

Hyperpolarization of Deuterated Metabolites via Remote Cross-Polarization and Dissolution Dynamic Nuclear Polarization

Basile Vuichoud,[†] Jonas Milani,[†] Aurélien Bernet,^{*,†} Roberto Melzi,[‡] Sami Jannin,^{*,†,§} and Geoffrey Bodenhausen^{†,||,⊥,∇}

[†]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

[‡]Bruker Italia S.r.l., Viale V. Lancetti 43, 20158 Milano, Italy

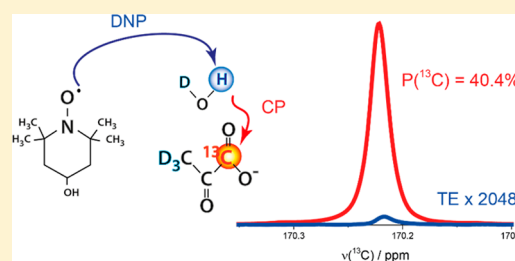
[§]Bruker BioSpin AG, Industriestrasse 26, 8117 Fällanden, Switzerland

^{||}Département de Chimie, Ecole Normale Supérieure, 24 Rue Lhomond, 75231 Paris, Cedex 05, France

[⊥]Université Pierre-et-Marie Curie, Paris, France

[∇]UMR 7203, CNRS/UPMC/ENS, Paris, France

ABSTRACT: In deuterated molecules such as [1-¹³C]pyruvate-*d*₃, the nuclear spin polarization of ¹³C nuclei can be enhanced by combining Hartmann–Hahn cross-polarization (CP) at low temperatures (1.2 K) with dissolution dynamic nuclear polarization (D-DNP). The polarization is transferred from remote solvent protons to the ¹³C spins of interest. This allows one not only to slightly reduce build-up times but also to increase polarization levels and extend the lifetimes *T*₁(¹³C) of the enhanced ¹³C polarization during and after transfer from the polarizer to the NMR or MRI system. This extends time scales over which metabolic processes and chemical reactions can be monitored.



INTRODUCTION

One of the most remarkable developments in nuclear magnetic resonance (NMR) in the last ten years is the invention of hyperpolarization by dissolution dynamic nuclear polarization (D-DNP).^{1,2} This offers a means to increase the sensitivity by several orders of magnitude. Molecular probes and nuclear spins of all kinds (⁶Li, ¹³C, ¹⁵N, ⁸⁹Y, and ¹²⁹Xe)^{3–6} may be enhanced in this fashion and then transferred and injected in vitro or infused in vivo in view of performing an ever-expanding range of studies, from the early detection of prostate cancer^{7,8} to the real-time observation of metal–ligand complexation,⁵ protein folding,⁹ and polymerization.¹⁰

Although spectacular enhancement factors can be achieved by D-DNP, applications are inexorably limited by the lifetime of the hyperpolarized magnetization, which is usually determined by the relaxation time *T*₁. Considerable efforts have been deployed to reduce polarization losses during sample transfer prior to infusion, in particular to provide access to slower and subtler chemical transformations such as slow metabolic processes. This is of particular interest in the context of in vivo metabolic imaging where more than 60 s may be required between dissolution and injection for filtration, pH adjustment, and quality control.⁸ Among various attempts and methods to extend the short-lived fate of spin polarization, the use of radical scavengers such as ascorbate (Vitamin C) offers a way of suppressing paramagnetic contributions to relaxation that arise from the inevitable presence of polarizing agents and paramagnetic oxygen¹¹ dissolved in solution. Another path is the storage of hyperpolarized longitudinal magnetization in the

form of long-lived states (LLS)¹² that usually involve pairs of inequivalent spins and can exhibit lifetimes up to 37 times *T*₁. LLS can either be populated in the solid prior to dissolution,¹³ or in the liquid after dissolution and before transfer to the NMR or MRI system.^{14–16} Recently the groups of M. Levitt and W. Warren have reported extraordinarily long *T*_{LLS} up to 13¹⁶ and 5 min,¹⁷ respectively, on suitably tailored molecules.

Whatever paths are chosen for extending the lifetimes of hyperpolarization, in many cases the mere presence of proton spins in the molecules may impose a severe erosion of our hyperpolarized ambitions. Nuclear spin–lattice relaxation driven by intramolecular dipolar interaction, in particular between a hyperpolarized ¹³C spin and ¹H spins, even when distant by two or more bonds, may offer efficient relaxation pathways. Intramolecular dipolar interactions also limit the usefulness of LLS. A simple solution to this problem consists in the partial or complete deuteration of the molecule of interest, such as the methyl groups in [3-¹³C]pyruvate¹⁸ or ¹⁵N-trimethylglutamine,¹⁹ or even the amine group in ¹⁵N-glutamine.²⁰ A good example of this approach is the use of ¹⁵N-trimethylphenylammonium-*d*₉ as a platform for designing a variety of hyperpolarized chemical probes, with *T*₁(¹⁵N) exceeding 800 s.²¹ The ¹³C polarization of such deuterated molecular probes can be efficiently performed in a direct manner with radicals with narrow ESR lines such as Trityl.

Received: December 4, 2013

Revised: January 6, 2014

Published: January 7, 2014

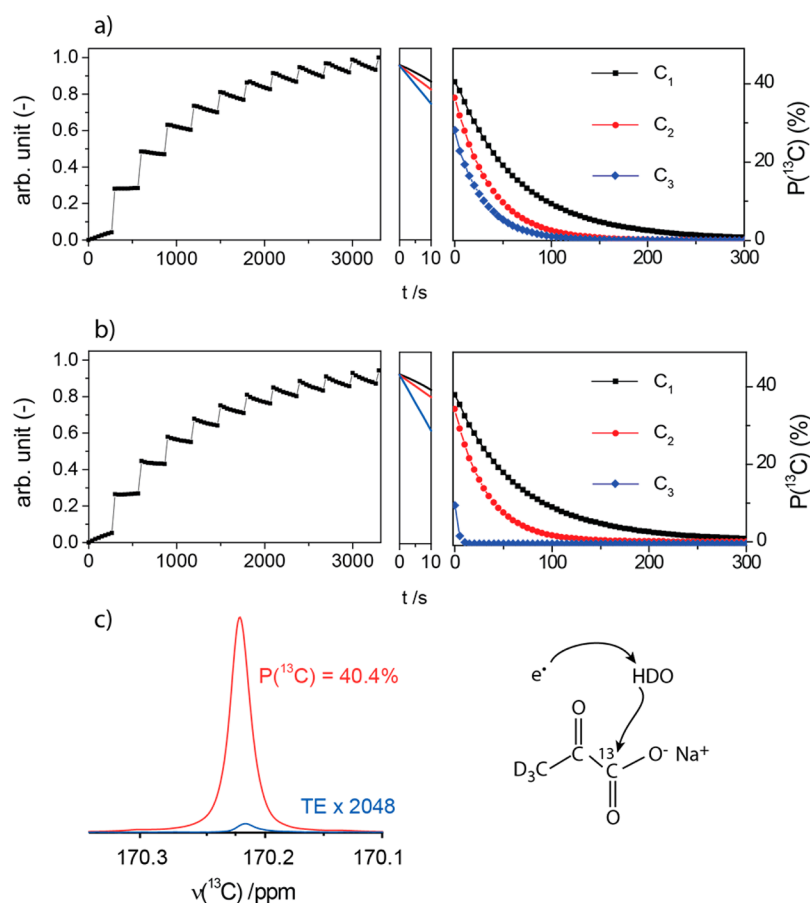


Figure 1. Hartmann–Hahn cross-polarization (CP) experiments performed on (a) deuterated and (b) nondeuterated $[1-^{13}\text{C}]$ pyruvate. The left panels show the build-up of the ^{13}C signals, measured every 37.5 s at 1.2 K using pulses with 5° nutation angles. An adiabatic CP contact²³ is established every 5 min, leading to nearly vertical jumps in the ^{13}C signals. The middle panels show relaxation during dissolution and transfer to the 7 T (300 MHz) NMR spectrometer. The transfer through a magnetic tunnel over 5 m, injection and settling require about 10 s. The right panels show the polarization decays of ^{13}C of carboxyl- (C_1), carbonyl- (C_2), and methyl- (C_3) groups after dissolution. The signal was measured at $T = 300$ K with 5° nutation pulses applied at 5 s intervals. (c) Spectrum of $[1-^{13}\text{C}]$ pyruvate- d_3 measured 10 s after dissolution with a single 5° nutation pulse, showing $P(^{13}\text{C}) = 40.4\%$ (red line), compared to a spectrum measured after complete relaxation to thermal equilibrium with 128 calibrated 90° nutation pulses, and rescaled by a factor 2048 (blue line).

Table 1. Polarization and DNP Enhancements after Dissolution, and Spin–Lattice Relaxation Times of ^{13}C Sites in Nondeuterated and Deuterated $[1-^{13}\text{C}]$ Pyruvate and $[1,2-^{13}\text{C}_2]$ Acetate at $B_0 = 7$ T and $T = 298$ K

	site	$P(^{13}\text{C})$ [%]	ϵ [–]	T_1 [s]
$[1-^{13}\text{C}]$ pyruvate	C^1	37.9 ± 0.8	49 000	69.3 ± 0.6
	C^2	34.2 ± 0.7	44 000	41.7 ± 1.0
	C^3	9.4 ± 0.2	12 000	4 ± 2
$[1-^{13}\text{C}]$ pyruvate- d_3	C^1	40.4 ± 0.4	52 000	68.0 ± 0.5
	C^2	36.4 ± 0.4	47 000	47.5 ± 0.8
	C^3	28.2 ± 0.3	36 000	39 ± 2
$[1,2-^{13}\text{C}_2]$ acetate	C^1	24.1 ± 1.8	31 000	66.7 ± 1.5
	C^2	5.7 ± 0.3	7 400	11.0 ± 0.3
$[1,2-^{13}\text{C}_2]$ acetate- d_3	C^1	19.6 ± 1.7	25 000	78.5 ± 0.5
	C^2	14.5 ± 2.9	18 600	35.4 ± 0.4

However deuteration may result in a decrease in DNP efficiency by as much as 40%,²² especially when deuterons are fully abundant in the solvent. In this contribution we demonstrate a general route for efficiently polarizing ^{13}C or ^{15}N spins in perdeuterated molecules by combining the inexpensive radical TEMPO with Hartmann–Hahn cross-polarization (CP) from diluted remote ^1H spins of the solvent.

EXPERIMENTAL METHODS

Most D-DNP experiments rely on the direct enhancement of the polarization of ^{13}C labeled molecules embedded in glassy frozen solutions doped with dilute (typ. 10 to 30 mM) polarizing agents (PA) with narrow ESR lines such as Trityl or BDPA at low temperatures (typ. $0.8 < T < 1.5$ K) and moderate magnetic fields (typ. $B_0 = 3.35$ T). Nuclear spin polarizations as high as $P(^{13}\text{C}) = 35\%$ were reported using Trityl radicals as PA.^{24,25} By increasing the polarizing magnetic field to $B_0 = 4.6$ T, polarization levels of $P(^{13}\text{C}) = 65\%$ were obtained,²⁶ however with polarizing time constants exceeding $\tau_{\text{DNP}} = 50$ min. In order to accelerate this process, we have recently implemented a strategy consisting in the rapid polarization of ^1H spins by microwave irradiation of PA's with broad ESR lines such as the nitroxyl radical TEMPOL followed by Hartmann–Hahn CP²⁷ transfer to ^{13}C .^{28–30} This method could be applied at $B_0 = 3.35$ or 6.7 T and low temperatures $T = 1.2$ K, resulting in $P(^{13}\text{C}) > 70\%$ in less than 20 min with doubly tuned solenoidal rf coils,³¹ or $P(^{13}\text{C}) = 45\%$ at 1.2 K in 20 min with Helmholtz coils that are compatible with dissolution, and $P(^{13}\text{C}) = 40\%$ after dissolution at 300 K.²³ We show herein how the same scheme can be readily applied for ^{13}C spins in perdeuterated molecules, provided that the concentration of

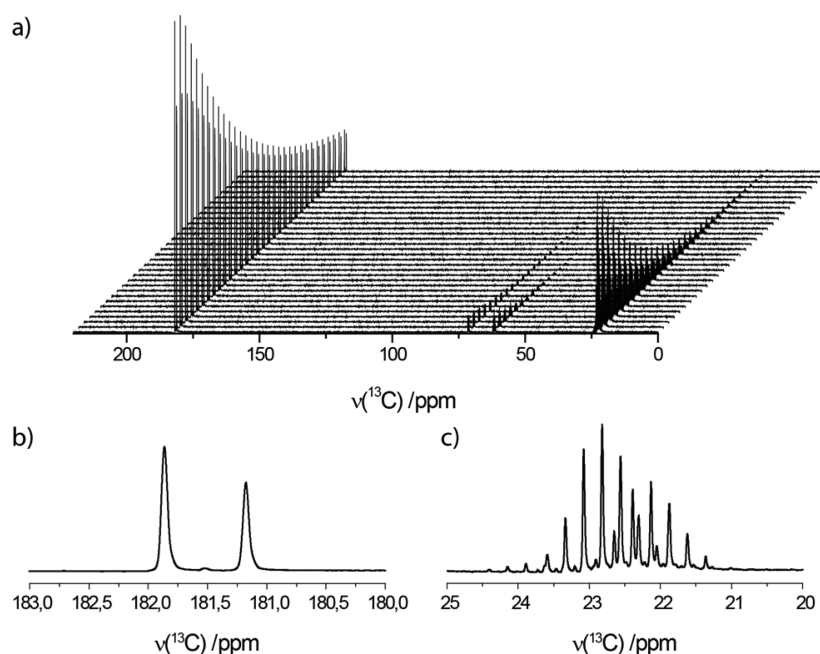


Figure 2. (a) Hyperpolarized ^{13}C NMR spectra of deuterated doubly enriched $[1,2-^{13}\text{C}_2]$ acetate- d_3 recorded every 5 s after dissolution-DNP, showing the effects of longitudinal relaxation $T_1(^{13}\text{C})$ at 7 T and 300 K. (b) NMR signals of the carboxyl C^1 and (c) NMR signals of the methyl C^2 , revealing asymmetries that are characteristic of very low spin temperatures.

protons in the surrounding glassy solvent is sufficient. Because of deuteration, the hyperpolarized perdeuterated molecules naturally exhibit longer relaxation time constants $T_1(^{13}\text{C})$, both during and after the transfer from the polarizer to the NMR or MRI system. As a proof of principle, we demonstrate our method for ^{13}C enriched $[1-^{13}\text{C}]$ pyruvate- d_3 , and for the $[2-^{13}\text{C}]$ and $[3-^{13}\text{C}]$ isotopologues of pyruvate- d_3 in natural abundance. This method could also be applied to many other molecules which benefit from perdeuteration: $[5-^{13}\text{C}]$ -glutamine,³² $[1,4-^{13}\text{C}_2]$ fumarate,³³ $[1-^{13}\text{C}]$ urea,³⁴ $[1-^{13}\text{C}]$ -DHA,³⁵ or even $[2-^{13}\text{C}]$ fructose.³⁶

Preparation of $[1-^{13}\text{C}]$ Pyruvate- d_3 . $[1-^{13}\text{C}]$ sodium pyruvate (Cambridge Isotope Laboratories; 111 mg, 1 mmol) was added to 20 mL D_2O 99.9% (Cambridge Isotope Laboratories). The pH of the solution was adjusted to 10 with 10 μL of 1 M NaOD (Sigma-Aldrich). The solution was stirred for one day at room temperature. The extent of the exchange reaction was monitored by ^1H and ^{13}C NMR after stopping the reaction by adding 1 M DCl (Sigma-Aldrich) to lower the pH to 7. The reaction produces $[1-^{13}\text{C}]$ pyruvate- d_3 with a yield >90%. The side product $[1,6-^{13}\text{C}]$ parapyruvate- d_6 was produced with a yield <10%. Since no further purification was needed for our purpose, the solution was directly lyophilized in order to remove deuterated water. A white powder was obtained. Further purification by chromatography may be desired for real-time metabolic imaging experiments.

Preparation of Samples for D-DNP. Solutions in D_2O :glycerol- d_8 (1:1) of 1.5 M $[1,2-^{13}\text{C}_2]$ sodium acetate and 1.5 M $[1-^{13}\text{C}]$ sodium pyruvate were doped with 50 mM TEMPOL (Sigma-Aldrich). Solutions in H_2O : D_2O :glycerol- d_8 (1:4:5) of 1.5 M $[1,2-^{13}\text{C}_2]$ sodium acetate- d_3 and 1.5 M $[1-^{13}\text{C}]$ sodium pyruvate- d_3 were also doped with 50 mM TEMPOL (Sigma-Aldrich).

D-DNP Experiments. DNP was performed at 1.2 K and 6.7 T in a home-built polarizer by applying CW microwave irradiation at $f_{\mu\text{w}} = 188.3$ GHz and $P_{\mu\text{w}} = 100$ mW. CP contacts

were established at intervals of 5 min with 30 W applied to the ^1H and 45 W to the ^{13}C channels (radio frequency amplitudes of 40 kHz on both channels). Five frozen beads of 10 μL each of the sample were mixed with 5 frozen beads of 10 μL each containing 3 M ascorbate in D_2O . They were dissolved together with 5 mL of D_2O (preheated to $T = 400$ K at $P = 1.0$ MPa) and intimately mixed in 700 ms, transferred in 3.5 s to a 7 T Bruker magnet through a 1.5 mm inner diameter PTFE tube in a 0.8 T magnetic tunnel, pressurized with helium gas at 0.6 MPa. After injection in 0.5 s into a 5 mm NMR tube containing 250 μL of D_2O to allow field-frequency locking prior to injection, 5° detection pulses were applied every 5 s to record the decay of the hyperpolarized ^{13}C signal.

RESULTS

A 1.5 M solution of sodium $[1-^{13}\text{C}]$ pyruvate- d_3 and 50 mM TEMPOL in D_2O : H_2O :glycerol- d_8 (4:1:5) has a ^1H concentration of ca. 11 M. About 50 μL of this solution were inserted in our polarizer operating at $B_0 = 6.7$ T and $T = 1.2$ K^{31,37} equipped with a doubly tuned saddle-coil probe operating at $f(^1\text{H}) = 285.23$ MHz and $f(^{13}\text{C}) = 71.73$ MHz.²³ The sample was irradiated at $f_{\mu\text{w}} = 188.3$ GHz (maximum of negative polarization transfer) with $P_{\mu\text{w}} = 100$ mW, and $^1\text{H} \rightarrow ^{13}\text{C}$ CP was performed with multiple adiabatic contacts as described elsewhere.²³ Figure 1a shows how the ^{13}C polarization of deuterated pyruvate can be increased stepwise by CP from remote solvent protons. If a CP contact is established every 5 min, the ^{13}C polarization builds up with a time constant $\tau_{\text{DNP}}(^1\text{H} \rightarrow ^{13}\text{C}) = 950 \pm 23$ s. This polarization build-up is comparable to the one observed with nondeuterated $[1-^{13}\text{C}]$ -pyruvate ($\tau_{\text{DNP}}(^1\text{H} \rightarrow ^{13}\text{C}) = 990 \pm 30$ s) under the same conditions (Figure 1b). The $[1-^{13}\text{C}]$ polarization in deuterated pyruvate measured after dissolution and transfer to a 300 MHz NMR spectrometer (Figure 1) is slightly higher ($P(^{13}\text{C}) = 40.4\%$) than in its nondeuterated counterpart ($P(^{13}\text{C}) = 37.9\%$) (see Table 1). The relaxation time $T_1(^{13}\text{C})$ of $[1-^{13}\text{C}]$ is almost

identical for deuterated and nondeuterated [$1\text{-}^{13}\text{C}$]pyruvate. On the other hand, [$2\text{-}^{13}\text{C}$] and [$3\text{-}^{13}\text{C}$] show significant extensions of $T_1(^{13}\text{C})$ upon deuteration respectively by factors 1.15 and 9.75. The obvious reason for this extension of $T_1(^{13}\text{C})$ is the absence of heteronuclear $^1\text{H}\text{-}^{13}\text{C}$ dipolar relaxation. In addition to the remarkable extension of $T_1(^{13}\text{C})$ in [$3\text{-}^{13}\text{C}$], we observed a significant improvement in nuclear polarization measured 10 s after dissolution. The polarization of the [$3\text{-}^{13}\text{C}$] was improved by a factor of 3 in the deuterated molecules after dissolution, simply because the polarization was better preserved during its transfer through “death valley” where the field drops to about 0.8 T despite our magnetic tunnel between the polarizer and the NMR magnet. Similar improvements are reported in Figure 2 and Table 1 for deuterated [$1,2\text{-}^{13}\text{C}_2$]acetate- d_3 and protonated [$1,2\text{-}^{13}\text{C}_2$]acetate.

DNP of deuterated molecules with low-gamma nuclei that are remote from solvent ^1H spins can benefit from a combination of efficient ^1H DNP and subsequent $^1\text{H} \rightarrow ^{13}\text{C}$ CP. This scheme can obviously also be applied to molecules that do not contain any proton spins. Because deuterated molecules exhibit long relaxation times, their enhanced polarization is better preserved during dissolution, transfer, and injection. Finally, for the exact same reasons, the enhanced polarization is available over longer time scales. We advocate the combination of deuteration with cross-polarization (CP), and we believe that other nuclei such as ^{15}N will benefit even more from this approach.²¹

AUTHOR INFORMATION

Corresponding Authors

*E-mail: aurelien.bornet@epfl.ch.

*E-mail: sami.jannin@epfl.ch. Fax: (+41) 21-693-94-35. Homepage: <http://lrmb.epfl.ch>.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Martial Rey and Dr. Pascal Miéville for valuable assistance. This work was supported by the Swiss National Science Foundation, the Ecole Polytechnique Fédérale de Lausanne (EPFL), the Swiss Commission for Technology and Innovation (CTI), Bruker BioSpin Switzerland AG, and the French CNRS.

REFERENCES

- (1) Abragam, A.; Goldman, M. Principles of Dynamic Nuclear-Polarization. *Rep. Prog. Phys.* **1978**, *41*, 395.
- (2) Ardenkjaer-Larsen, J. H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M. H.; Servin, R.; Thaning, M.; Golman, K. Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10158.
- (3) van Heeswijk, R. B.; Uffmann, K.; Comment, A.; Kurdzesau, F.; Perazzolo, C.; Cudalbu, C.; Jannin, S.; Konter, J. A.; Hautle, P.; van den Brandt, B.; Navon, G.; van der Klink, J. J.; Gruetter, R. Hyperpolarized Lithium-6 as a Sensor of Nanomolar Contrast Agents. *Magn. Reson. Med.* **2009**, *61*, 1489.
- (4) Sarkar, R.; Comment, A.; Vasos, P. R.; Jannin, S.; Gruetter, R.; Bodenhausen, G.; Hall, H.; Kirik, D.; Denisov, V. P. Proton NMR of ^{15}N Choline Metabolites Enhanced by Dynamic Nuclear Polarization. *J. Am. Chem. Soc.* **2009**, *131*, 16014.
- (5) Miéville, P.; Jannin, S.; Helm, L.; Bodenhausen, G. Kinetics of yttrium-ligand complexation monitored using hyperpolarized ^{89}Y as a model for gadolinium in contrast agents. *J. Am. Chem. Soc.* **2010**, *132*, 5006.
- (6) Comment, A.; Jannin, S.; Hyacinthe, J. N.; Miéville, P.; Sarkar, R.; Ahuja, P.; Vasos, P. R.; Montet, X.; Lazeyras, F.; Vallée, J. P.; Hautle, P.; Konter, J. A.; van den Brandt, B.; Ansermet, J. P.; Gruetter, R.; Bodenhausen, G. Hyperpolarizing Gases via Dynamic Nuclear Polarization and Sublimation. *Phys. Rev. Lett.* **2010**, *105*, 18104.
- (7) Golman, K.; in't Zandt, R.; Thaning, M. Real-time metabolic imaging. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 11270.
- (8) Nelson, S. J.; Kurhanewicz, J.; Vigneron, D. B.; Larson, P. E. Z.; Harzstark, A. L.; Ferrone, M.; van Criekinge, M.; Chang, J. W.; Bok, R.; Park, I.; Reed, G.; Carvajal, L.; Small, E. J.; Munster, P.; Weinberg, V. K.; Ardenkjaer-Larsen, J. H.; Chen, A. P.; Hurd, R. E.; Odegardstuen, L. I.; Robb, F. J.; Tropp, J.; Murray, J. A. Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [$1\text{-}^{13}\text{C}$]Pyruvate. *Sci. Transl. Med.* **2013**, *5*, 198ra108.
- (9) Chen, H. Y.; Ragavan, M.; Hilty, C. Protein Folding Studied by Dissolution Dynamic Nuclear Polarization. *Angew. Chem., Int. Ed. Engl.* **2013**, *52*, 9192.
- (10) Lee, Y.; Heo, G. S.; Zeng, H. F.; Wooley, K. L.; Hilty, C. Detection of Living Anionic Species in Polymerization Reactions Using Hyperpolarized NMR. *J. Am. Chem. Soc.* **2013**, *135*, 4636.
- (11) Miéville, P.; Ahuja, P.; Sarkar, R.; Jannin, S.; Vasos, P. R.; Gerber-Lemaire, S.; Mishkovsky, M.; Comment, A.; Gruetter, R.; Ouari, O.; Tordo, P.; Bodenhausen, G. Scavenging free radicals to preserve enhancement and extend relaxation times in NMR using dynamic nuclear polarization. *Angew. Chem., Int. Ed.* **2010**, *49*, 6182.
- (12) Carravetta, M.; Johannessen, O. G.; Levitt, M. H. Beyond the T1 limit: Singlet nuclear spin states in low magnetic fields. *Phys. Rev. Lett.* **2004**, *92*, 153003.
- (13) Tayler, M. C. D.; Marco-Rius, I.; Kettunen, M. I.; Brindle, K. M.; Levitt, M. H.; Pileio, G. Direct Enhancement of Nuclear Singlet Order by Dynamic Nuclear Polarization. *J. Am. Chem. Soc.* **2012**, *134*, 7668.
- (14) Vasos, P. R.; Comment, A.; Sarkar, R.; Ahuja, P.; Jannin, S.; Ansermet, J. P.; Konter, J. A.; Hautle, P.; van den Brandt, B.; Bodenhausen, G. Long-lived states to sustain hyperpolarized magnetization. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 18469.
- (15) Ahuja, P.; Sarkar, R.; Jannin, S.; Vasos, P. R.; Bodenhausen, G. Proton hyperpolarisation preserved in long-lived states. *Chem. Commun.* **2010**, *46*, 8192.
- (16) Pileio, G.; Bowen, S.; Laustsen, C.; Tayler, M. C. D.; Hill-Cousins, J. T.; Brown, L. J.; Brown, R. C. D.; Ardenkjaer-Larsen, J. H.; Levitt, M. H. Recycling and Imaging of Nuclear Singlet Hyperpolarization. *J. Am. Chem. Soc.* **2013**, *135*, 5084.
- (17) Feng, Y. S.; Theis, T.; Liang, X. F.; Wang, Q.; Zhou, P.; Warren, W. S. Storage of Hydrogen Spin Polarization in Long-Lived C-13(2) Singlet Order and Implications for Hyperpolarized Magnetic Resonance Imaging. *J. Am. Chem. Soc.* **2013**, *135*, 9632.
- (18) Barb, A. W.; Hekmatyar, S. K.; Glushka, J. N.; Prestegard, J. H. Probing alanine transaminase catalysis with hyperpolarized (CD3)-C-13-pyruvate. *J. Magn. Reson.* **2013**, *228*, 59.
- (19) Chiavazza, E.; Viale, A.; Karlsson, M.; Aime, S. N-15-Permethylated amino acids as efficient probes for MRI-DNP applications. *Contrast Media Mol. I* **2013**, *8*, 417.
- (20) Barb, A. W.; Hekmatyar, S. K.; Glushka, J. N.; Prestegard, J. H. Exchange facilitated indirect detection of hyperpolarized (ND2)-N-15-amido-glutamine. *J. Magn. Reson.* **2011**, *212*, 304.
- (21) Nonaka, H.; Hata, R.; Doura, T.; Nishihara, T.; Kumagai, K.; Akakabe, M.; Tsuda, M.; Ichikawa, K.; Sando, S. A platform for designing hyperpolarized magnetic resonance chemical probes. *Nat. Commun.* **2013**, *4*, 2411.
- (22) Lumata, L.; Merritt, M. E.; Kovacs, Z. Influence of deuteration in the glassing matrix on C-13 dynamic nuclear polarization. *Phys. Chem. Chem. Phys.* **2013**, *15*, 7032.
- (23) Bornet, A.; Melzi, R.; Linde, A. J. P.; Hautle, P.; van den Brandt, B.; Jannin, S.; Bodenhausen, G. Boosting Dissolution Dynamic Nuclear Polarization by Cross Polarization. *J. Phys. Chem. Lett.* **2013**, *4*, 111.
- (24) Ardenkjaer-Larsen, J. H.; Macholl, S.; Johannesson, H. Dynamic nuclear polarization with trityls at 1.2 K. *Appl. Magn. Reson.* **2008**, *34*, 509.

(25) Ardenkjaer-Larsen, J. H.; Leach, A. M.; Clarke, N.; Urbahn, J.; Anderson, D.; Skloss, T. W. Dynamic Nuclear Polarization Polarizer for Sterile Use Intent. *NMR Biomed.* **2011**, *24*, 927.

(26) Jóhannesson, H.; Macholl, S.; Ardenkjaer-Larsen, J. H. Dynamic Nuclear Polarization of [1-¹³C]pyruvic acid at 4.6 T. *J. Magn. Reson.* **2009**, *197*, 167.

(27) Hartmann, S. R.; Hahn, E. L. Nuclear Double Resonance in Rotating Frame. *Phys. Rev.* **1962**, *128*, 2042.

(28) Jannin, S.; Bornet, A.; Colombo, S.; Bodenhausen, G. Low-temperature cross polarization in view of enhancing dissolution Dynamic Nuclear Polarization in NMR. *Chem. Phys. Lett.* **2011**, *517*, 234.

(29) Bornet, A.; Melzi, R.; Jannin, S.; Bodenhausen, G. Cross Polarization for Dissolution Dynamic Nuclear Polarization Experiments at Readily Accessible Temperatures $1.2 < T < 4.2$ K. *Appl. Magn. Reson.* **2012**, *43*, 107.

(30) Batel, M.; Krajewski, M.; Dapp, A.; Hunkeler, A.; Meier, B. H.; Kozerke, S.; Ernst, M. Dissolution dynamic nuclear polarization efficiency enhanced by Hartmann-Hahn cross polarization. *Chem. Phys. Lett.* **2012**, *554*, 72.

(31) Jannin, S.; Bornet, A.; Melzi, R.; Bodenhausen, G. High field dynamic nuclear polarization at 6.7 T: Carbon-13 polarization above 70% within 20 min. *Chem. Phys. Lett.* **2012**, *549*, 99.

(32) Gallagher, F. A.; Kettunen, M. I.; Day, S. E.; Lerche, M.; Brindle, K. M. C-13 MR spectroscopy measurements of glutaminase activity in human hepatocellular carcinoma cells using hyperpolarized C-13-labeled glutamine. *Magn. Reson. Med.* **2008**, *60*, 253.

(33) Gallagher, F. A.; Kettunen, M. I.; Hu, D. E.; Jensen, P. R.; in't Zandt, R.; Karlsson, M.; Gisselsson, A.; Nelson, S. K.; Witney, T. H.; Bohndiek, S. E.; Hansson, G.; Peitersen, T.; Lerche, M. H.; Brindle, K. M. Production of hyperpolarized [1,4-C-¹³(2)]malate from [1,4-C-¹³(2)]fumarate is a marker of cell necrosis and treatment response in tumors. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 19801.

(34) von Morze, C.; Larson, P. E. Z.; Hu, S.; Keshari, K.; Wilson, D. M.; Ardenkjaer-Larsen, J. H.; Goga, A.; Bok, R.; Kurhanewicz, J.; Vigneron, D. B. Imaging of Blood Flow Using Hyperpolarized [C-¹³] Urea in Preclinical Cancer Models. *J. Magn. Reson. Imaging* **2011**, *33*, 692.

(35) Gallagher, F. A.; Kettunen, M. I.; Brindle, K. M. Biomedical applications of hyperpolarized C-13 magnetic resonance imaging. *Prog. Nucl. Magn. Reson. Spectrosc.* **2009**, *55*, 285.

(36) Keshari, K. R.; Wilson, D. M.; Chen, A. P.; Bok, R.; Larson, P. E. Z.; Hu, S.; Van Criekinge, M.; Macdonald, J. M.; Vigneron, D. B.; Kurhanewicz, J. Hyperpolarized [2-C-¹³]-Fructose: A Hemiketal DNP Substrate for In Vivo Metabolic Imaging. *J. Am. Chem. Soc.* **2009**, *131*, 17591.

(37) Comment, A.; van den Brandt, B.; Uffmann, K.; Kurdzesau, F.; Jannin, S.; Konter, J. A.; Hautle, P.; Wenckebach, W. T. H.; Gruetter, R.; van der Klink, J. J. Design and performance of a DNP prepolarizer coupled to a rodent MRI scanner. *Concepts Magn. Reson. B* **2007**, *31B*, 255.