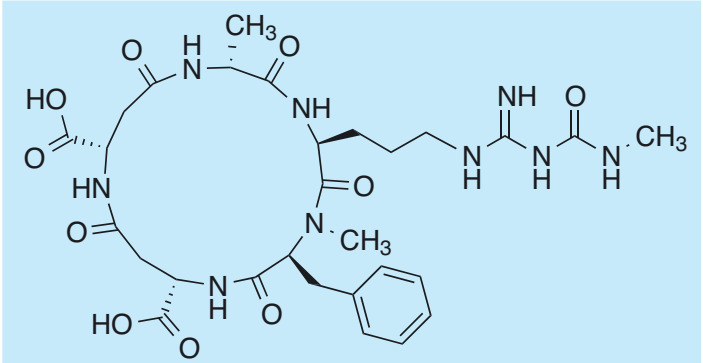
Argifin

1. Discovery, producing organism and structure[1-4,14,18)]

Argifin was isolated from the culture broth of *Clonostachys grammicosporopsis* FTD-0668 and found to be a chitinase inhibitor. It is a cyclic pentapeptide of cyclo(N^{ω}-(*N*-methylcarbamoyl)-L-arginyl-*N*-methyl-L-phenylalanyl-β-L-aspartyl-β-L-aspartyl-D-alanyl). The argifin-chitinase complex was resolved with the argadin-chitinase complex (see Argadin). The first total synthesis was achieved by Eggleston *et al*.[5)]

Gliocladium sp. FTD-0668
(*Clonostachys grammicosporopsis* FTD-0668)
Bar: 20 μm



Argifin

2. Physical data

White powder. $C_{29}H_{41}N_9O_{10}$; mol wt 675.70. Sol. in H_2O. Insol. in MeOH, acetone, $CHCl_3$.

3. Biological activity

1) Chitinase inhibition[2,4,6,7)] (see Argadin)

2) Argifin-chitinase complex[4,6,7)] (see Argadin)

3) Insecticidal activity[2)]

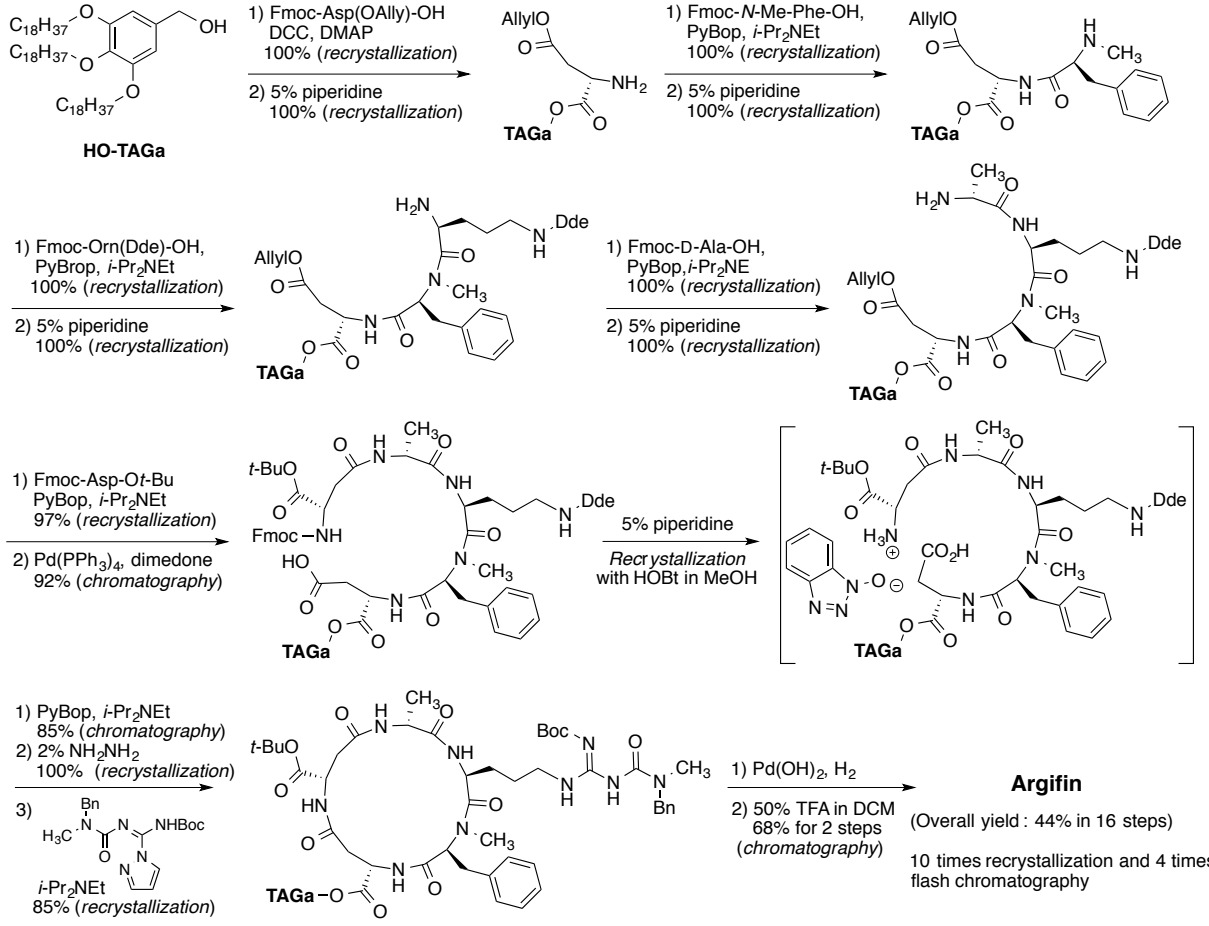
Argifin (20 μg) was injected into cockroach larvae in three separate trials and compared to mock injected controls. Mortality was assessed at 1, 5, and 20/23 days post injection. Three separate trials repeatedly showed the efficacy of argifin against larval stages of *Periplaneta americana* (American cockroach) and *Blattella germanica* (German cockroach). Argifin showed 73% mortality in cockroaches, while the mortality rate of the control was only 12% in *Periplaneta americana*. Most of the dead cockroach larvae showed no signs of molting. Cockroach larvae killed during molting showed new cuticle formation below the partially opened old cuticle. Furthermore, they were unable to leave the old exuva leading to their death shortly after sclerotization of the new cuticle.

4) Other biological activity[2)]

Argifin did not inhibit the growth of tested bacteria, yeast, or fungi at 10 μg/disc (paper disc method). Argadin also did not inhibit the growth of P388 or HL-60 cells at 25 μg/ml.

4. Total synthesis[5, 8-10,19,20)]

The total synthesis of argifin was reported by two groups. The following scheme is Ōmura's solution phase tag approach[10)]. (See Appendix-I)



5. Computer-aided rational molecular design from argifin[11-15)]

Efficient total synthesis of argifin could be applied to enable synthesis of analogs. In addition, the 3-D structure of argifin, in complex with chitinase B from *Serratia marcescens* (*Sm*ChiB), was resolved by X-ray analysis (see Argadin)[11)]. Rational molecular design of derivatives based on the structure of argifin led to production of novel derivatives, which showed great potential for inhibitory activity against *Sm*ChiB[12-15)].

6. The active framework of argifin and use of *in situ* click chemistry[14,16,17)]

The studies of argifin and its analogs by X-ray crystallography with various chitinases revealed that there are at least four conserved hydrogen-bond interactions between the N^{ω}-methylcarbamoyl-L-arginine moiety and the polar groups arrayed in the hydrolytic pocket of the family 18 chitinases. The remarkable fidelity of the hydrogen-bonding network between the chitinases and the argifin ligand implicates its critical role in revealing the micromolar to nanomolar range of inhibition. Ōmura's group thus focused on the design and simplification to azide-bearing N^{ω}-methylcarbamoyl-L-arginine substrate, as a smaller analogs of macrocyclic peptide natural product argifin, and the use of target-guided synthesis (TGS) depending on the 1,3-dipolar cycloaddition between the azide-molecules and the acetylenes, which is named as "*in situ* click chemistry" (Figure), for the screening of novel and more potent chitinase inhibitors[16)].

Ōmura's group also has determined the crystal structures of complexes of *Sm*ChiB with azide ligand (click precursor) and *syn*-triazole (generated *in situ*), together with the 3-component complex of [*Sm*ChiB]–[azide-ligand]–[mimic of alkyne-bearing quinoline-oxime fragment], respectively. The results of their X-ray analysis demonstrated that the assembly of azide ligand and alkyne-bearing quinolone-oxime within *Sm*ChiB is responsible for yielding a very potent *syn*-triazole inhibitor via an *in situ* click chemistry reaction, as visualized by X-ray crystallography.[17)]
Ōmura's strategy employed an azide substituent appended to an active domain excised, as it were, from the more complex natural macrocyclic peptide argifin. The *Sm*Chi, which in this case was specifically *Sm*ChiB, served as both mold and template for triazole formation between a unique pair of azide and alkyne fragments. In the process of *in situ* click chemistry, the highly exergonic nature of triazole formation makes the process completely irreversible, and thereby locks in unique information, a kind of embedded message of the encounter. More practically, it allowed us to discover a lead template for the discovery of a selective chitinase inhibitor directed toward the functions of *Sm*Chi, without the need for lengthy and costly analog syntheses.

7. References

1. [747] K. Shiomi *et al*., *Tetrahedron Lett*. **41**, 2141-2143 (2000)
2. [754] S. Ōmura *et al*., *J*. *Antibiot*. **53**, 603-608 (2000)
3. [755] N. Arai, *et al*., *J*. *Antibiot*. **53**, 609-614 (2000)
4. [805] D. R. Houston *et al*., *Proc*. *Natl*. *Acad*. *Sci*. *USA* **99**, 9127-9132 (2002)
5. I. Eggleston *et al*., *Bioorg*. *Med*. *Chem*. *Lett*. **15**, 4717-4721 (2005)
6. [891] F. V. Rao *et al*., *Chem*. *Biol*. **12**, 65-76 (2005)
7. [947] S. Ōmura & K. Shiomi, *Pure Appl*. *Chem*. **79**, 581-591 (2007)
8. I. M. Eggleston *et al*., *Org*. *Biomol*. *Chem*. **7**, 259-268 (2009)
9. [1034] T. Sunazuka *et al*., *Bioorg*. *Med*. *Chem*. **17**, 2751-2758 (2009)
10. [1107] T. Hirose *et al*., *Tetrahedron* **67**, 6633-6643 (2011)
11. [983] H. Gouda *et al*., *Bioorg*. *Med*. *Chem*. **16**, 3565-3579 (2008)
12. [1052] H. Gouda *et al*., *Bioorg*. *Med*. *Chem*. *Lett*. **19**, 2630-2633 (2009)
13. [1055] H. Gouda *et al*., *Bioorg*. *Med*. *Chem*. **17,** 6270-6278 (2009)
14. [1066] T. Hirose *et al*., *Proc*. *Jpn*. *Acad*., *Ser*. **B86**, 85-102 (2010)
15. [1079] H. Gouda *et al*., *Bioorg*. *Med*. *Chem*. **18**, 5835-5844 (2010)
16. [1031] T. Hirose *et al*., *J*. *Antibiot*. **62**, 277-282 (2009)
17. [1157] T. Hirose *et al*., *Proc*. *Natl*. *Acad*. *Sci*. *USA* **110**, 15892-15897 (2013)
18. T. Hirose, *Yakugaku Zasshi* **132**, 1001-1010 (2012)
19. M. J. Dixon *et al*., *Org*. *Biomol*. *Chem*. **7**, 259-268 (2009)
20. A. Andersen *et al*., *Chem*. *Biol*. **15**, 295-301 (2008)