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Communication

Application of $^{15}N_2$-Diazirines as a Versatile Platform for Hyperpolarization of Biological Molecules by d-DNP

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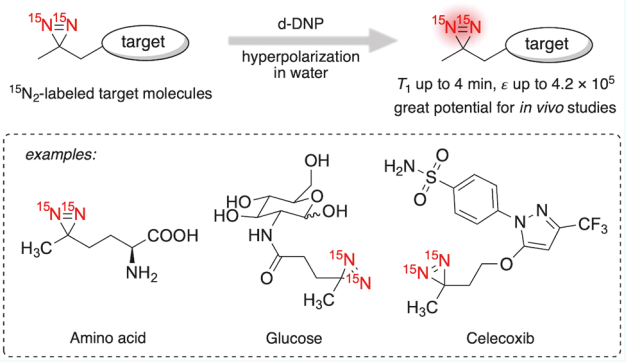
ABSTRACT: $^{15}N_2$-Diazirines represent an attractive class of imaging tags for hyperpolarized magnetic resonance imaging (HP-MRI), offering desirable biocompatibility, ease of incorporation into a variety of molecules, and ability to deliver long-lasting polarization. We have recently established hyperpolarization of $^{15}N_2$-diazirines in organic solvents using SABRE-Shield Enables Alignment Transfer to Heteronuclei (SABRE-SHEATH). Yet, the current challenge of SABRE-SHEATH in water, specifically poor polarization efficiency, presents a barrier in examining the practical use of $^{15}N_2$-diazirines for HP-MRI. Herein, we show that efficient polarization of diverse $^{15}N_2$-diazirine-labeled molecules in water can be readily achieved by dissolution dynamic nuclear polarization (d-DNP), a hyperpolarization technique used in clinical practice. Hyperpolarization by d-DNP also demonstrates greater enhancement for long-lasting ^{15}N signals, in comparison with SABRE-SHEATH. Various biologically important molecules are studied in this work, including amino acid, sugar, and drug compounds, demonstrating the great potential of $^{15}N_2$-diazirines as molecular tags in broad biomedical and clinical applications.



Magnetic resonance imaging (MRI) has received great attention in biomedical research and clinics as a noninvasive imaging technique that allows for identification, visualization, and characterization of physiological processes with high spatial and temporal resolution.[1] Yet, one of the most critical challenges of MRI is its low sensitivity arising from the intrinsically low magnetic energy of nuclear spins.[2] Low sensitivity is generally compensated by a high concentration of ^1H in the form of water and fat in the body, but scanning other MR-active isotopes with low natural abundance (e.g., ^{13}C and ^{15}N) is difficult to achieve.[3] By the hyperpolarization technique, signal sensitivity can be increased by several orders of magnitude. Particularly, hyperpolarized heteronuclei (e.g., ^{13}C and ^{15}N) often allow signal detection for extended time periods, enabling real-time *in vivo* detection of these heteronuclei for investigating dynamic metabolic and physiologic processes that were previously inaccessible to imaging.[3]

To develop novel strategies for hyperpolarized (HP)-^{15}N-MRI, we have recently reported a labeling strategy using the $^{15}N_2$-diazirine motif as a potential hyperpolarizable MRI tag with long polarization lifetime.[4,5] Diazirines, due to their small size, generally cause minimal effects on the biological activities of target molecules they are incorporated into.[6] Diazirines also show great stability in a wide range of chemical and biological conditions.[7,8] They have been used for photoaffinity labeling tags in biologically relevant molecules, which further exemplifies the compatibility of diazirines as imaging tags.[9−15] In addition, the $^{15}N−^{15}N$ moiety within $^{15}N_2$-diazirines is usually strongly coupled and close in chemical shifts, allowing support of the relaxation protected singlet state with long-lived polarization lifetimes.[16,17] To achieve such a strategy, we have first demonstrated that hyperpolarization of $^{15}N_2$-diazirines using SABRE-Shield Enables Alignment Transfer to Heteronuclei (SABRE-SHEATH) afforded excellent enhancement (ε) with long-lived (T_1) signals.[4,5] These results have established the feasibility of using the $^{15}N_2$-diazirine motif as an HP-MRI tag. Yet, hyperpolarization by SABRE has thus far been limited mostly to organic solvents such as methanol, because hyperpolarization in water leads to a decreased polarization level by orders of magnitude resulting from poor solubility of the polarizing partners in water.[18,19] This incompatibility presents challenges for translating this hyperpolarized ^{15}N-tagging method to *in vivo* applications and clinical practices.

To address this barrier, we decided to investigate hyperpolarization of $^{15}N_2$-diazirine-tagged biological molecules using

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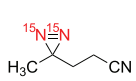
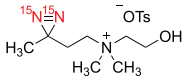
dissolution dynamic nuclear polarization (d-DNP). d-DNP is a hyperpolarization technique that is capable of polarizing almost any molecule of interest and is routinely used in preclinical studies.[20−22] Hyperpolarization occurs at low temperatures (∼1.4 K) by microwave irradiation in contact with a sample compound. The resulting hyperpolarized sample is then rapidly dissolved in superheated solvent (e.g., water), affording significantly enhanced nuclear polarization in an aqueous solution suitable for *in vivo* applications.[3,20]

In this work, we designed an array of $^{15}N_2$-diazirine-tagged compounds, including amino acid, sugar, and drug molecules. Using deuterated water as the dissolution solvent, we show that hyperpolarization of these compounds by d-DNP provided highly effective ^{15}N signal enhancements over 400,000-fold and long ^{15}N relaxation lifetimes up to 4 min at 1 T, thus paving the way for employing $^{15}N_2$-diazirines as promising molecular tags for biomedical and clinical imaging research.

RESULTS AND DISCUSSION

Our studies on hyperpolarization of $^{15}N_2$-diazirine-tagged molecules began with diazirines **1** and **2** (Table 1), which were previously reported to deliver long-lasting signals with 10^3-fold enhancement by SABRE-SHEATH hyperpolarization in D_4-MeOH.[4] Yet the presence of D_2O resulted in a ∼3.5-fold decrease in signal enhancement, as seen in $^{15}N_2$-diazirine-tagged choline derivative **2**. When we used the d-DNP method for their hyperpolarization in water, both compounds **1** and **2** afforded much higher levels of 10^4−10^5-fold enhancement, and long-lived ^{15}N-signal with comparable T_1 relaxation times, in comparison with SABRE-SHEATH (Table 1).

Table 1. Hyperpolarization of $^{15}N_2$-Diazirines 1 and 2 via SABRE-SHEATH and d-DNP Methods[a]

		 **1**	 **2**	
SABRE-SHEATH (in D_4-MeOH)[a]				*with D_2O*
	ε	7,100	6,800	2,000
	T_1 [min]	3.13 ± 0.17	3.70 ± 0.13	2.98 ± 0.32
d-DNP (in D_2O)[b]	ε	90,600	276,200	
	T_1 [min]	3.43 ± 0.11	2.36 ± 0.03	

[a]SABRE-SHEATH hyperpolarization conditions: diazirine (12.5 mM) in D_4-MeOH, Ir catalyst (0.125 mM), pyridine (1 mM) or D_2O (925 mM). Signal enhancement (ε) measured at 8.45 T and calculated by comparison to a reference of neat ^{15}N labeled acetonitrile at 8.45 T. T_1 measured at 1 T. Data from ref 4. [b]d-DNP hyperpolarization conditions: solution of diazirine (**1**, **2**) (500 mM) in DMSO/D_2O (volume ratio: 1/1), OX63 radical (15 mM), and Gd-DTPA (1 mM), dissolution solvent D_2O. Signal enhancement (ε) measured at 1 T and calculated by comparison to a reference of neat ^{15}N labeled acetonitrile at 1 T. T_1 measured at 1 T.

Based on the encouraging results of $^{15}N_2$-diazirines **1** and **2**, we investigated the generality and amenability of $^{15}N_2$-diazirines on complex biological molecules by d-DNP hyperpolarization. A range of $^{15}N_2$-diazirine-labeled compounds were prepared, including simple molecules (**3**−**4**) and biologically relevant molecules (**5**−**8**). The hyperpolarization efficiency of these molecules is shown in Table 2.

We first tested d-DNP hyperpolarization of $^{15}N_2$-diazirine-containing simple carboxylic acid (**3**) and alcohol (**4**). Both compounds provided ^{15}N-signal enhancement over ∼250,000-fold and long T_1 values. These results illustrate the functional group tolerability of diazirine compounds with d-DNP.

To demonstrate the adaptability of the $^{15}N_2$-diazirine tag on various target molecules, we chose to synthesize $^{15}N_2$-diazirine celecoxib analog **5**. Celecoxib is a known anti-inflammatory drug and $^{14}N_2$-diazirine-labeled celecoxib has been used for photoaffinity labeling studies to map the binding site of the drug.[23] Thus, $^{15}N_2$-diazirine celecoxib may be useful as an exogenous probe to study drug−enzyme interactions by HP-MRI. Hyperpolarization of celecoxib analog **5** by d-DNP provided signal enhancement over 114,000-fold and T_1 value over 4 min. Such significantly enhanced and long-lived signals are highly attractive for *in vivo* imaging applications, which also highlight efficient hyperpolarization and utility of the $^{15}N_2$-diazirine tag for studying and imaging drug molecules. Note that D_4-MeOH was used as a solvent in d-DNP experiment because of the poor solubility of $^{15}N_2$-celecoxib in D_2O.

We next examined the incorporation of $^{15}N_2$-diazirine into biological molecules and prepared $^{15}N_2$-photomethionine **6** as an example for a $^{15}N_2$-diazirine-tagged amino acid. Photoaffinity labeling studies with $^{14}N_2$-diazirine-tagged amino acids, including $^{14}N_2$-photomethionine, have shown diazirines to be biocompatible agents for metabolic studies.[11] Thus, we envisioned the use of $^{15}N_2$-diazirine-tagged amino acids as metabolic imaging probes for HP-MRI. Hyperpolarization of $^{15}N_2$-diazirine-tagged methionine **6** by d-DNP afforded high efficiency with ^{15}N signal enhancement over ∼293,000-fold and T_1 value close to 4 min in water. Amino acid derivatives labeled with MR active isotopes have been hyperpolarized for various bioimaging studies,[24,25] which denotes amino acid analogs as adaptable hyperpolarized imaging probes. Similarly, $^{15}N_2$-diazirine-incorporated amino acids such as **6** afford favorable polarization lifetime and may offer an innovative platform for hyperpolarized imaging applications.

We also prepared $^{15}N_2$-diazirine-tagged glucose molecules **7** and **8** for d-DNP studies. ^{13}C-labeled glucose has been extensively used for real-time imaging of glycolysis using HP-MRI, but it suffers from short polarization lifetime (e.g., T_1 for [U−^{2}H, U−^{13}C]-glucose is 12 s at 14.1 T).[21,26] d-DNP hyperpolarization of $^{15}N_2$-diazirine-tagged glucose derivatives **7** and **8** provided ample ^{15}N-signal enhancement over 400,000-fold and T_1 values over 3 min in water. As the feasibility of a HP-MRI probe is dependent on the relaxation time, the long T_1 lifetime of $^{15}N_2$-diazirine-tagged glucose molecules is an attractive attribute for their biomedical imaging applications. Moreover, **8** with a deuterated methyl group had a comparable hyperpolarization level to nondeuterated glucose derivative **7**, indicating that deuterium labeling has a negligible effect on $^{15}N_2$-diazirine hyperpolarization. Thus, efficient hyperpolarization of these glucose analogs in aqueous solution demonstrates their values as HP-MRI probes for tissue perfusion and targeting studies.

Finally, we compared hyperpolarization between d-DNP and the SABRE-SHEATH method for three selected diazirines (Table 2, **4**−**6**). Under SABRE-SHEATH conditions, $^{15}N_2$-diazirine-containing alcohol **4** was successfully hyperpolarized

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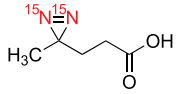
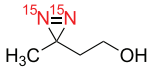
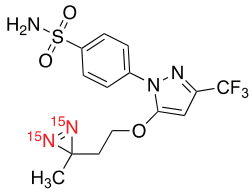
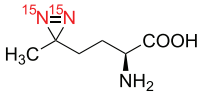
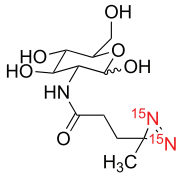
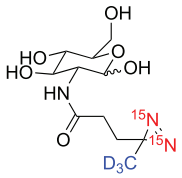
Table 2. Hyperpolarization of $^{15}N_2$-Diazirines—Enhancements (ε), Polarization levels (P), and ^{15}N T_1 Relaxation Times[a]

Diazirines	d-DNP[a]			SABRE-SHEATH[b]		
	ε[c]	P (%)	T_1[d] (min)	ε[c]	P (%)	T_1[d] (min)
 **3**	293,600	10.2	3.60 ± 0.04	–[e]	–	–
 **4**	248,800	8.6	3.81 ± 0.05	69,000	2.4	3.30 ± 0.04
 **5**	114,100[f,g]	3.9	4.10 ± 0.05	76,600	2.6	3.84 ± 0.05
 **6**	292,700	10.1	3.93 ± 0.07	6,400[h]	0.2	1.15 ± 0.16[h]
 **7**	408,600	14.1	2.97 ± 0.04	–[i]	–	–
 **8**	418,400	14.5	3.06 ± 0.04	–[e]	–	–

[a]d-DNP hyperpolarization conditions: solution of diazirine in DMSO/D_2O (See SI for details), OX63 radical (15 mM), and Gd-DTPA (1 mM). Dissolution solvent D_2O. [b]SABRE-SHEATH hyperpolarization conditions: diazirine (12.5 mM) in D_4-MeOH, Ir catalyst (0.125 mM), and pyridine (1 mM). [c]Signal enhancement (ε) measured at 1 T and calculated by comparison to a reference of neat ^{15}N labeled acetonitrile at 1 T. [d]T_1 measured at 1 T. [e]Not tested by SABRE-SHEATH polarization. [f]D_4-MeOH as a dissolution solvent. [g]Solution of diazirine in THF/toluene (see SI for details). [h]Polarization and T_1 affected by acidity of the compound solution.[27] (See SI for details). [i]Insoluble in D_4-MeOH.

in D_4-MeOH, exhibiting signal enhancement over 69,000-fold and T_1 value of 3.3 min, which is a similar trend to that observed for diazirines **1** and **2**.[4] SABRE-SHEATH hyperpolarization of celecoxib analog **5** in D_4-MeOH yielded lower polarization level ($P_{\text{SABRE-SHEATH}}$ = 2.6%; $P_{\text{d-DNP}}$ = 3.9%) and a comparable T_1 value, in comparison with d-DNP hyperpolarization of **5** in the same solvent. For the polarization of methionine analog **6** by SABRE-SHEATH, the signal enhancement (ε = 6400) was much lower, which was likely influenced by acidity of the compound solution.[27] An attempt to hyperpolarize glucose derivative **7** by SABRE-SHEATH was unsuccessful due to the insolubility of **7** in D_4-MeOH. These results suggested that hyperpolarization of diazirines by d-DNP is less limited by the chemical nature of the compound.[28]

CONCLUSION

In summary, we have investigated hyperpolarization of various $^{15}N_2$-diazirine-tagged biologically relevant molecules using d-DNP in aqueous solution. The $^{15}N_2$-diazirine-tagged compounds delivered signal enhancements over 400,000-fold and long ^{15}N relaxation lifetimes up to 4 min. The significantly enhanced signal and the long-lasting ^{15}N T_1 lifetimes of the $^{15}N_2$-diazirine moiety within these compounds in water offer the opportunity to observe ^{15}N signal for an extended period of time in biological systems, thus establishing $^{15}N_2$-diazirine tags

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suitable for *in vivo* applications. Compared to the SABRE-SHEATH method, hyperpolarization of $^{15}N_2$-diazirines with d-DNP offered higher polarization efficiency and comparable T_1 lifetimes. These results mark the promising potential of $^{15}N_2$-diazirines-labeled biological probes as useful hyperpolarized imaging tracers for various applications in biomedical and clinical research.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.bioconjchem.0c00028.

Synthesis and characterization of $^{15}N_2$-diazirines, hyperpolarization experimental methods, ^{1}H, ^{13}C, and ^{15}N NMR spectra and data analysis (PDF)

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§H. Park, G. Zhang, and J. Bae contributed equally to this work.

Notes

The authors declare no competing financial interest.

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(28) It should be noted that the polarization time in SABRE-SHEATH experiments (∼30 min) was shorter than the polarization time in d-DNP experiments (3.5 h), which could relate to the lower polarization levels of targeted molecules by SABRE-SHEATH methods. See polarization experimental details in the Supporting Information.

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