CHEMICAL SYNTHESSES AND BIOLOGICAL STUDIES OF AGELASTATIN A, A BIOACTIVE MARINE HETEROCYCLE GIFTED FROM NATURE

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Abstract – Agelastatin A, an alkaloid originally isolated from the marine sponge Agelas dendromorpha, has long been an attractive target of chemical synthesis due to its significant biological activity and unique chemical structure. The synthetic approaches to the agelastatin alkaloid have demonstrated the advances of new methodologies and strategies for accessing a highly functionalized polycyclic nitrogen heterocycle. The present article reviews synthetic endeavors on agelastatin A that have been made by various synthetic chemists as well as biological studies on the natural product and its analogues aimed at the development of medicinal resources.

CONTENTS
1. Introduction
2. Biological properties of agelastatin alkaloids
3. Development of synthetic routes to agelastatin A
4. Structure-activity relationship (SAR) studies on agelastatin aimed at developing medicinal resources
5. Conclusion

1. INTRODUCTION
Marine natural products constitute one of the most attractive medicinal resources due to their diverse biological profiles. Agelastatin A (1) belongs to a family of pyrrole-imidazole alkaloids that are known to exhibit various biological activities (Figure 1). Hence, this natural product has attracted considerable attention from synthetic and medicinal chemists since its discovery from nature. Agelastatins A (1) and B (2) were originally isolated by Pietra and co-workers in 1993 as bioactive constituents in the axinellid
sponge *Agelas dendromorpha* collected from the Coral Sea near New Caledonia.\textsuperscript{2a,b} The chemical structure of 1 was elucidated by NMR analysis, molecular-mechanics calculations, and exciton splitting analysis. In 1998, two new additional members, agelastatins C (3) and D (4), were discovered along with agelastatin A (1) by Molinski and co-workers from the Indian Ocean sponge *Cymbastela* sp.\textsuperscript{3} Later, Al-Mourabit’s group reported the isolation and identification of agelastatins E (5) and F (6) together with agelastatins A (1) and D (4) from the New Caledonian sponge *A. dendromorpha*.\textsuperscript{4} There are two proposals regarding the biogenesis of agelastatin A (1) (Scheme 1). The pathway proposed by Pietra involves a cascade cyclization of intermediate i,\textsuperscript{2a} and the route suggested by Al-Mourabit features the intermediacy of ii that is likely generated by the oxidation of the debromooroidin tautomer, followed by the formation of the C-ring prior to the B-ring cyclization.\textsuperscript{2b}

![Chemical structures of agelastatins](image1.png)

**Figure 1. Chemical structures of agelastatins**

![Scheme 1. Proposed biosynthesis of agelastatin A](image2.png)

**Scheme 1. Proposed biosynthesis of agelastatin A**
2. BIOLOGICAL PROPERTIES OF AGELASTATIN ALKALOIDS

The biological assessments of agelastatins A to F have revealed that agelastatin A (1) is the most potent and exhibits various biological activities, such as cytotoxicity,\textsuperscript{2a,4} insecticidal property,\textsuperscript{5} brine shrimp toxicity,\textsuperscript{3} and glycogen synthase kinase-3β (GSK-3β) inhibitory activity.\textsuperscript{5} The biological function of agelastatin A (1) at the molecular level was initially investigated by Hale and El-Tanani who discovered that agelastatin A (1) dramatically decreased β-catenin levels in cancer cells and inhibited cancer proliferation by arresting cell cycle at G2 phase.\textsuperscript{6} It was also found that agelastatin A (1) downregulated osteopontin (OPN) protein expression and inhibited OPN-mediated malignant cell invasion, adhesion, and colony formation \textit{in vitro}.\textsuperscript{6} The intriguing biological activities exerted by 1 unlike other congeners likely stem from its unique functionality and molecular architecture. The biological significance of 1 has thus stimulated keen interest in the total chemical syntheses, which are discussed in the following section.\textsuperscript{7}

3. DEVELOPMENT OF SYNTHETIC ROUTES TO AGELASTATIN A

A variety of synthetic routes to agelastatin A (1) have been established so far in either a racemic or an asymmetric manner. The routes can be classified in terms of their synthetic strategies that differ in the construction of the C-ring system at either the early or late stage of the routes. Described below are the total (and formal) syntheses of agelastatin A (1) that have appeared in the literature, listed in the order of publication year.

\textbf{Weinreb (1999):} The first total synthesis of (±)-agelastatin A (1) was accomplished by Weinreb and co-workers in 1999 (Scheme 2)\textsuperscript{8}. Their synthesis was initiated by a hetero Diels-Alder cycloaddition reaction between cyclopentadiene (7) and methyl N-sulfinylcarbamate 8 followed by phenylation with PhMgBr to afford allylic sulfoxide 9. Sulfoxide 9 was treated with hexamethylphosphorous triamide (HMPT) in EtOH under heating to induce a [2.3]-sigmatropic rearrangement and concomitant cyclization to generate an oxazolidinone, which was protected with Boc to give 10 in 99% yield. The Sharpless-Kresze allylic amination of 10 with 2-(trimethylsilyl)ethylsulfonyl (SES)-protected sulfodiimide 11 provided 12. Then, reductive N-S bond cleavage of 12 with NaBH₄ afforded an allylic amine derivative, which was assembled with pyrrole carboxylic chloride 13 to deliver compound 14. The SES group of compound 14 was removed by TBAF to give an oxazolidinone, which was subjected to hydrolysis with aqueous LiOH followed by oxidation with PDC to furnish enone 15. Cyclization of 15 was effected with Cs₂CO₃ and subsequent three-step manipulations involving desilylative bromination with NBS, Boc deprotection with trimethylsilyl iodide, and carbamoylation with methyl isocyanate furnished (±)-agelastatin A (1).
**Scheme 2. Weinreb’s total synthesis**

**Feldman (2002):** The Feldman synthesis commenced with chiral epoxide 16 (Scheme 3). Epoxide 16 was converted in five steps into stannylated alkyne 18, which, upon treatment with Stang’s hypervalent iodine reagent, i.e., PhI(CN)OTf, followed by sodium p-toluenesulfinate (NaTs), gave alkylidene carbene intermediate ii.

**Scheme 3. Feldman’s total synthesis**
Intermediate ii underwent diastereoselective sp$^3$C-H insertion to form sulfonylated cyclopentene derivative 19. Sulfonylated cyclopentene 19 was then subjected to nucleophilic amination with $o$-nitrobenzylamine ($o$-NBNH$_2$) followed by amidation with 2-pyrrole carboxylic acid chloride to give amide 20. The treatment of 20 with Cs$_2$CO$_3$ in MeOH promoted the cyclization of the B-ring, and the subsequent three steps that involved Swern oxidation, photolytic deprotection, and bromination with NBS furnished (-)-agelastatin A (1).

Hale (2003 and 2004): Hale and co-workers devised a chiron approach to (-)-agelastatin A (1) from Hough-Richardson aziridine 22 derived from D-glucose (Scheme 4).  

![Scheme 4. Hale's 1st generation synthesis](image)

Their route features a stereospecific and regioselective ring-opening of the aziridine with an azide ion to unambiguously establish a vicinal trans-diamino functionality. Aziridine 22 was thus transformed in ten steps into diene 28 via the mentioned construction of the trans-diamine (22$\rightarrow$23), protecting group manipulations (23$\rightarrow$25), a reductive olefination (25$\rightarrow$26), and the Julia-Kocienski olefination (26$\rightarrow$28). Diene 28 was subjected to a ring-closing metathesis (RCM) with a Hoveyda-Grubbs catalyst followed by
a base-induced cyclization to give cyclopentene derivative 29. Cyclopentene 29 was converted into known oxazolidinone 14 in two steps including Boc protection and amidation with acid chloride 13, thereby accomplishing the formal enantiospecific total synthesis of (-)-1.

In their 2nd generation synthesis, Hale and co-workers devised an alternative transformation of compound 29 into target natural product (-)-1 (Scheme 5).10b Carbamoylation of 29 with n-BuLi/N,N-benzylmethylcarbamoyl chloride afforded an oxazolidinone, which was acylated with known chloride 13 to give compound 30. Removal of the SES group under radical conditions followed by basic hydrolysis and oxidation gave compound 31. Further transformation of 31 via a bromination with NBS and a subsequent Hüning-base-mediated cyclization afforded intermediate 32, which was reductively converted into compound 21 under hydrogenation conditions. The final A-ring bromination led to the completion of the 2nd generation total synthesis of (-)-1.

Scheme 5. Hale’s 2nd generation total synthesis

**Davis (2005):** An RCM strategy was also successfully employed in the synthetic route to (-)-agelastatin A (1) reported by Davis and co-workers (Scheme 6).11 Diamine derivative 35 was initially prepared in 73% yield by the alkylation of ethyl (dibenzylamino)acetate 33 with imine 34. Then, diamine derivative 35 was reacted with lithium N,O-dimethylhydroxyamide to provide amide 36, whose N-sulfinylamino group was removed by treatment with TFA in MeOH to furnish an amine. The amine was immediately coupled with pyrrole-2-carboxylic acid to deliver amide 37 in 88% yield. Allylation of amide 37 and
base-mediated isomerization of the resultant β,γ-unsaturated ketone successfully afforded enone 38 in 85% yield. The RCM of 38 was effected with Grubbs 2nd generation catalyst to deliver cyclic enone 39. Treatment of enone 39 with Cs₂CO₃ in MeOH afforded 40 in 68% yield. Hydrogenation of a mixture of 40 and methyl isocyanate allowed debenzylation and concomitant carbamoylation to take place, furnishing 21 in 47% yield. The final bromination of the A-ring system with NBS in THF gave (-)-1 in 69% yield.

Scheme 6. Davis’s total synthesis

**Trost (2006):** Trost and Dong have reported enantioselective routes to agelastatin A (1) and its enantiomer based on the palladium-mediated asymmetric allylic alkylation (AAA) of meso-symmetric cyclopentene derivative 41 with chiral ligand 43 (Scheme 7).¹² The strategy features two complementary approaches where, by switching nucleophiles, i.e., 42 or 48 for the AAA, either enantiomer of agelastatin A was accessible. Thus, carbonate 41 was reacted with ester 42 in the presence of chiral ligand 43 to provide pyrrole 44 in 83% yield. Transformation of the ester moiety of 44 into N-OMe amide in two steps followed by cyclization with a palladium catalyst yielded cyclopentene derivative 45. Copper(I)-catalyzed aziridination of compound 45 gave aziridine 46, which underwent ring-opening with DMSO in the presence of In(OTf)₃ to give ketone 47 in 91% yield. The synthesis was accomplished in two more steps
including carbamoylation with methyl isocyanate and subsequent removal of the N-tosyl and methoxy groups with SmI$_2$ to yield (+)-ent-agelastatin A. When compound 41 was reacted with amide 48 under the same conditions, the cascade di-alkylation took place to directly afford tricyclic pyrrolospiperazinone ent-45 in 82% yield, which served as the key intermediate to access natural (-)-agelastatin A (1).

Scheme 7. Trost’s total synthesis

Ichikawa (2007): The [3.3] sigmatropic rearrangement sequence was successfully utilized in the synthesis of (-)-agelastatin A (1) by Ichikawa and co-workers (Scheme 8). Their synthesis commenced with chiral benzoate 50 prepared from L-arabinol. Allylic carbamate 52 was synthesized in eleven steps from benzoate 50 and subjected to the [3.3] sigmatropic rearrangement via allyl cyanate i that enabled a [1,3]-chirality transfer to establish a nitrogen-substituted stereogenic center in product 53. Acid treatment of 53 followed by an RCM using Grubbs 1st generation catalyst under heating in benzene afforded cyclopentene derivative 54. After a protection-deprotection sequence, resultant allylic alcohol 55 was again subjected to a second [3.3] sigmatropic rearrangement followed by trapping with trichloroethanol to deliver trichloroethoxy (Troc) carbamate 56 in 95% yield. Removal of the Troc group from 56 with
zinc/acetic acid in THF and subsequent amidation of the resultant amine with pyrrole 57 gave compound 58. Compound 58 was deprotected under acidic conditions to afford alcohol 59, which was further subjected to four-step manipulations involving IBX/DMSO oxidation, cyclization, hydrogenative carbamoylation with methyl isocyanate, and final bromination to yield (-)-agelastatin A (1).

**Scheme 8. Ichikawa’s total synthesis**

**Du Bois (2009):** Du Bois and Wehn accomplished an enantioselective synthesis of (-)-agelastatin A (1) that features a catalytic intramolecular aziridination of sulfamate 61 followed by ring-opening of the resultant aziridine with an azide ion to install a vicinal trans-diamino functionality in the B-ring (Scheme 9). Commercially available chiral lactam 60 was converted into sulfamate 61, which was treated with Rh$_2$(esp)$_2$/PhI(OAc)$_2$/MgO followed by NaN$_3$ to deliver oxathiazepane 62. Carboethoxylation of compound 62 and subsequent selenation gave selenide 63 in 93% yield. After the Paal-Knorr condensation of 63 with ketoaldehyde 64 to construct the A-ring, resultant selenide 65 was treated with
trimethylphosphine followed by trapping with methyl isocyanate to furnish a urea in 81% yield. The selenide group was oxidized with mCPBA to undergo selenoxide elimination, delivering alkene 66 in 89% yield. Then, the Lemieux-Johnson oxidation of 66 furnished a carbonyl functionality that spontaneously underwent cyclization to afford a hemiaminal in 81% yield. Two more steps involving lactamization and bromination produced natural product (-)-1.

Scheme 9. Du Bois’s total synthesis

**Wardrop (2009):** Wardrop’s synthesis features a [3.3] sigmatropic rearrangement of trichloroacetimidate, which is the so-called Overman rearrangement to access functionalized cyclopentene 71, wherein the trichloroacetamide functionality serves as a useful urea precursor (Scheme 10). Thus, imidate 68 that was prepared from alcohol 67 and trichloroacetonitrile was heated in xylene at reflux to provide amide 69 in 78% yield. Amide 69 was subjected to bromonium-mediated cyclization followed by dehydrobromination to produce cyclic imidate 70. The imidate was converted into phthalimide 71 via an acid hydrolysis with TsOH and a subsequent Mitsunobu reaction with phthalimide. Trichloroacetamide 71, a latent urea precursor, was subjected to amidation with BnMeNH/NaHCO$_3$ in DMF followed by introduction of a pyrrole group in two steps to furnish urea 72. Then, urea 72 was reacted with K$_2$CO$_3$ in MeOH followed by oxidation with IBX/DMSO to provide enone 73 in 91% yield. Enone 73 was cyclized with K$_2$CO$_3$ in DMSO under heating, giving rise to ketone 74. Debenzylation of 74 under hydrogenation conditions and subsequent A-ring bromination eventually gave (±)-agelastatin A (1).
Chida (2009): Chida and co-workers developed a sequential Overman/Mislow-Evans rearrangement of an allylic bistrichloroimidate to install the nitrogen-substituted stereogenic centers (Scheme 11).\textsuperscript{16} Their synthesis started with (-)-2,3-O-isopropylidene-D-threitol, which was converted into thiophenylidiol 75 in six steps. Diol 75 was transformed into diimidate 76, which was subjected to a [3.3] sigmatropic rearrangement in refluxing xylene to give diamide 77. Oxidation of 77 with mCPBA gave a sulfoxide that underwent rearrangement followed by reduction of the resultant sulfenyl ester with P(OMe)\textsubscript{3} to furnish allylic alcohol 78. An RCM of 78 with Grubbs 1st generation catalyst and subsequent cyclization delivered cyclopentene derivative 79, which was further converted into trichloroacetamide 81 in four steps. Trichloroacetamide 81 was reacted with amine 82 to furnish 2,4-dimethoxybenzylurea derivative 83, which was subjected to four more steps involving acid-mediated deprotection, IBX oxidation, cyclization, and final debenzylation with CAN to afford (-)-agelastatin A (1).
Movassaghi (2010): A unique synthetic approach inspired by the hypothetical biogenesis of the agelastatin framework was developed by Movassaghi and co-workers (Scheme 12). Unlike other routes reported so far, Movassaghi’s route features the construction of a C-ring system by cationic cyclization. Pyrrole derivative 84 derived from D-aspartic acid was subjected to bromination with NBS, carbamoylation, and reduction with NaBH₄ to generate bicyclic amide 85 in high yield with >99% ee. Compound 85 was transformed into keto-triazene 87 by thioesterification followed by Cu(I)-thiophene-2-carboxylate (CuTC)-mediated coupling with organostannyl reagent 86. The C-ring was successfully constructed under acidic conditions with MeSO₃H in aqueous MeOH to produce (-)-agelastatin A (1) along with its methyl acetal 89 in the ratio of 2:1. Application of a similar biomimetic synthetic strategy to compound 93 and transformation of 1 and 89 led to the comprehensive access to all agelastatin members (Scheme 13).
Scheme 12. Movassaghi’s total synthesis of agelastatins B to F

Scheme 13. Movassaghi’s total synthesis of agelastatins B to F
Hamada (2011): Hamada and co-workers devised an enantioselective route to (-)-agelastatin A (1), in which the nitrogen-substituted stereocenters were constructed by asymmetric aziridination of cyclopentenone (94) with N-tosyloxy benzyl tosylxycarbamate in the presence of chiral diamine catalyst 95 (Scheme 14). Thus, cyclopentenone (94) was converted into aziridine 96 with 95% ee in 75% yield by the diamine-catalyzed conjugate addition reaction of N-tosyloxy benzyl carbamate. Phenylselenation of aziridine 96 via trimethylsilylation with 97 followed by trapping of the resultant silyl enol ether afforded compound 98 in 75% yield. Carbonyl reduction with NaBH₄ and subsequent ring-opening with an azide anion gave azide 99 in 78% yield. After reduction of the azide group, a 4,5-dibromopyrrole carboxylic acid was connected to the resultant amine to deliver compound 100. The phenyl selenide was oxidatively removed by treating with H₂O₂ to furnish known compound 59, culminating in the formal synthesis of (-)-1.

Scheme 14. Hamada’s formal total synthesis

Maruoka (2012): In the synthesis route developed by Maruoka and co-workers, an enantioselective organocatalytic Mannich reaction of N-protected aminoacetaldehyde 102 was utilized to install the vicinal nitrogen functionality of (-)-agelastatin A (1) (Scheme 15). The Mannich reaction of aminoacetaldehyde 102 with imine 101 took place in a highly enantio- and syn-selective manner to give diamine 103 in 69% yield with 98% ee (syn/anti = 6.4:1). Diamine 103 was alkenylated with 1-bromopropene in the presence of NiCl₂/CrCl₂ in DMSO to provide allylic alcohol 104 in 73% yield. The Boc group was removed by treatment with TFA and subsequent coupling of the resultant amine salt with 4,5-dibromopyrrole carboxylic acid afforded compound 105 in 67% yield in two steps. The RCM of diene 105 was effected
using Hoveyda-Grubbs 2nd generation catalyst in toluene to deliver cyclopentenol derivative in 78% yield. A sequential IBX/DMSO oxidation and base-mediated cyclization protocol applied to the cyclopentenol derivative allowed the preparation of known ketone 106 via the intermediacy of i, leading to the formal synthesis of (−)-agelastatin A (1).

Scheme 15. Maruoka’s formal total synthesis

Romo (2012): Romo and Reyes reported a bioinspired total synthesis of (±)-agelastatin A (1) where a structural congener of keramadine (115), a potential biosynthetic precursor of 1, was used as the key scaffold for the construction of the tetracyclic architecture (Scheme 16). Their success in establishing the approach to 1 suggests that a biogenetic production of 1 likely initially takes place via C-ring formation followed by B-ring cyclization. The synthesis was started by condensing tartaric acid (107) with N-methylurea (108) to produce imidazolone acid 109 in 54% yield. Four-step transformations from 109, involving methyl esterification, p-toluenesulfonylethylation, hydride reduction, and allylic oxidation gave aldehyde 110 in 85% overall yield. Alkynyl homologation of aldehyde 110 with Ohira-Bestmann reagent 111 followed by acetalization with triethyl orthoformate under heating conditions delivered compound 112. N,O-transacetalization of 112 was carried out with pyrrolecarboxamide 48 in the presence of SnCl₄, and the methoxy group was reductively removed using SmI₂ to afford an amide. Hydrogenation of the amide under Lindlar conditions yielded cis-alkene 113, which was reacted with TFA to provide cyclized product 114 bearing a contiguous C/D-ring system. Deprotection of the N-Tse group using KHMDS and subsequent treatment of the resultant product with silica gel allowed acid-mediated B-ring formation to deliver desired (±)-agelastatin A (1) in 68% yield.
Batey (2013): Batey’s synthesis demonstrates a highly concise construction of a C-ring with requisite vicinal diamino functionality, enabling the shortest access so far to the natural product in its racemic form (Scheme 17).\(^{21}\) Cyclopentenone 118, a reasonable C-ring motif, was rapidly constructed by condensing 2-furaldehyde (116) and diallylamine (117) with the aid of lanthanide Lewis acid Dy(OTf)\(_3\). The reaction takes place through a domino condensation/ring-opening/Nazarov-like conrotatory \(\pi^4\) electrocyclization of the initially generated ring-opened Stenhouse salt i. Resultant unstable diamine 118 was immediately subjected to reduction by DIBAL followed by silylation with TBDPSCI to give 119. The palladium-catalyzed deallylation of 119 in the presence of \(N,N'\)-dimethylbarbituric acid afforded diamine hydrochloric salt in a multi-gram scale in 97% yield. After intensive screening of the reaction conditions and reagents, the regioselective amidation of 120 was proven successful by using 5-bromopyrrole carboxylic acid lithium salt 121, which is rarely used in a conventional amidation. Thus, diamine 120 was converted into allylic alcohol 123 via the 2-(2-pyridon-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.
(TPTU)-mediated amidation with 121 followed by carbamoylation with 122, a useful methyl isocyanate alternative, and concomitant desilylation with CsF. The endgame was completed in one pot by treating 123 with IBX/DMSO followed by mild heating after the addition of trifluoroethanol to afford (±)-agelastatin A (1) in 48% yield. It should be noted that the use of diisopropylethylamine in lieu of trifluoroethanol for the mentioned B-ring cyclization gave a poor yield of the desired product.

Liang (2017): Liang and co-workers developed an enantioselective approach to (+)-ent-agelastatins A (1) and B (2) through a late-stage construction of a C-ring, which is reminiscent of a biomimetic strategy (Scheme 18).22 Liang’s synthesis commenced with the preparation of pyrrole 126 in 52% yield by the Paal-Knorr condensation of L-aspartic acid diethyl ester (124) with pyruvate 125 followed by reduction with LiAlH₄. The Parikh-Doering oxidation of 126 and the subsequent one-pot Henry reaction with nitromethane smoothly took place to give alcohol 127 in 85% yield. A D-ring imidazolone motif was constructed via hydrogenation of the nitro group followed by carbamoylation with methyl isocyanate and Swern oxidation-cyclization sequence. Compound 128a was treated with NH₃ in MeOH to provide alcohol, which was oxidized by a modified Swern protocol to afford hemiaminal 129. Acid treatment of 129 in refluxing 0.1 N HCl and subsequent bromination led to (+)-ent-agelastatin A (1). Application of a similar strategy to another key intermediate 128b that was prepared from 127 through a hydrogenation/carbamoylation/bromination sequence also provided access to ent-(+)-agelastatin B (2).
Our total synthesis (2008, 2009, 2013, and 2018): We have developed four routes to (-)-agelastatin A (1). While the 1st generation route features thermal aziridination and subsequent ring-opening with an azide anion to furnish a vicinal diamino functionality\(^{23a}\), the three other routes are based on the implementation of nitrogen radical chemistry\(^{23b-d}\). Known alcohol 130 (> 99% ee) was converted into pyrrole 131 via the Mitsunobu esterification followed by the Clauson-Kaas pyrrole formation (Scheme 19).\(^ {23a}\)

Scheme 19. Our total synthesis: 1st generation strategy
Carbamoylation of the pyrrole with trichloroacetyl isocyanate and subsequent deacylation with acetic acid gave amide 132, which was transformed into azidoformate 133 in additional three steps. Application of heat to azidoformate 133 in a sealed tube gave aziridine 134, which was reacted with NaN₃ to furnish azide 135 in 61% yield. After hydrolysis of the nitrile group of 135 and subsequent hydrogenation of the azide group, the resultant amide was treated with HCl at 60 °C to successfully provide oxazolidinone 136 in good yield. Heating a mixture of 136 and aqueous methylamine solution in DMSO allowed the construction of a urea motif, thereby avoiding the use of toxic methyl isocyanate. Oxidation of 137 followed by bromination of the pyrrole ring led to (-)-1. Our 2nd and 3rd generation syntheses employed nitrogen radicals to manipulate the C-ring system, which circumvented the harsh heating conditions required in the 1st generation strategy (Scheme 20). Common intermediate 132 was converted into azidoformate 139a and N-tosyloxycarbamate 139b, respectively, both of which were subjected to a substoichiometric iron(II)-bromide-mediated redox transformation in the presence of Bu₄NBr salt as the bromide source. While this type of radical transformation was originally developed by Bach and co-workers who used catalytic FeCl₂ in combination with stoichiometric TMSCl to effect aminochlorination, our new reagent system consisting of FeBr₂/Bu₄NBr or FeCl₂/Bu₄NCl has expanded the scope of aminohalogenation reactions. It is worthy to mention that, besides our success, remarkable advances particularly in the area of enantioselective aminohalogenation were reported by Xu and co-workers. Resultant aminobromide 140 derived from either 139a or 139b was cyclized with NaH in DMF to furnish tetracyclic compound 136 in 91% yield. Further known three-step transformations were applied to 136, except the use of PDC as the oxidant that was proven more suitable in the large-scale transformation, leading to (-)-1.
As discussed in the next section, the structure-activity relationship (SAR) study on the agelastatin scaffolds required a ready access to various analogues. In this context, we were particularly interested in agelastatin analogues bearing various substituents at N1 position, whose comprehensive biological assessment had remained unexplored (Scheme 21). For this purpose, we decided to devise a new 4th generation strategy amenable to late-stage N1 functionalization that would enable a facile access to N1-modified analogues.\(^{23d}\) The 4th generation synthesis was initiated with carbamate 141, which was subjected to Boc deprotection followed by amidation of the resultant ammonium salt to provide amide 142. Hydrolysis of 142 with aqueous LiOH followed by PDC oxidation and Cs\(_2\)CO\(_3\)-mediated aza-Michael reaction provided tricyclic product 143. The silyl enolization of 143 with NaHMDS/TMSCl gave enol ether 144, which was treated with \(N\)-bromophthalimide (NBP) to deliver bromide 145. Then, bromide 145 was subjected to an S\(_{\text{t}}\)2’ radical azidation with a KMnO\(_4\)/BnEt\(_3\)NCl/TMSN\(_3\) reagent system to introduce the nitrogen functionality suitable for D-ring construction. Hydrogenation of resultant azide 146 gave an amine, which, upon one-pot carbamoylation with Batey reagent followed by desilylation with CsF, furnished debromoagelastatin A. Final bromination of debromoagelastatin A with NBS completed the 4th generation synthesis of (-)-1.

Scheme 21. Our total synthesis: 4th generation strategy

### 4. STRUCTURE-ACTIVITY RELATIONSHIP (SAR) STUDIES ON AGELASTATIN AIMED AT DEVELOPING MEDICINAL RESOURCES

SAR assessments of agelastatin analogues have been made independently by Pietra,\(^2^c\) Molinski,\(^2^6\) Movassaghi,\(^2^7\) Romo,\(^2^8\) and our group\(^2^9\) to validate the relevance of agelastatin A (1) as a promising
The earliest SAR study on agelastatin analogues that were semi-synthetically derived from 1 by Pietra and co-workers demonstrated that in vitro antiproliferative activities of agelastatin analogues were significantly affected by the structural modifications (Figure 2). All modifications including debromination at C13, acetylation/methylation of C5 hydroxy group, dimethylation at N3 and N9, dehydration of C5 hydroxy group, and removal of C2/C10 carbonyls significantly attenuated biological activity. While their study suggested that the agelastatin scaffold has a narrow window to explore new analogues, there was still room to devise new potent analogues by varying the N1 substituent.

Figure 2. In vitro cytotoxicity (IC_{50} values [μg/mL]) of agelastatin analogues against L1210 cancer cell line reported by Pietra and co-workers. Circles indicate the modified structures.

Our SAR studies on agelastatin analogues including N1-ethyl derivative 154 with C13-chlorine atom as well as C13/C14-dichlorinated analogue 155 have revealed that the structural modification of N1 and C13 substituents was tolerable in retaining the potent in vitro cytotoxicity. We have also demonstrated that the new agelastatin analogues potentially attenuate brain cancer, suggesting their in vivo therapeutic efficacy (Figure 3). In addition to brain cancer research, recent biological studies revealed that (-)-agelastatin A (1) attenuates age-related myocardial fibrosis and dysfunction in mouse models due to its anti-osteopontin activity, demonstrating the potential application of 1 to cardiac disorders.
While in line with the observations reported by the previous SAR studies where only C13 structural modification was tolerated, Molinski’s comprehensive SAR study led to the discovery of highly potent fluorinated C13-CF$_3$ analogue $\text{162}$ that exerts antiproliferative activity against chronic lymphocytic leukemia (CLL) cell lines (Figure 4). Fluorinated analogues $\text{162}$ and $\text{163}$ were synthesized by direct trifluoromethylation using NaSO$_2$CF$_3$/TBHP and difluoromethylation with Zn(SO$_2$CF$_2$H)$_2$/TBHP, respectively. Their studies provided an important insight into the role of the C13 substituent; an electron-withdrawing group with suitable steric effect similar to a bromine atom enhances the biological activity of agelastatin analogues.
Movassaghi and co-workers reported comprehensive SAR studies on agelastatin analogues that were prepared by their convergent synthetic strategies from common thioester 91, urea 164, and alcohols \( R^2 \text{OH} \) (Scheme 22).\(^{27}\) The synthetic approach was amenable to produce a wide range of analogues with varying N1 and C5 substituents, demonstrating the robustness of their synthetic strategies that further expanded the scope of derivatization of agelastatins. The analogues with D-ring modifications at N1 and C5 positions were evaluated by the three-dimensional co-culture assay for the effects of mammary fibroblasts on associated breast cancer cells. It has also been shown that agelastatin E (5), a natural congener, was superior to agelastatin A (1) in modulating fibroblast-mediated cancer invasion and metastasis at noncytotoxic doses. Their SAR studies led to the identification of potent new analogues 167, 168, and 169 bearing either a modified N1 or C5 substituent, which were found to be statistically equivalent to agelastatin E (5).

Scheme 22. General strategy to access new agelastatin analogues devised by Movassaghi and co-workers
Romo, Liu, and co-workers have also reported the derivatization of the agelastatin motif to develop new potent analogues (Scheme 23). Their SAR studies culminated in the discovery of C14-chlorinated analogue 171 that exerts potent cytotoxicity against various cancer cell lines including cervical cancer (HeLa), epidermoid carcinoma (A431), and primary human chronic lymphocytic leukemia (CLL) cells with low toxicity towards B and T cells. Analogue 171 was also proven to exhibit low serum protein binding. In addition, they developed trifunctional photo-affinity probe 174, which has lower cytotoxicity than 1 and is potentially useful for the identification of the cellular target of 1. They also demonstrated a possible application of alkoxyaminal derivative 175 as a prodrug that generates 1 under mild hydrolysis conditions.

While the origin of the cytotoxicity exerted by (-)-agelastatin A (1) had remained unclear until recently, its underlying mechanism of action at the molecular level was elucidated by Romo, Liu, and co-workers through a systematic top-down approach using a high-throughput chemical footprint method. They successfully identified the target protein to be ribosome with which (-)-1 interacts. The binding of (-)-1 to the ribosome peptidyl transferase A site leads to multiple conformational changes of ribosome peptidyl transferase center (PTC), thereby inhibiting protein synthesis responsible for the cytotoxicity. A 3.5 Å resolution crystal structure of the complex between 1 and the 80S eukaryotic ribosome from *S. cerevisiae* was obtained to validate the proposed interactions between the molecules. The crystallography suggests intermolecular associative forces that stem from π-halogen interaction of C13-bromine atom, and hydrogen bonding interactions of C5 hydroxy group, C2/C10 carbonyls, and N-H at N9 with ribosomal nucleic bases and amino acid residues are essential for exhibiting the biological activities. The above-mentioned interactions can reasonably rationalize the observed SARs reported by Pietra, Molinski,
Movassaghi, Romo/Liu, and our group. Given the intensive footprints associated with SARs described above, it can be concluded that while the agelastatin scaffold was found to have limited space to access potent medicinal leads with suitable pharmacokinetic properties, there is still room for the development of new analogues through structural modifications at N1 and C13 positions. Further studies on SARs of various analogues based on such structural modifications will pave the way for the development of new agelastatin-based medicinal leads.

5. CONCLUSION
The present article overviews the chemical syntheses and biological studies of marine alkaloid agelastatin A (1) that has, for a long time, attracted much attention from synthetic chemists. As seen in many other bioactive natural products, its unique chemical and biological properties have stimulated intensive efforts to access the natural alkaloid by chemical synthesis. Such efforts have culminated in the development of new means for forming multiple nitrogen-substituted stereogenic centers as well as constructing polycyclic molecular architecture. More recent studies have focused on the development of new medicinal agents based on the agelastatin alkaloid particularly applicable to the area of cancer research due to its significant ability to modulate protein expression. Future endeavors in this arena will promote further development of the synthetic organic and medicinal chemistry of this attractive heterocyclic nitrogen compound.

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REFERENCES


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