Hyperpolarized Molecules

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Use of Labile Precursors for the Generation of Hyperpolarized Molecules from Hydrogenation with Parahydrogen and Aqueous-Phase Extraction**

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Transfer of para-H_2 spin order to the products of hydrogenations with parahydrogen yields extraordinary enhancements of the NMR signals which, in theory, may reach values as high as 10^5 times the signal intensity of the corresponding derivatives obtained with normal H_2.[1,2] The possibility of polarizing heteronuclear resonances in molecules obtained from hydrogenation using parahydrogen is of huge interest for in vivo studies by MRI and MRS.[3–6] High hydrogenation rates, efficient removal of organic solvents, and catalyst from the aqueous solutions used for in vivo administration and slow loss of the polarized signal are the key determinants for a successful implementation of the method.[7,8]

Hyperpolarization is transferred from protons to heteronuclei through scalar coupling,[3,9] and most studies deal with systems containing ^{13}C-carbonyl resonances that are characterized by long T_1 values.[6,10–12]

To tackle the issue associated to the non-biocompatibility of the hydrogenation catalyst, its removal through a passage on a ion-exchange resin has been proposed.[13] However this step may cause a dramatic polarization loss, as the temporary immobilization of the substrate on the resin may result in markedly enhanced relaxation rates.

To avoid the step associated to the removal of the organic solvent, the use of water-soluble catalysts has been proposed. Recently, an important contribution has been given by using hollow fiber membranes that allow a continuous delivery of hydrogen gas to be obtained in aqueous solution at 3 bar.[15] However, to obtain a certain amount of polarized molecules within a few seconds that can be used for imaging purposes, high pressures (10 bar) are usually necessary while the amount of hyperpolarized product is often rather small, especially with biocompatible substrates.

Herein a new approach based on the use of hydrogeneable precursors of the hyperpolarized substrates of interest is reported. It relies on the fact that carbonyl-containing substrates can be obtained by precursors (anhydrides or esters) that are more suitable to the hydrogenation with parahydrogen than the derived molecules. Thus the substrates of interest are obtained from a precursor that is first hydrogenated with para-H_2 and then hydrolyzed upon a fast reaction with water in the phase-transfer step. Polarization is transferred from parahydrogen to the heteroatom in the organic phase and is maintained during the phase-transfer process.

The process is summarized in Figure 1. Both the precursor and the catalyst are not water soluble, and the reaction with para-H_2 is carried out in an organic solvent to yield a product

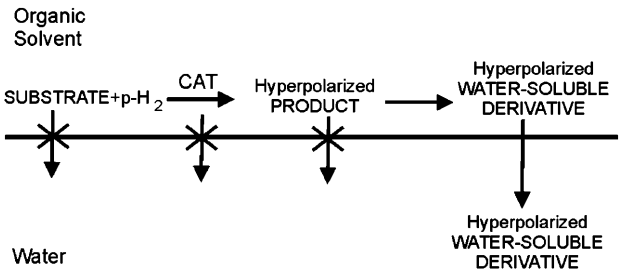
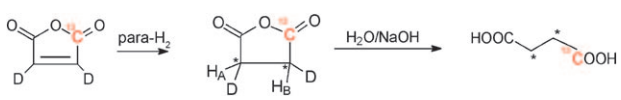


Figure 1. The parahydrogenation–hydrolysis–extraction procedure.

that, upon contact with water, transforms into the hyperpolarized substrate of interest. Furthermore, the hydrolysis step may be accelerated in the presence of a suitable enzyme.

The substrate chosen to test the proposed method is maleic anhydride, which affords succinic anhydride by hydrogenation. Succinic anhydride is then converted into succinic acid or succinate by hydrolysis (Scheme 1).

HP-succinate has been recently suggested[11] as a substrate for metabolic studies in tumor cells. When prepared from the parent fumaric acid, it has been shown that the amount of polarization observed on ^{13}C is strongly dependent on the pH value of its solutions.[14] In particular, for pH values close to the pKa of succinic acid, polarization transfer to ^{13}CO cannot be achieved owing to the indetermination in the ^{1}H–^{13}C coupling network caused by the exchange between succinic



Scheme 1. Formation of hyperpolarized 1-^{13}C-2,3-d_2-succinic acid obtained by parahydrogenation of 1-^{13}C-2,3-d_2-maleic anhydride.

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acid and succinate. Conversely, all the *J* coupling values of succinic anhydride are well defined,[16] allowing, in principle, a more efficient polarization transfer step. This feature is particularly relevant when the ^{13}C net magnetization is obtained by applying the pulse sequence described by Goldman and Johannesson.[17] This method requires an exact timing of pulses that is determined by the scalar coupling network between ^{13}C and parahydrogen protons.

To apply this method, a low-field NMR unit was designed and built in collaboration with Stelar/Invento. It consists in a 50 mT permanent magnet equipped with a $^{1}H/^{13}C$ double resonance wide bore probe that holds a Teflon reaction chamber (15 mL).

Hydrogenation, using para-H_2, of 1-^{13}C-2,3-d_2-maleic anhydride (100 mM) was pursued using [(1,4-bis(diphenylphosphino)butane)(1,5-cyclooctadiene) Rh^{I}]BF_4 (10 mM) as catalyst in $CDCl_3$ solution (a small amount of [D_6]acetone was still necessary for the activation of the catalyst). The reaction was carried out by spraying the solution into the reaction chamber previously pressurized with 4 atm of para-H_2 under ^{1}H-CW decoupling (5 s).

Under these experimental conditions, the hydrogenation of the unsaturated substrate led to a succinic anhydride concentration of about 40 mM (reaction yield 40%).[18] Figure 2a shows the ^{13}C spectrum of the obtained succinic anhydride.

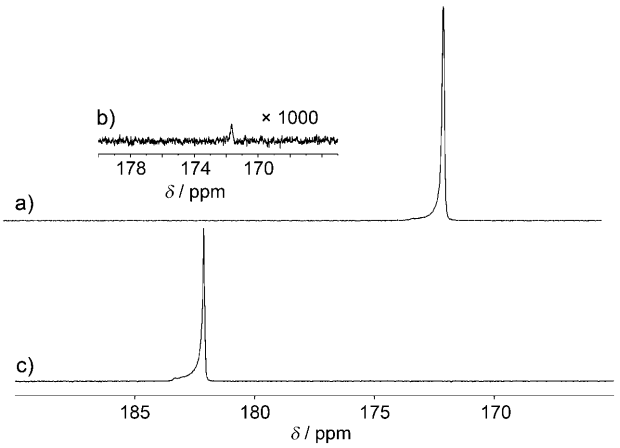

Figure 2. ^{13}C NMR spectra (14 T, RT) of a) hyperpolarized 1-^{13}C-2,3-d_2-succinic anhydride obtained by hydrogenation with para-H_2 of 1-^{13}C-2,3-d_2 maleic anhydride in $CDCl_3$/[D_6]acetone = 5:1 (1 scan, spectrum recorded immediately after parahydrogenation); b) equilibrium spectrum of succinic anhydride, and c) hyperpolarized 1-^{13}C-2,3-d_2-succinic acid obtained by parahydrogenation of 1-^{13}C-2,3-d_2-maleic anhydride in $CDCl_3$, successive hydrolysis, and extraction in basic D_2O.

The corresponding percentage of polarization can be calculated as described by Batthacharya et al.[8] First the signal enhancement was quantified in respect to the thermally polarized ^{13}C signal (Figure 2b); then, by using the expression $\eta_{t=0} = \eta_{obs} e^{-\tau/T_1}$, the η_0 value (corresponding to the enhancement factor at $t = 0$) was calculated. In the expression, τ is the time elapsed between the insertion of the sample into the spectrometer and the acquisition (10 s) and the T_1 is the longitudinal relaxation time of the carbonyl resonance that, at 14.1 T, was determined to be 22 s. On this basis, at 14.1 T, the polarization is 6.1%. However, considering that the para-H_2 enrichment is 52% (see the Experimental Section), the use of 98% para-H_2 would allow a further marked increment. By using the expression given by Bargon et al. [Eq. (1)],[1] (where *a* represents the para isomer part) one may estimate that, in the presence of 98% para-H_2 enrichment, the attainable polarization of the ^{13}C resonance of succinic anhydride would have been about 18.3%. This polarization is well above the 5% threshold for a hyperpolarized substrate to be considered for metabolic in vivo applications.[19]

$$\varepsilon = (1-4a)\,\frac{kT}{6\gamma\hbar B_0} \qquad (1)$$

The experiment was then repeated and hydrolysis of the succinic anhydride was obtained by quick addition of 0.4 mL of basic D_2O. The amount of NaOD dissolved in D_2O was calculated in order to obtain a succinate final solution having neutral pH (NaOD/maleic anhydride = 2.1:1). The mixture was then let to stand for few seconds in the test tube and the upper aqueous phase was transferred into the NMR tube for ^{13}C-spectrum acquisition. The signal intensity after aqueous phase extraction (Figure 2c) is about 70% of that observed in the organic phase (Figure 2a). Thus either an incomplete phase transfer process or the spontaneous polarization decay in the time of phase separation step do not appear to be limiting factors for the application of the herein reported procedure. Regarding in vivo use of hyperpolarized succinate, much attention has to be devoted to the presence of catalyst in the solution to be injected. As with this kind of metal complex, acetone is necessary for its activation, but a minimum amount was used to remain well below the toxic level in blood.[20]

A comparison between the polarization obtained for succinate when prepared from the precursor (succinic anhydride) polarized with parahdyrogen and that obtained for the same compound when directly formed from hydrogenation of fumarate in aqueous phase has been addressed. To do that, the enhancement attainable using the above mentioned apparatus depends on several factors (such as the volume of the reaction chamber, parahydrogen pressure, and pulse calibration) that are peculiar of the used instrumentation setup. It is quite likely that a change of one of those features (larger volume, higher pressure) would influence both chemical approaches (hydrogenation in aqueous medium and in organic phase) in the same way. Therefore, to compare the enhancement attainable by hydrogenation of the precursor of maleic anhydride with that obtained from direct hydrogenation in aqueous solution, the two approaches have been tested on the instrumentation developed in our laboratory. The reaction was carried out in aqueous phase by using the water-soluble catalyst [Rh(NBD)(diphos)]BF_4 (diphos = 4-Bis[(phenyl-3-propanesulfonate)phosphine]butane disodium salt, NBD = norbornadiene) (2.5 mM in D_2O) in phosphate buffer (pH 2.1). In fact, it had been reported[14] that scalar couplings between parahydrogen protons and carbon-

yl-^{13}C are optimal for polarization transfer at acidic pH conditions. Higher concentration of the catalyst may introduce further toxicity issues for the solutions to be used in vivo and, on the other hand, hydrogenation efficiency would not be markedly improved. Fumaric acid (5.9 mg, 25 mM solution) was then hydrogenated by spraying the solution in the reaction chamber charged with 4 atm of parahydrogen. The pulse sequence was calibrated using timing of pulses optimized with the *J* coupling values that had been reported[14] for succinate. In this case, the smaller amount of polarized product (4 mM) in respect to that obtained when the hydrogenation reaction is carried out in an organic solvent is due to the lower efficiency of hydrogenation in water. Furthermore, the attainable polarization (calculated on the equilibrium signal and extrapolated for 98% enriched parahydrogen) is about 11%. The resulting signal intensity for the product obtained upon hydrogenating fumaric acid in water is then less than 1/10 of that observed from the hydrolysis of succinic anhydride reported herein (see the Supporting Information). This is due both to a lower product concentration (4 mM), which is related to lower hydrogenation efficiency in water, and to the fact that when para-H_2 is added directly to the acid, scalar coupling values between ^{1}H and ^{13}C may not be optimal for polarization transfer to ^{13}C as discussed above.

In summary, the results reported herein have demonstrated that hydrogenation using para-H_2 of a precursor of the desired polarized probe has several advantages in respect to the direct reaction. The present method can be applied to molecules containing functional groups (anhydrides, esters) that are activated by the reaction with water. One key advantage deals with an improved hydrogenation efficiency, (in general, hydrogenation in organic solvent works much better than in water) and, besides succinate, the method can be exploited for generating other biologically interesting molecules.

The possibility of carrying out hydrogenation in an organic solvent instead of water allows a higher concentration of product to be attained, and overall it results in a marked enhancement of the polarized signal. The water-soluble probe is then obtained by a quick chemical reaction, leading to a catalyst-free water solution of the compounds of interest avoiding the use of high para-H_2 pressures and further manipulations of the hyperpolarized product with consequent polarization losses. This method may markedly widen the role of the PHIP method to generate HP molecules for MRI applications.

Experimental Section

Deuterated solvents and the catalyst [(1,4-bis(diphenylphosphino)-butane) (1,5-cyclooctadiene) Rh^I]BF_4 were purchased from Sigma–Aldrich, and $CDCl_3$ was dried over $CaSO_4$ and distilled before use. The catalyst [Rh(NBD)diphos]BF_4 was prepared according to the reported procedure.[8]

Ortho- and parahydrogen composition of the mixture used in the hydrogenation of maleic anhydride was ascertained by recording Raman spectra (Ranishaw microRaman, laser frequency 514 nm). To acquire these spectra, 4 bar of parahydrogen were collected in a NMR tube (10 mm) equipped with Young valve. From comparing the integrals of para ($\lambda = 354$ cm^{-1}) and ortho ($\lambda = 587$ cm^{-1}) signals, the mixture was determined to be 52% enriched in the para isomer.

The instrumentation setup used to carry out the hydrogenation reaction (built by STELAR/Invento) consists of a wide-bore 50 mT permanent magnet equipped with ^{1}H/^{13}C double-resonance probe operating at 2.27 MHz (^{1}H) and 0.57 MHz (^{13}C). Each channel is equipped with suitable elements for tuning in the suitable range and for impedance matching. A modified Stelar PC NMR multichannel NMR console was used to program and drive the experiments. The hydrogenation reaction was carried out into a Teflon reaction chamber (inner volume 15 mL) that was placed into the probe. The chamber was pressurized with 4 bar of hydrogen enriched in the para isomer. The solution of maleic anhydride (100 mM) and hydrogenation catalyst (7 mM) in 2 mL of $CDCl_3$/[D_6]acetone mixture (5:1) was collected in a syringe and sprayed mechanically into the reaction chamber. During the reaction (3–4 s), proton decoupling (WALTZ sequence) was applied, at the end of which the pulse sequence to transform the spin order of the added para-H_2 molecule into net ^{13}C magnetization started. The solution containing the polarized product was then collected outside the reaction chamber by exploiting the residual H_2 pressure and transferred to the 600 MHz Bruker Advance NMR spectrometer for the acquisition of the ^{13}C NMR spectrum. The flip angle calibration for the proton channel was carried out directly on the ^{1}H signal of 10 mL of water containing a paramagnetic complex [Gd(HPDO3 A)] (a commercially available MRI agent) that allows to minimize the repetition time between pulses and to speed up the calibration. The ^{13}C flip angle was optimized using the indirect calibration the method reported in ref. [8]. A solution of methyl-acetylenedicarboxylate (100 mM) (^{13}C enriched at one of the carboxylate positions) was polarized into a 14 T NMR spectrometer then quickly transferred into the 50 mT magnet where the excitation pulse was applied. Immediately afterwards, the sample is returned into the high-field spectrometer for ^{13}C signal acquisition. The pulse widths for ^{1}H and ^{13}C were 8 μs and 33 μs, respectively.

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