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Parahydrogen Allows Ultrasensitive Indirect NMR Detection of Catalytic Hydrogen Complexes

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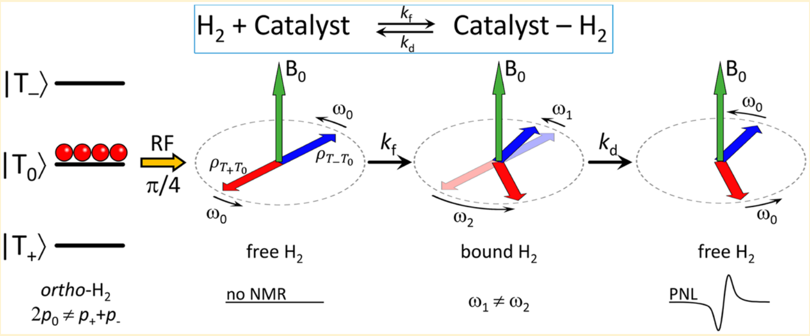
S *Supporting Information*



ABSTRACT: The ^{1}H NMR signal of dissolved molecular hydrogen enriched in parahydrogen (p-H$_2$) exhibits in the presence of an organometallic hydrogenation catalyst an unusual, partially negative line shape (PNL). It results from a strongly enhanced two-spin order connected to the population of the T_0 level of orthohydrogen (o-H$_2$). This two-spin order is made visible by a slow asymmetric exchange process between free hydrogen and a transient catalyst-hydrogen complex. By Only Parahydrogen Spectroscopy (OPSY) it is possible to selectively detect the two-spin order and suppress the signal from the thermal o-H$_2$. The intensity of the PNL can be strongly affected by the PArtially NEgative Line (PANEL) experiment, which irradiates a long narrow-band radio frequency (RF) pulse. When the RF is in resonance with the chemical shift values of the hydrogen bound to the elusive catalyst or of the free hydrogen, a strong intensity reduction of the PNL is observed. Numerical simulations of the experiments performed at 500 and 700 MHz proton frequency show that the indirect detection has at least 3 orders of magnitude higher sensitivity than the normal NMR experiment. A theoretical model, including reversible binding and $S - T_0$ evolution, is developed, which reproduces the NMR line shape, the nutation angle dependence and the dependence on the frequency of the irradiation field of the PNL and permits the determination of the proton chemical shift values and the sign of the scalar coupling in the transient NMR invisible complex where singlet−triplet conversion take place.

I. INTRODUCTION

The problem of the NMR detection and characterization of reaction intermediates, dihydrogen activation and transient species in organometallic catalyzed hydrogenation reactions is a long-standing challenging experimental and theoretical problem, going back to the original work of Wilkinson.[1,2] This field received a strong impetus by the development of Parahydrogen Induced Polarization (PHIP), developed in parallel by the groups of Eisenberg and Bargon[3] and Weitekamp.[4] This development resulted in a series of original papers (e.g., refs 5−7) and reviews (e.g., refs 8−10), in particular, from the Bargon and the Duckett group. After this initial period, the focus of the PHIP research shifted toward the sensitivity enhancement of NMR and MRI.

PHIP is a powerful method for enhancing weak NMR signals by exploiting spin order of parahydrogen (p-H$_2$, the H$_2$ molecule in its singlet state). The H$_2$ gas can be relatively easily enriched in the *para*-component; however, the resulting spin order does not produce an NMR signal. Therefore, in order to convert the spin order into enhanced NMR signals, an additional step is required to break the symmetry of the two protons. Symmetry breaking is performed by a suitable chemical process. Presently, PHIP exists in two main versions: (i) hydrogenation of a substrate molecule with an unsaturated

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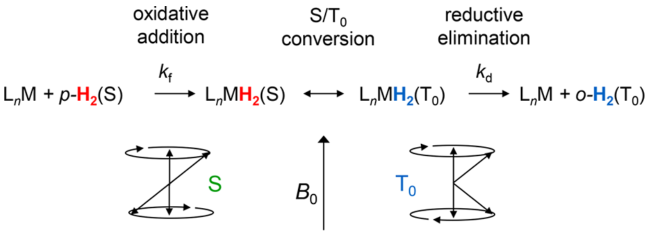
C−C bond so that in the reaction product the two protons originating from p-H_2 become nonequivalent;[11] (ii) spin order transfer from p-H_2 to a substrate molecule in a transient complex.[12] Both methods require a suitable catalyst, which usually is an organometallic complex L_nM (M, metal; L, ligand), which can bind p-H_2 and the substrate. The substrate is, however, often replaced by a second p-H_2 molecule. Hydrogen exchange between these two p-H_2 molecules causes a "chemical" p-H_2/o-H_2 conversion, which is in general faster than the magnetic spin conversion.[13,14] Therefore, it is often difficult to observe the magnetic conversion of a single p-H_2 molecule into a single o-H_2, which may also exhibit a spin polarization as illustrated in Scheme 1.

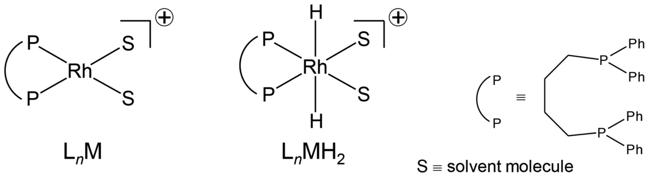
Scheme 1. Magnetic Field Induced Conversion of p-H_2 (Spin State S) to o-H_2 in Spin State T_0 According to Bargon et al.[15,16,a]



[a] After oxidative addition to a transition metal complex L_nM, where the two hydrogen nuclei exhibit different Larmor frequencies, the singlet state S with opposite phases of the nuclear spins is converted to the triplet sub-state T_0, where the two spins are in-phase.

First, such a conversion Scheme 2 requires binding of p-H_2 to L_nM by oxidative addition creating a short-lived transient intermediate dihydrogen complex or dihydride L_nMH_2. The formation of L_nMH_2 is characterized by the pseudo-first order rate constant k_f. Owing to their short lifetime and low concentration, L_nMH_2 may be invisible in solution state NMR experiments.

Scheme 2. Complex L_nM and Its Dihydride L_nMH_2 Generated from [1,4-Bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) Tetrafluoroborate by Hydrogenation



As proposed by Bargon et al.,[15,16] in L_nMH_2, the initial singlet S state is converted to the triplet state T_0 when the two dihydride nuclei experience different Larmor frequencies in an external magnetic field B_0. The formation of T_0 implies the creation of two-spin order in the form of a strongly enhanced nonthermal population.

We became interested in manifestations of nonthermally polarized o-H_2 while performing PHIP experiments on peptides,[17,18] where we noticed in some spectra[19] distortions of the NMR line of the dissolved hydrogen at about 4.5 ppm, leading to a partial negative line shape (PNL in the following). In some cases, where the hydrogenation reaction and the consumption of dissolved hydrogen were relatively slow, the intensity of the PNL line was very strong. Figure 1 presents a typical PHIP spectrum with a strong PNL signal visible at 4.53 ppm, taken during the hydrogenation of Fmoc-O-allyl-tyrosine by parahydrogen at elevated pressure. The appearance of the PNL is puzzling, because the signal of o-H_2, that is, of two equivalent protons, is expected to constitute a single line. Unusual distortions of o-H_2 signals released from transient dihydrides had been observed previously also by other groups,[20−22] but the origin of the PNL formation has not been discussed or remains unclear. During the preparation of this manuscript the Koptyug group[23] reported in a beautiful PHIP study PNL shapes of o-H_2 in the presence of a metal-free ansa-aminoborane catalysts (BorylCat). It was suggested that the PNLs might be caused by a one-sided release of o-H_2 from the catalyst-dihydrogen complex BorylCat-H_2.

In this report, we propose an experimental and theoretical analysis that quantitatively explains the nonthermal polarization of o-H_2 leading to the PNL phenomenon and shows that the effect is the result of the constantly ongoing slow exchange between the free and bound form of H_2 during the NMR experiment. We first show that there are two independent reaction channels, which can contribute to the PNL. In the first channel, the release of free o-H_2 from the intermediate - a one-sided reaction - can create the PNL, a possibility mentioned by Koptyug et al.[23] We discuss here a second channel that has not yet been considered previously, where the PNL of o-H_2 is created by a slow two-sided exchange of nonthermally populated free H_2 molecules with the bound L_nMH_2 intermediate. A detailed analysis shows that the latter channel dominates the production of the PNL under our experimental conditions. We also demonstrate that the PNL is not just a puzzling effect: by analyzing the PNL it is possible to reveal the spectral signature of the otherwise invisible intermediate complex by application of a long narrow-band RF-irradiation at the chemical shift of bound hydrogens and thus to detect these NMR invisible intermediates. Finally, we present a simple explanation of the occurrence of PNL in terms of slow-exchange signal shifts between free and bound H_2 and discuss the information provided by the PNL on the bound H_2 species, which is invisible under normal spectral conditions.

II. METHODS

Samples and Compounds. The study of the characteristic line shape caused by the interaction of dissolved p-H_2 with the catalyst [1,4-bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate was performed in acetone-d_6 (Scheme 2). Parahydrogen enriched hydrogen gas is prepared at 77 K with about 50% of p-H_2 spin-isomer. The hydrogen pressure during the bubbling with parahydrogen was between 2 and 5 bar. The exact conditions of each experiment (concentrations, bubbling pressure, measurement delays, etc.) are given in the figure captions.

Experiments with Parahydrogen. In all experiments, a fully automatic setup for hydrogen bubbling in situ controlled by the NMR spectrometer was employed. As a detailed description of the apparatus is beyond the scope of the present paper and we only briefly describe its relevant features: A gas (hydrogen or an inert gas, e.g., helium or nitrogen) is conducted from a source to the solution by a pipe system. The final thin plastic capillary is submerged into the solution directly in the NMR sample tube and is tightly fixed to the cap of the sample tube. Solenoidal magnetic valves operated from

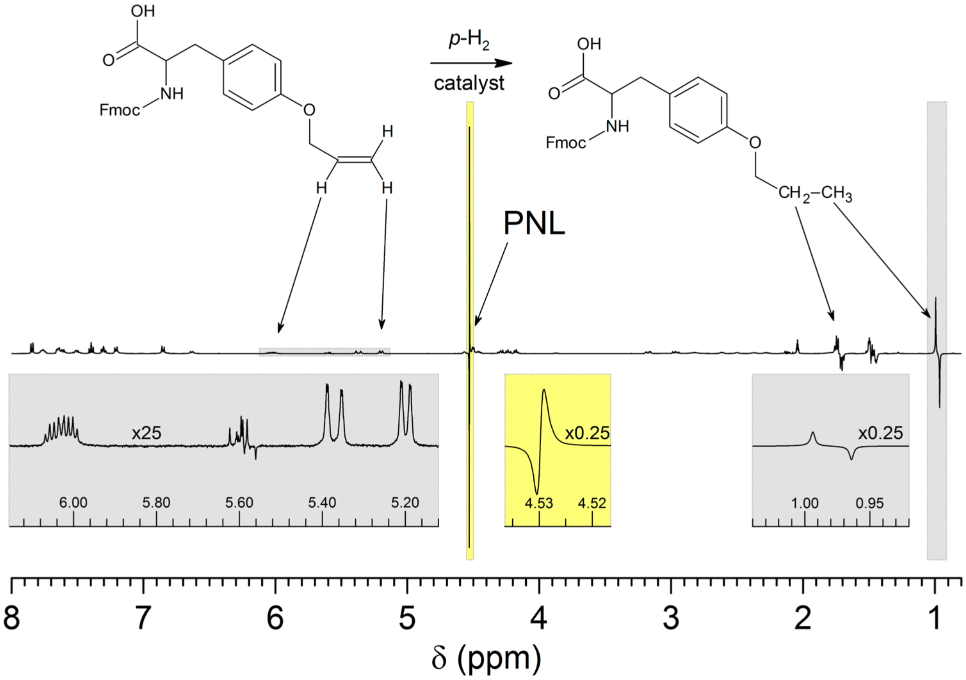


Figure 1. ^{1}H NMR PHIP spectrum recorded during the hydrogenation with p-H$_2$ of Fmoc-O-allyl-tyrosine (left) to Fmoc-O-propyl-tyrosine (right), showing a strong PNL signal at 4.53 ppm. Experimental conditions: concentration of substrate, 17 mM; catalyst, [1,4-bis(diphenylphosphino)-butane](1,5-cyclooctadiene) rhodium(I) tetrafluoroborate 4 mM; solvent, acetone-d_6; bubbling pressure, 5 bar; bubbling duration, 5 s; p-H$_2$ enrichment, 50%; delay after bubbling, 1.2 s; detection pulse, $\pi/4$; magnetic field, 11.7 T (500 MHz for protons).

the NMR pulse program control gas bubbling on the subsecond time scale. The whole system is closed; the bubbling pressure in a standard NMR screw-cap sample tube can be varied form atmospheric pressure up to 8 bar.

^{1}H NMR spectra were measured on a 500 and 700 MHz Bruker NMR spectrometer at magnetic fields of 11.7 and 16.4 T. All experiments were performed under standard PASADENA (bubbling of p-H$_2$ inside the magnet) conditions. Three different types of NMR experiments were performed:

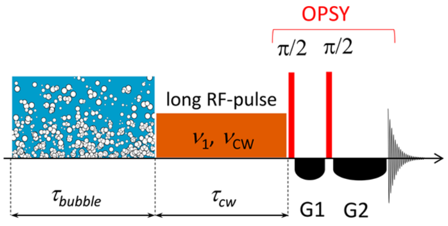
(1) Single pulse detection of PNL: after the PASADENA, the signal is detected by a single RF-pulse of variable detection flip angle, irradiated after a delay of 1s.

(2) OPSY[24] detection of PNL: after the PASADENA, the signal is detected by the OPSY sequence,[24] which selectively reveals the two-spin order of the spin system.

(3) PArtially NEgative Line (PANEL) experiment: Finally, we propose the PANEL experiment, which performs the OPSY detection of the PNL after the irradiation of a long narrow-band RF pulse, which is frequency swept, similar to classic cw-NMR.

The protocol for the PANEL experiment is depicted in Scheme 3. Nonthermal spin order formation is initiated by dissolving the H$_2$ gas enriched in its *para*-component. This is done by bubbling p-H$_2$ at elevated pressure through the catalyst solution for a time τ_{bubble}. The bubbling period is followed by the irradiation of an additional long narrow-band RF-pulse of duration τ_{CW}; having the frequency ν_{CW} and the amplitude ν_1. The detection of the NMR signal is performed with the OPSY sequence developed by Duckett and co-workers.[24] The latter has the advantage that it selectively detects the two-spin order by filtering out the thermal single-spin components. Note that the PANEL experiment can be easily implemented using any modern solution-NMR spectrometer, which is equipped with a source for bubbling parahydrogen through the sample.

Scheme 3. PANEL (PArtially NEgative Line) Experiment for the Indirect Detection of the Hydrogen Catalyst Complex[a]



[a]The sample is irradiated with a long narrow-band RF-pulse of the amplitude ν_1 for a time τ_{CW} between bubbling and detection. The irradiation frequency (ν_{CW}) of the RF-pulse is swept through the spectrum, similar to a cw-NMR experiment. The signal is detected by an OPSY block. Typical values of the experimental parameters: τ_{bubble} = 5 s, τ_{CW} = 2 s; parameters of the field gradients: G1:40 G/cm 1 ms, G2:40 G/cm 2 ms.

III. RESULTS AND DISCUSSION

Experimental Detection of the PNL and Related Phenomena. The typical appearance of the PNL is shown in Figures 1 and 2. The PNL appears during and after bubbling a catalyst solution with p-H$_2$. In general, since H$_2$ contains a pair of equivalent nuclear spins its spectrum is expected to have a single line. Here it is important to mention that the H$_2$ line can only show up when the NMR silent p-H$_2$ is converted to o-H$_2$. In turn, the signal of o-H$_2$ is expected to be a single absorptive line; however, this expectation is clearly not met, as the NMR line is a PNL, see Figure 2. Interestingly, the "out-of-phase" component (imaginary part of the spectrum) of the PNL also exhibits an unusual shape. Whereas thermal signals produce a Lorentzian line shape in the in-phase spectrum and dispersive line shape in the out-of-phase spectra, the PNL is apparently dispersive in the in-phase channel and has an

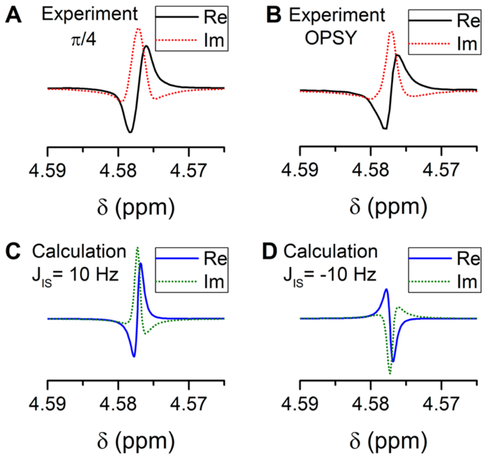


Figure 2. Real (Re, in phase) and imaginary (Im, out of phase) parts of ^{1}H NMR spectrum detected by a $\pi/4$ pulse (A) and an OPSY sequence (B) after bubbling p-H_2 through a catalyst solution (2 mM, acetone-d_6, bubbling pressure 3 bar, $\tau_{\text{bubble}} = 5$ s); simulation of ^{1}H NMR spectrum (C, D) with the model described in detail in the SI. Blue full lines show calculation at positive and negative values of $J_{IS} = 10$ Hz and $J_{IS} = -10$ Hz; $k_f = 1\ s^{-1}$, $k_d = 5000\ s^{-1}$. NMR frequency is 500 MHz.

unusual shape in the out-of-phase channel. The out-of-phase line of H_2 is apparently partly absorptive but has negative "wings", see Figure 2.

Further experiments showed that the PNL is only observed in the presence of catalyst and p-H_2 enriched, that is, nonthermally polarized H_2 in solution. The PNL is detectable for a number of various solvents of different chemical nature (see Figure S14 and Table S1 in SI). Since the catalyst enhances the para- to orthohydrogen conversion, the PNL disappears after establishing the thermal equilibrium between p-H_2 and o-H_2 with the characteristic time of about 5 s. Moreover, the PNL signal is apparently a single line having a dispersive shape without any substructure, for example by scalar interactions or small chemical shift differences (in our experiments the width of NMR line at half-height is about 0.5 Hz; hence, even a small splitting of an NMR line down to 0.1 Hz would be visible at the high S/N ratio of the signal).

As shown by the Ernst group,[25] partially negative line shapes of reaction products occur naturally when the latter are formed in a one-sided reaction. As the signal of o-H_2 is detected in our experiments, the most obvious process which could generate a dispersive like line shape is a one-sided release of o-H_2 from the catalyst. In this case, negative line components should be visible also when the experiments are performed with thermally polarized hydrogen (in the following abbreviated as n-H_2) instead of p-H_2. However, the n-H_2 does not show any unusual behavior: its NMR signal is just a normal absorptive line without any visible dispersive components. Thus, one can exclude such a mechanism.

To find out whether the PNL is caused by one-spin or two-spin order, we performed a nutation experiment (Figure 3), which measures the PNL intensity as a function of the flip angle φ. It reveals that the PNL intensity is proportional to sin 2φ. Such a pulse angle dependence is characteristic for two-spin order in a weakly coupled spin-pair. In contrast, for n-H_2, a sin φ dependence is expected. Since this result was indeed surprising, as such a nutation dependence is by no means expected from a pair of strongly coupled magnetically equivalent protons of H_2, we decided to perform an OPSY experiment, which filters all contributions from single-spin order from the spectrum. The OPSY results (Figure 2) corroborate the nutation experiment that the dispersive components of the line shape of the PNL are indeed caused by two-spin order. Hence, the flip angle dependence and the line shape of the PNL show that it is not related to the net-polarized signals of o-H_2 previously reported by Aime et al.[20]

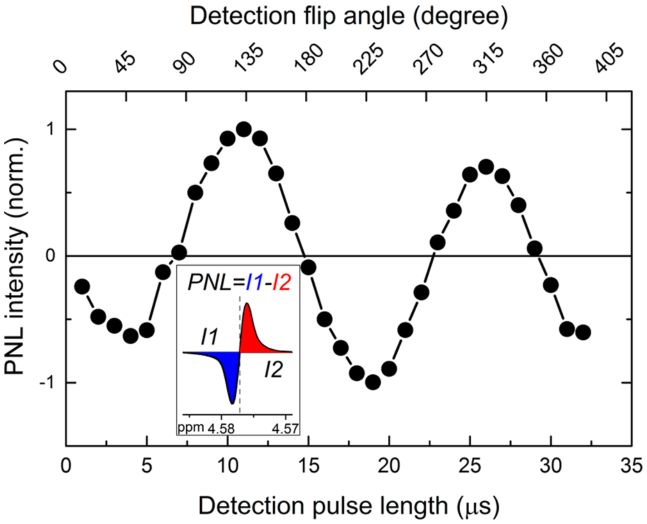
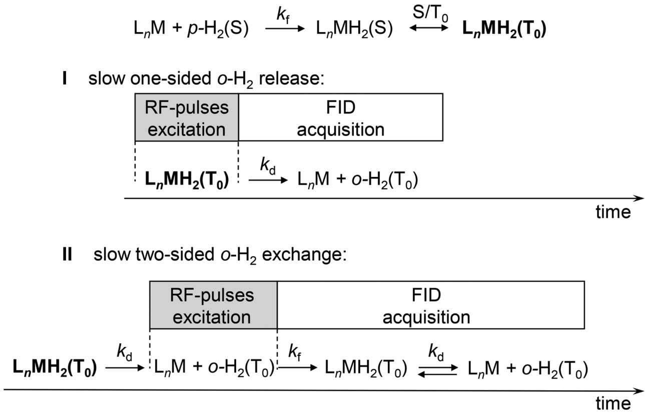


Figure 3. Typical flip angle dependence, that is, nutation pattern, of the PNL intensity. RF field strength 35 kHz (10 W), NMR frequency is 500 MHz. PNL intensity was measured as a difference between left (I1) and right (I2) integral (see insert). The dashed line, positioned at the maximum of the H_2 signal in the thermal spectrum, indicates the border between the two integrals (Experimental conditions: catalyst solution 3.3 mM in acetone-d_6, bubbling pressure p-H_2 3 bar).

Theoretical Analysis of the PNL. As shown above, the PNL is caused by two-spin order and only visible for dissolved nonthermally polarized H_2 molecules in the presence of dissolved catalyst. Based on this knowledge we can develop a complete model of the process, which does not need any special cross-relaxation mechanisms. Since neither the singlet state S of free-H_2 nor the singlet state of bound L_nMH_2 can contribute to an NMR signal, it follows that the necessary two-spin order cannot be created by the detection pulses or after the detection pulses. The reason is that, in the pure A_2 spin system of molecular hydrogen, the RF excitation of the T_0 state gives rise to two NMR signals with the same amplitude and opposite phase, which compensate each other at all times and do not give rise to any observable NMR signal. Thus, similar to the PHIP experiment, it is necessary to break the magnetic equivalence of the two hydrogen nuclei of the H_2 molecule, which is only feasible by forming a transient complex to the catalyst during the time of the NMR experiment. In this transient complex, the magnetic equivalence of the two hydrogen nuclei must be broken, for example, by different chemical shifts or by scalar couplings to nearby nuclei at the catalyst.

Thus, we have to postulate a two-step process according to Scheme 4. In the first step prior to the NMR pulses, a selective population enrichment of the T_0-triplet states of free and bound hydrogen is created by magnetic *para*/*ortho*-conversion. Such a magnetic mechanism, which mainly converts the singlet state S to the triplet T_0 state, resulting in a population enhancement p_0 of this triplet state, was first discussed by Bargon et al.[15,16] In

Scheme 4. Possible Reaction Channels for the Interaction of Nonthermally Polarized *o*-H_2 with the Catalyst (L_nM)



this mechanism, the free dissolved hydrogen molecules bind to the catalyst with the formation rate k_f and form either a dihydrogen or a dihydride complex. In this state, the singlet population is partially transferred to the T_0 state. Then, with the dissociation rate k_d, the hydrogen is released again from the complex, resulting in a growth of the population of the T_0 state of the free H_2.

Formation of *o*-H_2 in the T_0 state is not nearly the full story because such a triplet spin system does give an NMR spectrum of zero intensity. Therefore, a second step is required, in which the PNL is created from the population of the T_0 state. Here it is important to note that the PNL can be created only by those hydrogen molecules that interact with a catalyst molecule during the time of Free Induction Decay (FID) acquisition. The reason is that in those free H_2 molecules, which do not interact during the NMR experiment with the catalyst, the two hydrogen nuclei are magnetically equivalent and the two transitions $T_0 \rightarrow T_+$ and $T_0 \rightarrow T_-$ cancel exactly, since one is in absorption and the other in emission.

Thus, the entire NMR signal is visible in the PNL stems from those molecules, which were interacting in the course of the NMR detection with the catalyst, which renders the two hydrogen nuclei magnetically inequivalent. Here two possible pathways have to be distinguished.

- In the first pathway, the PNL signal is created by those hydrogen nuclei in the T_0 state that are bound to the catalyst at the time of the detection pulse. We note that this is the mechanism suggested by the Koptyug group.[23]
- In the second pathway, the PNL signal is created by those hydrogen molecules in the T_0 state that are free during the time of the NMR RF pulses, but interact during the time of the FID acquisition with the catalyst.

By the NMR pulse, visible NMR signals are created, which are then transferred by the dissociation rate k_d to the free hydrogen. Since the FID of the free hydrogen is initially zero and grows with a rate constant of k_d, both pathways will result in an apparently dispersive line shape of the PNL, as was discussed by the Ernst group for one-sided reactions.[25,26]

The following question arises: what are the relative efficacies of these pathways. In the case of the first mechanism, the NMR signal is proportional to the number $n(L_nMH_2)$ of hydrogen molecules bound to the catalyst (N_{H_2}: number of hydrogen molecules; K_{eq}: equilibrium constant of the reaction).

$$n(L_nMH_2) = N_{H_2}K_{eq} = N_{H_2}\frac{k_f}{k_d} \tag{1}$$

In the case of the second pathway, the total number of molecules $n(H_2 \rightarrow L_nMH_2)$ that are bound to the catalyst during the acquisition time of the FID (which can be approximated as two times the transversal relaxation time of the dissolved hydrogen molecules $\approx 2T_2$) is for an equilibrium strongly shifted to the free hydrogen ($K_{eq} \ll 1$)

$$n(H_2 \rightarrow L_nMH_2) \approx 2k_fN_{H_2}T_2 \tag{2}$$

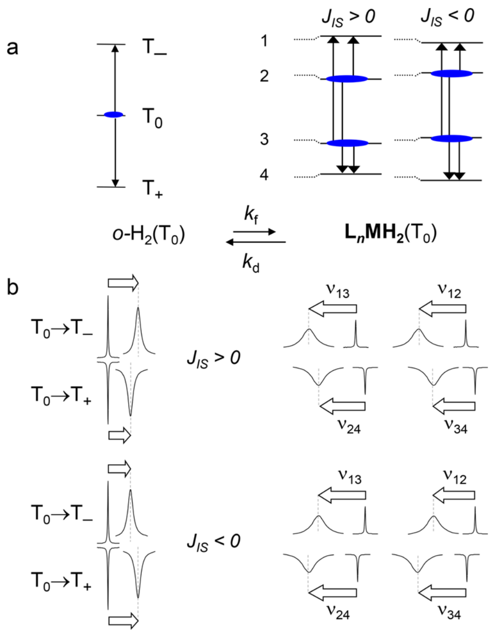
Their ratio is

$$\frac{n(L_nMH_2)}{n(H_2 \rightarrow L_nMH_2)} = \frac{1}{2k_dT_2} \tag{3}$$

Even for moderate decay rates on the order of 10^2 s^{-1} to 10^3 s^{-1} and typical transversal relaxation times on the order of 1 to 10 s, this ratio is between 0.005% and 0.5%. Thus, the first pathway can be excluded as a major contributor to the PNL and is not further discussed. Experiments with selective NMR excitation of nonthermally polarized *o*-H_2 clearly confirm that the PNL originates from the free form of H_2, see Supporting Information (SI page S11).

Simple Physical Picture of the PNL. In the following we summarize the quantitative analysis of the PNL phenomenon given in the SI, which is fairly technical, in a simple physical picture, which is illustrated schematically in Scheme 5. Scheme 5a depicts the energy level diagrams of the two exchanging two-spin systems in an external magnetic field, including the three triplet levels of free *o*-H_2 and the four levels of the AX spin

Scheme 5. Origin of the PNL Signal Based on the Nonthermally Polarized T_0-State of the *o*-H_2[a]



[a] (a) Energy level diagrams of exchanging *o*-H_2 and the AX spin system of two hydrogen spins in the complex L_nMH_2. (b) Associated spectral changes (schematically). Slow exchange line shifts are indicated by the length of horizontal arrows. They are artificially increased as compared to the associated exchange broadenings for the sake of clarity. For further explanation see text.

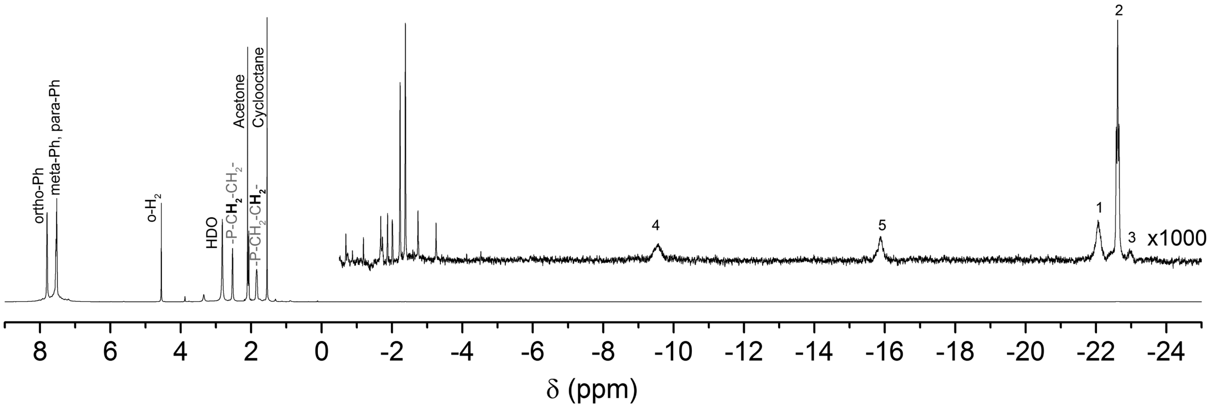


Figure 4. Thermal ^{1}H NMR spectrum accumulated after bubbling the catalyst solution by n-H_2 (catalyst concentration 3.3 mM in acetone-d_6). Spectrum was recorded with 512 scans measured at 700 MHz during 30 min. The inset displays the dihydrogen and dihydride region, magnified by a factor of 1000. Assignment of protons is shown on the plot. The lines at about −2 ppm are possible impurities. Signals 1, 2, and 3 are attributed to stable side products (possible rhodium hydrides).

system of the two hydrogen nuclei bound to the metal. We consider the case where p-H_2 has been used and where the reaction takes place in an external magnetic field under so-called "PASADENA"[11] conditions. We assume that S/T_0 mixing has already occurred and that o-H_2 has been released in the T_0 spin state according to Schemes 1 and 4. Thus, T_+ and T_- are not populated as indicated. On the other hand, for the intermediate L_nMH_2, only states **2** and **3** are populated in the case of the AX-spin system. In the absence of exchange, that would lead to positive lines for the transitions **1−2** and **1−3**, and negative lines for transitions **2−4** and **3−4**. When $J_{IS} > 0$, the transitions **1−2** and **1−3** give rise to lines at lower and the transitions **2−4** and **3−4** at higher field as illustrated on the right side of Scheme 5b. On the other hand, the transitions $T_0 \rightarrow T_+$ and $T_0 \rightarrow T_-$ of o-H_2 cancel each other.

If we switch on the exchange between the two species, the positive transitions **1−2**, **1−3**, and $T_0 \rightarrow T_+$ exchange with each other as well as the negative transitions **2−4**, **3−4**, and $T_0 \rightarrow T_-$. Now the well-established line broadening is operative, characterized for L_nMH_2 by k_d/π and for the two o-H_2 transitions by k_f/π.[27] It is rarely discussed, that even in the slow exchange limit there are small signal shifts of the exchanging lines toward each other, as depicted schematically in Scheme 5b by open arrows. These shifts are exaggerated for the sake of clarity; they are larger for the minor species and smaller for the major species. The shift direction is such that the frequency difference of the exchanging lines decreases. Moreover, the frequency difference is larger when the intrinsic chemical shift difference between the exchanging lines is smaller. When $J_{IS} > 0$, the positive signals are located closer to each other as compared to the negative lines. Therefore, the high-field shift of the positive $T_0 \rightarrow T_+$ is larger than of the negative $T_0 \rightarrow T_-$ transition, leading to a PNL with the high-field part positive and the low-field part negative. The contrary is true for the case $J_{IS} < 0$.

We note that the frequency differences are very small, much smaller than the frequency differences between the observed maxima and minima of the PNL. These differences depend on various parameters, such as the associated line intensities and the line widths. Therefore, it is easy to determine the sign of J_{IS} from the sign of the PNL, but not the absolute value of J_{IS} (these findings follow directly from eqs 23−24 in the SI, page S5).

This simple picture is corroborated by the detailed quantitative analysis of the second pathway, which is described in the Supporting Information (SI, page S6). The calculations show that the exchange between free and bound hydrogen creates two lines for the exchanging hydrogen molecules, which are high-field shifted relative to the position of the free hydrogen. Their amplitudes depend on the populations of the levels of the o-H_2 as

$$A_{1,2} = \frac{p_- - p_+}{2}\sin(\varphi) \pm \frac{2p_0 - (p_+ + p_-)}{4}\sin(2\varphi) \tag{4}$$

where p_+, p_0, and p_- are the initial populations of the three triplet states ($|T_+\rangle$, $|T_0\rangle$, $|T_-\rangle$).

This shows that they consist of a superposition of single-spin transitions with the same phase proportional to the population difference ($p_- - p_+$) and two-spin transition with opposite phases proportional to the population imbalance $\Delta p_0 = 2p_0 - (p_+ + p_-)$. In the case of thermal population $\Delta p_0 = 0$ of the three triplet sublevels, this value is zero and no PNL is created. From eq 4 it is evident, that the strongest PNL effect is obtained for a 45°-pulse and selective T_0 population

$$A_{1,2}\left(\frac{\pi}{4}\right) = \pm\frac{p_0}{2} \tag{5}$$

Thus, the PNL is a differential effect, where two Lorentzians of opposite phase are superimposed. Quantitative calculations (see SI, page S5) show that the frequency difference between the two transitions is on the order of mHz. For this reason, the major parts of the lines cancel and only a fraction of typically 0.1−0.5% (the actual amount will depend on experimental conditions, NMR line width, magnetic field strength, hydrogen pressure,) survives. However, owing to the high spin order due to the *para*-enrichment, which is on the order of unity, the NMR signal of the PNL is still on the order of the size of thermally polarized signals of the educts and solvent and thus easily detectable in NMR experiments. In the SI, an analytical solution for a weakly coupled AX-spin system in the complex is derived.

Application of the PNL Phenomenon for the Detection of Reaction Intermediates. Having realized this model, we first tried to find the supposed dihydride intermediate in the thermally polarized ^{1}H NMR spectrum. Figure 4 displays the ^{1}H NMR spectrum of a solution containing catalyst and dissolved H_2 measured at 700 MHz. The spectrum shows the signals of the dissolved hydrogen (4.5 ppm) and the catalyst at 7.8, 7.5, 2.5, and 1.9 ppm, plus some small signals at about −2 ppm, which are from impurities, but no sign of the proposed complex even after 30 min of accumulation. Longer accumulation was not possible due to degradation of the catalyst, visible by a change of the color of the solution and formation of stable side products. (Note that, after this time, the PNL signal is no longer observable.) During this process, we detected a decay of the H_2 signal because of chemical consumption. On the 700 MHz machine, we have detected five signals in the negative ppm. Immediately after introducing H_2 into solution, only signal **1** is visible, after few minutes signal **1** moves to the position **2**, and later, after a few hours, only signal **3** is visible, and the solution changed to dark color. In parallel, we observe a reduction of the H_2 signal due to the formation of stable side product **3**. Signals **1**, **2**, and **3** stay visible even after removing H_2 from solution by purging N_2, showing that they are all stable products and not transient catalytic intermediates. Two signals **4** and **5** are coming from a reaction product formed after bubbling by H_2. We were not able to characterize this compound. Further explanation of signals and degradation process of the catalyst is given in the Supporting Information (pages S12−S16).

This result shows that if this complex exists, its concentration is below the detection threshold of standard NMR. Therefore, we tried to detect the complex in a PHIP experiment, by measuring the spectra after bubbling with p-H_2, looking for the characteristic PHIP pattern expected for that kind of complexes in the region from −10 to −20 ppm. Again, no signals were observed (see Figure S12 in the SI) in this region.

Since the attempts to directly detect the presumed hidden complex were unsuccessful, we decided to try an indirect detection method of the proton-NMR signal. Inspired by the indirect detection experiments of Ahlquist et al.,[28] who monitored intermediates via ^{13}C NMR signals of the reaction products or the CEST (Chemical Exchange Saturation Transfer) technique,[29] which can be used to observe, for example, "invisible" excited protein states in the slow exchange regime,[30] we developed the PANEL experiment described above. It performs a selective narrow-band excitation of the ^1H-spins with stepwise variation of the excitation frequency (ν_{CW}), similar to a conventional cw-NMR spectrum. By OPSY the PNL intensity is monitored as a function of the excitation frequency (ν_{CW}). The pulse-sequence for that PANEL (PArtially NEgative Line) experiment is shown in Scheme 3.

Figure 5 compares the results of the ν_{CW} dependence of the PANEL experiment (B) for different amplitudes ν_1 of the narrow-band pulse excitation with simulations (C) of the PANEL experiment and a simulated conventional NMR spectrum (A). There are four minima in the resulting PNL intensity. Two of them are close to the position of the free hydrogen signal at 4.5 ppm. The other two minima are close to −15 ppm, that is, in the typical hydride region. Both the width and the depth of the minima depend strongly on the RF-amplitude ν_1. To prove that the signals in the region at −15 ppm are indeed caused by the interaction with parahydrogen with the catalyst, we performed a control experiment with n-H_2. While the resulting spectrum (Figure 5D) shows the signal at the position of free hydrogen at 4.5 ppm, the minima in the hydride region have disappeared. This proves that the application of p-H_2 is mandatory. From the simulations of these experimental curves (Figure 5C) it is possible to reveal the spectral signature of this complex (see quantitative explanation below).

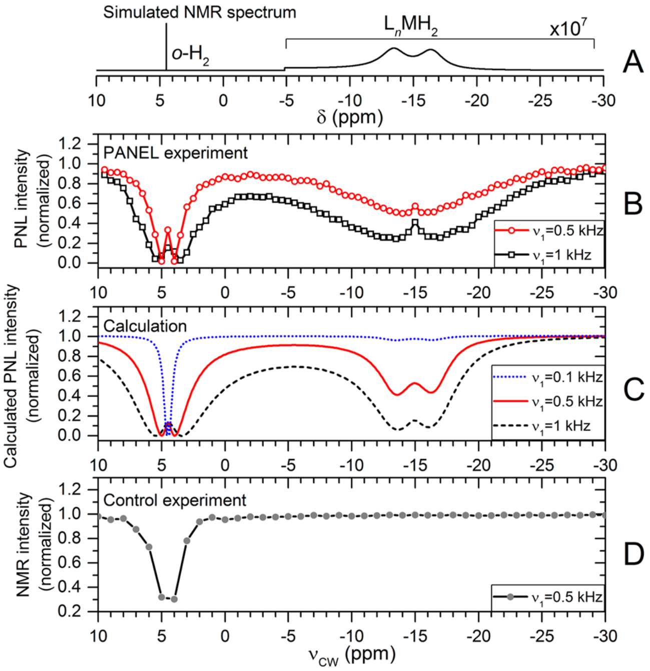


Figure 5. Results of the PANEL experiment. The normalized intensities of the PNL are shown as a function of the selective irradiation frequency of the narrow-band RF pulse ν_{CW}. (B) Experimental and (C) simulated PANEL data for the reaction with p-H_2; (D) control experiment with n-H_2 (details are given in SI, page S9). Additionally, the calculated thermal NMR spectrum of the postulated transient species L_nMH_2 and o-H_2 is shown in (A). Parameters of the calculation: $k_f = 1\ s^{-1}$, $k_d = 5000\ s^{-1}$, $J_{IS} > 0$ Hz (here $J_{IS} = 10$ Hz); $\delta_I = -16.5$ ppm, $\delta_S = -13.5$ ppm, T_1 and T_2 for H_2 and for the hydrogens of the L_nMH_2 complex are set to 1 s. NMR spectrometer frequency is 700 MHz.

For the quantitative analysis of this experiment, we made the following assumptions:

1. Only a single hydrogen-catalyst complex is relevant for both the PNL and the PANEL experiment.
2. The initial concentration of parahydrogen in the S state is much higher than the concentration of orthohydrogen in the T_0 state. Thus, mainly molecules that are in the S state will form a complex with the metal.
3. The length of the narrow-band RF-pulse is so long that the system goes into a quasi-stationary state (cw-condition). To verify this assumption, we did additional calculations with finite pulse lengths (not shown). These calculations showed that the quasi-stationary state is a good approximation for irradiation times up to about 3 s, where no saturation effects play a role.
4. The *para*/*ortho* ratio changes so slow that is not disturbed by the experiment and we can calculate a stationary limit.

Under these assumptions, a simple set of linear equations for the equilibrium density operator results, which can be solved algebraically or numerically (see SI, pages S2−S10). For the quantitative determination of the chemical shift values of the bound complex, we performed a numerical simulation of the frequency dependences, using the parameters shown in Table 1. The resulting curves (Figure 5C) are in very good agreement with the experimental results. Thus, with this experiment, we

Table 1. NMR and Kinetic Parameters of the Intermediate Organometallic Complex Responsible for the *para*/*ortho* Conversion[a]

parameter	value	range
complex formation rate, k_f (s^{-1})	1	0.5−2
complex dissociation rate, k_d (s^{-1})	5000	1000−10000
chemical shift of 1st hydrogen, δ_I (ppm)	−16.5	±0.1
chemical shift of 2nd hydrogen, δ_S (ppm)	−13.5	±0.1
mean value of chemical shifts, $\frac{\delta_I+\delta_S}{2}$ (ppm)	−15	±0.1
difference of chemical shifts, $|\delta_I - \delta_S|$ (ppm)	3	0.5−4
spin−spin coupling, J_{IS} (Hz)	+10	+0.5 − +500

[a] Here the most probable value and the acceptable range are summarized. Parameters were estimated from simulations of the PNL and the PANEL experiment (see Figures 2 and 5).

can reliably estimate the kinetic and NMR parameters of the elusive complex.

At this point it is important to note that in the dihydride intermediate the near degeneracy of the two transitions discussed above (see eq 5 and text below) is lifted. Thus, it is possible to individually saturate one of the transitions, which prevents the partial signal cancellation observed in the normal spectrum and results in a strong sensitivity enhancement.

Information about Intermediates from PANEL. The great advantage of the PANEL experiment is that it reveals information about the reaction intermediates, where singlet−triplet conversion occurs, which are otherwise very difficult or impossible to elucidate. In our case, it reveals both the chemical shift of the two protons (i.e., −16.5 and −13.5 ppm) in the intermediate complex (L_nMH_2) and the sign of their scalar coupling constant J_{IS} (positive).

The chemical shift values are typical for dihydride species[4,9,14,31] and exclude a bound dihydrogen species, as the latter would exhibit only a single averaged chemical shift. They are consistent with a slow hydrogen exchange with free dissolved dihydrogen. The positive sign of the coupling constants J_{IS} indicates that the HH distance is smaller than 2 Å.[32]

IV. SUMMARY AND CONCLUSIONS

In summary, ^{1}H NMR spectra of *p*-H_2 enriched hydrogen exhibit in the presence of an organometallic hydrogenation catalyst an unusual, partial negative line shape (PNL) of the NMR line of dissolved hydrogen.

By nutation and OPSY experiments it is shown that the dispersive component of the line shape is caused by two-spin order in the triplet state. The detailed experimental and theoretical analysis reveals that the PNL originates from the initial nonthermal spin order of parahydrogen, which is converted in an exchange process with the catalyst to a selective enrichment of the population of the T_0 state, that is, to nonthermally polarized orthohydrogen. At first glance, such a spin system does not give an NMR signal: it has two NMR transitions giving rise to two NMR lines, which exactly cancel each other. The theoretical analysis, employing Liouville-space formalism, shows that the PNL is a differential effect, caused by the superposition of two lines with slightly different chemical shift and opposing phase, created by the exchange of the free dissolved p-H_2 and a transient catalyst hydrogen complex. With the developed model, we are able to simulate the NMR line shape and the nutation angle dependence of the PNL and determine the sign of the scalar coupling in the intermediate as positive.

The quantitatively analyzed relatively complex spin dynamics are explained additionally in simple pictorial terms by correlating energy level diagrams of free H_2 and catalyst bound H_2. The simplified diagrams clearly show that the PNL signal comprises two lines with a small difference of resonance frequency and explain the resulting phase of these signals. Thus, we have established the mechanism of PNL formation in a theoretical approach as well as in a more feasible pictorial diagram.

Since no transient catalyst hydrogen complex was found in the NMR spectra of the system, we developed the PANEL experiment for the indirect detection of the elusive transient catalyst hydrogen complex, which revealed signals in the hydride region. From the simulation of PANEL data, the proton chemical shift values (−13.5 and −16.5 ppm) of the transient hydrogen-catalyst complex are obtained.

From the values of the proton chemical shifts and the positive sign of the scalar coupling, we can conclude that in our case a dihydride complex with Rh is the transient state responsible for the PNL and the para- to orthohydrogen conversion.

Quantitative estimations show that enhancement factors of 500−700 are obtained, depending on the strength of the external magnetic field. Thus, our results open up new applications for the investigation of catalytic complexes at very low concentrations.

Finally, we would like to mention that the PNL not only allows for an ultrasensitive detection of intermediates, but can also serve as a monitor for the spin-dynamics in intermediate complexes. The latter could be of interest for estimating the performance of organometallic complexes used for generating PHIP (or SABRE).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.7b01056.

> Quantitative analysis of the PNL employing Liouville space formalism, theoretical description, and calculation of the amplitudes of the PNL, analysis of the PANEL experiment for the detection of the transient intermediates employing Liouville space formalism, summary of the selective pulse excitation experiment, NMR spectra showing the activation and degradation process of the catalyst, kinetic data analysis of the signal intensity of the PNL signal, failure to direct detection of the intermediate complex with parahydrogen, comparative PANEL experiments, comparing *n*-H_2 and *p*-H_2, solvent dependence of the PNL signal, including ^{1}H NMR spectra, and main code of the applied pulse program for the PANEL experiment (PDF).

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Notes

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