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Synthetic Approaches to the Microtubule-Stabilizing Sponge Alkaloid Ceratamine A and Desbromo Analogues

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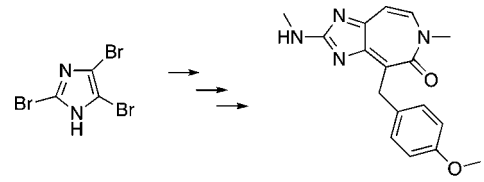

Desbromoceratamine A

Two synthetic approaches to the microtubule-stabilizing ceratamine alkaloids are described. The first approach involved attempts to graft an aminoimidazole moiety onto an azepine ring to form partially hydrogenated versions of the unprecedented aromatic imidazo[4,5-*d*]azepine core of the ceratamines. This route ultimately failed because it was not possible to aromatize the partially hydrogenated ceratamine intermediates. A second approach started with tribromoimidazole that was sequentially metalated and functionalized to efficiently generate a key imidazole intermediate containing vinyl bromide and amide functionalities. An intramolecular Buchwald vinyl amidation reaction converted this key intermediate into a bicyclic imidazo[4,5-*d*]azepine that was at the same oxidation state as the aromatic core of the ceratamines. The 2-amino functionality present on the imidazole ring of the ceratamines was installed using a Buchwald/Hartwig amination reaction on a 2-chloroimidazole precursor. Deprotection and aromatization resulted in the first synthesis of desbromoceratamine A (**55**) and desmethyldesbromoceratamine A (**60**). An unanticipated addition of atmospheric oxygen was encountered during deprotection of the imidazole ring in the last step of the synthesis leading to C-11 oxygenated ceratamine analogues as byproducts. Evaluation of the synthetic ceratamines in a TG3 cell-based assay for mitotic arrest revealed that the C-14 and C-16 bromine substituents in ceratamine A (**1**) play a major role in the antimitotic potency of the natural product. The synthetic route to ceratamine analogues has provided sufficient quantities of desbromoceratamine A (**55**) for testing in mouse models of cancer.

Introduction

The widespread clinical use of Vinca alkaloids, paclitaxel, and synthetic analogues of these natural products for treating cancer has provided unparalleled validation of tubulin as a molecular target for developing new anticancer drugs. Horwitz's seminal discovery that paclitaxel stabilizes microtubules and the subsequent success of paclitaxel in the clinic stimulated a vigorous worldwide search for additional natural product chemotypes possessing this important biological activity.[1] As a result, a substantial collection of microtubule-stabilizing natural products with widely divergent chemical structures has been discovered, and the new anticancer drug ixabepilone based on the epothilone scaffold has recently been approved for clinical use.[2,3] Nearly all of the known microtubule-stabilizing natural products were initially discovered because they were potent cytotoxins, and it was only subsequent investigation of their mechanism of action that revealed their abilities to arrest cells in mitosis and target tubulin. More recently, a cell-based assay has provided a sensitive and robust method to directly screen natural product extracts for the presence of compounds that arrest cells in mitosis.[4] Microscopic examination of tubulin in cells that have been arrested by a hit in the assay can often reveal if the active components are microtubule-depolymerizing or -stabilizing agents. In this way, the assay can be used as an

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(1) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665–667.

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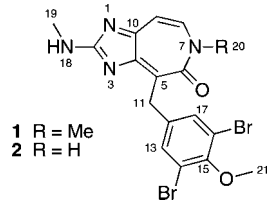
effective direct screen for microtubule-stabilizing natural products, and it enabled our discovery of caribaeoside,[5] the ceratamines,[6] and the nigricanosides.[7]

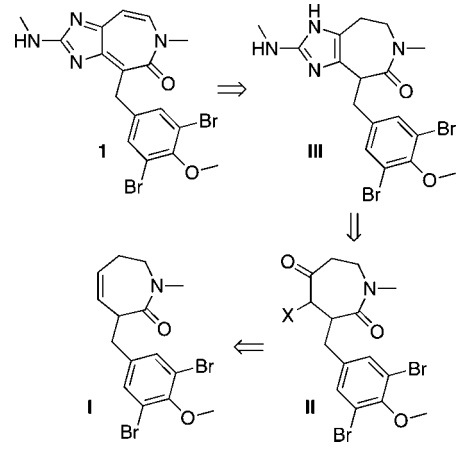


Ceratamines A (**1**) and B (**2**) are novel alkaloids isolated from the marine sponge *Pseudoceratina* sp. collected in Papua New Guinea.[6] The imidazo[4,5-*d*]azepine core heterocycle at the oxidation state found in the ceratamines has no precedent among known natural products or synthetic compounds. Ceratamines stabilize microtubules but do not bind to the taxol binding site, and they produce a novel mitotic arrest phenotype characterized by the presence of multiple pillar-like structures of tubulin.[8] The ability of ceratamines to stabilize microtubules and elicit a novel phenotype combined with their relative structural simplicity and lack of chiral centers makes them attractive anticancer drug leads. Scarcity of the natural products from the original collection and the potential negative environmental impact of recollecting additional source-sponge tissue created a need for a synthetic source of the ceratamines and analogues to enable further in vitro and in vivo evaluation of this new heterocyclic microtubule-stabilizing pharmacophore. We embarked on a total synthesis of the ceratamines to solve the supply problem, and in a recent communication we disclosed a route to a C-11 hydroxy ceratamine analogue that showed in vitro antimitotic activity.[9] However, challenges associated with scaling up this first generation route to a bioactive ceratamine and our failure to make analogues that were not oxygenated at C-11 led us to search for alternate methods to produce ceratamines. Herein, we describe the complete details of our synthetic efforts to make ceratamines, including description of a more efficient route to the 2-aminoimidazo[4,5-*d*]azepine heterocyclic core and the generation of new analogues lacking C-11 oxygenation that have revealed significant information about the SAR for the antimitotic pharmacophore.

Results and Discussion

Molecules containing a 2-aminoimidazole motif are often challenging to work with because of their poor solubility, poor chromatographic behavior, and unexpected reactivities.[10,11] Therefore, it was decided to introduce the 2-methylamino group at a late stage in all of our synthetic approaches to ceratamines. Our first approach to the imidazo[4,5-*d*]azepine ring system was to build the five-membered 2-aminoimidazole ring onto a suitably functionalized caprolactam ring and in the process install three of the four nitrogen atoms of the ceratamines in one operation (Scheme 1). We planned to construct partially hydrogenated versions of the imidazo[4,5-*d*]azepine ring system (i.e., **III**) and then take advantage of the putative aromaticity of the fully dehydrogenated heterocycle as a driving force to achieve the oxidation state found in the natural products.[12] 2-Aminoimidazoles have been formed in good yield via condensation of guanidine derivatives with α-halo ketones or α-pseudohalo ketones.[13] In order to implement this approach to ceratamine A, a suitably functionalized caprolactam such as **II** shown in the retrosynthesis in Scheme 1 was required, and it was anticipated that the α-halo ketone functionality in **II** could be formed from the corresponding alkene in the caprolactam **I**.

SCHEME 1



The key caprolactams **12** and **13** were prepared as shown in Scheme 2 as a first step in exploring the plan outlined in Scheme 1. Butenoic acid **3** was readily α-alkylated with benzylbromides **4** and **5** to give the carboxylic acids **6** and **7**, and butenylamine **9** was routinely prepared from butenylbromide **8** (see Supporting Information for experimental details). Coupling of butenylamine **9** with carboxylic acids **6** and **7** via their respective acid chlorides gave amides **10** and **11** in good yield. Ring-closing metathesis was carried out on **10** and **11** with Grubbs second-generation catalyst to yield caprolactams **12** and **13**, respectively.[14] Model lactam **12** was converted to the α-tosylketone **16** in low yield by sequential epoxidation, tosylation, and oxidation following a literature precedent (Scheme 3).[15] Reaction of the pseudohalo ketone **16** with guanidine in an attempt to form **17a** gave an intractable mixture of products. Attempts to form **17b** by treatment of **16** with *N*-acetylguanidine gave only starting material.

Further attempts to form the aminoimidazole ring from guanidine began with the formation of halohydrin **18** (Scheme 4). Lactam **13** was reacted with *N*-bromosuccinimide and water

(2) Examples. (a) Eleutherobin: Lindel, T.; Jensen, P. R.; Fenical, W.; Long, B. H.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744–8745. (b) Laulimalide: Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukam, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653–660. (c) Peloruside: Hood, K. A.; West, L. M.; Rouwe, B.; Northcote, P. T.; Berridge, M. V.; Wakefield, S. J.; Miller, J. H. *Cancer Res.* **2002**, *62*, 3356–3360. (d) Discodermolide: ter-Haar, E.; Kowalski, R. J.; Hamel, E.; Lin, C. M.; Longley, R. E.; Gunasekera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 243–250. (e) Epothilone: Bollag, D. M.; McQueney, P. A.; Zhu, J.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325–2333.

(3) Donovan, D.; Vahdat, L. T. *Oncology* **2008**, *22*, 408–416.

(4) Roberge, M.; Cinel, B.; Anderson, H. J.; Lim, L.; Jiang, X.; Xu, L.; Kelly, M. T.; Andersen, R. J. *Cancer Res.* **2000**, *60*, 5052–5058.

(5) Cinel, B.; Roberge, M.; Behrisch, H.; van Ofwegen, L.; Castro, C. B.; Andersen, R. J. *Org. Lett.* **2000**, *2*, 257–260.

(6) Manzo, E.; van Soest, R.; Matainaho, L.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2003**, *5*, 4591–4594.

(7) Williams, D. E.; Sturgeon, C. M.; Roberge, M.; Andersen, R. J. *J. Am. Chem. Soc.* **2007**, *129*, 5822–5823.

(8) Karjala, G.; Chan, Q.; Manzo, E.; Andersen, R. J.; Roberge, M. *Cancer Res.* **2005**, *65*, 3040–3043.

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(10) O'Malley, D. P.; Li, K.; Maue, M.; Zografos, A. L.; Baran, P. S. *J. Am. Chem. Soc.* **2007**, *129*, 4762–4775.

(11) Meketa, M. L.; Weinreb, S. M.; Nakao, Y.; Fusetani, N. *J. Org. Chem.* **2007**, *72*, 4892–4899.

(12) Shengule, S. R.; Karuso, P. *Org. Lett.* **2006**, *8*, 4083–4084.

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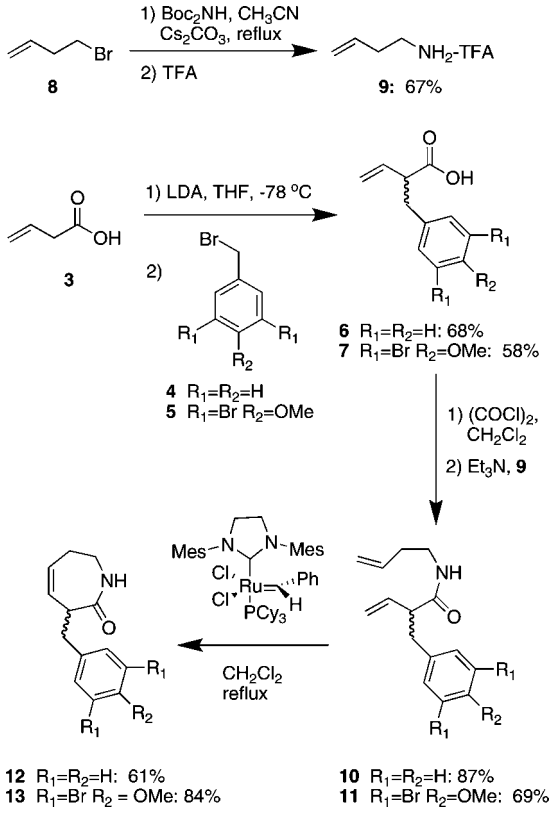
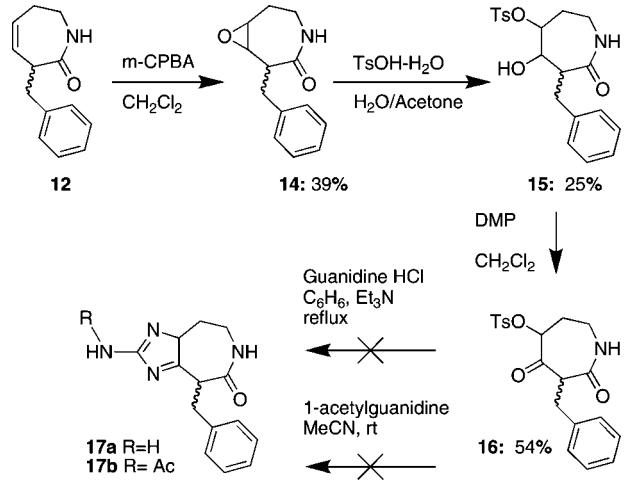
(14) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199–2238.

(15) (a) Chochrek, P.; Wicha, J. *Org. Lett.* **2006**, *8*, 2551–2553. (b) Martin, J. D.; Perez, C.; Ravelo, J. L. *J. Am. Chem. Soc.* **1985**, *107*, 516–518.

SCHEME 2



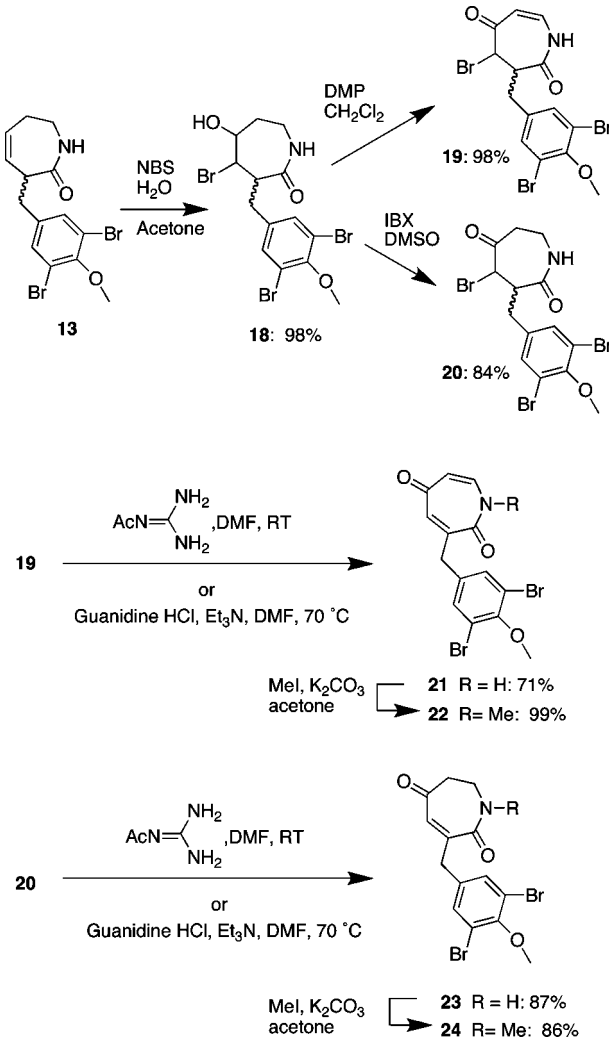
SCHEME 3



in acetone to yield **18** in excellent yield. Oxidation of **18** with 3 equiv of Dess−Martin periodinane (DMP) yielded the bromoenamide **19** in quantitative yield. The formation of **19** was advantageous, as it already possessed the requisite $\Delta^{6,7}$ olefin present in the ceratamines. This result was consistent with a report that the use of a closely related hypervalent iodine oxidant, iodoxybenzoic acid (IBX), is an efficient method for the one-pot conversion of alcohols, ketones, and aldehydes to α,β-unsaturated carbonyl compounds.[16] Interestingly, oxidation of **18** with IBX yields exclusively the C6−C7 saturated compound **20**. Multiple attempted reactions of these compounds with guanidine derivatives yielded only elimination products

(16) Nicolaou, K. C.; Zhong, Y.-L.; Baran, P. S. *J. Am. Chem. Soc.* **2000**, *122*, 7596–7597.

SCHEME 4



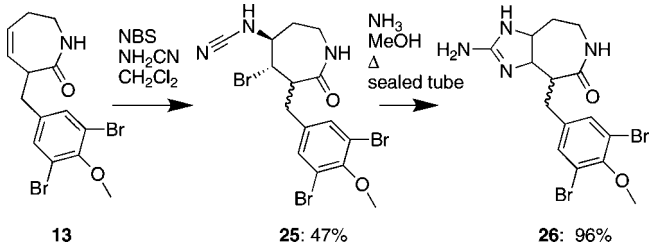
21 and **23**. Recognizing that these compounds would provide valuable insight into the pharmacophore of the ceratamines, caprolactams **21** and **23** were methylated at the amide nitrogen with MeI and K_2CO_3 to yield heterocycles **22** and **24**, which were evaluated for biological activity.

The failure of guanidine derivatives to condense with the cyclic α-halo ketones **19** and **20** prompted us to explore alternate methods to fuse the imidazole ring onto lactam **13**. Substituted guanidine derivatives have been prepared by the addition of amines to cyanamides.[17] We envisioned that treatment of an appropriate vicinal bromoalkylcyanamide with ammonia would give rise to a dihydro version of the 2-aminoimidazole ring of the ceratamines and dehydrogenation of this compound would give rise to the required aromatic imidazo[4,5-*d*]azepine. Cyanamide can be added to alkenes through a bromonium ion, and using this methodology the bromoalkyl cyanamide **25** was prepared in moderate yield as outlined in Scheme 5.[18] Treatment of cyanamide **25** with ammonia in MeOH in a sealed tube yielded the reduced ceratamine B analogue **26** in excellent yield. Attempting this reaction with methylamine or benzylamine failed to yield any of the desired substituted products. Completion of

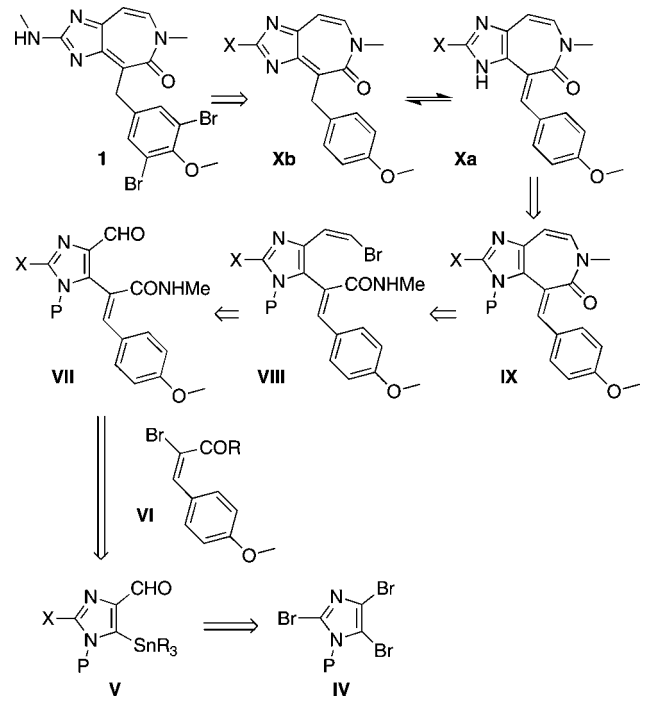
(17) Simig, G.; Lempet, K.; Tamás, J.; Czira, G. *Tetrahedron* **1975**, *31*, 1195–1200.

(18) Zhang, C.; Yang, R.; Chen, L.; Zhong, B.; Yun, L.; Wang, H. *J. Labelled Compd. Radiopharm.* **2004**, *41*, 583–590.

SCHEME 5



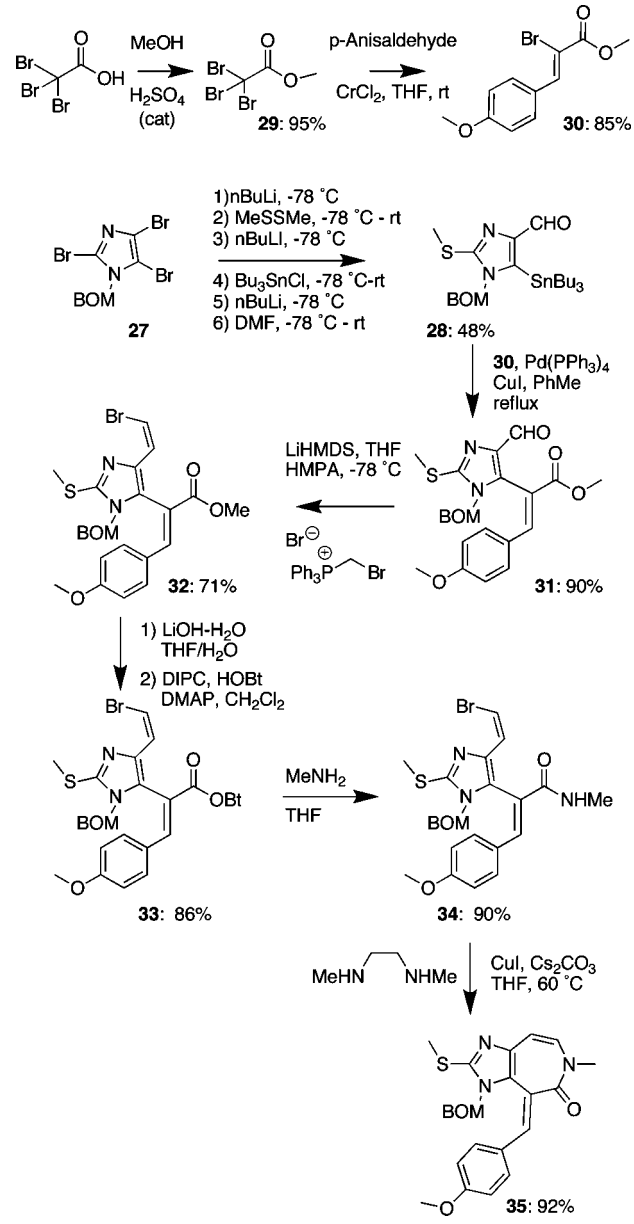
SCHEME 6



the synthesis of ceratamine B via this route was curtailed by the almost total intractability and chemical instability of **26**. Attempts to further purify the compound via RP-HPLC or oxidize it to an aromatic ceratamine resulted only in decomposition.

Our lack of success in grafting the 2-aminoimidazole ring onto a preexisting caprolactam prompted us to change strategy and explore avenues aimed at building a substituted caprolactam onto a preexisting imidazole as outlined in Scheme 6. This second approach made use of the fact that tribromoimidazoles can be selectively and sequentially metalated leading to trisubstituted imidazole intermediates of general structure **V**.[19] Once again, the plan was to convert X (-SMe or Cl) to an amino substituent near the end of the synthesis (**X** to **1**) to avoid the difficulties associated with handling 2-aminoimidazoles. The cornerstone of this approach to the ceratamines was to be the use of an intramolecular Buchwald vinyl amidation reaction to create the lactam ring (**VIII** to **IX**).[20] Implicit in our synthetic plan was the expectation that the deprotected intermediate **Xa** would spontaneously rearrange to the presumably more stable aromatic isomer **Xb**. The initial planning was also based on

SCHEME 7



the assumption that the bromine atoms in ceratamines A (**1**) and B (**2**) did not play a significant role in their antimitotic activity, making the desbromo analogues just as attractive targets as the natural products. This assumption stemmed from the observation that many sponge metabolites contain the dibromomethoxyphenyl moiety present in the ceratamines but do not show antimitotic activity. Therefore, at the outset it seemed likely that the novel imidazo[4,5-*d*]azepine core was the dominant feature of the ceratamine antimitotic pharmacophore.

The synthesis began with BOM-protected imidazole **27** prepared by treatment of tribromoimidazole with BOMCl and K_2CO_3 in DMF (Scheme 7). Metalation of **27** with 1 equiv of *n*BuLi, followed by quenching with MeSSMe first introduced the 2-thiomethyl moiety.[20] In the same pot, without workup, further metalations and quenchings following literature precedent[20b] installed the tributylstannane and aldehyde groups at C-5 and C-4, respectively, to form **28**. Cinnamic ester **30** was synthesized via Cr(II)-mediated condensation of anisaldehyde with tribromoester

(19) (a) Lipshutz, B. H.; Hagen, W. *Tetrahedron Lett.* **1992**, *33*, 5865–5868. (b) Groziak, M. P.; Wei, L. *J. Org. Chem.* **1991**, *56*, 4296–4300l. (c) Groziak, M. P.; Wei, L. *J. Org. Chem.* **1992**, *57*, 3776–3780. (d) Meketa, M.; Weinreb, S. *Tetrahedron* **2007**, *63*, 9112–9119.

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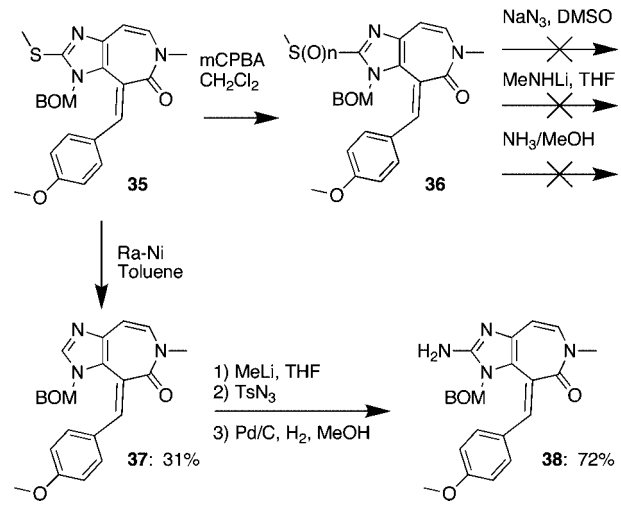
29.[21] Stille coupling between **28** and **30** in toluene yielded **31** in high yield.[22] HMBC correlations observed between the C-5 carbon resonance at δ 137.2 and both the alkene resonance at δ 8.10 and the BOM methylene resonances at δ 5.00/5.08 in **31** confirmed that the cinnamoyl residue was attached to C-5 of the imidazole as drawn in Scheme 7. *Z*-Vinyl bromide **32** was synthesized by reaction of the aldehyde **31** with (bromomethyl)triphenylphosphoniumbromide and lithium hexamethyldisilazide in the presence of HMPA.[23] Saponification of the methyl ester **32**, activation of the acid as the HOBt ester **33**, and displacement of the HOBt group with excess methylamine yielded the secondary amide **34**. The key step in the synthesis was the copper(I)-catalyzed ring closure of **34** to form the enamide **35** following a procedure developed by Buchwald.[24] A dilute THF solution of **34** was treated with CuI, Cs_2CO_3, and *N*,*N*′-dimethylethylenediamine at elevated temperatures to yield the 5,7-bicyclic system **35** in excellent yield.

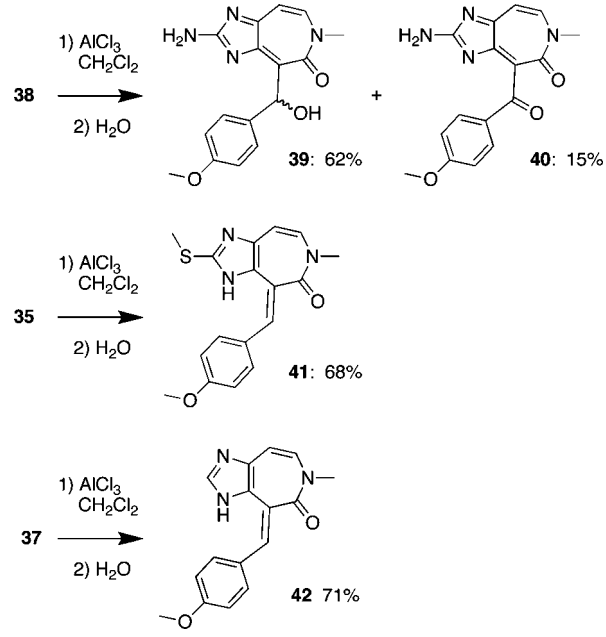
Enamide **35** was already at the oxidation state of the ceratamines so the only remaining transformations required to reach a desbromoceratamine were installation of the 2-amino functionality, removal of the BOM protecting group, and tautomerization to form the fully aromatic ceratamine heterocyclic system. Unfortunately, the 2-methylthio group in **35** proved to be resistant to substitution by a variety of nitrogen nucleophiles. Although oxidation of the methylthio group to the sulfoxide/sulfone **36** could be carried out, albeit in very poor yield due to competing oxidations, nucleophilic substitution of the oxidized 2-methylthio group failed as well (Scheme 8). In retrospect, this result was not surprising considering the documented need for electron-withdrawing substituents at C-4 or C-5 to promote nucleophilic aromatic substitution in the 2-position of an imidazole.[25]

The failure of nucleophilic substitution as an approach to install the 2-amino group shifted our attention to reactions between an electrophilic azide and a C-2 carbanion.[26] Compound **35** was reductively desulfurized with Ra-Ni to generate **37**, the precursor to the required imidazole C-2 carbanion (Scheme 8). This reaction was unsatisfactory for a number of reasons. The use of flammable Ra-Ni, large solvent volumes, adsorption of the compound to the metal surface, and over-reduction leading to low yields were all problems that characterized the transformation. Extensive experimentation with other desulfurizing agents failed to improve the yield or the ease of conducting the reduction. Nevertheless, a workable amount of **37** was generated, and following deprotonation with MeLi, quenching with multiple equivalents of TsN_3, and catalytic reduction of the azido group with H_2, the desired primary amine **38** was produced relatively cleanly. Attempted deprotection of the BOM group by hydrogenation or HCl-catalyzed hydrolysis either failed or resulted

SCHEME 8



SCHEME 9



in decomposition. However, treatment of **38** with $AlCl_3$ followed by H_2O workup gave the C-11 oxygenated ceratamine analogues **39** and **40** (Scheme 9).[27]

The addition of an oxygen atom at the C-11 position of the ceratamine skeleton in **39** and **40** during $AlCl_3$-mediated deprotection of **38** was totally unexpected, especially since $AlCl_3$-mediated removal of the BOM group in the 2-methylthio analog **35** and the 2-protio compound **37** did not result in isomerization to the fully aromatic system or oxygenation at the C-11 benzylic position (Scheme 9). It was clear that further investigation of these unexpected results was required to illuminate a solution to preventing the unwanted C-11 oxygenation of **38**. However, several low-yielding steps in the route to **38** shown in Scheme 8 made it difficult to generate sufficient material for mechanistic investigations of the $AlCl_3$-mediated deprotection or the eventual production of ceratamine analogues for biological evaluation by this method, so we set out to develop a more efficient synthesis of **38**.

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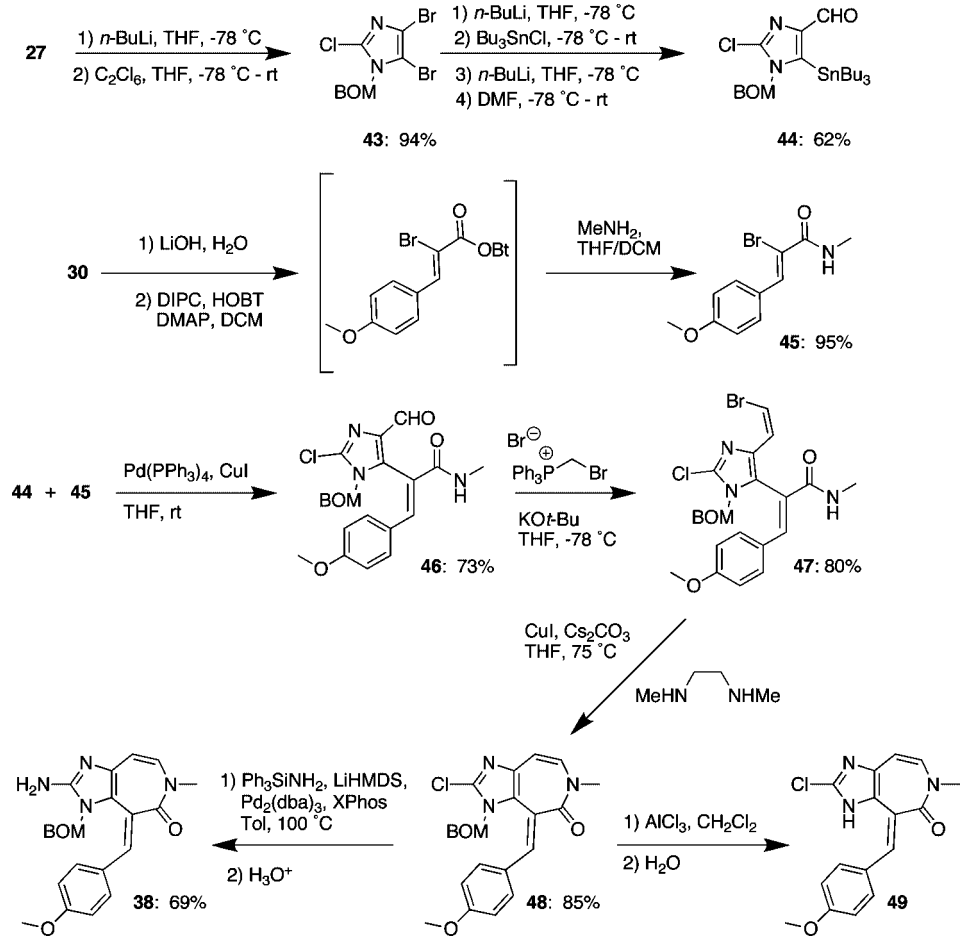
(27) Groves, J. K.; Cundasawmy, N. E.; Anderson, H. J. *Can. J. Chem.* **1973**, *51*, 1089–1098.

SCHEME 10



Our second generation route to the ceratamine skeleton was designed to exploit literature precedents suggesting that Pd(0)-catalyzed amination of the chloroimidazole **48** would be an efficient method to introduce the 2-aminoimidazole moiety.[28] We also anticipated that the synthetic flexibility imparted by the 2-chloro atom would allow for rapid structure diversification at an advanced stage of the synthesis and facilitate the SAR analysis of the ceratamine pharmacophore. Metalation of the BOM-protected tribromoimidazole **27**, followed by quenching with hexachloroethane, yielded crystalline chloride **43** in high yield (Scheme 10).[29] Further metalations and quenchings in the same pot installed the tributylstannyl and aldehyde groups to C-5 and C-4,[20b] respectively, to afford stannane **44**. The cinnamic amide **45** was prepared in quantitative yield via saponification of methyl ester **30**, in situ activation of the resulting acid, and displacement with excess methylamine. Bromide **45** was coupled with stannane **44** in THF at room temperature to yield aldehyde **46**. Conducting the Stille reaction in toluene at elevated temperatures scrambled the alkene geometry in **46**,[30] which did not occur during the Stille reaction between **28** and **30** (Scheme 7). Wittig olefination with (bromomethyl)triphenylphosphoniumbromide and potassium *tert*-butoxide in THF yielded the surprisingly insoluble *Z*-vinyl bromide **47** in good yield after precipitation from CH_2Cl_2 and chromatography of the remaining soluble materials. Ring closure of **47** via a Cu(I)-catalyzed Buchwald enamide reaction yielded the 2-chloroimidazo[4,5-*d*]azepine **48**. The structure of this compound was confirmed by single crystal X-ray diffraction analysis (Supporting Information). A palladium-mediated amination was then carried out using an ammonia surrogate in an attempt to form **38**.[31] Heating chloroimidazole **48**, triphenylsilylamine, and LiHMDS in the presence of $Pd_2(dba)_3$ and the Buchwald ligand 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) in toluene followed by acid hydrolysis of the triphenylsilyl group gave the primary amine **38**.[32]

With an efficient route to **38** in hand, we turned our attention back to the formation of **39** and **40**. The results shown in Scheme 9 suggested that oxygenation at the C-11 position of the ceratamine system occurs only upon $AlCl_3$-mediated deprotection of a system with an amino substituent at the 2-position of the imidazole ring. Less effective electron-donating substituents (MeS-, Cl-, and H-) do not lead to C-11 oxygenated products

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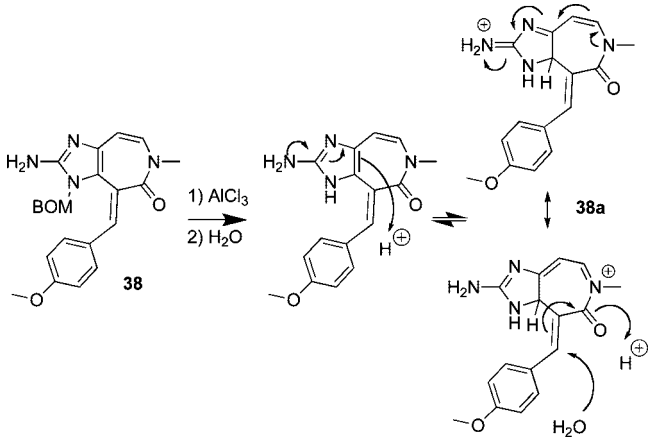
SCHEME 11



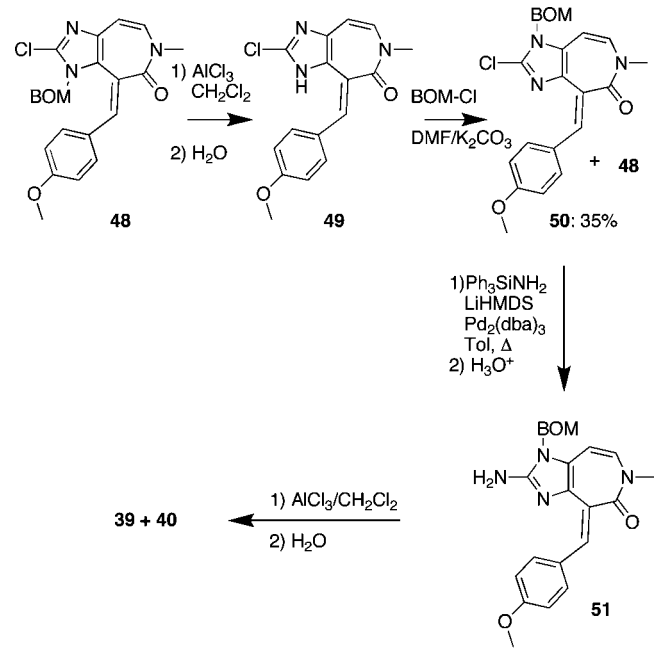
with aromatic imidazo[4,5-*d*]azepine core heterocycles (Scheme 9). The formal Michael addition of water to the C-11 position of **38** upon N-1 deprotection could perhaps be rationalized by the increased reactivity of the C-11 position upon C-4 ipso protonation available only to a 2-aminoimidazole containing compound in acidic media as shown in Scheme 11. However, upon deprotection of **38** with $AlCl_3$ followed by quenching with MeOH, only alcohol **39** and ketone **40** were formed. Furthermore, quenching the deprotection of **38** with ^{18}O-labeled water gave no indication of incorporation of ^{18}O into **39** or **40** as observed by ESI-MS or MALDI-MS. These results demonstrated that a simple Michael addition mechanism was not in operation and that the oxygen atoms present at C-11 in **39** and **40** must have had an alternate origin.

Another possible source of the C-11 oxygen atom in **39** and **40** was from the BOM protecting group via some intramolecular process that was occurring during the $AlCl_3$-mediated deprotection sequence. While there was no obvious mechanistic rationale for such a process, we nevertheless investigated this possibility by scrambling the regiochemistry of the BOM group as outlined in Scheme 12. Compound **48** was subjected to standard deprotection with $AlCl_3$ in CH_2Cl_2 followed by hydrolysis. Without isolation, the resulting imidazole **49** was treated with BOMCl and K_2CO_3 in DMF to yield a 1:1 mixture of compound **48** and its regioisomer **50**. The regioisomers were separated, and **50** was aminated as described above to give **51**. When **51** was deprotected with $AlCl_3$, only compounds **39** and **40** were isolated, eliminating the possibility that C-11 oxygenation resulted from some intramolecular process involving the BOM oxygen atoms. At this point it seemed most likely that the C-11 oxygen atoms in **39** and **40** were coming from atmospheric oxygen.

Since the synthetic goal was to produce ceratamine analogues that were not oxygenated at C-11, multiple attempts were made to deoxygenate **39** at C-11 via standard hydrogenolysis and Barton deoxygenation procedures, but all resulted in decomposition. Similarly, attempts to bypass C-11 oxygenation by carrying out a Buchwald/Hartwig amination on the already deprotected chloride **49** with an ammonia surrogate also failed to yield isolable products.

Next we sought to ameliorate the putative electron-donating effects of the aminoimidazole by acylation in order to forestall the oxygenation of C-11 during removal of the BOM group by $AlCl_3$. We reasoned that once the BOM protecting group had been removed, the acyl substituent could be cleaved or

SCHEME 12



transformed under conditions where atmospheric oxygen was excluded, which might result in isomerization and aromatization to the ceratamine system without C-11 oxygenation.

Reaction of chloroimidazole **49** with *N*-methylformamide in the presence of Cs_2CO_3 and $Pd_2(dba)_3$ using 2-di-*tert*-butylphosphino-3,4,5,6-tetramethyl-2′,4′,6′-triisopropyl-1,1-biphenyl **52** as a ligand and refluxing in toluene for 24 h gave the *N*-methylformamide derivative **53** in modest yield (Scheme 13).[33] Exposure of **53** to anhydrous $AlCl_3$ in CH_2Cl_2 cleanly yielded **54** as evidenced by TLC and HRESIMS. Although the ^1H NMR spectrum of **54** revealed a complicated dynamic behavior, likely due to the presence of rotamers and/or isomers, it was clear that tautomerization/oxidation had not occurred, and the compound presumably exists as drawn. Attempts to remove the formyl group in **54** by basic hydrolysis failed. However, hydrolysis of **54** in 1,4-dioxane and 6 N $HCl_{(aq)}$ gave the C-11 alcohol **56** as the major product after brief heating, and for the first time minor amounts of the desired desbromoceratamine **55** were also formed. After much experimentation, it was found that passage of dry $HCl_{(g)}$ through a solution of **54** in 50% 1,4-dioxane and water gave clean transformation of **54** to desbromoceratamine A (**55**) (Scheme 12) with the formation of only small amounts of **56**. The stream of HCl gas employed in this reaction presumably purged atmospheric oxygen, resulting in a dramatic reduction in the formation of the C-11 hydroxy analogue **56** and formation of desbromoceratamine as the major product, consistent with atmospheric oxygen being the source of C-11 oxygenation.

Analysis of the NMR data recorded for **55** in DMSO-d_6 revealed striking similarities with the NMR data recorded for ceratamine A (**1**) in the same solvent.[6] The spectra reveal two interconverting forms for both **1** and **55** in a ~3:1 ratio. COSY data showed that the C-19 methyl groups in both **1** and **55** are scalar coupled to the NH-18 proton in both forms, suggesting that in each case they are slowly interconverting rotamers about the C-2/N-18 bonds, consistent with the doubly vinylogous

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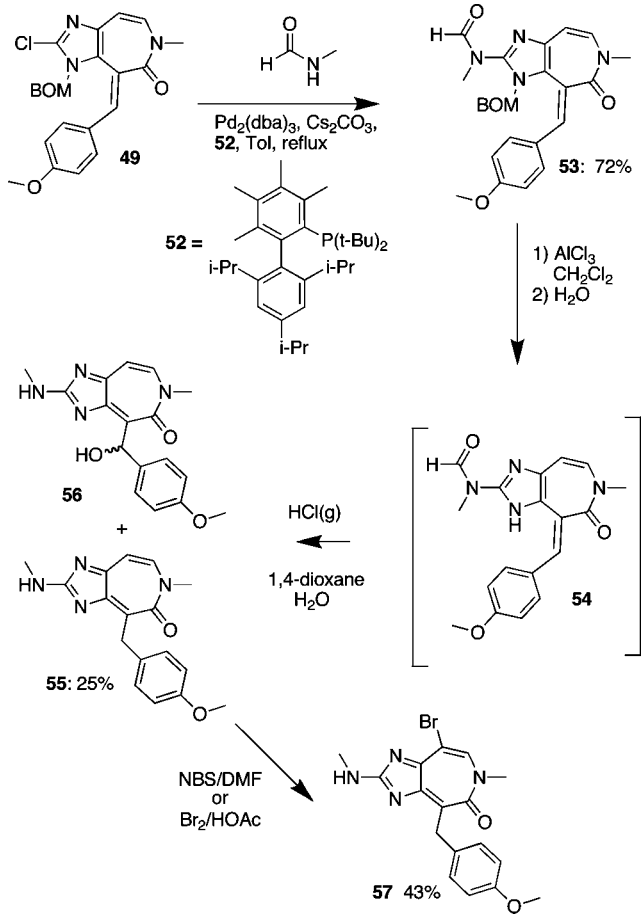
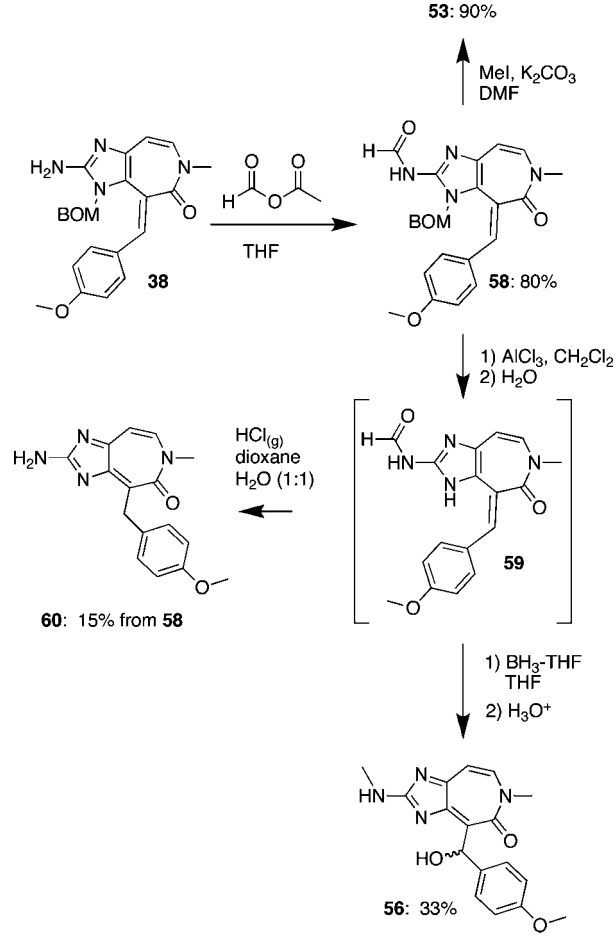
SCHEME 13



SCHEME 14



amide nature of the 2-*N*-methylamino substituents and the C-6 carbonyl in **1** and **55**. Variable temperature ^{1}H NMR experiments conducted on **55** in DMSO-d_6 showed a coalescence of the two forms at 400 °C (Supporting Information).

Desbromoceratamine A (**55**) was treated with 2 equiv of *N*-bromosuccinimide in DMF or 2 equiv of Br_2 in HOAc in an attempt to complete the synthesis of ceratamine A (**1**). However, both of these reaction conditions yielded only the 9-monobrominated product **57**, and use of a larger excess of bromine led to very complex mixtures from which none of the desired tribromoceratamine could be isolated (Scheme 13).

In an attempt to circumvent the long reaction times and relatively low yields involved in the transformation of **49** to **55**, the deactivating formyl group was added onto the more readily formed primary amine **38**. Reaction of **38** with acetic formic anhydride in THF for 24 h afforded **58** in good yield (Scheme 14).[34] Formamide **58** can be readily alkylated with MeI and K_2CO_3 in DMF to form **53**, which represents an improved method to make this critical desbromoceratamine precursor. Removal of the BOM group from **58** with $AlCl_3$ gave formamide **59**. Unfortunately, reduction of the *N*-formyl group in **59** with BH_3/THF followed by acidic aqueous workup again yielded alcohol **56** exclusively. However, desmethyldesbromoceratamine A (**60**) was cleanly formed when a stream of dry $HCl_{(g)}$ was bubbled through a solution of formamide **59** in 50% 1,4-dioxane and water.

Scheme 15 presents a mechanistic rationalization for the formation of C-11 oxygenated species during the synthesis of desbromoceratamines. When intermediates of general structure **XI** bearing a 2-amino substituent are exposed to strong acid, they can undergo ipso protonation at C-4 of the imidazole ring to give **XII**.[37] Analogues of **XI** with methylthio, proton, chloro, or *N*-formyl substituents at C-2 are less basic and not expected to as readily undergo this type of C-4 protonation. Loss of a proton from the 3-NH in **XII** gives the neutral species **XIII**. Reaction of **XIII** with atmospheric oxygen generates the radical **XIVa**, which is greatly stabilized by the aromatic imidazo[4,5-*d*]azepine core resonance structure **XIVb**. Reaction of **XIVb** with the hydroperoxide radical formed during the hydrogen abstraction step (**XIII** to **XIVa**), perhaps in a cage reaction, can lead to the hydroperoxide **XV** that can decompose to give the C-11 hydroxy (**39** or **56**) or keto (**40** or **61**) derivatives formed as the major products under most conditions. Alternatively, radical **XIVb** can abstract a hydrogen atom from some source to give the desired nonoxygenated analogues **55** and **60**. The formation of **55** and **60** may in fact result from a radical chain reaction where the proton source is the intermediate **XIII**. This mechanistic proposal provides an explanation for the variable yields of **39** and **55** that are formed when a dioxane/H_2O solution of **54** is treated with dry HCl gas. The relative amounts of oxygen and compound **54** present in the reaction mixture should determine

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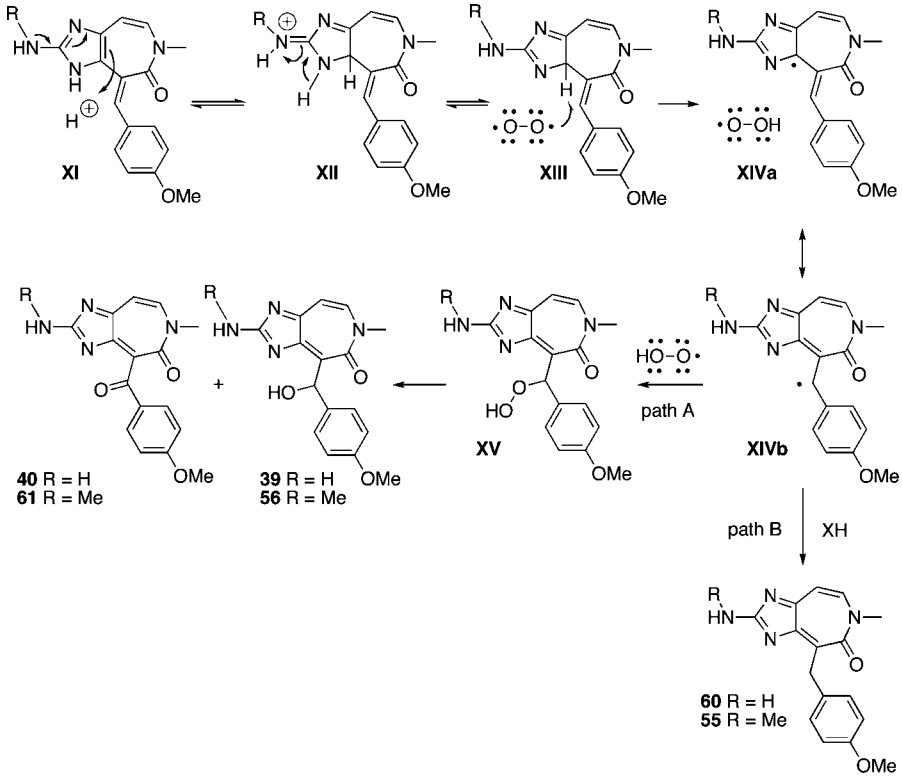
SCHEME 15



whether pathway A to C-11 oxygenated products or pathway B to nonoxygenated products will dominate.

Biological Activities of Synthetic Ceratamine Analogues. Synthetic analogues **22**, **24**, **26**, **39**, **40**, **41**, **42**, **55**, **56**, and **60** as well as natural ceratamine A (**1**) were evaluated for their ability to arrest cells in mitosis in the TG3 cell-based assay.[4] Compound **22**, which lacks the aminoimidazole ring in ceratamine A (**1**), showed evidence of weak mitotic arrest with a very low percentage of cells arrested in mitosis at concentrations above 40 μg/mL, and the dihydro analogue **24** and the partially reduced ceratamine **26** were not active (data not shown). These results indicated that the aminoimidazole ring is a required element in the ceratamine antimitotic pharmacophore and suggested that a fully planar (i.e., aromatic) imidazoazepine heterocycle is also essential. Both conclusions were supported by the observation that the analogues **41** and **42** lacking both the 2-*N*-methylamino substituent and an aromatic imidazoazepine heterocycle were completely inactive.

Figure 1 shows the TG3 assay data for ceratamine A (**1**), **39**, **40**, **56**, **60**, and desbromoceratamine A (**55**). The C-11 hydroxy analogue **39** shows antimitotic activity at ~10 μg/mL as previously reported,[9] but it does not generate a large percentage of cells arrested in mitosis compared with ceratamine A (**1**) and its activity falls off at higher concentrations. Desbromoceratamine A (**55**) shows the most promising activity of the synthetic analogues tested. At the highest concentration evaluated (50 μg/mL), **55** shows significant mitotic arrest (~20%), while **60**, which differs from desbromoceratamine A (**55**) simply by loss of the methyl substituent on the 2-amino group, showed no activity at the concentrations tested. The data in Figure 1 demonstrates that although the C-14 and C-16 bromine substituents in ceratamine A (**1**) are not essential for antimitotic activity, they make significant contributions to maximum potency (**1** versus **55**). Similarly, it is apparent that methylation of the 2-amino functionality is required for effective antimitotic activity (**55** versus **60**).

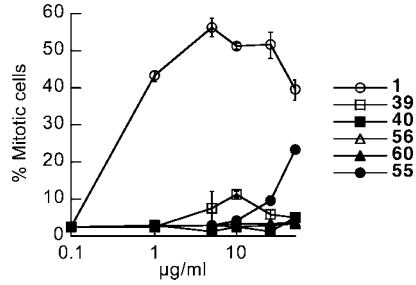
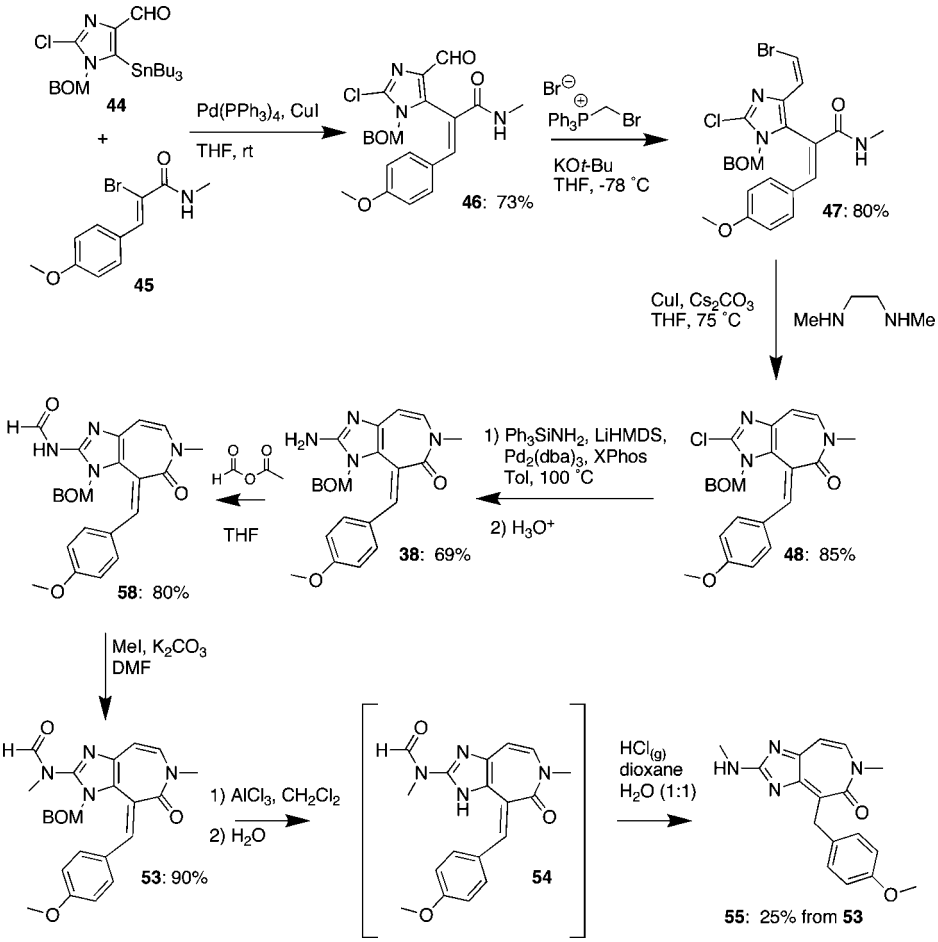


FIGURE 1. Antimitotic activity of natural ceratamine A (**1**) and the synthetic analogues **39**, **40**, **56**, **60**, and desbromoceratamine A (**55**) evaluated in a TG3 cell-based assay.[4]

Conclusion

The synthetic route shown in Scheme 16 has been developed for the preparation of the antimitotic compound desbromoceratamine A (**55**). This work features the first synthesis of an aromatic imidazo[4,5-*d*]azepine heterocycle, and it revealed an unexpected reaction between atmospheric oxygen and an imidazo[4,5-*d*]azepine precursor to the core of the ceratamines. The reaction with oxygen only occurred in precursors having an amino substituent at C-2 of the imidazole ring. A mechanistic rationalization (Scheme 15) suggests that the amino substituent is required to make the imidazole ring basic enough to undergo C-4 ipso protonation, which triggers the reaction with atmospheric oxygen and aromatization. The cornerstone of this first synthetic route to ceratamine analogues is the use of an intramolecular Buchwald vinyl amidation reaction to form the azepine ring, and the end game of the synthesis makes the first use of Buchwald/Hartwig amination to prepare 2-aminoimidazoles from a 2-chloroimidazole precursor. The discovery that the C-14 and C-16 bromine substituents in ceratamine A (**1**)

SCHEME 16



contribute in a major way to the potency of the antimitotic activity is noteworthy and was completely unanticipated. Efforts are currently ongoing in our laboratory to use this important SAR finding to guide the design and synthesis of new analogues that capture the potency of the natural product. Evaluation of desbromoceratamine A (**55**) in mouse xenograft models of human cancer are underway, and the results will be reported elsewhere.

Experimental Section

Additional details are available in Supporting Information.

1-Benzyloxymethyl-4,5-dibromo-2-chloro-1*H*-imidazole (43). Tribromide **27** (6.97 g, 16.4 mmol) was dissolved in 60 mL of dry THF. The solution was cooled to −78 °C, and *n*BuLi (10.3 mL, 1.6 M in hexanes, 16.4 mmol) was added slowly. The solution was stirred cold for 20 min, and then a solution of C_2Cl_6 (4.27 g, 18.04 mmol) in 10 mL of dry THF was added over a period of 6 min. The reaction was stirred at −78 °C for 10 min and then warmed to room temperature for an additional 20 min. The reaction was quenched with addition of H_2O, and the product was extracted into EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated. An initial 4.64 g (12.1 mmol, 74%) of the chloride could be recovered by crystallization from cold hexanes, and flash chromatography (hexanes/EtOAc) of the remaining residue yielded an additional 1.24 g (3.3 mmol, 20%) of product (94% yield overall).

1-Benzyloxymethyl-2-chloro-5-tributylstannanyl-1*H*-imidazole-4-carbaldehyde (44). Chloride **43** (3.60 g, 9.46 mmol) was dissolved in 36 mL of dry THF and cooled to −78 °C. *n*BuLi (5.91 mL, 1.6 M in hexanes, 9.46 mmol) was slowly added, and the solution was stirred cold for 20 min. Bu_3SnCl (2.55 mL, 9.46 mmol) was then added, and the solution was stirred at −78 °C for 10 min and then placed in a cold-water bath. This solution was stirred for 10 min and then recooled to −78 °C. *n*BuLi (5.91 mL, 1.6 M in hexanes, 9.46 mmol) was slowly added, and the solution was stirred for 20 min. DMF (2.0 mL) was then slowly added, the solution stirred at −78 °C for 10 min, and then the reaction was allowed to slowly warm to room temperature. The reaction was stirred for 10 min at room temperature and then quenched by addition of water. The reaction mixture was extracted into EtOAc, and the organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexanes/EtOAc) to yield stannane (**44**) (3.16 g, 5.85 mmol, 62%) as a colorless oil. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 0.89 (t, J = 7.3 Hz, 9H), 1.24 (m, 5H), 1.33 (m, 7H), 1.53 (m, 6H), 4.54 (s, 2H), 5.43 (s, 2H), 7.35 (m, 5H), 9.81 (s, 1H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 12.2, 14.4, 28.2, 29.9, 71.5, 76.6, 128.5, 129.0, 129.4, 137.5, 137.8, 144.8, 150.4, 187.5; ESIMS $[M + Na]^+$ calcd for $C_{24}H_{37}N_2O_2{}^{35}Cl^{116}SnNa$ 559.1459, found 559.1447.

2-Bromo-3-(4-methoxy-phenyl)-*N*-methyl-acrylamide (45). Ester (**30**) (10.95 g, 40.4 mmol) was dissolved in 30 mL of THF, 5 mL of H_2O, and 10 mL of MeOH. LiOH-H_2O (3.40 g, 80.8 mmol) was added, and the reaction was stirred vigorously at room temperature for 2 h. The reaction was then acidified with 1 M HCl and extracted into EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The resulting solid was redissolved in 70 mL of CH_2Cl_2. HOBt (8.2 g, 60.6 mmol) and DMAP (∼10 mg) were added, and the solution was cooled to 0 °C. DIPC (9.38 mL, 60.6 mmol) was slowly added, and the solution was stirred for 10 min at 0 °C. The reaction was warmed to room temperature and stirred for an additional 1 h. The reaction was then concentrated to dryness. The residue was then redissolved

in THF and treated with $MeNH_2$ (50 mL, 2.0 M in THF, 100 mmol) for 10 min. The reaction was then extracted into EtOAc and washed with 2 × 1 M HCl and 2 × saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexanes/EtOAc) to yield **45** (10.3 g, 38.4 mmol, 95%) as a colorless oil that crystallized upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96 (d, J = 4.8 Hz, 3H), 3.85 (s, 3H), 6.83 (s, br, 1H), 6.94 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H), 8.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 56.3, 113.3, 114.8, 127.5, 132.9, 137.9, 161.7, 164.2; ESIMS $[M + Na]^+$ calcd for $C_{11}H_{12}NO_2{}^{79}BrNa$ 291.9949, found 291.9940.

2-(3-Benzyloxymethyl-2-chloro-5-formyl-3*H*-imidazol-4-yl)-3-(4-methoxy-phenyl)-*N*-methyl-acrylamide (46). Stannane **44** (2.86 g, 5.30 mmol), amide **45** (1.58 g, 5.83 mmol), $Pd(PPh_3)_4$ (673 mg, 0.58 mmol), and CuI (544 mg, 2.86 mmol) were combined in 50 mL of dry THF and stirred at room temperature for 20 h. The reaction was then concentrated to dryness, redissolved in CH_2Cl_2, and filtered through Celite. The resulting solution was then concentrated, and the crude product was purified by column chromatography (hexanes/EtOAc) to yield **46** (1.70 g, 3.86 mmol, 73%) as a slightly yellow solid. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 2.82 (d, J = 4.8 Hz, 3H), 3.77 (s, 3H), 4.51 (s, 2H), 5.08 (d, J = 10.7 Hz, 1H), 5.22 (d, J = 10.7 Hz, 1H), 6.24 (s, br, 1H), 6.78 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 7.18 (m, 2H), 7.81 (m, 2H), 8.07 (s, 1H), 9.67 (s, 1H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 27.8, 56.3, 72.4, 74.5, 115.4, 118.3, 126.8, 128.6, 129.1, 129.4, 132.7, 137.2, 137.6, 137.9, 138.9, 144.8, 162.3, 166.1, 184.5; ESIMS $[M + Na]^+$ calcd for $C_{23}H_{22}N_3O_4{}^{35}ClNa$ 462.1197, found 462.1182.

2-[3-Benzyloxymethyl-5-(2-bromo-vinyl)-2-chloro-3*H*-imidazol-4-yl]-3-(4-methoxy-phenyl)-*N*-methyl-acrylamide (47). (Bromomethyl)triphenylphosphonium bromide (1.85 g, 4.24 mmol) was slurried in 50 mL of dry THF. Potassium *tert*-butoxide (476 mg, 4.24 mmol) was added, and the resulting yellow solution was stirred at room temperature for 10 min. The ylide solution was then cooled to −78 °C, and a 10 mL THF solution of aldehyde **46** (933 mg, 2.12 mmol) was added slowly. The reaction was stirred at −78 °C for 30 min and then allowed to warm to room temperature for an additional 20 min. The reaction was quenched with addition of water, and the product was extracted with EtOAc and then with 2 × CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The resulting crude residue was dissolved in 20 mL of CH_2Cl_2 and allowed to sit for 10 min. The resulting precipitate was filtered off and washed with 5 mL of CH_2Cl_2 to yield **47** (0.5 g, 0.97 mmol, 46%). An additional 370 mg (0.72 mmol. 34%) could be isolated from the remaining residue by column chromatography (hexanes/EtOAc) (80% yield overall). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 2.74 (d, J = 5.0 Hz, 3H), 3.78 (s, 3H), 4.46 (s, 2H), 5.00 (d, J = 10.8 Hz, 1H), 5.16 (d, J = 10.8 Hz, 1H), 5.72 (s, br, 1H), 6.31 (d, J = 8.3 Hz, 1H), 6.73 (d, J = 8.3 Hz, 1H), 6.80 (d, J = 8.9 Hz, 2H), 7.04 (d, J = 8.9 Hz, 2H), 7.16 (m, 2H), 7.80 (m, 3H), 8.07 (s, 1H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 27.6, 56.3, 72.0, 74.2, 107.1, 115.3, 118.5, 122.8, 127.1, 128.4, 128.9, 129.4, 129.7, 132.8, 135.5, 137.0, 137.4, 144.6, 162.2, 166.4; ESIMS $[M + Na]^+$ calcd for $C_{24}H_{23}N_3O_3{}^{35}Cl{}^{79}BrNa$ 538.0509, found 538.0499.

3-Benzyloxymethyl-2-chloro-4-(4-methoxy-benzylidene)-6-methyl-4,6-dihydro-3*H*-imidazo[4,5-*d*]azepin-5-one (48). Bromide **47** (682 mg, 1.31 mmol), CuI (50 mg, 0.262 mmol), and Cs_2CO_3 (853 mg, 2.62 mmol) were combined in 40 mL of dry THF. *N*,*N*′-Dimethylethylenediamine (57 μL, 0.524 mmol) was then added, and the solution was heated to 70 °C for 20 h. The reaction was cooled to room temperature and concentrated to dryness. The residue was redissolved in CH_2Cl_2 and filtered through Celite. The resulting solution was concentrated to dryness again and purified by column chromatography (hexanes/EtOAc) to yield **48** (486 mg, 1.11 mmol, 85%) as a pale yellow solid. A single crystal suitable for X-ray diffraction analysis was grown by infusion of MeOH into a solution of **48** in toluene. ^{1}H NMR (300 MHz, CD_2Cl_2) δ 3.27 (s, 3H), 3.77 (s, 3H), 4.35 (s, 2H), 4.57 (d, J = 11.5 Hz, 1H), 4.90 (d, J = 11.5 Hz, 1H), 6.13 (d, J = 9.0 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 6.77 (d, J = 8.6 Hz, 2H), 6.94 (d, J = 8.6 Hz, 2H), 7.28 (m, 6H); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 38.2, 55.4, 70.9, 73.2, 107.9, 114.4, 121.5, 123.5, 126.9, 127.9, 128.0, 128.4, 129.1, 130.5, 134.9, 136.1, 136.2, 136.9, 160.4, 169.3; ESIMS $[M + Na]^+$ calcd for $C_{24}H_{22}N_3O_3{}^{35}ClNa$ 458.1247, found 458.1242.

2-Amino-3-benzyloxymethyl-4-(4-methoxy-benzylidene)-6-methyl-4,6-dihydro-3*H*-imidazo[4,5-*d*]azepin-5-one (38) from Chloride 48. Chloride **48** (60 mg, 0.14 mmol), triphenylsilylamine (46.3 mg, 0.17 mmol), $Pd_2(dba)_3$ (13 mg, 0.014 mmol), and XPhos (CAS 564483-17-7; 16.2 mg, 0.034 mmol) were combined under Ar in 4.0 mL of dry toluene. LiHMDS (182 μL, 1.0 M in toluene, 0.182 mmol) was added, and the solution was heated to 100 °C for 1 h. The reaction was cooled to room temperature and diluted with EtOAc. Then, 1.0 M HCl was added, and the biphasic mixture was stirred rapidly for 10 min. The aqueous layer was basified with $NaHCO_3$ and extracted into EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (EtOAc/MeOH) to yield **38** (40 mg, 0.098 mmol, 69%) as a yellow solid. ^{1}H NMR (600 MHz, CD_2Cl_2) δ 3.26 (s, 3H), 3.76 (s, 3H), 4.19 (d, J = 11.5 Hz, 1H), 4.23 (d, J = 11.5 Hz, 1H), 4.45 (s, br, 2H), 4.52 (s, 2H), 6.03 (d, J = 8.9 Hz, 1H), 6.07 (d, J = 8.9 Hz, 1H), 6.77 (d, J = 8.9 Hz, 2H), 6.97 (d, J = 8.9 Hz, 2H), 7.18 (m, 3H), 7.28 (m, 3H); ^{13}C NMR (150 MHz, CD_2Cl_2) δ 38.7, 55.8, 70.8, 72.6, 109.1, 114.6, 119.2, 122.5, 127.8, 128.1, 128.5, 128.7, 128.9, 130.9, 131.9, 134.0, 137.2, 152.3, 160.3, 170.0; ESIMS $[M + Na]^+$ calcd for $C_{24}H_{24}N_4O_3Na$ 439.1746, found 439.1735.

***N*-[1-Benzyloxymethyl-8-(4-methoxy-benzylidene)-6-methyl-7-oxo-1,6,7,8-tetrahydro-imidazo[4,5-*d*]azepin-2-yl]-*N*-methyl-formamide (53).** Chloride **48** (208 mg, 0.48 mmol), Cs_2CO_3 (234 mg, 0.72 mmol), $Pd_2(dba)_3$ (22 mg, 0.024 mmol), and **52** (58 mg, 0.12 mmol) were combined in 4.5 mL of dry toluene. *N*-Methylformamide (43 mL, 0.72 mmol) was added, and the slurry was refluxed for 24 h. The reaction was then cooled to room temperature and concentrated to dryness. The residue was redissolved in CH_2Cl_2, filtered through Celite, and concentrated to give an orange oil. The crude product was purified by column chromatography (hexanes/EtOAc) to yield **53** (159 mg, 0.34 mmol, 72%) as a slightly yellow oil. ^{1}H NMR (600 MHz, CD_2Cl_2) δ 3.17 (s, 3H), 3.29 (s, 3H), 3.76 (s, 3H), 4.28 (s, 2H), 4.53 (d, J = 11.3 Hz, 1H), 4.59 (d, J = 11.3 Hz, 1H), 6.16 (d, J = 9.1 Hz, 1H), 6.21 (d, J = 9.1 Hz), 6.75 (d, J = 8.3 Hz, 2H), 6.88 (d, J = 8.3 Hz, 2H), 7.18 (m, 2H), 7.30 (m, 4H), 8.31 (s, 1H); ^{13}C NMR (150 MHz, CD_2Cl_2) δ 31.9, 37.6, 54.8, 70.4, 72.1, 107.5, 113.6, 121.1, 121.8, 126.3, 127.4, 127.5, 127.9, 128.7, 129.9, 133.9, 136.0, 145.6, 159.9, 161.7, 168.9; ESIMS $[M + K]^+$ calcd for $C_{26}H_{26}N_4O_4{}^{39}K$ 497.1591, found 497.1601.

4-(4-Methoxy-benzyl)-6-methyl-2-methylamino-6*H*-imidazo[4,5-*d*]azepin-5-one or Desbromoceratamine A (55). Formamide **53** (90 mg, 0.19 mmol) was dissolved in 10 mL of CH_2Cl_2. Anhydrous $AlCl_3$ (25 mg, 1.9 mmol) was added, and the slurry was stirred rapidly for 30 min. The solution was quenched with careful addition of satd $NaHCO_3$, and the crude reaction was extracted into EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated to yield **54**. ESIMS $[M + Na]^+$ calcd for $C_{18}H_{18}N_4O_3Na$ 361.1277, found 361.1275. The resulting product was used without further purification. The crude **54** was then dissolved in 25 mL of 1,4-dioxane and diluted with 25 mL of deionized water. Ar was then bubbled through the solution for 30 min. The solution was cooled to 0 °C, and anhydrous $HCl_{(g)}$ was bubbled through the solution for 1 min. The solution was then allowed to sit for an additional 2 min. The pH of the solution was then brought to neutral with addition of 5 M NaOH and then further basified with $NaHCO_3$. The reaction was extracted into EtOAc, and the bright yellow organic phase was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH) to

yield **55** (15 mg, 0.048 mmol, 25%, 2 steps) as a bright yellow solid. Compound **56** was also isolated from the product mixture (see below). The NMR showed a 3:1 mixture of isomers. Isomer 1 (major): ^{1}H NMR (600 MHz, DMSO) δ 3.07 (s, 3H), 3.53 (s, 3H), 3.66 (s, 3H), 4.26 (s, 2H), 6.39 (d, J = 10.0 Hz, 1H), 6.76 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 10.0 Hz, 1H), 8.51 (m, 1H); ^{13}C NMR (150 MHz, DMSO) δ 29.2, 35.2, 43.7, 54.9, 100.3, 113.3, 123.8, 129.9, 132.7, 142.4, 157.4, 160.0, 164.1, 169.7, 175.4. Isomer 2 (minor): ^{1}H NMR (600 MHz, DMSO) δ 3.06 (s, 3H), 3.56 (s, 3H), 3.66 (s, 3H), 4.21 (s, 2H), 6.52 (d, J = 9.7 Hz, 1H), 6.75 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 8.6 Hz, 2H), 7.83 (d, J = 9.7 Hz, 1H), 8.59 (m, 1H); ^{13}C NMR (150 MHz, DMSO) δ 29.3, 35.4, 43.9, 54.9, 100.5, 113.3, 123.1, 129.8, 132.4, 143.2, 157.3, 160.6, 163.9, 170.0, 176.1; ESIMS [M + Na]$^+$ calcd for $C_{17}H_{18}N_4O_2Na$; 333.1327, found 333.1319.

8-Bromo-4-(4-methoxy-benzyl)-6-methyl-2-methylamino-6*H*-imidazo[4,5-*d*]azepin-5-one (57). To a solution of **55** (1.8 mg, 0.006 mmol) in 0.5 mL of HOAc was added a solution of Br_2 (0.0116 mmol, 1.0 M in HOAc, 11.6 μL). The solution was stirred at room temperature for 1 h, and then the solvent was removed under vacuum. The crude compound was purified by column chromatography (CH_2Cl_2/MeOH) to yield **57** (1.0 mg, 0.0026 mmol, 43%) as a bright yellow solid. The NMR showed a 3:4 mixture of rotamers. Isomer 1: ^{1}H NMR (600 MHz, CD_2Cl_2) δ 3.25 (d, J = 5.3 Hz, 3H), 3.62 (s, 3H), 3.73 (s, 3H), 4.23 (s, 2H), 6.74 (d, J = 8.6 Hz, 2H), 7.36 (d, J = 8.6 Hz, 2H), 7.92 (s, 1H); ^{13}C NMR (150 MHz, CD_2Cl_2) δ 30.1, 36.4, 45.1, 55.2, 97.4, 113.7, 126.4, 129.1, 130.8, 143.9, 158.5, 163.2, 164.0, 167.8, 174.9. Isomer 2: ^{1}H NMR (600 MHz, CD_2Cl_2) δ 3.38 (s, 3H), 3.73 (s, 3H), 3.74 (s, 3H), 4.38 (s, 2H), 6.79 (d, J = 8.9 Hz, 2H), 7.41 (d, J = 8.9 Hz, 2H), 8.22 (s, 1H); ^{13}C NMR (150 MHz, CD_2Cl_2) δ 30.5, 35.9, 46.3, 55.2, 99.1, 114.2, 126.8, 130.9, 148.1, 158.5, 162.8, 163.0, 163.8, 166.9, 167.6; ESIMS [M + Na]$^+$ calcd for $C_{17}H_{17}N_4O_2{}^{79}BrNa$; 411.0433, found 411.0429.

***N*-[1-Benzyloxymethyl-8-(4-methoxy-benzylidene)-6-methyl-7-oxo-1,6,7,8-tetrahydro-imidazo[4,5-*d*]azepin-2-yl]-formamide (58).** To a slurry of sodium formate (215 mg, 3.16 mmol) in 3 mL of dry THF was added acetyl chloride (150 mL, 2.1 mmol). The slurry was then heated to 45 °C for 4 h. The slurry was cooled to room temperature, and a solution of **38** (40 mg, 0.1 mmol) in 1 mL of THF was added, followed by a catalytic amount of DMAP. The solution was then stirred at room temperature for 24 h. Hydrochloric acid (1 M) was added, and the solution was then stirred for 10 min. The solution was basified with the addition of saturated $NaHCO_3$, and the crude reaction mixture was extracted into EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield **58** (36 mg, 0.08 mmol, 80%) as a light yellow solid. ^{1}H NMR (600 MHz, DMSO) δ 3.22 (s, 3H), 3.73 (s, 3H), 4.26 (s, 2H), 4.31 (d, J = 11.5 Hz, 1H), 5.03 (d, J = 11.5 Hz, 1H), 6.14 (d, J = 9.1 Hz, 1H), 6.30 (d, J = 9.1 Hz, 1H), 6.85 (d, J = 8.0 Hz, 2H), 6.91 (d, J = 8.0 Hz, 2H), 7.17 (m, 3H), 7.26 (m, 3H), 8.82 (s, 1H), 10.88 (s, 1H); ^{13}C NMR (150 MHz, DMSO) δ 37.8, 55.2, 69.9, 71.2, 107.6, 114.3, 120.9, 126.6, 127.6, 127.7, 128.2, 129.0, 130.2, 132.0, 134.0, 136.9, 144.3, 159.6, 162.9, 168.6; ESIMS [M + Na]$^+$ calcd for $C_{25}H_{24}N_4O_4Na$; 467.1695, found 467.1694.

Methylation of 58 To Yield 53. A solution of **58** (4.6 mg, 0.01 mmol), K_2CO_3 (2.7 mg, 0.02 mmol), and MeI (1.0 mL, 0.016 mmol) in 0.5 mL of DMF was stirred at room temperature for 24 h. The solution was extracted into EtOAc and washed with 3 × H_2O. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexanes/EtOAc) to yield **53** (4.0 mg, 0.009 mmol, 90%) as a dull yellow solid.

4-[Hydroxy-(4-methoxy-phenyl)-methyl]-6-methyl-2-methylamino-6*H*-imidazo[4,5-*d*]azepin-5-one (56). Compound **58** (20 mg, 0.045 mmol) was dissolved in 1 mL of CH_2Cl_2. $AlCl_3$ (60 mg, 0.45 mmol) was added, and the resulting blood red slurry was stirred rapidly at room temperature for 30 min. The reaction was quenched by careful addition of saturated $NaHCO_3$ and extracted into EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated to yield **59**. ESIMS [M + Na]$^+$ calcd for $C_{17}H_{16}N_4O_3Na$ 347.1120, found 347.1128. This product was used without further purification. The crude compound **59** was then dissolved in 2.0 mL of dry THF. BH_3-THF (175 μL, 1.0 M in THF, 0.175 mmol) was added, and the solution was stirred for 1 h at room temperature. The reaction was then carefully quenched with the addition of 1.0 M HCl. The solution was basified with the addition of saturated $NaHCO_3$ and extracted into EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH) to yield **56** (5.0 mg, 0.015 mmol, 33%) as a bright yellow solid. The NMR showed a 3:1 ratio of rotamers. Only the major isomer is described. ^{1}H NMR (600 MHz, DMSO) δ 3.06 (d, J = 5.0 Hz, 3H), 3.55 (s, 3H), 3.69 (s, 3H), 6.26 (d, J = 11.3 Hz, 1H), 6.48 (d, J = 9.9 Hz, 1H), 6.80 (d, J = 8.6 Hz, 2H), 7.16 (s, br, 1H), 7.38 (d, J = 8.6 Hz, 2H), 7.80 (d, J = 9.9 Hz, 1H), 8.91 (d, J = 5.0 Hz, 1H); ^{13}C NMR (150 MHz, DMSO) δ 29.3, 43.7, 54.9, 72.6, 100.6, 113.2, 124.4, 127.3, 136.8, 143.6, 158.0, 163.8, 170.4, 174.5; ESIMS [M + Na]$^+$ calcd for $C_{17}H_{18}N_4O_3Na$ 349.1277, found 349.1279.

2-Amino-4-(4-methoxy-benzyl)-6-methyl-6*H*-imidazo[4,5-*d*]azepin-5-one (60). Compound **58** (25 mg, 0.056 mmol) was dissolved in 1 mL of CH_2Cl_2. $AlCl_3$ (74 mg, 0.56 mmol) was added, and the resulting blood red slurry was stirred rapidly at room temperature for 30 min. The reaction was quenched by careful addition of saturated $NaHCO_3$ and extracted into EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated to yield **59**. ESIMS [M + Na]$^+$ calcd for $C_{17}H_{16}N_4O_3Na$ 347.1120, found 347.1128. This product was used without further purification. Crude **59** was dissolved in 5 mL of 1,4-dioxane and diluted with 5 mL of deionized water. $HCl_{(g)}$ was bubbled through the solution for 1 min, and the solution was allowed to sit for an additional 2 min. The pH of the solution was brought to neutral with the addition of 5 M NaOH and then further basified by the addition of $NaHCO_3$. The reaction was extracted with EtOAc, and the aqueous phase was saturated with NaCl and extracted again with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH) to yield **60** (2.5 mg, 0.0084 mmol, 15%) as a bright yellow solid. Trace amounts of **39** were also present in the reaction mixture. ^{1}H NMR (400 MHz, DMSO) δ 3.53 (s, 3H), 3.66 (s, 3H), 4.22 (s, 2H), 6.43 (d, J = 9.8 Hz, 1H), 6.74 (d, J = 8.7 Hz, 2H), 7.21 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 9.8 Hz, 1H), 8.04 (s, br, 1H), 8.17 (s, br, 1H); ^{13}C NMR (100 MHz, DMSO) δ 36.8, 45.2, 56.4, 101.8, 114.8, 125.0, 131.3, 133.9, 144.0, 158.8, 162.2, 165.4, 171.6, 177.7; ESIMS [M + H]$^+$ calcd for $C_{16}H_{17}N_4O_2$ 297.1352, found 297.1354.

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Supporting Information Available: Experimental synthetic methods, ^{1}H and ^{13}C NMR spectra for all new synthetic compounds, and details of the X-ray diffraction analysis for compound **48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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