

## **Generation of Hybrid Polyketides through Combinatorial Biosynthesis of Polyketide Synthase (PKS) and Modification of Post-PKS Tailoring Steps**

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Polyketides are a class of structurally diverse natural products which possess a wide range of biological activities. These compounds are used throughout medicine and agriculture as antimicrobials, immunosuppressants, antiparasitics, and anticancer agents.

While structurally diverse, polyketides are assembled by a common mechanism of decarboxylative condensations of simple malonate derivatives by polyketide synthases (PKSs) in a manner very similar to fatty acid biosynthesis (Fig 1). After assembly by the PKS, tailoring enzymes such as glycosyltransferases, hydroxylases, or methyltransferases can then further modify the polyketide product. These post-PKS modifications are almost always necessary in order for the molecule to be bioactive.

Recent efforts to produce novel polyketides have opened the possibility of combining or altering individual domains or modules of PKS, a process known as combinatorial biosynthesis. Using this method, many successful examples of hybrid PKSs have been constructed and shown to produce unnatural polyketides.

We have shown the replacement of the pikromycin PKS in *S. venezuelae* by corresponding subunits from heterologous modular PKS resulted in the production of novel macrolides. For example, the replacement of the last module of the pikromycin PKS to tylGV, the part of the tylosin biosynthesis cluster, could produce novel polyketides (Fig 2).

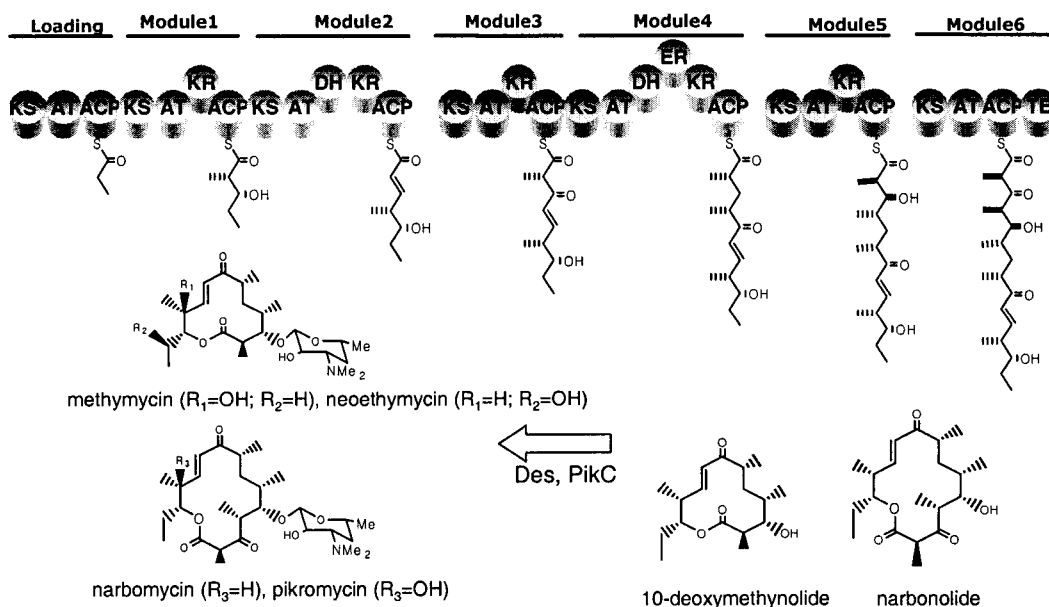
Also, combinatorial biosynthesis of further macrolactones modification steps such as hydroxylation and glycosylation could result in structural diversification of polyketides. As mentioned, these late-stage steps are crucial for the bioactivities of polyketides and are key to the structural diversity.

Highly flexible PikC hydroxylase from pikromycin biosynthesis cluster in *S. venezuelae* could show the abilities of hydroxylation of alternative polyketides (Fig 3). Also, EryF from erythromycin biosynthesis cluster in *Saccharopolyspora erythraea* and OleP from oleandomycin biosynthesis cluster in *S. antibioticus* could catalyze the hydroxylation of alternative polyketides (Fig 4).

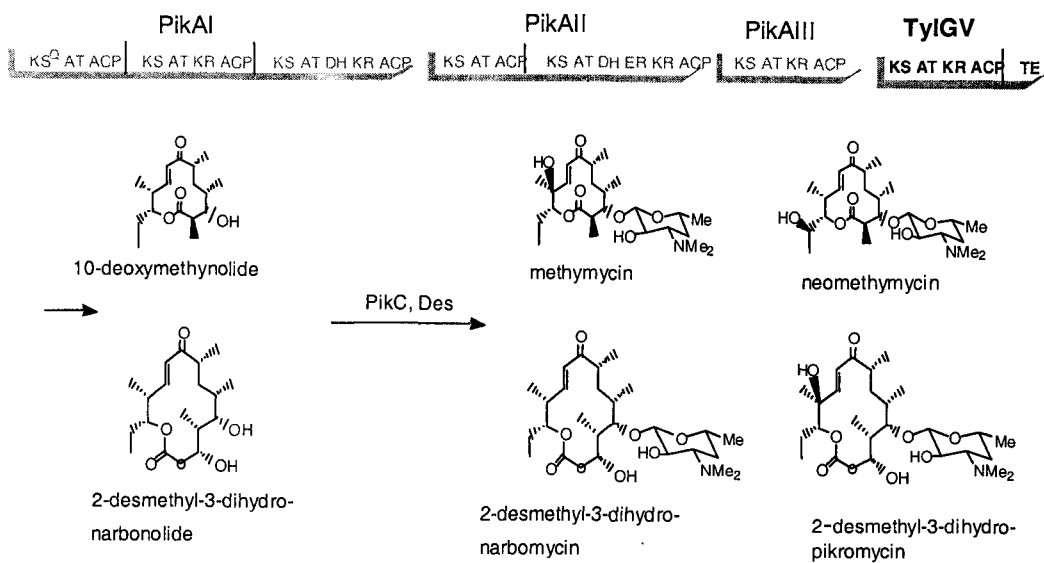
In another post-PKS tailoring step, glycosylation, to test the flexibility of the *S.venezuelae* glycosyltransferase, Des-VII, a host-vector system was developed.

This novel system could be utilized for the combinatorial biosynthesis of a wide range of deoxysugar moieties and attachment of the synthesized sugar to the aglycone substrate. The replacement of *Des* genes, which is the sugar biosynthesis genes from pikromycin biosynthesis cluster in *S. venezuelae*, by heterologous genes could produce the structurally modified macrolides.

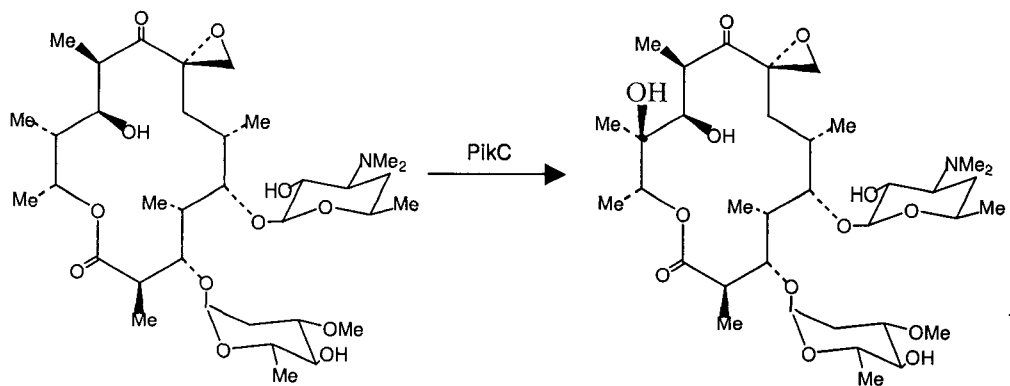
These results suggest that a wide variety of macrolide polyketides should be accessible by these approaches.



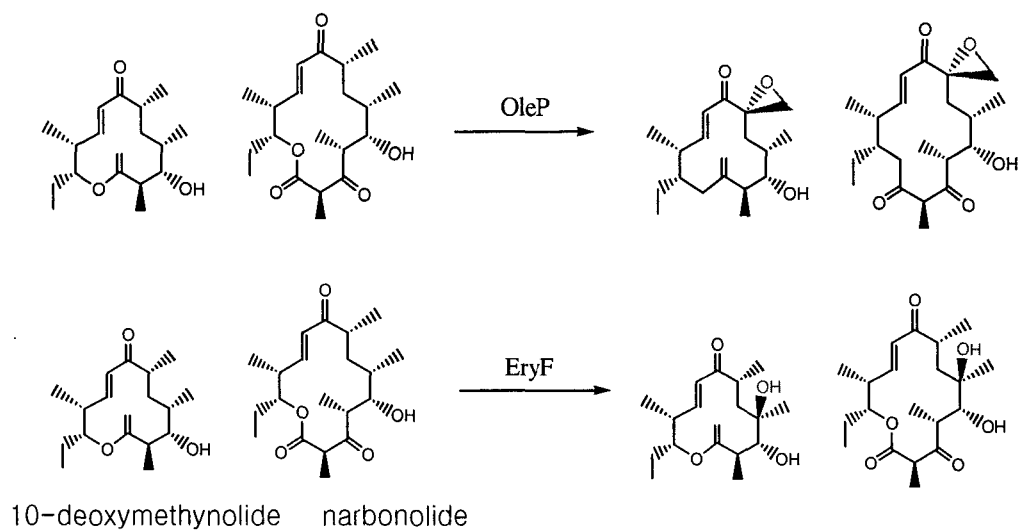
**Fig 1. Biosynthesis pathway of methymycin/pikromycin and structure of pikromycin module**



**Fig 2. Complementation of the *S. venezuelae* *pikA IV* deletion mutant by *tyIGV***



**Fig 3. The structure of oleandomycin and its hydroxylated form by PikC**



**Fig 4.** The expected hydroxylated structures of the aglycones from *S.venezueale* by the function of OleP and EryF