

Accepted Article

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Authors: Guannan Zhang, Johannes F.P. Colell, Thomas Glachet, Jacob R. Lindale, Vincent Reboul, Thomas Theis, and Warren S. Warren

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To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201904026
Angew. Chem. 10.1002/ange.201904026

Link to VoR: <http://dx.doi.org/10.1002/anie.201904026>
<http://dx.doi.org/10.1002/ange.201904026>

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Terminal Diazirines Enable Reverse Polarization Transfer from $^{15}\text{N}_2$ Singlets

Guannan Zhang,^{+[a]} Johannes F. P. Colell,^{+[a]} Thomas Glachet,^[b] Jacob R. Lindale,^[a] Vincent Reboul,^{+[b]} Thomas Theis,^{+[c]} and Warren S. Warren^{+[d]}

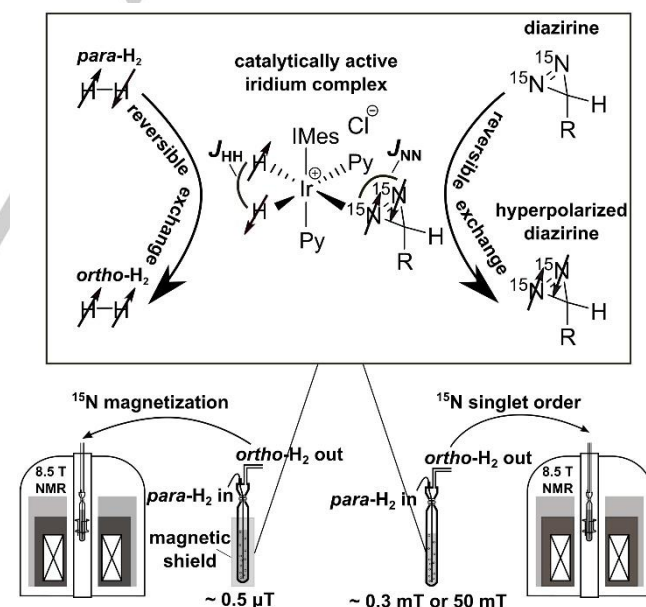
Abstract: Diazirine moieties are chemically stable and have been incorporated into biomolecules without impediment of biological activity. The $^{15}\text{N}_2$ labeled diazirines are appealing motifs for hyperpolarization supporting relaxation protected states with long-lived lifetimes. The (-CH $^{15}\text{N}_2$) diazirine groups investigated here are analogues to methyl groups, which provides the opportunity to transfer polarization stored on a relaxation protected (-CH $^{15}\text{N}_2$) moiety to ^1H , thus combining the advantages of long lifetimes of ^{15}N polarization with superior sensitivity of ^1H detection. Despite the proximity of ^1H to ^{15}N nuclei in the diazirine moiety, ^{15}N T_1 times of up to (4.6 ± 0.4) min and singlet lifetimes T_s of up to (17.5 ± 3.8) min are observed. Furthermore, we found terminal diazirines to support hyperpolarized $^1\text{H}_2$ singlet states in CH $_2$ groups of chiral molecules. The singlet lifetime of ^1H singlets is up to (9.2 ± 1.8) min, thus exceeding ^1H T_1 relaxation time (at 8.45 T) by a factor of ~ 100.

Introduction

$^{15}\text{N}_2$ labeled diazirines represent a promising class of molecular markers: they are biocompatible, they can sustain hyperpolarization for above an hour, and they hyperpolarize with extended variants of the simple Signal Amplification By Reversible Exchange (X-SABRE) hyperpolarization scheme. In X-SABRE, nuclear-spin singlet-order of *para*-H $_2$ is converted into observable magnetization. If substrates contain a pair of spin $\frac{1}{2}$ nuclei with a J -coupling comparable to the hydride-hydride coupling, the singlet order is transferred directly to a spin pair in the target molecule.^[1] Polarization transfer from *para*-H $_2$ to a target state occurs at Level-Anti-Crossings,^[2] where the optimal field depends on the target nucleus, the catalyst and the desired state. Transfer is mediated by the intramolecular J -coupling network of a polarization transfer catalyst (PTC), where a

transition metal establishes magnetic contact via a transient adduct of *para*-H $_2$ and substrate molecule. The method provides access to large levels of polarization in a short time at moderate / room temperature^[3] and to date, ^1H , ^{13}C , ^{15}N , ^{19}F , ^{29}Si , ^{31}P and ^{119}Sn have been hyperpolarized using SABRE.^[4,5]

In doubly ^{15}N -labeled diazirines either singlet order or ^{15}N polarization can be created (Scheme 1), where ^{15}N -magnetization is obtained by bubbling *para*-H $_2$ through a sample containing the PTC and diazirine at μT magnetic fields (the SABRE SHEATH method),^[6] whereas a ^{15}N – ^{15}N singlet state can be created at any field, as long as the (^{15}N – ^{15}N) chemical shift difference is smaller than the ^{15}N – ^{15}N J -coupling.^[7] In that limit the singlet remains an eigenstate and often exhibits a longer relaxation time than magnetization. The singlet can readily be converted into magnetization by simple field cycling schemes,^[8,9] where the chemical shift difference between the ^{15}N sites obtained by introducing a stereogenic center in proximity of the doubly ^{15}N -labeled diazirine evolves singlet states into detectable anti-Zeeman magnetization upon transfer to high field.



Scheme 1. Schematic representation of generation of hyperpolarized ^{15}N labeled terminal diazirines. IMes and Py represents [1,3-bis(2,4,6-trimethylphenyl)-imidazolyl] and pyridine ligands, respectively.

A new approach yielding terminal diazirines (-CHN $_2$) in only one step from α -amino acids has recently been demonstrated.^[10] Isotopically enriched diazirines were readily created using ^{15}N -ammonia and ^{15}N -labeled amino acids or unlabeled ones, where diazirines bearing a stereogenic center (asymmetric sulfur of the sulfoxide group) were synthesized from cysteine and methionine.

[a] Dr. G. Zhang,^[1] Dr. J. F. P. Colell,^[1] J. R. Lindale, Prof. W. S. Warren

Department of Chemistry
Duke University
Durham, NC, 27708 (USA)
E-mail: warren.warren@duke.edu

[b] T. Glachet, Dr. V. Reboul
Normandie Univ, LCMT, ENSICAEN, UNICAEN, CNRS
14000 Caen (France)
E-mail: vincent.reboul@ensicaen.fr

[c] Prof. T. Theis
Department of Chemistry
NC State University
Raleigh, NC 27695 (USA)
E-mail: ttheis@ncsu.edu

[d] Prof. W. S. Warren
Department of Physics, Radiology and Biomedical Engineering
Duke University
Durham, NC, 27708 (USA)

[†] These authors contributed equally to this work.

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These “terminal” diazirines replace methyl groups, one of the most commonly encountered chemical groups in biochemistry and beyond.

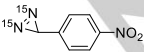
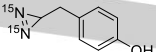
Since the strength of the NMR signal is proportional to γ^2 for spin $\frac{1}{2}$ nuclei^[11] (at the same polarization level), detection using a high γ nucleus (such as ^1H) results in much higher sensitivity. Previous studies have demonstrated major sensitivity gains by storage of hyperpolarization on a low γ heteronucleus with a long T_1 relaxation time followed by polarization transfer to protons for detection, providing up to $(\gamma_{\text{H}}/\gamma_{\text{X}})$ ($\text{X} = \text{heteronucleus}$) sensitivity gain for a given level of polarization.^[12–17] When the singlet lifetime is much larger than spin-lattice relaxation time, utilizing singlets for polarization storage is highly beneficial to track slow processes.^[18–25]

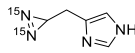
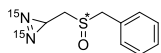
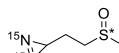
In this article, we focus on terminal diazirines as SABRE targets. The terminal diazirines depicted in Table 1 were chosen to probe various molecular motifs. In molecule (1) a terminal diazirine moiety is directly attached to an aryl group with a simple structure and J -coupling network. The tyrosine and histidine derivatives (2,3) represent non-chiral examples that were effectively synthesized with high yields.^[10] Diazirines (4,5) have a stereogenic center, which makes it possible to probe hyperpolarized long-lived singlet states and to permit transfer to ^1H . In the following, we will demonstrate a strategy to store polarization in hyperpolarized $^{15}\text{N}_2$ singlets and then convert the polarization from ^{15}N singlet order into ^1H magnetization for more sensitive detection. Furthermore, we show that polarization is transferred from ^{15}N singlets to ^1H pairs creating hyperpolarized ^1H singlet states, which exhibit significantly increased relaxation times.

Results and Discussion

^{15}N magnetization (Table 1) can be readily created using SABRE SHEATH by exposure of a sample to *para*- H_2 at a magnetic field of 0.5 μT (see Experimental Section in Supplementary information). Terminal diazirines exhibit long ^{15}N T_1 relaxation times in low fields (Table 1). Interpair dipolar relaxation is a major relaxation channel for many spin $1/2$ nuclei, but ^{15}N relaxation times in high field are significantly smaller due to the chemical shift anisotropy scaling with B_0^2 . It is noteworthy that the presence of ^1H in the diazirine moiety leaves ^{15}N T_1 relaxation almost unaffected when compared to previous studies.^[8,26]

Table 1. Enhancements ($\epsilon_{^{15}\text{N}}$), Polarization levels ($P_{^{15}\text{N}}$) and ^{15}N T_1 relaxation times at different magnetic fields for ^{15}N labeled terminal diazirines.

Entry	Substrate	$\epsilon_{^{15}\text{N}}^{[a]}$	$P_{^{15}\text{N}}$ [%] ^[a]	T_1 [s]
1 ^[c]		7100	1.98	23.1±1.6 @ 8.5 T 243.1±23.5 @ 3 G 234.4±14.9 @ 500 G
2		3300	0.92	28.3±0.5 @ 8.5 T 80.1±9.6 @ 3 G ^[d]

3		1000	0.27	34.8±2.6 @ 8.5 T 76.2±3.2 @ 3 G 103.0±4.7 @ 500 G
4		1200 ^[b]	0.34 ^[b]	15.2±1.3 @ 8.5 T 150.9±28.8 @ 3 G 274.4±21.9 @ 500 G T_1 : (17.5±3.8) min @ 3G
5		2250 ^[b]	0.63 ^[b]	32.3±3.8 @ 8.5 T 138.9±4.3 @ 3 G 149.0±9.0 @ 500 G T_1 : (8.5±0.4) min @ 3G

[a] Enhancements and polarization levels averaged over the two ^{15}N sites, relative to thermal magnetization at 8.45 T. Polarization was directly calculated from enhancement. [b] Polarization levels differ for the ^{15}N positions in a chiral diazirine (see Fig. 3a). [c] Substrate concentration is 3 mM due to low solubility. [d] $T_1(^{15}\text{N})$ relaxation at 500 G not measured.

Diazirines (1-3) do not contain a chiral group and accordingly ^{15}N nuclei are chemically and magnetically equivalent. Consequently, $^1J_{\text{NN}}$ is unobservable and the ^{15}N resonance is split by $^2J_{\text{NH}}$ for diazirine (1) (doublet) and by $^2J_{\text{NH}}$ and $^3J_{\text{NH}}$ for diazirines (2) and (3) resulting in doublet of triplets (Figure 1a-c). Diazirines (4,5) are racemic mixtures with *R,S* configuration of the asymmetric sulfur. The presence of the stereogenic center in the vicinity of the ^{15}N moiety (diazirine 4 = γ -chiral, diazirine 5 = δ -chiral) induces a chemical shift difference ($\Delta\delta$) between the ^{15}N positions (0.929 ppm and 0.232 ppm for diazirines (4) and (5), respectively, see Figure 1d-e; see Figure S1 in Supplementary information for the expanded views of asymmetric peak pattern for diazirines (4,5)). These spectra exhibit the characteristic roof effect for strong coupling ($x = \delta v_{\text{NN}}/J_{\text{NN}}$, $^1J_{\text{NN}} \sim 16$ Hz; $\delta v_{\text{NN}} = 33.9$ Hz, $x \sim 2$ for diazirine (4); $\delta v_{\text{NN}} = 8.5$ Hz, $x \sim 0.5$ for diazirine (5)). Exact values for J -coupling and chemical shift were obtained by simulation. For (4) and (5), we find 2.5 – 2.7 Hz for the $^2J_{\text{NH}}$ coupling, while the $^3J_{\text{NH}}$ couplings to ^1H in the nearest methylene functional group are on the order of 0.6 – 1.0 Hz (Figure 1).

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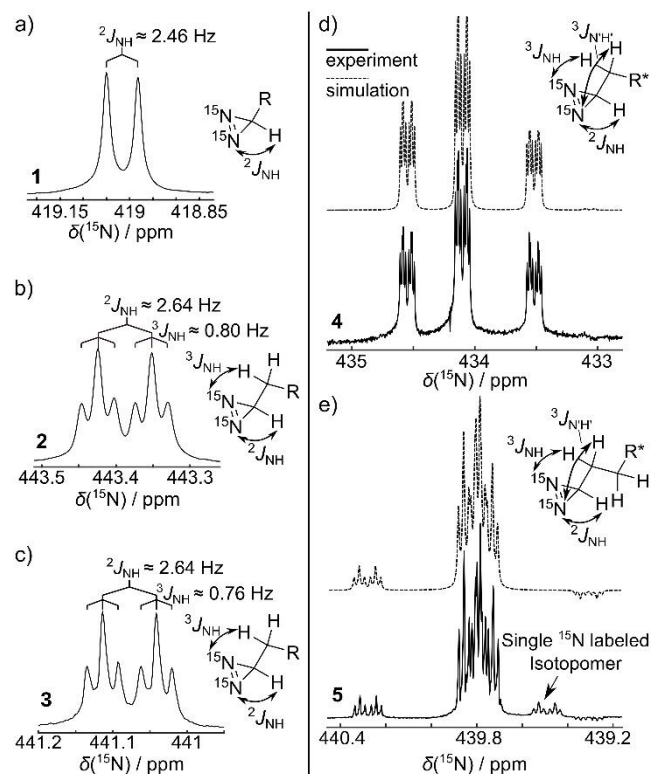


Figure 1. Experimental spectra of hyperpolarized ^{15}N magnetization created using the SABRE-SHEATH method for diazirines (1-5) and J -coupling analysis for diazirines (1-3) (a-c). (d,e) Hyperpolarized ^{15}N spectra of diazirines (4,5) were simulated using the Spinach Matlab extension. For diazirine (4), $^3J_{\text{NH}}=0.96$ Hz, $^3J_{\text{NH}}=1.01$ Hz, $^3J_{\text{NH}}=0.63$ Hz, $^3J_{\text{NH}}=0.65$ Hz. For diazirine (5), $^3J_{\text{NH}}=^3J_{\text{NH}}=^3J_{\text{NH}}=^3J_{\text{NH}}=0.83$ Hz. The asymmetric peak patterns in (d,e) can be explained by overlap of anti-phase spectra from ($S_{1z}-S_{2z}$) anti-Zeeman state and ($S_{1z}+S_{2z}$) state or chiral substrates exhibiting preferential coordination of the catalyst. We use $c_1 \cdot (S_{1z}+S_{2z}) + c_2 \cdot (S_{1z}-S_{2z})$ where c_1 over c_2 represents the ratio of ^{15}N signals generated from magnetization and singlet order, with $c_1 = 0.6$, $c_2 = 0.4$ for diazirine (4) and $c_1 = 0.7$, $c_2 = 0.3$ for diazirine (5), respectively (see text in Supplementary information for explanation).

The $^2J_{\text{NH}}$ coupling (2.5 – 2.7 Hz) in terminal diazirines are much smaller than 1J -couplings normally targeted in polarization transfer experiments (DEPT^[27]/INEPT^[28] and variants^[29–38] typically utilize 60 – 150 Hz 1J -couplings). Thus we investigated several established transfer schemes in a reverse mode (polarization transfer from the insensitive to the sensitive nucleus; see Figure S2 in Supplementary information), where the INEPT pulse sequence ($T_{\text{INEPT}} = (4 \times ^2J_{\text{NH}})^{-1}$) could be utilized most successfully to transfer polarization from ^{15}N to ^1H . The required delays are large, where $T_{\text{INEPT}} \approx 100$ ms. More sophisticated variants with larger number of pulses and duration suffer more from relaxation and multiple quantum coherence effects (diazirine (5), see Figure 2).

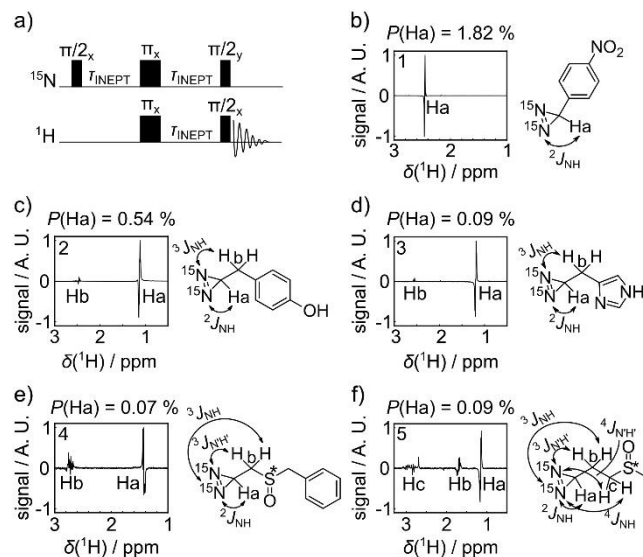


Figure 2. ^1H spectra after polarization transfer from hyperpolarized ^{15}N . a) Reverse INEPT pulse sequence used for polarization transfer (see Experimental Section in Supplementary information for parameters). (b)-(f) ^1H spectra of terminal diazirines (1-5) obtained using the reverse INEPT pulse sequence. From (b) to (f), ^1H polarization levels ($P(\text{Ha})$) are 1.82%, 0.54%, 0.09%, 0.07% and 0.09%, corresponding to polarization transfer efficiency 92%, 58%, 33%, 20% and 14%. The transfer efficiency is calculated by Eq. (1) (See Table 1 for ^{15}N polarization levels).

For the overall efficiency, initial nitrogen polarization levels, as well as relaxation times of source ^{15}N spins and target ^1H , are important. Evidently, the nitrogen polarization level is directly proportional to the fractional polarization maximally attainable on the protons and the efficiency of the polarization transfer is

$$\text{Transfer efficiency} = \frac{P_{1\text{H}}}{P_{15\text{N}}} \cdot 100\% = \frac{P_{1\text{H}}^{\text{thermal}} \cdot \epsilon_{1\text{H}}}{P_{15\text{N}}^{\text{thermal}} \cdot \epsilon_{15\text{N}}} \cdot 100\% \\ = \frac{\gamma_{1\text{H}} \cdot \epsilon_{1\text{H}}}{\gamma_{15\text{N}} \cdot \epsilon_{15\text{N}}} \cdot 100\% \quad [\text{Eq. (1)}]$$

Here $P_{1\text{H}}$ and $\epsilon_{1\text{H}}$ are polarization level and enhancement of detected ^1H sites after polarization transfer. $P_{15\text{N}}$ and $\epsilon_{15\text{N}}$ are polarization level and enhancement of ^{15}N nucleus hyperpolarized by SABRE-SHEATH. $P_{1\text{H}/15\text{N}}^{\text{thermal}}$ and $\gamma_{1\text{H}/15\text{N}}$ are equilibrium thermal polarization and gyromagnetic ratio of ^1H or ^{15}N , respectively. The polarization levels of ^1H after transfer are shown in Figure 2.

Enhancements and polarization transfer efficiencies scale with the structural complexity of the molecule, where the structurally simplest diazirine (1) affords a polarization transfer efficiency of 92 %, whereas the structurally more complex diazirines (2-5) afford 58 %, 33 %, 20 % and 14 %, respectively. For diazirines (2-5), which contain an additional methylene group (CH_2), the effects of $^3J_{\text{NH}}$ (0.6 – 1 Hz) and $^3J_{\text{HH}}$ lead to polarization transfer not only to the proton in the diazirine moiety, but also to the protons in the methylene groups of the molecule. This is particularly pronounced for the chiral diazirines (4,5), where lower symmetry increases the

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number of distinct J -couplings (see Figure S3 and S4) and additional pathways (multiple quantum coherences) lead to lowest polarization transfer efficiency to the target ^1H .

For diazirines (4,5), we find $^1J_{\text{NN}}$ between -16.4 Hz and -16.8 Hz in free substrates, which is roughly double the $^2J_{\text{HH}} = -(8-10)$ Hz coupling of the iridium bound hydrides. This value of $^1J_{\text{NN}}$ is sufficiently close to the $^2J_{\text{HH}}$ of catalyst bound hydrides to allow for hyperpolarization of the ^{15}N - ^{15}N singlet state ($\mathbf{S}_1 \cdot \mathbf{S}_2$) by direct transfer of spin-order ($I_1 \cdot I_2$) from *para*- H_2 . As previously mentioned, the nitrogen singlet gets populated at any (sufficiently low) magnetic field where the singlet remains an eigenstate,^[7] where evolution into magnetization at μT fields (the SABRE SHEATH methods) imposes a lower limit ($\approx 20 \mu\text{T}$) to the suitable magnetic field range. Upon transfer to very high field (8.45 T), the spin-exchange symmetry of the Hamiltonian is broken,^[39] allowing singlet-triplet transitions to transform hyperpolarized singlet order into observable anti-Zeeman magnetization ($S_{1z} - S_{2z}$) (Figure 3a,b). For non-magnetically equivalent nitrogen positions with a chemical shift difference $\Delta\nu$, the density matrix $\rho(\Delta\nu)$ evolves as

$$\rho(\Delta\nu) = \frac{1}{4} \mathbf{1} - S_{1z}S_{2z} - \frac{J_{\text{NN}}}{\sqrt{\Delta\nu^2 + J_{\text{NN}}^2}} (S_{1x}S_{2x} + S_{1y}S_{2y}) - \frac{\Delta\nu^2}{\sqrt{\Delta\nu^2 + J_{\text{NN}}^2}} \frac{1}{2} (S_{1z} - S_{2z}) \quad [\text{Eq. (2)}]$$

where $\mathbf{1}$ is the unity matrix, S_{iz} is the z -component of the operator of spin i , and J_{NN} is the ^{15}N - ^{15}N -coupling. In the limit of low field ($\Delta\nu = 0$) the prefactor of the last term is zero and the prefactor of the second term is equal to unity. The remaining term $(1/4)\mathbf{1} - (\mathbf{S}_1 \cdot \mathbf{S}_2)$ is the density matrix expression of a pure singlet. In the limit of $\Delta\nu \gg J_{\text{NN}}$, we obtain $(1/4)\mathbf{1} - S_{1z}S_{2z} - (1/2)(S_{1z} - S_{2z})$. The last term corresponds to observable anti-Zeeman magnetization as shown in Figure 3a.

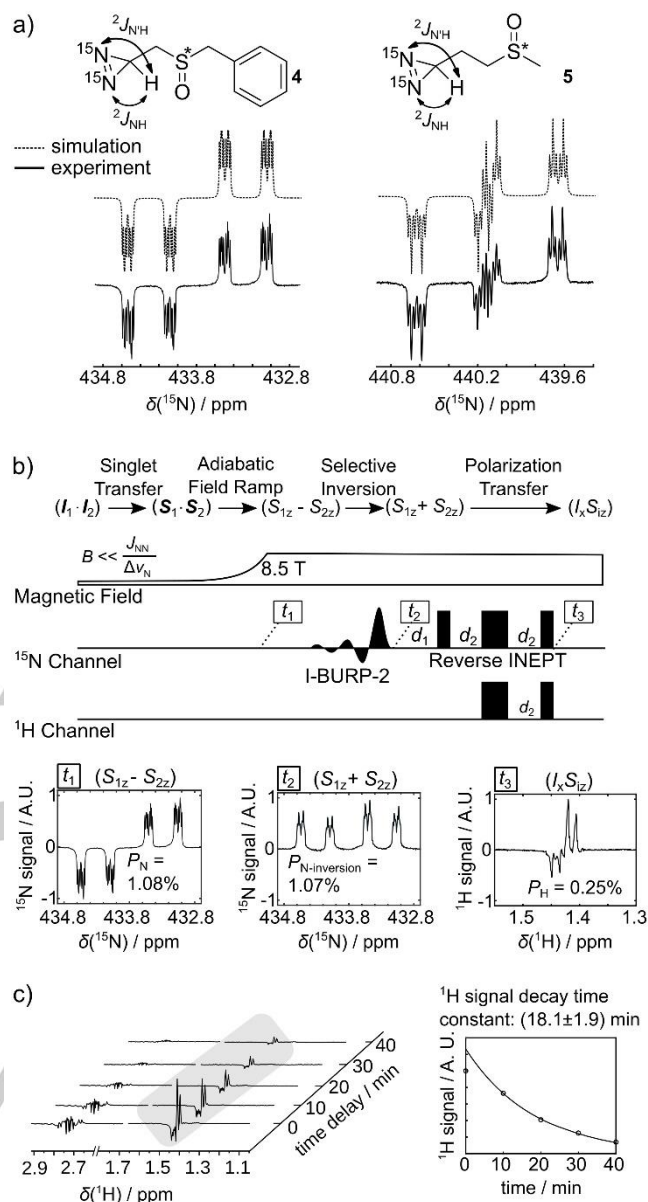


Figure 3. a) Experimental and simulated spectra for hyperpolarized ^{15}N anti-Zeeman order of diazirines (4,5). Singlet order was created at $B \sim 0.3$ mT. The spin system parameters for simulation are the same as shown in Figure 1. b) Polarization transfer from ^{15}N singlets to ^1H of diazirine (4). Top: Scheme for experimental procedure. Parameters are $d_1 = 60 \mu\text{s}$, $d_2 = (4 \times J_{\text{NH}})^{-1} = 96$ ms. Bottom: Experimental spectra of anti-Zeeman spin order ($S_{1z} - S_{2z}$) (t_1), in-phase magnetization ($S_{1z} + S_{2z}$) after selective inversion (t_2), ^1H spectra after using reverse INEPT sequence (t_3). c) Decay of hyperpolarized ^1H magnetization (shaded) stored as ^{15}N singlet order of diazirine (4). The ^1H signal decay time constant of (18.1 ± 1.9) min matches the ^{15}N singlet relaxation time T_s of (17.5 ± 3.8) min. The first data point was excluded from the fit due to continued singlet buildup (see Figure S5).

Singlet order has no dipole moment and is protected from interpair dipole relaxation, which constitutes a major relaxation channel. Thus, nuclear spin singlets constitute relaxation protected states that may exhibit decay time constants several times longer than T_1 . The relaxation time constants for singlet states of diazirines

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(4) and (5) were found to be (17.5 ± 3.8) min and (8.5 ± 0.4) min at 0.3 mT (Figure S5) with a $T_1 \sim 2.5$ min.

The anti-Zeeman order obtained after adiabatic transfer to high field are readily converted into ^{15}N magnetization ($S_{1z} + S_{2z}$) by frequency selective inversion of magnetization. Using the I-BURP-2 shaped pulse, developed to invert z-magnetization,^[40] and a pulse length of 100 ms (40.6 Hz excitation range), selective inversion can be achieved for diazirine (4) ($\delta v_{\text{NN}} = 33.9$ Hz at 8.45 T). Subsequently, polarization can be transferred to ^1H using a reverse INEPT sequence as described above (Figure 3b).

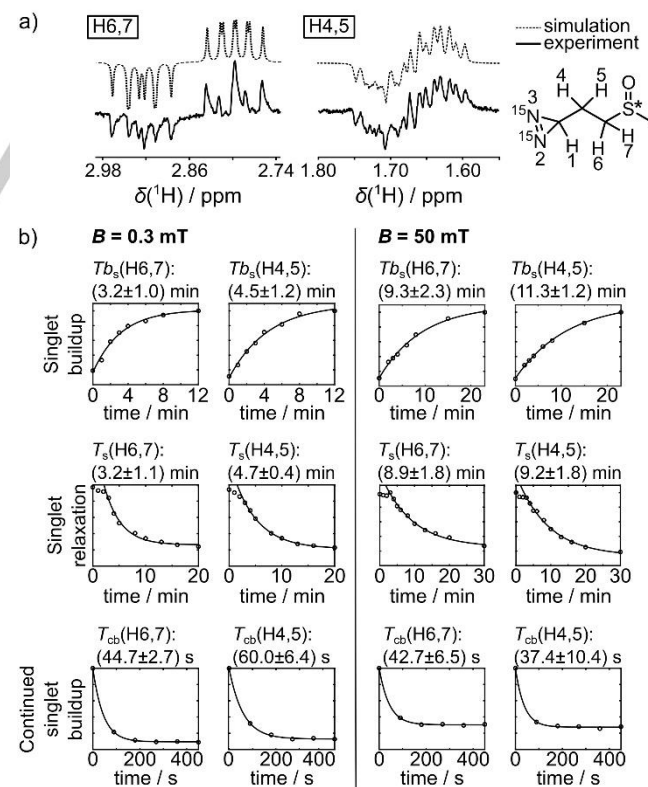
Polarization transfer from ^{15}N singlet to ^1H results in a polarization level of 0.25 % corresponding to a transfer efficiency of 23 %. Taking advantage of the long singlet lifetime of ^{15}N ($T_s = (17.5 \pm 3.8)$ min) within terminal diazirines, polarization can be stored on ^{15}N and transferred to ^1H at desired intervals (Figure 3c). This demonstrates the potential to incorporate a terminal diazirine into commonly used probes, which serve as a platform for monitoring slow processes over long time scales. Such processes include chemical reactions, diffusion, or metabolic flux that can be detected *in vitro* or *in vivo*. For example, a terminal diazirine moiety ($-\text{CH}^{15}\text{N}_2$) can be incorporated in proximity to a reactive site within a probe bearing a stereogenic center. The structural transformation of the probe results in a corresponding change in the NMR signal of the target nuclei, directly reporting on the process of interest. In addition, hyperpolarization can be stored on ^{15}N nuclei and then transferred to ^1H for detection, at time intervals of choice, as a reaction proceeds, thus offering the possibility to monitor processes in real-time.

Our analysis of the ^1H spectra and ^{15}N spectra of diazirines (4,5) and supporting Gaussian calculations revealed that the $^2J_{\text{HH}}$ between the ^1H spin pairs in the methylene (CH_2) moieties are comparable to the $^1J_{\text{NN}}$ couplings in the diazirine moiety ($^2J_{\text{H4,5}} = 15.0$ Hz, $^1J_{\text{NN}} = 16.4$ Hz for diazirine (4) and $^2J_{\text{H4,5}} = 15.0$ Hz, $^2J_{\text{H6,7}} = 13.3$ Hz and $^1J_{\text{NN}} = 16.8$ Hz for diazirine (5)). As equivalency of the hydride-hydride $^2J_{\text{HH}}$ coupling to $^1J_{\text{NN}}$ enabled direct transfer of singlet order from *para*- H_2 to the ^{15}N spin-pair, equivalency of $^2J_{\text{HH}}$ and $^1J_{\text{NN}}$ in the substrate should consequently enable the reverse process, that is singlet transfer from ^{15}N spin-pair to the nearest ^1H spin-pair. The stereogenic center renders both ^{15}N positions as well as the ^1H spins in the methylene moieties inequivalent and thus the chemical shift difference ($\Delta\delta_{\text{HH}}$) between the ^1H spin pairs will make ^1H singlets experimentally observable in full analogy to the ^{15}N spin pairs (see Eq. (2)).

A detailed study could be carried out using diazirine (5), which yields a very stable sample in the presence of PTC and affords consistent polarization levels on ^{15}N nuclei. We found that ^1H singlet order in methylene group ^1H spin-pair is indeed created by the same procedure applied for ^{15}N spin-pair singlet buildup (Figure 4a).

In diazirine (5) the chemical shift induced frequency separations δv_{H4H5} and δv_{H6H7} are on the order of the J_{HH} -coupling and accordingly we encounter a spin system with multiple strongly coupled spin pairs, in Poples' syntax an AXYFGMN system. For ^1H (6,7), where the stereogenic center is close, both $x = \delta v_{\text{H6H7}} / J_{\text{H6H7}}$ as well as $^3J_{\text{HH}}$ -couplings (H(6)/H(7) to H(4)/H(5)) can be accurately determined. The J -coupling network is relatively simple (ddd (d = doublet) for both H (6) and H (7)) giving rise to 16 peaks (see Figure S4). Precise analysis for ^1H (4,5) was difficult, as the stereogenic center is further away and resonances exhibit a dddd structure (d = doublet; t = triplet) (96 peaks) where δv_{H4H5} and J_{H4H5} were unknown. In addition, the ^1H spectrum with thermal polarization exhibits a very pronounced roof effect for ^1H (4,5) with an $x \sim 4$ times smaller than that of ^1H (6,7) (Figure S4).

If the initial singlet state is a singlet on ^1H (4,5), it is feasible to resolve the coupling network more accurately than with thermal polarization. If an anti-Zeeman state evolves from ^1H singlet order, all lines of each multiplet show similar amplitudes. As amplitudes of MR-lines are the product of transition probabilities and population differences, overpopulated levels may compensate for vanishing transition moments. This effect is commonly observed in *para*- H_2 and has been referred to as inverse roof effect.^[41] For ^1H (4,5) with their small x of ~ 0.85 and 96 distinct peaks, the inner lines of opposite amplitudes overlap, leading to partial cancellation of the signals as the overall width of the spectral pattern ($\approx \Sigma J$) is larger than the frequency separation (δv_{H4H5}). For ^1H (6,7) inner lines do not cancel, as $\delta v_{\text{H6H7}} > \Sigma J$ (Figure 4a).



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Figure 4. a) Experimental and simulated spectra for hyperpolarized ^1H singlet order of diazirine (5) (See Figure S4 for simulation parameters). The singlet order was created at $B \sim 0.3$ mT. Signal enhancements are ~ 5 for ^1H (4-7). All signals were detected at 8.45 T. (b) Time constants observed for ^1H singlet relaxation (T_s), singlet buildup (T_{b_s}) and continued singlet buildup (T_{cb}) at 0.3 mT and 50 mT. Since the singlet signal shows continued singlet buildup (T_{cb}) for up to 3 mins, data points from the first 3 mins for T_s measurements were excluded from the fitting.

Finally, we were able to probe the difference between T_1 times and T_s times for ^1H nuclei in prochiral methylene groups, which are extremely common in a variety of molecules. While T_1 for ^1H (4-7) is ~ 6 s (at 8.45 T) the decay time constants of hyperpolarized ^1H singlet order are (4.7 ± 0.4) min for ^1H (4,5) and (3.2 ± 1.1) min for ^1H (6,7) at 0.3 mT (Figure 4b). Interestingly, singlet relaxation times become larger at a higher field of 50 mT ((9.2 ± 1.8) min for ^1H (4,5) and (8.9 ± 1.8) min for ^1H (6,7)). Hence, the singlet relaxation time T_s at a low field is up to 100 times longer than T_1 at 8.5 T.

The five terminal diazirines investigated in the current study exhibit moderate enhancements and long T_1 relaxation times at low magnetic fields. Diazirine (1), with its simple molecular structure and J -coupling network, exhibits the highest polarization transfer efficiency from ^{15}N magnetization to ^1H . The presence of a stereogenic center in diazirines (4,5) offers the opportunity to support long-lived singlet state of ^{15}N nuclei and ^1H spin pairs. Due to the stereogenic center in diazirine (4), polarization can be transferred from relaxation protected ^{15}N singlet state to ^1H , allowing detection and regeneration of enhanced ^1H signal in terminal diazirines over a period of time governed by $T_s(^{15}\text{N})$.

Terminal diazirines exhibit a combination of chemical stability, long-lived ^{15}N polarization, and give access to superior sensitivity with ^1H detection, which is promising for biomolecular tagging strategies.^[8] Using the method demonstrated here, polarization can be stored on ^{15}N nuclei and then transferred to ^1H for detection. This enables tracking of metabolic reactions and visualization in real time. The lifetime of ^{15}N nuclei within a terminal diazirine moiety is up to (17.5 ± 3.8) min which is comparable or longer than some of the common Positron Emission Tomography (PET) radiotracers (e.g. $^{11}\text{C} = 20.4$ min; $^{15}\text{O} = 2.04$ min, $^{13}\text{N} = 9.97$ min),^[42] offering an increase in the observable time window.

Conclusion

In summary, the terminal diazirines, which are readily available via a convenient one-step synthesis, represent promising molecular tags for hyperpolarization. From a SABRE viewpoint, the lack of steric hindrance for terminal groups may be advantageous upon incorporation of diazirines in large molecules. Simple and symmetric terminal diazirines already allow for polarization transfer from relaxation protected ^{15}N singlet state to ^1H magnetization at desired time intervals by a combination of

routine methods (field cycling, selective inversion, polarization transfer), which overcomes the short T_1 lifetimes of ^1H and thus is of particular interest in the context of hyperpolarized contrast agents. In addition, the observation of long-lived ^1H singlet lifetimes in methylene groups is particularly fascinating, as it points to the possibility to create long-lasting ^1H singlet order on any prochiral moiety, which can be transformed into detectable anti-Zeeman spin order by a simple field cycling scheme. Finally, the long-lived hyperpolarization lifetime (17.5 ± 3.8) min of diazirine (4), combined with its feasibility of transferring polarization from ^{15}N singlet state to ^1H magnetization, makes it an interesting motif for future studies.

Storing polarization in a long-lived singlet state is of crucial relevance to the future of high end applications of hyperpolarized materials, as it renders longer time scales amenable to investigation thus enabling observation of processes, and the methodology demonstrated here points to an attractive approach to biomedical applications where ^1H MRI is a widely used modality for decades and the demonstrated singlet diffusion among aliphatic chains containing prochiral spin pairs is of particular note for such applications.

Acknowledgements

This work was supported by the National Science Foundation under grant CHE-1665090, by the NIH under grant R21EB026153, and by the National Institute of Biomedical Imaging and Bioengineering of the NIH under grant R21EB025313. The authors thank the CNRS, Normandie Université, Labex Synorg (ANR-11-LABX-0029), the Conseil Régional de Normandie and the European FEDER fundings for financial support.

Keywords: NMR spectroscopy • hyperpolarization • singlet states • polarization transfer • imaging agents

- [1] R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. Lopez-Serrano, D. C. Williamson, *Science* **2009**, *323*, 1708–1711.
- [2] A. N. Pravdivtsev, A. V. Yurkovskaya, H.-M. Vieth, K. L. Ivanov, R. Kaptein, *ChemPhysChem* **2013**, *14*, 3327–3331.
- [3] T. B. R. Robertson, R. E. Mewis, in *Annual Reports on NMR Spectroscopy*, Elsevier, **2018**, pp. 145–212.
- [4] M. J. Burns, P. J. Rayner, G. G. R. Green, L. A. R. Highton, R. E. Mewis, S. B. Duckett, *J. Phys. Chem. B* **2015**, *119*, 5020–5027.
- [5] J.-B. Hövener, A. N. Pravdivtsev, B. Kidd, C. R. Bowers, S. Glöggler, K. V. Kovtunov, M. Plaumann, R. Katz-Brull, K. Buckenmaier, A. Jerschow, F. Reineri, T. Theis, R. V. Shchepin, S. Wagner, P. Bhattacharya, N. M. Zacharias, E. Y. Chekmenev, *Angew. Chem. Int. Ed.* **2018**, *57*, 11140–11162.
- [6] T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, E. Y. Chekmenev, *J. Am. Chem. Soc.* **2015**, *137*, 1404–1407.
- [7] M. Carravetta, M. H. Levitt, *J. Chem. Phys.* **2005**, *122*, 214505.
- [8] T. Theis, G. X. Ortiz, A. W. J. Logan, K. E. Claytor, Y. Feng, W. P. Huhn, V. Blum, S. J. Malcolmson, E. Y. Chekmenev, Q. Wang, W. S. Warren, *Sci. Adv.* **2016**, *2*, e1501438–e1501438.
- [9] M. Carravetta, O. G. Johannessen, M. H. Levitt, *Phys. Rev. Lett.* **2004**, *92*, DOI 10.1103/PhysRevLett.92.153003.

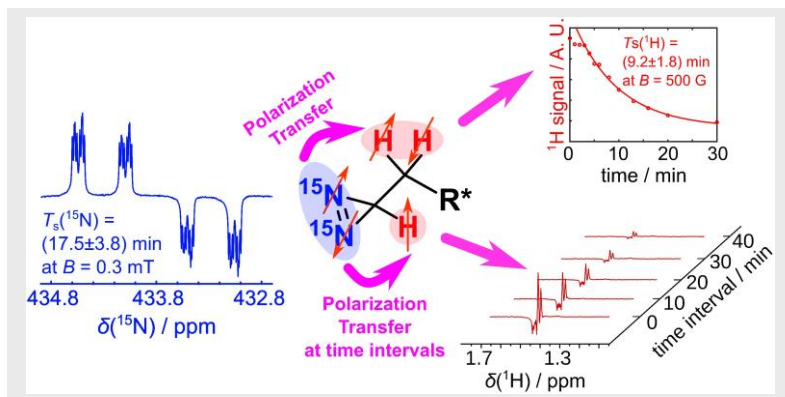
RESEARCH ARTICLE

- [10] T. Glachet, H. Marzag, J. F. P. Colell, N. Saraiva Rosa, G. Zhang, W. S. Warren, X. Franck, T. Theis, V. Reboul, Iodonitrene in Action: Direct Transformation of Amino Acids into Diazirines and $^{15}\text{N}_2$ -Diazirines and their Application as Hyperpolarized Markers, *submitted*.
- [11] J.-H. Ardenkjaer-Larsen, G. S. Boebinger, A. Comment, S. Duckett, A. S. Edison, F. Engelke, C. Griesinger, R. G. Griffin, C. Hilty, H. Maeda, G. Parigi, T. Prisner, E. Ravera, J. van Bentum, S. Vega, A. Webb, C. Luchinat, H. Schwalbe, L. Frydman, *Angew. Chem. Int. Ed.* **2015**, *54*, 9162–9185.
- [12] M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, E. Y. Chekmenev, *Contrast Media Mol. Imaging* **2014**, *9*, 333–341.
- [13] V. A. Norton, D. P. Weitekamp, *J. Chem. Phys.* **2011**, *135*, 141107.
- [14] J. A. Pfeilsticker, J. E. Ollerenshaw, V. A. Norton, D. P. Weitekamp, *J. Magn. Reson.* **2010**, *205*, 125–129.
- [15] E. Y. Chekmenev, V. A. Norton, D. P. Weitekamp, P. Bhattacharya, *J. Am. Chem. Soc.* **2009**, *131*, 3164–3165.
- [16] R. Sarkar, A. Comment, P. R. Vasos, S. Jannin, R. Gruetter, G. Bodenhausen, H. Hall, D. Kirik, V. P. Denisov, *J. Am. Chem. Soc.* **2009**, *131*, 16014–16015.
- [17] J. Wang, F. Kreis, A. J. Wright, R. L. Hesketh, M. H. Levitt, K. M. Brindle, *Magn. Reson. Chem.* **2018**, *79*, 741–747.
- [18] P. Ahuja, R. Sarkar, P. R. Vasos, G. Bodenhausen, *J. Am. Chem. Soc.* **2009**, *131*, 7498–7499.
- [19] J.-N. Dumez, J. T. Hill-Cousins, R. C. D. Brown, G. Pileio, *J. Magn. Reson.* **2014**, *246*, 27–30.
- [20] R. Sarkar, P. Ahuja, P. R. Vasos, G. Bodenhausen, *ChemPhysChem* **2008**, NA-NA.
- [21] G. Pileio, J.-N. Dumez, I.-A. Pop, J. T. Hill-Cousins, R. C. D. Brown, *J. Magn. Reson.* **2015**, *252*, 130–134.
- [22] R. Sarkar, P. R. Vasos, G. Bodenhausen, *J. Am. Chem. Soc.* **2007**, *129*, 328–334.
- [23] S. Cavadini, P. R. Vasos, *Concepts Magn. Reson. Part A* **2008**, *32A*, 68–78.
- [24] S. Cavadini, J. Dittmer, S. Antonijevic, G. Bodenhausen, *J. Am. Chem. Soc.* **2005**, *127*, 15744–15748.
- [25] A. Bornet, P. Ahuja, R. Sarkar, L. Fernandes, S. Hadji, S. Y. Lee, A. Haririnia, D. Fushman, G. Bodenhausen, P. R. Vasos, *ChemPhysChem* **2011**, *12*, 2729–2734.
- [26] K. Shen, A. W. J. Logan, J. F. P. Colell, J. Bae, G. X. Ortiz, T. Theis, W. S. Warren, S. J. Malcolmson, Q. Wang, *Angew. Chem. Int. Ed.* **2017**, *56*, 12112–12116.
- [27] D. T. Pegg, D. M. Doddrell, M. R. Bendall, *J. Chem. Phys.* **1982**, *77*, 2745–2752.
- [28] G. A. Morris, R. Freeman, *J. Am. Chem. Soc.* **1979**, *101*, 760–762.
- [29] O. W. Sørensen, *Prog. Nucl. Magn. Reson. Spectrosc.* **1989**, *21*, 503–569.
- [30] P. H. Bolton, *J. Magn. Reson.* **1980**, *41*, 287–292.
- [31] S. Wimperis, G. Bodenhausen, *J. Magn. Reson.* **1986**, *69*, 264–282.
- [32] T. J. Henderson, *J. Am. Chem. Soc.* **2004**, *126*, 3682–3683.
- [33] B. Jiang, N. Xiao, H. Liu, Z. Zhou, X. Mao, M. Liu, *Anal. Chem.* **2008**, *80*, 8293–8298.
- [34] A. V. Mäkelä, I. Kilpeläinen, S. Heikkinen, *J. Magn. Reson.* **2010**, *204*, 124–130.
- [35] V. S. Manu, A. Kumar, *J. Magn. Reson.* **2013**, *234*, 106–111.
- [36] A. Bax, *J. Magn. Reson.* **1984**, *57*, 314–318.
- [37] A. Bax, J. A. Ferretti, N. Nashed, D. M. Jerina, *J. Org. Chem.* **1985**, *50*, 3029–3034.
- [38] B. Baishya, C. L. Khetrpal, *J. Magn. Reson.* **2014**, *242*, 143–154.
- [39] M. H. Levitt, *Annu. Rev. Phys. Chem.* **2012**, *63*, 89–105.
- [40] H. Geen, R. Freeman, *J. Magn. Reson.* **1991**, *93*, 93–141.
- [41] P. Türschmann, J. Colell, T. Theis, B. Blümich, S. Appelt, *Phys. Chem. Chem. Phys.* **2014**, *16*, 15411–15421.
- [42] F. A. Gallagher, S. E. Bohndiek, M. I. Kettunen, D. Y. Lewis, D. Soloviev, K. M. Brindle, *J. Nucl. Med.* **2011**, *52*, 1333–1336.

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Guannan Zhang*, Johannes F. P. Colell*, Thomas Glachet, Jacob R. Lindale, Vincent Reboul,* Thomas Theis,* Warren S. Warren*

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Terminal Diazirines Enable Reverse Polarization Transfer from $^{15}\text{N}_2$ Singlets

Signal detection with superior sensitivity over long time scales: The terminal diazine groups ($-\text{CHN}_2$) represent a promising class of molecular markers, that provide the opportunity to transfer hyperpolarization stored on a relaxation protected ($-\text{CH}^{15}\text{N}_2$) moiety to ^1H , thus combining the advantages of long-lived lifetimes of ^{15}N polarization with superior sensitivity of ^1H detection. We further discuss terminal diazirines to support long-lived hyperpolarized $^1\text{H}_2$ singlet states in CH_2 groups of chiral molecules.