Efficient NMR Pulse Sequences to Transfer the Parahydrogen-Induced Polarization to Hetero Nuclei

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Received January 8, 1996[⊗]

Abstract: The PASADENA or PHIP effect originates from the breakdown of the parahydrogen symmetry when the two protons are found in magnetically inequivalent positions after hydrogenation. As a result, a signal enhancement of up to 10^4 in the proton NMR spectra can be observed. We introduce three polarization transfer techniques, namely PH-INEPT, PH-INEPT+, and INEPT($+\pi/4$), that are highly effective to record *in situ* hetero NMR spectra of parahydrogen-labeled compounds. A complete product operator treatment as well as experimental results for ^{13}C and ^{29}Si have been given. With these sequences we obtained a signal enhancement of 500 which allows *in situ* NMR investigations of hetero nuclear systems at natural abundance.

The parahydrogen labeling experiment under hydrogenation conditions has ever since its theoretical postulation[1] and experimental verification[2,3] been of great use for studying short-lived reaction intermediates during hydrogenations[4] and for mechanistic[5,6] investigations.

The PASADENA[1] (Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment) or PHIP (Parahydrogen Induced Polarization) effect originates from the breakdown of the p-H_2 symmetry when the two protons are found in magnetically inequivalent positions after hydrogenation. As a result, a signal enhancement (SE) of up to 10^4 in the proton NMR spectra can be observed. Recently, a few studies have attempted to transfer the p-H_2 polarization to hetero nuclei, employing conventional INEPT or INEPT+ pulse sequences,[7] or methods that exploit the nature of special spin systems.[8,9] In this article, we present an analysis of the product operator treatment of the p-H_2 magnetization with the goal to transfer the parahydrogen polarization to hetero nuclei. The pulse sequences derived yield a SE of about 500 which allows in situ NMR investigations of hetero nuclear systems at natural abundance.

When performing hydrogenations with p-H_2, two cases of different initial proton magnetization have to be distinguished: (a) hydrogenation and detection inside the NMR spectrometer (abbreviated by the acronym PASADENA) and (b) hydrogenation at low magnetic field outside the spectrometer and subsequently transporting the sample for detection into the spectrometer (termed ALTADENA[10] for Adiabatic Longitudinal Transport After Dissociation Engenders Net Alignment).

In addition to these two techniques, entirely different behavior with regard to polarization transfer (PT) during hydrogenations with p-H_2 is exhibited by strongly and weakly coupled systems.[8]

The density matrix of p-H_2 can be described by Cartesian product operators as $\sigma_{\text{parahydrogen}} = \mathbf{I}_1\mathbf{I}_2$. This density matrix is propagated by the Hamiltonian of the system. Performing this propagation with the weak coupling Hamiltonian and averaging, over the propagation time, the density matrix of the two former p-H_2 nuclei simplifies to $\sigma_{\text{PASADENA}} = I_{1z}I_{2z}$, in contrast to the initial density matrix for two hydrogen nuclei at thermal equilibrium, $\sigma_{\text{thermal}} = I_{1z} + I_{2z}$. In order to achieve polarization transfer from the two former p-H_2 nuclei to hetero nuclei, we substituted the initial ($\pi/2_x$) pulse in the standard INEPT sequence by ($\pi/4_y$), the first and the last pulse in the ^1H-channel now having the same phase. Applying this PH-INEPT (Para Hydrogen INEPT) sequence on the initial density matrix for PASADENA experiments yields signals on the hetero nuclei that are antiphase with respect to the J_{HX} couplings. Just as for the conventional INEPT sequence, the antiphase signals of the PH-INEPT sequence can be refocussed by employing an additional delay. The inphase signals that result from this so obtained PH-INEPT+ sequence can be decoupled for further signal enhancement, which facilitates the detection of small polarization signals. It should be noted that the PH-INEPT sequence does not transfer any proton magnetization stemming from systems at thermal equilibrium. Consequently, a PH-INEPT spectrum only displays signals stemming from the product just formed with p-H_2; the signals of the educt and product at thermal equilibrium are absent. This feature facilitates spectral analysis considerably. Scheme 1 displays the results of INEPT, PH-INEPT, and PH-INEPT+ applied on σ_{PASADENA} for simple three-spin systems consisting of the two former p-H_2 nuclei (I_1, I_2) and one hetero nucleus (S). Incorporation of additional protons and hetero nuclei does not alter the essential features of the product operator expressions derived. For all sequences other than INEPT, only terms that

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[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1996.

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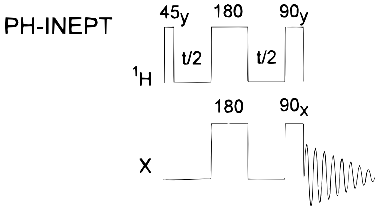
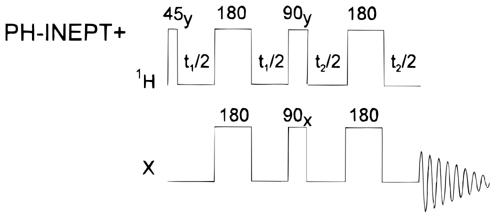
S0002-7863(96)00067-4 CCC: $12.00

Scheme 1

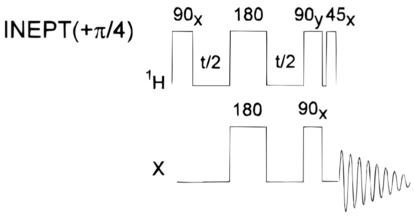
$$I_{1z}I_{2z} \xrightarrow{\text{INEPT}} \cos(\pi J_{1S}t)\cos(\pi J_{2S}t)\, I_{1y}I_{2y} - 2\sin(\pi J_{1S}t)\cos(\pi J_{2S}t)\, I_{1z}I_{2y}S_y + \sin(\pi J_{1S}t)\sin(\pi J_{2S}t)\, I_{1z}I_{2z} - 2\cos(\pi J_{1S}t)\sin(\pi J_{2S}t)\, I_{1y}I_{2z}S_y$$



$$I_{1z}I_{2z} \xrightarrow{\text{PH-INEPT}} \sin(\pi J_{12}t)\sin(\pi J_{1S}t)/2\; I_{1z}S_y + \sin(\pi J_{12}t)\sin(\pi J_{2S}t)/2\; I_{2z}S_y$$

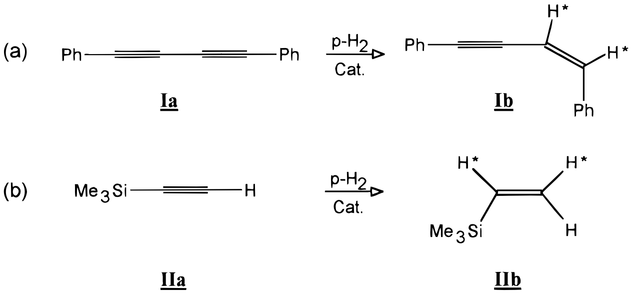


$$I_{1z}I_{2z} \xrightarrow{\text{PH-INEPT+}} \sin(\pi J_{12}t_1)\sin(\pi J_{1S}t_1)\sin(\pi J_{1S}t_2)\cos(\pi J_{2S}t_2)/4\; S_x + \sin(\pi J_{12}t_1)\sin(\pi J_{2S}t_1)\cos(\pi J_{1S}t_2)\sin(\pi J_{2S}t_2)/4\; S_x$$



$$I_{1z}I_{2z} \xrightarrow{\text{INEPT}(+\pi/4)} \sin(\pi(J_{1S} + J_{2S})\,t)\; I_{1z}I_{2z}S_y$$

Scheme 2



Ia **Ib**

IIa **IIb**

are observable on the hetero nucleus have been listed. In addition to the terms listed, the PH-INEPT+ sequence also generates antiphase signals on the hetero nucleus, which however are of no relevance when proton decoupling is employed. As can be seen from the product operator expression for the PH-INEPT sequence, the occurrence of a signal on the hetero nuclei crucially depends on the size of the $^1H-^1H$ coupling, J_{12}. For cases where J_{12} is small or non-existent, as is the case for some organometallic dihydrides or for all cases other than vicinal or geminal hydrogenations, this feature makes signal detection on the hetero nuclei with the PH-INEPT sequence difficult or impossible. There is however a simple remedy for this problem: As shown in Scheme 1, the conventional INEPT sequence yields trilinear terms of the kind $I_{1z}I_{2y}S_x$ when applied on the p-H_2 density matrix. Such terms are not detectable on the hetero nucleus. However, they can be transferred into detectable terms of the kind $I_{1z}I_{2z}S_x$ simply by applying an additional $\pi/4_x$ pulse on the protons after the conventional INEPT sequence. The entire sequence which we named INEPT($+\pi/4$) as well as its effects on the p-H_2 density matrix is displayed in Scheme 1. It is evident that polarization transfer using this sequence does not depend on the $^1H-^1H$ coupling. The last two pulses on the protons in the INEPT($+\pi/4$) sequence, namely ($\pi/2_y$) and ($\pi/4_x$), could be combined to a ($\pi/2_\phi$) pulse with the phase $\phi = 45°$, which however was not available on our spectrometer.

It has been shown that PT is also possible with the INEPT+ sequence,[7] which yields antiphase signals with p-H_2 systems and inphase signals with systems at thermal equilibrium. However, throughout all our experiments, the PH-INEPT family of sequences as well as the INEPT($+\pi/4$) sequence performed considerably better than the INEPT+ sequence.

All product operator transformations were evaluated using POMA,[11] a Mathematica[12] implementation of the product operator formalism. When performing PASADENA with strongly coupled spin systems, the very nature of the spin system enables PT from protons to hetero nuclei without any further spin manipulations being necessary. A simple $\pi/2$ pulse on the hetero nucleus is sufficient to observe hetero nuclear NMR spectra with a high degree of polarization.[8] We wish to emphasize that this effect does not occur for weakly coupled spin systems, e.g. for vinylsilane **IIb** as discussed in this study.

For an ALTADENA experiment,[10] the picture is completely different. At low field any proton network is strongly coupled. Consequently, hetero nuclear NMR spectra with a strong SE can be obtained directly with any spin system in an ALTADENA setup with a simple $\pi/2$ pulse on the hetero nuclei,[13] just as demonstrated for PASADENA experiments using strongly coupled systems. In some cases, such as the hydrogenation of norbornadiene to norbornene, we could observe polarization signals of the entire carbon skeleton of the hydrogenation product.

To support the theoretical predictions by experimental evidence, we have investigated the hydrogenation with p-H_2 of (a) 1,4-diphenylbutadiyne (**Ia**) to 1,4-diphenylbut-1-en-3-yne (**Ib**) and of (b) (trimethylsilyl)acetylene (**IIa**) to trimethylvinylsilane (**IIb**) using in both cases the rhodium catalyst [Rh(Ph-β-glup-OH)(COD)]BF_4.[14] To carry out the experiments, standard 5-mm NMR tubes were charged with either 30 mg (0.15 mmol) of **Ia** or 50 μL of **IIa** (0.35 mmol), 5 mg of the catalyst, and 500 μL of deoxygenated acetone-d_6. For PASADENA experiments, the NMR tube was connected to a glass tube about 50 cm in length and 8 mm in diameter. A glass capillary, 1 mm in diameter, was connected to the p-H_2 source via polyethylene tubing and inserted through the glass tube into the NMR tube. The glass capillary reached down to the bottom of the NMR tube, i.e. well inside the area of the receiver and transmitter coils. Despite the glass capillary in the detection zone, shimming proved little problem, i.e. a resolution <1 Hz could easily be achieved. No sample spinning was employed. Between subsequent scans, p-H_2, enriched to 50% over charcoal at 77 K, was bubbled through the solution for 7 s at ambient pressure. During acquisition, the hydrogen stream was interrupted. For the PH-INEPT, PH-INEPT+, and INEPT($+\pi/4$) experiments, the delay time *t* was optimized for each spin system using the product operator expressions in Scheme 1. For the

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[11] Güntert, P.; Schaefer, N.; Otting, G.; Wütrich, K. *J. Magn. Reson.* **1993**, *101*, 103; **1993**, *105*, 328.

[12] Mathematica is a registered trademark of Wolfram Research.

[13] This effect is hard to observe for quickly exchanging systems, i.e. for organometallic dihydrides, and for products with short relaxation times, i.e. short with respect to the time it takes to insert the sample into the spectrometer.

[14] Selke, R. *J. Organomet. Chem.* **1989**, *370*, 241. Selke, R.; Facklam, C.; Folken H.; Heller, D. *Tetrahedron: Asymmetry* **1993**, 369.

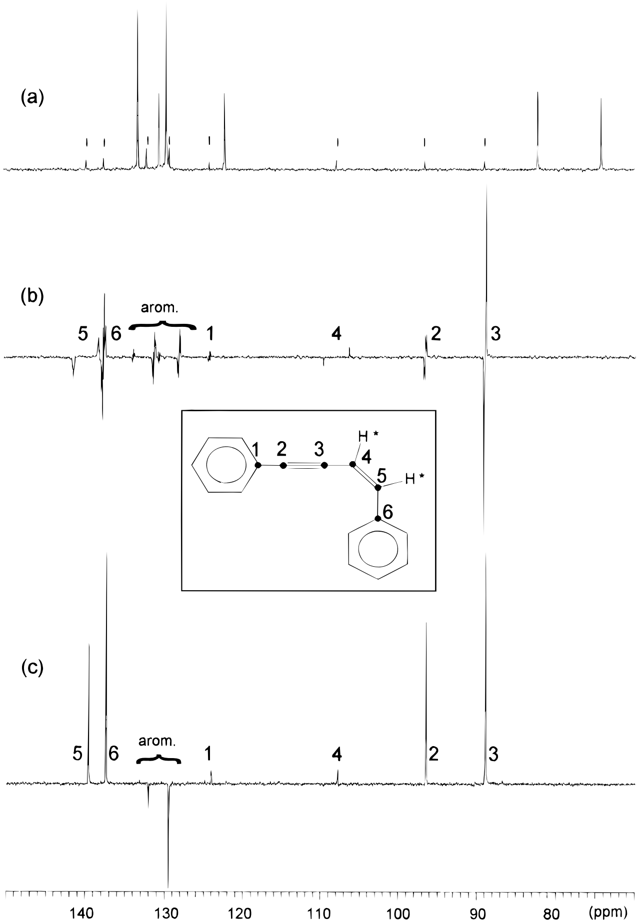

Figure 1. (a) $^{13}C-\{^{1}H\}$ spectrum of **Ia** and **Ib** obtained in 600 scans after the hydrogenation had proceeded for a total of 8 min hydrogenation time. The signals of **Ib** are marked with tick marks. Four signals of **Ib** are masked by the signal of **Ia** at 129.4 ppm. (b) ^{13}C-PH-INEPT spectrum of **Ib** (8 scans, $t = 0.042$ s) after a total of 1 min hydrogenation time under PASADENA conditions. (c) $^{13}C-\{^{1}H\}$-PH-INEPT+ spectrum (8 scans, $t_1 = t_2 = 0.042$ s) of **Ib** after a total of 1 min hydrogenation time under PASADENA conditions. The transitions are numbered as depicted in the inlet; "arom." symbolizes aromatic carbons. All spectra were apodized with a line broadening of 0.5 Hz.

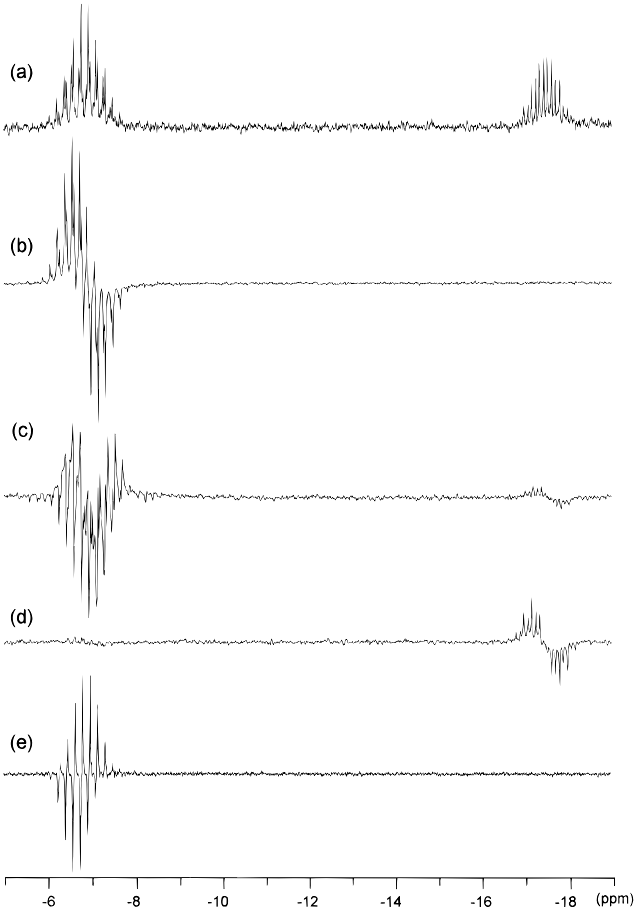

Figure 2. (a) ^{29}Si spectrum of **IIa** ($\delta = -17.5$ ppm) and **IIb** ($\delta = -6.5$ ppm) at thermal equilibrium. Spectrum reference is tetramethylsilane ($\delta = 0$ ppm). (b) ^{29}Si PH-INEPT spectrum (8 scans, $t = 0.032$ s) of **IIb** after a total of 1 min hydrogenation under PASADENA conditions. Note the complete absence of any signal belonging to **IIa**. (c) ^{29}Si INEPT($+\pi/4$) spectrum (8 scans, $t = 0.036$ s) of **IIb** after a total of 1 min hydrogenation time under PASADENA conditions. The signal of **IIa** is slightly visible. (d) ^{29}Si INEPT spectrum (16 scans, $t = 0.070$ s) after 8 min hydrogenation time (5 mg of **IIb** formed) and after the system had returned to thermal equilibrium. (e) ^{29}Si spectrum after the ALTADENA experiment using a $\pi/2$ (^{29}Si) pulse, single scan, spinning sample.

ALTADENA experiments, 5 mm screw cap NMR tubes with a PTFE/silicone septum were charged with 3 bar of para-enriched H_2 outside the magnet. The tubes were shaken vigorously and quickly inserted into the spectrometer for detection. The pulses were placed after the spinner had started and after the lock signal had returned to a normal level, i.e. after about 15 s. All experiments were carried out on a Bruker AC 200 NMR spectrometer.[15]

Figure 1a displays the thermal $^{13}C-\{^{1}H\}$ spectrum of **Ia**[16] and **Ib**[17] obtained after the hydrogenation had proceeded for 10 min by continously bubbling H_2 through the solution. The performance of the PH-INEPT sequence and its refocussed extension, PH-INEPT+, is demonstrated in Figure 1b,c. These spectra were each obtained in 8 scans and a total of 1 min hydrogenation time. From Figure 1a we derived that after 1 min 0.3 mg (1.5 μmol) of **Ib** had been formed, corresponding to a SE of about 300. Polarization is observed on the four carbon nuclei constituting the butane skeleton (marked 2−5 in Figure 1) as well as on the aromatic carbon nuclei (1, 6, "arom."). All signals in Figures 1b and 1c belong to **Ib**, the signals of the 90-fold excess of **Ia** being completely suppressed.

Figure 2a shows the ^{29}Si spectrum of **IIa**[18] and **IIb**,[19] and Figure 2b,c demonstrate the performance of the PH-INEPT and the INEPT($+\pi/4$) sequence, respectively. Both were obtained in 8 scans each and a total of 1 min hydrogenation time, corresponding to 0.6 mg (6 μmol) of **IIb** being formed. In agreement with the product operator expression displayed in Scheme 1, the PH-INEPT sequence yields an antiphase signal with intensities ($^{1}/_{2}$, $-^{1}/_{2}$), whereas the signal obtained with the INEPT($+\pi/4$) sequence diplays intensities of the kind ($^{1}/_{4}$, $-^{1}/_{2}$, $^{1}/_{4}$) as expected[20] of the term $I_{1z}I_{2z}S_y$. The PH-INEPT appears

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[15] On our spectrometer, the resonance frequencies of ^{1}H, ^{13}C, and ^{29}Si are 200, 50, and 39 MHz, respectively.

[16] Selected NMR data for **Ia** (acetone-d_6, room temperature): ^{13}C (50 MHz) δ 74.3, 82.2, 122.1, 129.4 (meta), 130.2 (para), 133.2 (ortho).

[17] Selected NMR data for **Ib** (acetone-d_6, room temperature): ^{1}H (200 MHz) δ 6.83 ($^{3}J_{HH} = 12.0$ Hz), 5.99. ^{13}C (50 MHz) δ 88.3 ($^{2}J_{CH} = 15.1$ Hz), 95.9 ($^{3}J_{CH} = 9.9$ Hz, $^{3}J_{CH} = 5.3$ Hz), 107.3 ($^{1}J_{CH} = 165.8$ Hz), 123.5, 129.0 ($^{1}J_{CH} = 158.6$ Hz, $^{3}J_{CH} = 7.3$ Hz, $^{3}J_{CH} = 12.3$ Hz), 131.6 ($^{1}J_{CH} = 169.5$ Hz), 136.9 ($^{2}J_{CH} = 11.8$ Hz, $^{2}J_{CH}$(aromatic) = 7.2 Hz), 139.1 ($^{1}J_{CH} = 159.1$ Hz, $^{3}J_{CH} = 4.9$ Hz).

[18] Selected NMR data for **IIa** (acetone-d_6, room temperature): ^{1}H (200 MHz) δ 2.80 ($^{3}J_{SiH} = 11.0$ Hz), 0.21 ($^{2}J_{SiH} = 7.2$ Hz). ^{29}Si (39 MHz) δ −17.5.

[19] Selected NMR data for **IIb** (acetone-d_6, room temperature): ^{1}H (200 MHz) δ 0.15 ($^{2}J_{SiH} = 6.7$ Hz), 6.20 ($^{3}J_{HH}$(trans) = 19.9 Hz, $^{3}J_{HH}$(cis) = 14.6 Hz, $^{2}J_{SiH}$(gem) = 6.5 Hz), 5.93 ($^{2}J_{HH}$(gem) = 4.3 Hz, $^{3}J_{SiH}$(trans) = 15.3 Hz), 5.69 ($^{3}J_{SiH}$(cis) = 8.7 Hz). ^{29}Si (39 MHz) δ −6.5.

to yield better results than the INEPT($+\pi/4$) in this case. Generally, the performance of both sequences should be comparable. The PH-INEPT sequence effects complete suppression of the educt signals, whereas the INEPT($+\pi/4$) sequence only reduces the signal intensities of the educt signals (I_zS_y) by a factor of $2^{1/2}$ as compared to the standard INEPT sequence. After a total of 8 min reaction time and after the sample had relaxed to thermal equilibrium, the spectrum in Figure 2d was recorded using the INEPT sequence. In this sample, **IIa** at −17.5 ppm is present in 9-fold excess with respect to **IIb** at −6.5 ppm, the latter being hardly visible. Comparing the spectrum in Figure 2b with 2d, we estimated a SE of 500 for the PASADENA/PH-INEPT experiment. It should be noted that no ^{29}Si polarization could be observed using a $\pi/2$ pulse after a PASADENA experiment. The reason for this is that **IIb** is a weakly coupled spin system at high field. Using ALTADENA conditions, **IIb** represents a strongly coupled spin system, which experiences strong cross relaxation from the p-H_2 protons to ^{29}Si. Spectrum e in Figure 2 has been recorded with a $\pi/2$ (^{29}Si) pulse following an ALTADENA experiment. The multiplet obtained appears to be in antiphase with respect to all couplings. Just as in the PH-INEPT experiments, the signal of **IIa** at −17.5 ppm is not visible. This, however, is due to the low sensitivity of ^{29}Si, and is not caused by any filtering technique.

Our results demonstrate that by employing standard NMR product operator theory, polarization transfer techniques for the unique p-H_2 magnetization $I_{1z}I_{2z}$ can be derived. The three sequences presented in this paper, namely PH-INEPT, PH-INEPT+, and INEPT($+\pi/4$), are highly effective in recording in situ hetero nuclear NMR spectra of p-H_2 labeled compounds. By choosing suitable delay times, the polarization was shown to be transferred to the entire carbon skeleton of the product molecule, thus considerably extending the applicability of p-H_2 labeling in organometallic research. Even with rudimentary optimization of the delay time, good PT has been achieved over a wide range of couplings in a single experiment, e.g. from 9 to 170 Hz for **Ib**. The ability to employ proton decoupling for the PH-INEPT+ sequence should prove a valuable feature. The INEPT($+\pi/4$) sequence is particularly suited for systems where the ^{1}H−^{1}H coupling is small or absent, as well as for systems where the two ^{1}H−X couplings are of similar size. We found all three sequences to work considerably better than the INEPT+ sequence employed previously for p-H_2 systems. An additional striking feature of the PH-INEPT family of sequences is its spin filter property. It is designed to exclusively transfer the magnetic label of p-H_2 to hetero nuclei, and suppresses all solvent and educt signals. This characteristic is extremely useful if unexpected product formation occurs. Due to its simplicity, the PH-INEPT family of sequences can be easily applied. It should be pointed out that all features presented in this article can also be employed for the editing of ^{1}H spectra.

Note Added in Proof: After acceptance of the present article, a few studies have been published that deal with the transfer of parahydrogen-induced polarization to heteronuclei or to other protons: (a) Duckett, S. B.; Barlow, G. K.; Partridge, M. G.; Messerle, B. A. *J. Chem. Soc., Dalton Trans.* **1995**, 3429. (b) Duckett, S. B.; Mawby, R. J.; Partridge, M. G. *Chem. Commun.* **1996**, 383. (c) Barkemeyer, J.; Bargon, J.; Sengstschmid, H.; Freeman, R. *J. Magn. Reson.* **1996**, *120*, 129. (d) Sengstschmid, H.; Freeman, R.; Barkemeyer, J.; Bargon, J. *J. Magn. Reson.* **1996**, *120*, 249.

Acknowledgment. Financial support from the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, the Volkswagenstiftung, and the Fonds der Chemischen Industrie is gratefully acknowledged. M.H. thanks the Konrad Adenauer Stiftung and J.N. the Studienstiftung des Deutschen Volkes for a graduate fellowship. We thank Prof. Dr. R. Selke, MPI Rostock, for a generous gift of the rhodium catalyst.

JA960067F

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