

Biosynthesis, Asymmetric Synthesis, and Pharmacology Including Cellular Targets of the Pyrrole-2-Aminoimidazole Marine Alkaloids

Ali Al-Mourabit,^{*1} Manuel A. Zancanella,² Supriya Tilvi,³ and Daniel Romo^{*4}

The pyrrole-2-aminoimidazole (P-2-AI) alkaloids are a growing family of marine alkaloids, now numbering well over 150 members, with high topographical information content. Their intriguing structural complexity, rich and compact stereochemical content, high N to C ratio (~1:2), and increasingly studied biological activities are attracting a growing number of researchers from numerous disciplines world-wide. This review surveys advances in this area with a focus on the structural diversity, biosynthetic hypotheses with increasing but still rare verifying experimental studies, asymmetric syntheses, and biological studies including cellular target receptor isolation studies of this stimulating and exciting alkaloid family.

¹ Centre de Recherche de Gif-sur-Yvette, Institut de Chimie des Substances Naturelles, UPR 2301, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

² the address of Manuel A. Zancanella

³ Bio-organic Chemistry laboratory, National Institute of Oceanography(CSIR), Dona Paula, Goa, India, 403 004

⁴ Department of Chemistry Texas A&M University College Station, TX 77842-3012

^{*}To whom correspondence should be addressed.

^a Email: Ali.Almourabit@icsn.cnrs-gif.fr

^d Email: romo@tamu.edu

1 Introduction

The ability of the exquisite biosynthetic machinery found in living organisms to first construct a simple building block with multi-faceted reactivity such as acetylCoA (**1**)/malonyl CoA(**2**), dimethylallylpyrophosphate (**3**), or shikimic acid (**4**) and utilize it to construct a plethora of fascinating chemical structures including terpenes, polyketides, and alkaloids respectively, is awe-inspiring (*e.g.* **7-10**, Figure 1). The energy expenditure in generating such complex structures, including biosynthesis of the complex biosynthetic enzymes required for their synthesis, suggests that these molecules must serve a purpose and indeed these genetically-encoded small molecules¹ are often associated with biological activity. The generation of the pyrrole-2-amino imidazole (P-2-AI) family of alkaloids, hypothesized to originate from clathroдин/oroidin (**5/6**) (Figure 1) (a.k.a. clathroдин- or oroidin-derived alkaloids), is another amazing example of such a feat of complex biosynthetic machinery found in marine sponges or their symbionts to construct an arsenal of fascinating and potentially therapeutically useful bioactive molecules (*e.g.* palau'amine (**10**)). While detailed molecular ecological studies of these compounds may ultimately reveal their utility for the producing organism, if any, the present utility of such natural products can be found in the resultant inspiration given to isolation chemists to tackle their isolation and structure elucidation, synthetic chemists in assaulting their synthesis, biochemists and both cell and molecular biologists to understand their bioactivity at the molecular level, and ultimately pharmaceutical companies to translate these natural products into potential drugs for chemotherapy to combat human disease.

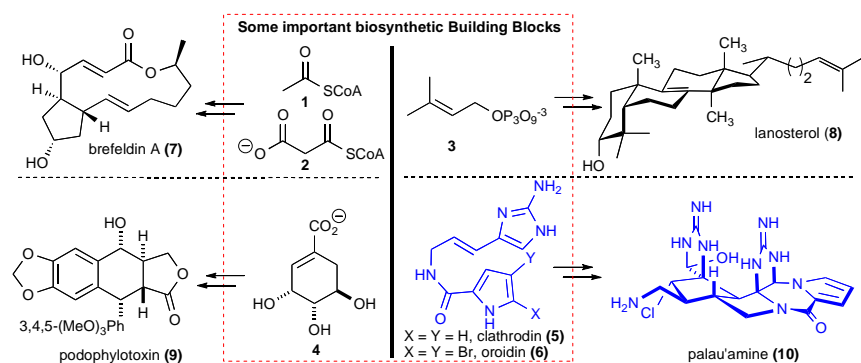


Fig. 1 Common biosynthetic building blocks including dimethylallylpyrophosphate (**3**), acetyl and malonyl CoA (**1,2**), shikimic acid (**4**) leading to the steroid lanosterol (**8**), the polyketide brefeldin A (**7**), and the alkaloid podophylotoxin (**9**), respectively and the subject of this review, new building blocks, clathroдин and oroidin (**5,6**), leading to complex P-2-AIs including palau'amine (**10**). Ali-
Check for For structure 3 $\text{OP}_3\text{O}_9^{-3}$ or $-\text{OP}_2\text{O}_5^{-3}$

The P-2-AI alkaloids constitute a biogenetically unique natural product family which to date have been exclusively isolated from marine sponges. Now numbering more than 150 members, the last decade has witnessed significantly increased interest in this alkaloid family along several lines of investigation due to the continued isolation of more complex members with novel and unique molecular architecture and biological activity. These marine alkaloids correlate well with the phylogenetic relationships within families of sponges belonging to Agelasida and Axinellida (Halichondrida) orders. In addition to further understanding of their ecological roles, the biosynthetic origin of the P-2-AIs remains a very intriguing question. The technically restricted use and low incorporation levels of isotopically labeled precursors employed in early sponge feeding experiments² left room for speculation on the biogenetic origin of early biosynthetic intermediates *e.g.* clathrocin (**5**) or oroidin (**6**). While biosynthetic details are completely lacking, biomechanistic analysis of isolated P-2-AIs and successful synthetic strategies premised on speculative biosynthetic pathways have provided some indirect evidence regarding biosynthetic feasibility. From the standpoint that inherent reactivity of biosynthetic intermediates may offer clues about actual biosynthetic pathways leading to various complex P-2-AIs, isolation of an increasing number of putative biosynthetic intermediates has also been extremely important in developing biosynthetic hypotheses. The evolution of their biogenetic pathways appears to be based in large part on the very interesting ambivalent reactivity embedded in the 2-aminoimidazole nucleus (Figure 2). Thus, great effort has been devoted to unveiling the mechanistic and stereochemical details of reactions involving these pivotal intermediates.

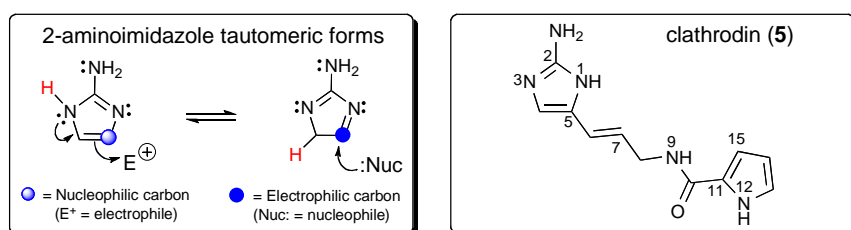


Fig. 2 Dual reactivity of the common 2-aminoimidazole nucleus found in the P-2-AIs metabolites and numbering used in this review of the postulated precursor, clathrocin (**5**), containing the 2-aminoimidazole nucleus.

Several reviews describing biosynthetic hypotheses of the so-called bromopyrrole or pyrrole-imidazole alkaloids isolated from *Agelas* genus (Agelasida order) were reviewed by Braekman in 1992.³ In this review, this family is named pyrrole-2-aminoimidazole (P-2-AIs), to highlight both participatory heterocycles and the contribution of the amino group to the reactivity pattern of the

imidazole ring.

In this review, we have introduced a numbering system that is meant to amplify the importance of the 2-aminoimidazole nucleus, which is key to the molecular diversity of the P-2-AIs (Figure 2). The carbon chain begins with the aminoimidazole and numbering continues to the branched pyrrole-2-carboxamide. This numbering system is also applied to dimeric structures and partially truncated natural products and atoms keep their original numbering for biomechanistic reasons. The use of trivial names is also adopted in some cases for practical reasons.

The chemical reactivity and hypothetical chemical pathways leading to complex P-2-AI metabolites were reported by Al-Mourabit in 2001.⁴ Extension of these hypotheses to include stereochemical relationships, especially in light of the structural revision of palau'amine (**10**) and extension to the recently isolated tetrameric P-2-AI members, was reported by Köck and Baran in a 2007 mini-review.⁵ Several review articles have compiled synthetic advances within the P-2-AI metabolites and partial descriptions of biosynthetic aspects of selected members of this family.⁶ The present review focuses on the structural diversity of the P-2-AIs in the context of discussing possible biosynthetic origins of these alkaloids and when available, substantiating synthetic studies that demonstrate the suggested reactivity. A review of only asymmetric synthesis of P-2-AIs is included giving the relevance to subsequent pharmacological and biological studies including scarce but growing structure-activity and biological receptor isolation studies, which are also described.

2 Structural Diversity, Proposed Biosynthetic Hypotheses, and Biosynthetic Studies of the Pyrrole-2-Aminoimidazole (P-2-AIs) Alkaloids

To conceive and eventually discover the operative overall biosynthetic pathways leading to the complex molecular architectures of these alkaloids, several lines of investigation have been undertaken. These studies are aimed at understanding the origin and the reactivity of simple precursors *e.g.* clathrocin (**5**), oroidin (**6**), debromodispacamide B (**11**) (Figure 3) and possible pathways for their conversion to higher order P-2-AIs. These include:

1. Biochemical investigations involving incorporation studies linking early precursors, presumably amino acids, to monomers like clathrocin or debromodispacamide and their brominated derivatives (*e.g.* oroidin) and incorporation studies of labeled putative intermediates into higher order P-2-AIs.

2. Chemical reactivity studies of presumed precursors including clathrocin, oroidin, or debromodispacamide B to higher order P-2-AIs including the exquisite control exerted by the unique multifaceted chemical behavior of the 2-aminoimidazole nucleus (*vide supra*).
3. Synthetic studies premised on presumed biosynthetic pathways ('bioinspired' syntheses) to determine the feasibility of such transformations while also providing unique and often highly concise routes to these complex molecules.

Based on the combination of newly isolated P-2-AI members and 'bioinspired' synthetic studies, highly analogous biosynthetic hypotheses have been posited by several groups that appear to be converging. The global biosynthetic pathways include formation of presumed building blocks (*e.g.* clathrocin (**5**) and oroidin (**6**)) from amino acid precursors and further transformation and elaboration of these building blocks into more complex P-2-AIs (Figure 3). Furthermore, degradation of more or less elaborated P-2-AI members could explain the isolation of several simpler P-2-AI derivatives. Hence, aminoimidazolyl propeneamine (AAPE) (**12**) is possibly a case in point for such degradation, as it is a "misleading" compound with respect to biosynthetic origins. While it was considered to be an early precursor for oroidin (**6**) through acylation with 4,5-dibromopyrrole-2-carboxylic acid, the pseudodipeptide route is favored over amide hydrolysis of oroidin.^{7,8} In addition, the bromopyrrole unit is found in other seemingly related but distinctly different natural products (*e.g.* clathramides and manzacidins) raising the possibility that these are shunt metabolites resulting from mixing of clathrocin-utilizing and alternative biosynthetic pathways (Figure 3).

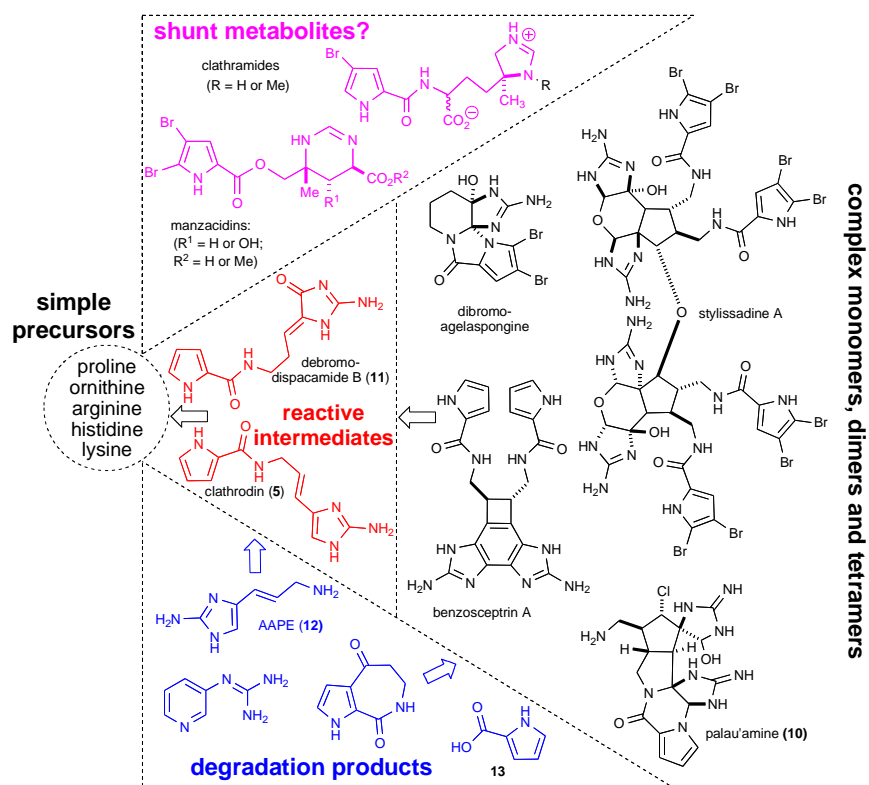
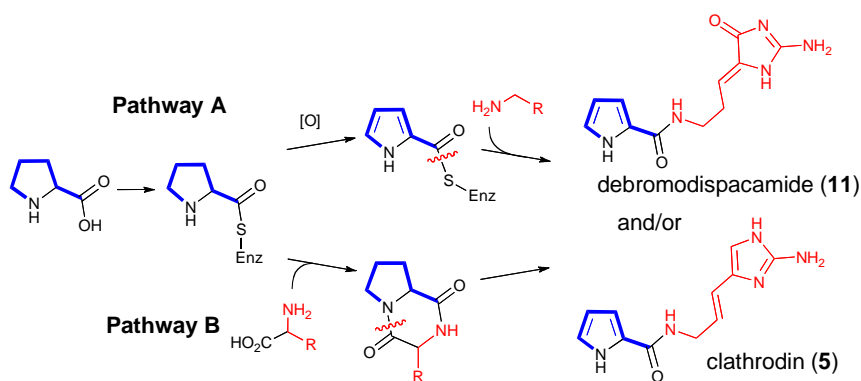


Fig. 3 Putative biosynthetic progression from simple amino acids to monomeric, dimeric, and tetrameric P-2-AIs, degradation products and possible shunt metabolites.

2.1. Biosynthesis from simple early precursors: pre-clathrocin events

The majority of reported biosynthetic hypotheses for formation of the P-2-AI building blocks, clathrocin (**5**) and its brominated derivatives, originate from proteinogenic amino acids. The postulated key steps rely on several presumed precursors isolated from phylogenetically related sponges or co-isolated together with other higher order P-2-AIs. While it is reasonable to speculate that the pyrrole-2-carboxylic acid unit (**13**) (Figure 3) is derived from proline oxidation (Scheme 1a),⁹ several alternative possibilities have been suggested for the origin of the 2-amino-5-(3-amino) propylimidazole unit including diketopiperazine intermediates (Scheme 1b).

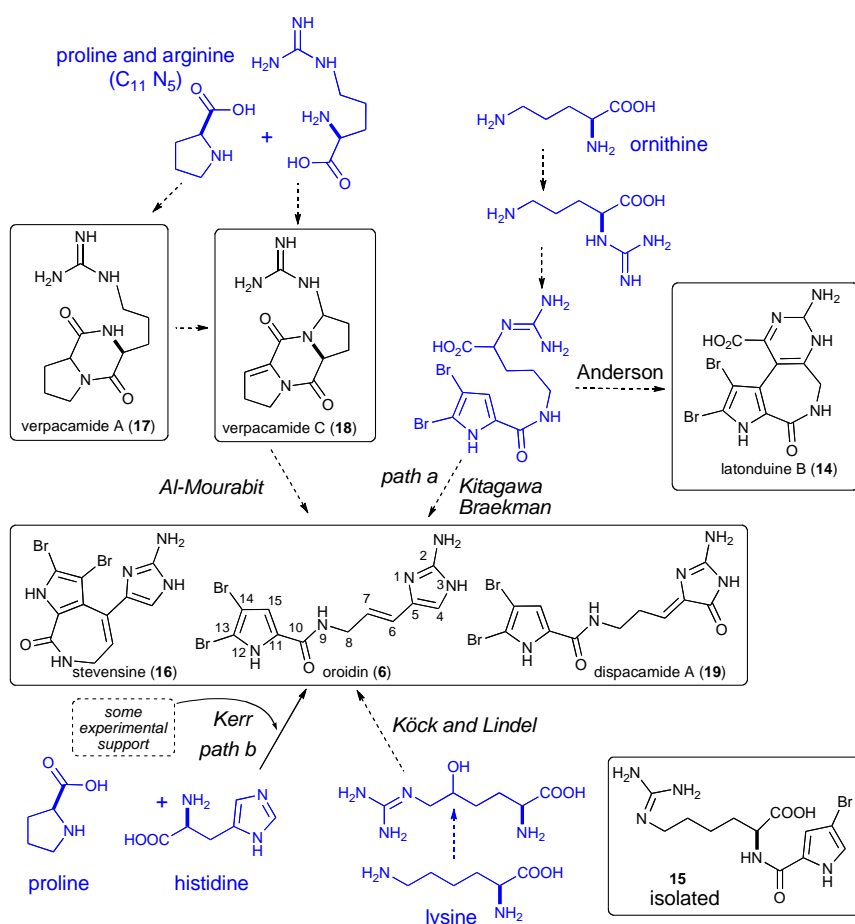


Scheme 1 Possible routes to debromodispacamide B (**11**) and clathrocin (**5**) via (a) **Pathway A**: pyrrole oxidation and nucleophilic addition to an activated acyl pyrrole (b) **Pathway B**: dipeptide-based synthesis through diketopiperazines and intramolecular rearrangements

Kitagawa, and later Braeckman³ proposed that proline, ornithine, and guanidine are probable precursors of both the bromopyrrole and 2-aminoimidazolinone moieties found in P-2-AIs. Following this proposal, Kerr reported the incorporation of the ¹⁴C-labeled proline, ornithine and histidine into the cyclized oroidin derivative stevensine (**16**) (Scheme 2).² How the incorporation of histidine into oroidin (**6**) could proceed remains an interesting question. Unfortunately, only low levels of radioactivity and specific incorporation levels were observed with amino acids in the Kerr experiment ([U-¹⁴C]-His, 1460 dpm (0.026%) and [U-¹⁴C]-ornithine, 1300 dpm (0.024%), and these results along with the lack of *in vivo* biosynthetic studies, still leaves the question unanswered. Based on some synthetic studies and the isolation of a C₁₁N₅ diketopiperazine, verpacamide C (**18**), Al-Mourabit and coworkers proposed proline or arginine as early precursors for both the pyrrole-carboxylic acid and the 2-aminoimidazole moieties.⁸ As there is no direct biochemical evidence for the structure of the early precursors involved in the formation of the 2-aminoimidazole and 2-aminoimidazolone motifs, hypotheses on the conversion of proline, ornithine, histidine, arginine, or lysine into the P-2-AI metabolites remain purely speculative at the present time. The ideas put forward with a particular focus on the origin of the 2-aminoimidazole moiety are summarized in Scheme 2.

In the Kitagawa/Braekman proposal suggesting ornithine as the precursor, it was envisioned that the α -amino acid function was transformed into 2-aminoimidazolinone precursors *via* a guanidine intermediate (Scheme 2, path a).^{3,7} This proposal was recently supported by the isolation of the latonduines (**14**)¹⁰ although the absence of a true 2-aminoimidazole nucleus leaves the question of their belonging to P-2-AI alkaloids open. In addition, the pyrrole-2-carboxylic acid is a very common acylating agent present in several marine organisms and

microorganisms, and its presence alone is certainly not sufficient evidence for a relationship with P-2-AI biosynthesis.¹¹ Based on the isolation of a pyrrole-lysine pseudo-dipeptide (**15**) (Scheme 2), Köck and Lindel proposed a lysine precursor in which the terminal amine was converted into a guanidine and then into a 2-aminoimidazole after oxidative hydroxylation.¹² Lindel subsequently synthesized aminohomohistidine from α -hydroxy-(*L*)-lysine demonstrating possible chemistry enabling synthesis of the 2-aminoimidazole moiety from an oxidized lysine motif.¹³ In addition, Lindel and coworkers reported the biomimetic synthesis of cyclooroidin itself in good yield.¹⁴ Following the first experimental biosynthetic study toward stevensine (**16**) performed by Kerr, further progress towards substantiating biomechanistic speculations experimentally is lacking and the quest to provide support for or against various proposals could uncover unexpected and thus intriguing biosynthetic processes. One such study involving the synthesis of ¹⁵N-labelled oroidin (**6**) has recently appeared (*vide infra*).¹⁵



Scheme 2 Proposed biosyntheses of the P-2-AIs building blocks

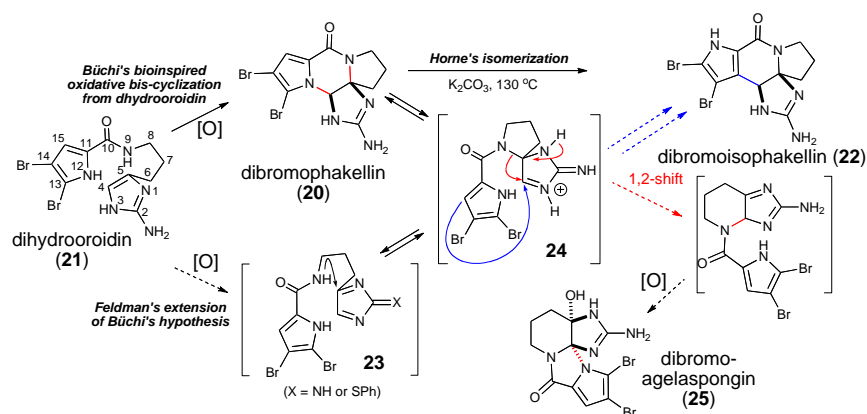
2.2. Post-clathrocin/oroidin biosynthetic events including controlled cyclizations

To date, no experimental biosynthetic studies have been reported testing the incorporation of linear derivatives like clathrocin (**5**) or oroidin (**6**) into polycyclized, complex P-2-AI members despite the almost assumed biosynthetic connection. However, recently Molinski and Romo reported the first investigations in this direction aimed at monitoring the incorporation of synthetic ¹⁵N-labeled oroidin (**6**) into complex P-2-AIs by both ¹⁵N NMR and FT-MS.¹⁵ While this initial report only described the sensitivity of these methods, planned sponge-feeding experiments may provide the first experimental evidence for the common assumption that clathrocin/oroidin are precursors to more complex P-2-AIs.

The hypothetical chemical pathways described below are primarily based on isolated P-2-AIs, the established reactivity of clathrocin/oroidin, and in some cases successful biomimetic or more accurately 'bioinspired' reaction sequences given that the biosynthesis of these natural products has not yet been elucidated.

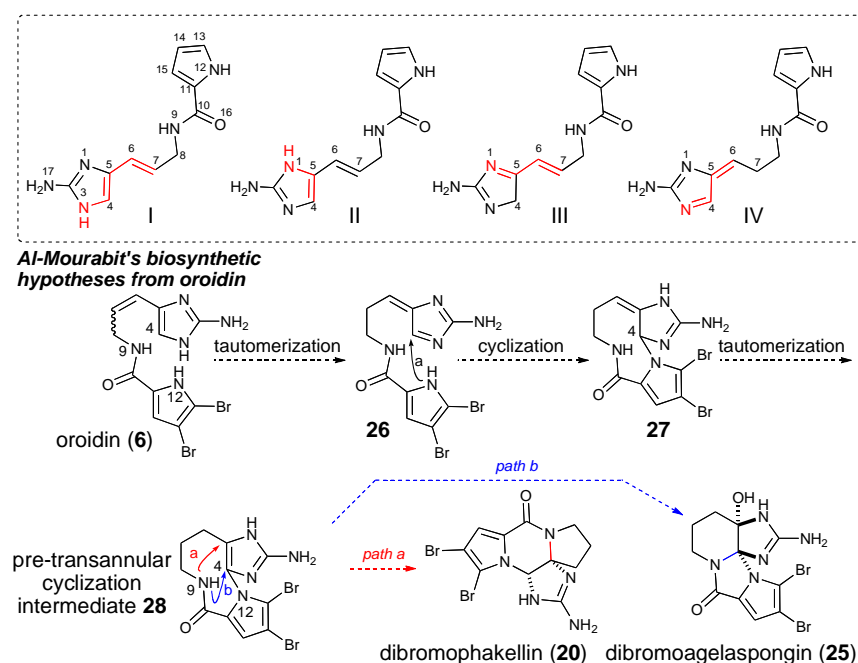
2.3. Polycyclic P-2-AI monomers

Since the landmark 'bioinspired' synthesis of dibromophakellin (**20**) from dihydrooroidin (**21**) by Büchi in 1982,¹⁶ oxidative strategies taking advantage of the reactivity of the 2-aminoimidazole nucleus have been widely employed for the synthesis of several P-2-AI monomeric members. In extensions of Büchi's seminal work, Horne reported improved conditions for oxidative cyclizations to phakellins and also the rearrangement of dibromophakellin (**20**) to dibromoisophakellin (**22**) at 130 °C involving a net N→C migration presumably involving an iminium intermediate **24**.¹⁷ Feldman utilized sulfur oxidation by way of a Pummerer reaction to access the core structures of dibromophakellstatin and dibromoagelaspongin and proposed a key spiro intermediate **24** that overlaps with biomechanistic speculations.¹⁸



Scheme 3 Büchi's landmark 'bioinspired' synthesis of dibromophakellin (**20**) and more recent optimizations and isomerizations/rearrangements

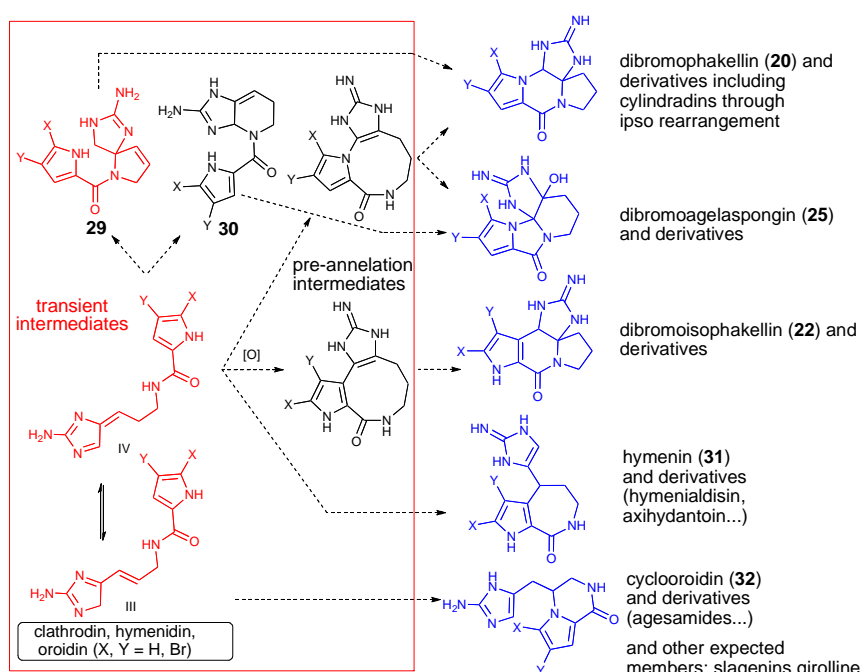
However, Horne, who nicely emulated Büchi's oxidative cyclization chemistry, also reported the non-propensity of spiro derivatives to cyclize. The landmark comprehensive analysis of the formation of monomeric and dimeric polycyclic P-2-AIs published in 2001 suggests a biosynthetic route starting from oroidin (**6**) itself and involving only proton-mediated isomerizations into tautomeric forms **I-IV** followed by intramolecular cyclizations (Scheme 4)



Scheme 4 Proton-mediated isomerization of clathrocin/oroidin and further polycyclizations: alternative biosynthetic hypothesis for the formation of dibromophakellin and dibromoagelaspongins

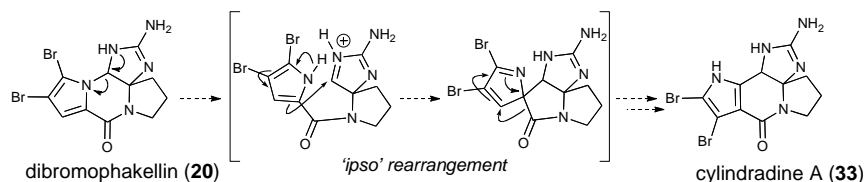
It was envisioned that cyclization of the pyrrole onto transient aminoimidazole tautomer **26** (tautomer type **IV**, Scheme 4) could generate bicyclic, spirocyclic, and macrocyclic pre-

transannular cyclization intermediates (e.g. **28**). Subsequent transannular cyclizations of the latter species could give rise to the tetracyclic phakellins and agelaspongins. Baran recently provided evidence for this hypothesis in a recent synthesis of palau'amine in which the "phakellin" substructure was forged through a transannular cyclization of a "macropalau'amine" precursor (*vide infra*).¹⁹ It is possible that macrocyclization/transannular cyclization induced by tautomer **IV** is arguably one of the major pathways followed in the formation of several polycyclic P-2-AI metabolites. Interestingly, the phakellin-expanded derivative **28** (Scheme 4) was also obtained by Sharma in 1978 as a degradation product of dibromophakellin (**20**).²⁰ Formation of partially cyclized intermediates like the hypothetical pre-phakellin spiro intermediate **29** and agelaspongins intermediate **30**²¹ can also be accounted for by similar mechanisms. In fact, tautomers **III** and **IV** (Scheme 5) form the basis and can account for a general chemical pathway involved in P-2-AI polycyclic monomers. Dibromoisophakellin (**22**), hymenin (**31**) and cyclooroidin (**32**) could be formed through a similar transient intermediate derived from oroidin (**6**). Cyclooroidin, agesamides A-B,²² and oxocyclostylidol²³ presumably arising from cyclization of the fleeting tautomer **III** are less frequent, likely due to the low intrinsic diversity derived from potential intramolecular cyclizations of this tautomer.



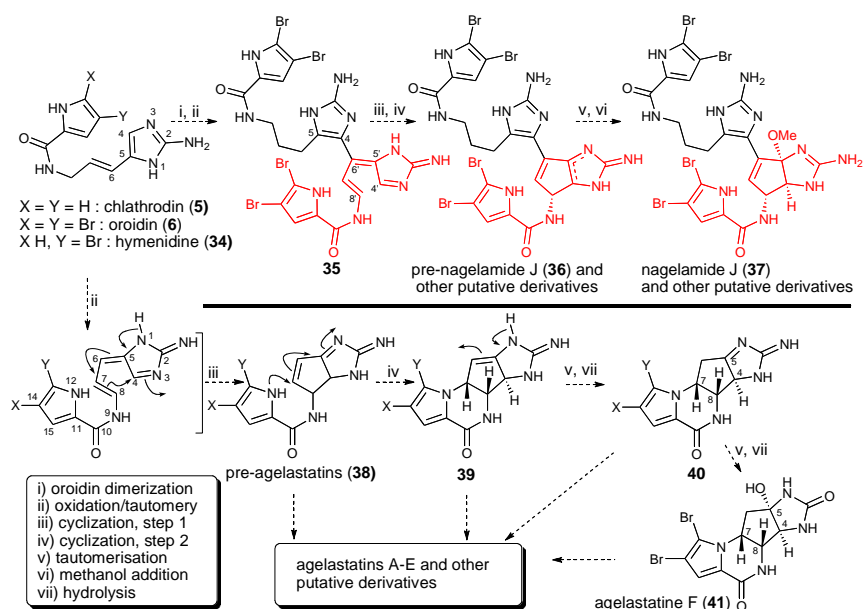
Scheme 5 Intramolecular cyclizations of presumed precursors including tautomers and pre-transannular intermediates leading to bi-, tri-, and tetracyclic monomers

Cylindradine A (**33**), potentially generated via an ‘ipso’ rearrangement of dibromophakellin (**20**) was also recently isolated (Scheme 6).²⁴



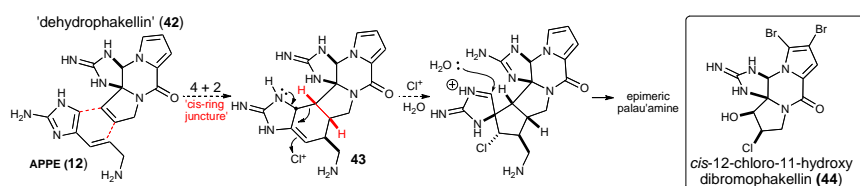
Scheme 6 ‘Ipso’ rearrangement of dibromophakellin (**20**) to cylindradine A (**33**)

The biologically and structurally interesting agelastatins A-F²⁵ are intriguing examples of P-2-AI alkaloids derived from C8-C4 and N12-C7 cyclizations (Scheme 7). A pre-agelastatin intermediate (**38**) generated by an initial C8-C4 cyclization proposed by Al-Mourabit⁴ is clearly supported by the recent isolation of the dimeric natural product nagelamide J (**37**) by Kobayashi.²⁶ The agelastatin pathway further demonstrates the general applicability of the biogenetic proposal based on tautomeric forms of clathrodrin/oroidin and the great potential for P-2-AI metabolites to undergo oxidative couplings.



Scheme 7 Proposed biogenesis of agelastatins and the related dimeric nagelamides [Ali correct Agelastatine F spelling to Agelastatin F]

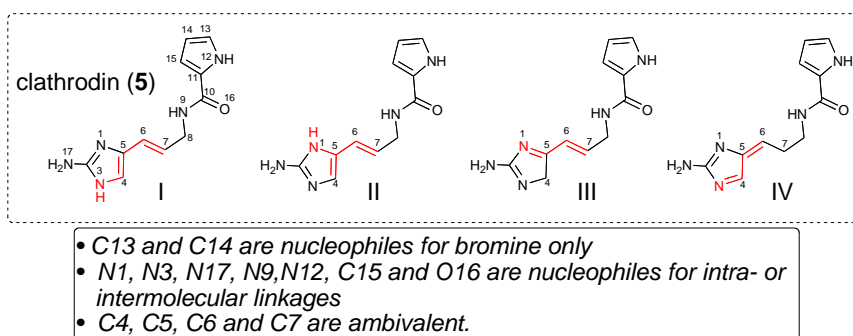
2.4. Polycyclic P-2-AI dimers



One of the first biosynthetic proposals for the generation of higher order P-2-AIs was advanced by Kinnel and Scheuer²⁷ who envisioned a Diels-Alder cycloaddition between a hypothetical 'dihydrophakellin' (**42**) dienophile and AAPE (**12**) (Scheme 8). A subsequent chloroperoxidase-initiated oxidation/ring-contraction event would deliver the hexacyclic core (**43**) of the originally assigned structure of palau'amine. Inspired by this hypothesis, successful Diels-Alder/oxidative ring contraction sequences on related systems were reported independently by Romo²⁸ and Lovely.²⁹ In addition, the isolation of *cis*-12-chloro-11-hydroxybromoisophakellin (**44**)³⁰ resulting from chlorohydration of the hypothetical 'dihydrophakellin' (**42**) would serve as a dienophile in the proposed Diels-Alder reaction. However, it should be noted that the *cis*-stereochemistry of the chlorohydrin found in this metabolite suggests chlorohydroxylation of a *trans*-alkene found in oroidin (**6**) followed by cyclization to the phakellin core and points to an alternative Diels-Alder process leading to the *trans*-ring fusion (*vide infra*). The relative stereochemistry of this phakellin chlorohydrin is identical to that of girolline (**73**, *vide infra*),³¹ presumably derived from chlorohydroxylation of AAPE (**12**).

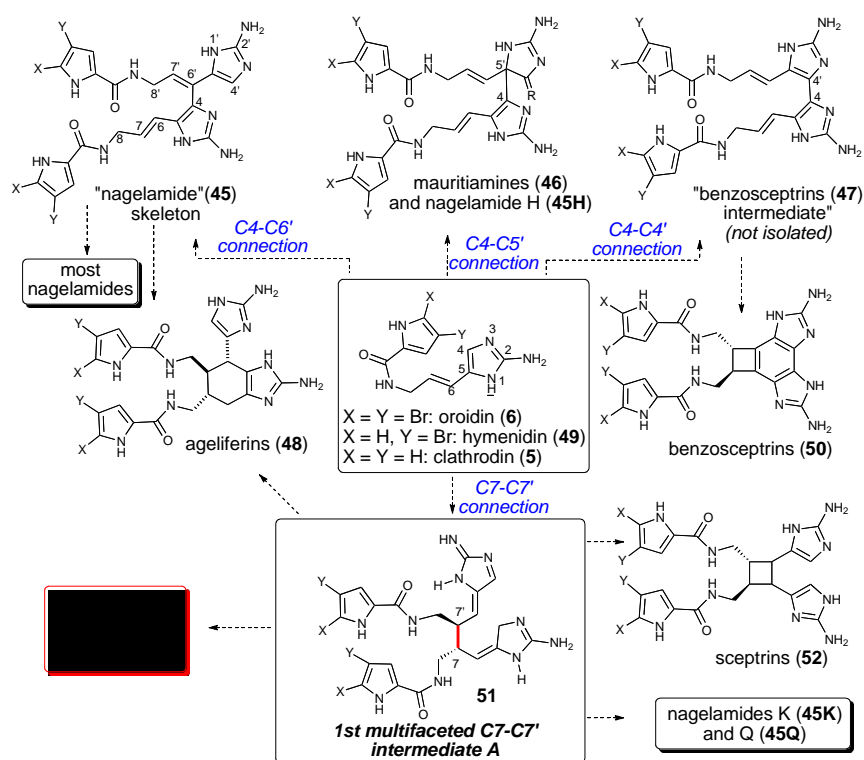
Scheme 8 Original Kinnel/Scheuer biosynthetic hypothesis for palau'amine involving a [4+2] cycloaddition/chlorination/ring contraction sequence. (Inset: An isolated phakellin derivative supporting the proposed dehydrophakellin.) [Ali---Change APPE to AAPE and dehydrophakellin to dihydrophakellin]

A universal chemical pathway for the formation of more complex, dimeric, polycyclic P-2-AI members known up to 2000, involving tautomeric forms **I-IV** of clathrocin (**5**) (and brominated derivatives), was proposed in 2001 by Al-Mourabit and Potier (Scheme 9). Analysis of the ambivalent reactivity of the central intermediate clathrocin (**5**) led a logical pathway that provides insights into the dimerization process of P-2-AIs members that extends from hypotheses already described above leading to bicyclic, tricyclic, and tetracyclic P-2-AIs.



Scheme 9 Tautomerism in the building blocks of the P-2-AIs

The decisive first C-C bond enables the possibility of numerous cascades of cyclization reactions and various modes of C-C and N-C dimerization involving tautomers **I-IV** (Scheme 9) with nucleophilic and electrophilic reactive positions dependent on the tautomer engaged in the dimerization step. In general, C13 and C14 are nucleophilic sites albeit exclusively towards halogenation, N1, N3, N17, N9, N12, C15 and O16 typically act as nucleophiles for intra- or inter-molecular cyclizations, while C4, C5, C6 and C7 are ambivalent. The dual reactivity of these latter reactive sites is then modulated by the pre-organization and presumed substrate-biosynthetic enzyme interactions resulting in the predominance of one of the transient tautomers **I** to **IV**. This reactivity then leads to the various dimerizations and further polycyclizations observed (Scheme 10).

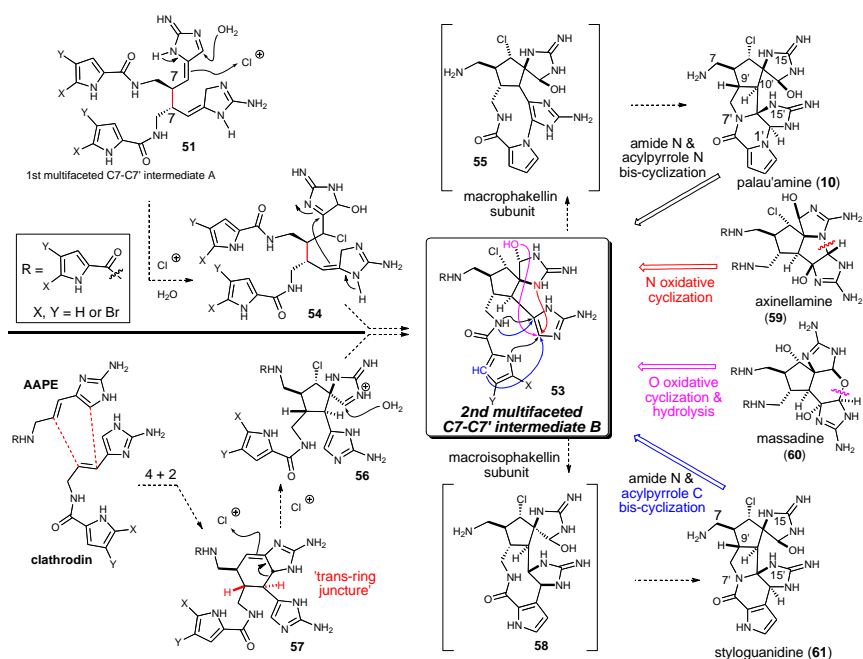


Scheme 10 Alternative “first-bond” dimerization pathways from clathrodine precursors

The fact that all the dimeric P-2-AI members isolated during the last decade fit in the general chemical pathway suggested for their formation is a testament to the soundness of this proposal. In addition, the revision of palau’amine’s relative configuration gave even more consistency to the overall hypothesis, revealing complete uniformity of stereochemical relationships within the P-2-AI dimer subclass (Scheme 11). The tremendous structural variety exhibited in the many dimers isolated in recent years provides a wealth of valuable clues on the mechanisms involved in their formation. Among the possible initial C-C dimerizations, a C7-C7’ dimer provides the highest potential for generating the greatest structural diversity, and when analyzing various congeners having a C7-C7’ connection, it becomes apparent that all could be derived from the 1st multi-faceted intermediate **51** (Scheme 10) and more advanced 2nd multi-faceted intermediate **53** (Scheme 11) via different modes of intramolecular oxidative cyclizations. Alternative initial C-C bond formations can give rise to the primary dimers mauritiamine (**46**) and the nagelamides (**45**) and the newly isolated polycyclic benzosceptrins (**47**) and stylissazoles (*vide infra*, Scheme 14). The synthesis of mauritiamine by Horne³² followed these biomimetic suggestions. The first C7-C7’ “intermediate A” **51** was also invoked in the formation of sceptrin, and the ageliferins (**48**) through a non-concerted mechanism (Scheme 10). Furthermore, the C7-C7’ “intermediate A” (**51**) could undergo an enzymatic chloro-hydroxylation to give chlorinated adduct (**54**), followed

by intramolecular cyclization generating the 2nd multifaceted C7-C7' intermediate B (**53**) (Scheme 11) which features the spirocyclic subunit common to numerous complex P-2-AIs. The different modes of proposed oxidative cyclizations summarized below, would then deliver the variety of complex molecular architectures observed in these metabolites.

It is important to note that the 2nd multifaceted intermediate **53**, a putative common precursor to several higher order P-2-AIs, could also be derived *via* a modified “Diels-Alder”/oxidative ring contraction sequence as originally proposed by Kinnel and Scheuer (*vide supra*, Scheme 8). However, the revision of the azabicyclo[3.3.0]octane core of palau'amine to the *trans*-ring fusion³³ requires that if a [4+2] cycloaddition does occur to give a cyclohexene adduct, delivering an ageliferin-type intermediate **57**, it must involve a non-cyclic dienophile (*e.g.* clathrocin) possessing *E* olefin stereochemistry (Scheme 11, *i.e.* **57**→**56**→**53**). This sequence would provide the observed *trans* relative stereochemistry in the [3.3.0] bicyclic core common to all higher order P-2-AIs including palau'amine (**10**), axinellamine (**59**), massadine (**60**), and the styloguanidines (**61**).



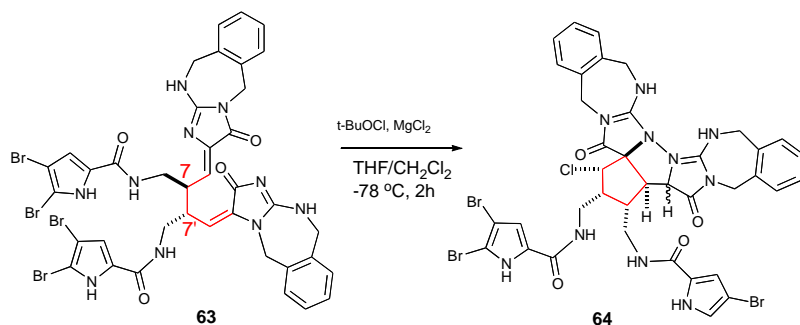
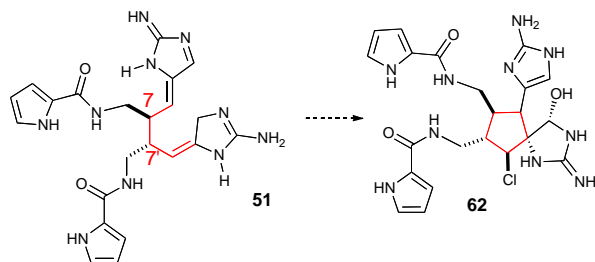
Scheme 11 Generation of molecular complexity in 'higher order' dimeric P-2-AIs from common multifaceted C7-C7' precursors (intermediate B, **53**)

The presence of similar structural motifs and functionalities in several P-2-AIs regardless of

their monomeric or dimeric nature suggests common biosynthetic transformations leading to their formation. A particular illustration is found in the palau'amines and the regioisomeric styloguanidines that could arise from macrocyclic fleeting intermediates ("macrophakellin" and "macroisophakellin" respectively in Scheme 11), which then collapse in a transannular fashion generating the strained *trans*-azabicyclo[3.3.0]octane core of these alkaloids. Variations of Al-Mourabit's postulated biosynthetic intermediate **B 53** were exploited by Baran for the synthesis of axinellamine A and B,³⁴ massadine chloride and massadine³⁵ and very recently for palau'amine, elegantly utilizing a "macropalau'amine" precursor (*vide infra*).

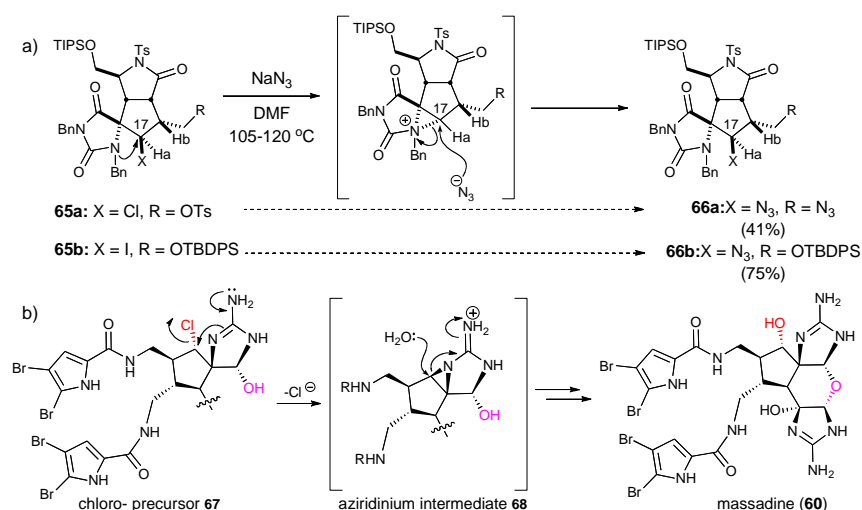
A C4-C6' connection and the following cascade were successfully performed recently by Harran, including the challenging chlorination event required for the cascade (Scheme 12).³⁶ The oxidative chlorination process formed the imbedded spirocyclopentane motif observed in palau'amine (**10**) and congeners. Harran's approach was based on the biosynthetic proposal invoking an initial C7-C7' bond formation (*e.g.* **51**) leading to dimeric dispacamide-type intermediates.³⁷ Interestingly, dispacamide-type dimers **63** indeed undergo an oxidative chlorination/cyclization event installing the highly advanced chlorinated cyclopentane intermediate **64**. The reaction sequence demonstrated the potential for chemoselectivity to initiate cyclization once the C7-C7' bond was installed.

1st multifaceted C7-C7' intermediate A
in scheme 11



Scheme 12 a) Proposed biosynthetic process for cyclopentane formation. b) Bioinspired approach by Harran mimicking this putative reaction

While massadine stands out in this sub-class as it is the only metabolite featuring a pyran core and a hydroxyl group in place of the otherwise ubiquitous chlorine, speculations on its biogenesis were rather scarce. In 2006, during studies toward palau'amine and congeners, the Romo group reported that an unexpected reaction of a cyclopentyl chloride **65a**, and subsequently a cyclopentyl iodide **65b**, with azide surprisingly proceeded with retention of configuration (Scheme 13).³⁸ This group concluded that a mechanism involving neighboring group participation of the spiroaminoimidazole might be in operation involving an aziridinium species. It was further proposed that a similar process could account for the biosynthesis of massadine from an earlier chlorinated precursor.

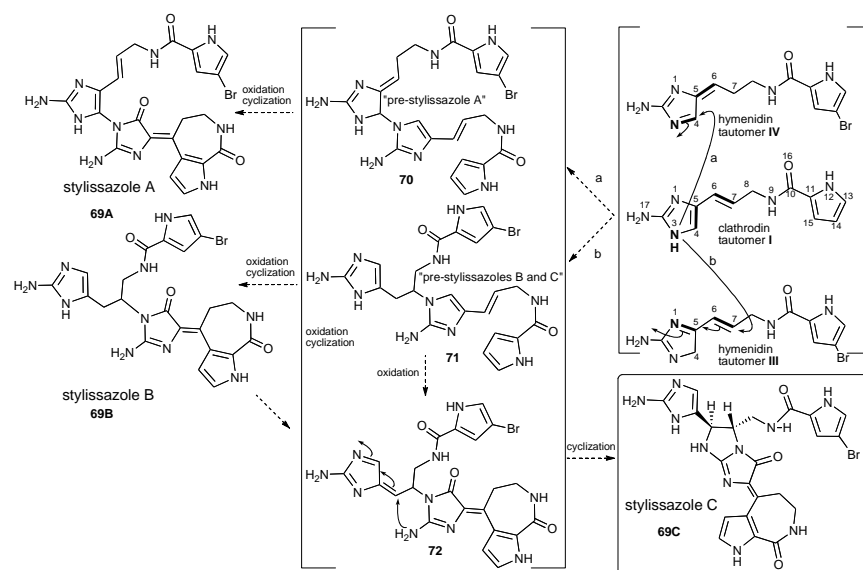


Scheme 13 a) Proposed aziridinium intermediate suggested by an observed retention of configuration displacement of chloride by azide. b) Romo's proposed biosynthesis of massadine based on this observation³⁷

In 2007 Baran and Köck provided evidence for this pathway by isolating the unprecedented massadine chloride and reported its rapid conversion into massadine in an aqueous medium at 60 °C. They also advanced a proposal for the reason this peculiar behavior is not observed in any other P-2-AI metabolites, namely due to the favorable geometrical disposition and the dihedral angle between the aminoimidazole nitrogen and the chlorine atom.³⁹ In addition, it could be envisioned that a similar mechanism is involved in the dimerization of massadine leading to the stylissazines, the only tetrameric metabolites that have been detected to date. This presumed 'biomimetic transformation' was also exploited by Baran in his total synthesis of massadine.

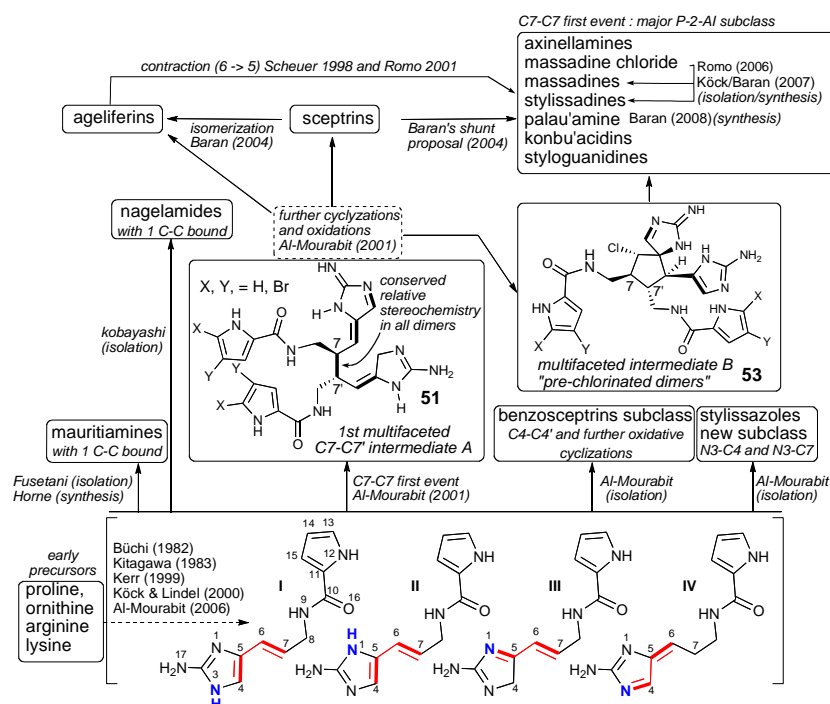
In 2010, isolation of three novel P-2-AI dimeric members, stylissazoles A-C (**69A-C**) demonstrate the first examples of dimerization involving solely N-C bond formation (Scheme 14).⁴⁰ These members provide another dimension to the molecular diversity of P-2-AI metabolites and further highlight the unique dual reactivity of the vinylogous 2-aminoimidazole precursors. The co-occurrence of stylissazoles A-C give biosynthetic clues to this new dimerization/oxidation sequence. Scheme 14 shows a proposed biogenesis suggesting that the building blocks of the molecule are clathrodin and the brominated hymenidin. The biogenetic scheme also suggests that stylissazole C might arise from stylissazole B or from a common "pre-stylissazole B/C". The pathway would presumably start from clathrodin (tautomer **I**) and hymenidin (tautomer **IV**) for stylissazole A and tautomer **III** for stylissazole B-C in a 1,2 or 1,4 aza-Michael fashion to produce the decisive first N3'-C4 and N3'-C7 bounds. The formation of

the N-C connected stylissazoles A-C dimers likely involves N-C bond construction in the first step.



Scheme 14 Proposed biogenetic pathway for stylissazoles A-C (**69A-C**)

The dimeric P-2-AI examples given here suggest that various modes of C-C and N-C dimerization involving tautomers **I-IV** are possible. Nucleophilic and electrophilic reacting centers can vary based on the tautomer engaged in the dimerization step. Unlike other classes of alkaloids, the group of P-2-AI alkaloids shows an unprecedented use of multifaceted intermediates that enable a high degree of complexity and molecular diversity (Scheme 15). The enzymatic stabilization leading to the selection of the reacting tautomers from relatively simple molecules is an interesting question to be answered. The dynamic hydrogen-bonding interactions of monomers like clathrocin with the host enzyme will likely unveil mechanistically interesting and highly ordered biochemical catalytic system.



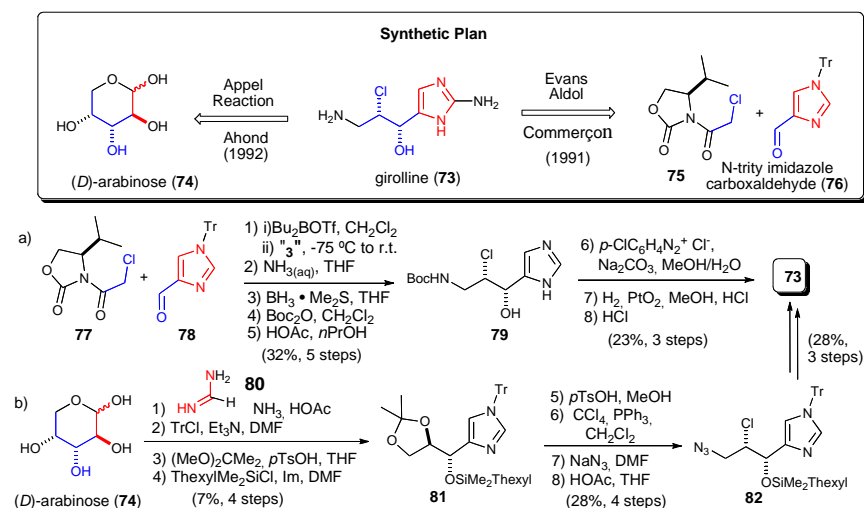
Scheme 15 Summary of biogenetic proposals for P-2-AIs dimers

3 Enantioselective Chemical Syntheses of the Pyrrole-Imidazole Alkaloids (P-2-AIs)

Given the immense structural diversity and architectural complexity of P-2-AIs, it is understandable that efforts toward the asymmetric, total synthesis of these marine alkaloids has been a very prolific domain of study for chemical synthesis. Numerous synthetic groups continue to be active in the area and a significant amount of progress has been achieved recently, including the first completed enantioselective synthesis of monomeric P-2-AIs and racemic syntheses of long elusive, complex dimeric alkaloids. A detailed and exhaustive account of these synthetic endeavors in this area goes beyond the scope of the present review, which has a different focus (*vide infra*). Furthermore, a majority of the synthetic work toward P-2-AIs is the subject of several recent reviews.^{5,41} However mention must be made of outstanding contributions by Overman,⁴² Harran, Horne, Otha,⁴³ Carreira,⁴⁴ Chen,⁴⁵ and Lovely,⁴⁶ Gin,⁴⁷ Gleason,⁴⁸ and Williams.⁴⁹ The enantioselective synthesis of natural products is paramount to subsequent studies of bioactivity including pharmacology, cellular receptor isolation, and structure-activity studies (SAR) for potential drug discovery and development. For this reason and due to space limitations, only enantioselective routes to P-2-AIs will be described with the exception of the recent synthesis of racemic palau'amine.

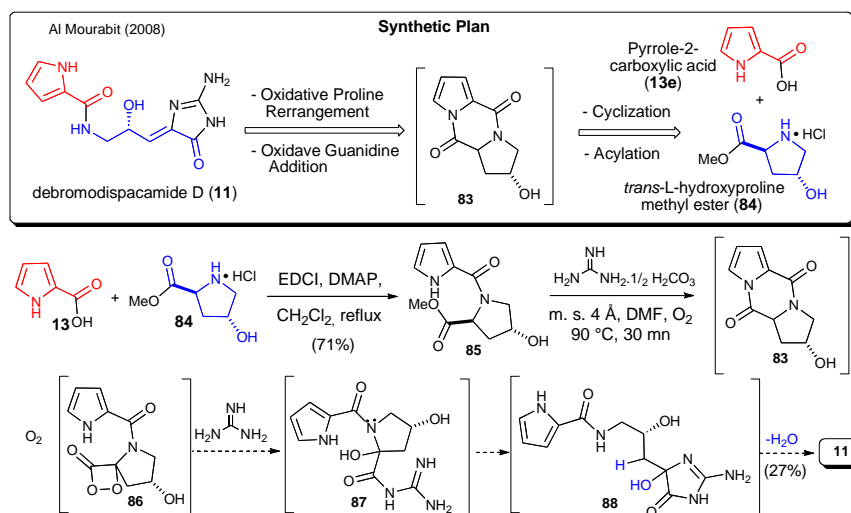
One of the earliest marine P-2-AIs isolated is girolline (**73**) (a.k.a. girodazole), which despite

lacking a pyrrole sub-unit is always included in any discussion about P-2-AIs as it is proposed to be derived from the same simple precursor, AAPE. Shortly after its isolation, synthetic efforts by Ahond and Commerçon led to the elucidation of the absolute configuration of girolline.⁵⁰ In his approach Commerçon⁵¹ employed an Evans' aldol strategy to establish the chlorohydrin relative and absolute stereochemistry (*i.e.* chlorohydrin **79**). Subsequently, Ahond and Poupat⁵² disclosed a chiral pool approach that generated the target stereochemical relationship *via* elaboration of *D*-arabinose (**80**).



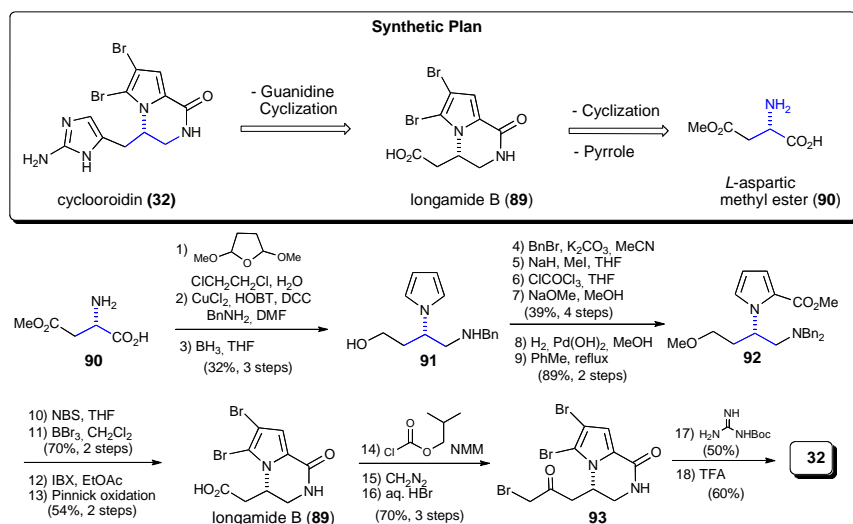
Scheme 16. Enantioselective syntheses of girolline (**73**, a.k.a. giradazole). a) Commerçon's aldol approach. b) Ahond's chiral pool strategy.⁵² Tr = triphenylmethyl; Boc = *t*-butoxycarbonyl, Im = imidazole, DMF = *N,N*-dimethylformamide, THF = tetrahydrofuran.

Many of the simpler "linear" P-2-AIs (*vide supra*) either lack stereogenic centers (*e.g.* oroidin and analogues) or are naturally occurring as racemates (*e.g.* midpacamide,⁵³ dispacamides C and D,⁵⁴ and tauroacidin B⁵⁵). Yet other hydroxylated metabolites which showed optical rotation were revealed, upon further investigation, to exist as a non-racemic mixture of enantiomers like tauroacidin and mukanadin A, which was isolated two years previously and found to be identical to dispacamide D.⁵⁶ Debromodispacamide D possessing a single carbinol stereogenic center was also isolated as a racemate, and its synthesis in optically active form began with hydroxyproline (Scheme 17)⁵⁷ and subsequent stability studies were instrumental in confirming that racemization does not occur during the purification process. The key step was an intriguing presumably biomimetic oxidative reaction that generated the aminoimidazolone moiety *via* the fragmentation of a proposed spirocyclic dioxetanone (**86**) intermediate.



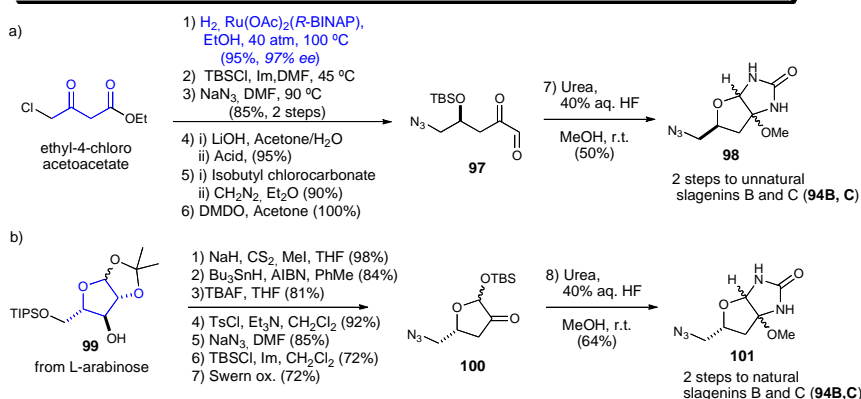
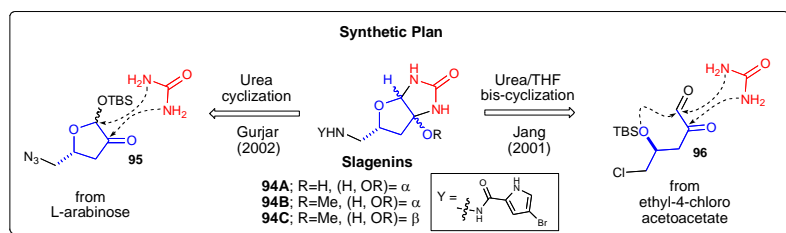
Scheme 17. Concise, enantioselective synthesis of debromodispacamide D by Al Mourabit using a biomimetic oxidation. EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, DMAP = *N,N*-dimethylaminopyridine, m.s. = molecular sieves, DMF = *N,N*-dimethylformamide

A presumed intramolecular oxidative cyclization also introduces elements of stereochemistry in cyclooroidin and the slagenins. After two racemic syntheses, the absolute configuration of natural cyclooroidin was confirmed by asymmetric synthesis in 2006 by Pelloux-Leon and Minassian,⁵⁸ expanding on their previous synthesis of the longamide B (**89**) related from (*L*)-aspartic acid methyl ester (**90**, Scheme 18).⁵⁹ After conversion of the carboxylate moiety of longamide B (**89**) into the corresponding mixed anhydride, sequential treatment with diazomethane and aqueous hydrogen bromide delivered the optically active bromoketone **93**. Subsequent reaction with Boc-guanidine directly generated the aminoimidazole ring, and cleavage of the carbamate protecting group delivered cyclooroidin (**32**).



Scheme 18. Enantioselective synthesis of cyclooroidin by Pelloux-Leon and Minassian. HOBT = *N*-hydroxybenzotriazole, DCC = dicyclohexylcarbodiimide, DMF = *N,N*-dimethylformamide, THF = tetrahydrofuran, NBS = *N*-bromosuccinimide. IBX = Iodoxybenzoic acid, TFA = trifluoroacetic acid

The absolute configuration of the slagenins was established by Jiang, who completed the first total synthesis of the antipodes of natural slagenins B and C (Scheme 19a).⁶⁰ After Noyori reduction of ethyl-4-chloroacetoacetate, the newly generated alcohol was protected as the TBS ether. Azide displacement of the terminal chloride was then followed by conversion of the ethyl ester to glyoxal **97**. One pot intermolecular urea cyclization/intamolecular THF annulation then generated a 50% yield of a 9:5 (α : β) mixture of inseparable diastereomeric bicyclic cores of the slagenins. Finally, azide reduction and acylation of the resulting primary amines with 4-bromopyrrole carboxylic acid delivered the slagenin optical antipodes, which could be separated by column chromatography.

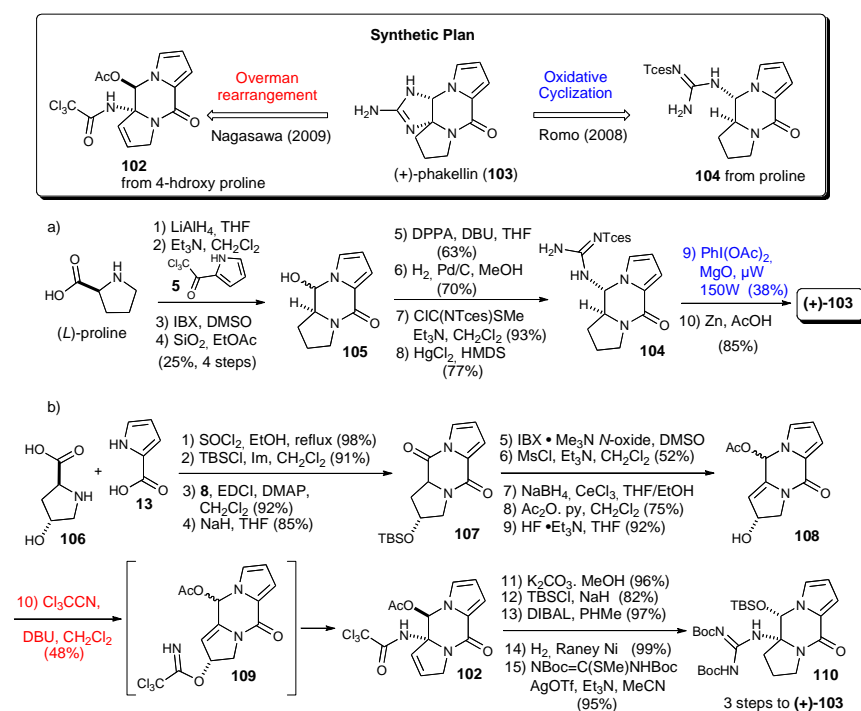


Scheme 19. Enantioselective syntheses of slagenins. a) Jiang's synthesis of the antipodes of slagenins B and C.⁶¹ b) Gurjar's synthesis of the natural enantiomers.⁶³ BINAP = binaphthyl; TBS = *t*-butyldimethylsilyl; Im = imidazole, DMF = *N,N*-dimethylformamide, DMDO = dimethyldioxirane; THF = tetrahydrofuran; AIBN = azoisobutyronitrile. TBAF = tetrabutylammonium fluoride; Ts = *p*-toluenesulfonyl.

The following year Jiang⁶¹ and Gurjar⁶² independently but nearly simultaneously arrived at the synthesis of the natural enantiomers starting from *L*-xylose (not shown) and *L*-arabinose (Scheme 19b) respectively. In Gurjar's approach starting from known *L*-arabinose derivative **99**, Barton-McCombie deoxygenation of the free hydroxyl, was followed by azide displacement on the activated primary alcohol and selective protection and oxidation to generate furanone **100**. Intermolecular urea cyclization in the presence of hydrogen fluoride and methanol, then generated an inseparable mixture of the bicyclic cores of natural slagenin B and C (**94B, C**). The natural products were then completed by installation of the pyrrole side chain as described above.

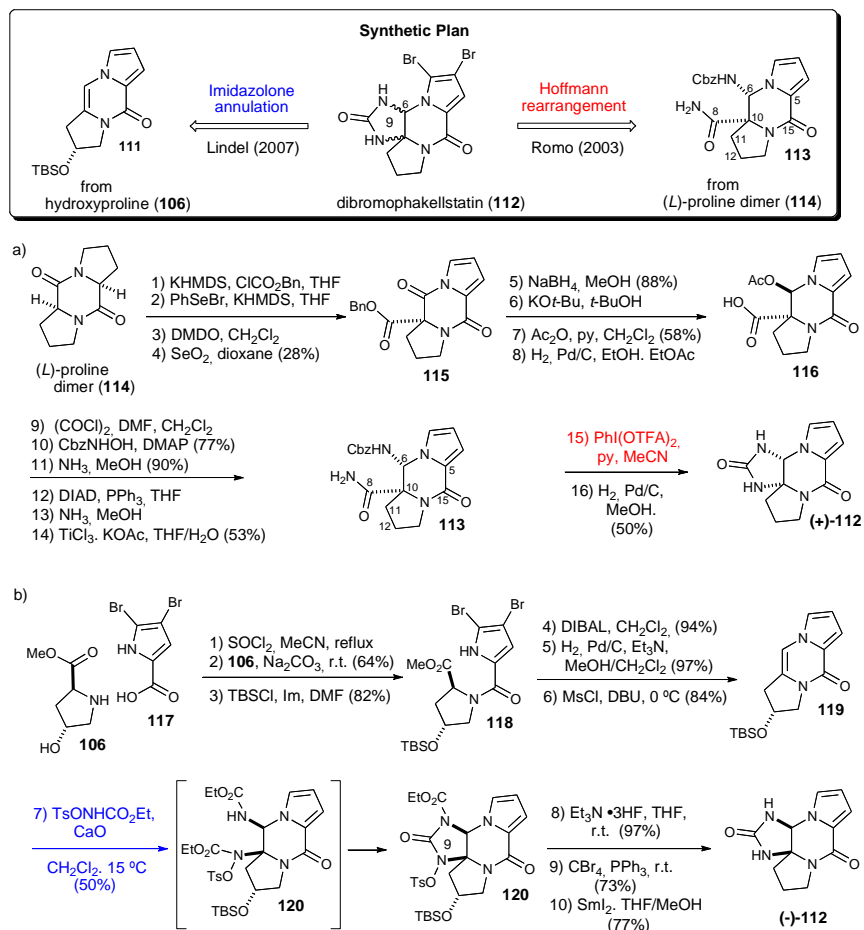
While no significant biological activity was initially reported for phakellin (**103**), several groups actively pursued its synthesis given that it is one of the simplest tetracyclic P-2-AI congeners and for at least two additional reasons. One driving force was the hypothesis first proposed by Sharma who isolated dibromophakellin in search of an elusive potent antibiotic activity found in extracts of *Phakellia flabellata*⁶³ and proposed by Kinnel and Scheuer to

possibly be due to phakellin given the greater bioactivity of palau'amine compared to dibromopalau'amine. A second driving force was the possibility of using developed phakellin annulation strategies in synthetic approaches toward palau'amine (see section V). The synthesis of (+)-phakellin by Romo⁶⁴ began with *L*-proline and trichloroacetylpyrrole to construct the tricyclic ketopiperazine. Installation of the pendant guanidine then set the stage for the key microwave-mediated oxidative cyclization leading to the formation of (+)-phakellin (**103**). The strategy employed by Nagasawa⁶⁵ for the synthesis of (+)-phakellin started with the construction of hydroxyketopiperazine (**107**) from 4-hydroxyproline (**106**) and pyrrole carboxylic acid (**13**). Overman rearrangement of the trichloroacetyl imidate transient intermediate (**109**) obtained from treatment with trichloroacetyl nitrile then installed the tertiary amide precursor (**102**) to the required guanidine (**110**). Intramolecular cyclization of pendant guanidine **110** finally delivered (+)-phakellin (**103**).



Scheme 20. Enantioselective syntheses of phakellin. a) Romo's synthesis of (+)-phakellin b) Nagasawa's synthesis of (+)-dibromophakellin. (IBX = iodoxobenzoic acid; DMSO = Dimethyl sulfoxide. DPPA = diphenylphosphoryl azide; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; THF = tetrahydrofuran, Tces = trichloroethylsulfonyl, HMDS = hexamethyldisilazane; TBS = *t*-butyldimethylsilyl; EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DMAP = dimethylaminopyridine; Ms = Methanesulfonyl; py = pyridine; DIBAL = diisobutylaluminum hydride; Boc = *t*-butoxycarbonyl, Tf = trifluoromethylsulfonyl)

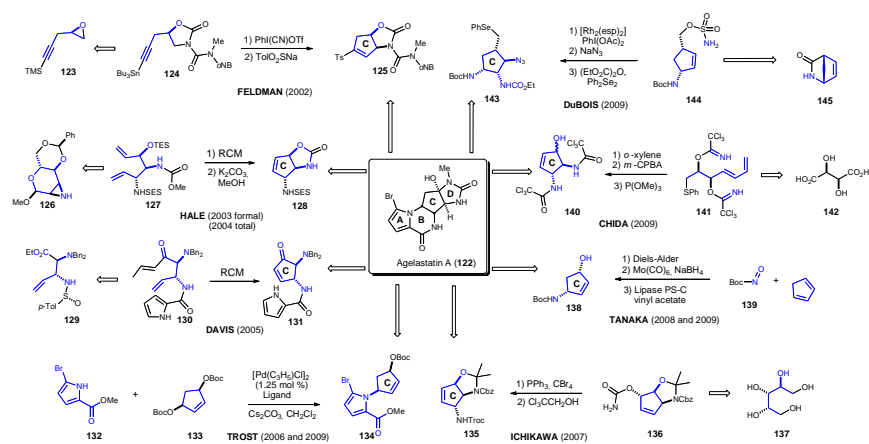
The tetracyclic phakellstatins, which appear to be hydrolyzed phakellins, have attracted significant interest because of their anticancer activity (*vide infra*) and since their isolation a number of racemic syntheses have been reported.^{16,66} The first asymmetric synthesis of (+)-



phakellstatin was completed by Romo in 2003 and led to the enantiomeric natural product (Scheme 21a).⁶⁷ The Romo synthesis employed desymmetrization strategy of a diketopiperazine (**114**) derived from dimerization of proline and a Hoffman rearrangement simultaneously introduced the C10-N9 bond and formed the final cyclic urea. The synthesis confirmed the absolute configuration of the natural product. In 2007, Lindel disclosed the completion of the synthesis of the natural (-)-enantiomer starting with the construction of optically active tricyclic enamide **7** (**118**) from hydroxyproline (**106**) and dibromopyrrole carboxylic acid (Scheme 21b). The key step involved a diastereoselective, intermolecular 3-component imidazolone cyclization with enamide **119** proceeding through a presumed transient bis-carbamate intermediate to deliver cyclic urea **121**. Deprotections, bromination, and deoxygenation delivered (-)-phakellstatin ((-)-**112**).⁶⁸

Scheme 21. Enantioselective syntheses of dibromophakellstatin. a) Romo's synthesis of (+)-phakellstatin. b) Lindel's synthesis of (-)-phakellstatin. KHMDS = potassium hexamethyldisilylazide, Bn = benzyl; DMDO = dimethyldioxirane; py=pyridine; Cbz = benzyloxycarbonyl; DMAP = dimethylaminopyridine, DIAD = diisopropylazodicarboxylate; TFA = trifluoroacetate; TBS = *t*-butyldimethylsilyl; DIBAL = diisobutylaluminum hydride, Ms = methanesulfonyl; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; THF = tetrahydrofuran; AIBN = azoisobutyronitrile; Ts = *p*-toluenesulfonyl. [Ali—120 number is twice..change to 121]

Of all monomeric P-2-AIs, the agelastatins have garnered the most synthetic interest and this is undoubtedly due to its potent antitumor activity. Eight asymmetric syntheses have been reported to date along with a comparable number of racemic approaches (Scheme 22).⁶⁹ While unique and creative, each synthesis features an appended acyl-pyrrole moiety, which enables a late-stage cyclization of the pyrrole and a pendant urea onto the C ring. These ring closures construct the B and D rings respectively, delivering the tetracyclic core of agelastatin. Thus, the primary differences in synthetic approaches are primarily the routes taken to the optically active, functionalized cyclopentane(C ring). Enantioselective strategies to agelastatin along with



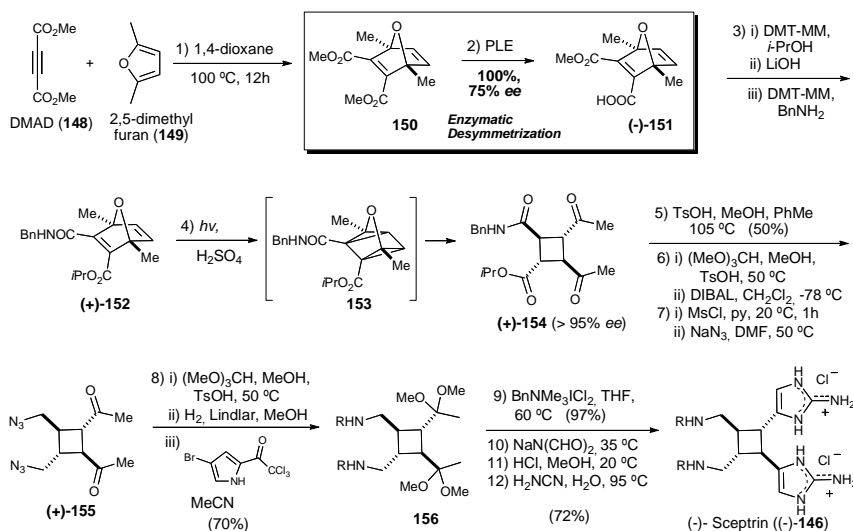
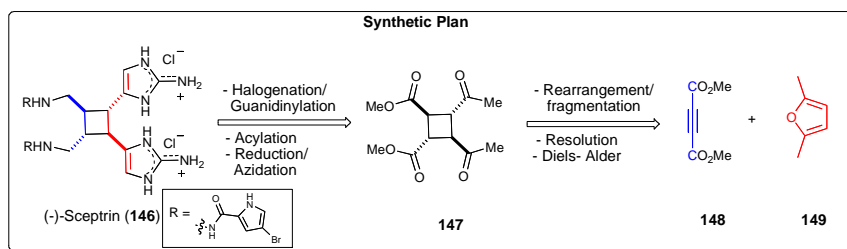
starting materials employed are summarized (Scheme 22) and briefly discussed below.

Scheme 22. Summary of enantioselective syntheses of agelastatin.¹⁹⁻³⁰

Feldman was the first to synthesize the natural enantiomers of agelastatin A and B.⁷⁰ In his strategy, the intramolecular oxidative cyclization of acetylene **123**, readily obtained from (*R*)-epichlorohydrin, generated cyclopentene **125** a precursor efficiently elaborated to the ABC ring system of the agelastatins. In 2003 Hale reported a formal synthesis of agelastatin A⁷¹ involving enantioselective construction of an advanced intermediate **126** previously reported as a racemate

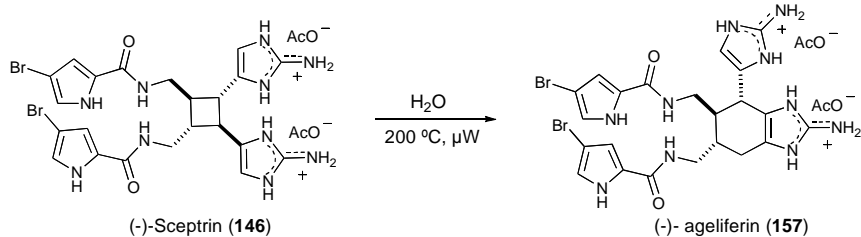
by Weinreb.⁷² Several manipulations of the known aziridine **126** led to diene **122**, which was readily converted to cyclopentene **7** *via* ring-closing metathesis (RCM). The following year, Hale and coworkers advanced cyclopentene **128** through a 9-step route generating several hundred milligrams of enantiomerically pure agelastatin A.⁷³ In Davis's 2005 synthesis the key intermediate cyclopentenone **131** was forged by alkylation of sulfinimine **129** and ring closing metathesis of the advanced allyl-enone **130**.⁷⁴ The asymmetric allylic alkylation (AAA) protocol reported by Trost in 2006,⁷⁵ and again in 2009⁷⁶ rapidly assembled the pyrrole-cyclopentene precursor **134** to the ABC ring system of agelastatin A. Remarkably, the target was then completed efficiently in only an additional 8 steps. In 2007 Ichikawa constructed the functionalized C ring **135** *via* a [3,3]-sigmatropic rearrangement of an allylic isocyanate (**136**), which proceeded very efficiently with complete transfer of chirality.⁷⁷ The synthesis disclosed by Tanaka and Yoshimitsu in 2008 commenced with the hetero Diels-Alder cycloaddition of cyclopentadiene and acylnitroso dienophile **139**, followed by reductive N-O cleavage and enzymatic resolution, which delivered the enantiomerically pure C ring precursor **138**.⁷⁸ The same approach was also utilized in their second generation synthesis completed the following year.⁷⁹ Also in 2009, the strategy employed by Chida featured an elegant sequential Overman/Mislow-Evans rearrangement of bis-trichloroimidate **141** followed by ring-closing metathesis that established cyclopentene **140**.⁸⁰

In 2009, Du Bois disclosed an 11-step synthesis of (-)-agelastatin A,⁸¹ hinging on a novel catalytic intramolecular aziridination method applied to sulfonamide **144** derived from heterobicyclic **145**, followed by sequential regioselective ring openings affording the completely functionalized C ring **143**.



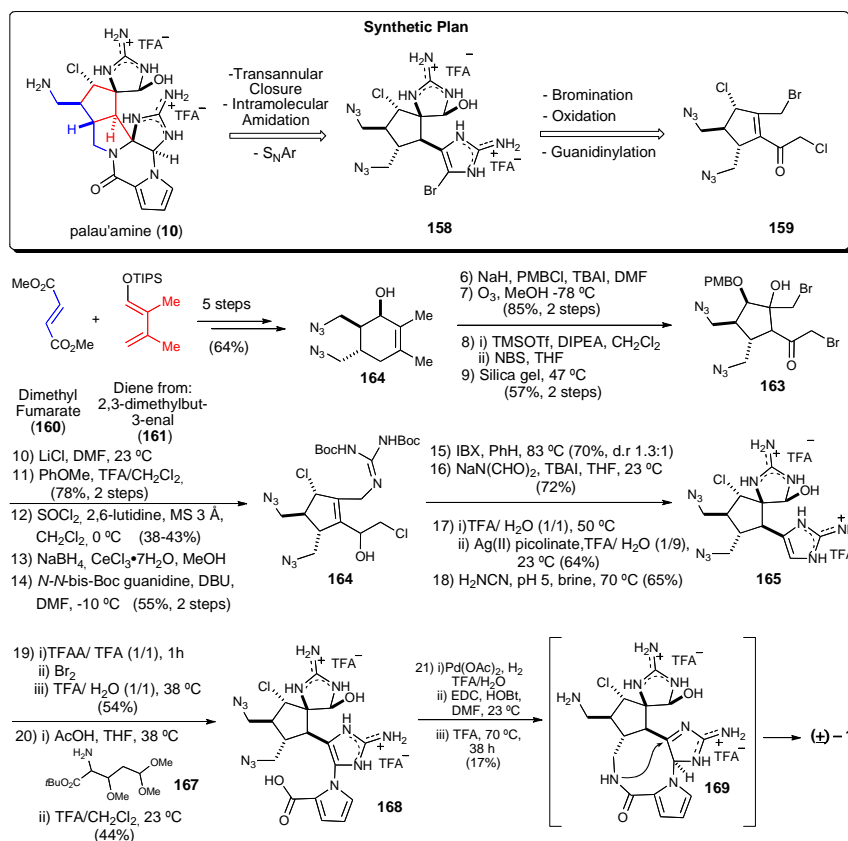
Scheme 23. Enantioselective synthesis of sceptrin (**146**) by Baran. PLE = pig liver esterase, DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride, TsOH = *p*-toluenesulfonic acid, DIBAL = diisobutylaluminum hydride, MsCl = methanesulfonyl chloride, DMF = *N,N*-dimethylformamide, THF = tetrahydrofuran.

Sceptrin (**146**), which bears an intriguing cyclobutane, is one of the simplest “dimeric” P-2-AIs and it has been proposed as the natural precursor of the more complex and highly sought-after members of the family (*e.g.* palau’amine). After two racemic syntheses appeared virtually simultaneously by Baran and Birman,⁸² the latter group revised their original approach and prepared the key optically active cyclobutane intermediate through the enzymatic resolution of the previously reported Diels Alder adduct **156**, followed by photo-induced oxaquadricyclane rearrangement (**152**→ **153**→**154**; Scheme 23). Introduction of the bromopyrrole side-chains and aminoimidazole annulation, then completed the first and only enantioselective synthesis of sceptrin (**146**) to date.⁸³ Expanding on early investigations in the Baran laboratory that led to the discovery that sceptrin could be induced to undergo ring expansion in an aqueous medium at high temperatures, this group disclosed the first enantioselective synthesis of (–)-ageliferin (**157**) and its optical antipode (not shown) from sceptrin (**146**, Scheme 24).⁸³



Scheme 24. Conversion of sceptrin (**146**) to ageliferin (**157**) by Baran.

Baran's recent synthesis of the long elusive palau'amine⁸⁴ represents an important advance in complex P-2-IA synthesis and sets the bar for future endeavors in this field. This synthesis, while racemic, is included to demonstrate another 'bioinspired' strategy involving a transannular stitching to form the strained [3.3.0] core from a macrocyclic precursor, originally posited by Al-Mourabit. Starting from the previously reported chlorocyclopentane precursor **164**, guanidine annulation and silver-mediated oxidation of the unprotected resulting aminoimidazole generated the spirocyclic core **165** of the target. Following construction of the pendant aminoimidazole, treatment with bromine and pyrrole precursor **167** followed by cyclization delivered bis-azide **168**. Following reduction of the azide functionalities, amide coupling with the pyrrole carboxylate moiety and the vicinal primary amine established a "macropalau'amine" advanced intermediate **169**. Finally, acid promoted transannular cyclization afforded the strained azabicyclo [3.3.0] core found in palau'amine (**10**). The inability to directly introduce the common pyrrole carboxylic acid moiety and to initiate biomimetic oxidative cyclizations for late-stage phakellin annulation is instructive.



Scheme 25. Racemic synthesis of palau'amine by Baran. PMBCl = *p*-methoxybenzyl chloride, TBAI = tetrabutylammonium iodide, DMF = *N,N*-dimethylformamide, TMSOTf = trimethylsilyl trifluoromethanesulfonate, DIPEA = diisopropylethylamine, NBS = *N*-bromosuccinimide, THF = tetrahydrofuran, TFA = trifluoroacetic acid, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, IBX = 2-iodoxybenzoic acid, TFAA = trifluoroacetic anhydride, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = *N*-hydroxybenzotriazole.

4 Biological Activity and Pharmacology Including Cellular Targets of the P-2-AI Alkaloids

Marine organisms and in particular sponges, and their associated symbiots, have become a fertile ground in the search for natural products that can combat cancer and viruses.⁸⁵ Notable among such 'leads' from marine sources are the nucleosides spongthymidine and spongouridine which led to the development of Ara-C, used clinically for leukemia and lymphoma, and Ara-A, an antiviral agent. More recently, the anticancer agents ecteineiasidin (ET-743) (PharmaMar) and bryostatin derivative (Eisai, Inc.) were approved as anticancer agents for clinical use.

In the course of isolating P-2-AIs, as with most natural products, bioassay guided fractionation is typically used to identify the most compelling and potent isolates possessing a particular

bioactivity. Some of the most interesting P-2-AI's from the standpoint of established bioactivity are described below. However, the initial bioactivity identified for a given natural product may not be the most important or the bioactivity with the greatest potential for drug development. It is for this reason that isolation and identification of exact cellular targets for natural products continues to be a very important goal in harvesting the full biological potential of isolated bioactive natural products. Unfortunately, in the case of P-2-AIs, very few direct cellular targets have been identified and therefore, this remains an important area for further research. However, some studies aimed at further elucidating details regarding the biological effects of several P-2-AIs including SAR studies have been reported and these are summarized below. In this section, the various P-2-AIs are categorized and described with respect to the bioactivity which has been most extensively studied.

4.1 Oroidin, Ageliferin, and Derivatives: Potential Antibacterial (Antibiofilm) and Antifouling Agents

The Melander group has extensively studied the potential of dibromoageliferin, oroidin, and related P-2AIs as inhibitors of bacterial biofilm formation, an important approach for combating bacterial infections.⁸⁶ A series of oroidin derivatives were prepared including 'reverse amide' derivatives **171** that maintained and in some cases exceeded the antibacterial activity of the native natural product (Scheme 100). *N*-alkylation of the pyrrole nitrogen leading to *N*-methyloroidin (*e.g.* **170**) was found to increase inhibition and dispersion activity of the bacterium *Acinetobacter baumannii*.⁸⁷

Ageliferin (**157a**), bromoageliferin (**157b**), and dibromoageliferin (**157c**) were initially reported by Rhinehart⁸⁸ and subsequently fully characterized structurally by Kobayashi and determined to be activators of actomysin ATPase.⁸⁹ Two simplified ageliferin derivatives, *trans*-ageliferin (\pm)-**172** (TAGE) and *cis*-ageliferin derivative (\pm)-**173** (CAGE) synthesized as racemates, inhibited the ability of *Pseudomonas aeruginosa* strains to form biofilms but interestingly displayed differential toxicity toward planktonic *P. aeruginosa* bacteria.⁹⁰ To date, the bioactivity of the optically active series has not been reported nor has the cellular target for these simplified ageliferin derivatives been described. Further simplified ageliferin derivatives removing all stereochemistry and leading to benzimidazole cores (*e.g.* **174**) were described to also have useful antibacterial activity.

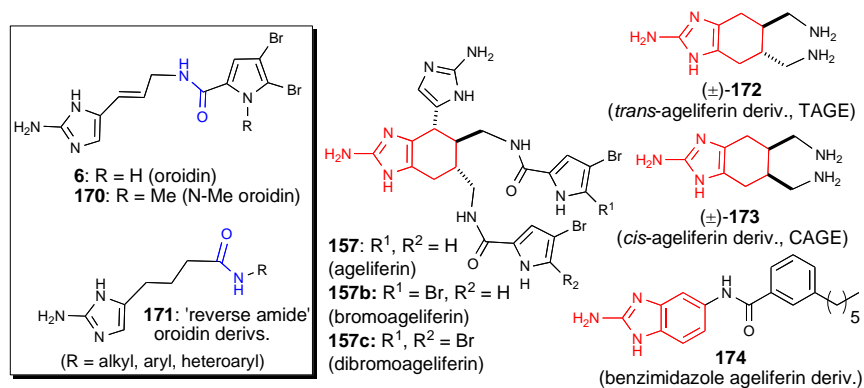


Fig. 4 (a) Structures of oroidin (**6**) and oroidin-inspired derivatives including 'reverse amide' derivatives displaying antibiofilm activity (b) Structure of the ageliferins and simplified derivatives demonstrating antibacterial activity [Ali---change 157 to 157a]

Other P-2-AIs Exhibiting Antibacterial and Antifouling Activity

A large number of P-2-AIs exhibit antibacterial activity towards various bacteria including *S. aureus*, *E. coli* and *B. subtilis*. Nakamuric acid (**176**) and its corresponding methyl ester (**177**) from *Agelas nakamurai* only exhibited antibacterial activity against *B. subtilis*, with 9 mm zones of inhibition. These P-2-AIs exhibit lower antibiotic activity compared to scep trin (**52**) and ageliferin (**157a**) and these preliminary SAR studies suggested that structural changes in the guanidine moiety of the dimer can alter bioactivity. The presence of the oxoguanidine in oxy scep trins and absence of the guanidine in nakamuric acid and its methyl ester leads to a substantial decrease in activity or complete loss of antibiotic activity. A non cyclized, keramadine (**175**) also showed activity against *S. aureus* and *B. subtilis*.⁹¹ A new oroidin dimer, mauritiamine (**46**), isolated from the marine sponge *Agelas mauritiana* exhibited moderate antibacterial activity against *Flavobacterium marinotypicum* with growth inhibitory zones of 10 and 15 mm at 10 µg/disk, respectively.⁹² Axinellamines B-D (**59B,59D**), was active against *Helicobacter pylori* at 1000 µM.⁹³ Kobayashi's group has isolated several dimeric bromopyrrole alkaloids nagelamides, ranging from nagelamide A-R (**46A-46R**) exhibiting antibacterial activity. Nagelamides A-H (**46A-46H**), nagelamide Q-R (**46Q-46R**), ageliferins (**157a-c**), and scep trin (**52**) showed antimicrobial activity against bacteria, *Micrococcus luteus*, *B. subtilis* and *E. coli* (Figure 5).^{94,95} Nagelamide O (**46O**) was weakly active against *B. subtilis*, *Micrococcus luteus* and *S. aureus* (MIC 33.3 µg/mL each).⁹⁶ Nagelamide J (**37**) was active against only *Staphylococcus aureus* (MIC, 8.35 µg/mL) and nagelamides K-L (**46L-46L**) against *Micrococcus luteus* (MIC, both 16.7 µg/mL).⁹⁷

Palau'amine (**10**) isolated from *Stylorella aurantium* displayed antibacterial activity against *S. aureus* and *B. subtilis* among other bioactivities (*vide infra*). The degree of bromination on the pyrrole was shown to affect the biological activity with the monobromo and dibromo derivatives found to be less active compared to the non-brominated congener. The structurally related styloguanidine (**61**) isolated from the sponge *Stylorella aurantium* from the Yap sea, showed inhibitory activity against chitinase, an enzyme responsible for hydrolysis of chitin, using the bacterium *Schwannella sp.* at 2.5 mg/disk. Chitin plays an important role in a wide variety of organisms ranging from nutrition to defense and control of ecdysis in arthropods, therefore chitinase inhibitors have potential as antifungal and insecticidal agents.⁹⁸ For example, the styloguanidines were found to inhibit the moulting of cyprid larvae of barnacles at 10 ppm demonstrating their potential as antifouling agents.

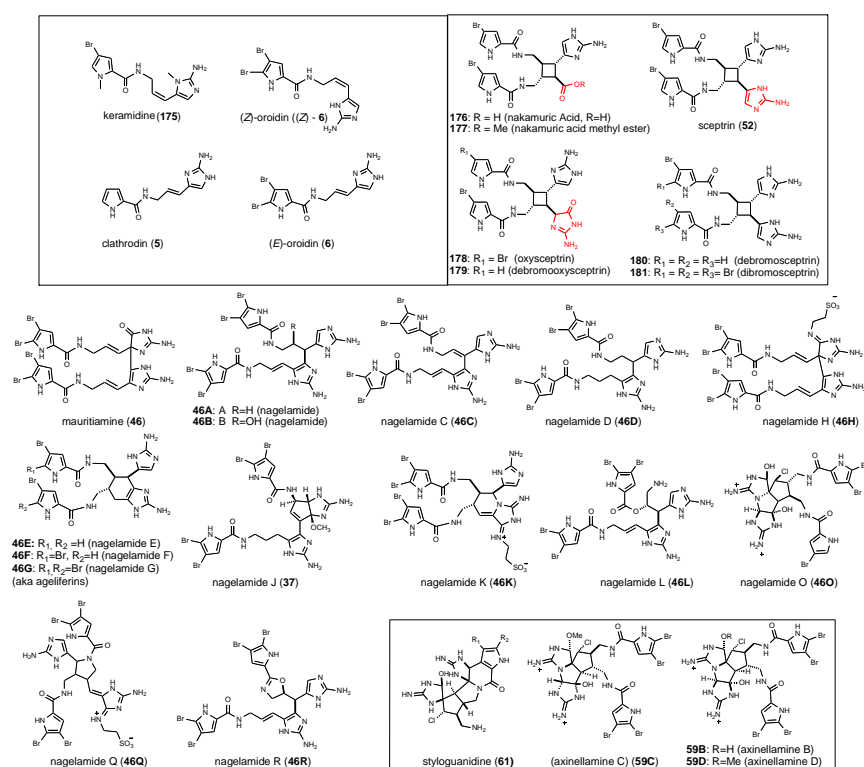


Fig. 5 Structures of P-2-AIs with antibacterial activity [Ali---46A and 46B, A and B in bracket; Nakamuric acid, delete R=H in bracket]

4.2 Girolline (aka girodazole): A Cellular Probe for Eukaryotic Protein Synthesis and a Potential Anticancer Agent

Girolline (**73**), also known as girodazole, was isolated from *Pseudaxinyssa cantharella* and *Axinella sp.* from New Caledonia in 1988.⁹⁹ In 1991, this simple P-2-AI, a result of apparent

chlorohydroxylation of 2-AAPE, was recognized as a potent anti-tumor agent and found to exhibit significant cytotoxicity *in vitro* against several tumor cell lines and *in vivo* against several murine grafted tumors: leukemia (P388, L1210), mammary adenocarcinoma (MA 16/C) and hitiocytosarcoma (M5076).¹⁰⁰ However, girolline did not exhibit any antitumour activity in a phase-I clinical trial, but had severe cardiovascular toxicity.¹⁰¹ Girolline is one of the first P-2-AIs for which a detailed molecular level understanding of its mode of action has been elucidated. Comparative studies revealed that, despite its relatively simple but potentially reactive structure, which embodies latent potential for epoxide or aziridine formation and thus potential action as an alkylating agent for example to nucleic acids, it was determined that girolline's anticancer activity was not a result of alkylation on DNA or RNA. Rather, it involved inhibition of the termination steps of protein synthesis *in vivo*.¹⁰² It is interesting to note that several marine sponge isolates including mycalamide A,¹⁰³ pateamine^{104,105}, and others are also potent inhibitors of protein synthesis, a primary cellular function for survival. Girolline induced G2/M cell cycle arrest in several tumor cell lines and immunochemical analysis revealed that polyubiquitinated p53 was accumulated in girolline-treated cells, while other polyubiquitinated cellular proteins were not accumulated, indicating that the effect of girolline is specific for p53. On the other hand, girolline did not inhibit proteasome activity *in vitro*, and accumulation of polyubiquitinated p53 was scarcely detected in the presence of leptomycin B, an inhibitor of nuclear export. Thus, it was proposed that girolline affects the step of recruitment of polyubiquitinated p53 to the proteasome, postulating that it might be a novel inhibitor against the ubiquitin-dependent proteolytic pathway.¹⁰⁶ To gain further insight into the mechanism of action of girolline *in vivo*, effects on translation and on cell cycle progression were investigated. Girolline neither induced an increase in translational read through nor affected the polysome profile of treated cells. Cell-cycle analysis shows that girolline induced a dose- and time-dependent arrest of cells in G2 phase. Altogether, these results suggest that girolline does not act on translation *in vivo*, but interferes with the control of the G2 checkpoint.¹⁰⁷ However, recently the crystal structure of girolline in the E binding site of the 50S ribosomal subunit of *Haloarcula Marismortui* (*Hma*) was reported,¹⁰⁸ an unprecedented site for antibiotic binding and shows hydrogen bonding with the hydroxyl, amino, and imidazole ring nitrogen of this target protein (Figure 6b).

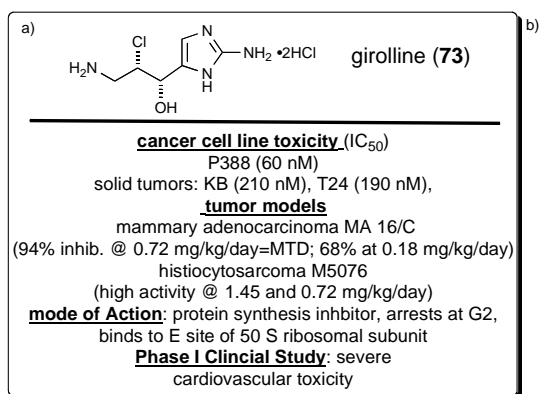


Fig. 6 (a) Structure and pharmacology of girolline (**73**, a.k.a. giradazole) (b) key interactions between girolline and E site binding domain of 50 S ribosomal subunit (*with permission from XX-still needed*)

4.3 Hymenialdisines: Novel Kinase Inhibitors of the Ras-MAPK Signaling Cascade as Potential Treatments for Osteoarthritis

10Z-Debromohymenialdisine (**33**), was first isolated in 1980 from the Caribbean sponge *Phakellia flabellata* (Figure 7a).¹⁰⁹ Its corresponding (**E**) isomer was isolated in 1996 from the sponge *Stylorella aurantium*.¹¹⁰ Debromohymenialdisine has been shown to slow joint deterioration and cartilage degradation associated with osteoarthritis in animal models and is currently under development as a promising new drug candidate.^{111,112} A number of reports centering on 10Z-debromohymenialdisine and the related analogue hymenialdisine¹¹³ have appeared describing the modulation of protein kinase C and the proinflammatory transcription factor, nuclear factor κ B (NF κ B).^{114,115,116} NF κ B is believed to be involved in the regulated gene expression of the inflammatory enzyme, cyclooxygenase (COX-II).¹¹⁷ Several structural congeners of the hymenialdisine sub-group of P-2-AIs displayed inhibitor activity in the Ras-MAPK signaling cascade.¹¹⁸ In addition, 10Z-debromohymenialdisine inhibited checkpoint kinases Chk1 and 2 with IC₅₀ values of 3 and 3.5 μ M, respectively. A detailed study of the inhibitory activity of hymenialdisine towards kinases including the synthesis of >60 analogs led to the identification of 11 new kinase targets for this class of P-2-AIs (Figure 7b).¹¹⁹ In addition, an X-ray structure of CDK2 complexed with hymenialdisine A has been reported and showed that this inhibitor occupied the ATP binding pocket. Importantly, this structure also revealed that the pyrrole bromine was pointing out of the binding pocket,¹²⁰ therefore two affinity resins were prepared by direct Sonogashira couplings at the bromopyrrole leading to amines **184** and **185** (control) which were then coupled to NHS-agarose resins (Figure 7c). Affinity

chromatography experiments led to the identification of six binding proteins including the known high affinity interacting kinases, GSK3 α/β and Mek1, in addition to two new interacting kinases, p90RSK and an unknown kinase thought to be an isoform of BIKe (BMP2-inducible kinase). (*Z*)-debromohymenialdisine and (*Z*)-hymenialdisine also proved to be active against human monocytic leukemia cells (MONOMAC-6) with IC₅₀ values of 2.4 and 0.2 $\mu\text{g/mL}$, respectively.¹²¹ The synthesis and utility of hymenialdisines as potential medicinal agents has been recently reviewed.¹²²

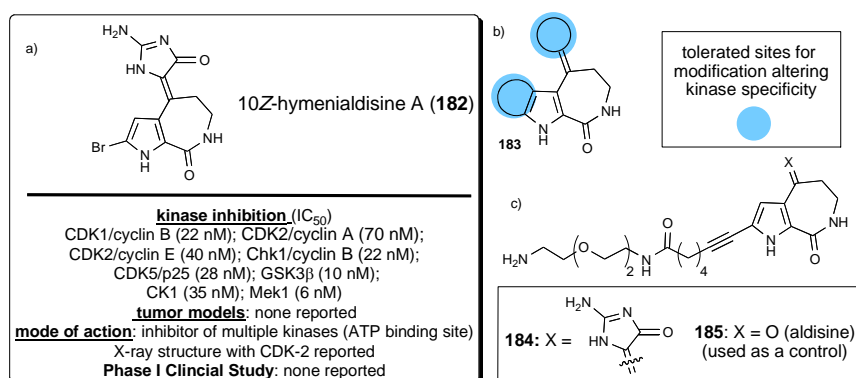


Fig. 7 (a) Structure of 10*Z*-hymenialdisine (**182**) and summary of pharmacology (b) derivatization sites for kinase specificity studies (c) amine derivatives **184/185** used for affinity chromatography experiments

4.3.1 Other P-2-AIs with Anticancer Potential but Unknown Cellular Targets

Several other P-2-AIs have been reported to have anticancer activity but details regarding their specific cellular targets are lacking. One potent anticancer P-2-AI is agelastatin A (**122**) and more extensive SAR data are available for this natural product due to isolation of several congeners and synthesis of derivatives. Agelastatin A (**122**)¹²³ was first isolated in 1993 by Pietra from *Agelas dendromorpha* and displays significant cytotoxicity against both the L1210 and KB tumor cell lines as well as notable activity against a doxorubicin-resistant L1210 leukemia strain.¹²⁴ (-)-Agelastatin A is 1.5 to 16 times more potent than *cis*-platin at inhibiting cell growth, its effects being most pronounced against human bladder, skin, colon, and breast carcinomas.¹²⁵ Additional congeners, agelastatins C (**122C**) and D (**122D**), were isolated by Molinski in 1998 from the West Australian axinellid sponge *Cymbastela* sp. and found to selectively inhibit GSK-3 (glycogen synthase kinase-3), a kinase that may be involved in the onset of Alzheimer's disease, with an IC₅₀ of 12 μM . Structure-activity relationship studies show

that unsubstituted OH-C(8a), H-N(5), and H-N(6) moieties are essential in the B/D transoid configuration for high cytotoxic activity. Reductive debromination of the pyrrole bromine also leads to a significant loss of potency. (-)-Agelastatin A showed a wide range of anti-cancer, antiangiogenic,¹²⁶ antimetastatic, and even insecticidal properties. Its potent cytotoxicity was also observed against a panel of human cancer cell lines (IC₅₀ 0.097-0.703 μM) providing important SAR data primarily by detailed and methodical studies by Hale and coworkers.¹²⁷ The latter studies revealed for the first time that agelastatin A down-regulates β-catenin expression while simultaneously up-regulating Tcf-4, causing the repression of osteopontin (OPN). Despite these promising biological activities, the cellular receptor of the agelastatins has not been reported and its potential as a drug candidate has not been fully explored.

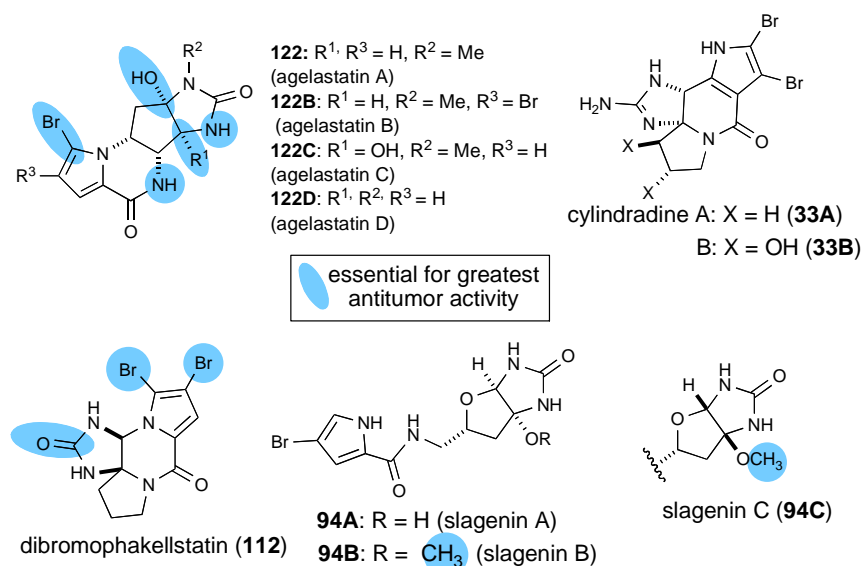


Fig. 8 P-2-AIs with anticancer potential but unknown cellular targets and summary of known SAR data

Dibromophakellstatin (**112**) from the Republic of Seychelles sponge *Phakellia mauritiana* was identified as the most significant cancer cell growth inhibitory substance. In the original isolation, phakellstatin showed antineoplastic activity against melanoma Sk-MEL-5 and colon KM20L2 cell lines (ED₅₀ 0.28 μM for both cell lines)¹²⁸ and antitumor activity was confirmed in another small panel of cancer lines by Lindel using racemic phakellstatin.^{129a} Namely, antitumor activity was found against SF-268 (brain cancer, ED₅₀ 1.4 μM), NCI-H460 (large cell lung cancer, 2.1 μM), and KM20L2 (colon cancer, 0.4 μM) cell lines. In subsequent studies, Lindel

reported potent cell growth inhibitory activity with dibromophakellstatin against a more extensive panel of 36 human cancer cell lines (ED_{50} 0.28-3.9 μM).^{129b} The greatest potency of racemic dibromophakellstatin was observed toward an ovarian (OVXF 899L, 0.60 μM , IC_{50}), a glioblastoma (CNXF 498NL, 0.93 μM), a non-small lung (LXF 529L (0.96 μM), and uterine UXF 1138L (1.21 μM) cancer cell lines. Importantly, Lindel also claimed that the selectivity profile of *rac*-dibromophakellstatin may be indicative of a novel mechanism of action. Limited structure-activity studies of dibromophakellstatin to date have indicated that the bromides are essential for activity since their removal led to dramatic loss of antitumor activity. In addition, the lack of anticancer activity of the phakellins suggests that the cyclic urea is also of importance for activity. Lindel identified a dichlorocyclopropane derivative of phakellstatin that exhibited more potent (average IC_{50} 7 μM against 36 cell lines tested), albeit less pronounced selectivity profile than dibromophakellstatin (average IC_{50} >30 μM). They also verified that only one enantiomer, (-)-phakellstatin, exhibited antitumor activity suggestive of a specific mechanism of action with a likely cellular macromolecular receptor.

Several other P-2-AIs have been reported as cytotoxic agents to various cancer cell lines. Slagenins B (**94B**) and C (**94C**), which share a bicyclic imidazolone with the agelastatins, exhibited cytotoxicity against leukemia L1210 cells in vitro (21 and 20 μM , respectively), however slagenin A (**94A**) exhibited greatly reduced activity (IC_{50} >29 μM , Figure 7).¹³⁰ This limited SAR data for the slagenins suggests that the presence of the methoxyaminal rather than a free carbinolamine is more important than ring fusion stereochemistry. Recently, cylindradine A (**33A**) and B (**33B**) isolated from *Axinella cylindratus* showed moderate inhibitory activity against the murine leukemia cell lines P388 with IC_{50} values of 20 and 77 μM , respectively. In the original isolation of palau'amine (**10**), it was reported to be active against P-388 (IC_{50} 0.1 $\mu\text{g/mL}^{-1}$), A549 (0.2 $\mu\text{g/mL}$), HT-29 (2 $\mu\text{g/mL}$) and KB (10 $\mu\text{g/mL}$) cell lines.

4.4 Scepterin and Related P-2-AI's: Antibacterial Agents Targeting Actin

One of the more structurally interesting and potent antibacterial P-2-AIs is scepterin (**146**), isolated from *Agelas scepterum* and exhibiting activity against *S. aureus* (MIC 15 $\mu\text{g/mL}$), *B. subtilis* and *Pseudomonas aeruginosa*. The activity was considerably greater than that recorded for oroidin.^{131,132} In one study, scepterin demonstrated a bacteriostatic rather than a bactericidal effect on exponentially growing *E. coli* cells.¹³³ In 1991, Keifer and coworkers reported diacetate salts of seven P-2-AI alkaloids from the Caribbean sponge, *Agelas conifera*. Among

these, debromosceptrin (**180**), sceptrin (**146**), ageliferin (**46E**), bromoageliferin (**46F**), dibromoageliferin (**46G**) inhibited growth of *B. subtilis* at 10 µg/disk with lower activities found for dibromosceptrin (**181**), debromooxysceptrin (**179**) and oxysceptrin (**178**). Sceptrin, ageliferin, and bromoageliferin were also found to have antibacterial activity against *E. coli* at 10 µg/disk.^{134,121} again reported an antibiotic effect of sceptrin and ageliferin against *B. subtilis*, *S. aureus*, and *E. coli*. at a concentration of 0.2 µmol/disk. Recently a binding protein of sceptrin from *E. Coli* was identified via a bidirectional affinity protocol.¹³⁵ The process involved the use of resins with bound *E. Coli* derived proteins to first partially purify crude natural product extracts to separate protein-interactive natural products from an *Agelas conifera* extract, and following random alkylation of alcohol/amine functional groups of the partially purified natural product mixture with a coumarin-derived immunoaffinity fluorescent (IAF) tag **186** via ring cleavage of an acyl aziridine, forward affinity co-immunoprecipitation in a sandwich assay with an antibody that recognizes the IAF tag was utilized to identify binding protein(s) (Figure 9). In this manner, a 40 kDa protein was identified as the actin equivalent protein in *E. Coli*, MreB, and is in accord with the observed bacteriostatic effect of sceptrin and its ability to disrupt bacterial cell wall biosynthesis leading to spheroblast formation. The structure of the IAF-tagged sceptrin **187**, which was not reported, would provide useful information regarding SAR of this P-2-AI subset by eliminating sectors of the natural product that do not bind to MreB. The full utility of this potentially powerful methodology for isolating cellular receptors of bioactive natural products, which does not require *apriori* structural information for a natural product, will be realized when an affinity resin for each protein in the human proteome becomes available. This will enable both a reverse affinity purification of crude natural product extracts for specific cellular protein target(s) of interest and a forward affinity purification using co-immunoprecipitation with the IAF tag.

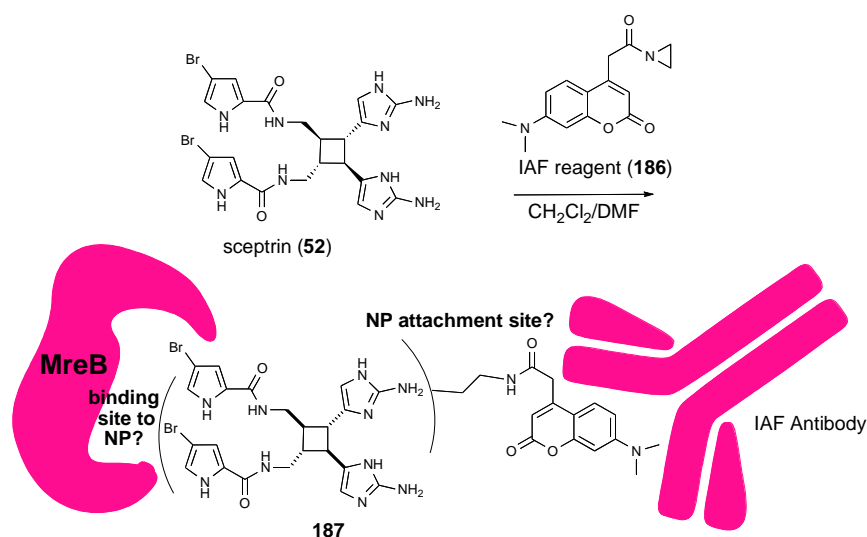


Fig. 9 Synthesis of a random (structure undefined) immunoaffinity fluorescence (IAF) tagged-scepterin derivative **187** and its use in a sandwich assay using an IAF antibody (blue) to identify MreB (red) as an *E. Coli* binding protein of scepterin.

4.5 Receptor Antagonists

Although the cyclic oroidin derivative dibromophakellin (**20**) was first isolated and characterized more than 30 years ago¹³⁶, no investigation into its mode of action were reported. Indeed no significant biological activity was initially associated with this simple P-2-AI (Figure 10). Only recently Quinn,¹³⁷ after re-isolation of dibromophakellin from the organic extract of the marine sponge *Acanthella costata*, discovered activity as an antagonist of the α_{2B} adrenoceptor, a subtype of G-protein coupled receptor affecting analgesia, sedation, hypotension, regulation of hypothermia, gastric protection and gastric motility. This points the importance of re-screening known natural products using new assays.

The recently isolated stylissadine A (**188A**) and B (**188B**),¹³⁸ the first “tetrameric” PIAs, derived from dimerization of massadine, were shown to be specific antagonists of the P2X₇ receptor with IC₅₀'s of 0.7 and 1.8 μM , respectively. Although the physiological function of P2X₇ remains unknown it was advanced that it may have a role in inflammation and immunomodulation.

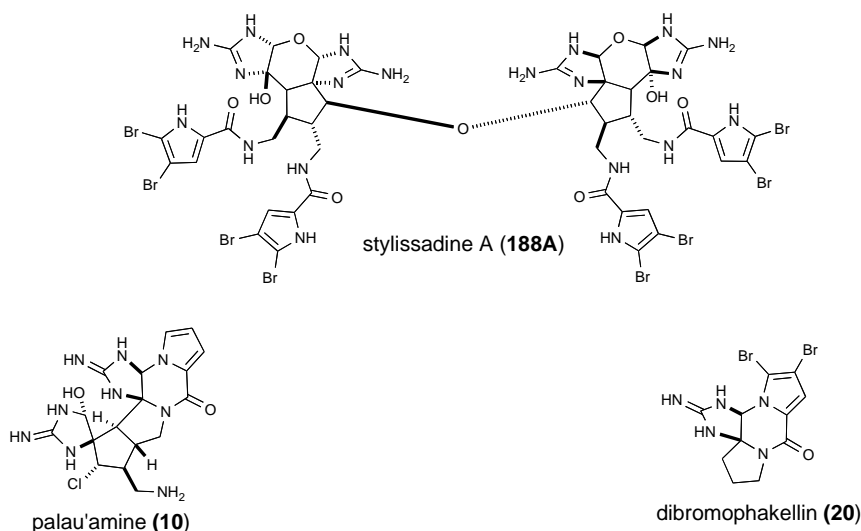


Fig. 10 Structures of receptor antagonists, styllisadine A (**188A**) and dibromophakellin (**20**) and the immunosuppressive agent, palau'amine (**10**)

4.6 Immunosuppressive Agents

At the time of its initial isolation, palau'amine (**10**) exhibited one of the most potent and interesting biological activities, namely immunosuppressive activity in the mixed lymphocyte reaction with an IC_{50} value of < 42.8 nM) coupled with low acute toxicity (LD_{50} 13 mg/kg (*ip* in mice) (Figure 10).^{Error! Bookmark not defined.} This P-2-AI was at one time undergoing preclinical studies.¹³⁹ A review article describing phage display also reported an attempted application of this technology to identify a cellular target for palau'amine, however the only isolated sequence corresponded to hNopp140, a presumed promiscuous non-specific binder, previously observed in phage display experiments and concluded to be an artifact.¹⁴⁰ No further reports have reconfirmed biological activity nor described new bioactivities for palau'amine.

4.7 Phosphatase Inhibitors

The dimeric bromopyrrole alkaloids, nagelamides A-C, G, and H (**46A-C,G,H**), were shown to inhibit PP2A (IC_{50} 12 to >50 μ M) a protein phosphatase involved in the regulation of numerous cellular functions, including muscle contractions, neurotransmission cell proliferation, carcinogenesis, and apoptosis (Figure 11a).

4.8 Other P-2-AIs Exhibiting Kinase Inhibition

Konbu'acidin A (**190**) was found to inhibit cyclin-dependent kinase 4 (CDK4), a protein tyrosine kinase involved in cell cycling events, with an IC_{50} of 27 μ M, however this activity appears decoupled from cytotoxicity since at this concentration no toxicity was observed with L1210 or KB cells ($IC_{50} > 27 \mu$ M; Figure 11b).¹⁴¹ Spongiacidins A and B (**189A, B**) closely related to the hymenialdisines) isolated from *Hymeniacidon* sp., inhibited *c-erbB-2* kinase (IC_{50} 21 and 18 μ M, respectively) and CDK4 (IC_{50} 80 and 37 μ M, respectively).¹⁴² However, spongiacidins C and D (**189C,D**) did not inhibit either kinase (IC_{50} , $>50 \mu$ g/mL). Taurocidins A and B (**191A,B**) isolated from the Okinawan sponge *Hymeniacidon* sp., also exhibited inhibitory activity against EGF receptor kinase and c-erbB2 kinase with IC_{50} in the 28-45 μ M range.¹⁴³

4.9 Massadine: A Protein Prenylation Inhibitor

Massadine (**60**)¹⁴⁴ isolated from the organic extract of the marine sponge *Stylissa* aff. *massa*, showed inhibitory activity of the geranylgeranyltransferase type I (GGTase I) enzyme, a Zn(II) metalloenzyme involved in protein prenylation of *Candida albicans* with an IC_{50} of 3.9 μ M (Figure 11c).

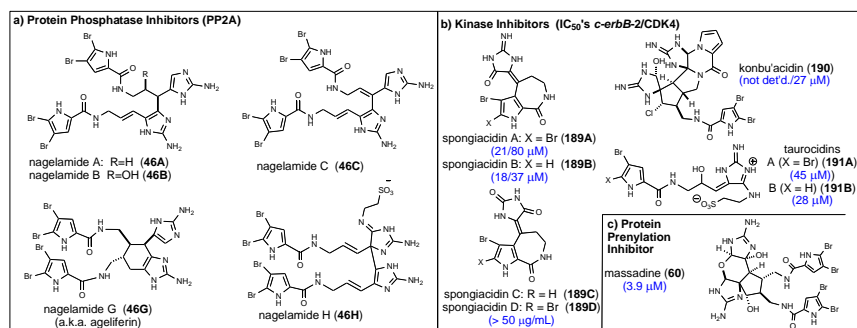


Fig. 11 Miscellaneous enzymatic inhibitory activities reported for several P-2-AIs including (a) phosphatase inhibitors, nagelamides A-C, G, and H (**46A-C,G,H**) (b) kinase inhibitors, konbu'acidin A (**190**) and spongiacidins A (**189A**) and B (**189B**), taurocidins A (**191A**) and B (**191B**) and (c) a protein prenylation inhibitor, massadine (**60**).

5 Summary and Future Outlook

Bioactive natural products (i.e. genetically encoded small molecules) continue to be an active gold mine for discoveries in synthetic strategy development and in biosynthesis leading to novel mechanisms and enzymatic machinery, for inspiration eliciting novel synthetic methods, and for

novel insights into cellular function and perturbation or inhibition of aberrant cellular machinery leading to potential drugs for human ailments. This is not surprising given that these genetically encoded small molecules have been designed to interact with various cellular receptors in addition to the biosynthetic enzymes involved in their synthesis and tailoring. It is our hope that this review has conveyed this to be true for the continually growing family of pyrrole-2-aminoimidazole (P-2-AI) alkaloids. However, what should also be clear to the reader is that we are only at the initial stages of discovery with respect to asymmetric syntheses important for further biological studies, biosynthetic studies, basic cell biology studies using these alkaloids, and drug discovery and development with respect to this family of alkaloids. While over 150 members of this family are known, the recent discovery of the benzosceptrins, styllisazoles, and styllissadines, an apparent dimer of massadine, clearly suggests that further bioassay-guided searching for novel members of this family will continue to be a rich source of 'gold.' Furthermore, the discovery of new bioactivities for 'old' P-2-AIs such as dibromophakellin demonstrates the importance of rescreening for novel bioactivities. The great utility of biosynthetic speculations guiding total synthesis endeavors to achieve high efficiency will continue to provide practical avenues for posing questions into structure-activity relationships especially with the more complex P-2-AIs, *e.g.* palau'amine.

In the future, it should be expected that insights into the actual biosynthetic machinery and synthetic strategies used by P-2-AI generating enzymes to generate the presumed universal precursors clathrocin/oroidin and their conversion to higher order P-2-AIs, *e.g.* ageliferin, axinellamines, will have a profound impact on the most practical ways to synthesize these alkaloids and derivatives enabling further research in basic cell biology and drug discovery. Furthermore, direct evidence for the numerous proposed biosynthetic pathways will undoubtedly uncover some interesting biosynthetic mechanisms and catalytic systems. In addition, with a number of new methods for elucidating cellular receptors for natural products being developed it should be expected that the number of P-2-AI/cellular receptor complexes known will increase dramatically in the coming years beyond the few (3) that are currently known. Undoubtedly, the future will clearly reveal additional novel enantioselective strategies, initial biosynthetic studies, and further pharmacological and cellular receptor isolation studies of this exciting family of natural products known as the pyrrole-2-aminoimidazole alkaloids.

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