Natural Product Synthesis

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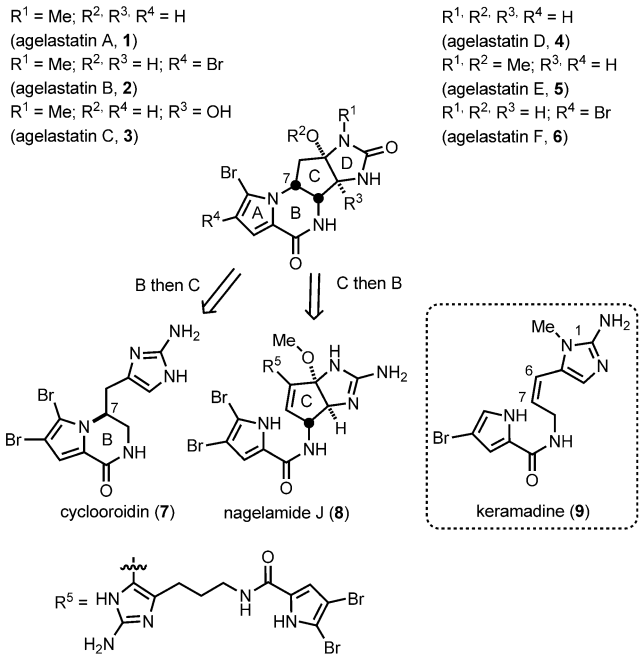
Bioinspired Total Synthesis of Agelastatin A**

*Jeremy Chris P. Reyes and Daniel Romo**

Agelastatin A (**1**) is a unique tetracyclic member of the growing family of pyrrole-2-aminoimidazole alkaloids (PAIs).[1] This marine alkaloid was first isolated by Pietra and co-workers in 1993 from the axinellid sponge *Agelas dendromorpha* and was contaminated with a small amount of a dibromo analogue, agelastatin B (**2**; Scheme 1).[2] In 1998, two other congeners, agelastatins C (**3**) and D (**4**), were isolated from the West Australian axinellid sponge *Cymbastela sp.* by Molinski and co-workers.[3] More recently, Al-Mourabit and co-workers isolated two additional members, agelastatins E (**5**) and F (**6**), from *Agelas dendromopha*.[4] The intriguing biogenesis of these alkaloids, which can be traced back to simple precursors, such as oroidin and clathrodin, has inspired several biosynthetic proposals but culminated in only one purely bioinspired and elegant total synthesis, which was recently described by Movassaghi et al.[5] The fact that there has only been one bioinspired total synthesis is surprising given that there are numerous total syntheses reported to date[5,6] spurred by its challenging structure and it being arguably the most bioactive member of the PAI family. Agelastatin A (**1**) was found to be highly cytotoxic to a panel of human-cancer cell lines (IC_{50}'s 97–703 nM),[7,8] potent in inhibiting osteopontin (OPN)-mediated neoplastic transformation and metastasis,[9] and potentially antiangiogenic,[7] antidiabetic,[10] and insecticidal.[3] The C ring, which contains all four stereogenic centers found in the molecule, is clearly the most challenging aspect of this molecule. In fact, most previous synthetic strategies focused on building the functionalized cyclopentane followed by late-stage construction of the B and D rings. As part of our efforts to investigate the biogenesis of PAIs,[11] we now report a concise bioinspired approach to agelastatin A that is premised on the isolation of the structurally related natural product, nagelamide J (**8**).



Scheme 1. The agelastatins and structurally related PAIs.

Two distinct and plausible biosynthetic presursors of agelastatin A that have been isolated to date are cyclooroidin (**7**)[12] and nagelamide J (**8**)[13] (Scheme 1). These natural products each support a different order of B-ring and C-ring formation in the biosynthesis of **1**. Although the absolute configuration of **8** is unknown, it is worth noting that the configuration of the C7 stereocenter in **7** is opposite to that of the corresponding center of agelastatin A. Movassaghi et al. recently described a strategy that was premised on the formation of an *ent*-cyclooroidin-like compound followed by C-ring cyclization to deliver (−)-agelastatin A.[5] In contrast, the synthetic strategy described herein highlights an alternative biogenetic pathway inspired by the nagelamide J structural motif, which supports a biogenetic sequence involving initial C-ring formation followed by B-ring formation.

Our bioinspired approach to agelastatin A was also guided by our previous experience with the ambivalent reactivity of the imidazolone moiety in our studies toward dimeric PAIs.[14] This reactivity is in stark contrast to what is commonly invoked for the biogenesis of these targets, wherein an oxidized imidazolone or aminoimidazole serves as an electrophile in subsequent cyclizations.[2a,15] These previous results led us to consider a different biosynthetic pathway, which proceeds through an *N*-acyliminium intermediate **13** that could lead to sequential C- and B-ring formation through a cascade process (Scheme 2). To this end, strategic disconnections at the C4–C8 and C7–N12 bonds revealed carbinolamide **10**; this intermediate is an oxidized version of keramadine (**9**),[16] which is another isolated natural product

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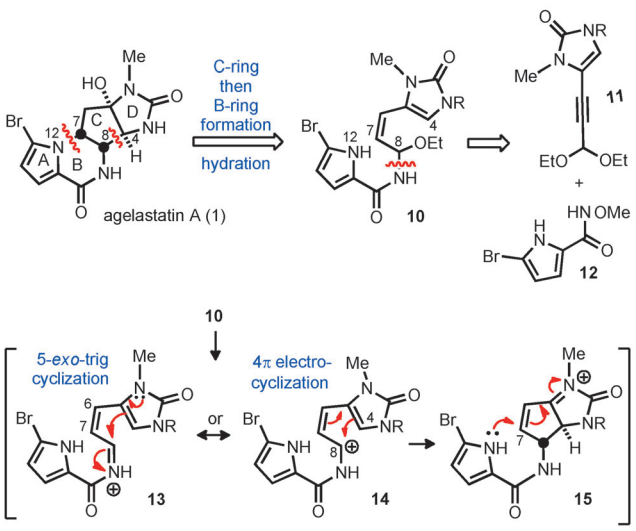


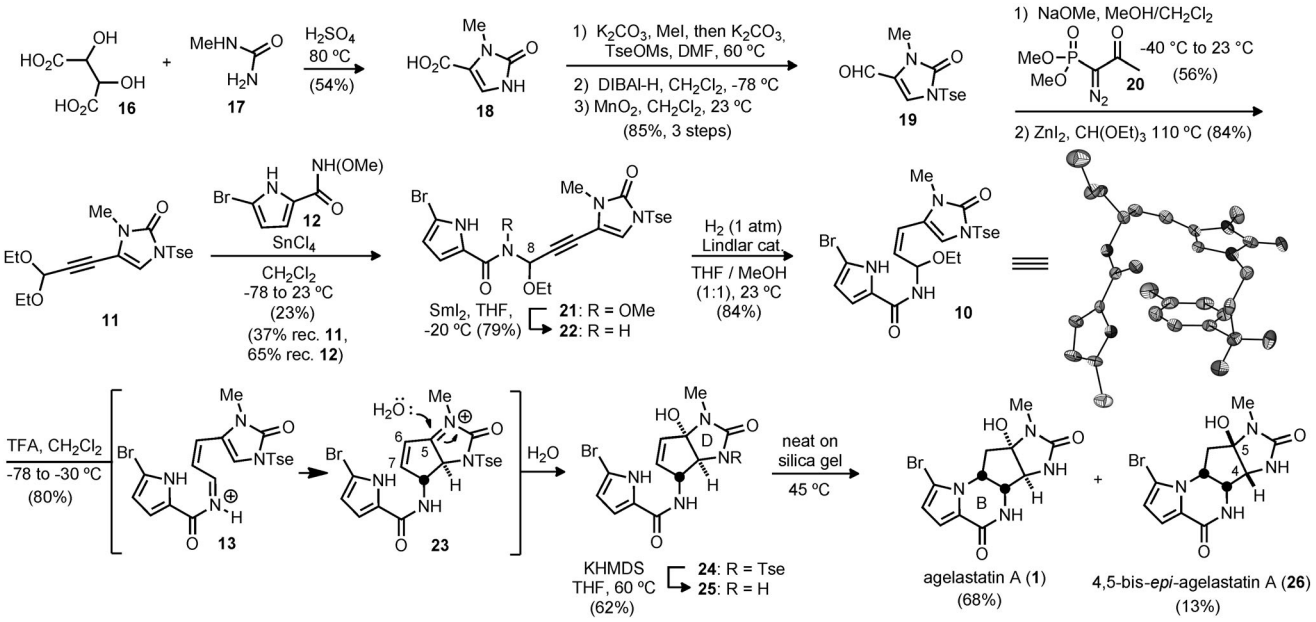
Scheme 2. Bioinspired retrosynthetic analysis of agelastatin A and proposed cascade process involving two cyclizations and proceeding via two *N*-acyliminium intermediates **13** and **15**.

that lends further credence to the biosynthetic proposal. Keramadine **9**, which was also isolated from an *Agelas* sponge, is the only isolated monomeric PAI known to possess a stable *Z*-configured olefin and notably bears the requisite *N*1-methyl group found in the agelastatins. We envisioned that a key *N*-acyliminium intermediate **13** could be derived from **10** by using a Brønsted or Lewis acid. The C6–C7 *Z* olefin was introduced to exert conformational bias and assist in the formation of the C4–C8 bond through a 5-*exo*-trig cyclization or via pentadienyl cation **14**, which is amenable to a Nazarov 4π electrocyclization.[17] The second *N*-acyliminium intermediate **15**, which is generated as a consequence of C-ring formation, could then be trapped by the adjacent pyrrole nitrogen atom to form the B ring. Following an acid-catalyzed hydration, this concise sequence would deliver agelastatin A.

To access the *N*-acyliminium precursor **10**, the imidazolone alkynyl acetal **11** and *N*-methoxy pyrrole amide **12**[6j] were ultimately identified as suitable coupling partners. The synthesis of imidazolone alkynyl acetal **11** commenced with known imidazolone acid **18**,[18] which is readily prepared from tartaric acid **16** and *N*-methyl urea **17** in one step (Scheme 3). Conversion of acid **18** into aldehyde **19** was accomplished in a three-step sequence involving a single-pot esterification and protection, reduction with DIBAl-H, and oxidation with MnO_2. The subjection of **19** to modified Seyferth–Gilbert conditions by using the Ohira–Bestmann reagent **20**[19] and sodium methoxide[20] in a 1:1 mixture of methanol and dichloromethane as solvent gave the desired alkyne. A subsequent Zn^{II}-mediated acetalization[21] furnished the targeted imidazolone alkynyl acetal **11**; this entire five-step sequence could be readily performed on multigram scale.

The key coupling of acetal **11** and known *N*-methoxy amido pyrrole **12**[5j] was achieved by activation with $SnCl_4$,[22] thus providing ethoxy carbinolamide **21** (Scheme 3). A substoichiometric amount (0.25 equivalents) of $SnCl_4$ and a 3 hour reaction time provided the best results in terms of avoiding further decomposition and enabling optimal recovery of both alkynyl acetal **11** and amido pyrrole **12**. The α effect associated with the *N*-methoxy group of the amide of **12** was critical for successful coupling because it engenders an amide nitrogen atom of higher nucleophilicity than the pyrrole nitrogen atom, thus enabling a chemoselective addition reaction.

Reductive cleavage of the *N*-methoxy group was accomplished using SmI_2[23] and following the designed late stage



Scheme 3. Bioinspired total synthesis of agelastatin A and 4,5-bis-*epi*-agelastatin A. In the crystal structure of **10**, hydrogen atoms have been removed for clarity and thermal ellipsoids are shown at 50% probability. DIBAl-H = diisobutylaluminium hydride, DMF = *N*,*N*-dimethylformamide, KHMDS = potassium hexamethyldisilazide, Ms = methanesulfonyl, TFA = trifluoroacetic acid, THF = tetrahydrofuran, Tse = *p*-toluenesulfonylethyl.

Lindlar reduction,[24] the desired cyclization precursor **10** was obtained. X-ray crystallographic analysis[25] of this intermediate confirmed that the coupling step involved the addition of the amide nitrogen atom (**11**→**21**) and not the pyrrole nitrogen atom. When the *Z* olefin **10** was subjected to a variety of Lewis and Brønsted acids, a deep-red solution was formed immediately; upon addition of water, nucleophilic attack occurred regioselectively at C5, thus delivering carbinolamine **24** a single diastereomer. When the deep-red solution was treated with EtOH, the corresponding ethoxy carbinolamine was formed (not shown). Formation of the C ring was quite facile because complete conversion occurred within 5 minutes; this ring formation also delivered three contiguous stereocenters with the relative configuration corresponding to the agelastatins and nagelamide J. Mechanistically, this process occurs either through a 5-*exo*-trig cyclization involving nucleophilic addition of the imidazolone moiety to the *N*-acyliminium moiety of **13** or through a Nazarov 4π electrocyclization of pentadienyl cation **14**, which is an alternative resonance form of **13**, involving the pseudoaromatic imidazolone.[17] However, the second cyclization, which was envisioned to complete the synthesis and involves nucleophilic addition of the pyrrole nitrogen atom to C7 of the α,β-unsaturated *N*-acyliminium **23**, did not proceed under a variety of reaction conditions, including prolonged reaction times, addition of non-nucleophilic bases, or exposure to elevated temperatures. Under most reaction conditions, the dark-red intermediate, which is presumably the acyl iminium **23**, was easily regenerated from **24** with both Lewis and Brønsted acids. This intermediate persisted until a nucleophile, for example water or methanol, was added; in the case of water being the nucleophile, carbinolamine **24** was reisolated. Conformational analysis of intermediate **23** suggests that the C6–C7 olefin is out of plane with respect to the *N*-acyliminium moiety by approximately 25°, thus resulting in a low degree of conjugation, which in turn is responsible for the low electrophilicity at C7.[26] This analysis may also explain why intermolecular addition of nucleophiles occurs exclusively at the more electrophilic C5 carbon atom. Moreover, DFT calculations predict that products arising from nucleophilic trapping at C7 were energetically disfavored by approximately 2 kcal mol^{-1} relative to those obtained upon trapping at C5.[27]

An alternative strategy to form the final B ring from bicyclic intermediate **24**, would be a transient base-induced opening of the cyclic urea (D ring) to a cyclopentenone followed by an aza-Michael addition and reformation of the cyclic urea. Interestingly, several previous syntheses of agelastatin A that required late-stage B-ring closure involved an enone intermediate, thus bolstering this idea further.[6a,h,i,k,o,q] The Tse protecting group on one of the urea nitrogen atoms was removed to enable greater conformational mobility and thus facilitate D-ring cleavage. After much experimentation, we serendipitously found that bicyclic intermediate **25** is readily converted into agelastatin A on silica gel[28] under solvent-free conditions with mild heating. 4,5-Bis-*epi*-agelastatin A (**26**) was observed as a byproduct of the cyclization and presumably arises from a retro-Nazarov reaction or a retro-5-*exo*-trig ring opening followed by recyclization. Attempted cyclizations with alternative substrates revealed that the absence of the Tse group and the presence of both the unprotected OH group at C5 and the bromine substituent at C13 of the pyrrole ring of **25** were essential for successful cyclization.

We were intrigued by the observed intense red color during the first cyclization event (**10**→**24**); this color change occurs immediately upon addition of acid even at low temperature. Given the absorption of light in the visible region, we suspected that the color may be due to a charge-transfer complex involving the *N*-acyliminium intermediate **23**. We used time-dependent density functional theory (TD-DFT) calculations incorporating B3LYP and X3LYP hybrid functionals to analyze excited states of this charged intermediate and simpler substructures.[29] We compared these values with those extracted from experimental UV/Vis spectra of this colored intermediate and related simpler substructures. These studies revealed that the red color of intermediate **23**, is likely due to a π→π* transition between the HOMO, which is composed mostly of orbital contributions from the bromopyrrole amide moiety, and the LUMO, which is composed mostly of orbital contributions from the *N*-acyliminium moiety (Figure 1).

In summary, we accomplished a concise total synthesis of agelastatin A (**1**) through two sequential, potentially biomimetic, cyclizations. The described sequential assembly of the C and B rings provides evidence for the proposed reactivity of a linear alkenyl imidazolone pyrrole, which leads to the agelastatins; the strategy complements other approaches to agelastatin that involve initial B-ring followed by C-ring formation.[5] C-ring formation, which sets three contiguous centers in a highly diastereoselective fashion, led to a reaction mixture with an intense red color, which we propose originates from a π→π* transition between the HOMO and LUMO of *N*-acyliminium intermediate **23**, a hypothesis, which is supported by TD-DFT calculations. The final B-ring

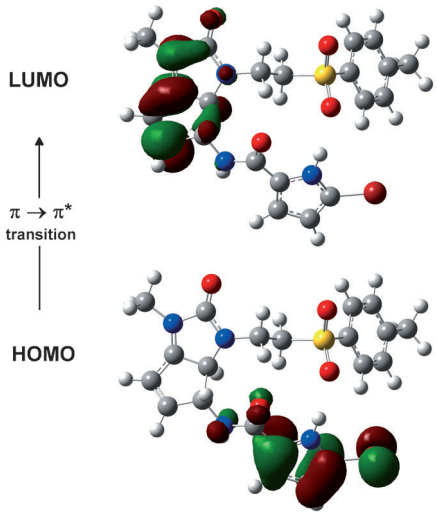


Figure 1. Calculated HOMO and LUMO of the red-colored *N*-acyliminium **23** and the π→π* transition, which based on TD-DFT calculations is proposed to be responsible for this color. Isovalue for surface = 0.04.

closure was uniquely successful under solvent-free conditions on silica gel with mild heating. The reaction sequence leading from an oxidized keramadine analogue **10** via a nagelamide J like intermediate **25** to agelastatin A is a provocative proposal for the biosynthesis of the agelastatins and inspired the concise route to the natural product reported herein. Further studies of the described strategy, including development of an enantioselective version,[30] are currently underway.

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[25] CCDC 849248 (**10**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[26] Molecular mechanics calculations were performed using Avogadro 1.0.0 software package (MMFF94s forcefield).

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[29] See the Supporting Information for computational details and both experimental and calculated UV/Vis spectra for intermediate **23** and related substructures.

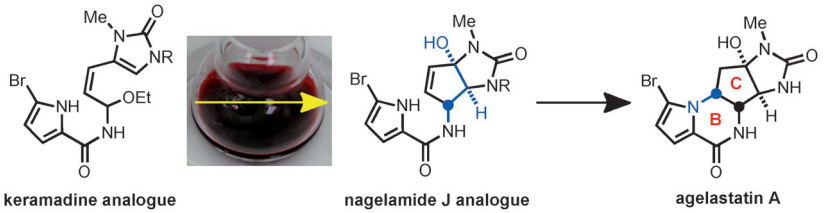
[30] In preliminary studies, the use of 1.1 equiv of (*R*)-TRIP, *(R)*-3,3′-bis(2,4,6-triisopropylphenyl)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (S. Hoffmann, A. M. Seayad, B. List, *Angew. Chem.* **2005**, *117*, 7590 – 7593; *Angew. Chem. Int. Ed.* **2005**, *44*, 7424 – 7427; T. Akiyama, Patent U.S. 0276329 A1, Dec 7, **2006**) to promote the Nazarov-type cyclization of vinylimidazolone **10** gave carbinolamine **24** and then, following deprotection of the Tse group, bicyclic adduct **25** in 63% *ee*. See the Supporting Information for further details.

Communications

Natural Product Synthesis

J. C. P. Reyes, D. Romo* ___ ▮▮▮▮–▮▮▮▮

Bioinspired Total Synthesis of Agelastatin A



A one-two punch: Two potentially biosynthetically relevant cyclizations of a keramadine analogue give agelastatin A (see scheme). A diastereoselective C-ring formation, which proceeds through a 5-*exo*-trig cyclization or a Nazarov cyclization of a red-colored *N*-acyliminium intermediate, generates the three contiguous stereocenters of the cyclopentane core. A silica gel assisted cyclization of a nagelamide J analogue gives agelastatin A.