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Research paper

Enhancing NMR of insensitive nuclei by transfer of SABRE spin hyperpolarization

Andrey N. Pravdivtsev [a,b], Alexandra V. Yurkovskaya [a,b], Herbert Zimmermann [c], Hans-Martin Vieth [a,d], Konstantin L. Ivanov [a,b,*]

[a] International Tomography Center SB RAS, Institutskaya 3a, Novosibirsk 630090, Russia
[b] Novosibirsk State University, Pirogova 2, Novosibirsk 630090, Russia
[c] Max-Planck-Institut für Medizinische Forschung, Jahnstrasse 29, Heidelberg 69120, Germany
[d] Institut für Experimentalphysik, Freie Universität Berlin, Arnimallee 14, Berlin 14195, Germany

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ABSTRACT

We describe the performance of methods for enhancing NMR (Nuclear Magnetic Resonance) signals of "insensitive", but important NMR nuclei, which are based on the SABRE (Signal Amplification By Reversible Exchange) technique, i.e., on spin order transfer from *para*hydrogen (H_2 molecule in its nuclear singlet spin state) to a substrate in a transient organometallic complex. Here such transfer is performed at high magnetic fields by INEPT-type NMR pulse sequences, modified for SABRE. Signal enhancements up to three orders of magnitude are obtained for ^{15}N nuclei; the possibility of sensitive detection of 2D-NMR ^{1}H-^{15}N spectra of SABRE complexes and substrates is demonstrated.

1. Introduction

SABRE (Signal Amplification By Reversible Exchange) [1,2] is a promising method for boosting weak NMR signals. SABRE exploits spin order transfer from *para*hydrogen (pH_2, the H_2 molecule in its singlet nuclear spin isomer) to a substrate, S, in a suitable transient Ir-based organometallic complex, see Scheme 1. As a result, the substrate acquires strong non-thermal spin polarization, also termed spin hyperpolarization, which can exceed the equilibrium (Boltzmann) polarization by several orders of magnitude. Accordingly, NMR signals of the substrate (both in its free form in solution and in the bound form at the SABRE complex) are strongly enhanced. The spin order transfer in the SABRE complex is efficient at low magnetic fields, notably, at spin Level Anti-Crossings (LACs) of the SABRE complex [3–5]; recently, it has been shown [6–9] that high-field SABRE becomes feasible when RF-excitation is used (in order to mimic the low-field conditions, i.e., to create LACs in the RF-rotating frame) or appropriate pulse sequences are exploited.

Despite the fact that the SABRE method is relatively new, several promising applications of this technique in NMR spectroscopy and imaging have already been demonstrated. Notably, applicability of high-field SABRE for trace analysis in complex mixtures has been shown [9–11]. At the same time, for many NMR applications it is of importance to record spectra not only for protons, but also for hetero-nuclei; furthermore, multi-dimensional hetero-nuclear NMR techniques [12] enable precise characterization of molecules and complexes. In order to perform SABRE experiments on hetero-nuclei and to record multi-dimensional spectra, efficient spin order transfer from pH_2 to spin-1/2 hetero-nuclei is a prerequisite. The feasibility of such a transfer directly at the high magnetic field of an NMR spectrometer has been demonstrated [7,8,13], but so far not the feasibility of running two-dimensional (2D) hetero-nuclear SABRE-enhanced NMR experiments.

In this work, we make use of pulse sequences based on the INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) method [14] in order to perform spin order transfer from pH_2 to hetero-nuclei (in this work, to ^{15}N) and to record 2D NMR spectra. Previously, it was shown [13] that INEPT-based methods are compatible with the SABRE method (although the SABRE technique was named differently in that paper). We demonstrate that the new techniques enable fast and efficient polarization transfer; moreover, polarization of hetero-nuclei can be generated in a continuous way. We also perform a detailed study of the resulting polarization on inter-pulse delays of the pulse sequences, which allow us to optimize the resulting signal enhancement and to determine parameters of SABRE complexes and to measure complex dissociation rates. Experimental studies are performed for pyridine, which is presently the most widely used SABRE substrate, and for imidazole, which also can be polarized by SABRE and used as a pH-sensitive probe [15].

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* Corresponding author at: International Tomography Center SB RAS, Institutskaya 3a, Novosibirsk 630090, Russia.

E-mail address: ivanov@tomo.nsc.ru (K.L. Ivanov).

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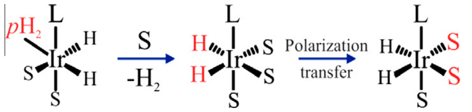


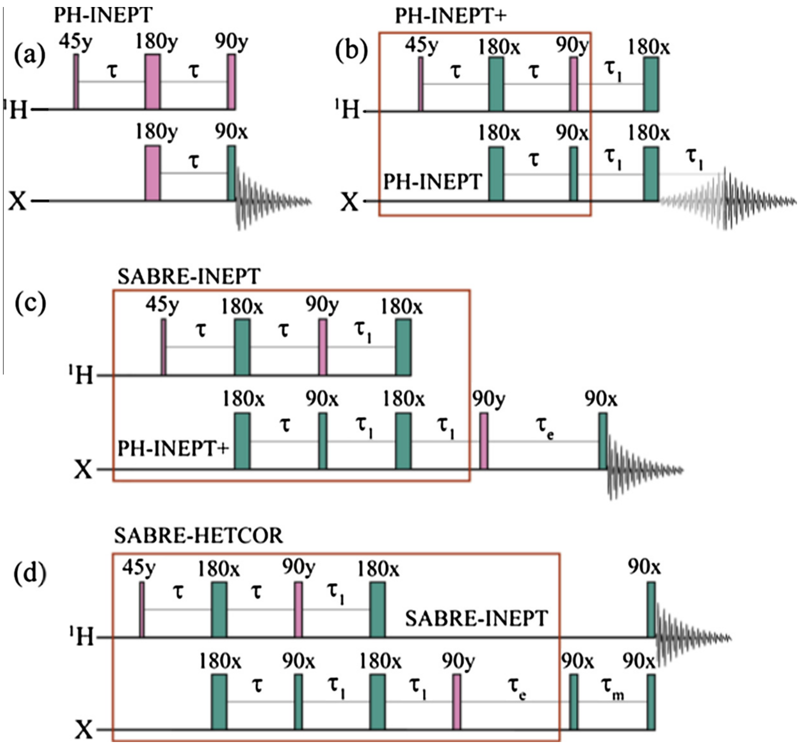
Scheme 1. Scheme of SABRE formation. pH_2 and substrate (S) transiently bind to an Ir-based complex (L = IMes) where spin order transfer takes places and the substrate gets hyperpolarized. Previously, different strategies have been used for nuclear spin order transfer (see text); here we exploit INEPT-based NMR methods for hyperpolarizing hetero-nuclei of S.

2. Materials and methods

The pulse sequences, used in our experiments, are presented in Scheme 2 in the order of increasing complexity: each pulse sequence contains the previous one as an initial building block. As we demonstrate below, the first three sequences (a, b and c) allow one to polarize hetero-nuclei; one can choose a specific pulse sequence in order to change the spectral pattern in a desired way or to polarize predominantly the free or bound substrate S. Here, we use two sequences, PH-INEPT and PH-INEPT+. In PH-INEPT+ an additional 180-degree pulse is used to prevent cancelation of the anti-phase signals. It was originally proposed for conventional Para-Hydrogen Induced Polarization (PHIP) [16,17] but later also exploited in SABRE [13]. The third sequence, originally proposed in Ref. [13] better suits for polarizing free SABRE substrates in solution; here we name it SABRE-INEPT. The last sequence, proposed here for the first time, is designed for 2D-NMR spectroscopy. Importantly, in the last sequence we introduce a variable delay τ_m before the last pulse, see Scheme 2d. This modification of SABRE-INEPT enables collecting 2D data by measurements with a stepwise incremented variable delay τ_m, which gives an access to the "indirect domain" [12] in 2D-NMR. We name this sequence SABRE-HETCOR because it enables heteronuclear correlated spectroscopy with signal amplification by hyperpolarization. In all sequences shown in Scheme 2, before applying the first NMR-pulse we initiated spin hyperpolarization by bubbling H_2 gas enriched in its para-component through the liquid sample located inside the NMR probe as described before [18]. The procedure of pH_2 bubbling (not shown in the Scheme 2) is automated and controlled by the NMR spectrometer [18]. Our previous studies [7] have shown that conditions for bubbling are perfectly reproducible for subsequent SABRE experiments, which can be repeated for at least several hundreds of times providing "continuous generation" of hyperpolarization at high field. As a result, the NMR signal intensity fluctuates only by about 5% for subsequent acquisitions. In both 1D and 2D experiments, we did not use any phase cycling and only applied pulses with phases reported in Scheme 2.

In our experiments we used a mixture of 26 mM of ^{15}N-pyridine (^{15}N-Py) as a SABRE substrate and 2 mM of IrIMesCODCl as a SABRE pre-catalyst (IMes = 1,3-bis(2,4,6-trimethylphenyl) imidazole-2-ylidene, COD = cyclooctadiene) [19] in methanol-d_4. Another sample that we used contained 90 mM of imidazole (Im) and 4 mM of IrIMesCODCl in methanol-d_4. Methanol-d_4 and ^{15}N-pyridine were purchased from Deutero GmbH, the pre-catalyst was synthesized using the procedure described in Ref. [20]. ^{15}N-labelled Im was synthesized as described before [21,22]. All samples were used without additional purification. *Para*hydrogen was prepared using a Bruker *para*hydrogen generator (providing H_2 gas with an enrichment of about 80% in pH_2) and stored in a gas-bottle prior to the experiments. Experiments were carried out on Bruker Ascend 400 MHz and 700 MHz spectrometers.

NMR enhancements were determined by comparison with the corresponding thermal ^{15}N-NMR signals. Such signals can only be obtained for free S in solution; for bound S, the NMR intensity



Scheme 2. Pulse sequences used in this work. PH-INEPT enables formation of multiplet polarization of the X-nuclei. PH-INEPT+ (containing PH-INEPT as a building block) converts this polarization into net polarization. SABRE-INEPT (containing PH-INEPT+ as a building block) creates net polarization on the free and bound substrate. Finally, SABRE-HETCOR (containing SABRE-INEPT as a building block) allows one to record a 2D heteronuclear ^{1}H-X NMR spectrum by varying spin evolution time between the last two pulses, τ_m. Flip angles and phases are specified for each pulse in the proton channel and X-channel; Free Induction Decay (FID) signals are indicated as decaying oscillations. Prior to each sequence pH_2 is bubbled through the solution for a few seconds (not shown), see text for further detail.