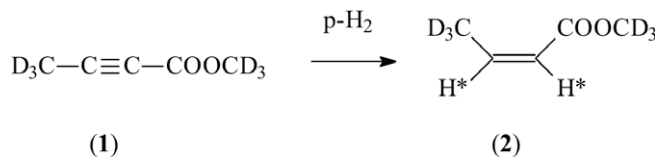


Fig. 1. Parahydrogenation reaction of perdeuterated methyl-butynoate.

- (3) The sample was kept at 50  $\mu\text{T}$  (or at 0.1  $\mu\text{T}$ ) for a time interval varying from 60 to 420 s, then a single scan  $^1\text{H}$  NMR spectrum (45° pulse) was acquired on a Bruker Advance 600 spectrometer.
- (4) The whole procedure was repeated for each time interval, at each magnetic field.

Fig. 2 reports the two series of  $^1\text{H}$  NMR spectra of the parahydrogenated product (2) acquired for increasing time delays at 50  $\mu\text{T}$  (left) or 0.1  $\mu\text{T}$  (right).

Polarization decay rates, one for each magnetic field, were obtained by interpolation of the polarized signal intensities as a function of time (Fig. 3). Using this method, the polarization decay constant obtained at 50  $\mu\text{T}$  was  $100 \pm 7$  s, while at 0.1  $\mu\text{T}$  it was only  $42 \pm 3$  s.

The relaxation constant ( $T_1$ ) measured by the inversion recovery sequence inside the spectrometer magnetic field resulted to be 21 s.

### 3. Discussion

All the parahydrogenation reactions were carried out in the ALTADENA way, therefore the parahydrogen singlet state is kept on the product molecule until the sample is adiabatically transferred into the spectrometer. According to reported theory [16], the polarized signal intensities (one positive  $I_z^{H1}$ , one negative  $-I_z^{H2}$ ) depend on the population of the singlet state (para state) with which they cor-

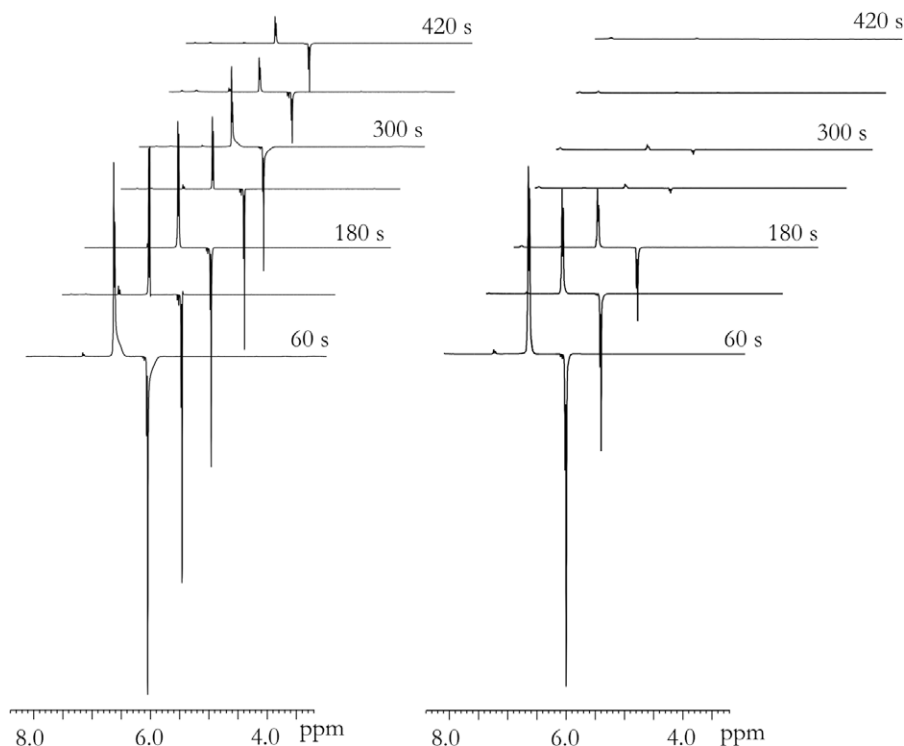


Fig. 2. Single scan  $^1\text{H}$  NMR spectra of parahydrogenated 2: the samples (one for each spectrum) were kept at 50  $\mu\text{T}$  (left) or at 0.1  $\mu\text{T}$  (right) for time delays varying from 60 to 420 s.

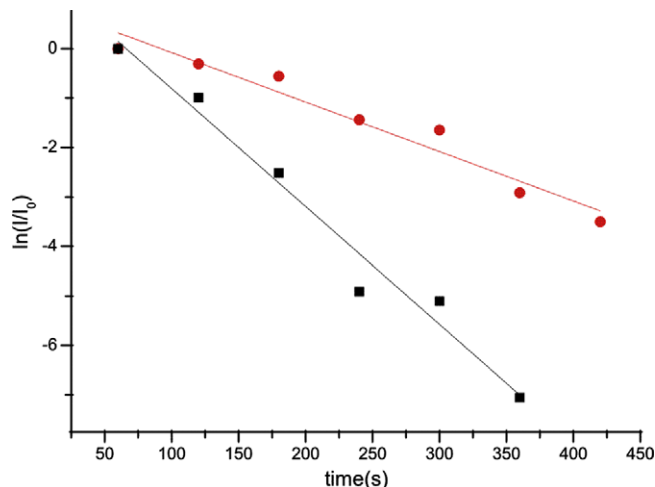


Fig. 3. Logarithmic plot of polarization  $I/I_0$  ( $I$ , mean longitudinal magnetization ( $I_{zH1}(t) - I_{zH2}(t) \rangle / 2$ ;  $I_0$ , mean longitudinal magnetization at 60 s) of molecule (2) as a function of time, at 50  $\mu\text{T}$  (red circles) and 0.1  $\mu\text{T}$  (black squares). (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

relate. Therefore the relaxation time constants obtained above can be referred to the relaxation rate of the singlet state.

In agreement with previous observations [9], the polarization decay constant obtained at 50  $\mu\text{T}$  was about five times the relaxation constant  $T_1$  measured using the inversion recovery sequence.

In the second experiment series, when samples were kept inside the magnetic field shield at 0.1  $\mu\text{T}$ , the polarization decay resulted faster than that observed at earth field (50  $\mu\text{T}$ ): this is rather unexpected as the singlet state formed by the two parahydrogen protons should be kept when the magnetic field is lowered.

To account for this result we need to consider the  $^2\text{H}$  nuclei of the methyl group: their scalar coupling with the two parahydrogen



$$\begin{bmatrix} H_{66} & H_{69} \\ H_{96} & H_{99} \end{bmatrix} = \frac{1}{2} \begin{bmatrix} +2\nu_D + \frac{J_{H1H2}}{2} & -J_{H1D} + J_{H2D} \\ -J_{H1D} + J_{H2D} & +2\nu_D - \frac{3}{2}J_{H1H2} \end{bmatrix}. \quad (7)$$

From the calculation of the eigenfunctions the following spin states were found:

$$\psi_4 = c_{11}\phi_4 + c_{12}\phi_7, \quad \psi_7 = c_{21}\phi_4 + c_{22}\phi_7$$

$$\psi_5 = \phi_5, \quad \psi_8 = \phi_8$$

$$\psi_6 = d_{11}\phi_6 + d_{12}\phi_9, \quad \psi_9 = d_{21}\phi_6 + d_{22}\phi_9.$$

The coefficient values are reported in Table 1.

Then, it is worth noting that at 50  $\mu$ T the spin states with different deuterium spin are not mixed.

At 0.1  $\mu$ T isotropic mixing between heteronuclei allows mixing between spin states with different deuterium spin. In this condition all the spin functions having the same total spin  $M$  are mixed, namely:

$$M = 0: \text{ mixed spin states } \phi_3\text{--}\phi_5\text{--}\phi_8\text{--}\phi_{10}.$$

$$M = 1: \text{ mixed spin states } \phi_2\text{--}\phi_4\text{--}\phi_7.$$

$$M = -1: \text{ mixed spin states } \phi_6\text{--}\phi_9\text{--}\phi_{11}.$$

Therefore in the Hamiltonian matrix

$$\begin{bmatrix} H_{11} \\ H_{22} & H_{24} & H_{27} \\ H_{33} & H_{35} & H_{38} & H_{3,10} \\ H_{42} & H_{44} & H_{47} \\ H_{53} & H_{55} & H_{58} & H_{5,10} \\ H_{66} & H_{69} & H_{6,11} \\ H_{72} & H_{74} & H_{77} \\ H_{83} & H_{85} & H_{88} & H_{8,10} \\ H_{96} & H_{99} & H_{9,11} \\ H_{10,3} & H_{10,5} & H_{10,8} & H_{10,10} \\ H_{11,6} & H_{11,9} & H_{11,11} \\ H_{12,12} \end{bmatrix} \begin{bmatrix} |\alpha\alpha\rangle|1\rangle \\ |\alpha\alpha\rangle|0\rangle \\ |\alpha\alpha\rangle|-1\rangle \\ \frac{1}{\sqrt{2}}|\alpha\beta + \beta\alpha\rangle|1\rangle \\ \frac{1}{\sqrt{2}}|\alpha\beta + \beta\alpha\rangle|0\rangle \\ \frac{1}{\sqrt{2}}|\alpha\beta + \beta\alpha\rangle|-1\rangle \\ \frac{1}{\sqrt{2}}|\alpha\beta - \beta\alpha\rangle|1\rangle \\ \frac{1}{\sqrt{2}}|\alpha\beta - \beta\alpha\rangle|0\rangle \\ \frac{1}{\sqrt{2}}|\alpha\beta - \beta\alpha\rangle|-1\rangle \\ |\beta\beta\rangle|1\rangle \\ |\beta\beta\rangle|0\rangle \\ |\beta\beta\rangle|-1\rangle \end{bmatrix} \quad (8)$$

the following sub-matrices can be identified:

$$\begin{bmatrix} H_{22} & H_{24} & H_{27} \\ H_{42} & H_{44} & H_{47} \\ H_{72} & H_{74} & H_{77} \end{bmatrix} = \frac{1}{2} \begin{bmatrix} -2\nu_H + \frac{J_{H1H2}}{4} & J_{H1D} + J_{H2D} & -J_{H1D} + J_{H2D} \\ J_{H1D} + J_{H2D} & -2\nu_D + \frac{J_{H1H2}}{2} & J_{H1D} - J_{H2D} \\ -J_{H1D} + J_{H2D} & J_{H1D} - J_{H2D} & -2\nu_D - \frac{3}{2}J_{H1H2} \end{bmatrix}, \quad (9)$$

$$\begin{bmatrix} H_{33} & H_{35} & H_{38} & H_{3,10} \\ H_{53} & H_{55} & H_{58} & H_{5,10} \\ H_{83} & H_{85} & H_{88} & H_{8,10} \\ H_{10,3} & H_{10,5} & H_{10,8} & H_{10,10} \end{bmatrix} = \begin{bmatrix} -\nu_H + \nu_D + \frac{J_{H1H2}}{4} & -\frac{J_{H1D}}{2} - \frac{J_{H2D}}{2} & \frac{\sqrt{2}}{4}(J_{H1D} + J_{H2D}) & \frac{\sqrt{2}}{4}(J_{H1D} - J_{H2D}) & 0 \\ \frac{\sqrt{2}}{4}(J_{H1D} + J_{H2D}) & \frac{3}{4}J_{H1H2} & 0 & \frac{\sqrt{2}}{4}(J_{H1D} + J_{H2D}) & 0 \\ \frac{\sqrt{2}}{4}(J_{H1D} - J_{H2D}) & 0 & -\frac{3}{4}J_{H1H2} & \frac{\sqrt{2}}{4}(J_{H1D} - J_{H2D}) & 0 \\ 0 & \frac{\sqrt{2}}{4}(J_{H1D} + J_{H2D}) & \frac{\sqrt{2}}{4}(J_{H1D} - J_{H2D}) & +\nu_H - \nu_D + \frac{J_{H1H2}}{4} - \frac{J_{H1D}}{2} - \frac{J_{H2D}}{2} & 0 \end{bmatrix}, \quad (10)$$

$$\begin{bmatrix} H_{66} & H_{69} & H_{6,11} \\ H_{9,6} & H_{99} & H_{9,11} \\ H_{11,6} & H_{11,9} & H_{11,11} \end{bmatrix} = \frac{1}{2} \begin{bmatrix} +2\nu_D + \frac{J_{H1H2}}{2} & -J_{H1D} + J_{H2D} & J_{H1D} + J_{H2D} \\ -J_{H1D} + J_{H2D} & +2\nu_D - \frac{3}{2}J_{H1H2} & +J_{H1D} - J_{H2D} \\ J_{H1D} + J_{H2D} & +J_{H1D} - J_{H2D} & +2\nu_H + \frac{J_{H1H2}}{2} \end{bmatrix}. \quad (11)$$

For example, the spin states obtained from the sub-matrix (9) are

$$\varphi_2 = c'_{11}\phi_2 + c'_{12}\phi_4 + c'_{13}\phi_7,$$

$$\varphi_4 = c'_{21}\phi_2 + c'_{22}\phi_4 + c'_{23}\phi_7,$$

$$\varphi_7 = c'_{31}\phi_2 + c'_{32}\phi_4 + c'_{33}\phi_7.$$

The values of all the coefficients are reported in Table 2.

From the calculation of the other spin functions it resulted that the para state is spread on more spin levels at 0.1  $\mu$ T than at earth field.

The increase of polarization decay rate observed at zero field might be due to the fact that, as evidenced in Fig. 5, deuterium transitions  $\varphi_2 \rightarrow \phi_1$ ,  $\varphi_3 \rightarrow \phi_2$ ,  $\varphi_{10} \rightarrow \phi_{11}$ ,  $\varphi_{11} \rightarrow \phi_{12}$  take place between spin states that are mixed, even if at very low extent, with the singlet state. Therefore quadrupolar relaxation might play a role in the polarization decay process at this field (more detailed calculations goes beyond the scope of the present work).

**Table 1**

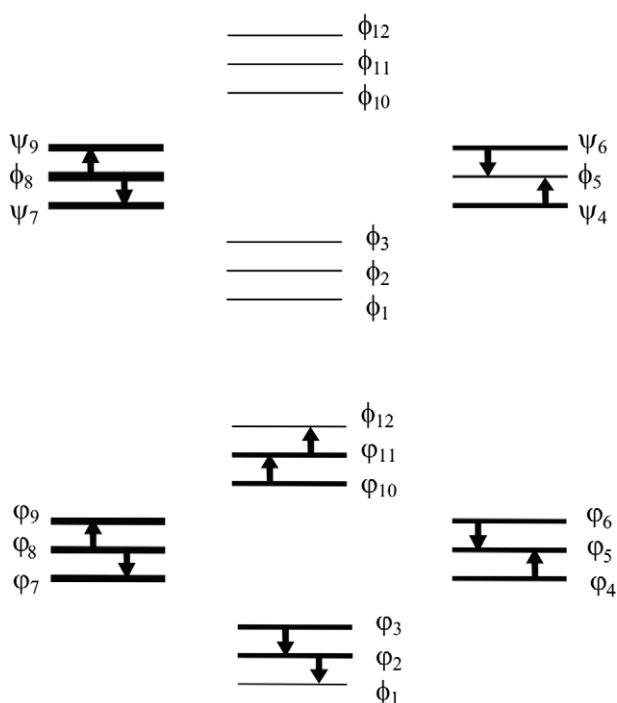
Coefficients of the linear combinations of spin states at 50  $\mu\text{T}$ . They are the eigenvectors of the matrices in Eqs. (5)–(7) calculated using Mathcad and the values  $J_{\text{H1H2}} = 11.0$  Hz,  $J_{\text{H1D}} = 1.27$  Hz,  $J_{\text{H2D}} = 0.33$  Hz,  $\nu_{\text{H}} = 2129$  Hz and  $\nu_{\text{D}} = 327$  Hz.

	$\alpha\alpha 1$	$\alpha\alpha 0$	$\alpha\alpha - 1$	$(\alpha\beta - \beta\alpha)1$	$(\alpha\beta - \beta\alpha)0$	$(\alpha\beta - \beta\alpha) - 1$	$(\alpha\beta + \beta\alpha)1$	$(\alpha\beta + \beta\alpha)0$	$(\alpha\beta + \beta\alpha) - 1$	$\beta\beta 1$	$\beta\beta 0$	$\beta\beta - 1$
$\varphi_1$	1											
$\varphi_2$		1										
$\varphi_3$			1									
$\varphi_4$				-0.9966			0.0818					
$\varphi_5$					1							
$\varphi_6$						-0.9991			0.0412			
$\varphi_7$				-0.0818			-0.9966					
$\varphi_8$								1				
$\varphi_9$						-0.0412			-0.9991			
$\varphi_{10}$										1		
$\varphi_{11}$											1	
$\varphi_{12}$												1

**Table 2**

Coefficients of the linear combinations of spin states at 0.1  $\mu\text{T}$ . They are the eigenvectors of matrices reported in Eqs. (9)–(11) calculated using Mathcad and the values  $J_{\text{H1H2}} = 11.0$  Hz,  $J_{\text{H1D}} = 1.27$  Hz,  $J_{\text{H2D}} = 0.33$  Hz,  $\nu_{\text{H}} = 4.26$  Hz and  $\nu_{\text{D}} = 0.67$  Hz.

	$\alpha\alpha 1$	$\alpha\alpha 0$	$\alpha\alpha - 1$	$(\alpha\beta - \beta\alpha)1$	$(\alpha\beta - \beta\alpha)0$	$(\alpha\beta - \beta\alpha) - 1$	$(\alpha\beta + \beta\alpha)1$	$(\alpha\beta + \beta\alpha)0$	$(\alpha\beta + \beta\alpha) - 1$	$\beta\beta 1$	$\beta\beta 0$	$\beta\beta - 1$
$\varphi_1$	1											
$\varphi_2$		0.9931		-0.0235			0.1149					
$\varphi_3$			-0.9907		0.0471			0.1270		-0.0120		
$\varphi_4$		0.0190		0.9990			0.0403					
$\varphi_5$			-0.0476		-0.9986			-0.0012		0.0233		
$\varphi_6$						-0.9993			-0.0303		0.0233	
$\varphi_7$		0.1158		0.0378			0.9926					
$\varphi_8$			-0.1266		0.0093			0.9731	-0.1922			
$\varphi_9$						-0.0335			0.9833		-0.1487	
$\varphi_{10}$			0.0138		0.0225			0.1922		0.9810		
$\varphi_{11}$						0.0185			0.1494		0.9886	
$\varphi_{12}$												1



**Fig. 5.** Spin states and their population at 50  $\mu\text{T}$  (top) and 0.1  $\mu\text{T}$  (bottom): the levels in bold are more populated than the others. The arrows represent  $^2\text{H}$  transitions.

#### 4. Conclusions

One of the main limit to the application of hyperpolarized molecules is given by polarization decay, whose rate is dictated by relaxation processes. Deuteration and low magnetic fields may be highly beneficial for limiting the polarization loss in parahydrogenated molecules providing that isotropic mixing between  $^1\text{H}$  and  $^2\text{H}$  does not take place. Therefore a careful inspection into  $^1\text{H}$  and  $^2\text{H}$  NMR parameters (scalar coupling and chemical shift) is necessary in order to select the proper magnetic field strength for storing  $^1\text{H}$  polarization for times much longer than  $T_1$  on the hyperpolarized molecule.

#### Acknowledgments

We gratefully acknowledge discussion with Prof. D. Canet. This work was carried out in the frame of the EU-NEST STREP project (No. 005032). Support from MIUR (Firb Project No. RBIP06293N) and EU-EMIL NoE is also acknowledged.

#### References

- [1] K. Golman, J.S. Petersson, Metabolic imaging and other applications of hyperpolarized  $^{13}\text{C}$ , *Acad. Radiol.* 13 (2006) 932–942.
- [2] K. Golman, L.E. Olsson, O. Axelsson, S. Mansson, M. Karlsson, J.S. Petersson, Molecular imaging using hyperpolarized  $^{13}\text{C}$ , *Br. J. Radiol.* 76 (2003) S118–S127.
- [3] P. Bhattacharya, E.Y. Chekmenev, W.H. Perman, K.H. Harris, A.P. Lin, V.A. Norton, C.T. Tan, B.D. Ross, D.P. Weitekamp, Towards hyperpolarized  $^{13}\text{C}$ -succinate imaging of brain cancer, *J. Magn. Reson.* 186 (2007) 150–155.

- [4] K. Golman, J.H. Ardenkjær-Larsen, J.S. Petersson, S. Mansson, I. Leunbach, Molecular imaging with endogenous substances, *PNAS* 100 (18) (2003) 10435–10439.
- [5] J.H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M.H. Lerche, R. Servin, M. Thaning, K. Golman, Increase in signal-to-noise ratio of >10,000 times in liquid state NMR, *PNAS* 100 (18) (2003) 10158–10163.
- [6] K. Golman, O. Axelsson, H. Johannesson, S. Mansson, C. Olofsson, J.S. Petersson, Parahydrogen-induced polarization in imaging: subsecond  $^{13}\text{C}$  angiography, *Magn. Reson. Med.* 46 (2001) 1–5.
- [7] M. Goldman, *Quantum Description of High Resolution NMR in Liquids*, Oxford University Press, New York, 2002, pp. 243–257.
- [8] S. Aime, R. Gobetto, F. Reineri, D. Canet, Polarization transfer from parahydrogen to heteronuclei: effect of H/D substitution. The case of AA'X and A<sub>2</sub>A<sub>2</sub>'X spin systems, *J. Magn. Reson.* 178 (2006) 184–192.
- [9] M. Carravetta, O.G. Johannessen, M.H. Levitt, Beyond the T<sub>1</sub> limit: singlet nuclear spin states in low magnetic field, *Phys. Rev. Lett.* 92 (2004) 153003–153007.
- [10] S. Cavadini, J. Dittmer, S. Antonijevic, G. Bodenhausen, Slow diffusion by singlet state NMR spectroscopy, *J. Am. Chem. Soc.* 127 (2005) 15744–15748.
- [11] M. Carravetta, M.H. Levitt, Long-lived nuclear spin states in high field solution NMR, *J. Am. Chem. Soc.* 126 (2004) 6228–6229.
- [12] T. Jonischkeit, U. Bommerich, J. Stadler, K. Woelk, H.G. Niessen, J. Bargon, Generating long-lasting  $^1\text{H}$  and  $^{13}\text{C}$  hyperpolarization in small molecules with parahydrogen-induced polarization, *J. Chem. Phys.* 124 (2006) 201109.
- [13] D. Canet, S. Bouguet-Bonnet, C. Aroulanda, F. Reineri, About long lived nuclear spin states involved in para-hydrogenated molecules, *J. Am. Chem. Soc.* 129 (2007) 1445–1449.
- [14] Propyne-d<sub>3</sub> was prepared according to the method reported by Kriz et al. (*Tetrahedron Lett.* 1965, 33, 2881). Then it was purified by distillation under reduced pressure and reduced to the sodium alkylide using the same process as described above. The sodium salt was filtered under inert atmosphere and suspended in freshly distilled THF. Carbon dioxide was bubbled into the solution for about one hour. The formed sodium salt of the butynoic acid was separated by filtration and dried. This salt was then suspended in methanol-d<sub>4</sub> and added to a solution of D<sub>2</sub>SO<sub>4</sub> in methanol-d<sub>4</sub> in a cold bath (0 °C). After 4 days stirring methyl-butynoate-d<sub>6</sub>- was extracted in diethylether, washed with water and a saturated solution of sodium carbonate, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under vacuum.
- [15] The parahydrogenation reactions were carried out in gas tight NMR tubes provided with Teflon Young valve. Each sample consisted in an acetone-d<sub>6</sub> solution (400 μl) of the catalyst [RhCOD(dppb)]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup> (10 mM) and the substrate (70 mM) butynoate methyl ester-d<sub>6</sub> (1). The NMR tubes were loaded with 4 atm parahydrogen. This catalyst is commonly used for selective reduction of triple bonds to double bonds (P.A. Chaloner, M.E. Esteruelas, L.A. Oro, *Homogeneous hydrogenation*, Kluwer Academic Publishers, 1994, Dordrecht, The Netherlands), therefore the hydrogenation conditions can be set in such a way that the reaction does not proceed to the formation of the completely saturated molecule (alkane). The parahydrogen pressure and the substrate–catalyst ratio used in our experiments fit these requirements and make the reaction to proceed only to the alkene formation. This molecule is the only hyperpolarized product observed in the reported spectra. Parahydrogen enrichment (50%) was obtained by equilibrating normal-H<sub>2</sub> at 77 K in the presence of activated charcoal as ortho–para conversion catalyst.
- [16] M.G. Pravica, D.P. Weitekamp, Net NMR alignment by adiabatic transport of parahydrogen addition products to high magnetic field, *Chem. Phys. Lett.* 145 (1988) 255–258.