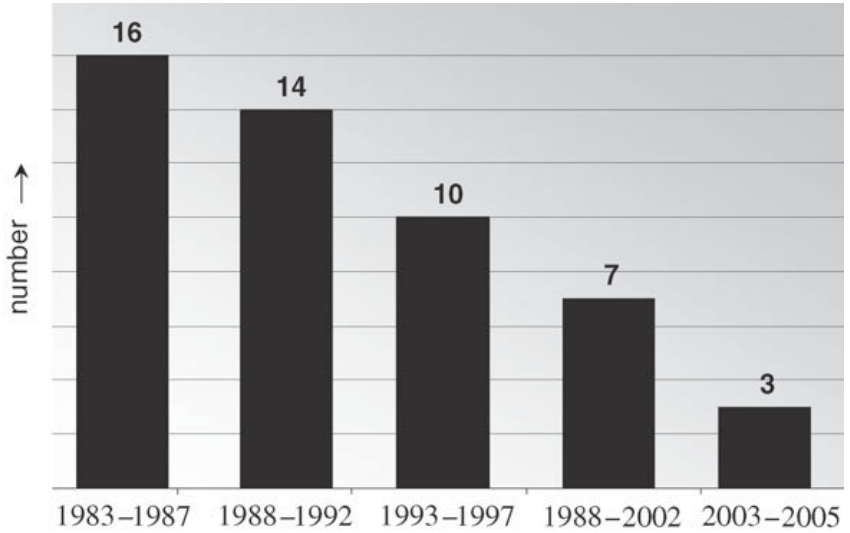
Antibacterial Drugs

Antibiotics: Commercial Aspects

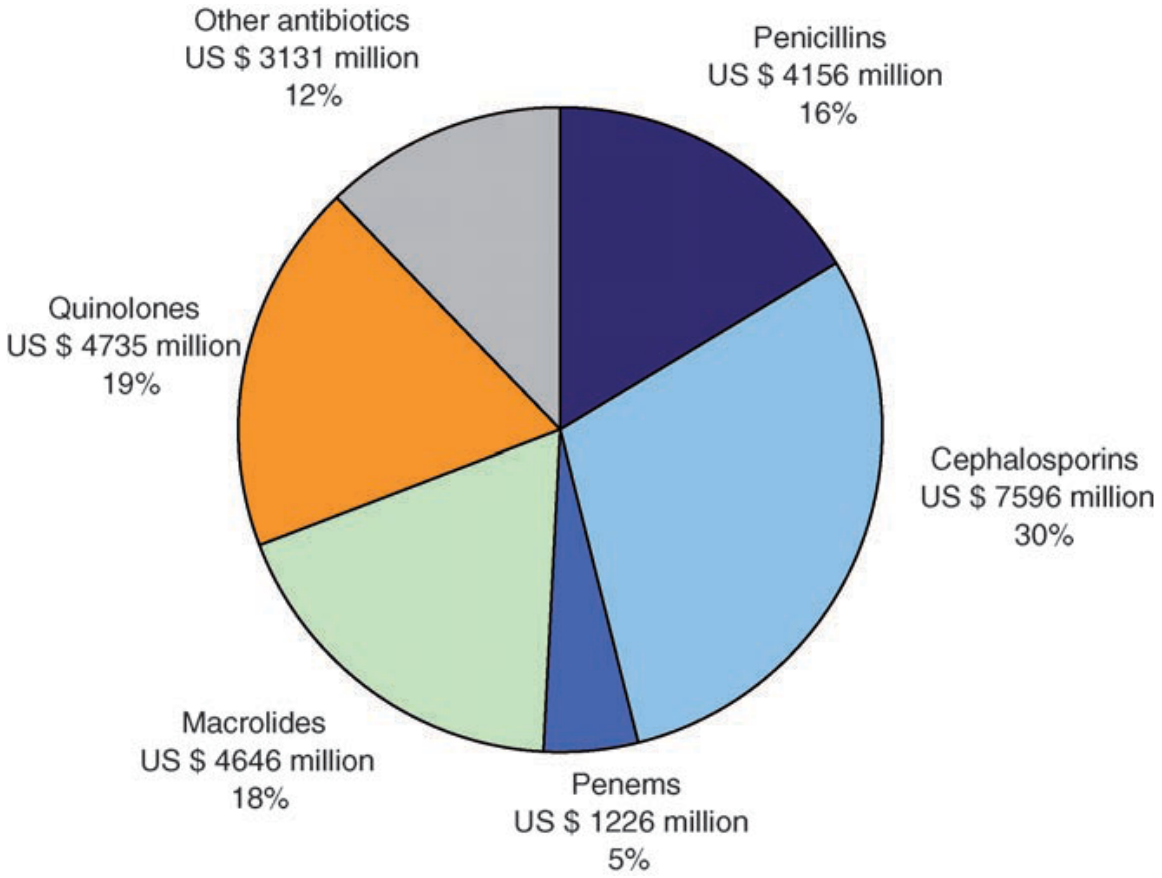
Table : Top 10 antibacterial companies by global sales of antibiotics in 2004 (Source: Wood Mackenzie[69]).

Rank	Company	US $ million
1	Pfizer	2938
2	GlaxoSmithKline	2425
3	Abbott	1657
4	Bayer	1346
5	Johnson & Johnson	1295
6	Hoffmann-La Roche	1142
7	Wyeth	846
8	Merck & Co.	704
9	Daiichi	687
10	Shionogi	678
Others		12064
Total		25782



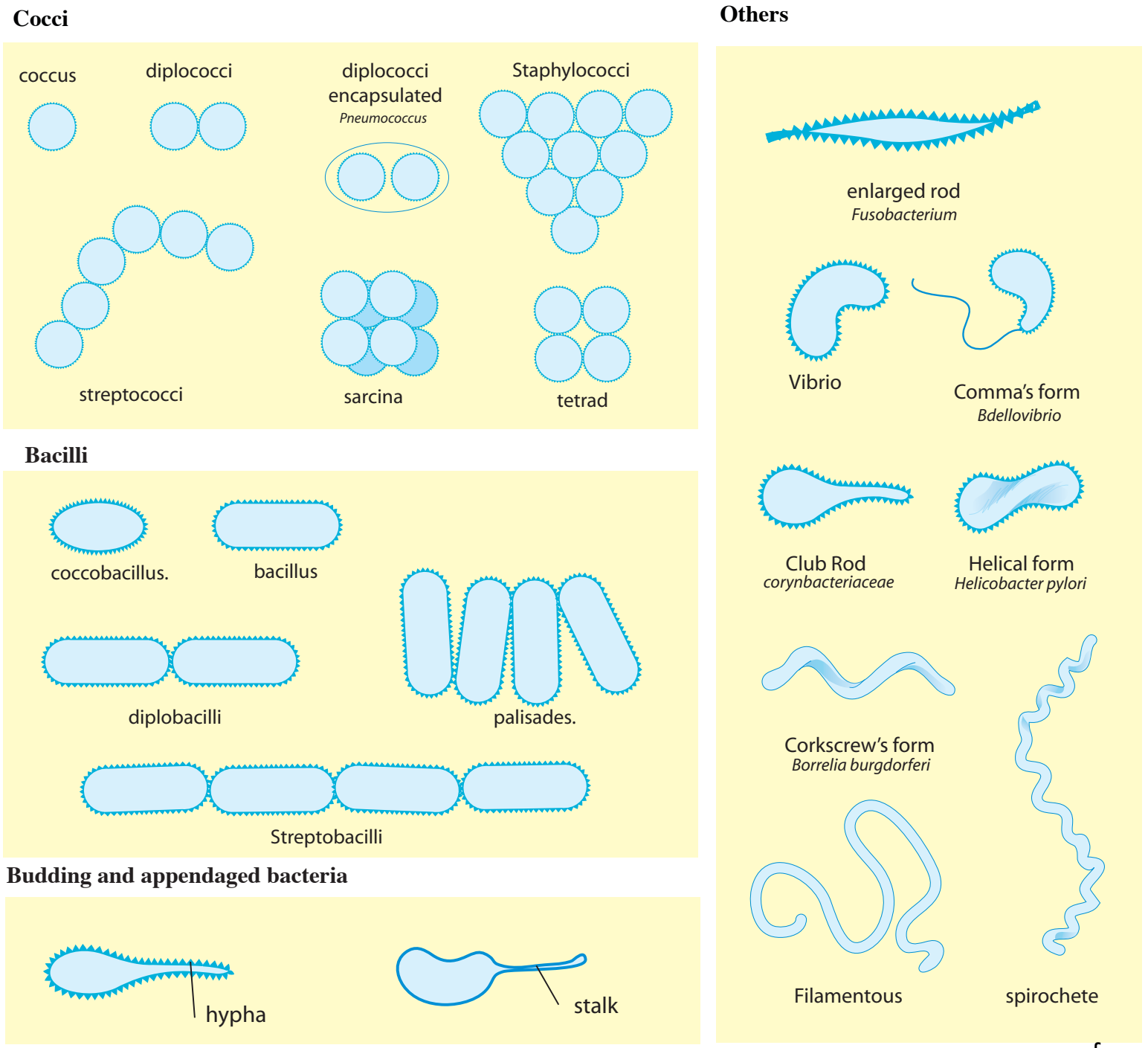
New antibacterial agents approved in the US.[62]

- Bacteristatics: Inhibit cell growth and cell division
- Bactericidals: Kill bacteria

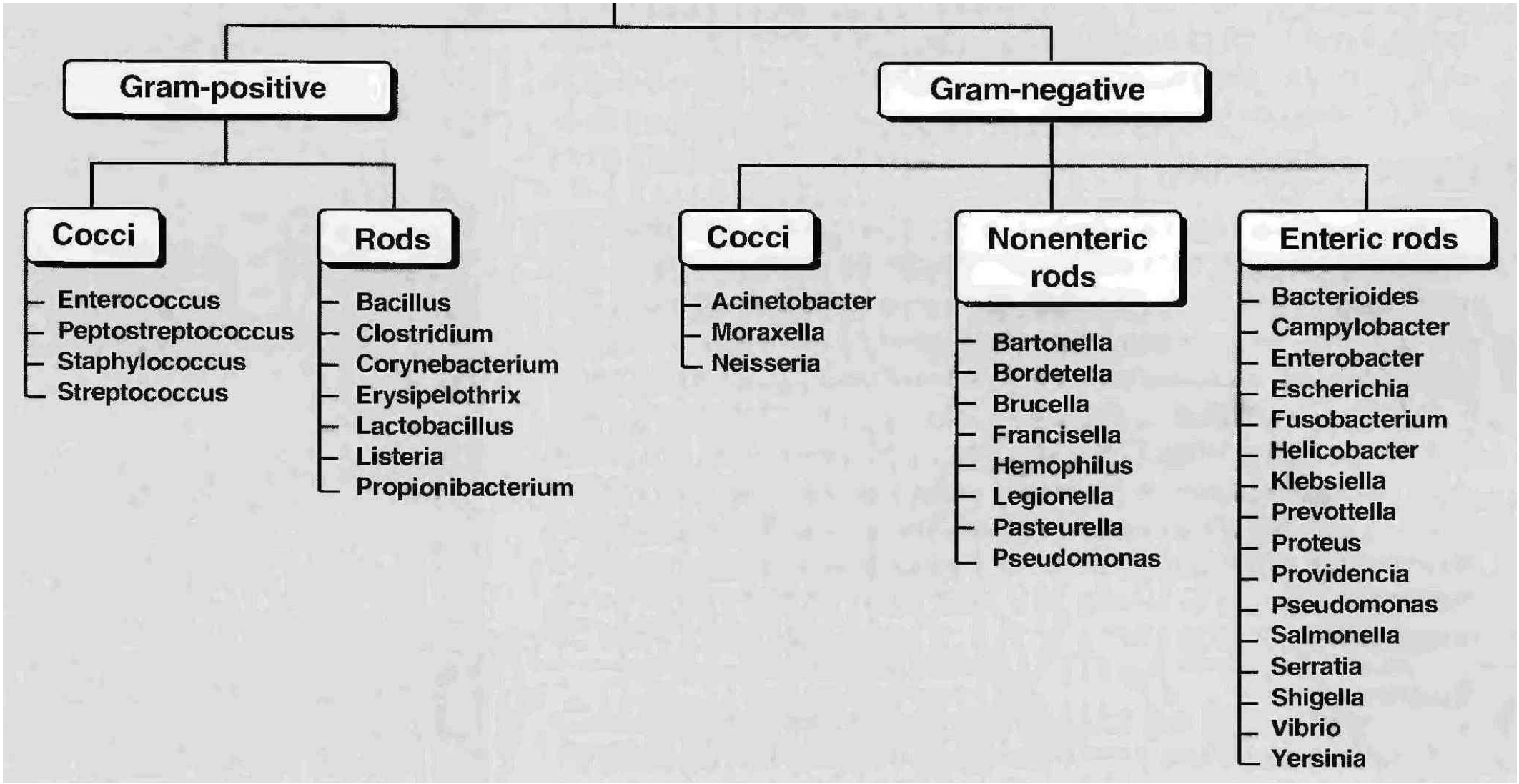


Global sales of the major antibacterial classes in 2004 (from Wood Mackenzie[69]).

Classification of bacteria according to shape



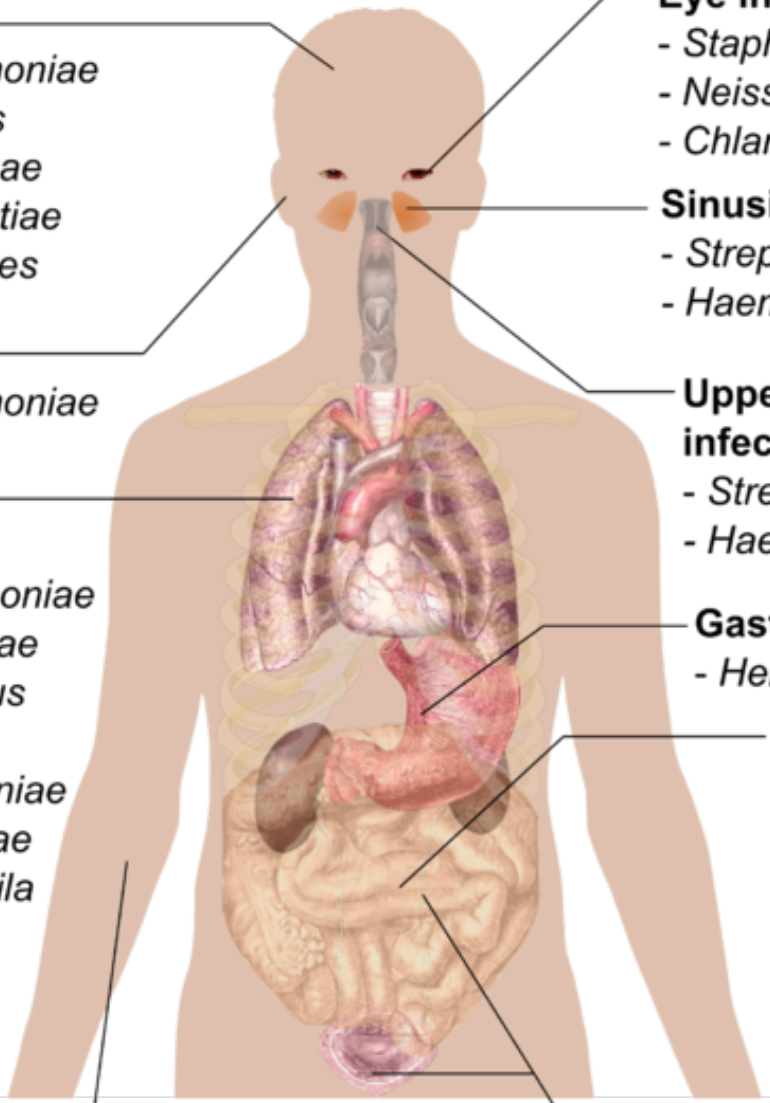
from:Wikipedia


Gram-positive
Gram-negative
Cocci
Rods
Cocci
Nonenteric rods
Enteric rods
Enterococcus
Peptostreptococcus
Staphylococcus
Streptococcus
Bacillus
Clostridium
Corynebacterium
Erysipelothrix
Lactobacillus
Listeria
Propionibacterium
Acinetobacter
Moraxella
Neisseria
Bartonella
Bordetella
Brucella
Francisella
Hemophilus
Legionella
Pasteurella
Pseudomonas
Bacterioides
Campylobacter
Enterobacter
Escherichia
Fusobacterium
Helicobacter
Klebsiella
Prevottella
Proteus
Providencia
Pseudomonas
Salmonella
Serratia
Shigella
Vibrio
Yersinia

Pathogen	Infectious Disease
Staphylococcus aureus	skin and wound infection, abscess, bacteremia, nosocomial pneumonia, endocarditis, toxic shock syndrome
Streptococcus pneumoniae	upper respiratory infection, pneumonia, otitis, sinusitis, meningitis
Streptococcus pyogenes	pharyngitis, tonsillitis, skin and soft-tissue infection, scarlet fever
Enterococcus faecalis	bacteremia, endocarditis, urinary-tract infection, peritonitis
Enterococcus faecium	bacteremia, endocarditis, peritonitis
Escherichia coli	bacteremia, urinary-tract and gastrointestinal infection
Klebsiella pneumoniae	hospital-acquired pneumonia, bacteremia
Proteus spp.	urinary-tract infection
Haemophilus influenzae	respiratory infection, otitis, sinusitis, meningitis
Moraxella catarrhalis	respiratory infection
Pseudomonas aeruginosa	nosocomial pneumonia, burn infection, bacteremia
Acinetobacter spp.	pneumonia in immuno-compromised patients
Mycobacterium tuberculosis	tuberculosis

Overview of

Bacterial infections



Bacterial meningitis
- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Streptococcus agalactiae*
- *Listeria monocytogenes*

Otitis media
- *Streptococcus pneumoniae*

Pneumonia
Community-acquired:
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Staphylococcus aureus*

Atypical:
- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*
- *Legionella pneumophila*

Tuberculosis
- *Mycobacterium tuberculosis*

Skin infections
- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Pseudomonas aeruginosa*

Sexually transmitted diseases
- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- *Treponema pallidum*
- *Ureaplasma urealyticum*
- *Haemophilus ducreyi*

Eye infections
- *Staphylococcus aureus*
- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*

Sinusitis
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*

Upper respiratory tract infection
- *Streptococcus pyogenes*
- *Haemophilus influenzae*

Gastritis
- *Helicobacter pylori*

Food poisoning
- *Campylobacter jejuni*
- *Salmonella*
- *Shigella*
- *Clostridium*
- *Staphylococcus aureus*
- *Escherichia coli*

Urinary tract infections
- *Escherichia coli*
- Other Enterobacteriaceae
- *Staphylococcus saprophyticus*
- *Pseudomonas aeruginosa*

from:Wikipedia

Medically relevant

gram-positive

- Bacillus - degrades complex macromolecules- dust, water, plants, animals fur
- Bacillus anthracis: Common in cattle, Bioterrorism
- Bacillus cereus:food, rice, potatoes, meat
- Clostridium perfringens: progressive, toxins diffuse to healthy tissue, Surgical, compound fractures, sores, septic abortions,gunshot wounds, crushing injuries with dirt

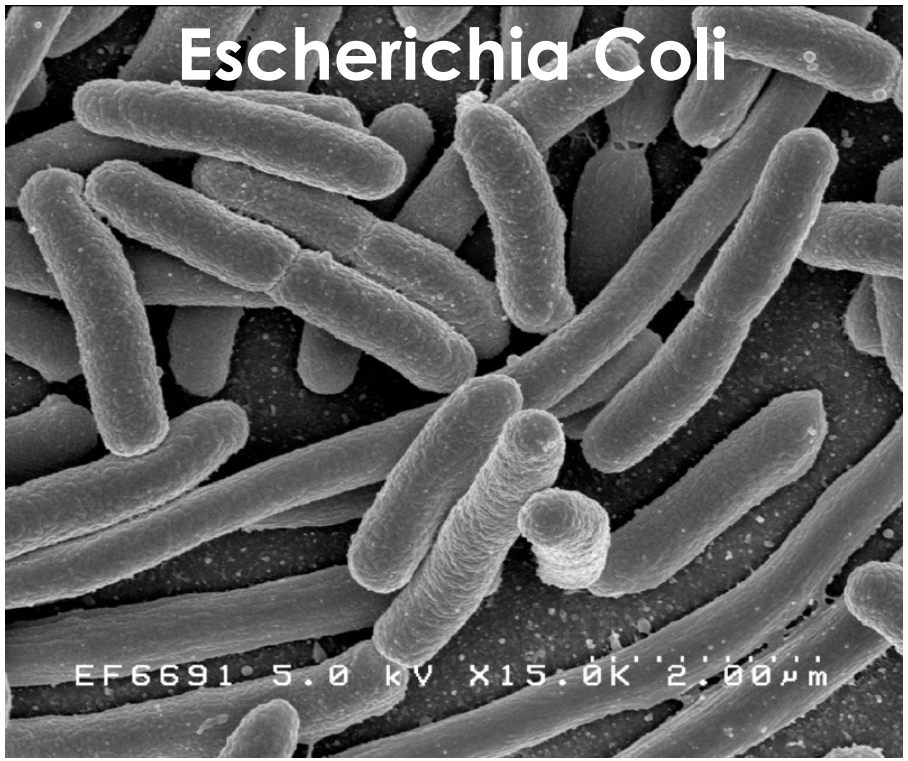
gram-negative cocci

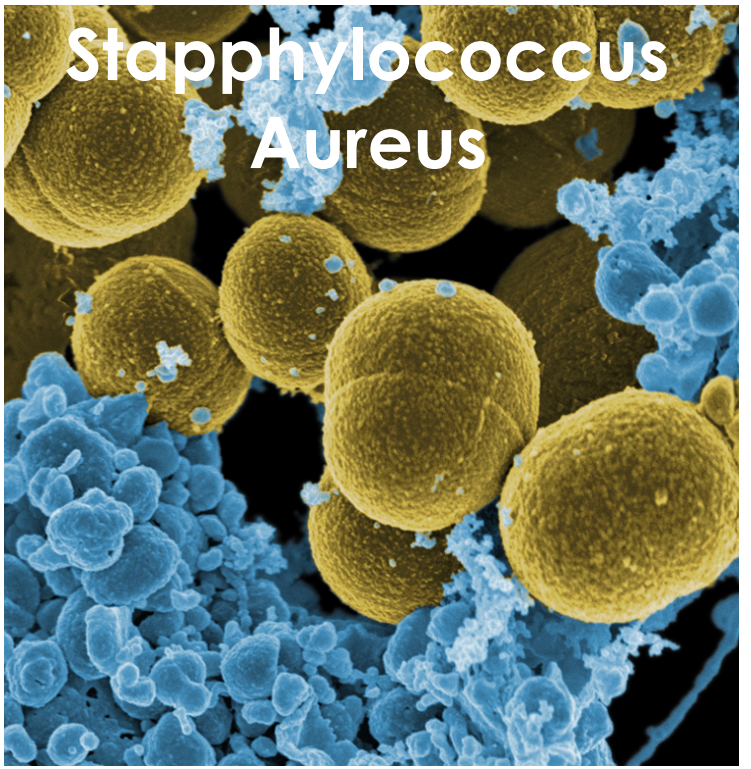
- sexually transmitted disease (Neisseria gonorrhoeae)
- meningitis (Neisseria meningitidis)
- respiratory symptoms (Moraxella catarrhalis).

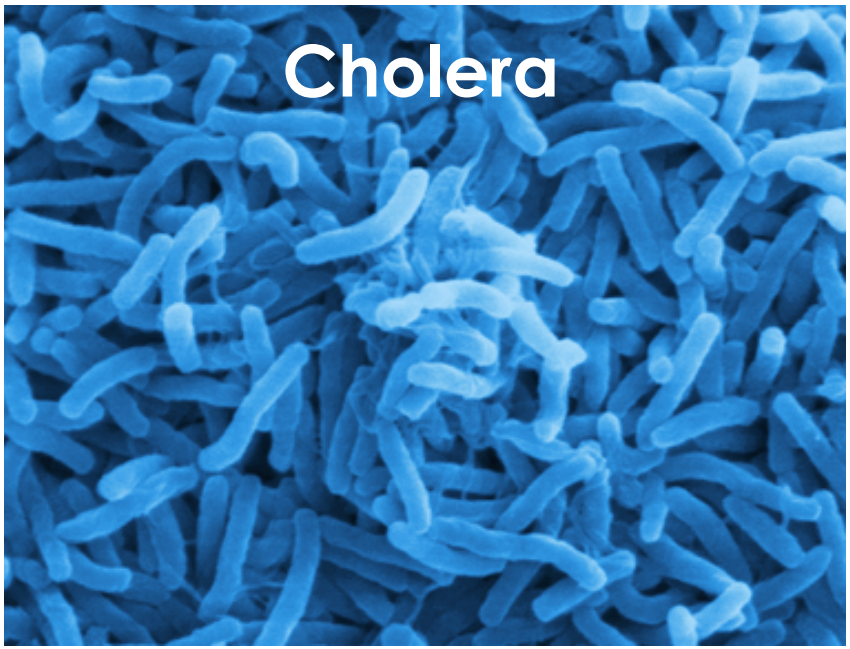
gram-negative bacilli

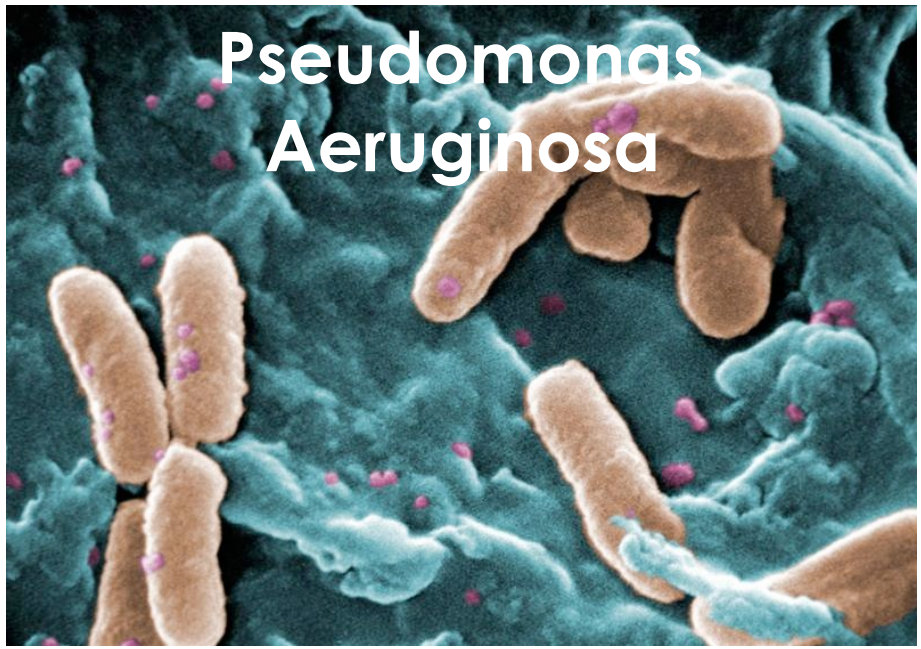
- respiratory problems (Hemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Pseudomonas aeruginosa)
- urinary problems (Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens)
- gastrointestinal problems (Helicobacter pylori, Salmonella enteritidis, Salmonella typhi

Bacteria under the electron microscope

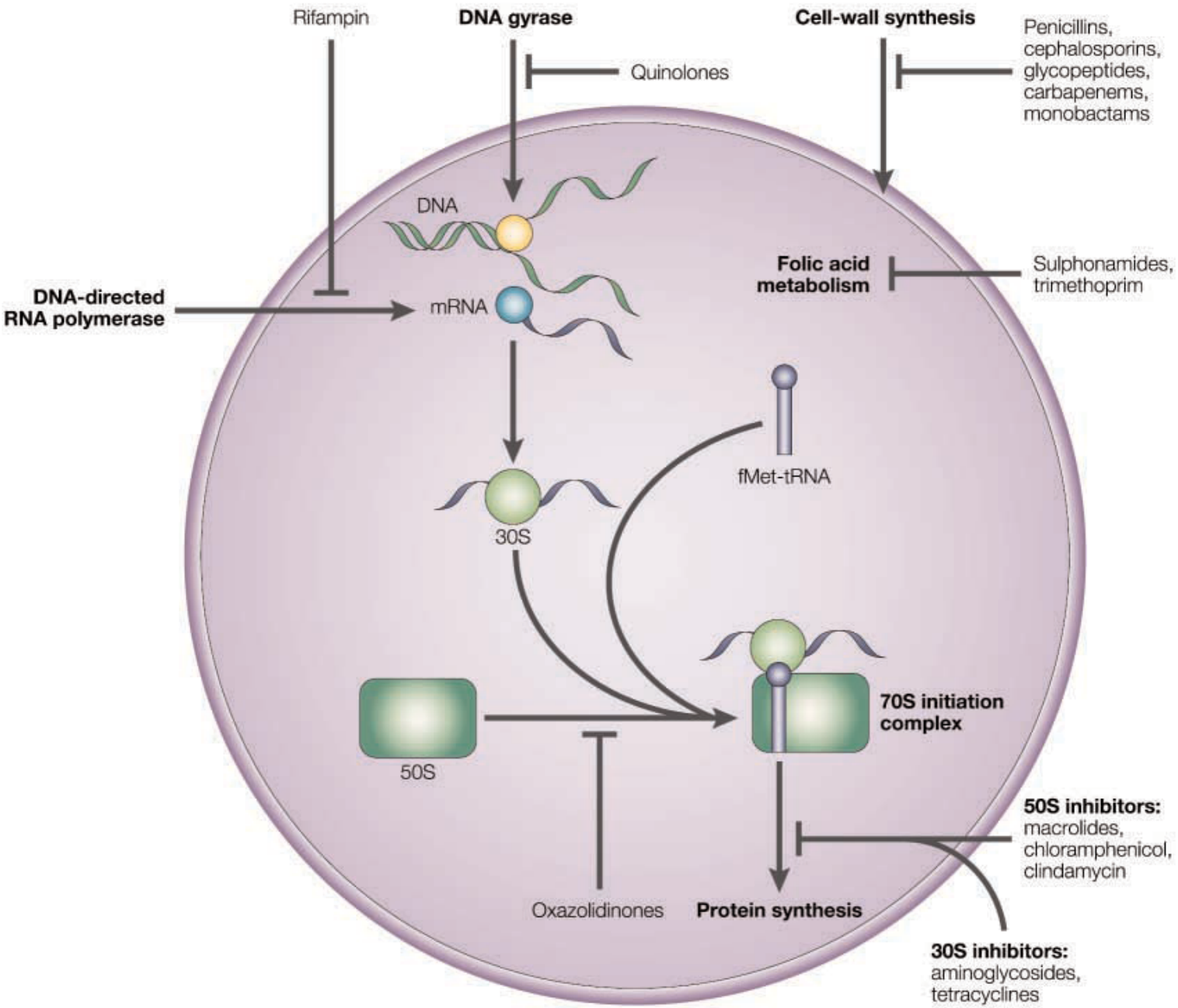








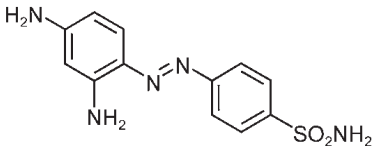
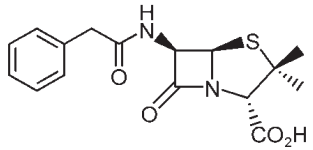
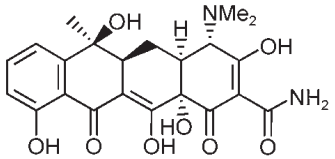
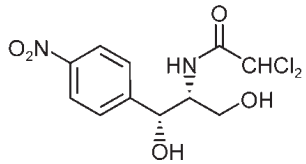
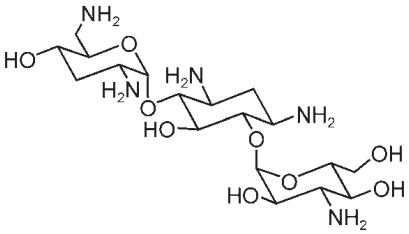
Strategy of Antibacterial Drugs

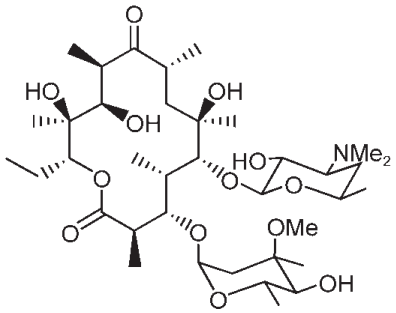
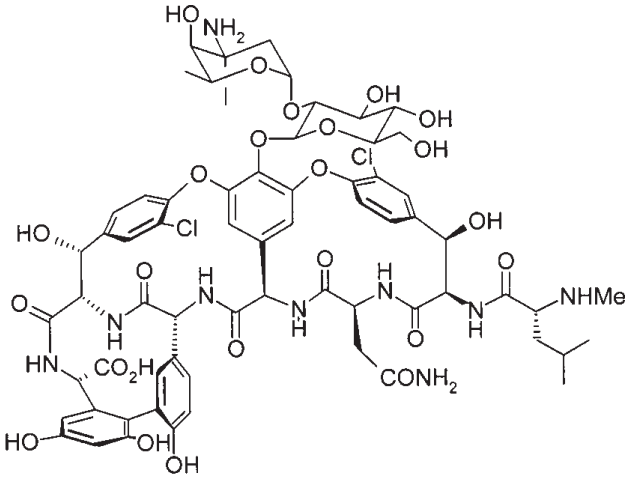
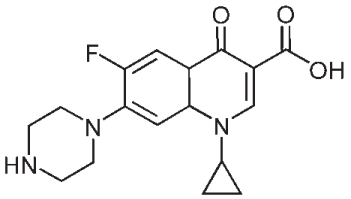


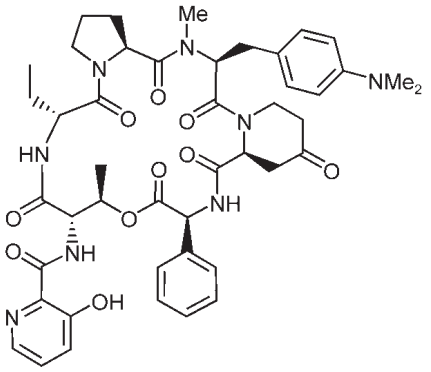
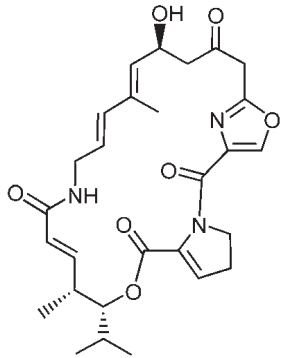
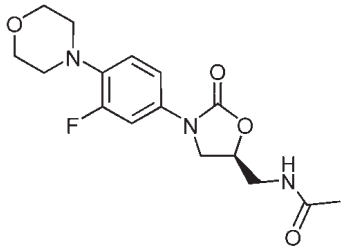
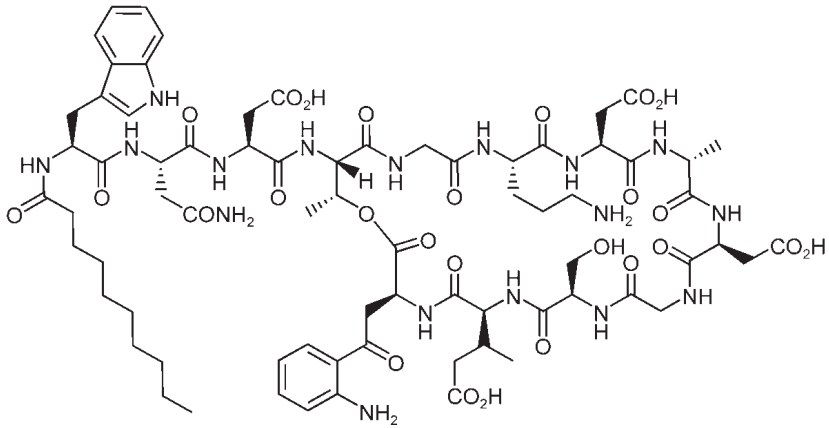
The mode of action of antibacterial compounds

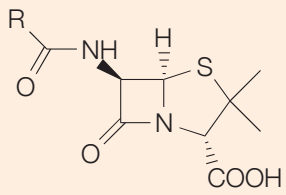
- Inhibition of metabolism (antimetabolites): Sulfonamides
- Inhibition of bacterial cell wall synthesis: Penicillins, Cephalosporins, Vancomycin
- Interaction with the plasma membrane: Polymyxin, Tyrothricin
- Disruption of protein synthesis: Rifamycins, aminoglycosides, tetracyclines, chloramphenicol
- Inhibition of nucleic acid transcription and replication: Nailidixic acid, proflavine

The introduction of antibacterials

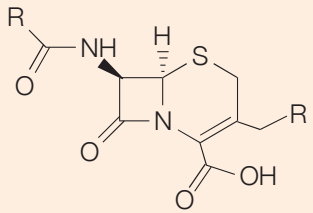
Year	Class	Target	Example	Structure
1935	**sulfonamide**	**folate pathway**	**prontosil**	 1
1940	**β-lactam**	**cell wall**	**penicillin G**	 2
1949	**polyketides**	**protein biosynthesis**	**tetracycline**	 3
1949	**phenylpropanoids**	**protein biosynthesis**	**chloramphenicol**	 4
1950	**aminoglycosides**	**protein biosynthesis**	**tobramycin**	 5

Year	Class	Target	Example	Structure
1952	**macrolides**	**protein biosynthesis**	**erythromycin A**	 6
1958	**glycopeptides**	**cell wall**	**vancomycin**	 7
1962	**quinolones (synthetic)**	**DNA replication**	**ciprofloxacin**	 8

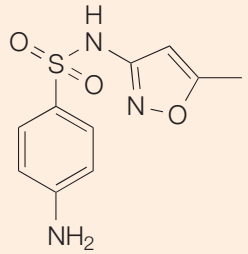
Year	Class	Target	Example	Structure
1962	streptogramins	protein biosynthesis	pristinamycin (I_A + II_A)	 9  10
…				
2000	oxazolidinones (synthetic)	protein biosynthesis	linezolid	 11
2003	lipopeptides	bacterial membrane	daptomycin	 12



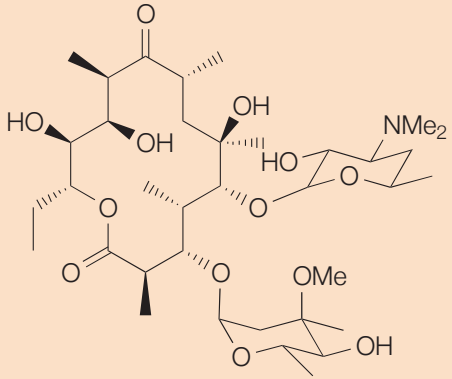
Penicillins



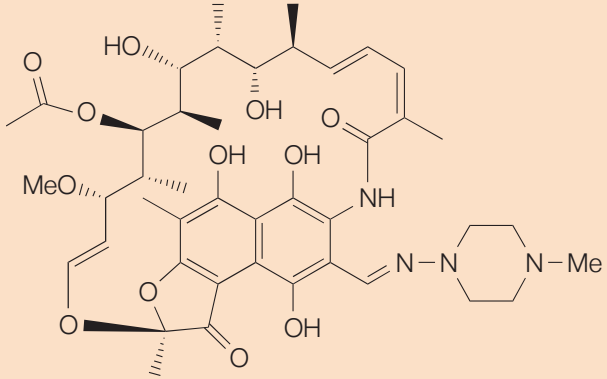
Cephalosporins



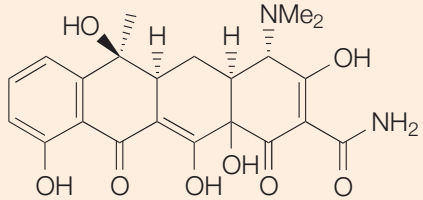
Sulphomethoxazole



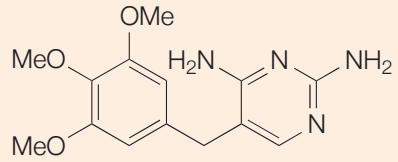
Erythromycin A



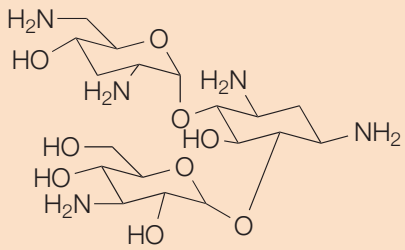
Rifampin



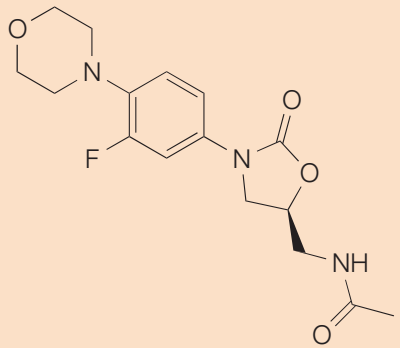
Tetracycline



Trimethoprim



Tobramycin



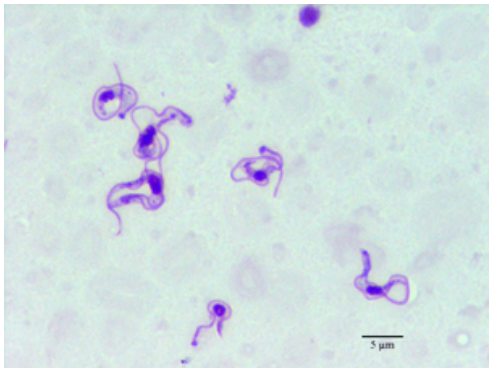
Linezolid

Table 1 | Main classes of antibiotics

Class	Examples
***β*-Lactams**	
Penicillins	Penicillin G, penicillin V, methicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, mezlocillin, piperacillin, azlocillin, temocillin
Cephalosporins	
First generation	Cepalothin, cephapirin, cephradine, cephaloridine, cefazolin
Second generation	Cefamandole, cefuroxime, cephalexin, cefprozil, cefaclor, loracarbef, cefoxitin, cefmetazole
Third generation	Cefotaxime, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefixime, cefpodoxime, ceftibuten, cefdinir
Fourth generation	Cefpirome, cefepime
Carbapenems	Imipenem, meropenem
Monobactams	Astreonam
***β*-Lactamase inhibitors**	Clavulanate, sulbactam, tazobactam
Aminoglycosides	Streptomycin, neomycin, kanamycin, paromycin, gentamicin, tobramycin, amikacin, netilmicin, spectinomycin, sisomicin, dibekalin, isepamicin
Tetracyclines	Tetracycline, chlortetracycline, demeclocycline, minocycline, oxytetracycline, methacycline, doxycycline
Rifamycins	Rifampicin (also called rifampin), rifapentine, rifabutin, bezoxazinorifamycin, rifaximin
Macrolides	Erythromycin, azithromycin, clarithromycin
Lincosamides	Lincomycin, clindamycin
Glycopeptides	Vancomycin, teicoplanin
Streptogramins	Quinupristin, dafloprístin
Sulphonamides	Sulphanilamide, *para*-aminobenzoic acid, sulfadiazine, sulfisoxazole, sulfamethoxazole, sulfathalidine
Oxazolidinones	Linezolid
Quinolones	Nalidixic acid, oxolinic acid, norfloxacin, pefloxacin, enoxacin, ofloxacin/levofloxacin, ciprofloxacin, temafloxacin, lomefloxacin, fleroxacin, grepafloxacin, sparfloxacin, trovafloxacin, clinafloxacin, gatifloxacin, moxifloxacin, sitafloxacin
Others	Metronidazole, polymyxin, trimethoprim

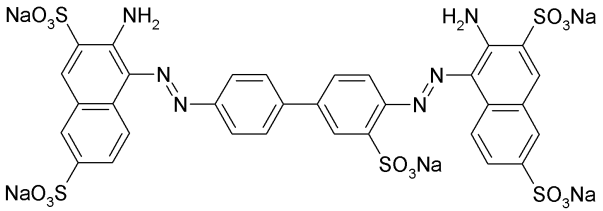
History of Antimetabolites

Paul Ehrlich developed staining dyes, and for example discovered mast cells. He tested more than 100 synthetic dyes for biological activity against *Trypanosoma equinum*, responsible for a disease that horses suffered from.



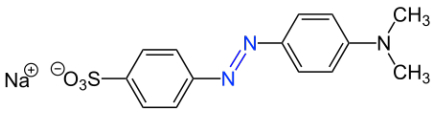
Trypanosoma equinum

The first active compound was **trepan red**



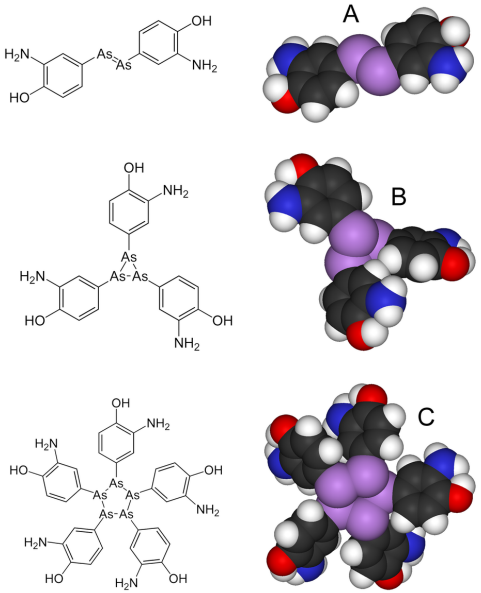
trepan red

trepan red belongs to the class of **azo dyes**



methyl-orange

salvarsan is a anti-syphilis compound that is chemically related

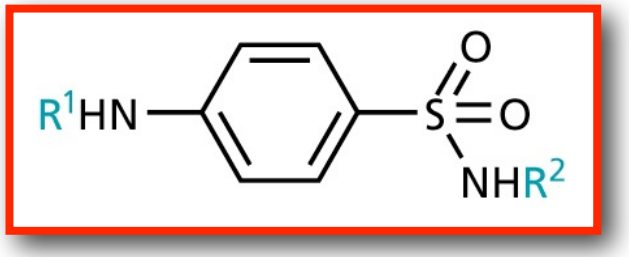


Hoechst was a company that produced ago dyes, and the discovery of these antibiotics triggered the transition to pharma industry !

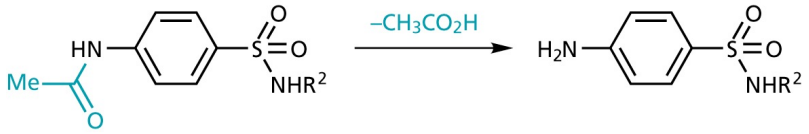
source: Wikipedia

Antimetabolites

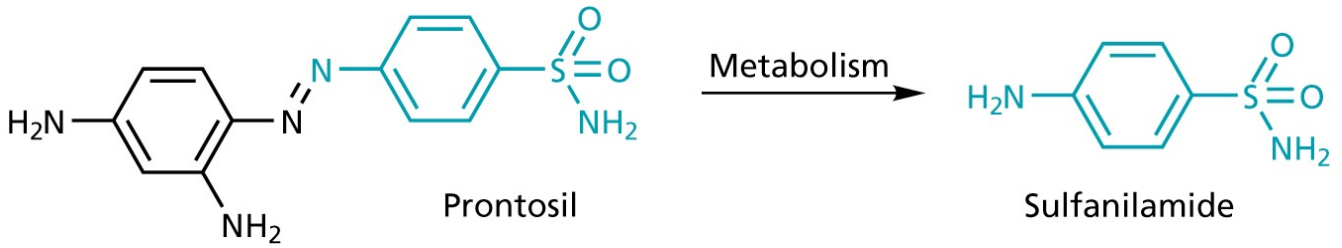
- **Sulfonamides**



- aromatic ring and sulfonamide required, ring must be para-substituted.
- the sulfonamide nitrogen must be primary or secondary
- R1 must be H (or acyl), R2 is variable



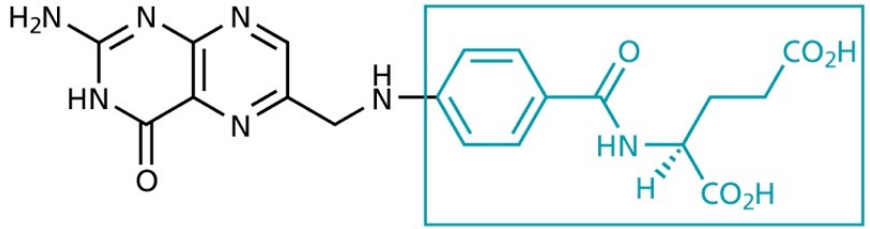
- active form can be formed in vivo (prodrugs)

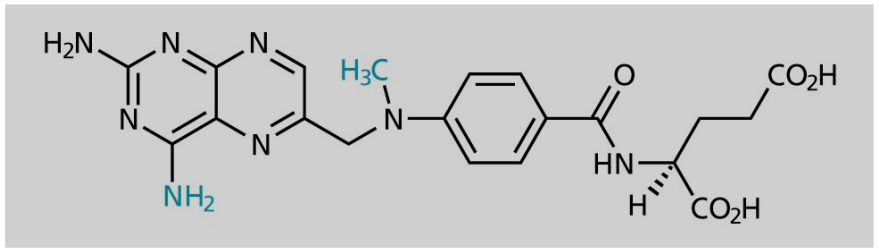


1932, Bayer

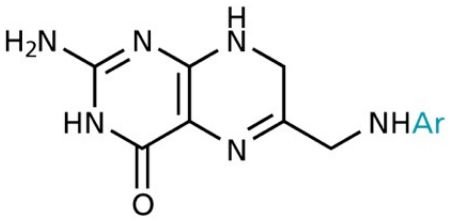
Antimetabolites

conversion of FH_4 to Methlyen-FH_4 by the serine **hydroxymethyltransferase**

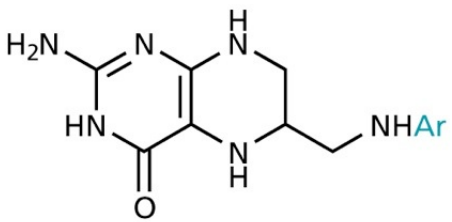




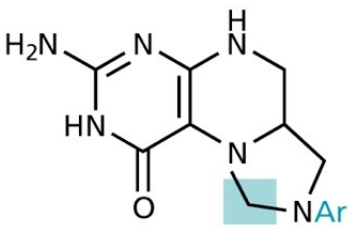
methotrexate



Dihydrofolate (FH_2)



Tetrahydrofolate (FH_4)

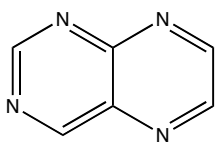


N^5,N^{10}-Methylene-FH_4

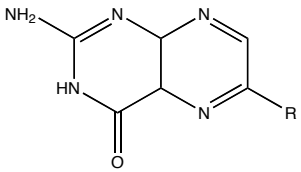
source of one-C unit for methylations of deoxyuridinemonophosphate (dUMP) to form deoxythymidinemonophosphate (dTMP)

Mode of action of sulfonamides

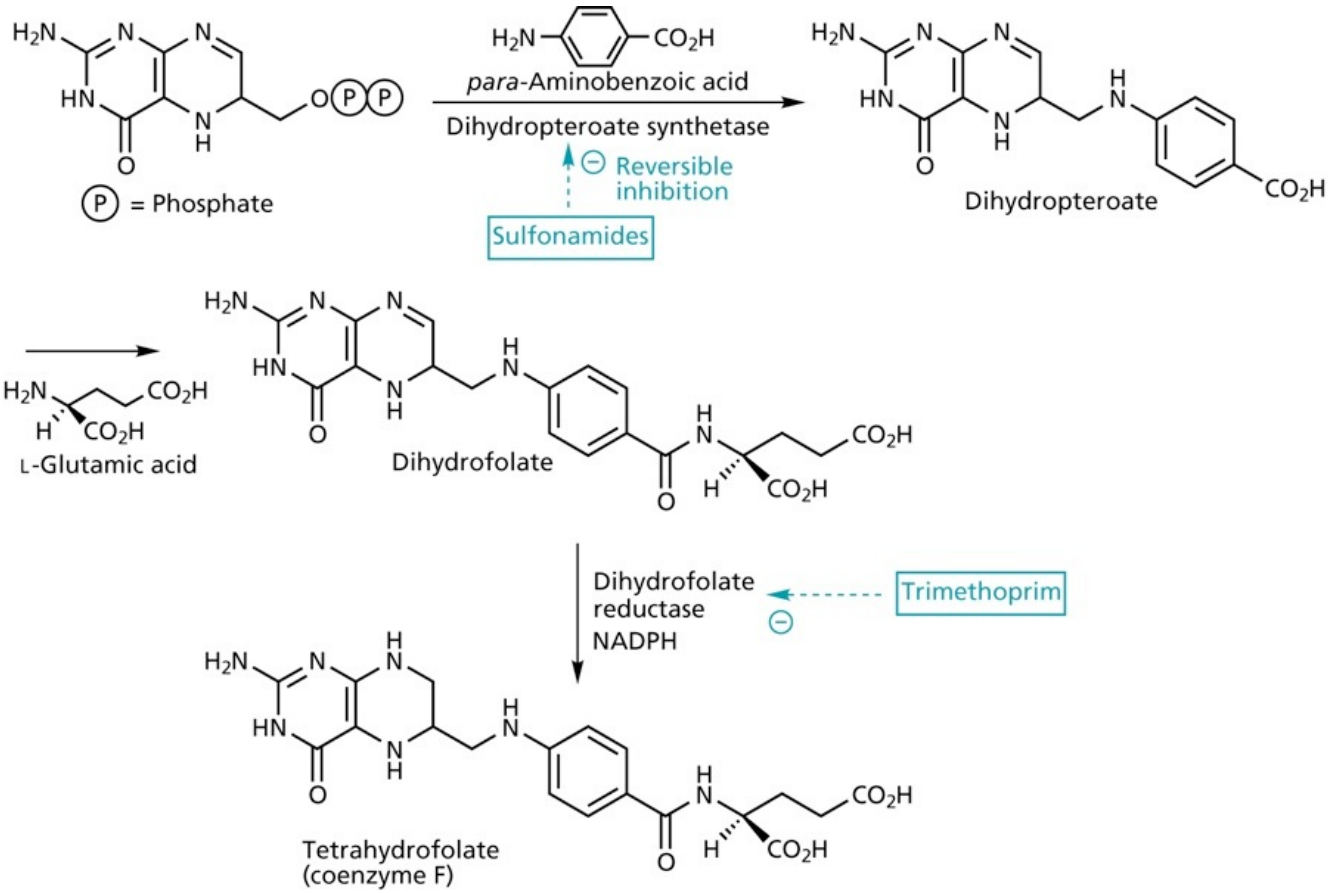
- sulfonamides **block the biosynthesis of tetrahydrofolate** in bacterial cells. Tetrahydrofolate provides C-1 units for the pyrimide biosynthesis required for DNA synthesis.
- Sulfonamides **mimick p-aminobenzoic acid**, a substrate for dihydro-pteroate synthetase.



Pteridine

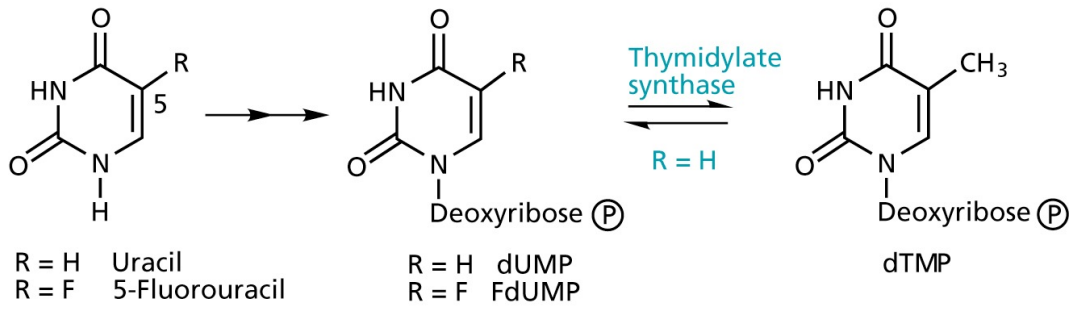


Pterin

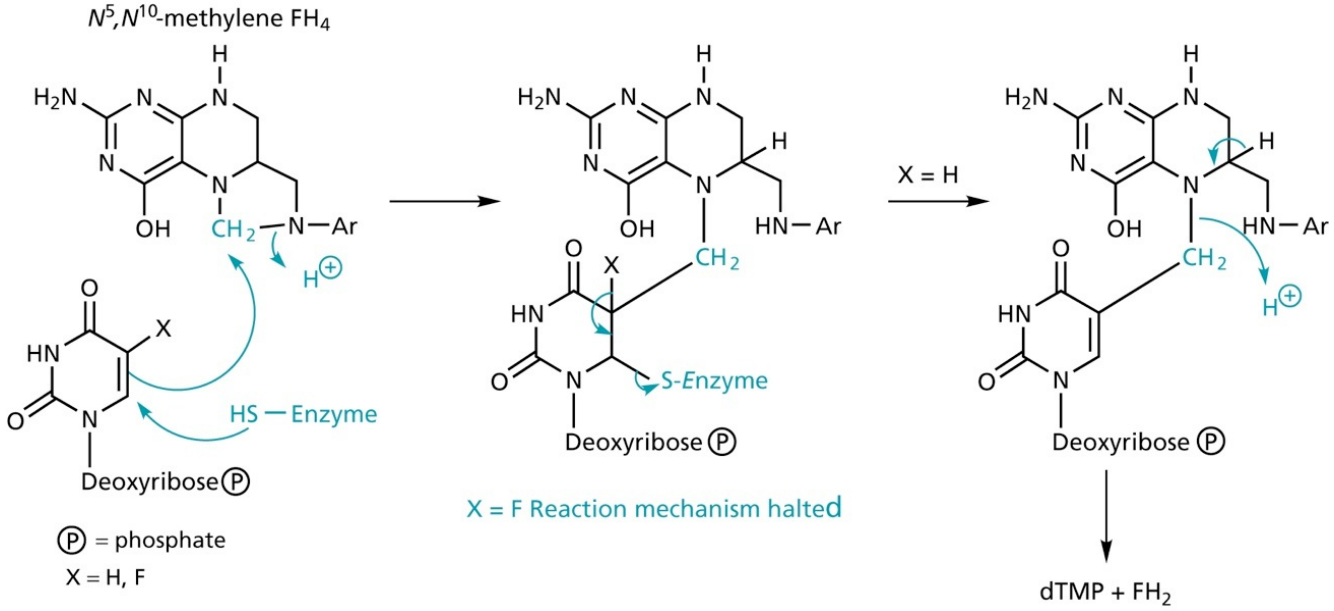


Antimetabolites

Thymidylate synthase inhibitors

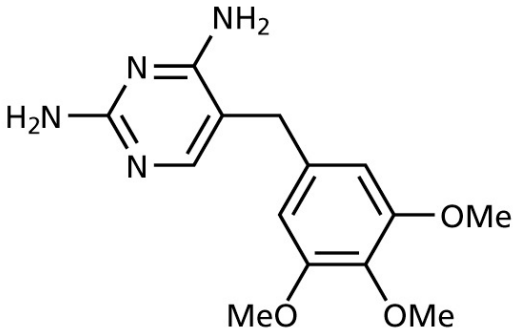


5-Fluorouracil acts as an suicide inhibitor



Other Antimetabolites

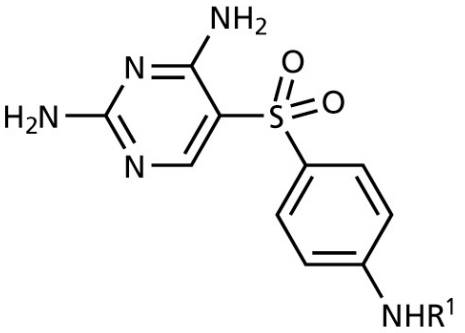
- Trimethroprim: Inhibits dihydrofolate reductase



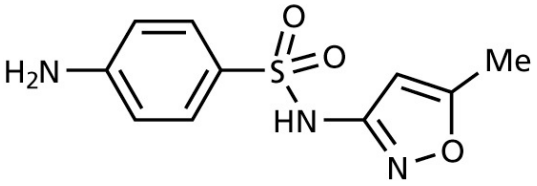
Trimethoprim (antimalarial)

Applications

- treatment of urinary tract infections
- eye lotions
- treatment of infections of mucous membranes
- treatment of gut infections

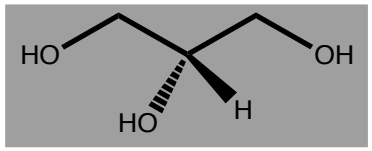


Sulfones (antileprosy)



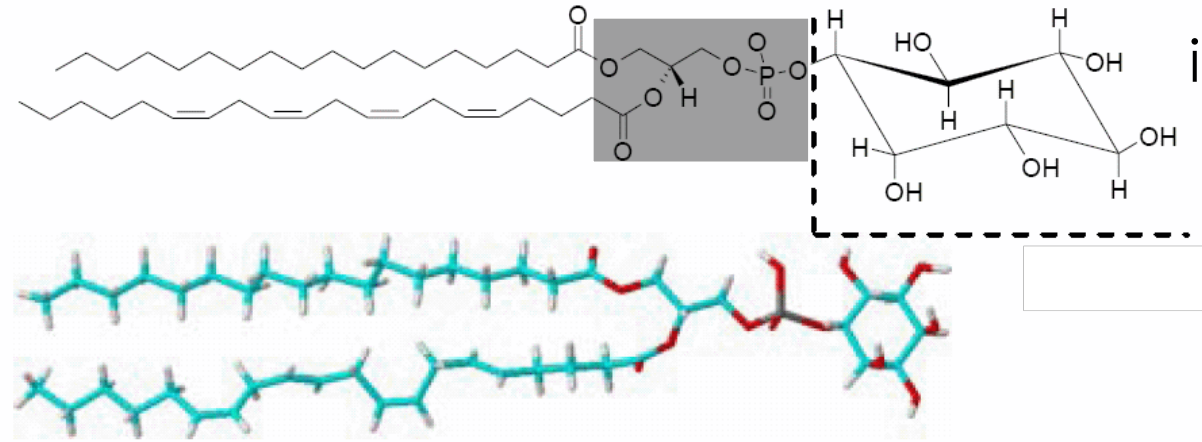
Sulfamethoxazole

Lipid components of mammalian membranes

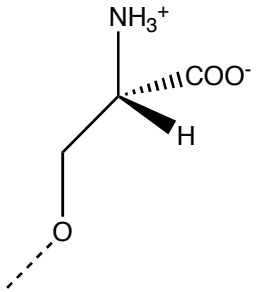


glycerol

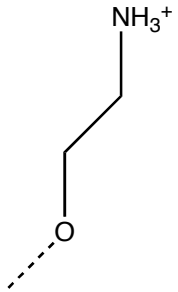
phosphatidylinositol



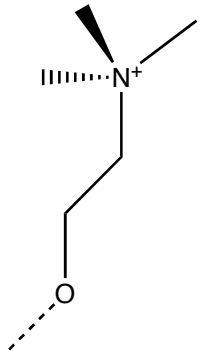
inositol



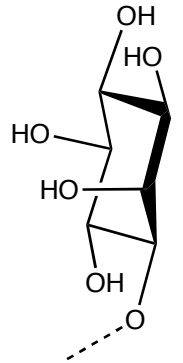
..serine



..ethanolamine



..choline

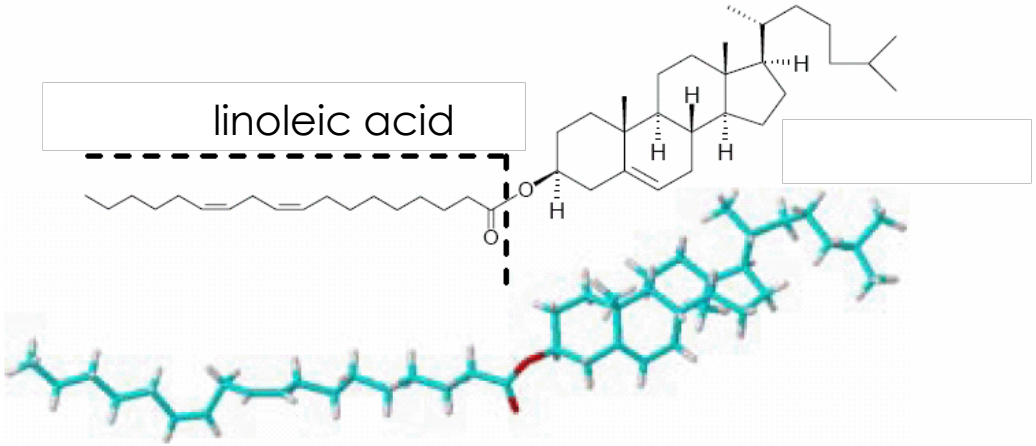


..inositol

total charge	-1	0	0	-1

cholesterol

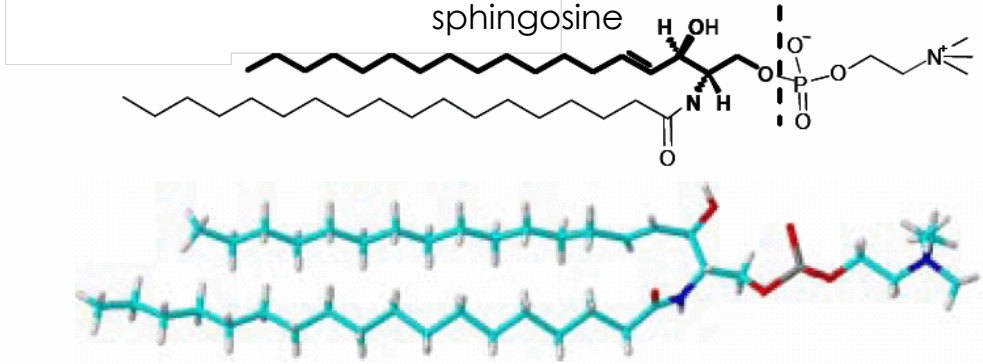
linoleic acid



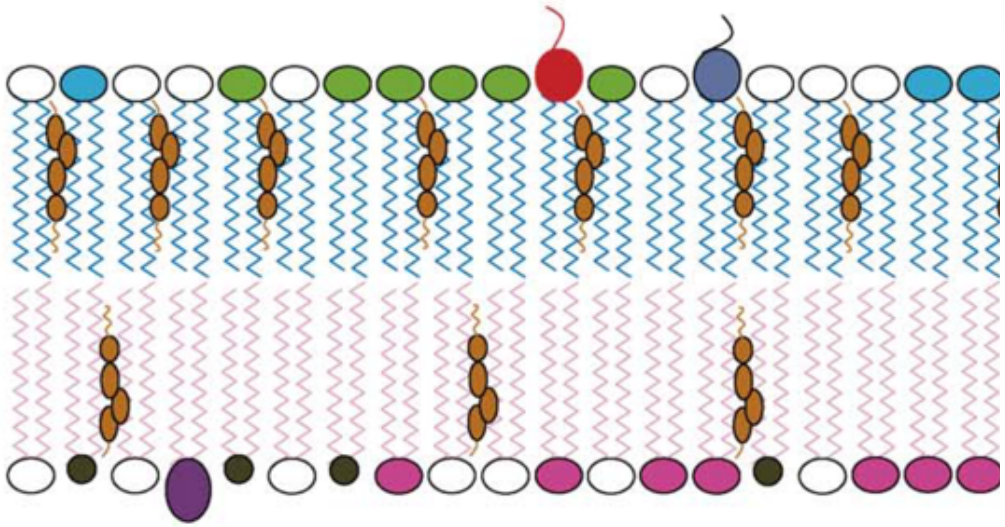
sphingomyelin

ceramide

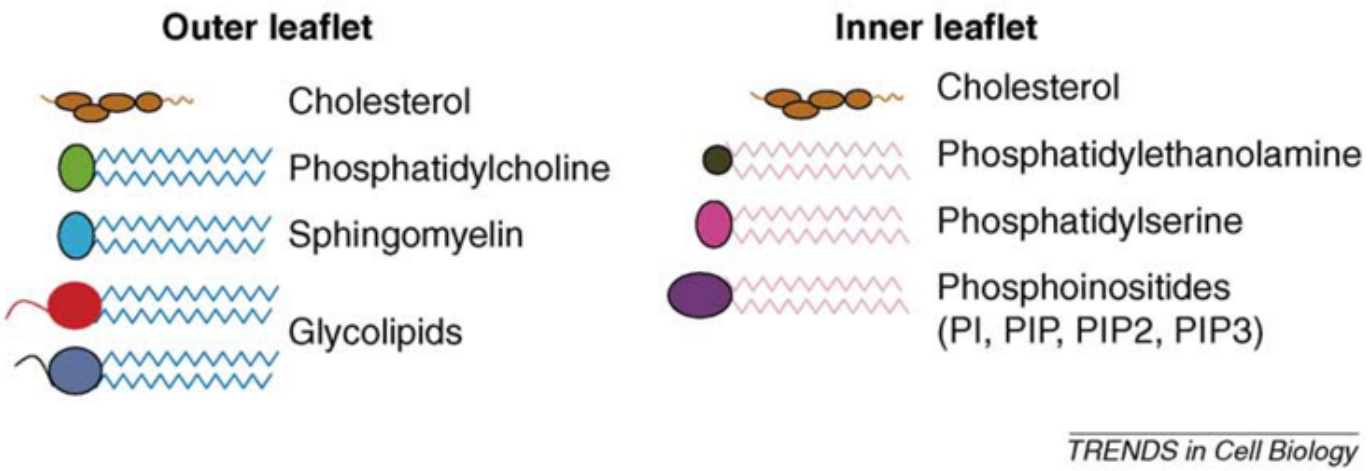
sphingosine



Lipid Asymmetry

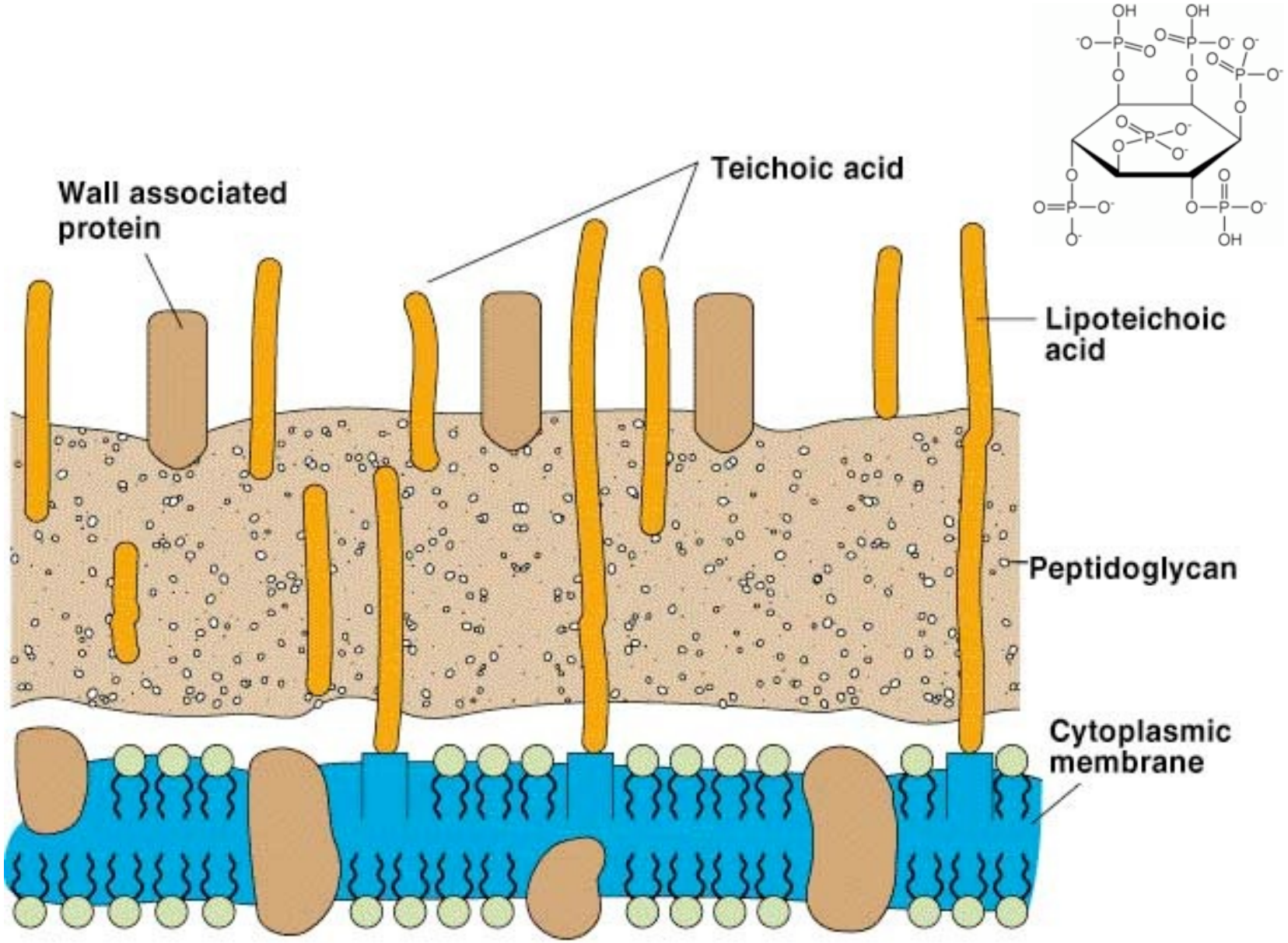


Membrane phospholipid	Percent of total membrane phospholipid	Distribution in membrane
		Inner monolayer 100 — 0 — Outer monolayer 100
Phosphatidylethanolamine	30	
Phosphatidylcholine	27	
Sphingomyelin	23	
Phosphatidylserine	15	
Phosphatidylinositol	5	
Phosphatidylinositol 4-phosphate		
Phosphatidylinositol 4,5-bisphosphate		
Phosphatidic acid		

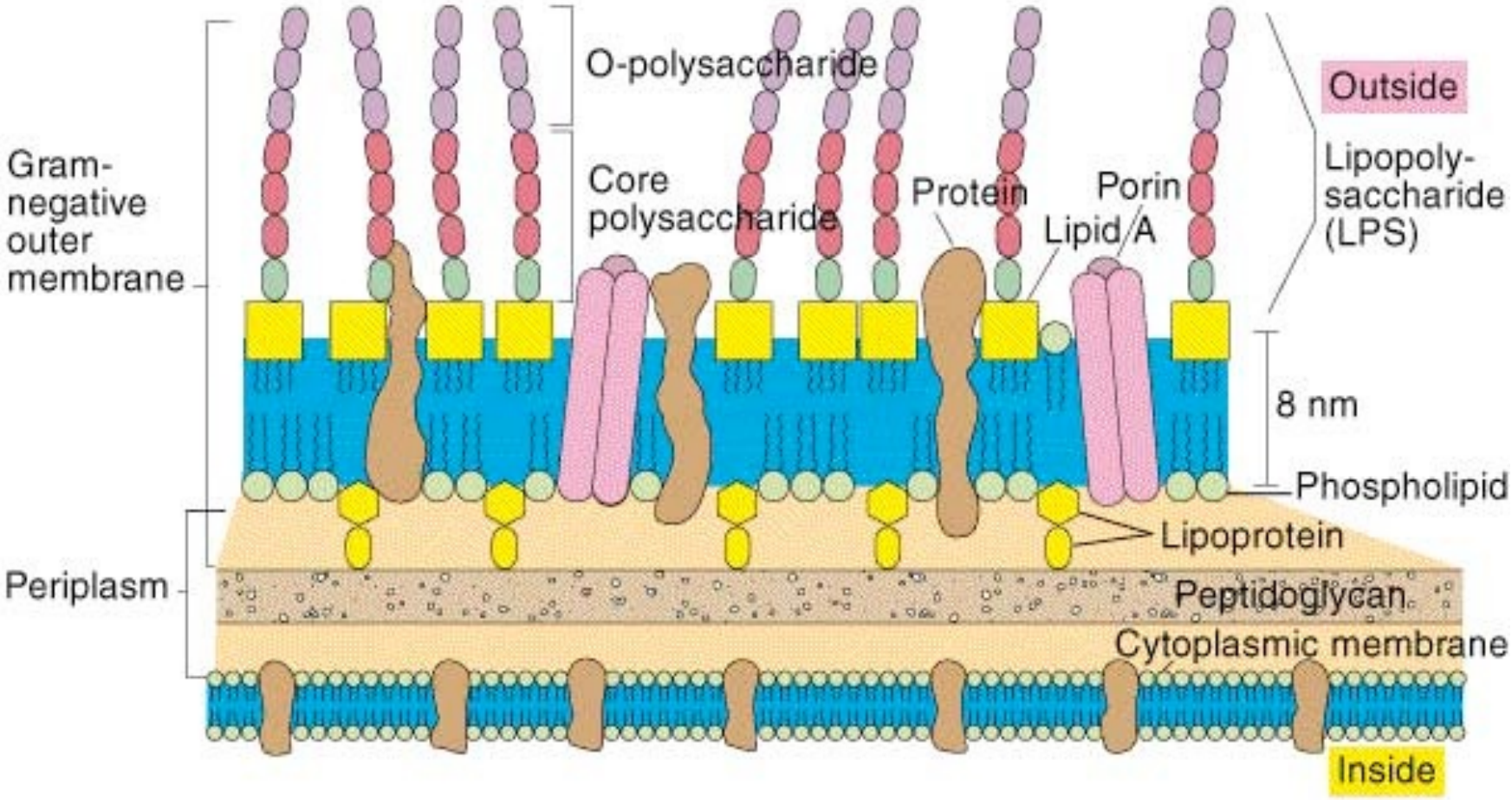


TRENDS in Cell Biology

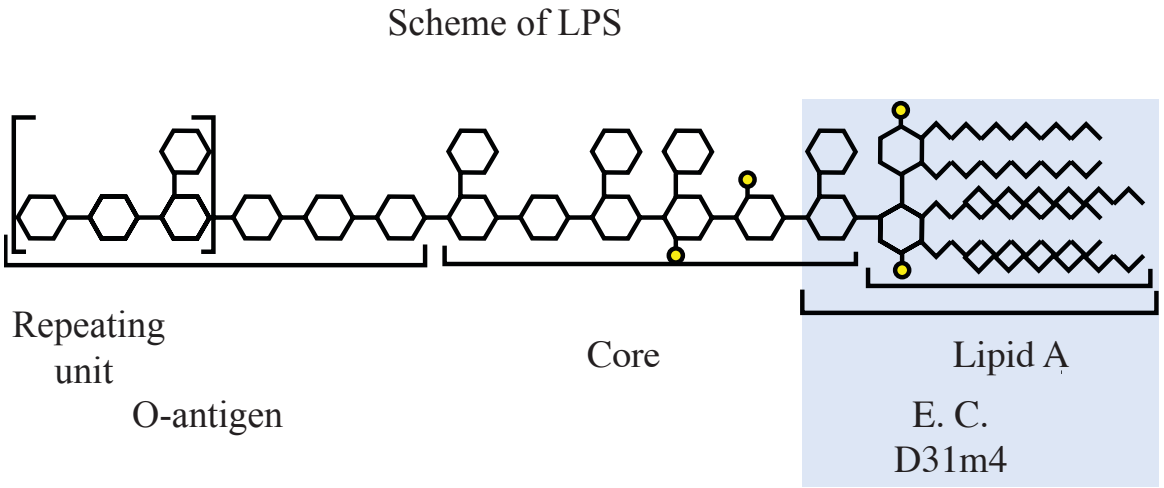
Cell wall of gram-positive bacteria

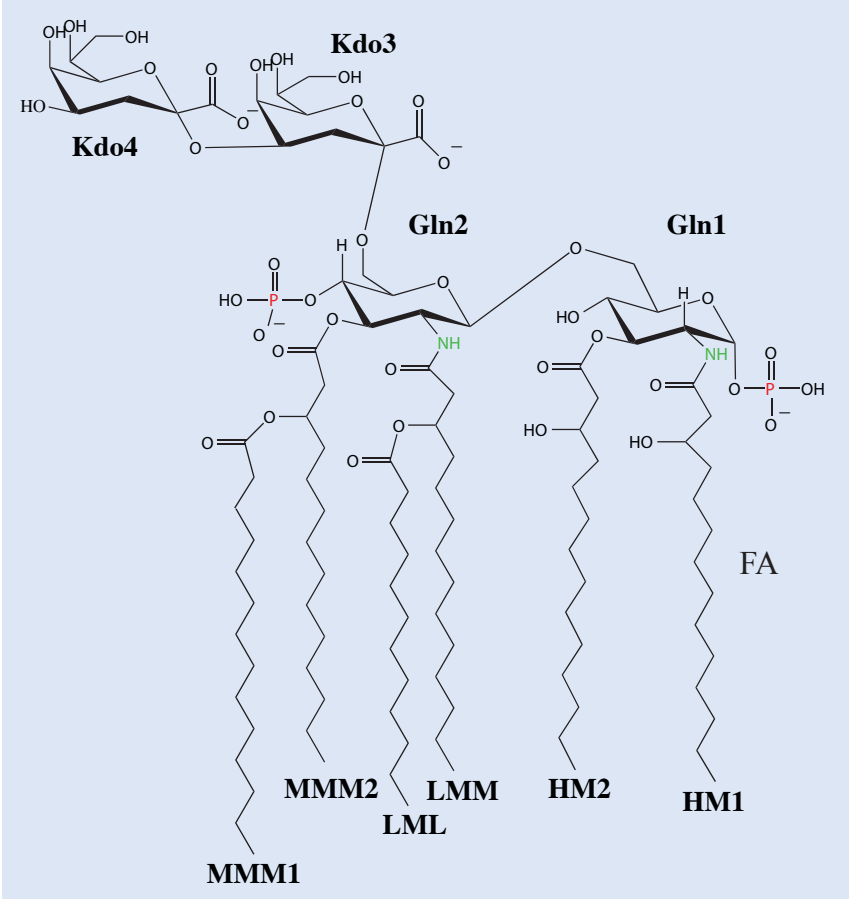


Cell wall of gram-negative bacteria

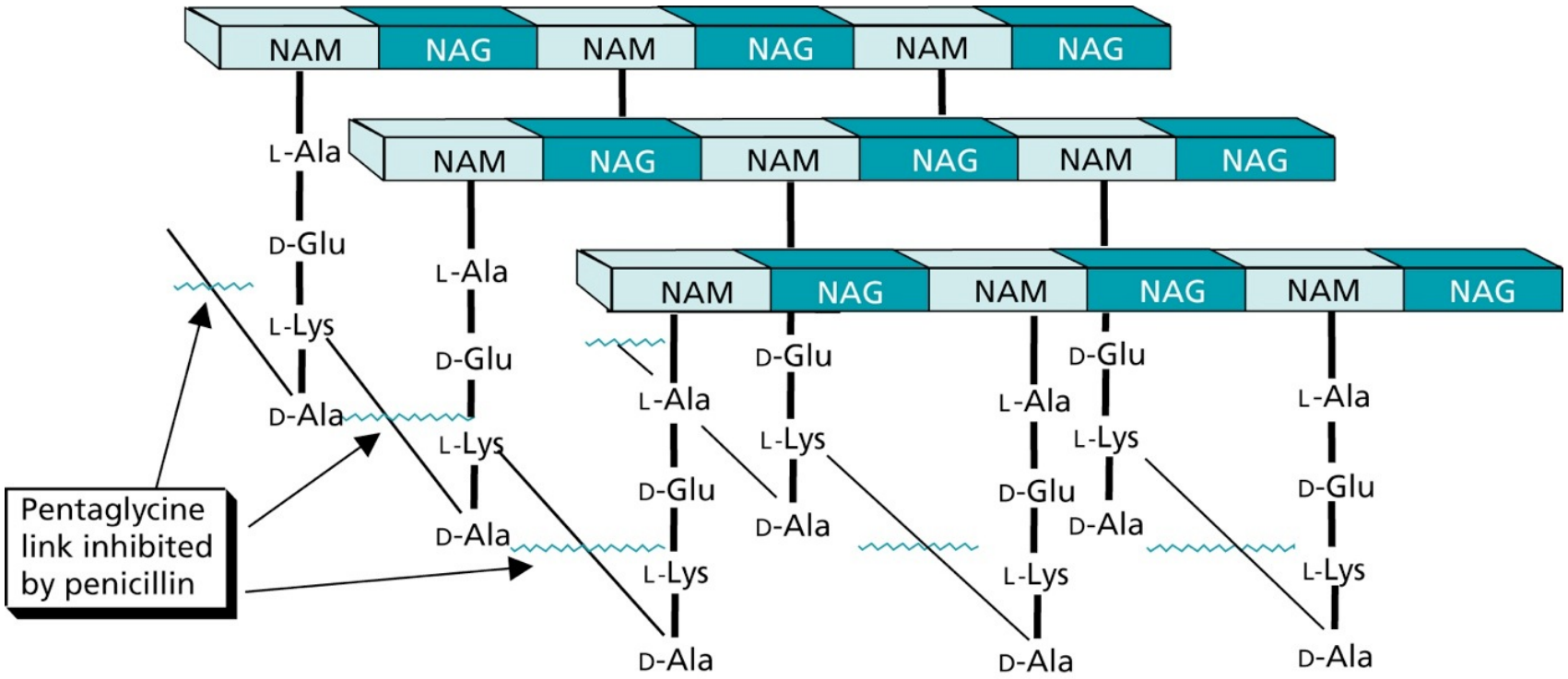


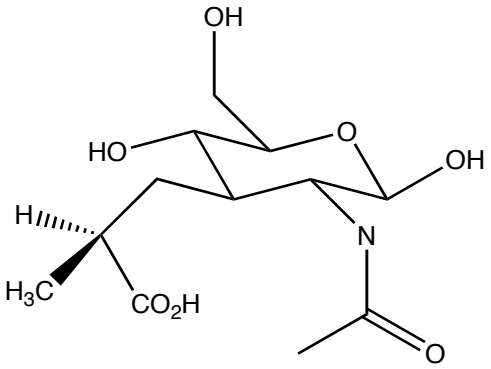
The Structure of Lipopolysaccharide (LPS)



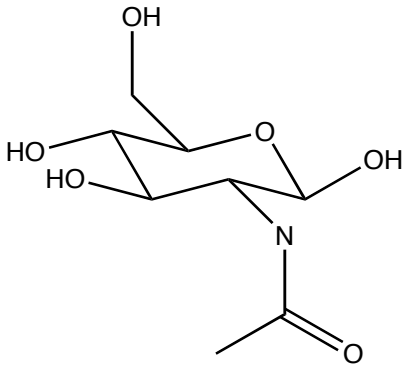


The peptidoglycan layer



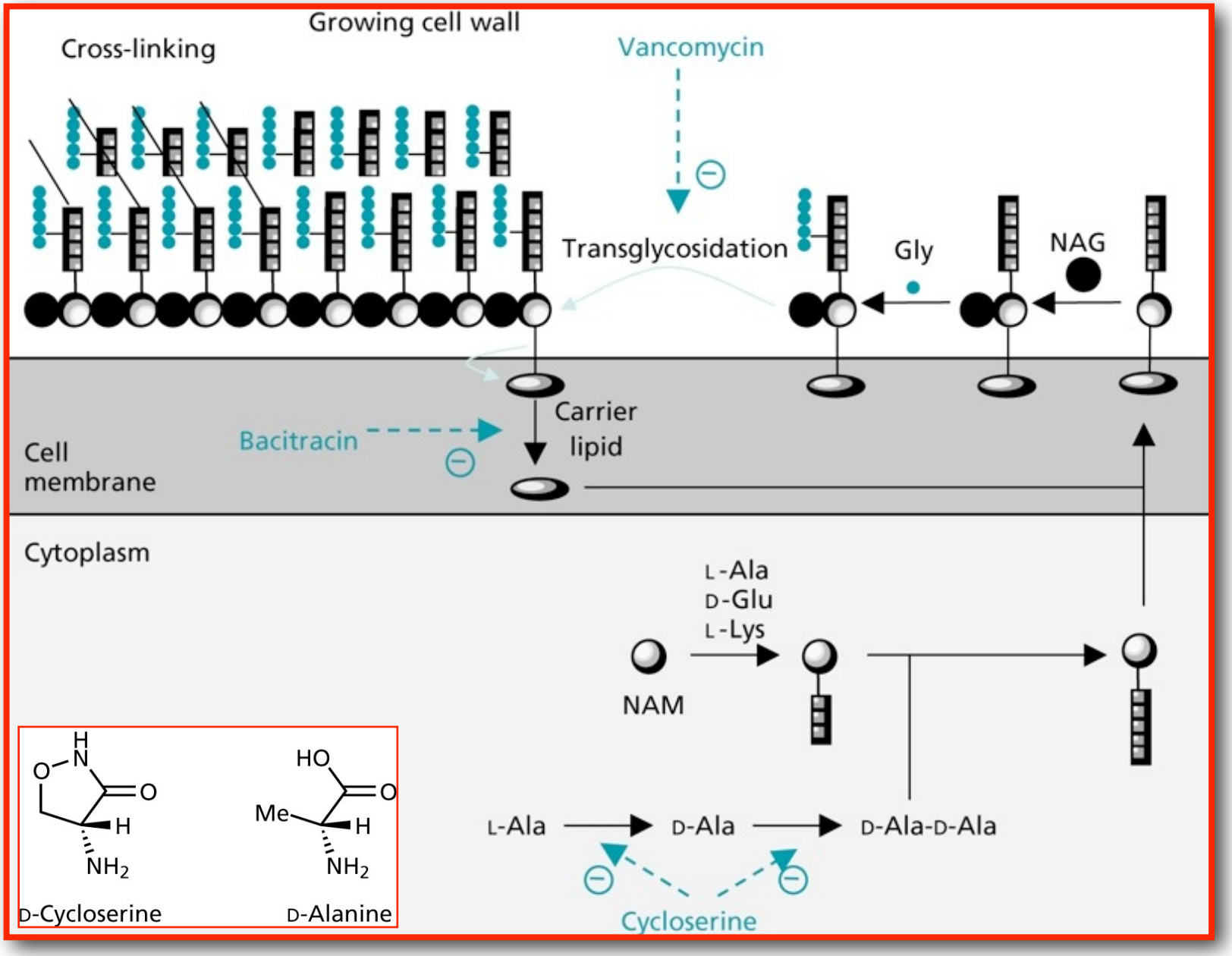


NAM



NAG

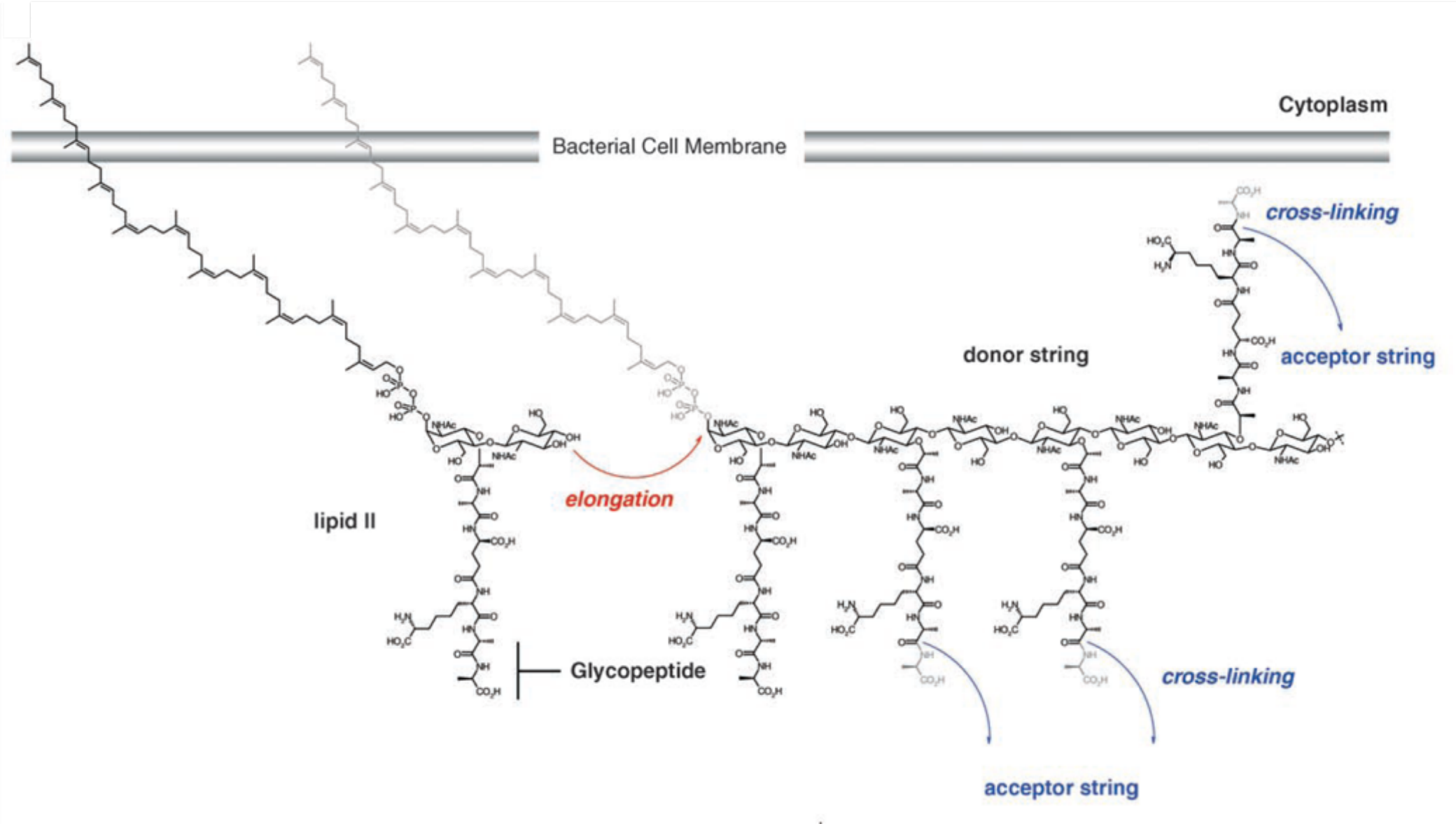
Cell Wall Biosynthesis



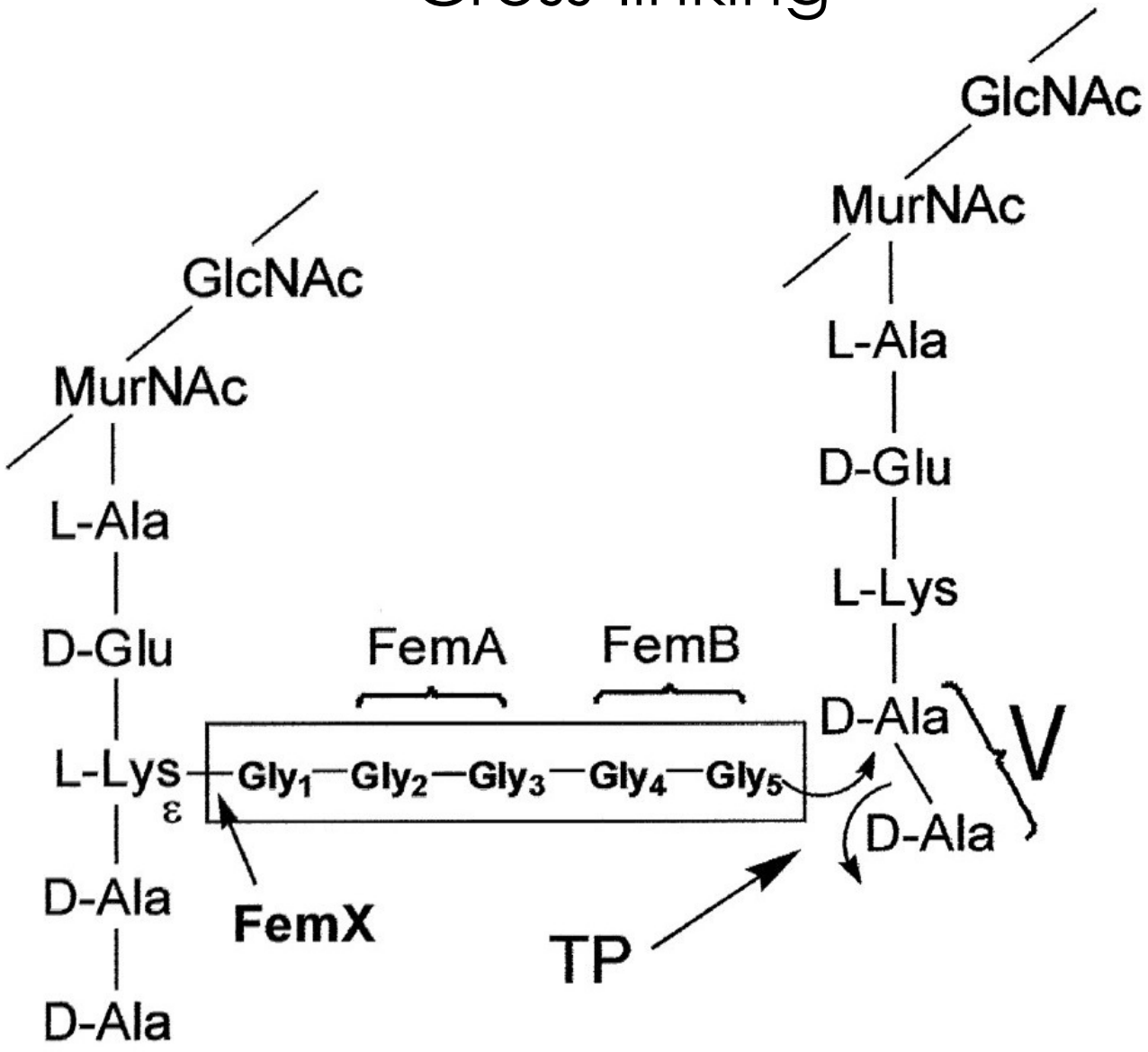
Cell Wall Biosynthesis

- Firstly, N-acetylmuramic acid (NAM) is linked to three amino acids (^{L}Ala-^{D}Glu-^{L}Lys)
- The tripeptide is then linked to ^{D}Ala-^{D}Ala
- The resulting pentapeptide glycopeptide is attached to a C55 carrier lipid by a translocase enzyme, and carried to the outer surface of the cell membrane
- In the following step N-acteylglucosamine (NAG) is added
- Afterwards a pentaglycine chain is linked to the peptide part
- A transglycosylase enzyme catalyses the attachment of the disaccharide building block to the growing cell wall, and releases the lipid carrier

Chain elongation



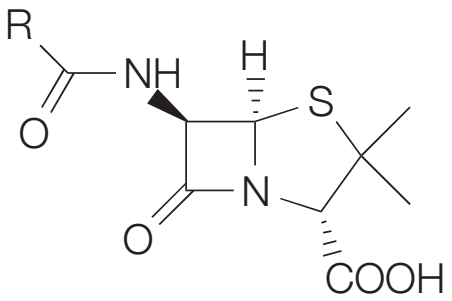
Cross-linking



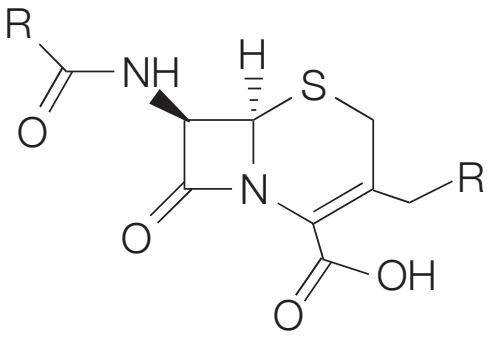
Discovery of Penicillin

- Alexander Flemming discovers in 1928 that a fungus grew on a bacterial plate containing staphylococci. Close to the fungus all bacteria were killed.
- Biotechnological production of penicillins was established during the second world war and helped saving the life of many soldiers.
- Fleming, Chain und Florey received the Nobel price 1945

Antibacterials that inhibit the cell wall synthesis



Penicillins

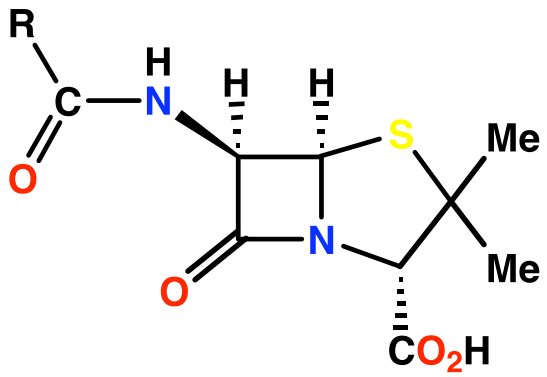


Cephalosporins

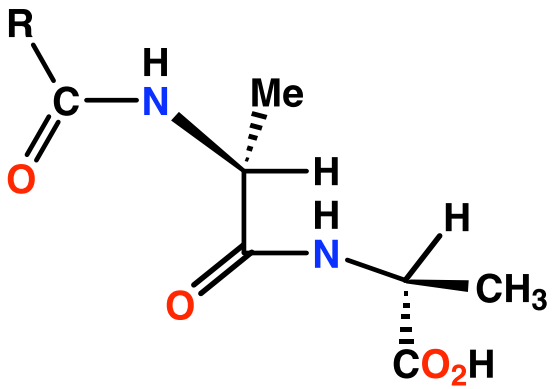
- Antibacterial agents which inhibit bacterial cell wall synthesis
- Discovered by Fleming from a fungal colony (1928)
- Shown to be non toxic and antibacterial
- Isolated and purified by Florey and Chain (1938)
- First successful clinical trial (1941)
- Produced by large scale fermentation (1944)
- Structure established by X-Ray crystallography (1945)
- Full synthesis developed by Sheehan (1957)
- Isolation of 6-APA by Beechams (1958-60) - development of semi-synthetic penicillins
- Discovery of clavulanic acid and β-lactamase inhibitors

Mechanism of action - bacterial cell wall synthesis

- Penicillin inhibits final crosslinking stage of cell wall synthesis
- It reacts with the transpeptidase enzyme to form an irreversible covalent bond
- Inhibition of transpeptidase leads to a weakened cell wall
- Cells swell due to water entering the cell, then burst (lysis)
- Penicillin possibly acts as an analogue of the L-Ala-γ-D-Ala portion of the pentapeptide chain. However, the carboxylate group that is essential to penicillin activity is not present in this portion

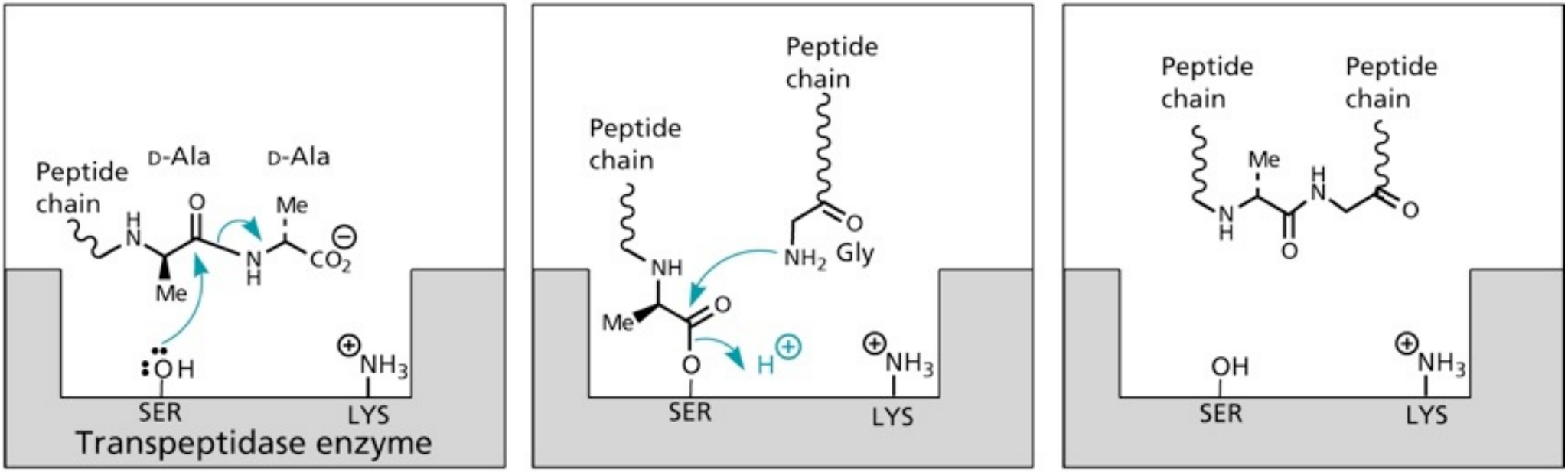


Penicillin

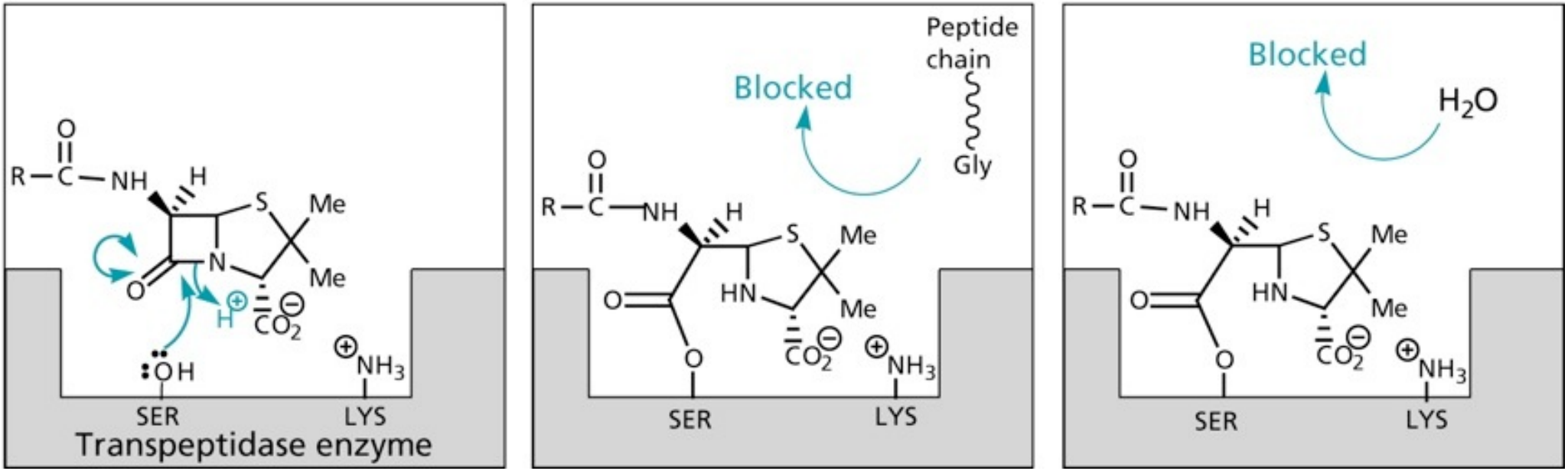


Acyl-D-Ala-D-Ala

(a) Transpeptidase cross-linking

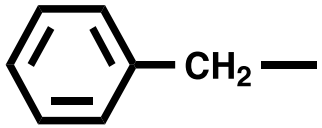


(b) Penicillin inhibition

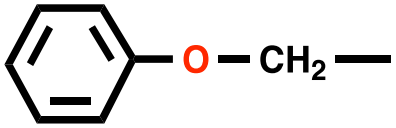


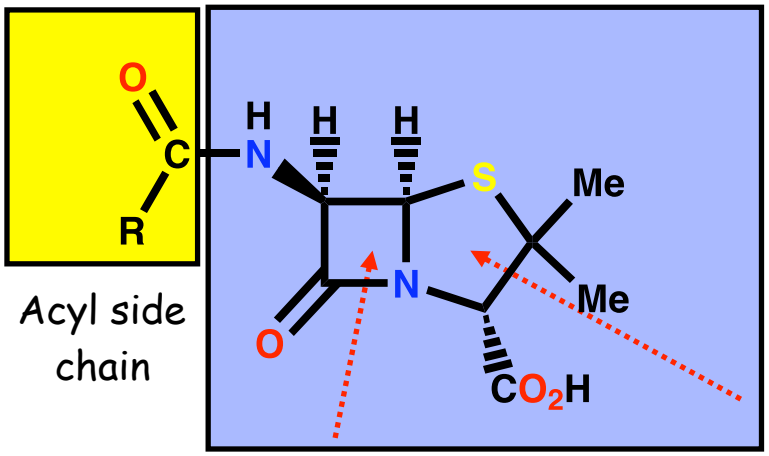
Penicillins

R = Benzyl



R=Phenoxymethyl penicillin (Pen V)





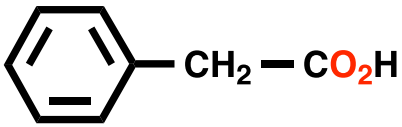
Acyl side chain

6-Aminopenicillanic acid (6-APA)

Thiazolidine ring

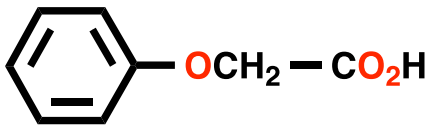
β-Lactam ring

Side chain varies depending on carboxylic acid present in fermentation medium



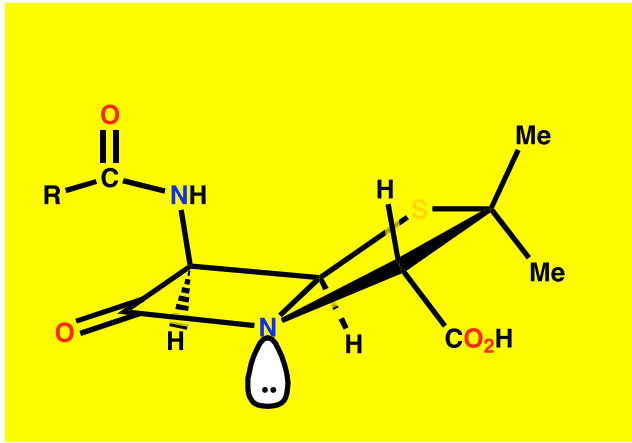
→ Penicillin G

present in corn steep liquor



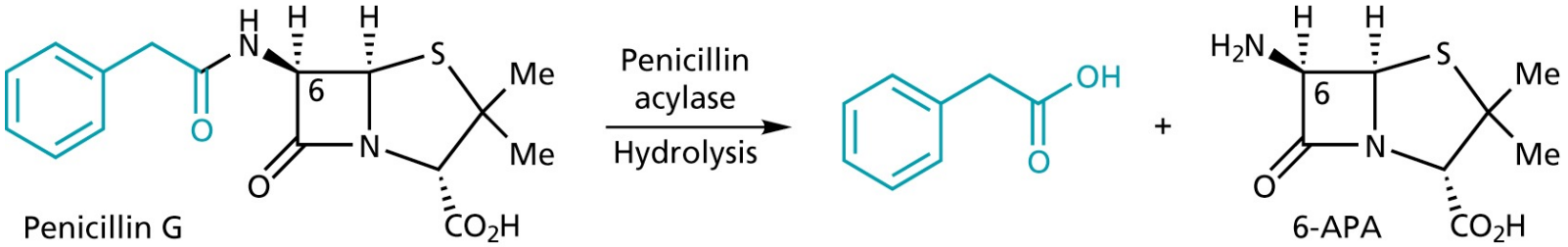
→ Penicillin V
(first orally active penicillin)

Shape of Penicillin G

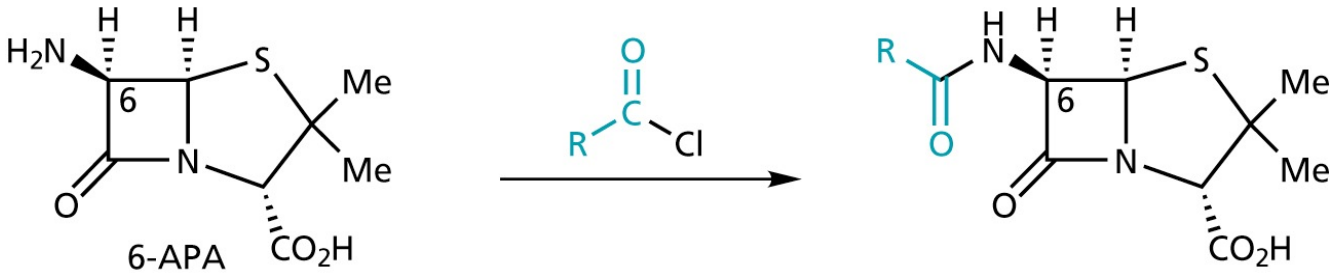


Synthesis of Penicillins

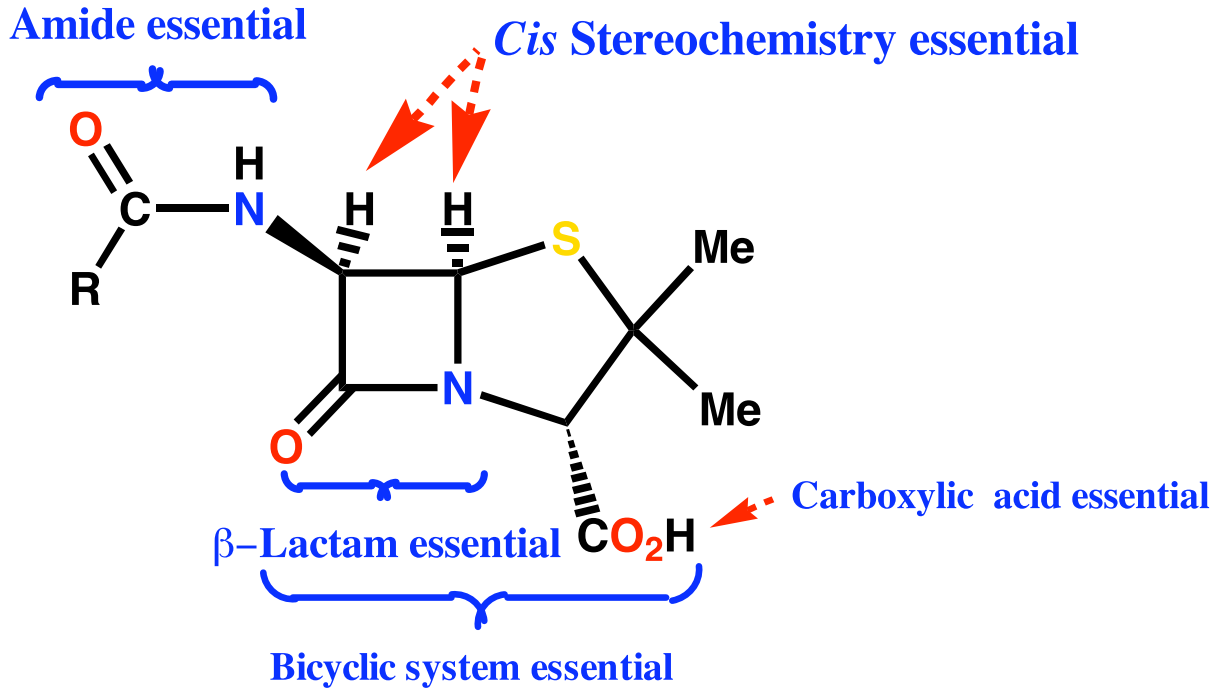
Penicillin G can be enzymatically converted into 6- aminopenicillanic acid (6-APA)



6-APA serves as a convenient starting material for the synthesis of other penicillins



SAR



Conclusions

- Amide and carboxylic acid are involved in binding
- Carboxylic acid binds as the carboxylate ion
- Mechanism of action involves the β-lactam ring
- Activity related to β-lactam ring strain (subject to stability factors)
- Bicyclic system increases β-lactam ring strain
- Not much variation in structure is possible
- Variations are limited to the side chain (R)

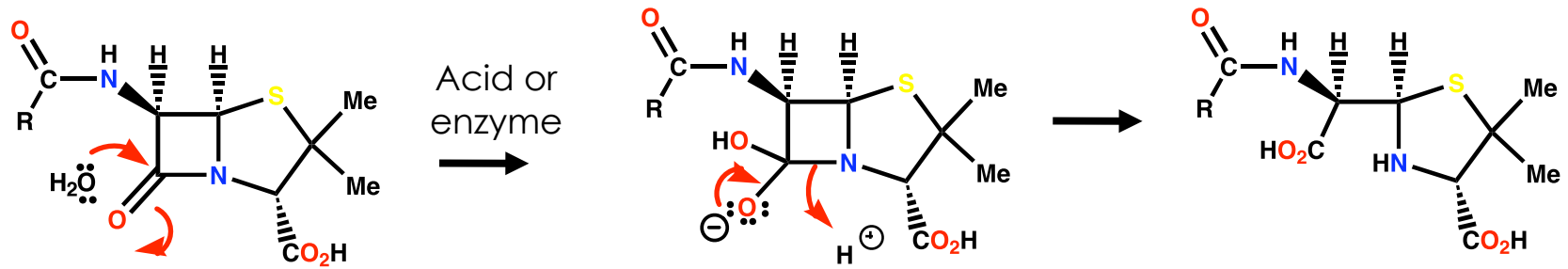
Resistance to Penicillins

- Gram -ve bacteria have a lipopolysaccharide (LPS) outer membrane preventing access to the cell wall
- Penicillins can only cross via porins in the outer membrane
- Porins only allow small hydrophilic molecules that can exist as zwitterions to cross
- High levels of transpeptidase enzyme may be present
- The transpeptidase enzyme may have a low affinity for penicillins (e.g. PBP 2a for S. aureus)
- Presence of β-lactamases
- Concentration of β-lactamases in periplasmic space
- Mutations
- Transfer of β-lactamases between strains
- Efflux mechanisms pumping penicillin out of periplasmic space

Problems with Penicillin G

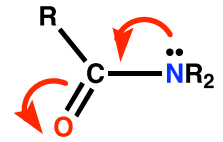
- It is sensitive to stomach acids
- It is sensitive to β-lactamases - enzymes which hydrolyse the β-lactam ring
- It has a limited range of activity

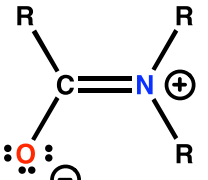
ring strain



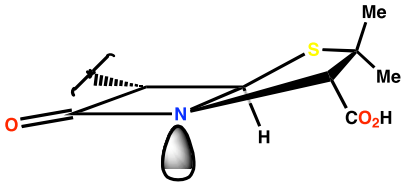
reactive carbonyl group

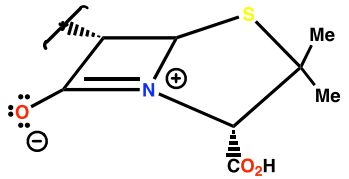
Tertiary amide



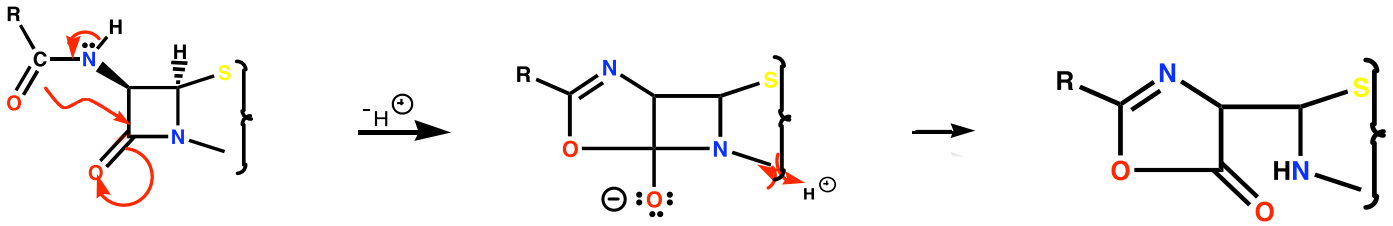


β-Lactam



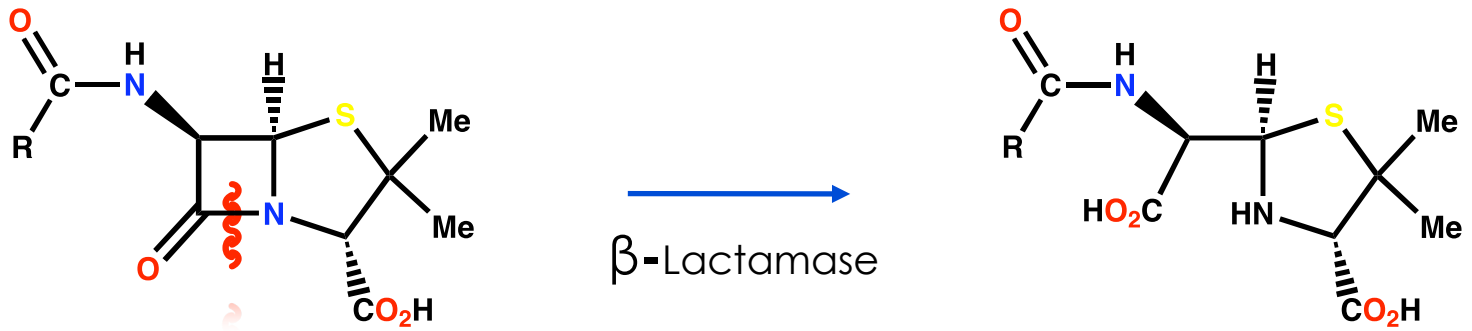


neighboring group part.



Sensitivity to β-Lactamases

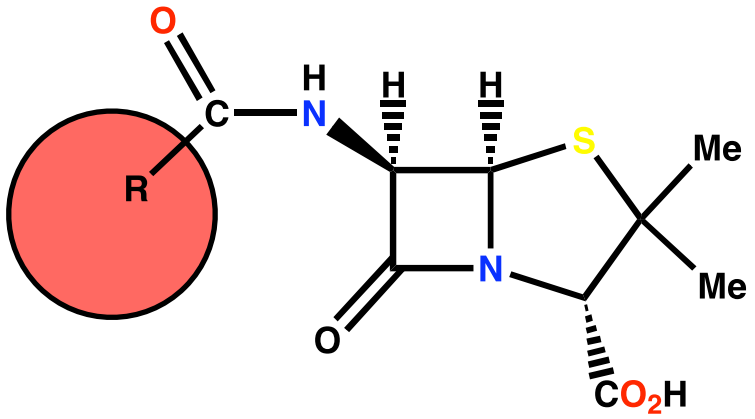
- Enzymes that inactivate penicillins by opening β-lactam rings
- Allow bacteria to be resistant to penicillin
- Transferable between bacterial strains (i.e. bacteria can acquire resistance)
- Important w.r.t. Staphylococcus aureus infections in hospitals
- 80% Staph. infections in hospitals were resistant to penicillin and other antibacterial agents by 1960
- Mechanism of action for lactamases is identical to the mechanism of inhibition for the target enzyme
- But product is removed efficiently from the lactamase active site



Sensitivity to β-Lactamases

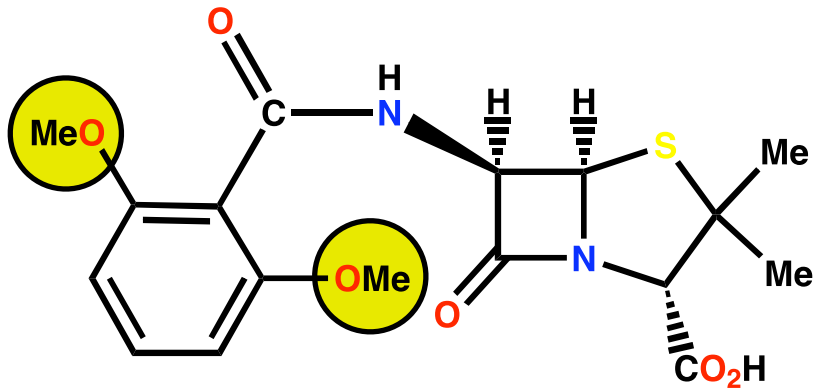
- Block access of penicillin to active site of enzyme by introducing bulky groups to the side chain to act as steric shields
- Size of shield is crucial to inhibit reaction of penicillins with β-lactamases but not with the target enzyme (transpeptidase)

Bulky group

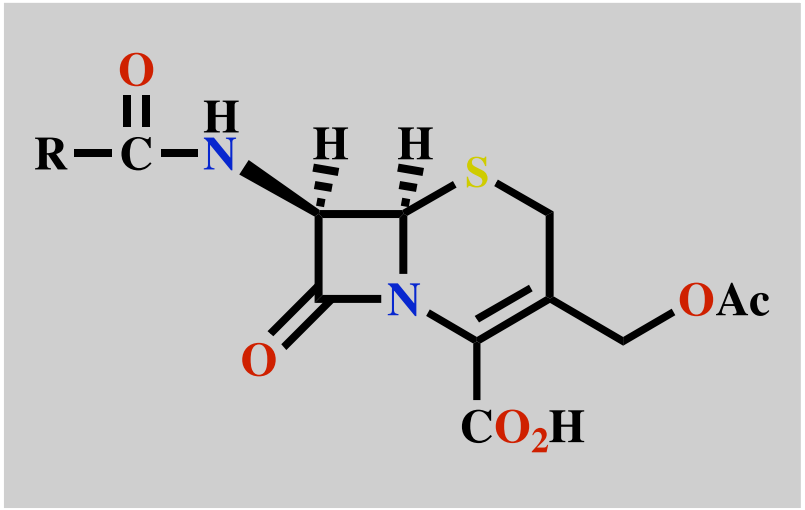


Methicillin (Beechams - 1960)

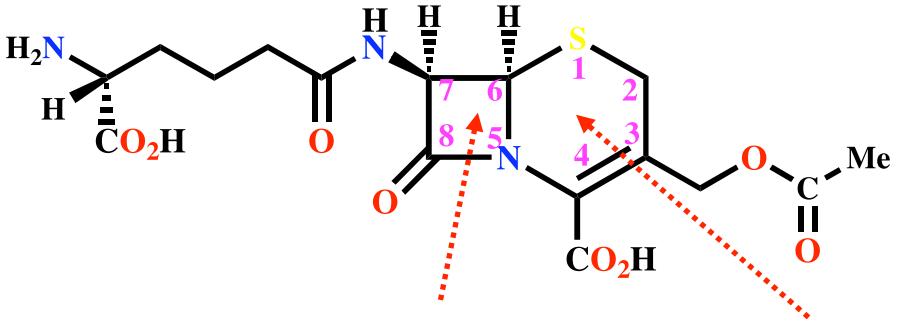
ortho groups important



CEPHALOSPORINS

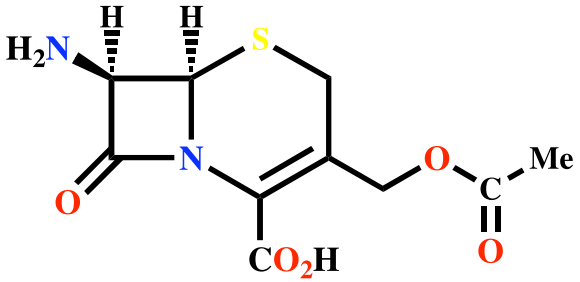


7-Aminoadipic side chain



β-Lactam ring

Dihydrothiazine ring



7-Aminocephalosporinic acid (7-ACA)

Properties of Cephalosporin C

Disadvantages

- Polar due to the side chain - difficult to isolate and purify
- Low potency, not absorbed orally

Advantages

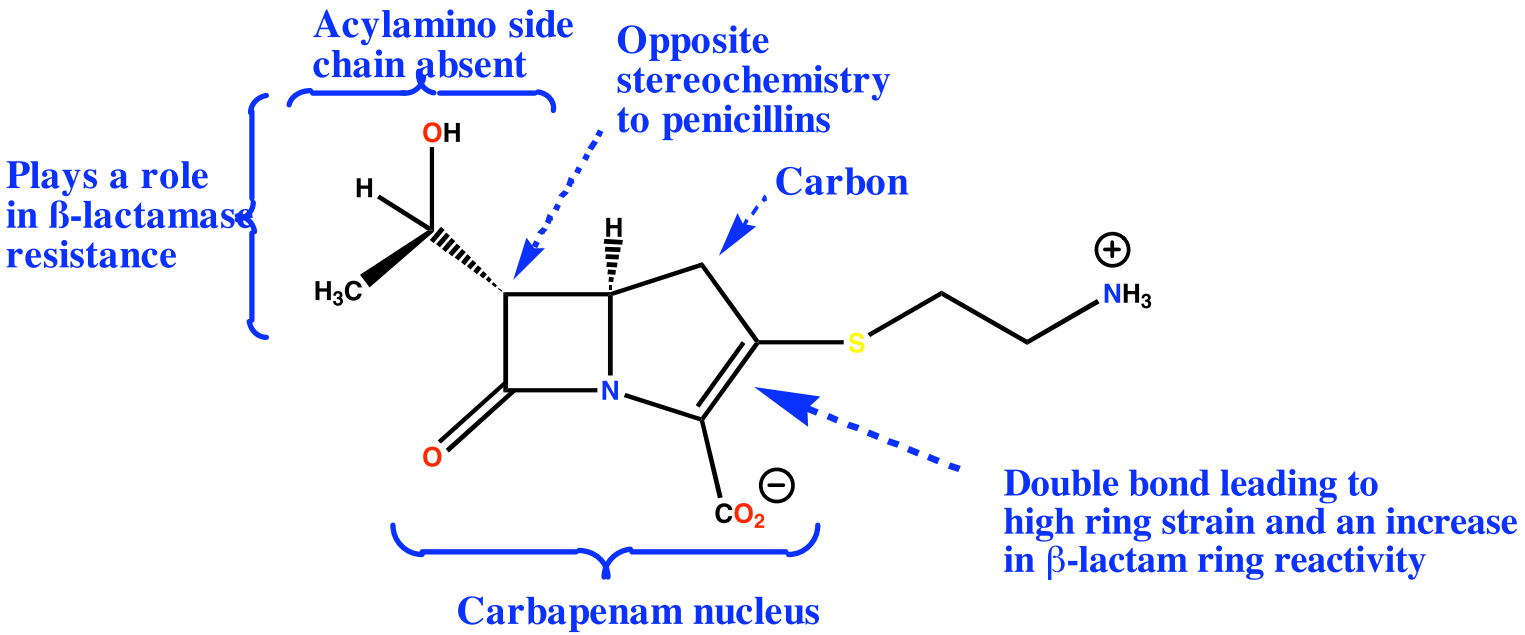
- Non toxic
- Lower risk of allergic reactions compared to penicillins
- More stable to acid conditions
- More stable to β-lactamases
- Ratio of activity vs Gram -ve and Gram +ve bacteria is better

SAR

- The β-lactam ring is crucial to the mechanism
- The carboxylic acid at position 4 is important to binding
- The bicyclic system is important in increasing ring strain
- Stereochemistry is important
- The acetoxy substituent is important to the mechanism
- Possible modifications
- 7-Acylamino side chain
- 3-Acetoxymethyl side chain
- Substitution at C-7

Newer β-Lactam Antibiotics

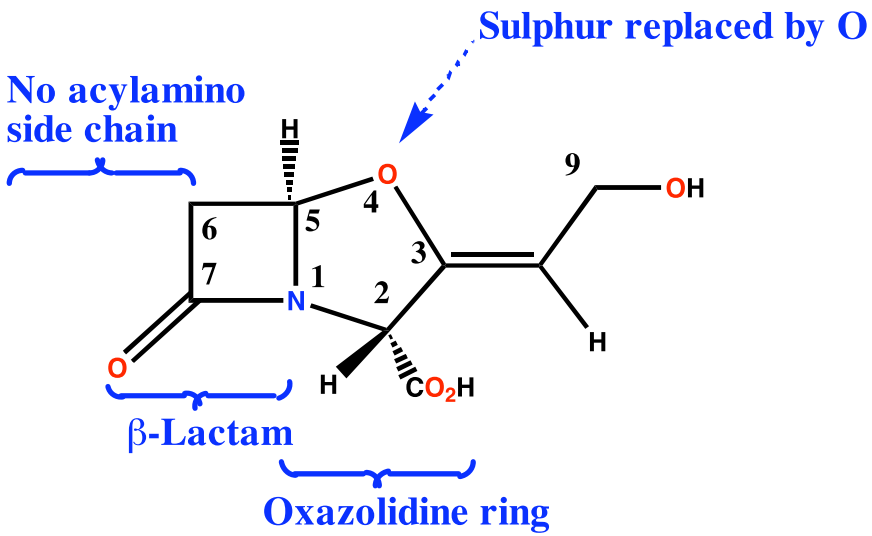
Thienamycin (Merck 1976) (from Streptomyces cattleya)



- Potent and wide range of activity vs Gram +ve and Gram -ve bacteria
- Active vs. Pseudomonas aeruginosa
- Low toxicity
- High resistance to β-lactamases
- Poor stability in solution (ten times less stable than Pen G)

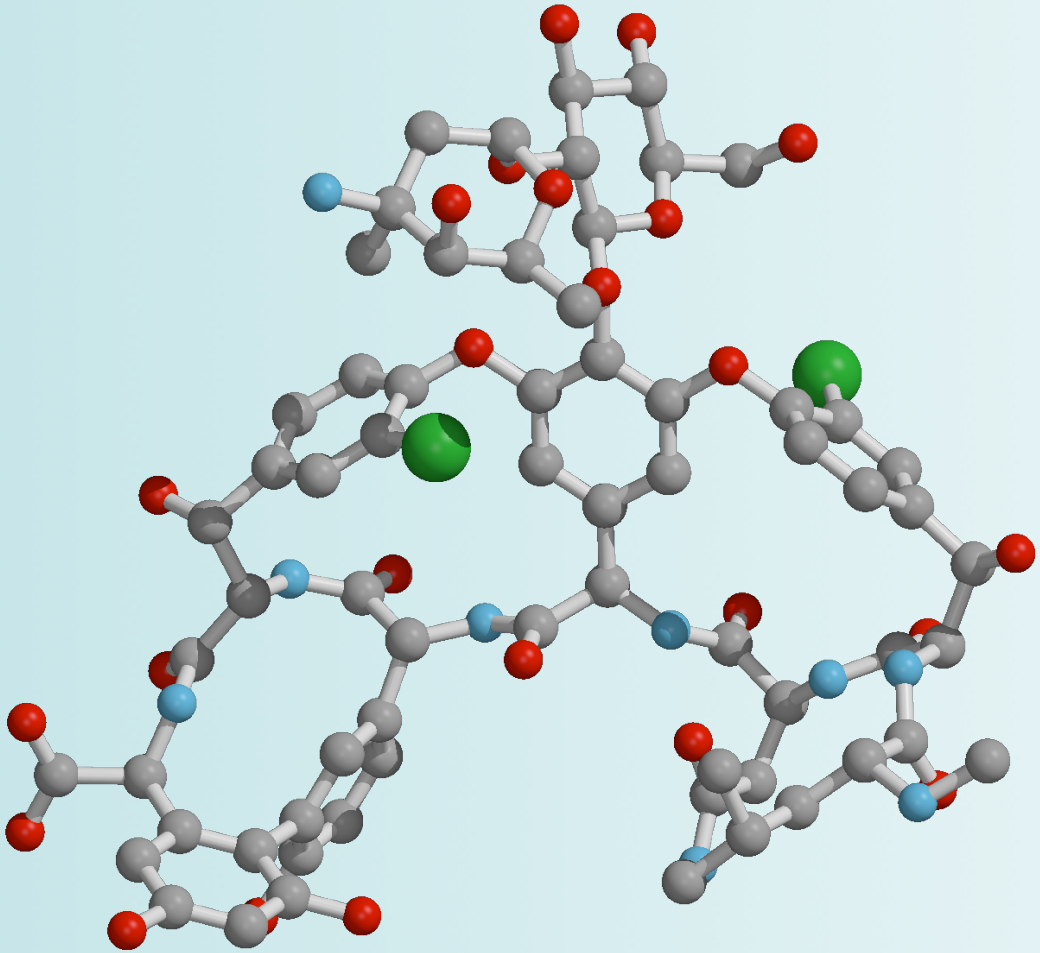
β-Lactamase Inhibitors

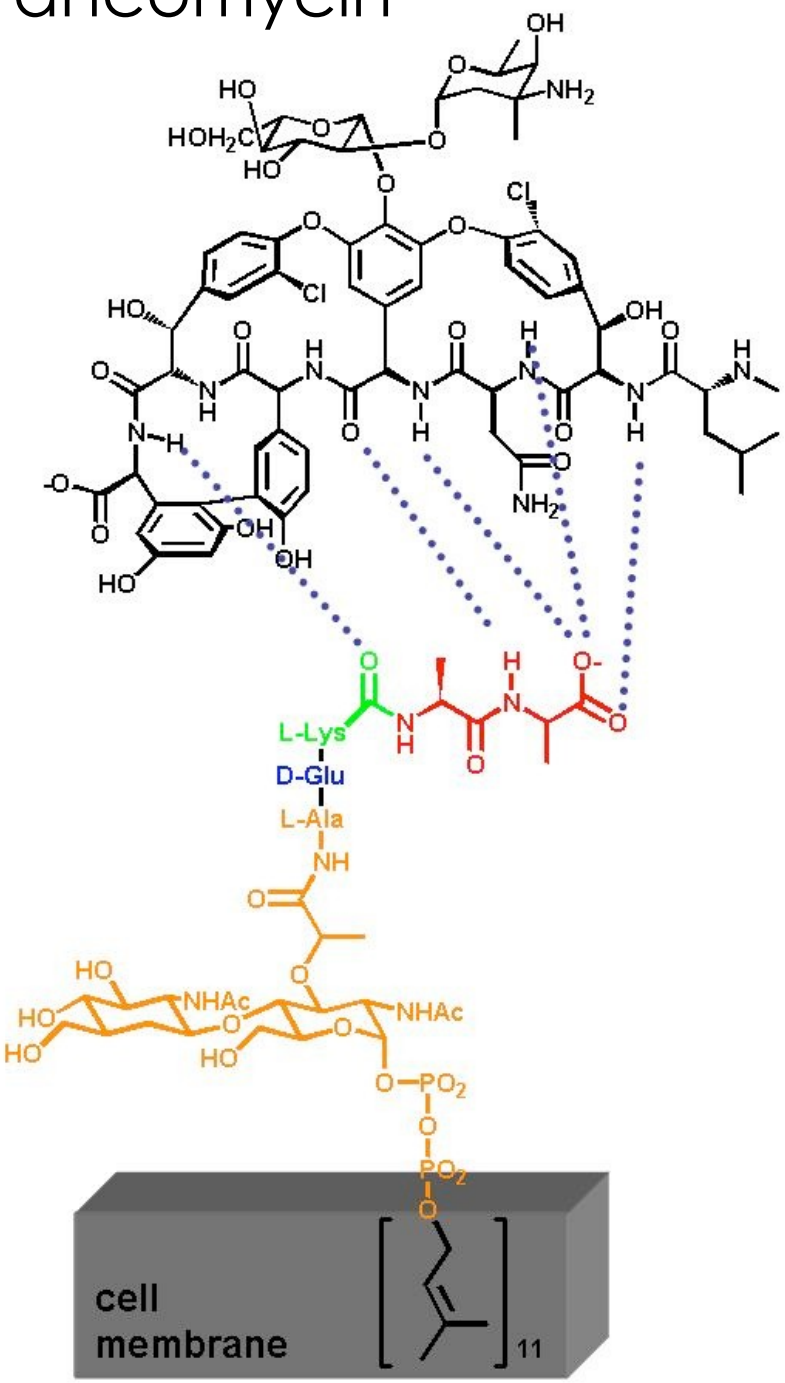
Clavulanic acid (Beechams 1976) (from Streptomyces clavuligerus)



- Weak, unimportant antibacterial activity
- Powerful irreversible inhibitor of β-lactamases - suicide substrate
- Used as a sentry drug for ampicillin
- Augmentin = ampicillin + clavulanic acid
- Allows less ampicillin per dose and an increased activity spectrum
- Timentin = ticarcillin + clavulanic acid

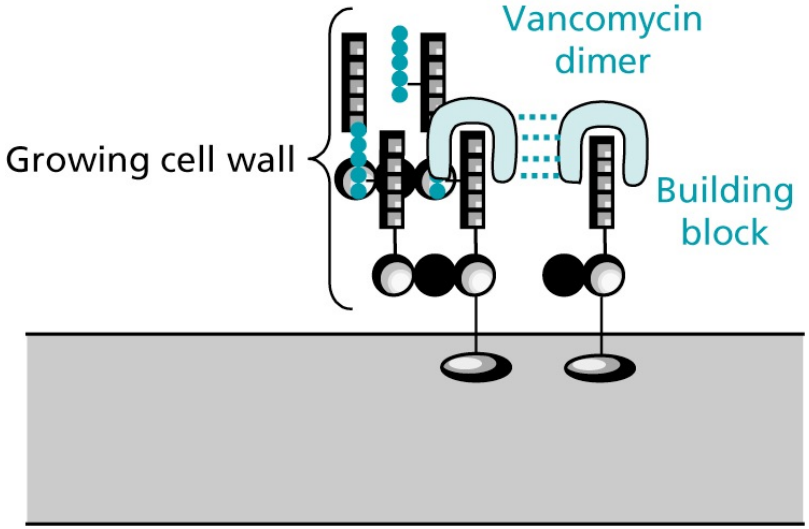
Glycopeptide antibiotics: Vancomycin

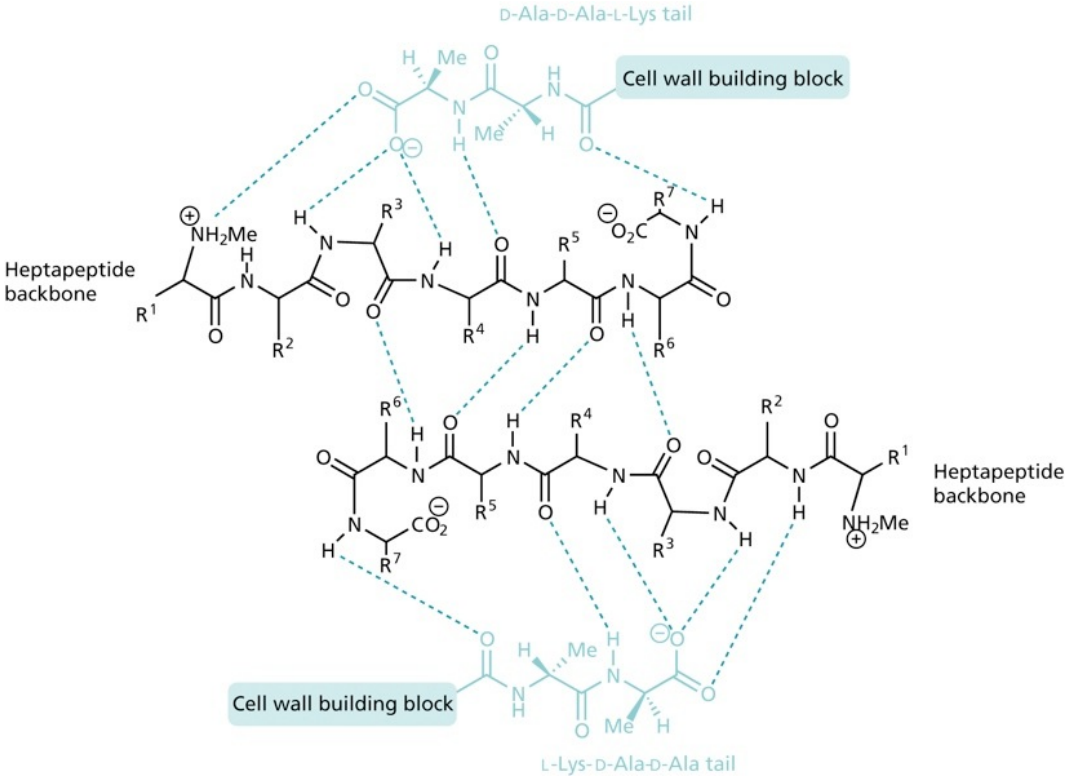




Hubbard & Walsh, Angew. Chem. Int. **2003**, 42, 731.

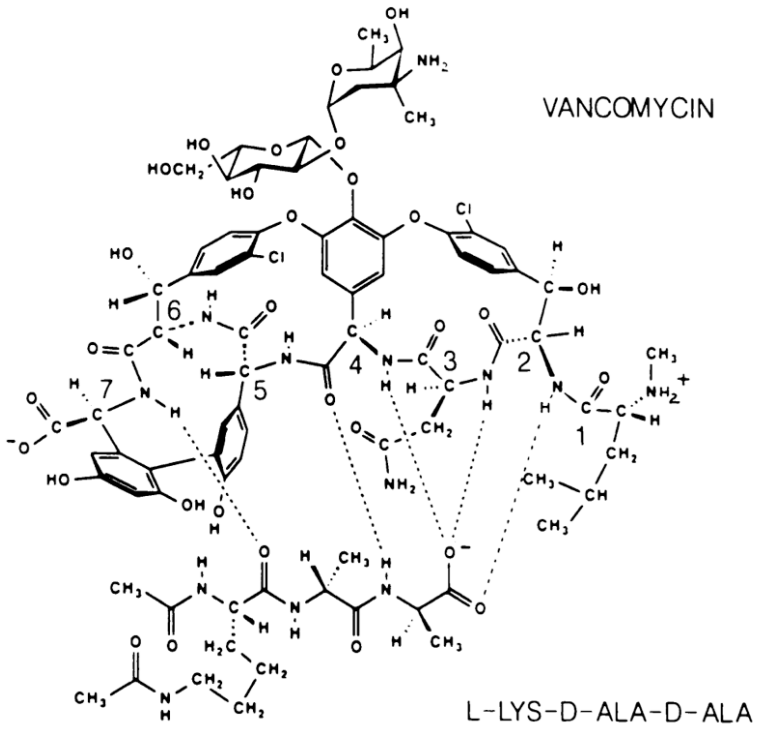
Inhibition of transglycosylation by Vancomycin

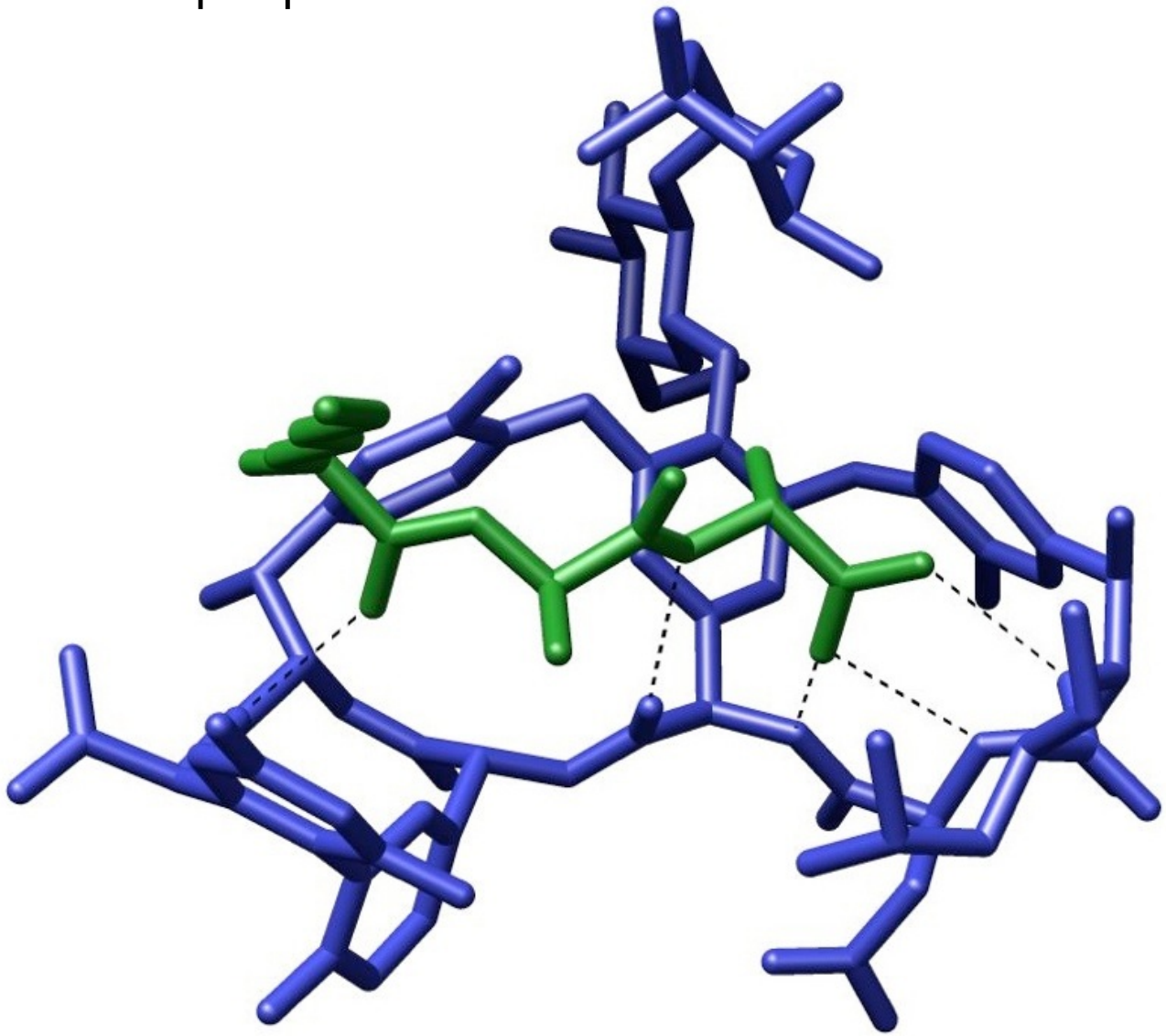




- Vancomycin forms tight hydrogen bonds with the ^{D}Ala-^{D}Ala terminal unit of the pentapeptide, thereby capping the pentapeptides
- Vancomycin can form rather stable head-to-tail dimers
- Due to the large size of Vancomycin it acts as a steric block preventing access from the transglycosylase and transpeptidase enzymes

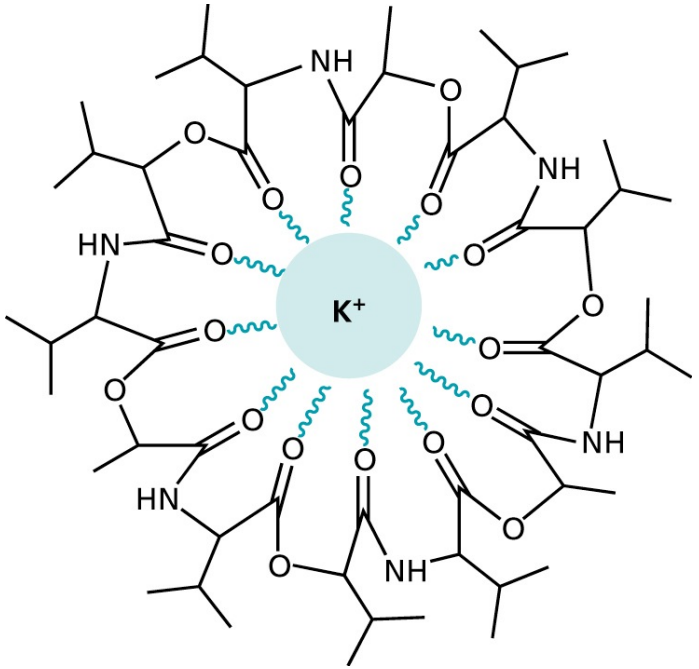
Structure of the complex between Vacomycin and a tripeptide

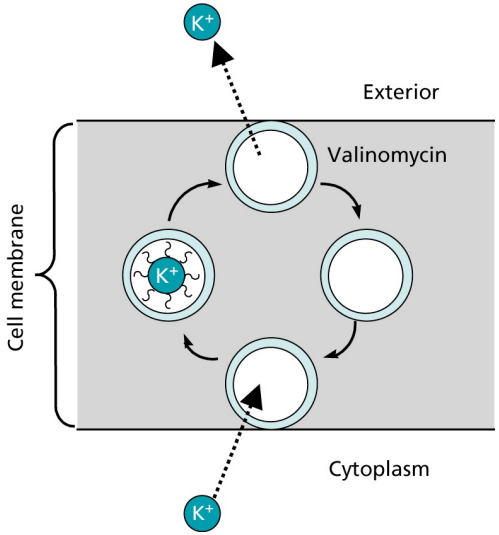




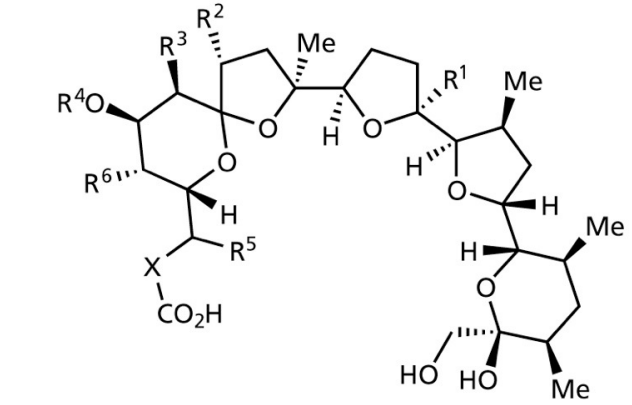
Knox et al., ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, **1990**, 1342-1347

Agents that act on the plasma membrane

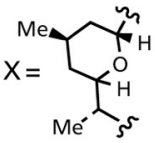




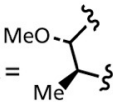
- **Valinomycin**

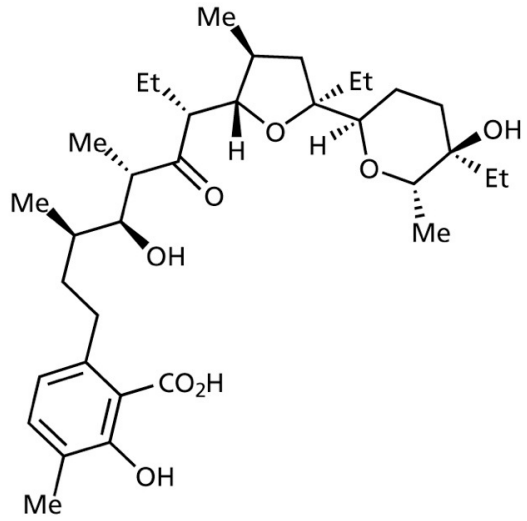


Nigericin R^1–R^4 = Me; R^5 = R^6 = H; X =



Monensin A R^1 = Et; R^2–R^4 = H; R^5 = R^6 = Me; X =

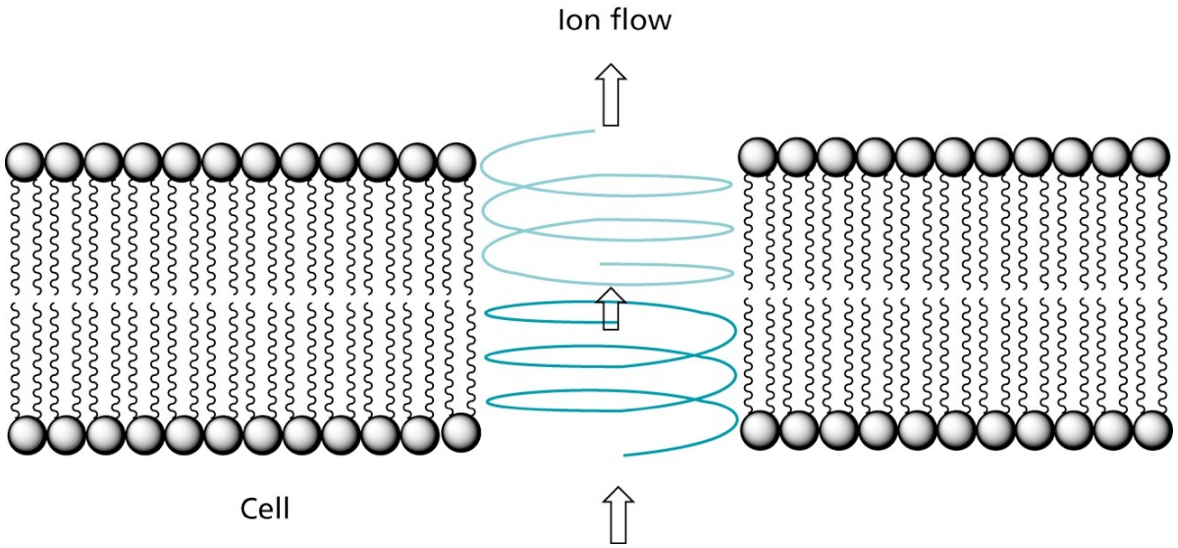


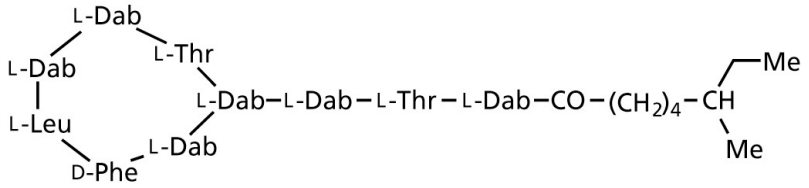


Lasalocid A

Ionophores such as **Valinomycin**, **Nigericin**, **Monensin A** and **Lasalocid A** complex ions and transport them in a non-regulated fashion through the membrane disturbing ion concentrations intra- and extracellularly.

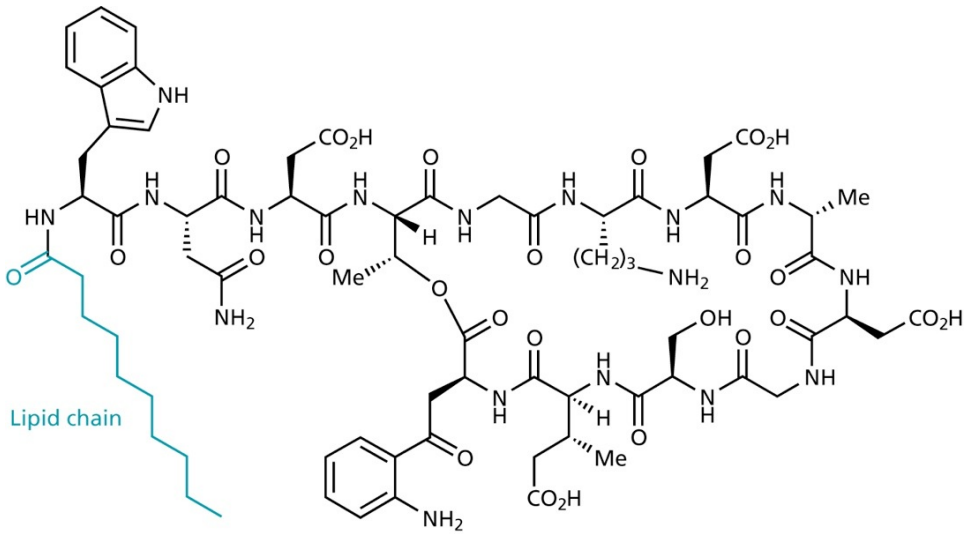
Gramicidin A forms channels that allow passing of ions, but also of nucleosides. Another such peptide is **Polymyxin B**, that binds with high selectivity to the bacterial plasma membrane





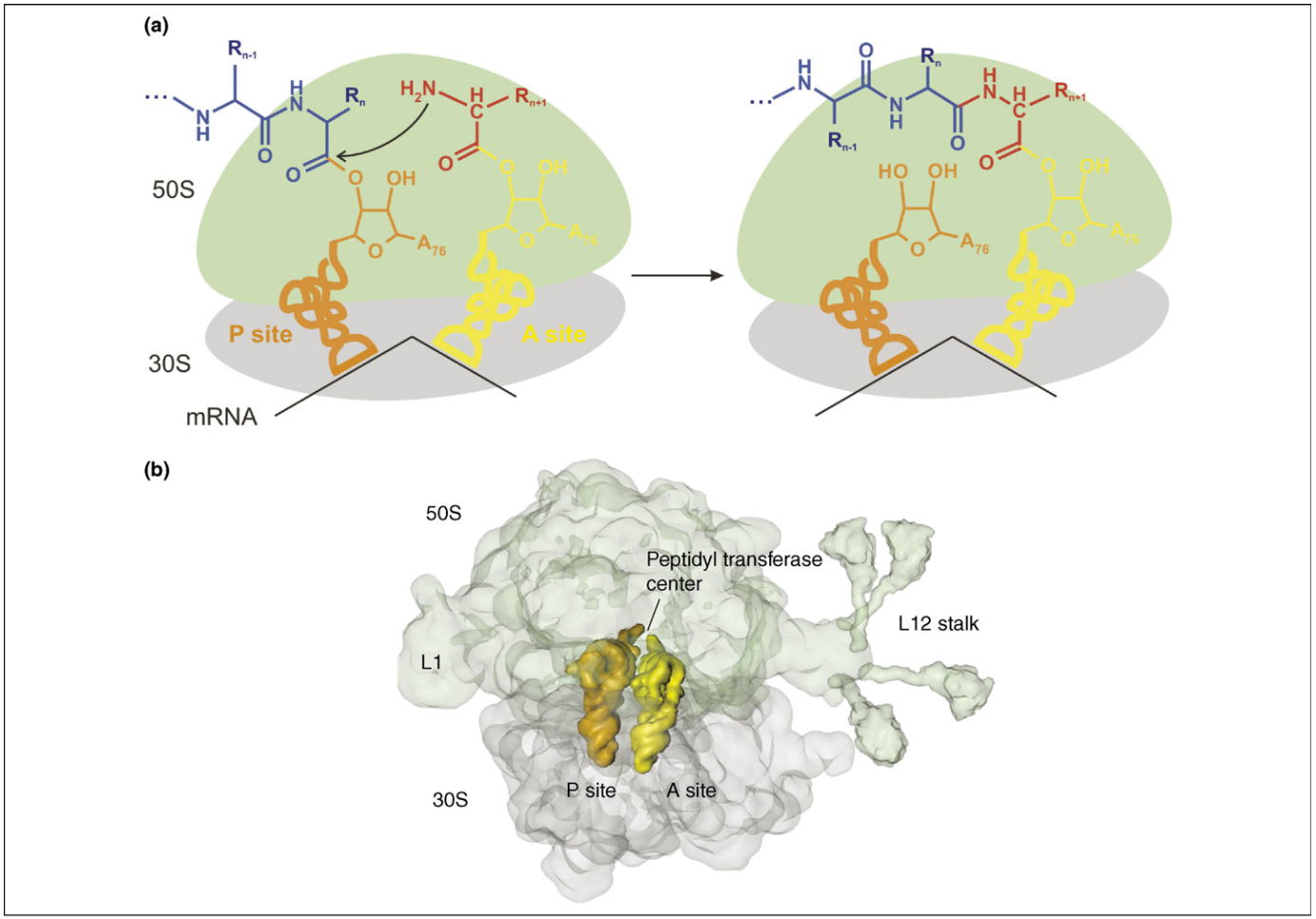
Polymyxin B

Recently, lipopeptides such as **Daptomycin** were discovered that disrupt the cell membrane

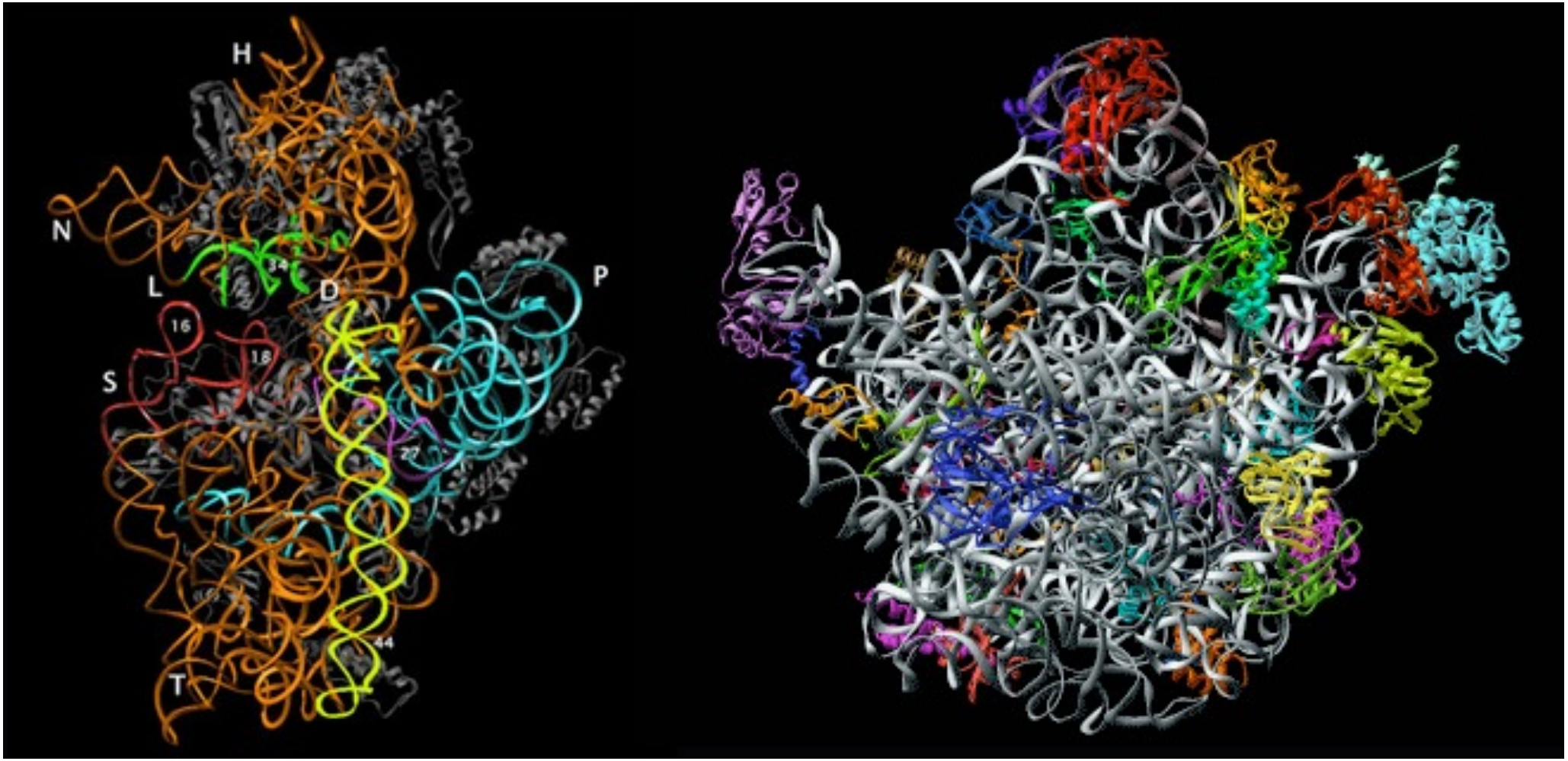


The ribosomes

- ribosomes are the location of protein biosynthesis
- ribosomes consist of both proteins and RNA
- the active site for peptide bond formation consists solely of rRNA
- it consists of a large (50S) and a small (30S) subunit



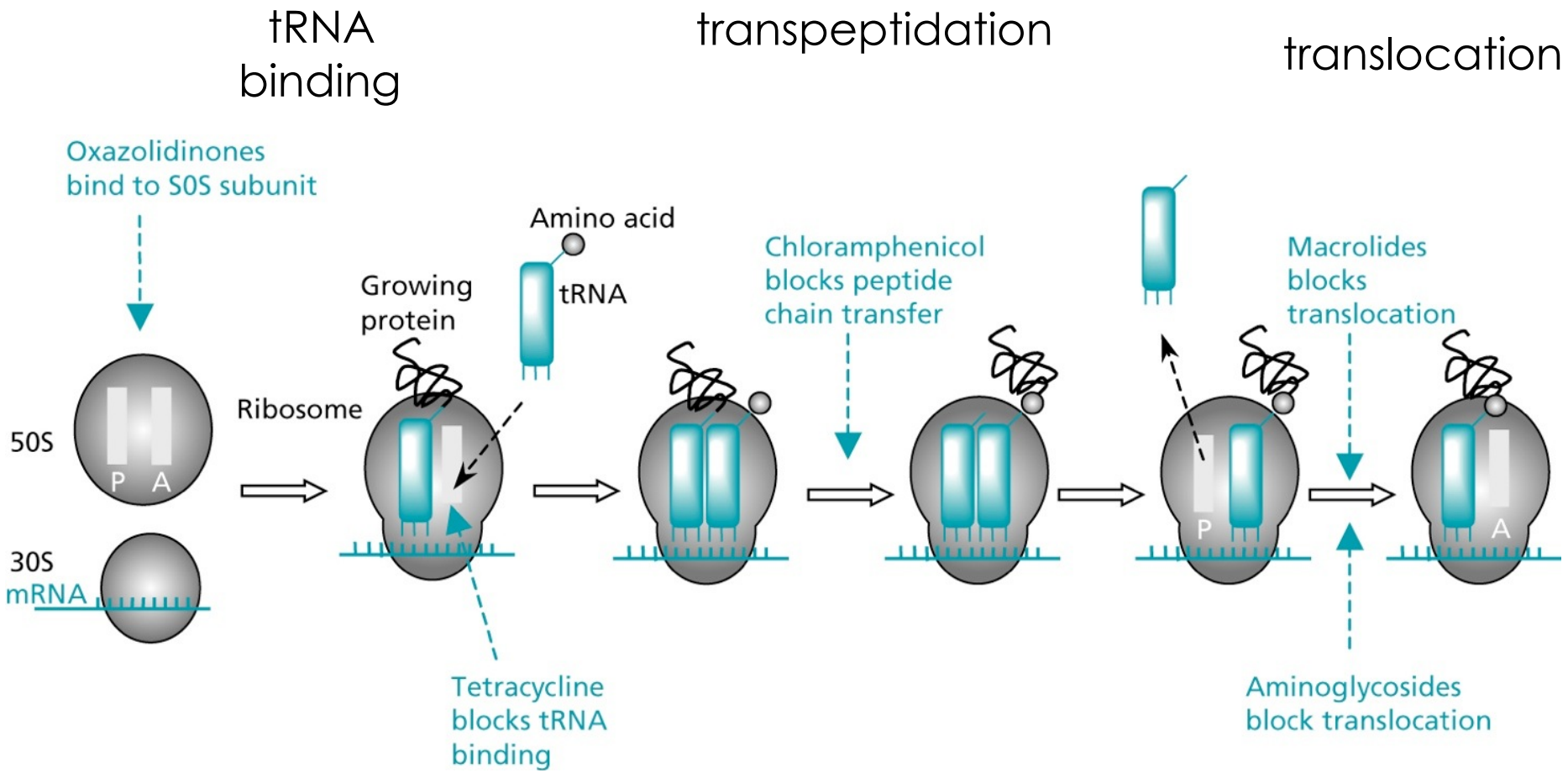
Ribosomes are made of RNA and proteins



Structure of the 30S subunit
from T. thermophilus

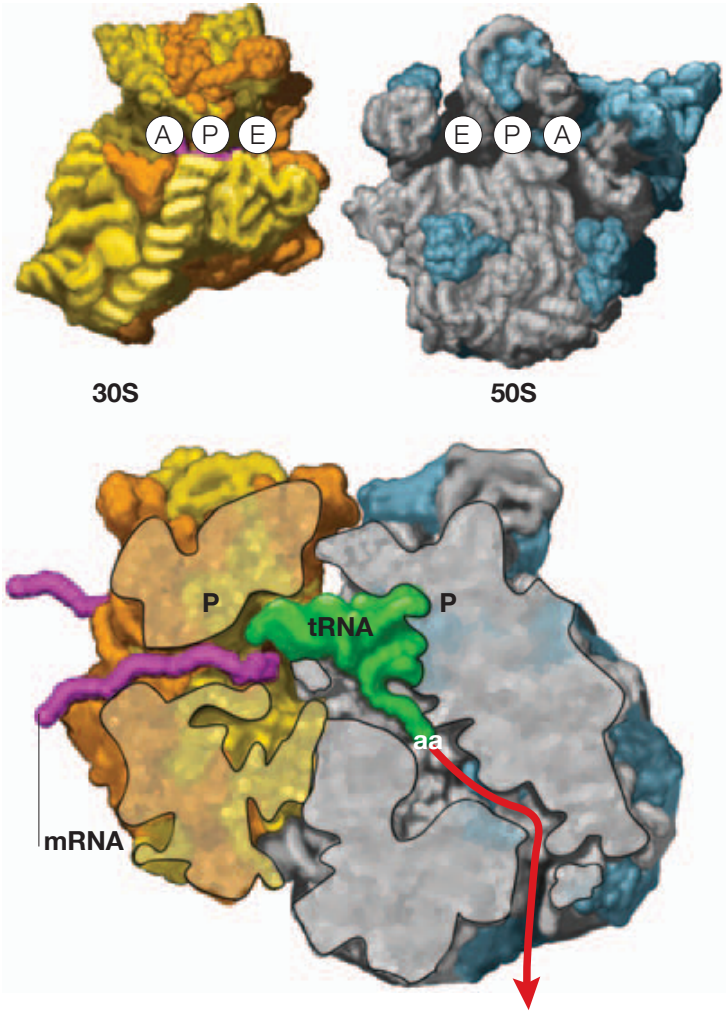
Structure of the 50S subunit
from D. radiodurans

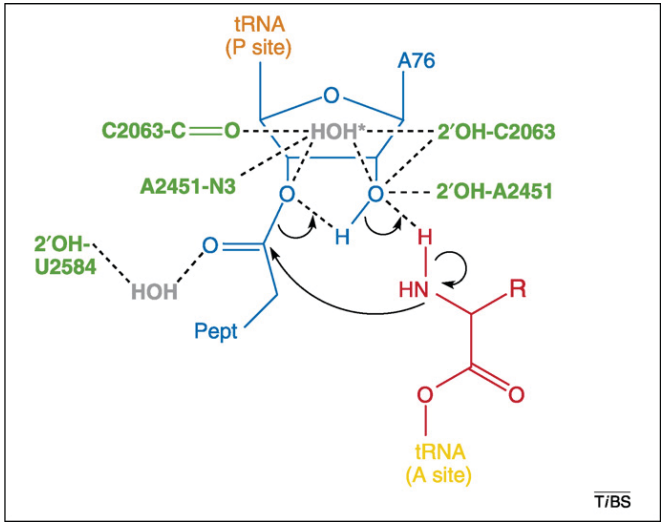
Interference with protein synthesis by blocking protein translation



Protein biosynthesis at the bacterial ribosome

(video)

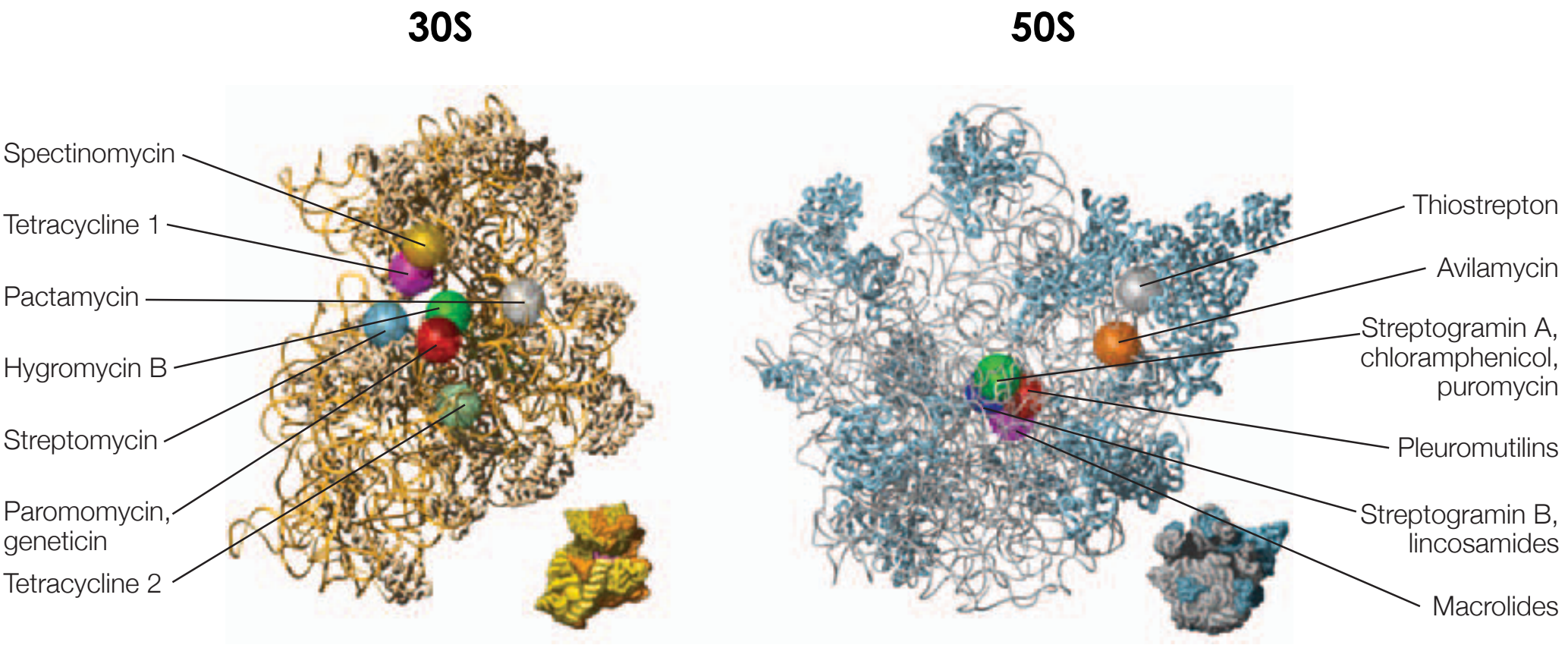




the ribosome employs entropic catalysis by positioning substrates, re-organizing water and providing an electrostatic network to stabilize reaction intermediates

- mRNA passes through two narrow channels on the 30S subunit to be displayed at the interface decoding site where it interacts with the tRNA anticodon
- Initially the start codon is translocated into the P site on the 30S subunit to interact with the initiator tRNA charged with Met
- the second mRNA codon , in the adjacent A site, accepts the next tRNA. Basepair matching is checked by the decoding site
- If it matches, the aminoacyl end of the tRNA is swung to the peptidyl-transferase centre, on the 50S subunit
- The ribosome then moves along the mRNA to bring the next codon into the aminoacyl (A) site. In addition, the tRNA holding the nascent peptide strand is translocated into the peptidyl (P) site
- At the end the full strand is moved into the E (exit) site, from where it is ejected

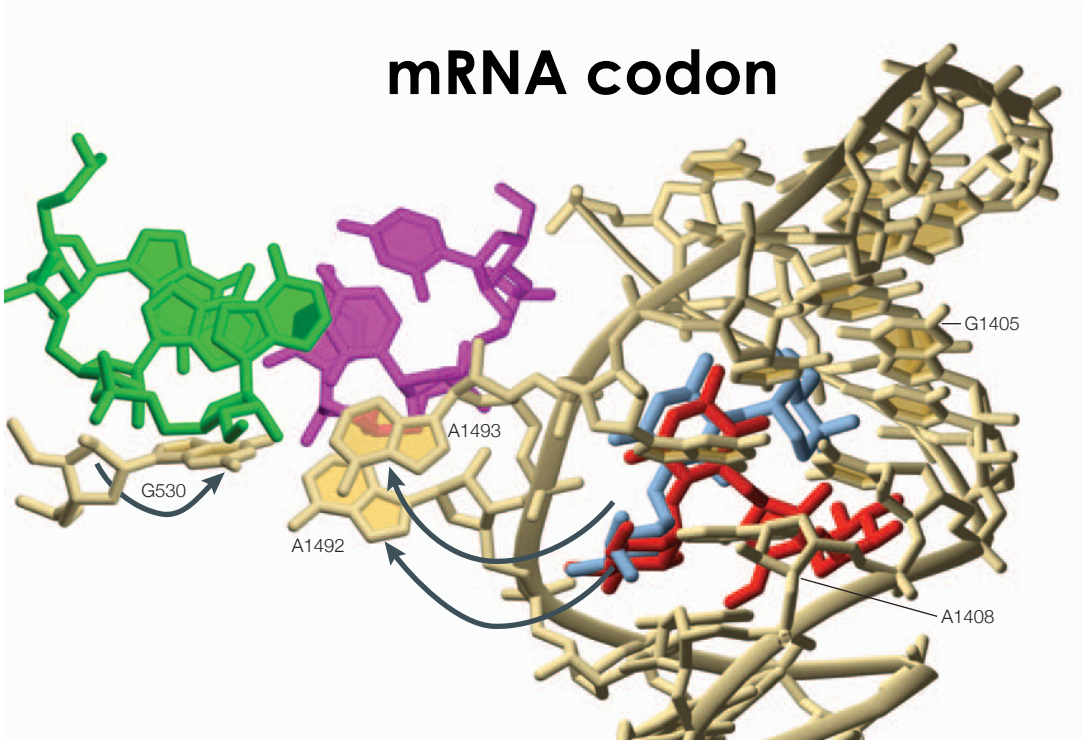
Binding sites for antibiotics



- the chain of the nascent polypeptide passes through a narrow tunnel on the 50S subunit, which is 100 Å in length, and runs from the peptidyltransferase centre to emerge from the back of the ribosome
- the macrolides bind in the narrow tunnel blocking exit of the nascent chain

Binding site on the 30S subunit

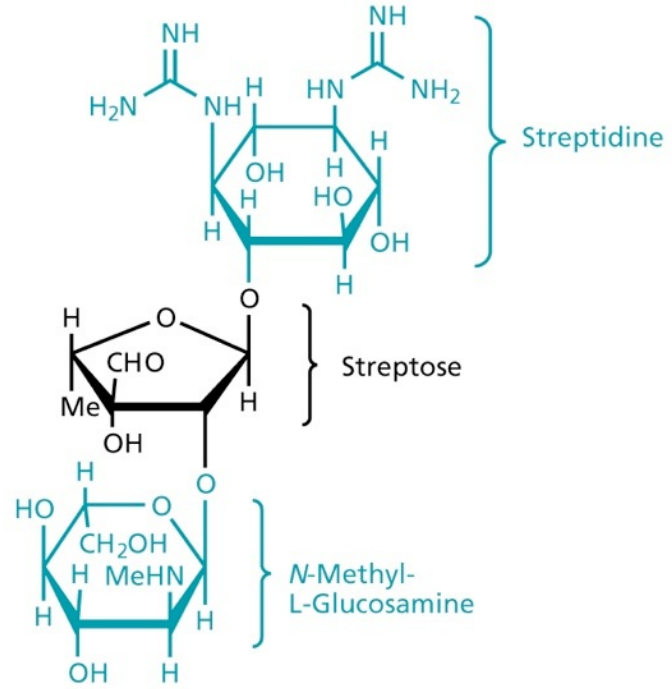
tRNA anticodon



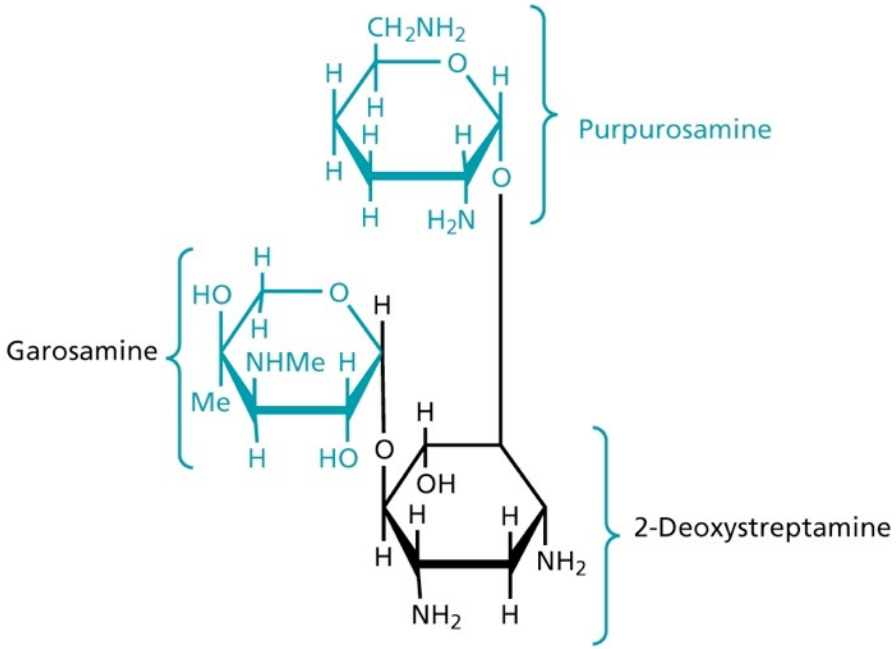
16S rRNA

- during protein synthesis nucleotides A1492 and A1493 are flipped out of the 16S rRNA helix to interact with the mRNA codon and its cognate tRNA anticodon at the ribosomal A-site
- some aminoglycosides antibiotics bind within the helix and induce a similar but not identical flip-out of A1493 and A1492.
- as a result basepair matching is NOT required leading to the addition of incorrect amino acids to the nascent chain.

Aminoglycosides



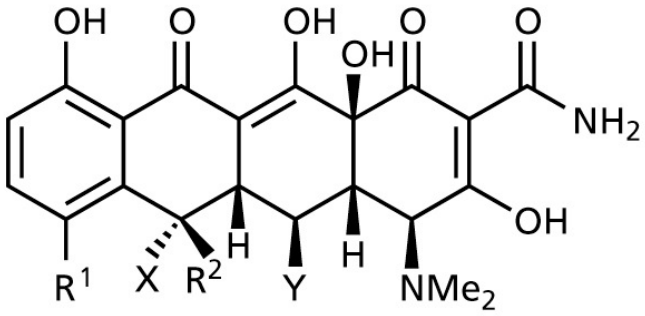
Streptomycin (from *Streptomyces griseus*)



Gentamicin C1a

- at physiological pH charged-> binding to lipopolysaccharide, phospholipids and permeabilize the membrane
- bind to the 30S ribosomal subunit, thereby preventing the movement along the mRNA, so that the triplet code cannot be read
- don't bind to human ribosomes
- polar molecules, need to be injected, unable to cross the BBB

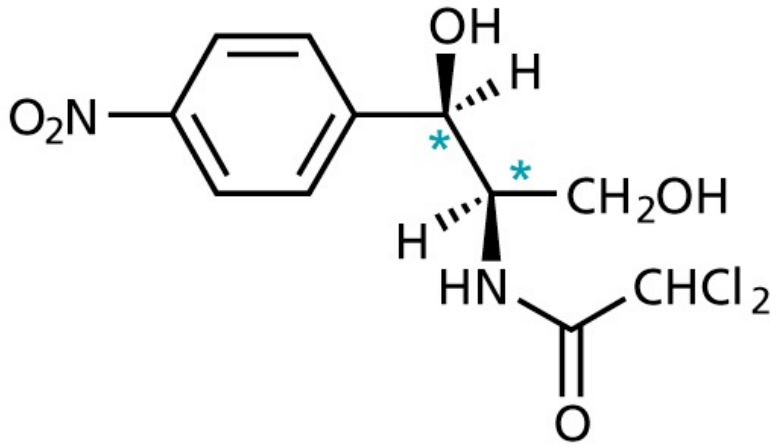
Tetracyclines



Chlortetracyclin (Aureomycin) (R^1 = Cl, R^2 = MeX = OH, Y = H)
Tetracycline (R^1 = H, R^2 = MeX = OH, Y = H)
Doxycycline (Vibramycin) (R^1 = H, R^2 = MeX = H, Y = OH)
Demeclocycline (R^1 = Cl, R^2 = H, X = OH, Y = H)

- bacteriostatic, widely prescribed
- broad-band antibiotic against gram-positive and gram-negative bacteria
- bind to **30S subunit** of ribosomes, and thereby prevent aminoacyl-tRNA from binding
- passes through membrane via porins
- inhibits also protein synthesis in mammalian cells, but more effective in bacteria

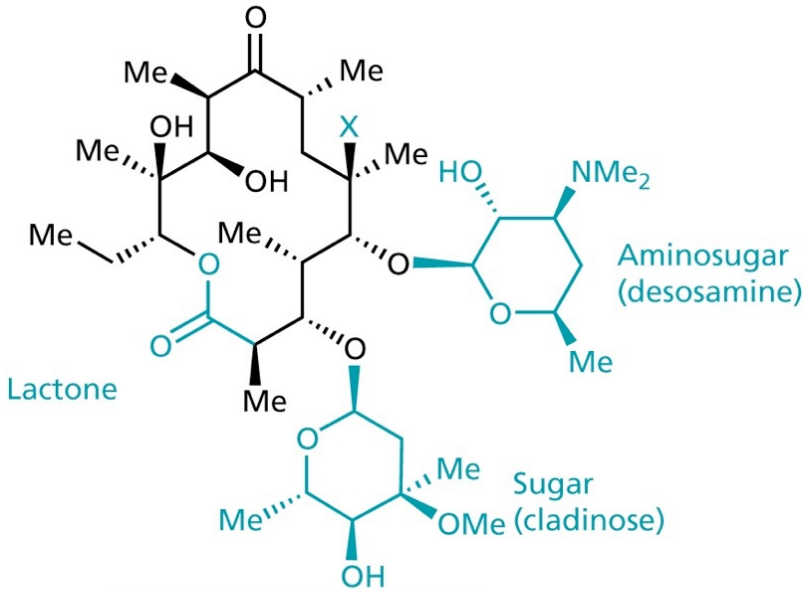
Chloramphenicol



Chloramphenicol

- binds to the **50S subunit** of the ribosomes, inhibiting the movement of the ribosomes along the mRNA
- used to cure eye infections and typhoid
- quite toxic
- bacteria that contain the gene for chloramphenicol transferase are resistant

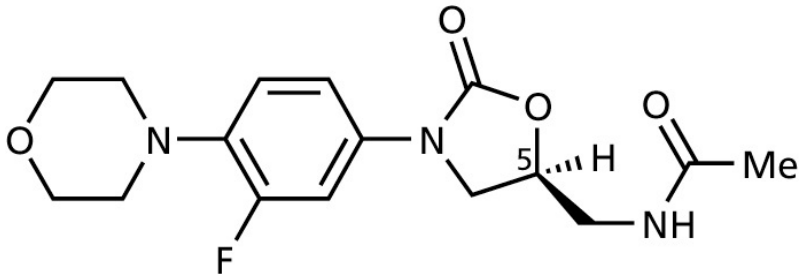
Macrolides



Erythromycin; X = OH
Clarithromycin; X = OMe

- one of the safest antibiotics
- binds to the 50S subunit of the ribosome, thereby inhibiting translocation
- can be orally taken
- acid sensitive

Oxazolidones



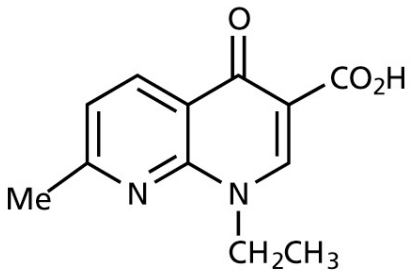
Linezolid

Before protein synthesis the 30S and the 50S subunits must associate to form the 70S ribosome. This step is inhibited

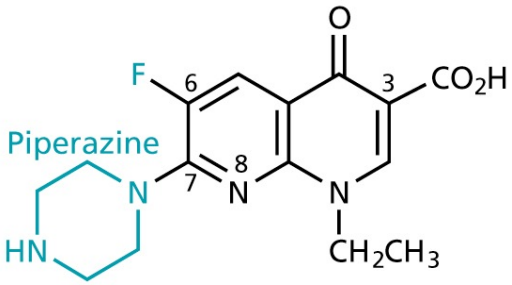
Antibiotics inhibiting nucleic acid transcription and replication:

Quinolones and fluoroquinolones

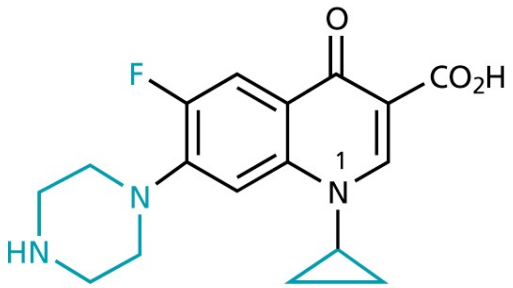
1st generation



Nalidixic acid



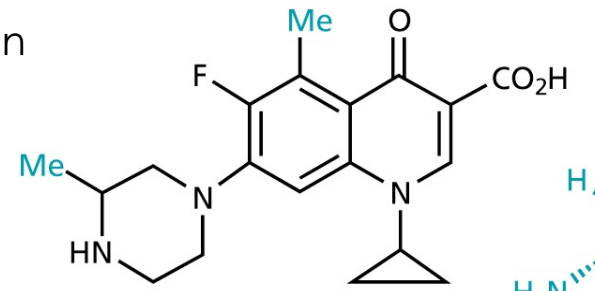
Enoxacin



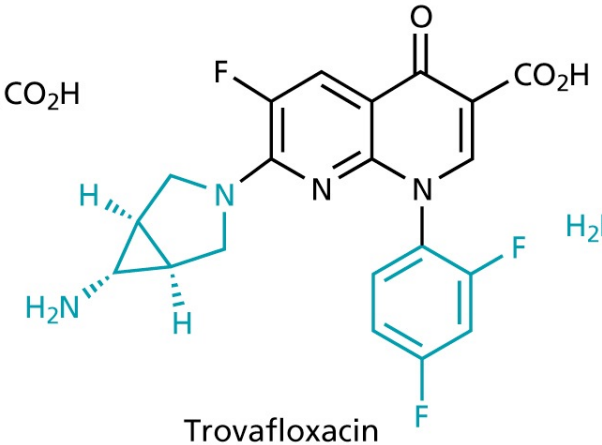
Ciprofloxacin

- broad-band antibiotics
- inhibit the replication and transcription of bacterial DNA by **inhibiting the topoisomerases**
- used for infections involving the urinary, respiratory and gastrointestinal tracts as well as against infections of skin, bone and joints

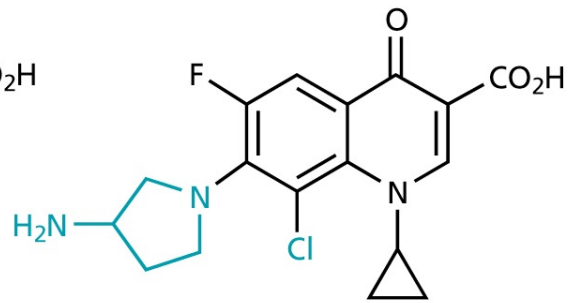
2nd generation



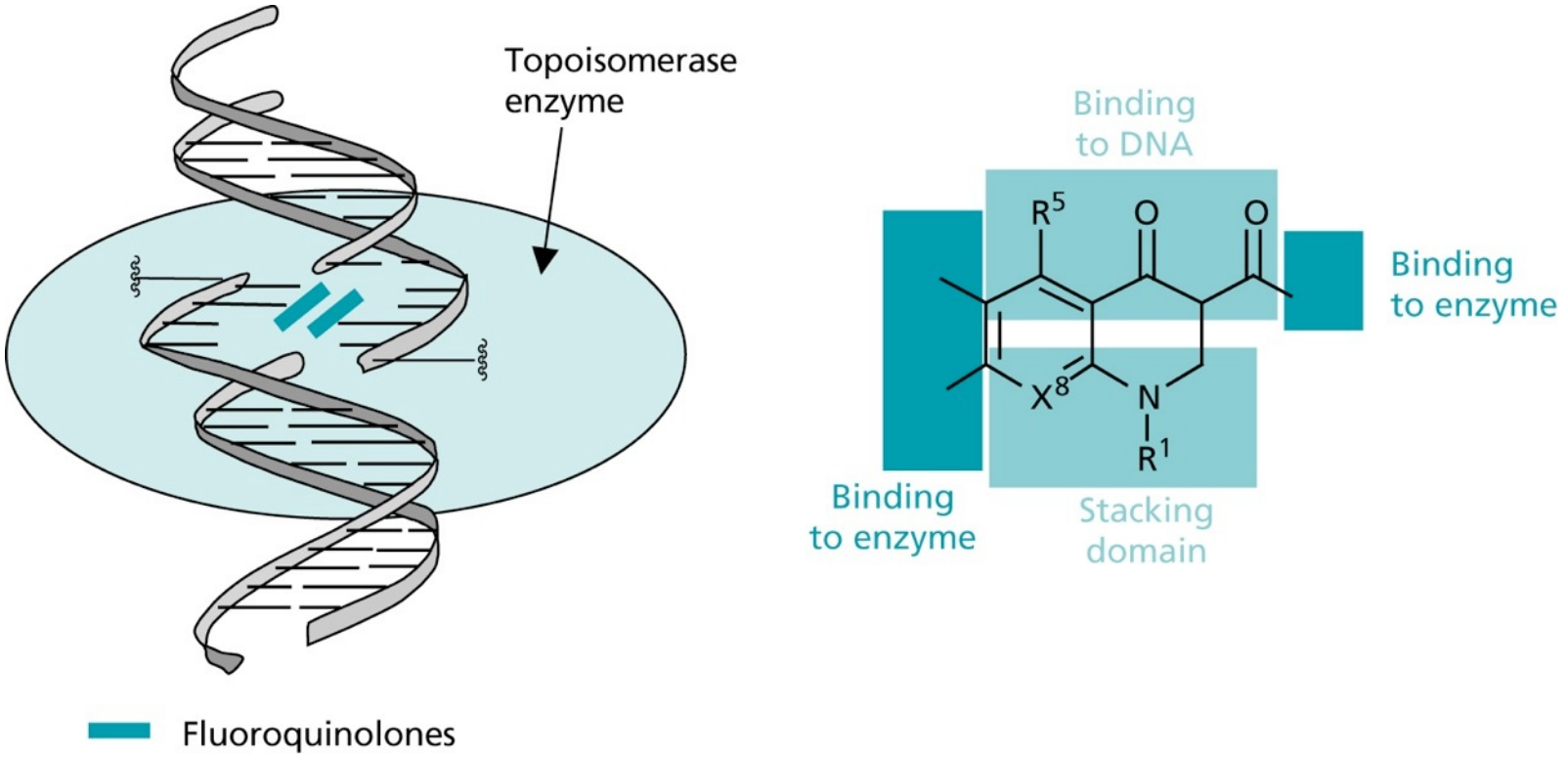
Grepafloxacin



Trovafloxacin

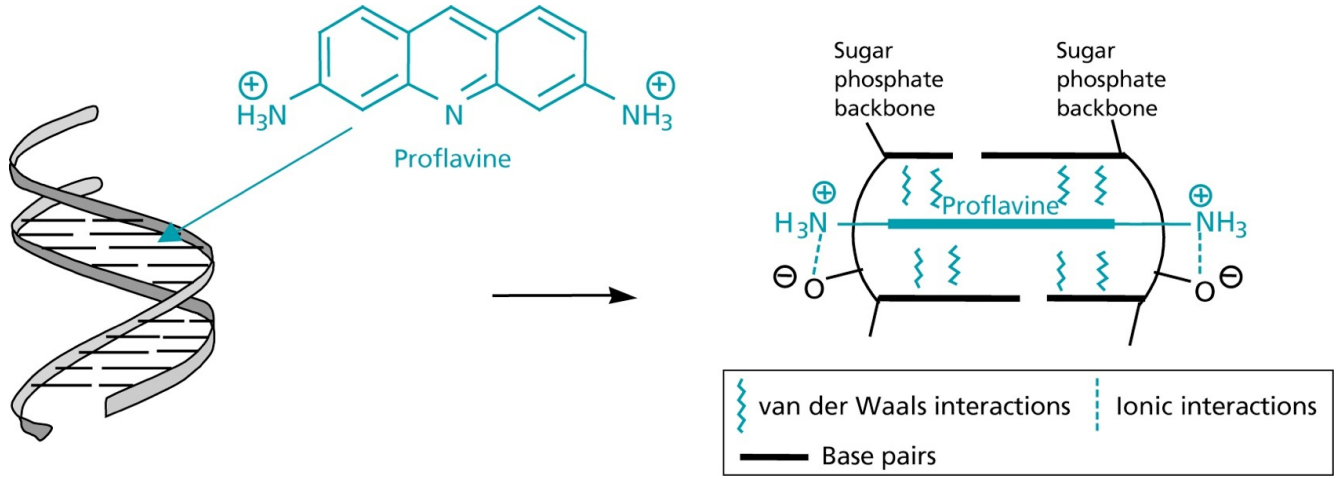


Clinafloxacin

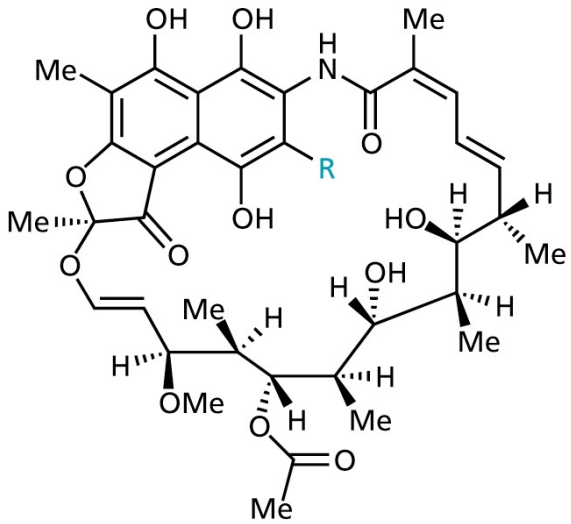


- during replication, supercoiling of the DNA is removed by helicases
- the created tension is released by **topoisomerases**, that cut break both strands of the DNA and rejoin them
- fluoroquinones inhibit topoisomerase IV in gram +ve bacteria with 1000 fold selectivity over the human enzyme
- in gram -ve bacteria, the topoisomerase II (**DNA gyrase**), that reintroduces supercoiling after replication and transcription is inhibited

Aminoacridines



- directly interact with bacterial DNA through intercalation
- thereby prevents replication and transcription (but toxic)



Rifamycins

- binds non-covalently to the bacterial DNA-dependent RNA polymerase
- does not attach to eukaryotic RNA polymerase
- bactericidal
- used to treat tuberculosis and staphylococci infections