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Synthesis of Antimitotic Analogs of the Microtubule Stabilizing Sponge Alkaloid Ceratamine A

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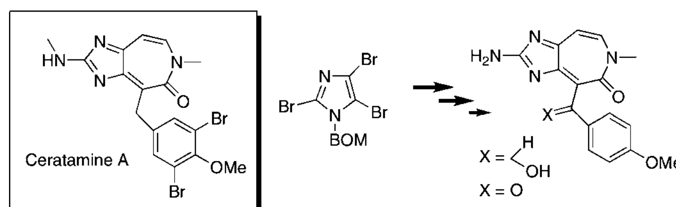
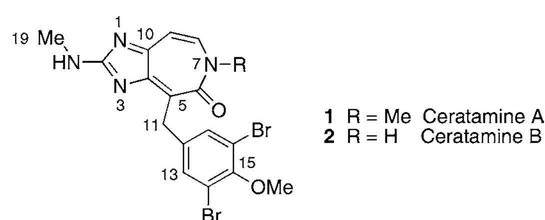
ABSTRACT



Antimitotic analogs of the microtubule stabilizing sponge alkaloid ceratamine A (1) have been synthesized starting from tribromoimidazole. A key step in the synthesis is the formation of the azepine ring via an intramolecular Buchwald coupling between a vinyl bromide and a *N*-methyl amide. This represents the first synthesis of a fully unsaturated imidazo[4,5,*d*]azepine. NMR data obtained for the synthetic ceratamine analogs has provided support for the structure assigned to the natural product.

Ceratamines A (**1**) and B (**2**) are antimitoic alkaloids isolated from the marine sponge *Pseudoceratina* sp. collected in Papua New Guinea.[1] The imidazo[4,5,*d*]azepine core heterocycle at the oxidation state found in the ceratamines appears to have no precedent among known natural products or synthetic compounds. Ceratamines stabilize microtubles but do not bind to the taxol binding site.[2] They produce an unusual antimitotic phenotype characterized by the formation of pillar-like tubulin structures that extend vertically from the basal cell surface and span the entire thickness of the arrested cells.

We embarked on a total synthesis of **1** to confirm the assigned structure and generate additional quantities of the natural product and analogs for further biological evaluation. Our retrosynthetic analysis of **1** is shown in Scheme 1. At



the outset, the plan was to install the C-2 methylamine and bromine atoms in the penultimate steps of the synthesis after the heterocyclic core had been assembled (i.e., **VIIb** to **1**), recognizing that desmethylamine and desbromoceratamines would also be useful for structure confirmation and biological evaluation. The key step in the assembly of the imidazo-[4,5,*d*]azepine core was to be an intramolecular Buchwald coupling between a vinyl halide and an amide in **V** to give **VI**.[3] It was anticipated that removal of the protecting group from the imidazole nitrogen in **VI** to give **VIIa** would set the stage for tautomerization to **VIIb**. NMR data obtained for the natural product **1** indicated that the fully unsaturated

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

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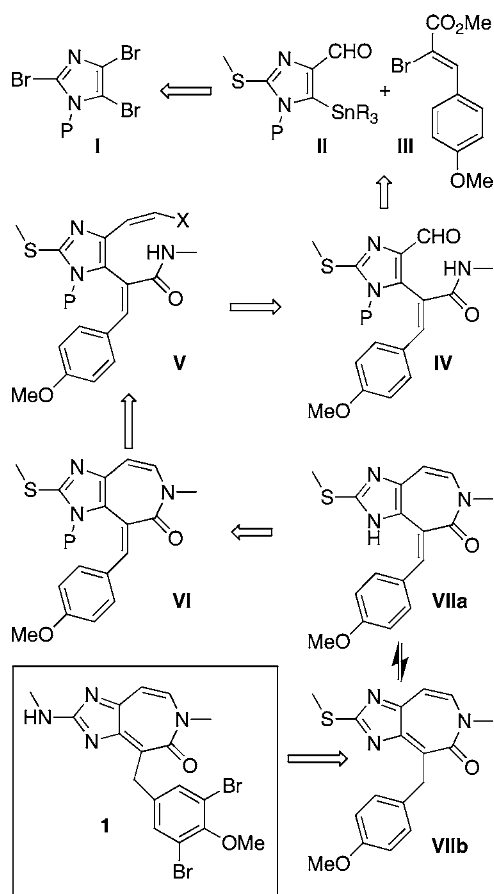
Scheme 1



imidazo[4,5,*d*]azepine core had aromatic properties, leading to the expectation that tautomeric equilibria for **VII** would strongly favor **VIIb**. The vinyl halide in **V** was to be generated from a Wittig reaction,[3b,4] and the amide was to come from the methoxycinnamic acid ester fragment **III**, after it was added to the imidazole **II** via a Stille coupling to give **IV**.[5] The stannylated imidazole intermediate **II** was to be prepared from commercially available tribrominated imidazole **I** (P = H) by sequential installation of BOM, methylsulfide, tributyltin, and aldehyde functionalities.[6]

The synthesis started with *N*-1 protection of tribromoimidazole by treatment with BOMCl and K_2CO_3 in DMF to give **3** (Scheme 2). Tribromoimidazole **3** was sequentially metalated and functionalized in a one-pot procedure following a literature precedent.[6] Thus, **3** was first metalated at

(3) (a) Jiang, L.; Job, G. E.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 3667−3669. (b) Meketa, M. M.; Weinreb, S. M. *Org. Lett.* **2007**, *9*, 853−855. (c) Toumi, M.; Couty, F.; Evano, G. *J. Org. Chem.* **2007**, *72*, 9003−9009.

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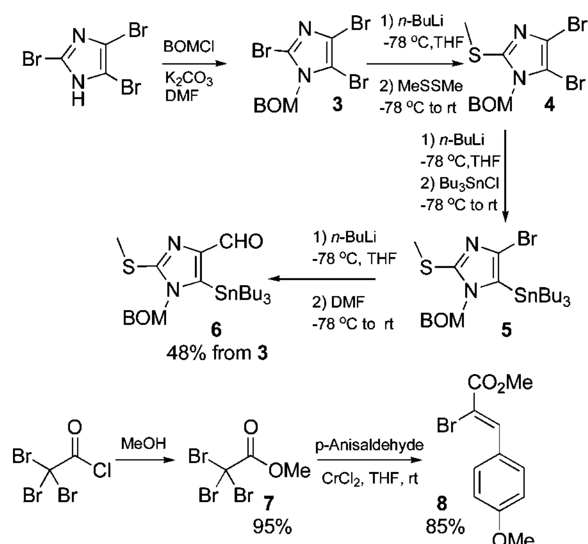
Scheme 2



C-2 with *n*-butyllithium followed by addition of dimethyldisulfide to generate the C-2 thiomethylimidazole **4**. In the same pot without workup, a second equivalent of *n*-butyllithium was added, resulting in metalation at C-5, and this product was reacted with tributyltin chloride to give the C-5 stannylated product **5**. Finally, addition of a third equivalent of *n*-butyllithium to **5** in the same pot gave metalation at C-4, and this lithiated species was reacted with DMF to generate the aldehyde **6**.

Synthesis of the cinnamic acid ester moiety required for the Stille coupling started with conversion of tribromoacetylchloride to the methyl ester **7** (Scheme 2). Condensation of the tribromo ester **7** with anisaldehyde mediated by $CrCl_2$ at rt gave the desired *Z*- methoxycinnamate **8** in excellent yield.[7] Stille coupling between the bromocinnamate **8** and the tributylstannylimidazole **6** gave the desired *E*-cinnamate **9** in good yield (Scheme 3). The aldehyde **9** was converted to the *Z*-vinyl bromide **11** using (bromomethyl)triphenylphosphoniumbromide and lithium hexamethyldisilazide.[8] Hydrolysis of the ester in **11** with aqueous lithium hydroxide gave the corresponding acid **12**. The carboxylic acid **12** was converted to the HOBt ester **13** by activation with DIPC followed by addition of HOBt. Treatment of the ester **13** with excess methylamine in THF/CH_2Cl_2 generated the *N*-methylamide **14** in good yield.

The key step in the synthesis was formation of the azepine ring. This was accomplished using Buchwald methodology involving a copper-catalyzed intramolecular coupling between the *Z*-vinylbromide and *N*-methyl amide functionalities in **14** to give the enamide **15** (Scheme 4). With the core skeleton of ceratamine A assembled, we turned our attention to installing the amino functionality at C-2. Following a Weinreb precedent, the methyl sulfide functionality in **15** was oxidized in poor yield to the corresponding sulfoxide

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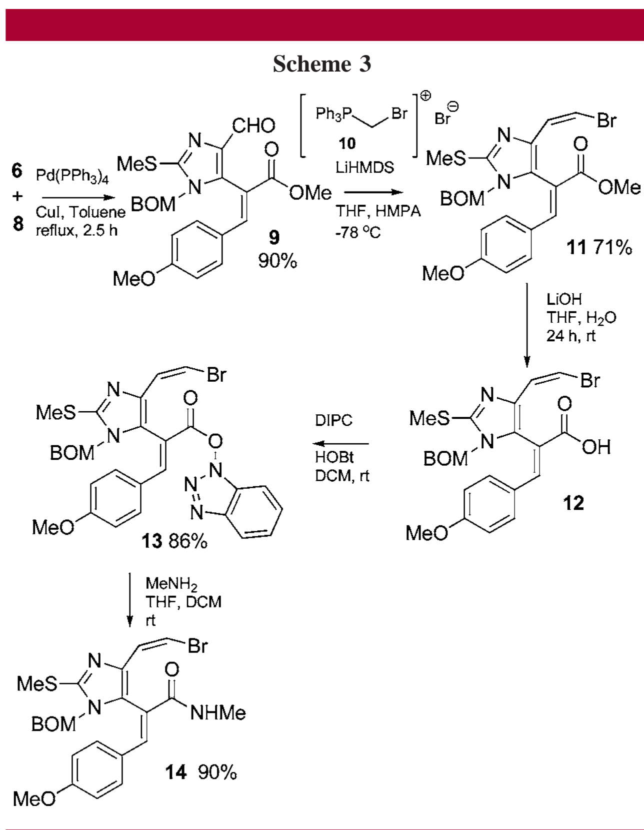
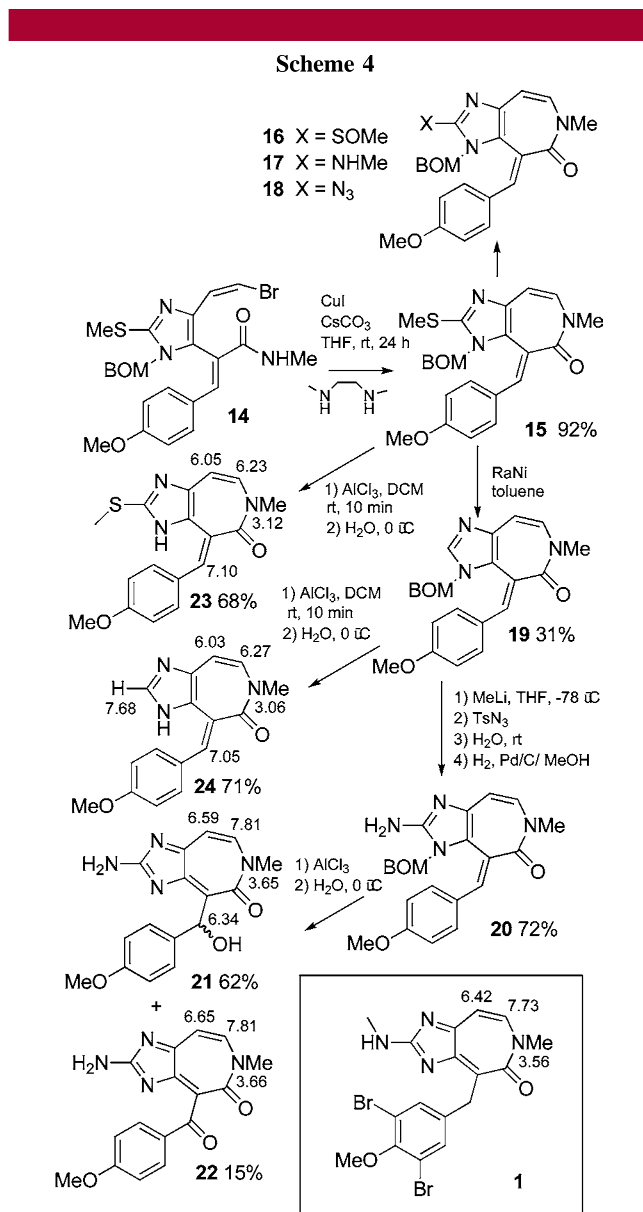
Scheme 3



Scheme 4



16 with *m*-chloroperbenzoic acid. All attempts to displace the sulfoxide in **16** with methyl amine in a sealed tube at high temperatures to give **17** or with azide to give **18** failed to give detectable products.

An alternate approach to introduction of an amine at C-2 started with the reductive removal of the methyl sulfide in **15** using Raney nickel to give **19**.[9] Deprotonation of **19** at C-2 with methyl lithium, followed by treatment of the carbanion with tosylazide[10] and subsequent workup with H_2O and reduction of the azide with H_2 and catalyst, proceeded cleanly to give the amine **20** (Scheme 4). Removal of the BOM protecting group from **20** with aluminum trichloride[6d] gave the ceratamine A analog **21** having a hydroxyl at C-11 and a small amount of the corresponding C-11 ketone **22**. Similarly, removal of BOM from **15** and **19** with aluminum trichloride[6d] gave the methylthio ceratamine analog **23** and analog **24** lacking the C-2 amino functionality, respectively. However, neither of the analogs **23** or **24** isomerized to give the putative aromatic tautomers (i.e., **VIIb**).

The generation of a C-11 alcohol in the ceratamine analog **21** during the $AlCl_3$ promoted deprotection of **20** and aqueous workup was unexpected. Formally, the OH can be introduced by Michael addition of water to the C-11/C-5/C-6 enamide followed by air oxidation of the resulting dihydroceratamine (Scheme 5). However, alcohol formation at C-11 is only observed in the deprotection of **20** and not in the deprotection of **15** or **19**. Therefore, the process appears to depend on the presence of an amino functionality at C-2, and it is probably mechanistically more complex than shown. The initial product from deprotection of **20** is colorless, too unstable to isolate, and turns the bright yellow, characteristic of the amino ceratamine chromophore in **21** and **22**, upon stirring in acidic water exposed to the air. This is consistent with air oxidation of a dihydroceratamine intermediate as shown in Scheme 5. Further air oxidation of **21** in the presence of strong acid (HCl) as proposed in Scheme 5 can lead to the C-11 ketone in **22**. Attempts to remove the BOM in **21** by hydrogenation or to remove the C-11 alcohol or brominate the methoxyphenyl ring in **21** via various methods have thus far all resulted in decomposition. These failures demonstrate that the synthesis will have to be redesigned to reach the natural products **1** and **2**.

The ^{1}H NMR data for compounds **21**, **22**, **23**, and **24** provided insight into the tautomeric equilibria and potential aromaticity in these ceratamine analogs (Scheme 4). Of

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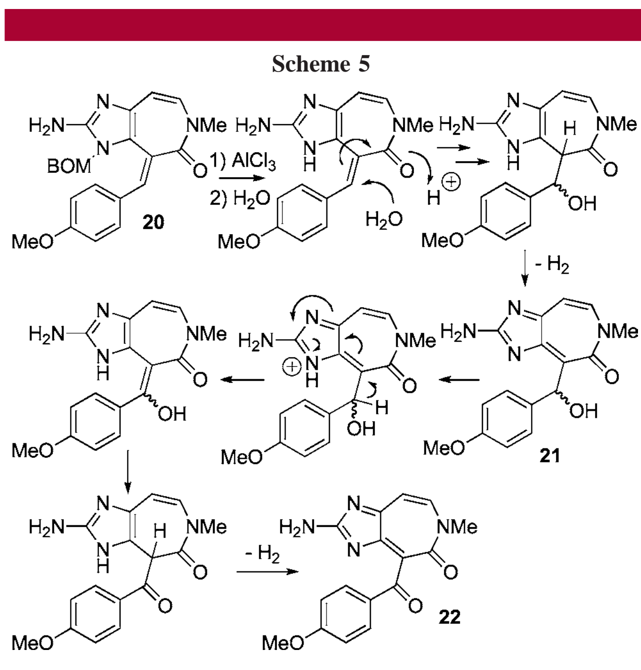
Scheme 5



particular interest were the resonances assigned to the enamide protons H-8 and H-9 and the protons on the benzylic carbon C-11. The H-8 enamide protons in **23** and **24** have chemical shifts (CD_2Cl_2) of δ 6.23 and 6.27, the H-9 protons have shifts of δ 6.05 and 6.03, and the H-11 protons appear as a singlets at δ 7.10 and 7.05, respectively, consistent with the presence of an *E* $\Delta^{5,11}$ alkene. The H-8 and H-9 resonances (CD_3OD) are shifted significantly downfield to δ 7.81 and 6.59 in **21** and to δ 6.65 and 7.89 in **22**, respectively, close to the corresponding shifts in ceratamine A (**1**) {DMSO-d_6 δ 7.73 (H-8), 6.42 (H-9)}. The *N*-methyl resonances have shifts of δ 3.12 and 3.06 in **23** and **24** compared with shifts of δ 3.65 and 3.66 in **21** and **22**, and δ 3.56 in **1**. H-11 appears as a singlet at δ 6.34 in the ^{1}H NMR spectrum of **21** and shows an HSQC correlation to a ^{13}C resonance at δ 75.3, consistent with a C-11 carbinol methine instead of a $\Delta^{5,11}$ alkene. ^{13}C assignments for the aminoimidazoazepine ring of **21**, based on HMBC and HSQC data, were in excellent agreement with the corresponding assignments for **1**.[11] The similarity between the ^{1}H and ^{13}C NMR assignments for the synthetic analog **21** and the natural product **1** provides strong support for the structure assigned to the natural product. Significant downfield shifts of the H-8, H-9 and *N*-Me resonances in **1**, **21**, and **22** compared with their counterparts in **23** and **24** can be attributed to the presence of a ring current, suggesting that the aminoimadzoazepine core in the ceratamines **1**, **21**, and **22** has aromatic character.

Ceratamine analogs **21** and **22** both showed antimitotic activity with potency close to ceratamines A and B in a cell-based assay (Supporting Information), demonstrating that Me-19 and the bromine atoms in **1** are not required for activity.[2] The analogs **23** and **24** were inactive, suggesting that either one or both of the aromatic imidazo[4,5,*d*]azepine heterocyclic core or an amino substituent at C-2 are required for antimitotic activity.

In summary, the synthesis of ceratamines **21** and **22** has confirmed the structures assigned to the natural products **1** and **2**, provided a route for the preparation of antimitotic ceratamine analogs for further biological evaluation, and revealed preliminary SAR for this novel microtubule-stablizing pharmacophore. The synthesis, which utilizes an intramolecular Buchwald reaction to assemble the azepine ring in the ceratamines, represents the first preparation of an aromatic imidazo[4,5,*d*]azepine heterocyle.

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Supporting Information Available: Experimental details and NMR spectra for compounds **6**, **9**, **11**, **13**, **14**, **15**, **19**, **20**, **21**, **22**, **23**, and **24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) **21**: δ 176.4 (C-2), 160.5 (C-4), 126.8 (C-5), 165.7 (C-6), 145.6 (C-8), 102.3 (C-9), 173.2 (C-10); **1**: δ 175.6 (C-2), 160.5 (C-4), 121.3 (C-5), 164.1 (C-6), 142.9 (C-8), 100.4 (C-9), 169.7 (C-10).