Rutas bio-sintéticas de antibióticos y toxinas

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Antibiotic Production

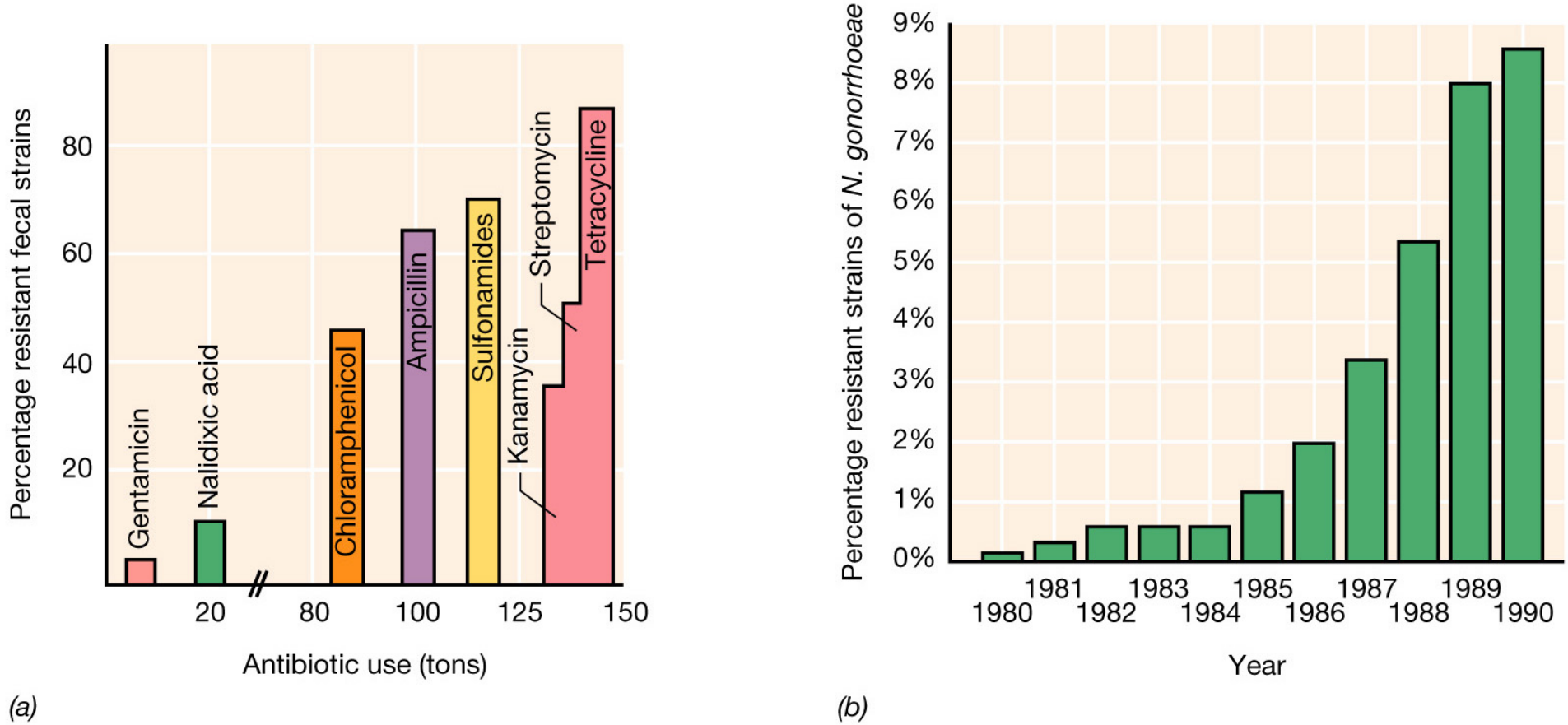
- There are approx. 8000 known antibiotics
- More than 100 are produced commercially by microbial fermentation
- 70% of these are produced by *Streptomyces* spp.
- Strain selection and development has resulted in very high product yields (e.g., 1100 g/L Penicillin)

Common antibiotics and their sources

Bacitracin	*Bacillus subtilis*
Cephalosporin(s)	*Cephalosporium* sp.
Chloramphenicol	*S. venezuelae*
Cycloheximide	*S. griseus*
Hygromycin	*S. hygromyces*
Penicillin	*P. chrysogenum*
Streptomycin	*S. griseus*
Tetracycline(s)	*S. aurofaciens*
Vancomycin	*S. orientalis*

Industrial evolution of penicillin production

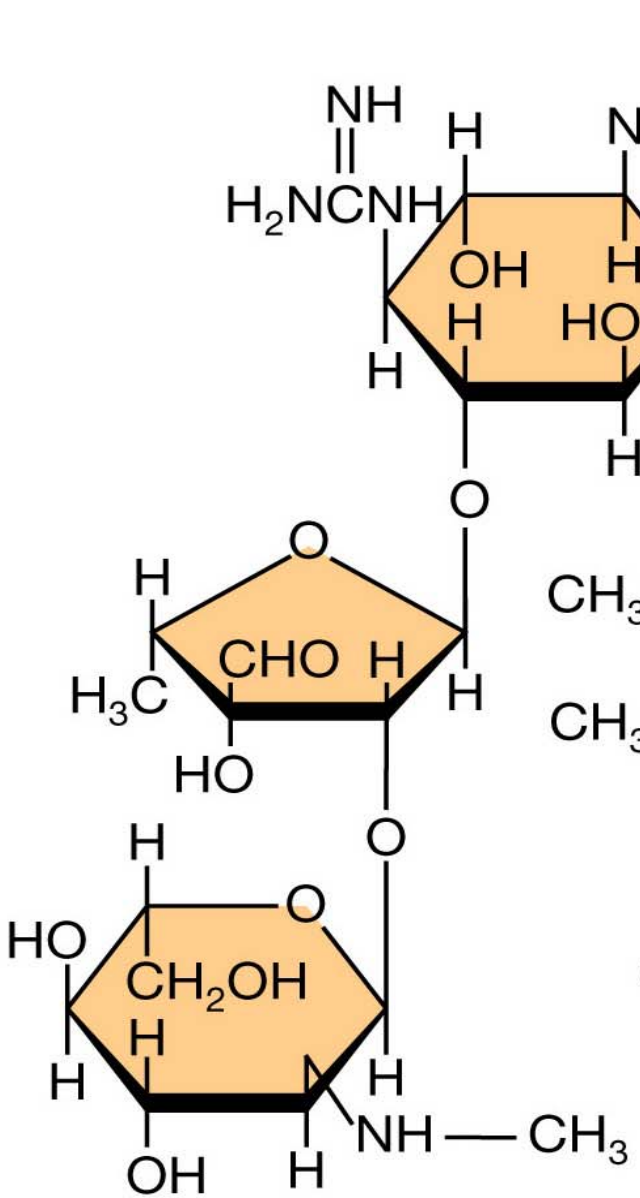
Date	Yield (units/mL)	Development
1929	2-20	Wild-type (P. notatum)
1941	40-80	Better WT
1943	80-100	New WT (P. chrysogenum)
1944	100-200	Colony selection
1944	300-500	X-irradiation
1945	800-1000	UV-irradiation
1949	1500-2000	Chemical mutagenesis
1951	2400	Chemical mutagenesis
1953	2700	Strain selection
1960	5000	Strain selection
1970	10000	Strain selection



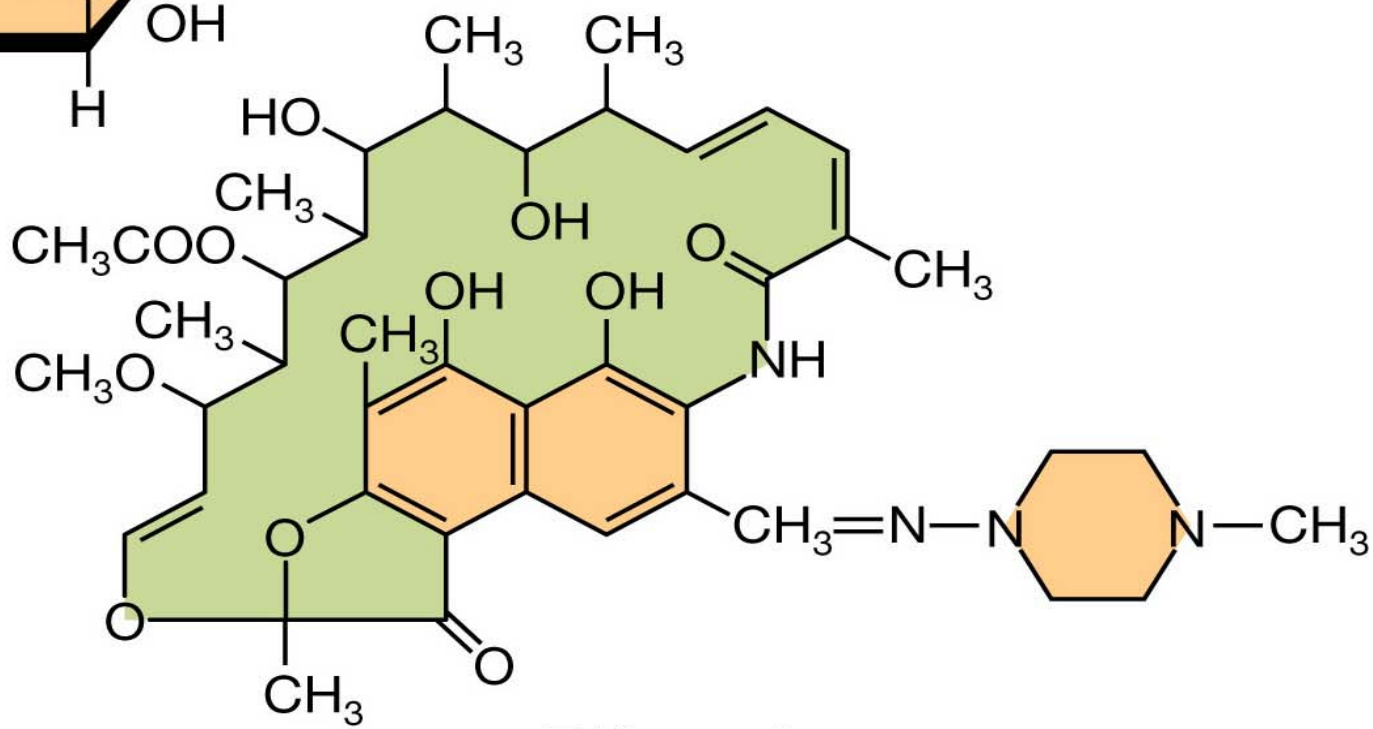
The emergence of antimicrobial drug-resistant bacteria. (a) Relationship between antibiotic use and the percentage of bacteria isolated from diarrheal patients resistant to the antibiotic. Those agents that have been used in the largest amounts, as indicated by the amount produced commercially, are those for which drug-resistant strains are most frequent. (b) Percentage of reported cases of gonorrhea caused by drug-resistant strains. The actual number of reported drug-resistant cases in 1985 was 9000. This number rose to 59,000 in 1990. Greater than 95% of the reported drug-resistant cases are due to penicillinase-producing strains of *Neisseria gonorrhoeae*. Since 1990, penicillin has not been recommended for treatment of gonorrhea because of emerging drug resistance. (Source: Centers for Disease Control, Atlanta, GA).

Representative structure

Classification of antibacterial chemotherapeutic agents according to chemical structure. A representative example is shown for each group.

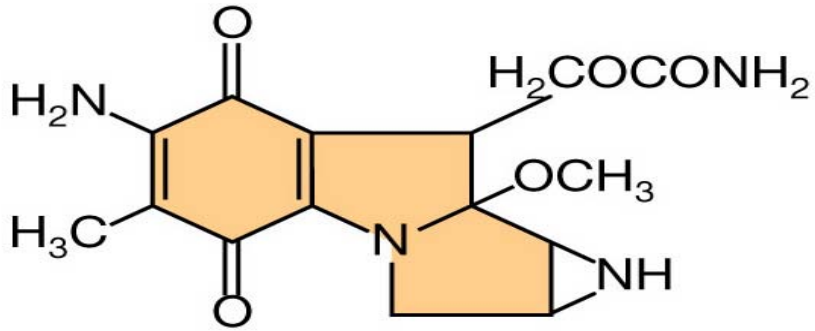


Streptomycin

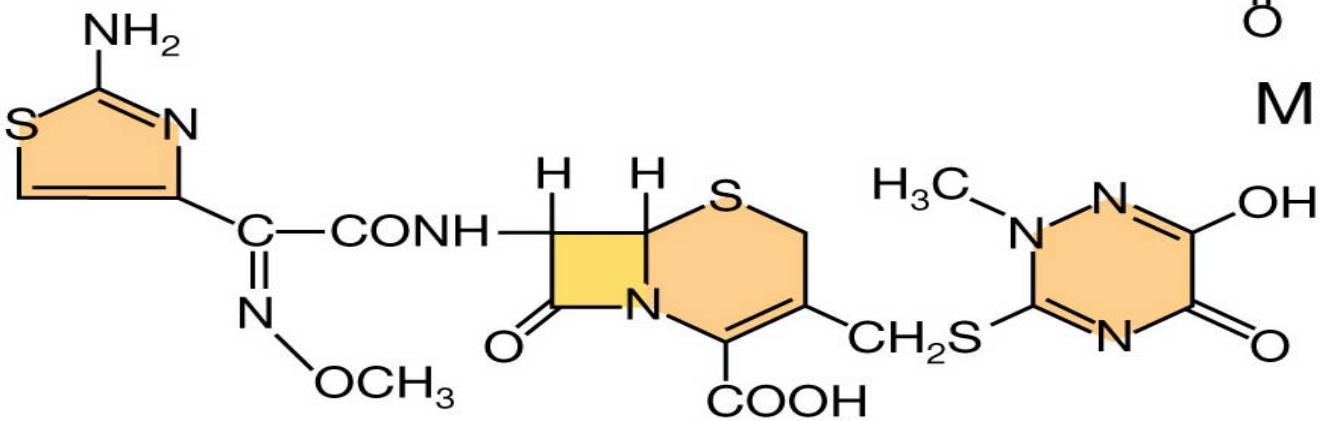


Rifampin

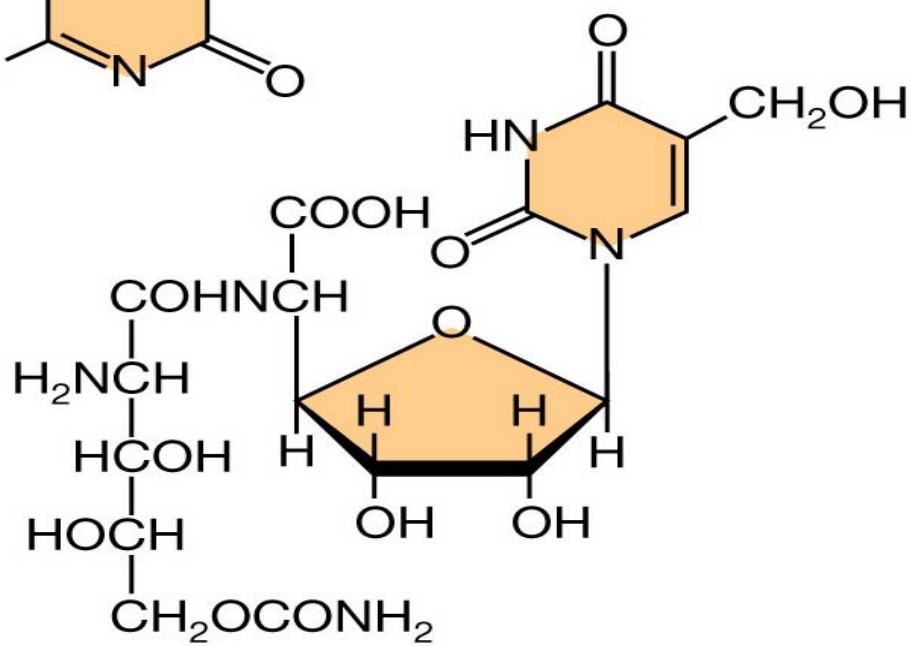
Representative structure



Mitomycin C



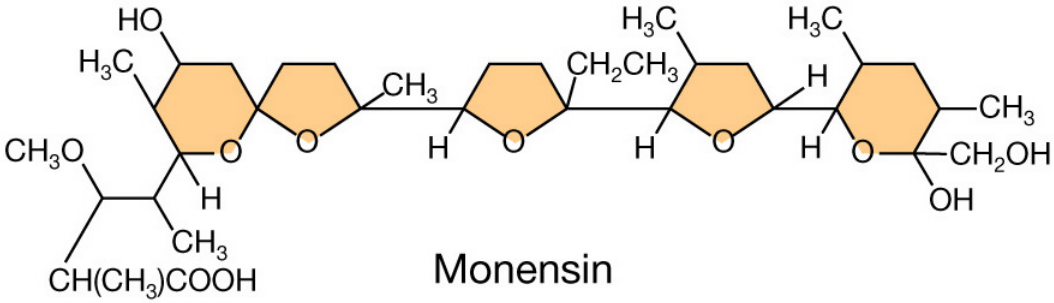
Ceftriaxone



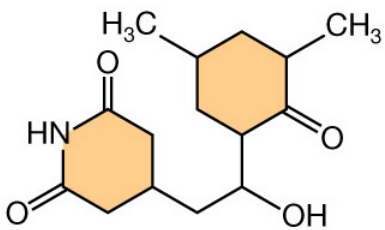
Polyoxin B

Classification of antibacterial chemotherapeutic agents according to chemical structure. A representative example is shown for each group.

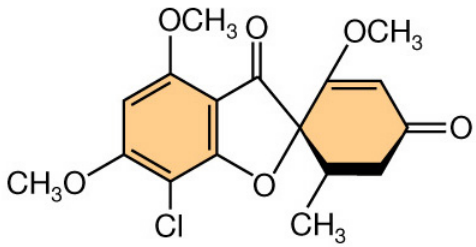
Representative structure



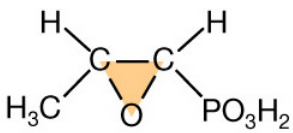
Monensin



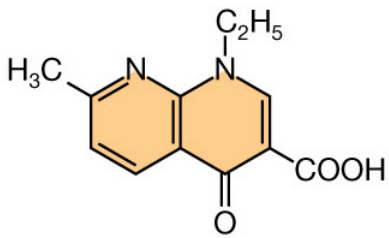
Cycloheximide



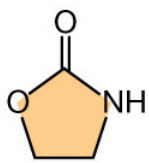
Griseofulvin



Fosfomycin

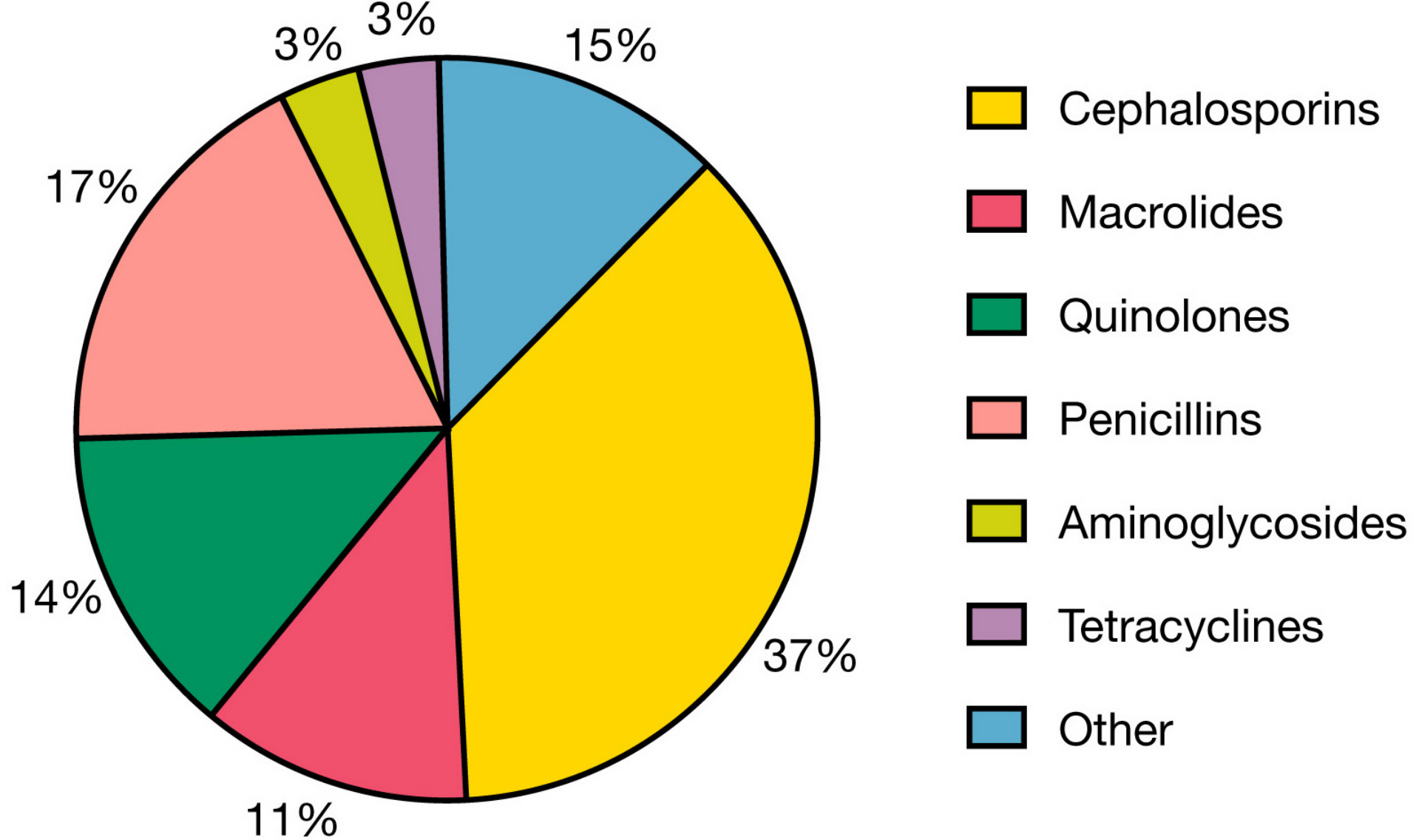


Nalidixic acid

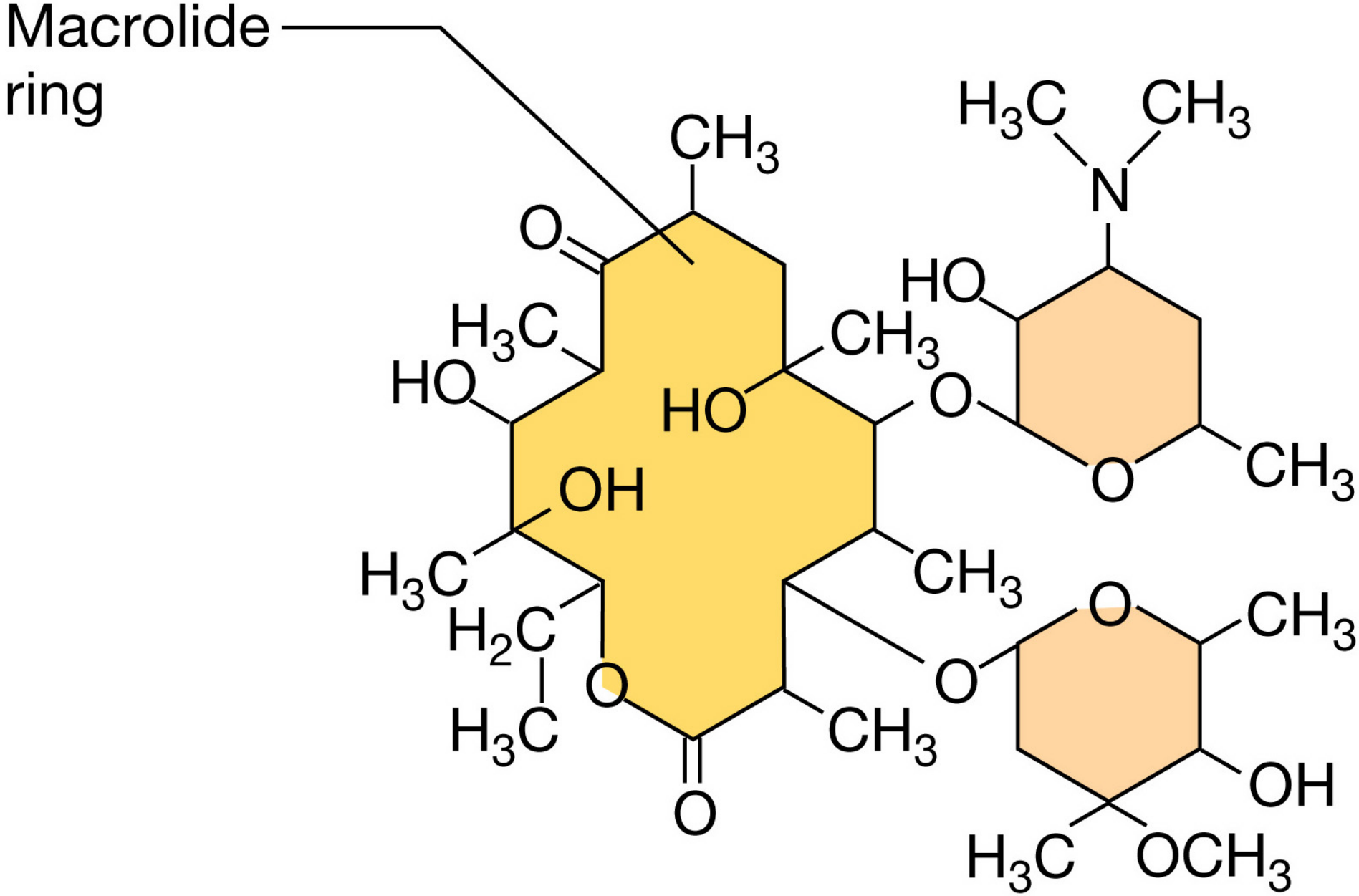


2-Oxazolidinone

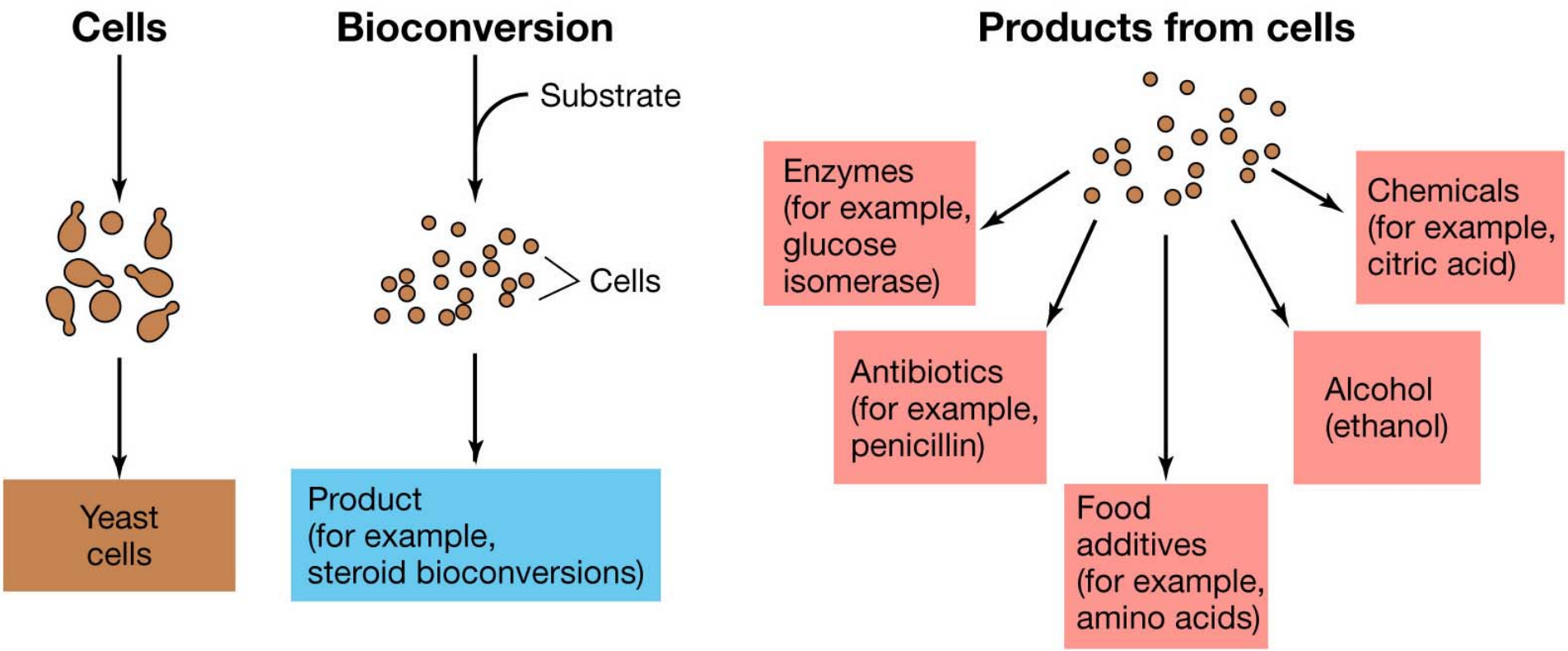
Classification of antibacterial chemotherapeutic agents according to chemical structure. A representative example is shown for each group.



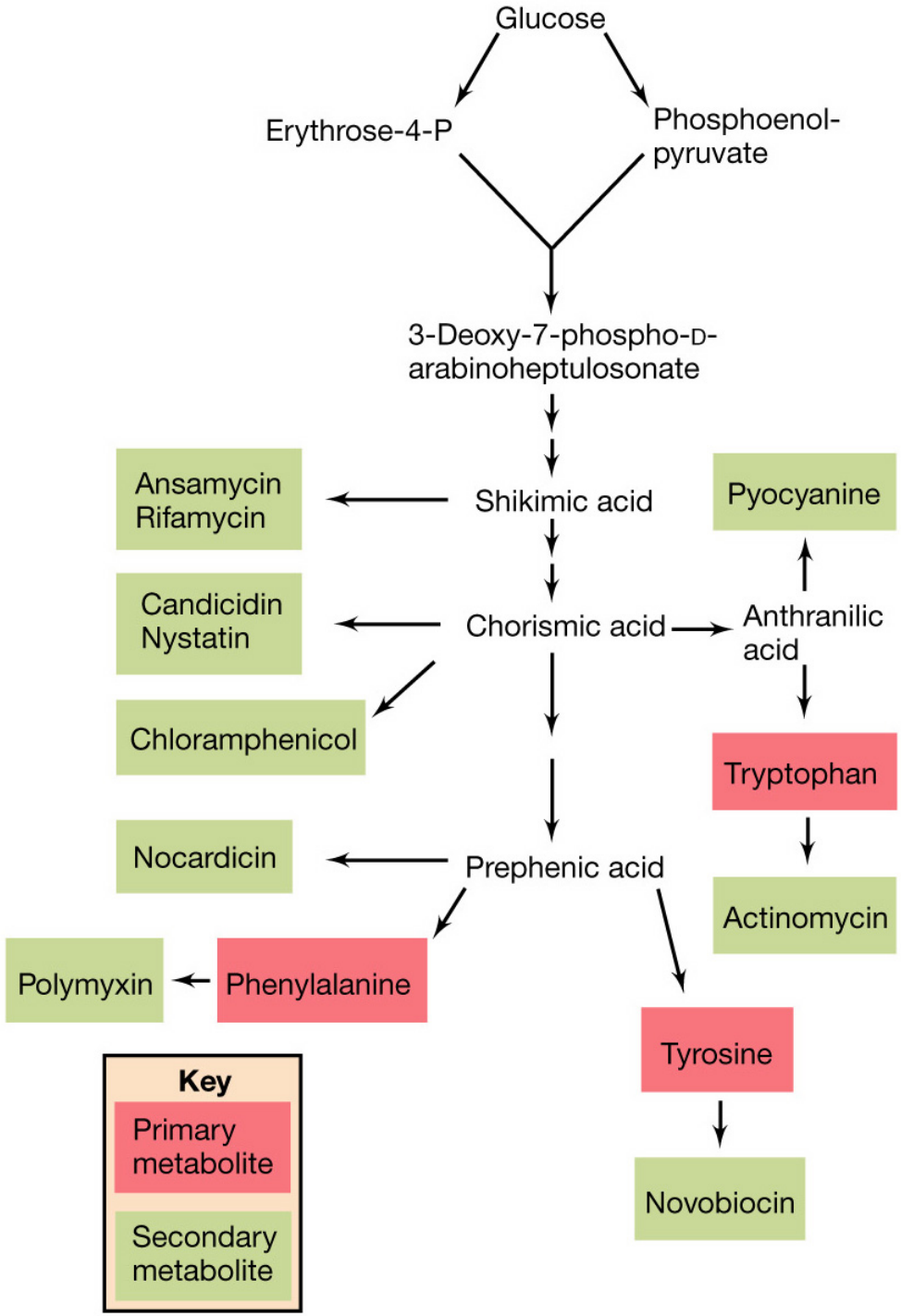
Annual worldwide production and use of antibiotics. Each year more than 500 metric tons of chemotherapeutic agents are manufactured.



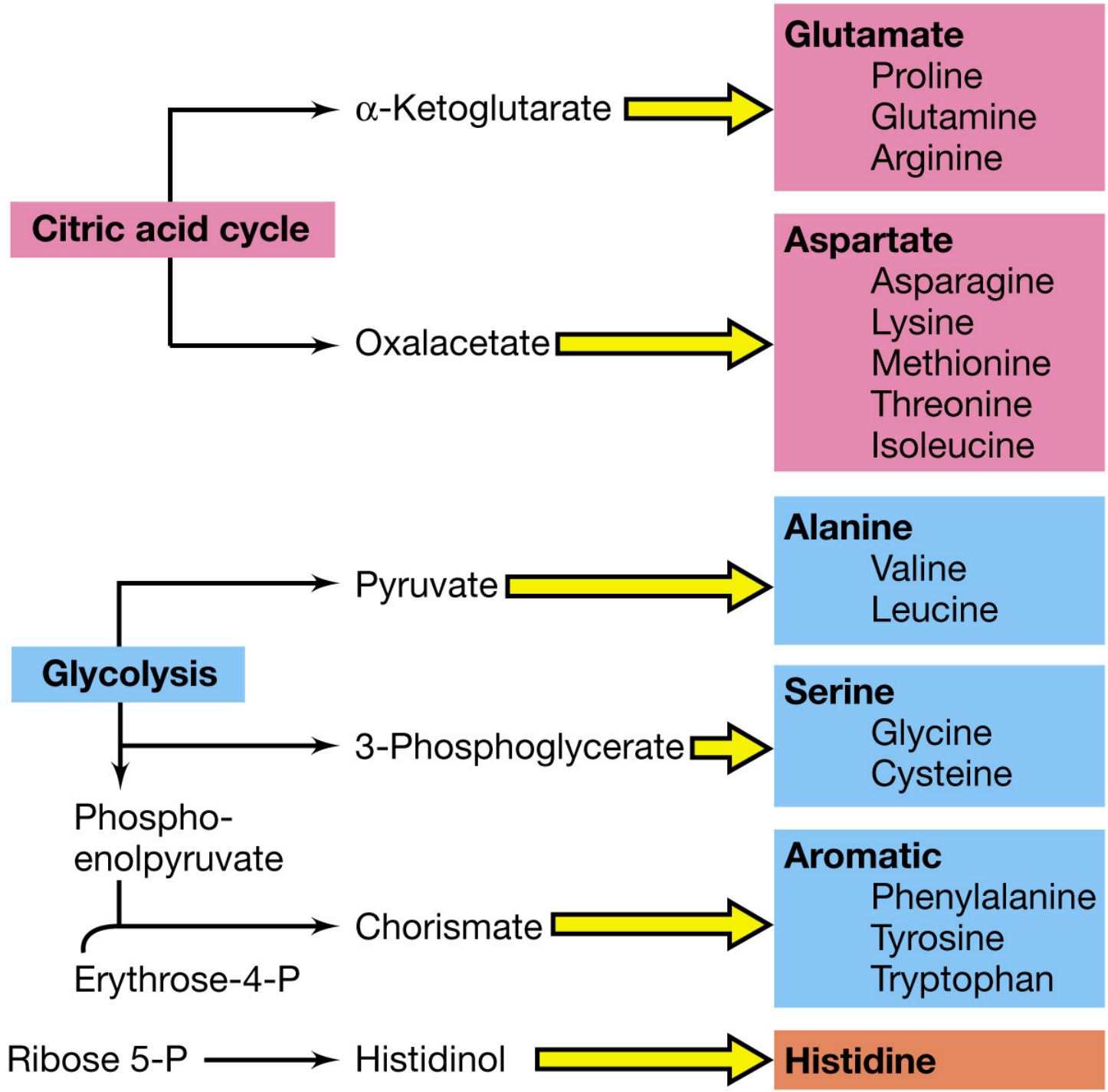
Structure of erythromycin, a macrolide antibiotic.



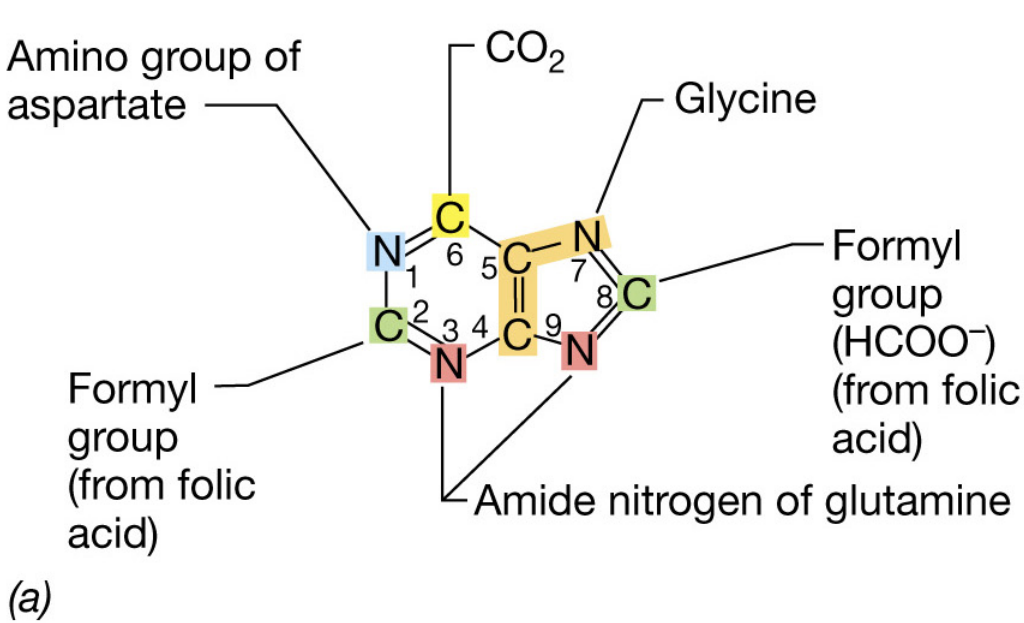
Products of industrial microbiology/biocatalysis. The products may be the cells themselves or products made from cells. In the case of bioconversion, cells are used to chemically convert a specific substance from one form to another.

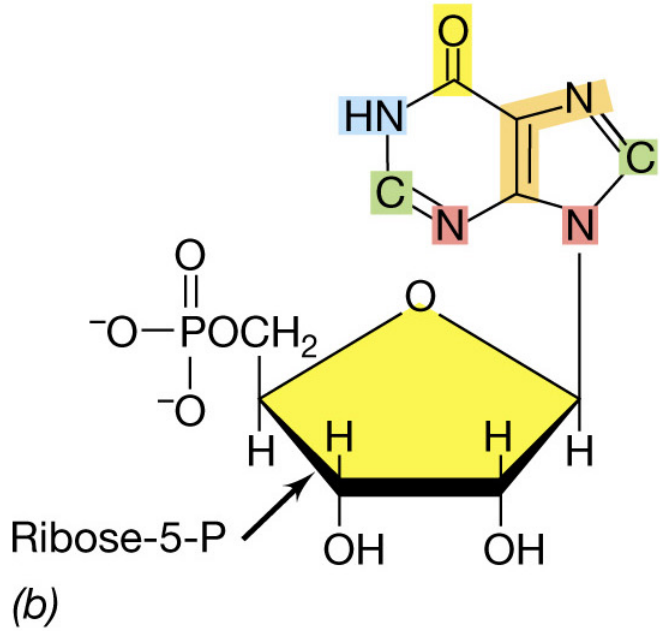


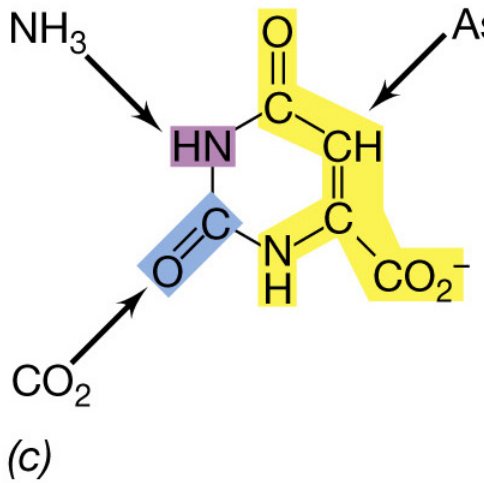
Relationship of the primary metabolic pathway for the synthesis of aromatic amino acids (Section 5.15) and formation of a variety of secondary metabolite antibiotics containing aromatic rings. This is a composite scheme of processes occurring in a variety of microorganisms: No one organism produces all these secondary metabolites, and many individual steps exist between amino acid and antibiotic in all cases.

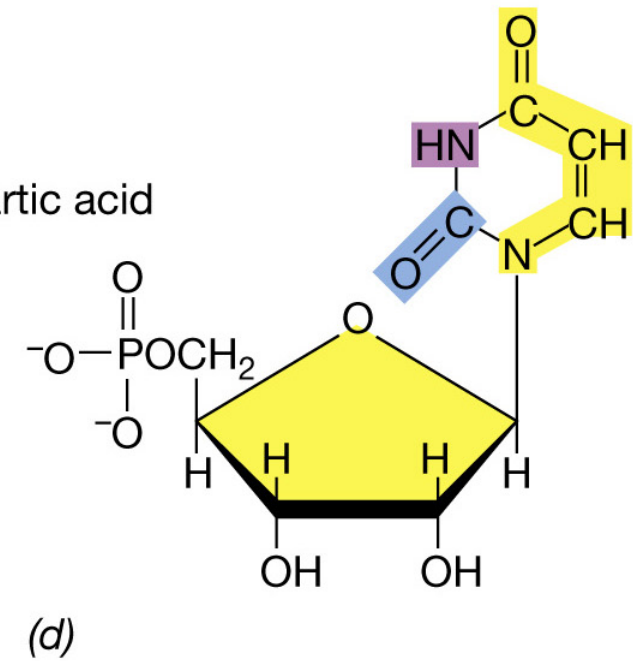


Amino acid families. Note how the carbon skeletons for most amino acids are derived from either the citric acid cycle or from glycolysis. Synthesis of the various amino acids in a family frequently requires many separate enzymatically catalyzed steps starting from the parent amino acid (shown in bold).

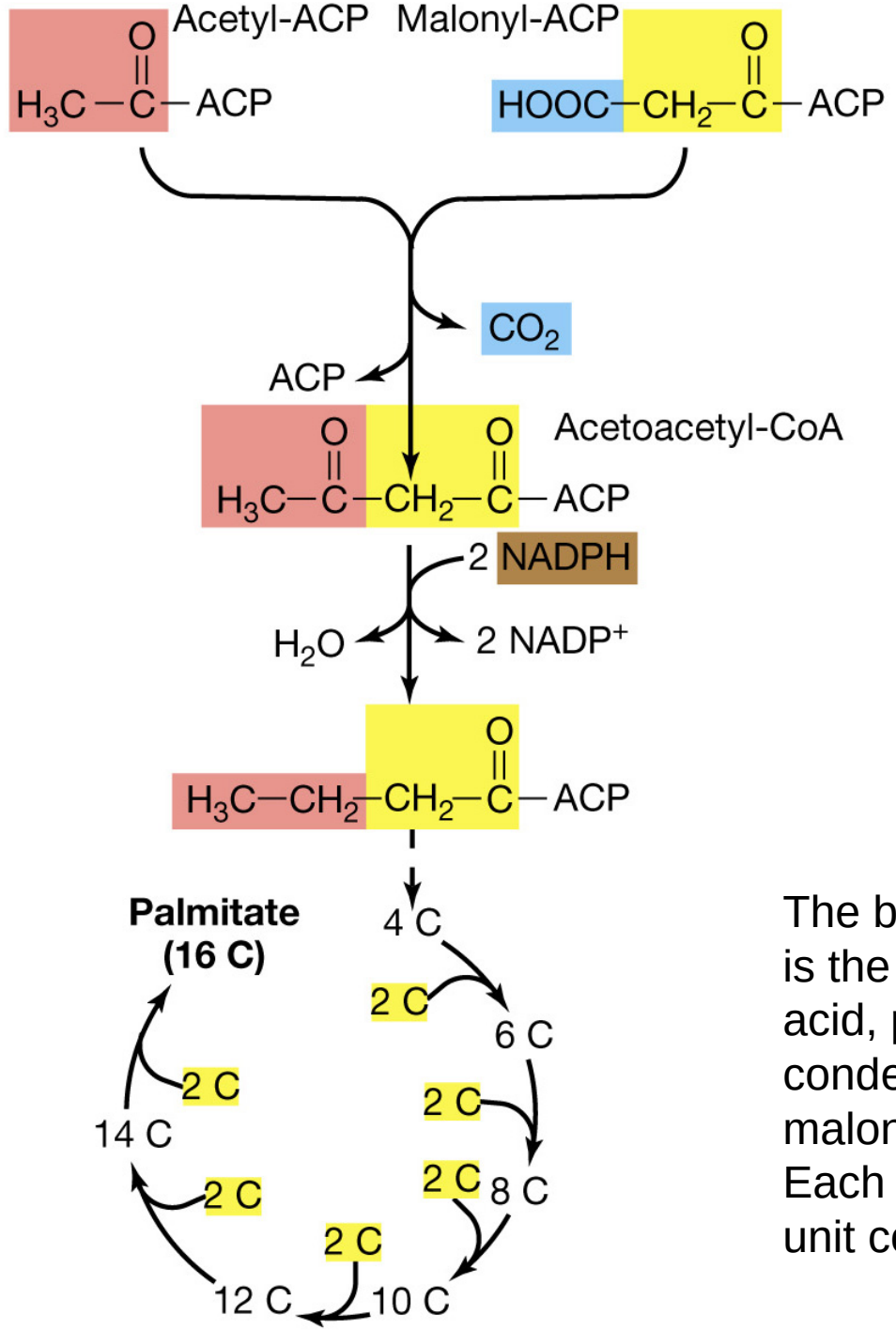




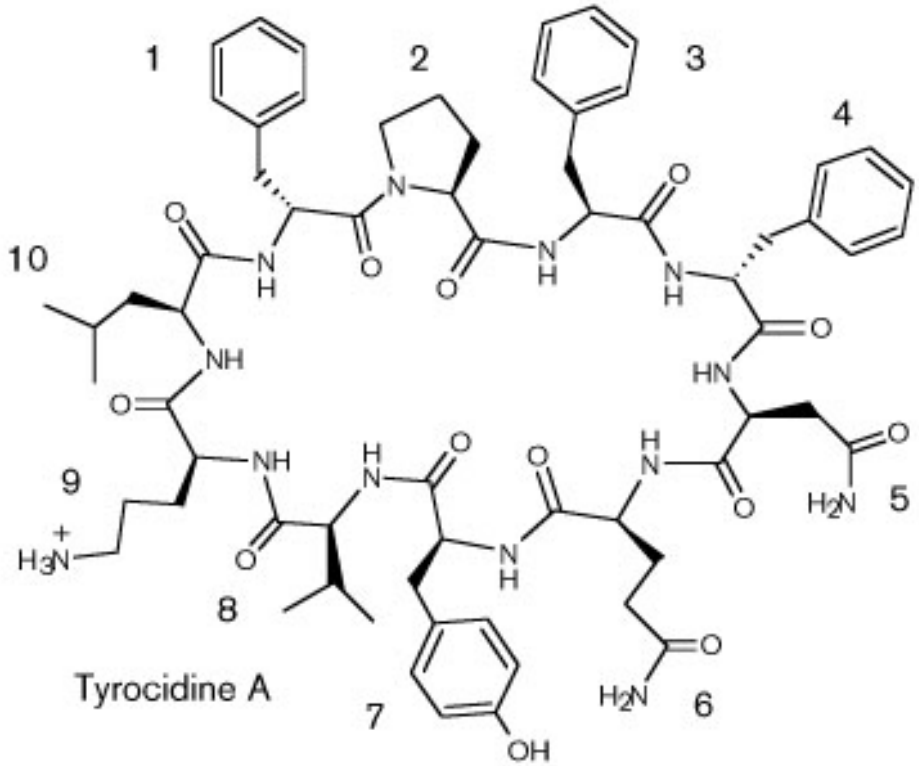




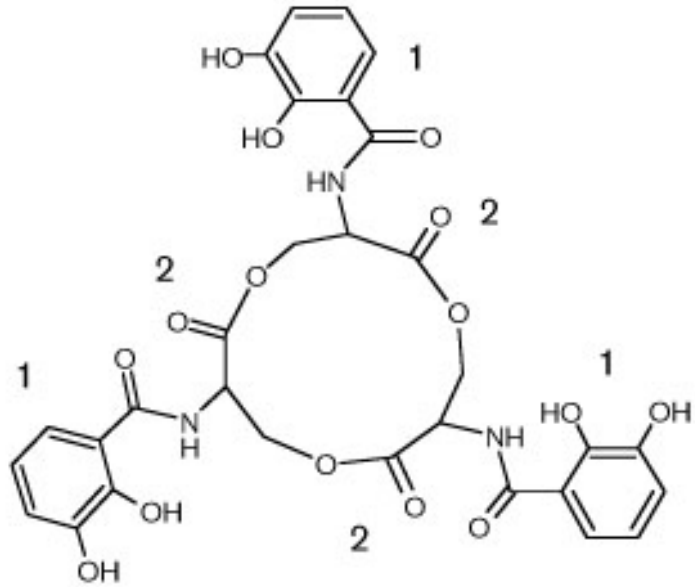
Biosynthesis of purines and pyrimidines. (a) The precursors of the purine skeleton. (b) Inosinic acid, the precursor of all purine nucleotides. (c) The precursors of the pyrimidine skeleton, orotic acid. (d) Uridylate, the precursor of all pyrimidine nucleotides. Uridylate is formed from orotate following a decarboxylation and the addition of ribose-5-phosphate.



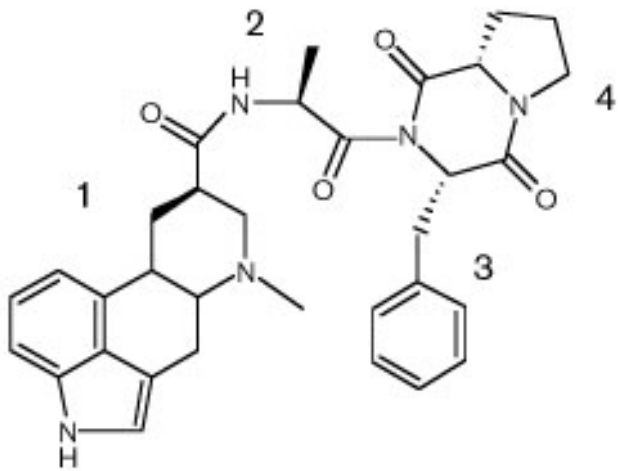
The biosynthesis of fatty acids; shown is the biosynthesis of the C16 fatty acid, palmitate (Figure 3.7). The condensation of acetyl-ACP and malonyl-ACP forms acetoacetyl-CoA. Each successive addition of an acetyl unit comes from malonyl-CoA.



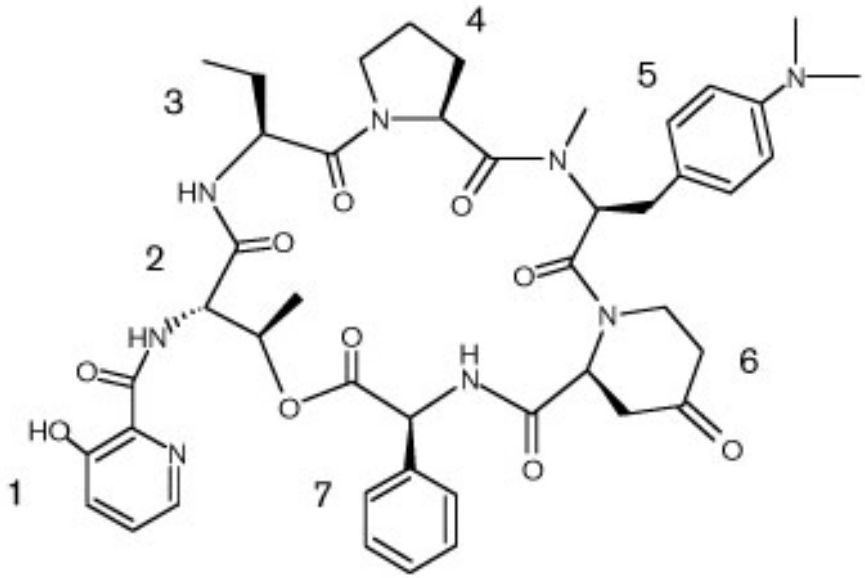
Tyrocidine A



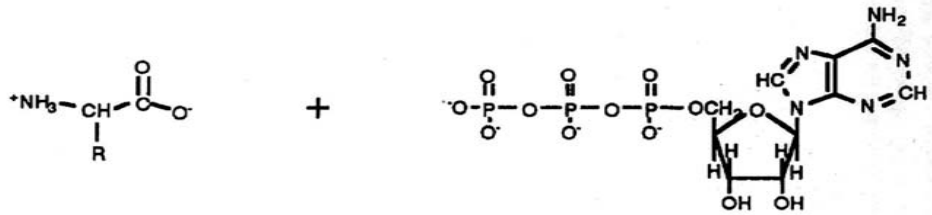
Enterobactin



Ergotamine



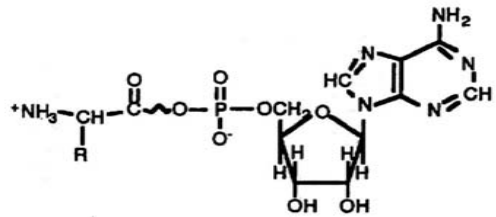
Pristinamycin I_A



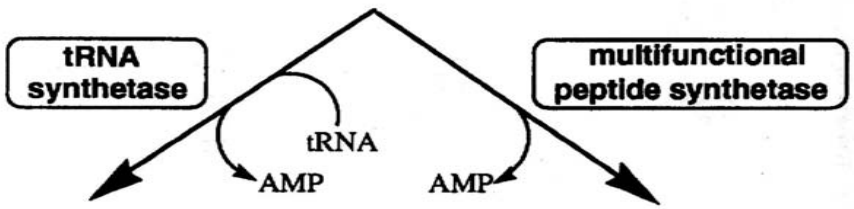
amino acid

ATP

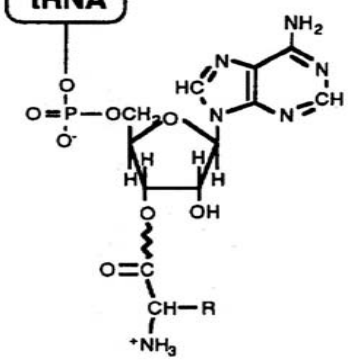
enzyme tRNA synthetase or multifunctional peptide synthetase



amino acyladenylate (enzyme-associated)

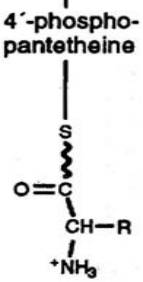


tRNA

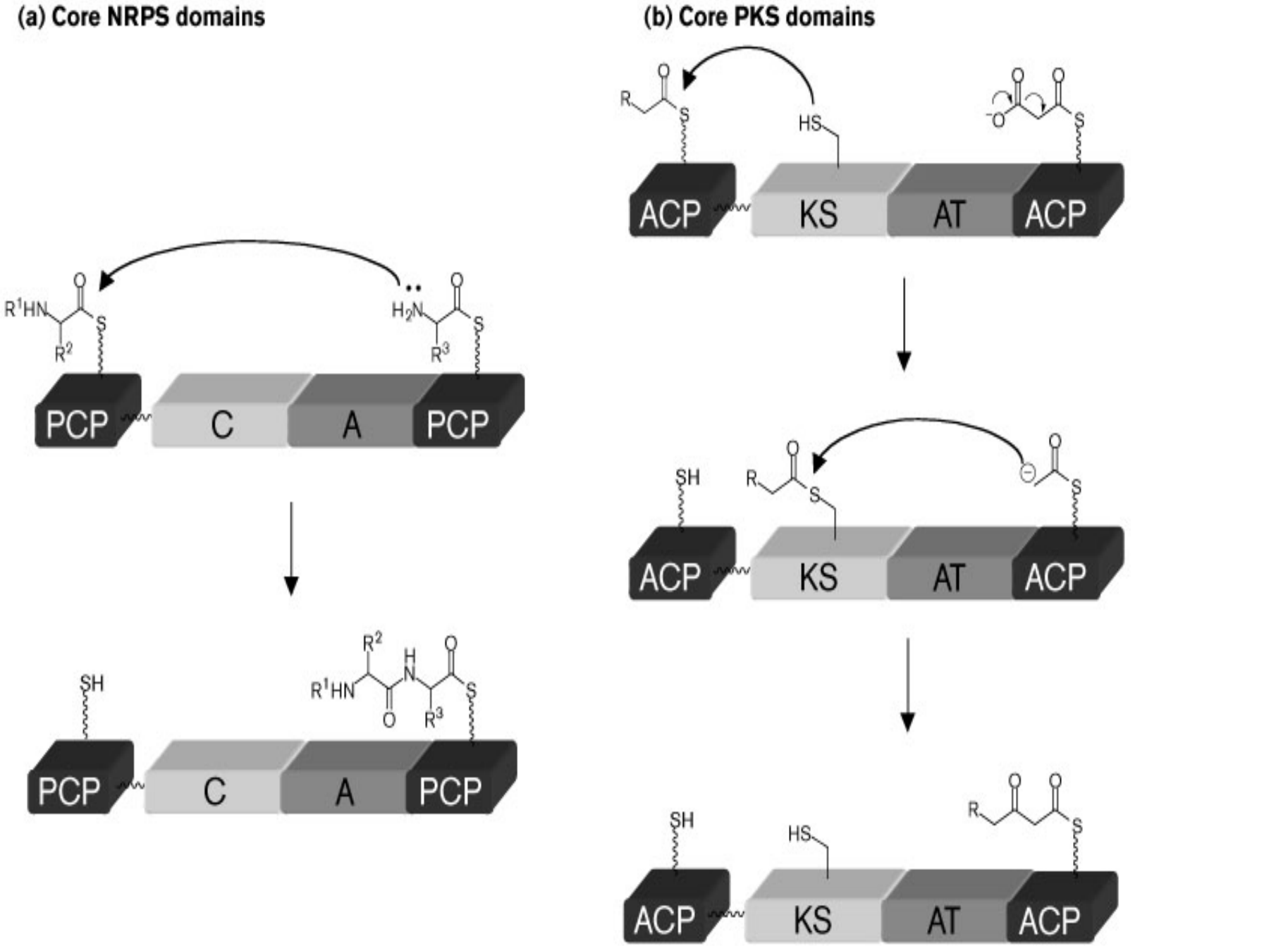


amino acyl-tRNA

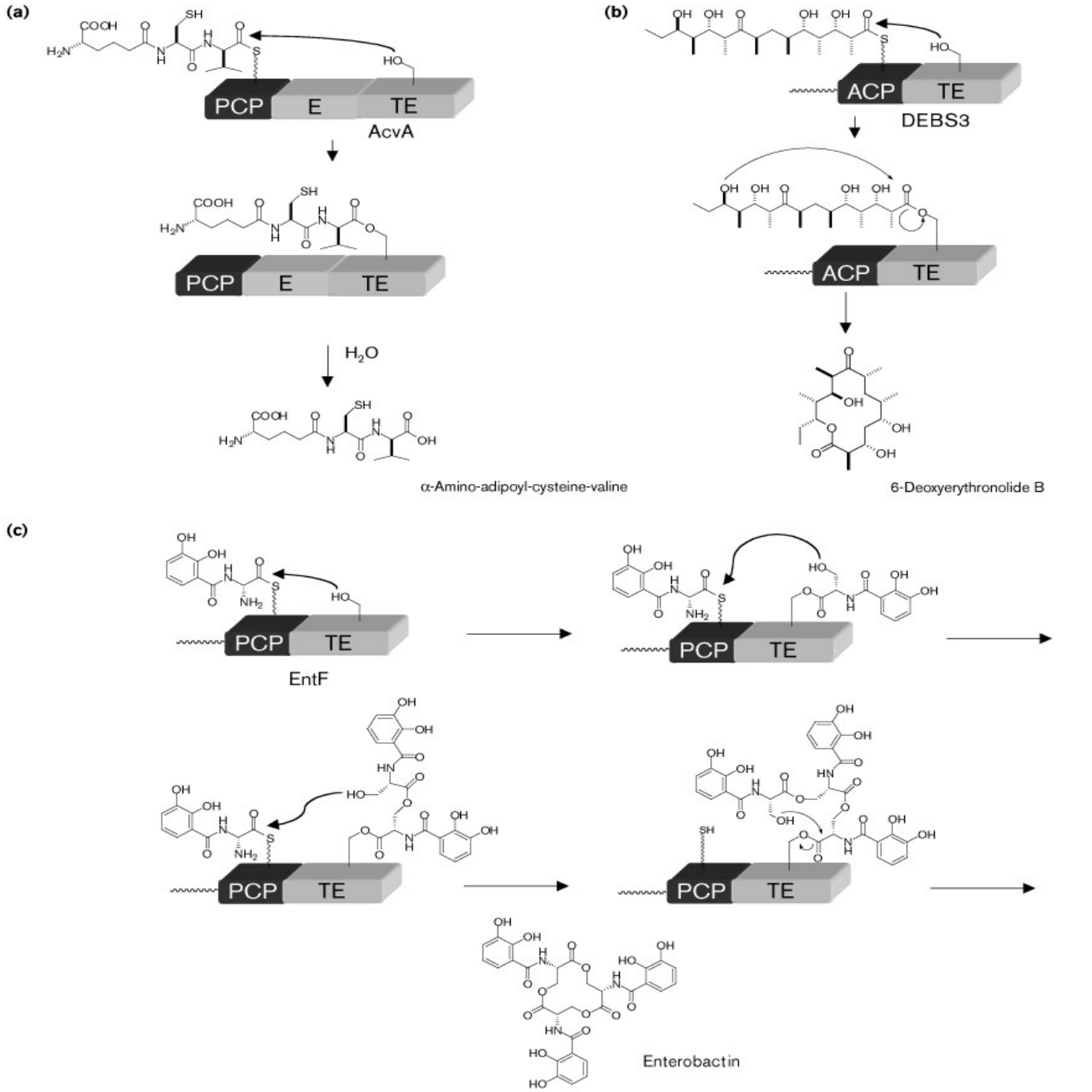
multi-enzyme

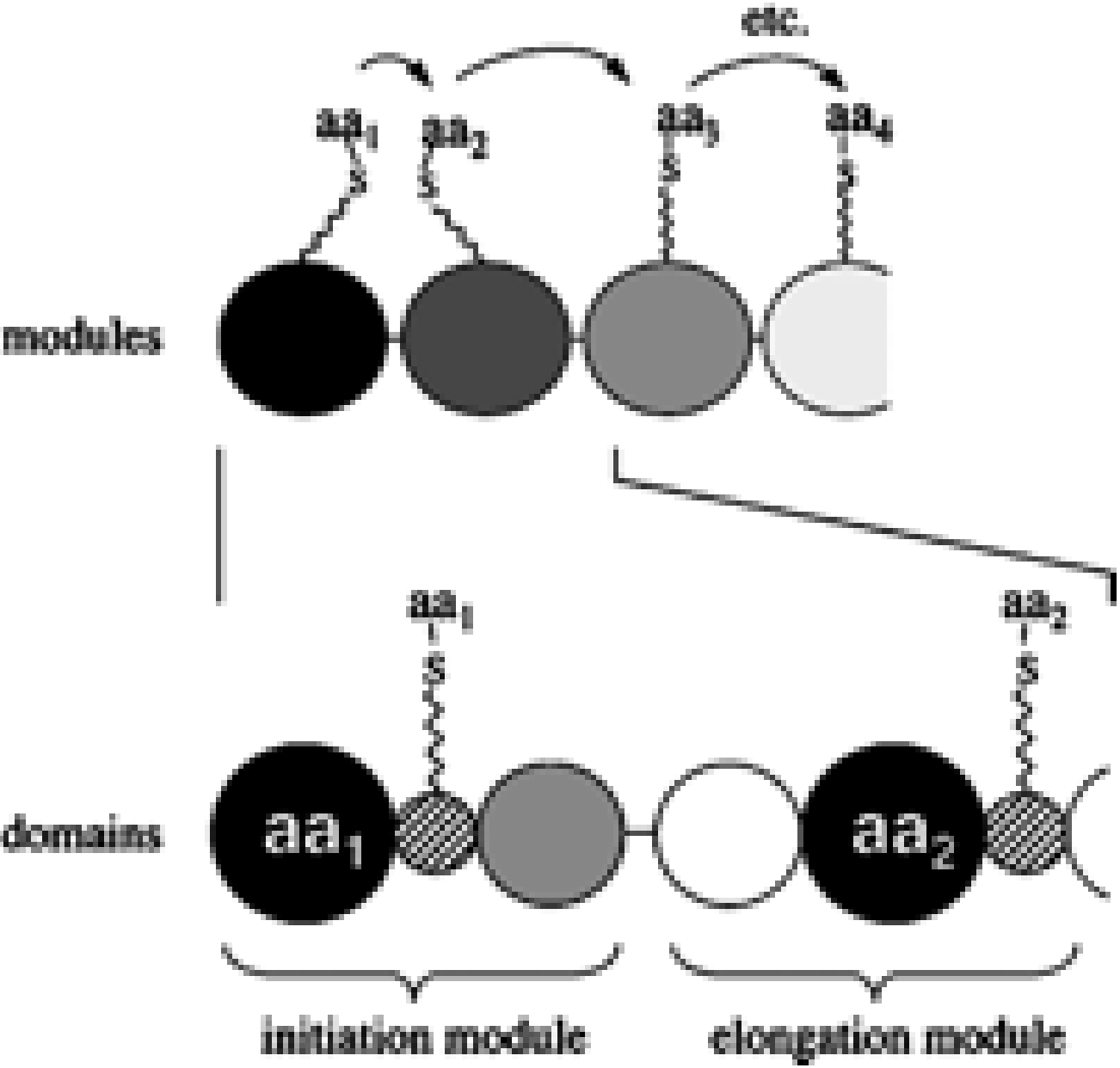


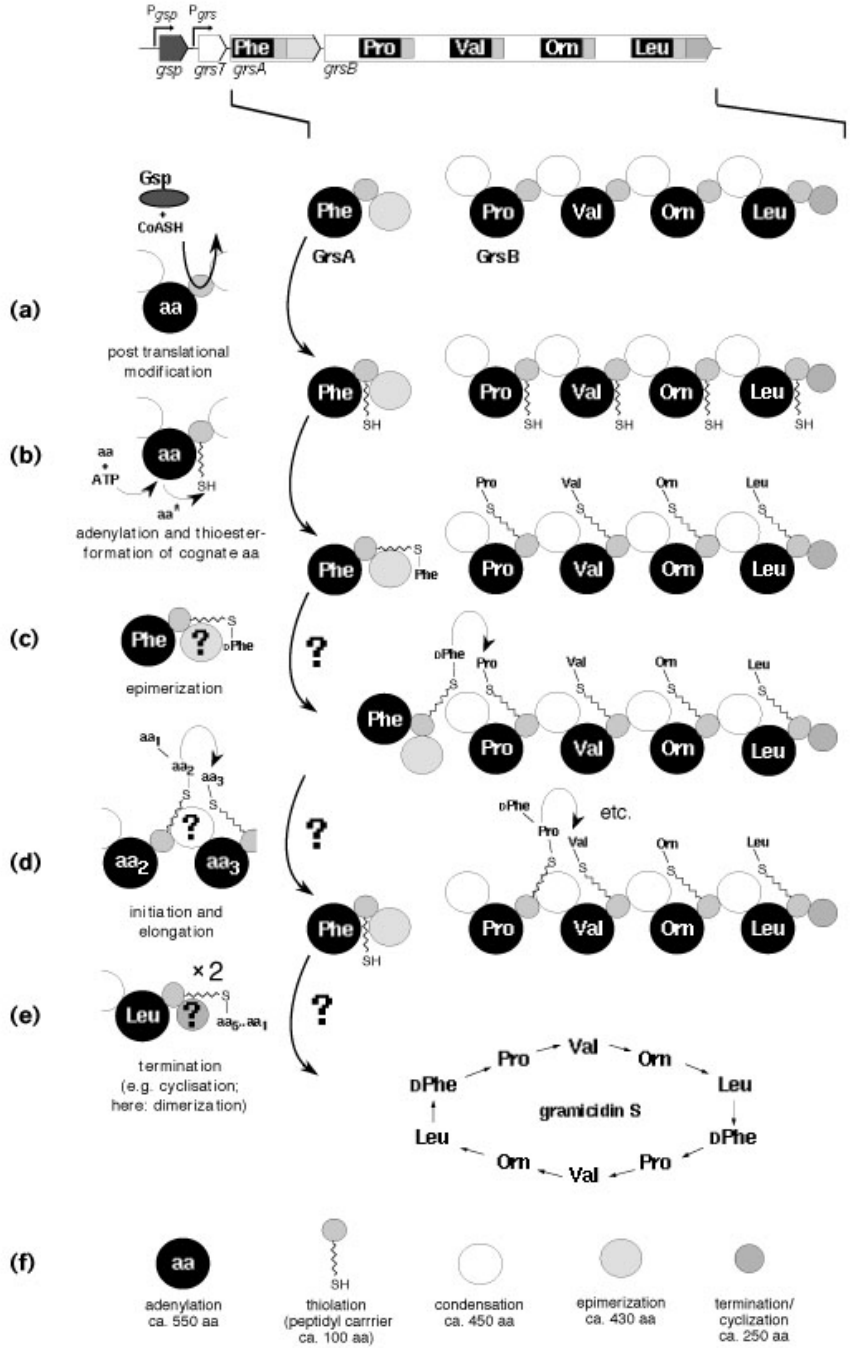
thioester bound amino acid



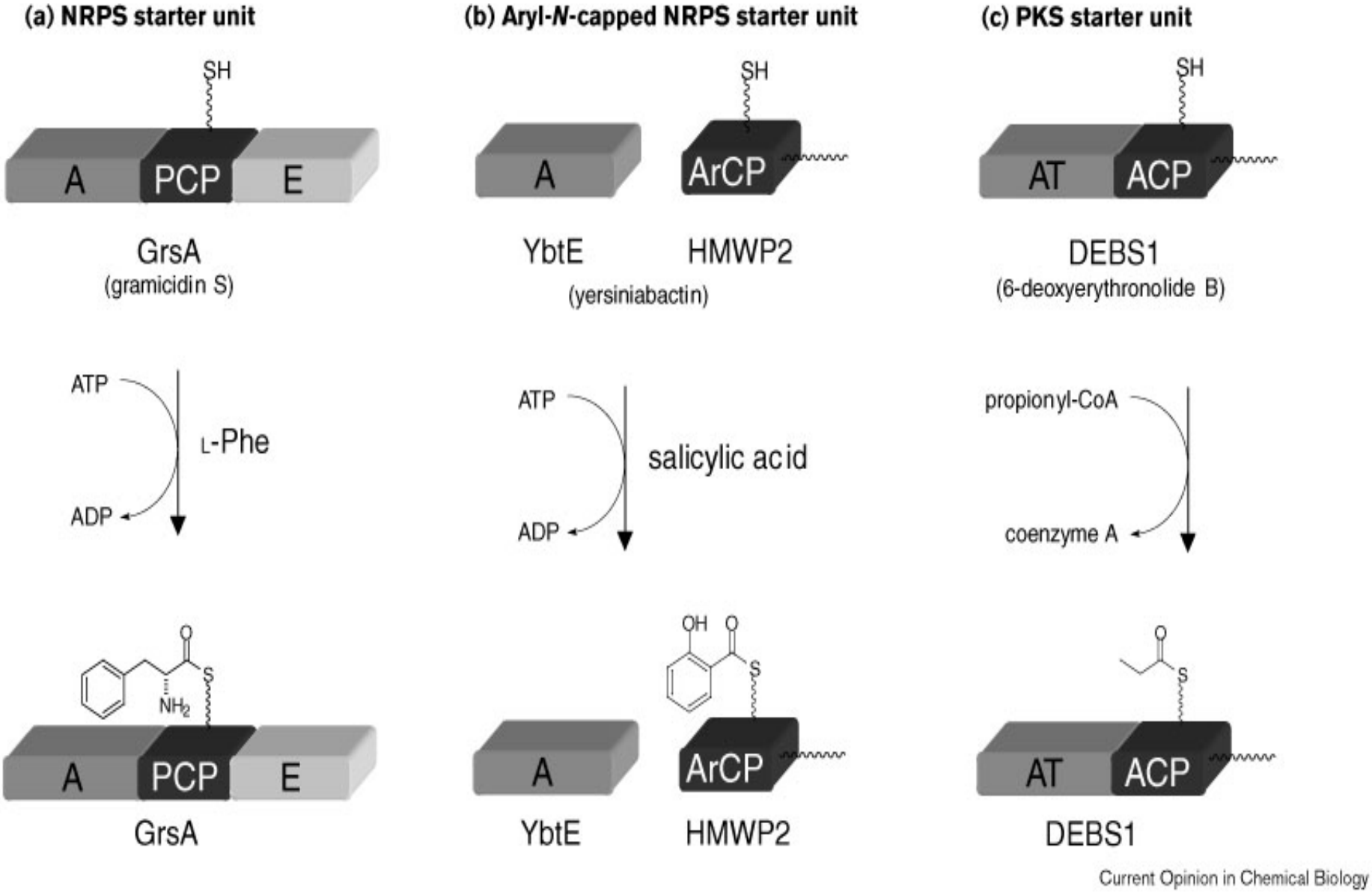
Current Opinion in Chemical Biology


(a)
PCP
E
TE
AcvA
H_2O
α-Amino-adipoyl-cysteine-valine
(b)
ACP
TE
DEBS3
6-Deoxyerythronolide B
(c)
EntF
Enterobactin

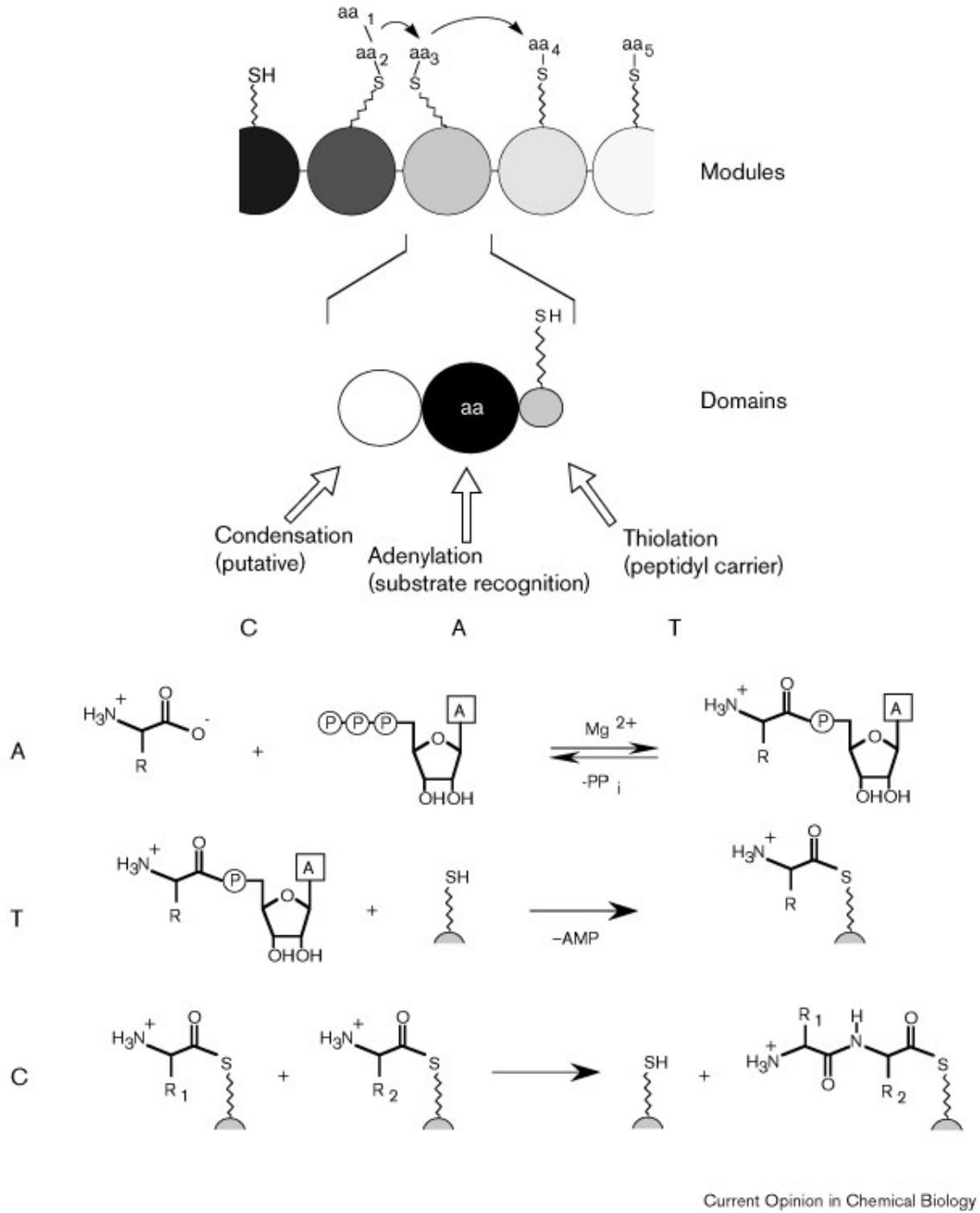

etc.
aa1
aa2
aa3
aa4
modules
aa1
aa2
domains
aa1
aa2
initiation module
elongation module



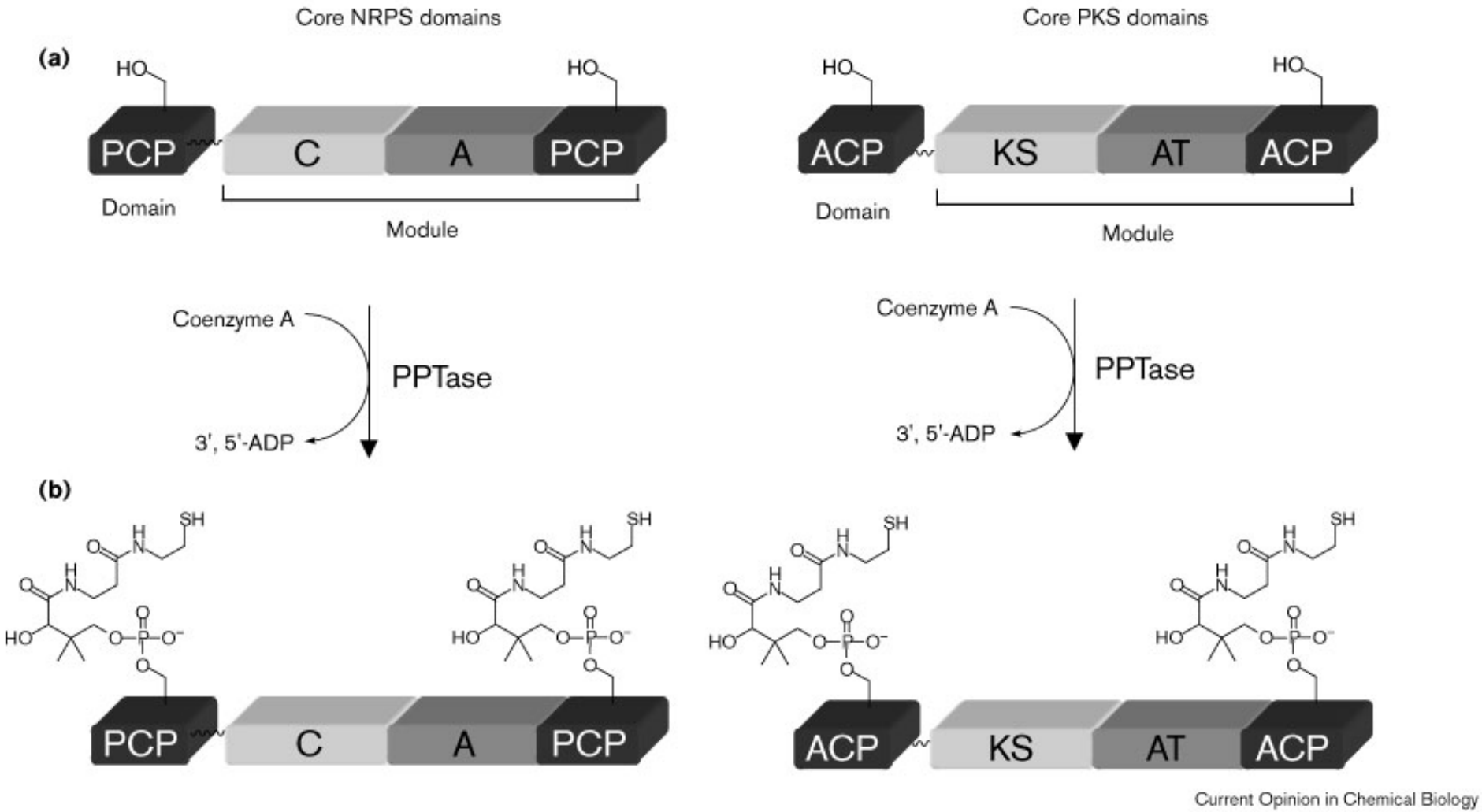
Current Opinion in Chemical Biology



Current Opinion in Chemical Biology



Current Opinion in Chemical Biology



Current Opinion in Chemical Biology

Type II PKSs are composed of discrete monofunctional enzymes (KS, ACP etc.), e. g. aromatic PKSs.

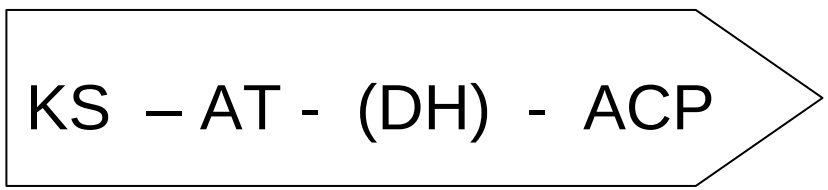
With **Type I PKSs** the enzymes are covalently linked.

Type I PKSs are composed of one or more multienzyme polypeptides.

Modular type I PKSs have a set of enzymes (KS-AT-reduction domains-ACP) for every cycle of chain extension.

Iterative type I PKSs have one set of enzymes that is used repeatedly.

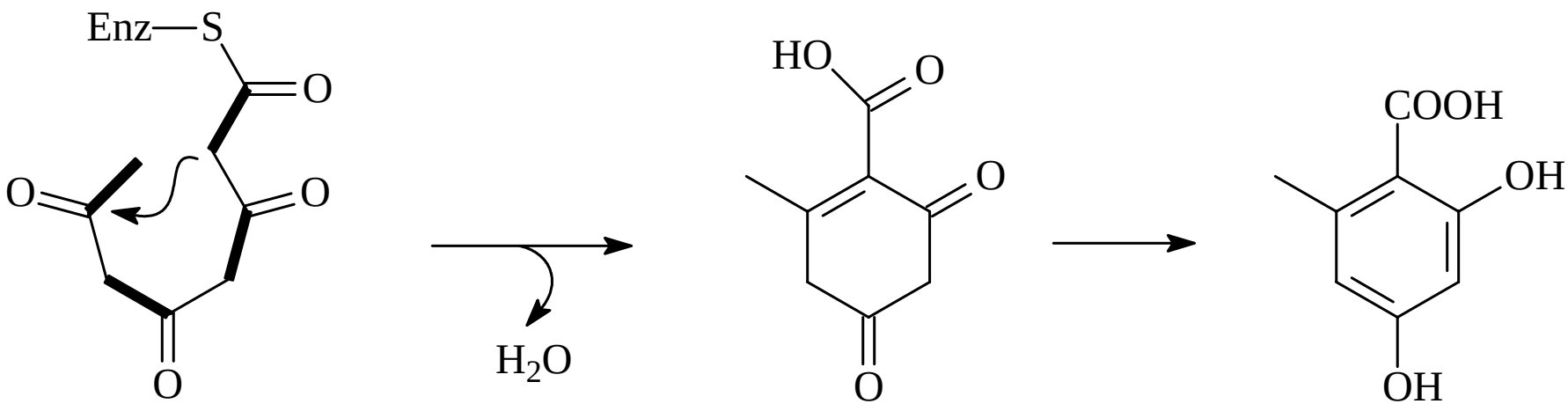
These systems make simpler polyketides than modular PKSs.



Orsellinic acid occurs in the antibiotic avilamycin, and the anticancer enediyne calicheamicin.

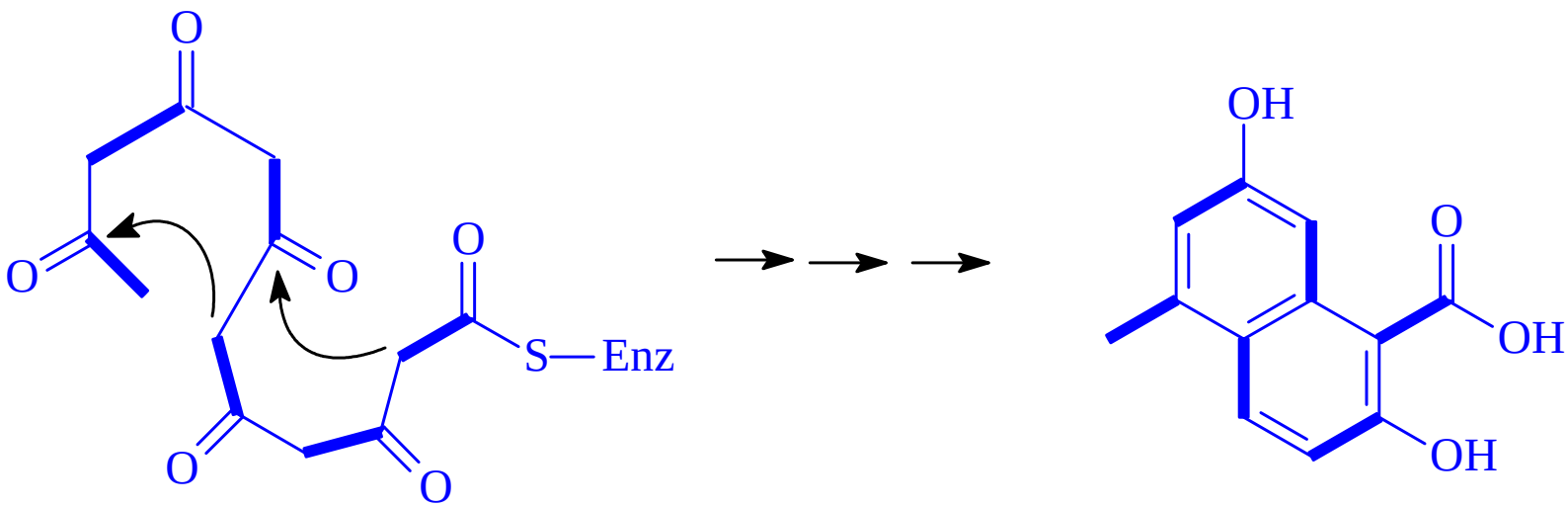
It is synthesised by an iterative (non-modular) type I PKS.

KS – AT - (DH) - ACP



Orsellinic acid

Napthalinic acid is also synthesised by an iterative type I PKS.

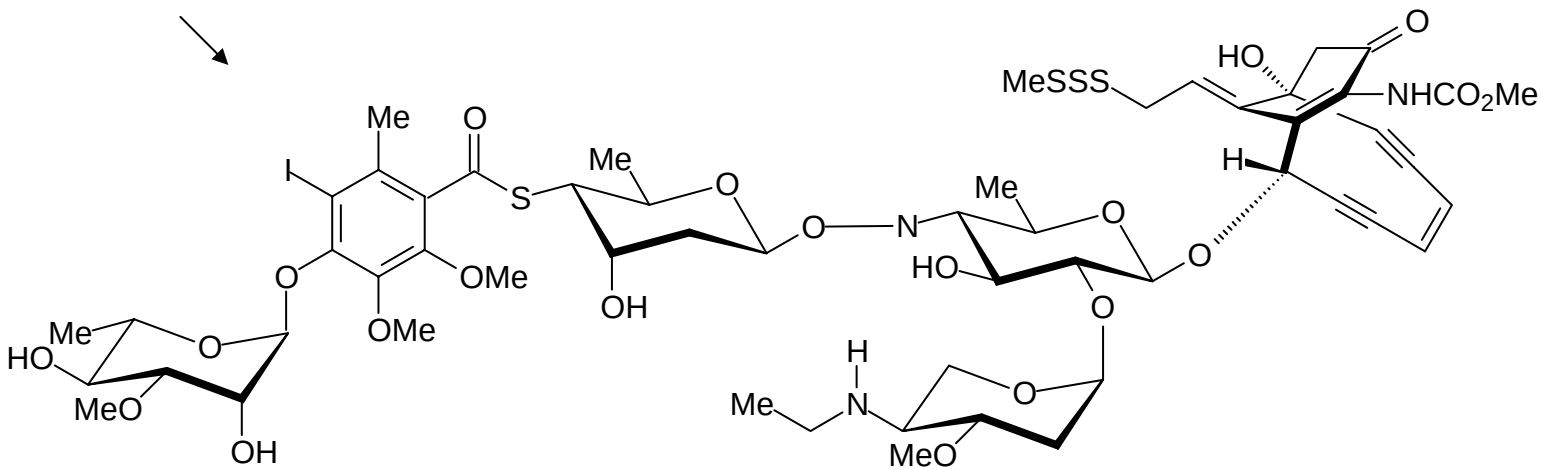


Enediyne antibiotics contain two polyketide-derived moieties.

Both are synthesised by iterative type I PKSs.

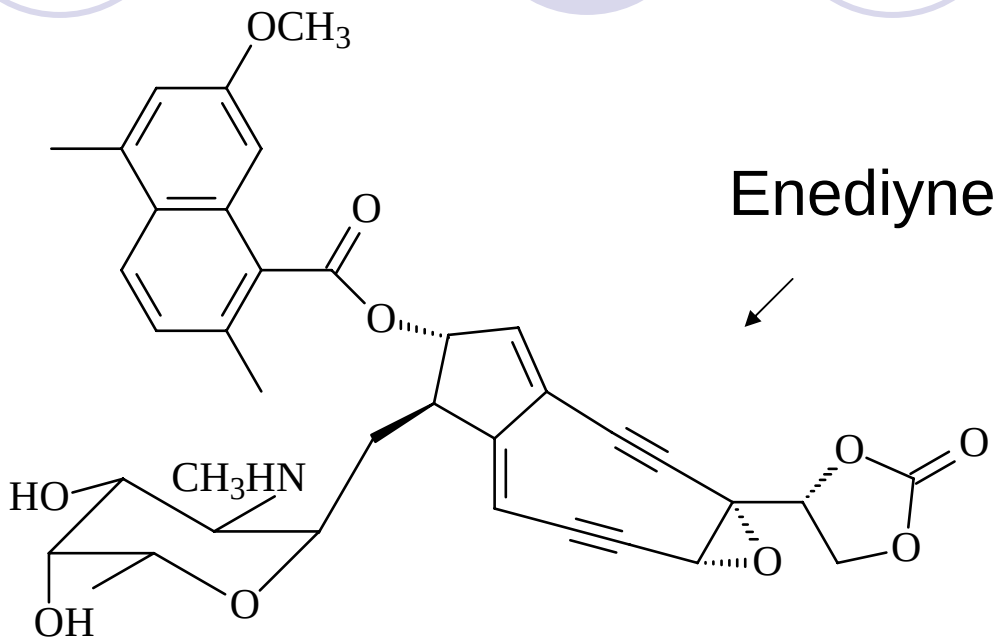
Enediyne core

Orsellinic acid



Calicheamicin

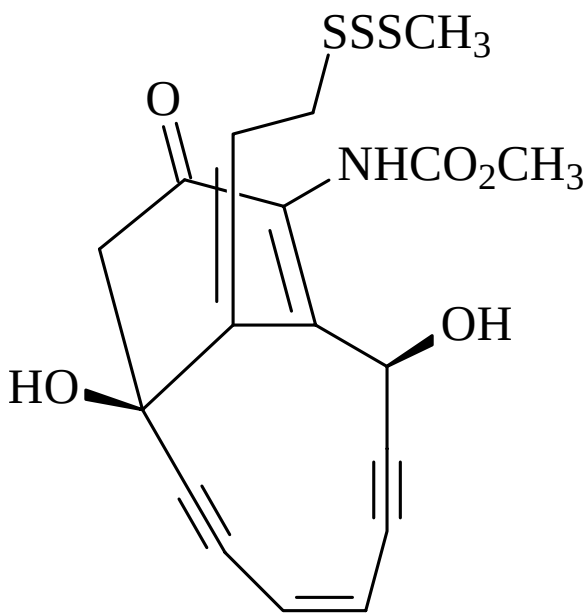
Napthalinic acid



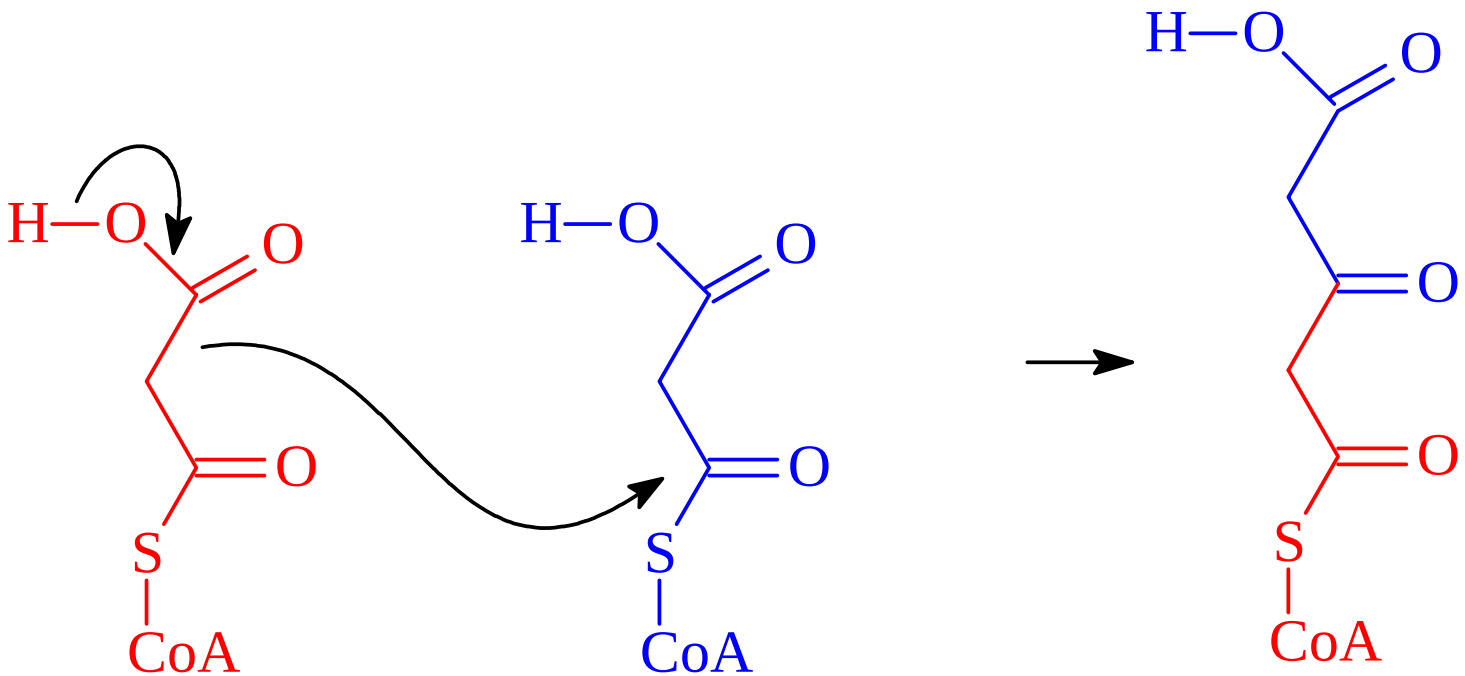
Enediyne core

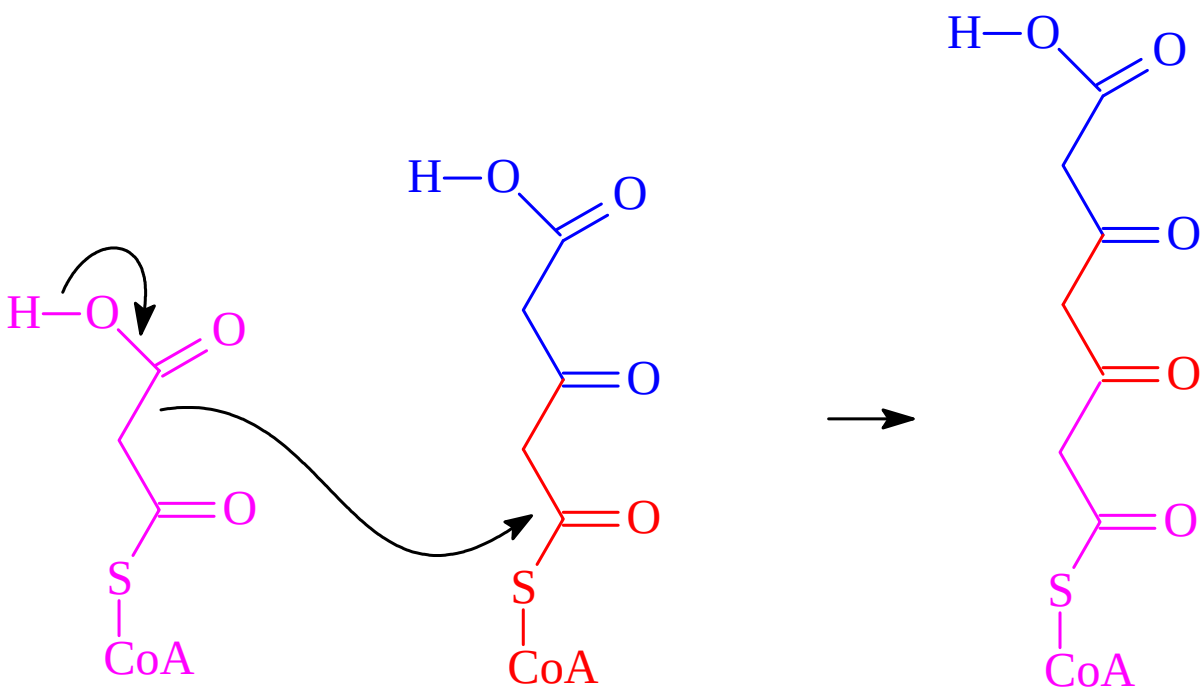
Enediyne C1029

Enediyne cores are derived from polyunsaturated acyl chains that are synthesised by iterative type I PKSs.

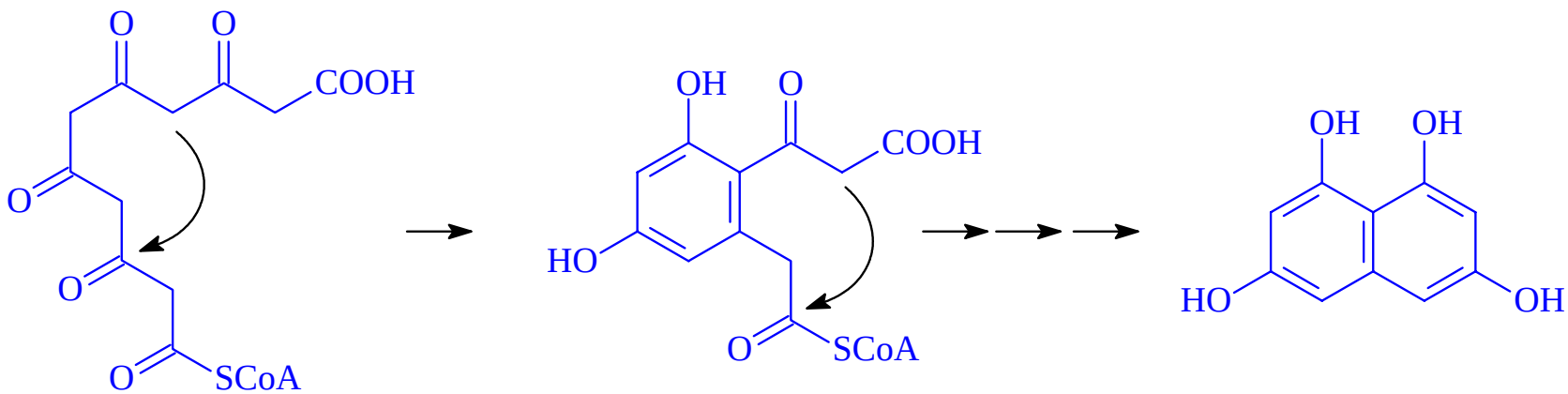


Type III PKSs are composed of KS domains only.
They assemble the polyketide chain on CoA pantetheine thiol (not ACP).




H—O
O
O
O
S
CoA
H—O
O
O
O
S
CoA
H—O
O
O
O
O
S
CoA

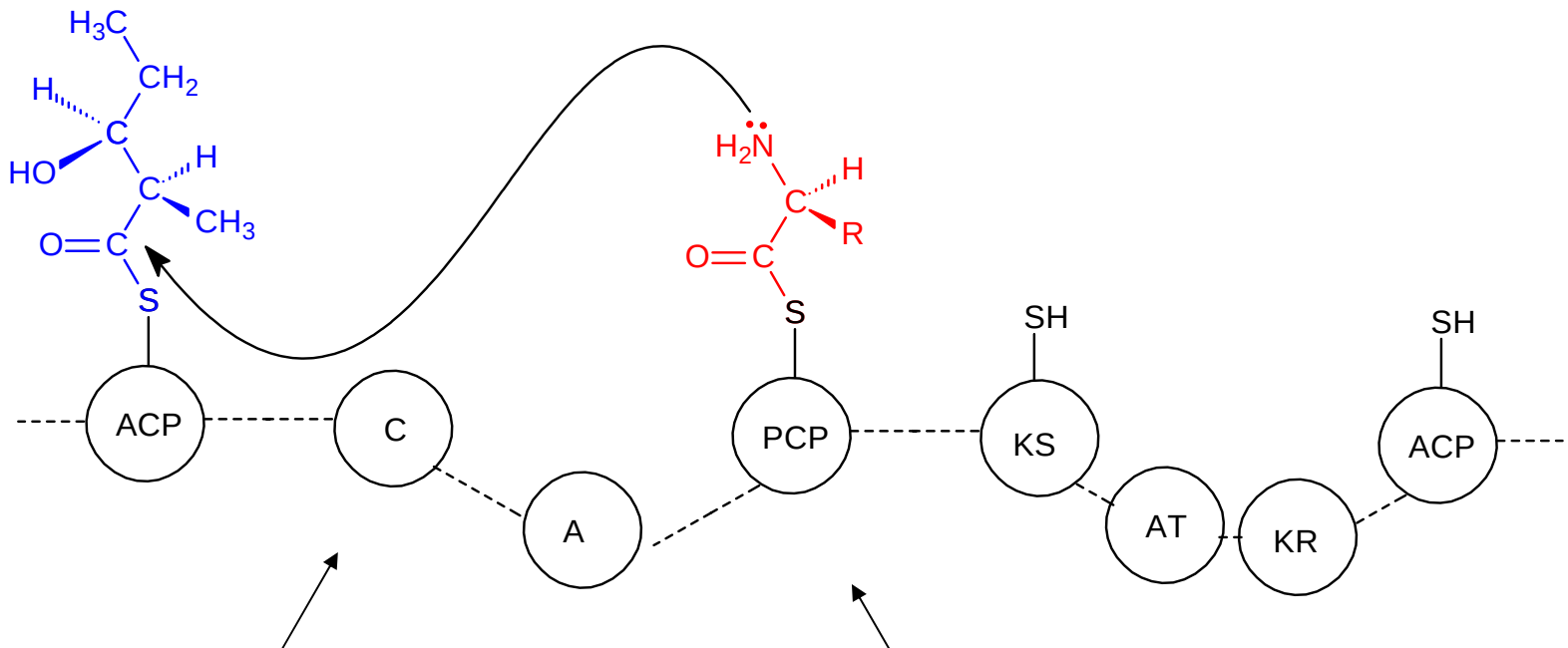
Type III PKSs synthesise pigments (e. g. flower colours), chalcones, flavonoids, flaviolins.



Flaviolin

Mixed PKS-NRPS (non-ribosomal peptide synthetase) systems

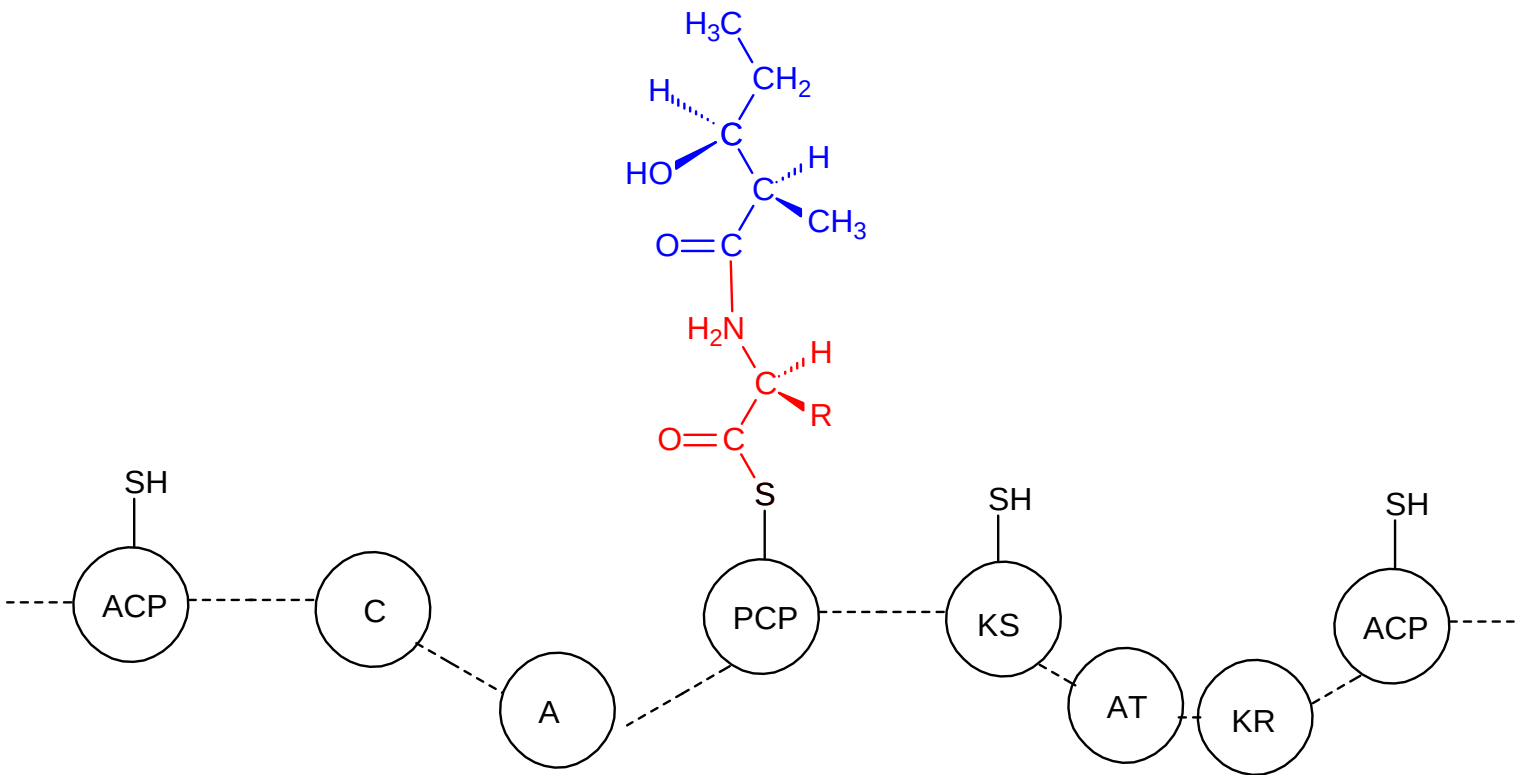
Modular PKSs that can use amino acid extenders as well as dicarboxylic acid extenders.



Condensation domain

AA activating domain

Peptidyl carrier protein (has pantetheine arm).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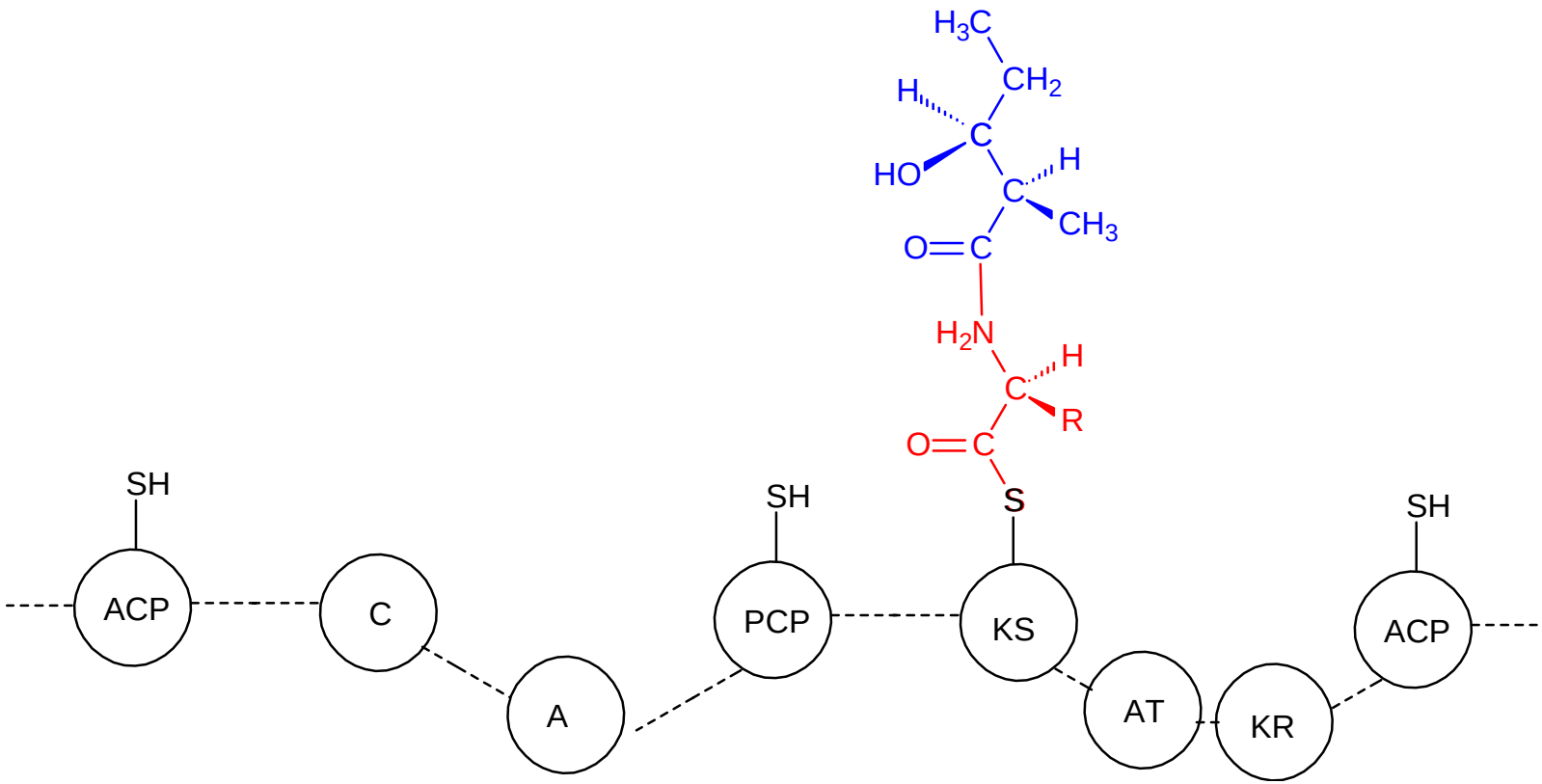
H
C
HO
H
C
CH3
O
C
H2N
H
C
R
O
C
S
SH
ACP
C
A
PCP
SH
KS
AT
KR
SH
ACP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C
HO
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C
CH3
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A
SH
PCP
KS
AT
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SH
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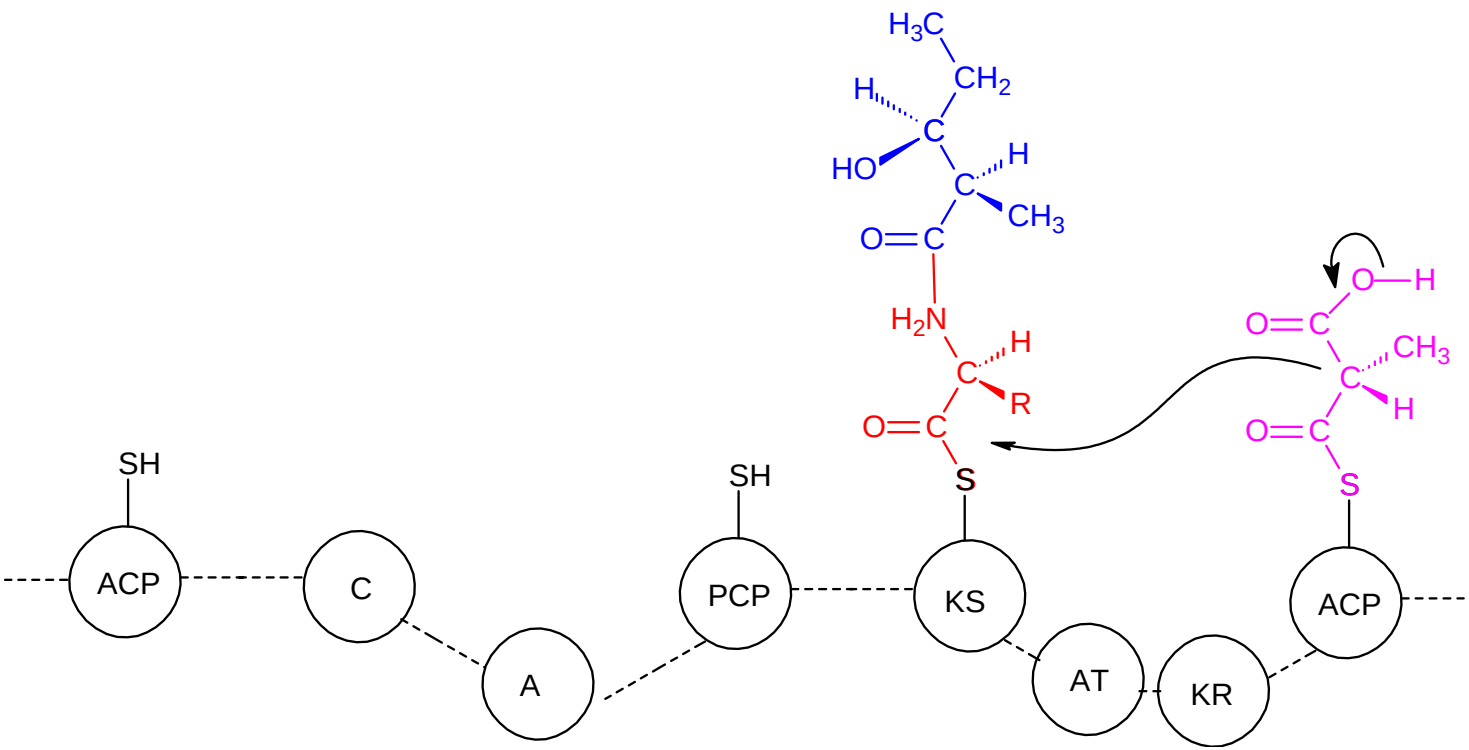
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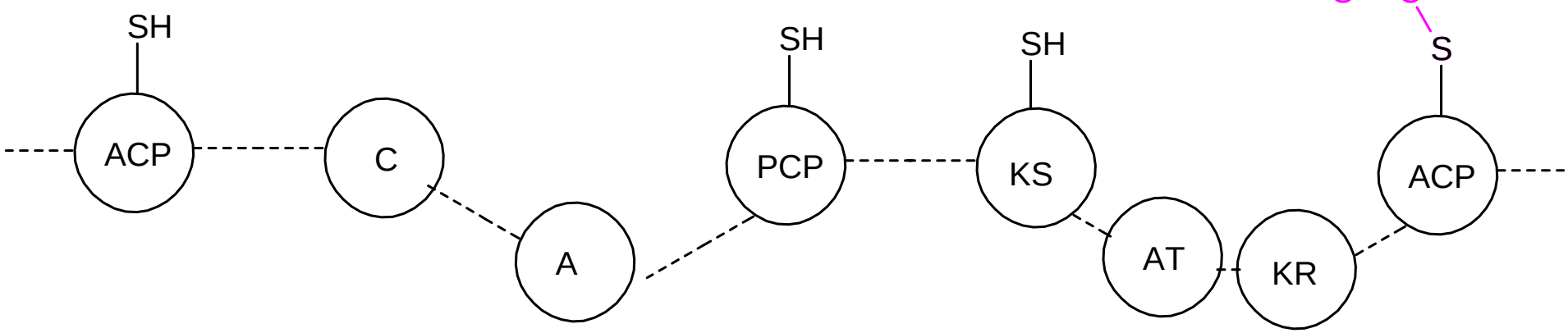
C
HO
H
C
CH3
O=C
H2N
H
C
R
O=C
S
O—H
O=C
CH3
C
H
O=C
S
SH
ACP
C
A
SH
PCP
KS
AT
KR
ACP

In principle, mixed PKS-NRPSs could make a greater diversity of products because there are potentially scores of different AA extenders.



Types of PKS

Type III (KS domains only, build chains on CoA)

Type II (composed of small discrete enzymes, synthesise aromatic polyketides.

Type I (covalently linked enzymes[e.g. KS-AT-X- Y-ACP])

Type I iterative – one set of enzymes is used repeatedly.
Relatively simple chains are made.

Type I modular – a set of enzymes for every cycle
includes mixed PKS-NRPSs.
Complex polyketides are made.
Can be reprogrammed.

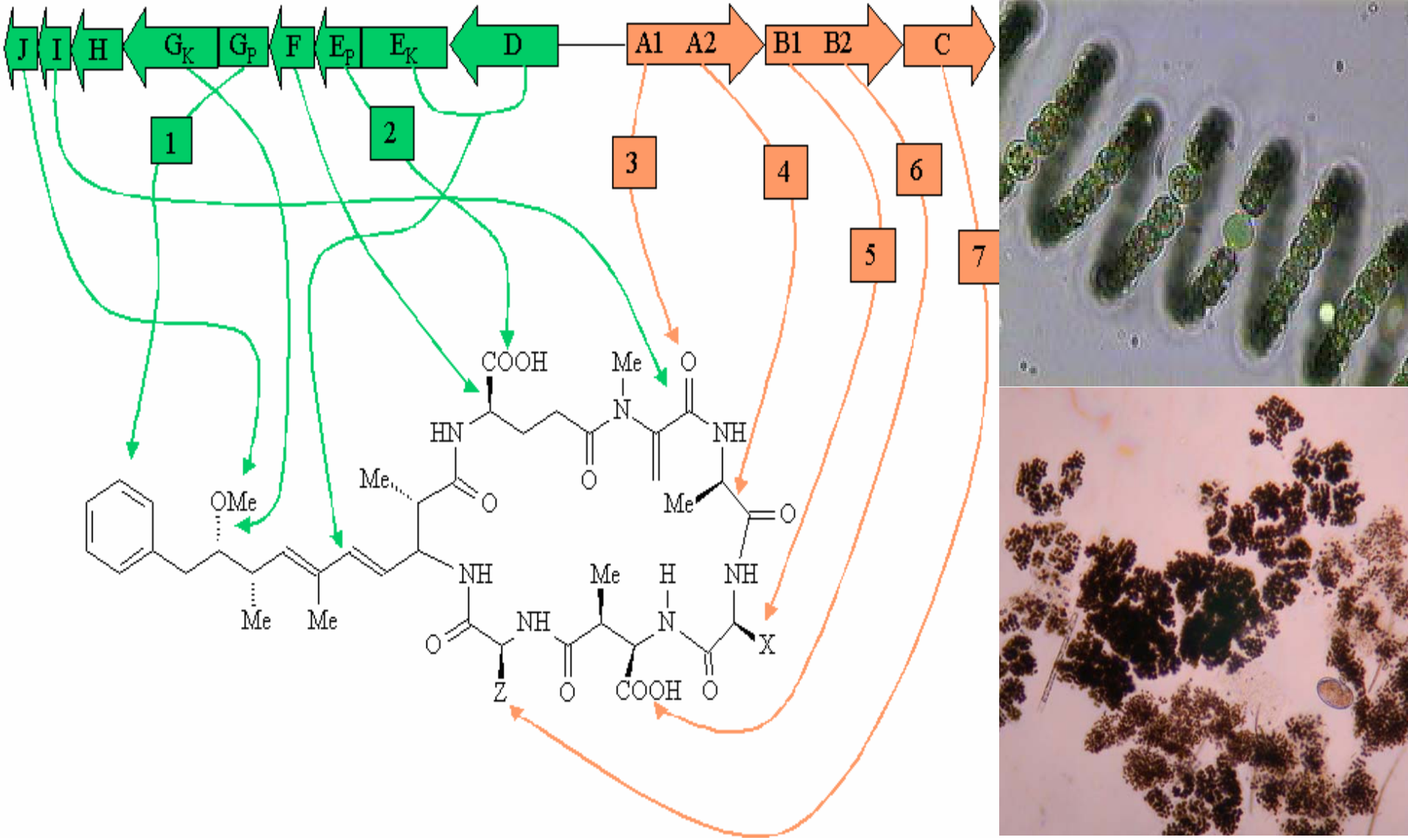
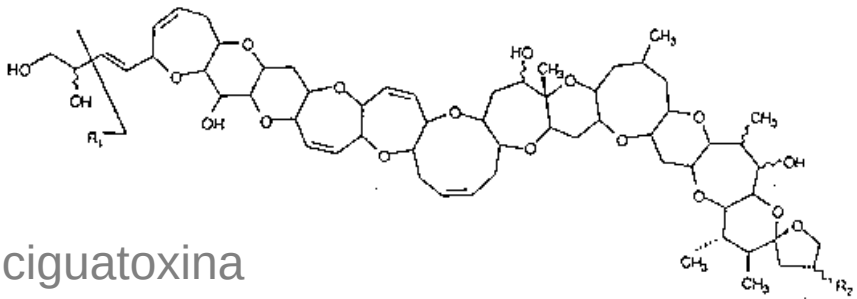


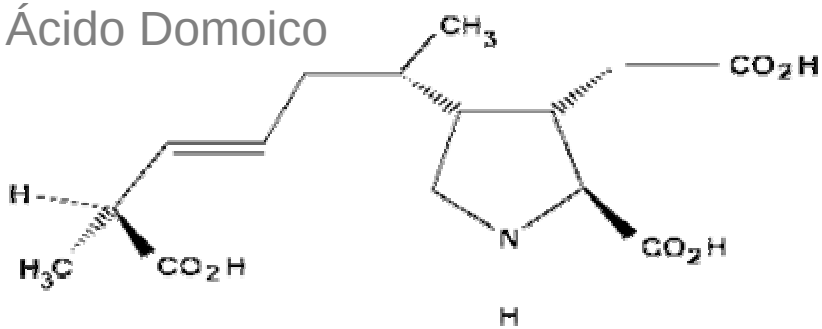
Figure 1: The structure of microcystin and its biosynthetic pathways (left). The gene organization is shown on the top. The amino acids in the X and Z positions represent variable amino acids. *Anabaena* (top right) and *Microcystis* (bottom) as seen under a microscope.

Toxinas



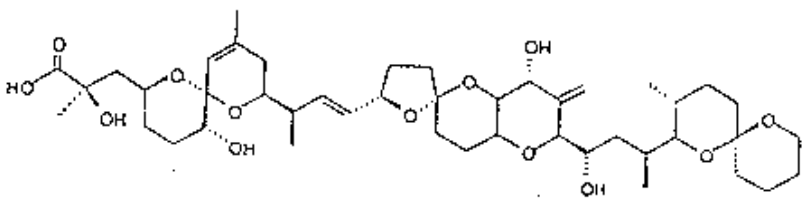
ciguatoxina

Ácido Domoico

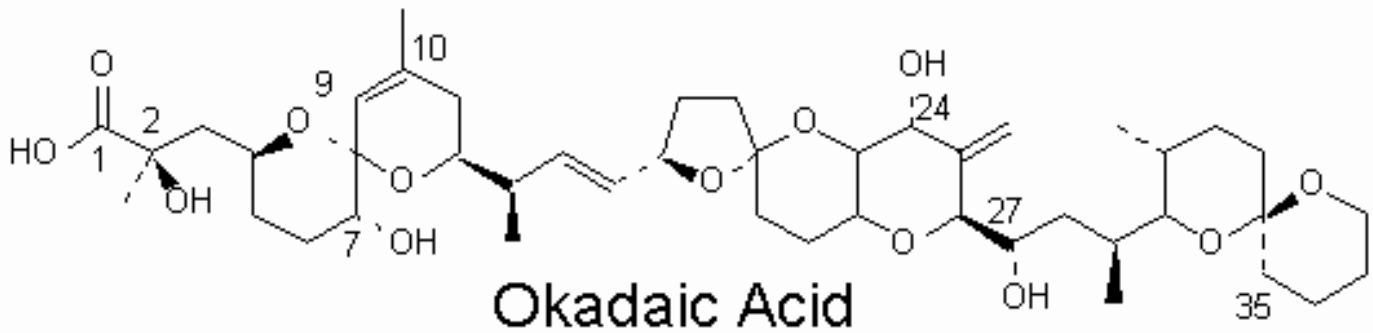


?

Ácido okadaico



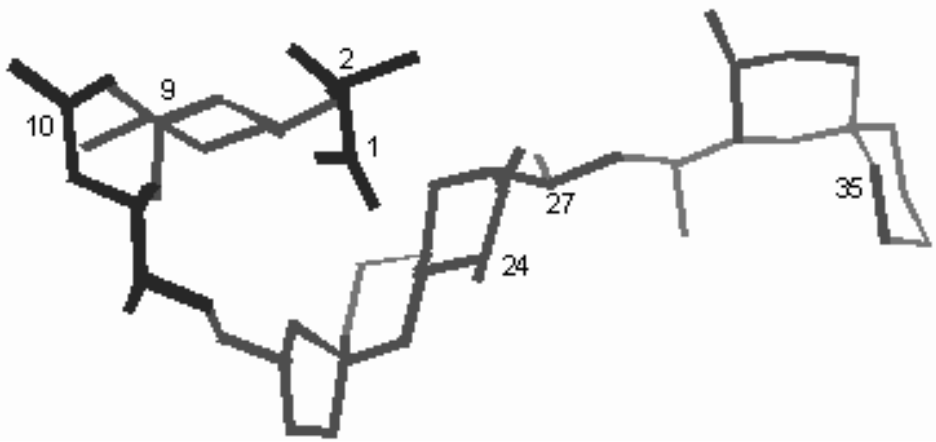
A



Okadaic Acid

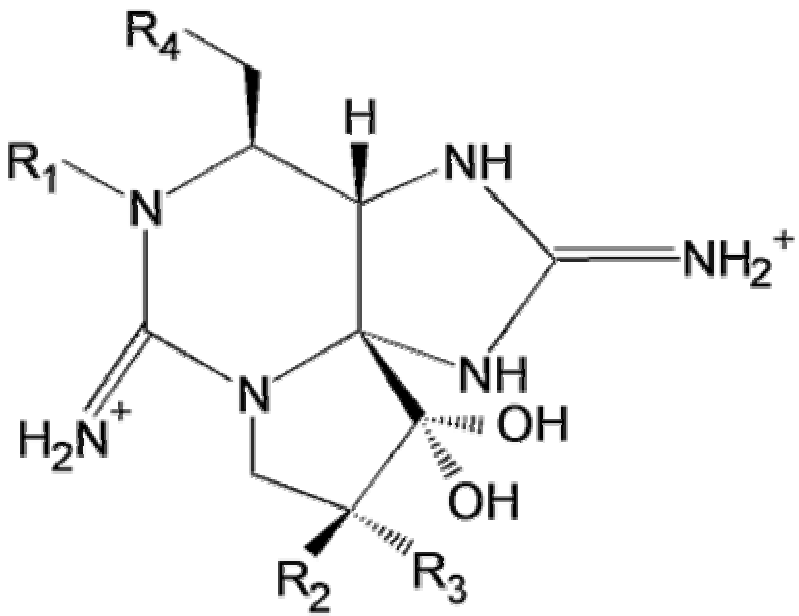
?

B

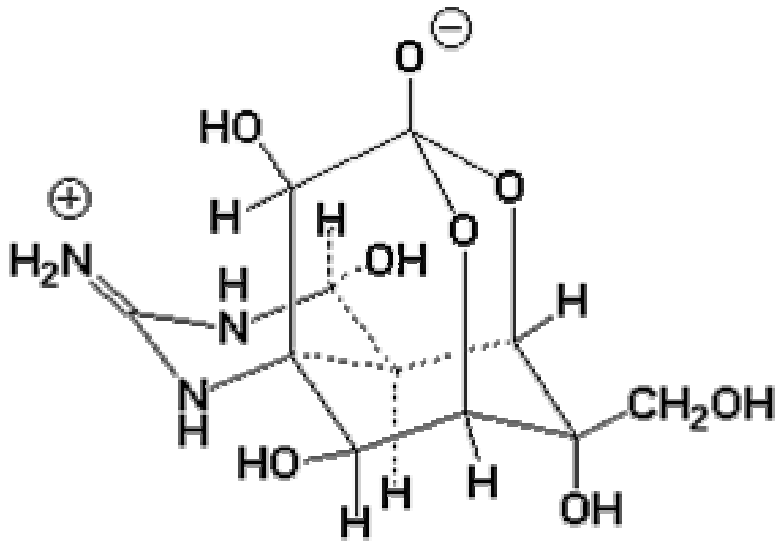


	IC_{50}
PP-1	20 nM
PP-2A	0.2 nM
PP-2B	5.0 μM

?



PSP toxins



Tetrodotoxin

Useful references.

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Current Opinion in Chemical Biology 2: 339- 345.

Polyketide biosynthesis beyond the type I, II and III PKS paradigms. Shen, B. 2003. Current Opinion in Chemical Biology 7: 285- 295.

Building block selectivity of polyketide synthases.
Liou, G. F. and Khosla, C. 2003. Current Opinion in Chemical Biology 7: 279-284.