

Synthesis of the Reported Structures for Kealiinines B and C

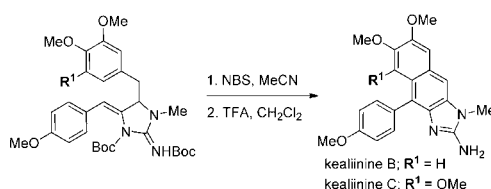
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ABSTRACT



Syntheses of the reported structures of kealiinines B and C have been executed. An intermolecular electrophile-induced cyclization of a pendant arene on an ene–guanidine affords the tetracyclic, oxidized naphthimidazole cores.

Marine sponges of the class Calcarea provide a large number of small molecule alkaloids containing a 2-aminoimidazole core with interesting biological activities (Figure 1).^{1,2} For example, naamine D has been shown to be a competitive inhibitor of iNOS.³ Naamidine A has shown antimetogenic effects by inhibiting EGF-stimulated DNA synthesis in the growth of squamous tumor cells as well as cell cycle arrest in the G1 phase.^{4,5} As a result, interest has emerged to incorporate this heterocyclic scaffold as a pharmacophore in biomedical research.

Within this class of natural products are several oxidized variants, including 14-hydroxynaamidines A and G⁶ which are hydroxylated at C14 and naamidine F⁷ which is isolated as the A-ring quinone. 2-Deoxy-2-aminokealiquinone⁸ is oxidized both in the A-ring and at C14.

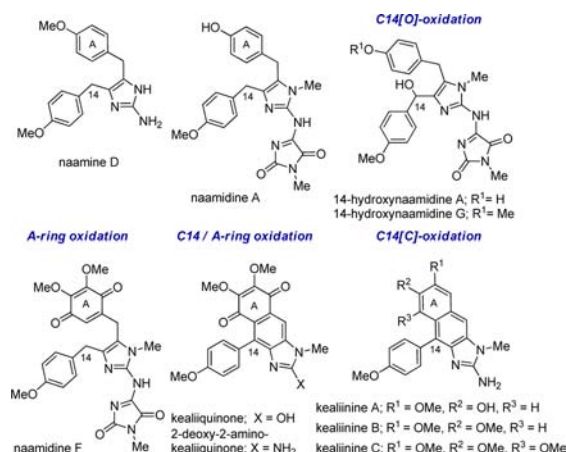


Figure 1. Representative Calcarea alkaloids.

In 2004, a chemical investigation of the marine sponge *Leucetta chagosensis* by Proksch and co-workers afforded the new imidazole alkaloids kealiinines A–C.⁹ Related to 2-deoxy-2-aminokealiquinone, these natural products are oxidized at C14 by virtue of a new C–C bond but remain at the hydroquinone oxidation state in the A-ring. Although their biosynthesis has not been studied, it is reasonable to

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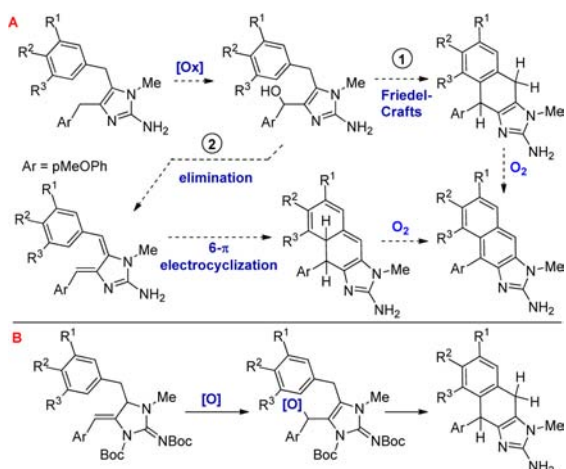
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Scheme 1. Plausible Biomimetic Strategy



suggest that the kealiinines are intermediates between kealiquinones and naamine derivatives by ring closure and aromatization.^{6,10} For example, naamines may be hydroxylated at C14 (Scheme 1, A). These intermediates may be subject to ionization to promote a Friedel–Crafts reaction (pathway 1). Oxidation would then provide the kealiinine skeleton. Alternatively, the hydroxylated intermediate may be subject to elimination (pathway 2) to give an intermediate which can undergo 6- π electrocyclicization and oxidation to give the kealiinines. This biosynthetic strategy has also been explored in the laboratory by both Ohta and Lovely to access these naphthimidazole cores.¹¹

Our strategy for synthesizing kealiinines B and C was also inspired by these hypothetical reactive intermediates that can be generated from the oxidized naamine (Scheme 1, B). If a cyclic ene–guanidine were reactive toward an electrophile, the intermediate formed would be reactive with a pendant nucleophile leading to Friedel–Crafts type alkylation or elimination/6- π electrocyclicization. Access to these ene–guanidines was envisaged to be straightforward with the development of 5-*exo-dig* selective propargylguanidine hydroaminations recently reported by both our group and Van der Eycken's group.^{12,13}

Synthesis of the propargylguanidine precursors was initiated with a Cu(I)-catalyzed three-component iminium–acetylide addition (A3-coupling)¹⁴ of 4-ethynylanisole,

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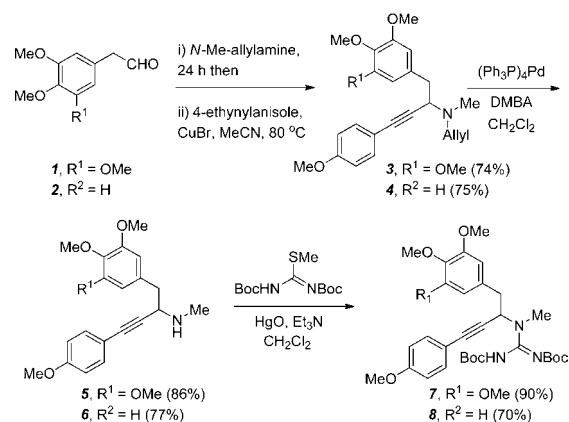
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Scheme 2. Substrate Preparation



N-methylallylamine, and the aryl acetaldehydes **1** or **2** (Scheme 2).¹⁵ Although this reaction required elevated heating for extended reaction times due to the formation of stable enamine intermediates, **3** and **4** could be obtained in good yields on multigram scale. Deallylation of these intermediates with Pd(0) gave the secondary propargylamines **5** and **6**.¹⁶ Guanylation of these intermediates with *S*-Me-*N,N'*-di-Boc-pseudothiourea and Hg(II) provided the propargylguanidines **7** and **8** in good yield.¹⁷

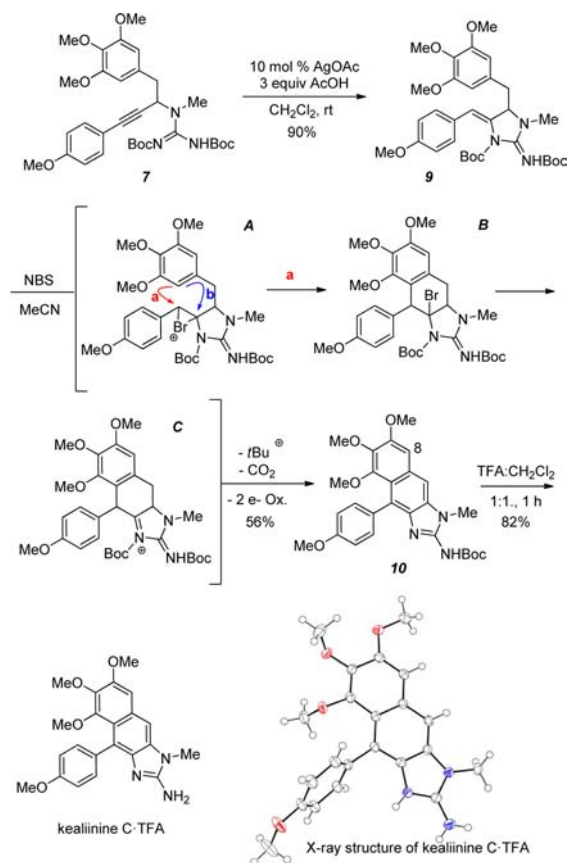
We initiated our cyclization studies with the trimethoxy-substituted arene for kealiinine C as it is symmetric (Scheme 2). To our surprise, cyclization of the propargylguanidine **7** gave **9** as a single regioisomer in good yield. Previous studies had shown that electron-rich alkynes (e.g., *p*MeOPh-) typically gave poor 5-*exo*/6-*endo* selectivity. Angle compression provided by substitution at the propargylic position presumably overrides this electronic selectivity through a Thorpe–Ingold effect. We next anticipated that activation of the ene–guanidine with NBS should generate the bromonium ion **A**. A key to this synthetic plan was that this bromonium ion can be opened via pathway a and not cyclization through pathway b to give intermediate **B**. While at first one might expect the formal products of a 5-*exo* cyclization (pathway b) to outcompete a 6-*endo* cyclization, there are several examples where the related electrophile-induced cyclizations are successful.¹⁸ Further, donation from the *p*-methoxy substituent might make this a formal 6-*exo* cyclization.¹⁹ In our experience, the ene–guanidines are not very nucleophilic, suggesting that donation of the neighboring nitrogen lone pair is hampered by steric compression. This gave us hope that pathway a might be competitive.

(15) Both aryl acetaldehydes are commercially available or obtainable by the LiAlH₄ reduction of the more readily available phenylacetic acid to the alcohol, followed by IBX oxidation to the aldehyde. See: Pelphrey, P. M.; Popov, V. M.; Joska, T. M.; Beierlein, J. M.; Bolstad, E. S.; Fillingham, Y. A.; Wright, D. L.; Anderson, A. C. *J. Med. Chem.* **2007**, *50*, 940.

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Scheme 3. Synthesis of Kealiinine C



To our delight, treatment of **9** with NBS in acetonitrile provided the cyclized, fully aromatic, and mono-Boc-protected intermediate **10** in 56% isolated yield. The only other compound isolated from this reaction was brominated at C8 (ca. 5%). While it does appear that the *p*-methoxyphenyl substituent on the ene-guanidine directs cyclization to give the 6-membered ring, we cannot rule out a 5-exo pathway followed by a pinacol rearrangement or that an elimination 6- π electrocyclization is operative. Either way, any potential intermediates must funnel to intermediate **C**, which is capable of losing a Boc group and undergoing a two-electron oxidation to give **10**. Deprotection of this intermediate gave kealiinine C in good yield (Scheme 3), and the structure was ultimately confirmed by X-ray crystallography.²⁰ In the isolation paper only ¹H NMR data in CD₃OD and DMSO-*d*₆ were reported for both kealiinines B and C. Comparison of our spectra to that reported in DMSO-*d*₆ shared very little agreement. However, the TFA salt of synthetic kealiinine C did match the natural product

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(19) A similar donation strategy has been used to favor 5-*endo-dig* over 5-*exo-dig* cyclizations. David, W. K.; Redfern, A. L.; Gilmore, J. *Tetrahedron Lett.* **1998**, *39*, 8909.

(20) This structure has been deposited at the Cambridge Crystallographic Database (CCDC No. 887954).

well in CD₃OD when referenced to the observed proton resonances for the natural product and not the solvent.

We next targeted kealiinine B to examine the regioselectivity of the oxidative cyclization relative to the A ring. Treatment of the propargyl guanidine **8** with AgOAc gave the ene-guanidine **11**, also as a single regioisomer (Scheme 4). Exposure to NBS again delivered a single cyclized, fully aromatic, and mono-Boc protected intermediate **12**. This was presumed to be the correct isomer for kealiinine B as H8 was a singlet. Presumably, attack of the bromonium ion is preferred by rotamer A as rotamer B would encounter more demanding steric interactions with the neighboring aromatic ring. Deprotection of **12** gave kealiinine B in 82% yield.

Again, the ¹H NMR data for synthetic kealiinine B did not match that reported for the natural compound in DMSO-*d*₆. The free base did not match either, but it was a crystalline solid which permitted us to unambiguously assign the structure of our synthetic material via X-ray crystallography.²¹ Having now realized that kealiinine C only matched the natural material in CD₃OD, we examined the TFA salt of kealiinine B in CD₃OD and when aligned to the reported peaks there was excellent agreement for all proton resonances except for that at H6 which differed by 0.27 ppm.

Still concerned about the spectroscopic data, the Proksch laboratory was generous enough to compare our synthetic sample with a small amount of natural material by HPLC. This revealed two things: (1) that the natural sample was actually an inseparable mixture of kealiinines B and C and (2) the compounds did coelute, suggesting they were indeed identical. We were also able to examine the original spectrum in CD₃OD for the natural products (a mixture of B and C) which revealed that H6 in kealiinine B is actually at 7.74 ppm not 7.90 ppm as originally reported.²² This is in good agreement with our synthetic material, and gave us confidence that the structures of the natural products are correct and agree nicely with our synthetic materials.

The fact that the spectra were still not in agreement in DMSO-*d*₆ was puzzling (most protons differing by 0.1–0.2 ppm). We hypothesized that (1) this might be due to a concentration effect; however, dilution experiments did not reveal a change in the chemical shifts of the protons, or (2) the presence of both natural products in the sample might give rise to unique intermolecular interactions which change the chemical shifts; however, titration of kealiinine C into a solution of B also showed no obvious change in chemical shifts. While we are confident, from the LC and ¹H NMR data in CD₃OD, that these are indeed the structures corresponding to the natural products, we must concede the possibility that alternate structures might exist that fit the DMSO-*d*₆ data with better agreement.

In the original isolation of kealiinines A–C only biological activity for kealiinine A was reported. This was reported in a brine shrimp lethality assay with an IC₅₀ = 20 μ g/mL.⁹ The activity of kealiinines B and C was not reported, likely due to the small amounts of isolated material and the fact

(21) This structure has been deposited at the Cambridge Crystallographic Database (CCDC No. 887953).

(22) See “Spectral comparisons” in the Supporting Information for more details.

Scheme 4. Synthesis of Kealiinine B

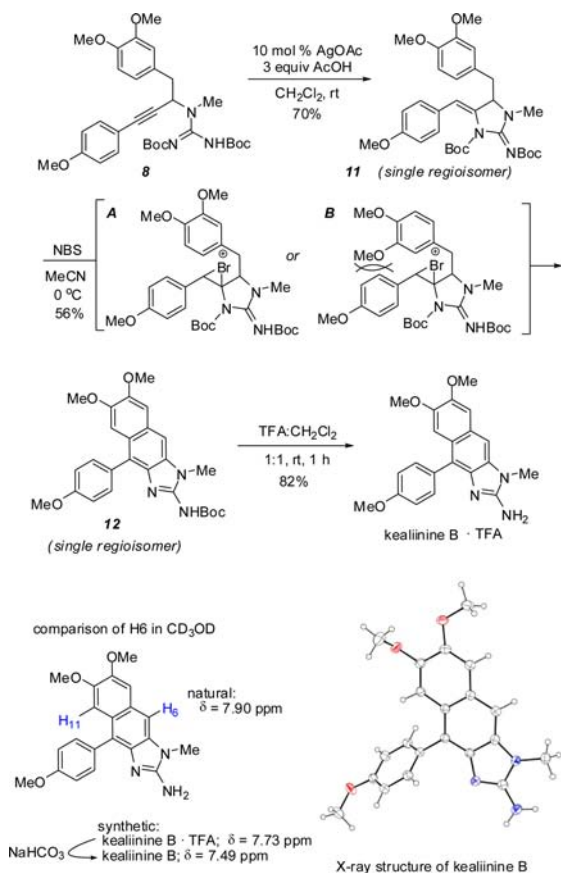
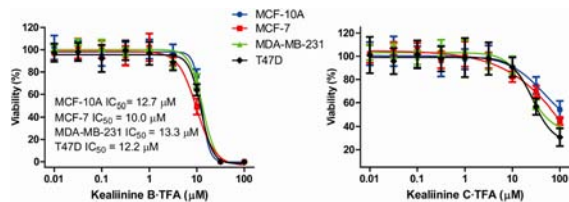


Figure 2. Antiproliferative activities.



that they were isolated as an inseparable mixture. We evaluated our synthetic kealiinines B and C against several breast cancer cell lines, including a normal breast cell line (MCF-10A), a luminal ER+ cell line (MCF-7), an aggressive luminal ER+ line (T47D), and a “basal-like” triple negative cell line (MDA-MB-231). Kealiinine B showed antiproliferative activity against all of these cell lines with IC_{50} 's ranging from ~ 10 to $13 \mu\text{M}$ (Figure 2). Kealiinine C,

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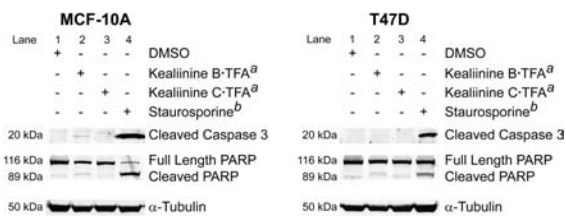


Figure 3. Interrogating caspase 3 activity.

however, did not appreciably inhibit proliferation at concentrations below $100 \mu\text{M}$.

The antiproliferative effects of members of this family have most extensively been studied for naamidine A, which is known to cause apoptosis through a caspase-3 dependent cascade after activation of the MAPK pathway.²³ We examined the similarity of kealiinines B and C's activity in this regard, and it appears that the antiproliferative effects arise from a caspase independent pathway (Figure 3). Treatment of MCF-10A or T47D cells, which are sensitive to caspase activation,²⁴ with $30 \mu\text{M}$ concentrations of kealiinine B or C for 48 h showed no evidence of caspase-3 cleavage or PARP cleavage, known hallmarks of caspase activation (lanes 2 and 3). Due to limited amounts of natural naamidine A, staurosporine was used as a positive control²⁵ and shows that indeed both cell lines are capable of undergoing caspase-3-dependent apoptosis (Lane 4).

In conclusion, we have demonstrated that ene-guanidines, derived from the hydroamination of propargylguanidines, are useful intermediates for the synthesis of the reported structures for kealiinines B and C via an electrophilic activation/cyclization sequence. The ability to prepare these compounds individually has allowed us to provide independent and comprehensive characterization for these purported natural products, as well as an independent evaluation of their antiproliferative activity. Current efforts are directed to understand the mechanism by which kealiinine B inhibits cell proliferation.

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Supporting Information Available. Experimental and characterization details, including ^1H and ^{13}C NMR spectra for all compounds. Biological protocols as well as Western Blots. This material is available free of charge via the Internet at <http://pubs.acs.org>

The authors declare no competing financial interest.