Development of MR active contrast agents via Parahydrogen Induced Polarization

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Abstract— **Parahydrogen Induced Polarization provides dramatic MR signal enhancement that can be exploited for molecular imaging. This method allows amongst others for Magnetic Resonance Imaging of ^{13}C and ^{15}N, which is usually constrained by the low MR sensitivity of these nuclei. By combining hydrogenation of barbiturates with parahydrogen under special experimental conditions (PASADENA under pressure) with a polarization transfer sequence we demonstrate the transfer of the initial ^{1}H polarization to ^{13}C. The polarization transfer yields a signal increase for ^{13}C of more than 1000. Hence, the role of certain target compounds such as anesthetics like the barbituric acid derivatives could be investigated using MRI techniques. Moreover, we present the first MRI experiments on antiphase PHIP polarized protons in the liquid phase using the model system hexyne/hexene. Our results show that it is possible to get images of good quality and high SNR with PHIP polarized protons and that no artifacts are created by the antiphase character of the ^{1}H signal.**

Keywords— **Magnetic Resonance Imaging, Hyperpolarization, contrast agent, molecular imaging**

I. INTRODUCTION

The applications of hyperpolarized contrast agents in MRI significantly increased during the last decade. They offer a great advantage over conventional contrast agents because they are the direct signal source and due not rely on implied T1, T2 or T2* changes on the surrounding liquid or tissue as classical Gd based contrast agents. A very exciting medical application of hyperpolarized molecules is molecular or metabolic imaging, i.e. to improve tumor diagnosis or therapy monitoring [1,2]. Malignant tissue can be differentiated from healthy organs on the basis of different metabolic pathways within the citrate cycle.

So far, medical imaging with hyperpolarized compounds was realized with ^{13}C or ^{15}N hyperpolarized substances, which have the advantage of very long T1 times and large chemical shift ranges. The hyperpolarization can be achieved via two different techniques, which were discovered many years before, namely Dynamic Nuclear Polarisation (DNP) and Parahydrogen Induced Polarisation (PHIP). Whereas DNP uses the large polarization of unpaired electrons, which is transferred to nuclear spins by microwave irradiation [3,4], PHIP is a technique to produce hyperpolarized samples via a chemical route [5,6]. It makes use of the parahydrogen symmetry breaking during homogeneously catalyzed hydrogenation of unsaturated substrates, creation of non-equivalent product protons and the re-insertion of parahydrogen spin information into the substrate molecule.

Parahydrogen which is the thermodynamically preferred spin isomer of the hydrogen molecule (as opposed to orthohydrogen) can be enriched by cooling under the effect of a paramagnetic catalyst (e.g. charcoal). After a subsequent homogeneous parahydrogenation reaction, PHIP NMR experiments lead to absorption and emission signals and a theoretical signal increase of up to 10^4, which is in practice limited by relaxation processes. If the hydrogenation is conducted at low magnetic field, followed by transfer into the NMR magnet and subsequent spectra acquisition, the experiment is referred to as Adiabatic Longitudinal Transport After Dissociation Engenders Nuclear Alignment (ALTADENA), leading to signals either in net absorption or emission. If the hydrogenation and NMR measurement are carried out in high field, it is termed Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment (PASADENA), leading to characteristic antiphase signals exhibiting both absorption and emission of the NMR resonances resulting from the respective protons.

In the case of PHIP, the parahydrogen atoms that are introduced in an unsaturated molecule carry the hyperpolarization in the first place. There arise two problems, if ^{1}H PHIP polarized molecules should be used for imaging purposes: the Parahydrogen Induced Polarization creates an antiphase proton signal, that might cause artifacts in the images and the T1 of protons is in the range of a few seconds for most molecules. Their polarization can be transferred to hetero nuclei by applying field cycling techniques or adequate pulse sequences [7]. In this paper, we present the synthesis and hyperpolarization of barbiturates. We demonstrate polarization transfer to ^{13}C in these molecules with adequate pulse sequences and in a proof-of-concept experiment the imaging of hyperpolarized antiphase ^{1}H signals using a model compound.

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II. MATERIALS AND METHODS

A. Parahydrogen enrichment and chemistry

Para-H_2 can be enriched by cooling under the influence of a paramagnetic catalyst (e.g. active charcoal). For this work 95% p-H_2 was obtained by using a closed-cycle cryostat (Advanced Research Systems, Macungie, PA, USA) for cooling down to 30K. It was easily stored for 2-3 days in transportable aluminium cylinders at 3.5 bar.

Barbituric acid derivatives were synthesized from urea and unsaturated malonic acid derivatives according to literature procedures, which were adapted to the corresponding substitution patterns [8]. For parahydrogenation reactions a commercially available rhodium catalyst system [Rh(COD)(dppb)]BF4 was used. All chemicals were purchased from Sigma-Aldrich, Acros or Merck and used without further purification.

10 mm NMR capillary tubes were filled with 10 mg catalyst, 0.28 mmol of the unsaturated barbiturate (or for the imaging experiments with 6.09 mmol hexyne) and 3 ml acetone-d6 under argon atmosphere and sealed with a septum cap. The "PASADENA under pressure" experiment was carried out at elevated temperature and pressure in order to enhance the conversion rate of the hydrogenation reaction. Before the experiment, the reaction tube was gently heated to 60°C in a water bath and then pressurized with 3.5 bar of para-enriched H_2. Subsequently, the tube was shaken above the bore of the magnet to start the hydrogenation and immediately inserted into the spectrometer or scanner.

B. NMR and MRI experiments

All spectroscopic experiments were performed on a Bruker AVANCE DRX 300 MHz spectrometer using 45° pulses. The high proton polarization was transferred to ^{13}C using the PH-INEPT+ [7]. The difference between the PH-INEPT+ sequence and a standard INEPT+ sequence is the length and the phase of the first pulse. All references were measured under the same conditions as the PHIP spectra including the same pulses, and the same receiver gain.

Imaging experiments were performed in a 1.5 Tesla imaging system (Magnetom Sonata, Siemens Medical, Erlangen, Germany). All filter algorithms were deactivated. The resonance frequency, transmitter and shim of the system were calibrated using a water phantom of the same size. In order to achieve a good filling factor a small loop coil (Siemens Medical, Erlangen, Germany) with 3 cm diameter was used.

III. RESULTS

Figure 1 presents a typical ^{1}H-PASADENA spectrum (top) and a reference spectrum (bottom) of 5-methyl-5-propargylbarbituric acid in acetone-d6 at ~50°C. The strong antiphase signal of the hyperpolarized protons can be easily recognized. The PHIP spectrum shows a signal enhancement of more than 400 compared to the reference.

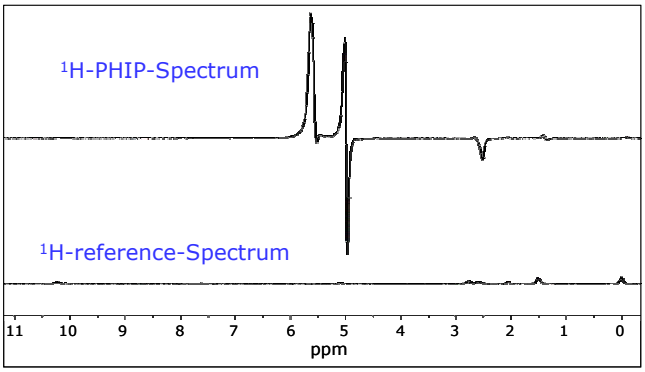

Fig. 1: ^{1}H NMR spectra upon para-hydrogenation of 5-methyl-5-propargylbarbituric acid. The enhancement of the PHIP spectrum is more than 400.

Figure 2 shows the ^{13}C PHIP-NMR spectra of 5-methyl-5-propargylbarbituric acid in acetone-d6 at ~50°C after applying the PH-INEPT+ sequence. The spectra was obtained 35 s after shaking the NMR tube with the reaction mixture charged with 3 bar of 95% enriched parahydrogen and subsequent insertion into the spectrometer. The conversion of the hydrogenation was nearly completed after shaking the reaction mixture twice. By using the PH-INEPT+ sequence with an delay of 15 ms a transfer of polarization to the resulting double bond and the vicinal carbons of the product was observed leading to a signal enhancement of up to 1000 for C3.

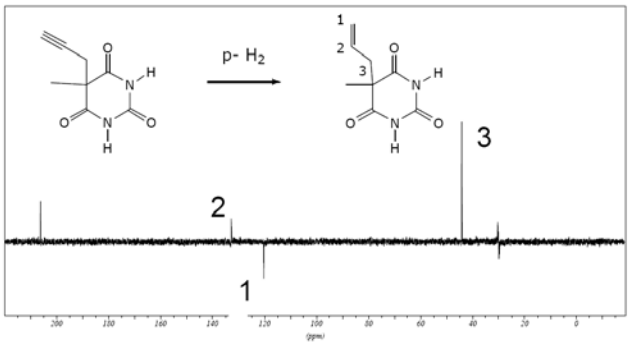

Fig. 2: ^{13}C PH-INEPT+ NMR spectra upon para-hydrogenation of 5-methyl-5-propargylbarbituric acid. The insert shows the molecular structure and the positions of the hyperpolarized carbons.

The imaging of hyperpolarized antiphase protons of hexyne is demonstrated in the FLASH images shown in Fig. 3. The images on the left were obtained under hyperpolarized conditions with different flip angles. The experiments on the right were recorded with a sample, which had no contact with parahydrogen at all and was therefore only thermally polarized. Because all the images are windowed equally the thermal image is not visible under these conditions and an image scaled with a factor of ten is provided. A very strong signal increase in the hyperpolarized compared to the thermal polarized images can be recognized that scales with the flip angles used in the centric reordered FLASH images, as expected. For very high flip angles (i.e. 45°) an artifact in the phase encoding direction occurs, that arises from differing amount of Z-magnetization for subsequent k-space lines, because a certain amount of polarization is destroyed by each rf pulse during the image acquisition.

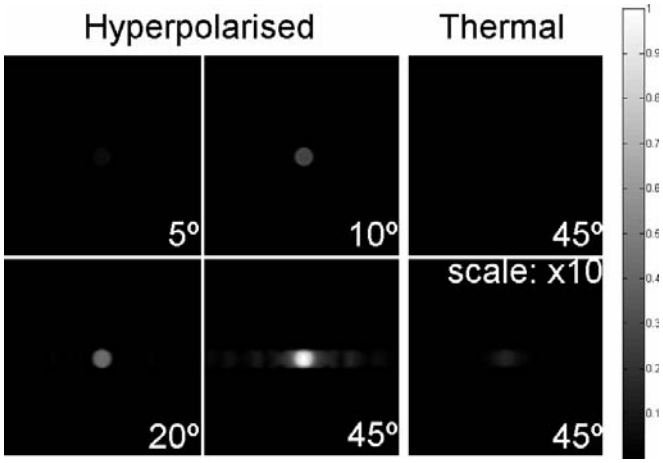

Fig. 3: ^{1}H-PHIP images of hyperpolarized hexene obtained with a centric reordered FLASH sequence. The flip angles are denoted in the corresponding images, TR and TE were the same for all images and set to 5.5 ms and 3.1 ms, respectively.

IV. CONCLUSIONS

It was shown before by our group that homogeneous hydrogenation of unsaturated barbituric acid derivatives with 50% parahydrogen yielded a substantial increase of the ^{1}H-NMR signals of the reaction products [8]. However, signal enhancement by randomly triggered polarization transfer to ^{13}C in the weak magnetic field could not be observed. Application of a closed-cycle cryostat setup for parahydrogen enrichment up to 95% together with effective INEPT-derived pulse sequences allowed for ^{13}C NMR signal enhancements up to 1000. These results may allow for in vivo MRI investigations of barbiturates.

Our results show that it is possible to get images of good quality and high SNR with PHIP polarized protons using a centric reordered FLASH sequence. However, artifacts can arise if high flip angles are employed that lead to large differences in the polarization of the sample between the acquisition of subsequent k-space lines. Hexyne is a very good candidate for the optimization of this kind of experiments, because its hydrogenation product is easy to polarize and may serve as a model compound for biocompatible molecules with long ^{1}H T_1 relaxation time, which could be utilized for in vivo MRI experiments.

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