Studies on the Role of AmbF in the Biosynthesis of the Divinylcyclopropanediyl Fragment of the Ambruticins

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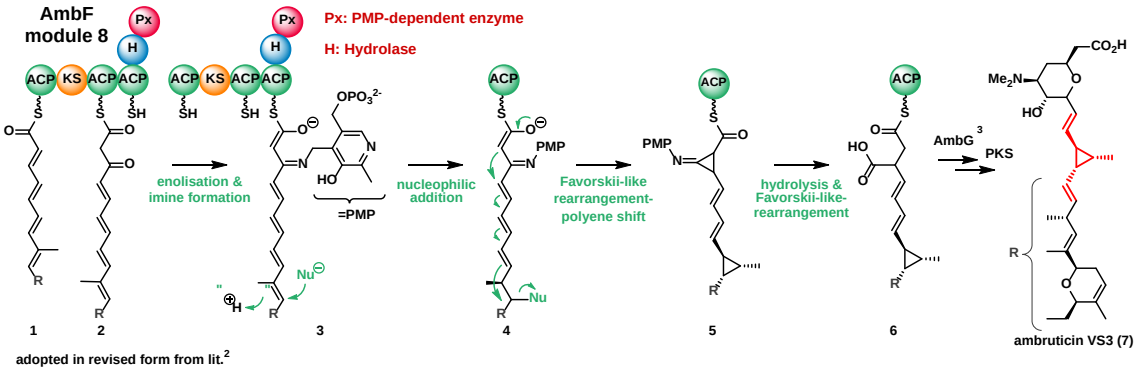
Ambruticins (**7**) are fungicidal polyketides, produced by the myxobacterium *Sorangium cellulosum* 10.[1] Julien *et al*. proposed their unusual biosynthetic pathway based on genetic analysis.[2] Significant parts of this pathway, however, remain enigmatic, thus requiring a thorough *in vitro* study on its enzymology.

We are particularly interested in the unprecedented, AmbF-catalysed formation of the divinylcyclopropanediyl fragment of the ambruticins (highlighted in red, see Scheme below for a putative pathway). This AT-free module contains an unusual number of three ACP domains along with the pyridoxamine phosphate (PMP) binding didomain (Px) and an H domain, which are probably responsible for polyene shift cyclopropanation and consecutive Favorskii-like rearrangement to **6**, which is then further processed by the FAAL-ACP didomain AmbG and downstream PKS moduls.[3]



To prove our biosynthetic hypothesis, we are following a multifaceted approach, consisting of *in vitro* reconstitution and isolation of sensitive intermediates for spectroscopic and spectrometric analysis. All relevant genes have been cloned and expressed. We will present results from the *in vitro* assaying of individual AmbF domains as well as their analysis by X-ray crystal structure analysis and bioinformatic methods. Furthermore, we will present our recently developed method for the short and versatile synthesis of the highly sensitive precursor surrogates.

[1] S. M. Ringel, M. von Stradtmann *et al.*, *J. Antibiot.* **1977**, *30*, 371. [2] B. Julien, C. D. Reeves *et al.*, *Chem. Biol.* **2006**, *13*, 1277–1286. [3] F. Hemmerling, K. Lebe, J. Wunderlich, F. Hahn, *ChemBioChem*, **2018**, *19*, 1006–1011.