Isotopic Labeling

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Efficient Synthesis of Molecular Precursors for Para-Hydrogen-Induced Polarization of Ethyl Acetate-1-^{13}C and Beyond

*Roman V. Shchepin, Danila A. Barskiy, Aaron M. Coffey, Isaac V. Manzanera Esteve, and Eduard Y. Chekmenev**

Abstract: *A scalable and versatile methodology for production of vinylated carboxylic compounds with ^{13}C isotopic label in C1 position is described. It allowed synthesis of vinyl acetate-1-^{13}C, which is a precursor for preparation of ^{13}C hyperpolarized ethyl acetate-1-^{13}C, which provides a convenient vehicle for potential in vivo delivery of hyperpolarized acetate to probe metabolism in living organisms. Kinetics of vinyl acetate molecular hydrogenation and polarization transfer from para-hydrogen to ^{13}C via magnetic field cycling were investigated. Nascent proton nuclear spin polarization ($\%P_H$) of ca. 3.3 % and carbon-13 polarization ($\%P_{13C}$) of ca. 1.8 % were achieved in ethyl acetate utilizing 50 % para-hydrogen corresponding to ca. 50 % polarization transfer efficiency. The use of nearly 100% para-hydrogen and the improvements of $\%P_H$ of para-hydrogen-nascent protons may enable production of ^{13}C hyperpolarized contrast agents with $\%P_{13C}$ of 20–50 % in seconds using this chemistry.*

Hyperpolarized (HP) magnetic resonance[1] is a rapidly growing field, which enables real-time metabolic imaging.[2] This is possible because nuclear spin polarization (P) of long-lived (on the order of a minute or more) ^{13}C sites in biologically relevant molecules can be enhanced transiently by 4–8 orders of magnitude[3] to the order of unity or 100 %. Dissolution dynamic nuclear polarization (d-DNP)[3a] is one of the leading hyperpolarization technologies, which has advanced into clinical trials,[4] and its success has been largely driven by a wide range of biomolecules amenable for efficient hyperpolarization. The alternative hyperpolarization technique of para-hydrogen induced polarization (PHIP)[5] has two advantages over d-DNP: 1) fast production speed of under 1 min versus tens of minutes[6] to several hours, and 2) it is significantly less instrumentation demanding.[7] Therefore, PHIP may potentially become an ultra-fast and low-cost hyperpolarization technique for affordable production of multiple doses of HP contrast agents within minutes.[8] However, unlike d-DNP, the PHIP technique relies on the pairwise addition of para-hydrogen (para-H_2) to an unsaturated precursor usually followed by polarization transfer from nascent protons to ^{13}C centers, with substantially longer P decay times (T_1) required for in vivo applications.[9] While a number of metabolic ^{13}C HP contrast agents have been developed for in vivo applications with $\%P_{13C} \geq 10\%$ in aqueous medium (e.g. succinate[2e,10] and phospholactate[3b,11]), PHIP remained a relatively restricted technology because of the chemical challenge of inserting para-H_2 adjacently to ^{13}C in molecular frameworks to yield metabolically relevant contrast agents: for example, acetate, pyruvate.[11a]

Recently PHIP using side arm hydrogenation (SAH) was demonstrated,[12] in which para-H_2 is added into vinyl moiety, and para-H_2-derived polarization is transferred to carboxylic ^{13}C atom. This is fundamentally possible, because in PHIP-SAH the ^{13}C atom is hyperpolarized by nascent protons three and four chemical bonds away using $^3J_{H-13C}$ and $^4J_{H-13C}$[12,13] rather than the $^2J_{H-13C}$ and $^3J_{H-13C}$ in the conventional PHIP approach.[9,14] As a result, PHIP-SAH significantly expands the reach of amenable-to-hyperpolarization biomolecules, including ethyl acetate-1-^{13}C, ethyl pyruvate-1-^{13}C, and potentially many others. Ethylation is not necessarily a drawback, because the produced HP contrast agent can be de-protected,[12] or used directly, because ethylation of carboxylic acids leads to better cellular[10b] and brain uptake.[15] The uptake in the brain is especially relevant to ethyl acetate, because acetate is one of a few metabolites directly utilized by the brain as a fuel source.[16]

Despite the potential of PHIP-SAH to revolutionize molecular imaging, it is faced with two fundamental challenges. First, an efficient synthesis of vinylated 1-^{13}C-carboxylates must be developed. Second, $\%P_{13C}$ of only 2.3 % (using on 92 % of para-H_2) was achieved by Cavallari et al.,[13] and a further significant $\%P_{13C}$ boost is required for in vivo applications. Hence, this work is focused on 1) developing an efficient synthetic procedure for production of vinyl acetate-1-^{13}C, and 2) investigating the field cycling polarization transfer process used in PHIP-SAH to improve $\%P_{13C}$.

A number of methodologies for the preparation of vinyl acetate with various isotopic labeling patterns have been described. Roberts et al.[17] developed a procedure based on the mercury-catalyzed reaction of ^{14}C-labeled acetylene and acetic acid. Similar methodology, based on stoichiometric amount of mercury ethoxide and acetyl chloride-D_3, was

[*] Prof. R. V. Shchepin, Dr. D. A. Barskiy, Dr. A. M. D. Coffey, Dr. I. V. Manzanera Esteve, Prof. E. Y. Chekmenev
Department of Radiology
Vanderbilt University Institute of Imaging Science (VUIIS)
Department of Biomedical Engineering
Vanderbilt-Ingram Cancer Center (VICC)
Vanderbilt University
Nashville, TN 37232 (USA)
Prof. E. Y. Chekmenev
Russian Academy of Sciences
119991 Moscow (Russia)
E-mail: eduard.chekmenev@vanderbilt.edu

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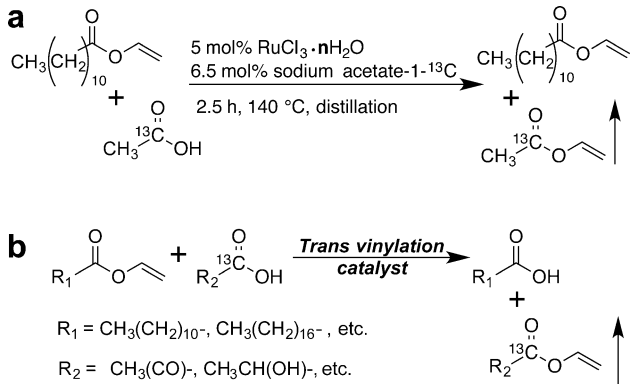
applied to the preparation of vinyl acetate-D_3 by Kim and Caserio.[18] Alternatively, Livshits and Isagulyants[19] showed an efficient reaction between the ^{14}C-labeled acetic acid and acetylene catalyzed by zinc acetate in the gas-phase. While vinyl acetate is industrially produced by vapor-phase acetoxylation of ethylene over Pd-based catalysts,[20] such large-scale gas-phase processes are poorly suited for significantly smaller-scale production of isotopically enriched vinyl acetate-1-^{13}C. Earlier variants of synthetic procedures with interexchange of vinyl groups were based on mercury catalysis,[21] which had some obvious disadvantages of toxicity and laborious workup. Another potential alternative is based on the recent advances in ruthenium-based catalysis.[22] However, the equilibrium between vinyl acetate-1-^{13}C and its unlabeled counterpart was not directly amenable to the preparation of vinyl acetate-1-^{13}C.

Replacement of natural abundance vinyl acetate (VA) by vinyl laurate and the application of distillation column allowed for convenient removal of vinyl acetate-1-^{13}C (VA-1-^{13}C) from the reaction mixture (Scheme 1 a). Owing to the flexible nature of the substrates participating in the vinyl exchange, this method of ^{13}C enrichment can be applied to a variety of potential PHIP targets, such as pyruvate and lactate derivatives (Scheme 1 b).



Scheme 1. a) Reaction scheme for the preparation of vinyl acetate-1-^{13}C (VA-1-^{13}C). b) Generalized scheme for preparation of potential targets with vinylated ^{13}C carboxyl groups. The vertical arrows indicate that the product leaves the mixture as a gas.

We utilized the Rh catalyst [Rh(NBD)(dppb)]BF_4 ([(bicyclo[2.2.1]hepta-2,5-diene)[1,4-bis(diphenylphosphino)butane]rhodium(I) tetrafluoroborate) for molecular addition of para-H_2 to VA, analogous to that used in the recent PHIP-SAH studies (Figure 1 a).[12,13] A previously developed setup for high-pressure experiments with para-H_2 was utilized (Supporting Information),[23] demonstrating that higher para-H_2 pressure significantly accelerates VA hydrogenation to EA (Figure 1 b). Therefore, the highest pressure achievable in this setup (ca. 7.2 bar) was used in further PHIP-SAH experiments. Pairwise addition of 50 % para-H_2 was performed in the Earth magnetic field (ca. 50 μT) and then the sample was quickly (ca. 2 s) adiabatically transferred to 9.4 T for HP ^{1}H NMR detection of nascent protons (corresponding to ALTADENA[24] conditions). The detected HP ^{1}H NMR signal initially rises (during fast product build-up) and then decreases (when the contribution of HP product relaxation overweighs formation of new HP

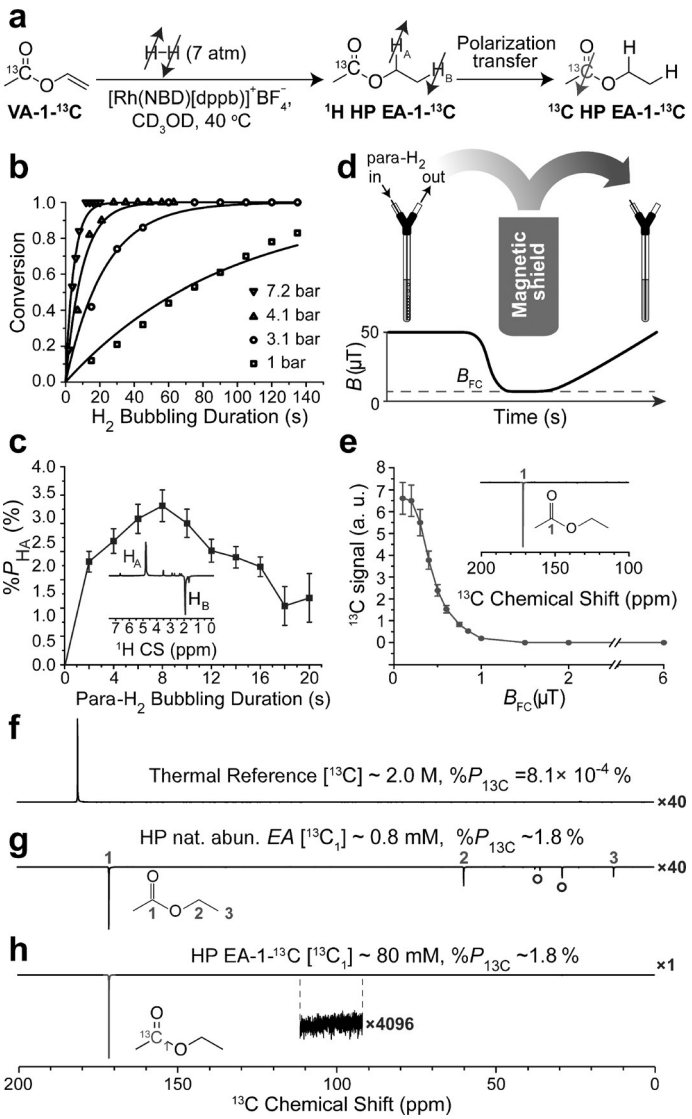


Figure 1. a) Molecular addition of para-H_2 to vinyl acetate-1-^{13}C (VA-1-^{13}C) followed by polarization transfer resulting in ^{13}C hyperpolarized ethyl acetate-1-^{13}C (^{13}C HP EA-1-^{13}C); b) Conversion profile for vinyl acetate (VA, 80 mM, in [D_4]MeOH, at ca. 40 °C maintained by the 9.4 T NMR spectrometer) hydrogenation reaction in four pressure regimes; c) Dependence of "H_A" signal of hyperpolarized ethyl acetate (^{1}H HP EA) on the para-H_2 bubbling duration at the Earth's magnetic field (resulting in ALTADENA[24]-type spectrum shown in inset); d) Top: schematic representation of the experimental setup for magnetic field cycling: hydrogenation is carried out at the Earth's magnetic field, the sample then is quickly moved inside a μ-metal shield (with magnetic field B_{FC}) and slowly transferred from the shield for subsequent NMR detection; bottom: schematic magnetic field profile during the field cycling; e) Dependence of HP 1–^{13}C NMR signal (shown in the insert) of ethyl acetate (^{13}C HP EA) on the B_{FC}; f) Thermal spectrum of ^{13}C signal reference sodium acetate-1-^{13}C (ca. 2.0 M); g) ^{13}C HP spectrum of natural abundance 80 mM ethyl acetate (^{13}C HP EA). Note the resonances labeled with ° correspond to HP ^{13}C resonances originating from the hydrogenation catalyst (Figure S7); h) HP ^{13}C spectrum of 80 mM ethyl acetate-1-^{13}C (^{13}C HP EA-1-^{13}C).

species) with the duration of para-H_2 bubbling (Figure 1c). The conditions corresponding to bubbling duration of about 8–10 s yielded maximum observed $\%P_H$ of 3.3% (equivalent to ^{1}H signal enhancement $\varepsilon_{1H} > 1000$ fold) and thus, were used to transfer polarization from HP nascent protons to 1-^{13}C using magnetic field cycling[9,12] (Figure 1d). In this approach, the pairwise para-H_2 addition is performed in the Earth's field, the sample is then quickly moved in a magnetic field $< 1\,\mu T$ (B_{FC}) and hyperpolarization is transferred to ^{13}C during slow (adiabatic) sample transfer back to the Earth's field (Figures 1c,d). B_{FC} adjustment of polarization transfer was carried out by measuring ^{13}C HP NMR signal of natural abundance EA (ca. 1.1% ^{13}C in each carbon site) produced by hydrogenation of 80 mM VA with para-H_2 (Figure 1e). In-shield field (B_{FC}) of 0.1–0.2 μT provided the maximal HP transfer efficiency, and therefore it was used in all subsequent polarization transfer experiments.

The maximum detected ε_{13C} in HP EA was over 2200 fold for the 1-^{13}C site corresponding to $\%P_{13C}$ of around 1.8% using 50% para-H_2 (Figure 1g). A similar $\%P_{13C}$ was also achieved for HP ^{13}C EA-1-^{13}C (Figure 1h). When compared to natural abundance HP EA, HP ^{13}C EA-1-^{13}C carries around a 90-times greater polarization payload owing to the 98% ^{13}C isotopic enrichment of the ^{13}C site. The enriched site was employed for ^{13}C 3D ultra-fast magnetic resonance imaging (MRI; Figure 2) showing the feasibility of high-resolution (pixel size of $0.5 \times 0.5 \times 4\,mm^3$ and ca. 2.5 s duration) molecular imaging with this contrast agent using 15.2 T Bruker MRI scanner.

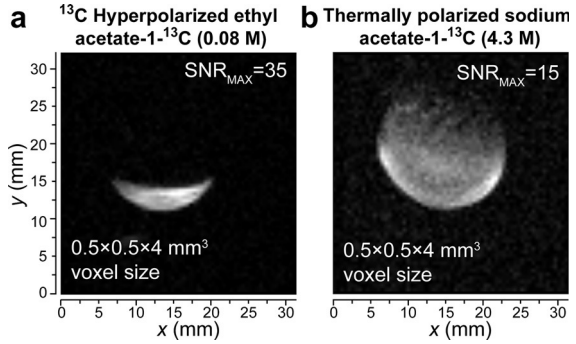


Figure 2. ^{13}C 3D MRI of a) a hollow spherical plastic ball partially filled with 80 mM HP EA-1-^{13}C and b) a plastic sphere (ca. 2.8 mL) filled with thermally polarized 4.3 M sodium acetate-1-^{13}C reference phantom. Both 3D true-FISP images were acquired using 15 mm outside-diameter (OD) round radio-frequency (RF) surface coil tuned to 163.4 MHz in 15.2 T small-animal Bruker MRI scanner (see Supporting Information for additional details). One representative slice is shown for each 3D image. SNR = signal to noise ratio.

If around 100% para-H_2[25] were to be employed, the effective $\%P_H$ and $\%P_{13C}$ would be tripled to yield 10% and 5.5% respectively. These values would more than double the reported pioneering results.[13] Moreover, the efficiency of polarization transfer from nascent para-H_2 protons to 1-^{13}C was approximately 50%, which is in quantitative agreement with previous theoretical simulation for similar spin system of 2-hydroxyethyl propionate-1-^{13}C.[14] The expected $\%P_{13C}$ of approximately 5.5% is clearly largely limited by the HP source of $\%P_H$ of around 10% in this study and most likely in recent work by Reineri and co-workers.[13] Therefore, future studies must focus on the $\%P_H$ increase and understanding factors leading to the polarization losses. There are three possible major $\%P_H$-reducing barriers: 1) the degree of pairwise addition of para-H_2 to the vinyl moiety,[5a,26] 2) nascent protons' P relaxation, and 3) singlet–triplet mixing of nascent protons.[14] The first challenge is not a fundamental barrier, because similar catalysts yielded $\%P_{13C}$ of around 30–50% on similar molecules[14,27] indicating that $\%P_H$ was 50–100%. On the other hand, the other two barriers have always been addressed via the use of high-pressure automated spray-injection PHIP polarizers[8,28] operating at elevated temperatures, and enabling ultra-fast substrate conversion (1–3 s and thereby minimizing the effects of spin-lattice relaxation) and ^{1}H decoupling that allows all molecules to retain the singlet states during the course of hydrogenation reaction.[14] While additional future studies investigating the reasons behind low $\%P_H$ in this and previous PHIP-SAH studies are certainly warranted, the use of such PHIP polarizers[8,28] will likely provide the remedy and can potentially yield $\%P_{13C}$ of up to 50% based on the efficiency of H→^{13}C polarization transfer demonstrated herein. The generality and flexibility of our *trans* vinylation approach will benefit future efficient preparation of other vinylated analogues of metabolically relevant compounds, such as lactate and pyruvate for PHIP-SAH.[2a,b] This would pave the way for the future in vivo imaging of metabolically impaired conditions such as cancer[2a,b] and brain[16] damage. In particular, future in vivo studies in animal models can be carried out with[8a] or without Rh-based PHIP catalysts removal (which are generally well tolerated by animals and cause no clinical toxicity[29]) in a manner similar to the previous use of HP succinate-1-^{13}C[2e,8b,10b] and HP 2-hydroxyethyl propionate.[9,27] The ultimate clinical translation will require employing Rh-based PHIP catalyst filtration/removal[8a] and improving of the filtration/removal step or the alternative use of improved heterogeneous PHIP catalysis.[26] Moreover, future in vivo translation of this work would require the use of water-soluble catalysts, which have been successfully employed in combination with high-pressure PHIP polarizers[8,28] to produce aqueous solution of HP succinate-^{13}C,[10a] phospholactate-1-^{13}C[3b] and others with $\%P_{13C}$ exceeding 15% and T_1 in excess of 40 s.

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Keywords: contrast agents · ethyl acetate · hyperpolarization · MRI · para-hydrogen induced polarization (PHIP)

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[1] a) A. Abragam, M. Goldman, *Rep. Prog. Phys.* **1978**, *41*, 395 – 467; b) B. M. Goodson, *J. Magn. Reson.* **2002**, *155*, 157 – 216; c) P. Nikolaou, B. M. Goodson, E. Y. Chekmenev, *Chem. Eur. J.* **2015**, *21*, 3156 – 3166.

[2] a) J. Kurhanewicz, D. B. Vigneron, K. Brindle, E. Y. Chekmenev, A. Comment, C. H. Cunningham, R. J. DeBerardinis, G. G. Green, M. O. Leach, S. S. Rajan, R. R. Rizi, B. D. Ross, W. S. Warren, C. R. Malloy, *Neoplasia* **2011**, *13*, 81 – 97; b) K. M. Brindle, *J. Am. Chem. Soc.* **2015**, *137*, 6418 – 6427; c) A. Comment, M. E. Merritt, *Biochemistry* **2014**, *53*, 7333 – 7357; d) K. Golman, R. in't Zandt, M. Thaning, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11270 – 11275; e) P. Bhattacharya, E. Y. Chekmenev, W. H. Perman, K. C. Harris, A. P. Lin, V. A. Norton, C. T. Tan, B. D. Ross, D. P. Weitekamp, *J. Magn. Reson.* **2007**, *186*, 150 – 155.

[3] a) J. H. Ardenkjaer-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning, K. Golman, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10158 – 10163; b) R. V. Shchepin, A. M. Coffey, K. W. Waddell, E. Y. Chekmenev, *Anal. Chem.* **2014**, *86*, 5601 – 5605.

[4] S. J. Nelson, J. Kurhanewicz, D. B. Vigneron, P. E. Z. Larson, A. L. Harzstark, M. Ferrone, M. van Criekinge, J. W. Chang, R. Bok, I. Park, G. Reed, L. Carvajal, E. J. Small, P. Munster, V. K. Weinberg, J. H. Ardenkjaer-Larsen, A. P. Chen, R. E. Hurd, L. I. Odegardstuen, F. J. Robb, J. Tropp, J. A. Murray, *Sci. Transl. Med.* **2013**, *5*, 198ra108.

[5] a) C. R. Bowers, D. P. Weitekamp, *Phys. Rev. Lett.* **1986**, *57*, 2645 – 2648; b) T. C. Eisenschmid, R. U. Kirss, P. P. Deutsch, S. I. Hommeltoft, R. Eisenberg, J. Bargon, R. G. Lawler, A. L. Balch, *J. Am. Chem. Soc.* **1987**, *109*, 8089 – 8091; c) C. R. Bowers, D. P. Weitekamp, *J. Am. Chem. Soc.* **1987**, *109*, 5541 – 5542.

[6] A. Bornet, R. Melzi, A. J. Perez Linde, P. Hautle, B. van den Brandt, S. Jannin, G. Bodenhausen, *J. Phys. Chem. Lett.* **2013**, *4*, 111 – 114.

[7] W. Jiang, L. Lumata, W. Chen, S. Zhang, Z. Kovacs, A. D. Sherry, C. Khemtong, *Sci. Rep.* **2015**, *5*, 9104.

[8] a) J.-B. Hövener, E. Chekmenev, K. Harris, W. Perman, L. Robertson, B. Ross, P. Bhattacharya, *Magn. Reson. Mater. Phys.* **2009**, *22*, 111 – 121; b) J.-B. Hövener, E. Chekmenev, K. Harris, W. Perman, T. Tran, B. Ross, P. Bhattacharya, *Magn. Reson. Mater. Phys.* **2009**, *22*, 123 – 134.

[9] K. Golman, O. Axelsson, H. Johannesson, S. Mansson, C. Olofsson, J. S. Petersson, *Magn. Reson. Med.* **2001**, *46*, 1 – 5.

[10] a) E. Y. Chekmenev, J. Hovener, V. A. Norton, K. Harris, L. S. Batchelder, P. Bhattacharya, B. D. Ross, D. P. Weitekamp, *J. Am. Chem. Soc.* **2008**, *130*, 4212 – 4213; b) N. M. Zacharias, H. R. Chan, N. Sailasuta, B. D. Ross, P. Bhattacharya, *J. Am. Chem. Soc.* **2012**, *134*, 934 – 943.

[11] a) R. V. Shchepin, A. M. Coffey, K. W. Waddell, E. Y. Chekmenev, *J. Am. Chem. Soc.* **2012**, *134*, 3957 – 3960; b) R. V. Shchepin, W. Pham, E. Y. Chekmenev, *J. Labelled Compd. Radiopharm.* **2014**, *57*, 517 – 524.

[12] F. Reineri, T. Boi, S. Aime, *Nat. Commun.* **2015**, *6*, 5858.

[13] E. Cavallari, C. Carrera, T. Boi, S. Aime, F. Reineri, *J. Phys. Chem. B* **2015**, *119*, 10035 – 10041.

[14] M. Goldman, H. Johannesson, *C. R. Phys.* **2005**, *6*, 575 – 581.

[15] R. E. Hurd, Y.-F. Yen, D. Mayer, A. Chen, D. Wilson, S. Kohler, R. Bok, D. Vigneron, J. Kurhanewicz, J. Tropp, D. Spielman, A. Pfefferbaum, *Magn. Reson. Med.* **2010**, *63*, 1137 – 1143.

[16] S. Blüml, A. Moreno-Torres, F. Shic, C. H. Nguy, B. D. Ross, *NMR Biomed.* **2002**, *15*, 1 – 5.

[17] J. D. Roberts, C. C. Lee, W. H. Saunders, *J. Am. Chem. Soc.* **1954**, *76*, 4501 – 4510.

[18] J. K. Kim, M. C. Caserio, *J. Am. Chem. Soc.* **1982**, *104*, 4624 – 4629.

[19] V. S. Livshits, G. V. Isagulyants, *Russ. Chem. Bull.* **1966**, *15*, 1801 – 1802.

[20] a) M. Kuhn, J. Jeschke, S. Schulze, M. Hietschold, H. Lang, T. Schwarz, *Catal. Commun.* **2014**, *57*, 78 – 82; b) S. Nakamura, T. Yasui, *J. Catal.* **1970**, *17*, 366 – 374.

[21] R. L. Adelman, *J. Org. Chem.* **1949**, *14*, 1057 – 1077.

[22] J. Ziriakus, T. K. Zimmermann, A. Pothig, M. Drees, S. Haslinger, D. Jantke, F. E. Kuhn, *Adv. Synth. Catal.* **2013**, *355*, 2845 – 2859.

[23] M. L. Truong, T. Theis, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, E. Y. Chekmenev, *J. Phys. Chem. C* **2015**, *119*, 8786 – 8797.

[24] M. G. Pravica, D. P. Weitekamp, *Chem. Phys. Lett.* **1988**, *145*, 255 – 258.

[25] B. Feng, A. M. Coffey, R. D. Colon, E. Y. Chekmenev, K. W. Waddell, *J. Magn. Reson.* **2012**, *214*, 258 – 262.

[26] a) K. V. Kovtunov, V. V. Zhivonitko, I. V. Skovpin, D. A. Barskiy, I. V. Koptyug, *Top. Curr. Chem.* **2013**, *338*, 123 – 180; b) O. G. Salnikov, K. V. Kovtunov, I. V. Koptyug, *Sci. Rep.* **2015**, *5*, 13930.

[27] L. E. Olsson, C.-M. Chai, O. Axelsson, M. Karlsson, K. Golman, J. S. Petersson, *Magn. Reson. Med.* **2006**, *55*, 731 – 737.

[28] a) K. W. Waddell, A. M. Coffey, E. Y. Chekmenev, *J. Am. Chem. Soc.* **2011**, *133*, 97 – 101; b) S. Kadlecek, V. Vahdat, T. Nakayama, D. Ng, K. Emami, R. Rizi, *NMR Biomed.* **2011**, *24*, 933 – 942.

[29] H. R. Chan, P. Bhattacharya, A. Imam, A. Freundlich, T. T. Tran, W. H. Perman, A. P. Lin, K. Harris, E. Y. Chekmenev, M.-L. Ingram, B. D. Ross in *17th ISMRM Conference, April 18 – 24, Vol. 17*, Honolulu, Hawaii, **2009**, p. 2448.

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