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The Chemistry and Biology of Guanidine Natural Products

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Covering: 2012 to 2014

Previous review: *Nat. Prod. Rep*., 2012, **29**, 1382.

The present review discusses the isolation, structure determination, synthesis, biosynthesis and biological activities of secondary metabolites bearing a guanidine group. Topics include non-ribosomal peptides, alkaloids, guanidine-bearing terpenes, polyketides and shikimic acid derivatives from natural sources. A critical analysis of some yet underdeveloped aspects of guanidine metabolites is also presented.

1 Introduction

This review completes twenty years of analysis of the literature on guanidine natural products.[1] The main achievements on the chemistry, biochemistry and biology of organic guanidine compounds during these two decades are very difficult to be comprehensively listed. A personal choice provides only a glance of the scientific challenges and of the aesthetics related to the molecular architecture of some natural guanidines.

The diversity of cyanobacterial toxins bearing arginine derivatives, or even guanidine groups derived from unique biosynthetic pathways, is very impressive. The discovery and continuous investigations on the identification, pharmacology and biosynthesis of these toxins has direct implications not only for the monitoring of water quality implied in human and animal health, but also in understanding the mechanism-of-action of these potent toxins, as well as in the knowledge of the ecology, phylogeny and evolution of toxic and non-toxic cyanobacteria. The impressive diversity of guanidine alkaloids in marine sponges, both PKS and NRPS derived, is an additional intriguing aspect of guanidine chemistry and biochemistry. There are only scarce investigations on the actual source, as well as on the biosynthetic pathways, of such alkaloids, which include structural motifs from simple arginine or agmatine residues and derivatives incorporated into terpenes and peptides, to the highly complex polycyclic frameworks of Palau'amine, massadines, ptilomycalins, crambescidins and related alkaloids. Sponge guanidines are truly unique. Last, but not least, the small, highly functionalized, intriguingly complex and potently active saxitoxins and tetrodotoxins still remain a subject of much research during the several decades after their identification. The 50th anniversary of the structure elucidation of tetrodotoxin was marked in 2014. However, its biosynthesis still remains unknown. The importance of tetrodotoxin is not only scientific: it also has significant implications in human health. Saxitoxins and tetrodotoxin are continuously monitored in sea food, in order to avoid human

intoxication. Some concern has been expressed related to the potential use of saxitoxin and tetrodotoxin as bioweapons.[2]

The interest on natural guanidines is obviously related to the very impressive achievements on the total synthesis of guanidine natural products. Also, major challenges are related to the isolation and identification of guanidine natural products, occurring in very complex and polar mixtures of difficult separation. The chemistry of guanidines has been highlighted by a recent special issue on "The Chemistry of Guanidine, Guanidinium, and Guanidinate Compounds" published in the *Australian Journal of Chemistry*.[3]

The structure of this review is slightly different from the previous ones, but keeps the essence in discussing the isolation of new compounds and their bioactivities, biosynthesis and total synthesis. Reviews of general interest that have been published between 2012 and 2014 include "Heterocyclic Superbases: Retrospective and Current Trends",[4] "2-Aminoimidazoles in Medicinal Chemistry",[5] "Biological activities of guanidine compounds, 2008 - 2012 update",[6] almost exclusively on the literature of patents, "Neuraminidase inhibitors for influenza B virus infection: Efficacy and resistance",[7] "Thiourea and Guanidine Derivatives as Antimalarial and Antimicrobial Agents",[8] "Guanidine Motif in Biologically Active Peptides",[9] "Guanidines: from classical approaches to efficient catalytic syntheses",[10] "On Guanidinium and Cellular Uptake",[11] "Recent progress in neuroactive marine natural products",[12] "Amidines, isothioureas, and guanidines as nucleophilic catalysts",[13] "Guanidine Organocatalysis",[14] "Fifteen Years of Cell-Penetrating, Guanidinium-Rich Molecular Transporters: Basic Science, Research Tools, and Clinical Applications"[15] and "Transition metal-catalyzed N-arylations of amidines and guanidines".[16]

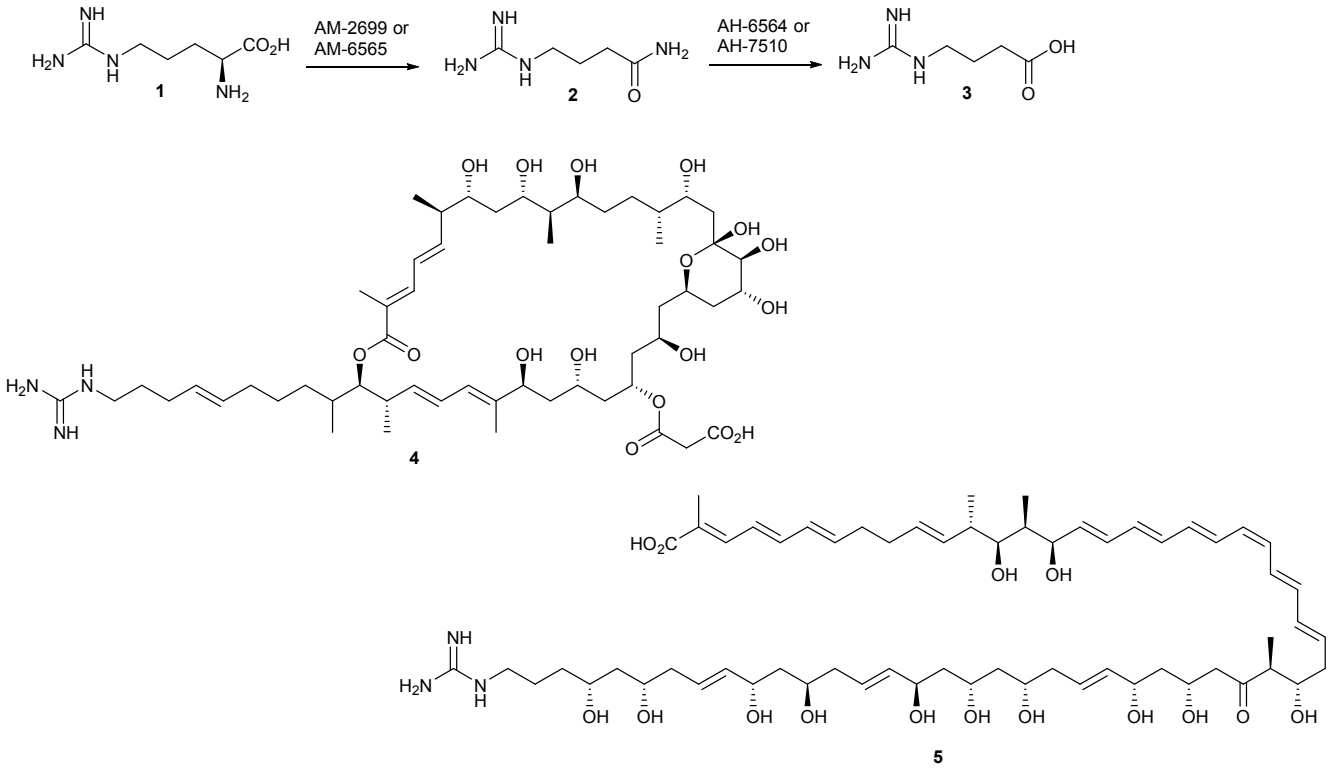
As usual, intensive research continues on guanidines as bases,[17-20] catalysts,[21-59] membrane transporters,[60-67] nucleotide mimetics,[68-71] peptide mimetics,[68,72-86] and as molecular probes.[87-90] Due to the very diverse and potent biological activities of synthetic guanidines, including anti-bacterial,[91-99]

anti-microbial,[100-103] anti-parasitic,[104-106] anti-viral,[107-110] as enzyme inhibitors,[111-133] and in several additional assays,[134-142] methods of guanidine synthesis continue to be developed.[143-166]

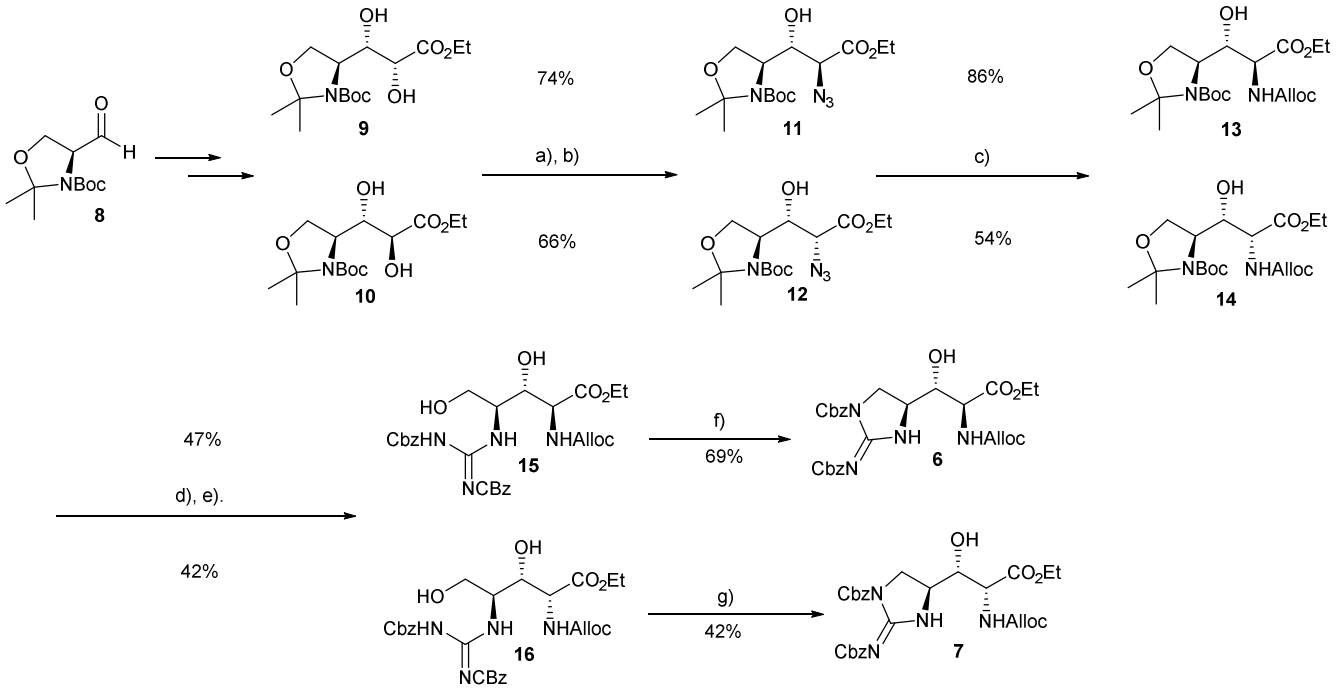
In the following sections, results from the investigation of natural guanidines during the period of 2012-2014 are presented and discussed.

2 Natural guanidines from microorganisms

Several years ago it was postulated that 4-guanidinobutanoic acid is the starter unit for the formation of guanidine-bearing macrocyclic polyketides which have been repeatedly isolated from Streptomycete, and present anti-fungal activity. It has been recently found that *Streptomyces violaceusniger* DSM 4137 genome harbors genes which encode the biosynthesis of 4-guanidinobutanoic acid (**3**) from L-arginine (**1**). Such genes are not located in a single cluster, but distributed in different loci. It has been possible to heterologously express them in *Escherichia coli* and to reconstitute the arginine to 4-guanidinobutanoic acid pathway *in vitro*. Two homologous genes expressed by *E. coli*, AM-2699 and AM-6565, converted L-[U-^{13}C,^{15}N]arginine (**1**) quantitatively into 4-guanidinobutanamide (**2**), confirmed by its isolation and ^{13}C NMR analysis. Feeding [U-^{13}C,^{15}N]-**1** and -**3** into cultures of *S. violaceusniger* DSM 4137 led to the isolation of labeled azalomycin F3a (**4**) and desulfoclethramycin (**5**). Furthermore, two additional homologous genes, AH-6564 and AH-7510, expressed enzymes that hydrolyzed **2** into **3**. Finally, the thioester of **3** was prepared in the presence of ATP and CoASH, clearly indicating **3** as the starting unit of a NRPS-PKS interchange system, leading to **4** and **5**.[167] Quite unexpectedly, a new arginine catabolic pathway leading to **3**, then to γ-aminobutyrate, has been found in the fungus *Kluyveromyces lactis*, and subsequently found spread among several fungal strains.[168]



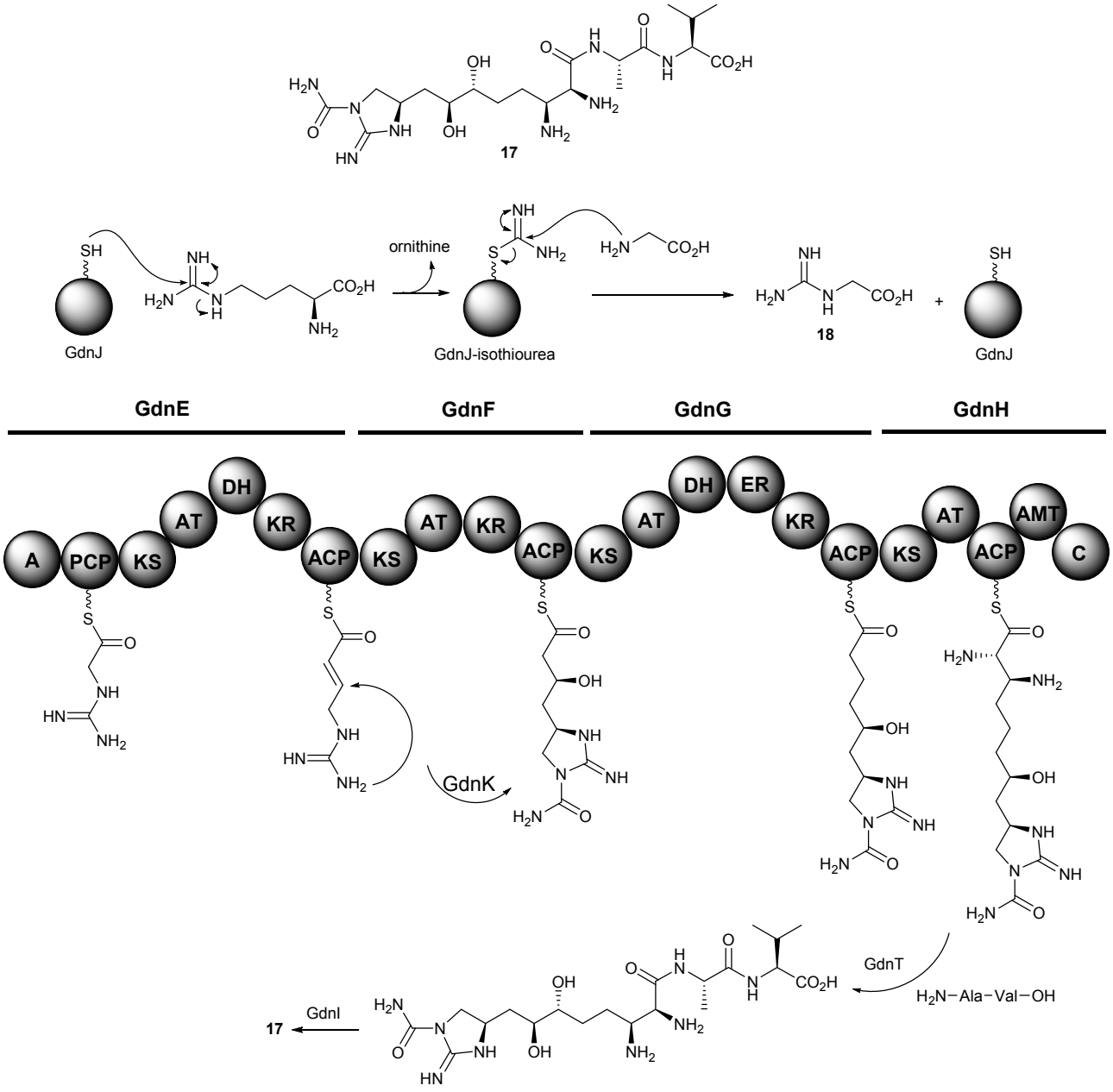
A new synthesis of protected L- and D-β-hydroxyenduracididines (**6** and **7**) has been accomplished (Scheme 1).[169] Garner's aldehyde (**8**) was the starting material, which was converted into **9** and **10** by a series of well described procedures. The following steps were developed identically for both stereoisomers, using thiocarbonyldiimidazole (TCDI) as the activating group of the α-hydroxy substituent in both **9** and **10**. After the azide reduction and protection of the corresponding amines **13** and **14**, acid-catalyzed deprotection and conversion into the corresponding protected guanidines, intermediates **15** and **16** were independently transformed into the final products by reaction with nosyl chloride (NsCl). Compounds **6** and **7** were obtained in 16% and 8% overall yield, respectively.



Scheme 1. Synthesis of *N*-Alloc-Cbz-bis-guanido-protected L- and D-β-hydroxyenduracididines (**6** and **7**). *Reagents and conditions*: a) TCDI, THF; b) NaN3, PPTS, DMF; c) Ph_3P, THF-H_2O, reflux AllocOSu, Et_3N; d) TFA-CH_2Cl_2, 0 ^oC; e) *N*,*N*'-dicarboxybenzyl-*S*-methylisothiourea, $HgCl_2$, Et_3N, THF; f) NsCl, pyridine; g) NsCl, Et_3N, CH_2Cl_2.

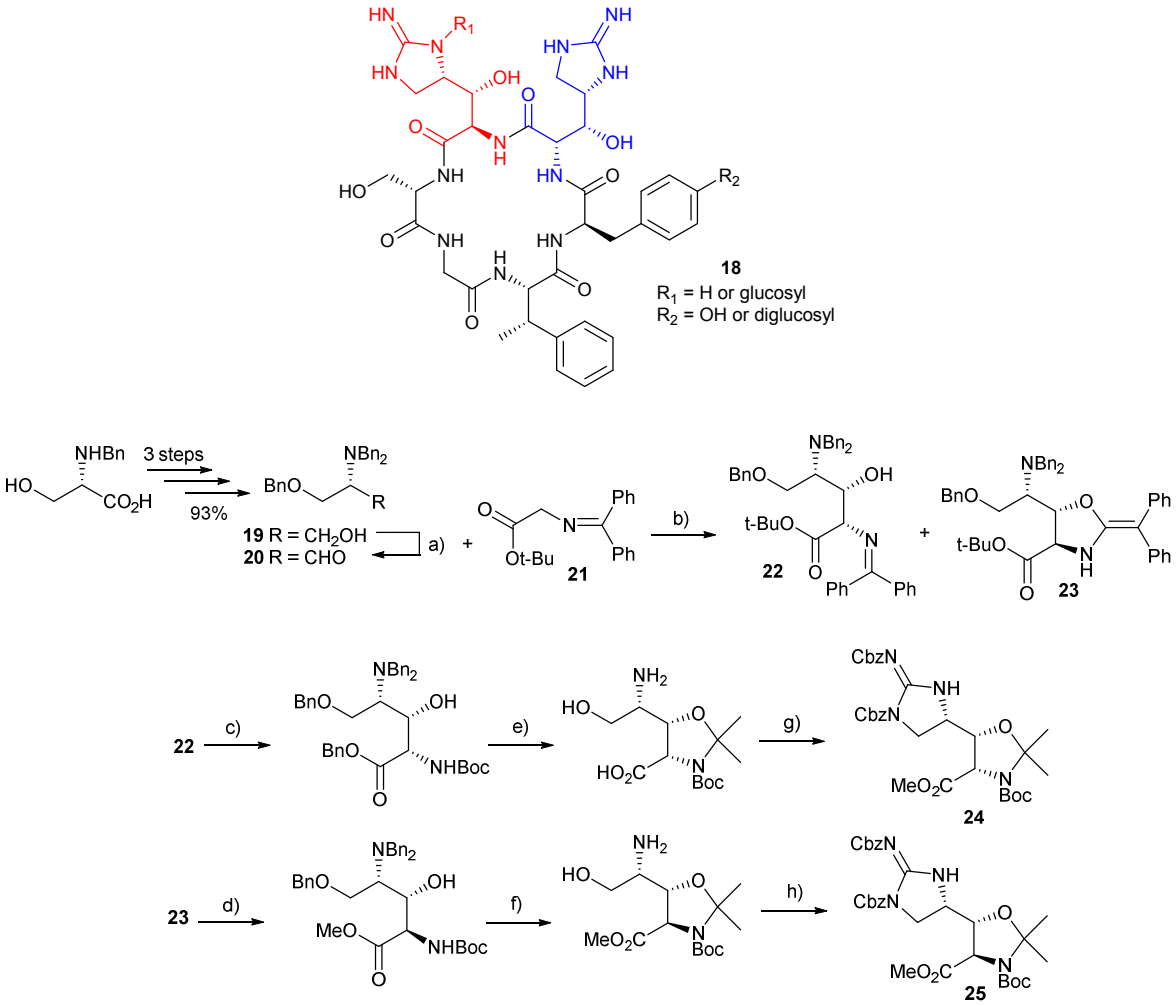
The biosynthesis of guadinomine B (**17**) was established.[170] After shotgun sequencing of the whole genome, cosmid library screening of *Streptomyces* sp. K01-0509, followed by BLAST analysis, a 3,000 bp contig was found to code an aminomalonyl-acyl carrier protein, assumed to participate in the biosynthesis of **17**. Further analysis indicated a NRPS-PKS gene cluster, starting with the formation of guanidinoacetate (**18**), which is subsequently recruited into the NRPS module. The GdnE module was assigned to the polyketide chain elongation and functionalization prior to the guanidine intramolecular cyclization. At this point, the cyclized guanidine moiety tailoring includes the capture of an ureido moiety from carbamoyl phosphate. The last module, GdnH, recruits the unusual extender unit aminomalonyl-ACP, derived from serine via a two steps oxidation, first by NAD^+ then by FAD. The

aminomalonyl-ACP is incorporated into the polyketide chain forming a α-amino-β-ketothioester, which undergoes a reductive amination in the aminotransferase domain (AMT) of GdnH. The further incorporation of the alanyl-valine dipeptide residue into the polyketide chain occurs after its formation and release. A final oxidation is promoted by a Fe(II)-dependent oxygenase. The guadinomine B gene cluster and biosynthesis is closely related to that of cylindrospermopsin.



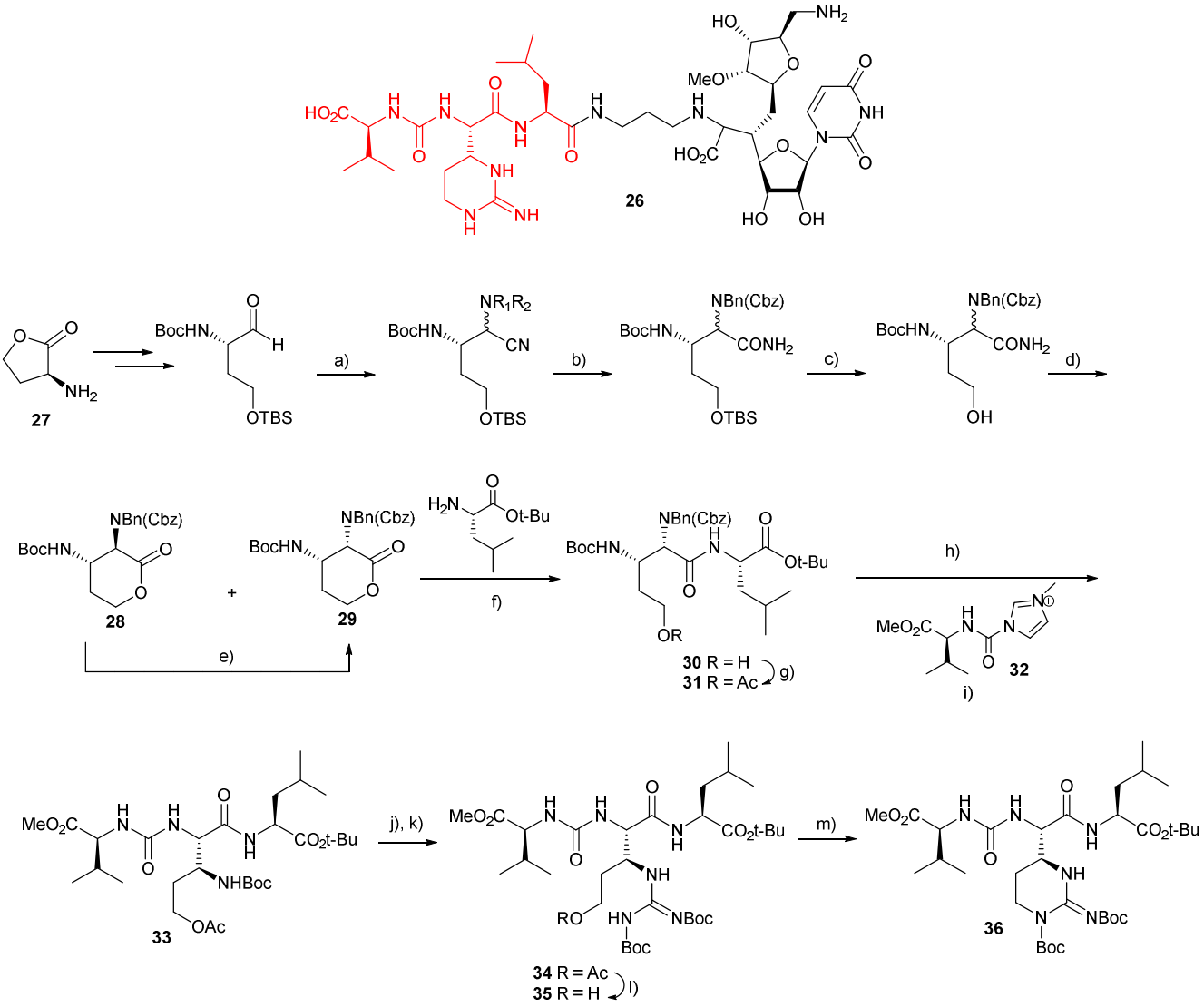
Scheme 2. Biosynthesis of guadinomine B (**17**).

A new synthesis of both α-amino-β-[4'-(2'-iminoimidazolinyl)]-β-hydroxypropionic acid (Aiha) moieties of mannopeptimycins (**18**) has been accomplished (Scheme 3).[171] Benzyl protected serine was employed as the starting material for the synthesis of both guanidine aminoacids, and it was efficiently converted to the alcohol **19** in three steps with 93% yield. Conversion of **19** to its corresponding aldehyde **20** preceded aldol condensation with the diphenyl imine **21**, to give the free alcohol **22** and the cyclized enol ether **23**, as anticipated. After chromatographic separation, both synthetic intermediates **22** and **23** were converted to the protected guanidine aminoacids **24** and **25** by a series of stepwise interconversions, before condensation between them with the additional aminoacid constituents of mannoeptimycins.



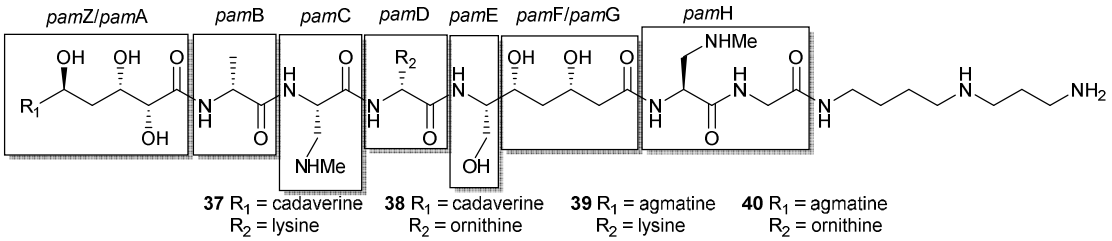
Scheme 3. Synthesis of protected α-amino-β-[4'-(2'-iminoimidazolinyl)]-β-hydroxypropionic acids **24** and **25**. *Reagents and conditions*: a) Swern oxidation; b) $LiN(TMS)_2$, THF, - 78 ^oC, SiO_2 chromat.; c) 1. TFA, Et_3SiH, H_2O; 2. Boc_2O, K_2CO_3; 3. BnBr, K_2CO_3 (74%, 2 steps); d) 1. TFA, Et_3SiH, H_2O; 2. Boc_2O, K_2CO_3; 3. MeI, K_2CO_3 (81%, 3 steps); e) 1. PPTS, 2-methoxyprop-1-ene; 2. $Pd(OH)_2$, H_2; f) 1. PPTS, 2-methoxyprop-1-ene; 2. Pd/C, HCO_2H, Et_3N; g) 1. *N*-triflyl-*N*',*N*''-dicarboxybenzylguanidine, Et_3N; 2. MeI, K_2CO_3 (64%, 3 steps); 3. MsCl, Et_3N, 88%; h) *N*-triflyl-*N*',*N*''-dicarboxybenzylguanidine, Et_3N; 2. MsCl, Et_3N (80%, 3 steps).

The ureidomuraymycidine tripeptide moiety (**36**) of muraymycin D1 (**26**) has been prepared (Scheme 4),[172] starting from (2*S*)-2-amino-γ-butyrolactone (**27**) via a stepwise interconversion into a mixture of lactones **28** and **29**. Eventually **28** could be quantitatively converted into **29**, which was coupled with acid-protected L-leucine, to give the dipeptide **30**. After the removal of the benzyl and carboxybenzyl protecting groups, the dipeptide **30** was coupled with the activated urea derivative of valine (**32**). Removal of the *tert*-butoxy carbonyl group of **33** preceded its conversion into the protected guanidine **34**. Acetyl group removal and intramolecular cyclization of the corresponding free alcohol gave the desired product **36**.



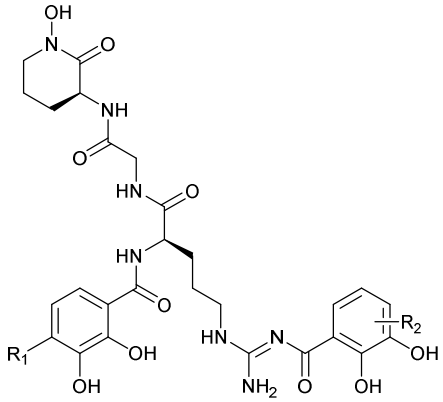
Scheme 4. Synthesis of the ureidomuraymycidine tripeptide moiety (**36**) of muraymycin D1 (**26**). *Reagents and conditions*: a) TMSCN, $MgSO_4$, CH_2Cl_2 (88%); b) (*E*)-acetaldehyde-oxime, $InCl_3$, toluene, 70 ^oC (84%); c) TBAF, AcOH, THF; d) toluene, reflux, (75%, 2 steps); e) DBU, toluene, rt, 1.5 h (100%) or pyridin-2(1*H*)-one, toluene, reflux, 3h; f) toluene, reflux (82%); g) Ac_2O, pyridine; h) H_2, Pd/C, AcOH, MeOH (80%); i) Et_3N, CH_2Cl_2/MeCN (87%); j) TFA, CH_2Cl_2; k) N,N'-diBoc-methylisothiourea, $HgCl_2$, Et_3N, DMF; l) $[t\text{-}Bu_2Sn(OH)Cl]_2$, MeOH (85%); m) DIAD, PPh_3, THF (76%).

Paenilamicins are hybrid NRPS/PKS antibiotics produced by the honey bee pathogen *Paenibacillus larvae*.[173] Production of the antibiotic mixture by *P. larvae* DSM25430 was followed by antibiotic-guided isolation, which led to a mixture of four paenilamicins A1 (**37**), A2 (**38**), B1 (**39**) and B2 (**40**), two of which (B1 and B2) have an agmatine residue. The antibiotics were identified by analysis of spectroscopic data. The paenilamicins gene cluster was identified within the genome of *P. larvae*, and presented a few particularities. The first module (*pam*A) is a flexible NRPS which activates both lysine or arginine for the dipeptide formation. The formation of D-alanine is performed by a racemase preceding D-Ala recruitment into the second NRPS module (*pam*B), after chain extension by two PKS modules. The unusual residue *N*-methyl-diaminopropionic acid was found to be loaded into de third module (*pam*C), as a rare example of a side chain aminoacid methylation by a methyl transferase, which is also recruited at the eighth module (*pam*H). The adenylation domain of the fourth NRPS module (*pam*D) also showed flexibility, in recruiting either lysine or ornithine into the antibiotic chain extension. Finally, a very unusual thioesterase release by reaction with spermidine is followed by the starting group hydrolysis at *pam*J, before formation of paenilamicin B1 (**39**). The paenilamicin antibiotic complex significantly inhibited the growth of a sister healthy strain, *P. larvei*, in the gut of mock larvae, showing that the mixture of paenilamicin antibiotics may impact honey bee survival.[173]

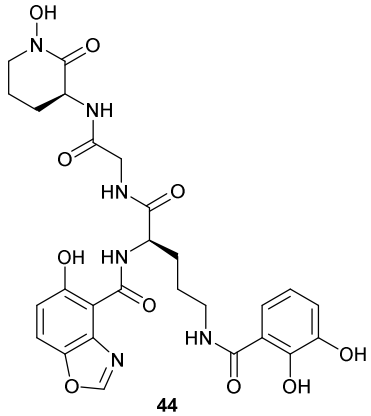


Cultivation of *Rhodococcus erythropolis* PR4 in iron-depleted medium led to the production of two new siderophores, heterobactins S1 (**41**) and S2 (**42**).[174] Heterobactin S2 was identified by analysis of spectroscopic data. Moreover, the correct structure of heterobactin A (**43**), previously isolated from

the culture medium of *R. erythropolis* IGTS8,[175] was also assigned since it was also produced by *R. erythropolis* PR4.[174] Both heterobactins S1 and S2 appear to be derived from sulfonation of heterobactin A in the presence of inorganic sulfate, which seems to be enzyme-catalyzed. Based on a distinct chromatographic behavior of **41** compared to **42**, and also by MS/MS analysis, it was assumed that **41** was sulfonated on a distinct position than **42**, not determined due to the small amount isolated of **41**. Genome analysis of *R. erythropolis* PR4 led to the identification of heterobactins gene cluster. An epimerization domain at the HtbG module is related to the presence of a D-arginine residue in the structure of heterobactins. Confirmation of gene cluster analysis was performed by gene deletions and heterologous expression in *E. coli*, enabling the proposed heterobactins biosynthesis (Scheme 5). The second HtbG module presents unusually arranged C-PCP-A modules, for the first time reported instead of a canonical C-A-PCP arrangement.



41 $R_1 = R_2 = H$
42 $R_1 = SO_3H$, $R_2 = H$
43 $R_1 = H$, $R_2 = SO_3H$



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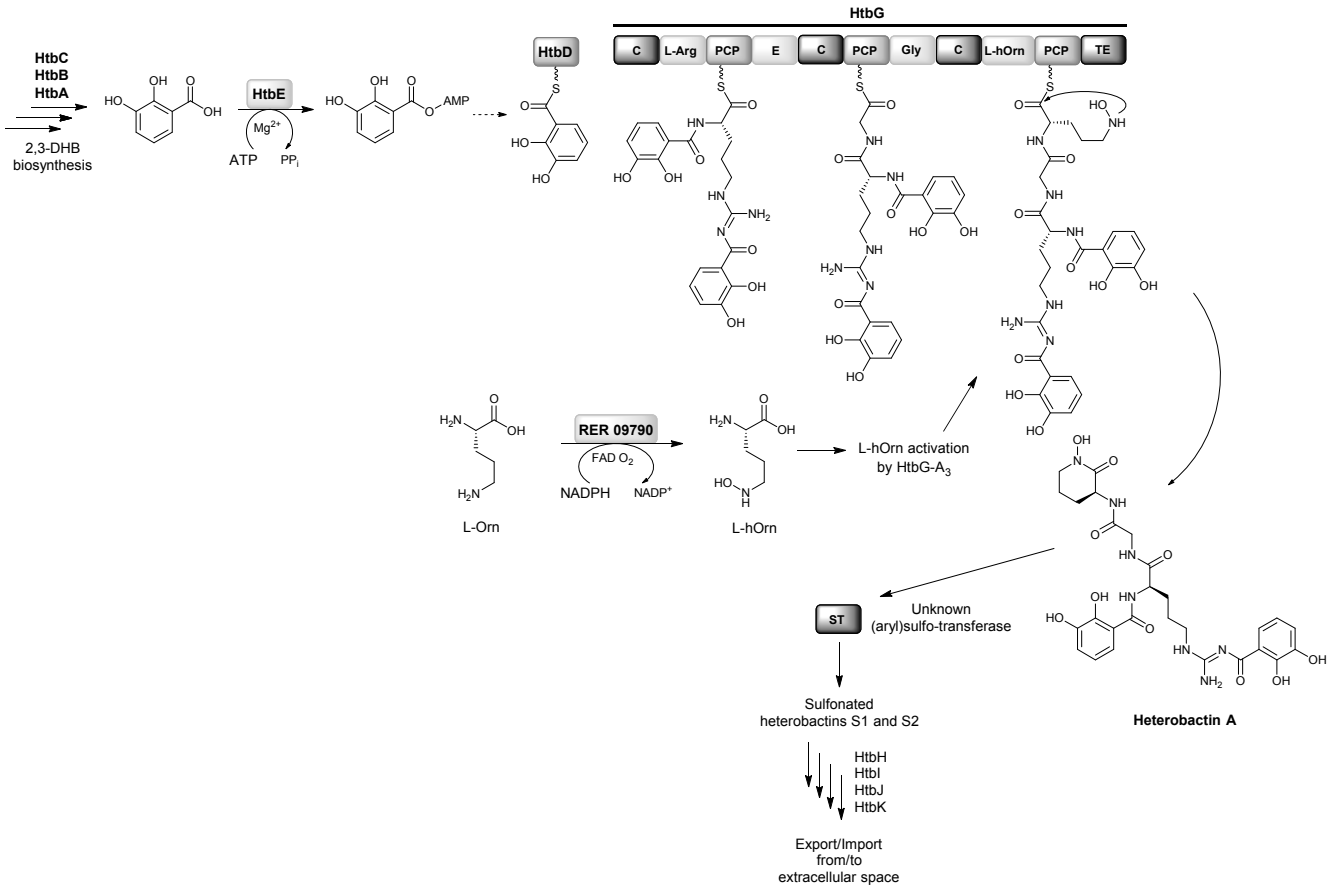
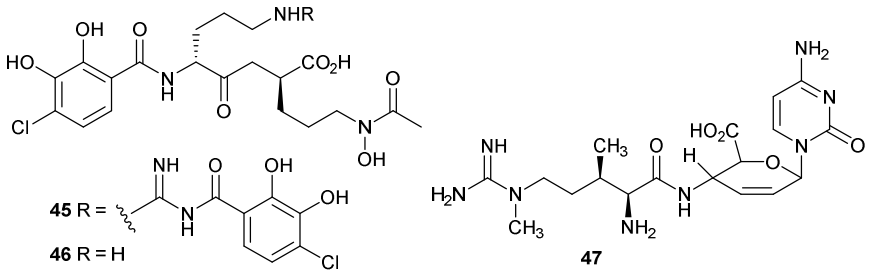
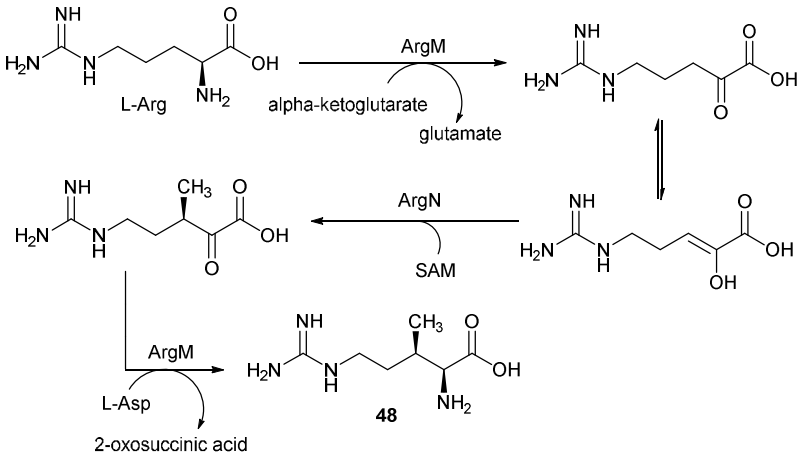


Scheme 5. Proposed biosynthetic assembly for heterobactins.

Somewhat related to heterobactins are chlorocatechelins A (**45**) and B (**46**), siderophores produced by *Streptomyces* sp. ML93-86F2.[176] The structure of **45** was established by extensive spectroscopic analyses, including MS/MS analysis and ^{13}C NMR hydrogen-deuterium exchange analysis in 60:1 DMSO-d_6/D_2O. The absolute configuration of aminoacids were assigned by Marfey's analysis. The structure of **46** was also established by extensive spectroscopic and chemical analyses. Spectrophotometric titration of **45** in the presence of $FeCl_3$ indicated a 1:1 Fe(III)/**45** ratio. Cyclic voltametric analysis of **45** in the presence of $FeCl_3$ gave a half wave potential ($E_{1/2}$) of -578 mV, higher than that of ferrioxamine ($E_{1/2}$ of -440 mV), indicating a higher affinity of Fe(III) for **45**. Chlorocatechelin A (**45**) was sponteneously converted into **46** under acidic conditions.



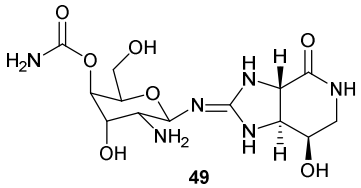
Biosynthesis of the β-methylarginine moiety of arginomycin (**47**) produced by *Streptomyces arginensis* NRRL 15941 has been elucidated.[177] The arginomycin (**47**) gene cluster (29 kb) was first heterogously expressed by *Streptomyces coelicolor*. Then, gene/enzyme individual assignment to each biosynthetic step was monitored by HPLC-MS. Results showed that a *S*-adenysylmethionine-dependent methyl transferase ArgN was preceded by transamination by the pyridoxal-phosphate-dependent aspartate-based transaminase ArgM in converting L-arginine into (2*S*,3*R*)-β-methylarginine (Scheme 6).



Scheme 6. Biosynthesis of (2*S*, 3*R*)-β-methylarginine by *Streptomyces arginensis* NRRL 15941.

Several steps of the biosynthesis of streptothricins (STs), with β-lysine oligomers of variable length attached to the 8-amino group of streptotrisamine (**49**) produced by *Streptomyces lavendulae*,

have been established (Scheme 7).[178] A 34 kbp gene cluster was identified and had its genes expressed by *Streptomyces lividans* TK 23. To the gene ORF18 was assigned the production of streptothrisamine (**49**), an unprecedented guanidine alkaloid devoid of the β-lysine oligomeric chain. The module ORF5 promotes adenylation of a β-lysine residue before it is loaded into ORF18, where the β-lysine oligomeric chain is extended. Additional β-lysine residues are loaded into ORF18 by the additional adenylation domain ORF19, which also promotes sequential condensation between the residues. After a suitable β-lysine oligomer extension, freely diffusible **49** is condensed to the β-lysine oligomeric chain by ORF18, in order to produce streptothricin C (**50**).



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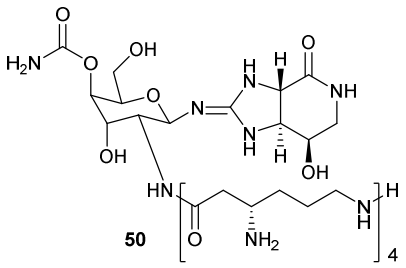

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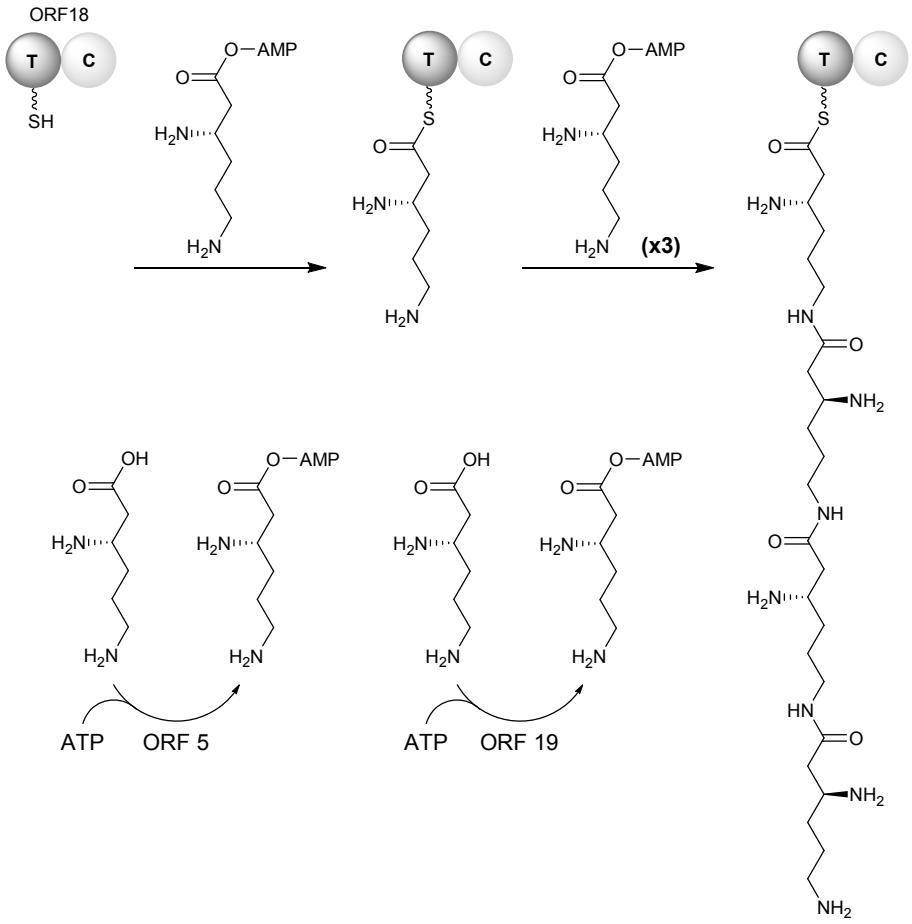
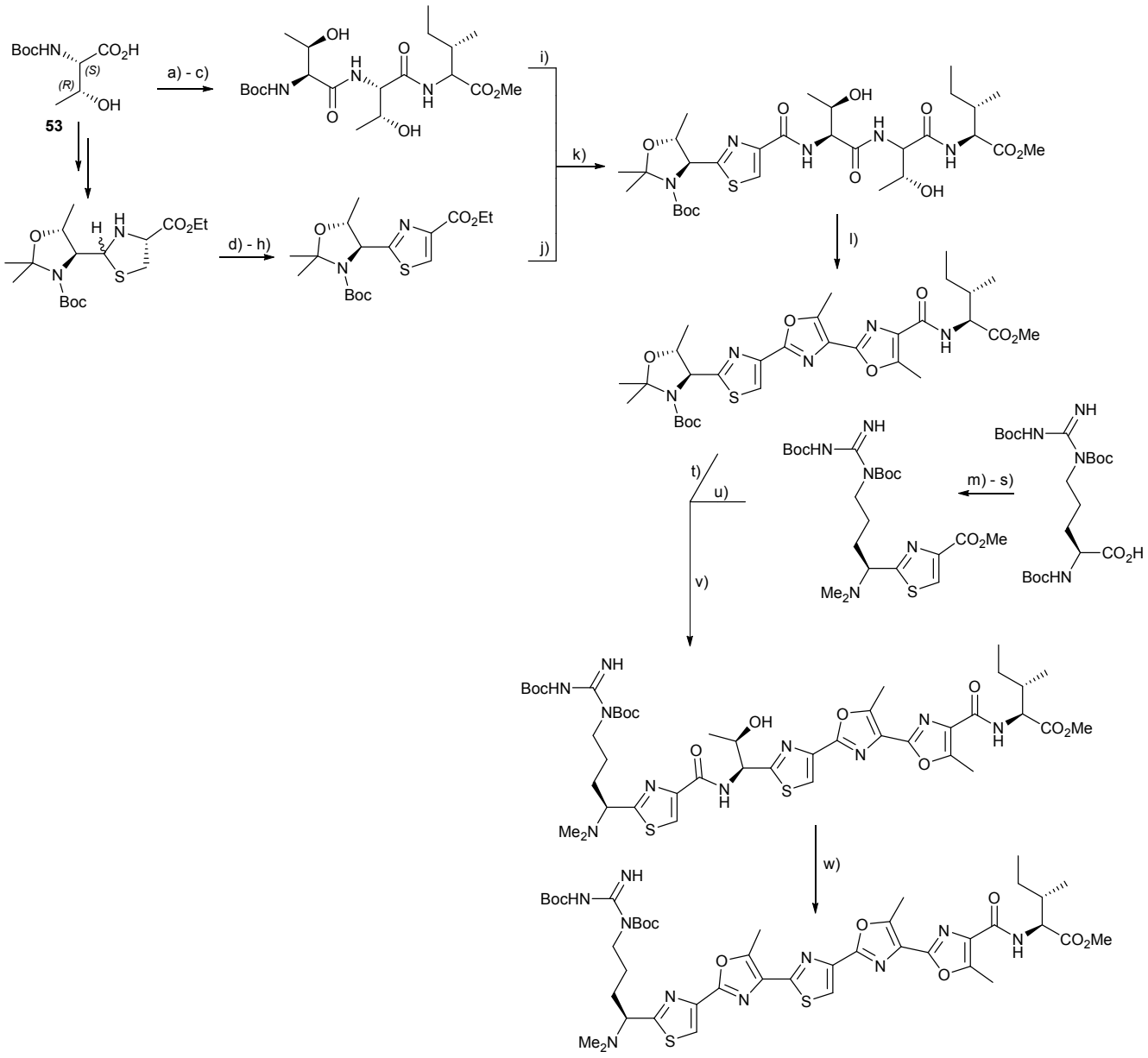


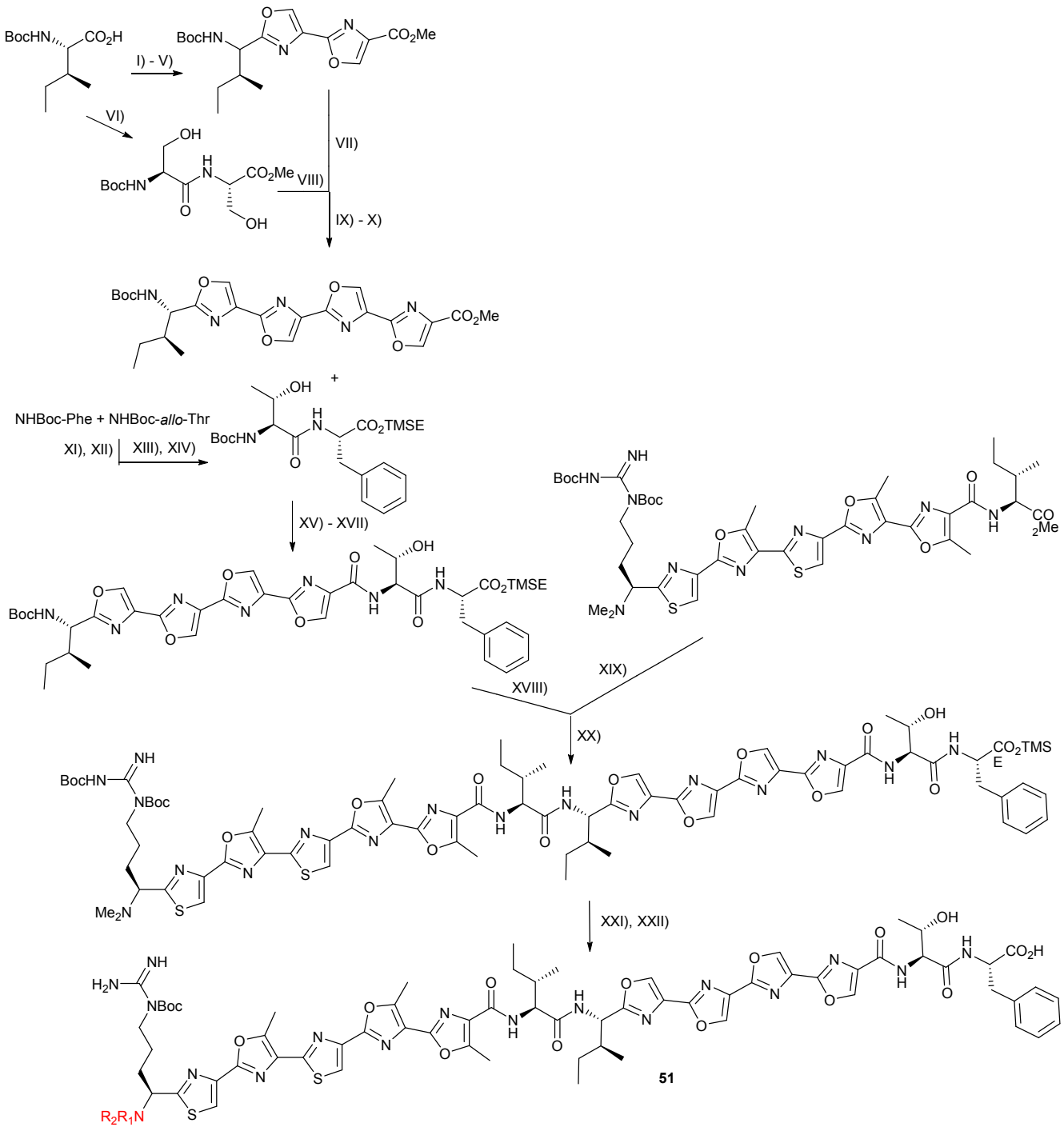
Scheme 7. Extension of the β-lysine oligomeric unit in streptothricins biosynthesis.

The total synthesis of plantazolicin A (**51**, R_1=R_2=Me) and B (**52**, R_1=R_2=H), isolated from the bacterium *Bacillus amyloliquefaciens* FZB42,[179a,b] has been achieved following a highly convergent approach, using an efficient strategy for the preparation of oxazoles and thiazoles and subsequent coupling (the synthesis of **51** is shown in scheme 8).[179c] The preparation of truncated analogues of plantazolicins enabled the investigation of structural requirements for the operation of BamL, an unusual methyltransferase that promotes the methylation of α-thiazole substituted arginine.[180]



Scheme 8. Total synthesis of Plantazolicin A (**51**). *Reagents and Conditions*: a) Ile-OMe·HCl, HOBt, EDCI, EtN(*i*-Pr)$_2$, CH_2Cl_2, rt, 19 h (99%); b) HCl, 1,4-dioxane, rt, 23 h; c) Boc-Thr-OH (**53**), same reagents as for a), 18 h, (79%, 2 steps); d) CH_3ONHCH_3·HCl, same as for a), 22 h; e) $CH_3C(OCH_3)_2CH_3$, PPTS, THF, reflux, 18 h, (86%, 2 steps); f) DIBAL-H, CH_2Cl_2, -78 ^oC, 1 h; g) Cys-OEt·HCl, $KHCO_3$, MeOH/H_2O/toluene (1:1:1), rt, 18 h, (83%, 2 steps); h) MnO_2, toluene, 80 ^oC, 24 h (59%); i) LiOH·H_2O, MeOH/H_2O (3:2), rt, 3 h; j) HCl, 1,4-dioxane, 30 min; k) same as for a), 18 h, (71%, 3 steps); l) Deoxo-Fluor, CH_2Cl_2, -20 ^oC, 2 h, then $BrCCl_3$, DBU, 5 d, 0 ^oC (64%); m)

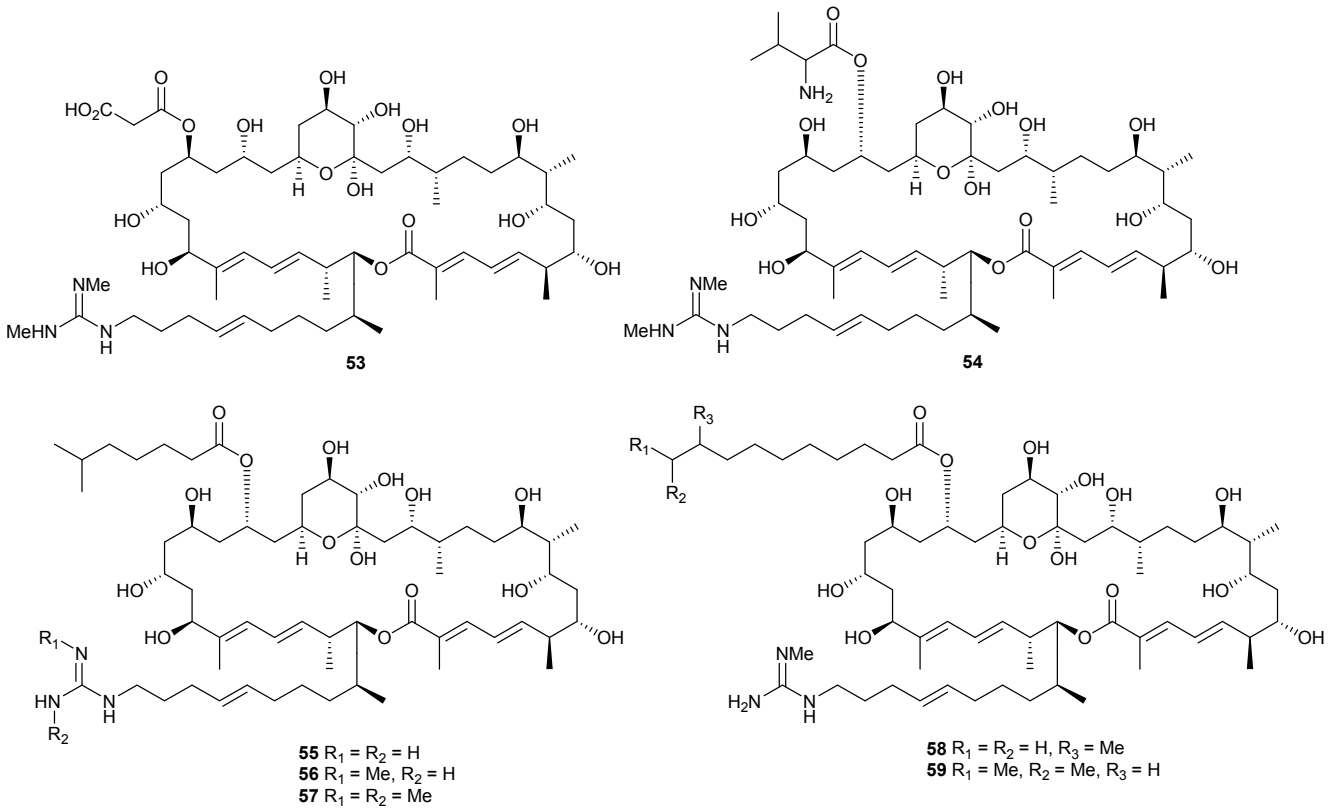
$CH_3ONHCH_3 \cdot HCl$, same as for a), 16 h (96%); n) DIBAL-H, CH_2Cl_2, -78 ^oC, 1 h; o) Cys-OMe·HCl, $KHCO_3$, MeOH/H_2O (2:1), rt, 41.5 h, (78%, 2 steps); p) MnO_2, toluene, 80 ^oC, 15 h (48%); q) HCl, 1,4-dioxane, rt, 1 h; r) formaldehyde (37% in H_2O), MeOH, rt, 1 h then $NaCNBH_3$, 15.5 h; s) Boc_2O, EtN(*i*-Pr)$_2$, CH_2Cl_2, rt, 48 h (35%); t) HCl, 1,4-dioxane, rt, 1 h; u) LiOH, THF/H_2O (1:1), 0 ^oC, 1.5 h; v) HATU, EtN(*i*-Pr)$_2$, CH_2Cl_2, DMF, 0 $^oC \rightarrow$ rt, 22 h (61%); w) Same conditions as l), 20 h (69%).



Scheme 8 (contd.). I) Ser-OMe·HCl, same as for a), 20 h (91%); II) Same conditions as l), 2-3 ^{o}C, 8 h (81%); III) LiOH·H_2O, THF/MeOH/H_2O (5:5:1), 0 ^{o}C → rt, 18 h; IV) Ser-OMe·HCl, same as for a), 20 h, (82%, 2 steps); V) Same conditions as l), 2–3 ^{o}C, 7 h (78%); VI) Ser-OMe·HCl, same as for a), 20 h (88%); VII) LiOH.H_2O, THF/MeOH/H_2O (10:6:1), 0 ^{o}C → rt, 2 h; VIII) HCl, 1,4-dioxane, 0 ^{o}C → rt, 3.5 h; IX) same as for a), 20 h, (61%, 3 steps); X) Deoxo-Fluor, CH_2Cl_2, -20 ^{o}C, 45 min, then

$BrCCl_3$, DBU, 0 ^oC, 24 h (77%); XI) $(CH_3)_3SiCH_2CH_2OH$, EDCI, DMAP, CH_2Cl_2, 0 ^oC → rt, 18 h (74%); XII) HCl, 1,4-dioxane, 0 ^oC → rt, 30 min; XIII) $NaHCO_3$, Boc_2O, H_2O, MeOH, rt, 15 h; XIV) HATU, EtN(*i*-Pr)$_2$, CH_2Cl_2, 0 ^oC → rt, 15 h, (81%, 3 steps); XV) $LiOH{\cdot}H_2O$, $CHCl_3$/MeOH/H_2O (9:3:1), 68 ^oC, 48 h; XVI) HCl, 1,4-dioxane, 0 ^oC → rt, 4 h; XVII) HATU, EtN(*i*-Pr)$_2$, CH_2Cl_2, DMF, 0 ^oC → rt, 18 h (77%, 3 steps); XVIII) LiOH, THF/H_2O (1:1), 0 ^oC, 2.25 h; XIX) HCl, 1,4-dioxane, 0 ^oC, 5 min, rt, 30 min; XX) HATU, EtN(*i*-Pr)$_2$, CH_2Cl_2, DMF, 0 ^oC → rt, 16 h; XXI) Deoxo-Fluor, CH_2Cl_2, -20 ^oC, 24 h (43%); XXII) TFA, rt, 2 h (59%). Deoxo-Fluor = bis(2-methoxyethyl)aminosulfur trifluoride.

Azalomycins belong to the polyene class of polyketide macrolides, which very often present an alkyl guanidine moiety.[1] Seven new azalomycins have been isolated from the growth medium of *Streptomyces* sp. 211726 strain, obtained from the rhizosphere of *Heritiera globosa* found in Wenchang, China. Only the relative stereochemistry of 25-malonyl demalonylazalomycin F_{5a} monoester (**53**), 23-valine demalonylazalomycin F_{5a} ester (**54**), 23-(6-methyl)heptanoic acid demalonylazalomycins F_{3a} ester (**55**), F_{4a} ester (**56**) and F_{5a} ester (**57**), 23-(9-methyl)decanoic acid demalonylazalomycin F_{4a} ester (**58**) and 23-(10-methyl)undecanoic acid demalony lazalomycin F_{4a} ester (**59**) have been established.[181] The antimicrobial activity of **53** – **59** has been evaluated against *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis* and *Eschzerichia* (*Escherichia*?) *coli*. Results showed sub-μg/mL activities against *S. aureus* and *E. coli*.



3 Saxitoxins and Tetrodotoxins

Research on saxitoxins and tetrodotoxins continues to be of much interest, not only due to the unique chemistry of these guanidine alkaloids, particularly for organic synthesis, but also because currently the actual biological role(s) of these guanidine alkaloids is under intense investigation. Reviews on saxitoxins and tetrodotoxins published between 2012 and 2014 include the general review "An Overview on the Marine Neurotoxin, Saxitoxin: Genetics, Molecular Targets, Methods of Detection and Ecological Functions",[182] the excellent, authoritative and comprehensive "Saxitoxin",[183] a short review on the "Synthesis of Saxitosins",[184] "Tetrodotoxin (TTX) as a Therapeutic Agent for Pain",[185] a summary on Nishikawa and Isobe's approach towards the synthesis of tetrodotoxin "Synthesis of Tetrodotoxin, a Classic but Still Fascinating Natural Product",[186] a broad review on "Tetrodotoxin: Chemistry, Toxicity, Source, Distribution and Detection",[187] as well as the focused on pharmacology and biophysics rather comprehensive review "The molecular mystique of tetrodotoxin".[188]

Additionally, two similar reviews, which present the concept of keystone biological molecules as those whose "actions disproportionately determine species assemblages and the rates of material and energy flow across entire [biological] communities", and include the possible biological roles and potential adaptative functions of textrodotoxin and saxitoxin, are "Community Ecology and the Evolution of Molecules of Keystone Significance"[189] and "Molecules of Keystone Significance: Crucial Agents in Ecology and Resource Management".[190]

Although, to the best of our knowledge, no new saxitoxin (**60**) derivative has been isolated between 2012 and 2014, a series of articles started to investigate the evolution of saxitoxins' biosynthesis in cyanobacteria and dinoflagellates, a subject that quickly became controversial.

The first of such investigations was published in 2011,[191] and was inadvertently ommited in our previous review within this series.[192] In order to address the phylogeny of saxitoxin genes, the gene *sxtA* served as a template for the mining analysis of *Alexandrium fundyense* CCMP1719 and *A. minutum* CCMP113 nuclear genome, using *in silico* transcriptome, RACE, qPCR and conventional PCR along with Sanger sequencing analysis. As for *A. fundyense* CCMP1719 genome analysis, the terminal aminotransferase domain was missing. Further analyses were not unambiguous, since dinoflagellate genomes are extremely large, over 60 times larger than the human haploid genome,[193] with a large GC content, sometimes with the presence of multiple copies, not necessarily identical,[191] making dinoflagellate genome analysis extremely challenging. However, saxitoxin biosynthetic genes *sxtA1* and *sxtA4* were found in fifteen STX-producing dinoflagellate species, even if the production of STX itself was not detected in some of the strains, possibly due to different reasons. The results indicated a non-bacterial producing system within dinoflagellates and suggested a horizontal gene transfer mechanism from ancient STX-producing cyanobacteria to dinoflagellates. However, several points remained open for further investigation, such as the understanding of the relationship of *sxtA* sequences in different dinoflagellate species as well as the non-amplification of *sxtA1* and *sxtA4* genes

detected in non-STX-producing dinoflagellate strains.[191] In a subsequent investigation by the same authors, the second gene of the saxitoxin cluster, *sxtG*, was used as a marker.[194] Complete *sxtG* mRNA transcripts from *A. fundyense* CCMP1719 and *A. minutum* CCMP113, as well as partial transcripts from five additional dinoflagellate strains were amplified and analyzed. The results confirmed a dinoflagellate origin of saxtitoxin genome. The gene *sxtG* has a wider distribution than *sxtA*, inclusive in non-STX-producing strains. The phylogenetic analysis of *sxtG* was less clear than of *sxtA*, because of the prevalent sequence conservation of the coding sequence between dinoflagellate species. No parallel could be estalished between *sxtG* and *sxtA* in *Alexandrium* spp. The horizontal gene transfer of distinct genes was considered as multiple events, however unlikely. Further questions remained opened, mainly due to the complexity of dinoflagellate genome analysis.[194]

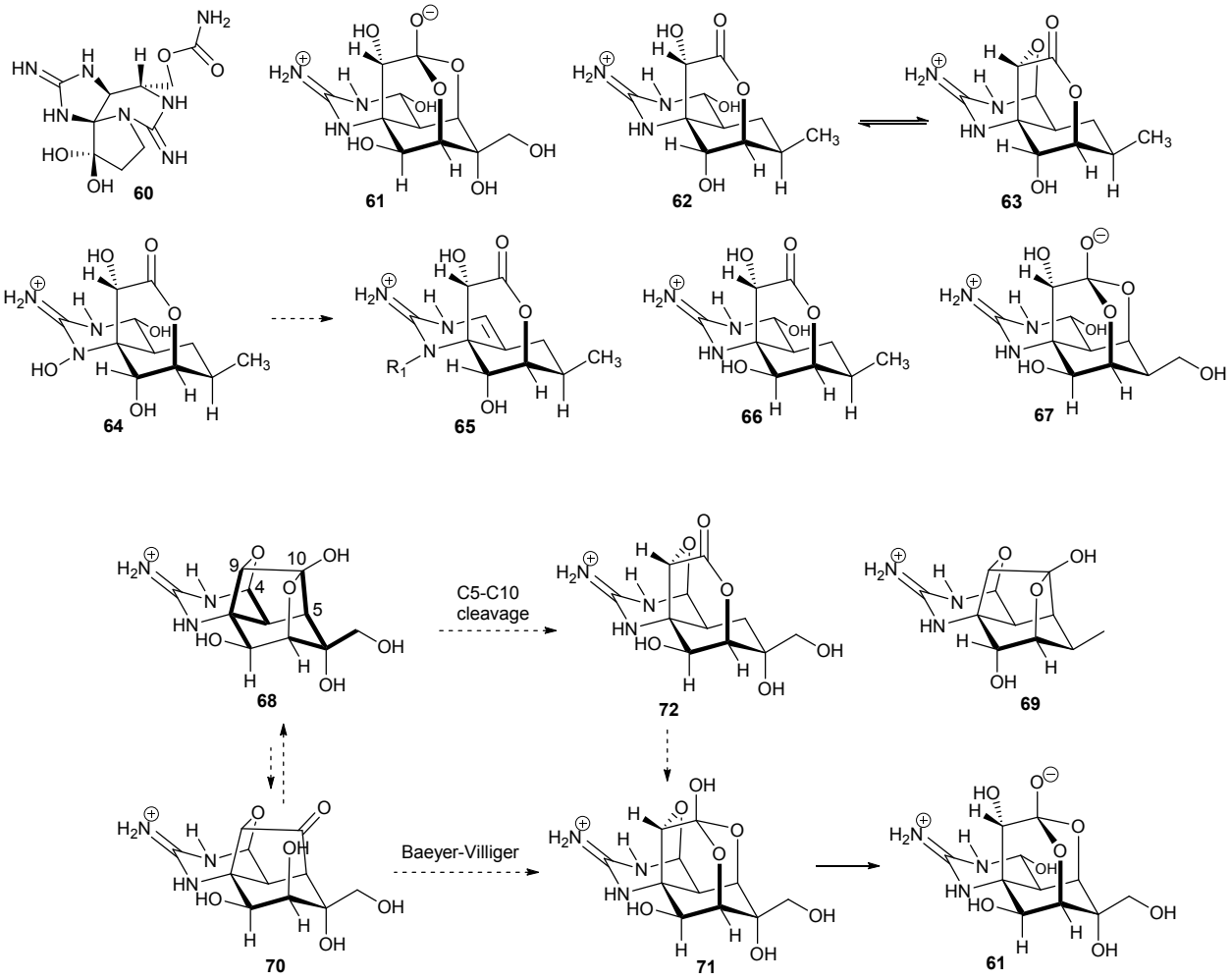
A related investigation by Hackett et al.[195] using transcriptome and phylogenetic analysis indicated that *sxtA* genes in dinoflagellates were acquired independently from cyanobacteria, since they did not show relationships to the cyanobacterial *sxtA* genes, such as not fused *sxtA*-related proteins and distant phylogeny. Moreover, a monophyletic relationship was observed for *sxtA*-related proteins in STX-producing dinoflagellates and cyanobacteria, separately. Similar results were observed for *sxtG*-related proteins. Questions were raised concerning the likeliness of the proteins involved in the three first steps of saxitoxin biosynthesis being unique, since these proteins likely present several homologs in distinct organisms. Moreover, genes *sxtA*, *sxtG* and *sxtB* appear to be of a common ancestor origin, event though not directly related to saxitoxin biosynthesis, since these genes are found in many non-STX producing organisms. Finally, it seemed possible that distinct saxitoxin biosynthetic pathways may be operative in cyanobacteria and dinoflagellates, suggesting that in the last organisms it may be facilitated by associated bacteria.[195]

Orr and collaborators authoritatively and comprehensively reviewed the overall results obtained by these investigations.[193] The authors proposed that a hypothesis of evolutionary convergence to

explains separate origins of saxitoxin biosynthetic machinery in both cyanobacteria and dinoflagellates was discarded, based on the similar homology of *sxt* genes found in these phylogenetically distant organism groups. A proposal that the saxitoxin biosynthetic machinery evolved once in cyanobacteria and was transferred massively to dinoflagellate strains was strongly supported by the results so-far obtained, but many questions remain unanswered related to genes loss and when the horizontal gene transfer occurred during dinoflagellate evolution.[193] Therefore, the origin of the saxitoxin biosynthetic genes still remains inconclusive.

The fascinating topic of saxitoxin biosynthetic machinery origin and evolution may well be related to the so-far completely unknown biosynthetic origin of tetrodotoxin (**61**). The occurrence of an increasing number of tetrodotoxin (TTX) derivatives in both pufferfishes and newts is very intriguing. Yotsu-Yamashita's group strategy to unveil the biosynthesis of TTX is the isolation of unprecedented TTX derivatives, which may be biosynthetic intermediates in the TTX biosynthetic pathway. Among the new TTX derivatives isolated between 2012 and 2014, 8-*epi*-5,6,11-trideoxyTTX (**62**), 4,9-anhydro-8-*epi*-5,6,11-trideoxyTTX (**63**), 1-hydroxy-8-epi-5,6,11-trideoxyTTX (**64**) and 1-hydroxy-4,4a-anhydro-8-epi-5,6,11-trideoxyTTX (**65**) were obtained from the newt *Cynops ensicauda popei*.[196] Tetrodotoxin **63** is the anhydro derivative of **62**, and both showed some interconversion, while **65** is the dehydration product of **64**. Analysis by HPLC-MS showed that compounds **62** – **65** had no occurrence in the tissues of the pufferfish *Fugu poecilonotus*, but TTX (**61**) is the major metabolite in both animals. Interestingly, 5,6,11-trideoxyTTX (**66**) is found only in *F. poecilonotus*. It was suggested that **66** is the biosynthetic precursor of TTX (**61**) in pufferfish, but only its 8-epimer **64** is found in newts. 6-DeoxyTTX (**67**) has been isolated from *Takifugu pardalis* and identified by analysis of spectroscopic data.[197] Compound **67** was subsequently detected by HPLC-HRMS in tissues of the snail *Nassarius glans*, of the Japanese blue-ringed octopus *Hapalochlaena* sp., and of the Australian blue-ringed octopus *H. maculosa*, and it was suggested as the direct putative biosynthetic precursor of TTX. 6-

DeoxyTTX (**67**) showed three-fold less potent blocking of the Na^+ voltage-gated channel than TTX. The highly unusual 4,9-anhydro-10-hemiketal-5-deoxyTTX (**68**) and 4,9-anhydro-8-epi-10-hemiketal-5,6,11-trideoxyTTX (**69**) have been isolated from the newt *Cynops ensicauda popei*.[198] The amounts obtained of **68** (0.2 mg) and of **69** (0.08 mg) enabled their complete structure analysis. HPLC-HRMS analysis of tissues of 22 newt species belonging to genera *Cynops*, *Paramesotriton*, *Taricha* and *Notophthalmus* indicated that the TTX derivative **68** is prevalent in all animals, while **69** is found only in some specimens of *C. e. popei*, and was considered as a shunt biosynthetic product of the TTX pathway. Based on the isolation of **68** and **69**, it was suggested a monoterpenoid biosynthesis for the TTX skeleton. Although the arrangement of the isoprene moieties was not clearly indicated in the original report, it is assumed as drawn herein. The new TTX derivative **68** would be a TTX precursor *via* its conversion into its 10-keto form (**70**), followed by a Baeyer-Villiger oxidation to 4,9-anhydroTTX (**71**) before its hydrolysis to TTX (**61**), or via 4,9-anhydro-5-deoxyTTX (**72**) and **71** to **61**. In any instance, the isolation of such minor and of restricted occurrence TTX derivatives provides a wider landscape on the TTX biosynthesis complexity, involving an array of oxidative reactions of particular interest to biosynthetic and synthetic chemists.



A considerable body of research on the ecology of TTX-bearing organisms has been reported, mainly related to toxic newts, but also to pufferfish, a marine mollusc and to terrestrial flatworms. The pufferfish *Takifugu rubripes* can smell tetrodotoxin with its olfactory apparatus, in the concentration range of 3.7 to 5.6 10^{-9} mol/L.[199] When maintained in captivity with a TTX-free diet, the opistobranch *Pleurobranchea maculata* levels of TTX diminish in time to an almost undetected level after 4 months, contrary to previous results observed for the newt *Taricha granulosa*, for which the levels of TTX increased over time.[200] In a more recent assessment of TTX maintenance in *T. granulosa*, a three years measurement in captivity showed a slow decrease, bot not complete lost, of TTX accumulation.[201] During this investigation, a single newt specimen was found to contain up to 28 mg of TTX, an amount

sufficient to kill 56 humans. The TTX amount in *T. granulosa* eggs varied considerably across years, but was not related with a reduction of the newt egg mass while in captivity.[201] On the other hand, the red-spotted newt *Notophthalmus viridescens* lost TTX when the animals were kept in captivity over six years.[202] The eggs of *T. granulosa* are preyed by the caddisfly larvae (*Limnophilus* spp., Trichoptera), and TTX is sequestered by the insect.[203] Curiously, insect larvae kept in laboratory maintain TTX levels in the absence of a TTX-bearing diet, presenting even an increase of the toxin levels.

Newts of the species *Cynops pyrrhogaster* were maintained in captivity during 10 years in order to assess the level of TTX in the animal tissues.[204] In total, 221 adult newts belonging to 27 populations were analyzed. A significant variation of TTX amounts was observed between populations, but not between sexes of the animals. Newts from mainland populations presented higher concentrations of TTX than an insular population. An explanation to the higher levels of TTX in mainland populations is related to the predation by mammals, which are less prone to be inhibited by aposematism than birds, the main predator of the news in the insular niche. However, during the time of the experiment, TTX levels of the insular population increased while of the mainland populations decreased. Additionally, an indirect correlation was observed between the body size and the amount of toxin. No correlation was observed between aposematic coloration due to the presence of carotenoids in newts tissues and the levels of TTX. Changes of TTX levels in mainland newt tissues over time was assigned to human impact, indicating that such changes are consequence of environmental modifications rather than due to genetic variation.[204] The intestinal parasites (Nematoda, Trematoda and Cestoda) of the newt *Notophthalmus viridescens* are tolerant to TTX and 6-epiTTX, even when the toxins are found in high levels, up to 33.7 μg/g.[205]

Tetrodotoxin has been found for the first time in terrestrial invertebrates, the flatworms *Bipalium adventitium* and *B. kewense*, invasive to USA from southeastern Asia.[206] Earthworms prey of

B. adventitium and *B. kewense* showed partial paralysis after being attacked, a behaviour that led to the discovery of TTX in the flatworms.

A quite interesting historical account on Professor Butch Brodie's research on the co-evolutionary arms race between toxic newts and garter snakes has been highlighted.[207] The snake *Thamnopsis sirtalis* is known to have developed resistance to TTX, a fact that explain the high levels of TTX production/accumulation within the body of the newt *Taricha granulosa*. This is one of the very few documented cases of coevolution between predators and prey, clearly mediated by a secondary metabolite, tetrodotoxin. Interestingly, Professor Brodie's investigations demonstrated that in regions where newts have low TTX levels, such as in Vancouver Island, sympatric garter snakes present low resistance to TTX, and vice-versa where newts have high levels of the toxins. In some snake populations the resistance level is so high that for those snakes it would be unfeasible to eat enough newts to get intoxicated.[207]

Contrary to pufferfish that are capable of smelling TTX, garter snakes are insensitive to TTX via chemoreception, using the forked tongue that brings chemicals to the vomeronasal organ. The snake *Thamnophis sirtalis* cannot detect or respond to TTX using the tongue. Rather, the snake has to attack the prey and get it into the grastrointestinal tract in order to either eat it or reject it when the TTX levels are too high.[208]

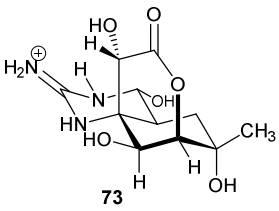
Biophysical constraints of the voltage-gated sodium channels determined the acquisition of TTX resistance by garter snakes during evolution.[209] Such resistance is likely a consequence of evolutionary convergence, since it has been observed independently in North America, Central/South America and in Asia. It has been estimated that TTX resistance appeared at least six times during evolution in snakes that prey on toxic animals. In garter snakes of these three continents, only 13 amino acid substitutions were found in proteins of sodium channels of snakes that consume TTX-bearing prey These amino acids form the vestibule (the ion-selectivity filter) and constitute an optimal environment

for Na^+ permeation, which is inhibited by TTX. Therefore, in TTX-resistant snakes, only slight mutations of the Na^+ channels arose in order not to compromise the channel's function. High levels of molecular convergence have been observed among pufferfish Na^+ channels as well as between pufferfish and snakes, but pufferfishes show a wider diversity of mutations than snakes. As a result of such analyses, it was suggested that "evolutionary constraints based on the functional consequences of amino acid replacements limit the spectrum of genetic variation available for adaptative evolution and shape the route to convergence".[209]

Parallel evolution of TTX-resistance in two voltage-gated sodium channel proteins of *Thamnophis sirtalis*, $Na_v1.6$ and $Na_v1.7$, has been observed.[210] Results showed that TTX-resistance in peripheral nerves arose earlier during evolution than in muscle, mainly because the levels of TTX to affect peripheral nerves is considerably smaller than to affect muscles. Mutations in $Na_v1.6$ and $Na_v1.7$ have been observed in both Oregon, California and Virginia populations of *T. sirtalis* to confer resistance, but mutations in $Na_v1.4$ have been observed only in *T. sirtalis* populations of the west US coast, where highly toxic populations of *Taricha* newts are found. It is suggested that resistance in $Na_v1.6$ and $Na_v1.7$ channels evolved specifically in response to selection promoted by the TTX levels in garter snake prey.[210] A scholar critical analysis on the evolution of voltage-gated sodium channels illustrates the many fascinating aspects of this essential physiological system in animals, with mention to the TTX resistance.[211] Among the various curious aspects which are mentioned, the fact that TTX-resistant amino acid substitutions in pufferfish and garter snakes voltage-gated sodium channels occurred multiple times during evolution deserves mention, as an illustration of how powerful the driving force of adaptation can be towards a favourable trait mediated by a secondary metabolite.

Total syntheses of (-)-5,11-dideoxyTTX (**73**),[212] 5-deoxyTTX (**74**),[213] 8-deoxyTTX (**75**),[213] 5,6,11-trideoxyTTX (**76**)[214] and of chiriquitoxin (**77**) have all been accomplished by similar approaches developed by Professor Nishikawa's group. The construction of chiriquitoxin amino acid side chain

was performed starting from **78**, an intermediate of TTX synthesis (Scheme 9). The trichloroacetyl protecting group in **78** was replaced by a benzyl carbamate in **79**, aiming to avoid the harsh conditions for the trichloroacetyl group deprotection later in the synthesis. It followed the TBS deprotection in aqueous HCl in EtOH and the Albert-Goldman oxidation to provide aldehyde **81** in 96% for this last step. The aldehyde group of **81** was condensed with the tricycloiminolactone **83** as a chiral glycine equivalent, in 69% yield. Subsequent transformations included the guanidylation at the amino group and the removal of alcohol protection groups, which required a series of transformations including a Pummerer rearrangement of sulfoxide **86**. The synthesis was completed by removal of the acid-sensitive protecting groups with simultaneous formation of lactone **89** prior to its hydrolysis with 0.1% pyridine in H_2O, to give chiriquitoxin (**77**).



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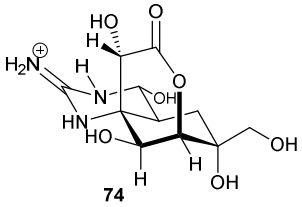

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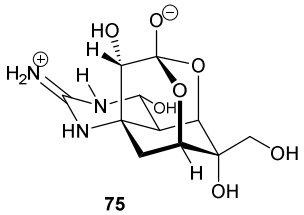

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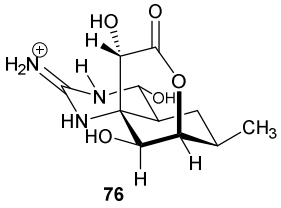

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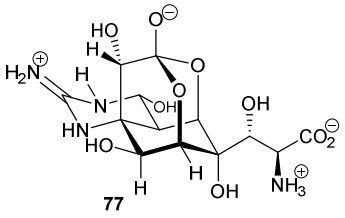

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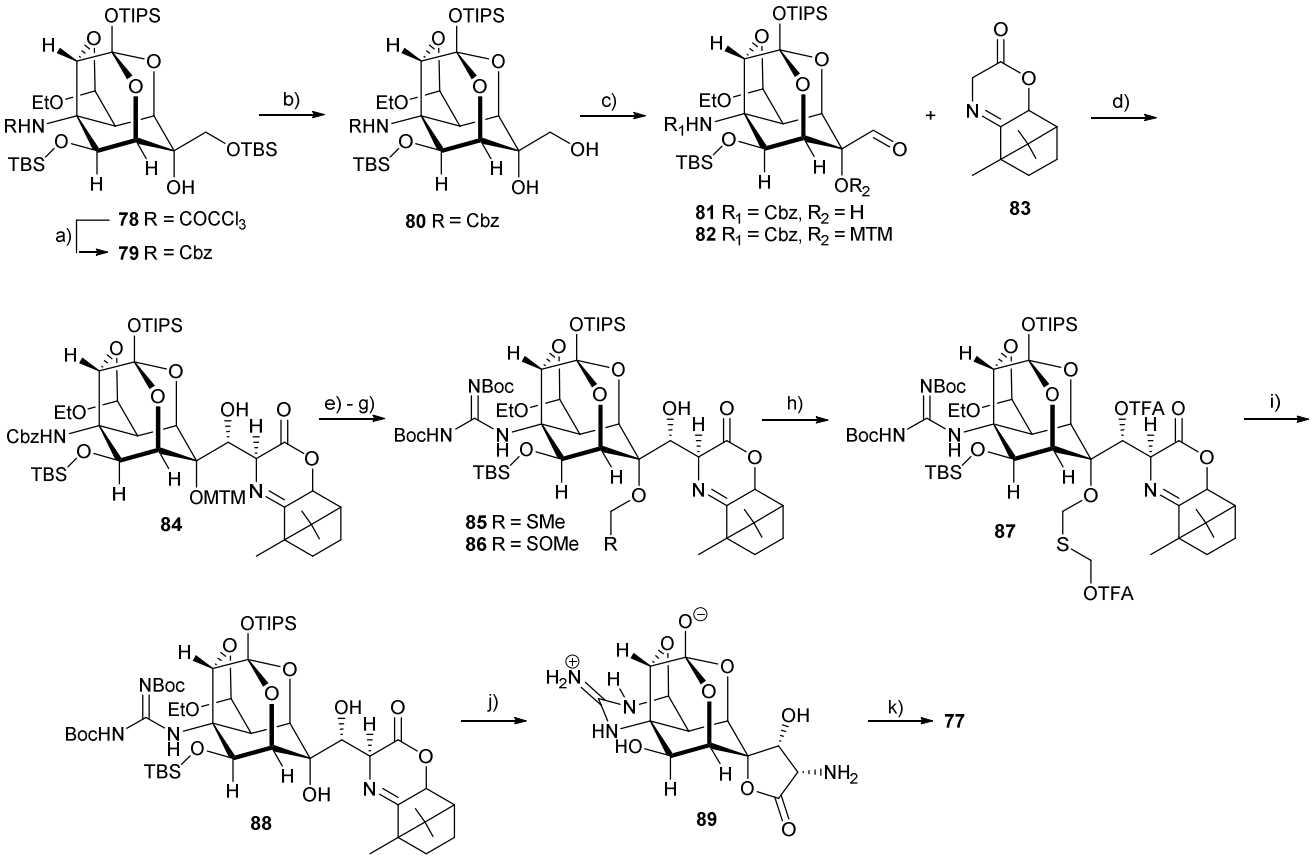


Scheme 9. *Reagents and conditions*: a) BnOH, Na_2CO_3, DMF, 150 ^oC, 2 h, 85%; b) 0.05N HCl aq., EtOH, r.t., 22 h, 51% (recovered starting material 33 %); c) DMSO, Ac_2O, r.t., 22 h, 96%; d) **83**, LDA, LiCl, THF, -20 ^oC, 40 min, 69%; e) H_2, Pd/C, EtOH, r.t., 17 h; f) BocN=C(SMe)NHBoc, $HgCl_2$, Et_3N, DMF, r.t., 20 min, 67% in 2 steps; g) mCPBA, CH_2Cl_2, 0 ^oC, 10 min; h) TFAA, 2,6-lutidine, CH_2Cl_2, 0 ^oC, 1.5 h; i) NH_3 aq., EtOH, 0 ^oC, 1 h, 37% in 3 steps; j) HF, H_2O, r.t., 14 h; k) TFA, H_2O, 40 ^oC, 4 days; l) pyridine, H_2O, 35 ^oC, 12 h, 41% for **77**, 28% for 4,9-anhydro-**77**.

4 Natural guanidines from cyanobacteria

Cyanobacterial guanidines are related to two major classes: arginine-derived in peptidic toxins and related metabolites, and non-peptidic guanidines, which are very often cyanotoxins as well. Recent reviews on this topic include the excellent “Cyanobacterial toxins: biosynthetic routes and evolutionary

roots"[216] and the related "Environmental conditions that influence toxin biosynthesis in cyanobacteria".[217]

Phylogenetic analysis of microcystins-producing *Microcystis aeruginosa* using maximum likelyhood (ML), Neighbor-Joining (NJ) and Bayesian phylogenetic reconstruction of three genes (*mcyA*, *mcyD* and *mcyG*) indicated a biogeographic distribution pattern for strains producing specific classes of such cyanotoxins. Australian and South African *M. aeruginosa* strains were shown to be in a common phylogenetic cluster independently of the marker gene. Strains from Asia present the highest genetic differentiation among the strains analyzed, particularly for genes *mycD* and *mycG*. However, other distinctions observed were not sufficiently significant in order to establish a clear biogeographic distibution pattern for microcystins-producing *M. aeruginosa*.[218]

Nostoc spp. bacteria associated with nodularin-producing cyanobacterial strains have nodularin biosynthetic genes, and were established as cyanobacterial endosymbionts. Nevertheless, not all nodularin biosynthetic genes were observed in *Nostoc* spp., or genes sequences showed to be different than observed in *Nodularia* spp. gene clusters, and some of the bacterial strains proved unable to produce nodularin. The results obtained were unclear to understand the evolution of nodularin gene cluster in *Nostoc* spp., requiring further analyses to clarify the observed trends.[219] Over 800 lichen specimens were screened for the *mcyE*, one of the genes encoding microcystins biosynthesis, showing that species belonging to genera *Peltigera*, *Nephroma*, *Sticta* and *Lobaria* presented microcystin biosynthesis. The distribution of *mcyE* gene in lichens correlates with *Nostoc* bacteria diversity in the same organisms, further indicating a possible relationship with an endosymbiontic production of such cyanotoxins.[220]

Microcystins and cylindrospermosin cyanotoxins have been detected in all major water reservoirs which supply large cities of the Brazilian northeastern region, clearly indicating an under-management of freshwater supply by local governments.[221] Proteomic analyses of microcystin-LR and

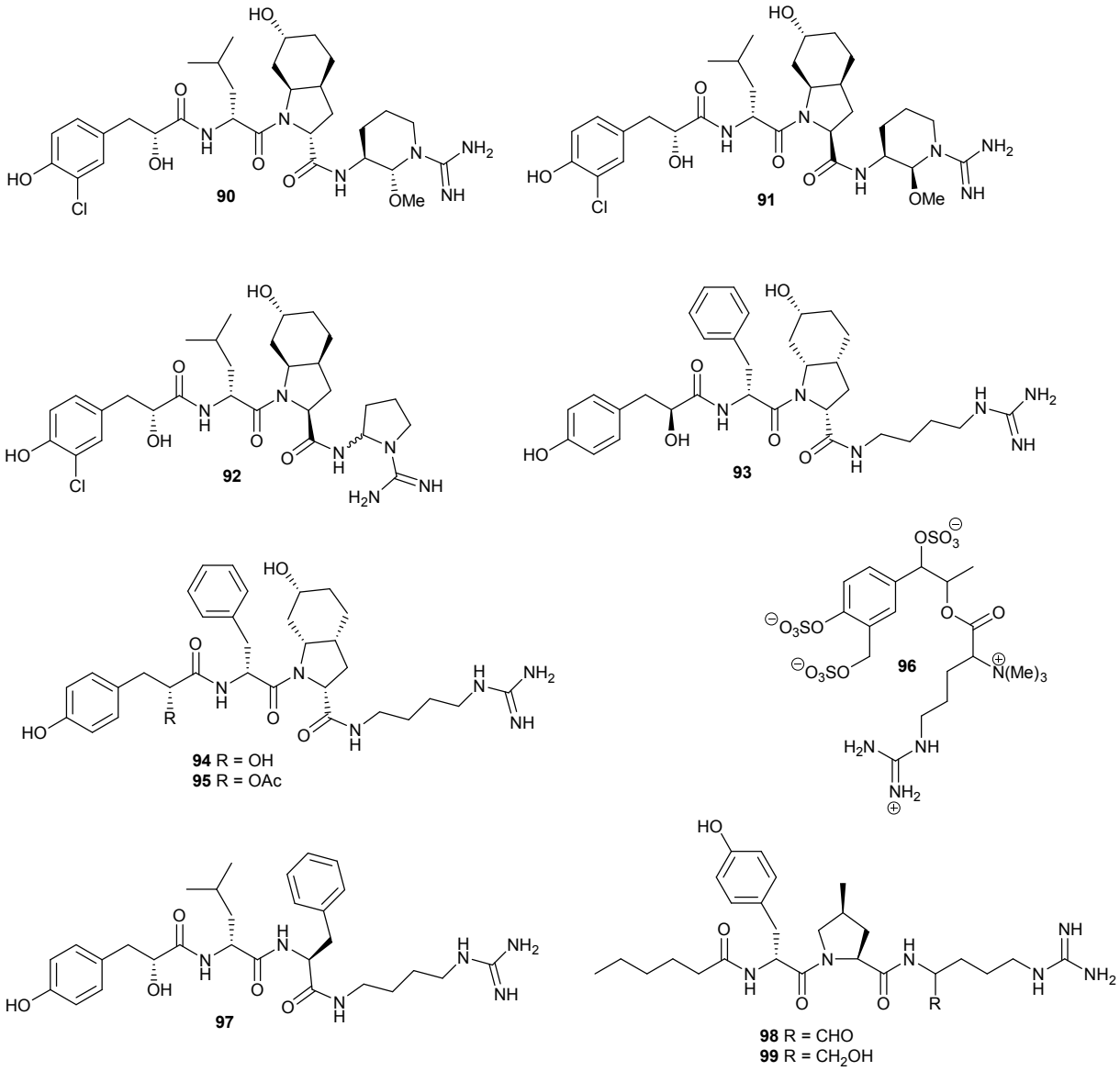
M. aeruginosa extracts effects on rice (*Oryza sativa*) physiology showed alterations in protein expression and biosynthesis, response to stress, and carbohydrate and nitrogen metabolism.[222] Furthermore, microcystin-LR is accumulated in rice grains grown in natural environment, but in levels lower than the tolerable daily intake established by the World Health Organization.[223] However, microcystins present some cumulative degree, and the effects on humans after long-term consumption of either water or food contamined with microcystins is not yet completely well known.[224] Incredibly, microcystins have been detected in 60 (1950) to 170 (1839) year old dried samples of cyanobacteria preserved in the herbaria of the Natural History Museum (London). Analyses were performed by HPLC-PDA, microcystin ELISA, MALDI-TOF MS and PCR for *mcyD* gene, showing sub-nanogram concentrations per mg of samples.[225]

Three new aeruginosins, LH650A (**90**), LH650B (**91**) and LH606 (**92**), have been isolated from a culture of *Microcystis* sp. IL-376 found in a fishpond near Kibutz Lehavot Habashan, Israel.[226] The relative configuration of the 2-carboxy-6-hydroxy-octahydroindole moiety in **90**-**92**, as well as of the 1-amidino-2-methoxy-3-aminopiperidine fragment in **90** and **91**, has been established by analysis of ROESY data. The absolute stereochemistry of the amino acid residues have been established by the Marfey's method, while the absolute configuration of the *o*-chloro-*p*-hydroxyphenyllactic acid moiety was determined by chiral HPLC analysis. Aeruginusins LH650A (**90**), LH650B (**91**) and LH606 (**92**) exhibited mild inhibition of chymotrypsin (IC_{50} at 37.9, 35.3 and 18.5 μM) and stronger inhibition of thrombin ((IC_{50} at 1.8, 1.8 and 2.5 μM).

An array of guanidine-bearing metabolites have been isolated from *Microcystis aeruginosa* TAU strain IL-347.[227] The aquous medium from which the cyanobacterial cells were harvested was processed by adsorption on XAD-2 and elution with MeOH, followed by separation on Sephadex LH-20 and purification by HPLC. This separation yielded the new aeruginosins KT608A (**93**), KT608B (**94**) and KT650 (**95**). The 70% MeOH extract of freeze-dried harvested cells gave, after a similar

separation procedure, microguanidine KT636 (**96**), aeruginosins KT608A (**93**), KT608B (**94**), and KT650 (**95**) and pseudoaeruginosin KT554 (**97**). The structures of compounds **93** – **97** have been established similarly as for **90** – **92**. Microguanidine KT636 (**96**) was not active as trypsin, thrombin or bovine amino petptidase inhibitor, and did not display cytotoxic activity against the HCT116 cancer cell line. Aeruginosins KT608A (**93**), KT608B (**94**) and KT650 (**95**) inhibited trypsin with IC_{50} at 1.9 1.3 and 19.9 μM, respectively. Pseudoaeruginosin KT554 (**97**) was essentially inactive in the same assay.[227]

Pseudoaeruginosins NS1 (**98**) and NS2 (**99**) are further members of the new pseudoaeruginosin family of modified peptides, which have been detected in 33 *Nodularia spumigena* strains isolated at the Baltic Sea.[228] In the case of compounds **98** and **99**, a hexenoic acid moiety in both compounds substitutes the tyrosine residue in **97**, while a tyrosine and a 4-methyl proline residue replace the leucine and the phenylalanine residue of **97**. The structures of **98** and **99** were first proposed based on extensive MS analysis, followed by total synthesis. The synthesis was accomplished starting from protected arginine, which was intramolecularly cyclized before its condensation to protected 4-methyl proline, then to protected tyrosine and finally to hexanoic acid, before the arginine des-cyclization and the peptide full deprotection. The synthetic compounds were subsequently analyzed by HPLC-MS and NMR, in order to assign the configuration of the L-Tyr and (2*S*,4*S*)-4-methyl-proline residues. The stereochemistry of the argininol or argininal residues could not be determined because both these arginine derivatives racemise quickly. Pseudoaeruginosin NS1 (**98**) binds to serine protease more effectively than **99**.[228]



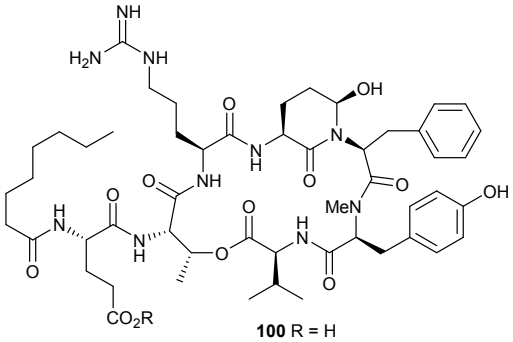
Among eight new micropeptins isolated from a *Microcystis* spp. bloom, four presented an arginine residue. Micropeptins LH1021 (**100**), LH1048 (**101**), LH1062 (**102**) and LH911A (**103**) were isolated from an assembly of several *Microcystis* species collected at a fishpond near Kibbutz Lehavot HaBashan, Israel. The structures of **100** - **102** have been established based on analysis of spectroscopic data, with HMBC and ROESY correlations to assign the connectivities between the amino acid residues. The absolute configuration of the amino acids were established after acid hydrolysis of the peptides followed by Marfey's analysis. The absolute configuration of the amino hydroxy piperidone

residue was assigned after the peptide oxidation with Jones' reagent followed by acid hydrolysis, which liberated L-glutamic acid, derivatization with Marfey's reagent and HPLC analysis. The diastereomeric configuration of L-threonine residue was assigned jointly by Marfey's and NMR analyses. A similar approach was used to identify micropeptin LH911A (**103**), in addition to the identification of the L-Ile residue based on NMR and comparison with literature data. Micropeptins **100**-**103** displayed similar inhibition of trypsin, in the range between 1.9 and 3.0 μM, except for **100**, essentially inactive against trypsin but the most active as chymotrypsin inhibitor, at 1.1 μM.[229] An additional micropeptin (**104**) has been isolated from *Microcystis aeruginosa* blooms in North Carolina's Cape Fear River, although no details of its structure elucidation were provided.[230]

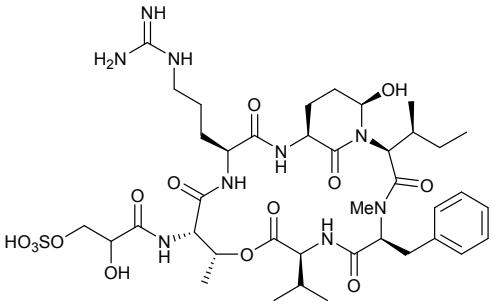
A prenylated peptide, autumnalamide (**105**), has been isolated from the cyanobacterium *Phormidium autumnale* and identified by analyses of NMR and extensive CID MS^2 fragmentation data.[231] The absolute configuration of the amino acids have been established after hydrolysis and derivatization with ethyl chloroformate in heptafluorobutanol, followed by bidimensional GC analysis. Conformational analysis and theoretical calculations using density functional theory at the B3LYP/6-31 level has been used to ascertain the configuration of the proline and of the prenylated arginine residues. Autumnalamide (**105**) indirectly blocks the Ca^{2+} influx in store operated calcium channels acting on mitochondria.[231]

Nostosins A (**106**) and B (**107**) are related peptide-derived metabolites isolated from a *Nostoc* sp. strain FSN collected at the Golestan Province of Iran.[232] The structures of both **106** and **107** have been asigned by extensive analysis of spectroscopic data, including MS data obtained from nostosins enriched with ^{15}N (by medium feeding with ^{15}N-urea). Nostosin A (**106**) inhibited trypsin with IC_{50} at 0.35 μM while nostosin B (**107**) was much less active, with IC_{50} at 55 μM. The trypsin inhibition by nostosin A was shown to be time-dependent. Nostosin A binds covalently to the catalytic serine S195 (by docking analysis), the argininal residue being the key active point of interaction with trypsin.[232]

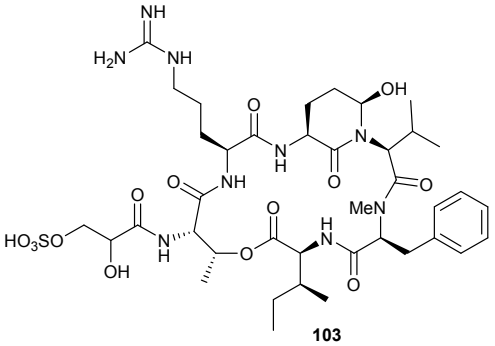
Two new cylindrospermosin analogues, 7-deoxy-desulfo-cylindrospermopsin (**108**) and 7-deoxy-desulfo-12-acetylcylindrospermopsin (**109**), have been isolated from *Cylindrospermopsis raciborskii* collected in Thailand, although no details of the collecting site were provided. The structures of both **108** and **109** have been determined by analysis of spectroscopic data, briefly presented and discussed.[233]



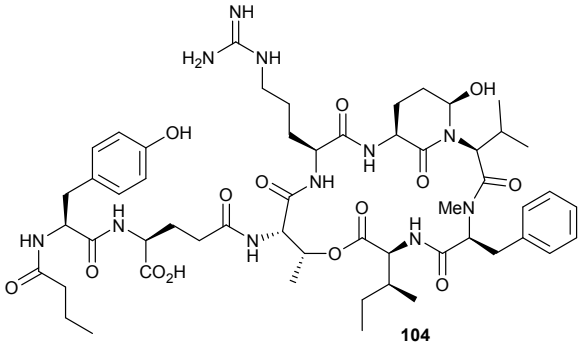
100 R = H
101 R = Me



102



103



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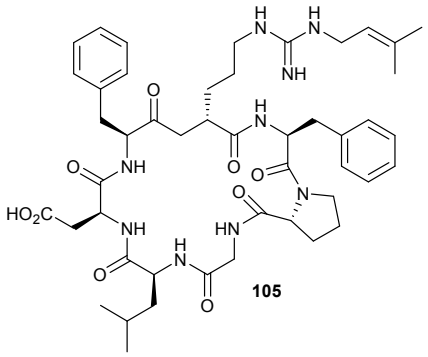
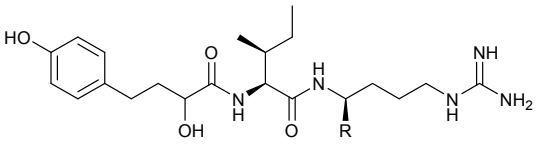
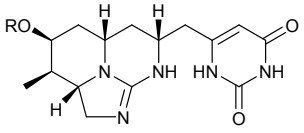


105



106 R = CHO
107 R = CH_2OH



108 R = H
109 R = Ac

5 Natural guanidines from marine invertebrates

5.1 Marine sponges

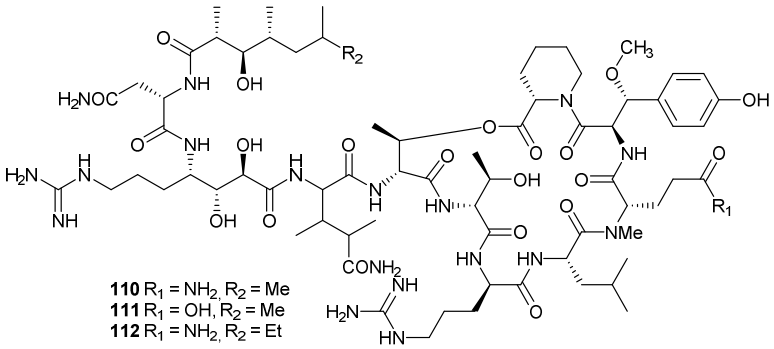
Marine sponges constitute the biological group with the largest diversity of guanidine metabolites, probably because sponge tissues comprise, in large extension, complex assemblies of microorganisms, which may be, at least partially, the actual source of such metabolites.

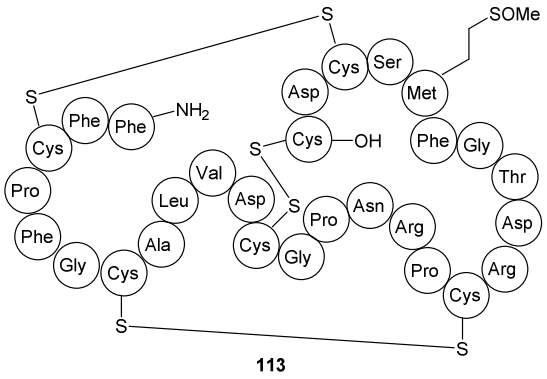
5.1.1 Peptides

Neamphamides B (**110**), C (**111**), and D (**112**) have been isolated from the marine sponge *Neamphius huxleyi* by HPLC-MS guided isolation.[234] NMR analysis of **110** free base was performed in MeOH-d_3, while electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry analysis (ESI-FTICR-MS^n) by direct insertion provided extensive fragmentation data to support the structure of **110**. The absolute configuration of amino acids was established by Marfey's analysis, but not for β-OMeTyr, which had its relative configuration assigned as *erythro*-β-OMe-D-Tyr using the Murata's method and comparison with literature data. The relative configuration of 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (Agdha) and 3-hydroxy-2,4,6-trimethylheptanoic acid (Htmha) residues were established by *J*-based NMR configurational analysis as (2*R**,3*R**,4*S**)-Agdha and (2*R**,3*R**,4*R**)-Htmha, respectively. The structures of **111** and **112** were assigned using a similar strategy as for **110**. The cytotoxic activity of peptides **110** – **112** were evaluated against human lung adenocarcinoma cells (A549), cervical adenocarcinoma cells (HeLa), prostate adenocarcinoma cells (LNCaP and PC3) as well as against the noncancer human cell lines embryonic kidney (HEK 293) and neonatal foreskin fibroblasts (NFF). Neamphamides B (**110**) showed more potent cytotoxic activity against HeLa cells at 110 ± 8.1 nM, while neamphamides C (**111**) was more active against PC3 at 110 ± 4.1 nM, and neamphamide D (112) against HEK cells at 88 ± 13 nM.[234]

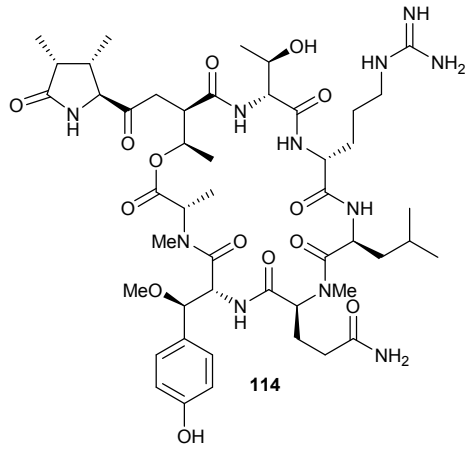
A series of neopetrosiamides (e.g., neopetrosiamide B, **113**) have been prepared in order to evaluate the cell invasion activity on the highly metastatic human breast cancer cell line MDA-MB-

231. The sulfoxide group proved to be unnecessary for such activity, while disulfide bonds replacement let to completely activity loss.[235]

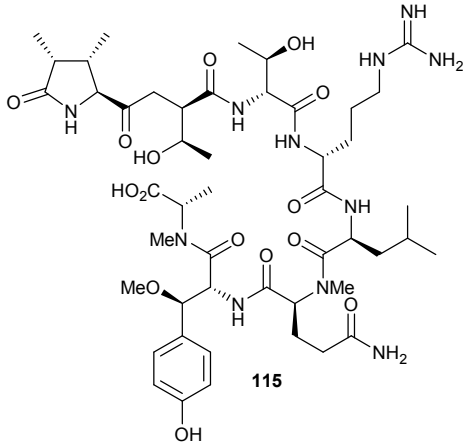




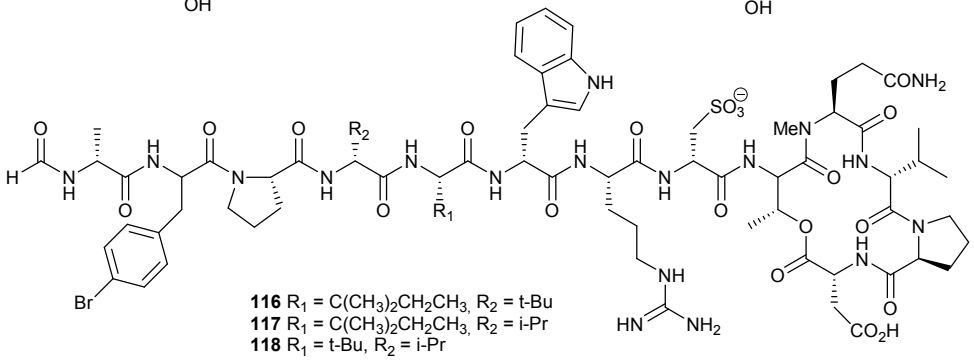
Callipeltins B (**114**) and M (**115**), previously isolated from the marine sponge *Callipelta* sp., have been synthezised using solid-phase peptide synthesis of FMOC protected aminoacids, followed by lactonization with *N*,*N*'-diisopropylcarbodiimide and DMAP.[236] Polydiscamides B (**116**), C (**117**) and D (**118**) have also been synthezised, using solid-phase/Fmoc protected aminoacids to generate the cyclodepsipeptide **119** and the linear **120** moieties. Coupling **119** and **120** was accomplished using a ligation-oxidation procedure with a solution of guanidine hydrochloride, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid and *N*-methylpyrrolidone in order to stabilize both peptides, and thiophenol as a catalyst, at 37 ^{o}C during 16 h. After HPLC purification, the thiol group of the cysteine residues was oxidized with performic acid followed by deprotection with 90:5:5 TFA/iPr_3SiH/H_2O to afford the peptides **116** – **118** (Scheme 10).



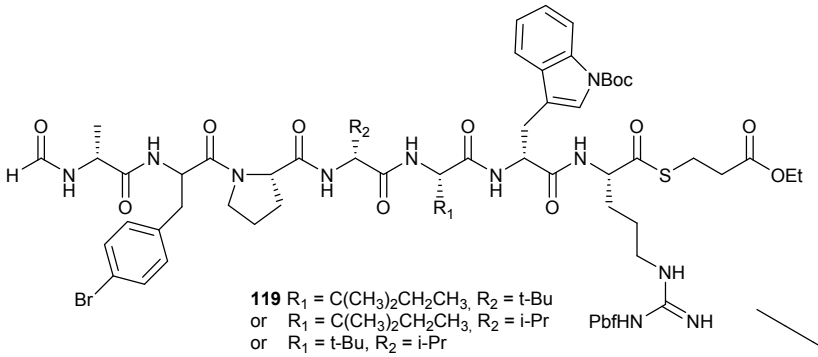
114



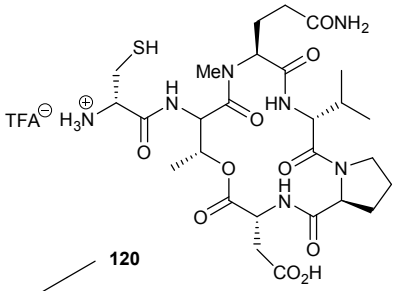
115



116 R_1 = $C(CH_3)_2CH_2CH_3$, R_2 = t-Bu
117 R_1 = $C(CH_3)_2CH_2CH_3$, R_2 = i-Pr
118 R_1 = t-Bu, R_2 = i-Pr

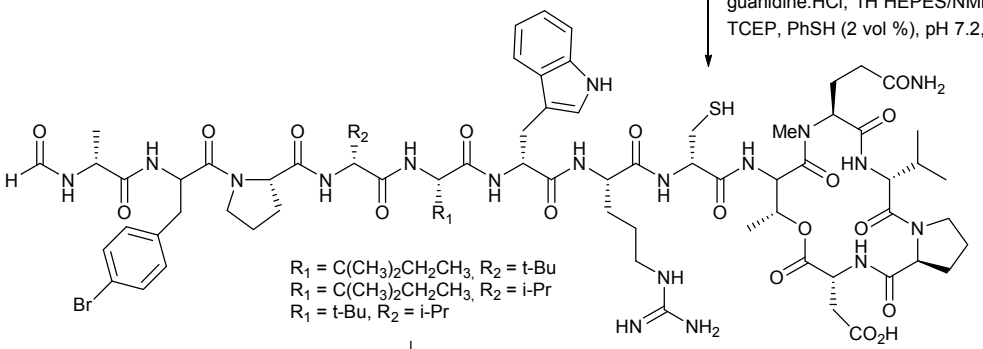


119 R_1 = $C(CH_3)_2CH_2CH_3$, R_2 = t-Bu
or R_1 = $C(CH_3)_2CH_2CH_3$, R_2 = i-Pr
or R_1 = t-Bu, R_2 = i-Pr



120

guanidine.HCl, 1H HEPES/NMP (1:1 v/v)
TCEP, PhSH (2 vol %), pH 7.2, 37 °C, 16 h



R_1 = $C(CH_3)_2CH_2CH_3$, R_2 = t-Bu
R_1 = $C(CH_3)_2CH_2CH_3$, R_2 = i-Pr
R_1 = t-Bu, R_2 = i-Pr

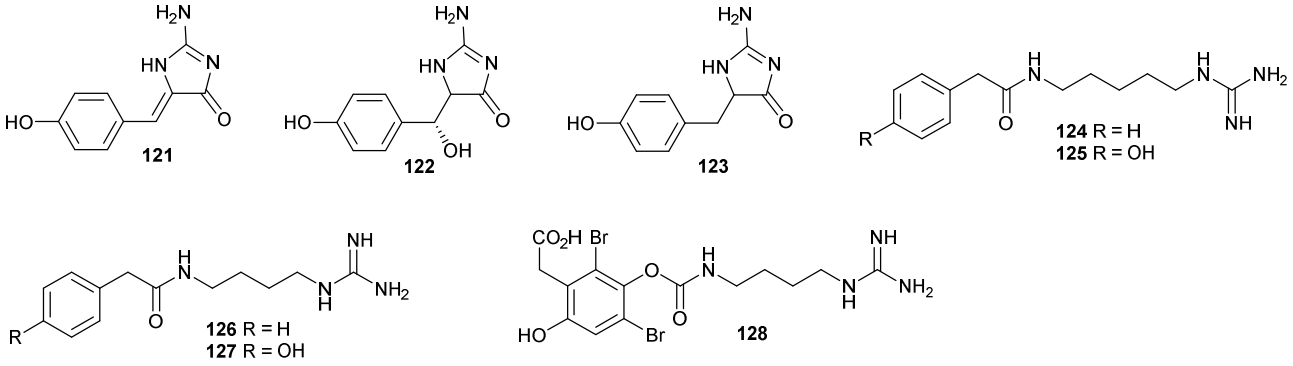
1. 1 vol % aq. performic acid, 0 °C, 1 h
2. TFA/i-Pr_3SiH/H_2O (90:5:5), 1 h

116, **117** and **118**

Scheme 10. Final steps of the synthesis of polydiscamides B (**116**), C (**117**) and D (**118**). *Reagents and conditions*: a) guanidine.HCl, 1H HEPES/NMP (1:1 v/v) TCEP, PhSH (2 vol %), pH 7.2, 37 ^{o}C, 16 h; b) 1. 1 vol % aq. performic acid, 0 ^{o}C, 1 h; 2. TFA/*i*-Pr_3SiH/H_2O (90:5:5), 1 h.

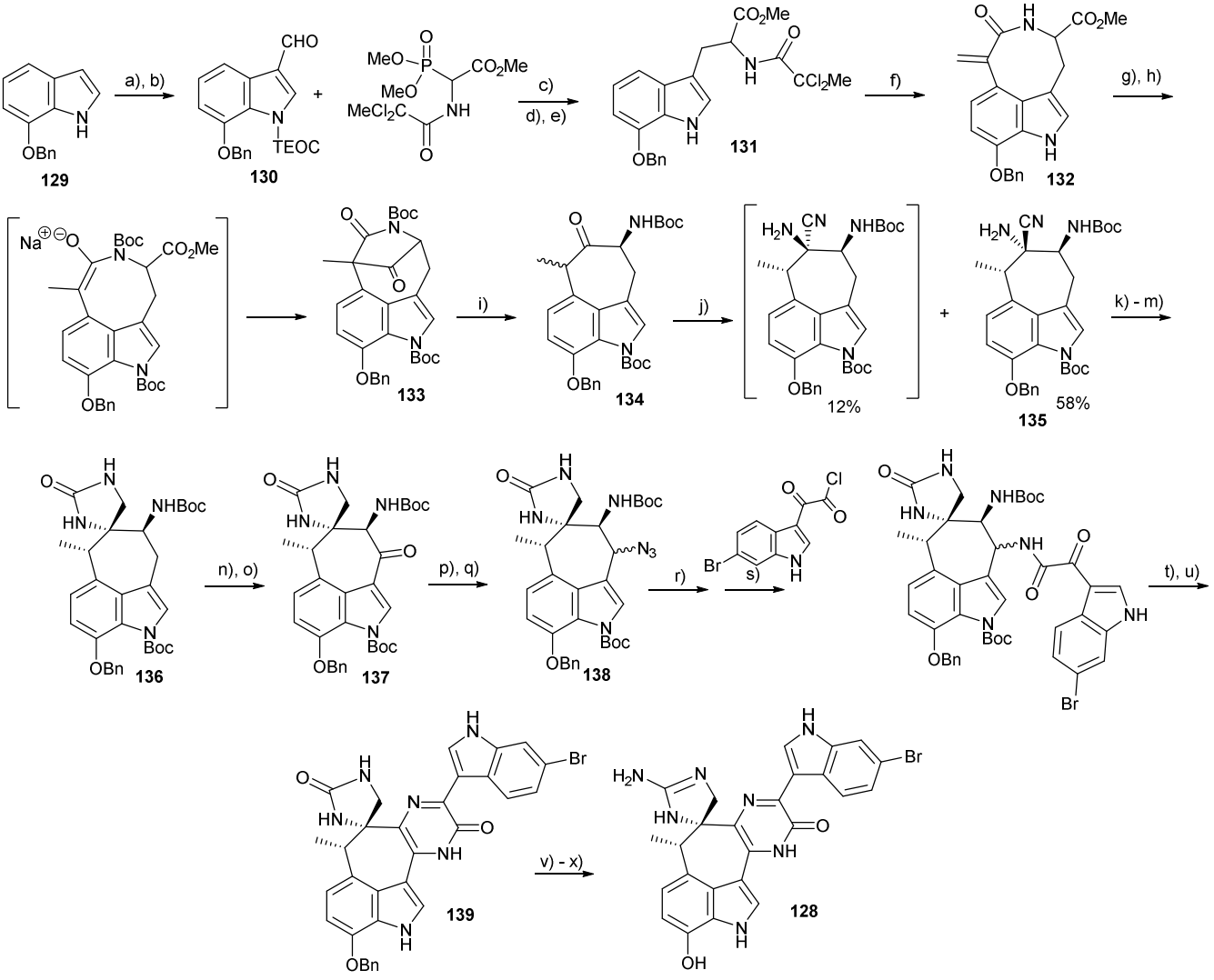
5.1.2 Alkaloids

Phorbatopsins A – C (**121** – **123**) have been isolated from the sponge *Phorbas topsenti* and identified by analysis of spectroscopic data.[238] Compounds **121** – **123** showed antioxidant activity comparable to ascorbic acid. Biologically inactive iotrochotadines A – D (**124** – **127**) have been isolated from an *Iotrochota* sp. sponge using a NMR-guided procedure isolation and identified by analysis of spectroscopic data.[239]



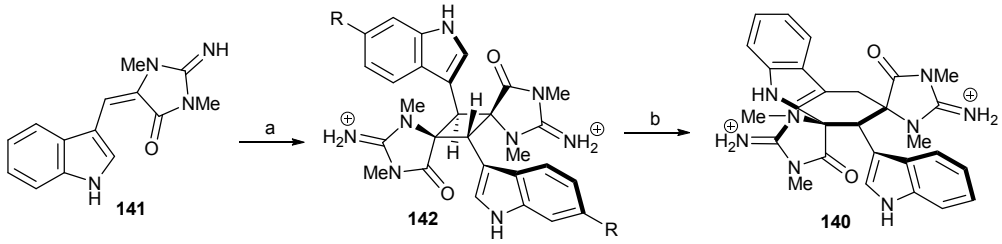
The total synthesis of dragmacidin E (**128**) was accomplished (Scheme 11),[240] starting from 1*H*-indol-7-ol (**129**) which was protected and formylated to give aldehyde **130**, itself coupled with methyl 2-(2,2-dichloropropanamido)-2-(dimethoxyphosphoryl)acetate aiming to obtain the protected tryptophan derivative **131**. Intermediate **131** was prepared to a Witkop photocyclization after its reduction and removal of the trimethylsilylethyl carbamate protecting group. The cylized product **132** was then subjected to the alkene reduction and concomitant Dieckmann cyclization. Cleavage of the lactam bridge of **133** gave **134** as a mixture of diastereomers. The Strecker reaction on **134** was

achieved under strict temperature control (< 80 ^{o}C), with freshly prepared NH_3 in MeOH. The desired stereoisomer **135** was obtained in larger yield. It followed a series of transformations including carbamoylation, nitrile reduction and cyclization, to give the urea **136** which had its structure and relative configuration confirmed by X-ray diffraction analysis. The tetracyclic indole derivative **136** was subjected to oxidation to the ketone **137**, converted to the azide **138**, followed by reduction and condensation with 2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride. It followed a cyclization to provide the dihydropyrazinone **139**, which was converted into dragmacidin E (**128**) in a three step conversion. The total synthesis ended in 25% overall yield over 25 steps.



Scheme 11. a) $POCl_3$, DMF; b) TEOC–$OC_6H_4NO_2$, NaH, THF (89%, 2 steps); c) DBU, CH_2Cl_2 (83%); d. H_2 (1250 psi), RhCl $(PPh_3)_3$, PhMe, MeOH; e) Bu_4NF, THF (86%, 2 steps); f) hν, MeCN; g. Boc_2O, DMAP, MeCN; h) N-Selectride, THF (57%, 3 steps); i) LiOH, H_2O, THF (90%); j) NH_4Cl, NH_3/MeOH sealed tube, then TMSCN; k) MeOCOCl, K_2CO_3, THF; l) $CoCl_2$, $NaBH_4$, MeOH; m) LiOH, THF, H_2O (67%, 3 steps)); n) DDQ, H_2O, THF; o) Boc_2O, DMAP, MeCN (63%, 2 steps); p) $NaBH_4$, MeOH; q) $(PhO)_2PON_3$, DBU, PhMe, DMF (78%, 2 steps); r) $NiCl_2$, $NaBH_4$, MeOH; s) Et_3N, CH_2Cl_2 (57%); t) TFA, CH_2Cl_2, aq $NaHCO_3$; u) DDQ, 1,4-dioxane (65%); v) Me_3OBF_4, $NaHCO_3$, EtOAc; w) NH_3, MeOH, sealed tube; x) TMSI, MeCN (25%, 3 steps).

Tubastrindole B (**140**) has been synthezised from aplysinopsin (**141**) via a photodimerization to give **142** (R = H) followed by a rearrangment cascade in aqueous TFA (Scheme 12).[241] Dictazole B (**142**, R = Br) was also prepared using the same strategy.[242]



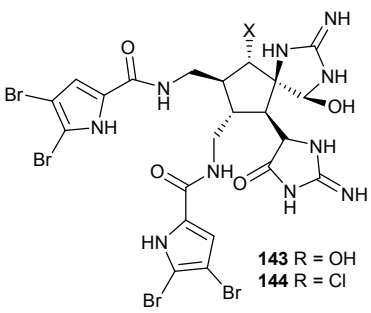
Scheme 12. Synthesis of (±)-tubastrindole B (**140**). *Reagents and conditions*: a) hν, 14 h, CuOTf-toluene complex, DMF; b) microwaves, 110 ^oC, 85 s, TFA, H_2O.

Donnazoles A (**143**) and B (**144**) have been isolated from the marine sponge *Axinella donnani*.[243] Structures of **143** and **144** have been established by analysis of spectroscopic data and by comparison with literature data, while the absolute configuration of both alkaloids was assigned by exciton chirality method circular dichroism spectroscopy and by comparison with the CD spectrum of

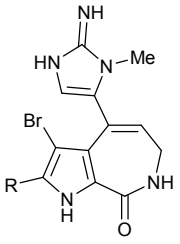
sceptrin. Donnazoles A and B have the same cyclopentane substituted moiety as Palau'amine, suggesting the existence of an universal biochemical pathway for the biosynthesis of these alkaloids, which seems plausible.[243] Related alkaloids, 12-*N*-methyl-stevensine (**145**), 12-*N*-methyl-2-debromostevensine (**146**), 3-debromolatonduine B methyl ester (**147**) and 3-debromolatonduine A (**148**), have been isolated from the sponge *Stylissa* sp.[244] The structures of **145** – **148** have been established by analysis of spectroscopic data, and 12-*N*-methyl-stevensine (**145**) showed the most potent inhibitory activity (86%) of L5178Y mouse lymphoma cells proliferation, at 10 μg/mL. Agelamadins A (**149**) and B (**150**) have been isolated from a marine sponge of the genus *Agelas* as racemates and displayed moderate antimicrobial activity against *Bacillus subtilis*, *Micrococcus luteus* and *Cryptococcus neoformans*.[245] Nagelamides U – Z (**151** – **156**) are further members of this series, which appears to be never ending, and show how versatile can be a biochemical pathway leading to an astonishing variety of new carbon skeletons. Nagelamides U – W (**151** – **153**) have been isolated from the sponge *Agelas* sp. collected off Kerama Islands in Okinawa (Japan),[246] and identified by analysis of spectroscopic data. Alkaloids **151** and **153** displayed antifungal activity against *Candida albicans*, at 4 μg/mL. A biogenetic pathway has been proposed for negelamides U and V (**151** and **152**), from taurodiscapamide. However, an alternative pathway involving guanidinylation of ornithine and subsequent condensation with taurine cannot be rulled out. Nagelamides X – Z (**154** – **156**) have been isolated from the same sponge species, and also identified by analysis of spectroscopic data.[247] Analysis by chiral HPLC indicated that alkaloids **154** and **155** have been isolated as racemic mixtures, while alkaloid **156** was the most active one against a series of microbial pathogens.

Palau'amine (**157**) and dibromophakellin (**158**) showed modulation of the proteolytic activity of the human proteasome and of the immunoproteasome.[248] Natural (-)-Palau'amine (**157**) showed a more pronounced activity than its racemate, being the most active inhibitor of the chymotryptic-like (CT-L, IC_{50} 2.5 μM) and caspase-like (Casp-L, IC_{50} 1.6 μM) catalytic sites of the proteasome.

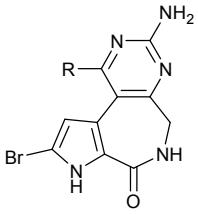
Dibromophakellin (**158**) was ca. 10 fold less active than (-)-**157**, while its urea analogue was completely inactive. (-)- Palau'amine also reduced the proteolytic activity of the human immunoproteasome, with IC_{50} at 2.3 μM, twice as active as its racemate. The activity is due to Palau'amine itself and not to possible degredation products, which are formed at pH > 7.5. Moreover, Palau'amine binds irreversibly to the 20S proteasome, induces the accumulation of ubiquitinylated proteins and reduces the IκBα degradation and, consequentially, the NF-κB release. The ensemble of the results observed for (-)-Palau'amine clearly reflects its proteasome inhibitory action.[248]



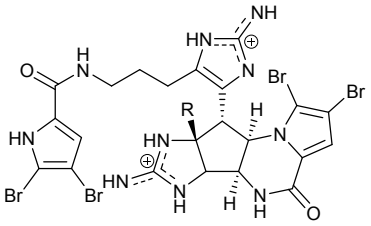
143 R = OH
144 R = Cl



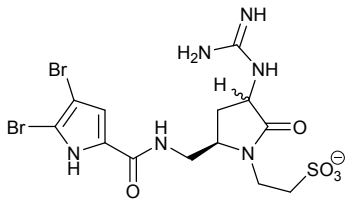
145 R = Br
146 R = H



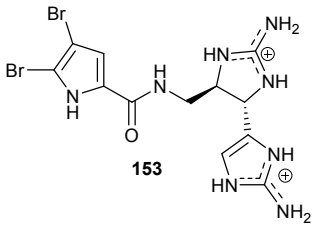
147 R = CO_2Me
148 R = H



149 R = OMe
150 R = OH



151 R = β-H
152 R = α-H



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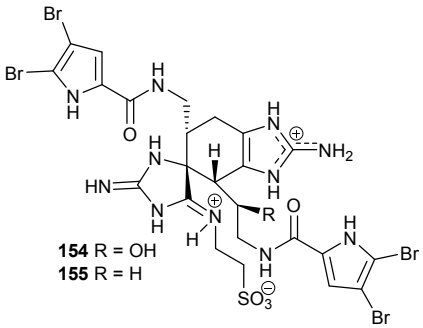
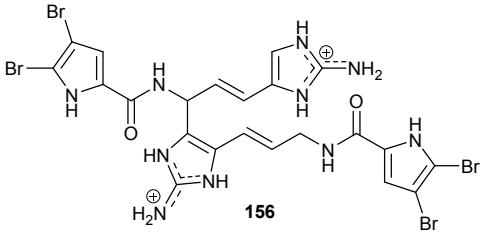


154 R = OH
155 R = H



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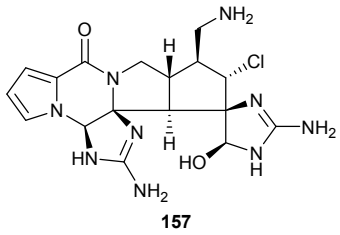

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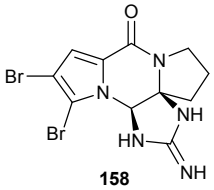
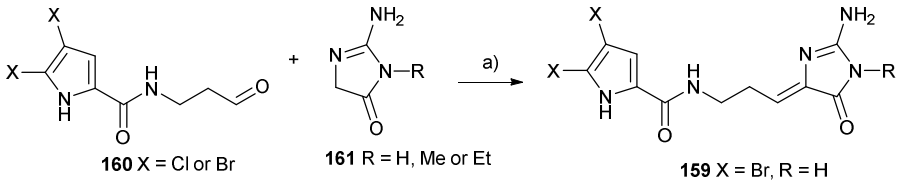


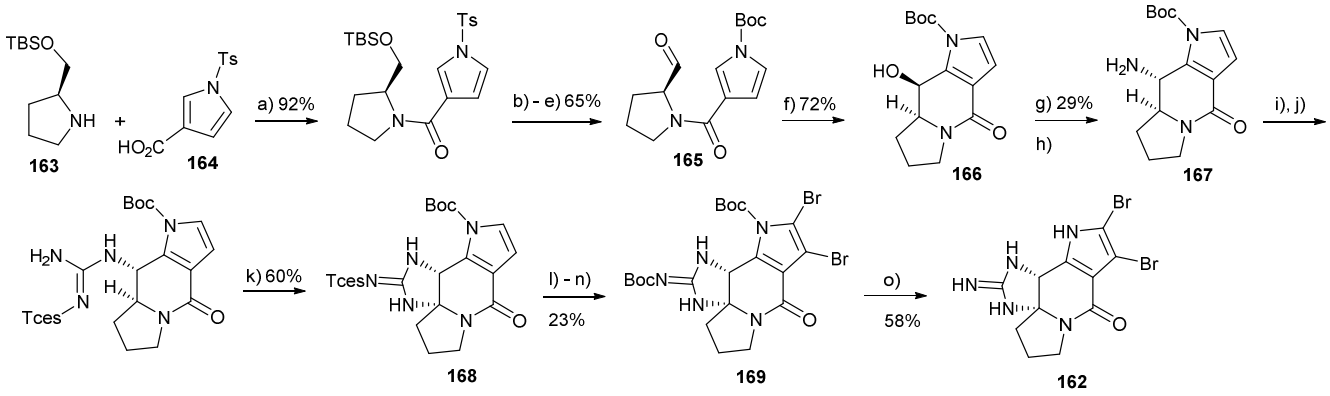
158

A new convergent synthesis of discapamide (**159**) and of a series of derivatives has been developed.[249] The key step in the new approach is a Knoevenagel condensation between aldehydes (**160**) and 2-aminoimidazolin-4-ones (**161**), usually referred as an Erlenmeyer-Plöchl reaction (Scheme 13).



Scheme 13. Synthesis of discapamide (**159**). *Reagents and conditions*: AcONa, AcOH, 120 ^{o}C, microwaves, 20 min, or piperidine, EtOH, 60 ^{o}C, microwaves, 30 min.

A new total synthesis of (+)-cylindradine (**162**) was achieved,[250] starting from the condensation of pyrrolidine **163** with the tosyl-protected 1*H*-pyrrole-3-carboxylic acid (**164**), followed by the product conversion to the aldehyde **165**. Friedel-Crafts cyclization of **165** with camphorsulfonic acid gave the pyrrol-lactam **166** as the major steroisomer. After the alcohol of **166** conversion into the amine group of **167**, via the corresponding azide, guanidine preparation was optimized with *S*-methyl *N*-(2,2,2-trichloroethoxysulfonyl)carbonchloroimidothioate. The guanidine cyclization was also optimized with distinct oxidants. The cyclic, protected guanidine **168** had a protecting group exchanged before bromination of the pyrrole moiety, to give **169** as the direct precursor of (+)-cylindradine A (**162**).



Scheme 14. Synthesis of (+)-cylindradine (**162**). *Reagents and conditions*: a) EDCI, DMAP, CH_2Cl_2, rt; b) KOH, MeOH, 0 ^{o}C; c) Boc_2O, Et_3N, CH_2Cl_2, rt; d) TBAF, THF, rt; e) IBX, DMSO, 60 ^{o}C; f) CSA, MeCN, rt; g) DPPA, DBU, toluene, rt; h) PMe_3, H_2O, toluene, 0 ^{o}C; i) *S*-methyl *N*-(2,2,2-trichloroethoxysulfonyl)carbonchloroimidothioate, Et_3N, CH_2Cl_2, 0 ^{o}C; j) NH_3/MeOH, $HgCl_2$, MeCN;

k) (diacetoxyiodo)benzene, MgO, MeCN; l) $Pd(OH)_2$, H_2, MeOH/EtOAc, rt; m) Boc-ON, Et_3N, H_2O/ CH_2Cl_2; Br_2, n) $NaHCO_3$, CH_2Cl_2, rt; o) TFA, CH_2Cl_2, rt.

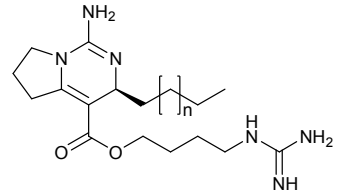
Genomic, phylogenetic, metagenomic, fluorescence *in situ* hybridization and transmission electron microscopy analyses indicated that a single bacterial operational taxonomic group dominates the tissues of the marine sponge *Crambe crambe*,[251] a species which is well known for the accumulation of a number of guanidine-bearing alkaloids, such as crambescins and crambescidins.

Isolation of what appears to be the whole homologue series of crambescins A, B and C have been performed.[252] The overall structure of the compounds was the same as previously reported, while the actual size of the alkyl chains has been established by MS/MS analysis. Homologues of crambescin A2 (**170**), as well as crambescin A1 (**171**), didehydrocrambescin A1 (**172**), a series of crambescin B1 homologues (**173**), and of crambescin C1 homologues (**174**), have all been identified. The absolute stereochemistry of the crambescin series was established by analysis of electronic circular dichroism spectra.[252] Crambescidin 816 (**176**) is the most active antifungal agent against *Saccharomyces cereviseae*, when compared with crambescidin 800 (**175**) and crambescidin 830 (**177**), with a minimal inhibitory concentration to inhibit 50% of fungal growth at 0.557 μM.[253] The same study indicated that **176** displayed its cytotoxic activity by increasing the cell size of *S. cereviseae*, disrupting the mitochondrial membrane potential, and inducing apoptosis. The mechanism of crambescidin 816 (**176**) cytotoxicity on human liver HepG2 cancer cells was also investigated.[254] The alkaloid inhibits the cell cycle at G_0/G_1 phase, by downregulating the expression of cyclins A and D and cyclin-dependent kinases 2 and 6, but increased the expression of cyclin-dependent kinase inhibitors A and D. Crambescidin 816 (**176**) disrupted the attachment of cells to the substrate, leading to cell cycle arrest in G1, and also inhibited the constitution of stress fibres participating in cell adhesion, motility and morphogenesis, affecting cell migration directly.

The marine sponge *Monanchora pulchra* continues to be a source of additional, unique guanidine alkaloids, differentiated from the ones isolated from *Monanchora* and *Crambe* species found in Mediterranean and Atlantic locations. A sample of *M. pulchra* collected by dredging at 46°07,0 N; 150°02,1 E, at a depth of approximately 150 m, provided monanchomycalins A (**178**) and B (**179**).[255] Both compounds were identified by analysis of spectroscopic data and also by reduction of **178** with $NaBH_4$, to give a polyketide devoid of the ether bridges along with the free hydroxyspermidine residue. Both **178** and **179** displayed cytotoxic activity against HL-60 human leukemia cells, with IC_{50} values of 120 and 140 nM, respectively. Monanchomycalin C (**180**) is an additional member of this class, which could be named as 20-methyl ptilomycalin A, identified by analysis of spectroscopic data.[256] Alkaloid **180** displayed cytotoxic activity against MDA-MB-231 cells with IC_{50} at 8.2 mM. Pulchranins A – C (**181**-**183**) are the simpler members of the Poescilosclerida guanidine alkaloids isolated so-far. These simple alkyl-substituted guanidines have also been isolated from *M. pulchra*,[257,258] and probably constitute the putative precursors of the complex, polycyclic alkaloids isolated from these sponges. While the structures of **181**-**183** have been established by analysis of spectroscopic data, the absolute configuration of pulchranin A (**181**) was assigned after ozonolysis and reduction with $NaBH_4$, followed by Mosher ester analysis of the corresponding diol. The absolute configuration of **182** and **183** was assigned by comparison with that of **181**.[258] Pulchranin A inhibited Ca^{2+} release in rTRPV1-CHO cells,[257] and is a more efective inhibitor of TRPV1, TRPV3 and TRPA1 channels than pulchranins B and C.[258]

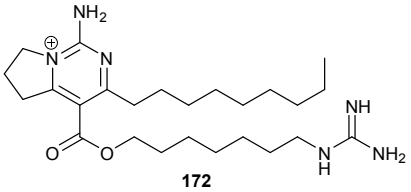
Uroposidins A (**184**) and B (**185**) are guanidine alkaloids bearing an unprecedented skeleton, also isolated from *Monanchora pulchra*. The structures were assigned by analysis of spectroscopic data and the absolute configuration by Mosher ester analysis of **184**, after its transformation into its methyl ester. Uroposidin A (**184**) promotes increase in the production of nitric oxide in macrophages at 1.0 μM.[259]

Netamines H – N (**186** – **192**) have been isolated from the sponge *Biemna laboutei* collected at Salary Bay, Madagascar.[260] The structure of the compounds have been established by analysis of spectroscopic data, as well as by reduction of netamine H (**186**) into netamine A, also reported for the first time as a natural product (previously isolated as a peracetyl derivative). All compounds exhibited moderate to good antimalarial activity against *Plasmodium falciparum*. Mirabilin K (**193**) has been isolated from the marine sponge *Acanthella cavernosa*, along with the known mirabilin G (**194**) and netamine M (**195**).[261] Mirabilin G (**194**) and netamine M (**195**) inhibited TPA-induced degradation of PDCD4, with EC_{50} values at 1.8 and 2.8 μg/mL. PDCD4 (programmed cell death 4) is a tumor suppressor which acts inhibiting protein translation by interacting with the eukaryotic translation initiation factors eIF4A and eIF4G.



170 crambescin A2 series (n = 8, 9 and 10)

171



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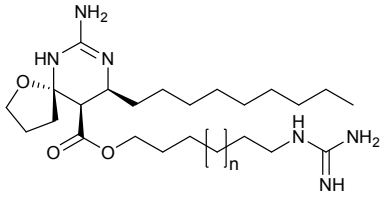
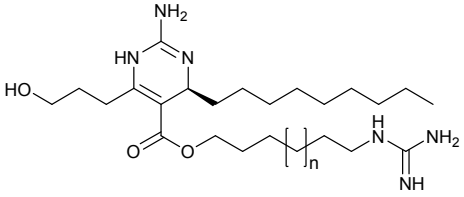
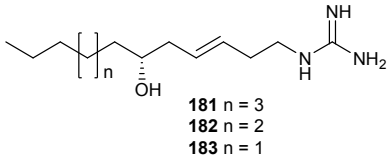


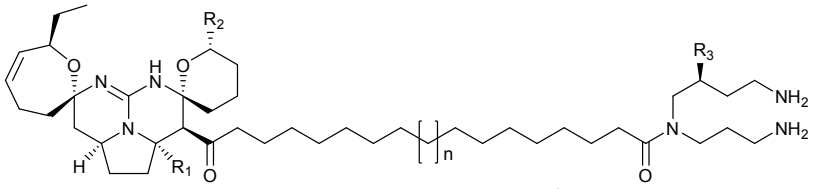
173 crambescin B1 series (n = 1, 2 and 3)



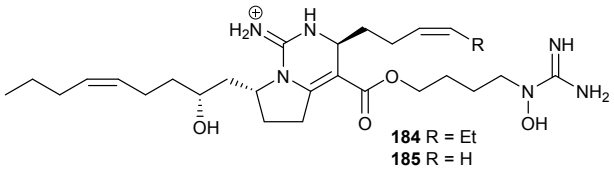
174 crambescin C1 series (n = 1, 2 and 3)



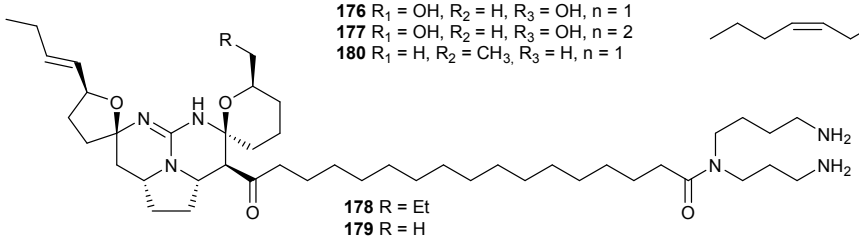
181 n = 3
182 n = 2
183 n = 1



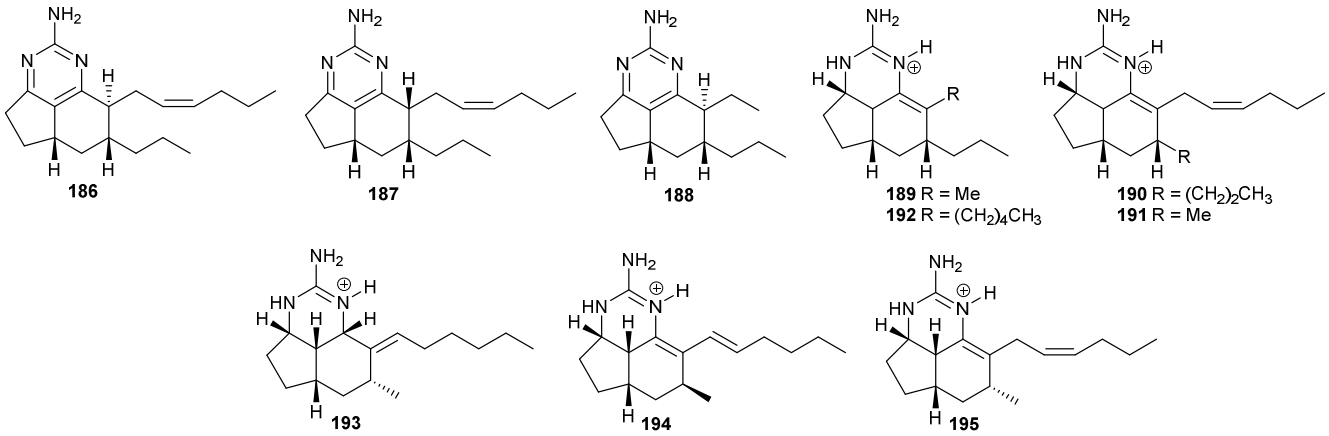
175 R_1 = H, R_2 = H, R_3 = OH, n = 1
176 R_1 = OH, R_2 = H, R_3 = OH, n = 1
177 R_1 = OH, R_2 = H, R_3 = OH, n = 2
180 R_1 = H, R_2 = CH_3, R_3 = H, n = 1



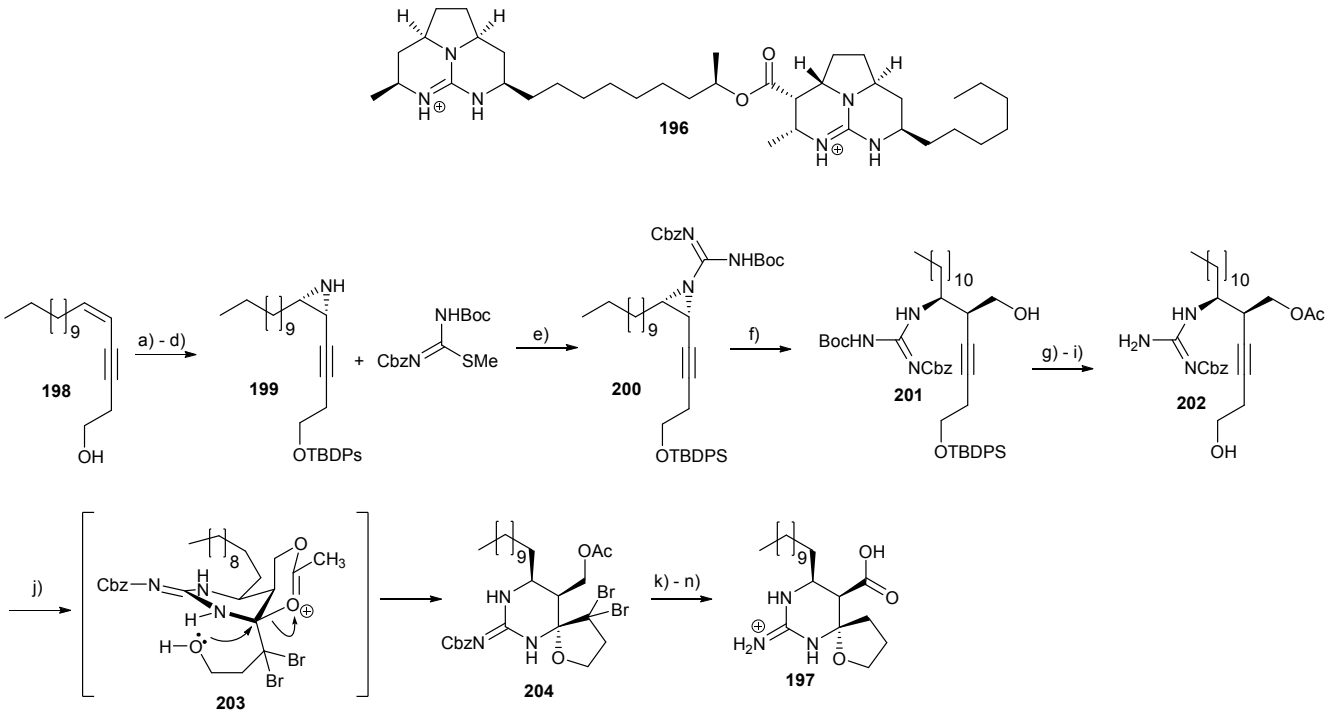
184 R = Et
185 R = H



178 R = Et
179 R = H



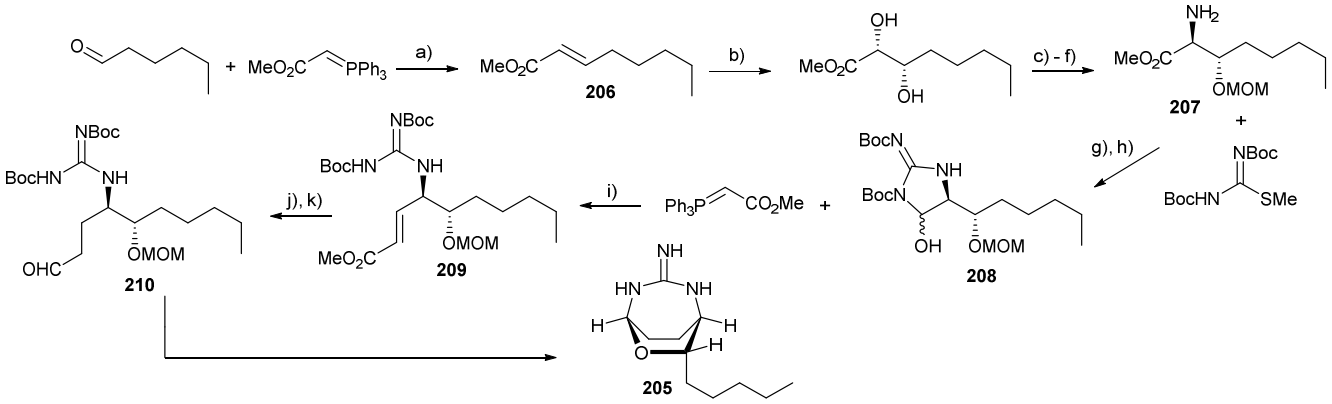
Analogues of batzelladine F (**196**) have been synthezised in order to evaluate their antiviral activity as inhibitors of the enzyme reverse transcriptase (RT) of HIV-1.[262] Although batzelladine F itself has not been evaluated in the same batch of assays, making difficult a comparison with the activity profile of the natural product, some of the analogues showed good inhibitory activity of HIV-1 RT and low cytotoxicity. Crambescin B carboxylic acid (**197**) has been synthezised because of its molecular architecture, related to that of tetrodotoxin.[263] The aziridine **199** was prepared from (*Z*)-heptadec-5-en-3-yn-1-ol (**198**) by a series of stepwise interconversions. The guanidine **200**, obtained in 91% from **199**, was reacted with formalin with indium iodide and $Pd(PPh_3)_4$, to give the alcohol **201** as a single stereoisomer (dr ≥ 95%). Although subsequent conversions could be performed with the unprotected alcohol, its esterification with acetic anhydride provided a better substrate (**202**) for the following reaction sequence. Thus, cyclization of **202** promoted by pyridinium bromide perbromide under alkaline conditions, through the hypothezised intermediate **203**, yielded the cyclized guanidine **204** as the sole product. A further series of stepwise interconversions, including a debromination, followed by protecting groups removal and oxidation to the corresponding carboxylic acid, gave the desired carboxylic acid derivative of crambescin B (**197**). The alkaloid **197** was tested as inhibitor on voltage-gated sodium channel, and presented an ED_{50} of 42 ± 25 nM, only nine fold larger that TTX.



Scheme 15. Synthesis of crambescin B carboxylic acid (**197**). *Reagents and conditions*: a) mCPBA, $NaHCO_3$, CH_2Cl_2, 71%; b) NaN_3, MeOH; c) PPh_3, DMF, rt to 80 ^oC; d) TBDPSCl, imidazole, CH_2Cl_2, 56% (3 steps); e) $HgCl_2$, Et_3N, DMF, 91%; f) $Pd(PPh_3)_4$, InI, formalin, THF, HMPA, 75%; g) Ac_2O, Et_3N, DMAP, CH_2Cl_2; h) TBAF, THF, 92% (2 steps); i) TFA, CH_2Cl_2; j) $PyHBr_3$, K_2CO_3, CH_2Cl_2-H_2O; k) Et_3B, n-Bu_3SnH, toluene, 97%; l) DIBAL, CH_2Cl_2, -78 ^oC, 87%; m) H_2, Pd-C, MeOH; n) Jones, acetone, 0 ^oC, 32% (2 steps).

A new synthesis of (+)-monanchorin (**205**) has been accomplished (Scheme 16).[264] Starting from hexanal subjected to a Wittig reaction with methyl triphenylphosphoranylidene to give methyl (*E*)-oct-2-enoate (**206**), which was subjected to a series of stepwise reactions towards the amino ester (**207**). The substrate **207** was guanydilated before guanidine cyclization to give the hemiaminal of the protected guanidine **208**. A second Witting reaction with **208** yielded the α,β-unsaturated ester **209**,

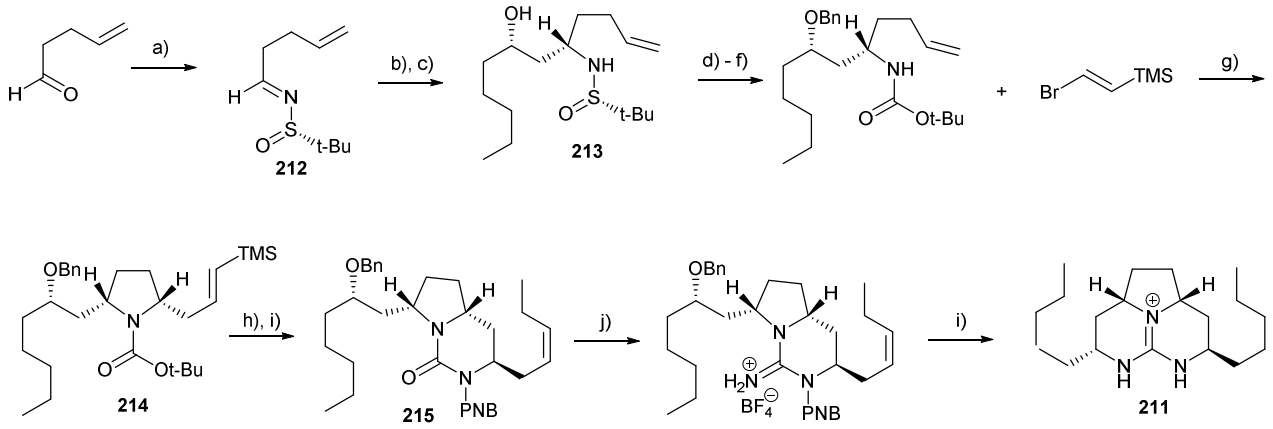
which was reduced both at the double bond and at the ester group to the aldehyde **210**, before cyclization in acidic medium to give the natural alkaloid **205**.



Scheme 16. Synthesis of (+)-monanchorin (**205**). *Reagents and conditions*: a) CH_2Cl_2, rt, 17 h, 85%; b) AD-Mix-a, t-BuOH/H_2O (1:1), $MeSO_2NH_2$ (1 eq.), 0 ^oC, 96 h (75 - 84%, 92% ee); c) $SOCl_2$ (1.2 eq), EtOAc, Δ, 3 h, then MeCN, 0 ^oC, cat $RuCl_3.xH_2O$ (0.073 mol %), $NaIO_4$ (2.37 eq.), H_2O, rt, 3 h (91%); d) NaN_3 (1.94 eq.), Me_2CO/H_2O (10:1), rt, 1.5 h, then 20% aq. H_2SO_4/Et_2O (1:1), 48 h (89%); e) MOMCl (4.3 eq.), *i*-Pr_2NEt (7.4 eq.), CH_2Cl_2, 50 ^oC, 24 h; f) H_2 (1 atm), 20% $Pd(OH)_2$, EtOAc, 1 h; g) DMF, Et_3N (3.01 eq.), $AgNO_3$ (1.09 eq.), 0 ^oC, 4 h, then rt, then 20 h (76%, 3 steps); h) *i*-Bu_2AlH (3.1 eq.), PhMe, - 78 ^oC, 35 min. (79%); i) CH_2Cl_2, rt, 18 h (64%); j) H_2 (1 atm), 10% Pd/C (wet), EtOAc, 16 h (91%); k) *i*-Bu_2AlH (2.5 eq.), PhMe, - 75 ^oC, 35 min.; l) TFA (40 eq.), CH_2Cl_2, Δ, 4d (40%, 2 steps).

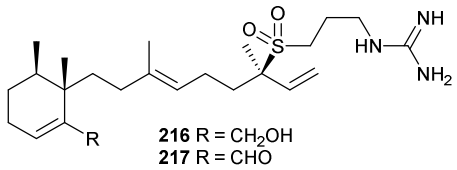
A new synthesis of merobatzelladine (**211**) has also been reported (Scheme 17).[265] The starting material pent-4-enal was converted into the sulfinamide **212**, which was condensed with 2-heptanone to give the chiral ketone in very good yield and diastereomeric ratio. It followed a series of stepwise interconversions, first with the asymmetric reduction to the alcohol **213**, which was protected before transformation of the sulfinamide group into a carbamate, with excellent enantiomeric excess. A key step involving a stereoselective carboamination generated the pyrrolidine **214**. Further transformation

and preparation of the substrate to a second carboamination led to the urea **215**, also in excellent yield and diastereomeric ratio. A series of subsequent transformations, including guanidine cyclization via a Mitsunobu reaction and final deprotection, provided tha natural alkaloid **211** in 6.7% overall yield. This approach is remarkable in its conception, in constructing the guanidine group step-by-step, a strategy rarely used in the synthesis of guanidine alkaloids.



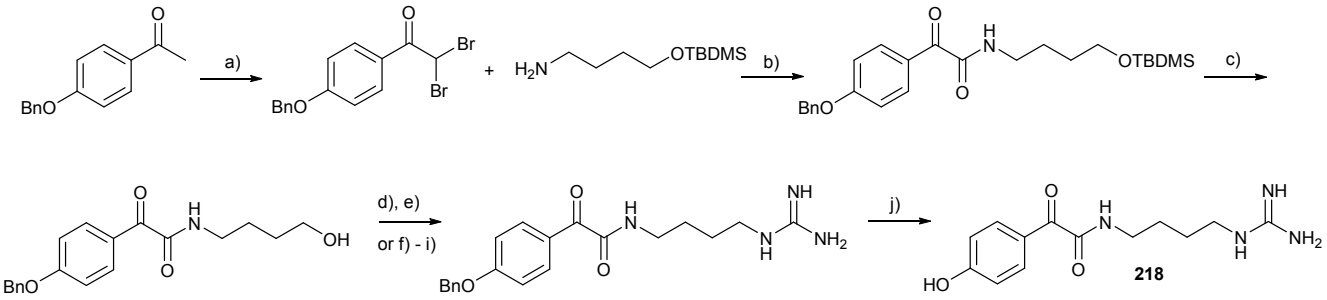
Scheme 17. a) 2-methylpropane-2-sulfinamide, $[Ti(OEt)_4]$ (87%); b) 2-heptanone, KHMDS, 81% (> 20:1 d.r); c) $NaBH_4$, $CeCl_3$ (3:1 d.r, 63%); d) NaH, BnBr; e) HCl; f) Boc_2O (91%, 3 steps); g) 2 mol % $[Pd_2(dba)_3]$, 8 mol % P(2-furyl)$_3$, NaOtBu, xylenes, 140 ^oC (68%, >20:1 d.r.); h) TFA, then PMBNCO (72%); i) (*Z*)-1-bromobut-1-ene, 3 mol% $[Pd_2(dba)_3]$, 12 mol% $PCy_3.HBF_4$, NaOtBu, toluene, 110 ^oC (91%, >20:1 d.r.); j) $POCl_3$, then NH_3 aq., then $NaBF_4$ workup (89%); i) H_2, Pd/C, then PPh_3, DIAD, then TFA (43%, 3 steps).

(–)-Agelasidines E (**216**) and F (**217**) have been isolated from the sponge *Agelas citrina*.[266] While the structures of both **216** and **217** have been established by analysis of spectroscopic data, the absolute configuration of both compounds have been elegantly determined by a combination of degradative and chemical correlations, as well as by global estimations of molar rotation, [ϕ], by employing the principle of optical superposition, proposed by van't Hoff and Le Bel over 100 years ago.



5.2 Other marine invertebrates

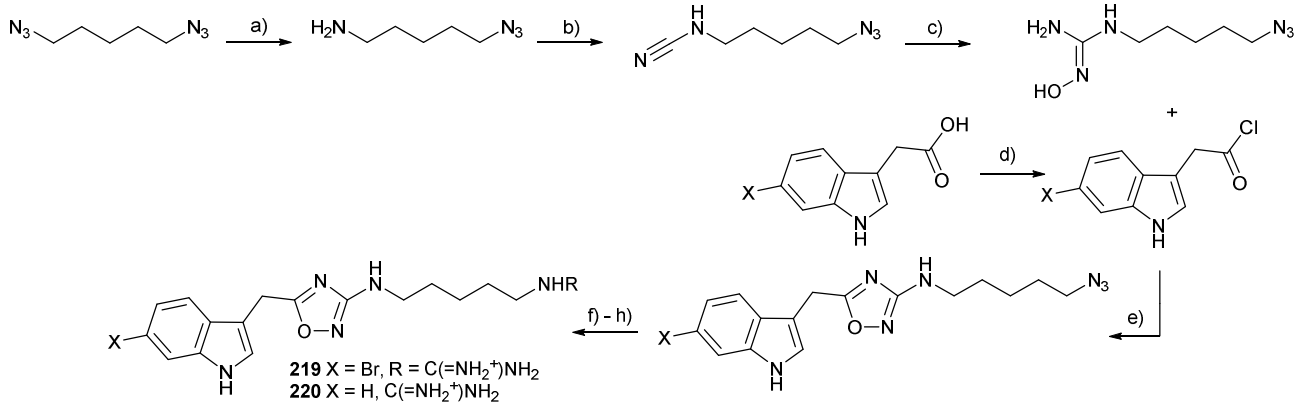
The first total synthesis of *N*-(3-guanidinopropyl)-2-(4-hydroxyphenyl)-2-oxoacetamide (**218**), previously isolated from the hydroid *Campanularia* sp., has been reported.[267] The synthesis was developed starting from 1-(4-(benzyloxy)phenyl)ethan-1-one, via two concurrent series of stepwise interconversions (Scheme 18).



Scheme 18. Synthesis of *N*-(3-Guanidinopropyl)-2-(4-hydroxyphenyl)-2-oxoacetamide (**218**). *Reagents and conditions*: a) Br_2, dioxane (73%); b) NaI, K_3PO_4, sulfolane, *t*-BuOOH (55%); c) TBAF, THF, 25-30 ^oC, 3 h (93%); d) di-*N*,*N*'-Boc-guanidine, Ph_3P, DIAD, THF, 0 ^oC, then rt, 48 h (19%); e) CH_2Cl_2, TFA, 0 ^oC then rt, 3 h (90%); f) MsCl, Et_3N, CH_2Cl_2, 0 ^oC then rt, 3 h; g) NaI, N_3, DMF, 55-60 ^oC, 3 h (85%); h) Ph_3P, H_2O, EtOAc, 30 ^oC, 1 h, then 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine, Et_3N, CH_2Cl_2, 0 ^oC, rt, 3h (65%); i) TFA, CH_2Cl_2, 0 ^oC then rt, 3 h (95%); j) HBr, AcOH or TMSBr, CH_2Cl_2 (80%).

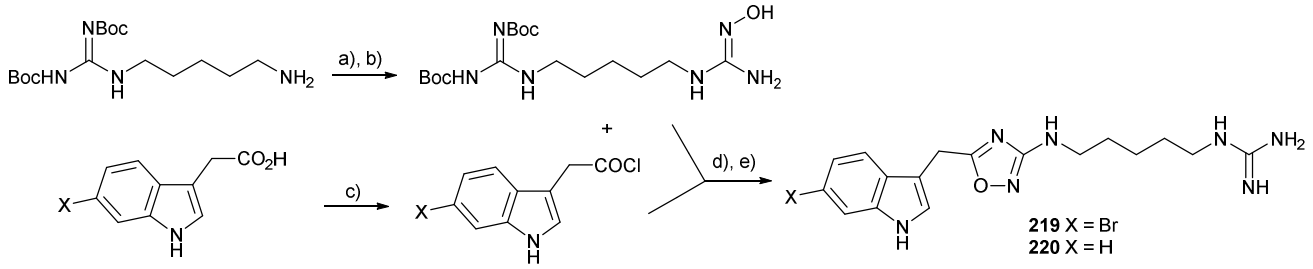
Four syntheses of phidianidines A (**219**) and B (**220**) have been reported between 2012 and 2014. These metabolites, the first ones bearing a 1,2,4-oxadiazole moiety, have been isolated from the

Opistobranch mollusk *Phidiana militaris*. Phidianidines displayed sub-μM cytotoxic activity against HeLa human epithelial cervical cancer and C6 rat glioma tumor cell lines, as well as to normal human epithelial kidney (HEK293) cells. Lin and Snider synthesis of **219** and **220** started from 1,5-diazidopentane and proceeded via a series of stepwise interconversions to give both phidianidines in 19% overall yield (Scheme 19).[268] Brogan, Stoops and Lindsley synthesis of both **219** and **220** proceeded via a very similar approach, but using *tert*-butyl (5-aminopentyl)carbamate instead of 5-azidopentan-1-amine (in Scheme 19) as the mono-protected diamine derivative to be condensed with both 2-(6-bromo-1*H*-indol-3-yl)acetic acid and 2-(1*H*-indol-3-yl)acetic acid, before subsequent transformations to give **219** and **220** in overall yields from 5 to 8%.[269] Manzo *et al*.[270] route to both **219** and **220** was essentially identical to that of Brogan, Stoops and Lindsley. It must be mentioned that the three first syntheses of **219** and **220** were published in 2012. Finally, the synthesis of **219** and **220** by Buchanan, Petersen and Chamberland was developed by an alternative strategy, in 6 to 7% overall yield (Scheme 20).[271]



Scheme 19. Lin and Snider synthesis of phidianidines A (**219**) and B (**220**). *Reagents and conditions*: a) PPh_3, 25 ^oC, 30 h, Et_2O, EtOAc, 5% HCl (85%); b) BrCN, $NaHCO_3$, CH_2Cl_2, H_2O, 25 ^oC, 1 h; c) $NH_2OH.HCl$, K_2CO_3, EtOH, 25 ^oC, 5 h; d) oxalyl chloride, DMF (cat.), CH_2Cl_2, 0 ^oC; e) Et_3N, CH_2Cl_2, 25 ^oC, 2 h then $ClCH_2CH_2Cl$, 80 ^oC, 2 h; f) $NH_4^+HCO_2^-$, Zn, MeOH, 25 ^oC, 7 h (40%, 3 steps); g) *N*,*N*'-

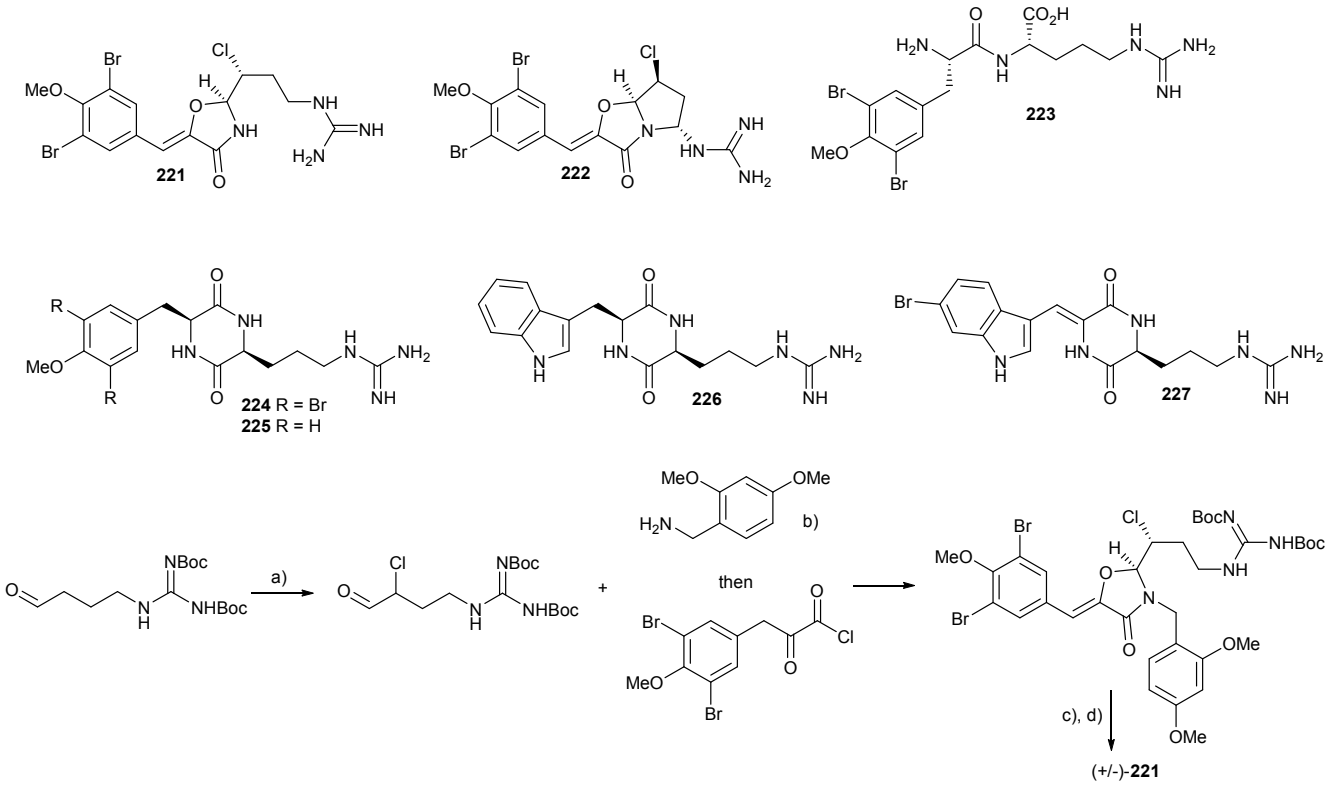
di-Boc-*S*-methyl isothiourea, Et_3N, $AgNO_3$, DMF, 0 ^oC, 2 h, 25 ^oC, 5 h (61%); h) CH_2Cl_2/TFA (10:1), 25 ^oC, 8 h (93%).



Scheme 20. Buchanan, Petersen and Chamberland synthesis of phidianidines A (**219**) and B (**220**). *Reagents and conditions*: a) BrCN (1.2 equiv), $NaHCO_3$ (6.0 equiv), H_2O/CH_2Cl_2, 0 ^oC, 0.5 h, then, rt, 1 h (72%); b) $NH_2OH.HCl$ (1.2 equiv), K_2CO_3 (3.0 equiv), EtOH, rt, 3 h (61%); c) oxalyl chloride (3.0 equiv), DMF (cat.), CH_2Cl_2, 0 ^oC, 1.5 h; d) CH_2Cl_2, rt, 3 h, then $ClCH_2CH_2Cl$, 83 ^oC, 2 h (14-15%); (e) TFA/ CH_2Cl_2 (1:10 v/v), rt, 8 h (99%).

Synoxazolidinones A (**221**) and C (**222**) have been isolated from the ascidian *Synoicum pulmonaria*, collected at Rya, off the Norwegian coast.[272] Both **221** and **222**, as well as derivatives **223-226** of barettin (**227**), previosuly isolated from the sponge *Geodia baretti*, have been assayed against a panel of marine bacteria and marine microalgae, and also as inhibitors of *Balanus improvisus* settlement, organisms directly involved in both micro and macrofouling processes. The synthetic derivatives **223**-**226** are overall more active than the natural products **221** and **222** on both the adhesion and growth of microalgae. However, only the natural products **221** and **222** were capable of inhibiting the adhesion of the barnacle *Balanus improvisus*. Gram-negative marine bacteria were less sensitive to the synthetic derivatives **223**-**226**, but the bacterial growth was significantly affected. The overall activities of compounds **223**-**226** were comparable to that of the commercial antifoulant 4,5-dichloro-2-octylisothiazol-3-one.[272] The absolute configuration of **221** and of **222** has been re-assigned after a very

detailed and careful analysis by electronic circular dichroism, vibrational circular dichroism and Raman optical activity.[273] The results indicated that synoxazolidinone A (**221**) has a 6*Z*,10*S*,11*R* configuration, while synoxazolidinone C (**222**) has either (6*Z*,10*S*,11*R*,13*R*) or (6*Z*,10*S*,11*S*,13*R*) configuration. The synthesis of racemic **221** has been accomplished via stepwise interconversions in 19% overall yield (Scheme 21), as well as the synthesis of racemic synoxazolidinone B, the deschloro analogue of **221**.[274] Synthetic (±)-**221** showed moderate antimicrobial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Acinetobacter baumannii*.

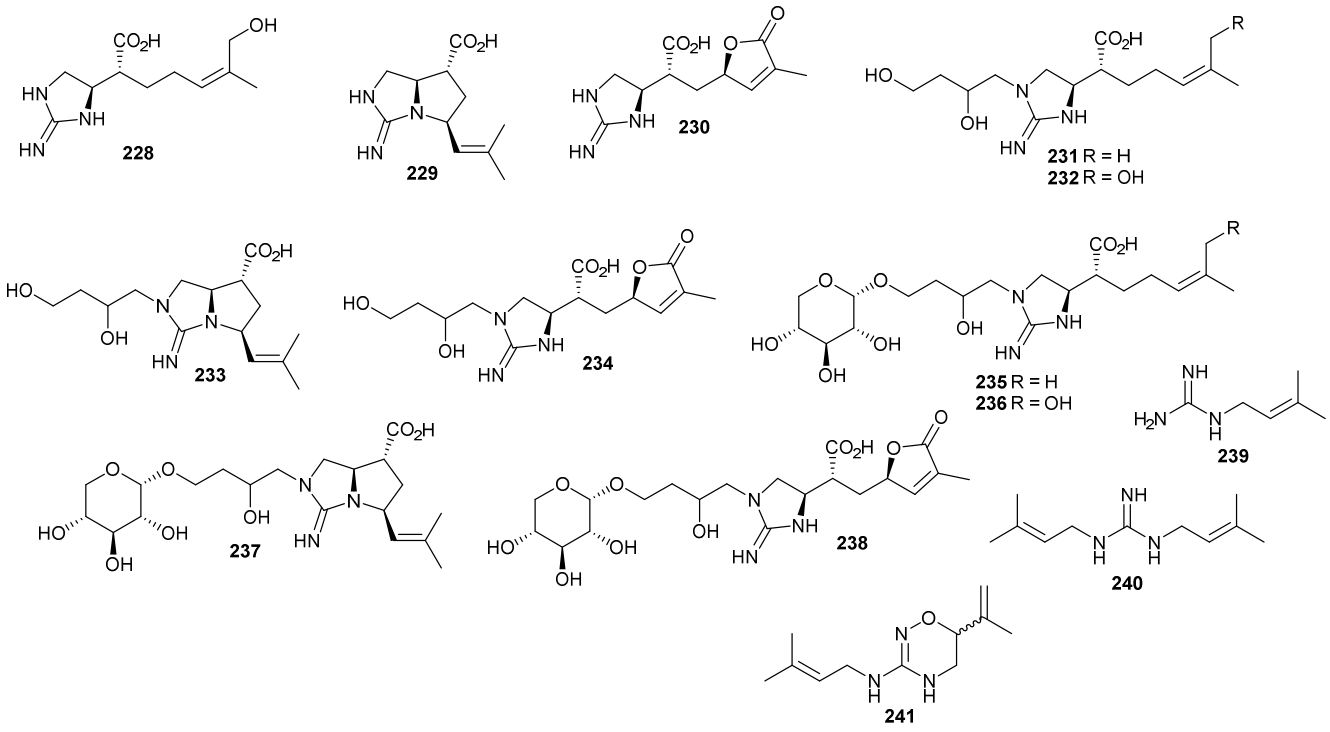


Scheme 21. Synthesis of synoxazolidinone A (**221**). *Reagents and conditions*: a) L-proline (1.1 eq.), *N*-chlorosuccinimide (1.3 eq.), CH_2Cl_2, 0 ^oC; b) $MgSO_4$, $CHCl_3$, rt to 0 ^oC; c) TFA, CH_2Cl_2, rt, 2 h; d) TFA 40 ^oC, 48 h.

6 Natural guanidines from higher plants

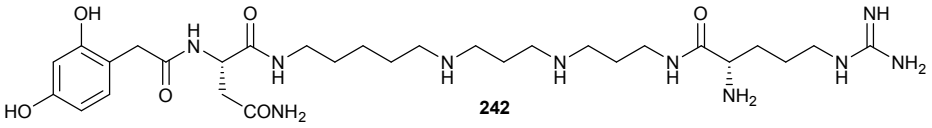
Plumbagines A−G (**228**−**234**) and plumbagosides A−D (**235**−**238**) have been isolated from *Plumbago zeylanica*.[275] The structures of these guanidine alkaloids have been established by analysis of spectroscopic data, while the absolute configuration of **228** was assigned by X-ray diffraction analysis and the configurations of the remaining compounds were assigned by comparison with circular dichroism spectrum of **228** and with that of (23*R*)-3-oxo-9β-lanosta-7,24-dien-26,23-olide. The sugar moiety of **235**-**238** was identified as α-D-xylose. None of the alkaloids **228**-**238** displayed cytotoxic activity against PANC-1 (human pancreatic carcinoma) and MDA-MB-231 (human breast carcinoma) cell lines.[275]

Among a series of natural and synthetic prenylated guanidines, galegine (**239**) and pterogynidine (**240**) isolated from *Pterogyne nitens* showed the most potent, yet mild, antibacterial activity against *S. aureus* strains, including two methicillin-resistant ones, in the range between 20 and 31 μM.[276] Alchornedine (**241**) has been isolated from *Alchornea glandulosa* and identified by analysis of spectroscopic data. Alkaloid **241** displayed antiparasitic activity against *Trypanosoma cruzi* trypomastigotes, with IC_{50} at 93 μg/mL, as well as against *T. cruzi* amastigotes with IC_{50} at 27 μg/mL. The cytotoxic activity of **241** on mice conjunctive cells NCTC was observed with an IC_{50} of 50 μg/mL.[277]



7 Natural guanidines from terrestrial animals

A series of derivatives of argiotoxin-636 (**242**), have been synthezised in order to evaluate the activity as selective inhibitors of the *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subfamilies of ionotropic glutamate (iGlu) receptors. Correlations could be established for the two receptors sub-families, indicating that the secondary amino group in the polyamine chain closer to the aromatic moiety led to a more effective inhibition of NMDA receptors, while a secondary amino group nearer to the amino acid polar chain led to a more effective inhitibion of AMPA receptors. Therefore, derivative ArgTX-75 (**243**) was developed as a more potent and selective ligand to the NMDA receptor, leading to a further optimization exemplified by the indole derivative (**244**). On the other hand, ArgTX-48 (**245**) was found to be a more potent and very selective ligand to the AMPA receptor, which could be optimized to the *ortho*-phenol derivative **246**. All inhibitors optimized had ligand affinities in the nM range.[278] Further optimization of the AMPA receptor inhibitory selectivity and potency of **244** led to the development of the hydroxyindole **247**, with inhibitory activity on AMPA receptors at 15 nM.[279]



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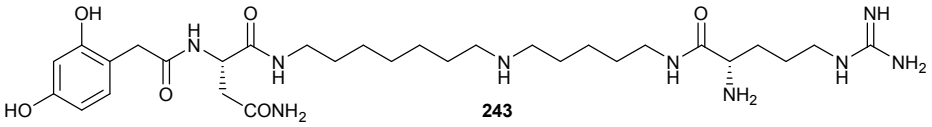

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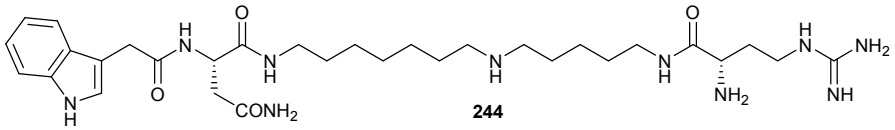

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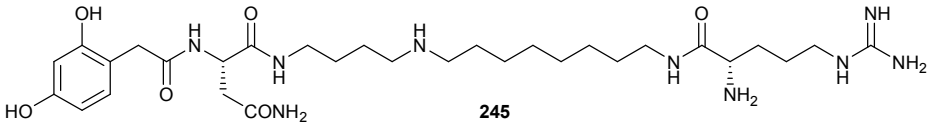

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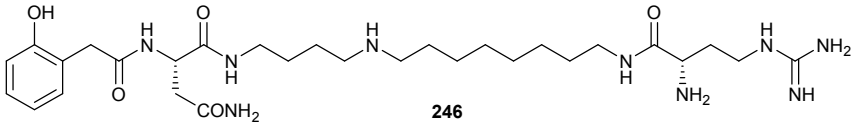

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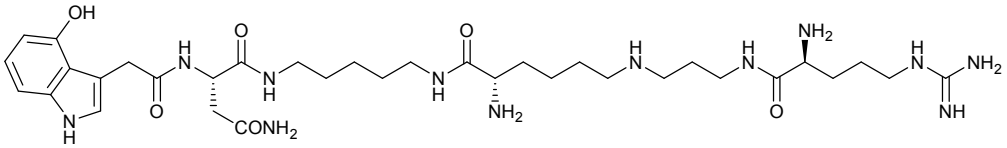
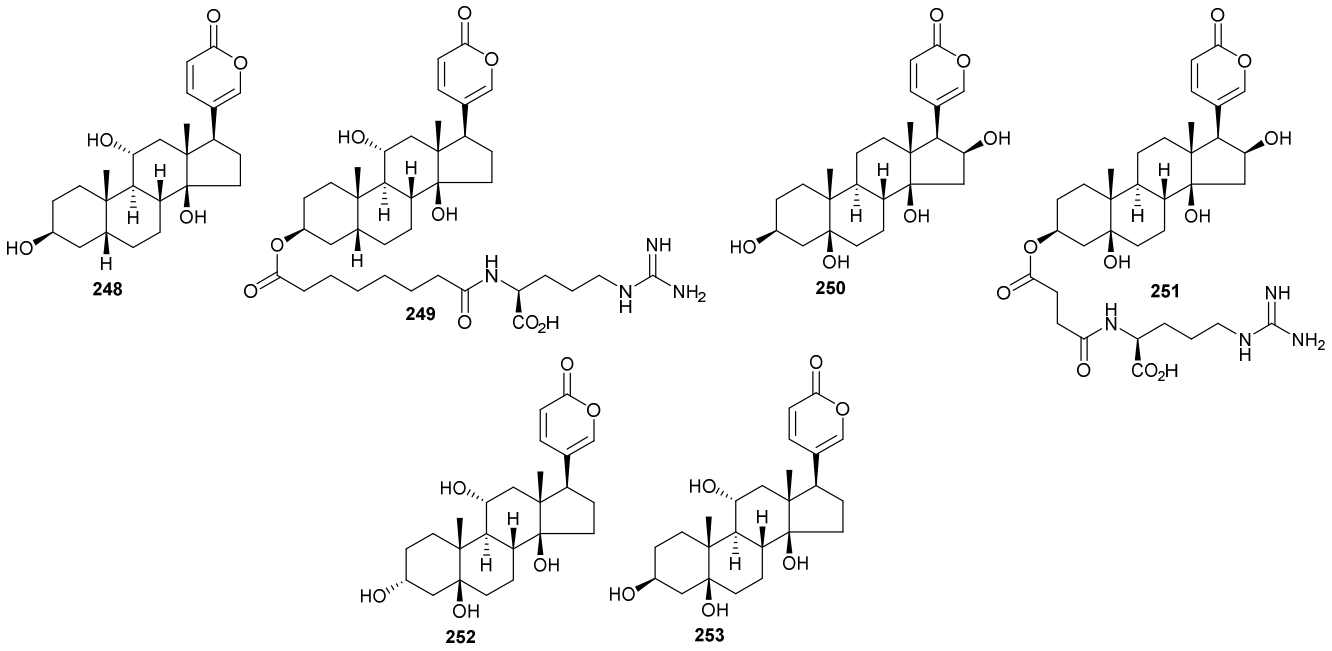


247

The Asian snake *Rhabdophis tigrinus* preys on the toad *Bufo japonicus*, and captures bufadienolides which are accumulated in the snake's nuchal glands.[280] Hatchlings of *R. tigrinus* fed with toads presented a much higher diversity of bufadienolides than the individuals fed with fish or with frogs devoid of bufadienolides. *R. tigrinus* dams (female parents) with abundant variety and quantity of bufadienolides produced offspring also containing the same compounds. Dams fed with toads late in gestation produced offspring with a higher variety of bufadienolides, as late as 12 days priori to oviposition when embryos were already enclosed in shelled eggs. Eggs treated with 0.5 mg of bufo toads extracts absorbed the compounds, which were not retained in the egg shells or membranes

but likely accumulated in the embryos. The major compounds accumulated by the snakes were gamabufotalin (**248**), which also occurs in bufo toads or may be derived from bufotoxin T3 (**249**), and **250**, which appears to be a product of the hydrolysis and hydroxylation of bufotoxin T11 (**251**). In order to investigate the biotransformations promoted in the snake's tissues, different bufotoxins were administered individually in snake food. For example, when bufotoxin T3 (**249**) was given to the snake, compounds **248**, **252** and **253** were found in the snake's nuchal gland. Bufotoxins, i.e., bufadienolides bearing a suberoylarginine side chain, were never found in the snake's nuchal gland, a finding which might be related to the fact that bufotoxins are less toxic than bufadienolides. Finally, snakes from Kinkasan Island (Japan), which were devoid of bufadienolides due to the absence of toads in the ecological niche, rapidly accumulated the compounds when fed with the toads. On the other hand, snakes from Ishima Island usually bearing bufadienolides because toxic toads are abundant in that location, did not loose the compounds completely after a diet devoid of toads, presumably because the metabolites were accumulated in early life by maternal provisioning.[280]



8 Conclusion

Research into the chemistry and biology of guanidine-related metabolites during the last twenty years provided a very impressive number of biologically active new metabolites, with unique structures and modes-of-action. Further investigations on the biosynthesis of such compounds revealed some unique pathways for the formation of these metabolites, very often derived from arginine as the source of the guanidine group. The development of synthetic strategies for the preparation of natural and unnatural guanidines enabled the access to a number of complex skeletons, exemplified by the bromopyrrole alkaloids of marine sponges, some of which have the most complex structures of natural guanidines isolated so-far.

The presence of the guanidine group in these metabolites is very often directly related to their biological activities, as exemplified by the above mentioned bufotoxins/bufadienolides. Such particular properties reveals that the molecular mode-of-action of guanidine metabolites is not only related to a possible role as detergent-like compounds, but can also be more specific, in many cases still unknown. A deeper investigation of natural guanidines modes-of-action should demonstrate the actual role and importance of the guanidine group for the chemistry and pharmacology of these metabolites.

However, the biosynthesis of a number of guanidine secondary metabolites still remains essentially conjectural. Such is the case of sponge alkaloids with very complex scaffolds, indicating that possibly a number of enzyme-regulated pathways remains to be uncovered and investigated. Clearly, the discovery of additional guanidine natural products, the knowledge of their biology, which also includes the actual function of such compounds, remain a fascinating aspect to be continuously investigated.

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