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CHEMICAL SYNTHESES 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

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).[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.[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.[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*.[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**,[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.[2b]

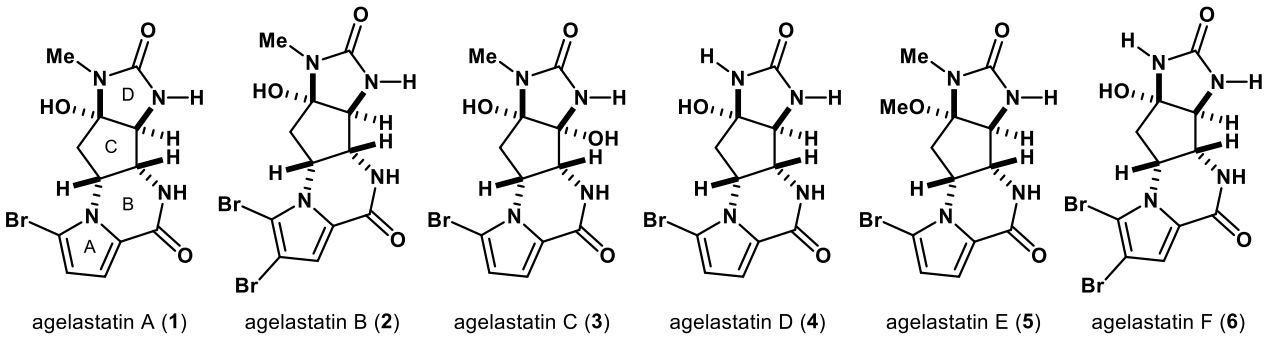
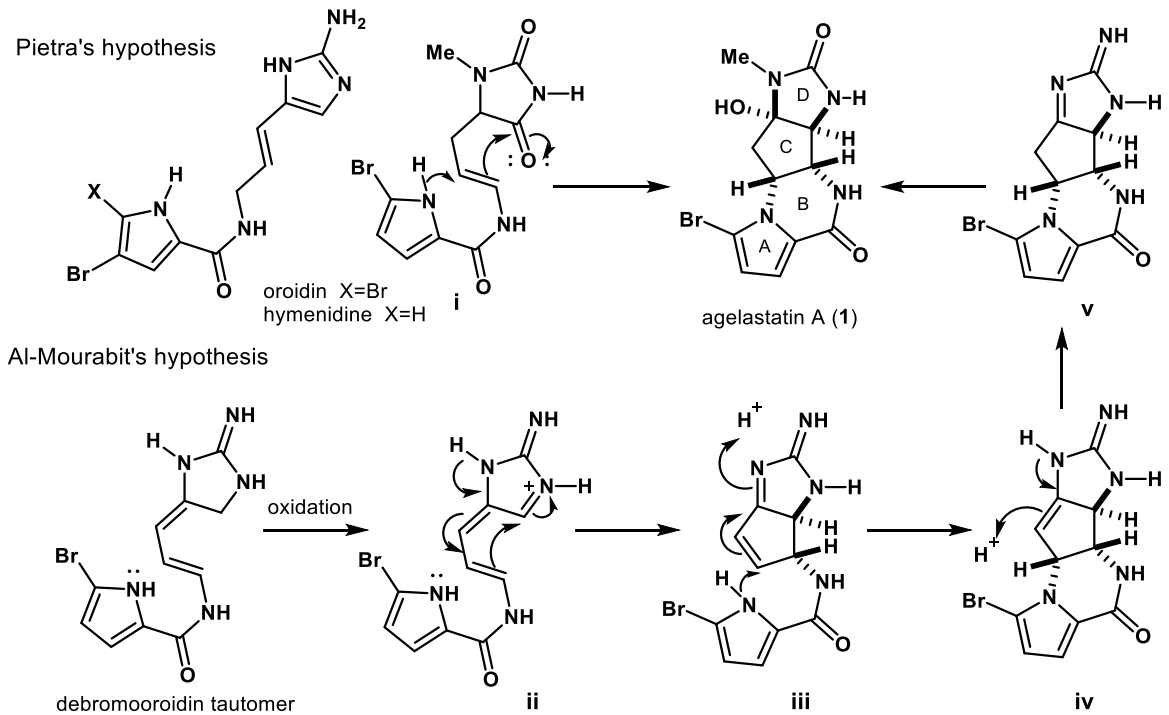


Figure 1. Chemical structures of agelastatins



Scheme 1. Proposed biosynthesis of agelastatin A

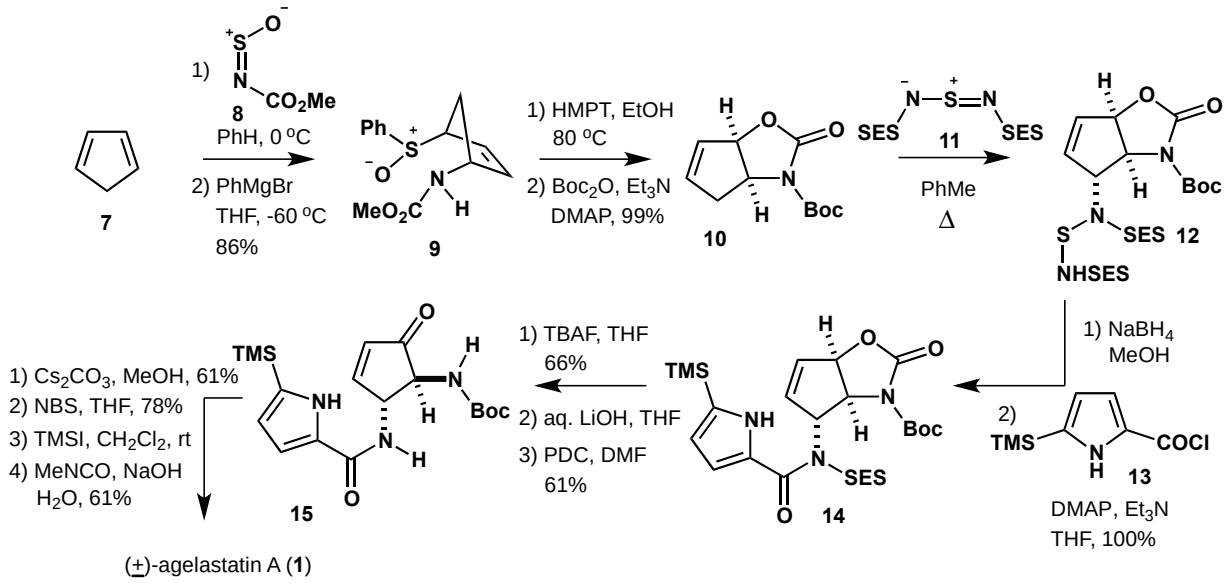
2. BIOLOGICAL PROPERTIES OF AGELASTATIN ALKALOIDS

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,[2a,4] insecticidal property,[3] brine shrimp toxicity,[3] and glycogen synthase kinase-3β (GSK-3β) inhibitory activity.[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.[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 *in vitro*.[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.[7]

3. DEVELOPMENT OF SYNTHETIC ROUTES TO AGELASTATIN A

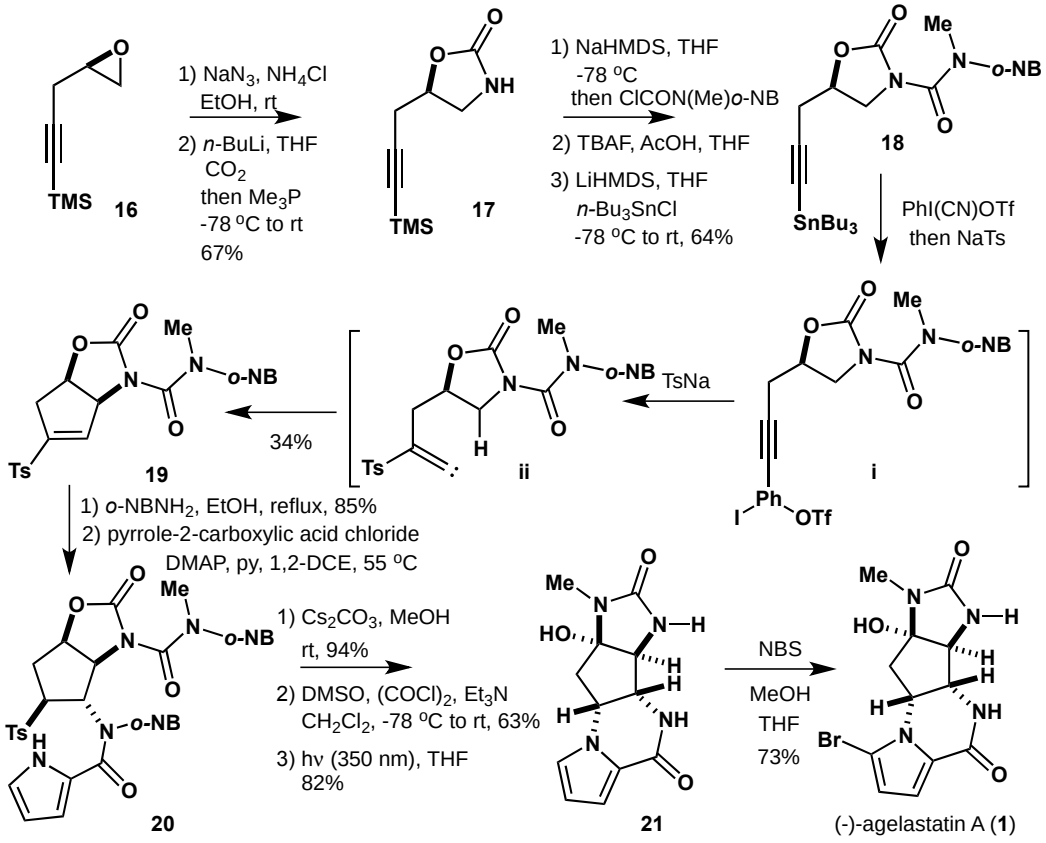
A variety of synthetic routes to agelastatin A (**1**) have been established so far in either a racemic or an asymmetric manner. The routes can be classified in terms of their synthetic strategies that differ in the construction of the C-ring system at either the early or late stage of the routes. Described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

Weinreb (1999): The first total synthesis of (±)-agelastatin A (**1**) was accomplished by Weinreb and co-workers in 1999 (Scheme 2).[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 $NaBH_4$ 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 Cs_2CO_3 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

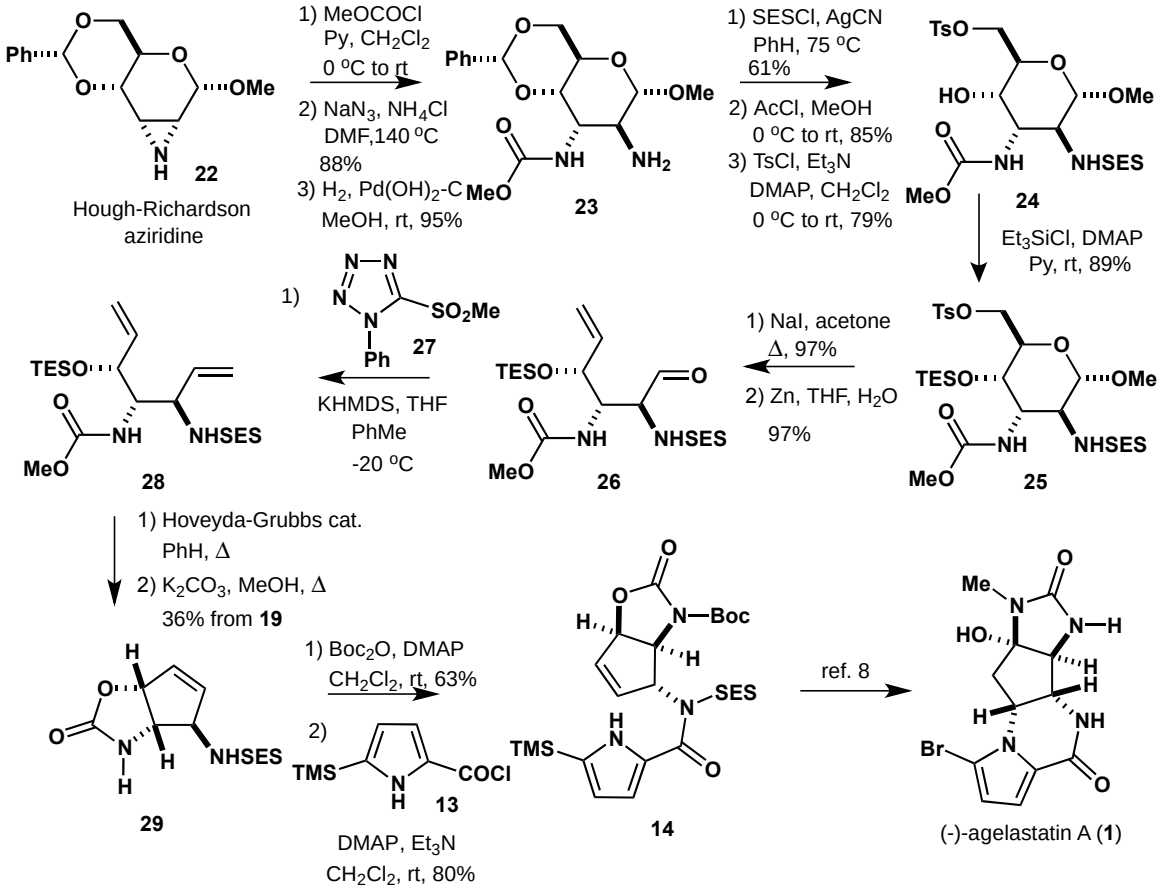
Feldman (2002): The Feldman synthesis commenced with chiral epoxide **16** (Scheme 3).[9] Epoxide **16** was converted in five steps into stannylated alkyne **18**, which, upon treatment with Stang's hypervalent iodine reagent, i.e., PhI(CN)OTf, followed by sodium *p*-toluenesulfinate (NaTs), gave alkylidene carbene intermediate **ii**.



Scheme 3. Feldman's total synthesis

Intermediate **ii** underwent diastereoselective sp^3C-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_2CO_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).[10a]

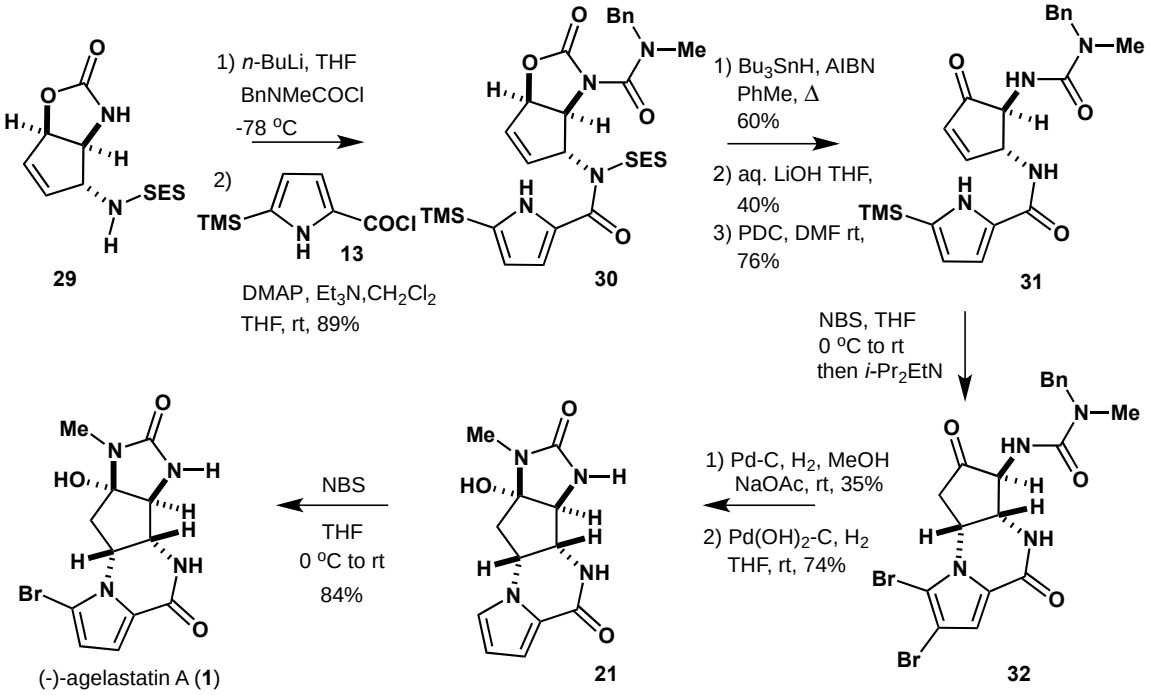


Scheme 4. Hale's 1st generation synthesis

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**→**23**), protecting group manipulations (**23**→**25**), a reductive olefination (**25**→**26**), and the Julia-Kocienski olefination (**26**→**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**.

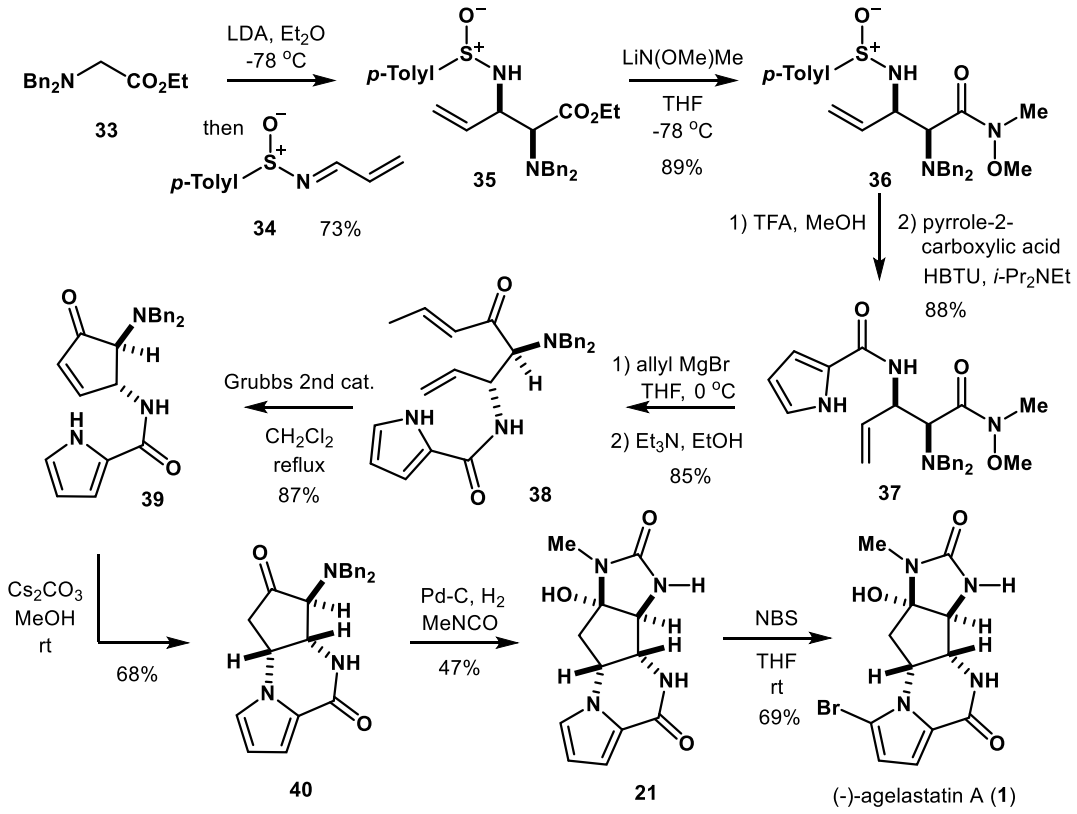
In their 2nd generation synthesis, Hale and co-workers devised an alternative transformation of compound **29** into target natural product (-)-**1** (Scheme 5).[10b] Carbamoylation of **29** with *n*-BuLi/*N,N*-benzylmethylcarbamoyl chloride afforded an oxazolidinone, which was acylated with known chloride **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 Hünig-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

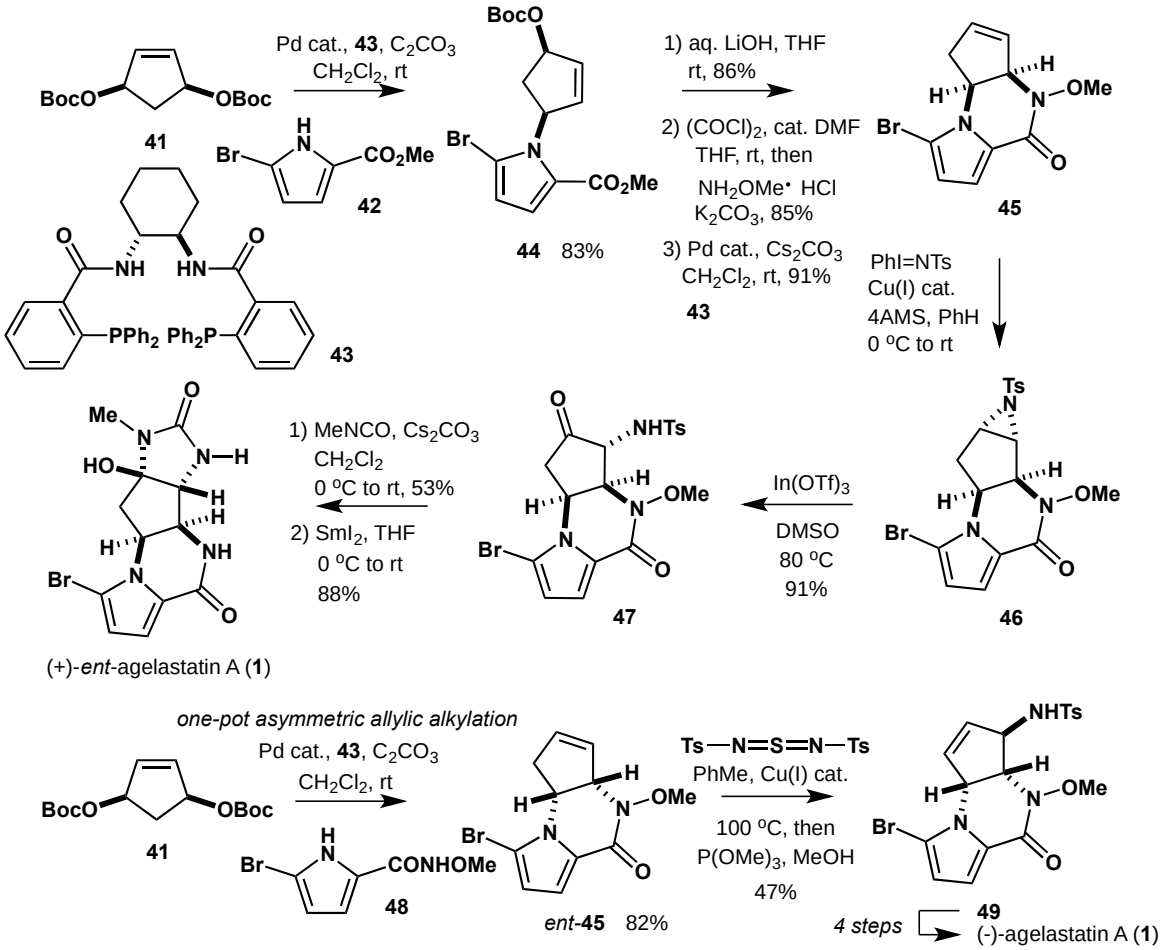
base-mediated isomerization of the resultant β,γ-unsaturated ketone successfully afforded enone **38** in 85% yield. The RCM of **38** was effected with Grubbs 2nd generation catalyst to deliver cyclic enone **39**. Treatment of enone **39** with Cs_2CO_3 in MeOH afforded **40** in 68% yield. Hydrogenation 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

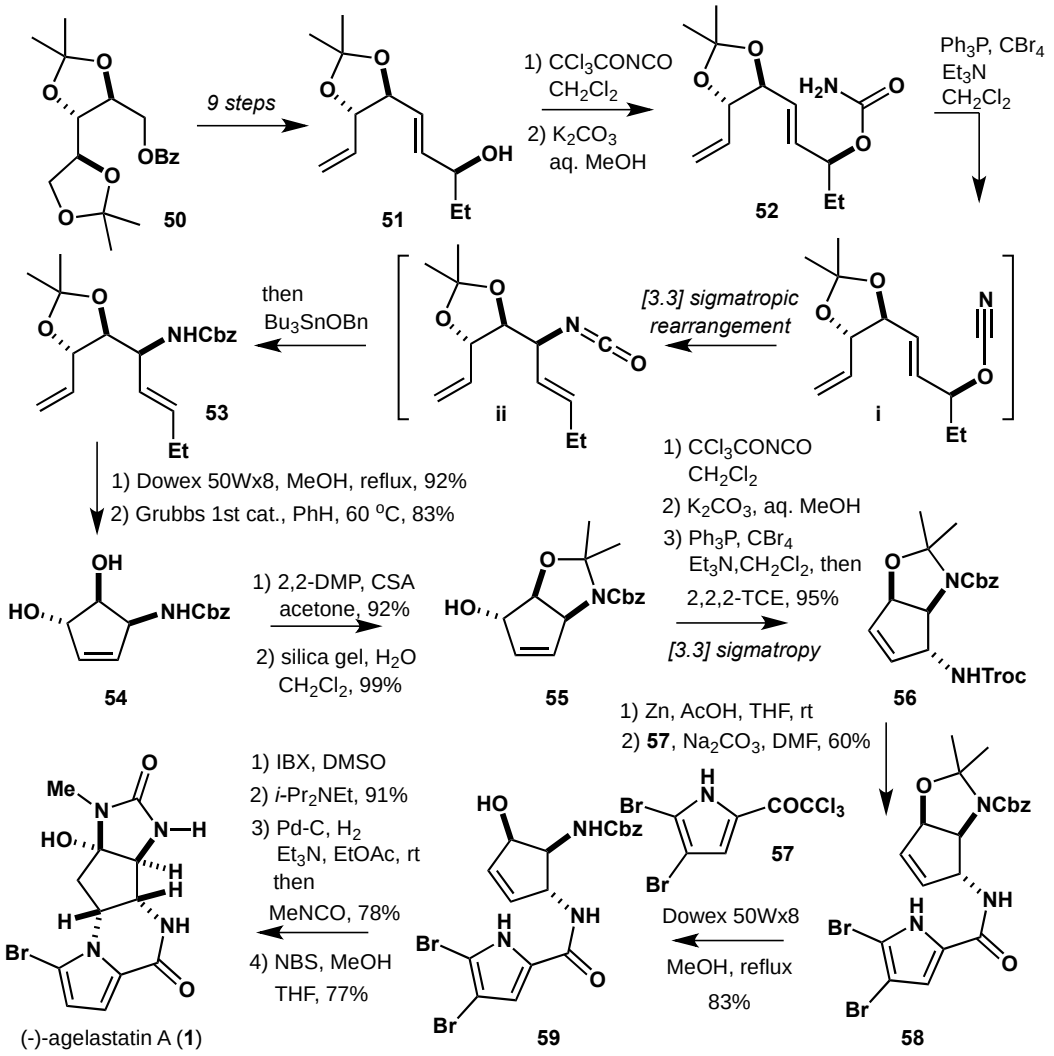
including carbamoylation with methyl isocyanate and subsequent removal of the *N*-tosyl and methoxy groups with SmI_2 to yield (+)-*ent*-agelastatin A. When compound **41** was reacted with amide **48** under the same conditions, the cascade di-alkylation took place to directly afford tricyclic pyrrolopiperazinone *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).[13] 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

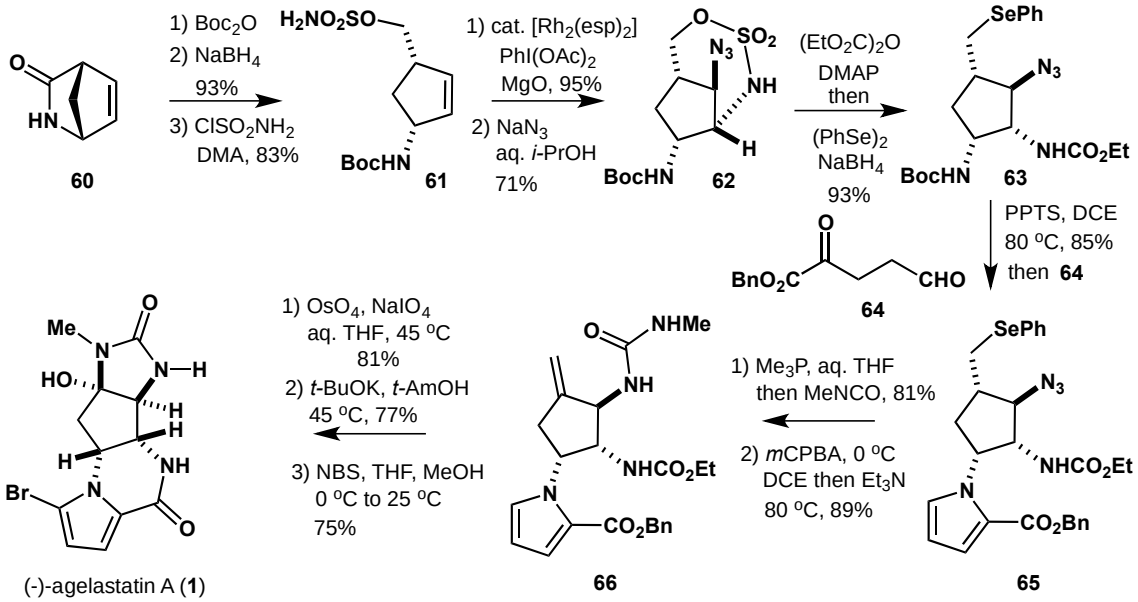
zinc/acetic acid in THF and subsequent amidation of the resultant amine with pyrrole **57** gave compound **58**. Compound **58** was deprotected under acidic conditions to afford alcohol **59**, which was further subjected to four-step manipulations involving IBX/DMSO oxidation, cyclization, hydrogenative carbamoylation with methyl isocyanate, and final bromination to yield (-)-agelastatin A (**1**).



Scheme 8. Ichikawa's total synthesis

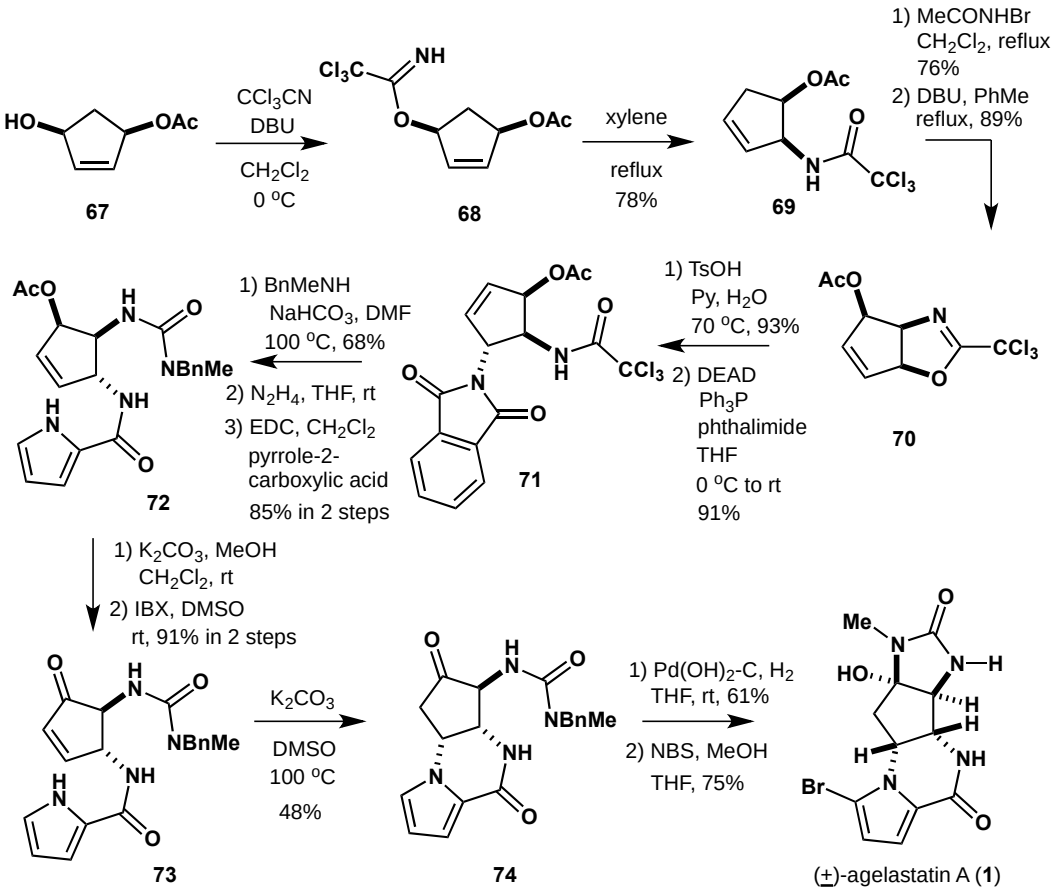
Du Bois (2009): Du Bois and Wehn accomplished an enantioselective synthesis of (-)-agelastatin A (**1**) that features a catalytic intramolecular aziridination of sulfamate **61** followed by ring-opening of the resultant aziridine with an azide ion to install a vicinal *trans*-diamino functionality in the B-ring (Scheme 9).[14] 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 *m*CPBA 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**.



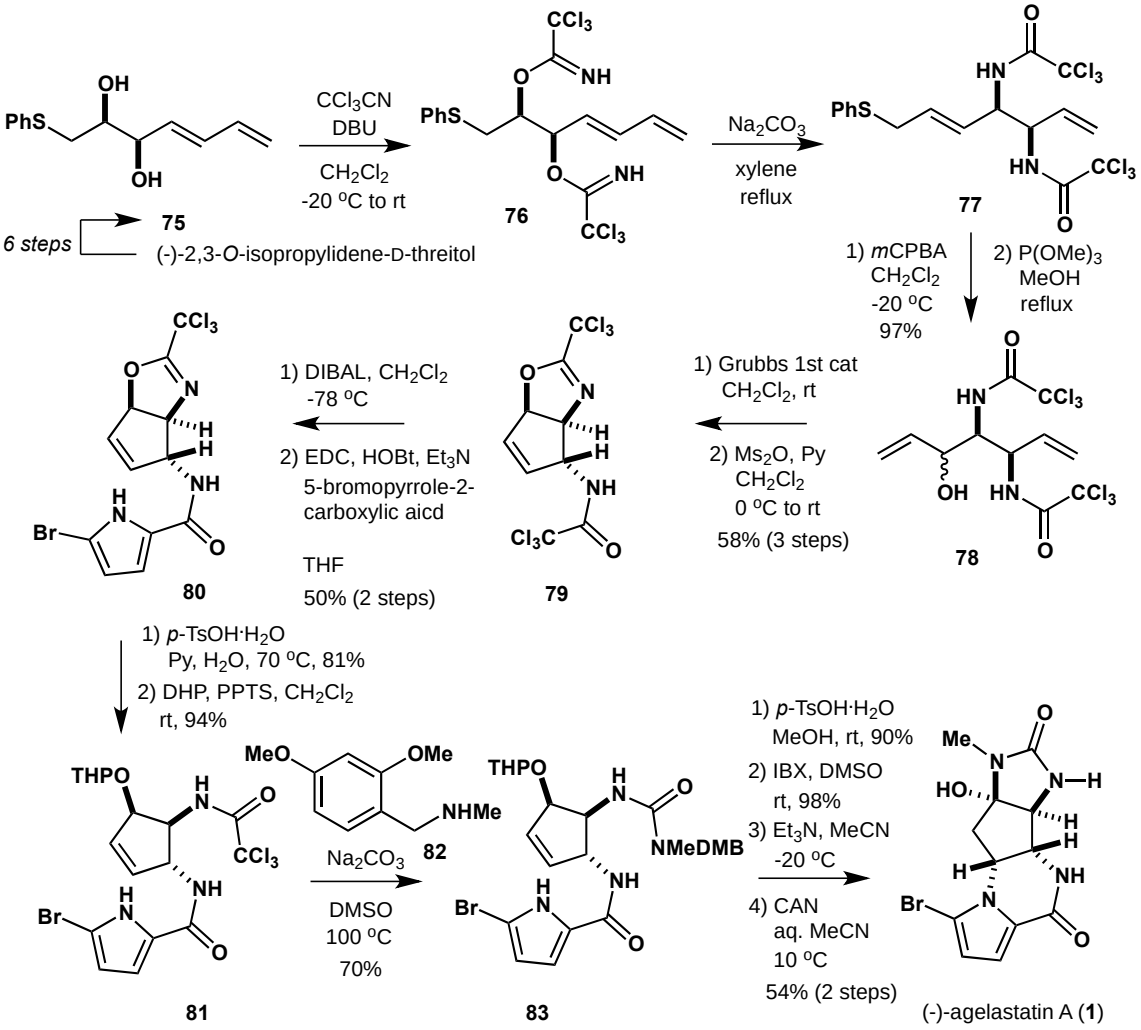
Scheme 9. Du Bois's total synthesis

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).[15] 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_2CO_3 in MeOH followed by oxidation with IBX/DMSO to provide enone **73** in 91% yield. Enone **73** was cyclized with K_2CO_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**).



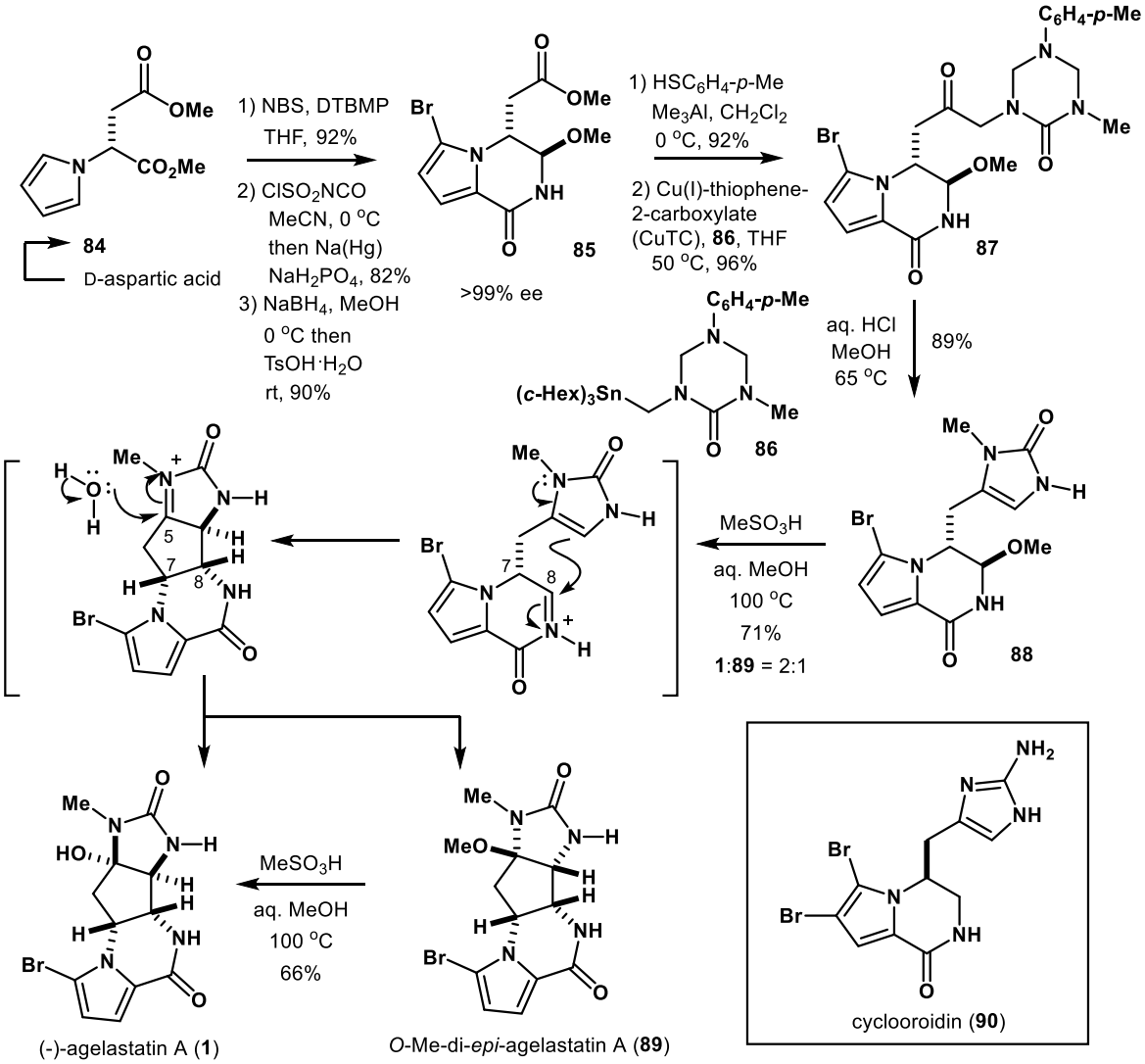
Scheme 10. Wardrop's total synthesis

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).[16] Their synthesis started with (-)-2,3-*O*-isopropylidene-D-threitol, which was converted into thiophenyldiol **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 *m*CPBA gave a sulfoxide that underwent rearrangement followed by reduction of the resultant sulfenyl ester with $P(OMe)_3$ 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**).

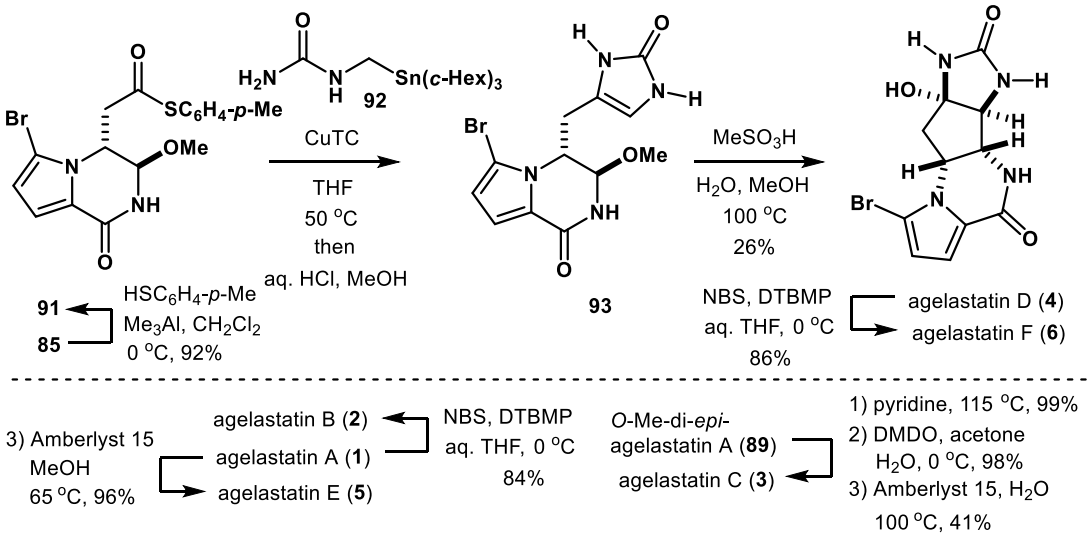


Scheme 11. Chida's total synthesis

Movassaghi (2010): A unique synthetic approach inspired by the hypothetical biogenesis of the agelastatin framework was developed by Movassaghi and co-workers (Scheme 12).[17] 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 $NaBH_4$ to generate bicyclic amide **85** in high yield with >99% ee. Compound **85** was transformed into keto-triazone **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 $MeSO_3H$ 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).

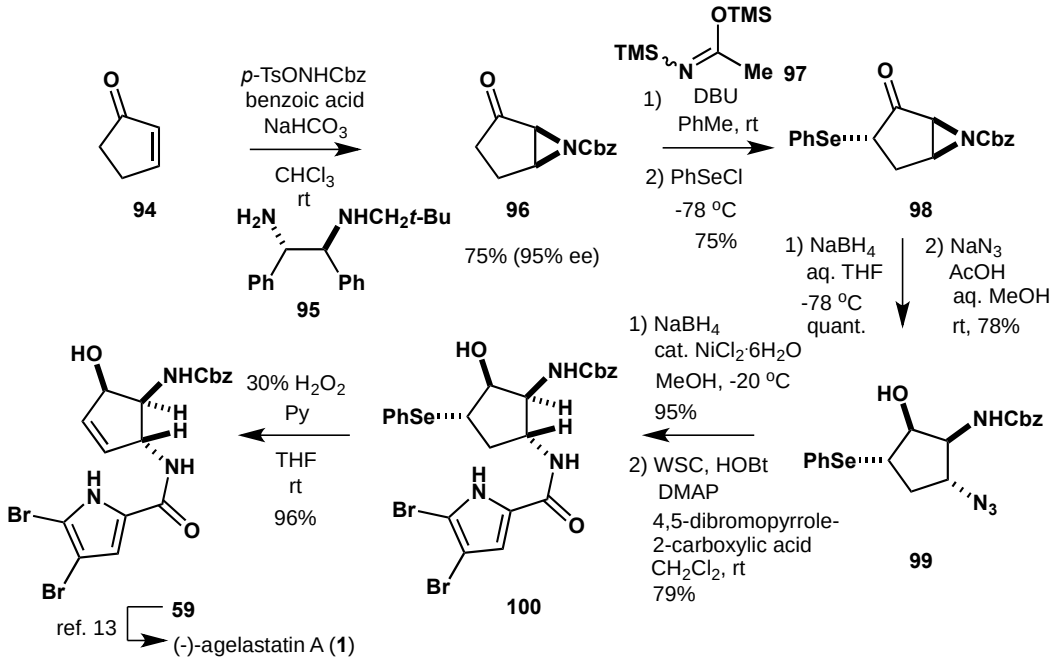


Scheme 12. Movassaghi's total synthesis



Scheme 13. Movassaghi's total synthesis of agelastatins B to F

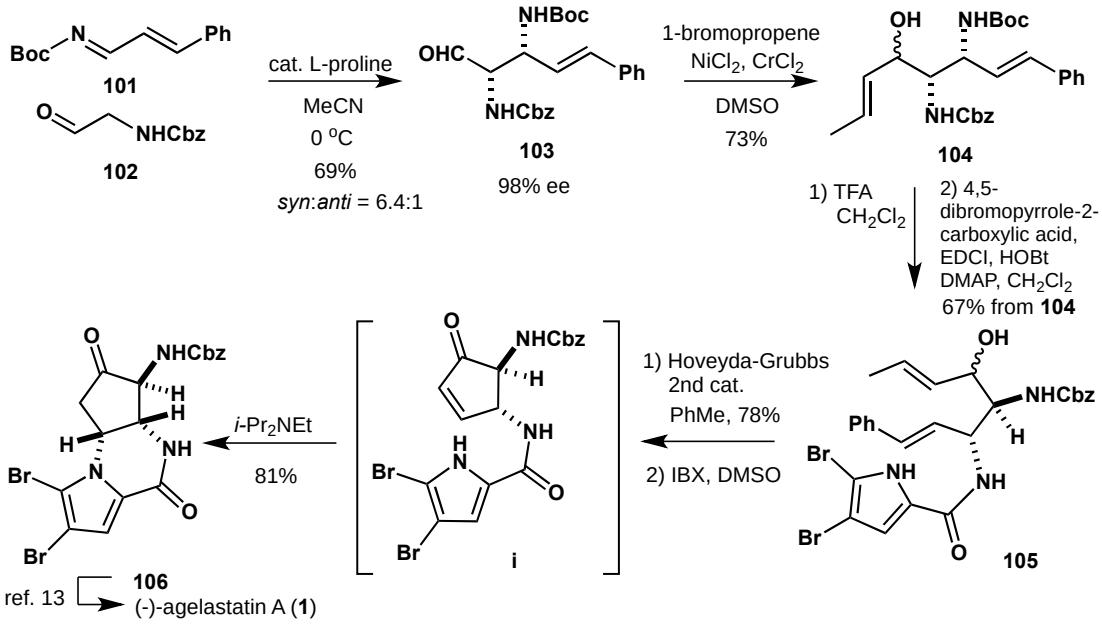
Hamada (2011): Hamada and co-workers devised an enantioselective route to (-)-agelastatin A (**1**), in which the nitrogen-substituted stereocenters were constructed by asymmetric aziridination of cyclopentenone (**94**) with *N*-tosyloxy benzyl tosyloxycarbamate in the presence of chiral diamine catalyst **95** (Scheme 14).[18] 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 $NaBH_4$ 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 H_2O_2 to furnish known compound **59**, culminating in the formal synthesis of (-)-**1**.



Scheme 14. Hamada's formal total synthesis

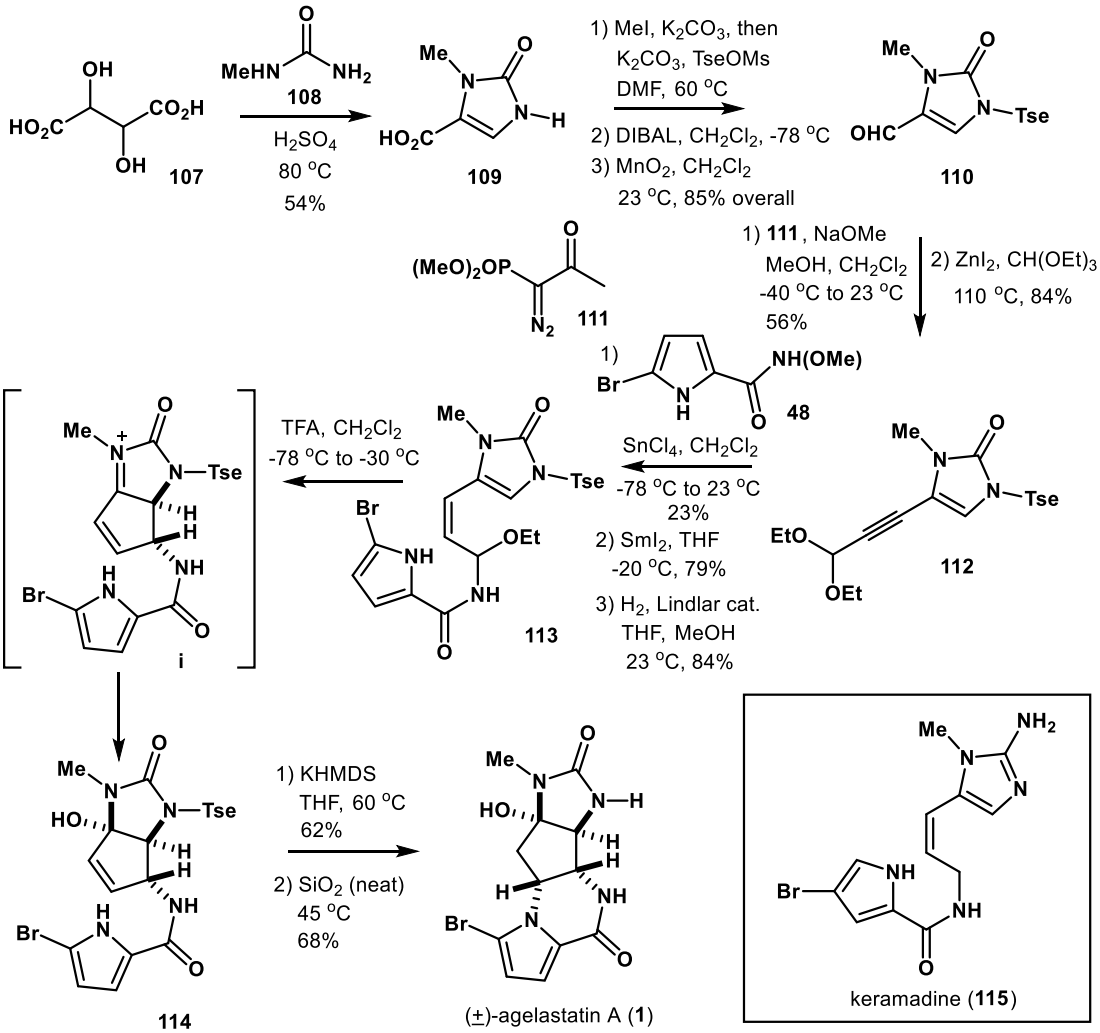
Maruoka (2012): In the synthesis route developed by Maruoka and co-workers, an enantioselective organocatalytic Mannich reaction of *N*-protected aminoacetaldehyde **102** was utilized to install the vicinal nitrogen functionality of (-)-agelastatin A (**1**) (Scheme 15).[19] The Mannich reaction of 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 $NiCl_2/CrCl_2$ 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

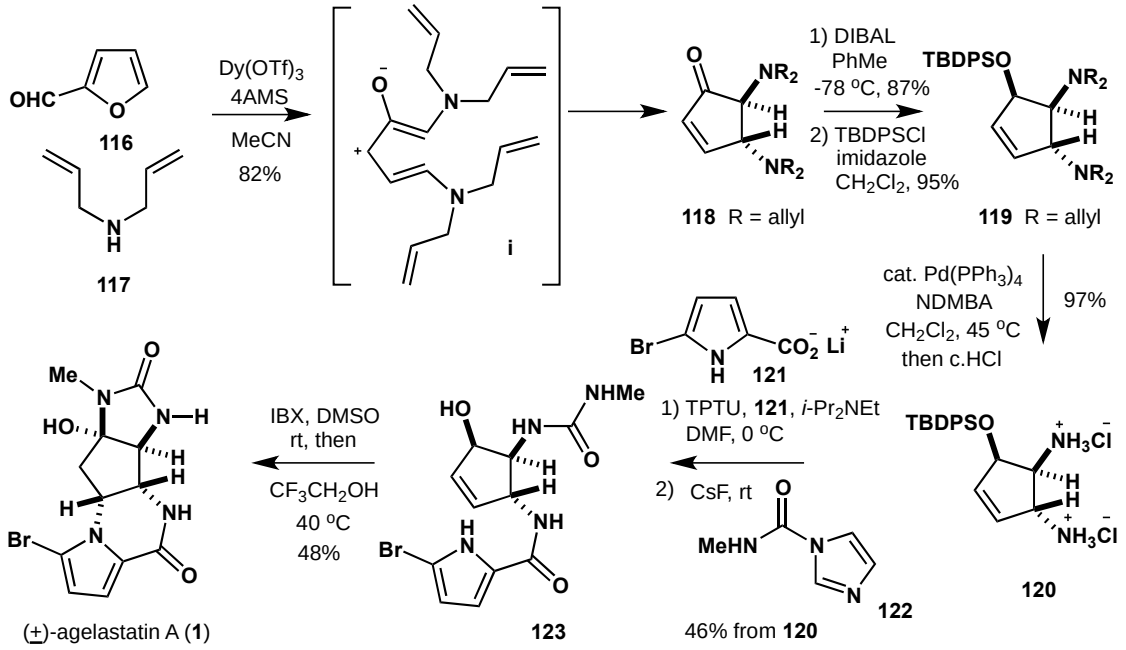
Romo (2012): Romo and Reyes reported a bioinspired total synthesis of (±)-agelastatin A (**1**) where a structural congener of keramadine (**115**), a potential biosynthetic precursor of **1**, was used as the key scaffold for the construction of the tetracyclic architecture (Scheme 16).[20] Their success 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 $SnCl_4$, and the methoxy group was reductively removed using SmI_2 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.



Scheme 16. Romo's total synthesis

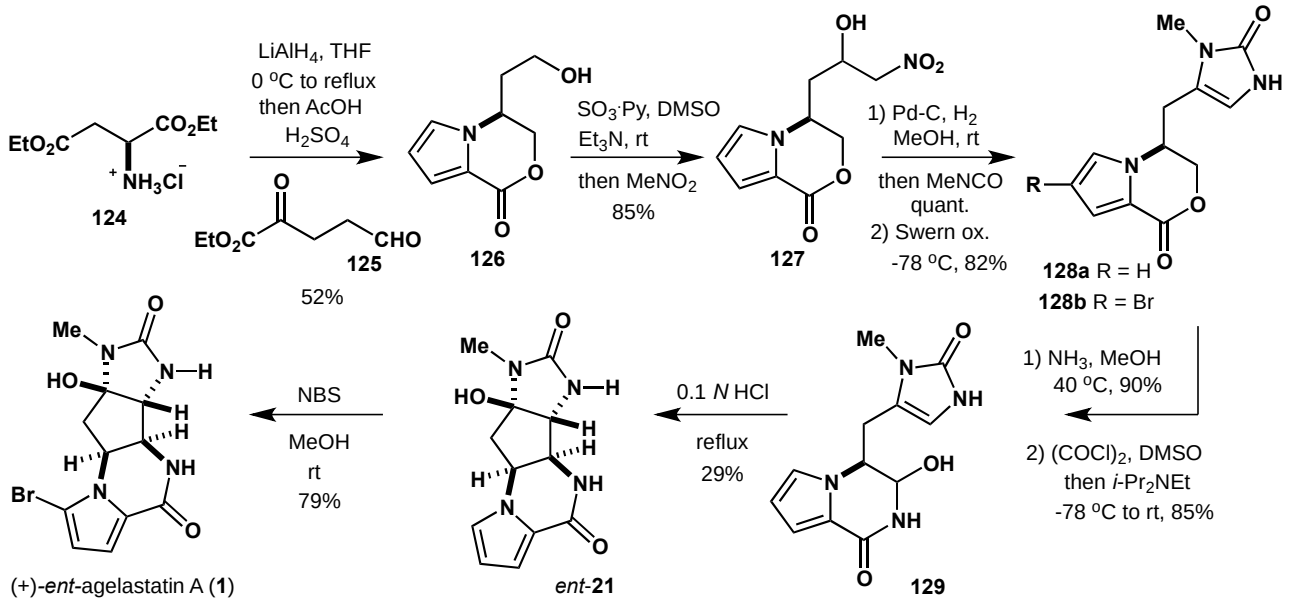
Batey (2013): Batey's synthesis demonstrates a highly concise construction of a C-ring with requisite vicinal diamino functionality, enabling the shortest access so far to the natural product in its racemic form (Scheme 17).[21] Cyclopentenone **118**, a reasonable C-ring motif, was rapidly constructed 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 $\pi 4_a$ 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 TBDPSCl 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.



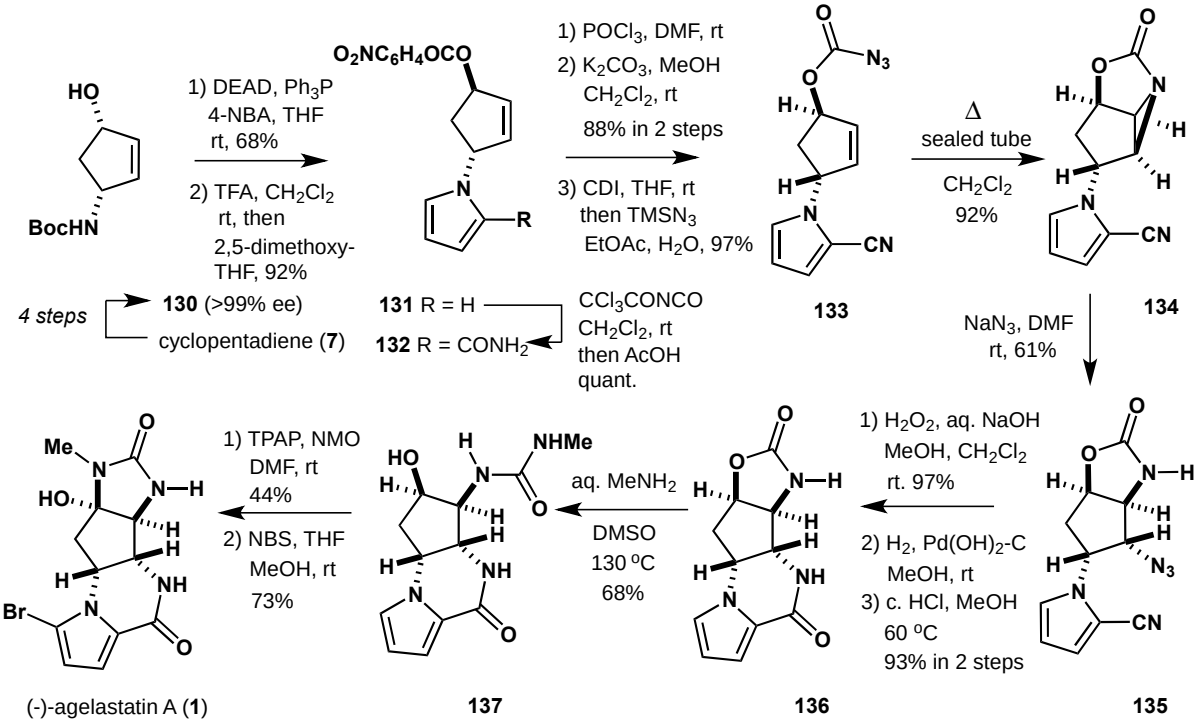
Scheme 17. Batey's total synthesis

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 $LiAlH_4$. 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 NH_3 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**).



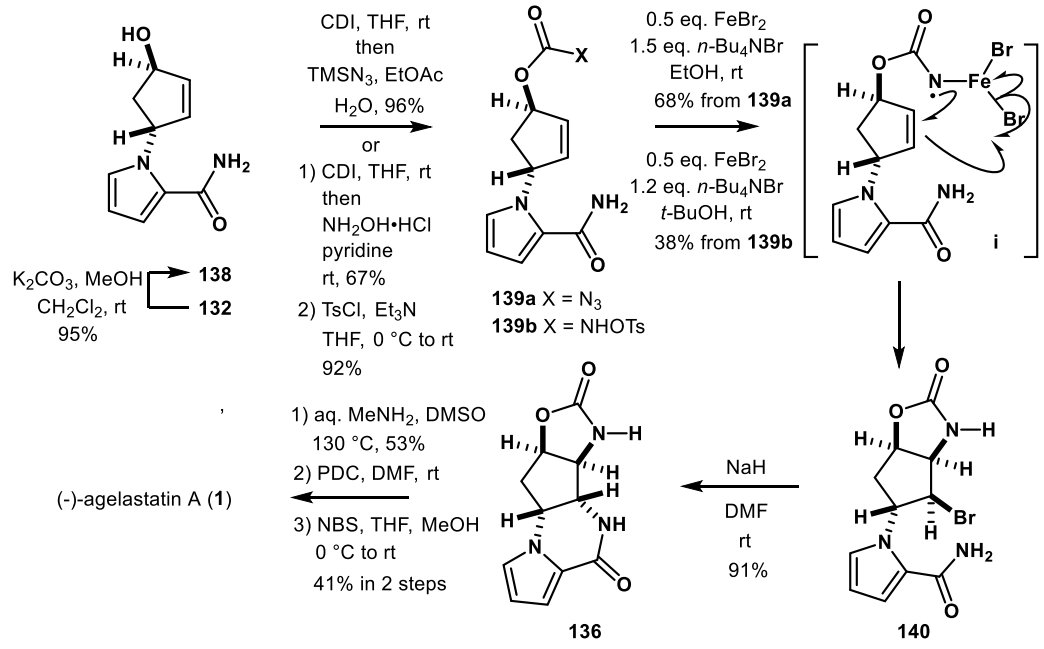
Scheme 18. Liang's total synthesis

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]



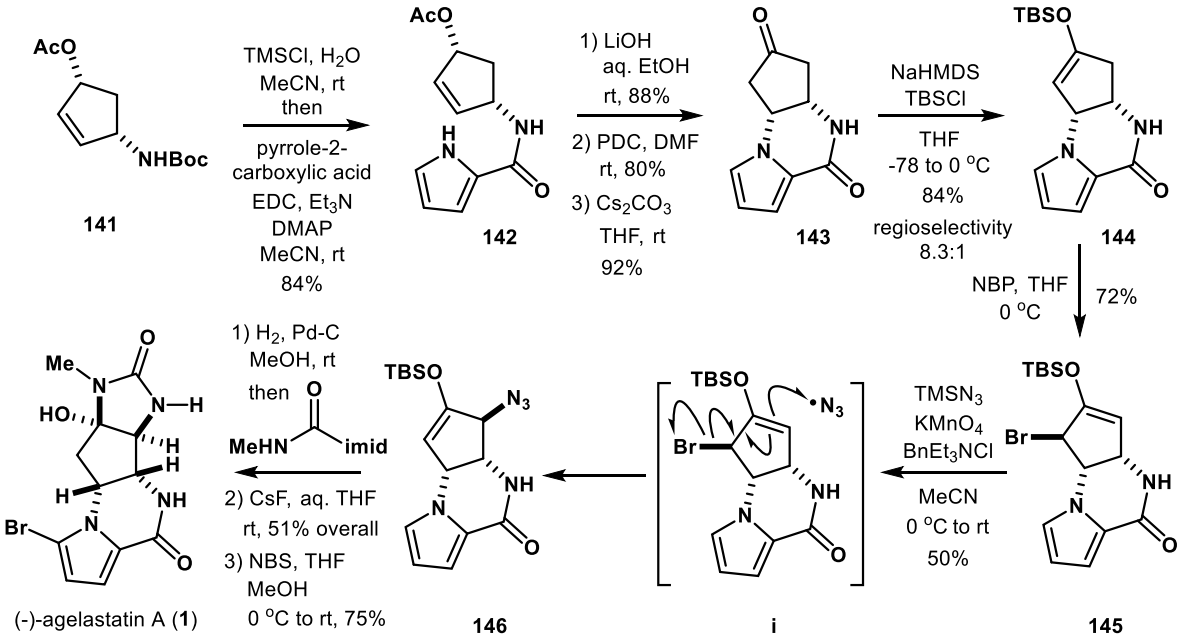
Scheme 19. Our total synthesis: 1st generation strategy

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 NaN_3 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).[23b,c] 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 Bu_4NBr salt as the bromide source. While this type of radical transformation was originally developed by Bach and co-workers[24] who used catalytic $FeCl_2$ in combination with stoichiometric TMSCl to effect aminochlorination, our new reagent system consisting of $FeBr_2/Bu_4NBr$ or $FeCl_2/Bu_4NCl$ 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.[25] 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**.



Scheme 20. Our total synthesis: 2nd and 3rd generation strategies

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_2CO_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_H2' radical azidation with a $KMnO_4/BnEt_3NCl/TMSN_3$ 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,[2c] Molinski,[26] Movassaghi,[27] Romo,[28] and our group[29] to validate the relevance of agelastatin A (**1**) as a promising

anticancer agent. 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).[2c] 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.

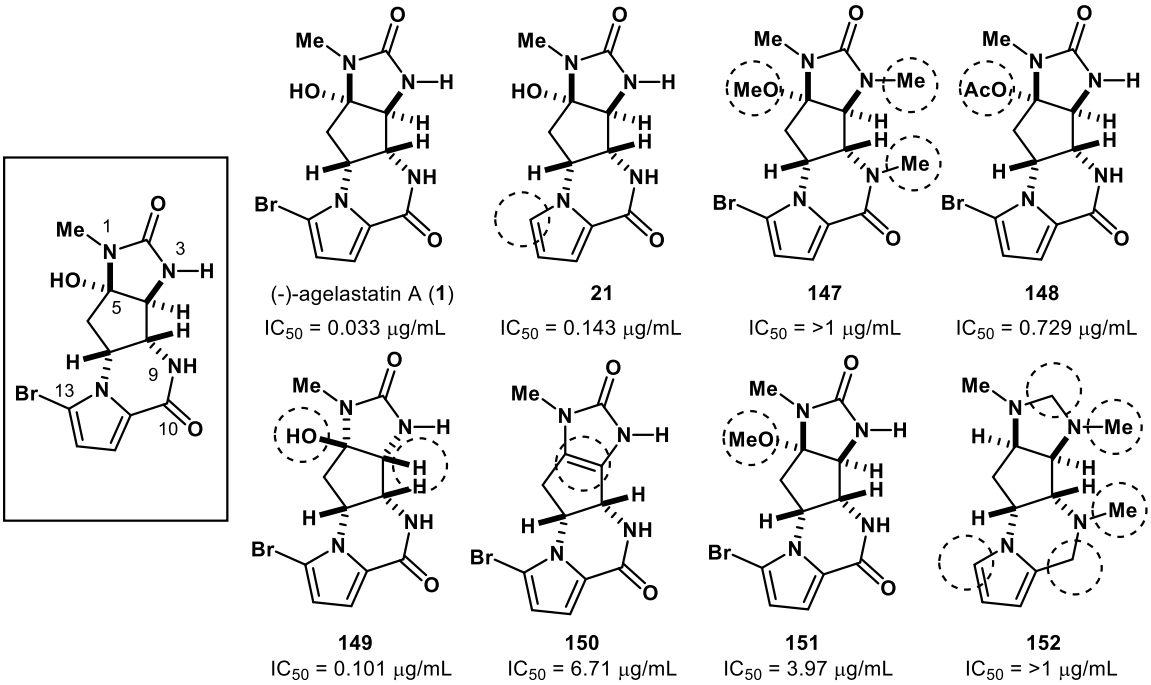
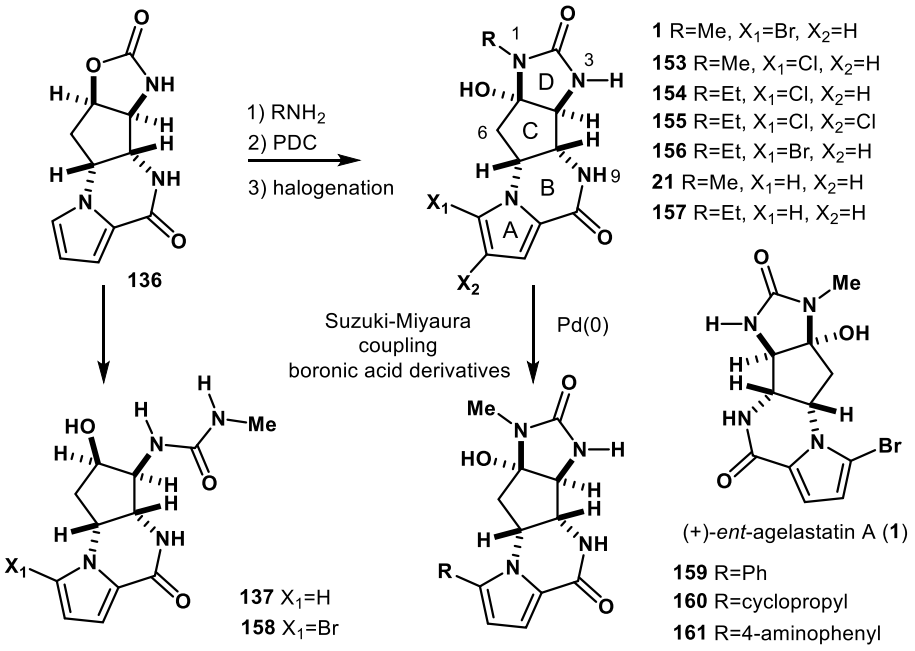


Figure 2. *In vitro* cytotoxicity (IC_{50} values [μg/mL]) of agelastatin analogues against L1210 cancer cell line reported by Pietra and co-workers. Circles indicate the modified structures.

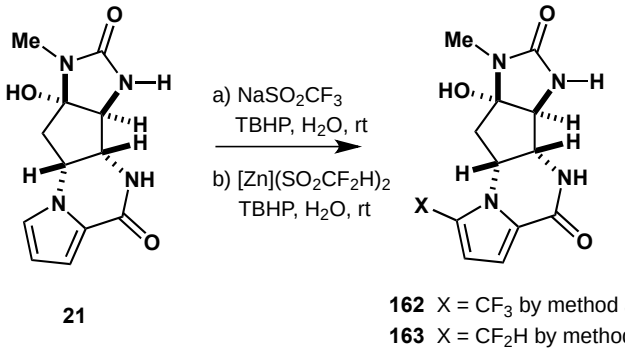
Our SAR studies on agelastatin analogues including N1-ethyl derivative **154** with C13-chlorine atom as well as C13/C14-dichlorinated analogue **155** have revealed that the structural modification of N1 and C13 substituents was tolerable in retaining the potent *in vitro* cytotoxicity. We have also demonstrated that the new agelastatin analogues potentially attenuate brain cancer, suggesting their *in vivo* therapeutic efficacy (Figure 3).[29] 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.[30]



compound	cytotoxicity (IC_{50}, μM)			compound	cytotoxicity (IC_{50}, μM)		
	DU145	Raji	MDA-MB-132		DU145	Raji	MDA-MB-132
1	2.3	0.14	2.8	**157**	>10	———	———
136	ND	>10	>10	**159**	6.0	3.8	1.5
153	1.2	0.18	0.08	**160**	5.0	0.40	1.0
154	2.6	0.30	0.28	**161**	>10	———	———
155	1.4	0.62	0.44	**137**	ND	>10	>10
156	>10	———	———	**158**	ND	>10	>10
21	>10	3.16	3.0	(+)-*ent*-**1**	>10	———	———

Figure 3. Our SAR studies on agelastatin analogues

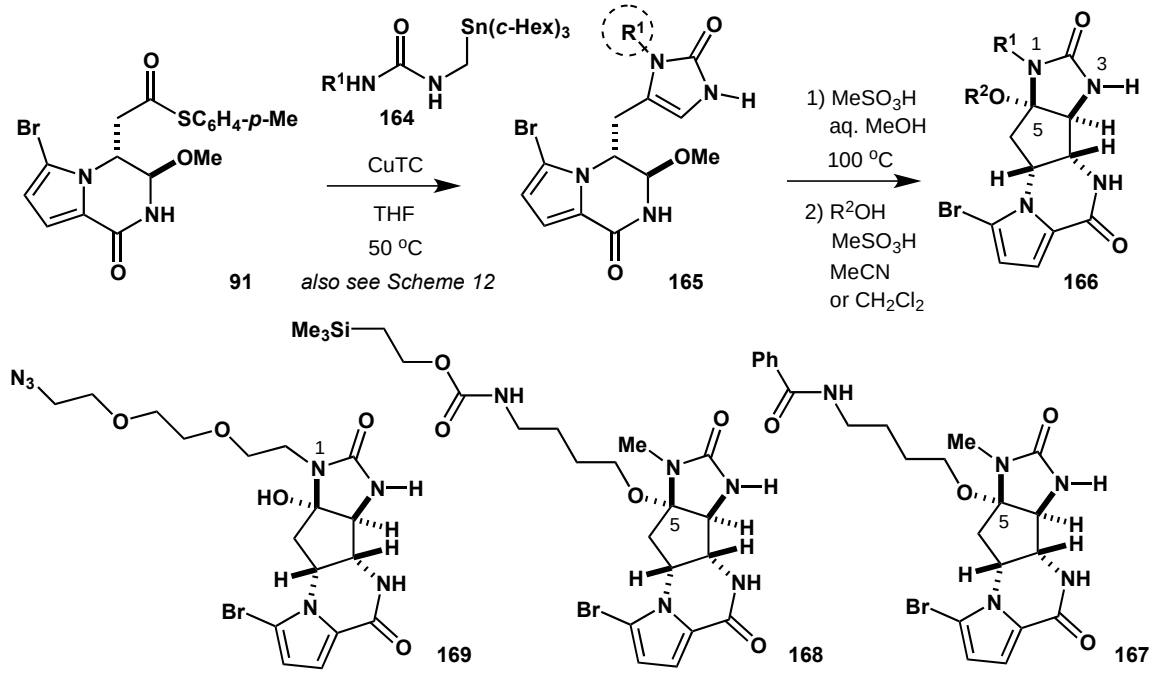
While in line with the observations reported by the previous SAR studies where only C13 structural modification was tolerated, Molinski's comprehensive SAR study led to the discovery of highly potent fluorinated C13-CF_3 analogue **162** that exerts antiproliferative activity against chronic lymphocytic leukemia (CLL) cell lines (Figure 4).[26] Fluorinated analogues **162** and **163** were synthesized by direct trifluoromethylation using $NaSO_2CF_3$/TBHP and difluoromethylation with $Zn(SO_2CF_2H)_2$/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.



compound	*in vitro* cytotoxicity EC_{50} (mM)			
	CLL1	CLL2	JVM-2	HeLa
(-)-agelastatin A (**1**)	0.31	0.16	0.28	0.11
162 X = CF_3	0.43	0.064	0.66	0.15
163 X = CF_2H	13.0	5.44	4.66	4.01

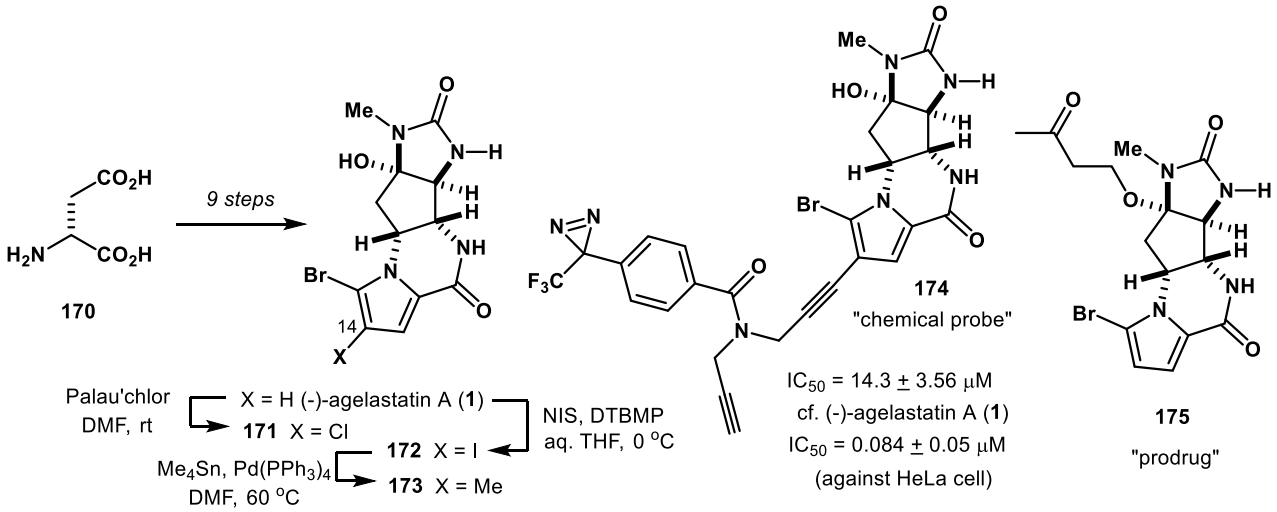
Figure 4. C13-fluoromethyl agelastatin analogues devised by Molinski and co-workers

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^2OH) (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).[28] 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.



Scheme 23. SAR study by Romo and co-workers

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.[31] 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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