Bioconjugate Chemistry

Communication

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Efficient Synthesis of Nicotinamide-1-^{15}N for Ultrafast NMR Hyperpolarization Using Parahydrogen

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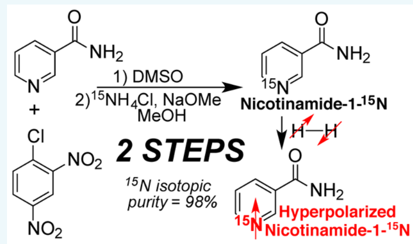
Supporting Information

ABSTRACT: Nicotinamide (a vitamin B_3 amide) is one of the key vitamins as well as a drug for treatment of *M. tuberculosis*, HIV, cancer, and other diseases. Here, an improved Zincke reaction methodology is presented allowing for straightforward and scalable synthesis of nicotinamide-1-^{15}N with an excellent isotopic purity (98%) and good yield (55%). ^{15}N nuclear spin label in nicotinamide-1-^{15}N can be NMR hyperpolarized in seconds using parahydrogen gas. NMR hyperpolarization using the process of temporary conjugation between parahydrogen and to-be-hyperpolarized biomolecule on hexacoordinate iridium complex via the Signal Amplification By Reversible Exchange (SABRE) method significantly increases detection sensitivity (e.g., >20 000-fold for nicotinamide-1-^{15}N at 9.4 T) as has been shown by Theis T. et al. (*J. Am. Chem. Soc.* **2015**, *137*, 1404), and hyperpolarized in this fashion, nicotinamide-1-^{15}N can be potentially used to probe metabolic processes in vivo in future studies. Moreover, the presented synthetic methodology utilizes mild reaction conditions, and therefore can also be potentially applied to synthesis of a wide range of ^{15}N-enriched N-heterocycles that can be used as hyperpolarized contrast agents for future in vivo molecular imaging studies.



NMR hyperpolarization increases nuclear spin polarization (P) by several orders of magnitude above equilibrium thermal polarization of nuclear spins achieved by high-field magnets (3−21 T).[1−3] This significant P increase enables concomitant gains in detection sensitivity. Small biomolecules with sufficiently slowly relaxing nuclear spins of ^{13}C and ^{15}N atoms (i.e., with T_1 on the order of tens of seconds or more) can be hyperpolarized and used in vivo to probe metabolism and function.[4−6] Isotopic enrichment of these slowly relaxing spins is mandatory to maximize the payload of hyperpolarization for MRI detection.[5] During one decade, hyperpolarized NMR and MRI progressed from a proof-of-principle concept[7−11] to clinical trials in human volunteers.[12,13]

The use of ^{15}N sites in hyperpolarized MRI[14] has a translational advantage over ^{13}C based hyperpolarized contrast agents: spin−lattice relaxation time can be significantly longer, up to tens of minutes vs approximately 1 min.[5,15] Moreover, we and others have recently demonstrated an additional advantage of hyperpolarization process speed: ^{15}N sites of N-heterocyclic compounds can be hyperpolarized in seconds via very simple and instrumentationally nondemanding approach of signal amplification by reversible exchange (SABRE[16]) in shield enables alignment transfer to heteronuclei (SABRE-SHEATH)[17−20] and other RF-based approaches.[21,22] While ^{15}N enrichment of prototype molecule ^{15}N-pyridine can be achieved by several techniques,[23] this compound itself has no significant biomedical relevance.

On the other hand, substituted pyridine-based ^{15}N-heterocycles represent key biomolecules and therefore can be potentially employed as molecular contrast agents. For example, they can be utilized for pH[24] and ion[25] sensing. Nicotinamide-1-^{15}N (vitamin B_3 amide), in particular, is potentially an attractive molecular imaging target, because it has low in vivo toxicity: LD50 of nicotinamide is 1.6 g/kg with intravenous injection in dogs.[26] Nicotinamide is a safe active ingredient for treatment of hyperlipidemia in doses of up to 2 g/day,[27] and it is was generally well tolerated at up to 8 g single dose in human volunteers in a dose-escalating study for treatment of Friedreich's ataxia.[28] The latter high dose corresponds to ∼0.8 mmol/kg dose, which is 8−25 times greater than the 0.03−0.1 mmol/kg dose used in a recent successful clinical MRI trial with hyperpolarized ^{13}C-pyruvate injection.[12] Furthermore, encouraging reports on indirect proton detection of ^{13}C[29,30] and ^{15}N[31,32] hyperpolarized compounds, which increase the detection sensitivity by several-fold compared to direct ^{13}C or ^{15}N detection, can significantly reduce the required dose of hyperpolarized contrast agent (to below 0.1 mmol/kg) required for image acquisition or can be utilized to significantly improve spatial or temporal resolution of future in vivo studies with hyperpolarized ^{15}N-nicotinamide.

More importantly, nicotinamide is used as a drug or a molecular framework for other drugs offering a wide range of potential applications in biomedicine.[33] For example, it was used directly for treatment of *M. tuberculosis*, HIV[33] and

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cancer,[34] traumatic brain injury,[35] and its two modifications (isoniazid and pyrazinamide) are the only two small-molecule drugs currently used for *M. tuberculosis* treatment.[33] Other potential imaging applications related to the differential uptake of hyperpolarized ^{15}N heterocycles (including nicotinamide) can include detecting tuberculosis drug resistance to pyrazinamide[36] and isoniazid[37] and detecting tumors.[38] The agent uptake or pH sensing typically happens on a scale of seconds,[39] because these are relatively fast metabolic processes, which are certainly compatible with slow relaxing hyperpolarized ^{15}N spins.[14,15,25]

Because of the low natural abundance (~0.3%) of NMR active ^{15}N nuclear spin, efficient isotopic enrichment is essential for the use of nicotinamide as a ^{15}N hyperpolarized contrast agent. A previously published procedure for nicotinamide-1-^{15}N synthesis[40] was based on the Zincke[41,42] two-step methodology. During the first reaction step the Zincke salt is formed, followed by ring opening and displacement by ammonia (or a primary amine) in the second step. The generalized scheme for synthesis of this class of compounds is shown in Figure 1A.

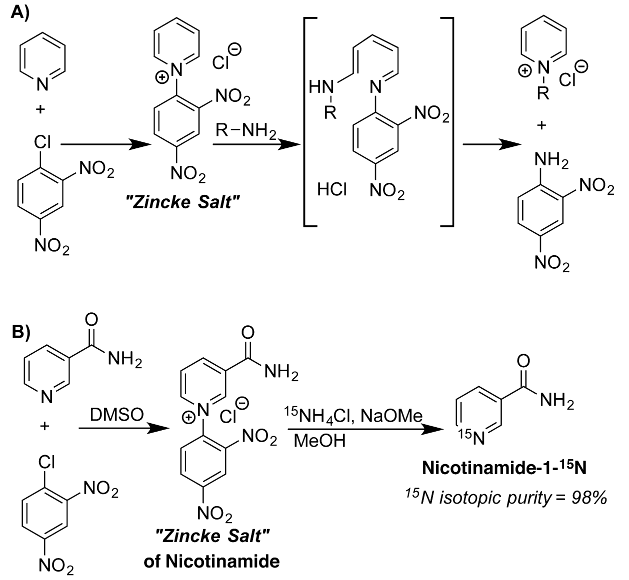

Figure 1. (A) Mechanism of Zincke reaction. (B) Reaction scheme for the improved preparation of nicotinamide-1-^{15}N.

Unfortunately, this methodology[40] based on our experience produced only fair ^{15}N isotopic enrichment (66%[18] to 85%). Similar results were obtained by Burgos et al.[43] forcing a tedious second round of isotopic enrichment (i.e., the 87% ^{15}N enriched product obtained after first round of isotopic enrichment was used again and the entire synthetic procedure was repeated) in order to achieve final 98% isotopic purity. It could be speculated that the accuracy of instrumentation technique used in ref 40 in 1977 was likely insufficient to properly determine the percentage of ^{15}N isotopic enrichment. Decomposition of nicotinamide-Zincke salt (back to the unlabeled nicotinamide) during its purification as well as during slow replacement reaction most likely caused the substantial loss of the isotopic purity. Here, we present a significant improvement for both reaction steps of this methodology.

Despite some recent advances of the Zincke reaction,[44] its first step is often performed by melting neat starting materials at elevated temperatures (>100 °C). Several attempts to conduct this reaction with nicotinamide either by heating the reaction mixture with a mantel or microwave irradiation as heat sources were made. In all cases, production of a large amount of resin was observed, which required a substantial amount of purification. In order to circumvent this problem, we utilized anhydrous solvents and mild, preferably room temperature, conditions.

First, nicotinamide solubility (semiquantitative) in several commercially available anhydrous solvents was investigated (Table S1). Due to the high solubility of nicotinamide and 2,4-dinitrochlorobenzene in anhydrous dimethyl sulfoxide (DMSO), it was chosen as a reaction medium for the Zincke salt formation. During the preliminary experiments, nicotinamide and 2,4-dinitro-chlorobenzene in the molar ratio 1:3 were dissolved in anhydrous DMSO. After 3 days, 66% conversion of nicotinamide to its corresponding Zincke salt was observed with no side or decomposition products accompanying the transformation (Figures S1 and S2). While overall conversion can be improved by increasing reagent concentration and reaction time, the purity of the conversion was very high. This in turn allowed us to simplify purification procedure and to decrease the handling time of the relatively unstable Zincke salt. Therefore, Zincke salt of nicotinamide was prepared by mixing nicotinamide and 2,4-dinitrochlorobenzene in the molar ratio 1:3 at their maximum concentration and allowing them to react for 5 days (Figure 1B), followed by a rapid reaction mixture decanting into anhydrous acetone in order to remove excess of the starting material. In order to further minimize possible decomposition of the Zincke salt, substoichiometric amounts of $^{15}NH_3$, prepared from ammonium-^{15}N chloride ($^{15}NH_4Cl$, 98% ^{15}N, Sigma-Aldrich-Isotec, P/N 299251) and sodium methoxide, were used instead of previously described $^{15}NH_4Cl$/triethylamine (Et_3N) system.[40] Purification involved several cycles of filtration with activated carbon, solvent evaporation, and pH adjustment followed by recrystallization. Absence of laborious flash-chromatography additionally makes this procedure highly scalable—a welcomed advantage for biomedical applications. The final product nicotinamide-1-^{15}N was produced with a 55% yield and the ^{15}N isotopic purity of 98% (Figure 1B), which was estimated by means of high-resolution mass spectrometry (Figure S7). Note that 98% product isotopic purity corresponds to a theoretical maximum, because reagent ($^{15}NH_4Cl$) isotopic purity was 98%. While the undesirable loss of ^{15}N isotope label (in the form of approximately one $^{15}NH_4Cl$ equivalent per every equivalent of nicotinamide-Zincke salt) is unavoidable in the presented methodology, the ease of preparation and high isotopic purity of the product compensates for the cost of this relatively inexpensive ($^{15}NH_4Cl$ costs less than \$20 per gram) ^{15}N isotope enrichment source.

In ^{15}N SABRE-SHEATH hyperpolarization, activated by H_2[45] Ir-IMes SABRE catalyst[46] forms a hexacoordinate complex with nicotinamide-1-^{15}N and parahydrogen (*para*-H_2), Figure 2A. Chemical exchange of equatorial nicotinamide-1-^{15}N and *para*-H_2 in μT magnetic fields[19] enables spontaneous polarization transfer from nascent *para*-H_2 singlet. Importantly, SABRE-SHEATH hyperpolarization of activated complex requires only several seconds.[18,19] For example, ^{15}N signal (ε_{15N}) and P_{15N} enhancement by ~7300-fold is shown in Figure 2. We note that 50% *para*-H_2 gas was employed here resulting in 1/3 of the maximum effect. If ~100% *para*-H_2 gas was utilized, ε_{15N} would be effectively tripled to ~22 000-fold

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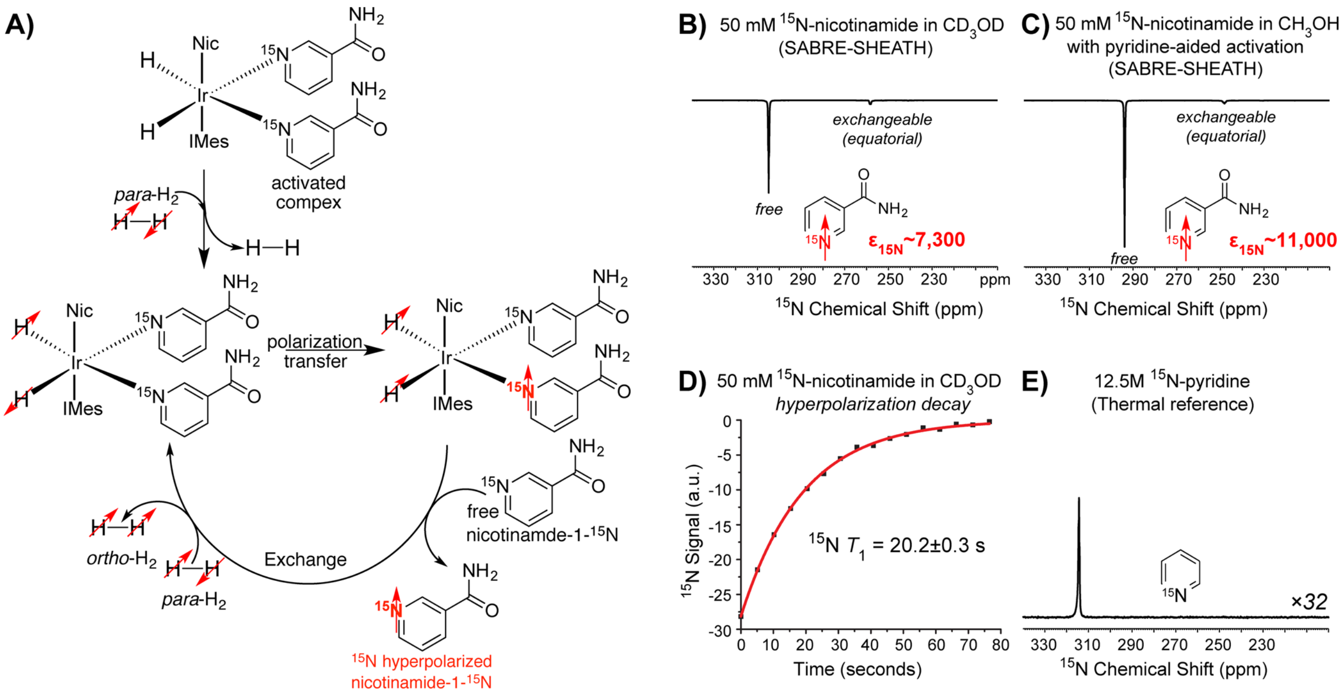

Figure 2. (A) Schematics of ^{15}N SABRE-SHEATH hyperpolarization process[18,19] for nicotinamide-1-^{15}N. Note that Nic stands for axial nicotinamide-1-^{15}N not participating in SABRE process, and IMes stands for 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene. (B) ^{15}N NMR spectroscopy of ^{15}N SABRE-SHEATH of a 50 mM sample of ^{15}N-nicotinamide (^{15}N enrichment of ∼98%) in methanol-d_4 using 3 mM of activated IMes catalyst. (C) ^{15}N NMR spectroscopy of ^{15}N SABRE-SHEATH of a 50 mM sample of ^{15}N-nicotinamide (^{15}N enrichment of ∼98%) in methanol using 3 mM of IMes catalyst activated using pyridine (see SI for details). Enhancements ε_{15N} of ∼7300-fold and ε_{15N} of ∼11 000-fold were achieved, respectively, in B and C in comparison to E. (D) ^{15}N T_1 signal decay of hyperpolarized nicotinamide-^{15}N in methanol-d_4. (E) Spectrum of thermally polarized sample of 12.5 M ^{15}N-Py (note the vertical axis is scaled by 32-fold). The spectra of HP nicotinamide-1-^{15}N show the expected resonances of free and catalyst-bound species.[18]

corresponding to $\%P_{15N}$ of ∼ 7.2%. The latter number is in quantitative agreement with $\%P_{15N}$ ∼ 7% reported earlier for nicotinamide with 66% ^{15}N enrichment.[18] While the efficient hyperpolarization of ^{15}N-nicotinamide for future in vivo experiments requires further hardware advances (e.g., improving the efficiency of *para*-H_2 mixing) and advances in chemistry to make hyperpolarized material biologically compatible to take full advantage of increased enrichment levels demonstrated here,[17] the fundamental parameters of polarization transfer efficiency from *para*-H_2 to ^{15}N spins and the ^{15}N polarization enhancements appear to be approximately the same for ∼66% enriched ^{15}N,[18] and the ∼98% ^{15}N enriched nicotinamide (Figure 2B) under nearly identical experimental conditions and preparation protocols. Therefore, the payload of produced hyperpolarized ^{15}N magnetization is effectively increased by ∼49% when using 98% ^{15}N enriched nicotinamide vs the previously used 66% ^{15}N enriched one. Furthermore, we additionally report that ^{15}N SABRE-SHEATH hyperpolarization efficiency for nicotinamide can be further improved through the use of SABRE catalyst activation with natural abundance pyridine and the use of natural abundance methanol vs deuterated methanol-d_4 employed in the original SABRE-SHEATH demonstration (ref 18 and Figure 2B). Figure 2C shows the ^{15}N spectrum of hyperpolarized ^{15}N enriched (98% enrichment) nicotinamide with ε_{15N} ∼ 11 000-fold, which was achieved using 50% *para*-H_2. If nearly 100% *para*-H_2 was utilized, ε_{15N} would be effectively tripled to ∼33 000-fold corresponding to $\%P_{15N}$ ∼ 11%. As a result of this improvement, the effective payload of produced hyperpolarized ^{15}N magnetization is further increased by ∼50% compared to the previously employed procedure at the same nicotinamide and catalyst concentrations. The reported ε_{15N} and $\%P_{15N}$ do not take into account T_1 relaxation losses, which likely occurred,[53,54] because ^{15}N T_1 of nicotinamide is only 20.2 ± 0.3 s at 9.4 T (Figure 2D)—in accord with a previous report of 22 ± 0.3 s in aqueous solution at the same magnetic field strength.[24] Overall, the results presented in this study show that the hyperpolarization payload of ^{15}N nicotinamide is ∼2.2 times greater than that previously shown.[18]

^{15}N SABRE-SHEATH has been successfully applied to several pyridine-based ^{15}N-heterocycles to date including nicotinamide-1-^{15}N,[18] which can potentially be used for pH sensing[24] or as a reporting molecular probe to study HIV, *M. tuberculosis*, and others. Furthermore, because conventional proton SABRE of isoniazid and pyrazinamide,[47] SABRE in aqueous media,[45,48,49] and heterogeneous SABRE[50,51] have been successfully demonstrated, the ^{15}N SABRE-SHEATH method can be likely extended to these[47] and other important biomolecules[52] for biomedical applications.

In summary, we have developed a straightforward, scalable method of preparation of isotopically pure (98% ^{15}N) nicotinamide-1-^{15}N. Because of mild conditions developed for the Zincke salt formation, this methodology is likely applicable to a wide range of potential ^{15}N-hyperpolarized contrast agents containing N-heterocycles such as the drug isoniazid,[47] potent in vivo pH sensor 2,6-lutidine,[24] ion sensors,[25] and others.

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ASSOCIATED CONTENT

Supporting Information

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Experimental details; NMR spectra; HR-MS spectra (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Nikolaou, P., Goodson, B. M., and Chekmenev, E. Y. (2015) NMR Hyperpolarization Techniques for Biomedicine. *Chem. - Eur. J. 21*, 3156−3166.

(2) Green, R. A., Adams, R. W., Duckett, S. B., Mewis, R. E., Williamson, D. C., and Green, G. G. R. (2012) The theory and practice of hyperpolarization in magnetic resonance using parahydrogen. *Prog. Nucl. Magn. Reson. Spectrosc. 67*, 1−48.

(3) Goodson, B. M. (2002) Nuclear magnetic resonance of laser-polarized noble gases in molecules, materials, and organisms. *J. Magn. Reson. 155*, 157−216.

(4) Brindle, K. M. (2015) Imaging Metabolism with Hyperpolarized ^{13}C-Labeled Cell Substrates. *J. Am. Chem. Soc. 137*, 6418−6427.

(5) Kurhanewicz, J., Vigneron, D. B., Brindle, K., Chekmenev, E. Y., Comment, A., Cunningham, C. H., DeBerardinis, R. J., Green, G. G., Leach, M. O., Rajan, S. S., et al. (2011) Analysis of Cancer Metabolism by Imaging Hyperpolarized Nuclei: Prospects for Translation to Clinical Research. *Neoplasia 13*, 81−97.

(6) Comment, A., and Merritt, M. E. (2014) Hyperpolarized Magnetic Resonance as a Sensitive Detector of Metabolic Function. *Biochemistry 53*, 7333−7357.

(7) Ardenkjaer-Larsen, J. H., Fridlund, B., Gram, A., Hansson, G., Hansson, L., Lerche, M. H., Servin, R., Thaning, M., and Golman, K. (2003) Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. *Proc. Natl. Acad. Sci. U. S. A. 100*, 10158−10163.

(8) Day, S. E., Kettunen, M. I., Gallagher, F. A., Hu, D. E., Lerche, M., Wolber, J., Golman, K., Ardenkjaer-Larsen, J. H., and Brindle, K. M. (2007) Detecting tumor response to treatment using hyperpolarized C-13 magnetic resonance imaging and spectroscopy. *Nat. Med. 13*, 1382−1387.

(9) Golman, K., Axelsson, O., Johannesson, H., Mansson, S., Olofsson, C., and Petersson, J. S. (2001) Parahydrogen-induced polarization in imaging: Subsecond C-13 angiography. *Magn. Reson. Med. 46*, 1−5.

(10) Golman, K., in't Zandt, R., and Thaning, M. (2006) Real-time metabolic imaging. *Proc. Natl. Acad. Sci. U. S. A. 103*, 11270−11275.

(11) Branca, R. T., He, T., Zhang, L., Floyd, C. S., Freeman, M., White, C., and Burant, A. (2014) Detection of brown adipose tissue and thermogenic activity in mice by hyperpolarized xenon MRI. *Proc. Natl. Acad. Sci. U. S. A. 111*, 18001−18006.

(12) 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., et al. (2013) Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized 1-C-13 Pyruvate. *Sci. Transl. Med. 5*, 198ra108.

(13) Mugler, J. P., and Altes, T. A. (2013) Hyperpolarized ^{129}Xe MRI of the human lung. *J. Magn. Reson. Imaging 37*, 313−331.

(14) Cudalbu, C., Comment, A., Kurdzesau, F., van Heeswijk, R. B., Uffmann, K., Jannin, S., Denisov, V., Kirik, D., and Gruetter, R. (2010) Feasibility of in vivo N-15 MRS detection of hyperpolarized N-15 labeled choline in rats. *Phys. Chem. Chem. Phys. 12*, 5818−5823.

(15) Nonaka, H., Hata, R., Doura, T., Nishihara, T., Kumagai, K., Akakabe, M., Tsuda, M., Ichikawa, K., and Sando, S. (2013) A platform for designing hyperpolarized magnetic resonance chemical probes. *Nat. Commun. 4*, 2411.

(16) Adams, R. W., Aguilar, J. A., Atkinson, K. D., Cowley, M. J., Elliott, P. I. P., Duckett, S. B., Green, G. G. R., Khazal, I. G., Lopez-Serrano, J., and Williamson, D. C. (2009) Reversible Interactions with para-Hydrogen Enhance NMR Sensitivity by Polarization Transfer. *Science 323*, 1708−1711.

(17) Shchepin, R. V., Truong, M. L., Theis, T., Coffey, A. M., Shi, F., Waddell, K. W., Warren, W. S., Goodson, B. M., and Chekmenev, E. Y. (2015) Hyperpolarization of "Neat" Liquids by NMR Signal Amplification by Reversible Exchange. *J. Phys. Chem. Lett. 6*, 1961−1967.

(18) Theis, T., Truong, M. L., Coffey, A. M., Shchepin, R. V., Waddell, K. W., Shi, F., Goodson, B. M., Warren, W. S., and Chekmenev, E. Y. (2015) Microtesla SABRE Enables 10% Nitrogen-15 Nuclear Spin Polarization. *J. Am. Chem. Soc. 137*, 1404−1407.

(19) Truong, M. L., Theis, T., Coffey, A. M., Shchepin, R. V., Waddell, K. W., Shi, F., Goodson, B. M., Warren, W. S., and Chekmenev, E. Y. (2015) ^{15}N Hyperpolarization By Reversible Exchange Using SABRE-SHEATH. *J. Phys. Chem. C 119*, 8786−8797.

(20) Zhivonitko, V. V., Skovpin, I. V., and Koptyug, I. V. (2015) Strong ^{31}P nuclear spin hyperpolarization produced via reversible chemical interaction with parahydrogen. *Chem. Commun. 51*, 2506−2509.

(21) Theis, T., Truong, M., Coffey, A. M., Chekmenev, E. Y., and Warren, W. S. (2014) LIGHT-SABRE enables efficient in-magnet catalytic hyperpolarization. *J. Magn. Reson. 248*, 23−26.

(22) Pravdivtsev, A. N., Yurkovskaya, A. V., Vieth, H.-M., and Ivanov, K. L. (2014) Spin mixing at level anti-crossings in the rotating frame makes high-field SABRE feasible. *Phys. Chem. Chem. Phys. 16*, 24672−24675.

(23) Whaley, T. W., and Ott, D. G. (1974) Syntheses with stable isotopes: Pyridine-^{15}N. *J. Labelled Compd. 10*, 283−286.

(24) Jiang, W., Lumata, L., Chen, W., Zhang, S., Kovacs, Z., Sherry, A. D., and Khemtong, C. (2015) Hyperpolarized ^{15}N-pyridine Derivatives as pH-Sensitive MRI Agents. *Sci. Rep. 5*, 9104.

(25) Hata, R., Nonaka, H., Takakusagi, Y., Ichikawa, K., and Sando, S. (2015) Design of a hyperpolarized ^{15}N NMR probe that induces a large chemical-shift change upon binding of calcium ions. *Chem. Commun. 51*, 12290−12292.

(26) Bergmann, F., and Wislicki, L. (1953) The Pharmacological Effects of Massive Doses of Nicotinamide. *Br. J. Pharmacol. Chemother. 8*, 49−53.

(27) Guyton, J. R., Blazing, M. A., Hagar, J., et al. (2000) Extended-release niacin vs gemfibrozil for the treatment of low levels of high-density lipoprotein cholesterol. *Arch. Intern. Med. 160*, 1177−1184.

(28) Libri, V., Yandim, C., Athanasopoulos, S., Loyse, N., Natisvili, T., Law, P. P., Chan, P. K., Mohammad, T., Mauri, M., and Tam, K. T. (2014) Epigenetic and neurological effects and safety of high-dose nicotinamide in patients with Friedreich's ataxia: an exploratory, open-label, dose-escalation study. *Lancet 384*, 504−513.

(29) Mishkovsky, M., Comment, A., and Gruetter, R. (2012) In vivo detection of brain Krebs cycle intermediate by hyperpolarized magnetic resonance. *J. Cereb. Blood Flow Metab. 32*, 2108−2113.

(30) Truong, M. L., Coffey, A. M., Shchepin, R. V., Waddell, K. W., and Chekmenev, E. Y. (2014) Sub-second Proton Imaging of ^{13}C Hyperpolarized Contrast Agents in Water. *Contrast Media Mol. Imaging 9*, 333−341.

(31) Norton, V. A., and Weitekamp, D. P. (2011) Communication: Partial polarization transfer for single-scan spectroscopy and imaging. *J. Chem. Phys. 135*, 141107.
(32) Sarkar, R., Comment, A., Vasos, P. R., Jannin, S., Gruetter, R., Bodenhausen, G., Hall, H., Kirik, D., and Denisov, V. P. (2009) Proton NMR of N-15-Choline Metabolites Enhanced by Dynamic Nuclear Polarization. *J. Am. Chem. Soc. 131*, 16014−16015.
(33) Murray, M. F. (2003) Nicotinamide: An Oral Antimicrobial Agent with Activity against Both Mycobacterium tuberculosis and Human Immunodeficiency Virus. *Clin. Infect. Dis. 36*, 453−460.
(34) Chen, A. C., Martin, A. J., Choy, B., Fernández-Peñas, P., Dalziell, R. A., McKenzie, C. A., Scolyer, R. A., Dhillon, H. M., Vardy, J. L., Kricker, A., et al. (2015) A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention. *N. Engl. J. Med. 373*, 1618−1626.
(35) Goffus, A. M., Anderson, G. D., and Hoane, M. R. (2010) Sustained delivery of nicotinamide limits cortical injury and improves functional recovery following traumatic brain injury. *Oxid. Med. Cell. Longevity 3*, 145−152.
(36) Raynaud, C., Lanéelle, M.-A., Senaratne, R. H., Draper, P., Lanéelle, G., and Daffé, M. (1999) Mechanisms of pyrazinamide resistance in mycobacteria: importance of lack of uptake in addition to lack of pyrazinamidase activity. *Microbiology 145*, 1359−1367.
(37) Sriprakash, K. S., and Ramakrishnan, T. (1970) Isoniazid-resistant Mutants of Mycobacterium tuberculosis H 37 RV: Uptake of Isoniazid and the Properties of NADase Inhibitor. *J. Gen. Microbiol. 60*, 125−132.
(38) Dietrich, L. S., and Ahuja, J. N. (1970) Uptake of Nicotinic Acid-Cl4 and Nicotinamide-C14 by Ascites Cells in Vitro*. *J. Biol. Chem. 238*, 1544−1547.
(39) Gallagher, F. A., Kettunen, M. I., Day, S. E., Hu, D. E., Ardenkjaer-Larsen, J. H., in't Zandt, R., Jensen, P. R., Karlsson, M., Golman, K., Lerche, M. H., et al. (2008) Magnetic resonance imaging of pH in vivo using hyperpolarized C-13-labelled bicarbonate. *Nature 453*, 940−943.
(40) Oppenheimer, N. J., Matsunaga, T. O., and Kam, B. L. (1978) Synthesis of ^{15}N-1 nicotinamide. A general, one step synthesis of ^{15}N labeled pyridine heterocycles. *J. Labelled Compd. Radiopharm. 15*, 191.
(41) Zincke, T., Heuser, G., and Möller, W. (1904) I. Ueber Dinitrophenylpyridiniumchlorid und dessen Umwandlungsproducte. *Justus Liebigs Ann. Chem. 333*, 296−345.
(42) Zincke, T., and Würker, W. (1905) Ueber Dinitrophenylpyridiniumchlorid und dessen Umwandlungsproducte. Ueber die Einwirkung aliphatischer Amine auf Dinitrophenylpyridiniumchlorid. *Justus Liebigs Ann. Chem. 341*, 365−379.
(43) Burgos, E. S., Vetticatt, M. J., and Schramm, V. L. (2013) Recycling Nicotinamide. The Transition-State Structure of Human Nicotinamide Phosphoribosyltransferase. *J. Am. Chem. Soc. 135*, 3485−3493.
(44) Cheng, W.-C., and Kurth, M. J. (2002) The Zincke Reaction. A Review. *Org. Prep. Proced. Int. 34*, 585−608.
(45) Truong, M. L., Shi, F., He, P., Yuan, B., Plunkett, K. N., Coffey, A. M., Shchepin, R. V., Barskiy, D. A., Kovtunov, K. V., Koptyug, I. V., et al. (2014) Irreversible Catalyst Activation Enables Hyperpolarization and Water Solubility for NMR Signal Amplification by Reversible Exchange. *J. Phys. Chem. B 118*, 13882−13889.
(46) Cowley, M. J., Adams, R. W., Atkinson, K. D., Cockett, M. C. R., Duckett, S. B., Green, G. G. R., Lohman, J. A. B., Kerssebaum, R., Kilgour, D., and Mewis, R. E. (2011) Iridium N-Heterocyclic Carbene Complexes as Efficient Catalysts for Magnetization Transfer from para-Hydrogen. *J. Am. Chem. Soc. 133*, 6134−6137.
(47) Zeng, H., Xu, J., Gillen, J., McMahon, M. T., Artemov, D., Tyburn, J.-M., Lohman, J. A. B., Mewis, R. E., Atkinson, K. D., Green, G. G. R., et al. (2013) Optimization of SABRE for polarization of the tuberculosis drugs pyrazinamide and isoniazid. *J. Magn. Reson. 237*, 73−78.
(48) Zeng, H., Xu, J., McMahon, M. T., Lohman, J. A. B., and van Zijl, P. C. M. (2014) Achieving 1% NMR polarization in water in less than 1 min using SABRE. *J. Magn. Reson. 246*, 119−121.
(49) Hövener, J.-B., Schwaderlapp, N., Borowiak, R., Lickert, T., Duckett, S. B., Mewis, R. E., Adams, R. W., Burns, M. J., Highton, L. A. R., Green, G. G. R., et al. (2014) Toward Biocompatible Nuclear Hyperpolarization Using Signal Amplification by Reversible Exchange: Quantitative in Situ Spectroscopy and High-Field Imaging. *Anal. Chem. 86*, 1767−1774.
(50) Shi, F., Coffey, A. M., Waddell, K. W., Chekmenev, E. Y., and Goodson, B. M. (2014) Heterogeneous Solution NMR Signal Amplification by Reversible Exchange. *Angew. Chem., Int. Ed. 53*, 7495−7498.
(51) Shi, F., Coffey, A. M., Waddell, K. W., Chekmenev, E. Y., and Goodson, B. M. (2015) Nanoscale Catalysts for NMR Signal Enhancement by Reversible Exchange. *J. Phys. Chem. C 119*, 7525−7533.
(52) Moreno, K. X., Nasr, K., Milne, M., Sherry, A. D., and Goux, W. J. (2015) Nuclear spin hyperpolarization of the solvent using signal amplification by reversible exchange (SABRE). *J. Magn. Reson. 257*, 15−23.
(53) Barskiy, D. A., Pravdivtsev, A. N., Ivanov, K. L., Kovtunov, K. V., and Koptyug, I. V. (2016) A simple analytical model for signal amplification by reversible exchange (SABRE) process. *Phys. Chem. Chem. Phys. 18*, 89−93.
(54) Mewis, R. E., Fekete, M., Green, G. G. R., Whitwood, A. C., and Duckett, S. B. (2015) Deactivation of signal amplification by reversible exchange catalysis, progress towards in vivo application. *Chem. Commun. 51*, 9857−9859.

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