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New Hyperpolarized Contrast Agents for ^{13}C-MRI from Para-Hydrogenation of Oligooxyethylenic Alkynes

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Abstract: Two alkyne derivatives, which contain one and two oligooxyethylenic chains respectively, showed to be good substrates for para-hydrogenation reactions, yielding the corresponding hyperpolarized alkenes in good yields. A suitable theory has been developed to account for the observed results, fully explaining the different para-H_2 induced effects observed upon the para-hydrogenation of symmetrically and asymmetrically substituted alkynes in ALTADENA and PASADENA modes. The oligooxyethylenic substituent provides good water solubility to the para-hydrogenated symmetrical derivative. ^{13}C-MR *in vitro* images of the latter derivative were obtained both in acetone and in water solutions (130 mM), using the ALTADENA procedure and after application of the field cycling procedure which allows acquisition of an in-phase ^{13}C carbonyl resonance. The finding that the hydrogenated product is water-soluble in contrast to the parent alkyne which is not allows for the pursuit of a fast phase-transfer separation from the organic solvent, the unreacted substrate, and the catalyst to obtain a "ready-to-use" water solution suitable for further *in vivo* MRI applications.

Introduction

Many efforts have been devoted in recent years to the development of MR-hyperpolarization procedures, mainly driven by the potential use of hyperpolarized molecules in Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS).[1−3] In this context, Dynamic Nuclear Polarization (DNP)[9−14,22,23] and Para-Hydrogen Induced Polarization (PHIP)[7,15−21,29−39] methods are currently under intense scrutiny for the preparation of ^{13}C enriched hyperpolarized substances, for which interesting applications such as ^{13}C-MRI contrast agents have already been anticipated. With this kind of contrast agent, images are acquired by the direct detection of the ^{13}C nucleus, whose endogenous signal is practically zero. The absence of a background signal allows us to obtain images with a high signal-to-noise ratio (SNR), where the contrast is given by the difference in signal intensity between regions reached by the hyperpolarized ^{13}C molecule and uninvolved zones.[8,9]

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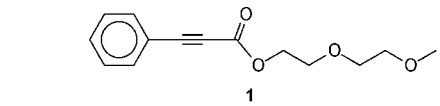
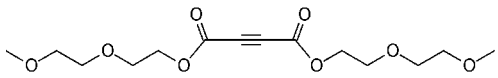
Chart 1. Chemical Structures of **1** and **2**



1



2

The DNP hyperpolarization procedure can be in principle applied to any molecule, provided that efficient methods for the rapid dissolution of the hyperpolarized substrate and the separation of the paramagnetic agent are available. Conversely, the use of the PHIP method requires hydrogenable substrates and implies the use of hydrogenation catalysts which must be removed before the *in vivo* administration. Its main advantage relies on the fact that it does not require the very low temperatures used in the DNP procedure, thus resulting in a simpler and cheaper methodology.

To produce a ^{13}C hyperpolarized contrast agent using this approach, an unsaturated substrate is necessary (usually a triple bond containing molecule, that is efficiently para-hydrogenated in the presence of a suitable catalyst), with an adjacent carbonyl group, whose ^{13}C resonance is characterized by a long T_1 value (which limits the polarization loss due to relaxation). Polarization is transferred to the carbonyl carbon nucleus through scalar coupling with protons from para-hydrogen.[7,34−38]

In the first papers dealing with ^{13}C-PHIP,[36,37] the effect was reported only for symmetric molecules, in which the para-H_2 symmetry was broken by the presence of the heteronucleus. Successively, it was observed that polarization transfer to heteronuclei can also be obtained on asymmetric molecules, providing that hydrogenation takes place at a low magnetic field (ALTADENA experiment).[35]

Herein we report the synthesis and parahydrogenation experiments of two novel substrates, the asymmetrical 2-(2-methoxyethoxy)ethyl phenylpropiolate (**1**) and the symmetrical bis[2-(2-methoxyethoxy)ethyl]acetylenedicarboxylate (**2**) (Chart 1), with the aim of obtaining an in-depth understanding of the potential of these species as ^{13}C hyperpolarized contrast agents. A theoretical explanation of the difference between ^{13}C hyperpolarized spectra of symmetrical and asymmetrical substrates in ALTADENA and PASADENA experiments is also provided.

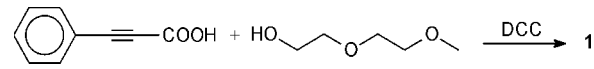
The (oligo)oxyethylenic chains were introduced into the alkynic substrates to obtain water-soluble para-hydrogenated products, as aqueous contrast agent solutions are needed for *in vivo* MRI applications. Up to now only one water-soluble substrate has been reported as a ^{13}C hyperpolarized contrast agent, namely 2-hydroxyethylacrylate, which upon hydrogenation yields 2-hydroxyethylpropionate.[17−21]

One aim of the present work was to improve the methodology by making the solvent elimination step faster. It was found that **2** affords an hydrogenated product which is soluble both in organic solvents and in water, thus allowing the hydrogenation process to be carried out in an organic solvent solution (where the homogeneous cationic Rh catalyst is more efficient and H_2 is more soluble) and then transfer of the hyperpolarized product to the aqueous phase.

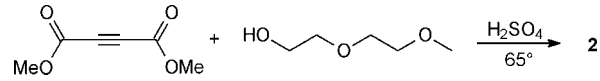
Results and Discussion

2-(2-Methoxyethoxy)ethyl phenylpropiolate (**1**) was synthesized by the direct esterification of phenylpropiolic acid with diethylene glycol monomethyl ether (Scheme 1; see Experimental Section for details). Bis[2-(2-methoxyethoxy)ethyl]acetylenedicarboxylate (**2**) was synthesized by the transesterification of dimethylacetylenedicarboxylate with diethyleneglycol monomethyl ether, in the presence of H_2SO_4 as catalyst (Scheme 2; see Experimental Section for details).

Scheme 1. Synthesis of **1**



Scheme 2. Synthesis of **2**



Both **1** and **2** are hydrogenated quite readily by the Rh catalyst [bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate. Typically, when the reactions were carried out in a 5 mm NMR tube containing the substrate (50 ÷ 150 mM) and catalyst (17 mM) and in the presence of 4.0 atm of H_2 (solvent acetone-d_6), yields of ∼85% were obtained.

In Figure 1a the ^{13}C NMR spectrum obtained upon para-hydrogenation of **2** (**2a**, Scheme 3), acquired in the PASADENA procedure, is reported. It shows two absorption/emission signals centered at 165.53 ppm (carbonyl carbon atoms) and at 132.21 ppm (olefinic carbon atoms), respectively.

The PASADENA experiment carried out under the same experimental conditions on the asymmetrical substrate **1** did not afford any ^{13}C enhanced signal (spectrum not shown). Conversely, a PHIP effect in the ^{13}C spectrum of **1a** was detected (see Scheme 3) when the para-hydrogenation was carried out in an ALTADENA experiment, as depicted in Figure 2a.

On substrate **2** the two H atoms from the para-H_2 molecule are transferred to chemically equivalent positions, while on **1** the two H atoms are added to chemically different sites. Chemical symmetry plays a role when the parahydrogenation

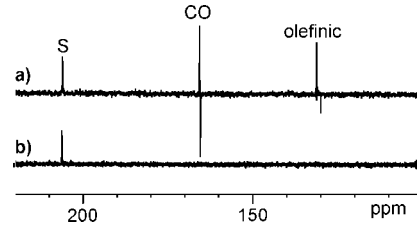
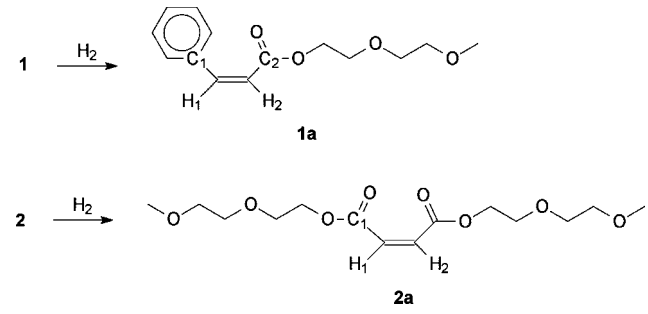


Figure 1. Single scan ^{13}C NMR spectra (in the 100−220 ppm region; 14.1 T, acetone-d_6, 298 K) of **2a** obtained upon para-hydrogenation of **2** in the PASADENA experiment, recorded (a) immediately after para-hydrogenation and (b) after relaxation (5 min). S indicates the solvent signal.

Scheme 3. Hydrogenation of **1** and **2**



1a

2a

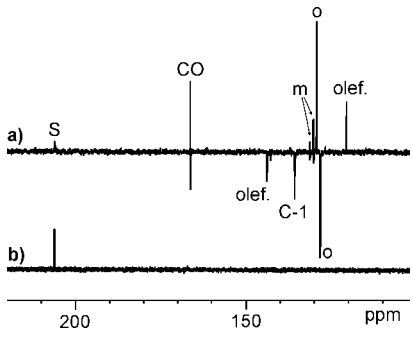


Figure 2. Single scan ^{13}C NMR spectra (in the 100−220 ppm region; 14.1 T, acetone-d_6, 298 K) of **1a** obtained upon para-hydrogenation of **1** in the ALTADENA experiment, recorded (a) immediately after para-hydrogenation and (b) after relaxation (5 min). S denotes the solvent signal; o and m indicate the ortho and meta positions of the aromatic ring, respectively.

takes place in PASADENA conditions; otherwise, when the ALTADENA experiment is carried out, both ^{13}C signals *J*-coupled with the parahydrogen protons in **1a** (the carbonyl at 166.31 ppm and the C-1 aromatic peak at 135.60 ppm) result in being polarized, even though to different extents. This is the result of the scalar coupling pattern, as it will be discussed in detail in the theoretical portion.

Interestingly, the olefinic (143.17 and 120.07 ppm) and the C-1 aromatic carbon atoms of **1a** are only slightly enhanced, while strong polarization is observed for the ortho aromatic carbon at 128.62 ppm (doublet absorption/emission), and a weaker effect is detected for the meta carbon (130.75 ppm). Polarization on these carbon atoms is not the result of scalar coupling with para-hydrogen protons, but it arises from an indirect pathway that involves the aromatic protons. Polarization is indeed detected on both ortho and meta aromatic protons in the ^{1}H NMR spectrum of **1a** (Figure 3), as they are involved in a dipolar interaction with the olefinic ones.

Both **1** and **2** are very soluble in organic solvents but not in water, in spite of the presence of ethoxylic groups. However, upon hydrogenation, **2a** shows good water solubility (~600 mM). This feature allows the para-hydrogenation reaction to be carried out in an acetone solution and then transfer of the alkene molecule into the aqueous phase for *in vivo* administration. This property allows bypassing the time-consuming procedure for solvent/catalyst elimination and dissolution in water, thus making this compound a particularly interesting ^{13}C hyperpolarized contrast agent for MRI applications.

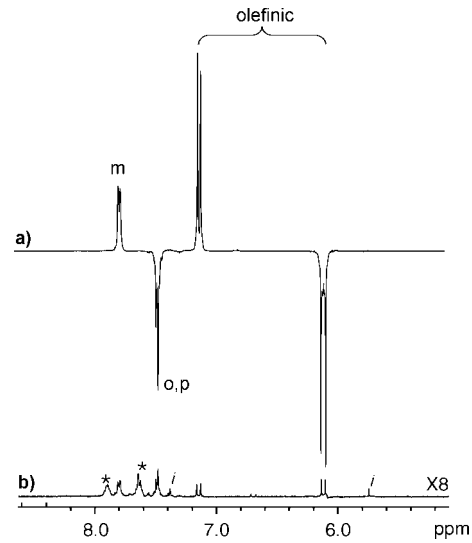


Figure 3. Single scan ^{1}H NMR spectra (9.4 T, acetone-d_6, 298 K) of **1a** obtained upon para-hydrogenation of **1** in the ALTADENA experiment, recorded (a) immediately after para-hydrogenation and (b) after relaxation (5 min). Trace b has been multiplied × 8; *i* denotes an impurity; * denotes catalyst's signals.

Table 1. Carbonyl ^{13}C Relaxation Times of **2a**

solvent	O_2 present	field (T)	T_1 (s)
acetone-d_6	no	14.1	20.0
D_2O	yes	14.1	6.3
D_2O	yes	9.4	11.1
D_2O	yes	7.0	16.0
D_2O	yes	0.5	33[a]

[a] Value estimated from the CSA and dipolar contributions.

As mentioned above, the relaxation rate of the hyperpolarized CA is crucial for the acquisition of *in vivo* ^{13}C images. Therefore the T_1 of the **2a** ^{13}C carbonyl carbon atom was measured under different conditions (solvent, magnetic field strength, in the presence and absence of oxygen). The obtained data are reported in Table 1. The relatively large molecular size of **2a** shortens T_1 with respect to the parent dimethylmaleate. In fact, the carbonyl carbon atom T_1 (measured in acetone-d_6 solutions at 14.1 T) is 20 s for **2a** and 40 s for dimethylmaleate, respectively. By measuring T_1 at different field strengths it was possible to obtain an estimate of dipolar and CSA contributions, the latter being the dominant term. From these data it is possible to anticipate a T_1 of 33 s at 0.5 T (corresponding to a field value still used in clinical MRI). The observed T_1 values appear to be long enough to allow the detection of ^{13}C images, even after its transfer into the aqueous phase and in the presence of oxygen.

For the acquisition of ^{13}C images of hyperpolarized **2a**, ^{13}C labeled **2** (^{13}C enriched on one of the carbonyl groups) was synthesized (see Experimental Section).

Hyperpolarization effects observed on the ^{13}C resonances of parahydrogenated molecules, in both ALTADENA and PASADENA experiments, stem from longitudinal two spin order terms ($I_z^H I_z^C$) and result in adsorption/emission signals that, as such, can not be exploited in the ^{13}C image acquisition, as their resultant is practically zero. Thus it is necessary to convert the longitudinal spin order into longitudinal magnetization (I_z^C)

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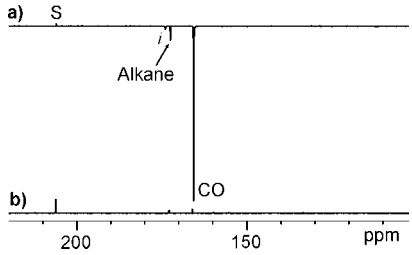

Figure 4. Single scan ^{13}C NMR spectrum (14.1 T, acetone-d_6, 298 K) of ^{13}C labeled **2a** obtained upon para-hydrogenation of ^{13}C-**2** (ALTADENA) recorded (a) immediately after para-hydrogenation and application of the field cycling procedure and (b) after relaxation (5 min). The signal at 172.48 ppm is attributed to the fully hydrogenated alkane carbonyl group; *i* denotes an impurity; S indicates the solvent signal. Signal enhancement in spectrum (a) is about 250.

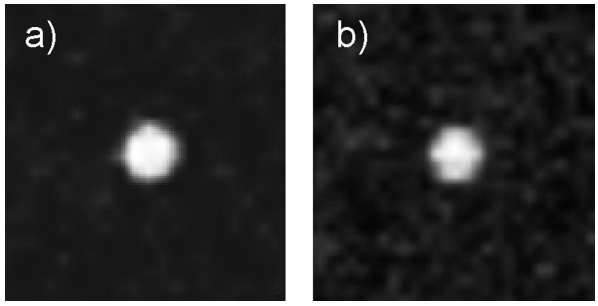

Figure 5. ^{13}C single shot RARE images of 5 mm NMR tubes containing hyperpolarized ^{13}C labeled **2a** (axial projections). (a) Tube containing 130 mM HP-^{13}C- **2a** in degassed acetone-d_6; (b) tube containing a D_2O 130 mM solution of HP-^{13}C-**2a** (residual acetone-d_6 in the solution is ~4% after the fast distillation procedure).

before proceeding to the MRI acquisition. This can be achieved by a magnetic field cycling procedure, consisting of two asymmetric transformations of the magnetic field: in the first passage the sample is quickly (non-adiabatically) thrown from a 50 μT (earth's field) to nearly zero (0.1 μT) magnetic field, and then it is slowly brought back (adiabatically) to the earth's magnetic field. The whole process takes only a few seconds.

The ^{13}C spectrum in Figure 4, obtained after application of the magnetic field cycle on ^{13}C labeled **2a**, shows an in-phase enhanced ^{13}C signal (165.53 ppm) related to the carbonyl ^{13}C atom, while the polarization on olefinic carbons is lost, probably due to the faster relaxation of these nuclei caused by dipolar interaction with directly bound protons. The corresponding ^{13}C image of the NMR tube containing HP ^{13}C-**2a** was then acquired, and it is reported in Figure 5a. The estimated concentration of the hydrogenated product in the solution was 130 mM.

A good polarization level was maintained even after acetone elimination (achieved by fast vacuum distillation, as described in detail in the Experimental Section) and dissolution of the residue in D_2O. The ^{13}C enhancements in the high resolution spectra of acetone-d_6 (figure 4) and D_2O (spectrum not shown) solutions were 250 and 50, respectively. The lower value in D_2O is due to polarization loss caused by relaxation during nebulization, acetone distillation, and transfer of the sample from the distillation apparatus to the NMR tube and due to the relaxation enhancement in the aqueous solution, as demonstrated by the comparison between the measured T_1 values in acetone-d_6 and D_2O (see Table 1). The increased relaxation rate in water is mainly due to the presence of oxygen but also to a lower molecular mobility (longer τ_c due to increased viscosity).

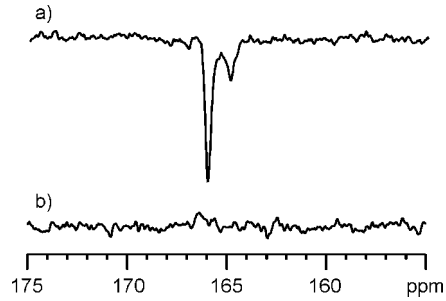

Figure 6. Single scan ^{13}C spectrum (100−220 ppm region; 14.1 T, 298 K) of ^{13}C labeled **2a** extracted in D_2O after para-hydrogenation of ^{13}C-**2** (ALTADENA, $CDCl_3$/acetone-d_6 6:1) and application of the field cycling procedure, recorded (a) immediately after phase separation; (b) after relaxation (5 min). The two different signals at 165.99 and 164.82 ppm are attributed to **2a** dissolved in D_2O (13 mM) and in $CDCl_3$ (still present in trace, 117 mM), respectively.

Nevertheless, the signal enhancement observed in D_2O is still sufficient to allow acquisition of the ^{13}C image of a tube containing a D_2O solution of the para-hydrogenated compound (130 mM), which is reported in Figure 5b.

The change in the solubility properties between the parent alkyne **2** and its hydrogenated derivative **2a** has been exploited to set up a novel method for separating the hyperpolarized product from the reaction mixture. Upon carrying out the para-hydrogenation reaction in a solvent not miscible with water, such as chloroform or dichloromethane, one may obtain an aqueous solution of the hyperpolarized product simply by phase transfer to a small amount of water added upon completion of the para-hydrogenation reaction. Thus the nonhydrogenated substrate, the catalyst, and the organic solvent will not pollute the aqueous phase. The possibility of attaining the hyperpolarized product solution in a single step will be highly advantageous, as the time-consuming solvent and catalyst elimination, during which polarization decays, is avoided. This goal was accomplished in the present work by performing the para-hydrogenation of **2** in a $CDCl_3$/acetone-d_6 6:1 mixture (a low fraction of acetone is necessary for maintaining the catalyst activity) in the NMR tube and then quickly adding some D_2O, shaking the tube, and let it stand for a few seconds to allow phase separation. The D_2O phase was then transferred into a second tube for NMR detection. The ^{13}C NMR spectrum of an aqueous solution of **2a** obtained by this procedure is shown in Figure 6a. Of course the signal enhancement (~100) is lower than that obtained in the acetone-d_6 solution (Figure 4), due to polarization decay occurring during the phase-transfer step.

Still a small amount of chloroform solution is present, thus yielding an additional signal for **2a** dissolved in the latter phase at 164.82 ppm. When comparing it to the peak from the aqueous phase (165.99 ppm), one must take into account that its intensity reflects the higher solubility of **2a** in chloroform (117 mM) than in water (13 mM). Thus, from the intensity ratio between the two different signals it is possible to estimate that the residual chloroform amount in the aqueous phase is ~3%.

Although the detected signal enhancement appears sufficient for image acquisition, further improvements are expected to make this methodology, based on the differential solubility of the para-hydrogenated molecule and the parent substrate, the technique of choice for the acquisition of ^{13}C-MR images of hyperpolarized molecules.

Theory: Polarization Transfer to ^{13}C in Symmetrical and Asymmetrical Molecules. Polarization transfer from para-hydrogen protons to ^{13}C is mainly driven by $^{1}H-^{13}C$ scalar

coupling in the para-hydrogenation products.[39] As experimentally observed, para-H_2 addition to chemically different sites as in molecule **1** allows ^{13}C polarization to be obtained only when it takes place out of the spectrometer (ALTADENA experiment), while para-hydrogenation of the symmetric molecule **2** leads to heteronuclear polarization in both ALTADENA and PASADENA experiments. In the latter case the loss of the singlet state, necessary to exploit para-hydrogen hyperpolarization, is due to asymmetrical coupling of the two protons to the ^{13}C nucleus. The difference between the two cases is of general applicability, and it can be demonstrated by describing the evolution of the para-hydrogen density operator σ_{para} on the product molecule using an approach, introduced by Canet et al.,[40,41] based on the assumption that a steady-state condition is reached by the para-H_2 density operator after addition to the substrate. This method, more convenient than others that require the solution of the evolution equation, is summarized below.

The para-H_2 density operator is described by eq 1,[42] and the hydrogenation reaction can be considered as a sudden change of the Hamiltonian governing the two spins system.

$$\sigma_{para} = \frac{E}{4} - I_z^H I_z^H - I_x^H I_x^H - I_y^H I_y^H \tag{1}$$

Thus the evolution of the density operator σ_{para} upon addition to the substrate is given by the Liouville−von Neumann equation

$$\frac{d\sigma_{para}}{dt} = -i[H, \sigma_{para}] \tag{2}$$

where H is the product molecule Hamiltonian.

After reaction completion, parahydrogen reaches a new steady state (σ_{stat}) on the product molecule, such that

$$\frac{d\sigma_{stat}}{dt} = -i[H, \sigma_{stat}] = 0 \tag{3}$$

which contains, besides the para-H_2 product operators (eq 2), other terms that, deriving from parahydrogen evolution, are polarized. Hyperpolarization is transferred according to σ_{stat} as follows:

$$\sigma_{stat} = \frac{E}{4} - \sum_j a_j^{stat} G_j \tag{4}$$

where

$$G_j = \prod_{i=1}^{h} S_i^{(j)}$$

with S_i is E^i, I_x^i, I_y^i, or I_z^i and a_j^{stat} represent the contribution of each G_j product operator to the steady state.

Therefore eq 3 becomes eq 5,

$$\sum_j [H_J, G_j] a_j^{stat} = 0 \tag{5}$$

where only the J-Hamiltonian is considered, as the Zeeman part does not effect para-H_2. This equation means that a certain term G_j is polarized if it does not commute with H_J.

^{13}C Hyperpolarization in ALTADENA and PASADENA Experiments. Let us consider the parahydrogen addition to a symmetrical substrate such as **2**. Only one of the two carbonyl groups contains a ^{13}C atom; therefore the symmetry of the two hydrogen atoms is broken by asymmetric coupling with it and an AA′X spin system is formed. In this case the strong coupling condition between protons is maintained inside the spectrometer. On the contrary, with an asymmetrical substrate such as **1** the two parahydrogen protons are added in chemically different environments and strong coupling between protons is achieved only outside the magnet, when an ALTADENA experiment is carried out.

As a consequence the J-Hamiltonian that transforms parahydrogen after its addition to a symmetric substrate results in eq 7 in both ALTADENA and PASADENA experiments, while with asymmetric molecules it results in eq 7 only in the latter case.

$$H = J_{H1H2}(I_z^{H1} I_z^{H2} + I_x^{H1} I_x^{H2} + I_y^{H1} I_y^{H2}) + J_{H2C1} I_z^{H2} I_z^{C1} + J_{H1C1} I_z^{H1} I_z^{C1} \tag{7}$$

The same expression can be written for the other ^{13}C nucleus (C_2); in fact only one position at a time can be occupied by a ^{13}C atom because of its low natural abundance.

If a PASADENA experiment is performed, the Hamiltonian by which para-hydrogen is transformed is

$$H_{asymm}^{PASADENA} = J_{H1H2} I_z^{H1} I_z^{H2} + J_{H2C1} I_z^{H2} I_z^{C1} + J_{H1C1} I_z^{H1} I_z^{C1} \tag{8}$$

As assessed above, a product operator is polarized if it does not commute with H. We focus on the longitudinal order $I_z^{H2} I_z^{C1}$ which gives the antiphase doublets observed in ^{13}C polarized spectra. This term is invariant with respect to eq 8, while applying Hamiltonian of eq 7 one obtains

$$[H, I_z^{H2} I_z^{C1}] = \frac{1}{2} J_{H1H2} I_y^{H1} I_x^{H2} I_z^{C1} - \frac{1}{2} J_{H1H2} I_x^{H1} I_y^{H2} I_z^{C1} \tag{9}$$

In other words the longitudinal magnetization term does not commute with $(I_x^{H1} I_x^{H2} + I_y^{H1} I_y^{H2})$, which is related to the strong coupling condition achieved in ALTADENA experiments or in PASADENA carried out on symmetric molecules. On the contrary, in PASADENA experiments on asymmetric molecules the strong coupling condition is not obtained.

The reported method allowed us to derive that the amount of polarization transfer toward ^{13}C is proportional to the ratio $R_{J,C1} = (J_{H2C1} - J_{H1C1})/J_{H1H2}$,[40] which is the same as that obtained by Bargon for symmetric molecules.[36] Being the coupling constants in **1a** $J_{H2,C1} = 10.0$ Hz, $J_{H1,C2} = 15.8$ Hz, $J_{H1,C1} = -1.8$ Hz, $J_{H2,C2} = -2.5$ Hz, and $J_{H1,H2} = 12.6$ Hz, then $R_{J,C1} = 0.94$ and $R_{J,C2} = 1.45$: this means that polarization is expected to be higher on the carbonyl signal with respect to the aromatic one. This is indeed the case. Hyperpolarization observed on the aromatic ortho ^{13}C carbon cannot be due to scalar coupling with para-hydrogen protons. The antiphase doublet clearly refers to the term $I_z^{Cortho} I_z^{Hortho}$, and polarization on H_{ortho} (seen in Figure 3) must be transferred from parahydrogen protons by means of dipolar interaction.

Considering the J coupling values of **2a** ($J_{H2,C1} = 24.0$ Hz, $J_{H1,C1} = -2.5$ Hz, $J_{H1,H2} = 12.0$ Hz), an $R_{J,C1}$ value of 2.20 is obtained; i.e. polarization transfer to the carbonyl ^{13}C atom in **2a** is expected to be more efficient than that in **1a**. This is in

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(43) A higher signal enhancement was observed in the ALTADENA experiment without application of the field cycling with respect to that observed when the field cycling was used (shown in Figure 4) because in the second case some polarization was lost during the procedure.

accordance with the observed carbonyl signal enhancements obtained in ALTADENA experiments without field cycling, which are 1500 for **2a** (spectrum not shown) and 800 for **1a** (Figure 1), respectively.

Conclusions

The possibility of obtaining ^{13}C-PHIP on asymmetrical molecules only when the para-hydrogenation reaction takes place at low magnetic field strength (ALTADENA experiment), while with symmetrical substrates this is observed even when para-hydrogenation occurs at high magnetic field strength (PASADENA experiment) was elucidated by means of the steady-state density operator approach. This method allowed to directly demonstrate why the strong coupling condition between protons is necessary to ^{13}C polarization transfer.

The symmetrical molecule **2** represents a good step along the way of applying para-hydrogenated substrates in MRI as ^{13}C hyperpolarized agents. In fact, in spite of its size, the carboxylate resonance in **2a** displays a relatively long T_1, reaching a value of 33 s at the field strenght 0.5 T. Furthermore, it is easily soluble in acetone, where it can be hydrogenated in high yields, and its hydrogenated product **2a** is also soluble in water, thus making its use possible for *in vivo* MRI applications. Good *in vitro* ^{13}C images were obtained from solutions of the para-hydrogenated **2a** product either in acetone or in D_2O at 7 T.

2a is characterized by water solubility and signal enhancement similar to those observed under the same experimental conditions for the already reported 2-hydroxyethylpropionate (derived from para-hydrogenation of 2-hydroxyethylacrilate).[17−21] However, it shows some advantages, namely:

(a) The ^{13}C relaxation time of **2a** is somewhat longer than that of 2-hydroxyethylpropionate (20 s for **2a**, 15 s for 2-hydroxyethylpropionate in degassed acetone-d_6 solutions at 14.1 T), possibly because in **2a** the dipolar relaxation is less efficient due to the minor number of protons adjacent to the carbonyl group, and because the OH group in 2-hydroxyethylpropionate can give rise to intermolecular interactions, slowing down the reorientation process and thus shortening T_1.

(b) **2a** shows a lower lipophilicity with respect to 2-hydroxyethylpropionate, as demonstrated by the measurement of the partition coefficient (P) in water/n-octanol of both substances (see Experimental Section). Since lipophilicity is commonly accepted as an indirect measure of toxicity,[44,45] one may conclude that **2a** is expected to be less toxic than 2-hydroxyethylpropionate.

Finally, the change in solubility properties between the parent alkyne **2** and its hydrogenated derivative **2a** represents an important result in view of its potential *in vivo* applications. In fact para-hydrogenation of this kind of molecules can be carried out in an organic solvent not miscible with water, and the parahydrogenated compound can be extracted from the reaction mixture simply by water addition. The occurrence of a phase separation provides a quick route to "ready-to-use" solutions (catalyst- and organic-solvent-free) for *in vivo* administration.

Experimental Section

Solvents were stored over molecular sieves and purged with nitrogen before use. Hydrogen was produced by a CLAIND generator, model HG300. $^{13}CO_2$ (99% enriched) was purchased from Eurisotop, France.

NMR spectra were recorded on a Bruker Avance 600 spectrometer operating at 600 MHz for the proton and 150.9 MHz for ^{13}C. For the detection of the PHIP effect single scan acquisitions were carried out. Single scan ^{13}C images were obtained on a Bruker Avance 300 spectrometer (7 T) equipped with a Microimaging probe, using the RARE sequence, with a RARE factor of 32, matrix 32 × 32, echo time 8.465 ms.

^{13}C relaxation times were measured using the inversion recovery pulse sequence.

Para-enriched hydrogen (52%) was prepared storing H_2 over Fe_2O_3 at 77 K for 1 h.

Para-hydrogenation reactions in ALTADENA conditions were carried out in a 5 mm NMR tube equipped with a Young valve. [Bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate (5 mg) was dissolved in acetone-d_6 (0.4 mL) and activated by reaction with H_2. Normal H_2 was then replaced by the para-H_2 enriched mixture (4 atm), after addition of the substrate (0.02 mmol for ^{1}H NMR spectra or 0.06 mmol for ^{13}C NMR spectra/images acquisition). The hydrogenation was carried out by shaking the tube for 10 s (yields = 80% for **1a** and 85% for **2a**). For the acquisition of an in phase ^{13}C resonance, magnetic field cycling was applied to the hydrogenated sample: this was pursued by quickly inserting the tube into a μ-metal shield (field strength 0.1 μT) and then slowly removing the shield (the entire field cycling procedure required 3−5 s). The sample was finally inserted into the spectrometer for acquisition of the high resolution NMR spectrum.

Para-hydrogenation reactions in PASADENA conditions were carried out by bubbling para-H_2 directly into the NMR tube for ∼10 s (inside the spectrometer). The tube contained 0.4 mL of an acetone-d_6 solution of activated catalyst and substrate at the same concentrations as those in the ALTADENA experiments. The bubbling was then stopped, and the NMR spectra were immediately acquired.

For MR image acquisitions, the ALTADENA procedure was applied. After completing the magnetic field cycle, the 5 mm NMR tube was inserted into the NMR spectrometer and the ^{13}C image in pure acetone was rapidly acquired.

Elimination of acetone was achieved by means of a fast distillation procedure. After the field cycling step, the tube containing the acetone solution of the para-hydrogenated sample was opened and the solution was transferred into a syringe containing 0.75 mL of degassed D_2O and then injected through a capillary into a flask connected to a vacuum pump. Nebulization occurred, and the more volatile acetone solvent was pumped off, while the remaining water solution was collected on the bottom of the flask. Argon was then inflated into the flask, and the solution was forced into a plastic tube by pressure, thus being transferred into the tube for MR acquisition. Residual acetone-d_6 in the collected solution was ∼4%, as determined by ^{2}D spectroscopy.

Samples for water extraction were prepared by para-hydrogenating **2** in $CDCl_3$/acetone-d_6 (6:1) in the NMR tube equipped with the Young valve (same procedure and conditions as for pure acetone samples). After the field cycling application, the tube was quickly opened and 0.4 mL of degassed D_2O were added; the tube was shaken vigorously for 3 s and then allowed to stand for 5 s, during which phase separation occurred. By means of a syringe, the water solution was transferred into another NMR tube. Due to the short procedure times, some residual $CDCl_3$ (about 3%) was present in the water solution. The quantity of **2a** transferred into the water phase was estimated to be about 10% of the total (corresponding to a final concentration in water of ∼13 mM).

Measurement of the partition coefficient (P) in water/n-octanol for **2a** and 2-hydroxyethylpropionate was carried out by a mi-

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croshake-flask procedure.[46] 40 mg of **2a** or 2-hydroxyethylpropionate were dissolved in 2 mL of phosphate buffer (0.01 M, pH = 7.4) saturated *n*-octanol and mixed with 2 mL of *n*-octanol saturated phosphate buffer at 25 °C. The mixture was vigorously shaken for 3 h, and then the two phases were separated and centrifuged individually. The concentration in each phase was determined by UV photometry. The partition coefficient P was calculated as $P = C_{n\text{-octanol}}/C_{\text{buffer}}$, obtaining $P_{\mathbf{2a}} = 0.371 \pm 0.03$ and $P_{2\text{-hydroxyethylpropionate}} = 2.388 \pm 0.08$.

Synthesis of 1. Pyridine (25 mL) was introduced into a round bottomed flask, and phenylpropiolic acid (1.000 g, 6.84 mmol) and diethyleneglycol monomethyl ether (1.116 g, 9.28 mmol) were added. A solution of *N*,*N*-dicyclohexylcarbodiimide (DCC, 1.806 g, 8.75 mmol) in pyridine (5 mL) was then slowly dropped into the flask, and the mixture was shaken at RT for 24 h. An aqueous solution of HCl 10% was then added (pH = 5), and the solution was extracted with three portions of diethylether (30 mL). The organic phase was dried over sodium sulfate, filtered, and rotary-evaporated. The product was purified by column chromatography with a petroleum ether/diethylether 9.9/0.1 mixture as eluent, yielding 900 mg of **1** as a colorless oil (yield 45%). ^{1}H NMR (acetone-d_6): δ = 7.63 (d, 2H), 7.53 (dd, 1H), 7.48(d, 2H), 4.43 (t, 2H), 3.65 (t, 2H), 3.57 (t, 4H), 3.45 (t, 2H), 3.26 (s, 3H); ^{13}C{^{1}H}-NMR (acetone-d_6): δ = 153.89 (1C), 133.40 (2C), 131.59 (1C), 129.53 (2C), 119.89 (1C), 86.16 (1C), 81.04 (1C), 72.37 (1C), 70.96 (1C), 69.03 (1C), 58.57 (1C).

Synthesis of 2. Dimethyl acetylenedicarboxylate (400 mg; 2.8 mmol) was added to a mixture formed of diethyleneglycol monomethyl ether (10 mL) and H_2SO_4 (0.5 mL). The reaction mixture was stirred under nitrogen at 65 °C for 8 h. The solution was then cooled to RT and diluted with diethyl ether (20 mL), and then it was washed twice with H_2O and once with brine. The organic layer was dried over sodium sulfate and rotary-evaporated. The residue was purified by column chromatography (petroleum ether/EtOAc 7:3; 3:7). Pure **2** was obtained as a colorless oil (264 mg; yield: 30%; R_f [petroleum ether/EtOAc 6:4] = 0.2). ^{1}H NMR (acetone-d_6): δ = 4.51 (t, 4H), 3.85 (t, 4H), 3.72 (t, 4H), 3.62 (t, 4H), 3.43 (s, 6H); ^{13}C{^{1}H}-NMR (acetone-d_6): δ = 151.93 (2C), 75.41 (2C), 66.58 (2C), 68.70 (2C), 70.76 (2C), 72.31 (2C), 58.52 (2C).

Synthesis of ^{13}C-Labeled Acetylenedicarboxylate. Propiolic acid (2.0 mL, 32.5 mmol) was placed in a flask containing THF (200 mL, freshly distilled from sodium and benzophenone). The solution was cooled at 0 °C, and then a solution of *n*-butyllithium in hexane (1.6 M, 40.6 mL, 65 mmol) was slowly added. The solution was then cooled at −50 °C, and air was removed. A $^{13}CO_2$ (99%) cylinder was connected to the reaction flask, and the gas was bubbled into the solution at RT. The $^{13}CO_2$ uptake was monitored by a digital manometer connected to the reaction flask. The obtained yellow Na acetylenedicarboxylate salt was filtered, washed three times with THF, and dried under vacuum. A suspension of the salt (3.64 g, 28.9 mmol) in methanol (20 mL) was then prepared and was added to a solution of 96% sulfuric acid (6.6 mL) in methanol (10 mL) in an ice bath (0 °C). The solution was then warmed to RT and stirred for 4 days. Water (∼50 mL) was added, and the ester was extracted with diethylether (5 × 40 mL). The organic fraction was washed with water and a saturated sodium bicarbonate solution and then dried over $CaCl_2$. The dryer was removed by filtration, and the solvent was evaporated under vacuum. Dimethyl acetylenedicarboxylate was purified by column chromatography on silica gel eluting with a petroleum ether/ethyl acetate (95:5) mixture. Yield 44%. ^{1}H NMR (methanol-d_4): δ = 3.90; ^{13}C{^{1}H}-NMR (methanol-d_4): δ = 153.45 (2C), 75.66 (2C, dd, $J_{13C,13C}$ = 72.3 Hz; 17.7 Hz), 54.75 (2C).

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