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Similarity of SABRE field dependence in chemically different substrates

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ABSTRACT

The Non-Hydrogenative *Para*hydrogen-Induced Polarization (NH-PHIP) technique, which is referred to as Signal Amplification by Reversible Exchange (SABRE), has been reported to be applicable to various substrates and catalysts. For more detailed studies, pyridine was mainly examined in the past. Here, we examined several pyrazole derivatives towards their amenability to this method using Crabtree's Catalyst, which is the polarization transfer catalyst that is best documented. Additionally, the dependence of the signal enhancement on the field strength, at which the polarization step takes place, was examined for pyridine and four different pyrazoles. To achieve this, the polarization step was performed at numerous previously determined magnetic fields in the stray field of the NMR spectrometer. The substrate dependence of the field dependence proved to be relatively small for the different pyrazoles and a strong correlation to the field dependence for pyridine was observed. Reducing the number of spins in the catalyst by deuteration leads to increased enhancement. This indicates that more work has to be invested in order to be able to reproduce the SABRE field dependence by simulations.

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1. Introduction

Ever since its discovery, NMR spectroscopy has proven to be an extremely versatile technique to examine the structure and dynamics of a large variety of different chemical compounds. The greatest drawback of the technique, however, is its low sensitivity, mainly caused by the relatively small population difference in the Boltzmann distribution of nuclear spin states found at thermal equilibrium. Considerable technical efforts have hence been undertaken to increase the sensitivity of NMR by implementing, for example, more powerful spectrometers exhibiting stronger magnetic fields or, alternatively, cryogenic probe technology. In addition, a number of different methodological approaches have been introduced to create non-thermal spin state distributions (so-called hyperpolarization), including Dynamic Nuclear Polarization (DNP) [1], or spin exchange optical pumping of noble gases [2]. Among the few methods employed to increase NMR sensitivity in a chemical way, the so-called *Para*hydrogen Induced Polarization (PHIP) has attracted wide attention. Here, hydrogenation reactions are performed using H_2 gas enriched in *para*hydrogen, the nuclear spin isomer of the dihydrogen molecule representing a nuclear singlet state. Upon addition of the *para*hydrogen molecule to the substrate, the non-Boltzmann distribution of nuclear spin states is introduced into the hydrogenation product and can subsequently be detected as enhanced absorptive and/or emissive NMR signals, usually being several orders of magnitude more intense as compared to the thermally polarized case [3,4].

For many years, it was believed that the *para*hydrogen polarization method required an unsaturated precursor molecule to be hydrogenated. Recently, however, Duckett and co-workers reported on a significant extension of the PHIP technique by allowing *para*hydrogen-induced polarization to a substrate molecule without the necessity of hydrogenation [5–8]. This achievement was accomplished by exploiting the temporary association of the *p*-H_2 with the substrate molecule through a metallo-organic complex at low magnetic fields. The acronym Signal Amplification by Reversible Exchange (SABRE) was chosen to describe this phenomenon. Several small molecules have been polarized successfully with this technique [9,10].

The formation of the metallo-organic complex, an iridium complex derived from Crabtree's Catalyst $[Ir(cod)(PCy_3)(py)]^+$, is shown in Scheme 1. The pyridine ligands *trans* to the hydrides are labile and exchange with free pyridine molecules in solution, acting as substrates in the polarization transfer process [7]. During the lifetime of the complex, the polarization transfer takes place via

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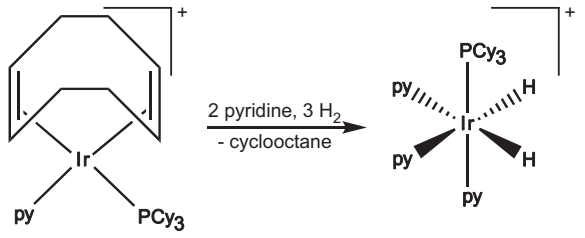

Scheme 1. Reaction scheme of the formation of the active catalyst. The pyridine ligands in the product complex are replaced when different substrates are examined. (py = pyridine, PCy_3 = tricyclohexyl phosphine).

isotropic mixing and, after ligand exchange, the former pyridine ligands remain in solution exhibiting strongly enhanced signals in the respective NMR spectra [7].

The recent theoretical description of the SABRE technique came to the conclusion that the efficiency of the polarization transfer depends on the size of the scalar couplings and the precession frequency differences in the active iridium complex [11]. Moreover, as the precession frequencies depend linearly on the magnetic field strength, a strong dependence of the enhancement on the magnetic field present during the polarization step was discovered. Additionally, the exchange rates of p-H_2 and the substrate molecules are important for the transfer efficiency as was concluded from experiments performed at different temperatures [8].

Since primarily pyridine has been investigated in more depth especially for field dependent SABRE, we here explore the amenability of pyrazole molecules to SABRE enhancement and its field dependence. We specifically chose pyrazoles, since they are a potential biomarker for Parkinson's and other diseases due to their properties of binding protein aggregates. One of them, anle138b, was found to be specifically active [12,13]. As this molecule is derived from pyrazole and has no accessible unsaturated precursor, the examination of pyrazole, and some of its methyl- and phenyl-substituted derivatives with the newly reported SABRE technique is of particular interest to us. Accordingly, we set out to investigate a series of pyrazole compounds performing the SABRE polarization transfer at varying magnetic fields.

2. Results

The structures and numbering schemes for the substrates that were examined in this study are displayed in Fig. 1. Due to proton–deuterium exchange upon addition of the deuterated solvent, the NH proton was undetectable for all examined pyrazole derivatives. Furthermore, enhanced pyridine resonances stemming from replaced pyridine ligands of the catalyst appeared in the spectra of the pyrazole derivatives and congested the aromatic region. To obtain more intense substrate resonances, the catalyst to substrate ratio was chosen to be 1:10 even though the highest enhancements were reported for a ratio of 1:5 [7]. Hence, the enhancements given here are not the highest attainable in the unmodified SABRE procedure.

Pyridine was employed as a substrate in all previous publications on SABRE [5–8,14] and was chosen as a reference substance. The polarization field dependence curves of the proton enhancement are shown in Fig. 2 (black data points). As can be seen, the signal enhancement is on the order of several hundreds, hence reproducing the previously reported values [5]. Largest enhancements for the protons in *ortho* and *para* position were found at 13 mT, while the *meta* protons displayed highest intensities at a polarization field of 3–4 mT. Both field strengths correspond, however, to maxima in the polarization field dependence curves of all

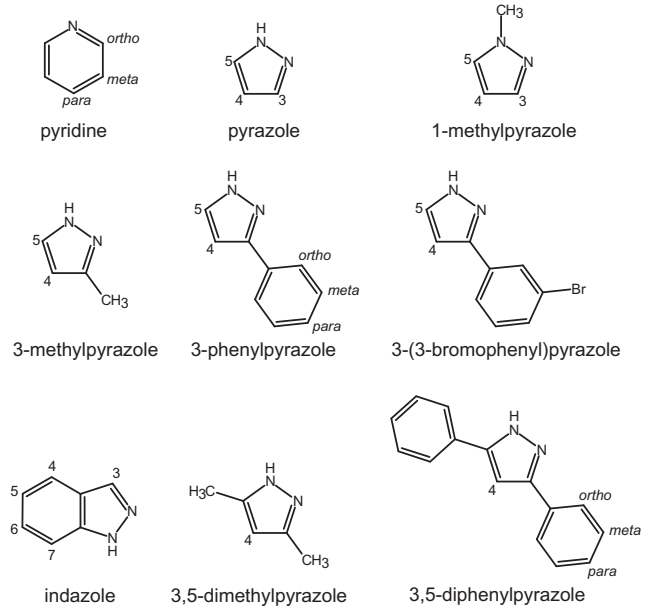

Fig. 1. Molecular structures and numbering schemes of the substrates examined in this work.

pyridine protons, yet the absolute values differ significantly. A change in sign is observable between the two extrema.

Since three molecules of pyridine are bound to the active complex and will most probably have to share the coherence transferred from the *para*hydrogen, we investigated the impact of replacing two of them by fully deuterated pyridine and having only one protonated pyridine bound. This can be achieved by a 1:10 ratio of protonated:fully deuterated pyridine. Due to the lower number of spins receiving the *para*hydrogen polarization, a significant increase in signal enhancement up to 1400 fold, a more than twofold increase compared to the standard SABRE experiment at 13 mT, can be observed for the protons in *ortho* position (Fig. 2: red data points), while the *meta* protons experience only a slightly deviating enhancement compared to the fully protonated pyridine. The values for the *para* protons remain almost unchanged.

Enhancement of pyrazole signals was two orders of magnitude, yet significantly smaller than for pyridine, as can be seen in Fig. 3. Maxima in the polarization field dependence curves were again obtained for field strengths of 3–4 and of 12–14 mT, respectively, thereby showing a similar behavior as in the aforementioned pyridine case. In the corresponding NMR spectra, the resonances show a phase change of 180° when comparing the two maxima (Fig. 4), as was mentioned above for pyridine and as is observable for all spins of all substrates examined in this work. Interestingly, the OH resonance of methanol also shows an enhancement and yields negative intensities in the range from 10 to 16 mT (see Fig. 4). The complete field dependence of its enhancement is given in Fig. 5.

Resonance overlap of the pyridine signals with those of protons 3 and 5 of 1-methylpyrazole prevented their adequate examination. However, the polarization field dependence of the proton in position 4 and the methyl protons was determined and is displayed in Fig. 6. The signal enhancement maxima are in the region of 3–4 mT and 12–14 mT, respectively, for both of the examined resonances. Notably, the observed enhancement for the aromatic proton is lower than that of its counterpart in the unsubstituted pyrazole. Furthermore, the enhancement for the methyl protons is even lower, gaining intensity only by one order of magnitude.

The field dependences for proton 4 and the CH_3 group of 3-methylpyrazole were also determined and the magnitude of the

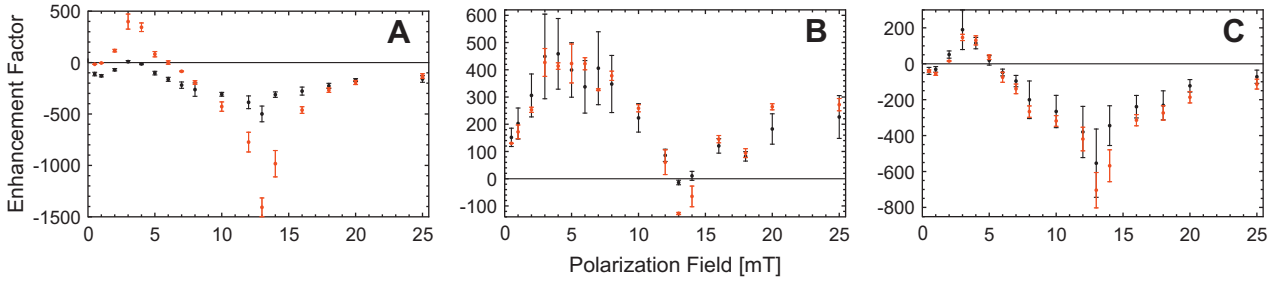

Fig. 2. Enhancement factors for pyridine depending on the strength of the polarization field. Graphs A, B and C show values determined by integration for the *ortho*-, *meta*- and *para*-protons, respectively. Black data points were obtained using regular pyridine, whereas red data points represent results from measurements with deuterated/protonated pyridine at a ratio of 10:1. Enhancement is measured compared to the Boltzmann signal.

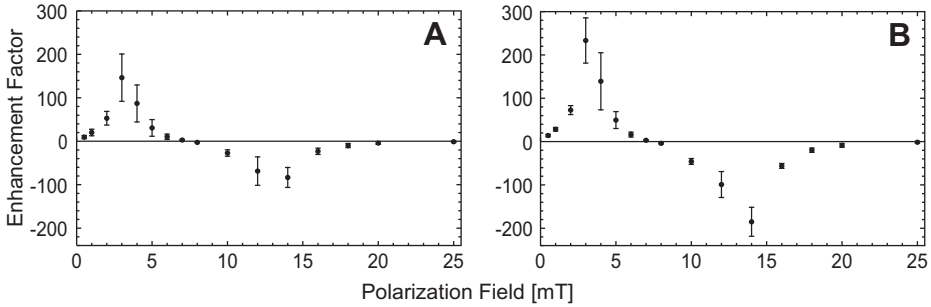

Fig. 3. Polarization field dependence of the signal enhancement for the protons in pyrazole. A displays the values for the protons in 3 and 5 position, while B gives values for the proton in position 4. Enhancement is measured compared to the Boltzmann signal.

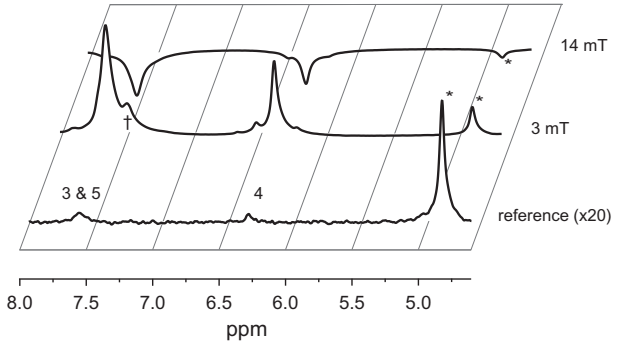

Fig. 4. SABRE spectra of pyrazole recorded after polarization at two different fields. The numbers in the spectra indicate the corresponding protons of the substrate. Solvent and pyridine resonances are marked with asterisks and daggers, respectively.

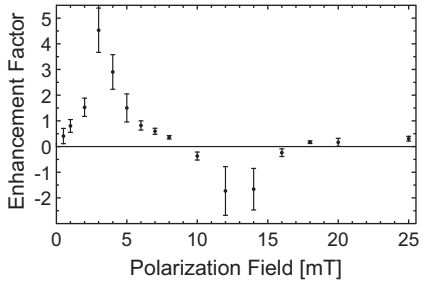

Fig. 5. Polarization field dependence of the enhancement factor of the solvent's hydroxy resonance in pyrazole spectra. Enhancement is measured compared to the Boltzmann signal. A strong correlation between this figure and Fig. 3 is clearly apparent.

enhancements was found to be similar to the *N*-methylated analog, with only slight deviations in the field strengths of the maxima (see Supplementary data for field dependence graphs). Additionally, the aforementioned enhancement of the solvent's OH resonance was also observable for this substrate.

For 3-phenylpyrazole, only the field dependence of proton 4 could be examined (see Supplementary data for field dependence graph), which exhibits a strong resemblance to the other substrates described above. Notably, no signal enhancement was observed for the phenyl ring protons, indicating insufficient polarization transfer to the six-membered ring. The solvent's OH resonance was also enhanced as described earlier.

Polarization of 3-(3-bromophenyl)pyrazole was achieved using the standard SABRE procedure. The obtained spectra resemble those of 3-phenylpyrazole, as the protons of the pyrazole ring experience signal enhancement, while the phenyl protons showed no increase in signal intensity. Due to this similarity, the field dependence is expected to be analogous to the aforementioned derivative and was not determined.

For indazole, a twofold signal enhancement was observed for the proton in position 3, while the protons of the annulated phenyl ring showed no signs of polarization transfer. As the enhancement is much smaller compared to the molecules described above, the field dependence was not examined.

The field dependence of 3,5-dimethylpyrazole (not shown) exhibits a significant difference to those described above, as polarization transfer could not be observed for magnetic fields up to 5 mT. At higher fields, signals enhanced by up to one order of magnitude and with a negative intensity were observed for the methine proton, while the methyl resonances showed no signal enhancement.

For 3,5-diphenylpyrazole, no polarization transfer was observed. Additionally, no enhanced pyridine resonances were observable, indicating formation of an inactive iridium complex following the hydrogenation of the cyclooctadiene ligand.

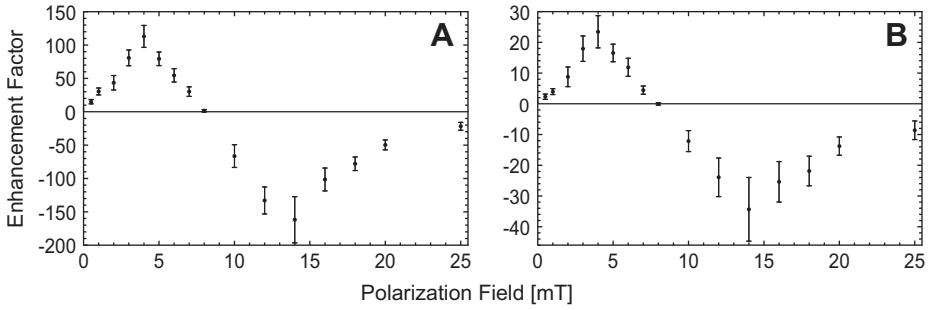

Fig. 6. Polarization field dependence of the enhancement factors of proton 4 (A) and the methyl group (B) of 1-methylpyrazole. Enhancement is measured compared to the Boltzmann signal.

Indicated by the slower decoloration of the sample after reduction of the protective group, the hydrogenation rate is much smaller in the presence of the diphenylated pyrazole and the solution keeps a light yellow color. Multiple hydride resonances in the region from −19.5 to −21.5 demonstrate the ability of the formed complexes to bind hydrogen, yet they seem incapable of polarization transfer.

3. Discussion

3.1. Polarization of new SABRE substrates

The enhancement curves for 1-methylpyrazole and 3-methylpyrazole show that protons of methyl groups are polarized to a significantly smaller extent as compared to the methine protons of the pyrazole ring. Similarly, polarization of protons of a phenyl ring attached to the pyrazole molecule in position 3 was not achieved at all, as the results for 3-phenylpyrazole and 3-(3-bromophenyl)pyrazole demonstrate.

In the case of the methyl groups, the reduced enhancements could partially be attributed to larger relaxation rates compared to the spins directly attached to the pyrazole ring. Spin–lattice relaxation times (T_1) were determined (see Table. 1) and exhibit significantly smaller values for CH_3 groups. This does, however, not apply for the protons of phenyl rings as their relaxation rates do not significantly differ from those of the pyrazole ring, thereby ruling out relaxation as a cause for the lower degree of enhancement.

The size of the scalar couplings within the active complex and the substrate molecule provide a more likely explanation for the phenomenon. Between a former *para*hydrogen spin and a substrate molecule, the largest H–H coupling can be expected to be to a proton which is attached to the carbon atom adjacent to the binding nitrogen. As this occurs over four bonds, the size of the *J*-coupling can be assumed to be approximately 1 Hz or lower, thereby limiting the rate of polarization transfer to the aromatic ring. The proton–proton couplings within the ring system are considerably larger, thereby facilitating a quick distribution of the polarization between the proton spins of the heterocyclic ring.

In methyl group carrying derivatives, coupling from ring protons to the CH_3 groups occurs across a quaternary carbon (or a tertiary nitrogen in 1-methylpyrazole) resulting in a ^4J coupling with a smaller magnitude. The efficiency of the polarization transfer might be limited by the size of this coupling resulting in a significantly lower enhancement of the methyl groups.

In the phenylated derivatives, coupling between the proton spins of the two aromatic ring systems has to occur across two quaternary carbons resulting in a considerably smaller ^5J coupling. As it seems, efficient transfer of polarization from one ring to the other cannot be achieved via such a small coupling resulting in vanishing enhancement for the proton resonances of the phenyl ring.

3,5-Dimethylpyrazole was the only examined substrate which showed a significant deviation from the enhancement curves determined for the other substrates: No signal enhancement was observable for the two methyl groups and up to a field strength of 5 mT, no signal enhancement was observable for the methine proton, either. At higher fields, this signal exhibited negative intensities, yet with an enhancement factor significantly smaller compared to 3-methylpyrazol which is only monosubstituted. The lack of a proton attached to carbon 3 and 5 exhibits the most substantial difference to the remaining substrates. Therefore only ^5J couplings from the *para*hydrogen nuclei to the spins of the examined substrate are present, requiring a stronger field to facilitate transfer of polarization. The efficiency of this transfer is, however, very limited.

3,5-Diphenylpyrazole was not polarized with the standard SABRE procedure. Also, no enhanced pyridine resonances were observed in the spectra, implying that no formation of an active polarization transfer catalyst occurred. The steric hindrance due to the bulky phenyl groups might prevent efficient binding to the iridium complex or, alternatively, cause the formation of a complex with a strongly deviating ligand conformation, which is not capable of either polarization transfer or effective ligand exchange. Polymerization of the iridium complexes due to a lack of suitable substrates [15] might serve as a probable explanation.

Although polarization transfer to indazole was observed, only proton 3 experienced a detectable signal enhancement. The enhancement was, however, very small. This observation can possibly be attributed to the insufficient stability of the iridium complex, as the efficiency of the polarization transfer depends, among

Table 1
Spin lattice relaxation times (T_1) of protons for selected substrates.

Substrate	Pos 3	Pos 4	Pos 5	Methyl	*Ortho*	*Meta*	*Para*
Pyridine	—	—	—	—	5.85 ± 0.09s	6.04 ± 0.09s	6.61 ± 0.12s
Pyrazole	5.89 ± 0.09s	5.53 ± 0.07s	5.89 ± 0.09s	—	—	—	—
1-Methyl-pyrazole	6.27 ± 0.08s	6.23 ± 0.08s	6.69 ± 0.10s	4.98 ± 0.07s	—	—	—
3-Methyl-pyrazole	—	5.55 ± 0.07s	5.85 ± 0.08s	3.45 ± 0.05s	—	—	—
3,5-Dimethyl-pyrazole	—	5.33 ± 0.08s	—	3.07 ± 0.05s	—	—	—
3,5-Diphenyl-pyrazole	—	2.43 ± 0.03s	—	—	2.61 ± 0.04s	2.48 ± 0.02s	3.05 ± 0.07s

T_1 values were determined using the same concentrations as were used for the recording of the SABRE spectra. Dashes indicate that no such proton is present in the substrate.

other things, on the lifetime of the formed complex. Changes to the complex might help to increase the obtainable enhancement.

Two possible explanations can be given for the observation of the enhanced hydroxy resonances in the NMR spectra of some of the substrates. In a straightforward approach, the solvent molecule acts as a substrate and binds directly to the active polarization transfer catalyst, thereby gaining additional intensity. Since this OH enhancement is not observable for pyridine, an indirect transfer seems more likely. For pyrazoles, the NH atom will be polarized being located directly next to the binding site of pyrazole with iridium (Fig. 7). Since this proton exchanges efficiently with the solvent's hydroxy proton, these protons will pick up the enhancement, which we observed in the spectrum. The strong correlation between Figs. 3 and 5 further supports this mechanism.

3.2. Determination of the polarization field dependences

The determined enhancement curves of pyrazole and its derivatives in the range from 0.5 to 25 mT are qualitatively almost identical. Even though the magnitude of the enhancement factors varies for different substrates, all of the derived curves exhibit a maximum in the range between 3 and 4 mT. Additionally, the minima, corresponding to maximum enhancements with a 180° phase shift compared to the reference, are found with only small variations in the range from 13 to 15 mT and the zero-transitions are in a small range around 8 mT. This applies to all the examined proton resonances, namely those ^{1}H nuclei directly attached to the pyrazole ring, as well as the methyl protons.

Strikingly, the enhancement curves of pyridine also reveal a maximum between 3 and 4 mT and a maximum negative enhancement at a field of 13 mT. A comparison of the field dependence curves of the *ortho* and *para* protons in pyridine with those obtained for the pyrazole derivatives shows that the pyridine spectra can be described as being shifted on the *y*-axis, as they are not centered around the *x*-axis, but start and end with negative signal intensities. The field dependence for the *meta* proton behaves differently, as the curve shows two maxima between 3 and 4 mT and at 7 mT, as well as two minima at between 13 and 14 mT and at 18 mT.

Further comparison of the obtained field dependence curves of pyridine with those proposed by Duckett and co-workers reveals two differences: first, a discrepancy between the field strengths for highest enhancement in the calculated [11] and experimentally determined curves is observed. This can possibly be attributed to the simplifications made for the model complex used in the calculations.

More substantial, however, is the difference in the curve shape between the calculated and the experimentally determined polarization field dependence curves. The calculated field dependence of the magnetization enhancement, which was detected in the course of our experiments, namely R_Z and T_Z in the nomenclature of the theoretical description [11], exhibit three maxima and two minima in the observed field range, whereas all but one of the experimentally determined curves show only one maximum and one minimum. Furthermore, the computed amplitudes show no change in sign over the examined polarization field range (compare [11] to Figs. 2, 3, 5 and 6. s1 and s2).

We simulated the polarization transfer but could not find parameters under which the experimentally observed curves were reproduced. Part of this may be due to the inadequacy of the used spin systems, since all protons in the complex might have to be taken into account, which are too many in number to be treated with simulation software currently available.

In order to test if the size of the spin system has an impact on the field dependence curves, we conducted experiments, in which pyridine, which acted as a substrate, was diluted with its deuterated derivative to achieve a 1:10 ratio. We found, that for these spin systems, on average reduced by 10 protons, a significant increase in signal enhancement can be observed for the resonance of the substrate's *ortho* protons. Similar findings were made by Duckett et al. in their recent publication in which they used partially deuterated pyridine [8]. Notable, however, is the fact that the field strengths for the maxima in the determined field dependence curves remain unchanged by the reduction of the spin system and the induced asymmetry of the active complex, when only one protonated substrate is coordinated.

We conclude that a further reduction of the spin system in necessary to be able to conduct reliable theoretical calculations and therefore defer the simulation of the field dependences to later work.

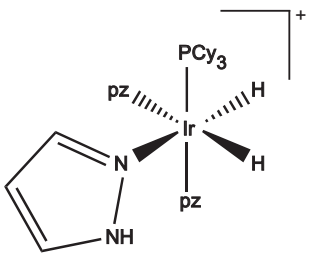

Fig. 7. Supposed structure of the active polarization transfer catalyst when pyrazole is employed as a substrate. (pz = pyrazole, PCy_3 = tricyclohexyl phosphine).

4. Conclusion

In this paper, we report the polarization of seven pyrazole derivatives using the SABRE *para*hydrogen polarization technique. The compounds examined include the unsubstituted pyrazole itself as well as selected mono- and disubstituted derivatives. For the monosubstituted compounds, a signal enhancement of several hundreds is found for the aromatic protons attached to the pyrazole ring. Protons of methyl groups showed signal amplification by one order of magnitude, while protons of phenyl rings attached to the pyrazole did not gain additional polarization. In the annulated pyrazole derivative indazole, only one proton resonance showed a substantial, i.e. twofold, enhancement. 3,5-dimethylpyrazole revealed differences to the other pyrazoles as the aromatic proton could only be polarized at higher field strengths, while the methyl groups could not be polarized at all. 3,5-Diphenylpyrazole with its bulky phenyl rings, however, proved to be an inappropriate substrate for the unmodified SABRE procedure as it did not exhibit any enhancement, even not of pyridine upon adding *para*hydrogen to the sample solution. Future research will be dedicated to the modification of the technique (synthesis of new catalysts), to open this polarization method to a bulkier substrate class. As Duckett et al. have recently shown [8], there is still room for improvement for this relatively new polarization method.

Polarization field dependence curves for the enhancement of four of the not previously reported substrates as well as the known substrate pyridine were determined in the range from 0.5 to 25 mT. Our results show that the field strengths for the maximum signal enhancements depend only mildly on the structure of the examined substrate, if a ^4J coupling from *para*hydrogen spins to a substrate ^{1}H nucleus exists, and can be found between 3 and 4 mT, as well as between 13 and 15 mT. Substrates with only ^5J couplings to the hydride ligands seem to exhibit a deviating behavior. This will be investigated in the future. By substitution of most of the pyridine by deuterated pyridine, we also saw that all the protons have to be taken into account if one wants to understand the field dependence of the SABRE effect.

In summary, we have shown that pyrazoles can be enhanced with the SABRE approach. The field dependences of the enhancements are surprisingly similar irrespective of the molecules used and do not follow the theoretical predictions [11]. More computational work is therefore necessary to understand the enhancement mechanism with the goal to optimize metallo-organic complexes for molecules that cannot be enhanced with the present technique.

5. Experimental

5.1. Synthesis

The syntheses were performed under a nitrogen atmosphere using the Schlenk-technique. Substances sensitive to water or air were handled in a dry argon glove box. The applied solvents were dried and degassed appropriately prior to their use. All required chemicals were purchased from Sigma Aldrich and used without further purification. $[Ir(cod)(py)_2]BF_4$ and $[Ir(cod)(PCy_3)(py)]BF_4$ were synthesized according to literature methods [16,17].

5.2. Preparation of parahydrogen

*Para*hydrogen was prepared by cooling hydrogen gas to 33 K using a closed circuit Joule–Thomson cryostat with helium gas as a coolant. Activated charcoal was utilized as a catalyst to facilitate the spin transition to obtain 96% enriched p-H_2 [18].

5.3. Sample preparation

All chemicals were purchased from Sigma Aldrich, except for pyridine-d_5 and methanol-d_4 from Deutero GmbH, and used without purification.

Samples were prepared by dissolving 1 mg of the precatalyst $[Ir(cod)(PCy_3)(py)]BF_4$ in 400 μL of perdeuterated methanol followed by an addition of 200 μL of a 67 mM stock solution of the substrate in methanol-d_4 (catalyst/substrate ratio 1:10). The solution was transferred into a 5 mm Wilmad quick pressure valve NMR tube and degassed in three freeze–pump-thaw cycles. At a pressure of 3.9 bar, *para*hydrogen was applied to the tube, which was subsequently shaken to dissolve the gas and to create the active catalyst $[Ir(H)_2(PCy_3)(substrate)_3][BF_4]$. p-H_2 was then replenished by freezing and evacuating the sample, followed by application of fresh p-H_2 at 3.9 bar. After thawing in a water bath, the sample was ready for use.

5.4. Experimental procedure

Spectra were recorded on an Oxford 300 MHz wide bore spectrometer, operating at 7.05 T and a proton resonance frequency of 299.38 MHz. To determine the polarization field dependence of the signal enhancements, the samples were polarized at various field strengths in the stray field created by the spectrometer, which had been carefully determined using a Hall probe prior to the measurements. Before the polarization step, it was made sure that the sample was not disturbed to minimize dissolution of p-H_2 and polarization transfer at random fields.

Polarization of the substrates was achieved by shaking the sample tube for approx. 5 s to dissolve the p-H_2 from the head space above the solution. During this step, a slight field variation due to the length of the sample tube was inevitable. At higher fields, this effect increased, as a result of the nature of the stray field. The sample was then rapidly inserted into the spectrometer (~5 s) while making sure to reproduce the sample's trajectory as precisely as possible for each measurement. During this transfer period, polarization transfer to a certain extend at shifting fields was unavoidable, as substrate, catalyst and residual p-H_2 could still interact. Acquisition of a proton spectrum using a 90° rf pulse was performed immediately after the sample rested in the spectrometer.

This procedure was repeated three times at each field strength. After each set of three measurements, the p-H_2 was replenished as described above to restore the starting conditions for the next set of measurements. Two reference spectra were recorded at different points of the measurement cycle after keeping the sample inside the spectrometer for 10 min, making sure the hyperpolarization had decayed completely.

5.5. Evaluation

Signal intensities were determined by two-point integration in MestReNova 6.2.0. In case of signal overlap, the implemented line fitting tool was used to deconvolute the unresolved or overlapping resonances. Enhancement factors were determined by division of the average intensity of the polarized signals by the average intensity of the Boltzmann signals.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2011.11.001.

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