# Drugs acting on bacterial protein biosynthesis

- Proteins are very essential for most of the bacterial metabolic functions as well as for cell integrity.
- Bacterial cell uses ribosomes to synthesize proteins.
- Targeting protein biosynthesis will produce bactericidal agents in most of the cases.
- Why targeting the bacterial protein synthesis will be selective:
  - Different diffusion rates between bacterial and mammalian cells.
  - Structural differences between bacterial and mammalian ribosomes

# Antibacterial agents which impair protein synthesis: translation

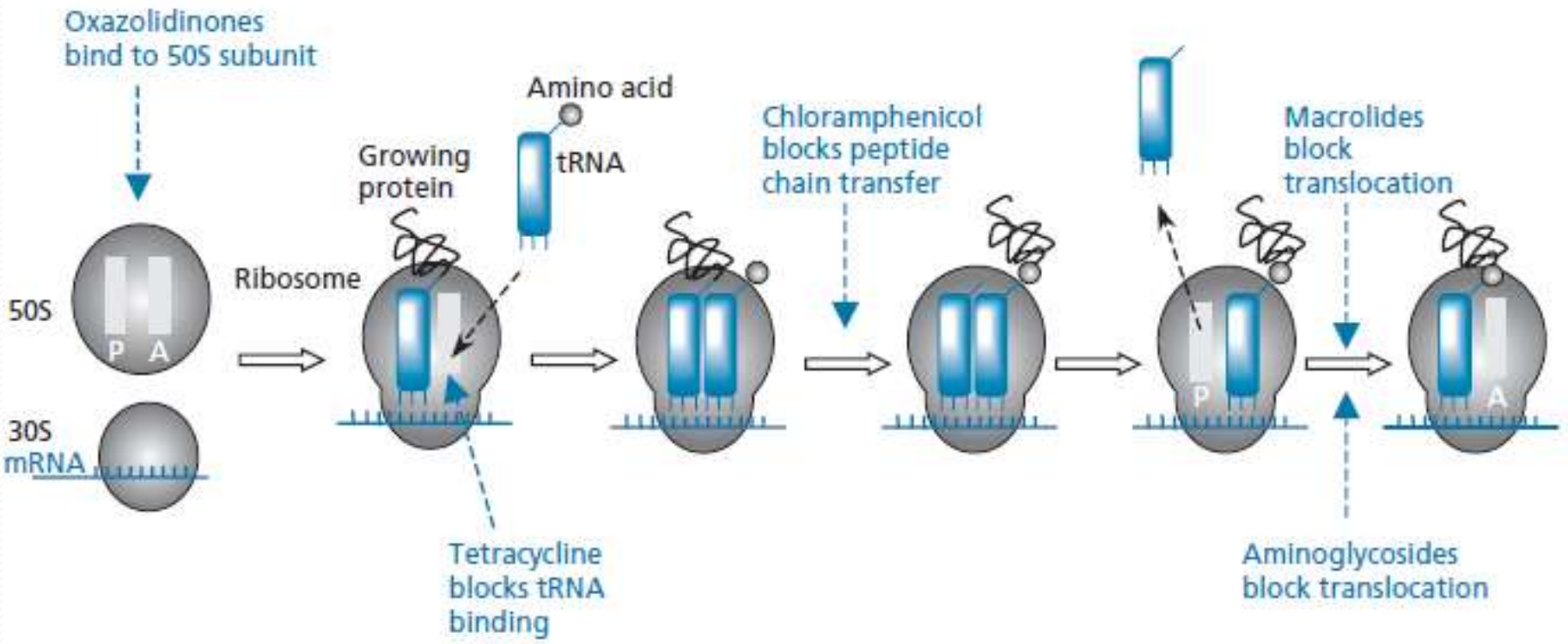



FIGURE 19.64 Stages at which antibacterial agents inhibit translation.

# Aminoglycosides

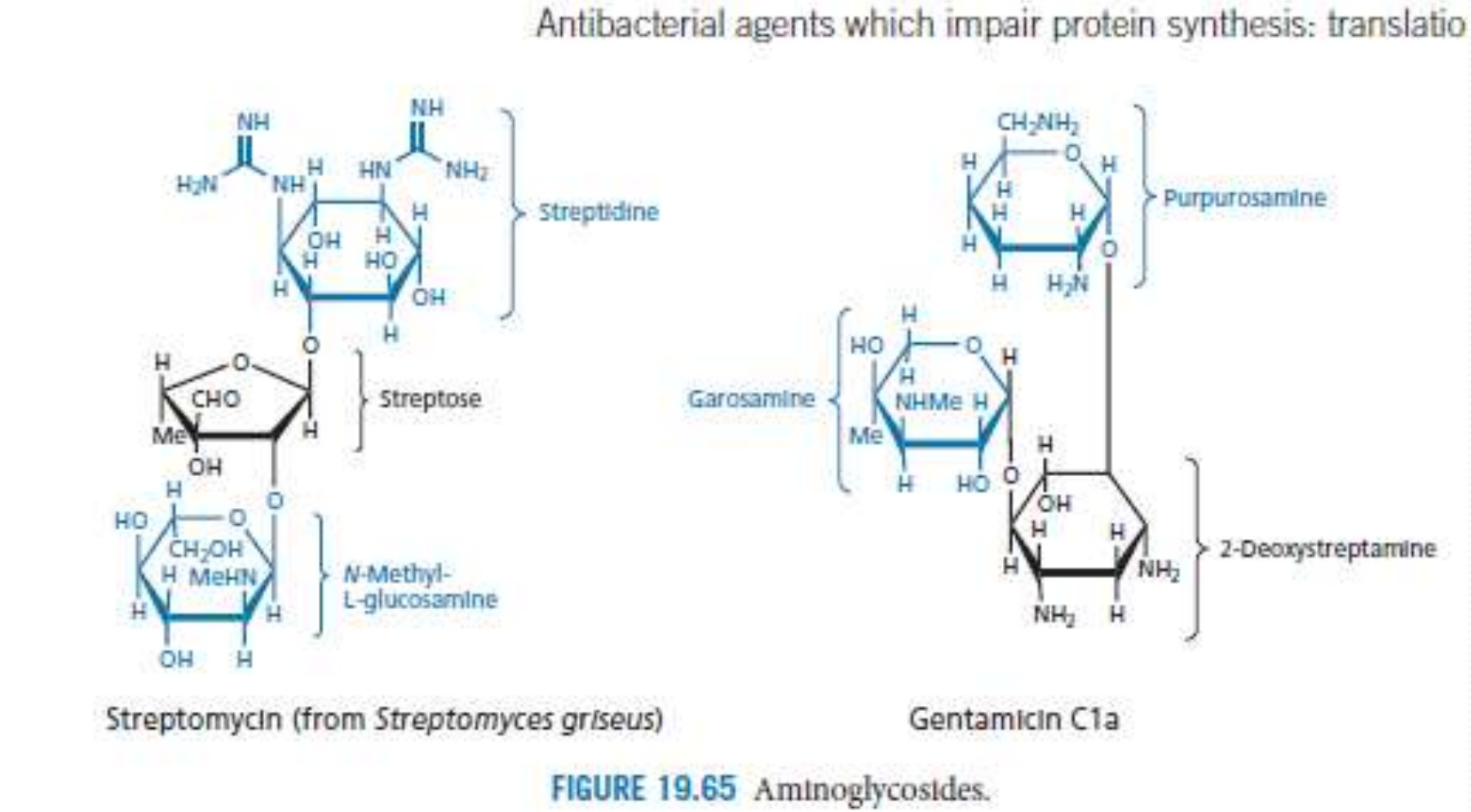



FIGURE 19.65 Aminoglycosides.

# Aminoglycosides

- **Streptomycin** was isolated from the soil microorganism *Streptomyces griseus* in 1944 and is example of an aminoglycoside—a carbohydrate structure which includes basic amine groups.
- Streptomycin was the next most important antibiotic to be discovered after penicillin and a variety of other aminoglycosides have been subsequently isolated from various organisms, for example **gentamicin .**

- The aminoglycosides work best in slightly alkaline conditions. At pH 7.4, they have a positive charge that is beneficial to activity by aiding absorption through the outer membrane of Gram-negative bacteria.

# Aminoglycosides

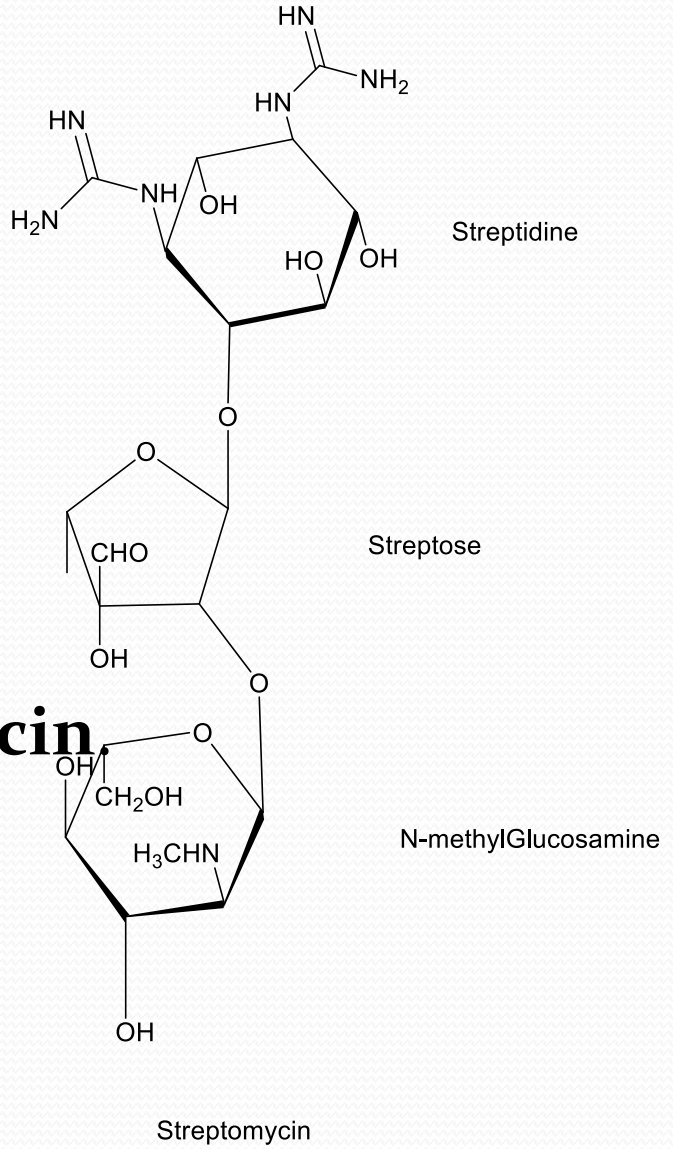
- An ionic interaction takes place with various negatively charged groups on the outer surface of the cell membrane which displaces **magnesium and calcium** ions.
- These ions normally act as **bridges between lipopolysaccharides**, and their displacement results in rearrangement of cell membrane components to produce pores through which an aminoglycoside can pass.
- The drug then crosses the cell membrane by an energy-dependent process and is trapped inside the cell where it accumulates to relatively high concentrations.

# Aminoglycosides

- Binding to bacterial ribosomes now takes place to inhibit protein synthesis.
- The binding is specifically to the 30S ribosomal subunit and prevents the movement of the ribosome along mRNA so that the triplet code on mRNA can no longer be read.
- In some cases, protein synthesis is terminated and the shortened proteins end up in the cell membrane. This can lead to a further increase in cell permeability, resulting in an even greater uptake of the drug.
- Aminoglycosides are bactericidal rather than bacteriostatic and it is thought that their activity may be due to their effects both on the ribosomes and the outer cell membrane.
- Because the ribosomes in human cells are different in structure from those in bacterial cells, they have a much lower binding affi nity for the aminoglycosides, whichexplains the selectivity of these drugs.

# Aminoglycosides Chemical structure

- Most of them have three rings, some of which are amino sugar other are simple sugar molecules, either pentose or hexose.
- Some have four sugar molecules such as **neomycin** and **paromomycin**
- Generally the pentose do not have the amino substituents, only the hexoses have.



# Aminoglycosides Chemical structure

- Highly polar chemical structure.
- Strongly basic compounds…. Form polycationic species at physiological pH.
- Normally given as sulfate salts to improve their water solubility.
- Most of them, especially the sulfate salts are highly water soluble which means:
  - Well distributed compounds.
  - Do not reach CNS (why?).
  - Do not reach bone, fatty or connective tissues (poorly vascularized tissues).
  - Given parentally.

# Combination of aminoglycosides and penicillins

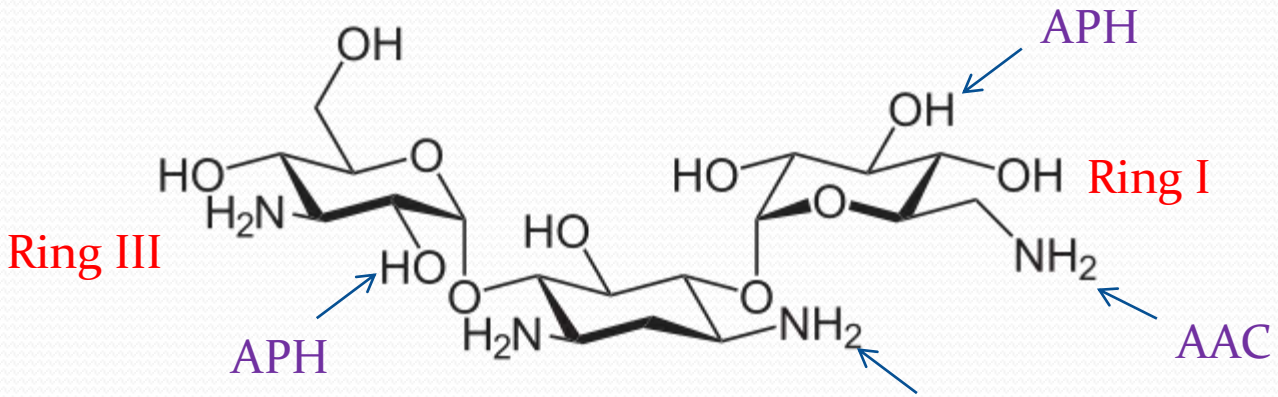
- Mainly used for *Ps. Aeruginosa* infections.
- The commonly used combinations:
  - Carbenicillin + Gentamicin.
  - Penicillin G + streptomycin for Enterococci infections (endocarditis).
- Here penicillins will destroy the integrity of the cell wall that will help the aminoglycosides to reach ribosomes and inhibit protein biosynthesis.
- Penicillins and aminoglycosides can't be put together in the same vial (why?)

# Mechanism of bacterial resistance

- Bacteria has two major resistant mechanisms to aminoglycosides:
  1. Affecting aminoglycosides uptake by the cell: mainly adapted by *Ps. aeruginosa* by making some sort of mutation in the energy dependant transport system required to get such compounds inside the cell.
  2. Inactivating aminoglycosides by changing the chemical structure, especially the essential functional groups for activity.

# Inactivating enzymes

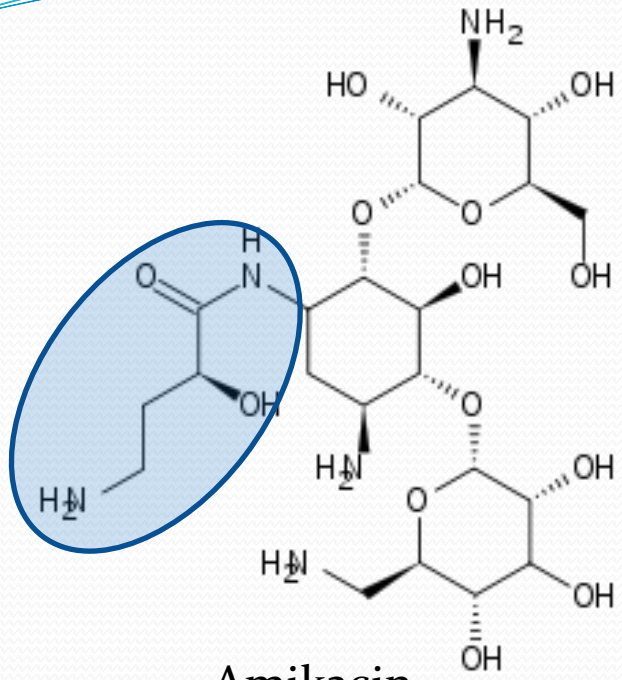
- Nine different types of aminoacetyl transferase (AAC): responsible for acetylating the amino groups in the structure from ring I and II.
- Aminoglycoside Phosphotransferase (APH): phosphorylate the hydroxyl groups from ring I and III.



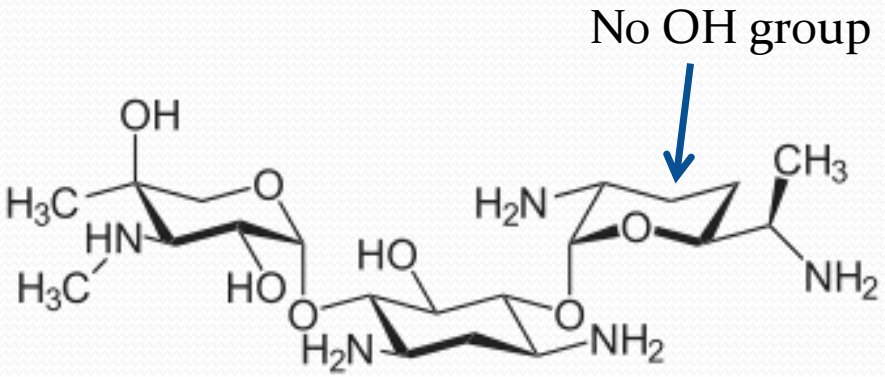

Kanamycin A

# Inactivating enzymes

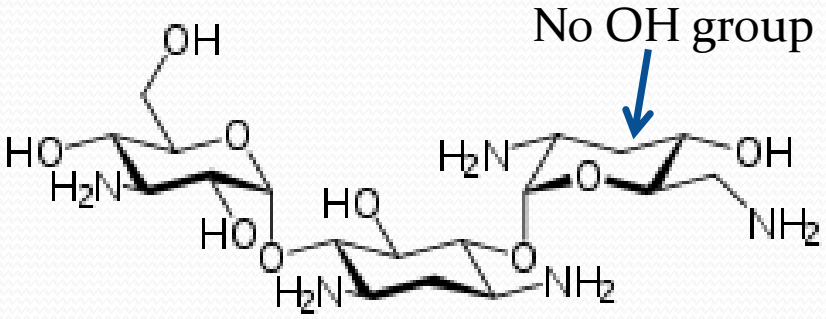
- New derivatives have been made to overcome the enzymatic inactivation by these enzymes.
- Removal of the functional group susceptible to attacking by the inactivating enzymes can lead to more active agents against the resistant strains
  - Gentamicin and tobramycin lack the 3′ OH in ring I which make them resistant to APH.
  - Amikacin has the 3-$NH_2$ being acylated (by L-hydroxyaminobuteroyl amide (L-HABA)), that makes it more resistant to AAC at this site.



Amikacin




Gentamicin




Tobramycin

# Antibacterial agents which impair protein synthesis: translation

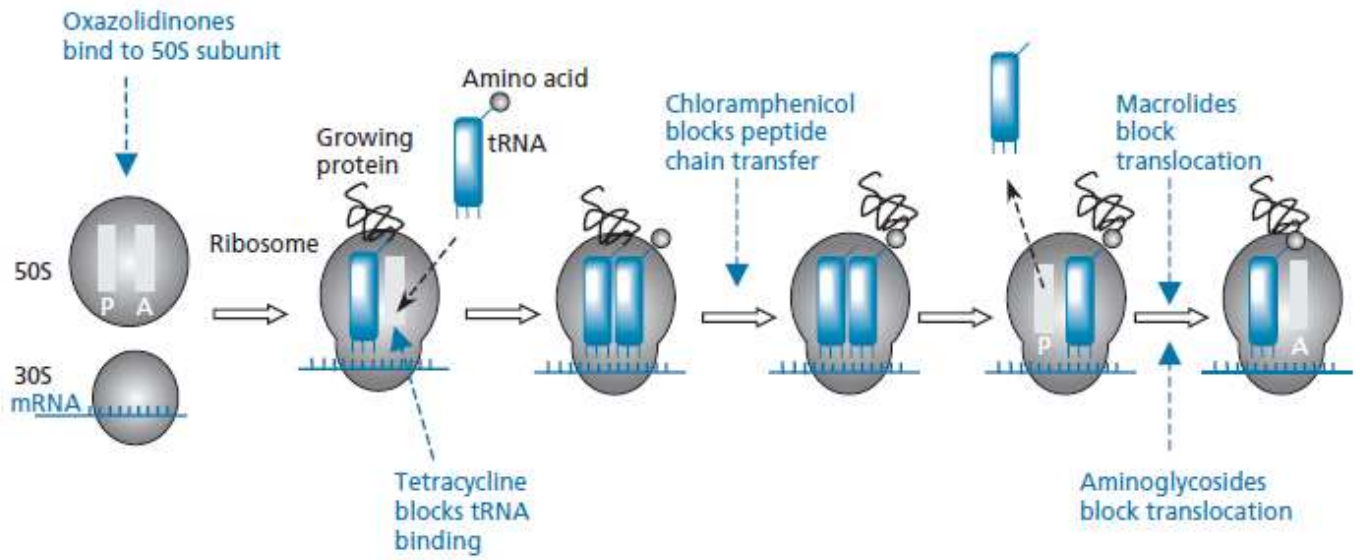
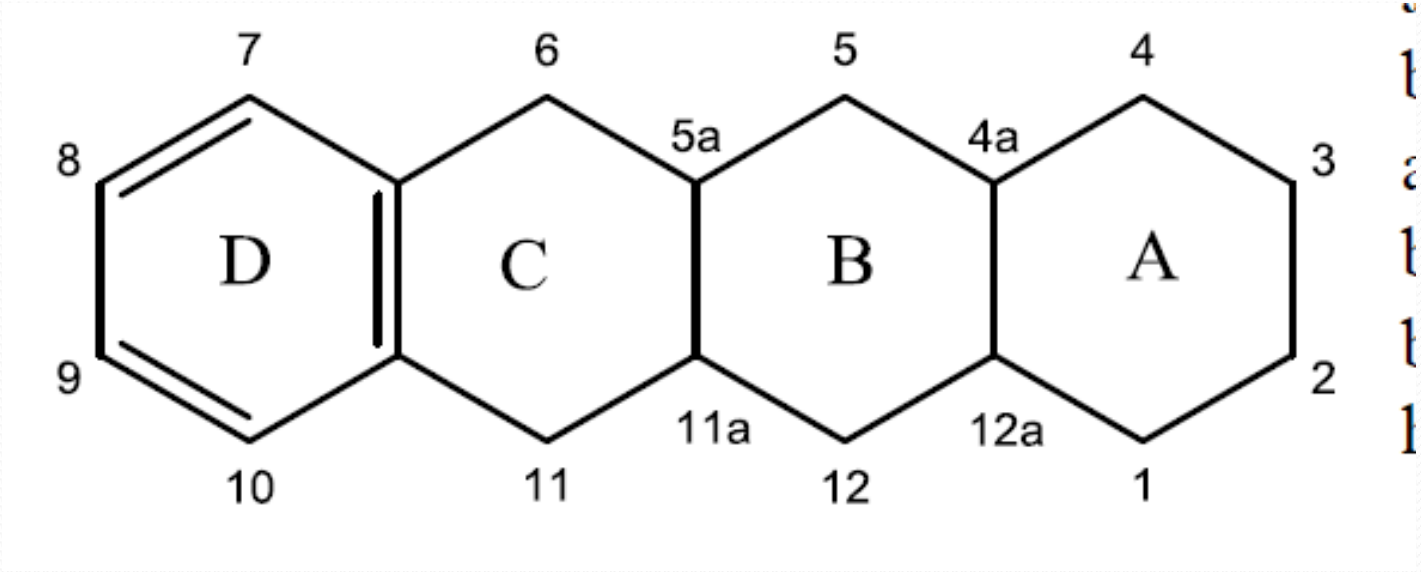



FIGURE 19.64 Stages at which antibacterial agents inhibit translation.

# Tetracyclines

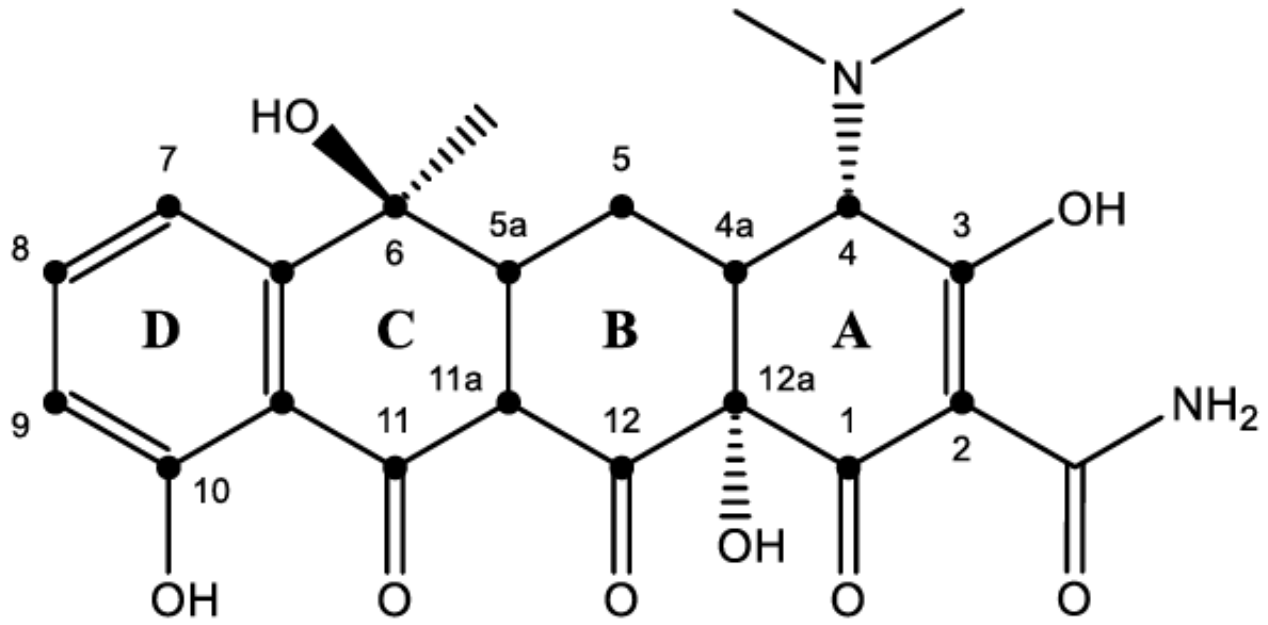
- The tetracyclines are bacteriostatic antibiotics which have a broad spectrum of activity and are the most widely prescribed form of antibiotic after penicillins.
- They are also capable of attacking the malarial parasite. One of the best known tetracyclines is **chlortetracycline** ( **aureomycin** ) which was isolated in 1948 from a mudgrowing microorganism in Missouri called *Streptomyces aureofaciens* — so-called because of its golden colour.
- Further tetracyclines, such as **tetracycline** and **doxycycline** have been synthesized or discovered.
- The tetracyclines inhibit protein synthesis by binding to the 30S subunit of ribosomes and preventing aminoacyl-tRNA from binding. This stops the further addition of amino acids to the growing protein chain. Protein release is also inhibited..

Junction atoms between ring C and D aren't numbered because they aren't to be substituted (already have 4 bonds)



# It's clearly composed of 4 rings which are essential according to SAR.

- Junction atoms between ring C and D aren't numbered
- because they aren't to be substituted (already have 4 bonds)



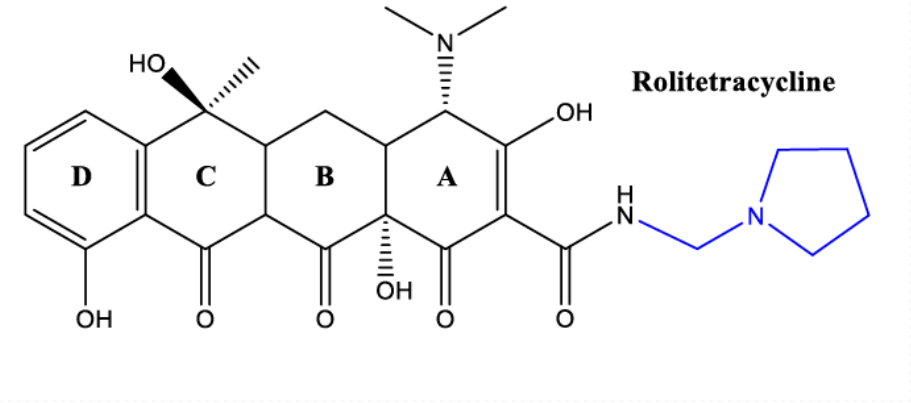

Tetracycline

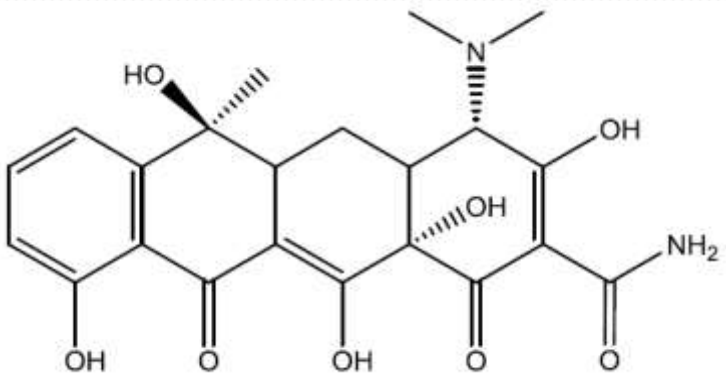
# Tetracyclines

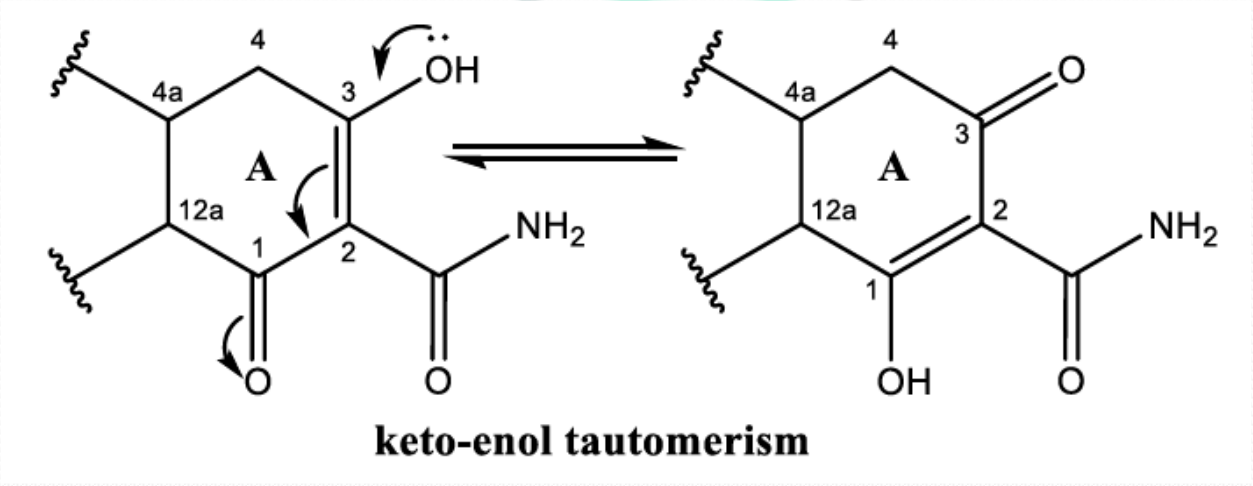
- In the case of Gram-negative bacteria, tetracyclines cross the outer membrane by passive diffusion through the porins. Passage across the inner membrane is dependent on a pH gradient, which suggests that a proton- driven carrier is involved.
- Selectivity is due to the ability of bacterial cells to concentrate these agents faster than human cells. This is fortunate because tetracyclines are capable of inhibiting protein synthesis in mammalian cells—particularly in mitochondria.

# Add our essential groups to these rings one by one to get to Tetracycline :

- RING A:
- 1. Ketone group: (any modification: weather reduction or substitution) will inactivate it.
- 2. Double bond: (essential) & Amide (also essential), if amide is converted to either
- carboxylic acid or ester,or just substituted it, it will lose it's activity.
- Rolitetracycline is substituted on the amide group, but that is only because it's a
- prodrug (the substitution will be cleaved), (we will talk about it next lecture).
- 3. Hydroxy group: the relationship between the Hydroxy on C3,the Amide on C2 and
- the Ketone on C1must allow them to be freely enolizable (freely flowing electrons)



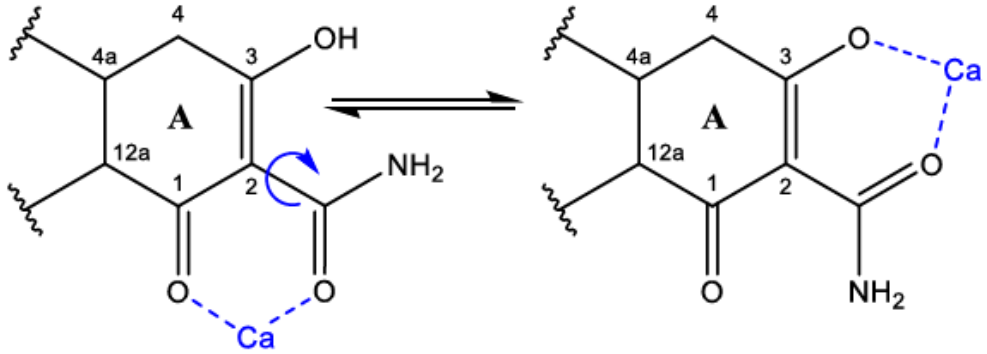






keto-enol tautomerism

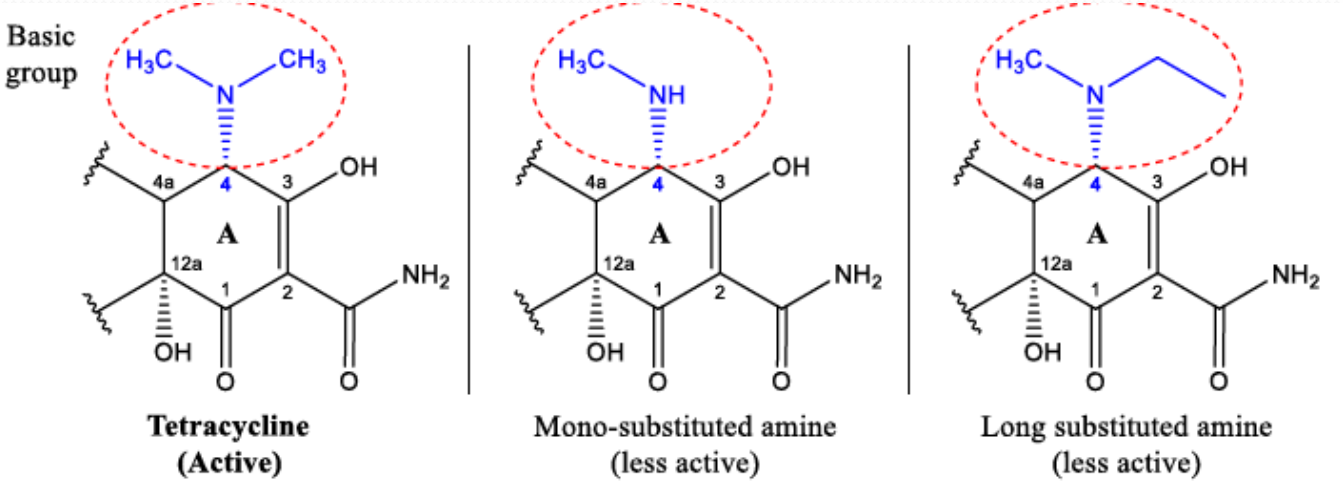
Tetracyclines are the **broadest** Spectrum antibacterial agents ever, and they are characterized by strong chelation (with transition metals like Ca, Fe, Mg,
Zn, Ag) which is dependent on the free flow of electrons, the chelation system is composed of hydroxyl and carbonyl

**Chelation**



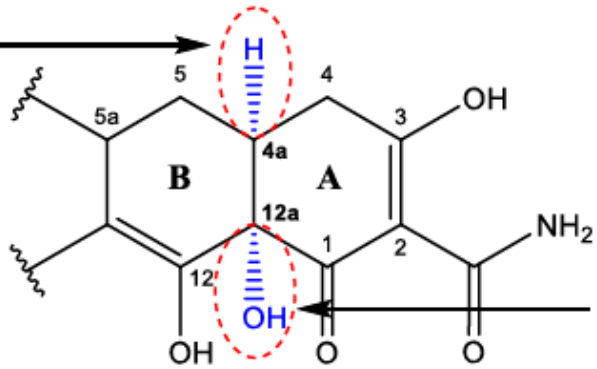

carboxylic acid (pka=4), its pka is 3.3 which is actually more acidic than carboxylic acid.

- At position 4 there is an **amine** which must be below the plane, the nitrogen of the amine must be di-substituted with a short substitution

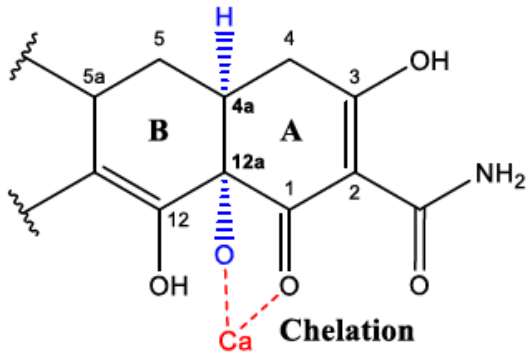


- Fd

**Position 4a:**
- H below th plane
is essential for activity

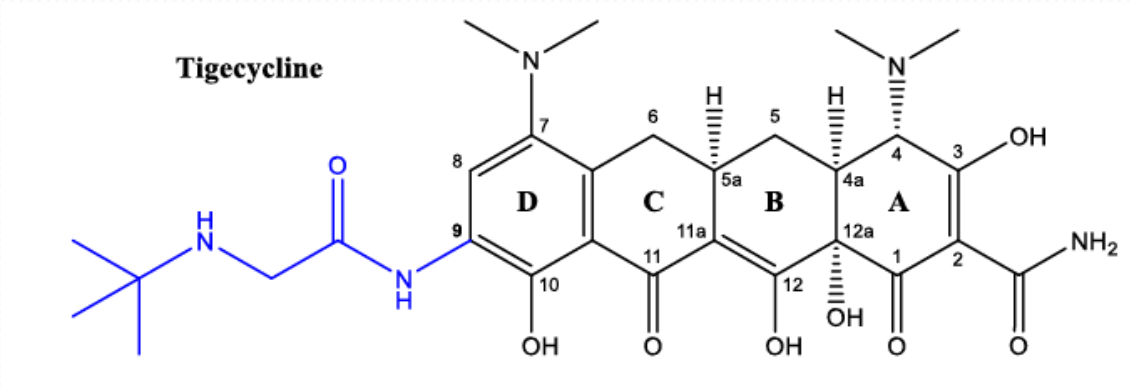



**Position 12a:**
- OH below th plane
- unsubstituted
is essential for activity



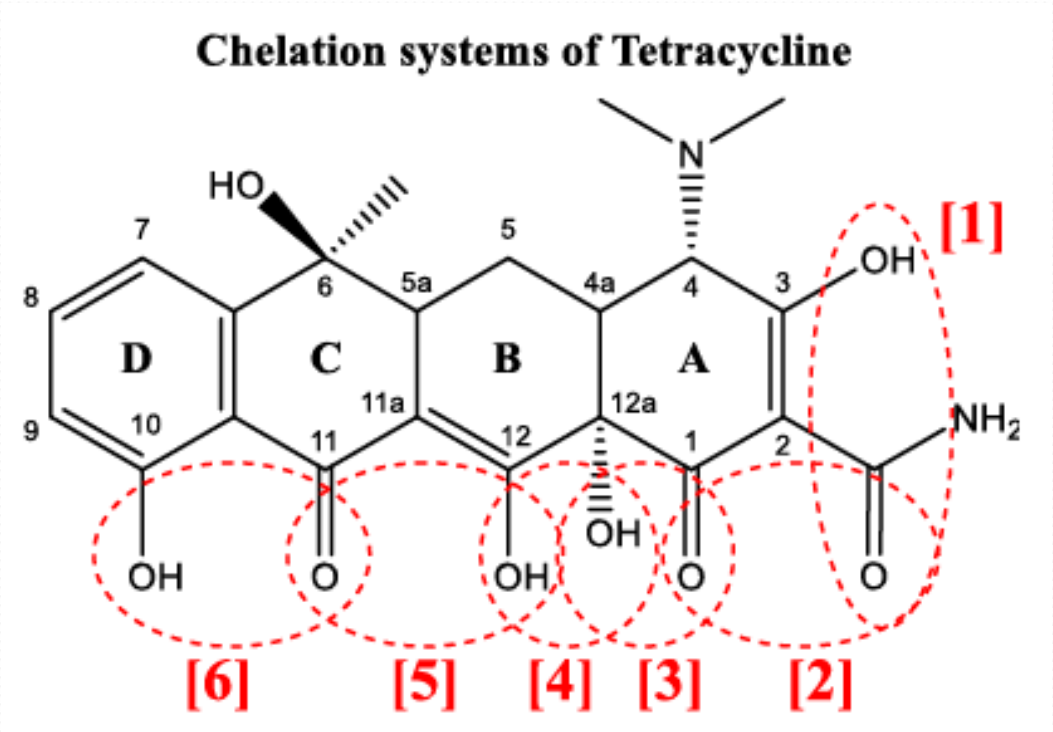

**Chelation**

- Position 9 was recently substituted in 2006 to produce **glycidyl tetracyclines**, from which is the famous drug **Tigecycline**, which has a glycine derivative side chain on position 9, the reason behind this discovery is that resistance to tetracycline is widespread, tetracyclines has broad spectrum and also covers atypical bacteria (like
- chlamydia and mycobacteria which are small in size, a bit larger than viruses, and they're very hard to eradicate). Tigecycline is sometimes called second generation tetracycline, which has broad spectrum without significant resistance, that's why it's a reserved antibiotic as injections in hospitals and not found in community pharmacies, and its use is limited to avoid developing resistance



# Mechanism of action

- D



# Mechanism of action

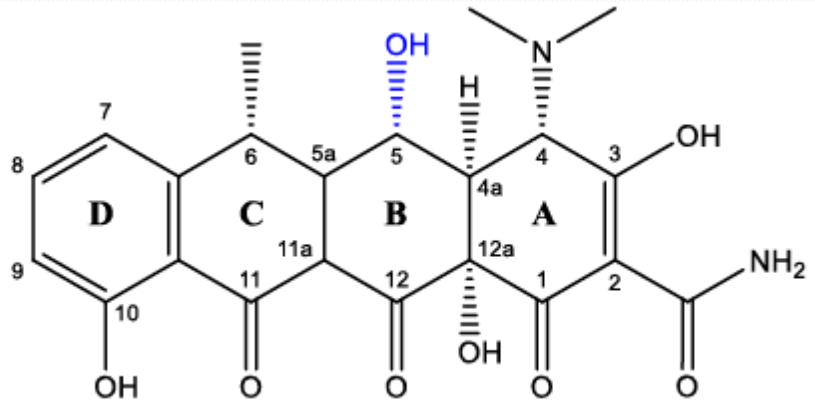
- Tetracycline work as antibacterial by inhibiting protein synthesis, by in fact binding to a ribosomal subunit called 30s which is a protein complexed with RNA that contains Mg as a stabilizing factor, on the surface of 30s subunit there is a binding packet for tetracycline where tetracycline form chelation with the Magnesium inside. It can form 6 chelations.
- When it is chelated with magnesium the reaction becomes irreversible (coordinate bond).
- That's what makes tetracyclines **potent protein synthesis inhibitors**.
- However it is **bacteriostatic**! The agents that work at the level of the protein are usually bacteriostatic agents, what does bacteriostatic means? It means that it does not kill the
- bacteria it only inhibits its growth and slow down its rapid differentiation providing sufficient time for immune system to deal with infection and get rid of it.

# Mechanism of action

- The idea of bacteriostatic agents makes us cautious when using such agents to deal with serious life threatening conditions such as septicemia or endocarditis in which we need very quick and potent bactericidal activity, so we use penicillins or aminoglycosides with a gram-negative activity in such serious cases.

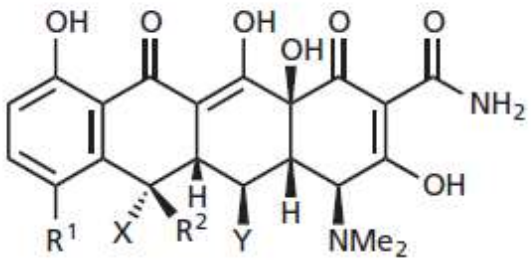
- One of the **current uses** of Tetracyclines is **acne** such as **Doxycycline**, acne is a mix of infections that needs a broad-spectrum antibiotics to deal with such as doxycycline.

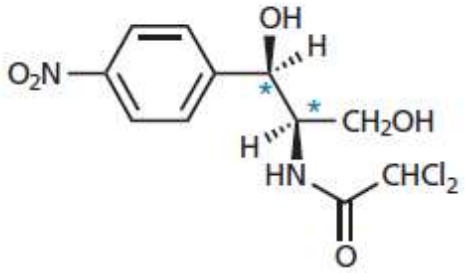
**Doxycycline**



# Tetracyclines

Tetracyclines should be avoided for young children and pregnant mothers as they can bind to developing teeth and bone leading to tooth discolouration.




Chlortetracycline (Aureomycin) ($R^1$ = Cl, $R^2$ = Me, X = OH, Y = H)
Tetracycline ($R^1$ = H, $R^2$ = Me, X = OH, Y = H)
Doxycycline (Vibramycin) ($R^1$ = H, $R^2$ = Me, X = H, Y = OH)
Demeclocycline ($R^1$ = Cl, $R^2$ = H, X = OH, Y = H)

Chloramphenicol

**FIGURE 19.66** Tetracyclines and chloramphenicol. The asterisks indicate asymmetric centres.

# Chloramphenicol

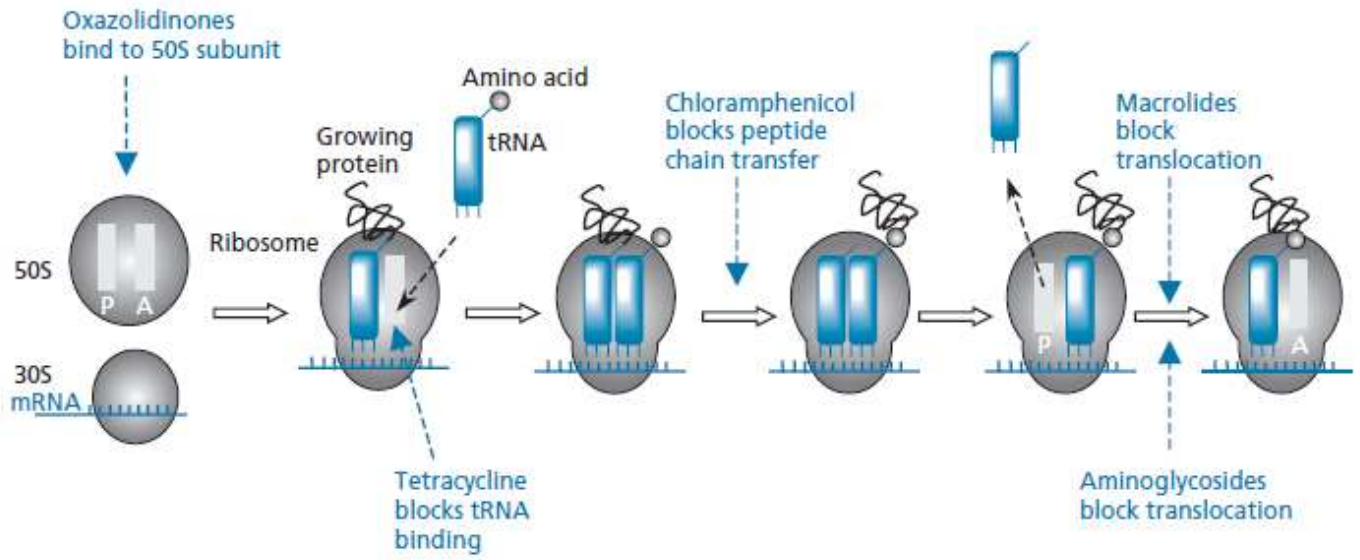
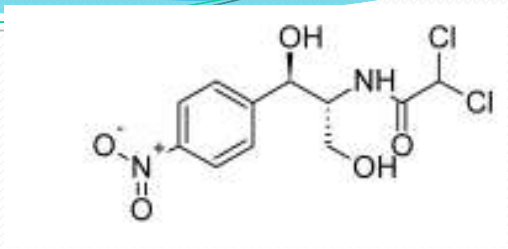


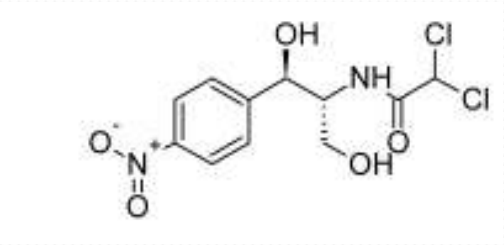

**FIGURE 19.64** Stages at which antibacterial agents inhibit translation.

# Chloramphenicol



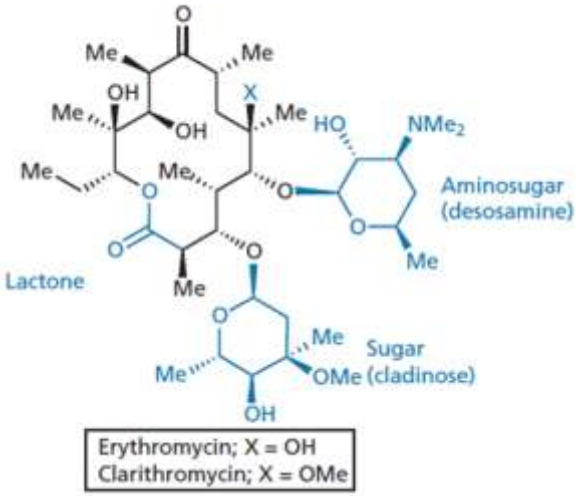

- Chloramphenicol was originally isolated from a microorganism called *Streptomyces venezuela* found in a field near Caracas, Venezuela.
- It is now prepared synthetically and has two asymmetric centres. **Only the *R,R* -isomer is active**.
- Chloramphenicol binds to the 50S subunit of ribosomes and appears to act by inhibiting the movement of ribosomes along mRNA, probably by inhibiting the peptidyl transferase reaction by which the peptide chain is extended.

# Chloramphenicol




- Since it binds to the same region as **macrolides** and **lincosamides** , these drugs **cannot** be used in combination.
- The nitro group and both alcohol groups are involved in binding interactions.
- The dichloroacetamide group is also important, but can be replaced by other electronegative groups.
- Chloramphenicol is quite toxic and the nitro substituent is thought to be responsible for this.
- The drug is metabolized inadequately in babies leading to a combination of symptoms described as the **gray baby syndrome** , which can be fatal

# Macrolides



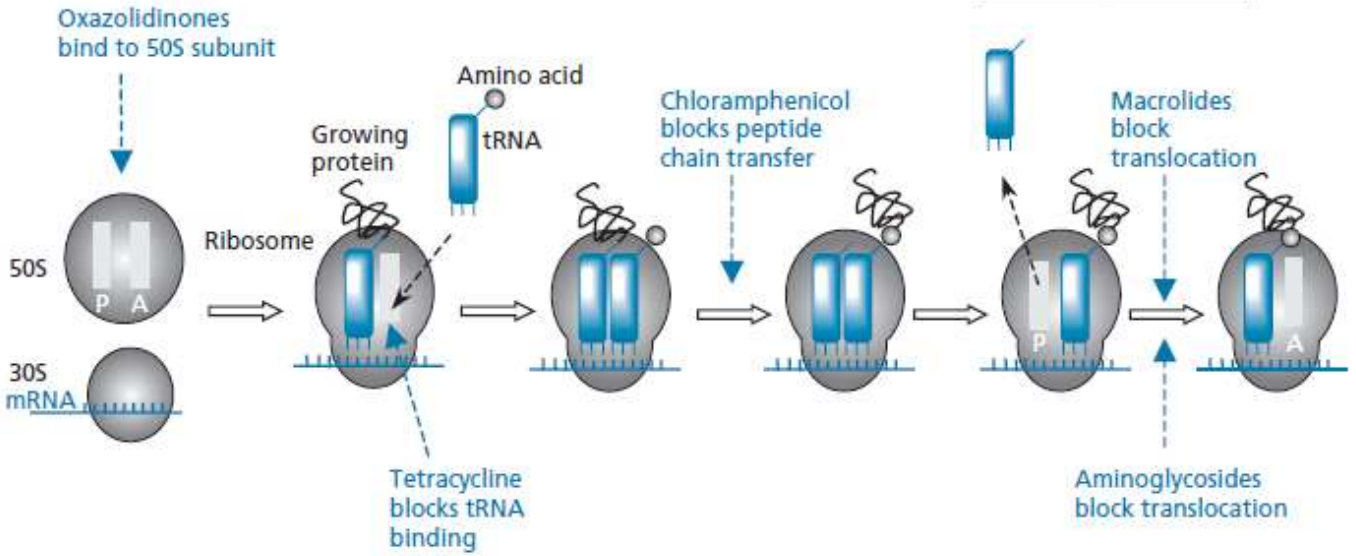
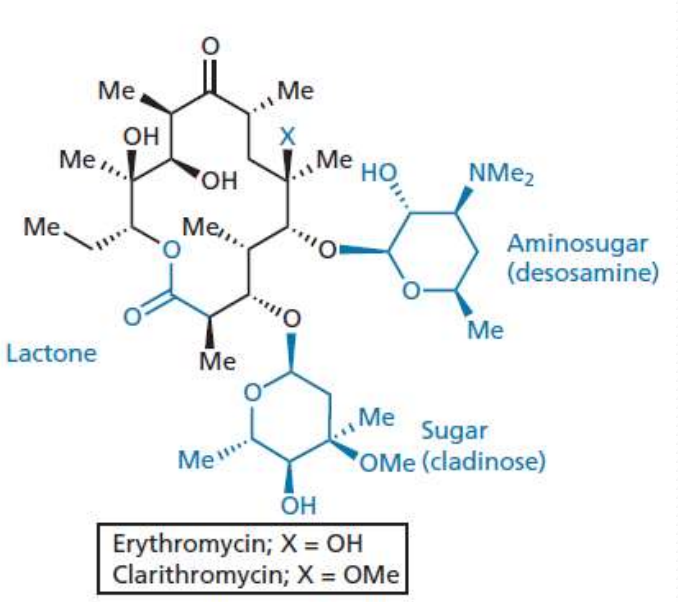




**FIGURE 19.64** Stages at which antibacterial agents inhibit translation.

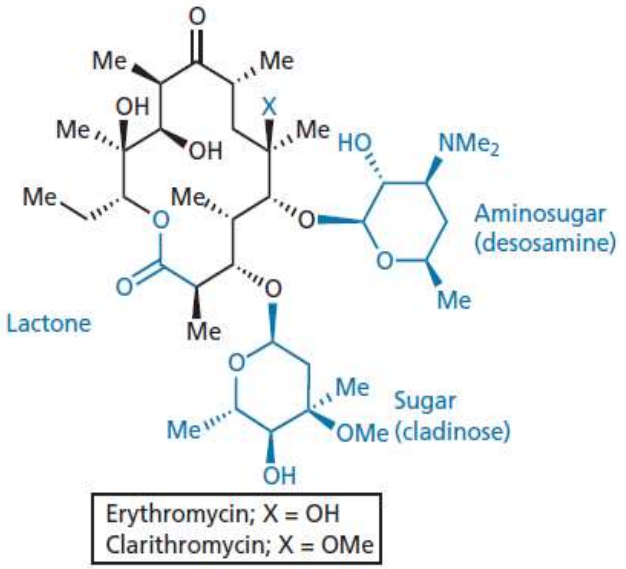
# Macrolides

- **Macrolides** are bacteriostatic agents. The best-known example of this class of compounds is **erythromycin**
- **Erythromycin** metabolite was isolated in 1952 from the soil microorganism *Streptomyces erythreus* found in the Philippines, and one of the safest antibiotics in clinical use.
- The structure consists of a **14-membered macrocyclic lactone ring** with a sugar and an amino sugar attached.
- The sugar residues are important for activity.



# Chemistry of Macrolides

- They all share 3 common features:
- 1. Large ring (12, 14, 16 atoms)
- 2. A ketone group (sometimes conjugated to double bond)
- 3. Glycosidically linked to amino sugars.
- Some tomes another neutral sugar is conjugated to the lactone ring
- The amino sugars causes macrolides to be basic thus useful salts are prepared from them
- Macrolides are inactivated by heat, strong acids, and strong bases



# Macrolides (Erythromycin)

- Erythromycin is unstable to stomach acids, but can be taken orally in a tablet form. The formulation of the tablet involves a coating that is designed to protect the tablet during its passage through the stomach, but which is soluble once it reaches the intestines (enterosoluble).
- The acid sensitivity of erythromycin is due to the presence of a ketone and two alcohol groups which are set up for the acid-catalysed intramolecular **formation of a ketal**.

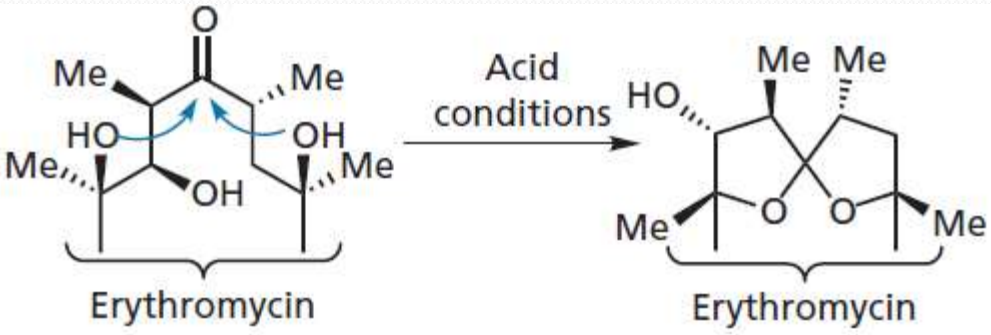


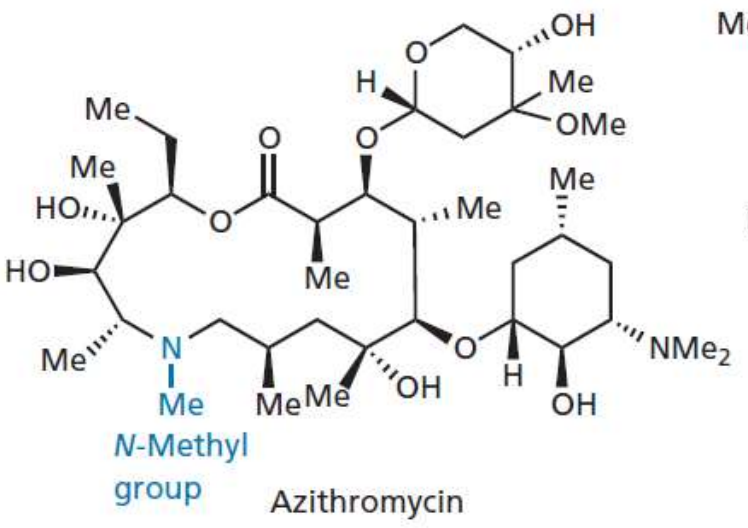

**FIGURE 19.68** Intramolecular ketal formation in erythromycin.

# Macrolides

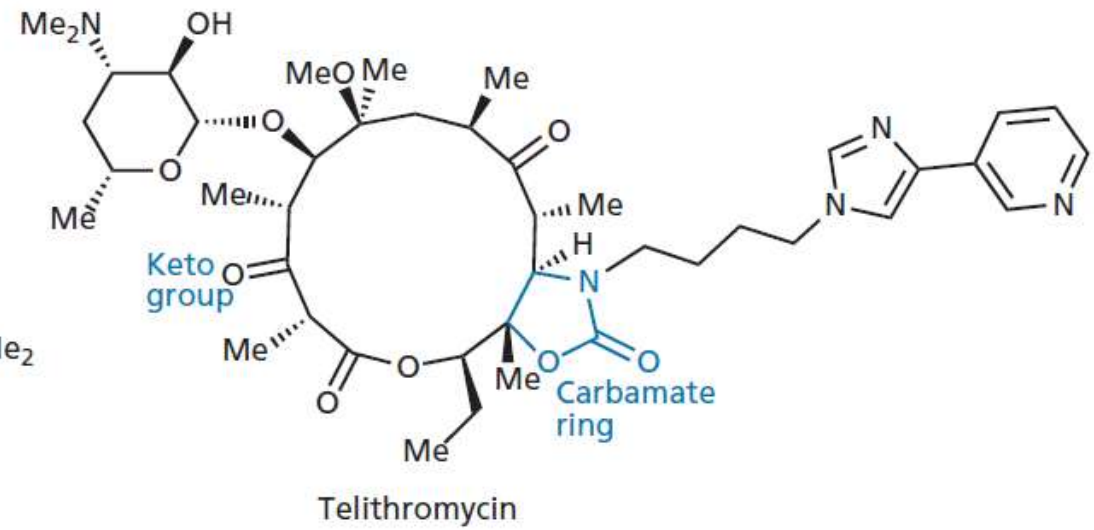
- One way of preventing this is to protect the hydroxyl groups. For example, **clarithromycin** is a methoxy analogue of erythromycin which is **more stable to gastric juices** and has improved oral absorption. **Another method** of increasing acid stability is to increase the size of the macrocycle to a 16-membered ring. (Erythromycin is 14 membered ring)

# Acid resistant Macrolides

- **Azithromycin** contains a **15-membered macrocycle** where an *N* -methyl group has been incorporated into the macrocycle. It is one of the world's best-selling drugs.
- **Telithromycin** is a semi-synthetic derivative of erythromycin and reached the European market in 2001. The cladinose sugar in erythromycin has been replaced with a keto-group and a carbamate ring has been fused to the macrocyclic ring. The two hydroxyl groups that cause the intramolecular ketal formation in erythromycin have been masked, one as a methoxy group and the other as part of the carbamate ring.



Azithromycin

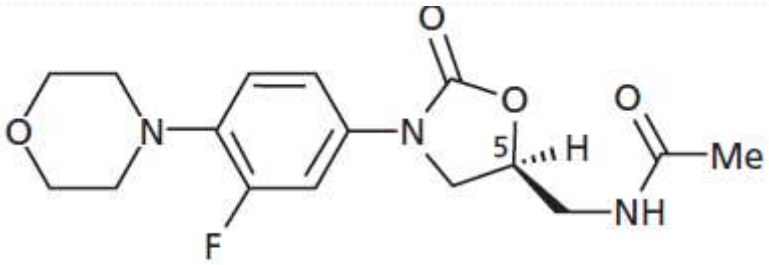


Telithromycin

# Macrolides spectrum of activity

- **Erythromycin** has an antibacterial spectrum that is similar to penicillins and can be used as an alternative to penicillins for those patients having penicillin allergies. It has been used against penicillin-resistant staphylococci, but newer penicillins are now preferred for these infections owing to increased resistance against erythromycin. It is very useful for the treatment of respiratory infections, including whooping cough and Legionnaires' disease. It can also be used to treat syphilis and diphtheria, as well as oral and skin infections. Topically, it can be used for the treatment of acne.
- **Clarithromycin** has slightly greater activity than erythromycin, with fewer gastrointestinal side effects. Therefore, it is often prescribed instead of erythromycin. Clarithromycin is one of the drugs used in the treatment of ulcers caused by the presence of *Helicobacter pylori* .
- **Azithromycin** is slightly less active than erythromycin against Gram-positive infections, but is more active against Gram-negative infections, including *H. influenza* —against which erythromycin shows poor activity.
- **Telithromycin** has a similar spectrum of activity to other macrolides. It should only be used for specified infections such as pneumonia, tonsillitis, and sinusitis.
- **Resistance to macrolides is due to** effective efflux mechanisms which pump the drug back out the cell. The ribosomal target site may also change in character such that binding is weakened. Enzyme-catalysed modifications can also occur.

# Oxazolidinones




Linezolid

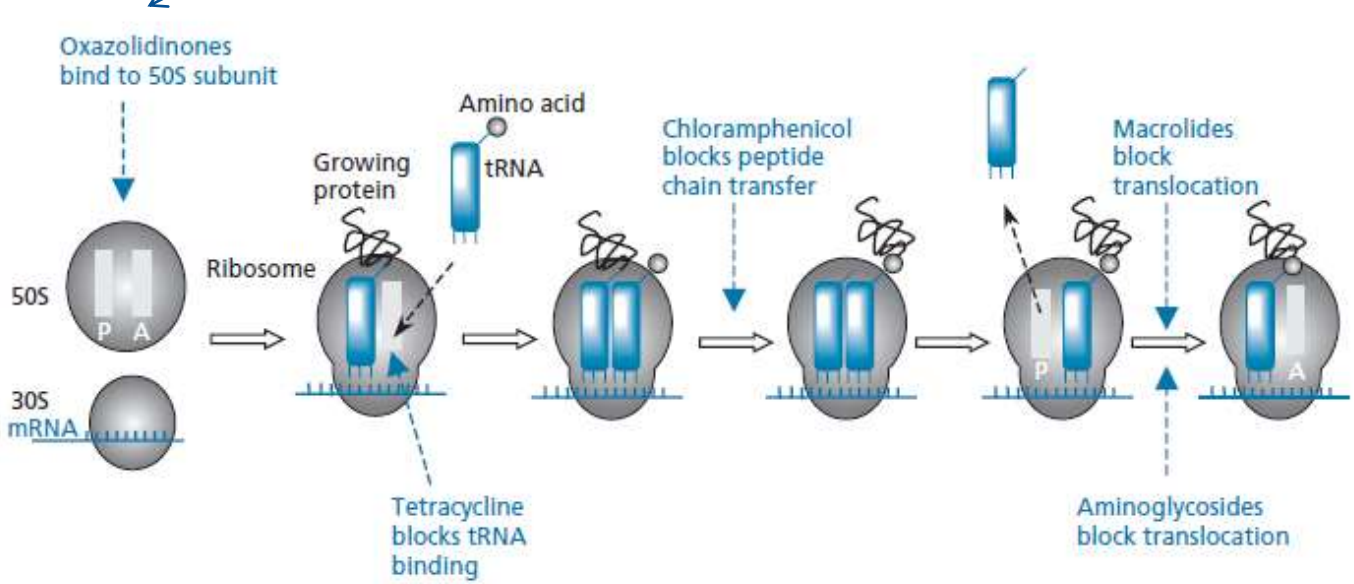



**FIGURE 19.64** Stages at which antibacterial agents inhibit translation.

# Oxazolidinones

- The **oxazolidinones** are a new class of synthetic antibacterial agents discovered in recent years.
- They inhibit protein synthesis at a much earlier stage than previous agents, and, consequently, do not suffer the same resistance problems.
- The oxazolidinones bind to the 50S ribosome and prevent protein synthesis. As a result, translation cannot even start.
- **Linezolid** was the first of this class of compounds to reach the market in 2000, and by 2010, it was netting sales of £716 million

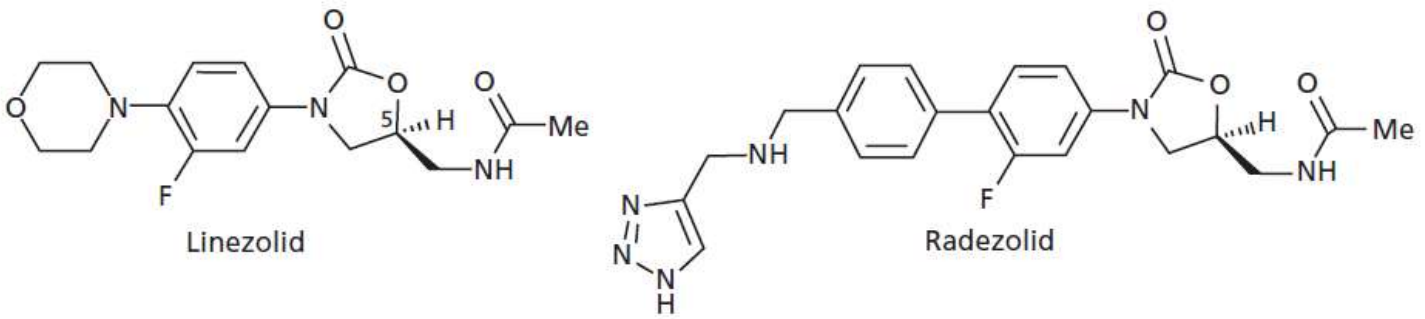



FIGURE 19.70 Oxazolidinones.

# The glycopeptides: vancomycin and vancomycin analogues

- is a narrow-spectrum bactericidal glycopeptide produced by a microorganism called *Streptomyces orientalis* found in Borneo and India.
- Aptly, its name is derived from the verb ‘to vanquish'. Vancomycin was introduced in 1956 for the treatment of infections caused by penicillin-resistant *S. aureus* , but was discontinued when methicillin became available.
- It has since been reintroduced and is now the main stand-by drug for treating MRSA. Vancomycin and related glycopeptides are often the last resort in treating patients with drug-resistant infections.
- As such, they have become extremely important and a great deal of research is currently being carried out in this area

# Vancomycin and its binding interactions to the L-Lys-D-Ala-D-Ala moiety.