Synthesis of ^{15}N-Pyridines and Higher Mass Isotopologs via Zincke Imine Intermediates

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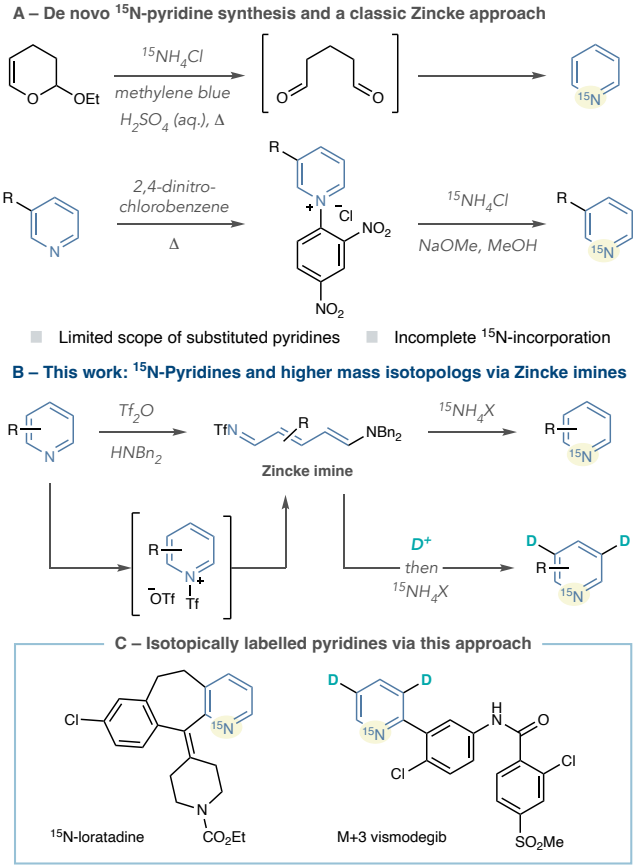
ABSTRACT: Methods to incorporate stable radioisotopes are integral to pharmaceutical and agrochemical development. However, despite the prevalence of pyridines in candidate compounds, methods to incorporate ^{15}N-atoms within their structures are limited. Here, we present a general approach to pyridine ^{15}N-labeling that proceeds via ring-opening to *N*Tf-Zincke imines and then ring-closure with commercially available $^{15}NH_4Cl$ salts. This process functions on a range of substituted pyridines, from simple building block-type compounds to late-stage labeling of complex pharmaceuticals, and ^{15}N-incorporation is >95% in most cases. The reactivity of the Zincke imine intermediates also enables deuteration of the pyridine C3- and C5-positions, resulting in higher mass isotopologs required for LCMS analysis of biological fluids during drug development.

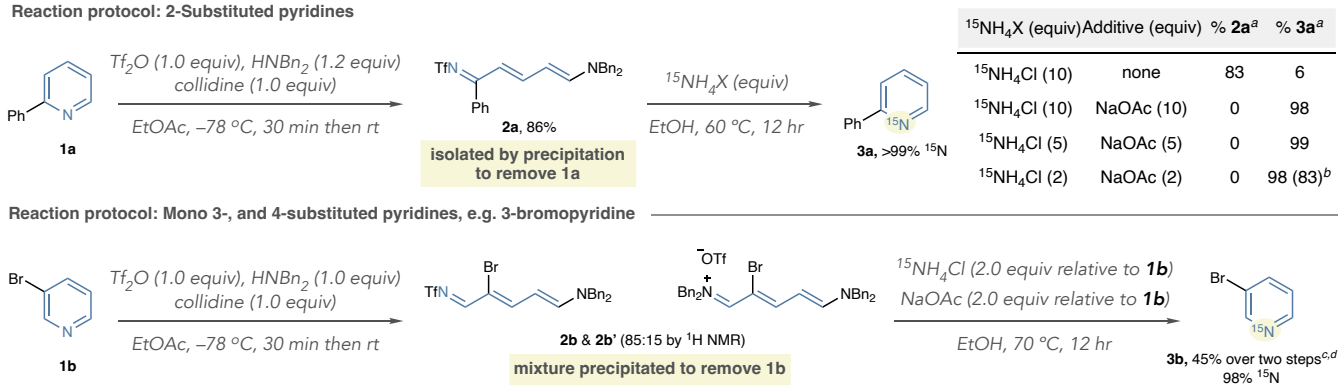
Incorporating stable radioisotopes into organic molecules has implications across various scientific disciplines and is particularly relevant to drug and agrochemical development. Stable mass isotopologs of candidate compounds are required at several stages of development, including structure elucidation, absorption, distribution, metabolism, excretion, and toxicology (ADMET) studies.[1-3] They are also used as mechanistic probes in conjunction with NMR and mass spectrometry.[4-6] Therefore, a subfield of chemical synthesis dedicates efforts to developing methods that incorporate stable isotopes into organic molecules, and two strategies dominate. First, *de novo* synthesis incorporates isotopic labels into pharmacologically relevant motifs by combining simpler precursors.[7] Second are isotope exchange reactions involving C–H bonds or functional groups on the periphery of molecules.[8-14] A remaining challenge is to isotopically exchange atoms inside heterocycles, which avoids multi-step *de novo* synthesis. Here, we report a process that converts ^{14}N-pyridines into their ^{15}N-isotopologs by ring-opening to Zincke imine intermediates and ring-closing using $^{15}NH_4Cl$.[15] This method functions on various substitution patterns, is effective on complex pyridines, and provides excellent ^{15}N-incorporation in the majority of cases. Deuteration of the C3- and C5-positions is also viable to produce higher M+2 and M+3 pyridine isotopologs.

The prevalence of pyridines and related azines in pharmaceutical and agrochemical compounds means that their isotopologs are relevant to the applications described above.[16-19] Additionally, techniques are emerging that specifically use ^{15}N-azines, such as the SABRE-SHEATH approach, where spin hyperpolarization phenomena have the potential for bioimaging.[20-22] However, current methods to incorporate ^{15}N-atoms within their structure are limited. Scheme 1A shows a *de novo* approach under oxidative conditions using tetrahydropyran derivatives as glutaraldehyde surrogates;[23] applying this method beyond pyridine would require extensive synthetic effort to append substituents within the starting material. Chukanov and Chekmenev used classic Zincke chemistry to form a small number of ^{15}N-pyridines.[24, 25] Installing the dinitroaryl

Scheme 1. Current Methods to Synthesize ^{15}N-Pyridines and an Approach via Zincke Imine Intermediates.



Scheme 2. Development of Two General Protocols for Pyridine ^{15}N-Labeling



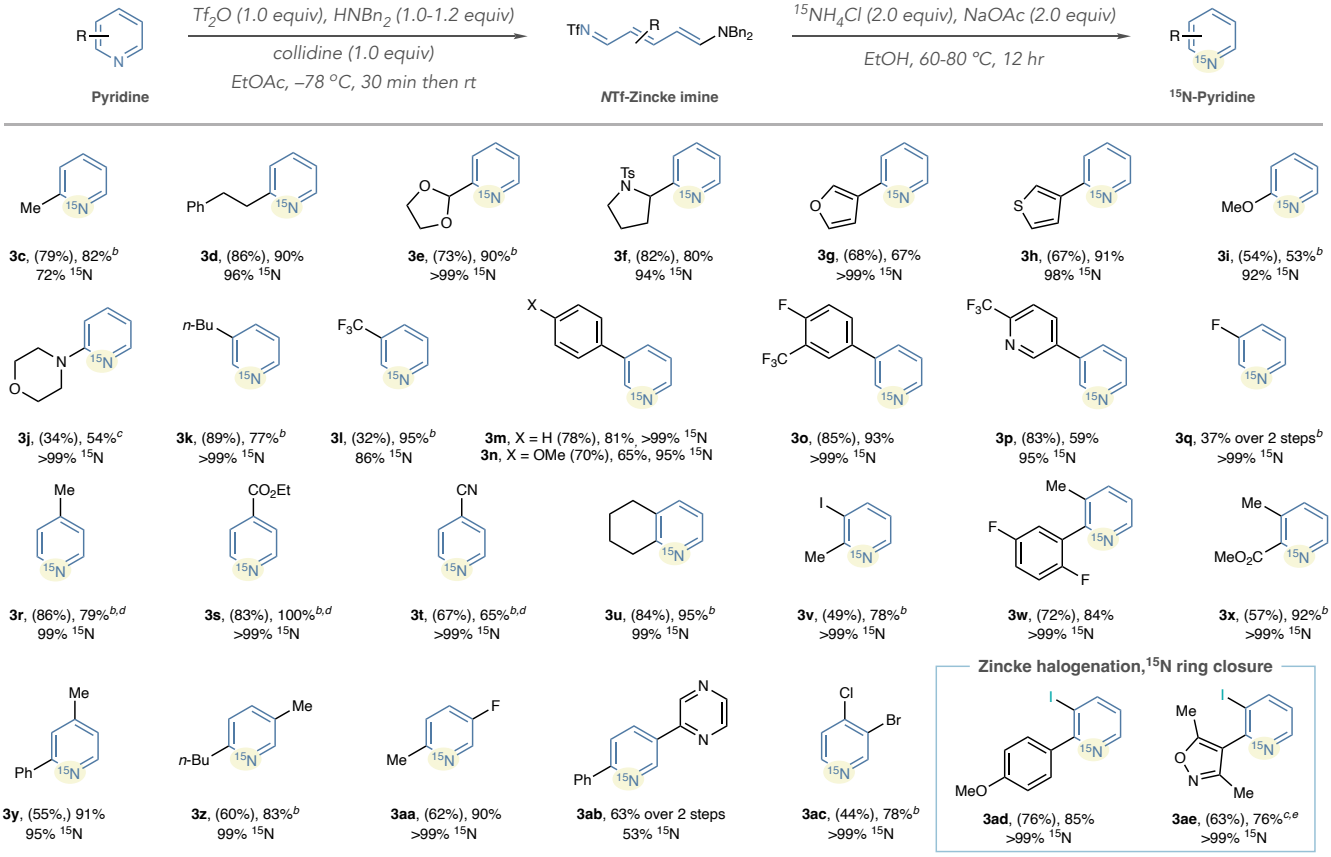
$^{15}NH_4X$ (equiv)	Additive (equiv)	% **2a**[a]	% **3a**[a]
$^{15}NH_4Cl$ (10)	none	83	6
$^{15}NH_4Cl$ (10)	NaOAc (10)	0	98
$^{15}NH_4Cl$ (5)	NaOAc (5)	0	99
$^{15}NH_4Cl$ (2)	NaOAc (2)	0	98 (83)[b]

[a]Yields determined by ^{1}H NMR analysis using mesitylene as an internal standard. [b]Isolated yield. [c]^{1}H NMR yield reported due to the volatility of **3b**. [d]An analytically pure sample of **3b**·HCl was obtained in 30% yield.

N-activating group in this approach limits the scope significantly as the process does not tolerate 2-position substituents and generally excludes applications such as late-stage ^{14}N to ^{15}N-exchange.[26] Furthermore, they observed incomplete ^{15}N-incorporation during the subsequent ring-opening ring-closing sequence.

Our group has a longstanding interest in the reactivity of *N*Tf-azinium salts, and Scheme 1B shows our proposed method to synthesize ^{15}N-pyridines.[27-30] In 2022, we disclosed that *N*Tf-activated pyridines undergo facile ring-opening with dibenzylamine to form *N*Tf-Zincke imines as part of a method for 3-selective halogenation.[15, 31] Ring-closing with $^{15}NH_4X$ salts would be a trivial way to incorporate the ^{15}N-atom, *and was obvious to us based on our previous work*. There are three distinct advantages of this approach.

Table 1. ^{15}N Labeling of Building Block-Type Pyridines via Zincke Imines[a,b,c,d,e]



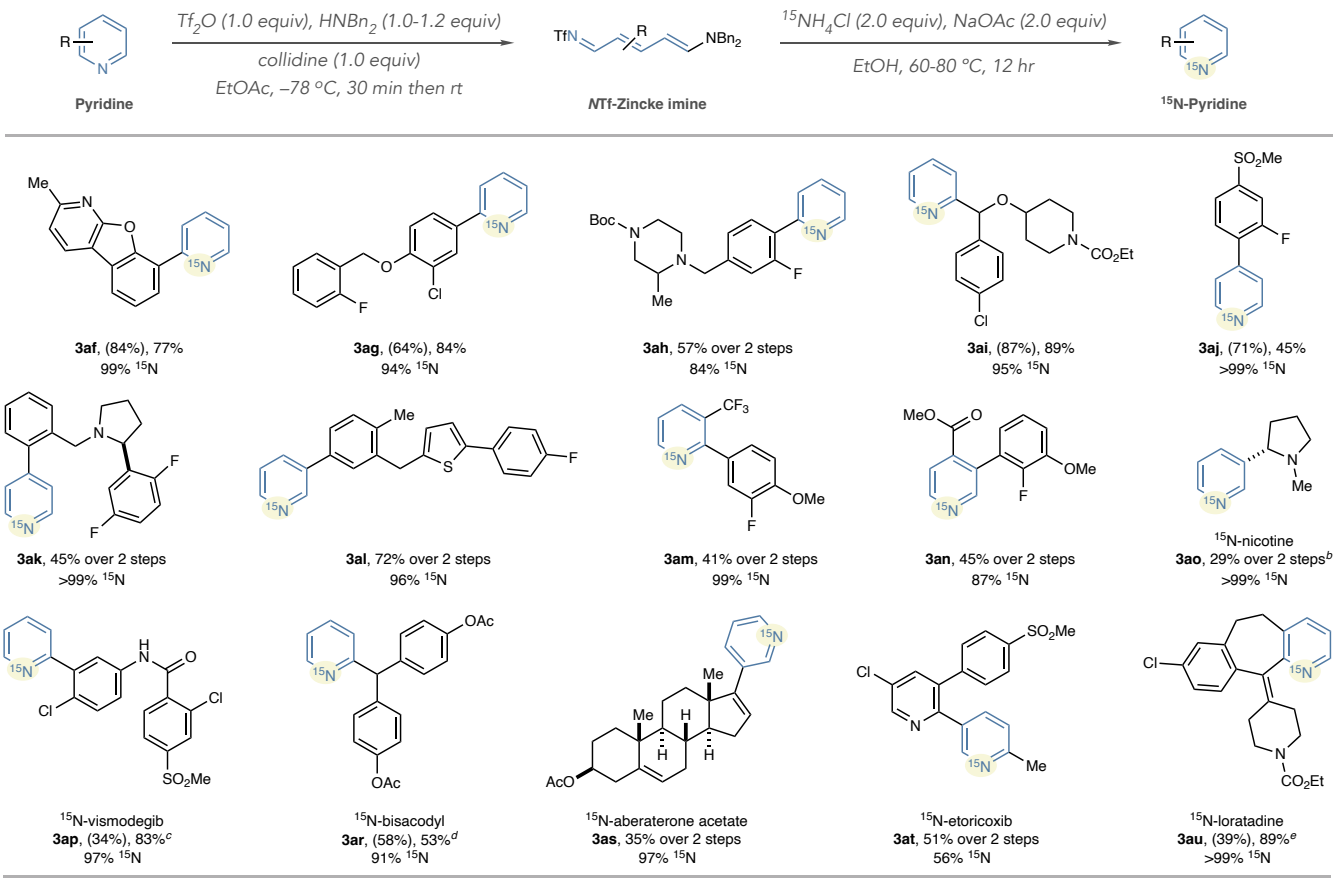
[a]Isolated yield of products are shown. Yields in parentheses are those of the isolated Zincke imines. [b]^{1}H NMR yields reported with reference to an internal standard due to product volatility. [c]Recyclized for 48 hours. [d]Reaction used 2.0 equiv of Bn_2NH and recyclized at 60 ºC for 1 hour. [e]Recyclized with 4.0 equiv $^{15}NH_4Cl$ and 4.0 equiv NaOAc.

First, the scope of pyridines is large, with 2-substitution tolerated and late-stage applications on complex pyridine-containing drugs are viable. Second, $^{15}NH_4X$ salts are relatively inexpensive as ^{15}N-sources and, based on our understanding of the ring-closing mechanism, we expected high levels of ^{15}N-incorporation. Third, deuterating the electron rich C3- and C5-positions in the Zincke imines prior to ring closure would result in higher mass pyridine isotopologs, which are required for *in vivo* ADMET studies (*vide infra*). Scheme 1C shows examples of pyridine isotopologs obtainable via this approach.[32]

Reaction Development. Scheme 2 shows two reaction protocols for ^{15}N-labeling that vary based on the pyridine substitution pattern. For 2-substituted pyridines, such as 2-phenylpyridine **1a**, our previously reported ring-opening conditions using Tf_2O, dibenzylamine, and collidine at low temperatures are appropriate.[15, 31] Washing the reaction mixture with an aqueous solution of $CuSO_4$ removes dibenzylamine, and a significant advantage of this approach is the solubility properties of Zincke imines that precipitate when we add the organic extracts to hexanes. Isolating **2a** in this fashion ensures that unlabeled **1a** does not advance into the subsequent ring-closing step. Our previous protocol to ring close Zincke imines used ten equivalents of NH_4OAc in EtOH at 60 ºC. Here, we preferred $^{15}NH_4Cl$ as the least expensive radiolabeled ammonium salt, but the yield of ^{15}N-pyridine **3a** was poor. However, adding an equimolar amount of NaOAc to the reaction formed **3a** in excellent yield. Scheme 2 also shows that the reaction performs equally well using two equivalents of $^{15}NH_4Cl$ and NaOAc. We isolated **3a** with >99% ^{15}N-incorporation, showing that the net isotope exchange process can occur with near perfect fidelity. In cases where precipitation of Zincke imines occurs with minor impurities, we elected on a protocol that adds two equivalents of $^{15}NH_4Cl$ and NaOAc relative to the pyridine starting material and recorded yields over two steps (*vide infra*).

In preliminary studies, we occasionally observed mixtures of Zincke imines and iminium salts during the ring-opening stage for pyridines with certain substitution patterns (Scheme 2). Specifically, we have witnessed this outcome for mono 3- and 4-substituted pyridines, with electron-withdrawing groups increasing the tendency to form iminium salts. For example, when ring-opening 3-bromopyridine **1b**, an 85:15 mixture of Zincke imine **2b** and iminium salt **2b'** formed. In this case, we isolated the mixture of **2b** and **2b'** via precipitation. In the ring-closing step, we used two equivalents of $^{15}NH_4Cl$ and NaOAc relative to **1b**, efficiently forming ^{15}N-3-bromopyridine **3b** with 98% isotopic incorporation.

Table 2. ^{15}N Labeling of Drug-Like Fragments and Pharmaceuticals via Zincke Imines[a,b,c,d]



[a]Isolated yield of products are shown. Yields in parentheses are those of the isolated Zincke imines. [b]^1H NMR yields reported with reference to an internal standard due to product volatility. [c]Recyclized with 4.0 equiv of $^{15}NH_4Cl$ and 4.0 equiv of NaOAc. [d]Recyclized for 1 hour. [e]Recyclized for 48 hours with 10.0 equiv of $^{15}NH_4Cl$ and [e]Recyclized for 48 hours with 10.0 equiv of $^{15}NH_4Cl$ and 10.0 equiv of AgOAc.

Results and Discussion. We next investigated the scope of building block pyridines with these two protocols in this isotope exchange process (Table 1). In most of the examples tested, we measured >95% ^{15}N in the pyridine products, with a significant proportion measured at >99%. In a small number of cases, ^{15}N-incorporation was lower indicating that competing mechanisms can occur that do not fully replace the initial ^{14}N-atom. Starting with 2-alkyl derivatives, we found that the process tolerated methyl and phenethyl substituents, an acetal, and a protected pyrrolidine (**3c-3f**). Furan and thiophene heterocycles are also viable at the 2-position, as are 2-methoxy and 2-amino derivatives (**3g-3j**). Examples **3k** and **3l** show that the sequence accommodates alkyl and trifluoromethyl groups at the 3-position. Similarly, a series of 3-(hetero)aryl groups worked well (**3m-3p**), with **3p** forming with complete regiocontrol. As well as 3-bromopyridine shown in Scheme 2, we also obtained a ^{15}N-version of 3-fluoropyidine in reasonable yield over two steps (**4q**).

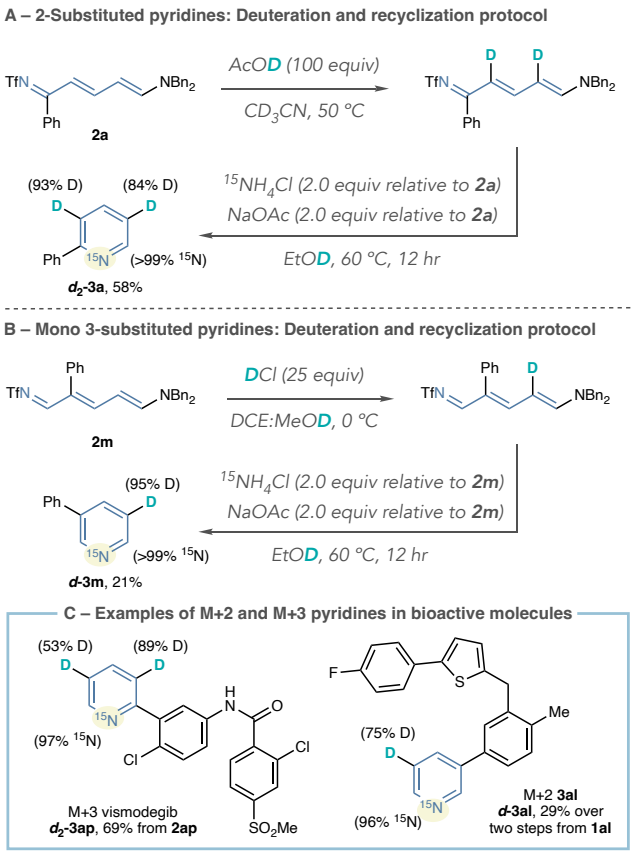
Mono 4-substituted pyridines are also compatible (**4r-4t**). Next, we tested a series of disubstituted pyridines and found successful outcomes with 2,3-, 2,4- 2,5- and 3,4-substitution patterns containing a range of useful functional handles and carbon-bearing groups (**4u-4ac**). Lastly, selective C3-halogenation of intermediate Zincke imines followed by ring closure is also effective in forming 2,3-disubstituted pyridines **4ad** & **4ae**.[15]

In the next part of this study, we tested a collection of drug-like molecules and pharmaceutical compounds in this labeling process (Table 2). These molecules are generally more complex than the building block pyridines in Table 1 and can possess multiple heterocycles and reactive sites. To access ^{15}N-labeled pyridines in these advanced molecules, practitioners typically process simpler ^{15}N-pyridines through multi-step sequences. This effort is laborious and potentially unfeasible if an appropriate ^{15}N-pyridine is not readily available. Such drawbacks can preclude applications such as SABRE-SHEATH discussed previously. Examples **4af-4ah** are 2-arylpyridine derivatives resembling drug fragments and are easily obtainable from the parent ^{14}N-compound. We also constructed a ^{15}N-version of a derivative of the antihistamine bepotastine (**4ai**). Monosubstituted pyridines with groups at the 3- and 4-positions also work well (**4aj-4al**), as do 2,3-disubstituted examples **4am** & **4an**. It was also possible to synthesize ^{15}N-nicotine via this approach (**4ao**). We then successfully applied the two-step process on a series of drug compounds and formed ^{15}N-isotopologs of vismodegib, bisacodyl, zytiga, etoricoxib, and loratadine (**4ap-4au**).

In the final part of this study, we deuterated the intermediate Zincke imines prior to ring closure to obtain M+2 and M+3 pyridine isotopologs. During *in vivo* ADMET studies involving biofluids such as blood plasma, ion suppression can occur that can introduce substantial inaccuracies and limit quantitative analysis. Higher mass isotopologs, with ion traces that do not overlap with the parent compound, serve as internal standards and alleviate these problems.[3, 33, 34] While these protocols normally require isotopologs with +4 mass units or higher, having sets of M+2 and M+3 pyridines is still valuable. Combined with other labeling methods, practitioners can introduce additional mass units into distinct portions of a candidate's structure. Adding isotopic labels at later stages of drug development can also facilitate this effort and avoid recourse to extensive synthesis from simpler precursors.

Scheme 3 shows a preliminary study that revealed two reaction protocols that incorporate deuterium atoms into Zincke imine intermediates. For 2-substituted pyridines, we tested Zincke imine **2a** and found that stirring in AcOD at 50 ºC hours deuterates the electron-rich C3- and C5-positions with reasonable level of D-incorporation (Scheme 3A). During this process, we observed minor amounts of recyclization to 2-phenylpyridine with incomplete deuterium incorporation; precipitating **2m** removes this partially unlabeled material and subjecting the crude material to ring closure under the standard protocol forms ***d*$_2$-3a** with reasonable levels of D- and ^{15}N-incorporation, and in 21% yield. For mono 3-substituted pyridines, we used a modified deuteration procedure (Scheme 3B). Stirring adduct **2d** in an EtOD/DCE solution of DCl at 0 ºC results in complete deuteration at C5; we again precipitated the deuterated intermediate to remove minor amounts of partially deuterated pyridine in the reaction mixture, and recyclization with $^{15}NH_4Cl$ formed ***d*-3m** with excellent isotopic purity. Scheme 3C shows two examples of complex pyridine-containing products that we converted into M+2 and M+3 higher-mass isotopologs using the above protocols. We obtained ***d*$_2$-3ap** from vismodegib in good yield over two steps ***d*-3al** from its respective Zincke imine in reduced efficiency. Further investigations will focus on increasing the levels of

Scheme 3. Deuterium and ^{15}N Labeling Sequences for [M+2] and [M+3] Pyridines



[a]Isolated yields are shown. [b]Percentage deuterium incorporation measured by ^{1}H NMR spectra. [c]Percentage ^{15}N incorporation values extrapolated from values from Tables 1 & 2.

deuterium incorporation and expanding this protocol to other complex pyridine-containing structures.

In summary, we have developed a process that ring opens ^{14}N-pyridines and then incorporates ^{15}N-atoms into their structure during a ring-closing step. This simple protocol uses common reagents and $^{15}NH_4Cl$, a commercially available source of ^{15}N-atoms and generally results in >95% isotope incorporation. The process functions on building block pyridines with various substitution patterns and functional groups and is viable for late-stage ^{15}N-incorporation in complex pyridine-containing molecules. We extended this process to label pyridine C3- and C5-positions with deuterium atoms, resulting in higher mass isotopologs that are valuable for *in vivo* ADMET studies. Given that simple ammonia sources function in this protocol, the chemistry should translate to ^{13}N labeling for PET tracer applications.

ASSOCIATED CONTENT

Supporting Information

(Experimental procedures and spectral data.

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Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡†These authors contributed equally. (match statement to author names with a symbol)

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ABBREVIATIONS

ADMET, absorption, distribution, metabolism, excretion and toxicology.

REFERENCES

(1) Elmore, C. S.; Bragg, R. A. Isotope Chemistry; A Useful Tool In The Drug Discovery Arsenal. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 167-171. DOI:10.1016/j.bmcl.2014.11.051

(2) Dalvie, D. Recent Advances in the Applications of Radioisotopes in Drug Metabolism, Toxicology and Pharmacokinetics. *Curr. Pharm. Des.* **2000**, *6*, 1009-1028. DOI:10.2174/1381612003399941

(3) Hesk, D.; McNamara, P. Synthesis of Isotopically Labelled Compounds at Schering-

Plough, an Historical Perspective. *J. Label. Compd. Radiopharm.* **2007**, *50*, 875-887. DOI:10.1002/jlcr.1424

(4) Theillet, F. X. In-Cell Structural Biology by NMR: The Benefits of the Atomic Scale. *Chem. Rev.* **2022**, *122*, 9497-9570. DOI:10.1021/acs.chemrev.1c00937

(5) Lehmann, W. D. A Timeline of Stable Isotopes and Mass Spectrometry in the Life Sciences. *Mass. Spectrom. Rev.* **2017**, *36*, 58-85. DOI:10.1002/mas.21497

(6) James, E. I.; Murphree, T. A.; Vorauer, C.; Engen, J. R.; Guttman, M. Advances in Hydrogen/Deuterium Exchange Mass Spectrometry and the Pursuit of Challenging Biological Systems. *Chem. Rev.* **2022**, *122*, 7562-7623. DOI:10.1021/acs.chemrev.1c00279

(7) Gevaert, K.; Impens, F.; Ghesquiere, B.; Van Damme, P.; Lambrechts, A.; Vandekerckhove, J. Stable Isotopic Labeling in Proteomics. *Proteomics* **2008**, *8*, 4873-4885. DOI:10.1002/pmic.200800421

(8) Hesk, D. Highlights of C (sp^2)-H Hydrogen Isotope Exchange Reactions. *J. Labelled. Comp. Radiopharm.* **2020**, *63*, 247-265. DOI:10.1002/jlcr.3801

(9) Labiche, A.; Malandain, A.; Molins, M.; Taran, F.; Audisio, D. Modern Strategies for Carbon Isotope Exchange. *Angew. Chem. Int. Ed.* **2023**, *62*, e202303535. DOI:10.1002/anie.202303535

(10) Kong, D.; Munch, M.; Qiqige, Q.; Cooze, C. J. C.; Rotstein, B. H.; Lundgren, R. J. Fast Carbon Isotope Exchange of Carboxylic Acids Enabled by Organic Photoredox Catalysis. *J. Am. Chem. Soc.* **2021**, *143*, 2200-2206. DOI:10.1021/jacs.0c12819

(11) Kang, Q.-K.; Shi, H. Catalytic Hydrogen Isotope Exchange Reactions in Late-Stage Functionalization. *Synlett* **2022**, *33*, 329-338. DOI:10.1055/a-1354-0367

(12) Atzrodt, J.; Derdau, V.; Kerr, W. J.; Reid, M. C-H Functionalisation for Hydrogen Isotope Exchange. *Angew. Chem. Int. Ed.* **2018**, *57*, 3022-3047. DOI:10.1002/anie.201708903

(13) Atzrodt, J.; Derdau, V.; Fey, T.; Zimmermann, J. The Renaissance of H/D Exchange. *Angew. Chem. Int. Ed.* **2007**, *46*, 7744-7765. DOI:10.1002/anie.200700039

(14) Dorsheimer, J. R.; Rovis, T. Late-Stage Isotopic Exchange of Primary Amines. *J. Am. Chem. Soc.* **2023**. DOI:10.1021/jacs.3c09442

(15) Boyle, B. T.; Levy, J. N.; de Lescure, L.; Paton, R. S.; McNally, A. Halogenation of the 3-Position of Pyridines Through Zincke Imine Intermediates. *Science* **2022**, *378*, 773-779. DOI:10.1126/science.add8980

(16) Vitaku, E.; Smith, D. T.; Njardarson, J. T. Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles Among U.S. FDA Approved Pharmaceuticals. *J. Med. Chem.* **2014**, *57*, 10257-10274. DOI:10.1021/jm501100b

(17) Baumann, M.; Baxendale, I. R. An Overview of the Synthetic Routes to the Best Selling Drugs Containing 6-Membered Heterocycles. *Beilstein J. Org. Chem.* **2013**, *9*, 2265-2319. DOI:10.3762/bjoc.9.265

(18) Zakharychev, V. V.; Kuzenkov, A. V.; Martsynkevich, A. M. Good Pyridine Hunting: A Biomimic Compound, a Modifier and a Unique Pharmacophore in Agrochemicals. *Chem. Heterocycl. Compd.* **2020**, *56*, 1491–1516.

(19) Josephitis, C. M.; Nguyen, H. M. H.; McNally, A. Late-Stage C-H Functionalization of Azines. *Chem. Rev.* **2023**, *123*, 7655-7691. DOI:10.1021/acs.chemrev.2c00881

(20) Truong, M. L.; Theis, T.; Coffey, A. M.; Shchepin, R. V.; Waddell, K. W.; Shi, F.; Goodson, B. M.; Warren, W. S.; Chekmenev, E. Y. ^{15}N Hyperpolarization by Reversible Exchange Using SABRE-SHEATH. *J. Phys. Chem. C* **2015**, *119*, 8786-8797. DOI:10.1021/acs.jpcc.5b01799

(21) Shchepin, R. V.; Barskiy, D. A.; Coffey, A. M.; Theis, T.; Shi, F.; Warren, W. S.; Goodson, B. M.; Chekmenev, E. Y. ^{15}N Hyperpolarization of Imidazole-$^{15}N_2$ for Magnetic Resonance pH Sensing via SABRE-SHEATH. *ACS Sens.* **2016**, *1*, 640-644. DOI:10.1021/acssensors.6b00231

(22) Kovtunov, K. V.; Kovtunova, L. M.; Gemeinhardt, M. E.; Bukhtiyarov, A. V.; Gesiorski, J.; Bukhtiyarov, V. I.; Chekmenev, E. Y.; Koptyug, I. V.; Goodson, B. M. Heterogeneous Microtesla SABRE Enhancement of ^{15} N NMR Signals. *Angew. Chem. Int. Ed.* **2017**, *56*, 10433-10437. DOI:10.1002/anie.201705014

(23) Whaley, T. W.; Ott, D. G. Syntheses with stable isotopes: Pyridine-^{15}N. *J. Labelled Comp.* **1974**, *10*, 283-286. DOI:10.1002/jlcr.2590100212

(24) Chukanov, N. V.; Kidd, B. E.; Kovtunova, L. M.; Bukhtiyarov, V. I.; Shchepin, R. V.; Chekmenev, E. Y.; Goodson, B. M.; Kovtunov, K. V.; Koptyug, I. V. A Versatile Synthetic Route to the Rreparation of ^{15}N heterocycles. *J Labelled. Comp. Radiopharm.* **2019**, *62*, 892-902. DOI:10.1002/jlcr.3699

(25) Shchepin, R. V.; Barskiy, D. A.; Mikhaylov, D. M.; Chekmenev, E. Y. Efficient Synthesis of Nicotinamide-1-^{15}N for Ultrafast NMR Hyperpolarization Using Parahydrogen. *Bioconjug. Chem.* **2016**, *27*, 878-882. DOI:10.1021/acs.bioconjchem.6b00148

(26) Cheng, W. C.; Kurth, M. J. The Zincke Reaction. A Review. *Org. Prep. Proced. Int.* **2002**, *34*, 585-608. DOI:10.1080/00304940209355784

(27) Hilton, M. C.; Dolewski, R. D.; McNally, A. Selective Functionalization of Pyridines via Heterocyclic Phosphonium Salts. *J. Am. Chem. Soc.* **2016**, *138*, 13806-13809. DOI:10.1021/jacs.6b08662

(28) Dolewski, R. D.; Hilton, M. C.; McNally, A. 4-Selective Pyridine Functionalization Reactions via Heterocyclic Phosphonium Salts. *Synlett* **2018**, *29*, 8-14.

(29) Koniarczyk, J. L.; Hesk, D.; Overgard, A.; Davies, I. W.; McNally, A. A General Strategy for Site-Selective Incorporation of Deuterium and Tritium into Pyridines, Diazines, and Pharmaceuticals. *J. Am. Chem. Soc.* **2018**, *140*, 1990-1993. DOI:10.1021/jacs.7b11710

(30) Zhang, X.; Nottingham, K. G.; Patel, C.; Alegre-Requena, J. V.; Levy, J. N.; Paton, R. S.; McNally, A. Phosphorus-Mediated sp2-sp3 Couplings for Selective C–H Fluoroalkylation of Complex Azines. *Nature* **2021**, *594*, 217-222. DOI:10.1038/s41586-021-03567-3

(31) Selingo, J. D.; Greenwood, J. W.; Andrews, M. K.; Patel, C.; J., N. A.; Pio, B.; Shevlin, M.; Phillips, E. M.; Maddess, M. L.; McNally, A. A General Strategy for *N*–(Hetero)aryl Piperidine Synthesis Using Zincke Imine Intermediates. **2023**. DOI:10.26434/chemrxiv-2023-68575

(32) At the conclusion of this project, we became aware of three other approaches for pyridine ^{15}N labeling: a) Bartholomew, G.L.; Kraus, S.L.; Karas, L.J.; Carpaneto, F.; Bennett, R.; Sigman, M.S.; Yeung, C. S.; Sarpong, R. ^{14}N to ^{15}N Isotopic Exchange of Nitrogen Heteroaromatics through Skeletal Editing. ChemRxiv. Cambridge: Cambridge Open Engage; 2023. DOI: 10.26434/chemrxiv-2023-30dtw. b) Tolchin Z, Smith J. ^{15}NRORC: An Azine Labeling Protocol. ChemRxiv. Cambridge: Cambridge Open Engage; 2023. DOI: 10.26434/chemrxiv-2023-cb5lg. c) Feng, M.; Norlöff, M.; Guichard, B.; Kealey, S.; Thuéry, P.; Gee, A.; Feuillastre,S.; Audisio, D. Pyridine-based Strategy towards Nitrogen Isotope Exchange. ChemRxiv. Cambridge: Cambridge Open Engage; 2023. DOI: 10.26434/chemrxiv-2023-r0xn7 ed.

(33) Annesley, T. M. Ion Suppression in Mass Spectrometry. *Clin. Chem.* **2003**, *49*, 1041-1044. DOI:10.1373/49.7.1041

(34) Loh, Y. Y.; Nagao, K.; Hoover, A. J.; Hesk, D.; Rivera, N. R.; Colletti, S. L.; Davies, I. W.; MacMillan, D. W. C. Photoredox-Catalyzed Deuteration and Tritiation of Pharmaceutical Compounds. *Science* **2017**, *358*, 1182-1187. DOI:10.1126/science.aap9674