Rutas bio-sintéticas de antibióticos y toxinas

Dra. Mónica Vásquez
Lab. Biotecnología
INTA

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*

Some clinically important antibiotics			
Antibiotic	**Producer organism**	**Activity**	**Site or mode of action**
Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria	Wall synthesis
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum	Wall synthesis
Griseofulvin	*Penicillium griseofulvum*	Dermatophytic fungi	Microtubules
Bacitracin	*Bacillus subtilis*	Gram-positive bacteria	Wall synthesis
Polymyxin B	*Bacillus polymyxa*	Gram-negative bacteria	Cell membrane
Amphotericin B	*Streptomyces nodosus*	Fungi	Cell membrane
Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria	Protein synthesis
Neomycin	*Streptomyces fradiae*	Broad spectrum	Protein synthesis
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria	Protein synthesis
Tetracycline	*Streptomyces rimosus*	Broad spectrum	Protein synthesis
Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria	Protein synthesis
Gentamicin	*Micromonospora purpurea*	Broad spectrum	Protein synthesis
Rifamycin	*Streptomyces mediterranei*	Tuberculosis	Protein synthesis

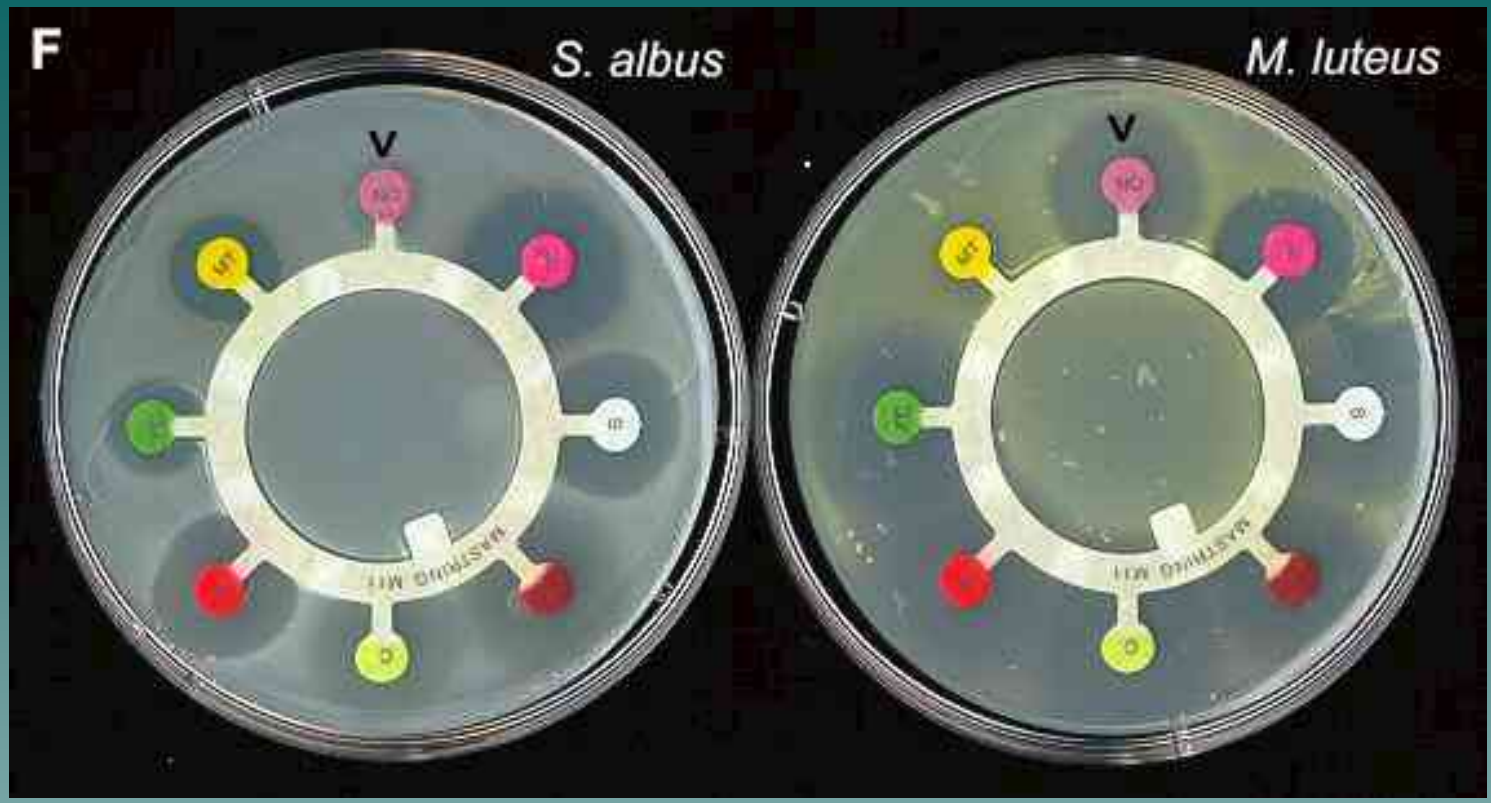


Figure F. Antibiotic-sensitivity testing. Petri dishes were spread-inoculated with *Staphylococcus albus* (white growth) or *Micrococcus luteus* (yellow growth) before antibiotic assay "rings" were placed on the agar surface. The coloured disks at the end of each spoke of the rungs are impregnated with different antibiotics. Clockwise from the top (arrow) these are: Novobiocin, Penicillin G, Streptomycin (white disk), Tetracycline, Chloramphenicol, Erythromycin, Fusidic acid (green disk) and Methicillin. Clear zones of suppression of bacterial growth around the individual antibiotic disks are evidence of sensitivity to these antibiotics.

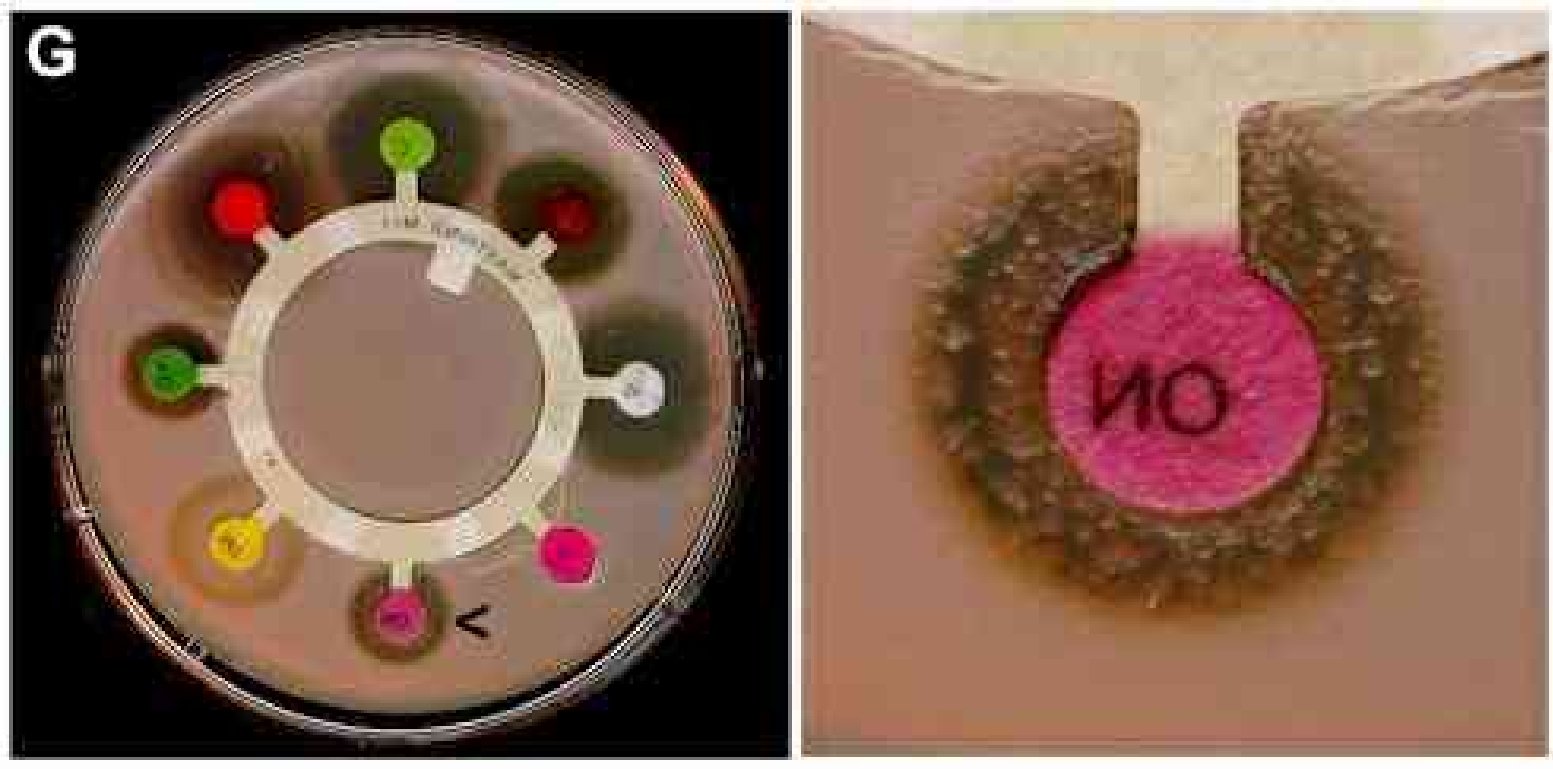


Figure G. Effects of different antibiotics on growth of a *Bacillus* strain. The right-hand image shows a close-up of the novobiocin disk (marked by an arrow on the whole plate). In this case some individual mutant cells in the bacterial population were resistant to the antibiotic and have given rise to small colonies in the zone of inhibition.

➢One of the most important examples concerns multiple-resistant strains of ***Staphylococcus aureus*** in hospitals.

➢ Some of these strains cause serious nosocomial (hospital-acquired) infections and are resistant to virtually all the useful antibiotics, including **methicillin**, **cephalosporins** and other **beta-lactams** that target peptidoglycan synthesis, the **macrolide antibiotics** such as **erythromycin** and the **aminoglycoside antibiotics** such as **streptomycin** and **neomycin**, all of which target the bacterial ribosome.

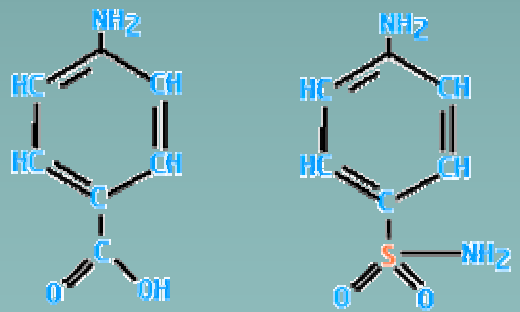
➢The only compound that can be used effectively against these staphylococci is an older antibiotic, **vancomycin**, which has some undesirable effects on humans. Recently, some clinical strains of *S. aureus* have developed resistance to even this compound.

Types of antibiotics

Sulfa Drugs and Folic Acid Analogs

Sulfanilamide was the first antibacterial agent. Many other sulfa drugs (such as sulfamethoxazole) have since come into use.

Both bacteria and their human hosts require folic acid for nucleic acid synthesis (it is converted into purines and thymidine) as well as protein synthesis (precursor of the amino acids methionine and glycine)
However, bacteria **synthesize** their folic acid starting with **para-aminobenzoic acid** (**PABA**), while we must ingest our folic acid already formed; that is, for us it is a **vitamin**.
Sulfanilamide, and the other sulfa drugs, are analogs of PABA; they compete with PABA and, when chosen, block the synthesis of folic acid.
Mammals ignore PABA and its analogs and thus can tolerate sulfa drugs.

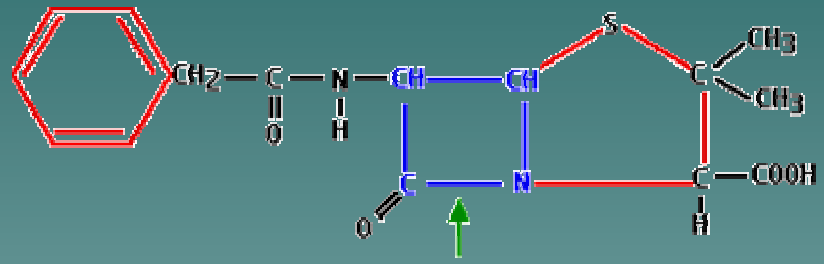


Para-aminobenzoic Acid (PABA)

Sulfanilamide

Sulfa Drugs

The **beta-lactams** get their name from the characteristic ring structure — shown here in blue — that they all share. (The green arrow shows the bond that is broken by the beta-lactamases that are synthesized by many penicillin-resistant bacteria.)



Penicillin G

The beta-lactam antibiotics bind to and inhibit enzymes needed for the synthesis of the peptidoglycan wall. While they have little effect on resting bacteria, they are lethal to dividing bacteria as defective walls cannot protect the organism form bursting in hypotonic surroundings.

Aminoglycosides

Examples are:
streptomycin
kanamycin
neomycin
gentamycin

The 70S bacterial ribosome differs in several ways from the 80S eukaryotic ribosome. The aminoglycosides bind to the **30S** subunit of the bacterial ribosome and interfere with the formation of the initiation complex cause misreading of the mRNA.

Tetracyclines

These are natural products derived from soil actinomycetes or their semi-synthetic derivatives. Examples:
chlortetracycline (trade name = "aureomycin")
oxytetracycline (trade name = "terramycin")
doxycycline

Tetracyclines bind to the **30S** subunit of the bacterial ribosome. They prevent the transfer of activated amino acids to the ribosome so protein synthesis is halted.

Macrolides, Lincosamides, Streptogramins

All these antibiotics bind to the 23S rRNA molecule in the large (**50S**) subunit of the bacterial ribosome where they block the elongation of the growing peptide chain.

Because of their similar action, the development of antibiotic resistance to one usually extends to all the others.

Fluoroquinolones

Ciprofloxacin (Cipro®), levofloxacin and moxifloxacin are examples. Cipro is the preferred antibiotic for people who have been intentionally exposed to anthrax, although some other antibiotics appear to be equally effective.

The fluoroquinolones block the action of two bacterial topoisomerases — enzymes that relieve the coils that form in DNA when the helix is being opened in preparation for
replication or
transcription or
repair
The topoisomerases in eukaryotes are not affected.

Others

Polypeptides
The most common of these are the **polymixins**.
They behave as detergents, increasing the permeability of the membranes that encase bacteria and causing the contents of the bacterial cell to leak out.

Rifampin
This semi-synthetic antibiotic binds to the bacterial **RNA polymerase** and prevents it from carrying out its role in transcription. Its affinity for the equivalent eukaryotic enzyme is much lower. Rifampin is also known as rifampicin.

Mupirocin
This antibiotic blocks the action of the bacterial **isoleucine tRNA synthetase**, the enzyme responsible for attaching the amino acid isoleucine (Ile) to its tRNA in preparation for protein synthesis, so protein synthesis is inhibited. It spares the equivalent eukaryotic enzyme.

Cycloserine
Cycloserine inhibits synthesis of the bacterial cell wall but by a different mechanism than the beta-lactam antibiotics discussed above. Cycloserine is an analog of **D-alanine** and blocks the incorporation of D-alanine into the peptide bridges in the bacterial cell wall (look back). It is derived from an actinomycete.

Aminocyclitols
These products of another actinomycete achieve their effect by interfering with the **30S** subunit of the bacterial ribosome. **Spectinomycin** (trade name = Trobicin) is an example. It is particularly effective against the gonococcus, the bacterium that causes the sexually-transmitted disease (STD) gonorrhea.

Glycopeptides

Glycopeptides also interfere with the synthesis of the bacterial cell wall but by a different mechanism than the beta-lactams.

Vancomycin is a widely-used glycopeptide in the U.S. It binds to the **D-alanines** on the precursors of the peptidoglycan cross bridges preventing their cross-linking (look back). It has become the antibiotic of last resort as resistance to the other antibiotics has become more and more common.

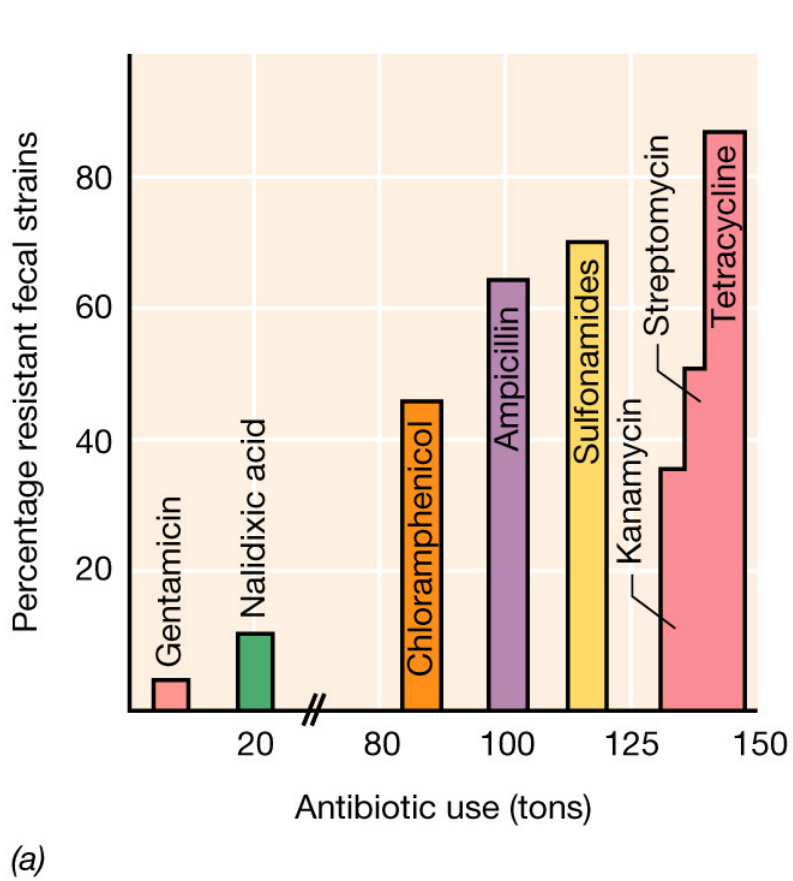
Oxazolidinones

The first of these new antibiotics, **linezolid** (trade name = **Zyvox**), was approved by the U.S. Food and Drug Administration on 19 April 2000. It is effective against many gram-positive bacteria that have developed resistance to the older antibiotics.

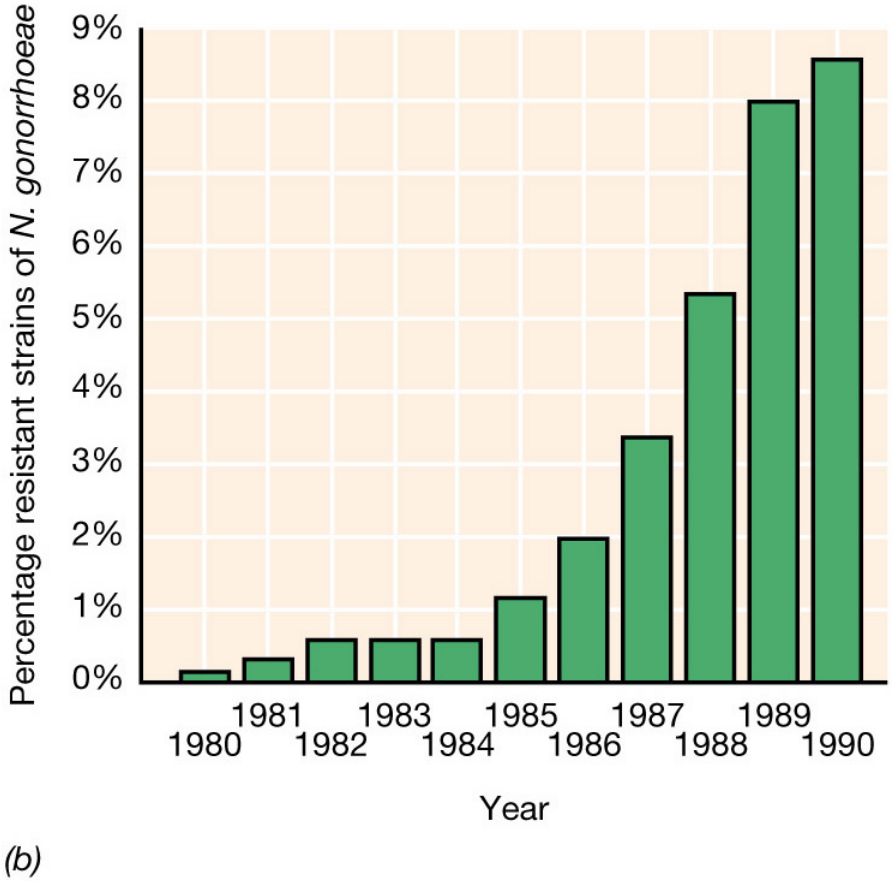
Zyvox attacks a previously-unexploited chink in the bacterium's armor: the proper assembly of the complex of **fMet-tRNA** with the messenger RNA (**mRNA**) and the two ribosomal subunits. Eukaryotes do not begin translation with fMet.

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



(a)



(b)

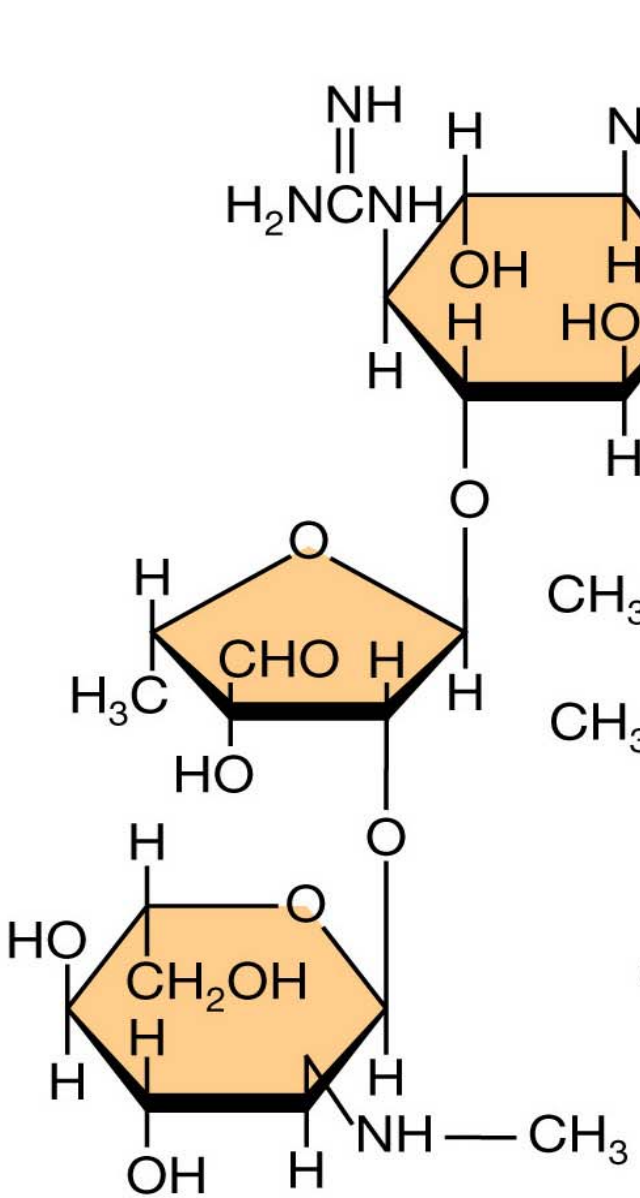
The emergence of antimicrobial drug-resistant bacteria.

(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.

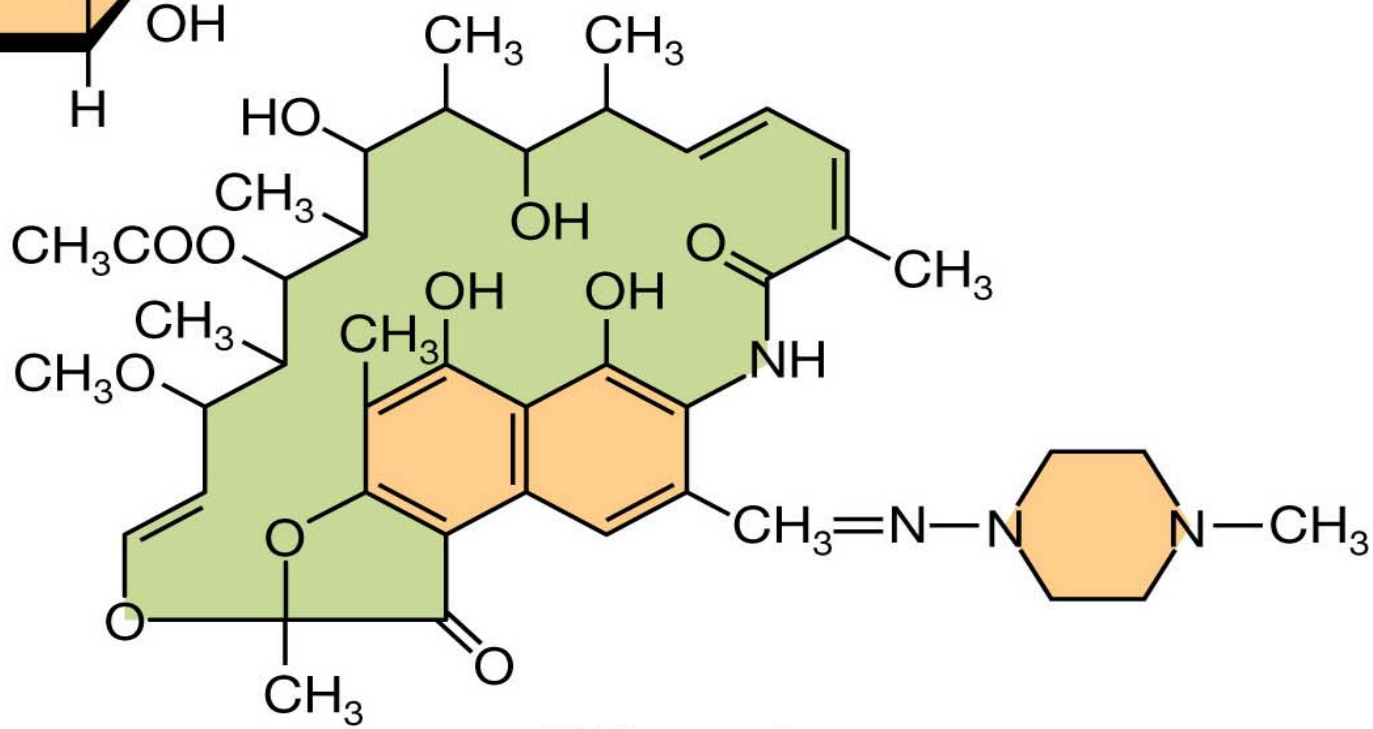
(b) Percentage of reported cases of gonorrhea caused by drug-resistant strains. The actual number of reported drug-resistant cases in 1985 was 9000. This number rose to 59,000 in 1990. Greater than 95% of the reported drug-resistant cases are due to penicillinase-producing strains of *Neisseria gonorrhoeae*. 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.

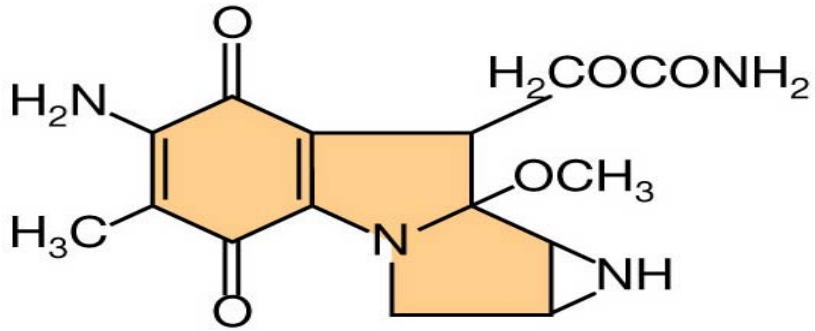


Streptomycin

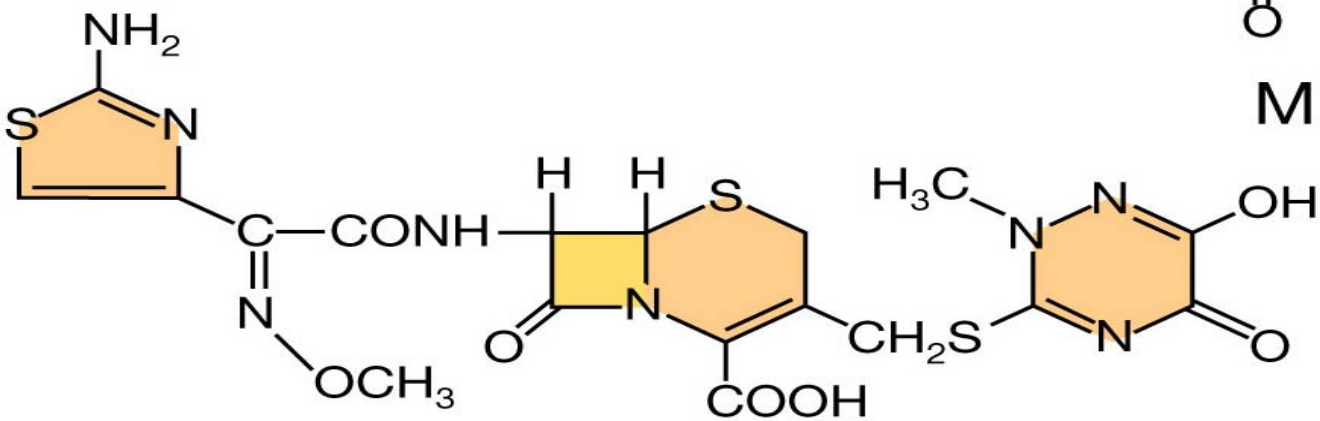


Rifampin

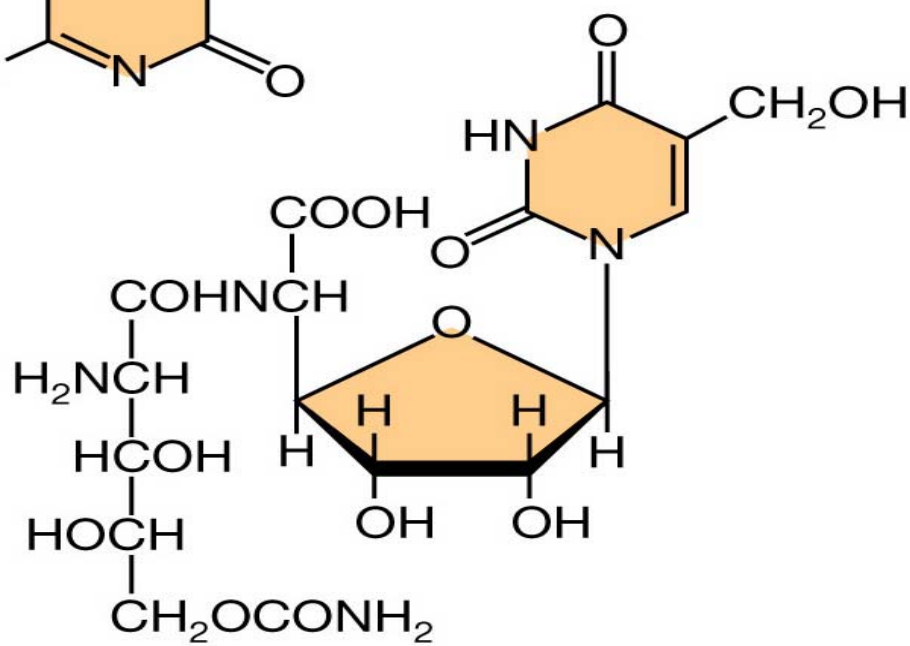
Representative structure



Mitomycin C



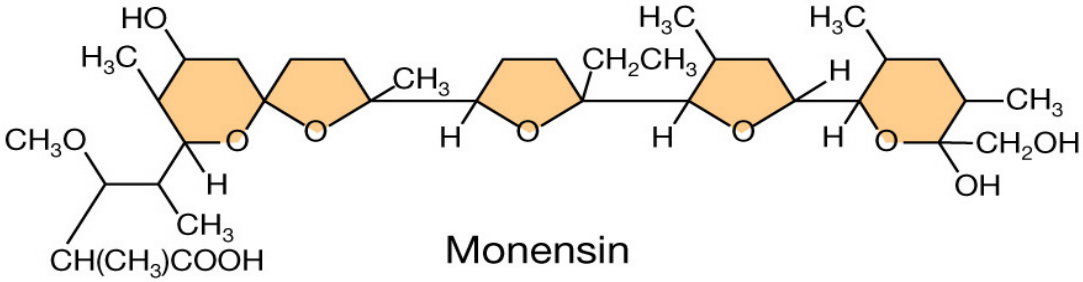
Ceftriaxone



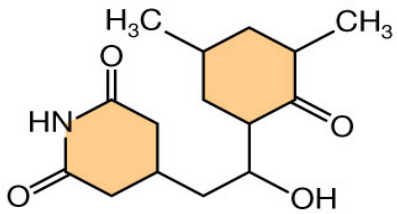
Polyoxin B

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

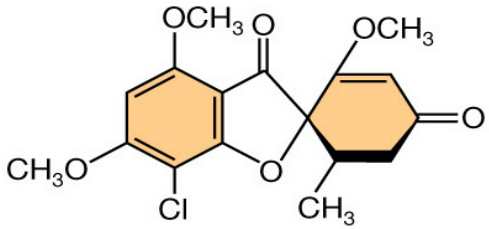
Representative structure



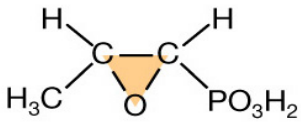
Monensin



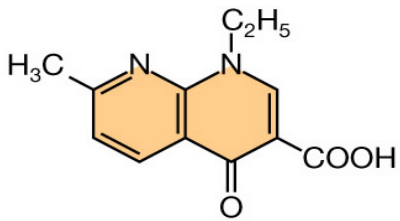
Cycloheximide



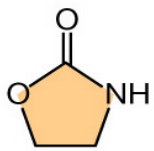
Griseofulvin



Fosfomycin

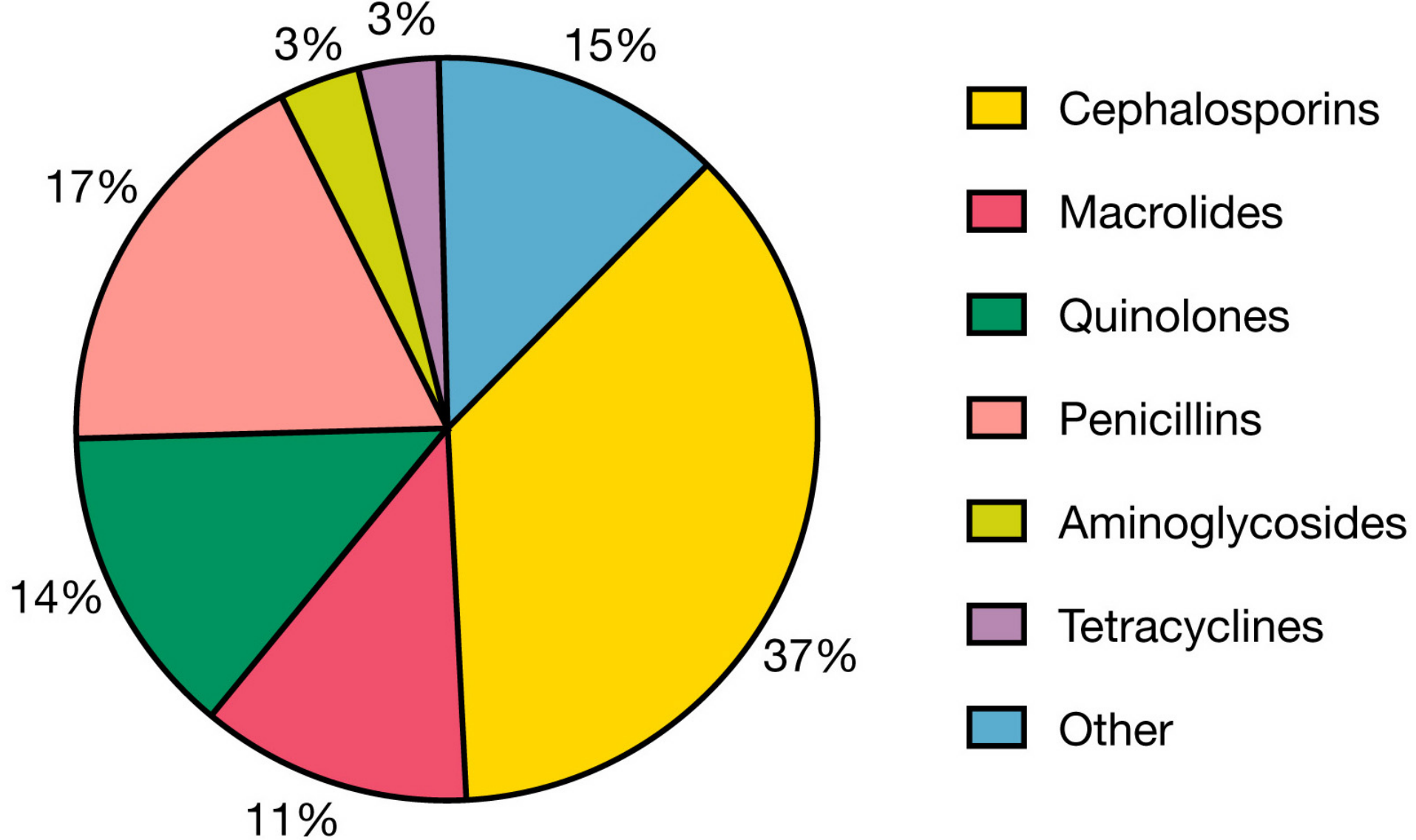


Nalidixic acid

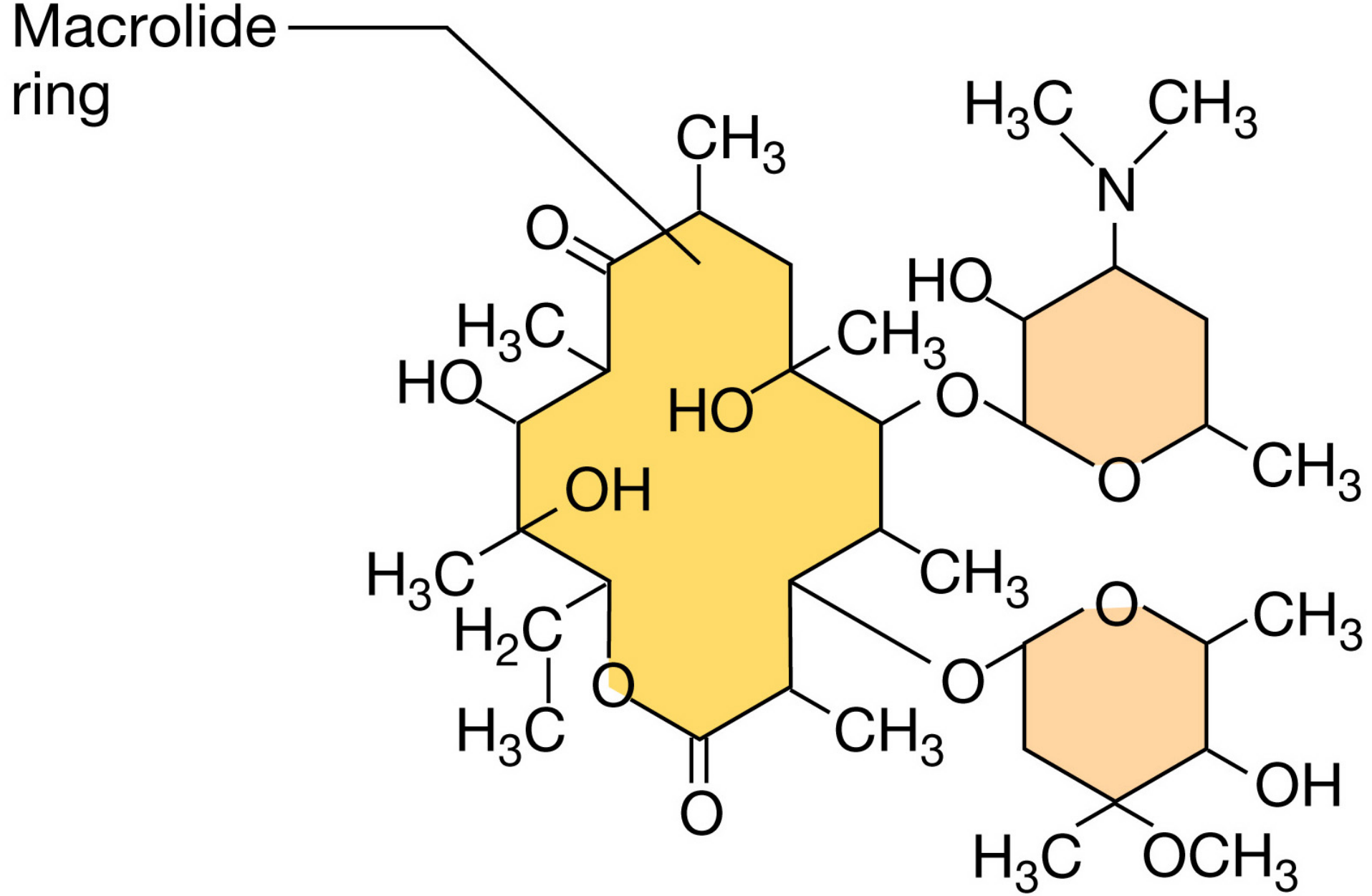


2-Oxazolidinone

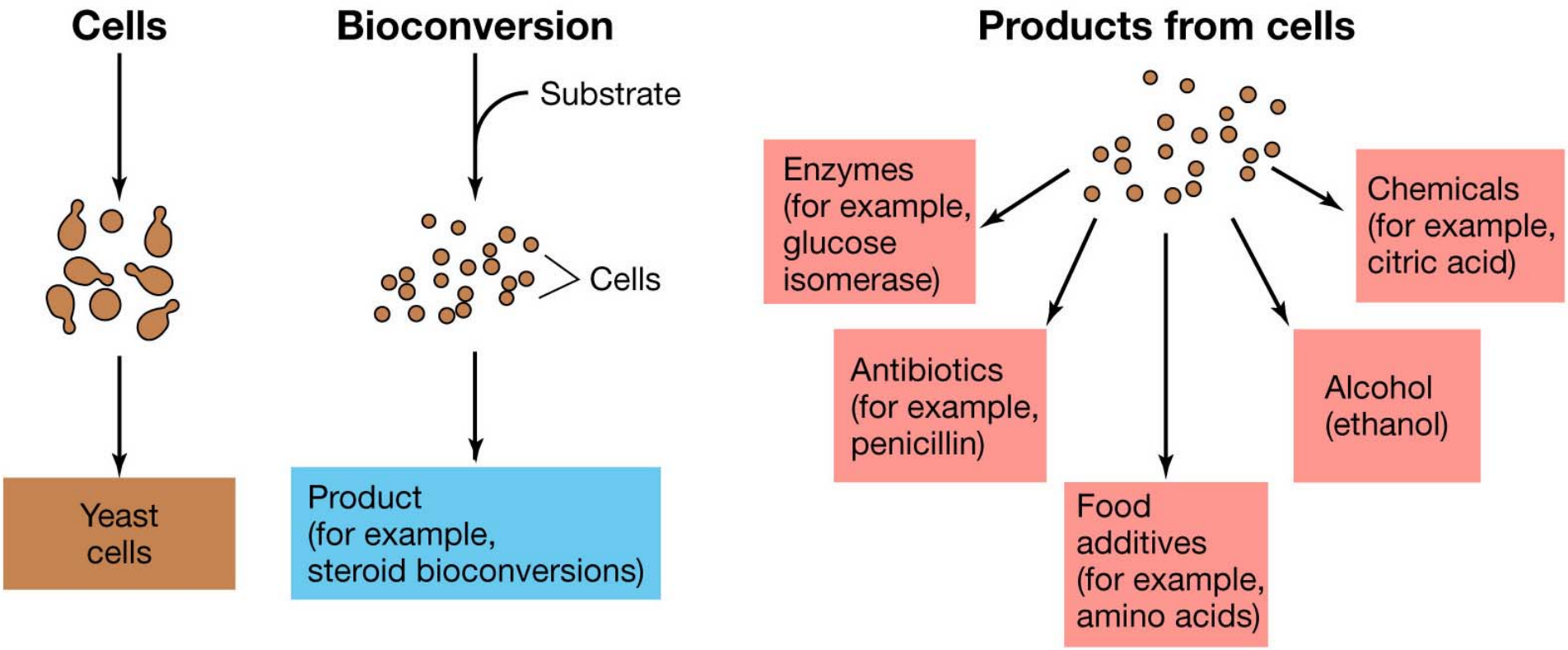
Antibacterial chemotherapeutic agents according to chemical structure.



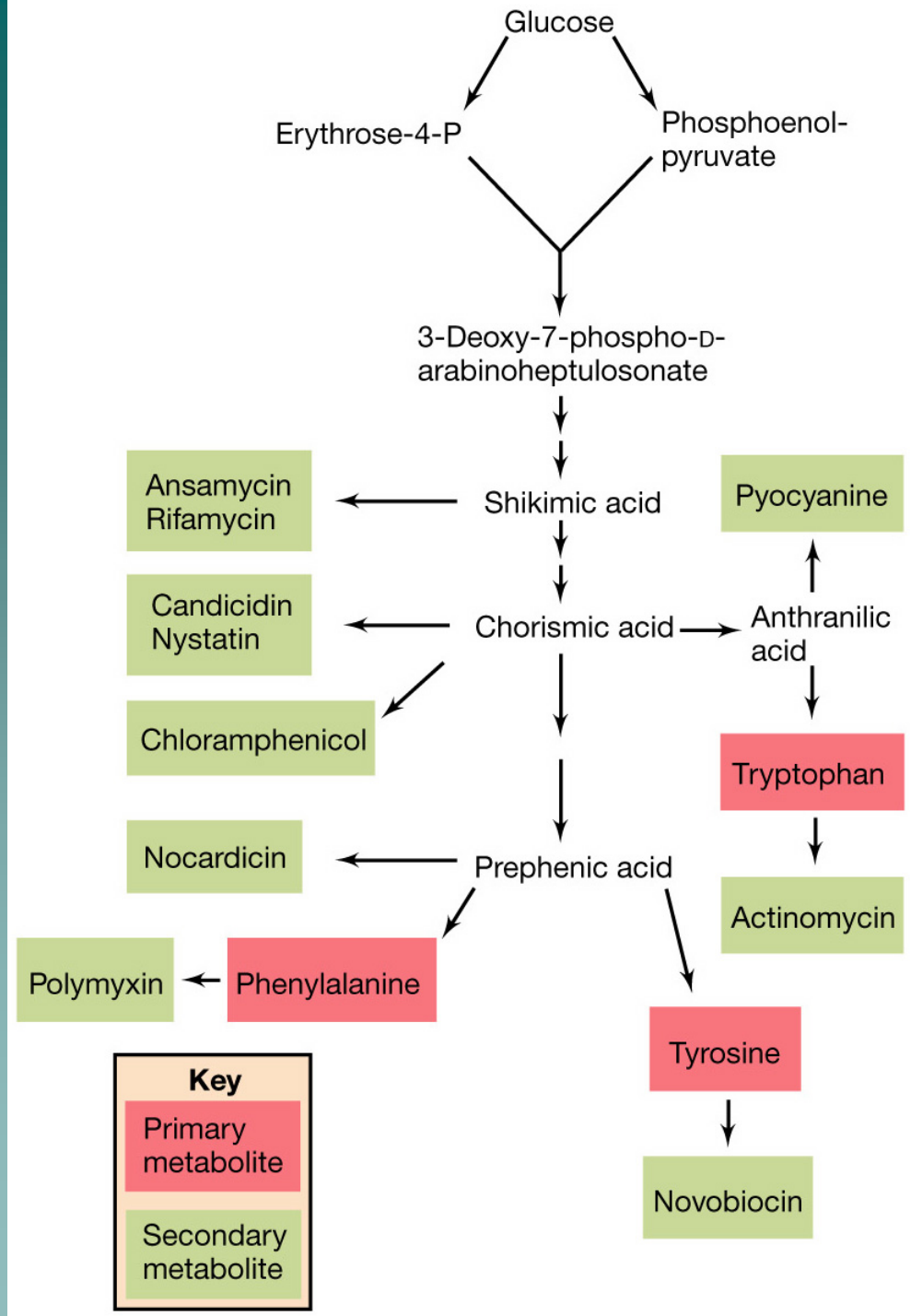
Annual worldwide production and use of antibiotics. Each year more than 500 metric tons of chemotherapeutic agents are manufactured.



Structure of erythromycin, a macrolide antibiotic.

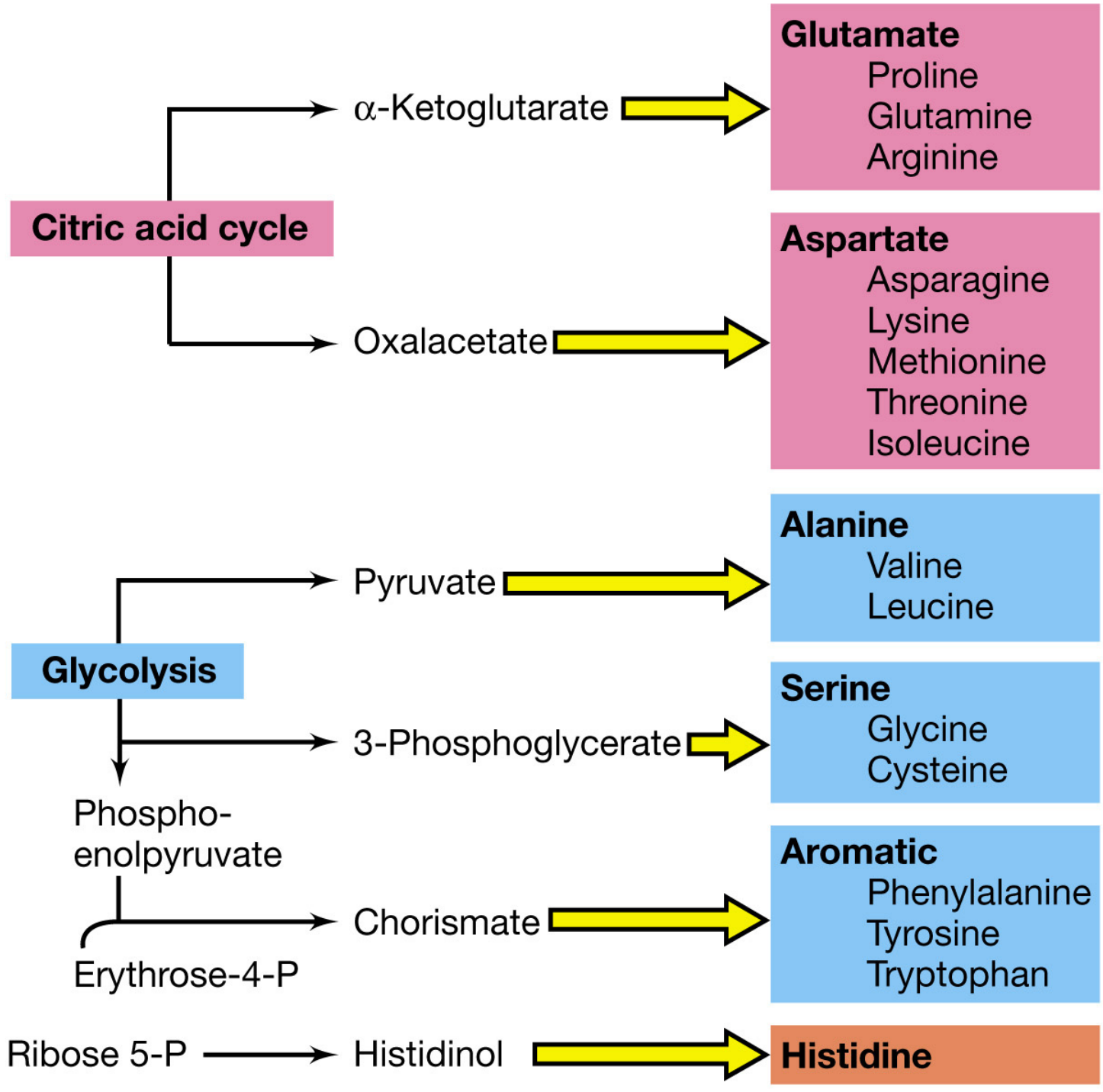


Products of industrial microbiology/biocatalysis. The products may be the cells themselves or products made from cells. In the case of bioconversion, cells are used to chemically convert a specific substance from one form to another.



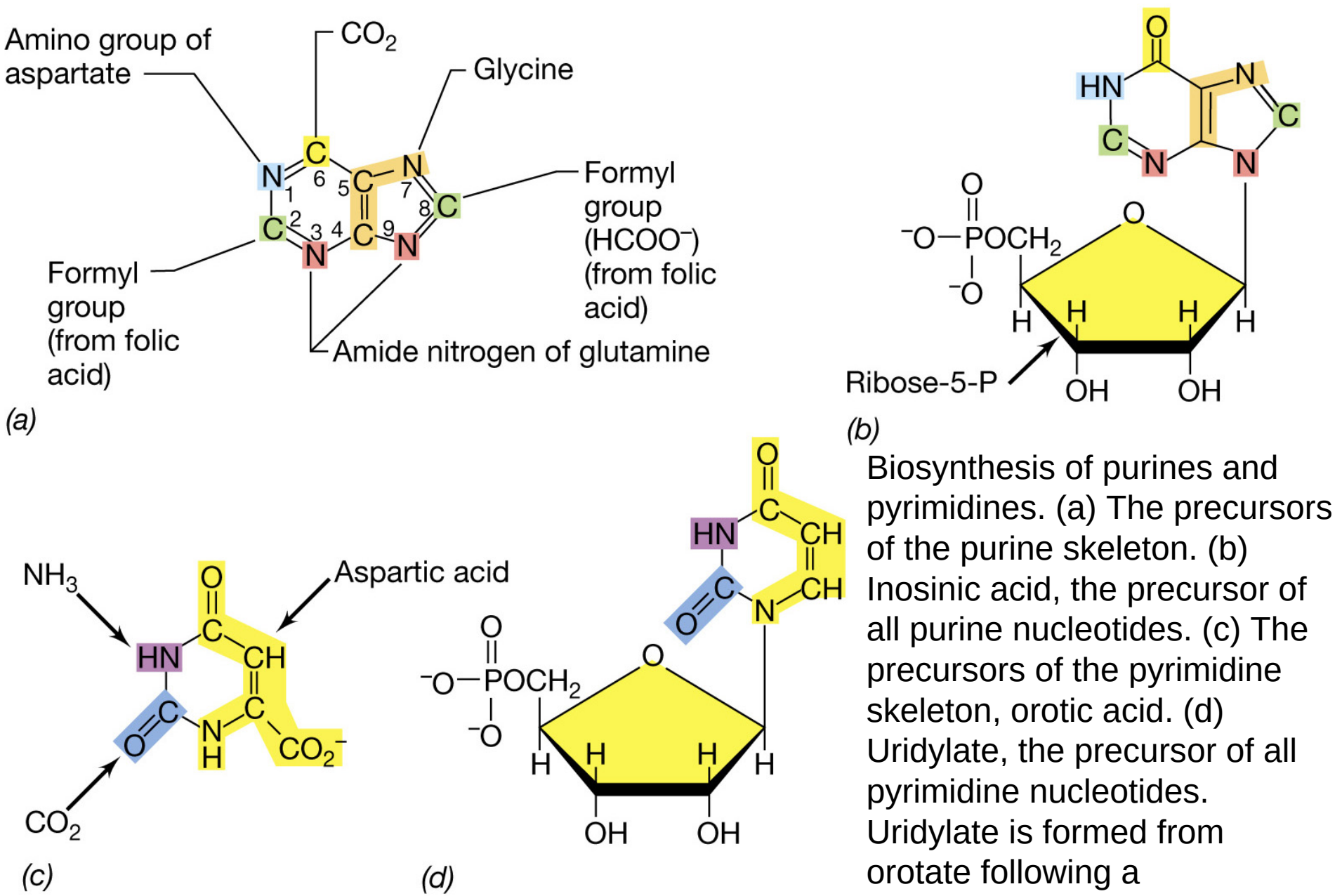
Relationship of the primary metabolic pathway for the synthesis of aromatic amino acids and formation of a variety of secondary metabolite antibiotics containing aromatic rings.

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.

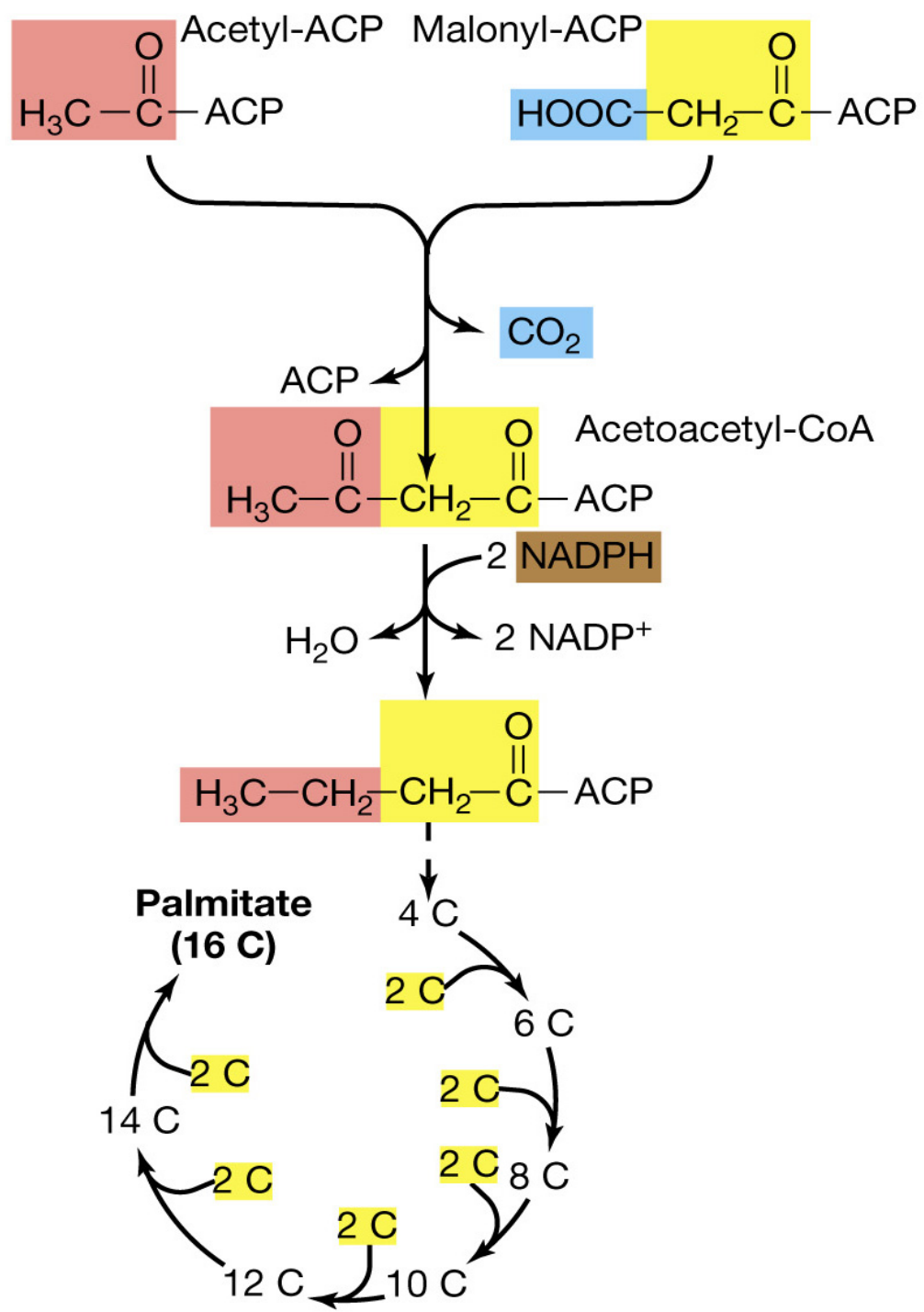


Amino acid families.

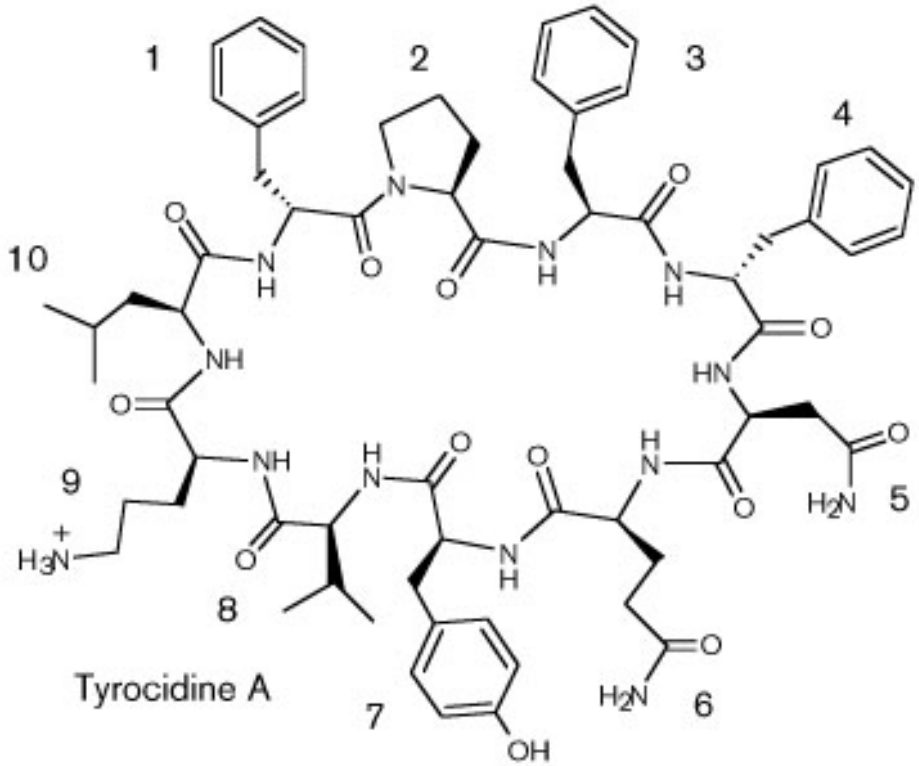
Note how the carbon skeletons for most amino acids are derived from either the citric acid cycle or from glycolysis. Synthesis of the various amino acids in a family frequently requires many separate enzymatically catalyzed steps starting from the parent amino acid (shown in bold).



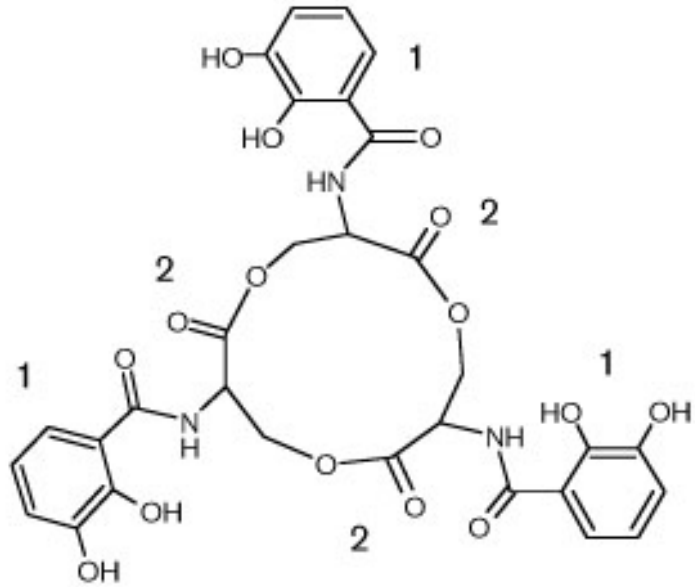
Biosynthesis of purines and pyrimidines. (a) The precursors of the purine skeleton. (b) Inosinic acid, the precursor of all purine nucleotides. (c) The precursors of the pyrimidine skeleton, orotic acid. (d) Uridylate, the precursor of all pyrimidine nucleotides. Uridylate is formed from orotate following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



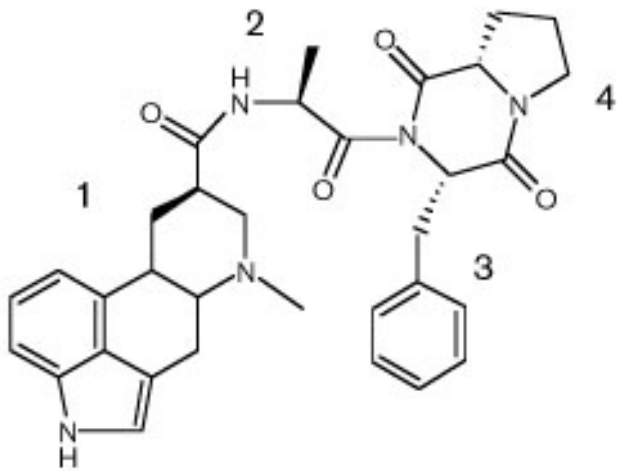
The biosynthesis of fatty acids; shown is the biosynthesis of the C16 fatty acid, palmitate. The condensation of acetyl-ACP and malonyl-ACP forms acetoacetyl-CoA. Each successive addition of an acetyl unit comes from malonyl-CoA.



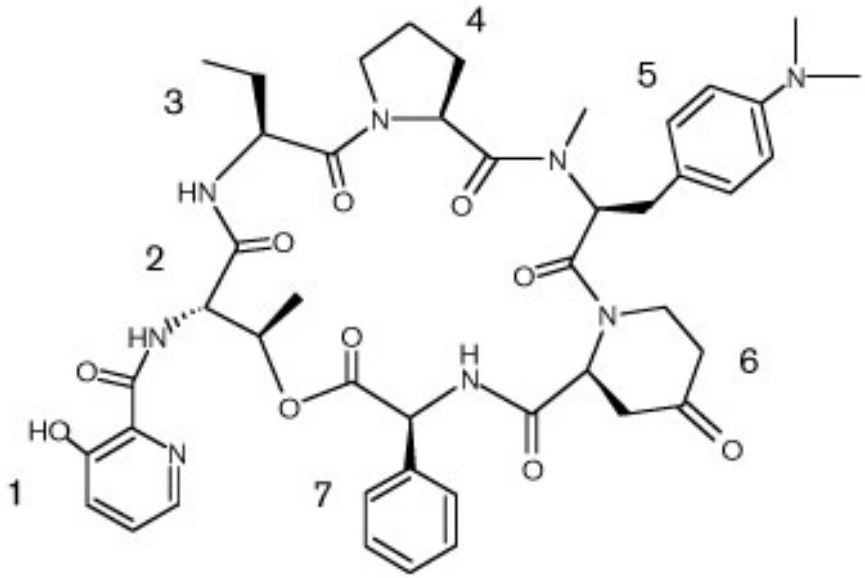
Tyrocidine A



Enterobactin



Ergotamine

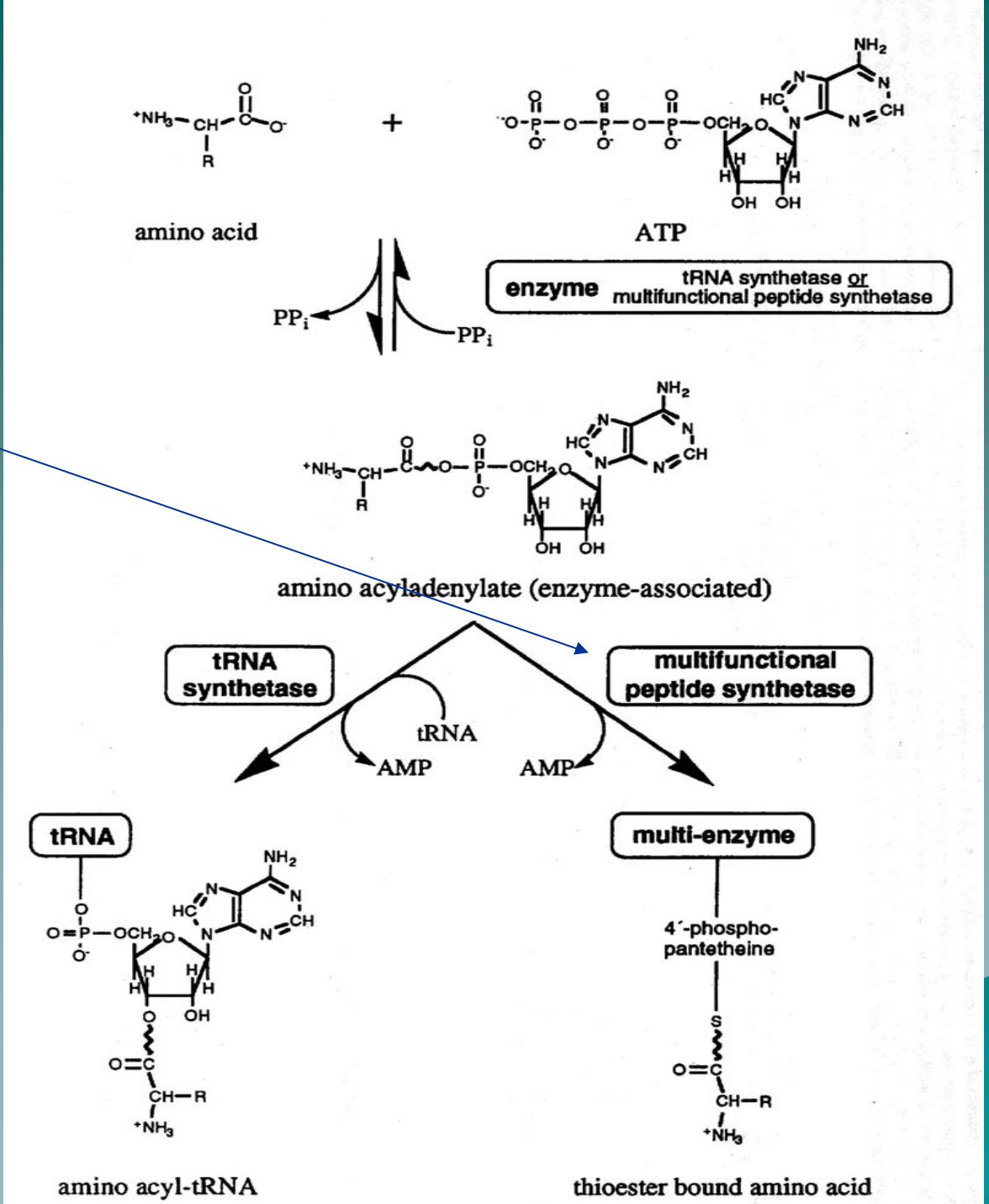


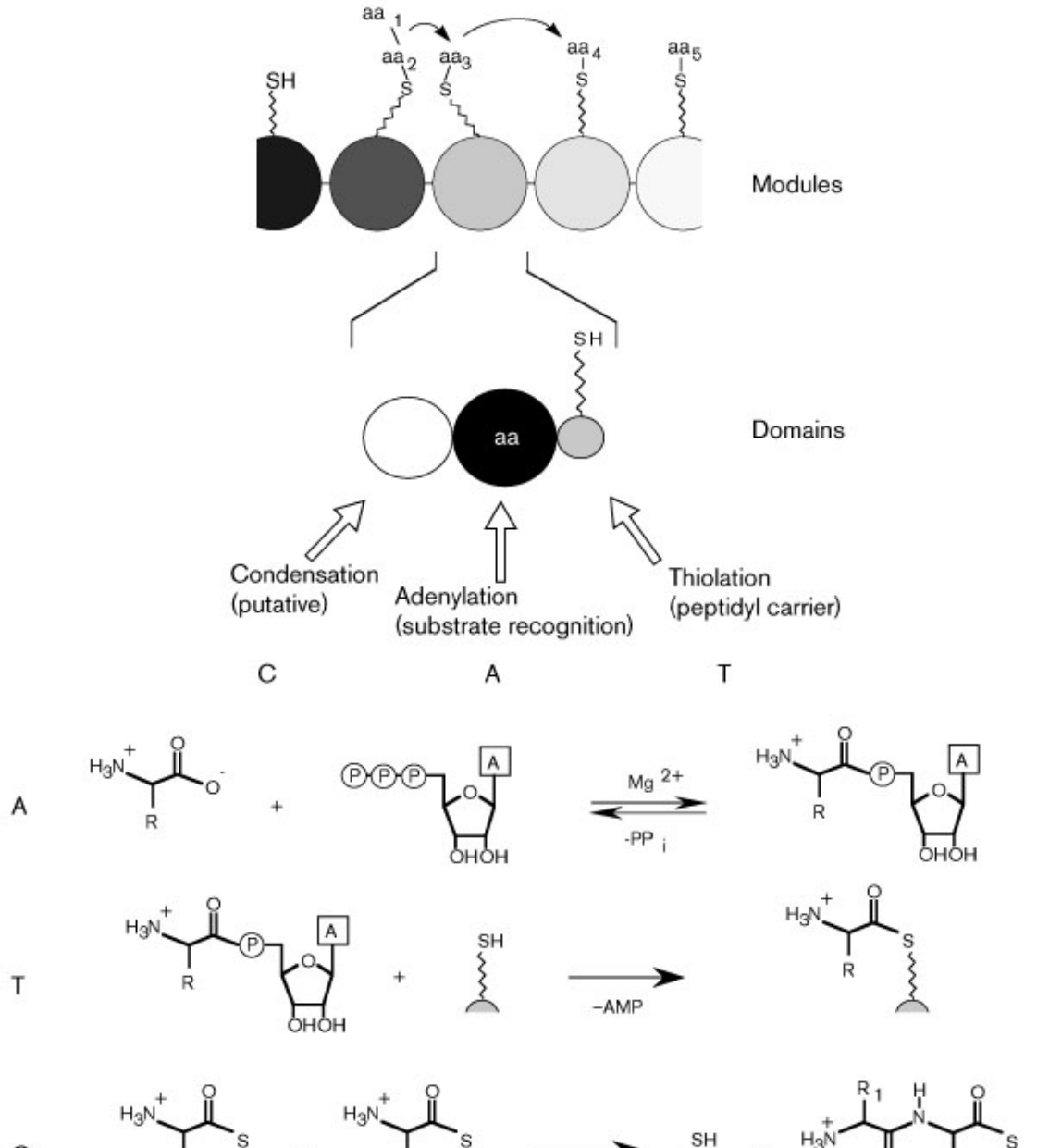
Pristinamycin I_A

- ¿how is the biosynthesis of those antibiotics?

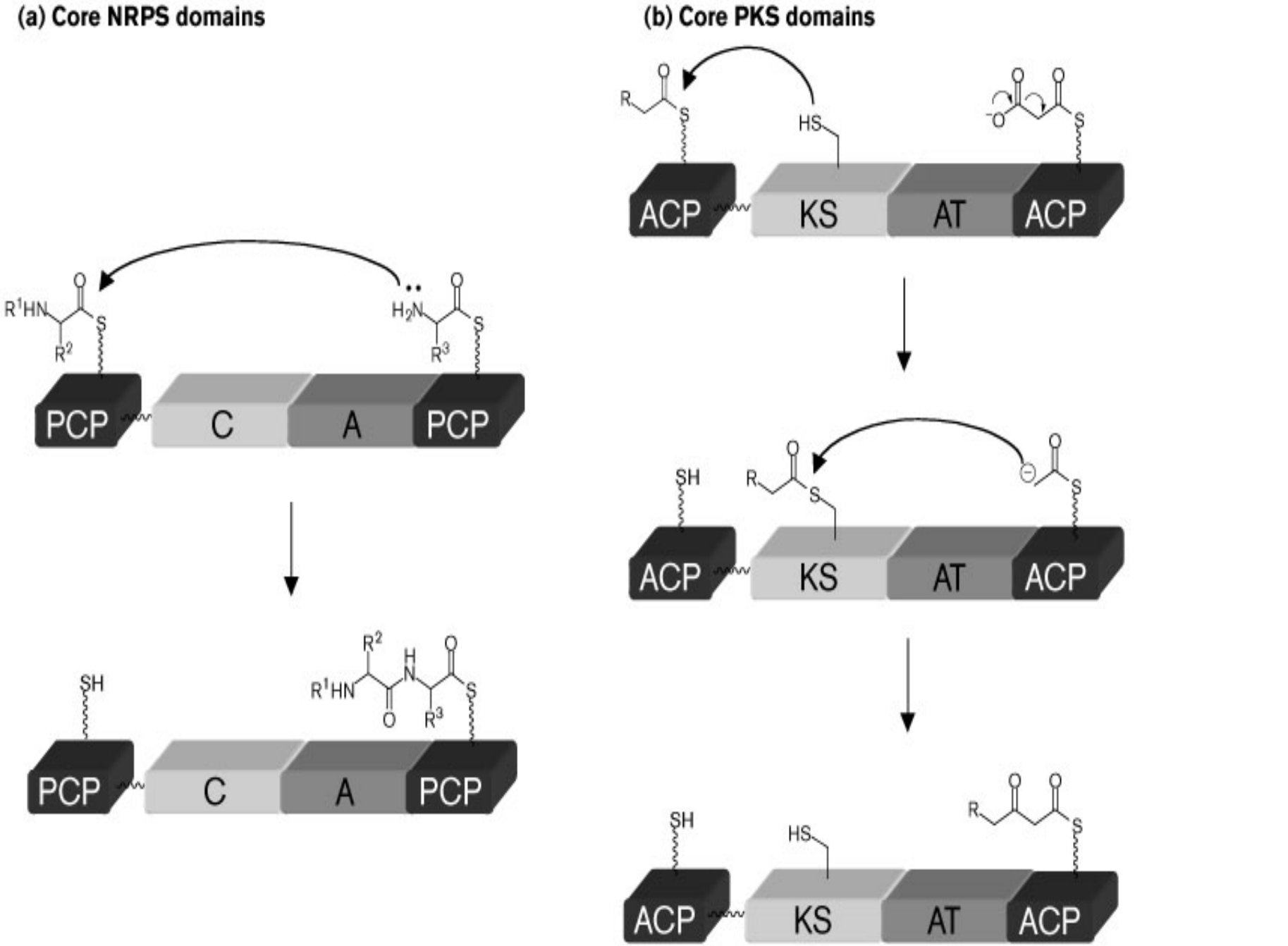
- Non ribosomal peptide synthetase
- Polyketide synthase

Non ribosomal peptide synthetase





Current Opinion in Chemical Biology



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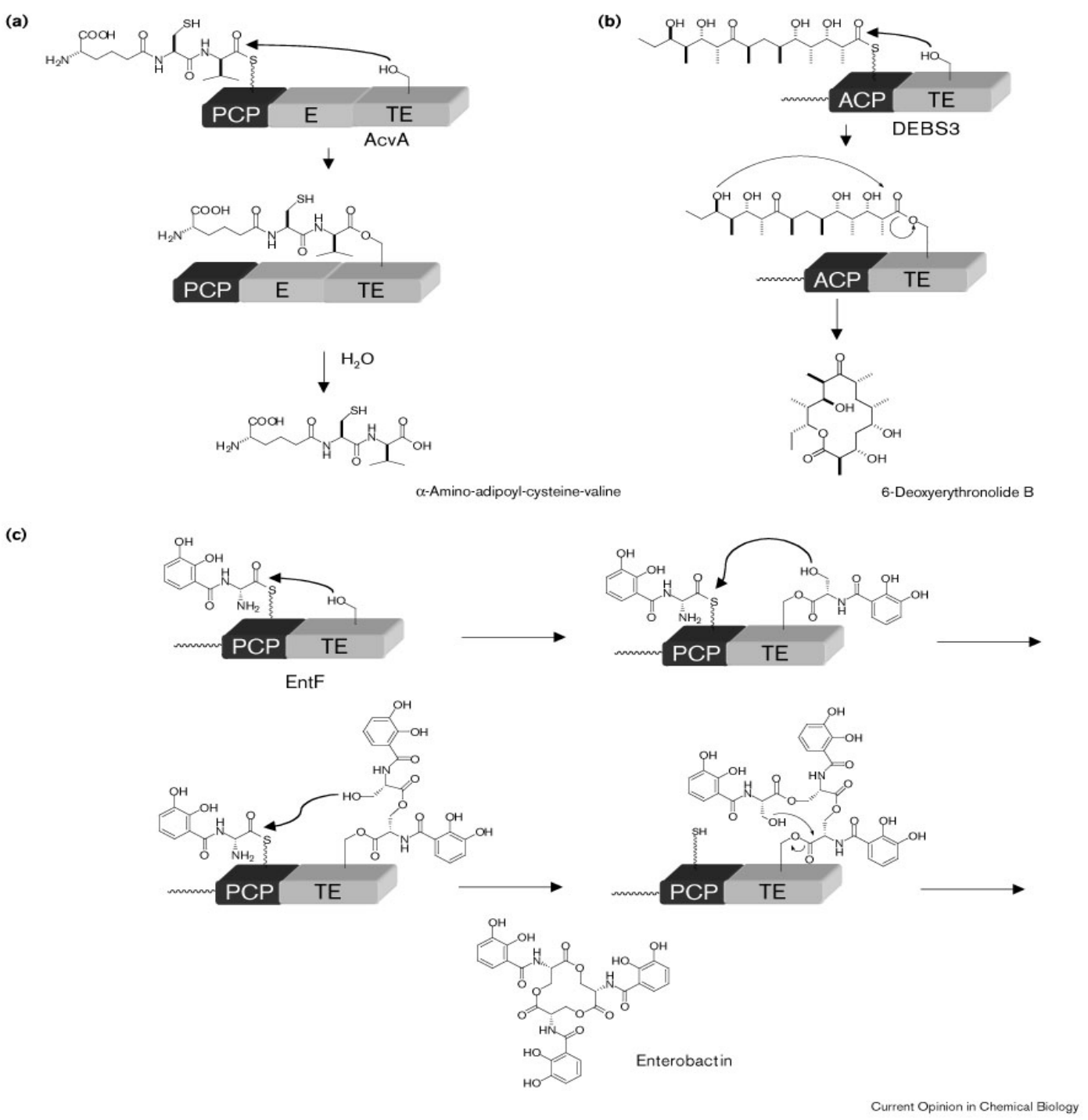

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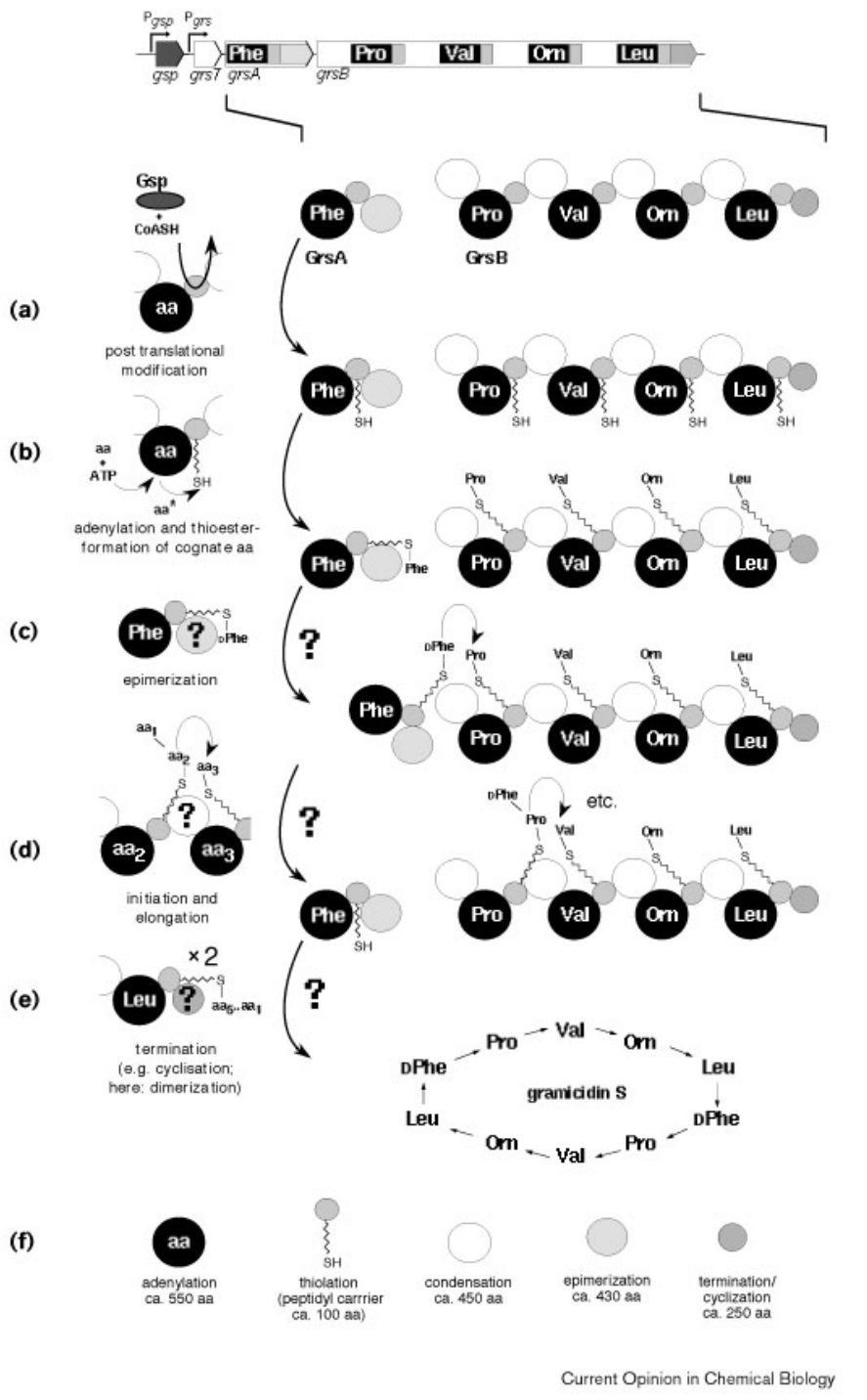

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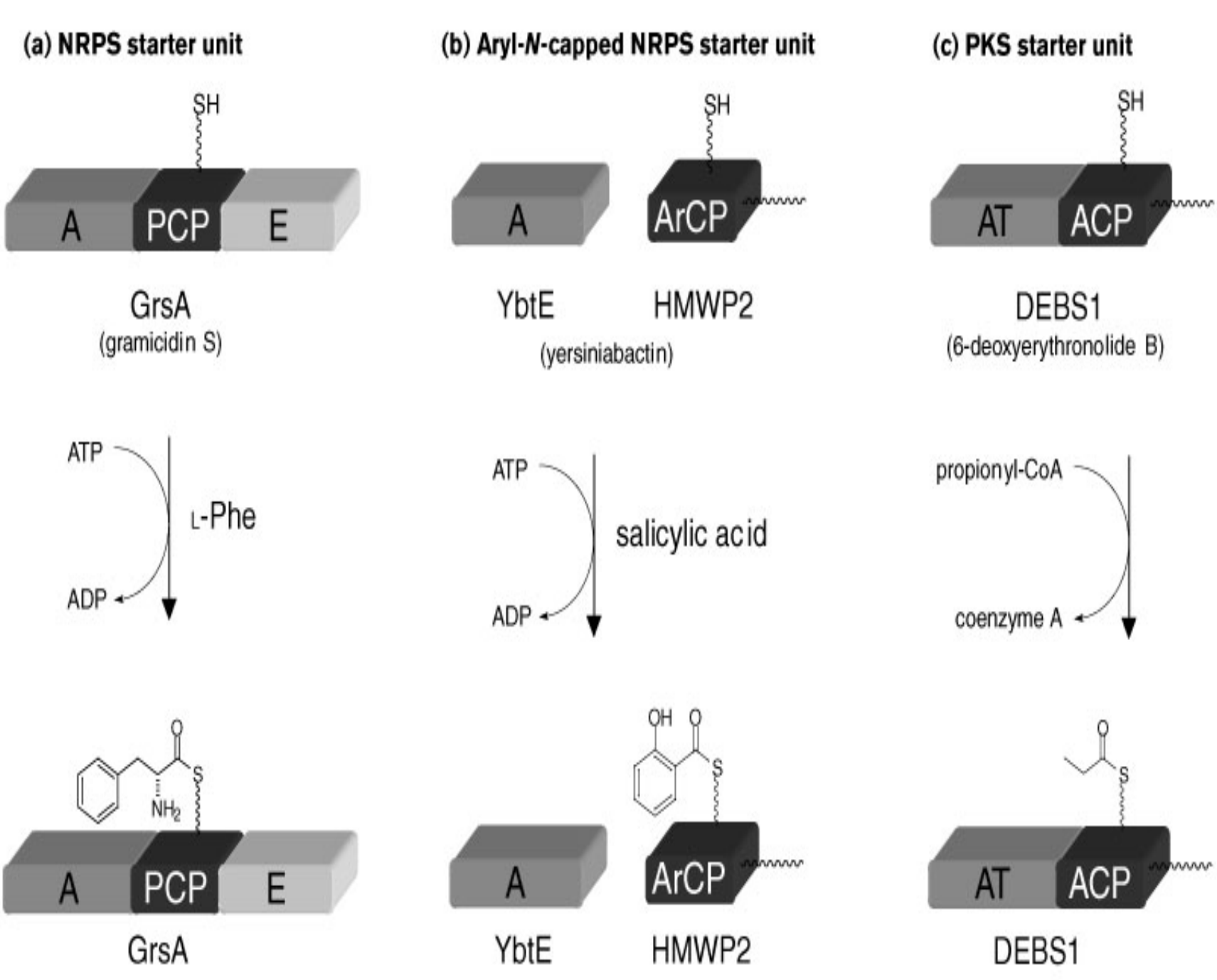
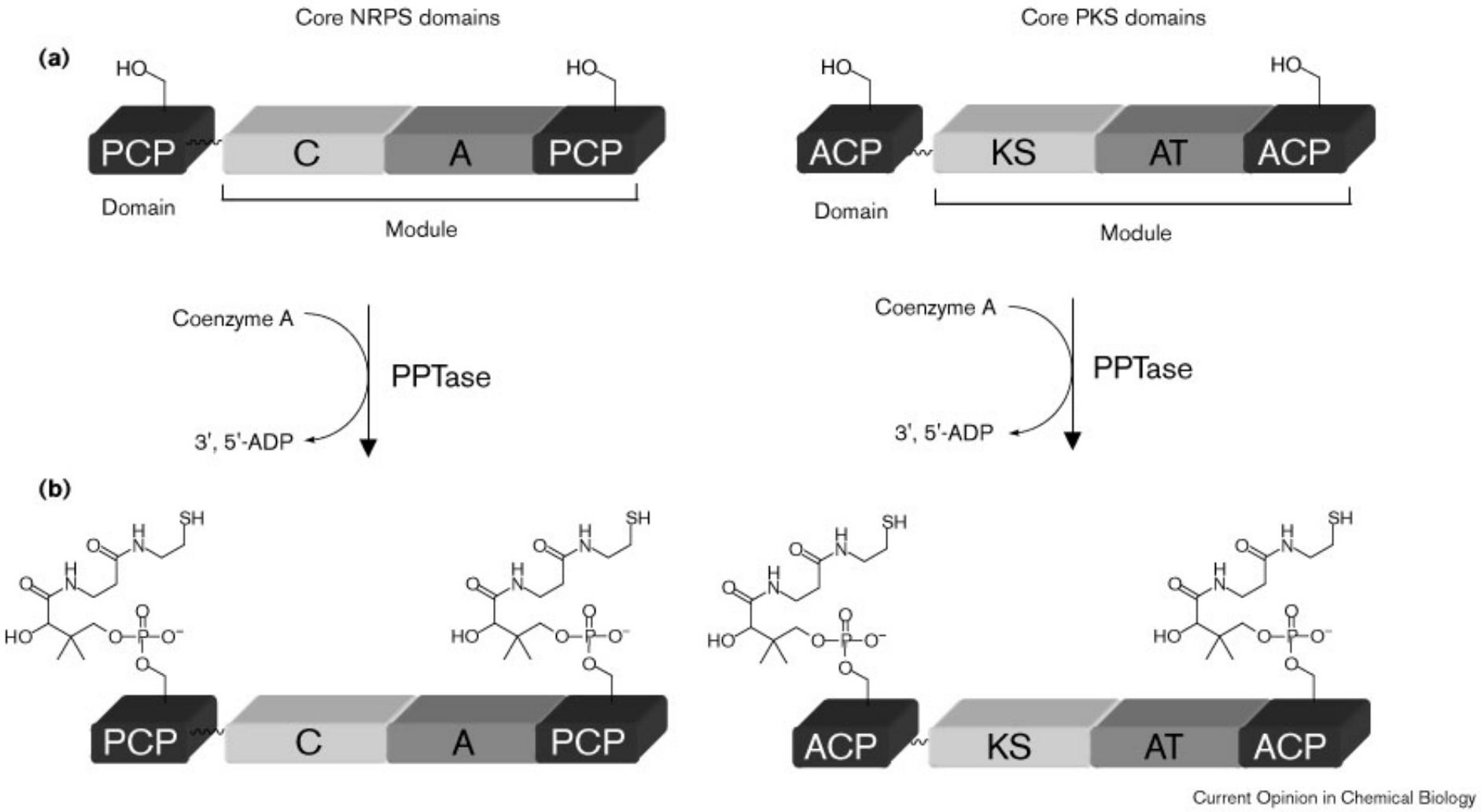


Current Opinion in Chemical Biology


Core NRPS domains
Core PKS domains
(a)
HO
PCP
C
A
PCP
Domain
Module
ACP
KS
AT
ACP
Coenzyme A
PPTase
3', 5'-ADP
(b)
SH
Current Opinion in Chemical Biology

Polyketide synthase

PKSs Tipo II están compuestas de enzimas discretas y monofuncionales (KS, ACP etc.), ejemplo PKSs aromaticos.

PKSs Tipo I las enzimas están covalentemente unidas.

PKSs Tipo I están compuestas de una o mas polipéptidos multienzimas

PKSs Modulares tipo I tienen un set de enzimas (KS-AT-dominios de reducción -ACP) para cada ciclo de extensión de la cadena.

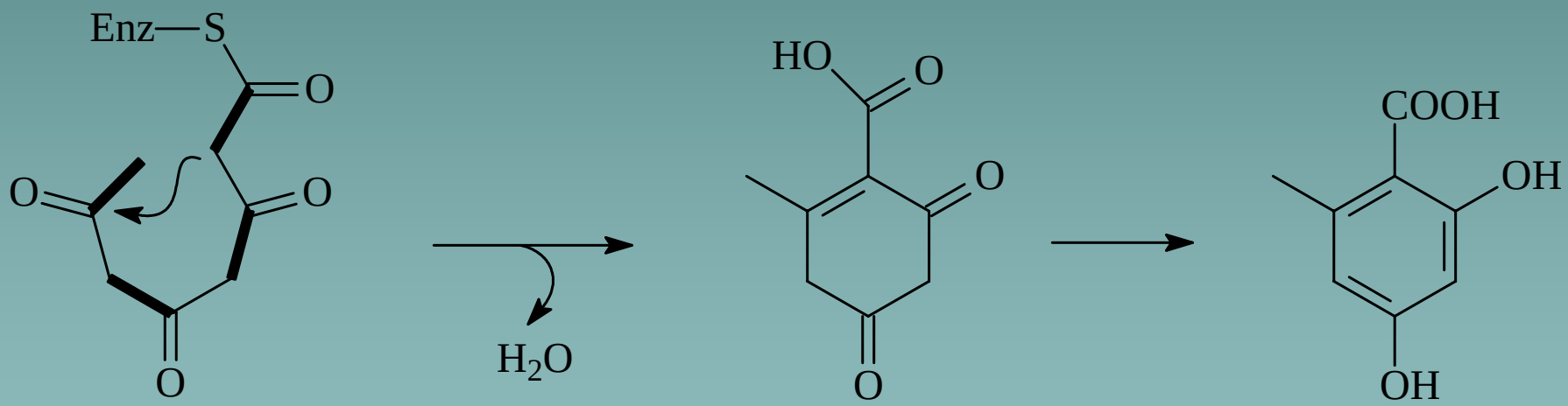
Iterative type I PKSs have one set of enzymes that is used repeatedly.

These systems make simpler polyketides than modular PKSs.

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

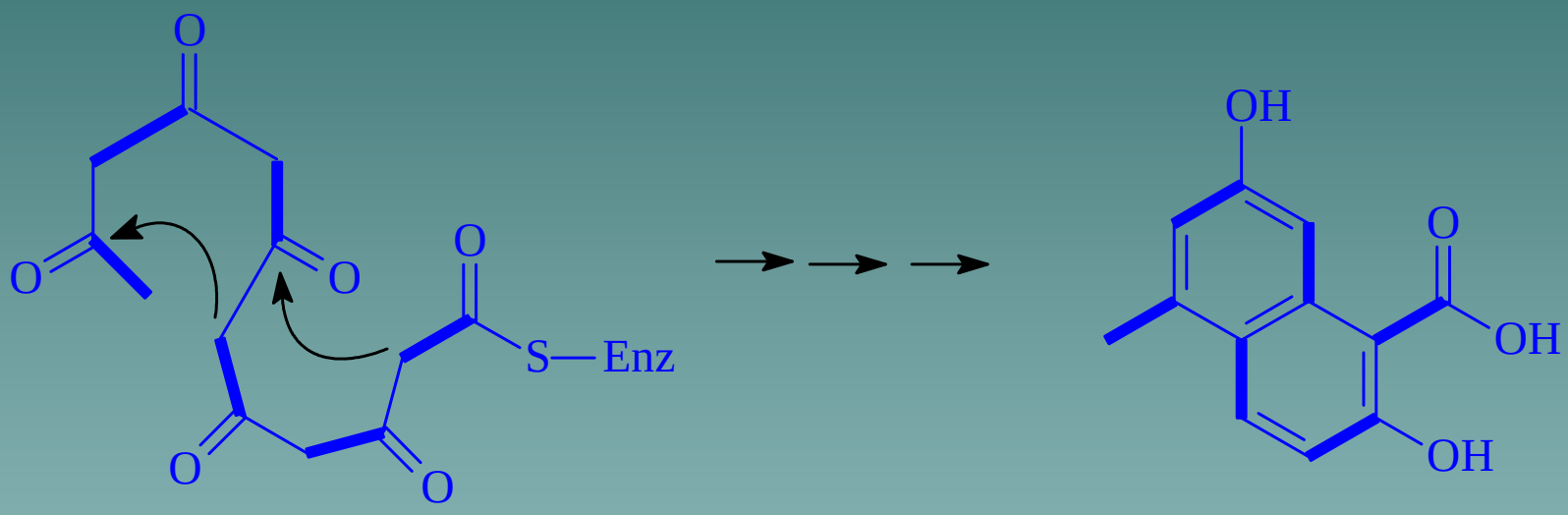
It is synthesised by an iterative (non-modular) type I PKS.

KS – AT - (DH) - ACP



Orsellinic acid

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

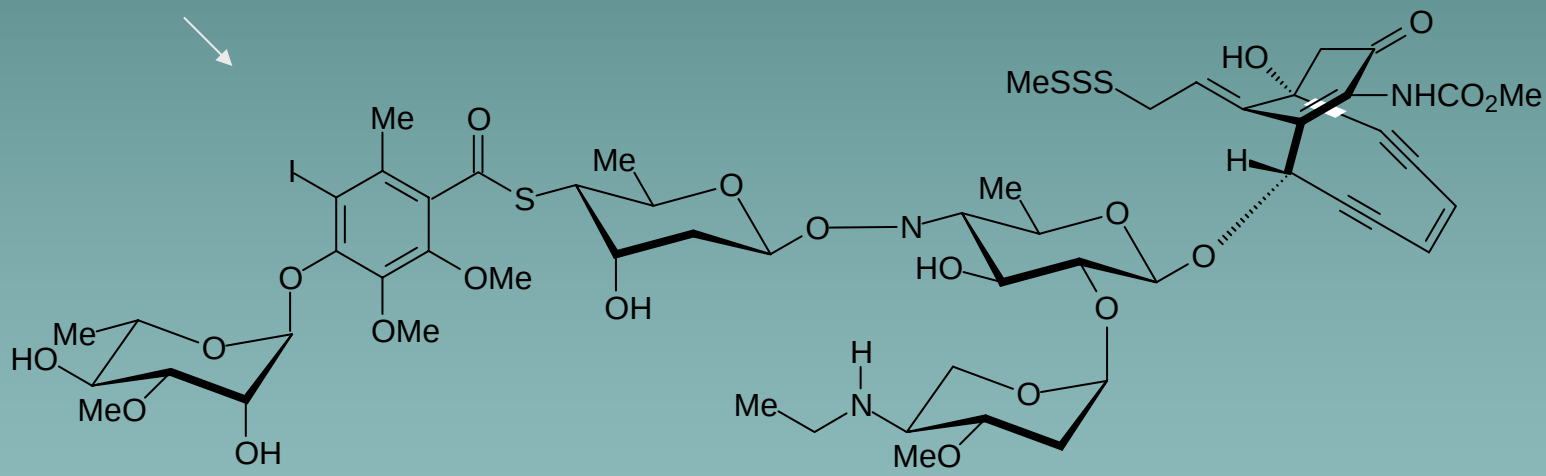


Enediyne antibiotics contain two polyketide-derived moieties.

Both are synthesized by iterative type I PKSs.

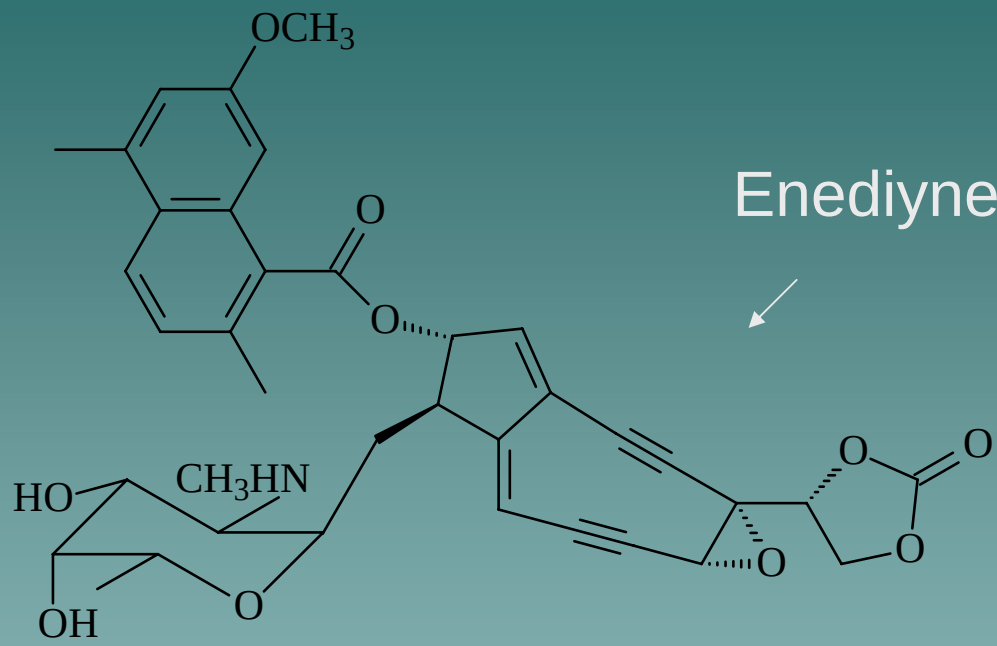
Enediyne core

Orsellinic acid



Calicheamicin

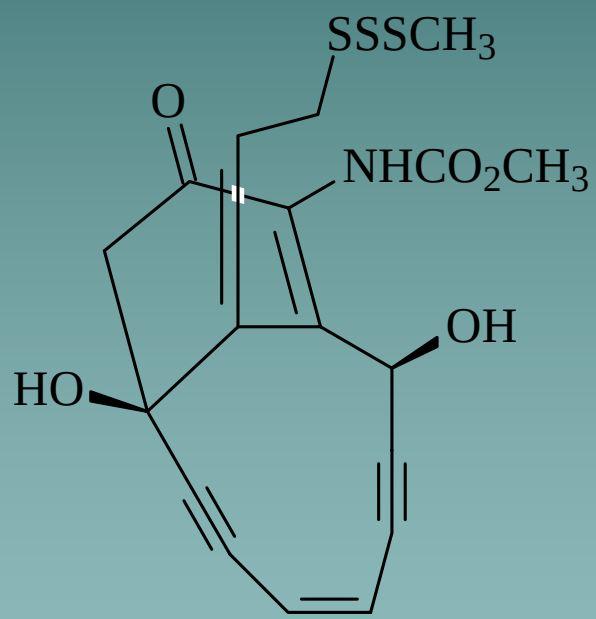
Napthalinic acid



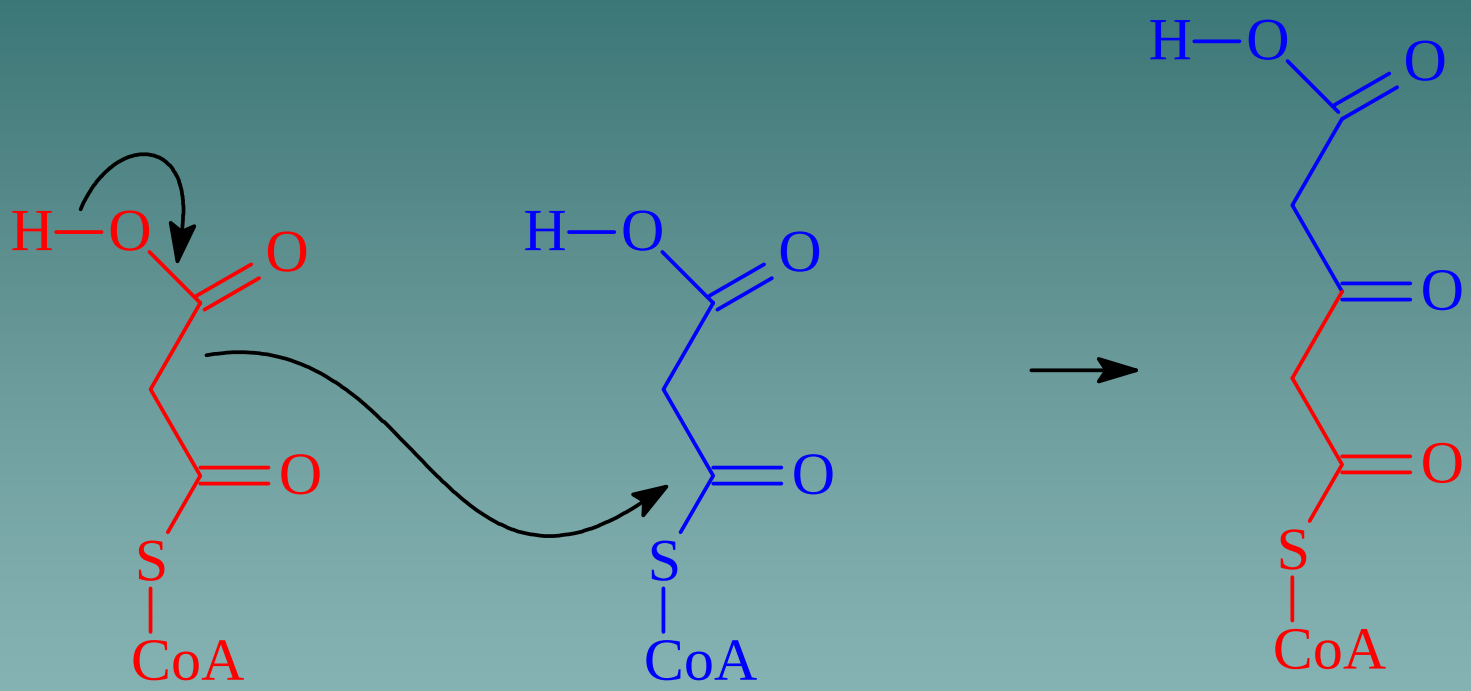
Enediyne core

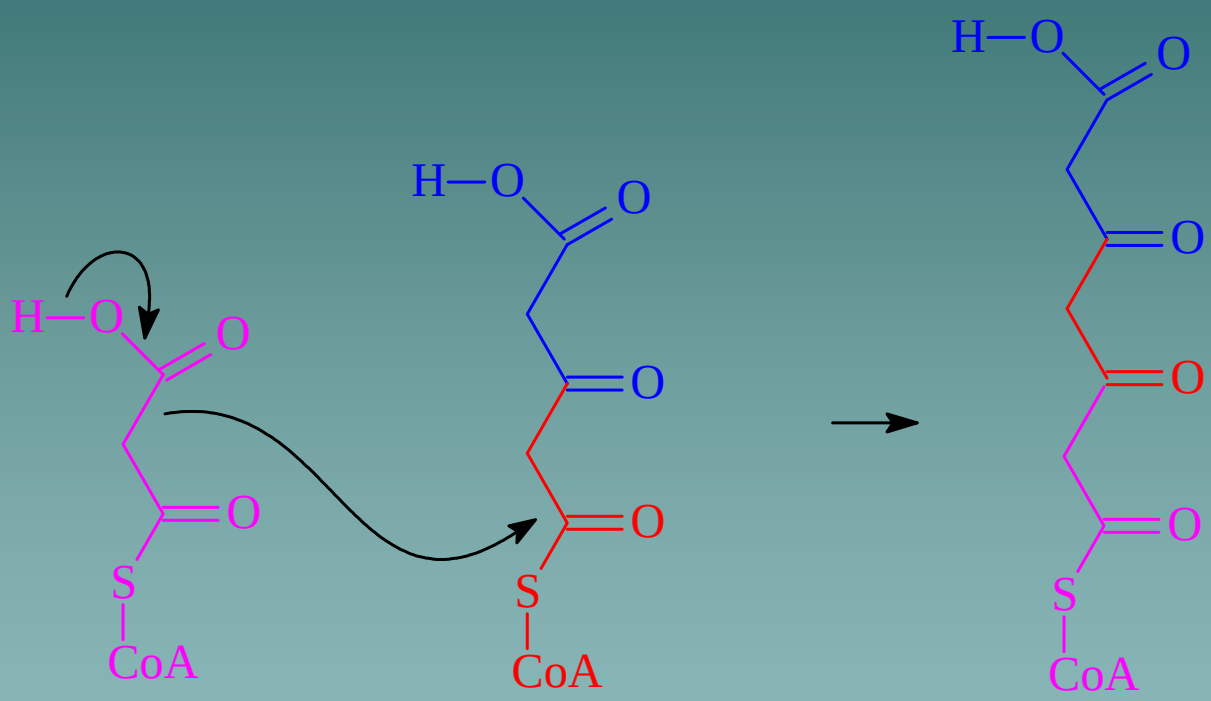
Enediyne C1029

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

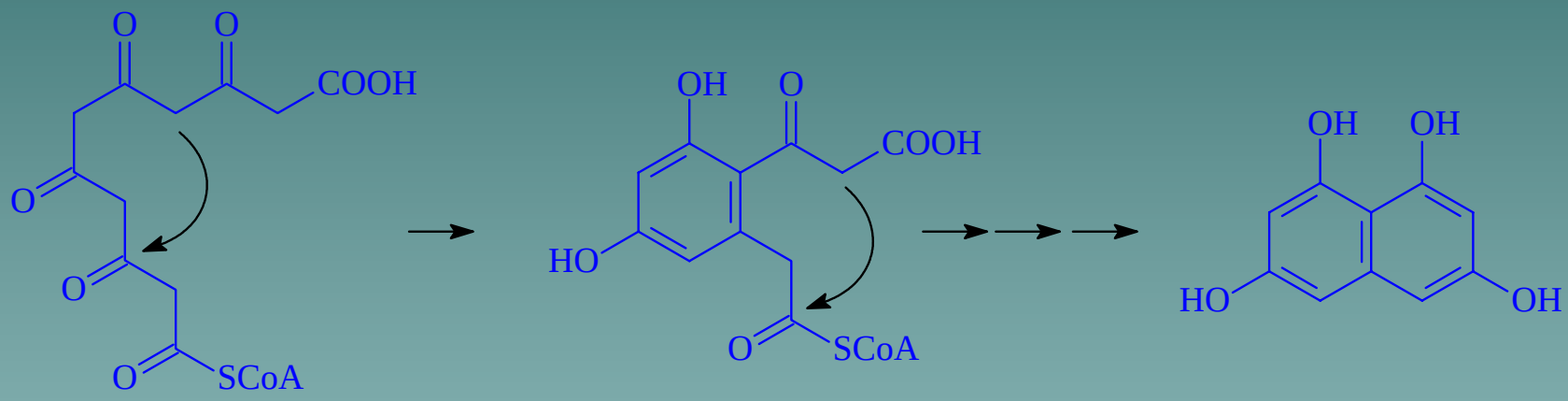


Type III PKSs are composed of KS domains only.
They assemble the polyketide chain on CoA pantetheine thiol (not ACP).




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

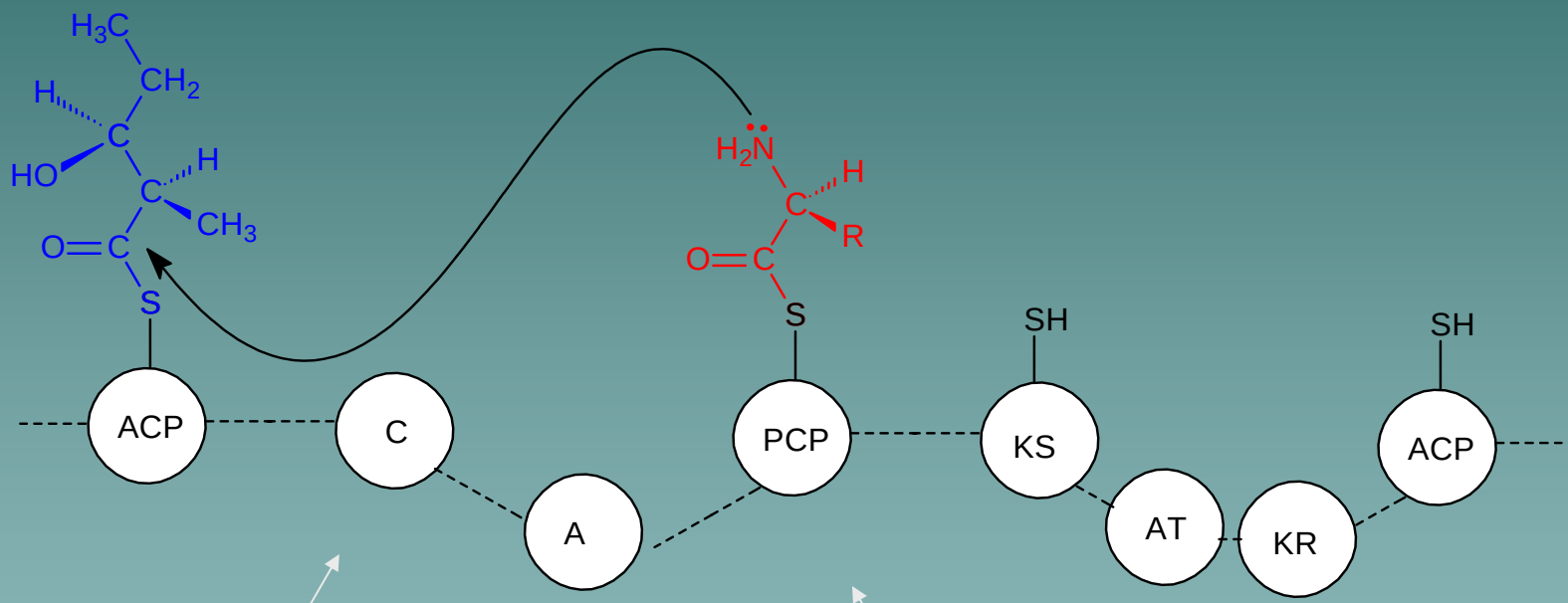
Type III PKSs synthesise pigments (e. g. flower colours), chalcones, flavonoids, flaviolins.



Flaviolin

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

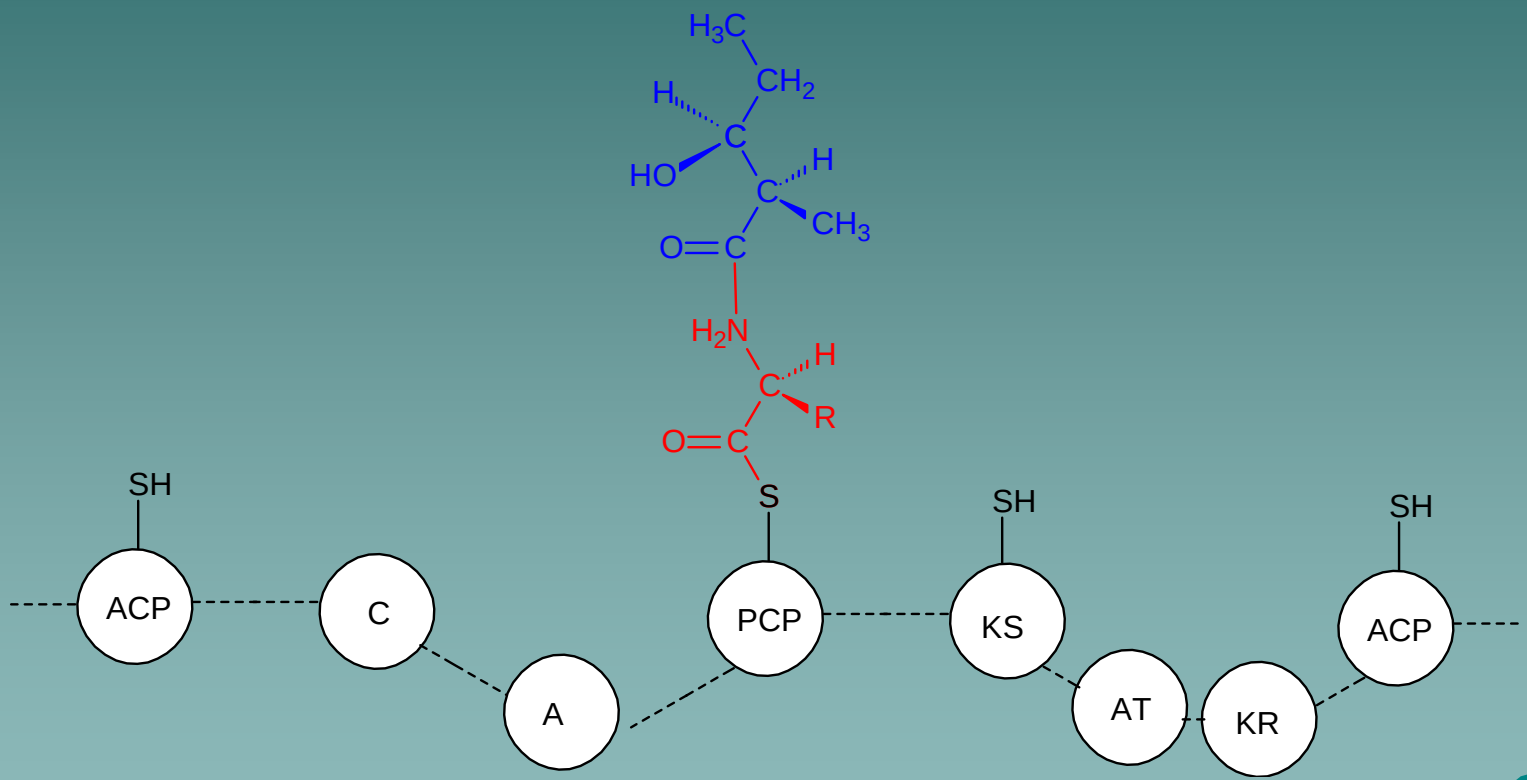
Modular PKSs that can use amino acid extenders as well as dicarboxylic acid extenders.

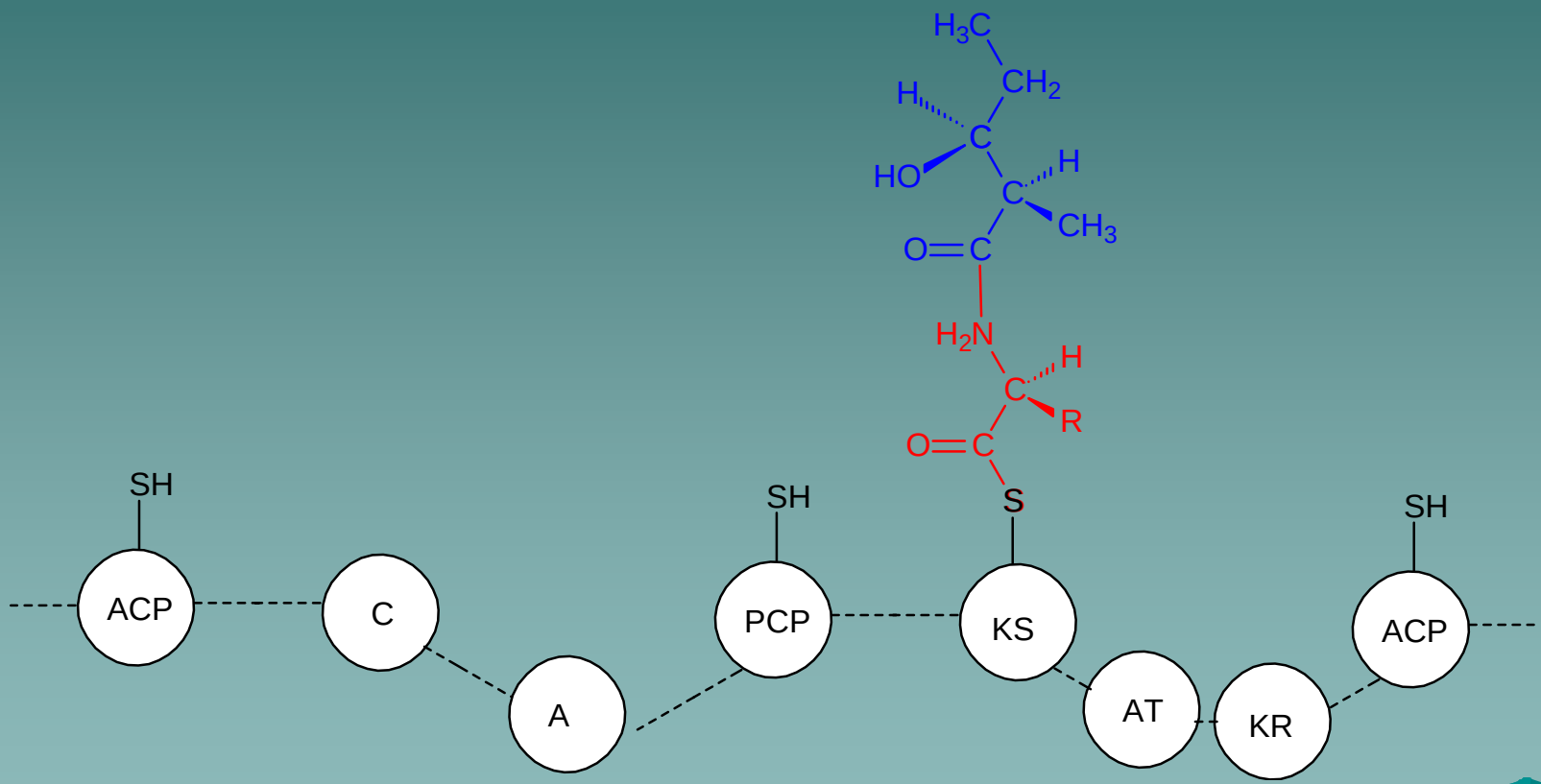


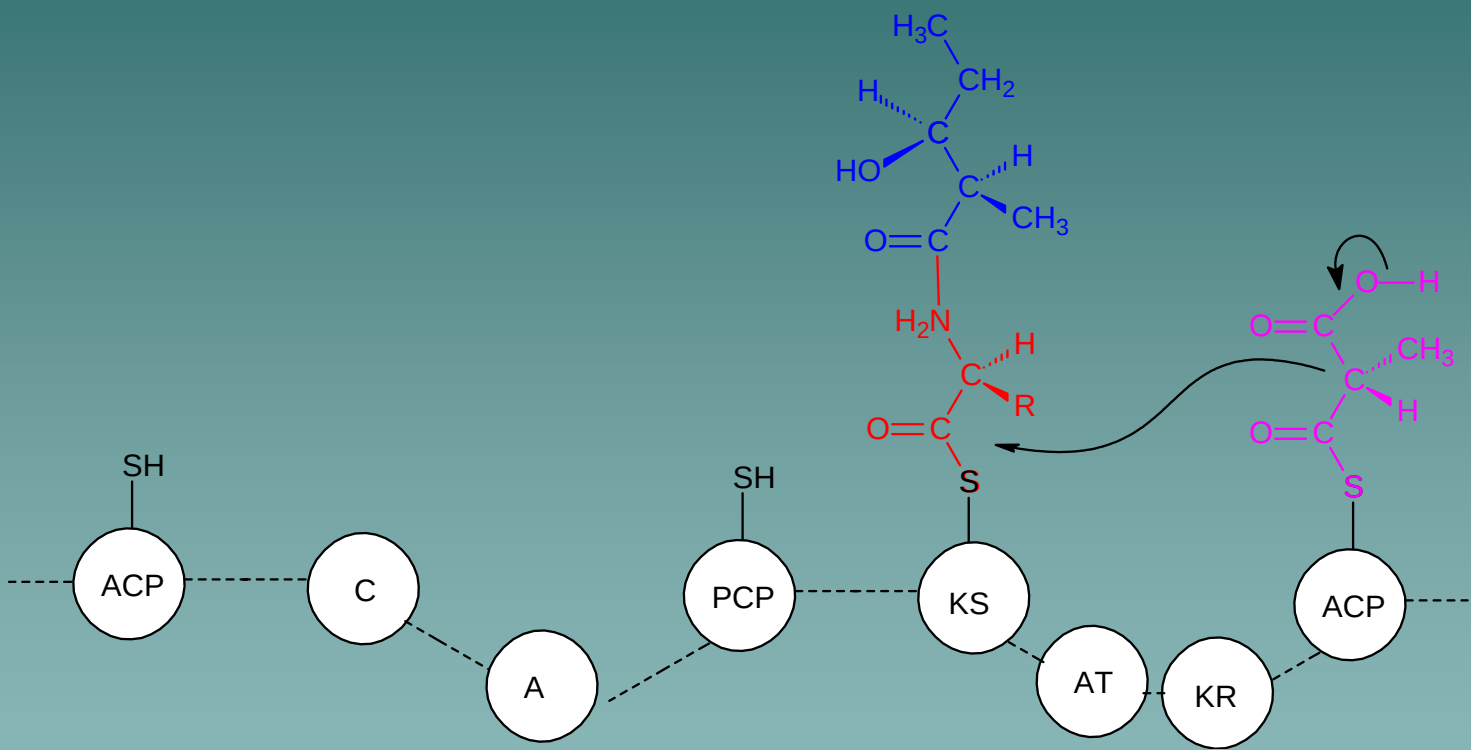
Condensation domain

AA activating domain

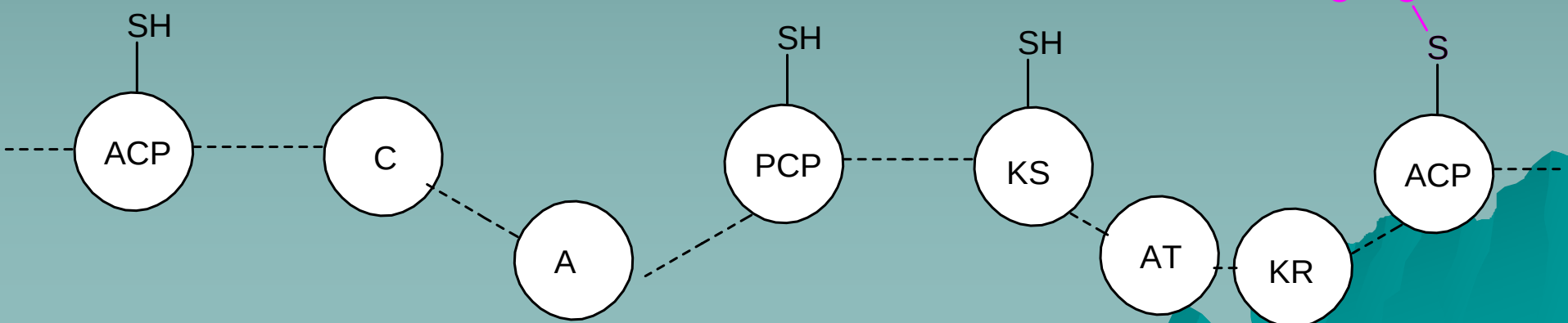
Peptidyl carrier protein (has pantetheine arm).

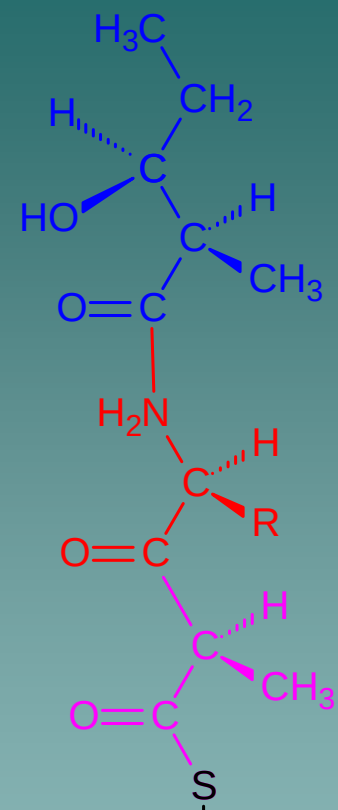

H3C
CH2
H
C
HO
H
C
CH3
O
C
H2N
H
C
R
O
C
S
SH
ACP
C
A
PCP
SH
KS
AT
KR
SH
ACP


H3C
CH2
H
C
HO
H
C
CH3
O
C
H2N
H
C
R
O
C
S
SH
ACP
C
A
SH
PCP
KS
AT
KR
SH
ACP


H3C
CH2
H
C
HO
H
C
CH3
O=C
H2N
H
C
R
O=C
S
SH
ACP
C
A
SH
PCP
KS
AT
KR
O—H
O=C
CH3
C
H
O=C
S
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.

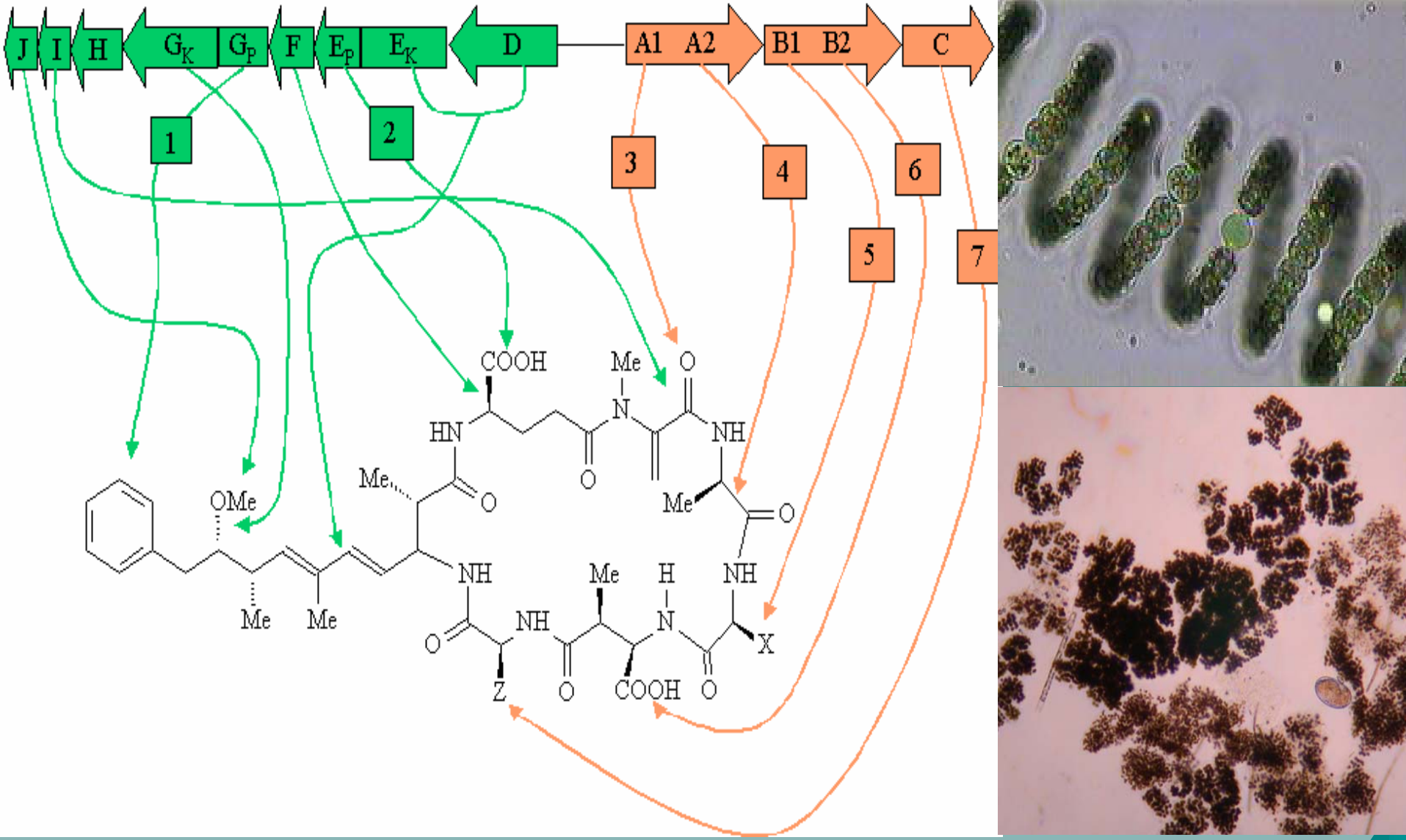
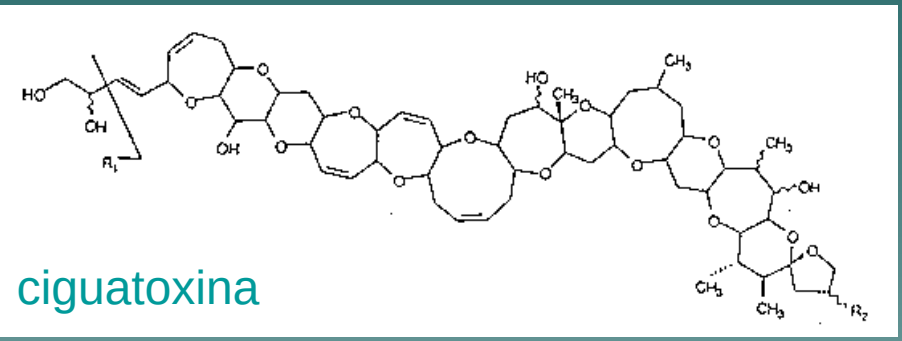


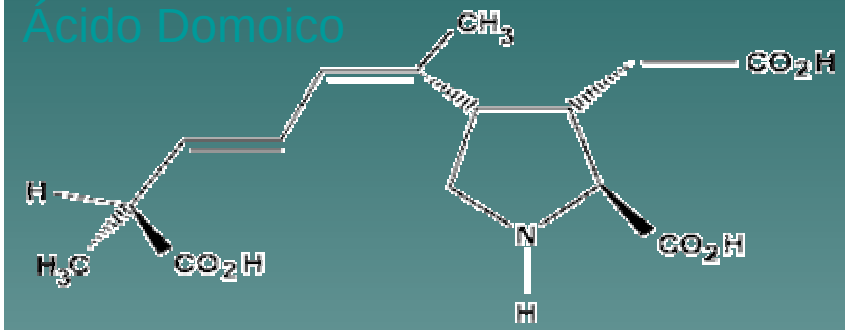
Figure 1: The structure of microcystin and its biosynthetic pathways (left). The gene organization is shown on the top. The amino acids in the X and Z positions represent variable amino acids. *Anabaena* (top right) and *Microcystis* (bottom) as seen under a microscope.

Toxinas

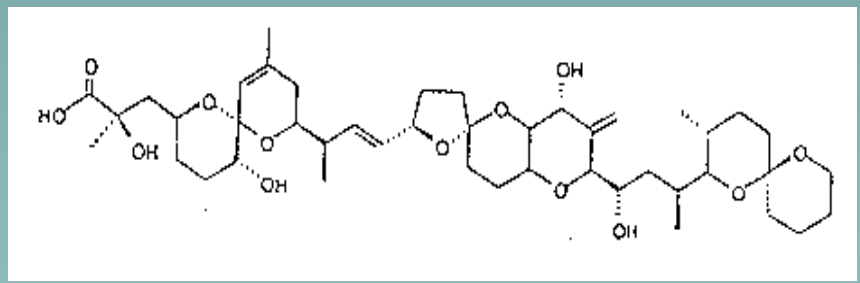


ciguatoxina

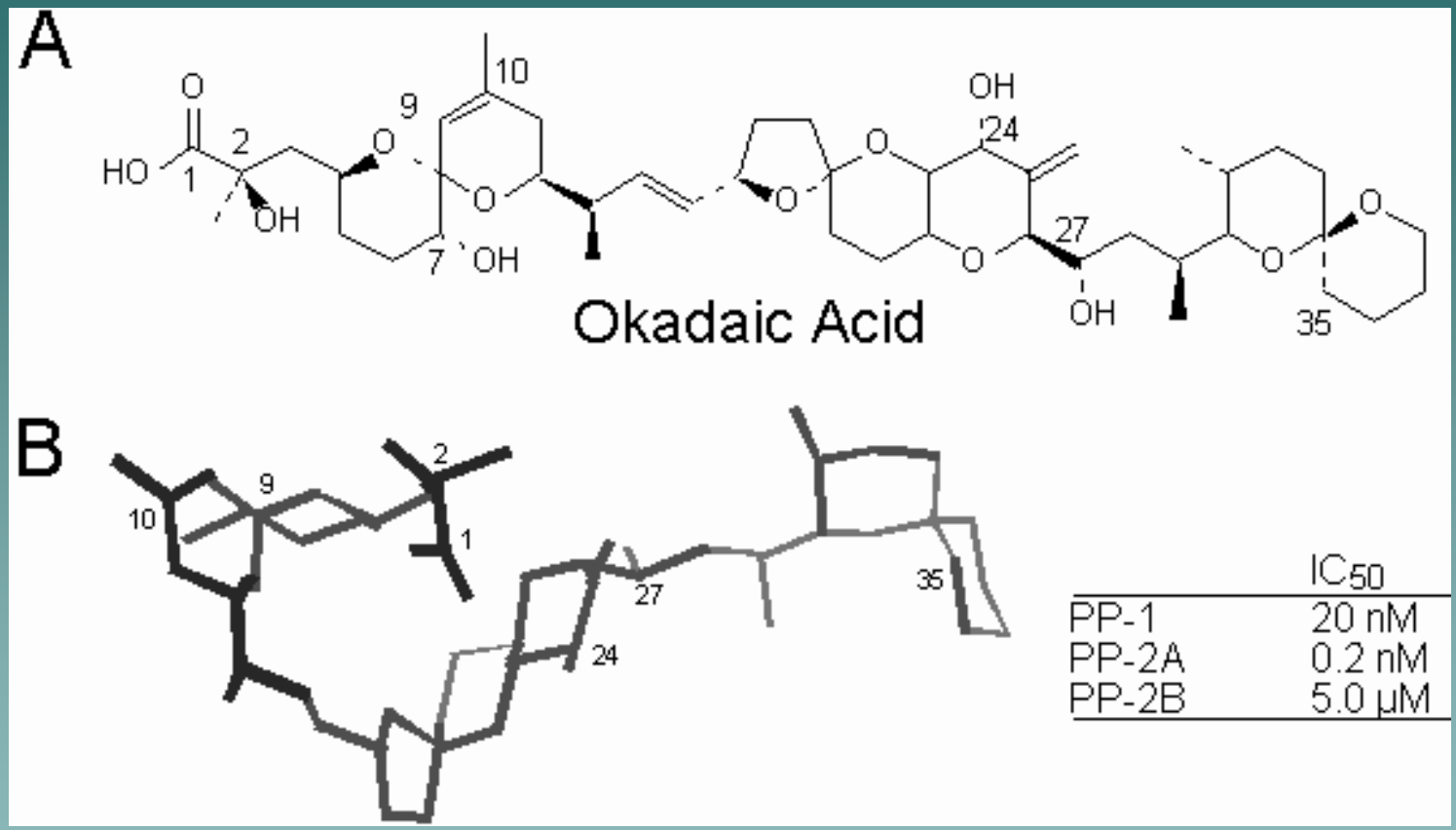
Ácido Domoico



Ácido okadaico

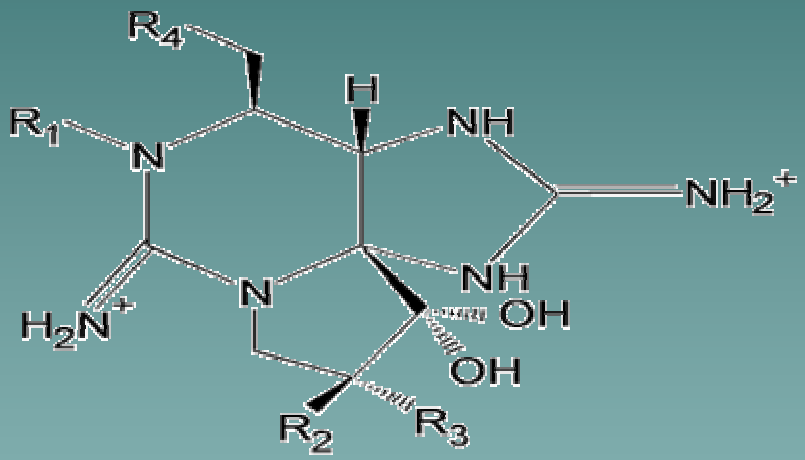


?



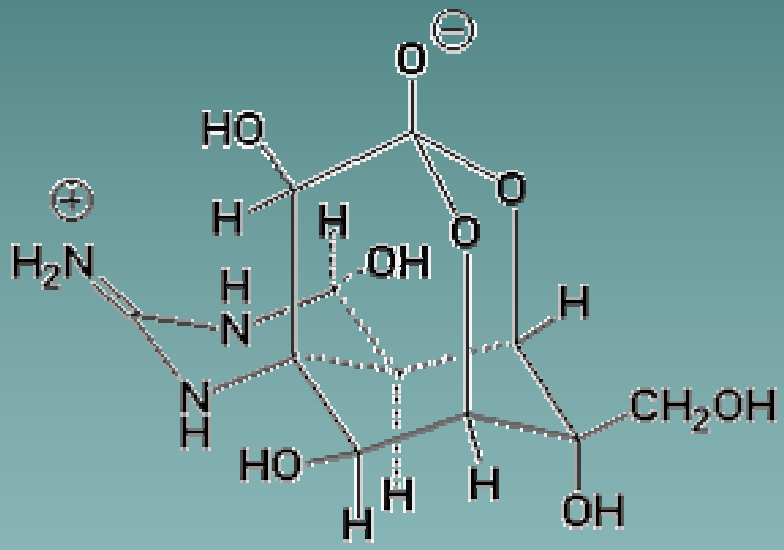
	IC_{50}
PP-1	20 nM
PP-2A	0.2 nM
PP-2B	5.0 μM

?



PSP toxins

?



Tetrodotoxin

Useful references.

Combinatorial biosynthesis of erythromycin and complex polyketides. Staunton, J. 1998.
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.

Antibiotics: action, origin and resistance by C. Walsh 2003

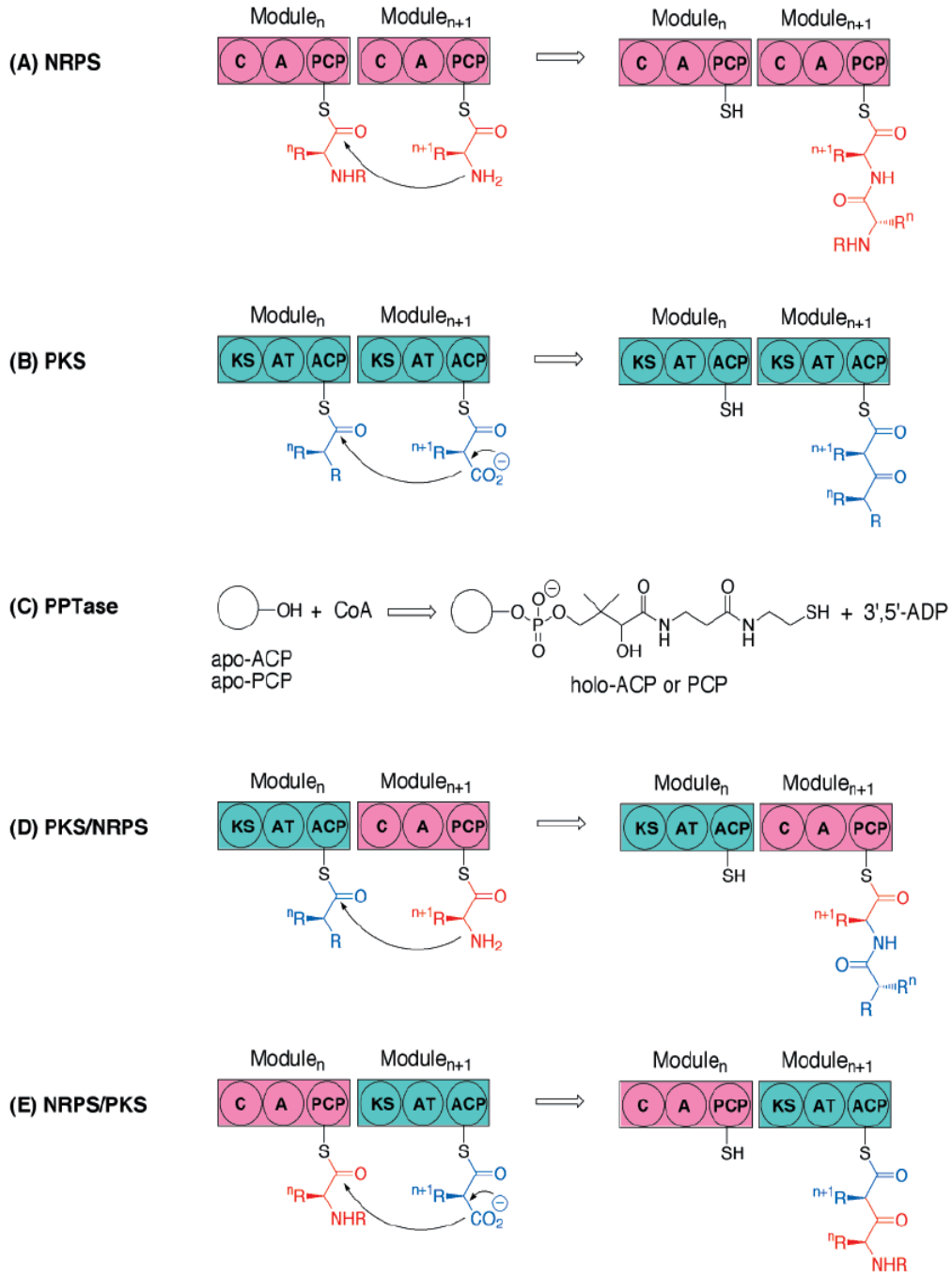
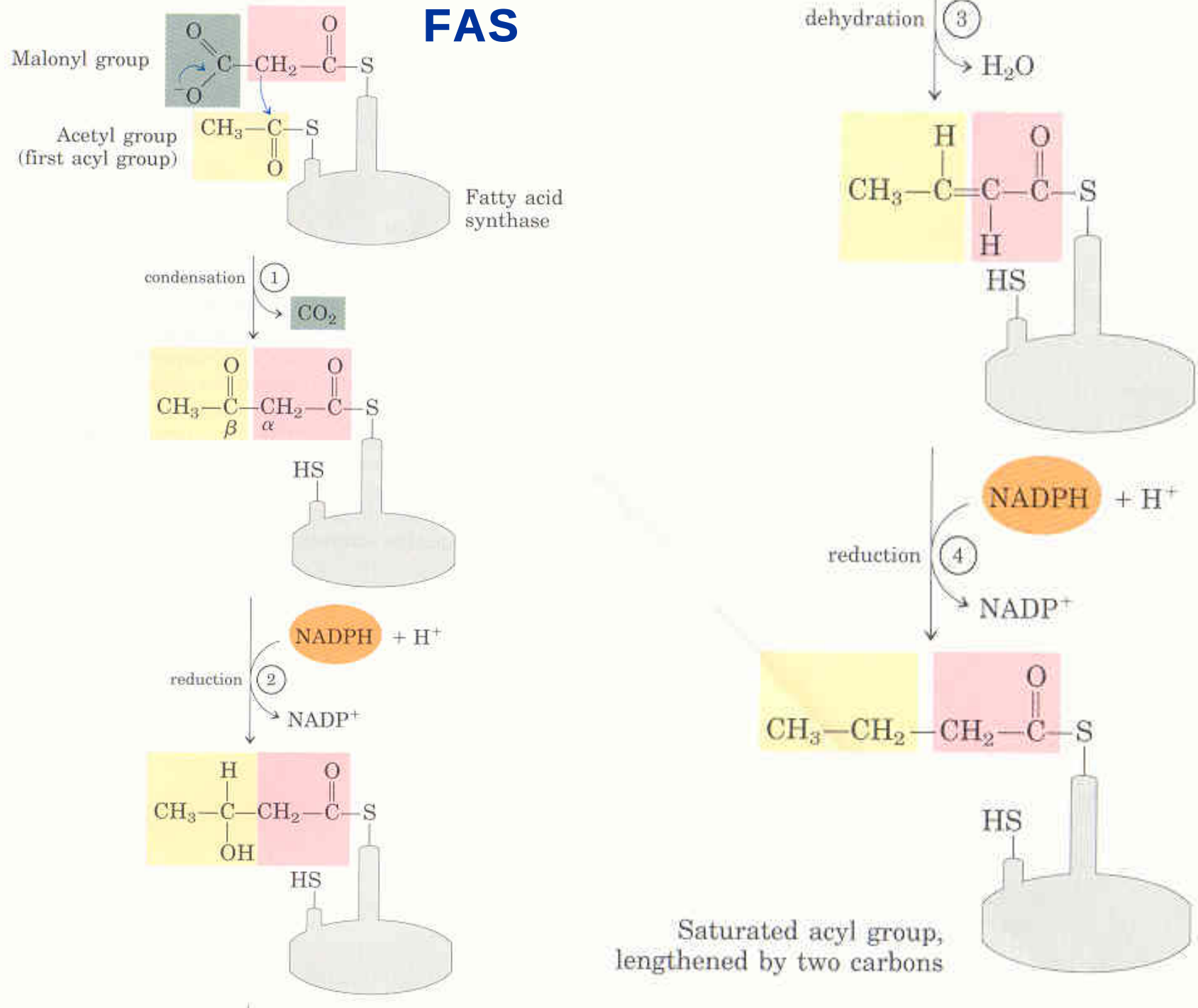
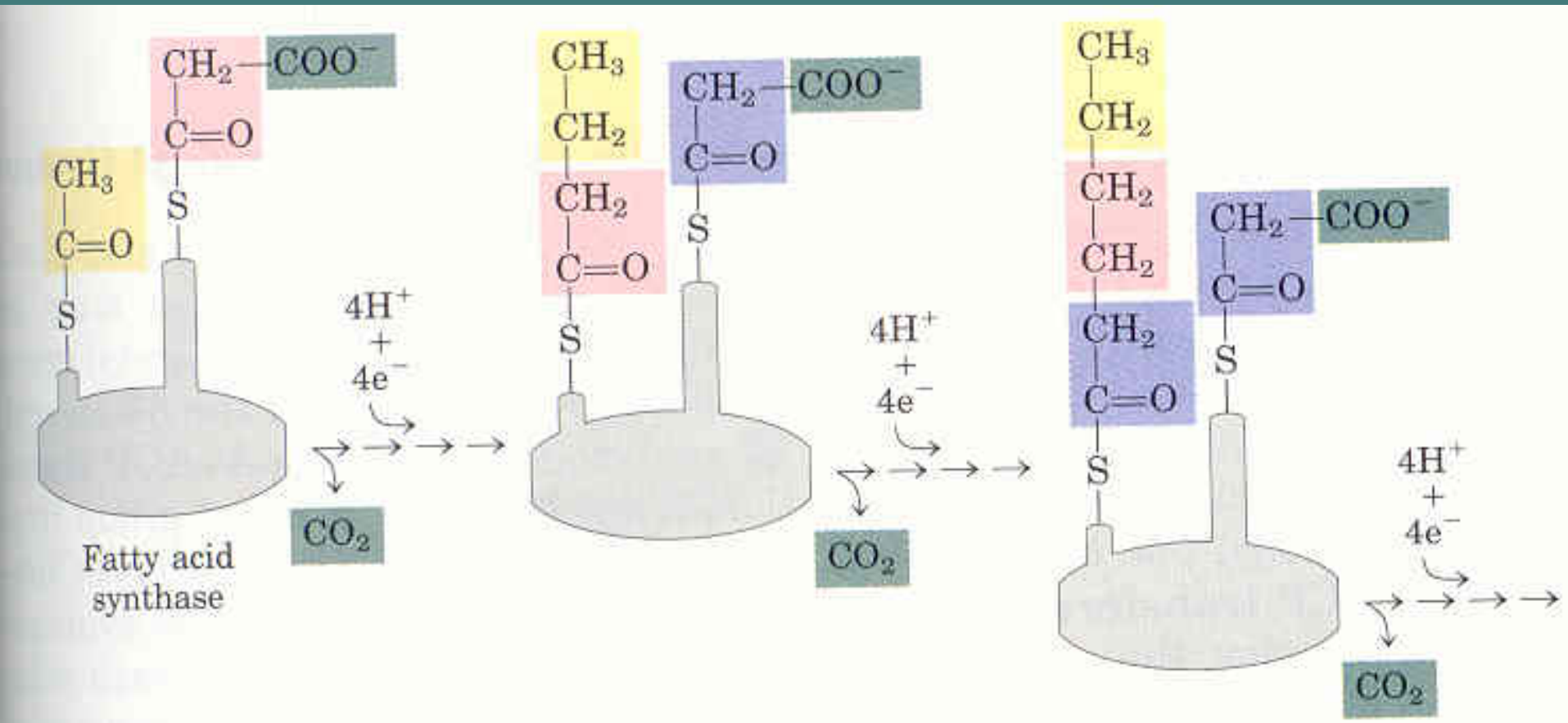


Figure 2. Modular organization of NRPS (A), PKS (B), hybrids PKS/NRPS (D) and NRPS/PKS (E), and post-translational modification of apo-ACP or apo-PCP into holo-ACP and holo-PCP by a PPTase (C). Hypothetical NRPS (pink) and PKS (blue) modules are shown with core domains. A, adenylation; ACP, acyl carrier protein; AT, acyltransferase, C, condensation; KS, ketoacyl synthase; PCP, peptidyl carrier protein.

FAS


Malonyl group
Acetyl group
(first acyl group)
Fatty acid
synthase
condensation
1
CO_2
reduction
2
NADPH
+ H^+
$NADP^+$
dehydration
3
H_2O
reduction
4
NADPH
+ H^+
$NADP^+$
Saturated acyl group,
lengthened by two carbons

FAS: elongation



FAS: elongation