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Heterocyclic Surgery for Isotopic Labeling

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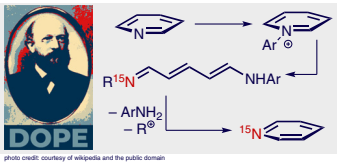
This manuscript is dedicated to the memory of Zachary A. Tolchin



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Abstract Recent developments in the isotopic labeling of heteroarenes may prove to be useful in the realms of biomedical science, materials chemistry, and fundamental organic chemistry. The use of the age-old Zincke reaction, or tactical variants thereof, has become particularly utilitarian in effecting single-atom nitrogen replacement in various azines to generate their desired isotopologues. This chemistry can be synthetically leveraged at an early stage for diversity-oriented heterocyclic labeling of pharmaceuticals and/or natural products. Additionally, given the prevalence of saturated azacycles in biologically relevant molecules, access to these isotopologues becomes relevant through dearomative retrosynthetic analysis from the corresponding ^{15}N-labeled heteroarenes.

Key words pyridine, azine, isotope, labeling, Zincke, dearomatization, ANRORC

Prof. Joel M. Smith was born and raised in Raleigh, NC as the son of a physician and a secondary school chemistry instructor. He earned his BSc in chemistry and music in 2010 at Furman University in Greenville, SC while performing total synthesis research under the guidance of Prof. Brian C. Goess. His graduate studies were performed as an NSF-GRFP Fellow under the mentorship of Prof. Neil K. Garg at the University of California, Los Angeles and focused on complex alkaloid total synthesis. Following his graduation in 2015, he then conducted postdoctoral research as an Arnold O. Beckman Postdoctoral Scholar in the lab of Prof. Phil S. Baran at Scripps Research where he endeavored in the total synthesis of terpenoid natural products and Ni-catalyzed cross-coupling reactions. He began his independent career as an assistant professor in 2018 at Florida State University where his lab focuses on the concise synthesis of complex molecules and the invention of novel chemical reactions.

1 Introduction

Heterocyclic chemistry has had a lasting impact on the fields of medicinal chemistry, materials science, and organometallic chemistry, amongst many others. Recently, subfields such as metabolomics and magnetic resonance imaging (MRI) have demanded more precise isotopic labeling as a means of characterizing the behavior of heterocyclic carbogens.[1,2] However, prior to recently, access to valuable isotopically labeled ^{15}N-heterocycles have remained rare and have limited advances in these translational subfields.[3] This Synpact article describes our lab's and others' utilization of the Zincke reaction to effect the isotopic labeling of azines. Furthermore, these advances are contextualized and summarized within plethora of useful reactions that expand the palette of heterocyclic functionalizations and transmutations that have greatly expanded practitioners' ability to easily synthesize and modify useful azine chemotypes.

In 1904 at the University of Marburg, Prof. Theodor Zincke disclosed a seminal discovery regarding an 'aniline replacement' in pyridinium salts (Scheme 1A).[4] Following activation of pyridine with 1-chloro-2,5-dinitrobenzene via nucleophilic aromatic substitution, Zincke discovered a peculiar behavior of these salts (**1**) upon treatment with 2

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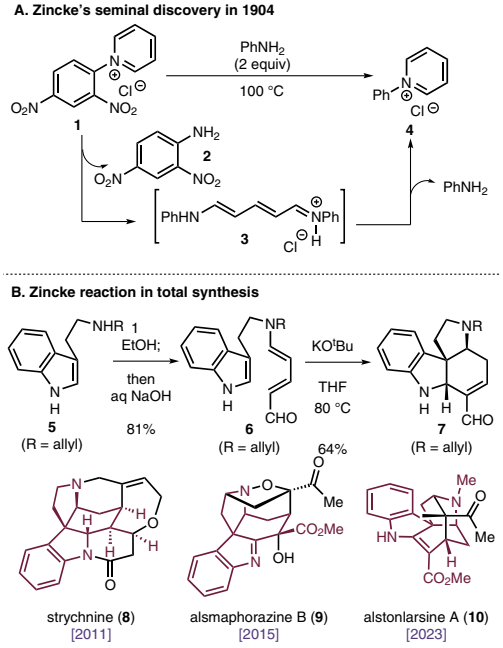
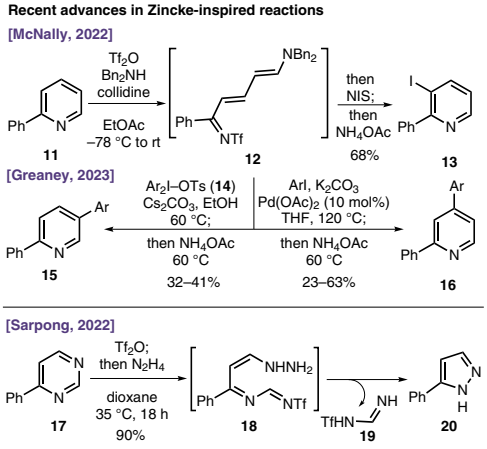


Scheme 1 (A) The original Zincke reaction demonstrating nitrogen replacement. (B) Examples of Zincke salts leveraged in complex molecule synthesis by Vanderwal.



Scheme 2 Zincke chemistry has had a resurgence in recent years, lending itself to novel heterocyclic functionalization and transmutation.

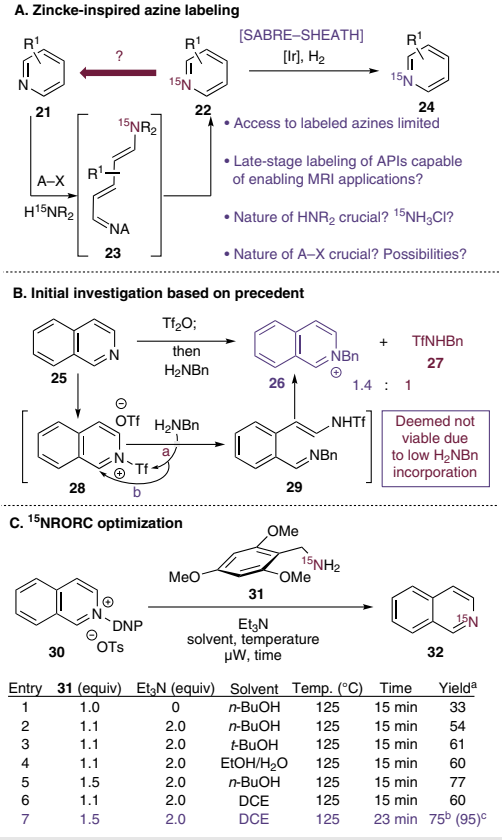
equivalents of aniline. A ring opening of the pyridinium by initial dearomative addition of aniline then promoted further fragmentation of the ring followed by further displacement of 2,4-dinitroaniline with aniline to generate iminium **3** as an intermediate. When the reaction was conducted at 100 °C, extrusion of one aniline equivalent was observed concomitant with the rearomatization of **3** to give *N*-phenylpyridinium chloride (**4**). This 'aniline replacement' was one of the first reactions of its kind, characterizing what was later to be known as an ANRORC-type (addition of nucleophile ring opening ring closing) reaction mechanism in the canon of heterocyclic chemical transforms.[5] Over 100 years after its discovery, the Zincke reaction has even earned an honored role in complex molecule total synthesis. Perhaps in some of the best examples from this century,[6] the Vanderwal group leverage a ring-opening reaction of **1** with tryptamine **5** to generate dienal **6** after basic imine hydrolysis (Scheme 1B). This intermediate was then treated with KO*t*-Bu at elevated temperature to promote a [4+2] cycloaddition and prototropic rearrangement towards enal **7**.[7,8] This sequence comprised a facile entryway to the core of various monoterpenoid indole alkaloids, such as strychnine (**8**)[9] and more recently isolated natural products like alsmaphorazine B (**9**)[10] and alstonlarsine A (**10**).[11] From a strategic standpoint, the Zincke salt (**1**) contained all necessary oxidation to enable a nearly isohypsic synthesis of these indole alkaloid core structures.[12] Given the sustained relevance of Zincke-type aziniums to mainstay heterocyclic motifs, many chemists continued inquiring as to how these malleable intermediates might be manipulated in several adventitious and/or practical synthetic avenues.

Recently, several research groups have leveraged Zincke-type reactivity for the strategic functionalization of pyridines or conversion to alternative heterocyclic motifs (Scheme 2). For example, McNally and co-workers demonstrated a selective C3-halogenation of pyridines **11** through activation and ring opening to Zincke imines such as **12**.[13] While the parent pyridines are electron-poor and relatively intractable for electrophilic halogenation reactions, the 'unraveled' dienamine variant serves as a nucleophilic surrogate with a much higher HOMO. In practice, treatment of **12** with NIS followed by ring closure with NH_4OAc afforded the iodinated pyridine **13** in 68% overall yield. This procedure that involves the activation of azines with triflic anhydride and strategic ring opening with dibenzyl amine to afford imine **12** laid the groundwork for other groups to leverage its adventitious reactivity. For example, one year later, the same intermediate **12** was demonstrated to undergo regiodivergent arylation by Greaney and co-workers.[14] Treatment of **12** with aryl iodoniums **14** promoted substitution at the nucleophilic C5-position to give products like **15**. In contrast, Heck-type arylation with **12** gave C4-arylated products **16** following rearomatization with NH_4OAc following with McNally's reported protocol. Through a similar activation mode, Sarpong and co-workers utilized pyrimidine **17** as precursor to its 'dehomologated' pyrazole counterpart **20** in 2022.[15] Following activation of **17** with triflic anhydride and addition of hydrazine, this resulted in the formation of ring-opened intermediate **18**. Subsequent condensation of the hydrazine's terminal N promoted displacement of amidine **19** generating **20** in excellent yield (90%). This formal ring contraction uniquely demonstrated how the pyrimidine could be dearomatized and thus manipulated as if it were a 1,3-dicarbonyl synthon typically employed

in classical pyrazole synthesis. Following these contributions, amongst others, it seemed a renaissance of Zincke-type chemistry was already afoot.

2 Our Lab's Development of the ^{15}NRORC Reaction

The inspiration for our lab's utilization of the Zincke reaction for azine ^{15}N labeling originated in ongoing efforts to elucidate marine alkaloid biosynthetic pathways. With the aim of generating isotopically labeled substrates for biosynthetic feeding studies, we hypothesized that the ^{15}N-labeled pyridines **22** might be suitable for identification of downstream metabolites by mass spectrometry and NMR. From a design standpoint, we thought that 'surgically' excising the nitrogen of the azine **21** through an ANRORC-type mechanism would enable this formal addition of one neutron to the heterocycle (Scheme 3A).[16] This would require the incorporation of an appropriately functionalized ^{15}N-labeled amine to generate an intermediate such as **23** that would be poised for a subsequent selective ring closure to deliver **22**. If successful, this synthetic process would also open avenues in magnetic resonance imaging (MRI) through a process called SABRE-SHEATH, in which labeled azines **22** can be spin-polarized at the ^{15}N center to afford MRI-active azines **24**.[2,17] Until this work, synthetic access to various labeled azines had been limited, stunting the breadth of this potentially useful technique. At the outset of our work, however, we anticipated that the reaction design was going to be crucial to its success. The main two factors that had to be considered in our lab's approach were (1) the nature of the activating agent used in the formation of a putative Zincke imine, and (2) the source of ^{15}N to be used in the replacement of the azine nitrogen.



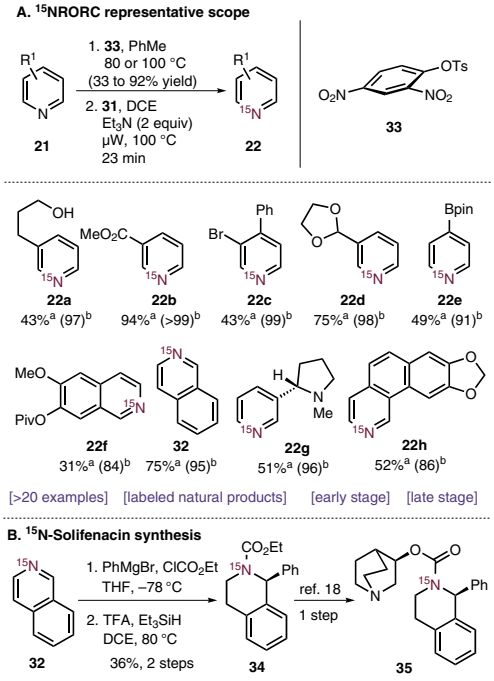
Entry	**31** (equiv)	Et_3N (equiv)	Solvent	Temp. (°C)	Time	Yield[a]
1	1.0	0	*n*-BuOH	125	15 min	33
2	1.1	2.0	*n*-BuOH	125	15 min	54
3	1.1	2.0	*t*-BuOH	125	15 min	61
4	1.1	2.0	EtOH/H_2O	125	15 min	60
5	1.5	2.0	*n*-BuOH	125	15 min	77
6	1.1	2.0	DCE	125	15 min	60
7	1.5	2.0	DCE	125	23 min	75[b] (95)[c]

Scheme 3 (A) Inspiration and strategic considerations in design of transformation. (B) Initial observation with Tf_2O activation. (C) Optimization of desired labeling reaction. [a] Yield determined by ^{1}H NMR spectroscopy with internal standard. [b] Isolated yield. [c] Percentage of ^{15}N incorporation.

Per the aforementioned precedent, azine activation using Tf_2O was first investigated (Scheme 3B).[13] Upon treatment of isoquinoline (**25**) with Tf_2O, azinium **28** was easily generated and subject to attack by benzylamine. The desired pathway (path b) would putatively result in a ring-opened intermediate **29** and subsequent condensation to deliver the desired azinium **26**. Although some of this product was generated, the competing mechanistic pathway (path a) was just as prevalent resulting in the synthesis of triflimide **27** in a nearly equal quantity. As the intention was to use a labeled benzylamine or variant thereof, activation with Tf_2O was not seen as a viable agent in our hands for attaining high degrees of isotopically labeled azines through this 'surgical' N exchange. Notably, while we were not successful in engineering a Tf_2O-based activation protocol, other concurrent contributors in this area including McNally, Sarpong, and Audisio were able to utilize this activation method to successfully implement the same desired ^{15}N azine labeling (*vide infra*).

Subsequently, we opted for the more traditional dinitroarene activation and utilized isoquinoline as our test substrate for the desired atomic exchange (see tosylate **30**, Scheme 3C). Additionally, with an interest in facilitating fragmentation of the benzyl group attached to the labeled amino unit, three methoxy groups were introduced such that this group would spontaneously ionize in the reaction to directly afford the labeled azine (see **31**). Several conditions were surveyed including parameters inclusive of solvent, temperature, time, and equivalency of Et_3N under microwave irradiation. It was initially found that Et_3N was essential to obtaining reasonable yields of **32** (entry 1) in addition to the utilization of either *n*-BuOH or DCE as a solvent (entries 2–6). A constant temperature of 125 °C proved imperative for cleavage of the TMB moiety, and 1.5 equivalents of **31** were crucial for the reaction to proceed to completion after 23 min (entry 7). Although a higher yield was obtained with *n*-BuOH as solvent (entry 5), purification issues with this solvent as a contaminant allowed for DCE to emerge as the preferred medium of the reaction. In the end, **32** was cleanly obtained in 75% yield and with 95% isotopic incorporation as determined by HRMS. Furthermore, re-

placement of **31** with $^{15}NH_4Cl$ as the ^{15}N source under these conditions resulted in poor yield (22%) and ^{15}N incorporation (56%).



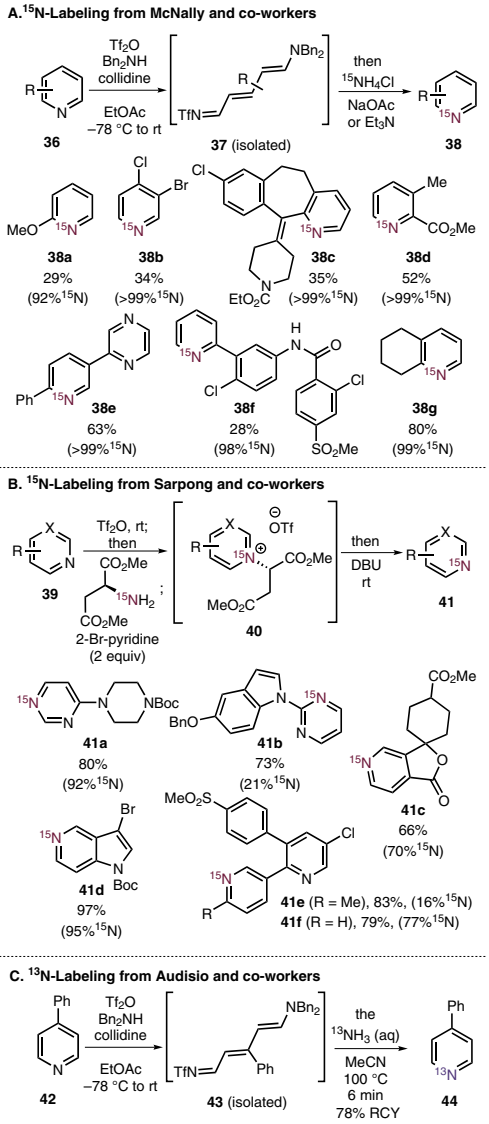
Scheme 4 (A) Scope of azine-labeling protocol; (B) dearoamative synthesis of the ^{15}N-labeled API solifenacin

The scope of the ^{15}NRORC reaction was evaluated under these optimized reaction conditions (Scheme 4A).[16] Activation of various azines **21** with tosylate **33** resulted in the formation of intermediate Zincke salts in a yield range of 33% to 92% depending upon the substrate. Treatment of these salts with amine **31** under the optimized conditions resulted in the generation of a selection of labeled products **22**, to which a small selection is shown in Scheme 4A. Various functionalities were tolerated as substituents including an alcohol (**22a**), an ester (**22b**), a halogen (**22c**), a boronic ester (**22d**), and an acetal (**22d**). Bicyclic substrates were also successful (**22f**, **32**) in addition to natural products like nicotine (**22g**) and toddisoquinoline (**22h**). Importantly, these latter examples demonstrated the ability for late-stage ^{15}N azine labeling which would likely be applicable to the application of this method to various APIs with azine moieties. To demonstrate the method's further utility, we employed a dearomative synthesis of ^{15}N-solifenacin (**35**) starting from the labeled isoquinoline (**32**). Activation of **32** with ethyl chloroformate followed by addition of phenylmagnesium bromide afforded an intermediate dihydroisoquinoline (Scheme 4B). Reduction of this dihydroisoquinoline with TFA in the presence of Et_3SiH afforded intermediate **34** in 36% yield over 2 steps. This compound has a known conversion into **35** through direct displacement of the ethoxy group of the carbamate.[18] Beyond the synthesis of labeled compounds such as **35**, it is anticipated that this chemistry will be useful in understanding the isoforms of variously formulated APIs through ^{15}N NMR applications, which is work currently ongoing in our group.

3 Other Recent Azine-Labeling Methods

Several other research groups investigated the ^{15}N-labeling of azines concurrently with our efforts (Scheme 5). Based on their initial work in pyridine halogenation, McNally and co-workers were able to ^{15}N label various pyridines through their Tf_2O-based Zincke reactivity platform. Under this method, the same activation of pyridine starting materials **36** was operable to afford their corresponding ring-opened intermediates **37**. Following isolation of **37** from the starting pyridine, exposure of this intermediate to $^{15}NH_4Cl$ resulted in the formation of the labeled product **38**. This two-step procedure has a broad scope of pyridines that could undergo labeling including monocyclic substrates such as **38a**, **38b**, and **38d** that were labeled with 92%, >99%, and >99% ^{15}N-isotopic incorporations, respectively. Notably, each of these examples are pyridines with electron-withdrawing substituents and/or C2 substitution (**38a**,**d**). Furthermore, pyridine rings could be selectively labeled in fused substrates (**38g**) with high yield and isotopic incorporation in addition to selective pyridine labeling in substrates with multiple heteroaromatic moieties (**38e**). Lastly, one of the best advantages to this method is its ability to enable late-stage functionalization of drug molecules. To this end, the synthesis of ^{15}N-loratidine was accomplished in 35% yield and >99% ^{15}N incorporation over the two steps, and ^{15}N-vismodegib was also generated in 28% yield and with 98% isotopic incorporation. Also concurrent with McNally's work, Sarpong and co-workers were able to activate substituted pyrimidines **39** with Tf_2O and perform a nitrogen displacement with ^{15}N-dimethylaspartic acid in the presence of 2-bromopyridine to form the intermediate pyrimidinium **40**.[19] This intermediate underwent mild elimination with DBU to afford the labeled product **41**. While the ^{15}N incorporation for this process was much more modest (Scheme 5B), the broad labeling of pyrimidines was an added benefit to the scope of azines that could undergo this atomic 'swap'. Thus, pyrimidines such as **41a** and **41b** were synthesized with 92% and 21% ^{15}N incorporation, respectively. In applying this protocol to various pyridines, once again triflate activation enabled the successful incorporation of the ^{15}N nucleus into an azaindole (**41d**), a fused tricycle pyridine (**41c**), and two tricyclic bypridines (**41e** and **41f**). Notably, the more sterically hindered of these two substrates (**41e**) had reduced ^{15}N incorporation at 16% compared with 77% for the latter. Lastly, the Audisio[20] group reported a nearly identical protocol to McNally at the same point in time. While much of the scope is quite similar to

that of the McNally group one additional entry of note was their incorporation of a ^{13}N radioactive label into a parent azine (Scheme 5C). In the event, 4-phenylpyridine (**42**) was converted into imine **43** in analogous fashion to McNally followed by treatment with aqueous $^{13}NH_3$ (generated via cyclotron) at elevated temperature to produce **44** with a 78% radiochemical yield (RCY) after 6 min of reaction.

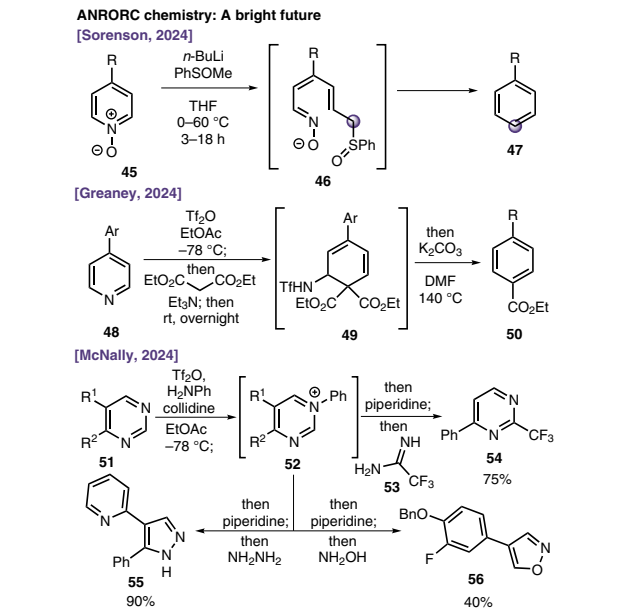


Scheme 5 Recently described azine-labeling methods from (A) McNally, (B) Sarpong, and (C) Audisio reported concurrent with the ^{15}NRORC protocol.

All four of these concurrent azine-labeling methods have important complimentary advantages in their respective scopes. With the ^{15}NRORC protocol, the two-step procedure tolerates functionality like alcohols and dialkyl amides that may not be amenable to azine activation with triflic anhydride. That being said, there are limitations to the activation of azines with arenes associated with the electronic structure and substitution patterns of the azine inputs. Products such as **38a–g** would be inaccessible do to their inability to be activated under classical Zincke conditions due to their electron-poor nature and/or substitution at the 2-position. This scope issue is greatly addressed by the triflate activation and ring-opening popularized by McNally and co-workers. The labeling efforts of Sarpong importantly addressed the isotopic labeling of pyrimidines in addition to pyridines, a useful expansion of the heterocyclic breadth of this tactic. However, as compared with the other methods, not forcing an azine ring opening with an amine like Bn_2NH diminished the degree of isotopic incorporation through this tactic. Sarpong, however, was able to partially circumvent this issue through separation of aziniums of structure **40** followed by subsequent elimination. When separated, azinium **40** could be converted into **41** with ^{15}N incorporations improving by an average of 45%. With regard to ^{15}N sourcing, the utilization of $^{15}NH_4Cl$ ($74.30/g, Cambridge Isotope Laboratories) by McNally and Audisio has a cost advantage of the designer ^{15}N surrogate used in the ^{15}NRORC reaction (**31**) and the use of the ^{15}N-dimethylaspartate by Sarpong ($558/g for amino acid, Cambridge Isotope Laboratories).

4 Expanded ANRORC Utilization

Even recently, work from several groups has expanded upon Zincke-inspired ANRORC chemistry for the transmutation of various aromatic moieties (Scheme 6). For example, earlier this year, Sorenson and co-workers were able to generate arenes **47** from their corresponding pyridinium *N*-oxides **45** through nucleophilic dearomatization of a lithiated sulfoxide anion to generate a ring-opened intermediate such as **46**.[21,22] Subseqent condensation of the sulfoxide α-center and extrusion of all the heteroatomic moieties led to a net N to C replacement. Greaney has reported a similar transformation, but through employment of the azine activation with Tf_2O.[23] Following a similar ANRORC pathway, conversion of **48** to intermediate **49** following dearomatization with diethylmalonate proceeds smoothly to then allow for a decarboxylative rearomatization to forge benzoate **50** in good yield. McNally has recently utilized pyrimidines **51** as 1,3-dicarbonyl synthons to transmutate these heterocycles into several other congeners.[23] A selection of the several transformations from this report are shown in Scheme 6. Following activation of **51** and ANRORC substitution with aniline to generate pyrimidinium **52**, ring opening with piperidine and condensation with amidine **53** generated pyrimidine **54** in 75% yield (one-pot protocol). The pyrimidinium **52** could also be formally intercepted with hydrazine to synthesize pyrazole **55** and undergo substitution with hydroxylamine to generate oxazole **56** in 40% yield.



Scheme 6 Recently described chemistry from Sorenson, Greaney, and McNally on ANRORC-based arene transmutation.

5 Conclusion and Outlook

While Zincke set the stage for the power of ANRORC chemistry in the canon of organic synthesis, we have seen its recent renaissance in the 21st century lay the groundwork for impactful scientific discovery. From total synthesis to heterocyclic transmutation to isotopic labeling, the surgical precision that these transformations offer certainly benefit the toolbox of the academician in addition to the practitioners of translational molecular discovery. It is of little doubt that Zincke's legacy will continue to earn a considerable role in the future of synthetic heterocyclic chemistry. Indeed, it has enabled access to molecular motifs that, at least in the context of ^{15}N-labeled azines, have remained largely elusive, or intractable until recently.

Conflict of Interest

The authors declare no conflict of interest.

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