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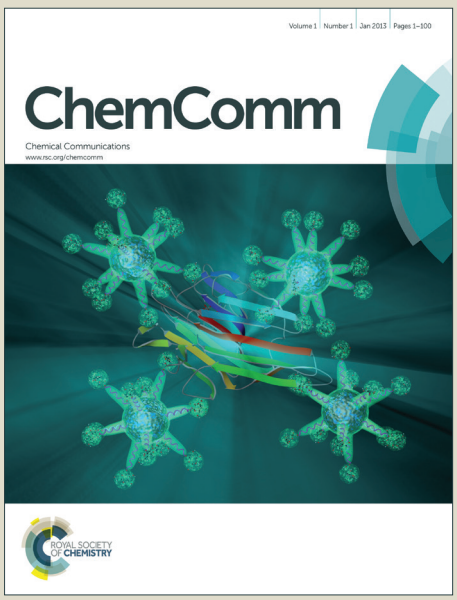

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The Origins of Cyanobactin Chemistry and Biology

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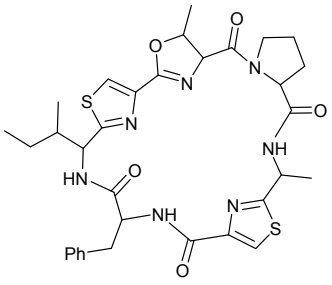
The correct structure of the symmetrical cyanobactin, ascidiacyclamide, was published in Chemical Communications by Hamamoto *et al* in 1983.[1] The cyanobactin family of compounds are cyclic peptides with modifications including azole/azoline rings, D-stereocentres and in some cases prenyl groups. Although related compounds were isolated earlier by Ireland *et al*,[2,3] two of the three published structures later had to be corrected.[1,4] Hamamoto's ascidiacyclamide structure assisted the understanding of the chemistry, bioactivity, biological origin and biosynthesis of this group of compounds. Cyanobactins are of interest as new chemotypes for the treatment of a range of diseases, in particular those in which extended binding sites are implicated.

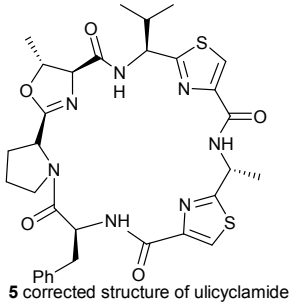
The realisation that not all drug targets can be modulated by Lipinski-compliant small molecules has led the pharmaceutical industry to revisit macrocycles and cyclic peptides of natural origin.[5] These mid-sized molecules are able to interact with extended binding sites not amenable to modulation by small molecules and have distinct advantages over the alternative biological therapeutics such as native proteins and antibodies. The marine environment is an excellent source of cyclic peptides, with some, such as the seasquirt-derived Aplidin (plitidepsin) now in phase III clinical trials for the treatment of myeloma. The discovery and manufacture of such cyclic peptides with potent and selective bioactivity has always relied on the interplay between natural product and synthetic chemists. More recently, a detailed understanding of the biosynthesis of the cyanobactins has led to their production using chemoenzymatic[6] and cell-based methods[7].

The original isolation of a cyanobactin, ulicyclamide (incorrectly assigned as **1**) and ulithiacyclamide (correctly assigned as **2**), from the Indo-Pacific ascidian (seasquirt) was carried out by Ireland and Scheuer and published in 1980.[2] In 1982 new cyanobactins, patellamides A-C were isolated from the Indo-Pacific ascidian (seasquirt) *Lissoclinum patella*, and their core structure was assigned as shown for patellamide A (incorrectly assigned as **3**) using NMR and MS techniques.[3] A year later, Hamamoto appeared to have isolated a similar material from the same organism, but reported its structure as **4** (scheme 1), with a distinct skeleton.[1] Shortly after this, Ireland and co-workers corrected the structure of

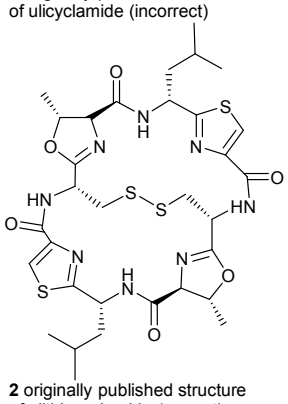
ulicyclamide to from **1** to **5**.[4] It is clear that the building blocks of **1/5** and **3/4** are nearly identical, but their sequence differs. Subsequent synthesis[8] and a crystal structure[9] indicated that structures in which the heterocycles were interspersed with peptide bonds as in **2**, **4** and **5** are correct. Confirmation of the structural skeleton of ulicyclamide and ascidiacyclamide is consistent with a proposed biogenesis in which the Thr/Ser and Cys residues are heterocyclised to form oxazolines and thiazoles respectively.



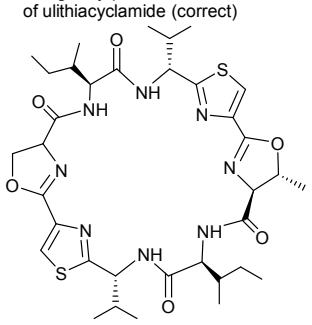
1 originally published structure of ulicyclamide (incorrect)



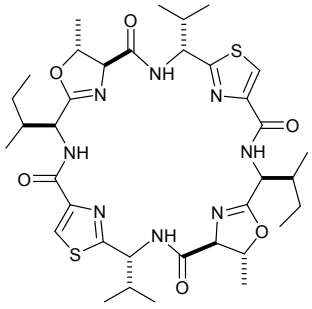
5 corrected structure of ulicyclamide



2 originally published structure of ulithiacyclamide (correct)



3 originally published structure of patellamide A (incorrect)



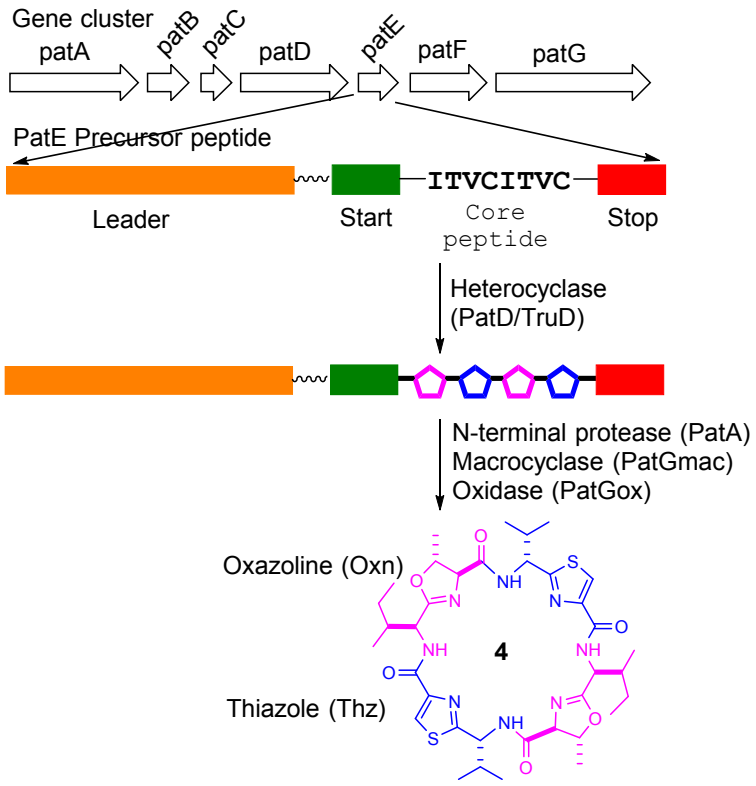
4 correct structure of a patellamide (ascidiacyclamide)

Currently, over 30 cyanobactins belonging to this structural family such as the patellamides, lissoclinamides, tawicyclamides and others have been isolated and characterised. Initial interest was in their biological activity, with several being highly cytotoxic but non-selective and others shown to inhibit the Pgp drug efflux system.[10] Their conformations aroused interest as did their ability to bind to transition metals which led to speculation about the biological role of these compounds. Recent studies by Comba have shown that the dicopper

complexes of patellamide derivatives are able to act as carbonic anhydrase mimics, suggesting that this might be their biological role in the producing organism.[11]

Increased understanding of natural product biosynthesis coupled with the fact that cyanobactins with related structures were being discovered in cyanobacteria led to the suggestion that the true origin of the cyanobactins in *Lissoclinum patella* was in fact its cyanobacterial symbiont, *Prochloron* sp. Separating *Prochloron* cells from the host ascidian and analysing their metabolite profile did not yield conclusive results as the cyanobactins were present in both suggesting that material was being translocated between symbiont and host.[12] A proposal that the cyanobactins were made by a non-ribosomal peptide synthetase could not be confirmed using molecular methods.[13]

Advances in gene sequencing and cloning methodology led two groups to confirm the proposed cyanobacterial origin of the cyanobactins using two complementary approaches. Our own approach relied on shotgun cloning of *Prochloron* gDNA into an *E. coli* host and confirming the heterologous production of the cyanobactins by LC-MS.[14] The use of *Prochloron* whole genome sequence data led Schmidt and co-workers to discover the gene cluster for the cyanobactins and showed it to be a post-translationally modified ribosomal peptide.[15] The cyanobactins are formed when a ribosomally encoded precursor peptide, containing a core peptide which eventually forms the product cyanobactin, is modified by a series of tailoring enzymes to install azoline rings, form the macrocycle, and oxidise the thiazolines to thiazoles (Scheme 1).[10] Curiously, no epimerase was found in the gene cluster to invert the stereocentres adjacent to the thiazoles, thus confirming an earlier suggestion that this process is spontaneous and driven by the more stable conformation of the final product cyanobactin containing D-stereocentres in these two residues.[16]



Scheme 1. Biosynthesis of the patellamide cyanobactins occurs via the action of a series of tailoring enzymes on the core peptide forming part of a larger precursor peptide. Installation of heterocycles occurs first followed by proteolysis, formation of the macrocycle, spontaneous epimerisation and finally oxidation of the thiazolines to thiazoles.

Detailed investigations into the gene cluster showed that the genes *patB* and *patC* were not essential to the formation of the cyanobactins. The precursor peptide could be engineered to contain non-native sequences, could be elongated and could incorporate non-amino acid portions, as long as the final residue was cyclic (heterocycle/proline).[17, 18] Promiscuity of the tailoring enzymes was also shown by incorporating unnatural amino acids in cyanobactins using Schultz's technology.[7] Biochemical studies were able to identify the some mechanistic parameters of the tailoring enzymes and delineated their flexibility, but structural understanding was still lacking. Crystal structures of three of these enzymes: TruD (heterocyclase specific for Cys)[19]; PatA[20,21] and PatGmac (macrocyclase)[21,22] have now been solved and provide an insight into these uniquely flexible enzymes which may find biotechnological application to generate a large range of highly modified cyclic peptides. Generating chemical diversity in the size range being demanded by the pharmaceutical industry to modulate extended binding sites may now be a viable option using chemoenzymatic or cell-based approaches. This may eventually lead to the discovery of new pharmacophores with the potential to treat immune disorders, inflammation, and treat drug

resistant cancers.[23] As an example, cyanobactins with selenazoles have been shown to be effective blockers of the mouse Pgp drug efflux pump,[24] and such compounds can now be made via a chemoenzymatic approach[6].

Hamamoto's delineation of the ascidiacyclamide structure in 1983 was followed by a series of steps which improved synthetic methodology to these compounds and allowed their bioactivity and potential ecological role to be defined. It also supported reasonable biogenetic proposals which were critical in identifying the *Prochloron* symbiont as the true cyanobactin producer and led to a more detailed understanding of how these complex cyclic peptides are formed ribosomally by enzymatic tailoring of a precursor peptide. The flexibility and promiscuity of these enzymes and their analogues from related species means the time is now ripe for their biotechnological exploitation to generate the complex chemical diversity required by the pharmaceutical industry to create new chemotypes for the treatment of human diseases with complex aetiologies.

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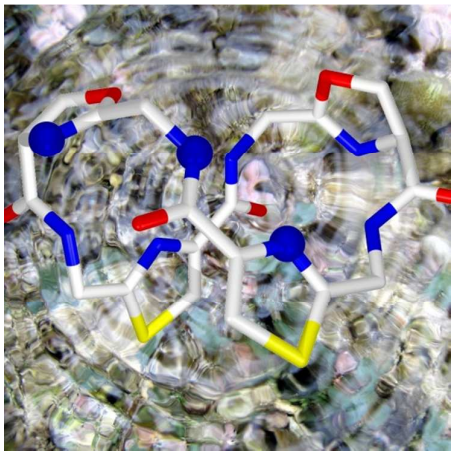

Novelty Sentence: Publication of the correct structure for ascidiacyclamide, a symmetrical patellamide, led to studies on the application and origins of these ribosomally produced compounds.