Total Synthesis of Peramine, a Defensive Alkaloid Produced by Endophytic Fungi of Cool Season Grasses Possessing Anti-Insect Properties

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Total Synthesis of Peramine, a Defensive Alkaloid Produced by Endophytic Fungi of Cool Season Grasses Possessing Anti-Insect Properties

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Chemistry in Department from The College of William and Mary

by

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May 2, 2016
Abstract

The total synthesis of peramine, a pyrrolopyrazine natural product containing a guanidine functionality, is described. Peramine is an anti-insect defensive alkaloid produced by fungi endophytic to cool season grasses. Peramine is one of four classes of alkaloid produced by these endophytic fungi and production of this compound is widespread across fungal-grass mutualisms. Unique to our synthesis of peramine is an enolate alkylation of a pyrrole-fuse diketopiperazine and an amenable route to derivatives of peramine. Our synthesis is comprised of seven steps and three chromatographic separations, making it as efficient as the previous two syntheses and better with respect to total yield.
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Acknowledgements

First I would like to thank Professor Jonathan Scheerer, for giving me the opportunity to work in his lab three years ago and mentoring me every step of the way. Even though this project has had its moments where I thought it would never be completed, I am grateful that he was always available to help and guide me. Additionally, I give my thanks to Professor Robert Hinkle, Professor Lisa Landino, and Professor Joshua Puzey for being a part of my honors committee. I would also like to thank the rest of the William and Mary chemistry faculty for creating a stellar educational environment and making my time here truly remarkable.

I would also like to thank my fellow honors students, Jacob Robins and Jill Williamson, for sharing this journey with me in completing our theses. I know I would not have been able to finish this without each of them. I would like to thank the rest of my lab mates in the Scheerer group as well, for helping me towards me successes and assisting me during my many failures. Thanks to Kyujin Kim and Nick Angello who worked over the summer with me and helped give the lab spirit. Thank you Margaret Olesen for being a good friend, I wish you the best of luck in your future endeavors and thank you Jonathan Perkins for making Adv. Inorganic Chemistry so much more enjoyable. Also, thanks to Jenna Landen, Ryan Perry, Elizabeth Kelley, and Skylar Norman. I hope you all enjoy the rest of your time at William and Mary and I wish you the best of success.
To my parents, Lisa and Chris Nelli, for always believing in my abilities and pushing me to continually strive for more. Without their love and support I would have never accomplished this undertaking.
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Chapter One

Grass-Endophyte Mutualisms

Several cool season grasses of the family Poaceae (e.g. perennial rye grasses or tall fescue) contain fungal endophytes of the family Clavicipitaceae (Ascomycota). A group of these fungal endophytes, called epichloae, form mutualistic symbioses with members of the grass subfamily Poöideae and are comprised of the sexual genus *Epichloë* and the asexual genus *Neotyphodium*. These symbioses are highly specific; one specie of epichloae is typically only compatible with one genus of host plant as a result co-phylogenetic evolution (*Epichloë*) or a complex process of interspecific hybridizations involving the combinations of two or sometimes three ancestors (*Neotyphodium*).\(^1\) Both of these genera live all of their lifecycle intercellularly in the host plant (Figure 1.1) and asymptotically infect all aboveground tissues excluding the roots. Transmission to the next generation is typically done by vertical transmission, whereby the fungus colonizes the host seed. This colonization, just like the infection of the mature host plant, is asymptomatic and causes little to no damage to the host seed and future mature plant. The *Epichloë*, unlike the *Neotyphodium*, can also propagate to a new host via horizontal transmission, where the fungus suppresses host plant growth and produces spore-bearing stromata in order to spread to neighboring plants via wind dispersal. However, very few species of *Epichloë* exhibit this behavior as most spread via vertical transmission (Figure 1.2).\(^ {1,2,3} \)

**Figure 1.1:** *Neotyphodium* hyphae growing in between the cells of a rye grass host.

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In return for shelter, nutrients, and transmission to the next generation, epichloae provide a number of benefits to their hosts including increased biomass, increased tillering, access to limiting soil nutrients, increased resistance to drought, and production of secondary defense metabolites against herbivores. However, the fungal-grass association is not always mutualistic, and it has been proposed that instead a continuum exists between mutualism and parasitism. Under high nitrogen supply, endophyte concentration was reduced by 40% in three different Neotyphodium-ryegrass (Lolium perenne) associations. In high sugar cultivar, the endophyte concentration was reduced by 50%, and these two effects were additive resulting in a 75% reduction. In addition, defensive alkaloid production was reduced under both increased nitrogen supply and high sugar cultivar. Thus, in conditions where the host plant has limited access to nutrients and resources, it may only be tolerating the presence of fungal endophytes in return for the net benefits provided by the epichloae.

**Figure 1.2**: Life cycles of the epichloae; a diagram of horizontal transmission (sexual transmission) carried out by a few *Epichloë* species and vertical transmission (asexual transmission) carried out by all *Neotyphodium* species and most *Epichloë* species. 

1
Secondary Metabolites of Fungal Endophytes

Four classes of endophyte secondary metabolites exist: indole diterpenes, ergot alkaloids, lolines, and peramine (Figure 1.3). Each class of alkaloid is derived from amino acid precursors, although the biosynthetic pathways to each of the four classes are independent of each other. Most fungal endophytes produce alkaloids belonging to at least one class, with select species producing a combination of two or three classes. However, no fungal isolate has been found to produce metabolites from all four classes.⁷

Figure 1.3: The four classes of alkaloids: peramine, indole diterpenes (represented here by lolitrem B), 1-aminopyrrolizidine alkaloids (lolines), and ergot alkaloids (represented here by ergovaline).

The indole diterpenes and ergot alkaloids possess both anti-insect and antivertebrate activities, and are well studied because of their detrimental effects on grazing livestock, such as ryegrass staggers and fescue toxicosis.⁸ The ergot alkaloid family can be divided into clavines, simple amides of lysergic acid, or ergopeptines. These alkaloids interact as agonists or antagonists for serotonin, dopamine, adrenaline, or noradrenaline receptors in mammals, resulting in a range of activities including vasoconstriction, uncontrolled muscle contractions, and disturbances in the central nervous system. Insects contain homologous neurotransmitters to mammals that are also affected by ergot alkaloids, and can exhibit feeding deterrence, delayed development, and increased
mortality when fed ergot alkaloids at levels found in endophyte-infected grasses.\textsuperscript{7} Indole diterpenes are largely known for their strong tremorgenic activity in mammals, such as ryegrass staggers caused by lolitrem B.\textsuperscript{9} Like ergot alkaloids, the indole diterpene family contains a diverse set of intermediates and spur products.\textsuperscript{10} The lolines and peramine are active against invertebrates, but differ in strength of action. Lolines exhibit overt toxicity to insects as well as nematodes, and the effects are comparable to nicotine when applied topically or ingested.\textsuperscript{11} Lolines exist in various congeners, generated by the presence or absence of methyl, formyl, or acetyl groups, depending on the active genes present in the endophyte fungus. The lolines are aminopyrrolozidine alkaloids that are derived from homoserine and proline joined in a non-peptide manner.\textsuperscript{7} Peramine is a single compound as opposed to a family of compounds and is derived from arginine and a proline precursor. Peramine is an insect feeding deterrent, but is not acutely toxic or a contact poison.\textsuperscript{12,13} There is no evidence of peramine possessing any mammalian toxicity.\textsuperscript{14}

**Peramine Background and Activity**

Peramine was first identified in extracts of perennial ryegrass (*Lolium perenne*) that were found to contain the *N. lolii* endophytic fungus.\textsuperscript{15} It was known that the presence of fungal endophytes, namely ergot alkaloids, in tall fescue grasses had an adverse effect on livestock performance. The effect on insects was elucidated through feeding studies on endophyte-infected grass and endophyte-free grass.\textsuperscript{12,15} Peramine was found to be very active against Argentine Stem weevil, as adult Stem weevils chose to feed on endophyte-free grass rather than endophyte-infected grass.\textsuperscript{15} However, the feeding deterrence of peramine is not universal. In a choice-feeding assay conducted on four aphid species (*Rhopalosiphum padi, Schizaphis graminum, Rhopalosiphum maidis, and Sitobion avenae*), the presence of a peramine-producing endophyte (*Acremonium coenophialum*) in tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) deterred aphid feeding, but the deterrence was dependent on the species of aphid and species of host plant. *R. maidis* was deterred by endophyte-infected perennial ryegrass to a greater extent than by endophyte-infected tall fescue, while *R. padi* and *S. graminum* were not deterred by endophyte-infected perennial rye grass even though they were deterred by endophyte-
infected tall fescue. *S. avenae* was unaffected by both endophyte-infected perennial rye grass and tall fescue. In addition, tests with the larvae of tobacco hornworm *Manduca sexta* and tobacco budworm *Heliothis virescens* showed no consistent response in feeding behavior when fed diets containing either ground plant material or plant extracts from endophyte-infected tall fescue, as compared to similar preparations of the endophyte-free tall fescue control.12 Another example, the larvae of the Japanese beetle *Popillia japonica*, shows no deterrence when feeding on the roots of endophyte-infected perennial ryegrass, however this may be because the fungal endophyte is absent from the host plant roots. Still, other herbivorous insects exhibit feeding deterrence towards endophyte-infected perennial ryegrass including eight species of the sod webworm (*Crambus* spp.) and several species of billbugs (*Sphenophorus* sp.).16 This moderate, and sometimes lacking, feeding deterrence across several insect species is interesting because peramine is the most widely distributed alkaloid class across epichloae mutualisms, as twenty-six out of forty-four (59%) grass-endophyte symbiota tested contained peramine.10

**Figure 1.4:** Example of a choice feeding assay conducted on adult stem weevils.17 Leaf blade samples are perennial rye grass infected with wild-type *E. festucae* (1), endophyte-free ryegrass (2), and two peramine-deficient mutant endophyte strains (3 and 4). As can be seen below, ryegrasses that are deficient in endophytes capable of peramine production are more susceptible to adult stem weevil feeding than the wild type.
Why would the *perA* gene be highly conserved across fungal endophyte species\(^\text{10}\) when the defensive capabilities extended to the host plant are modest? Further, peramine contains five nitrogen atoms, making the production of this alkaloid potentially costly on the host plant when nutrients are scarce. One possible answer could lie in the fact that peramine is primarily found in the leaf and guttation fluid of host plants, as the alkaloid is water soluble and traverses the host plant’s phloem.\(^\text{18,19,20}\) This allows the alkaloid to protect tissues remote from endophyte infection and protect against phloem feeders, such as aphids.\(^\text{20}\) Also, peramine’s presence in the leaf guttation fluid could conceivably allow protection against sensitive insects without the leaf cuticle being broken.\(^\text{7}\) However, peramine concentrations within the host plant vary depending on the age of plant tissue, tissue-type, reproductive status, and vegetative state (Figure 1.5). Peramine concentration is higher in younger vegetative tissue of perennial ryegrass than older vegetative tissue, and concentrations significantly increase in regrowth of tissue as a result of clipping. This leaf clipping was done to keep the ryegrass in a vegetative state, and likely simulates tissue damage by herbivore feeding. There is no peramine present in the roots during any season or maturity phase, reflecting the lack of endophyte colonization of belowground tissues. This is especially interesting considering that peramine traverses the plant’s phloem, which means it should theoretically be present in the root. Concentrations are lower in leaf and guttation fluid during reproductive development, which is mostly due to high concentrations of peramine contained in the seed.\(^\text{18}\) Consequently, this may provide a fitness advantage during germination for endophyte-infected seeds over endophyte-free seeds.
Previous studies have demonstrated that plant secondary metabolites can cascade up the food chain to higher trophic levels and a recent study by Fuchs et al. demonstrated that peramine cascades as well. Predacious ladybird larvae of three species (*Coccinella septempunctata*, *Adalia bipunctata*, and *Harmonia axyridis*) were fed *R. padi* aphids reared on *Lolium perenne* grass infected by the fungal endophyte *N. lolii* (known to produce lolines and indole diterpenes as well as peramine) and peramine concentrations were measured (Figure 1.5). Although peramine was present in the aphid predators, however it did not lead to ladybird death or a performance reduction. However, the *H. axyridis* larvae experienced a prolonged pupal stage, which could lead to a loss of fitness due to the vulnerability present during this developmental period. While these endophyte-infected plants also contained the indole diterpene lolitrem B, this alkaloid did not accumulate in the aphids or the ladybirds, thus the effects observed were solely due to the effects of peramine. The authors of this study also found that peramine concentrations are lower in young grass as compared to older grass, as the study reared aphids on six-week old grass (young grass) that had peramine concentrations of <0.1 µg/g as compared to two-year old grass (old grass) that had peramine concentrations >10 µg/g. Previous studies have shown that concentrations of peramine in grass above 10 µg/g promote feeding deterrence.
in insects, while concentrations below 3 µg/g show no effect on invertebrates.\textsuperscript{23} One explanation for the disparity between young and old endophyte-infected grass is that endophytic fungi grow more slowly than the host grass.\textsuperscript{24} Alkaloid concentrations in endophyte-infected grass also correlate with mass of fungal mycelia,\textsuperscript{6} so hyphae density increases linearly with plant age and concentration of peramine.

**Figure 1.6:** Peramine concentrations (Mean ± S.D. µg/g) measured in food chain cascade based on six-week old grass (young grass) infected with *N. lolii* endophytic fungus.\textsuperscript{22}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.6.png}
\end{figure}

**Peramine Biosynthesis**

Unlike the other alkaloid classes, peramine is synthesized by one multifunctional nonribosomal peptide synthetase (NRPS) as opposed to a complex biosynthetic pathway. The peramine NRPS enzyme PerA is the product of a single gene, *perA*, which encodes seven enzymatic domains.\textsuperscript{11} Generally, NRPS enzymes have a modular structure containing a minimal repeating unit of adenylation, thiolation, and condensation domains. Addition of further domains such as *N*-methylation, epimerization, and reductase domains creates a wide spectrum of possible modifications for amino acid substrates.\textsuperscript{17}

Tanaka *et al.* proposed that 1-pyrroline-5-carboxylate, L-arginine, and S-adenosylmethionine are the three precursors utilized by the PerA enzyme for the entire
biosynthesis of peramine. 1-pyrroline-5-carboxylate (the immediate precursor of L-proline) and L-arginine make up the structure of peramine while S-adenosylmethionine acts as a methyl donor. PerA is a two-module NRPS enzyme containing an N-methylation domain, and Tanake et al. posed two hypotheses on the composition of the modules. The first hypothesis proposed that module 2 catalyzes the adenylation, thiolation, and methylation of arginine, since the α-NH₂ of arginine is methylated in peramine. Module 1 was predicted to catalyze the adenylation and thiolation of 1-pyrroline-5-carboxylate. In this case, the single condensation domain would form the peptide bond between the proline precursor and arginine. Finally, the C-terminal reductase domain would be responsible for the reduction, cyclization, and release of the N-methylated dipeptide. The second hypothesis proposed that the condensation domain of PerA catalyzes the peptide bond between 1-pyrroline-5-carboxylate and arginine. The methylation domain would catalyze the N-methylation of the α-amino group of arginine, and the reductase domain would be responsible for the reduction of the thioester and cyclization to form an iminium ion, resulting in release from PerA. Deprotonation of the intermediate and air oxidation of the pyrroline ring would yield peramine.¹⁷ This hypothesis is consistent with reported chain termination steps for other NRPSs,²⁵ making the second hypothesis the more likely scheme for peramine biosynthesis.

The complete biosynthesis, based upon the second hypothesis of Tanaka et al., is described in **Scheme 1.1**. The first step is the association of the PerA enzyme with 1-pyrroline-5-carboxylate, followed by the peptide coupling with L-arginine to give the dipeptide 1. Methylation of the α-amino group of 1 is catalyzed by the N-methylation domain of PerA and donation of a methyl group from S-adenosylmethionine, to yield product 2. Next, the thioester of 2 is reduced by the reductive domain of PerA to give 3, which immediately cyclizes to the iminium ion 4 and is released from the PerA enzyme. Air oxidation of 4 produces peramine.¹⁷
**Scheme 1.1:** Proposed biosynthetic pathway for peramine.\textsuperscript{17} The shaded circle represents the NRPS multifunctional enzyme PerA.

Although peramine had been identified in cultures of *N. lolii* and *E. typhina*,\textsuperscript{13,26} indicating that this alkaloid was a fungal secondary metabolite, there was no experimental data on the biosynthesis of this compound. Thus, in order to confirm that *perA* is essential for peramine biosynthesis, Tanaka *et al.* prepared a mutant form of an *E. festucae* endophyte strain that contained a replacement for the *perA* gene. In a choice-feeding bioassay, plant material containing the mutant *perA* gene was as susceptible to feeding as endophyte-free plants, while the wild-type containing plant material was almost completely avoided. In addition to confirming that peramine was a fungal produced
alkaloid, it also confirmed that peramine was responsible for adult Argentine stem weevil feeding deterrence.\textsuperscript{17}

A second form of \textit{perA} (\textit{perA-ΔR*}) exists with a missing sequence for the C-terminal reductase domain, likely encoding a non-functional enzyme instead. According to Schardl \textit{et al.}, this type of deletion is common among different epichloae species,\textsuperscript{10} again raising the question as to why the domain for PerA is so highly conserved across several species of fungal endophytes.

\section*{Conclusion}

Of the four alkaloid classes produced by epichloae-grass mutualisms, peramine is intriguing because of its widespread production across fungal associations coupled with its mild feeding deterrence across several insect species\textsuperscript{10,12,15,16} and lack of mammalian toxicity.\textsuperscript{14} Peramine is widely distributed throughout the host plant phloem and is present in the plant seed,\textsuperscript{18,19,20} signifying a fitness advantage to the host plant that is worth the production of this alkaloid despite the resource cost of construction. A further facet of peramine worth exploring is whether an enhanced biological response exists from a combination of peramine with another fungal alkaloid, since fifteen (34\%) of forty-four grass-endophyte mutualisms that contained peramine also produced at least one other alkaloid.\textsuperscript{10} Thus, there is significant interest in synthesizing peramine to illuminate some of these questions. Although there are two previous syntheses of peramine that will be described in Chapter Two, we completed a more efficient synthesis that is also more amenable to producing peramine derivatives.
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Chapter Two

Previous Syntheses of Peramine

The first synthetic route to peramine (1) was completed by Dumas,1 two years after the alkaloid was first isolated by Rowan and co-workers and characterized as the diacetylated derivative,2 and the second route was completed two years later by Margaret Brimble and Daryl Rowan.3 Key to both routes is construction of peramine’s pyrrolopyrazine core, a feature that is unique from the other fungal alkaloid classes, followed by functionalization of the alkyl side chain towards installation of the guanidine group.

In the Dumas synthesis, the first step was determining how the formation of the hetero bicyclic ring would be initiated. 2-(trichloroacetyl)pyrrole was chosen based on precedence of facile N-alkylation on this substrate4 and 1-bromo-5-chloro-2-pentanone (2) was selected as the alkylating agent because it could readily be prepared from commercially available 5-chloro-2-pentanone.5 However, this bromination was known to be nonselective and produces both 2 and 3, but Dumas reasoned that 2 would be a more reactive alkylating agent than 3. Therefore, if the pyrrole was used as the limiting reagent towards the formation of 4, then product arising from 3 would be minimized. In practice, bromination of 2-(trichloroacetyl)pyrrole produced a roughly 2:1 mixture with the desired product 2 being the major component in 62% yield (Scheme 2.1). Treatment of 2-(trichloroacetyl)pyrrole (as a limiting reagent to 2) with the crude bromination product and potassium carbonate produced the lactone 4 in an isolated 62% yield by flash chromatography, with no alkylation product between the pyrrole and 3 observed. On a larger scale, the intermediate alkylation product 5 was also observed and the isolated yield of lactone 4 fell to 25%. Re-exposure of compound 5 to the alkylation conditions however converted it the lactone 4. Treatment of either 4 or 5 with methylamine followed by exposure to aqueous hydrochloric acid gave the pyrrolopyrazine 6, with both treatments giving roughly a 50% yield isolated by trituration.1
With the pyrrolopyrazine core complete, the second half of the synthesis was the functionalization of the alkyl chain in order to install the guanidine necessary for peramine. To do this, Dumas first converted chloro compound 6 to the primary amine 8 utilizing the Ing-Manske modification of the Gabriel synthesis\(^6\) (Scheme 2.2). This was done by \(N\)-alkylating phthalimide potassium salt with the alkyl halide of 6 and compound 7 was recrystallized in 81% yield. The primary amine 8 was then revealed via reflux of 7 in hydrazine hydrate followed by acidic work-up and recrystallization, with an 84% yield.\(^1\)

Following formation of the primary amine, Dumas installed the guanidine group via an \(N\)-alkylation of 8 with 2-methyl-2-thiopseudourea sulfate to give 1 Sulfate, which was recrystallized in 49% yield (Scheme 2.3). Completion of the synthesis was achieved by basic ion exchange chromatography, converting 1 Sulfate to the free base 1 in 98% yield.
and 5% total yield over the seven steps. Dumas then converted 1 to the diacetate in order to confirm the characterization of 1 \textit{N,N-Diacetate} made by Rowan and co-workers.

**Scheme 2.3:** Completion of Dumas' synthesis of Peramine and the diacetate derivative.\(^1\)

Brimble and Rowan sought an alternative strategy to the synthesis of peramine, utilizing a conjugate addition of a pyrrole to a nitro-olefin for the key \textit{N}-alkylation needed for construction of the pyrrolopyrazine core (\textbf{Scheme 2.4}). Thus, the first step was the preparation of the nitroalkene 9 following precedence in the literature,\(^7\) as a mixture of stereoisomers from methyl nitroacetate, acetic anhydride, and triethyl orthoformate (with no yield reported). Nitroalkene 9 then underwent conjugate addition with methyl pyrrole-2-carboxylate to produce compound 10 as a mixture of stereoisomers in 82% yield that were not separated. Treatment of 10 with sodium borohydride and cobalt(II) chloride reduced the nitro group to the amine in 11 and was isolated as a mixture of stereoisomers in 62% yield by flash chromatography. Reduction via catalytic hydrogenation over 5% palladium on charcoal was attempted as well because of difficulties from scaling up the sodium borohydride reduction, but it resulted in hydrogenolysis to give methyl pyrrole-2-carboxylate as the major product so the previous method was maintained. Following this reduction, 11 was refluxed in toluene to promote cyclization to the lactam 12 and was purified by flash chromatography in 88% yield. Upon treatment of lactam 12 with excess potassium hydride in tetrahydrofuran (THF), the ethoxy group was eliminated to give pyrrolopyrazine 13, which was purified by flash chromatography in 80% yield. The
pyrrolopyrazine core was then completed via elimination then \( N \)-methylation using potassium hydride, followed by addition of methyl iodide to produce 14 isolated in 76% yield by flash chromatography. Attempts to affect the \( N \)-methylation step concurrently with the elimination step produced less clean results than each step carried out individually.\(^3\)

**Scheme 2.4:** Brimble and Rowan’s synthesis of the pyrrolopyrazine core.\(^3\)

Similar to the Dumas synthesis, the second half of Brimble and Rowan’s synthesis involved the construction and functionalization of the alkyl side chain (Scheme 2.5). First, the methyl carboxylate of 14 was reduced to primary alcohol, using sodium borohydride to give 15 recrystallized in 72% yield. The primary alcohol of 15 was the transformed to the nitrile 17 via an intermediary bromination step. Conventional bromination methods of an alcohol, such as phosphorus tribromide or triphenylphosphine dibromide proved unsuccessful. Instead, the bromide 16 was generated *in situ* using methanesulphonyl chloride and triethylamine in THF at -60 °C followed by addition of excess lithium bromide as the reaction mixture was slowly warmed to -40 °C. The unstable bromide 16 was isolated and then quickly added to a solution of cyano(methyl)cuprate at -40 to -20 °C in THF to afford the nitrile 17, which was purified by flash chromatography to give a 57% yield from the alcohol 15. The nitrile 17 was then reduced to the primary amine 8 using sodium borohydride and cobalt(II) chloride and isolated in 62% yield via reverse phase chromatography as the formate salt. The synthesis was completed by the \( N \)-alkylation of amine 8 with 3,5-dimethyl-1-guanidinopyrazole nitrate and triethylamine, to give peramine in an isolated 30% yield by reverse-phase chromatography. The total synthesis was completed in eleven steps with an overall yield of 2%.\(^3\)
Scheme 2.5: Completion of Brimble and Rowan’s synthesis of Peramine.³

Our Synthesis

Similar to the previous syntheses of peramine, our strategy centered on construction of the pyrlopyrazine core followed by functionalization of the alkyl side chain. However, in our route we pursued an enolate alkylation of a pyrrole-fused diketopiperazine (DKP) 18 (Scheme 2.6) as this would potentially be competent with a variety of electrophilic substrates. In addition, we reasoned that enolization and alkylation of 18 would allow a small number of analogs of peramine to be prepared with minimal effort. From an alkylation product such as 19, the correct oxidation state could be installed by reduction of the acyl pyrrole and dehydration of a stable intermediate pyrrolyl carbinol 20.⁸ We chose to install the guanidine residue as the bis-Boc protected derivative 22, which could easily be converted to peramine upon exposure to acid. Additionally, we hoped that 22 would have more favorable physical properties than the unprotected natural product such as partitioning into organic solvents, and would likely be amenable to normal phase chromatographic purification.
Scheme 2.6: Our synthetic strategy, centered on an enolate alkylation of a pyrrole-fused diketopiperazine.

Our synthesis was initiated with the amide bond formation between sarcosine methyl ester HCl salt and pyrrole-2-carboxylic acid, with 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and hydroxybenztriazole (HOBt) to afford a mixture of 23 and a small amount (10-20%) of cyclized product, DKP 18 (Scheme 2.7). The mixture was heated at reflux in toluene with NEt₃ in order to promote condensation and complete conversion of 23 into DKP 18, which was recrystallized to provide pure material in 60% yield.

Scheme 2.7: Synthesis of the pyrrole-fused diketopiperazine.

The next step was the alkylation of DKP 18 (Scheme 2.8). The derived enolate of DKP 18, which was generated with LiHMDS at -78 °C, was poorly soluble in tetrahydrofuran (THF) and precipitated from the reaction mixture. Addition of allyl bromide to the solution accompanied by slow warming to 0 °C produced bis-alkylation product 25 in preference over the desired mono-alkylation product 24, in addition to unreacted starting material DKP 18 (entry A). We reasoned that the observed preference was correlated with poor enolate solubility, and if an equilibrium between the enolates of 24 and 25 was operational, then the presumed greater solubility of the derived enolate of
24 could favor a second alkylation to give rise to 25 as the major product. Optimization of reaction conditions such as solvent, concentration, stoichiometry, and temperature allowed isolation of the mono-alkylation product 24 in up to 30% isolated yield (entry B). Use of Cs$_2$CO$_3$ as a base improved the alkylation, leading to an increase in the isolated yield of 24 (entry C). In practice, the reaction procedure using Cs$_2$CO$_3$ (1 equiv) in THF at room temperature was operationally simple to execute, and the product mixture was purified by chromatography.

**Scheme 2.8: Alkylation of acyl pyrrole with allyl bromide.**

Next, DKP 24 was reduced with LiBH$_4$ in THF at 0 °C, delivering the stable intermediate pyrrolyl carbinol 26 as a single diastereomer, presumably via addition of hydride to the less hindered face of the acyl pyrrole.$^8$ Formation of 26 was a very clean reaction and no purification was necessary. Dehydration of 26 with methansulfonic acid (MsOH) at 0 °C gave the pyrrolopyrazine 27. This reaction was also very clean with no purification required, and 27 was submitted to the hydroboration step. Using 9-BBN and subsequent oxidation (H$_2$O$_2$, NaOH, 23 °C, 20h), the terminal alkene in 27 was transformed to the primary alcohol 28. The mixture was purified by chromatography to deliver 53% yield over the three steps. The primary alcohol 28 was then converted into the protected guanidine residue in 22 using a Mitsonobu reaction. Although the Mitsonobu reaction was competent with 30 and either diethyl or diisopropyl azodicarboxylate (DEAD or DIAD),
isolation of analytically pure 22 was complicated by contamination with the resulting hydrazine byproducts, which had both similar solubility and retention times on silica gel. No reaction was observed with resin-bound azodicarboxylate, however use of di-2-methoxyethylazodicarboxylate (29) was effective in the reaction. The derived hydrazine was both more soluble and more polar than product 22 ($\Delta R_f = 0.2$), theoretically allowing facile separation and isolation of 22. In practice, bis-Boc peramine (22) conveniently precipitated from the reaction mixture and was cleanly obtained in 70% yield. Removal of the Boc protection was facilitated by trifluoroacetic acid (TFA) in CH$_2$Cl$_2$ (23 °C, 2h) and gave the TFA salt of peramine (1) in 94% yield. Spectrometric data for 1 are in agreement with data provided by a certified vendor. Our synthesis was completed with an overall yield of 11%, and was recently published in the Journal of Natural Products.

**Scheme 2.9:** Completion of our synthesis of Peramine.

In conclusion, our synthesis of the natural product peramine was conducted in seven steps with a total of three chromatographic separations from pyrrole 2-carboxylic acid. Compared to the Dumas synthesis, our route is equally efficient in terms of step-count and chromatographic purifications but more competitive with respect to total yield (Table 2.1). Compared to the Brimble and Rowan synthesis, our synthesis is both more efficient.
and more competitive. Our strategy is also distinct from the previous approaches as it features an enolate alkylation of pyrrole-fused diketopiperazine and partial reduction of the acyl pyrrole functionality, making our approach more amenable to the preparation of peramine analogs. In addition, a ready of supply of peramine is beneficial for conducting future feeding studies comparing peramine in conjunction with other fungal alkaloids in testing improved anti-insect activity.

Table 2.1: Comparison between our synthesis and the previous syntheses of peramine.

<table>
<thead>
<tr>
<th>Synthesis</th>
<th>Number of Steps</th>
<th>Number of Chromatographic Purifications</th>
<th>Total Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dumas, 1988</td>
<td>7 steps</td>
<td>2</td>
<td>5%</td>
</tr>
<tr>
<td>Brimble and Rowan, 1990</td>
<td>11 steps</td>
<td>7</td>
<td>2%</td>
</tr>
<tr>
<td>Nelli and Scheerer, 2016</td>
<td>7 steps</td>
<td>3</td>
<td>11%</td>
</tr>
</tbody>
</table>

Future Directions

With the synthesis of the natural product completed, we sought to begin the next step of producing peramine analogs. Based upon the commercial use of N-nitro guanidine functionalities in neonicotinoid pesticides, we aimed to synthesize a similar derivative of peramine. Neonicotinoids are selectively toxic to insects, with a similar mode of action to nicotine, and cause death within a few hours of contact (Figure 2.1). Thus, a N-nitro derivative of peramine might possess increased anti-insect properties.
The synthesis of \( N \)-nitro peramine (Scheme 2.10) was conducted using the primary alcohol 28 as the starting material, which had to be transformed to the primary amine 8 in order to undergo an \( N \)-alkylation, similar to the methodology of the Dumas synthesis of peramine. However, we installed the phthalimide using a Mitsonobu reaction instead, as there was precedence for such a transformation on a primary alcohol.\(^{13}\) Use of DMEAD allowed easy isolation of 7 from the Mitsonobu by-products via flash chromatography in 76% yield. The primary amine 8 was then revealed upon reflux of 7 in hydrazine hydrate and ethanol, followed by aqueous workup. Amine 8 was purified in 82% yield by extraction of the hydrazine by-products from the organic layer, followed by addition of sodium hydroxide to the aqueous layer to partition the now free-based amine 8 to the organic phase. Treatment of amine 8 with \( N \)-nitro thiourea 31, which was synthesized via nitration of S-methylisothiourea sulfate, gave the \( N \)-alkylation product of \( N \)-nitro 1 in 59% yield by trituration, with 37% yield over the three steps. Future plans include synthesis of more peramine derivatives, as well as insect feeding assays on those derivatives to detect anti-insect activity.

Scheme 2.10: Synthesis of \( N \)-nitro Peramine.
Experimental Section

**2-Methyl-2,3-dihydropyrrolo[1,2-a]pyrazine-1,4-dione (18).** The following procedure is modified from the literature.\(^{14}\) A dry flask was charged with a stir bar, sarcosine methyl ester·HCl (8.54 g, 61.0 mmol, 1.2 equiv), CH\(_2\)Cl\(_2\) (50 mL), and dimethylformamide (50 mL). The slurry was rapidly stirred, and EDC (11.71 g, 61.0 mmol, 1.2 equiv), NEt\(_3\) (9.27 mL, 61.0 mmol, 1.2 equiv), and HOBt (8.24 g, 61.0 mmol, 1.2 equiv) were added in succession. Pyrrole 2-carboxylic acid (5.60 g, 50 mmol, 1.0 equiv) was added to the reaction mixture in four portions (~1.4 g each) over 1 h. After stirring at rt for an additional 2 h, the reaction mixture was poured into a separatory funnel containing EtOAc (120 mL) and HCl (140 mL, 1.0 M). The organic layer was removed, and the aqueous portion was extracted with additional EtOAc (2 × 120 mL). The combined organic portions were washed with saturated aqueous NaHCO\(_3\) (140 mL) and brine (140 mL), dried (Na\(_2\)SO\(_4\)), filtered, and concentrated. The resulting brown residue (10.35 g) was dissolved in PhMe (40 mL) and NEt\(_3\) (6 mL). The reaction flask was fitted with a reflux condenser, flushed with N\(_2\) (15 min), and heated at reflux for 16 h. Following concentration of the reaction mixture, an oily brown solid was obtained. Recrystallization from PhMe (~10 mL) afforded the desired diketopiperazine 18 (4.93 g, 60% yield) as yellow needles. Characterization data are provided in CDCl\(_3\) to supplement existing literature\(^{14}\): mp 146.0–147.2 °C; IR (neat) 3126, 2900, 1724, 1641, 1575, 1335 cm\(^{-1}\); \(^1H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.48 (dd, \(J_1 = 1.9\) Hz, \(J_2 = 3.1\) Hz, 1H), 7.11 (dd, \(J_1 = 1.9\) Hz, \(J_2 = 3.5\) Hz, 1H), 6.51 (t, \(J = 3.5\) Hz, 1H), 4.37 (s, 2H), 3.12 (s, 3H); \(^{13}C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 161.0, 155.6, 125.8, 118.7, 118.5, 115.7, 53.3, 32.8.
3-Allyl-2-methyl-2,3-dihydropyrrolo[1,2-a]pyrazine-1,4-dione (24). A dry flask was charged with a stir bar and diketopiperazine 18 (1.50 g, 9.0 mmol). The substrate was dissolved in THF (30 mL, 0.3 M) prior to the addition of Cs₂CO₃ (2.98 g, 9.0 mmol, 1.0 equiv) in one lot followed by allyl bromide (0.79 mL, 9.0 mmol, 1.0 equiv). The reaction mixture was rapidly stirred for 20 h at rt under N₂. The reaction was diluted with water (80 mL) and saturated aqueous NH₄Cl (80 mL) and extracted with EtOAc (3 × 80 mL). The combined organic portions were washed with brine (80 mL), dried (Na₂SO₄), filtered, and concentrated. Purification by flash chromatography on silica gel (elution: 25–100% EtOAc in hexane) afforded the desired allyl diketopiperazine 24 (1.0 g, 4.95 mmol, 55% yield) as a light yellow solid: mp 62.0–64.0 °C; IR (neat) 3145, 2968, 1718, 1641, 1577, 1413, 1400, 1332 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.74 (dd, J₁ = 1.6 Hz, J₂ = 3.1 Hz, 1H), 7.07 (dd, J₁ = 1.5 Hz, J₂ = 3.1 Hz, 1H), 6.49 (t, J = 3.1 Hz, 1H), 5.54–5.43 (m, 1H), 5.14 (dd, J₁ = 1.1 Hz, J₂ = 17.0 Hz, 1H), 5.06 (dd, J₁ = 0.7 Hz, J₂ = 10.15 Hz, 1H), 4.36 (t, J = 4.3 Hz, 1H), 3.12 (s, 3H), 2.84 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 164.1, 156.1, 129.1, 125.7, 121.1, 118.3, 118.0, 115.6, 63.6, 36.7, 31.6; MS (ES) calcd for C₁₁H₁₂N₂O₂⁺Na [M + Na] 227.0791, found 227.0792.

3-Allyl-4-hydroxy-2-methyl-3,4-dihydropyrrolo[1,2-a]-pyrazin-1(2H)-one (26). Based on the method reported by Evans and co-workers⁸, allyl diketopiperazine 24 (150 mg, 0.74 mmol) was dissolved in THF (7.4 mL, 0.1 M) and cooled to 0 °C. A solution of LiBH₄ in THF (~3 M, 0.74 mL, 3 equiv) was added dropwise over 10 min. After stirring for 3.5 h at 0 °C, the reaction was quenched with pH 7 buffer (0.1 M potassium phosphate, 10 mL), diluted with water (15 mL), and extracted with EtOAc (5 × 15 mL). The combined
organic portions were washed with brine (20 mL), dried (Na₂SO₄), filtered, and concentrated to afford 26 (151 mg, 99% yield) as a colorless powder, which was used directly without purification in the next reaction. The isolated yield and analytical data are reported for material judged >95% pure as determined by ¹H NMR spectroscopy: mp 108.0–110.0 °C; IR (neat) 3153, 2927, 1608, 1544, 1467, 1456, 1417, 1392 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.96 (t, J = 1.5 Hz, 1H), 6.81 (dd, J₁ = 1.6Hz, J₂ = 3.7Hz, 1H), 6.20 (t, J = 2.8Hz, 1H), 5.80 (m, 1H), 5.60 (br s, 1H), 5.52 (br s, 1H), 5.21 (dd, J₁ = 1.5 Hz, J₂ = 17 Hz, 1H), 5.15 (d, J = 10.1 Hz, 1H), 3.70 (m, 1H), 2.96 (s, 3H), 2.58 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 160.0, 133.4, 122.5, 121.3, 119.3, 114.2, 110.3, 77.4, 61.7, 32.6, 31.4; MS (ES) calcd for C₂₂H₂₆N₄O₄+Na [M × 2 + Na]⁺ 435.2003, found 435.2003.

3-Allyl-2-methylpyrrolo[1,2-a]pyrazin-1(2H)-one (27). Pyrrolyl carbinol 26 (151 mg) was dissolved in CH₂Cl₂ (2.4 mL) and cooled to 0 °C. An equal volume of MsOH (2.4 mL) was added, and the reaction mixture was stirred for 1 h at 0 °C, diluted with water (10 mL), and extracted with EtOAc (4 × 20 mL). The combined organic portions were washed with saturated aqueous NaHCO₃ (30 mL) and brine (30 mL), dried (Na₂SO₄), filtered, and concentrated to afford 27 (149 mg, 97% yield) as an off-white solid, which was used directly without purification in the next reaction. The isolated yield and analytical data are reported for material judged >95% pure as determined by ¹H NMR spectroscopy: mp 83.0–85.0 °C; IR (neat) 3107, 2947, 1674, 1624, 1531, 1477, 1423, 1375, 1348 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.06 (d, J = 3.9 Hz, 1H), 7.02 (t, J = 1.9 Hz, 1H), 6.82 (s, 1H), 6.52 (m, 1H), 5.96–5.86 (m, 1H), 5.26 (dd, J₁ = 1.2 Hz, J₂ = 10.2 Hz, 1H), 5.19 (dd, J₁ = 1.6 Hz, J₂ = 17.2 Hz, 1H), 3.44 (s, 3H), 3.27 (dd, J₁ = 0.8 Hz, J₂ = 5.1 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 157.0, 133.3, 126.6, 123.2, 118.5, 117.4, 112.2, 109.6, 106.7, 34.6, 28.9; MS (ES) calcd for C₁₁H₁₂N₂O+Na [M + Na]⁺ 211.0842, found 211.0844.
3-(3-Hydroxypropyl)-2-methylpyrrolo[1,2-a]pyrazin-1(2H)-one (28). The allyl pyrrolopyrazine 27 (149 mg, 0.79 mmol, 1.0 equiv) was dissolved in THF (3.16 mL, 0.25 M), and a solution of 9-BBN in THF (0.5 M, 4.74 mL, 3 equiv) was added. The reaction was stirred for 2 h at rt, after which time consumption of 27 was apparent by TLC. The reaction was cooled to 0 °C, and NaOH (1.0 M, 8.0 mL, 3.3 equiv relative to B) and H₂O₂ (35 wt %/vol, 0.86 mL, 4 equiv relative to B) were added. The reaction was allowed to warm to rt overnight with stirring. The reaction mixture was transferred to a separatory funnel with EtOAc (15 mL) and saturated aqueous NaHCO₃ (15 mL). The organic layer was removed, and the aqueous portion was extracted with additional EtOAc (2 × 15 mL). The combined organic portions were washed with brine (25 mL), dried (Na₂SO₄), filtered, and concentrated. The resulting residue was purified by flash chromatography on silica gel (elution: 0–10% MeOH in EtOAc) to afford the desired alcohol 28 (87.7 mg, 0.43 mmol, 53% yield over 3 steps) as a white powder: mp 123.2–125.0 °C; IR (neat) 3360, 3224, 3109, 2922, 2850, 1672, 1618, 1595, 1558 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.05 (d, J = 4.3 Hz, 1H), 7.01 (t, J = 1.6 Hz, 1H), 6.83 (s, 1H), 6.52 (m, 1H), 3.77 (t, J = 6.3 Hz, 2H), 3.47 (s, 3H), 2.65 (t, J = 7.0 Hz, 2H), 1.87 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 157.2, 128.0, 123.0, 117.4, 112.2, 109.6, 106.0, 61.2, 31.0, 29.0, 26.9; MS (ES) calcd for C₁₁H₁₄N₂O₂+Na [M + Na]⁺ 229.0947, found 229.0947.
3-(3-(1,3-Bis(tert-butoxycarbonyl)guanidine))-2-methyl-pyrrolo[1,2-a]pyrazin-1(2H)-one (22). The pyrrolopyrazine alcohol 28 (88 mg, 0.43 mmol, 1.0 equiv), PPh$_3$ (168 mg, 0.64 mmol, 1.5 equiv), and 1,3-bis(tert-butoxycarbonyl)guanidine (30) (166 mg, 0.64 mmol, 1.5 equiv) were added to a dry flask and dissolved in THF (4.3 mL, 0.1 M). DMEAD (29) (150 mg, 0.64 mmol, 1.5 equiv) was added, and the reaction mixture was stirred for 30 min, at which time a precipitate was observed. The reaction mixture was filtered, and 22 (133 mg, 70% yield) was collected as a colorless solid. The isolated yield and analytical data are reported for material judged >95% pure as determined by $^1$H NMR spectroscopy: mp 197.2–199.0 °C; IR (neat) 3377, 3269, 3128, 2976, 2929, 1724, 1681, 1637, 1604, 1504 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 9.39 (s, 1H), 9.19 (s, 1H), 7.05 (d, J = 2.4 Hz, 1H), 7.04 (s, 1H), 6.96 (s, 1H), 6.52 (t, J = 2.4 Hz, 1H), 3.99 (t, J = 7.4 Hz, 2H), 3.48 (s, 3H), 2.57 (t, J = 7.4 Hz, 2H), 1.92 (m, 2H), 1.51 (s, 18H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 163.7, 160.5, 157.1, 154.7, 127.7, 123.0, 117.5, 112.0, 109.6, 105.9, 84.1, 78.9, 43.9, 28.9, 28.3, 28.0, 27.9, 27.2; MS (ES) calcd for C$_{22}$H$_{33}$N$_5$O$_5$+Na [M + Na]$^+$ 470.2373, found 470.2373.

3-(3-Guanidine)-2-methylpyrrolo[1,2-a]pyrazin-1(2H)-one (Peramine, 1·(HO$_2$CCF$_3$)) (1). Bis-boc-peramine 22 (20.8 mg, 0.045 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (1.0 mL) and cooled to 0 °C. TFA (1.0 mL) was added, and the cooling bath was removed after 5 min. The reaction was stirred for 2 h at rt. The reaction mixture was first concentrated under a stream of N$_2$ and more thoroughly under reduced pressure to provide the TFA salt of 1 (20.2 mg, 0.043 mmol, 94% yield) as a colorless, amorphous solid. The isolated yield
and analytical data are reported for material judged >95% pure. NMR spectroscopic data below are in agreement with data for 1·HNO₃ provided by BDG Synthesis: mp 119.2–122.0 °C; ¹H NMR (400 MHz, DMSO) δ 7.80 (br s, 1H), 7.60–6.90 (br s, 4H), 7.33 (t, J = 2.4 Hz, 1H), 7.24 (s, 1H), 6.82 (dd, J₁ = 1.1 Hz, J₂ = 3.95 Hz, 1H), 6.50 (m, 1H), 3.36 (s, 1H), 3.21 (dt, J₁ = 6.3 Hz, J₂ = 7.0 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 1.76 (m, 2H); ¹³C NMR (100 MHz, DMSO) δ 156.8, 155.9, 127.7, 122.3, 118.2, 111.8, 108.5, 105.7, 40.1, 28.5, 27.1, 26.7; MS (ES) calcd for C₂₄H₃₅N₁₀O₂ [M × 2 + H]⁺ 495.2939, found 495.2939.

2-(3-(2-methyl-1-oxo-1,2-dihydropyrrolo[1,2-a]pyrazin-3-yl)propyl)isoindoline-1,3-dione (7). The following procedure is modified from the literature. The pyrrolopyrazine alcohol 28 (80mg, 0.42 mmol, 1.0 equiv), PPh₃ (131.2 mg, 0.50 mmol, 1.2 equiv), and phthalimide (73.6 mg, 0.50 mmol, 1.2 equiv) were added to a dry flask, dissolved in THF (4.2 mL, 0.1 M) and cooled to 0 °C. DMEAD (29) (117.1 mg, 0.5 mmol, 1.2 equiv) was added and the reaction mixture was allowed to warm to rt. The reaction mixture was stirred for 4 h until consumption of 28 was apparent by TLC, at which point the reaction was stopped and was concentrated. The resulting residue was purified by flash chromatography on silica gel (elution: 30-100% EtOAc in hexane) to afford 7 (109.0 mg, 0.32 mmol, 76% yield) as a white powder. NMR spectroscopic data below are in agreement with existing literature: ¹H NMR (400 MHz, CDCl₃) δ 7.85 (m, 2H), 7.73 (m, 2H), 7.02 (m, 2H), 6.92 (d, J = 0.7 Hz, 1H), 6.50 (dd, J₁ = 2.6 Hz, J₂ = 3.9 Hz, 1H), 3.82 (t, J = 6.9 Hz, 2H), 3.44 (s, 3H), 2.59 (t, J = 7.5 Hz, 2H), 2.03 (br quintet, 2H).
3-(3-aminopropyl)-2-methylpyrrolo[1,2-a]pyrazin-1(2H)-one (8). The following procedure is modified from the literature.\textsuperscript{1} The phthalimide 7 (97 mg, 0.29 mmol, 1.0 equiv) was dissolved in EtOH (2.89 mL, 0.1M) and stirred. Hydrazine hydrate (40 µL, 0.87 mmol, 3.0 equiv) was added and reaction mixture was heated at reflux for 30 min. The mixture was then cooled to rt, and then 1 N HCl was added, followed by heating back to reflux for 2 min until a clear solution was obtained. The solid that precipitated was removed by filtration and washed with water, and the filtrate was concentrated. The filtrate was extracted with CHCl\textsubscript{3} (1 x 10 mL) and aqueous layer was washed with brine (10 mL). The aqueous layer was extracted again (5 x 10 mL) and these organic fractions were set aside. 1 N NaOH was then added to aqueous layer in proportion to HCl, and aqueous layer was extracted with CHCl\textsubscript{3} (6 x 10 mL). These organic fractions were collected, dried (Na\textsubscript{2}SO\textsubscript{4}), filtered, and concentrated. Product 8 was purified by separation during extraction (48.4 mg, 0.24 mmol, 82% yield) as a white solid. NMR spectroscopic data below are in agreement with existing literature:\textsuperscript{1} \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \textdelta 7.05 (dd, J\textsubscript{1} = 1.5 Hz, J\textsubscript{2} = 4.0 Hz, 1H), 7.01 (dd, J\textsubscript{1} = 1.5 Hz, J\textsubscript{2} = 2.5 Hz, 1H), 6.82 (d, J = 0.8 Hz, 1H), 6.52 (dd, J\textsubscript{1} = 2.5 Hz, J\textsubscript{2} = 4.0 Hz, 1H), 3.47 (s, 3H), 2.83 (t, J = 6.9 Hz, 2H), 2.59 (br t, J = 7.7 Hz, 2H), 1.75 (br quintet, 2H), 1.43 (br s, 3H).

methyl nitrocarbamimidothioate (31). The following procedure is modified from the literature.\textsuperscript{15} Mixture of fuming nitric acid and sulfuric acid was cooled to -10 °C. S-methyl isothiourea sulfate (4.0 g, 14.37 mmol, 1.0 equiv) was added in portions over an hour. The reaction mixture was stirred for 1 h at 0 °C, then poured onto 60 mL of ice/water mix, and the white product was filter off under vacuum. The filter cake was washed with 7.5 mL
water, 3 mL cold ethanol, and 4.5 mL diethyl ether. The white solid was dried at rt in vacuo to give 2.38g of 31. NMR spectroscopic data was in agreement with existing literature.\textsuperscript{15}

\((E)-2-(3-(2\text{-}methyl\text{-}1\text{-}oxo\text{-}1,2\text{-}dihydropyrrolo[1,2-\text{a}]pyrazin\text{-}3\text{-}yl)propyl)-1\text{-}nitroguanidine (N\text{-}nitro 1)\). The following procedure is modified from the literature.\textsuperscript{16} Amine 8 (48.0 mg, 0.23 mmol, 1.0 equiv) and N-nitro thiourea 31 (42.8 mg, 0.12 mmol, 0.5 equiv) are dissolved in EtOH (2.3 mL, 0.1 M) in a dry flask. The reaction mixture is heated to reflux and stirred for 3 h, after which consumption of 31 was apparent by TLC. Trituration of the reaction mixture with CHCl\textsubscript{3} gave N-nitro 1 as a brownish solid (40 mg, 0.14 mmol, 59% yield). The isolated yield and analytical data are reported for material judged >95% pure as determined by \textsuperscript{1}H NMR spectroscopy: \textsuperscript{1}H NMR (400 MHz, DMSO) \(\delta 8.61 \text{ (s, 1H)}, 7.97 \text{ (br s, 1H)}, 7.33 \text{ (dd, } J_1 = 2.7 \text{ Hz}, J_2 = 1.5 \text{ Hz, 1H)}, 7.27 \text{ (br s, 1H)}, 6.81 \text{ (m, 1H)}, 6.50 \text{ (dd, } J_1 = 3.9 \text{ Hz}, J_2 = 2.7 \text{ Hz, 1H)}, 3.35 \text{ (d, } J = 8.7 \text{, 3H)), 3.26 \text{ (q, } J_A = 6.7 \text{ Hz, } J_B = 6.6 \text{ Hz, 2H)}, 2.58 \text{ (t, } J = 7.5 \text{ Hz, 2H)}, 1.79 \text{ (quintet, } J_1 = 7.5 \text{ Hz, } J_2 = 14.9 \text{ Hz, 2H)); }^{13}\text{C NMR (100 MHz, DMSO) }\delta 159.1, 155.9, 127.8, 122.3, 118.2, 111.7, 108.5, 105.7, 28.5, 26.8.
References


(10) BDG synthesis (New Zealand) provides authentic analytical reference standards of 1.


Appendix

Supporting Information for Chapter Two

General Experimental Procedures: All reactions were carried out under an atmosphere of nitrogen in flame-dried or oven-dried glassware with magnetic stirring. Acetonitrile, THF, toluene, and Et₂O were degassed with argon and purified by passage through a column of molecular sieves and a bed of activated alumina. CH₂Cl₂ was distilled from CaH₂ prior to use. All reagents were used as received unless otherwise noted. Flash column chromatography was performed using silica gel (230-400 mesh). Analytical thin-layer chromatography was performed on 60 Å glass plates. Visualization was accomplished with UV light, anisaldehyde, ceric ammonium molybdate, potassium permanganate, or ninhydrin, followed by heating. Infrared spectra were recorded using an FTIR spectrophotometer. ¹H NMR spectra were recorded on a 400 MHz spectrometer and are reported in ppm using solvent as an internal standard (CDCl₃ at 7.26ppm) or tetramethylsilane (0.00ppm). Proton-decoupled ¹³C NMR spectra were recorded on a 100 MHz spectrometer and are reported in ppm using solvent as an internal standard (CDCl₃ at 77.0 ppm; DMSO-d₆ at 39.5ppm). All compounds were judged to be homogeneous (>95% purity) by ¹H and ¹³C NMR spectroscopy unless otherwise noted as mixtures. Mass spectra data analysis was obtained through positive electrospray ionization (ICR-MS w/NaCl).
$\text{DMSO-}^6_{\partial} 400\text{MHz}$

$\text{DMSO-}^6_{\partial} 100\text{MHz}$

$\text{MeN} \quad \text{N} \quad \text{O} \quad 1 \quad \text{NH}_2 \quad \text{N} \quad \text{NH}_2 \quad \text{F}_3 \text{CCO}_2 \text{H}$

$\text{NCH}_3 + \text{solvent}$