

CHEM-E4109

MODERN METHODS IN **BIOCATALYSIS**

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*chapter #9: muta- & semisynthesis*

29.3.2022

[www.deskalab.com](http://www.deskalab.com)

**Jan Deska**  
**Bioorganic**  
**Chemistry**

# CHALLENGES IN BIOCATALYSIS

excellent tools for the selective preparation of functional  
small building blocks

but

general lack of applicability for late-stage complex  
synthetic targets

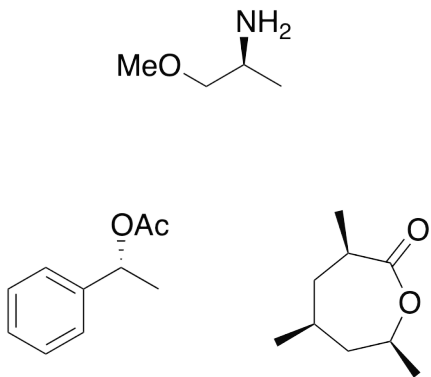
# CHALLENGES IN BIOCATALYSIS

excellent tools for the selective preparation of functional small building blocks

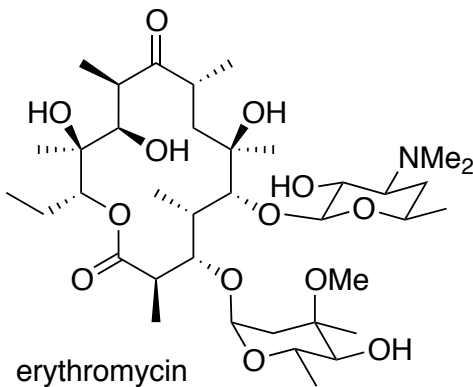
but

general lack of applicability for late-stage complex synthetic targets

typical products in biocatalysis



typical products in biosynthesis



# CHALLENGES IN BIOCATALYSIS

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natural products as excellent scaffolds for the synthesis of pharmaceuticals  
or even good candidates as drug molecules themselves

but

disadvantages isolation:

- natural sources often provide only marginal concentrations of the desired compound
- no diversity, no analogues

disadvantages total synthesis:

- complexity of the target often requires multiple complex chemical steps  
which limits the overall achievable yield

# TODAY'S MENU

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second session on "challenges"

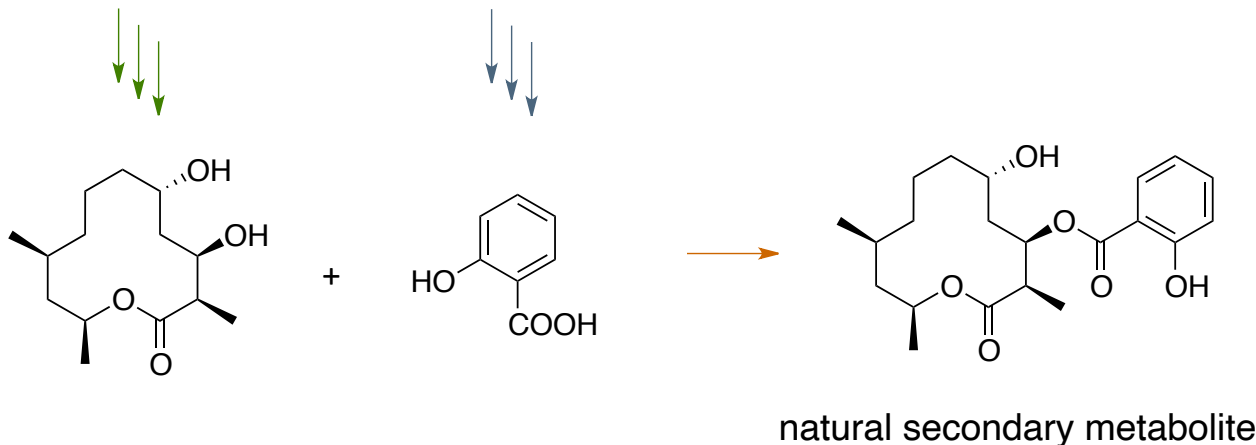
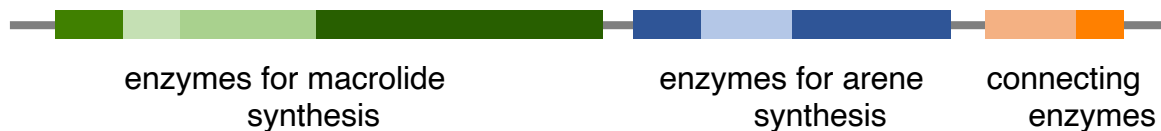
potential solution: exploit biosynthesis for synthetic organic purposes

- mutasynthesis
- semisynthesis
- metabolic engineering

# Manipulated biosynthesis

gene encodes biosynthesis and interconnection of organic building blocks

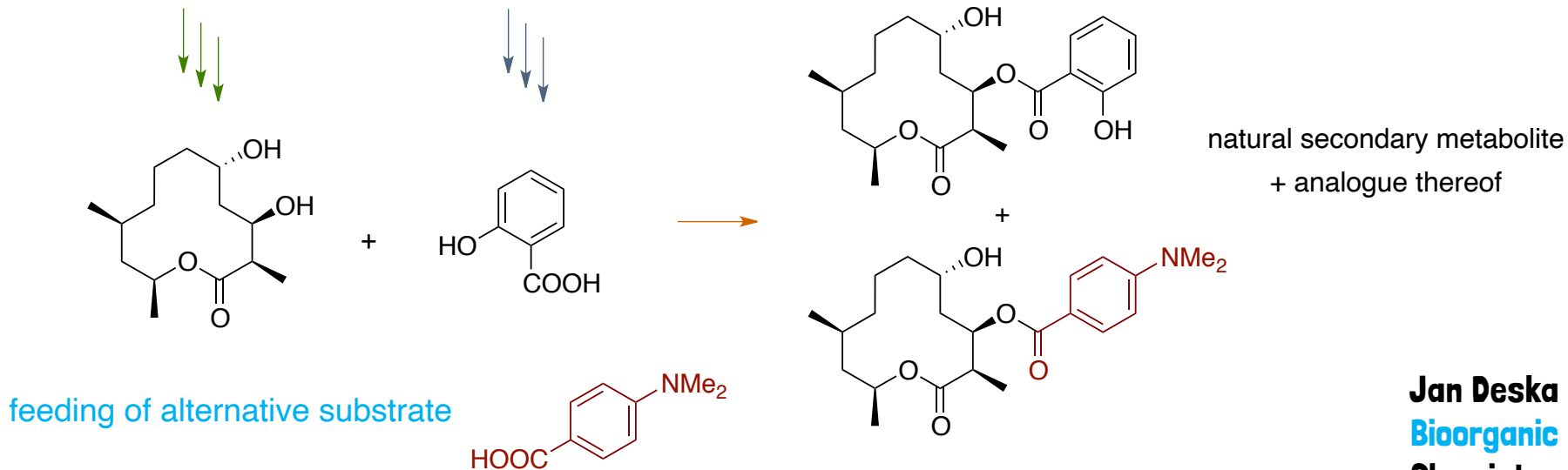
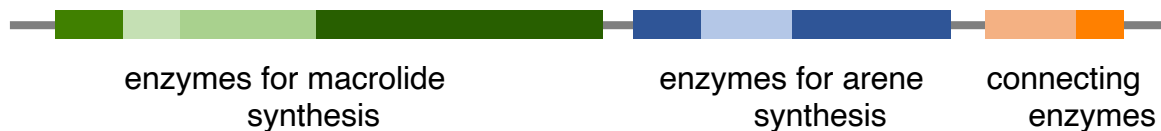
snapshot of gene encoding a polyketide natural product



# Manipulated biosynthesis

precursor-directed biosynthesis

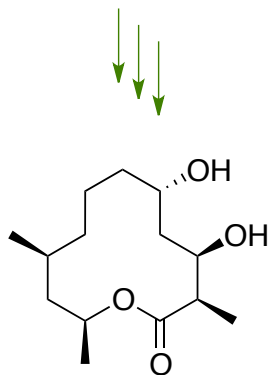
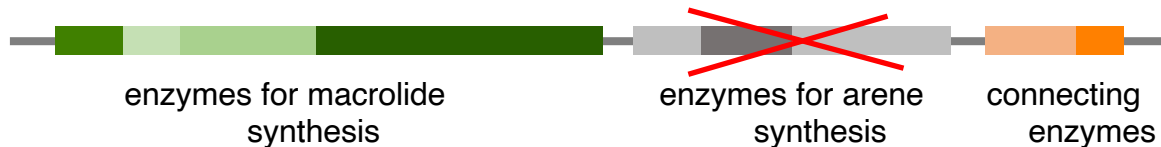
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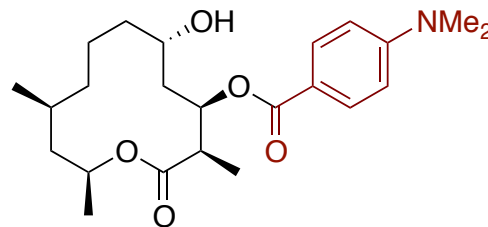
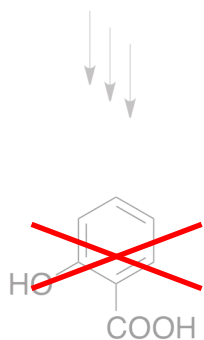
# Manipulated biosynthesis

mutasynthesis

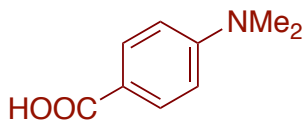
blocked mutant: snapshot of gene encoding a polyketide natural product



+



feeding of alternative substrate



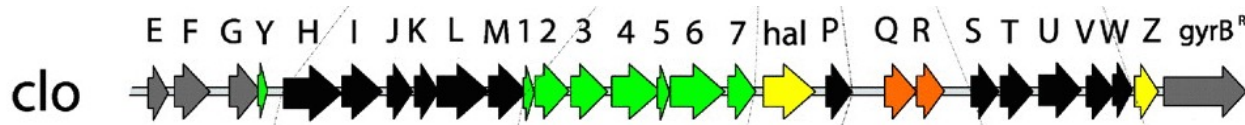
selective biosynthesis of natural product analogue



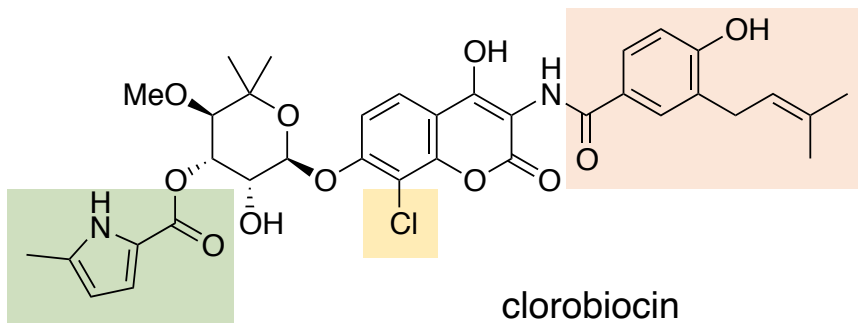
# Mutasyntesis

aminocoumarin antibiotics

- *clo* gene encodes for clorobiocin biosynthesis in *Streptomyces roseochromogenes*



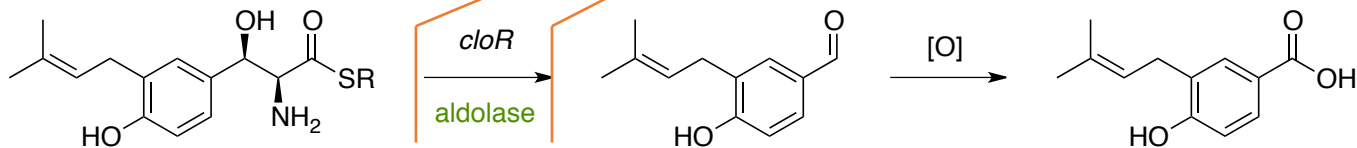
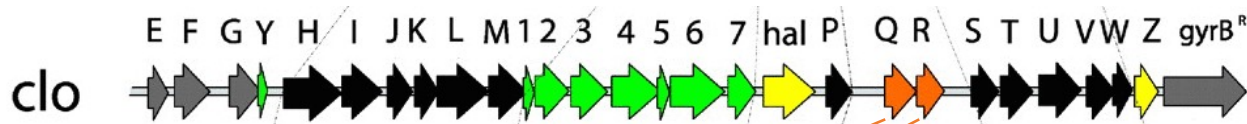
- novobiocin and clorobiocin potent against methicillin-resistant *Staphylococcus* strains



# Mutagenesis

aminocoumarin antibiotics

- *clo* gene encodes for clorobiocin biosynthesis in *Streptomyces roseochromogenes*

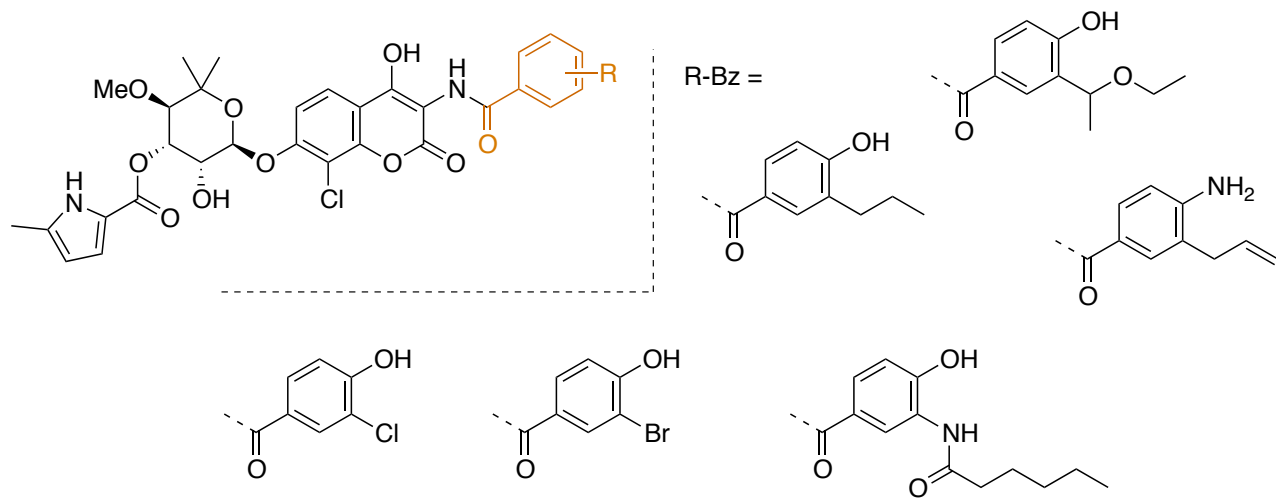


*cloR* responsible for prenylhydroxybenzoate synthesis

# Mutasyntesis

aminocoumarin antibiotics

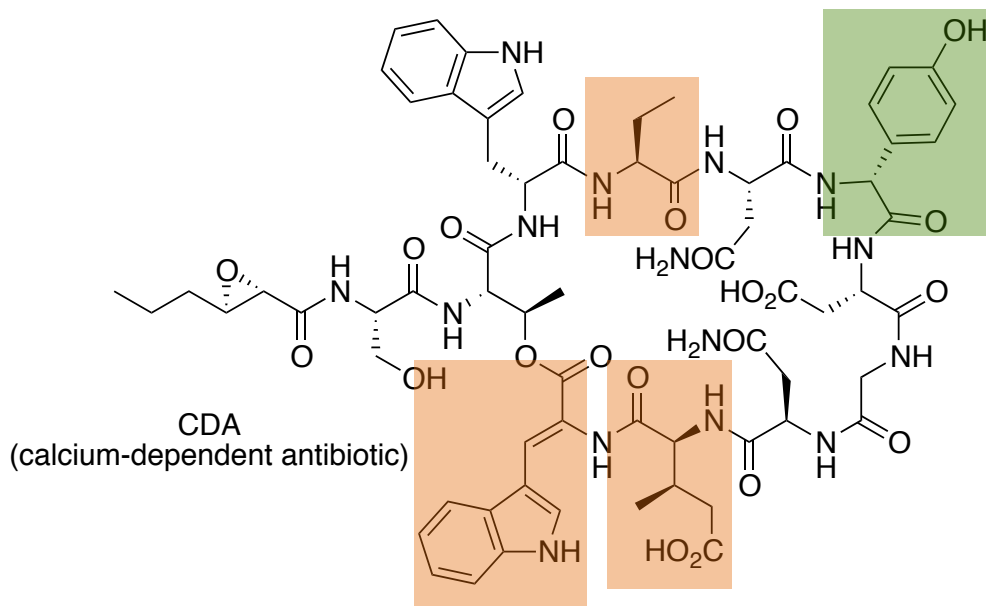
- *S. roseochromogenes*  $\Delta cloR$  blocked mutant unable to produce clorobiocin
- in presence of hydroxybenzoates, *S. roseochromogenes*  $\Delta cloR$  strain restores production including biosynthesis of natural product analogues



# Mutasyntesis

non-ribosomal peptides

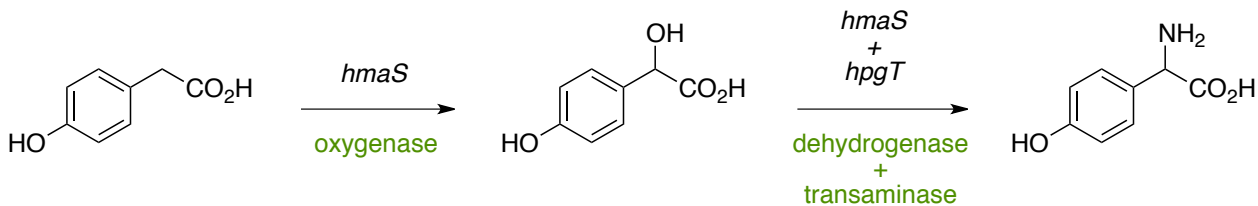
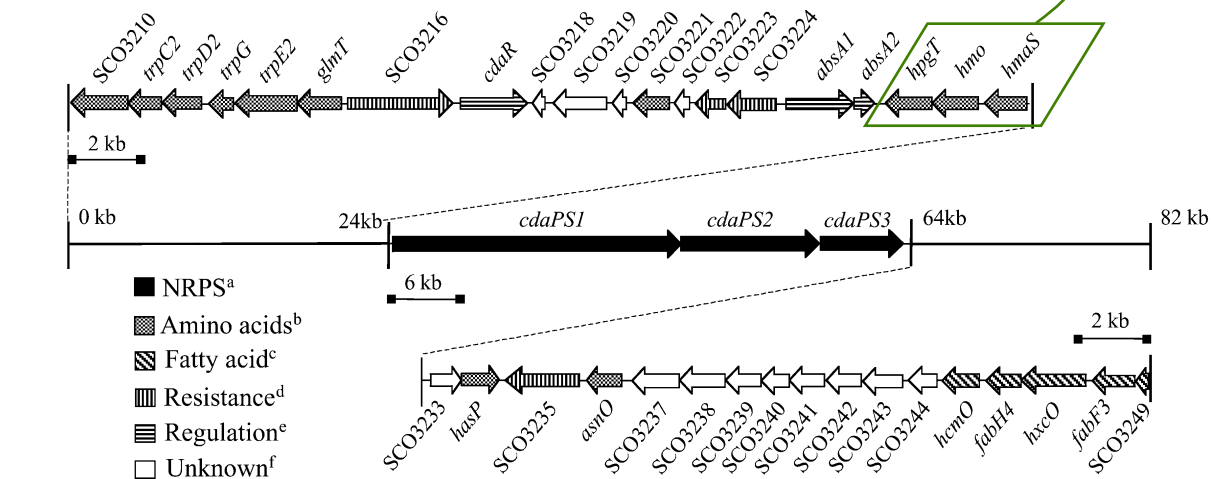
- complex cyclic peptides featuring numerous non-natural amino acids
- requires non-ribosomal peptide synthetase + amino acid production genes



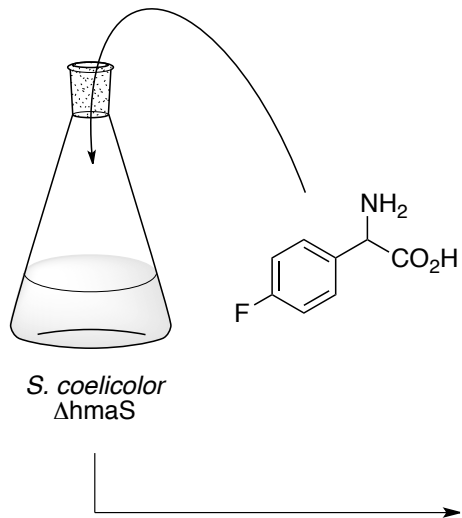
# Mutasythesis

3 enzymes produce 4-hydroxyphenylglycine

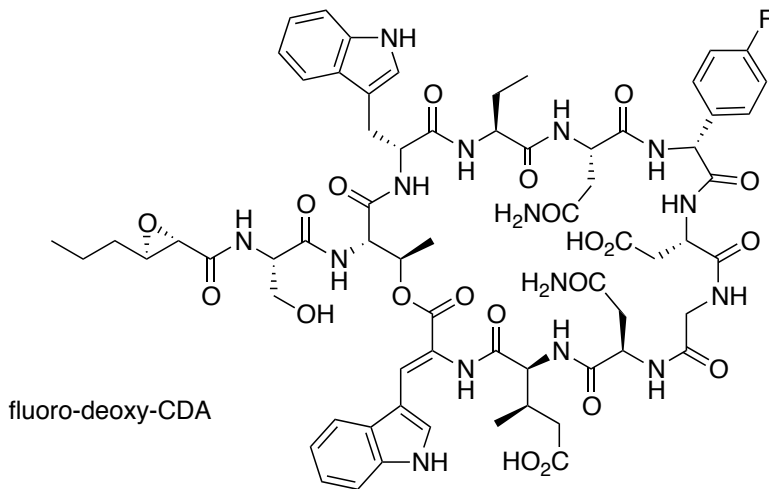
non-ribosomal peptides



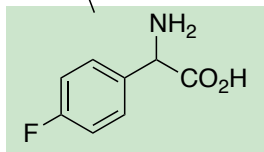
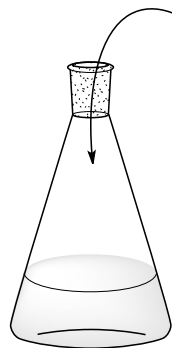
# Mutasythesis



knock-out mutant produces fluoro-CDA



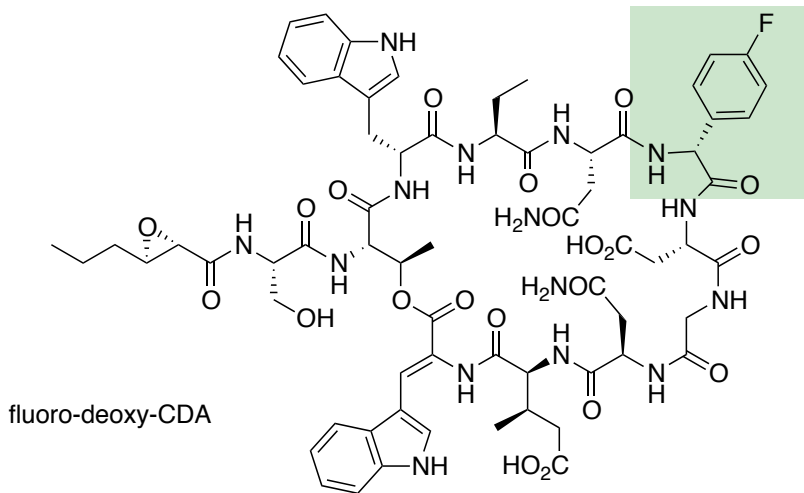
# Mutagenesis



*S. coelicolor*  
 $\Delta$ hmsA



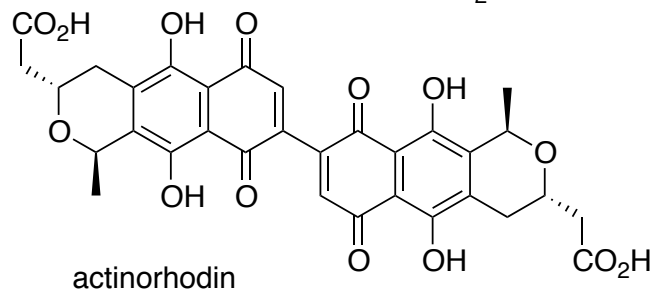
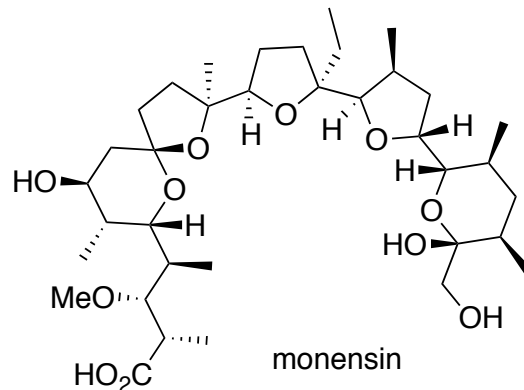
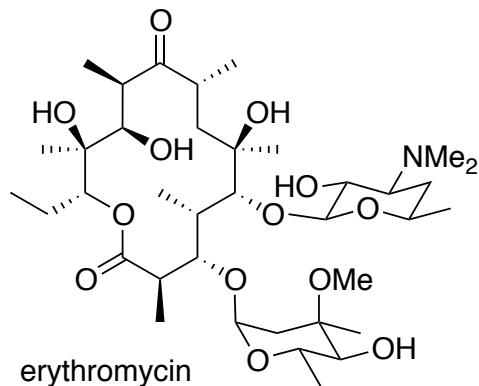
knock-out mutant produces fluoro-CDA



# Mutasyntesis

polyketides

- vastly complex family of natural products
- structural features can include both highly functionalized aliphatics as well as aromatics



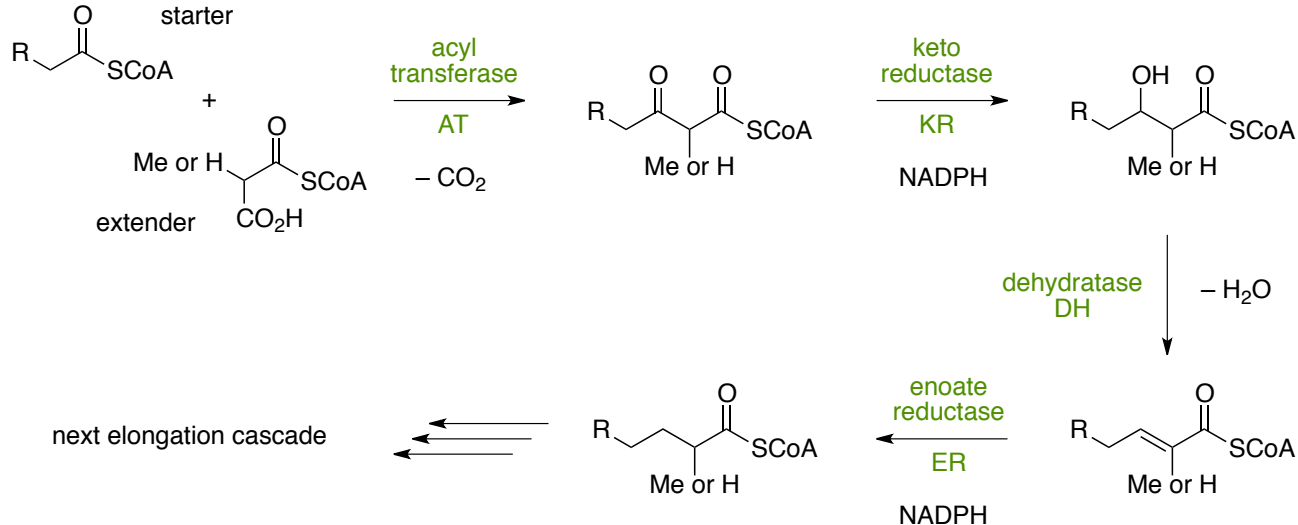


# Mutasyntesis

polyketides

- biosynthesis for all different polyketides based on one very simple principle
- ✓ Claisen condensation + follow-up chemistry,
- ✓ all located in Polyketide Synthase multienzyme complex (PKS)

fatty acid synthase

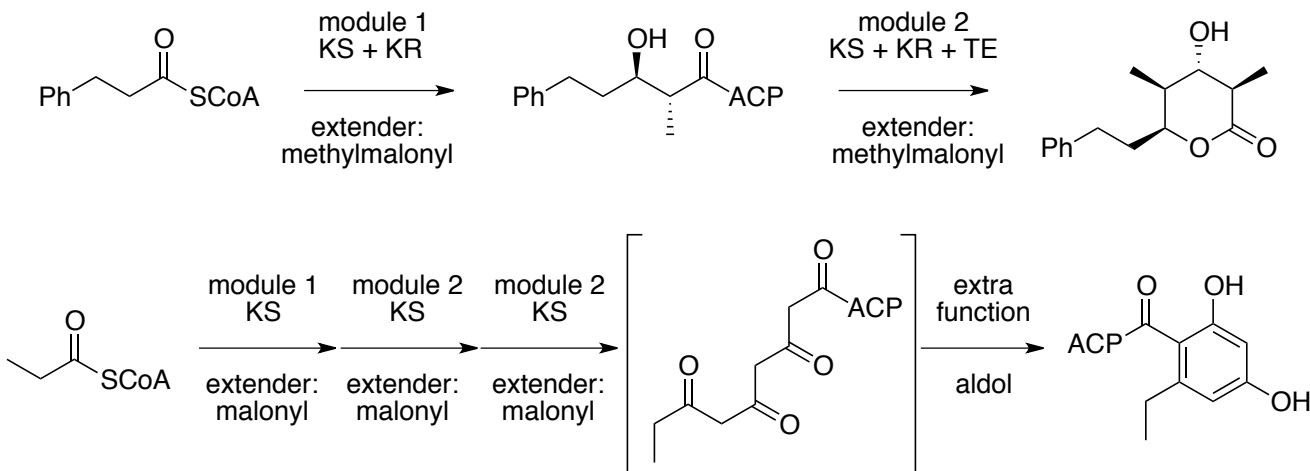


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polyketide synthase: various modules with optional KR/DH/ER activities

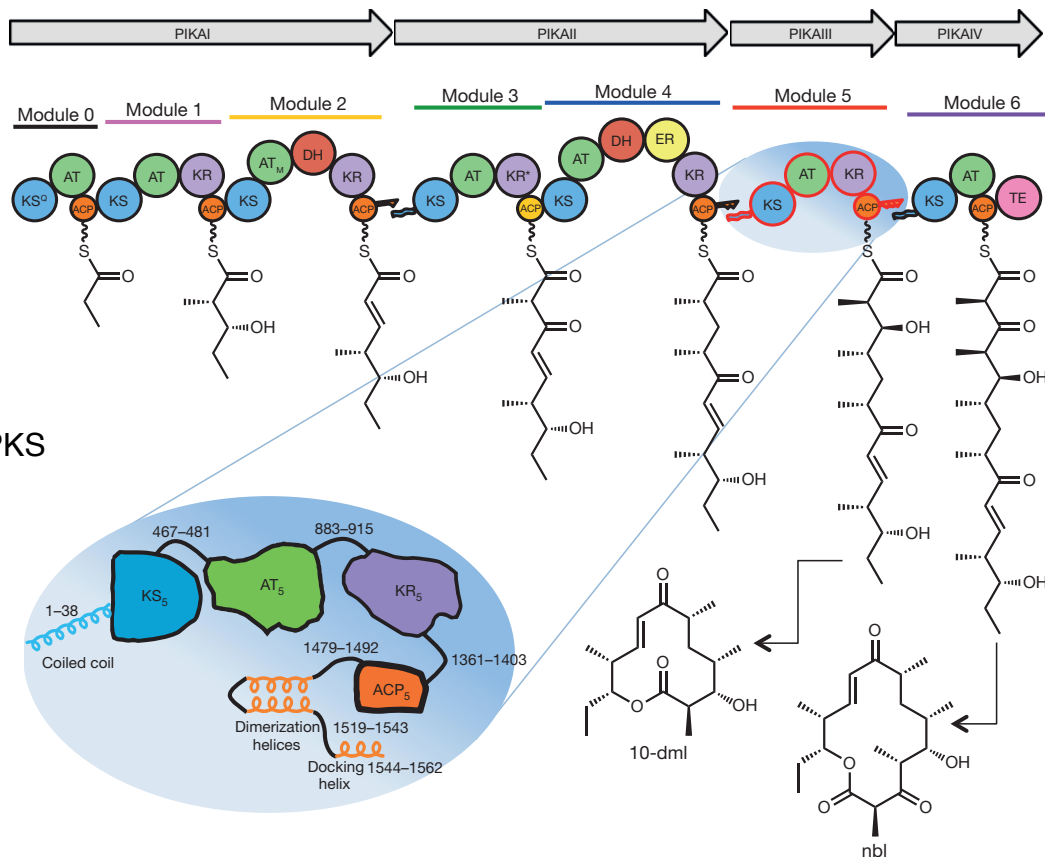


KS = ketosynthase

TE = thioesterase

# Mutasyntesis

polyketides



# Mutasynthesis

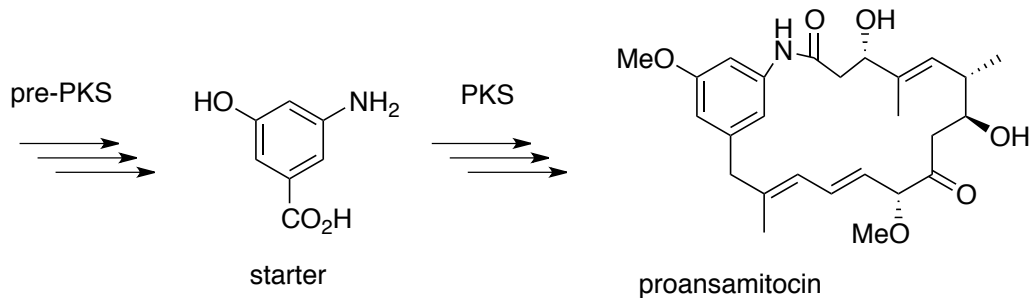
polyketide synthase: various modules with optional KR/DH/ER activities

points of interference for polyketide mutasynthesis

- pre-PKS (early stage mutasynthesis):
  - ✓ modified starter units (precursor directed or via knock-down)
- PKS mutasynthesis:
  - ✓ knock-down or manipulation of single units within modules
  - ✓ control of degree of saturation, methylation or chiral centres
- post-PKS modifications:
  - ✓ many functionalizations (halogenations, methylations, etc) occur after the actual PKS
  - ✓ control over degree of functionalization

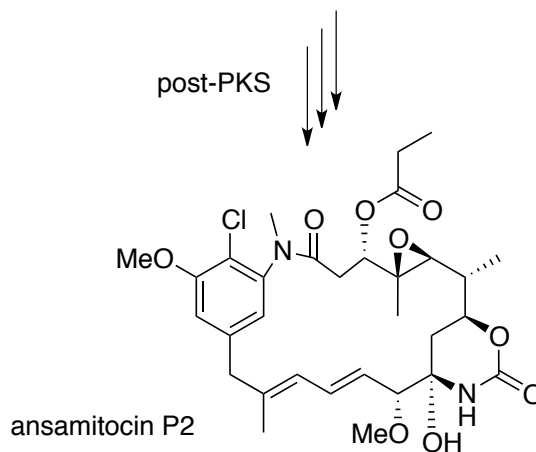
# Mutasyntesis

## ansamitocin mutasyntesis



### required steps:

- aminohydroxybenzoate synthesis
  - PKS (7 extensions)
  - post PKS: 1) chlorination
- 2) O-methylation
  - 3) carbamylation
  - 4) O-methylation
  - 5) epoxidation
  - 6) N-methylation

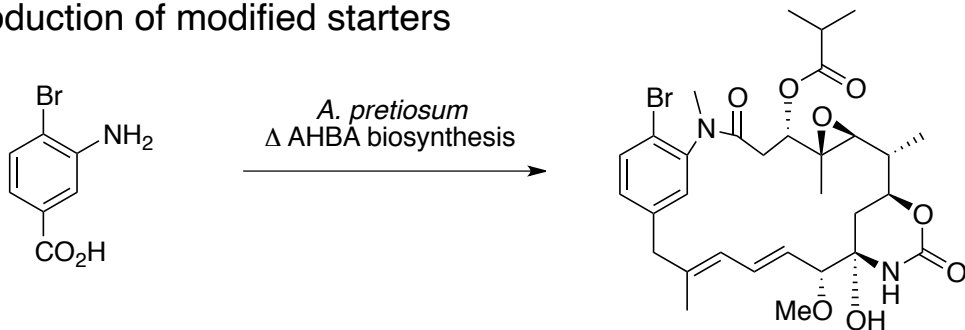


# Mutasyntesis

ansamitocin mutasyntesis

*Actinosynnema pretiosum* AHBA blocked mutant

✓ introduction of modified starters

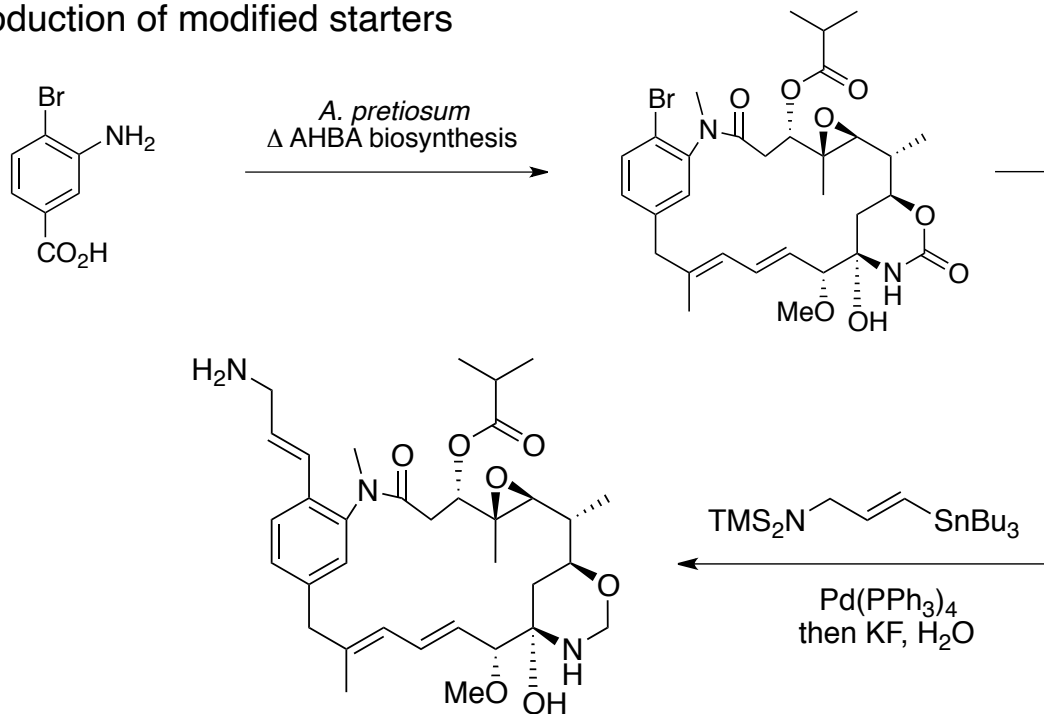


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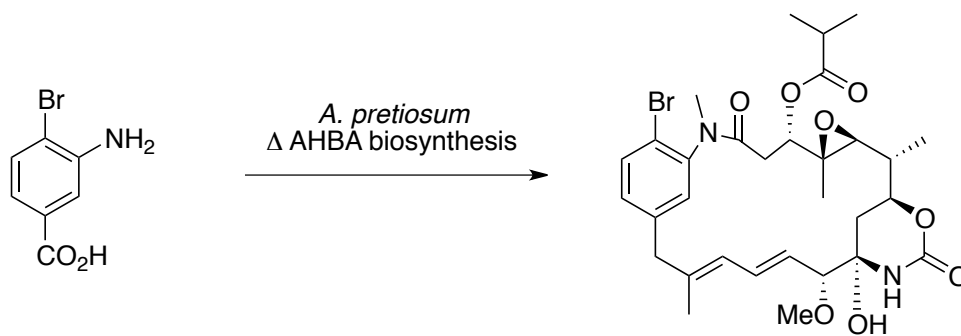
ansamitocin mutasyntesis

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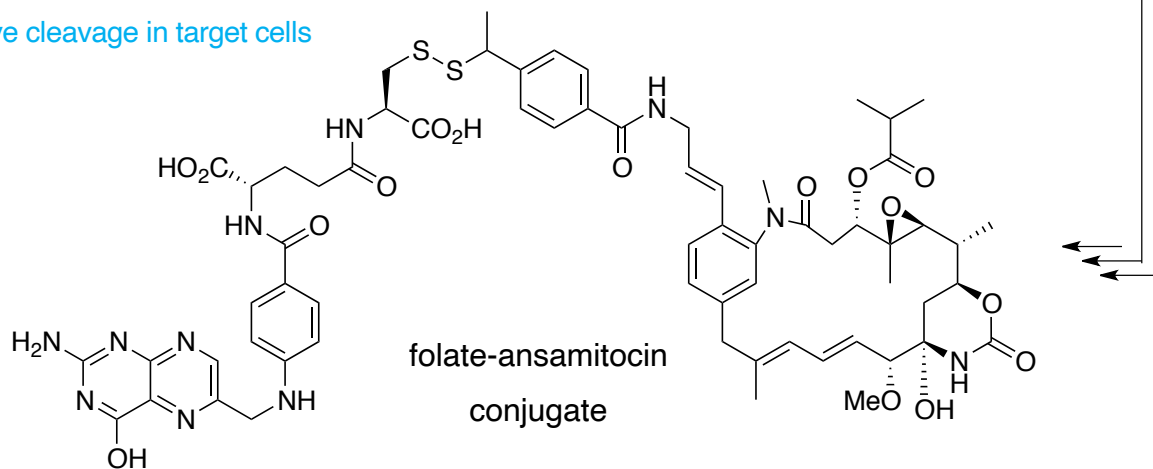
✓ introduction of modified starters



# Mutasyntesis



selective cleavage in target cells



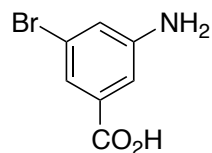
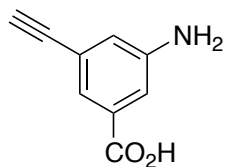
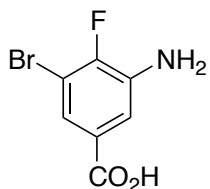
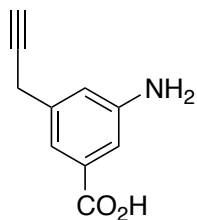
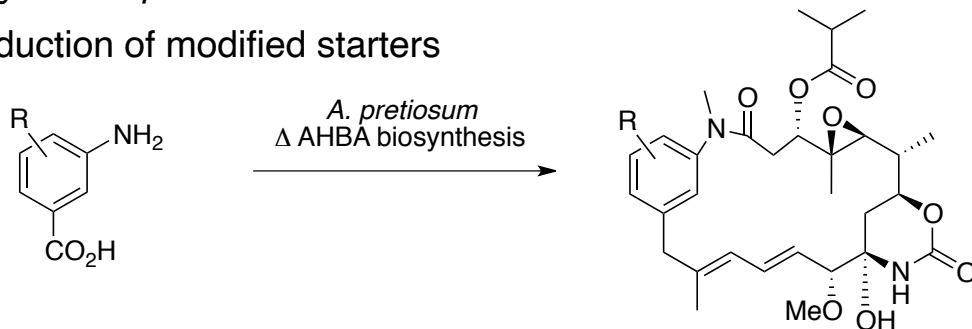


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ansamitocin mutasyntesis

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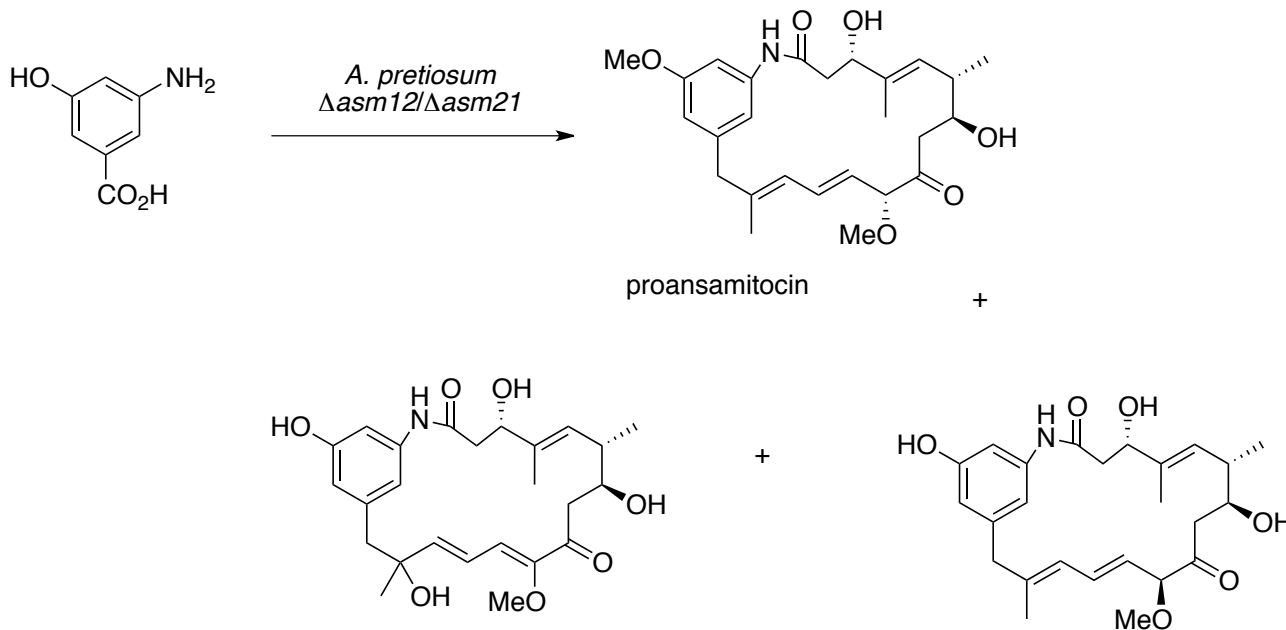
# Mutasyntesis

ansamitocin mutasyntesis

*Actinosynnema pretiosum* *asm12/asm21* blocked mutant

✓ *asm12* encodes chlorination, *asm21* encodes carbamoylation

✓ interruption of the post-PKS functionalization



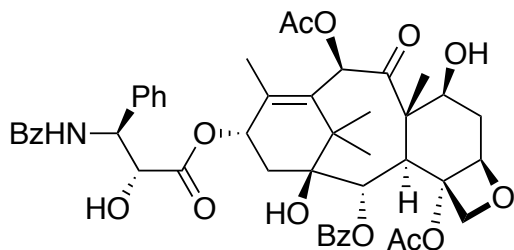


# Semisynthesis

- sometimes, compounds are too complex to be synthesized but too scarce to be isolated from natural sources.
- Therefore, biosynthetic intermediates are isolated and used as starting material for "fine-tuning" through organic synthesis
- ✓ e.g. synthetic penicillins, paclitaxel,...

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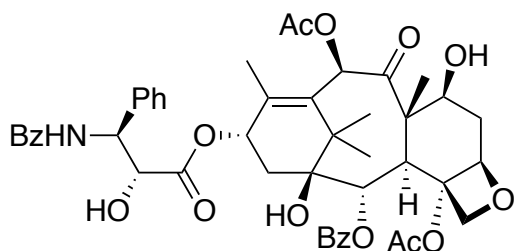
paclitaxel (Taxol)

highly potent tubulin binder

very successful anti-cancer drug

# Semisynthesis

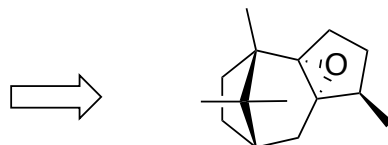
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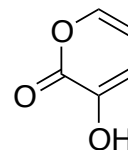
highly potent tubulin binder

very successful anti-cancer drug



Holton (1994)

48 steps from patchoulene  
oxide



Nicolaou (1994)

46 steps from very basic  
compounds

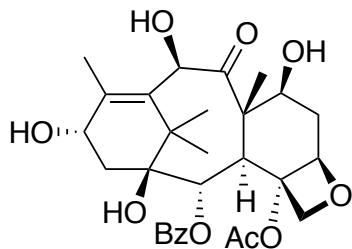
Holton *et al.*, *J. Am. Chem. Soc.* **1994**, *116*, 1599.

Nicolaou *et al.*, *Nature* **1994**, *367*, 630.

# Semisynthesis

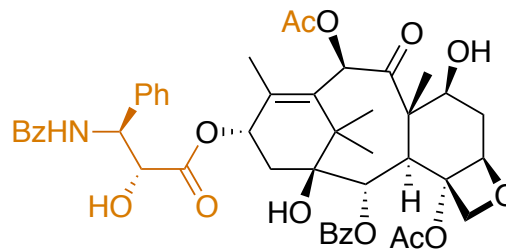
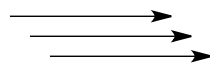
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semisynthetic process by Bristol-Myers Squibb



10-deacylbaccatin III

found in reasonable amounts in  
the bark of the European yew

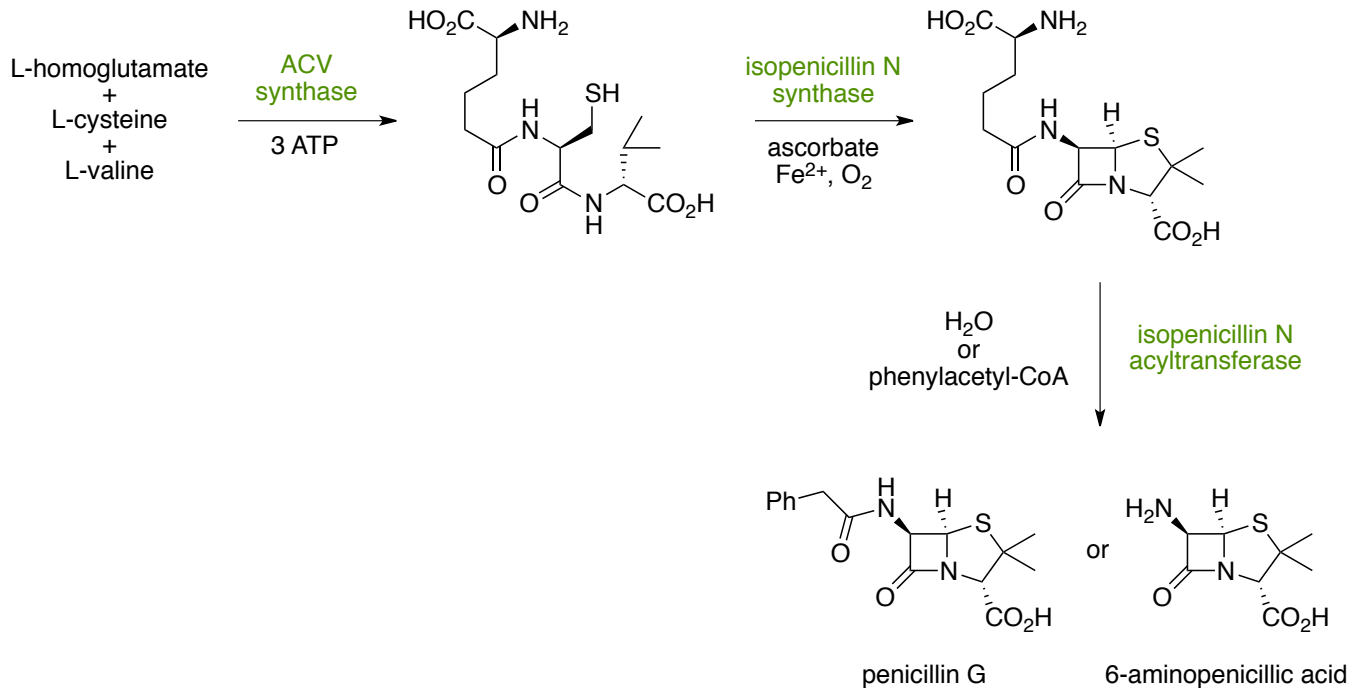


paclitaxel (Taxol)

found in traces in the bark  
of the Pacific yew

# Semisynthesis

industrial production of cillin-type antibiotics

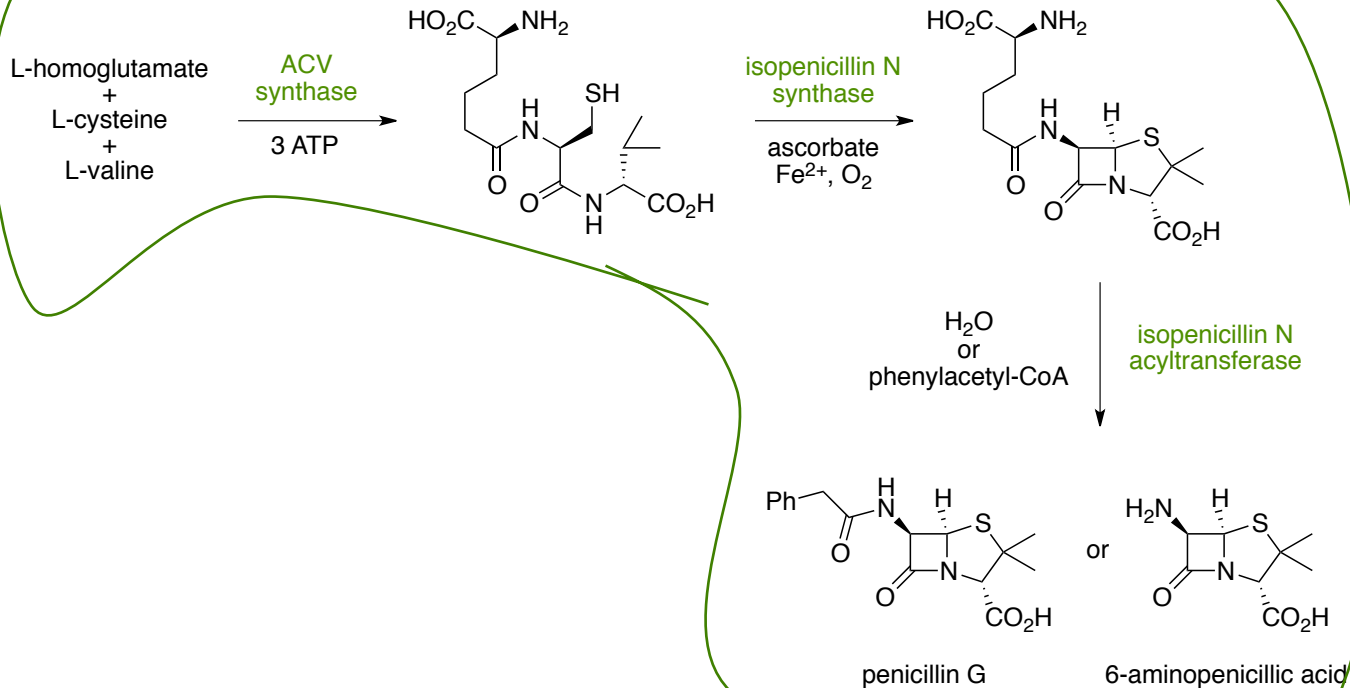




# Semisynthesis

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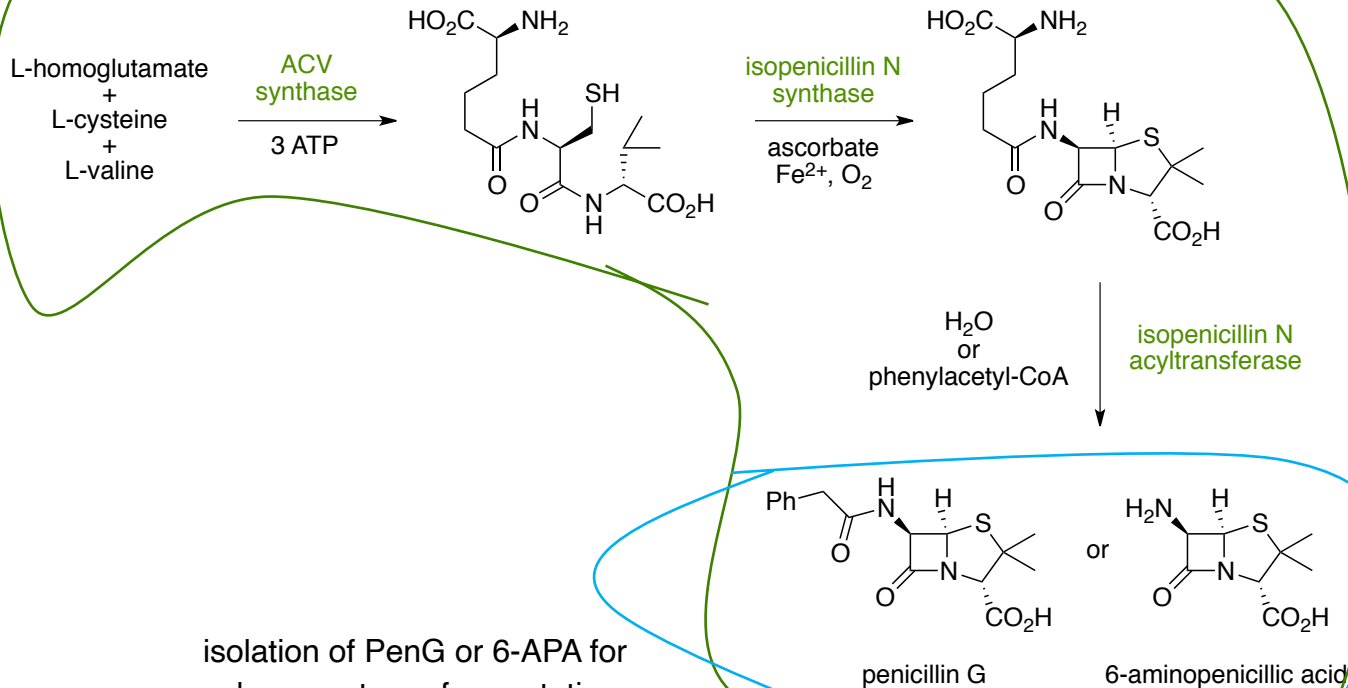
*Penicillium sp.*



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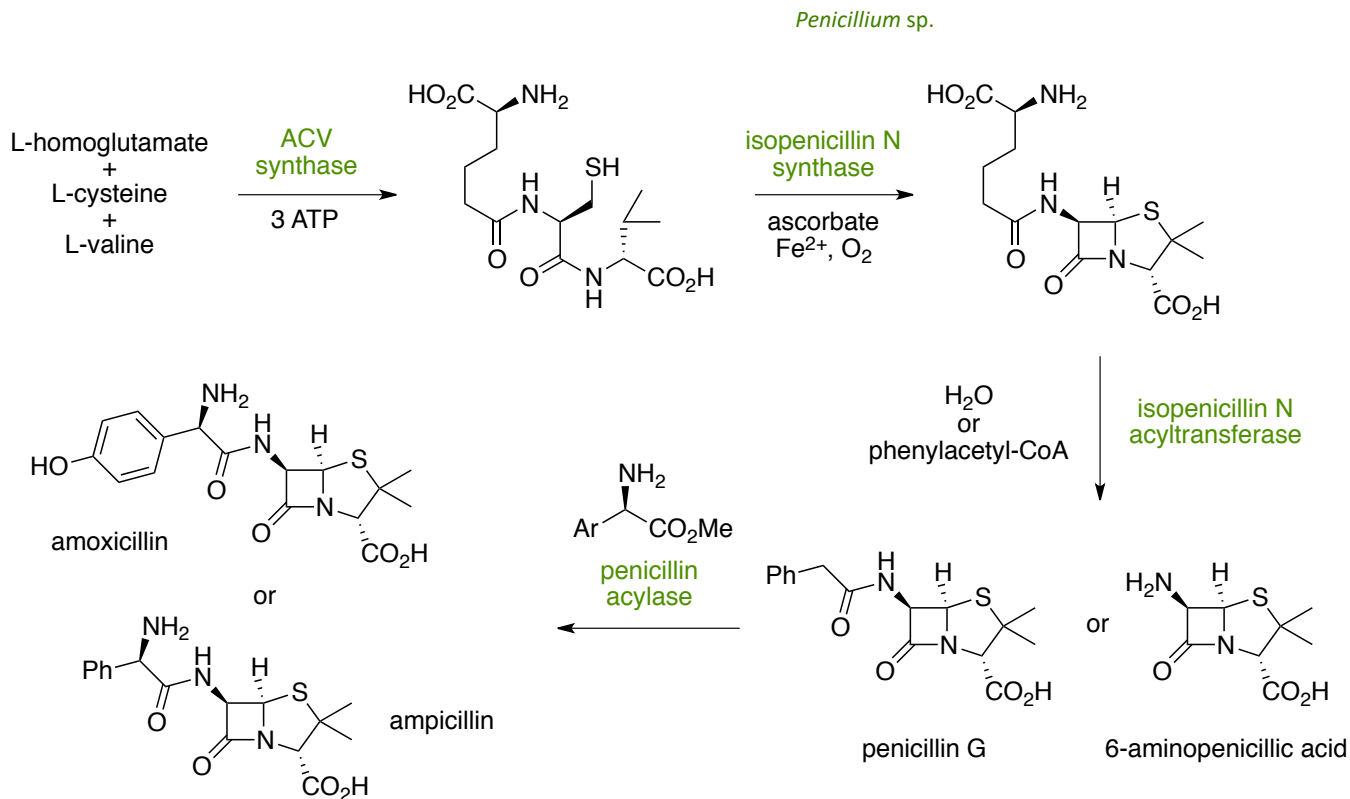
*Penicillium sp.*



isolation of PenG or 6-APA for subsequent non-fermentative synthesis

# Semisynthesis

industrial production of cillin-type antibiotics



# Metabolic engineering

modulation of pathways in microbial organisms

- up- or downregulation of endogenous processes
  - creation of transgenic organisms carrying alien DNA
- 
- ✓ fine-tuning of the bugs to provide desired products in higher yield
  - ✓ or, deviate from the natural products to something the bug wouldn't want to produce otherwise

# Metabolic engineering

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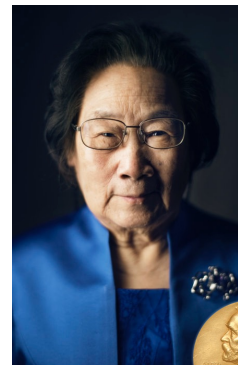
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poster child example: production of anti-malarial artemisinin

- original producer: *Artemisia annua* (sweet wormwood)
- artemisinin represents the benchmark medication against malaria
- limited natural supply demands alternative producers

- ✓ semisynthetic approach: via artemisinic acid in *S. cerevisiae*



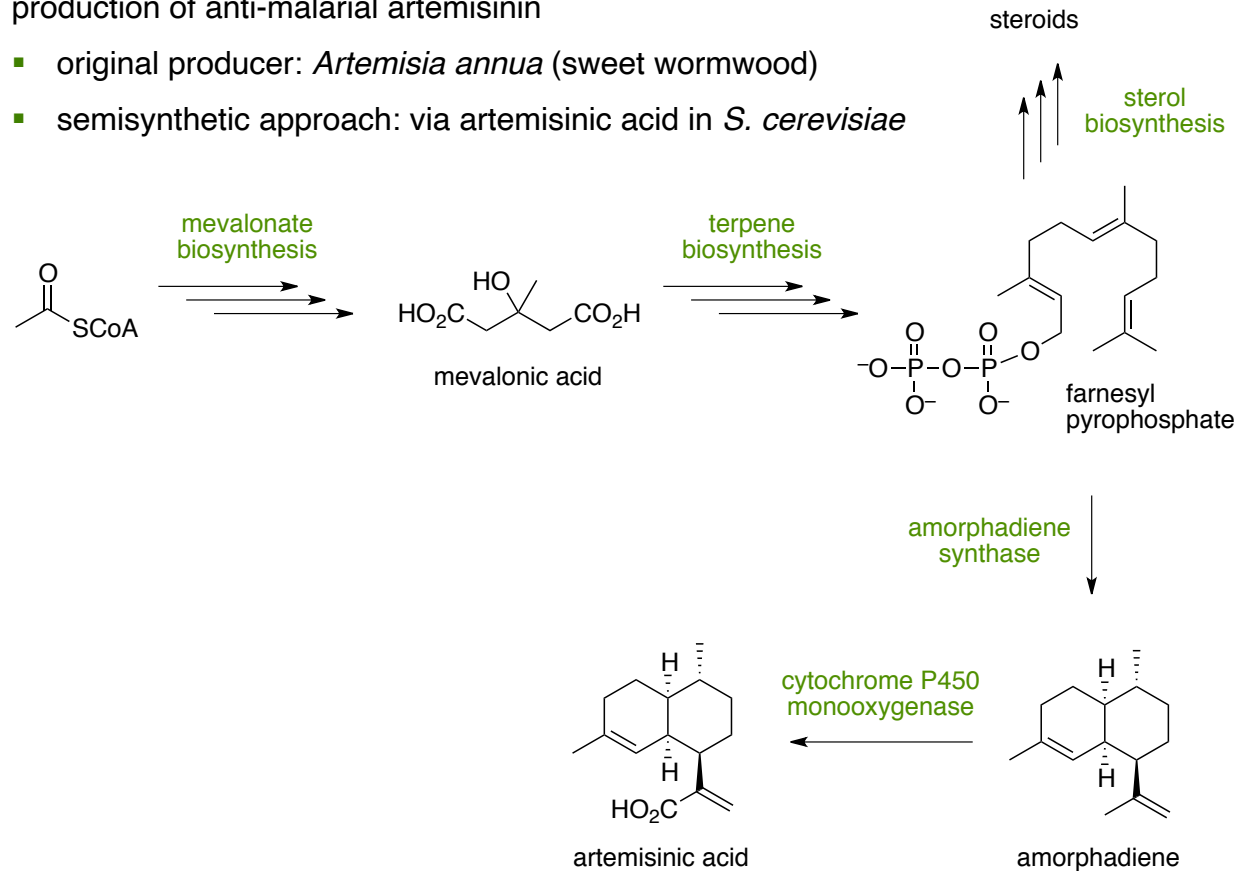
Youyou Tu

Nobel Prize in  
Medicine, 2015

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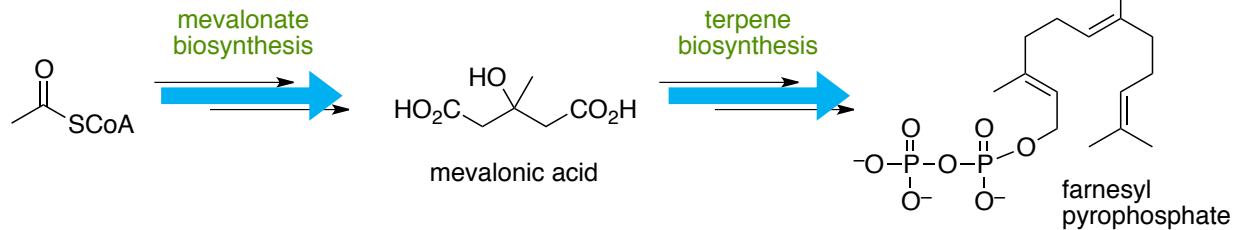


Ro, Paradise, Ouellet, Fisher, Newman, Ndungu, Ho, Eachus, Ham, Kirby, Chang, Withers, Shiba, Sarpong, Keasling, *Nature* **2006**, 440, 940-943.

# Metabolic engineering

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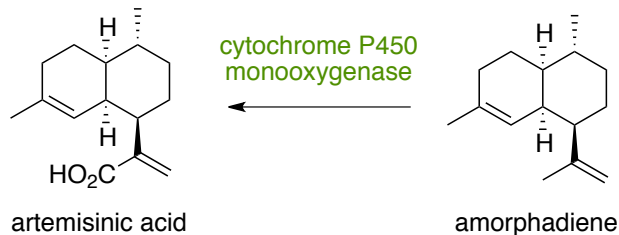
steroids

sterol biosynthesis

metabolic engineering requires:

- 1) upregulation of farnesyl synthesis

amorphaadiene synthase

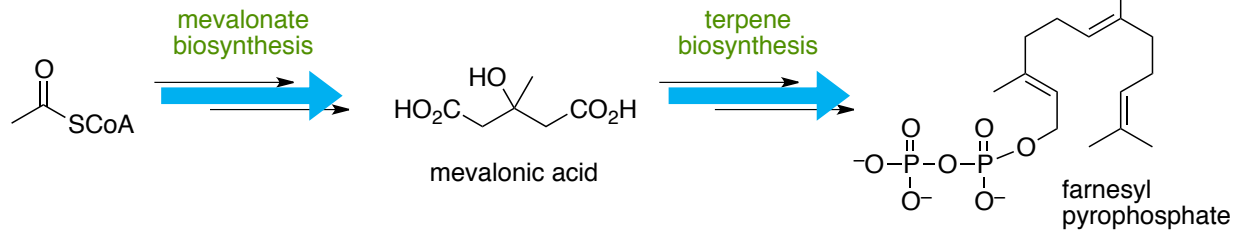


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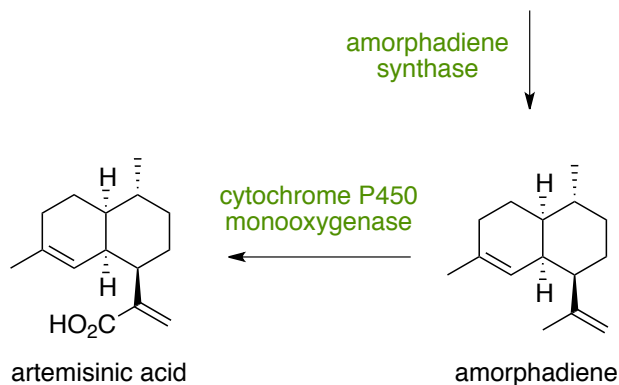
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2. downregulation of sterol synthesis



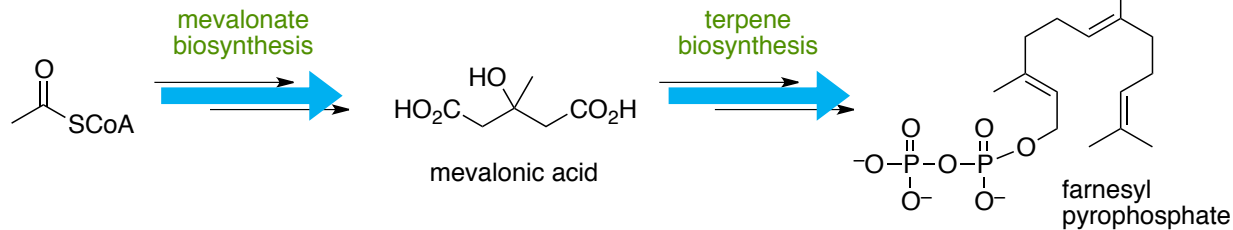
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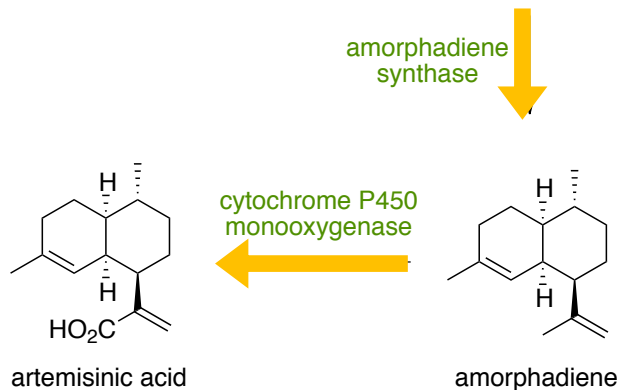
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metabolic engineering requires:

1. upregulation of farnesyl synthesis
2. downregulation of sterol synthesis
3. expression of synthase
4. expression of P450

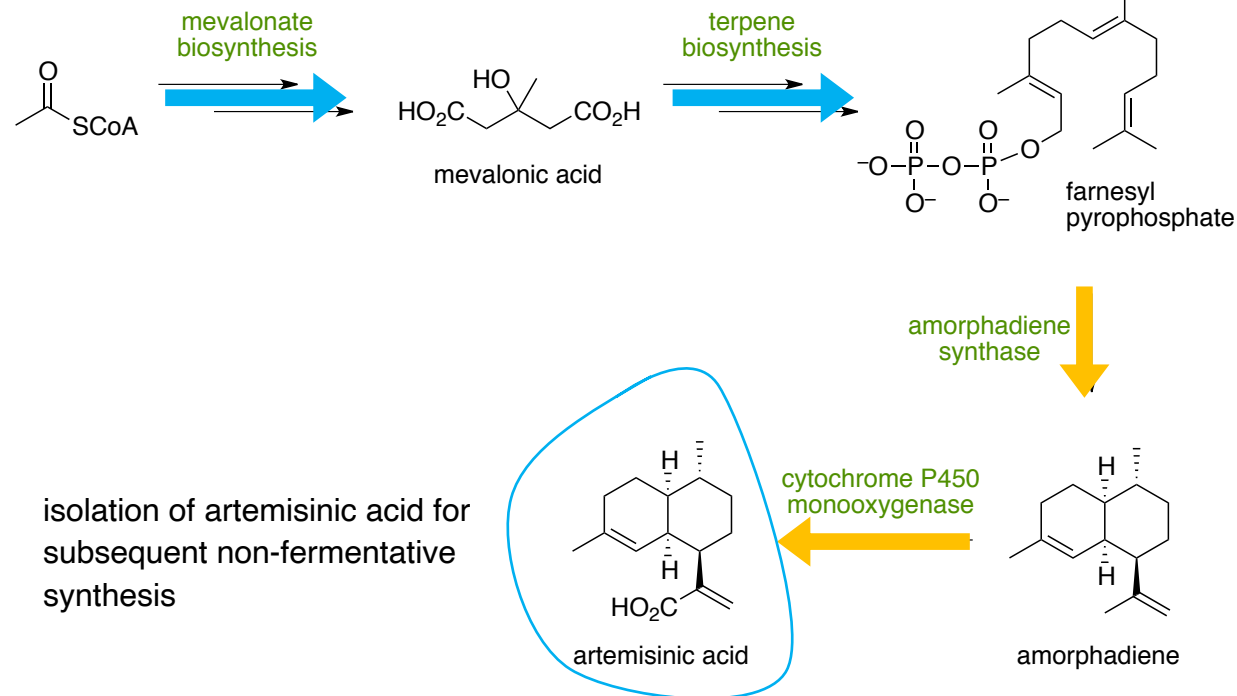


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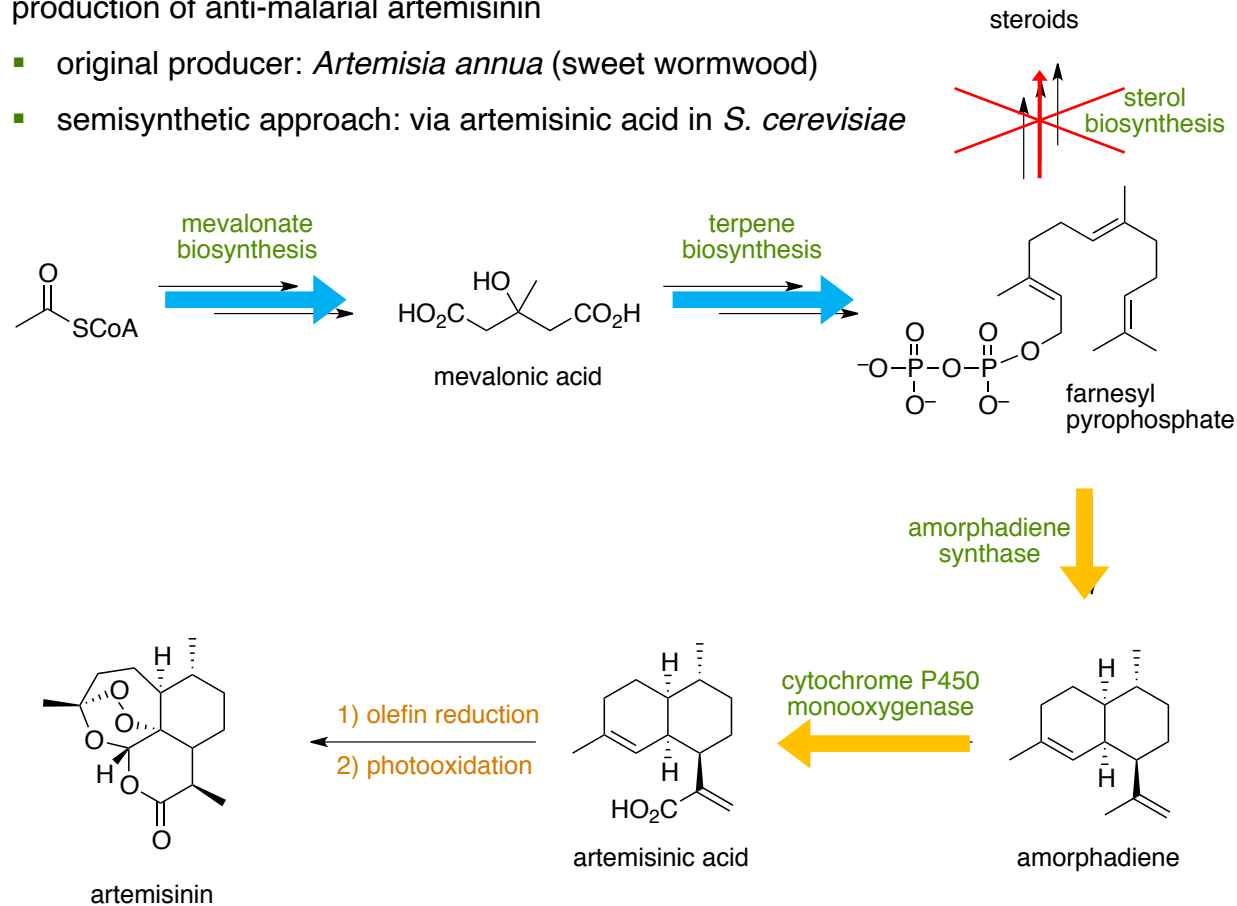
Ro, Paradise, Ouellet, Fisher, Newman, Ndungu, Ho, Eachus, Ham, Kirby, Chang, Withers, Shiba, Sarpong, Keasling, *Nature* **2006**, 440, 940-943.

isolation of artemisinic acid for subsequent non-fermentative synthesis

# Metabolic engineering

production of anti-malarial artemisinin

- original producer: *Artemisia annua* (sweet wormwood)
- semisynthetic approach: via artemisinic acid in *S. cerevisiae*



Ro, Paradise, Ouellet, Fisher, Newman, Ndungu, Ho, Eachus, Ham, Kirby, Chang, Withers, Shiba, Sarpong, Keasling, *Nature* **2006**, 440, 940-943.

# ~~That's nice, right? But how far can you go?~~

Synthetic Biology & Metabolic Engineering can...

- ✓ effectively regulate pathways that are intrinsic in life
- ✓ access structures that already found somewhere in nature

Synthetic Biology & Metabolic Engineering fails to...

- provide solutions for truly synthetic targets
- offer bio-based solutions for many traditional chemistries
- engage in anything that lacks precedence in biosynthesis