^{15}NRORC: An Azine Labeling Protocol

Zachary A. Tolchin and Joel M. Smith*

Department of Chemistry and Biochemistry, Florida State University, 95 Chieftan Way, Tallahassee, FL 32306

ABSTRACT: A practical method for the synthesis of ^{15}N-labeled azines with a high degree of isotopic enrichment is described. Activation of azine heterocycles with an electron-deficient arene allows for the facile substitution of the nitrogen atom with a specifically designed ^{15}N-labeled reagent that undergoes a canonical ANRORC-type mechanism. A wide range of azines can be converted to their corresponding ^{15}N isotopologs using this method, and it also allows for dearomative access to reduced heterocyclic congeners. A short dearomative formal synthesis of ^{15}N-solifenacin is accomplished as well to demonstrate a practical application of this method for generating labeled pharmaceuticals.

Nitrogenous heterocycles continue to be common structural units in various subfields of chemical science. Azines (**1**) and their redox congeners (**2**) find prominence in materials applications, agrochemicals, and prescribed pharmaceutical ingredients.[1] Thus, "isotopologs" of these heterocycles have become important for their roles both in structural biology[2] as well as pharmacology and metabolomics (See Figure 1A).[3–6] In metabolomics applications, often protons are substituted for deuterium or tritium atoms, but site-selective precision in these processes can be lacking depending on the substrate.[7,8] Substitution of carbon atoms with ^{13}C atoms can also be accomplished, but their installation often requires *de novo* synthesis from ^{13}C feedstocks like $^{14}CO_2$[9] or K^{13}CN.[10] Lastly, ^{15}N isotopologs (see **3**) have had some of the most limited attention due to the requirements of *de novo* synthesis from $^{15}NH_3$ or corresponding ammonium salts.[11] This synthetic restriction has hindered the utility of ^{15}N labels in many synthetic contexts, demanding novel strategies for its introduction, ideally at early and later stages.

One important application of ^{15}N-labeled azines is in the recently developed SABRE-SHEATH technology. Through binding of a ^{15}N-azine (**3**) to an Ir catalyst also bound to paramagnetic hydrogen, a nuclear spin polarization is induced on the ^{15}N center (see **4**).[12–14] The relatively long lifetime of this spin polarization holds promise for this phenomenon to be leveraged in MRI imaging applications. However, limited synthetic access to these specifically-labeled variants of biologically relevant azines has potentially slowed the development of this impactful imaging technique.[15] Herein, we describe a precise and controlled ^{14}N to ^{15}N atomic substitution protocol for the generation of variously substituted azine isotopologs.

Our initial investigations into these substitution reactions were inspired by classical ANRORC (Addition of Nucleophile, Ring Opening, Ring Closing) reactions described in the heterocyclic chemistry literature.[16] One ANRORC variant first pioneered by Zincke in the early 1900s relies on azine activation followed by subsequent substitution with amine nucleophiles.[17–19] For example, when isoquinoline (**5**) was heated in the presence of 2,4-dinitro-1-chlorobenzene, an intermediate N-aryl isoquinolinium is generated that is poised for the ANRORC Zincke-type substitution with benzylamine to produce the corresponding isoquinolinium **6** in high yield.[20] Our hypothetical reaction design involved a similar process (Figure 1C) in which an activated azinium (**7**) would undergo addition with an appropriately substituted ^{15}N primary amine. Subsequent ring-opening (see **9**), ring-closure, and aniline extrusion would afford azinium **10**. At this point, the R_1 substituent would ideally ionize to generate the corresponding ^{15}N-labeled azine, keeping in mind that its electronic nature would be important towards its spontaneous self-immolation to generate **3**.

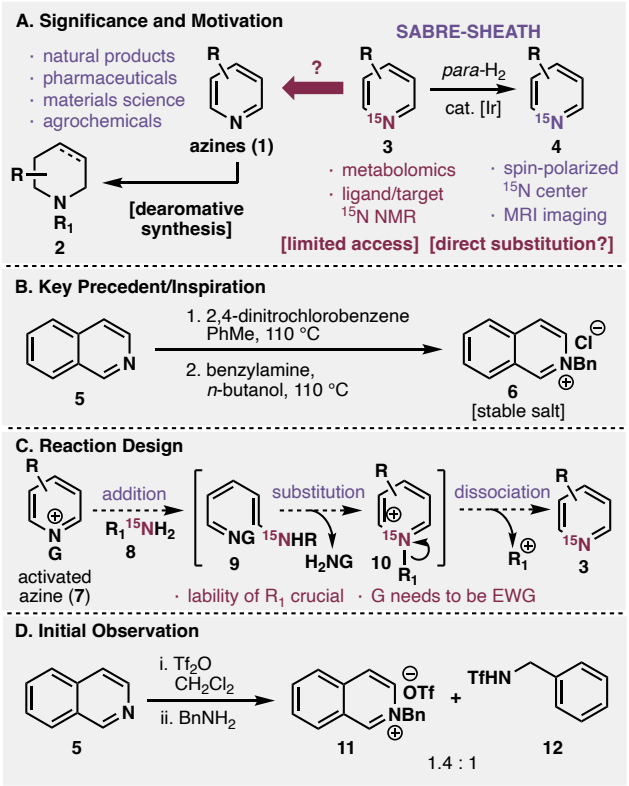
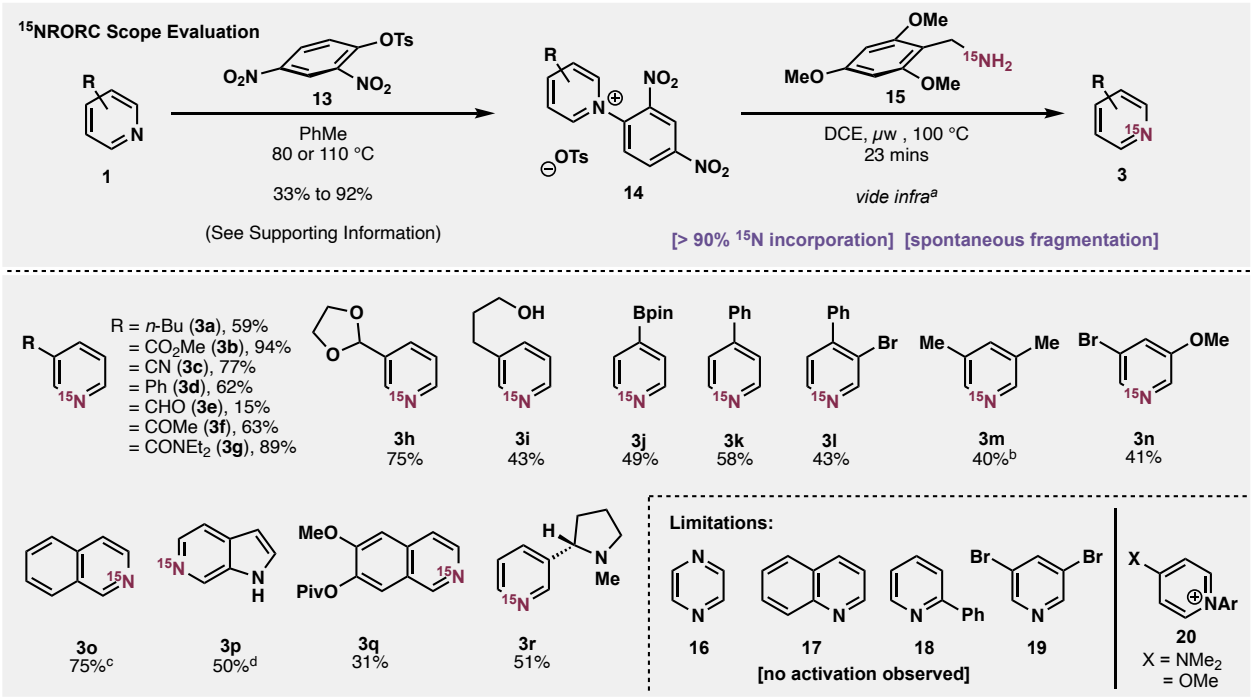


Figure 1. (A) Importance of ^{15}N labeling based on synthetic and translational applications. (B) Classical Zincke reaction. (C) hypothetical ^{15}NRORC transformation and design challenges. (D) Investigation of azine activation with Tf_2O and displacement.

Scheme 1. Scope and limitations of the ^{15}NRORC displacement procedure.



Notes: [a] isolated yields [b] product yield was low due to volatility [c] 1 mmol scale [d] isolated as the TMB salt.

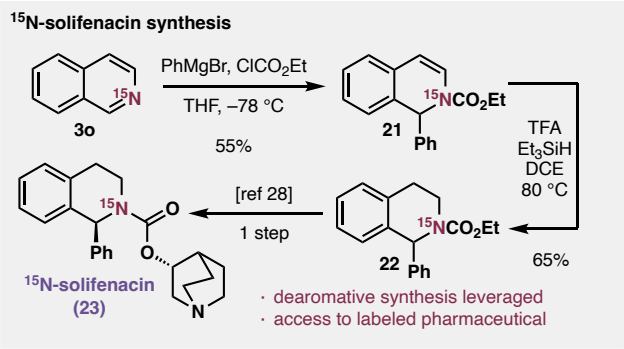
Initially intrigued by recent reports by Toscano,[21] Sarpong,[22,23] and McNally[24] that leveraged mild azinium generation by triflation with triflic anhydride, we evaluated it in the context of this proposed ANRORC reaction design (Figure 1D). While activation of isoquinoline (**5**) was mild and effective with Tf_2O, addition of benzylamine produced a 1.1:1.4:1 ratio of **5**, **11**, and **12**, respectively. This revealed that, while mildly effective at displacing triflamide, transfer of triflate to the alkyl amine was equally viable, reducing the overall likelihood for high ^{15}N isotopic enrichment through ANRORC displacement. After much surveying of activation methods, a modified Zincke-type activation with tosylate **13** (see Scheme 1) proved most effective across a relatively broad range of azine substrates.[25]

Regarding the ^{15}N surrogate used in the displacement, 15N-2,4,6-trimethoxybenzylamine (**15**) was selected for its inherent lability after aniline displacement in the Zincke reaction. The electron-rich nature of the arene putatively weakens the benzylic C–N bond of azinium **10**, promoting extrusion of the desired azine product *in situ*. This amine, and its ^{14}N congener were surprisingly unreported in the chemical literature, however it can be easily prepared in three steps from the relatively inexpensive $^{15}NH_4Cl$ ($71/g) in 62% overall yield (See Supporting Information). After thorough optimization of the depicted sequence on isoquinoline (**5**), it was discovered that treatment of the *N*-arylated isoquinolinium with **15** in DCE under microwave irradiation at 100 °C for 23 minutes delivered the desired isotopically-labeled isoquinoline (**3o**) in 75% yield and with >98% ^{15}N enrichment.

With an established sequence for this ^{15}NRORC substitution, the scope of azines was evaluated (Scheme 1). Activation of azines (**1**) with tosylate **13** afforded aziniums (**14**) in yields varying from 33 to 92%. Most of these salts were easily isolable by filtration and/or trituration before being treated with **15** at elevated temperature under microwave irradiation. The use of microwaves has been well established to expedite these types of transformations beyond conventional heating.[26] A variety of pyridines bearing C3 substitution underwent the sequence smoothly, including simple alkyl substituents such as butyl (**3a**) in addition to those bearing an alcohol (**3i**) and an acetal (**3h**). Electron-withdrawing substituents were also tolerated at C3, generally affording higher yields in the ^{15}N substitution reaction. Pyridines bearing ester (**3b**), amide (**3g**), nitrile (**3c**), and ketone (**3f**) functionalities all underwent labeling with 63-94% yields. The one exception to this trend with 3-formylpyridine, which only underwent the displacement in 15% yield. A substantial amount of the corresponding imine resulting from condensation with **3e** was also formed in this reaction, but eluded isolation from the crude mixture. Perhaps not surprisingly, an aryl substituent at C3 (see **3d**) also underwent smooth ^{15}N substitution in 62% yield. Pyridines with substitution at C4 (e.g. **3j** and **3k**) also provided their labeled products, and various 3,4 (**3l**) and 3,5-disubstituted pyridines (**3m** and **3n**) also delivered the corresponding ^{15}NRORC products, but in slightly reduced yield.

More elaborate substrates also underwent activation and substitution. As mentioned previously, isoquinoline (**5**) could be isotopically substituted in 75% yield (see **3o**) while a dioxygenated variant (**3q**) could also be obtained in modest yield. Of note, concurrent work from our lab has elaborated this latter isoquinoline to the natural opioid (–)-thebaine and derivatives, demonstrating that this method can indeed allow access to isotopologs of biomedically relevant molecular targets. Azaindole **3p** underwent this sequence in 50% yield and, of particular importance is the pyridyl labeling of nicotine with similar efficiency (see **3r**). This last example is of great importance as it demonstrates a "late-stage" labeling event for a biomedically important natural product. Intriguingly, no reactivity is observed on the pyrrolidine nitrogen, indicating a relatively high chemoselectivity for the pyridine nucleus in this sequence.

Scheme 2. Formal synthesis of ^{15}N-solifenacin



There are noteworthy limitations to this method as well. Azines with multiple N atoms like pyrazine (**16**) or multiple electron withdrawing groups (e.g., **19**) did not undergo activation with **13** to form the corresponding aziniums. Activation also was unsuccessful on quinoline (**17**) and 2-phenylpyridine (**18**) indicating that steric encumbrance of the nitrogen also perturbs azinium formation. For some pyridiniums bearing heteroatomic substitution at C4 (**20**, NMe_2 or OMe), activation with **13** proceeded well to give the corresponding aziniums (**14**), but the ^{15}NRORC substitution did not proceed, giving complex mixtures with hypothesized displacement of the C4 substituent in an uncontrolled manner.

The synthesis of ^{15}N-labeled bioactive compounds is an ancillary goal of this work. Since dearomative synthetic strategies have shown great promise towards the efficient synthesis of bioactive compounds,[27] access to the above labeled azines is therefore further underscored. To explore this principle, a short formal synthesis of the drug ^{15}N-solifenacin was implemented as depicted in Scheme 2. Labeled isoquinoline **3o**, accessed on 1 mmol scale, was first activated with ethyl chloroformate and then treated with phenylmagnesium bromide to generate dihydroisoquinoline **21** in 55% yield. Alkene reduction with TFA and Et_3SiH at elevated temperature then afforded tetrahydroisoquinoline **22** in good yield. The intermediate is reported to be a direct precursor to solifenacin (**23**) through carbamate substitution with 3-quinuclidinol.[28]

In conclusion, we have developed a ^{15}N labeling protocol (^{15}NRORC) for the facile and formal incorporation of a neutron to the nitrogen nucleus of various azines. This method applies to early-stage functionalization of important building blocks for natural product synthesis (e.g. **3q**) and rationally-designed pharmaceuticals (e.g. **3o** to **23**). In addition, the late-stage labeling of molecules like nicotine (**3r**) stands as a proof-of-concept for the application of this method in the generation of labeled APIs. It is anticipated that the platform established by this method will lay the groundwork for the future development of fundamental chemistry while paving the way for the synthesis of chemical probes both for translational metabolomics and therapeutically relevant imaging techniques.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures and relevant spectral data (PDF)

AUTHOR INFORMATION

Corresponding Author

*** Joel M. Smith** – *Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, 32306, United States;* ORCID: 0000-0002-1108-4751; Email: smith@chem.fsu.edu

Co-Author

Zachary A. Tolchin – *Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, 32306, United States*

Author Contributions

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