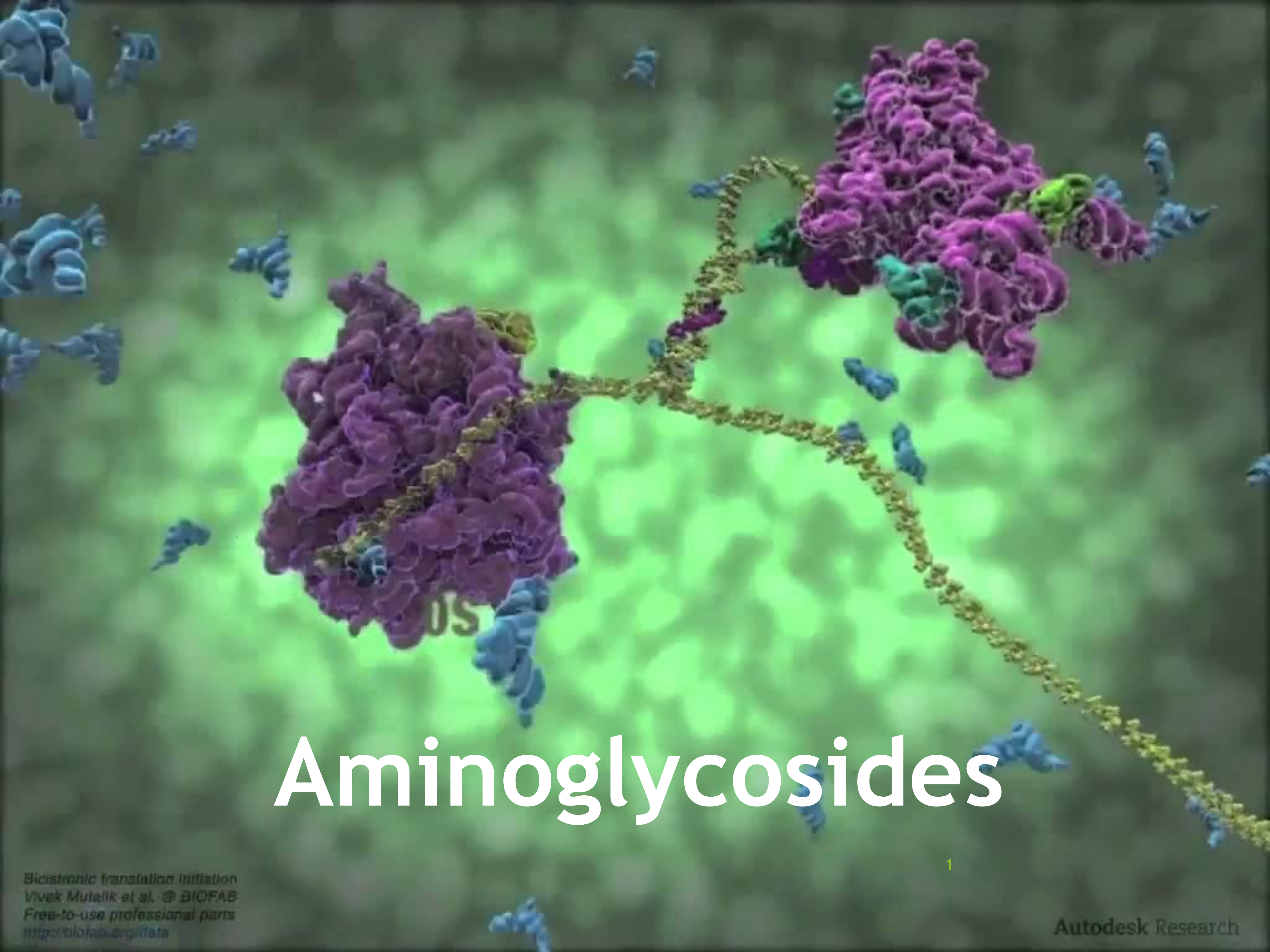


Aminoglycosides

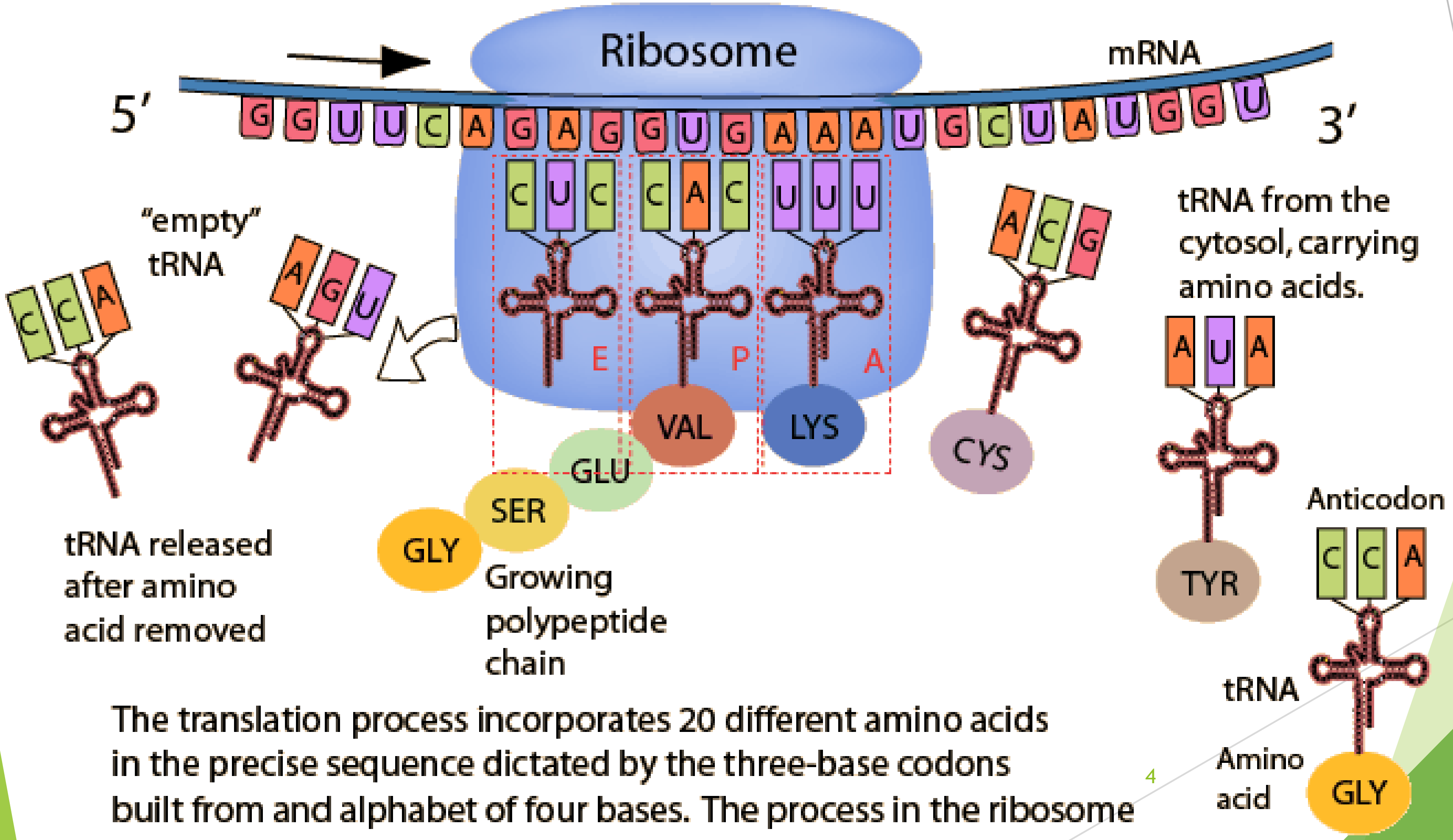
Antibiotics inhibit protein synthesis

- Proteins are super-important molecules and their sequences represent the information encoded in the DNA.
- The reading of the information from DNA, formation of the message (mRNA) and the translation of message to protein (by ribosomes) represents targets for inhibiting protein synthesis.
- Inhibition of protein synthesis is usually fatal for cells (bactericidal)
- How to achieve selectivity toward bacterial cells for antibiotics that inhibition protein synthesis?
  - Different diffusion rates between bacterial and mammalian cells.
  - Structural differences between bacterial and mammalian ribosomes

- Bacterial protein synthesis can be divided into four phases:
  - **Initiation:** where a functionally competent ribosome is assembled in the correct place on an mRNA ready to commence protein synthesis.
  - **Elongation:** whereby the correct amino acid is brought to the ribosome, is joined to the nascent polypeptide chain, and the entire assembly moves one position along the mRNA.
  - **Termination:** which happens when a stop codon is reached, there is no amino acid to be incorporated and the newly-synthesized polypeptide is released from the ribosome.
  - **Disassembly:** whereby a special factor binds to the ribosome so that it can release the mRNA and tRNA that is still bound to it and so that it can be recycled in another round of protein synthesis.

Steps for protein synthesis within ribosome

- Eukaryotic ribosome is bigger 80S (60S+40S)



The translation process incorporates 20 different amino acids in the precise sequence dictated by the three-base codons built from and alphabet of four bases. The process in the ribosome builds the polypeptide chains tha will become proteins.

Antibiotics inhibits the ribosome function

- The bacterial ribosome is a 70S particle (30S subunit + 50S subunit)
- 30S binds mRNA and initiates protein synthesis
- 50S binds aminoacyl tRNA and catalyze peptide bond formation.

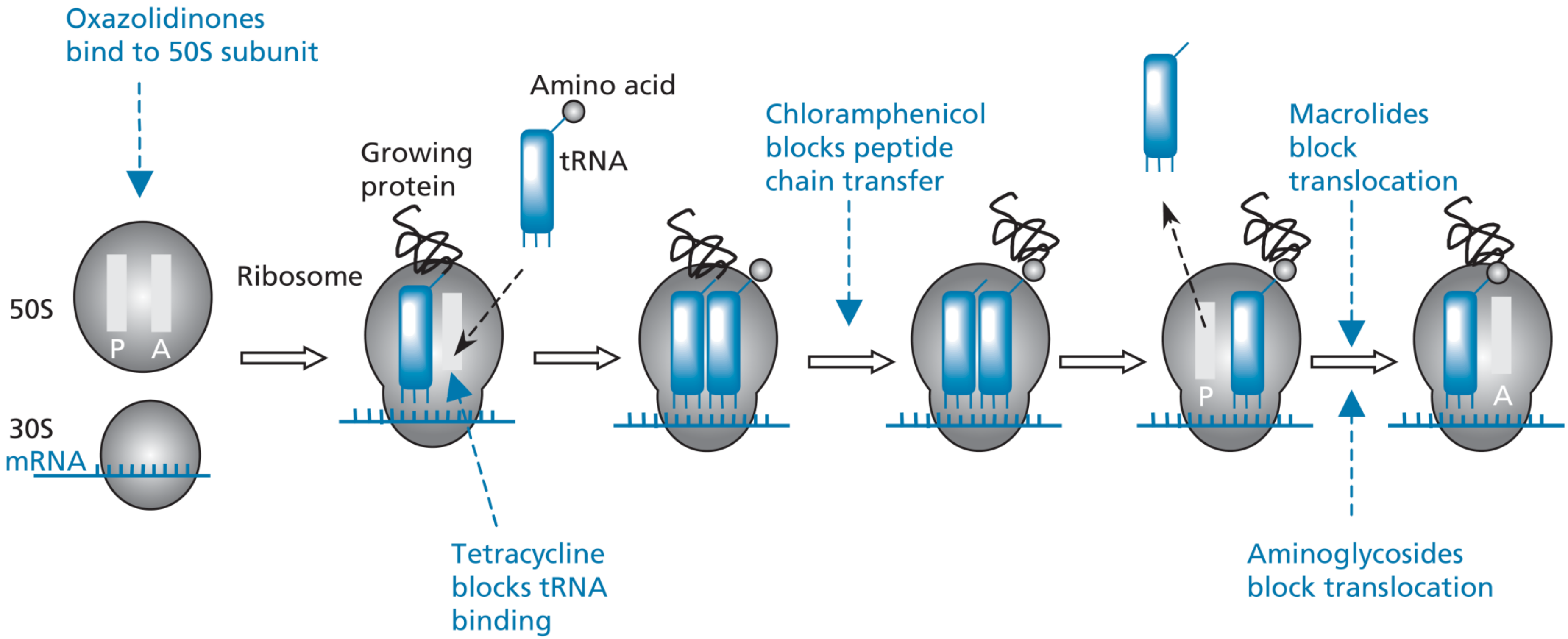
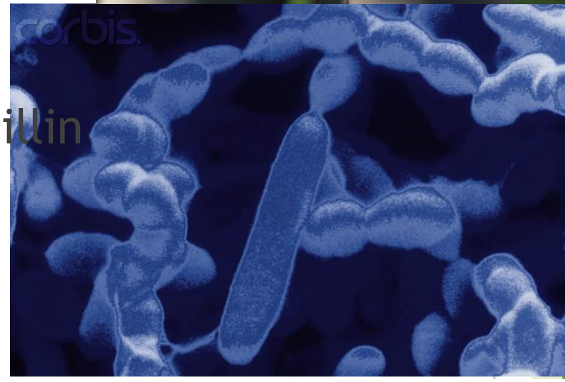
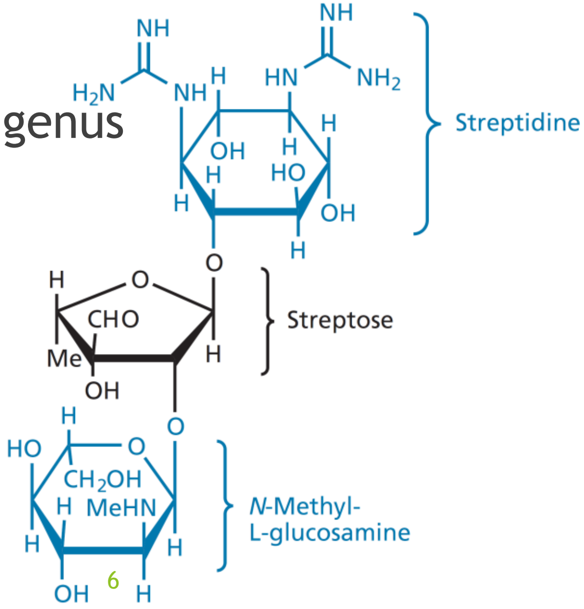


FIGURE 19.64 Stages at which antibacterial agents inhibit translation.

Aminoglycosides

- Isolated from Soil bacteria *Streptomyces griseus* in 1944.
- It is aminoglycoside (carbohydrate + amine).
- The next important antibiotic discovered after penicillin
- Best work at alkaline pH ➔ +ve charge ➔ ↑ absorption through outer membrane of Gram-ve
- Binds to 30S subunit of ribosome
- Used to treat aerobic Gram –ve bacteria
- Other aminoglycosides isolated from *Streptomyces* genus include —kanamycin, neomycin, paromomycin, gentamicin, tobramycin and Spectinomycin (the latter is different chemically and microbiologically
- Sisomicin, and amikacin are semisynthetic aminoglycoside





Streptomycin (from *Streptomyces griseus*)

NDC 39822-0706-1

LATEX FREE STOPPER

Streptomycin for Injection USP

1 gram*/ vial

FOR INTRAMUSCULAR USE

℞ only

X-GEN PHARMACEUTICALS INC

*This vial contains Sterile Streptomycin Sulfate USP equivalent to 1 gram Streptomycin.
See package insert for reconstitution instructions.

USUAL DOSAGE CONSULT PACKAGE INSERT

STORE DRY POWDER AT CONTROLLED ROOM TEMPERATURE 20° to 25°C (68° to 77°F) [see USP Controlled Room Temperature].

Approx. Conc. mg/mL	Volume (mL) to be added
200	4.2
250	3.2
400	1.8

Sterile reconstituted solutions should be protected from light and may be stored at controlled room temperature up to 7 days. **Protect from light. Retain in carton until time of use.**

Manufactured for: X-GEN Pharmaceuticals Inc Big Flats, NY 14814

Made In USA

STRP-VL-00

Non Varnish Area

Aminoglycosides (Cont.)

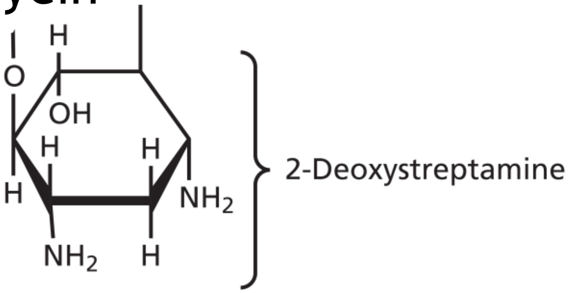
- Poor absorption (<1%), Kanamycin, Neomycin and Paromycin used for treatment of GIT infections
- Cause otoxicity and nephrotoxicity if used systematically

Chemistry

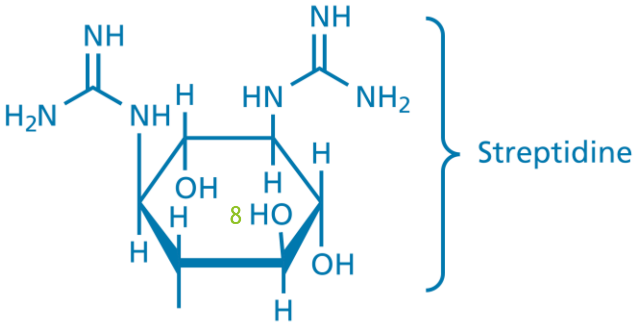
- Named aminoglycosides: aminosugars and aminocyclitols linked glycosidically.
- Aminoglycosides usually composed of 3 rings,
  but sometimes 4 such as in neomycin and paromomycin
- Aminoglycosides rings are:

1. At least 1 aminohexose and may have pentose (without amine) e.g. streptomycin, neomycin and paromomycin
2. A highly substituted 1,3-diaminocyclohexane central ring:

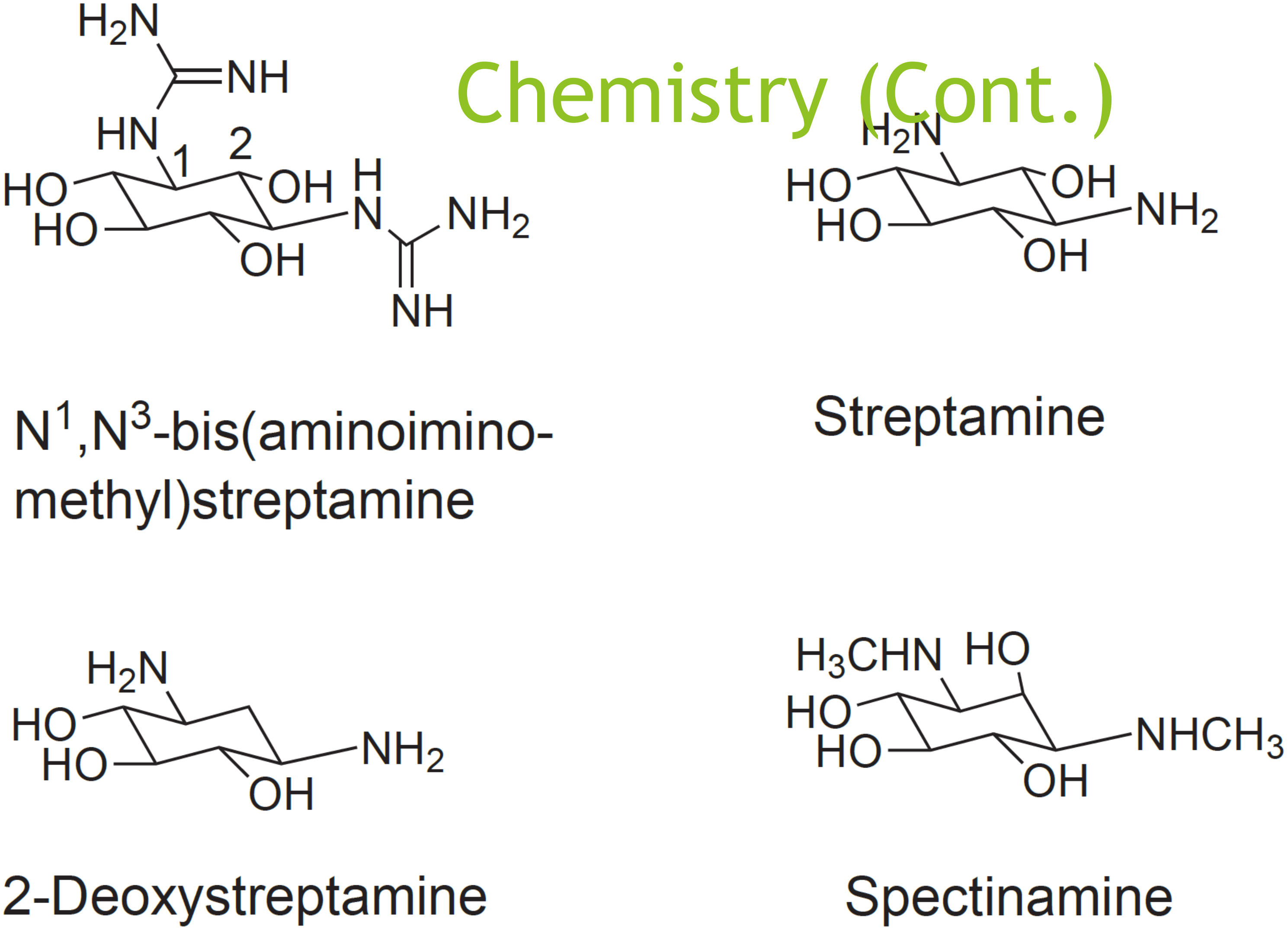
Deoxystreptamine in kanamycin, neomycin, gentamicin and tobramycin



Streptadine in streptomycin



Chemistry (Cont.)



N^1,N^3-bis(aminoiminomethyl)streptamine

Streptamine

2-Deoxystreptamine

Spectinamine

FIGURE 33.25 1,3-Diaminoinositol moieties present in aminog
cosides.

Chemistry (Cont.)

- Strong basic exist as polycations at physiological pH
- Their inorganic acid salts (e.g. sulfates) are very water soluble at all pH values
- Due to the polarity, they distribute well into most body fluids but **NOT** to CNS, bone, fatty and connective tissues.
- Concentrated in kidney, excreted by glomerular filtration and not metabolized *in vivo*
- Their aqueous solution is stable for autocalving
- chemistry, spectrum, potency, toxicity, and pharmacokinetics of these agents are a function of the specific identity of the diaminocyclitol and arrangement and identity of the attachments.

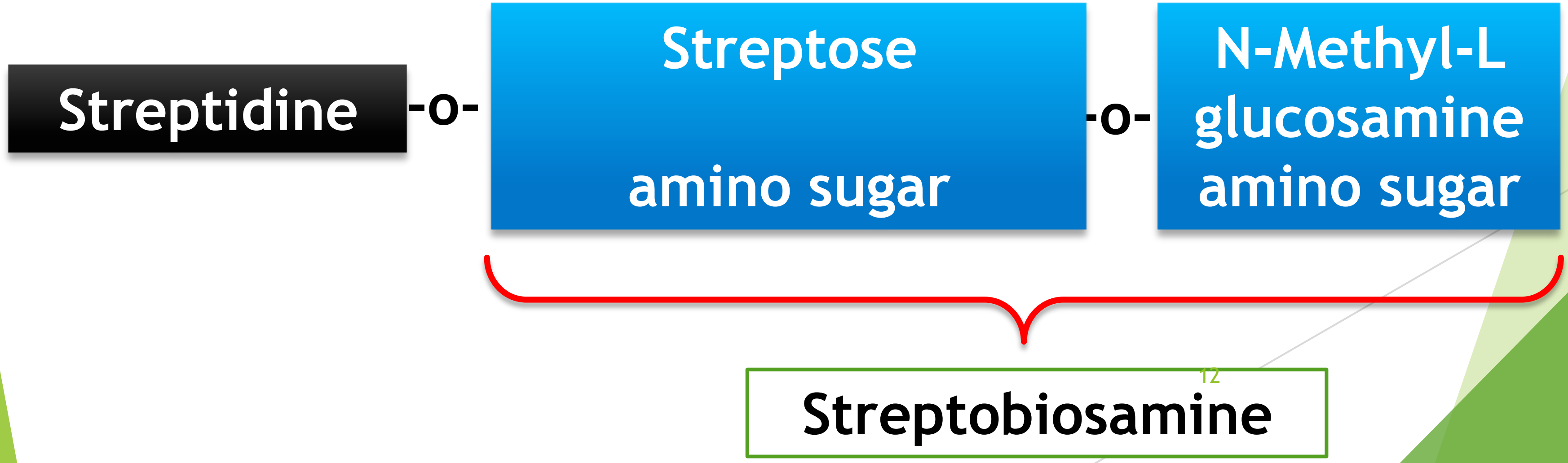
- **Aminoglycoside (AGL)**
  - **Stretomyces Suffix -mycin**
    - **Streptomycin**
    - **Paromomycin**
    - **Neomycin**
    - **Tobramycin**
  - **Micromonospora Suffix -micin**
    - **Gentamicin**
    - **Amikacin**
    - **Netilmicin**

Chemistry (Cont.)

Aminoglycosides Structure

Amino sugar -o- 2-deoxystreptamine -o- Amino sugar

Streptomycin structure



Streptidine -o- Streptose

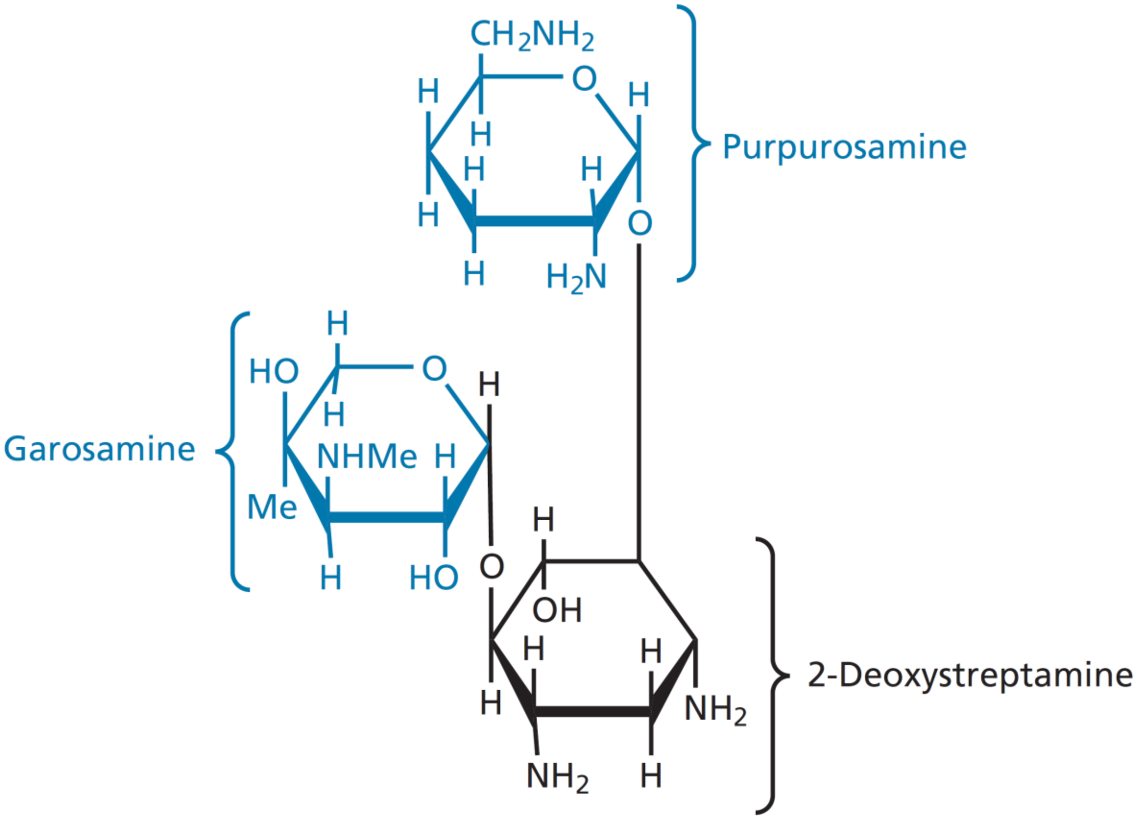
amino sugar -o- N-Methyl-L glucosamine amino sugar

Streptobiosamine

Chemistry

Aminoglycosides Structure

Amino sugar -o- **2-deoxystreptamine** -o- **Amino sugar**



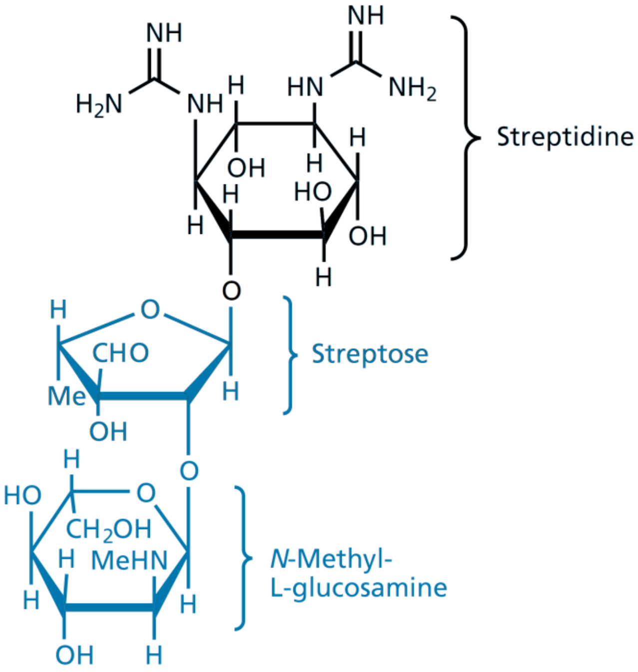
Gentamicin C1a

Chemistry

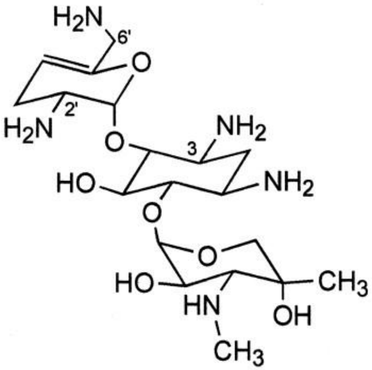
Streptomycin structure

Streptidine -o- **Streptose amino sugar** -o- **N-Methyl-L glucosamine amino sugar**

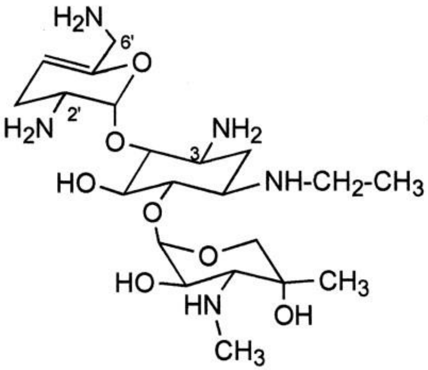
Streptobiosamine



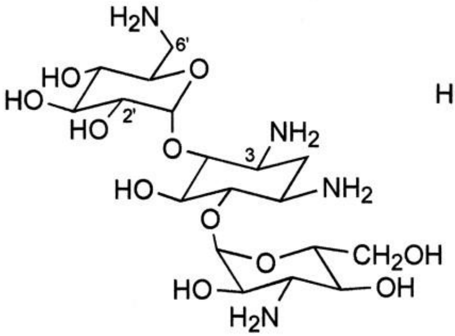
Streptomycin (from *Streptomyces griseus*)



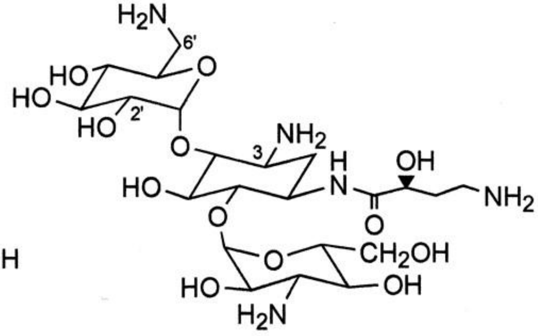
Sisomicin



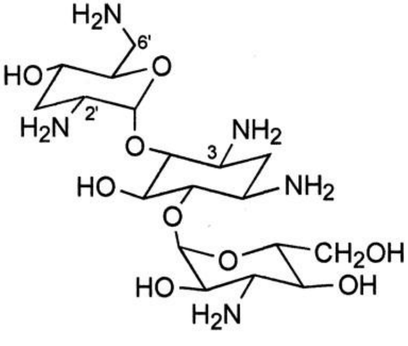
Netilmicin



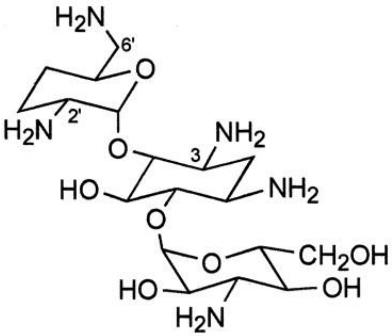
Kanamycin A



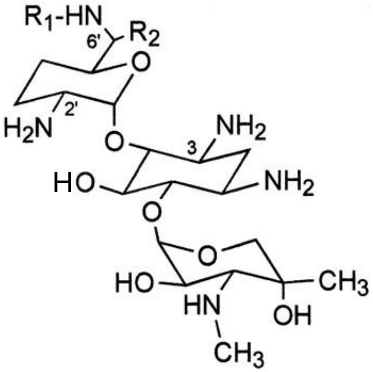
Amikacin



Tobramycin

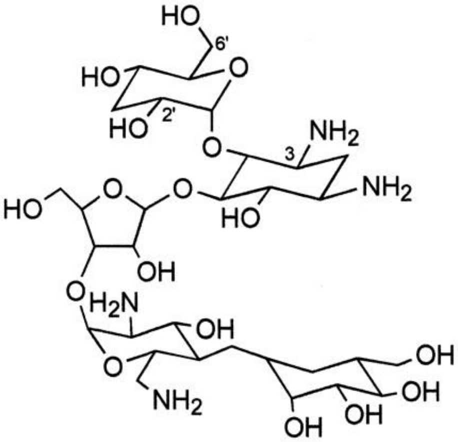


Dibekacin



Gentamicin Complex

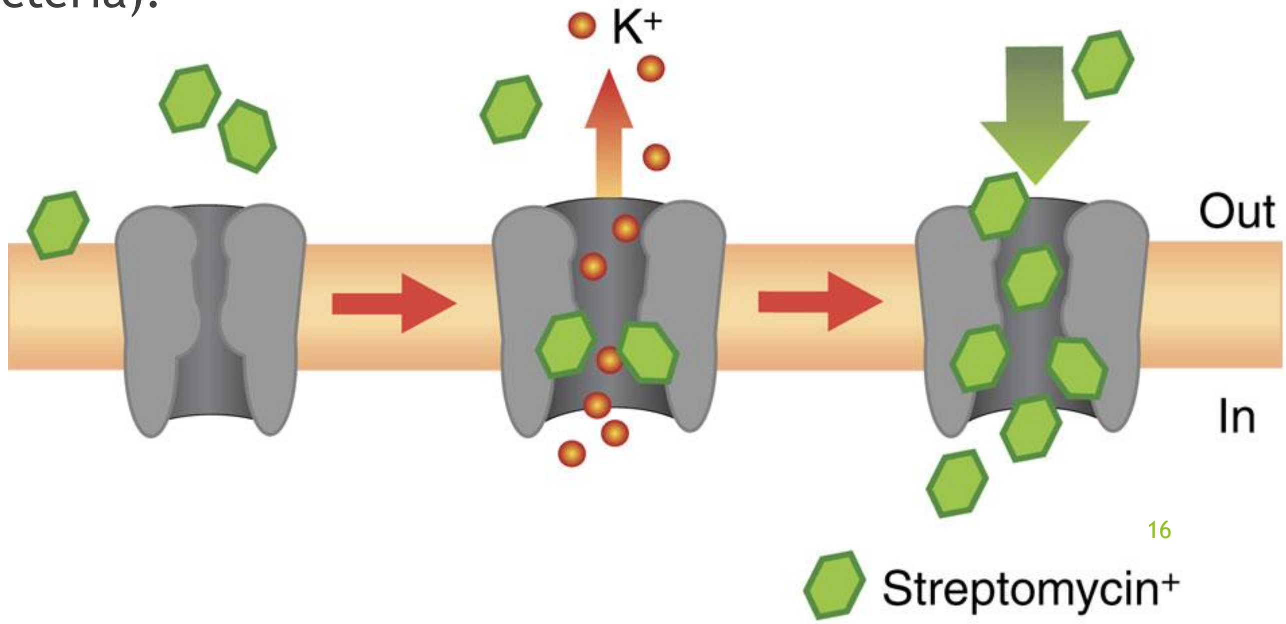
C_1: $R_1 = R_2 = CH_3$
C_{1a}: $R_1 = R_2 = H$
C_2: $R_1 = H$, $R_2 = CH_3$



Lividomycin A

Entrance of aminoglycosides into bacterial cell

- Aminoglycosides are highly polar
- Aminoglycosides bind initially to external lipopolysaccharides and diffuse into the cells in small amounts.
- The uptake process is inhibited by Ca^{+2} and Mg^{+2} ions. These ions are, then, partially incompatible therapeutically.
- Passage through the cytoplasmic membrane is dependent on respiration-driven active-transport (which is ascent in anaerobic bacteria).



Spectrum of activity

- Active against aerobic Gram-ve bacilli
- Less active against aerobic Gram-ve and Gram+ve cocci than ß-lactams
- Inactive against anaerobic bacteria (because aminoglycosides need oxygen dependent transport system to get inside the cell).
- The commonly used combinations:
  Aminoglycosides + ß-lactams = synergism
  (*damaged cell wall increases penetration of aminoglycosides*)
  - Carbenicillin + Gentamicin.
  - Penicillin G + streptomycin for Enterococci infections (endocarditis).
- Have good activity against *P. aeruginosa* especially in combination with penicillins.
- Some are widely used for skin and eye infections for local antibacterial actions such as neomycin and Gentamicin.
- Some are used for GIT infections such as paromomycin and tobramycin.

AG+Pen are **INCOMPATIBLE** if mixed in same syringe

Incompatibility of AG + Pen

- Aminoglycosides are incompatible with certain β-lactam antibiotics. The two drugs react with each other. For example *N*-acylation on C-l of gentamicin takes place by the β-lactam antibiotic, thus inactivating both antibiotics

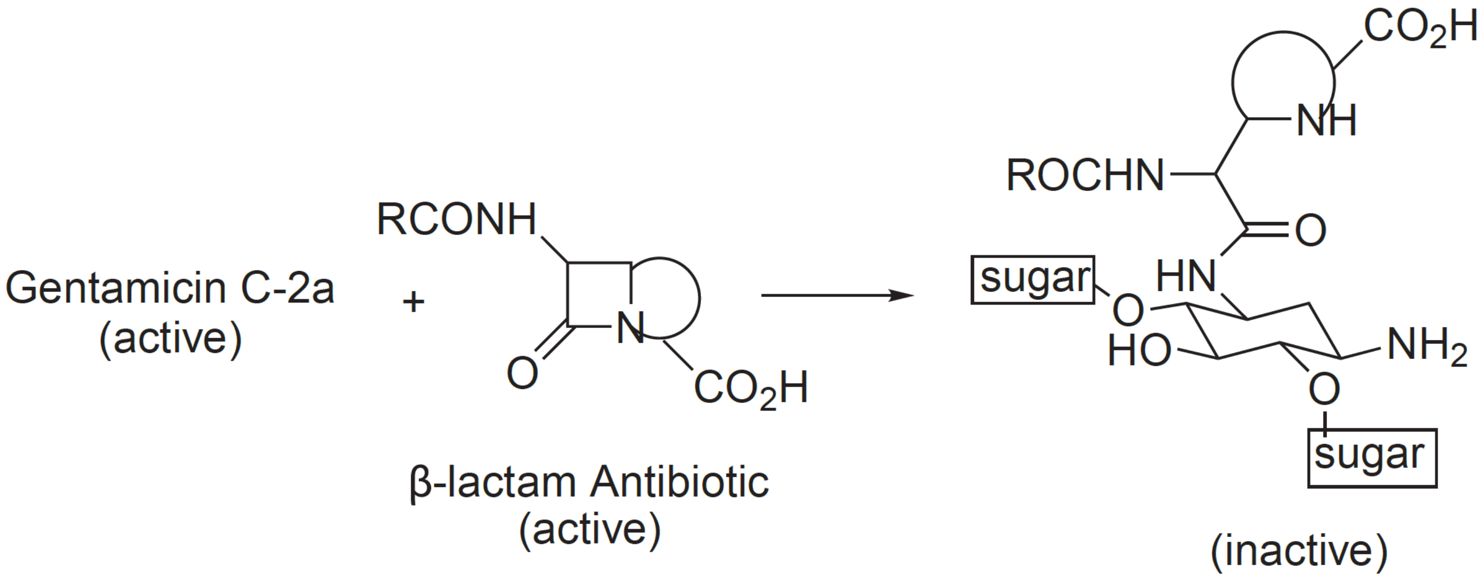


FIGURE 33.27 A chemical drug–drug incompatibility between gentamicin C-2a and β-lactams.

Mechanism of action

- Bind to 30S subunit of bacterial ribosome, thus
  - inhibit the initiation of protein synthesis
  - inhibit fidelity of translation of genetic message → wrong protein
- Deoxystreptamine-containing aminoglycosides (misreading at low doses and inhibition of initiation at high doses)
- Streptomycin-containing aminoglycosides (misreading and inhibition of initiation)
- Spectinomycin (inhibition of initiation only)
- All aminoglycosides are bacteriostatic (at lower dose) and bactericidal at higher dose, except spectinomycin which is only bacteriostatic.
- At high concentrations, eukaryotic protein biosynthesis can also be inhibited

Resistance

- Bacteria has two major resistant mechanisms to aminoglycosides:
  1. Mutation at energy dependent transport system which affects aminoglycosides uptake by the cell (e.g. *Ps. Aeruginosa)*
  2. Mutation at the ribosomal 30S subunit
  3. Inactivating aminoglycosides by changing the chemical structure, especially the essential functional groups for activity.
- Mainly in enterobacteriaceae family of bacteria
- Some strains are resistant to streptomycin, kanamycin and gentamycin
- Resistant strain produce enzymes that modify aminoglycosides' terminal amines and hydroxyls by acetylation, phosphorelation or adenylylation
- The sensitivity of an aminoglycoside to inactivating enzymes affects the spectrum of its antibacterial activity.

Resistance (Cont.)

the most important cause of resistance is enzymically catalyzed inactivation of aminoglycosides. More than 50 aminoglycoside-inactivating enzymes have been reported, however all catalyze only three major types of reactions:

1. **N-Acetylation** of vulnerable amino groups using acetyl coenzyme A as the acetyl donor. The N-acetyl transferases comprise the largest group of aminoglycoside-inactivating enzymes.
2. **O-Adenylylation** invoving the transfer of an AMP residue from ATP to certain hydroxyl groups. The O-adenylyl transferases form the smallest group of aminoglycoside-inactivating enzymes.
3. **O-Phosphorylation** of hydroxyl groups with ATO acting as the phosphate donor.

Resistance (Cont.)

Aminoglycoside-inactivating enzymes include:

a) N-acetyl transferases (NAT) which acetylates:

$6'$-NH_2 of Ring I
3-NH_2 of Ring II
$2'$-NH_2 of RingI

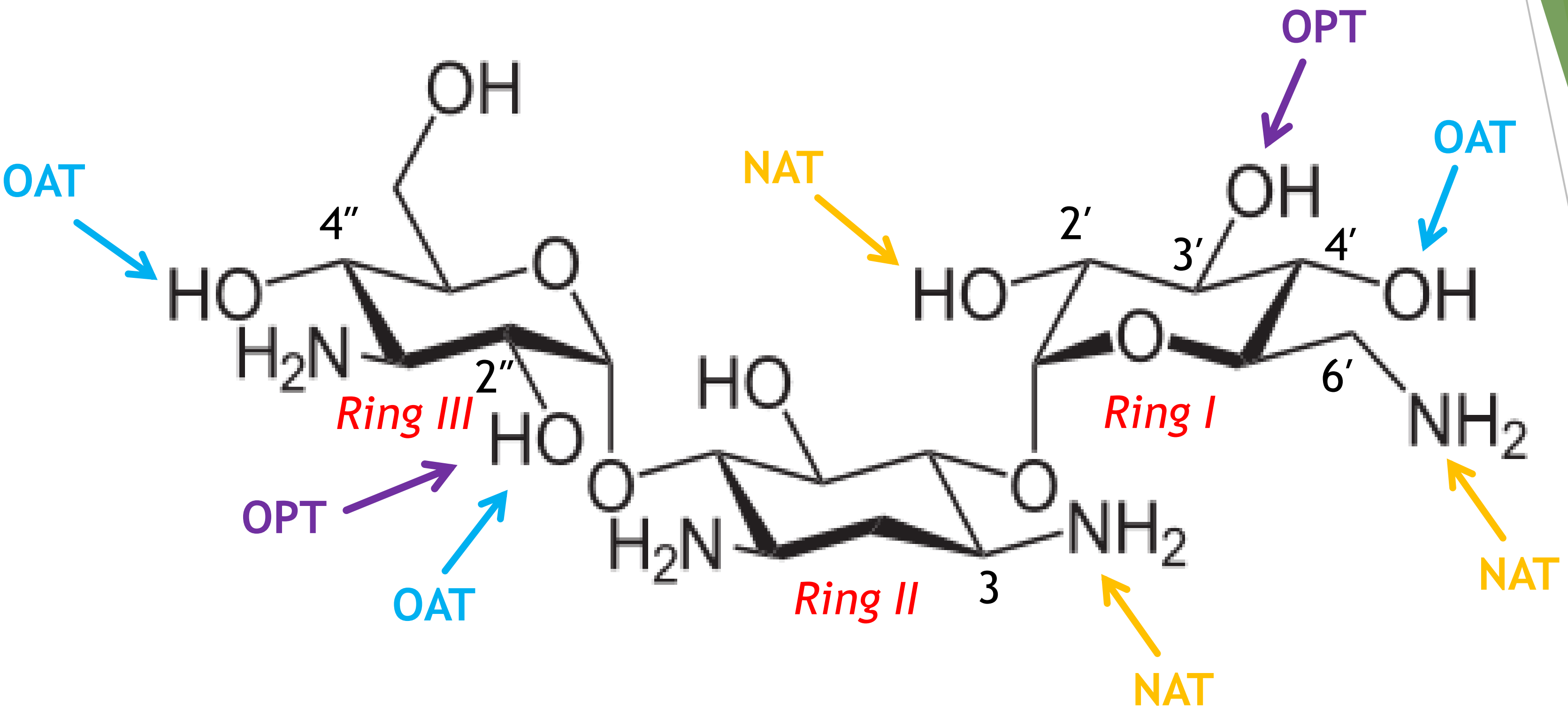
b) O-Phosphoryl transferees (OPT): which phosphorylates

3′-OH of Ring I
2″-OH of Ring III

c) O-Adenylyl transferees (OAT): which adenylates

2″-OH of Ring III
4′-OH of Ring I
4″-OH of Ring III

Resistance (Cont.)



Kanamycin A

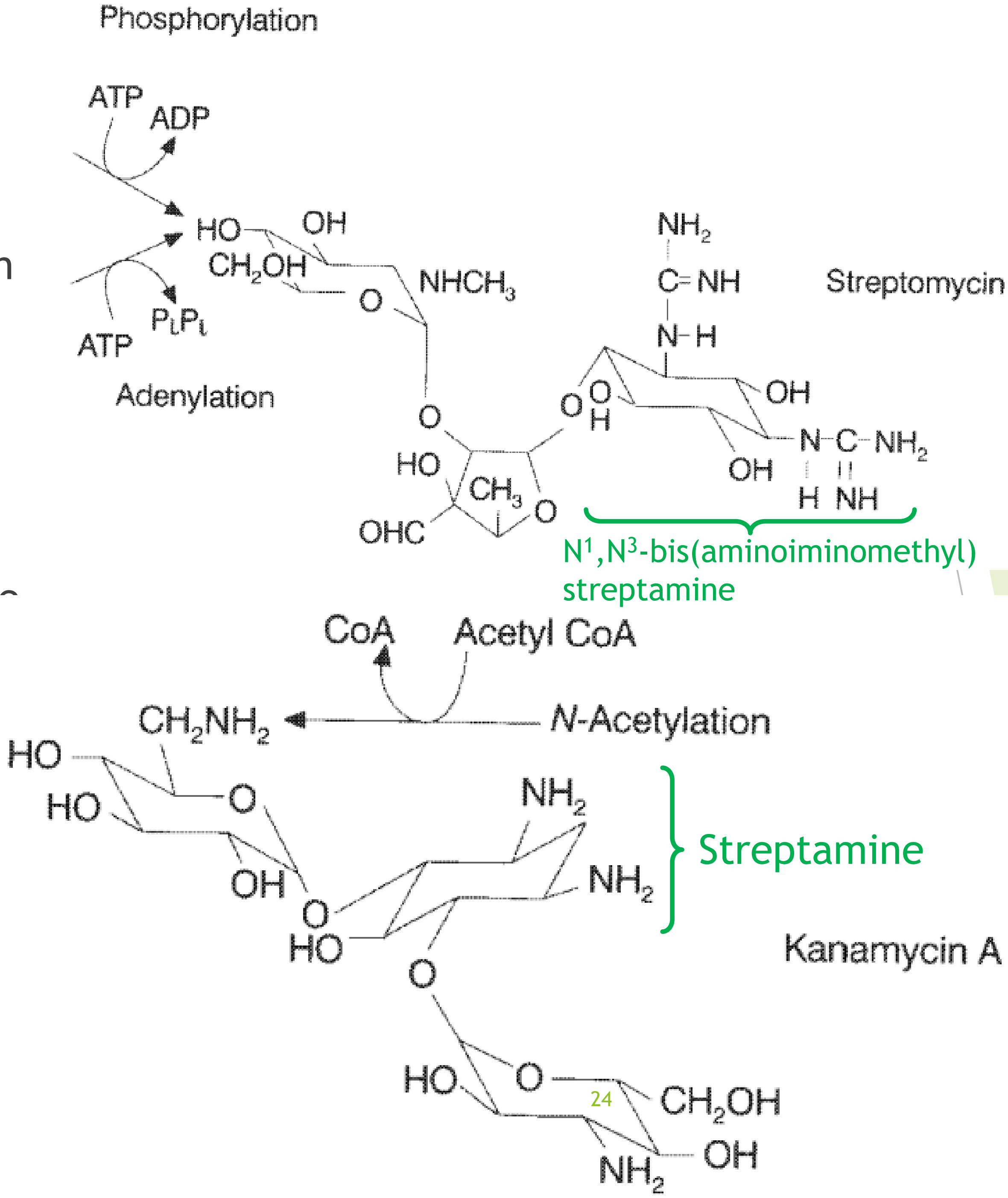
NAT → 6′, 3 and 2′ amines

OPT → 3′ and 2″ hydroxyls

OAT → 2″, 4′ and 4″ hydroxyls

Resistance (Cont.)

- Streptomycin is **NOT** subject to *N*-acetylation due to 1° amine of N1,N3-bis(aminoiminomethyl) streptamine
- Kanamycim A is subject to *N*-acetylation due to 1° amine of streptamin
- Both kanamycin A and streptomycin are inactivated by O-adenylylation and O-phosphorylation



N^1,N^3-bis(aminoiminomethyl) streptamine

Streptamine

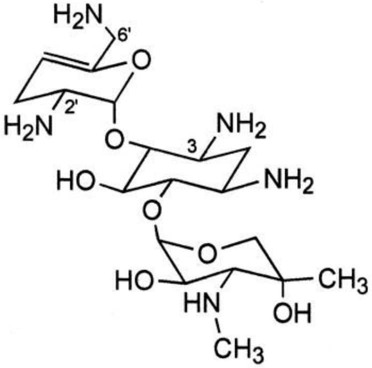
Resistance (Cont.)

Q) How to overcome the enzymatic inactivation of AG by molecular design?

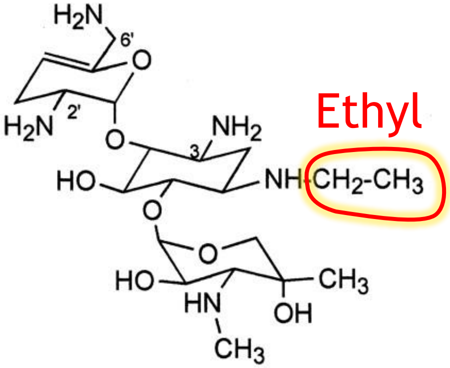
A) Modifying the valunerable functional groups, for example:

- 1° amine → 2° amine (resistant to N-acetylation e.g. Gentamycin C1 but NOT others)
- Removal of 3′-OH, 4′-OH or both in ring I (resistant to phosphorylation e.g. gentamycin and tobramycin but NOT kanamycin)
- 2°equatorial → 3°axial orientation for 4″-OH (resistant to adenylation e.g. gentamicins but NOT kanamycin)
- 1-NH2 is acylated by bulky group. L-hydroxyaminobuteroyl amide (L-HABA) to be more resistant to acetylation as well as to adenylation and phosphorylation at distant sites (2′ and 3′) (e.g amikacin). Similarly N-ethyl for Netilmicin

Examples of aminoglycosides

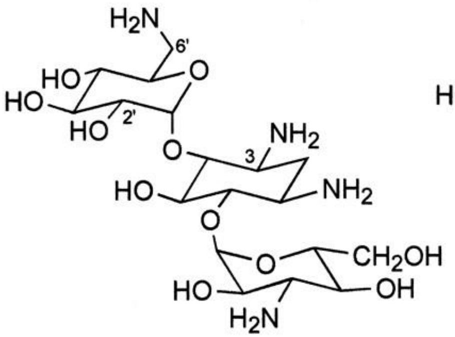


Sisomicin

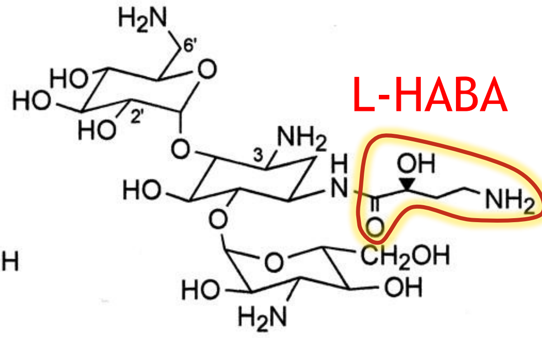


Ethyl

Netilmicin



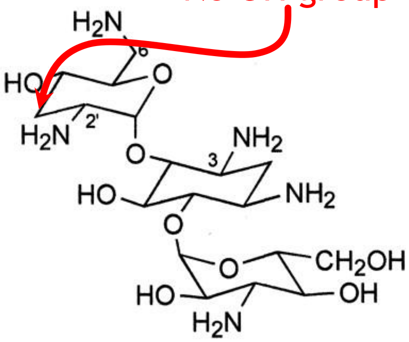
Kanamycin A



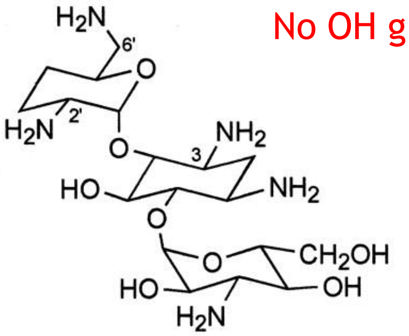
L-HABA

Amikacin

No OH group



Tobramycin



Dibekacin

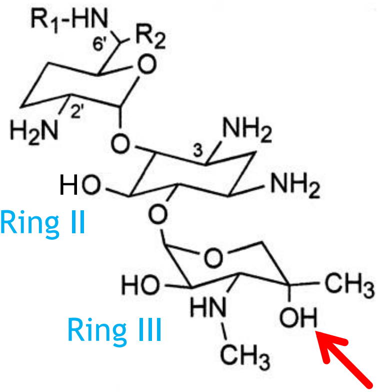
2° amine

No OH group

Ring I

Ring II

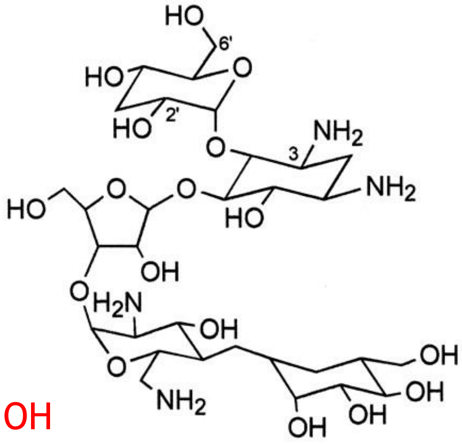
Ring III



Axial OH

Gentamicin Complex

C_1: $R_1 = R_2 = CH_3$
C_{1a}: $R_1 = R_2 = H$
C_2: $R_1 = H$, $R_2 = CH_3$

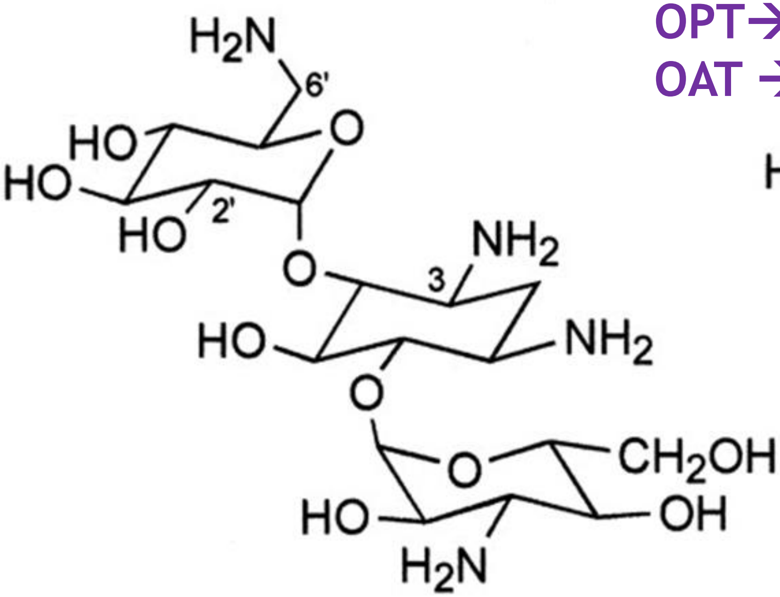


Lividomycin A

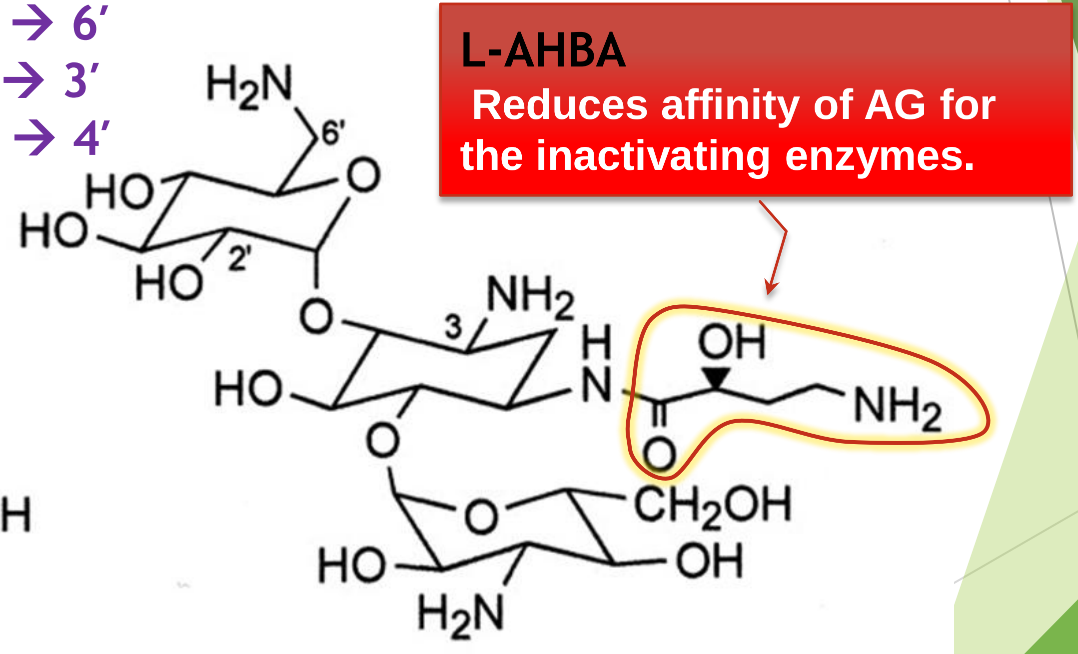
Resistance (Cont.)

- The most significant breakthrough yet achieved in the development of aminoglycosides is the amikacin, which is L-aminohydroxybutyric acid (L-AHBA) derivative of kanamycin A)

- Similar activity to kanamycin C
- Resist all AG inactivating enzymes except:
  NAT → 6′
  OPT→ 3′
  OAT → 4′



Kanamycin A



L-AHBA

Reduces affinity of AG for the inactivating enzymes.

Amikacin

Adverse effects

- Toxicities include:
  1. Disrupt functions mediated by eighth cranial nerve (hearing, vertigo).
  2. Kidney tubular necrosis ➔ decrease in glomerular function
  3. Curare-like neuromuscular blockade (may be due to competitive inhibition of calcium ion-dependent acetylcholine release at neuromuscular junction)
- Symptoms of toxicity may be delayed after injury

SAR for aminoglycosides

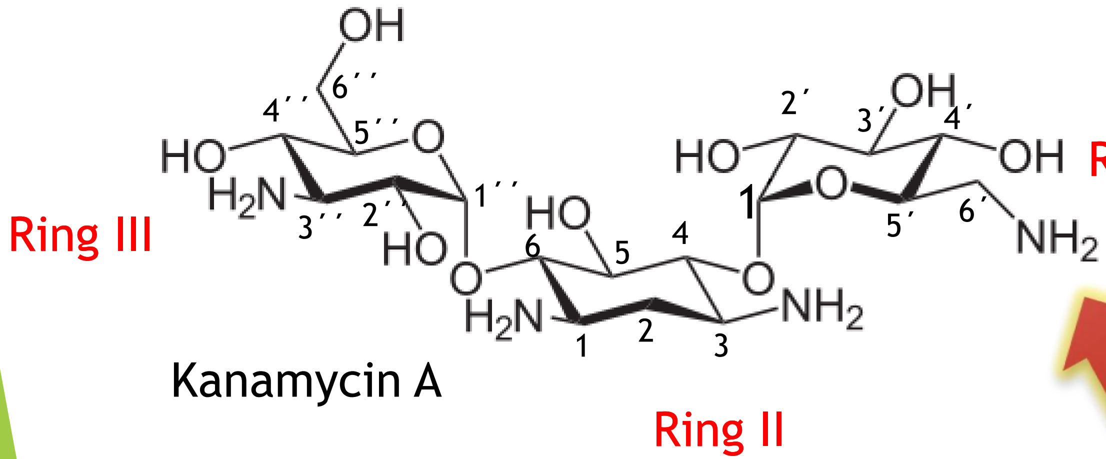
- The important structural characteristics responsible for interaction of aminoglycosides with rRNA of 30S subunit include:

1. The substituent at position 6′ of ring I,
2. The number of protonated amino groups of ring I
3. The linkage between the sugar rings and the central deoxystreptamine moiety (ring II)

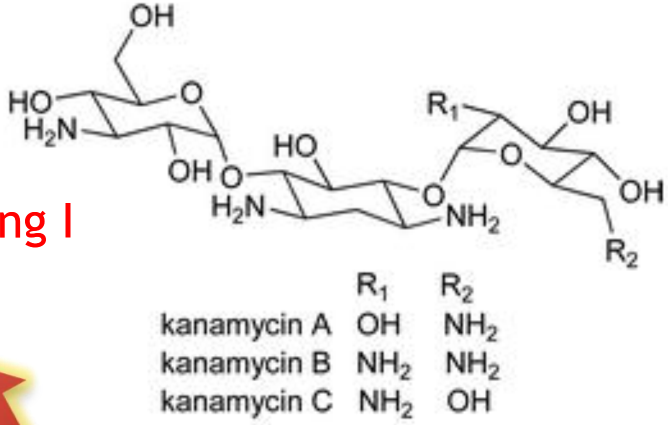
SAR for aminoglycosides (Ring I)

Ring I: is crucially important for characteristic broad-spectrum antibacterial activity

1. is crucially important for characteristic broad-spectrum antibacterial activity
2. Is the primary site for inactivating enzymes
3. Amino functions at 6′ and 2′ are important for activity: thus kanamycin B (6′-amino, 2′-amino) is more active than kanamycin A (6′-amino, 2′-hydroxyl) which is more active than kanamycin C (6′-hydroxyl, 2′-amino)



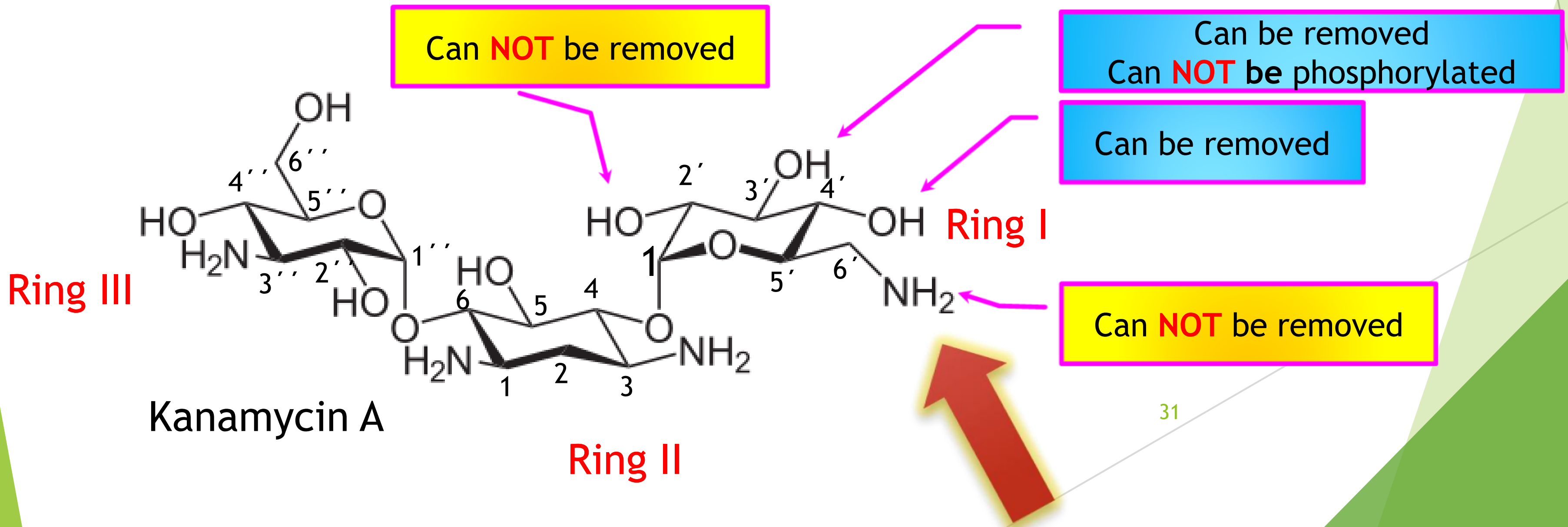
Kanamycin A



	R₁	R₂
kanamycin A	OH	NH₂
kanamycin B	NH₂	NH₂
kanamycin C	NH₂	OH

SAR for aminoglycosides (Ring I) cont.

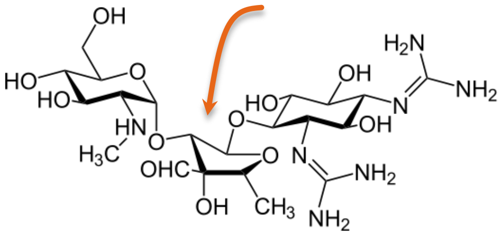
4. Methylation at either 6′-carbon or 6′-amino confers resistance to enzymatic acetylation and NOT lower antibacterial activity (e.g. gentamycin C1)
5. Removal of 3′-hydroxyl or 4′-hydroxyl or both in kanamycins (e.g. 3′,4′-dideoxykanamycin B) or getamicins (e.g. netilmicin) confers resistance to phosphorylation and does NOT reduce antibacterial activity
6. Phosphorylation of 3′-hydroxyl reduces binding to 30S subunit



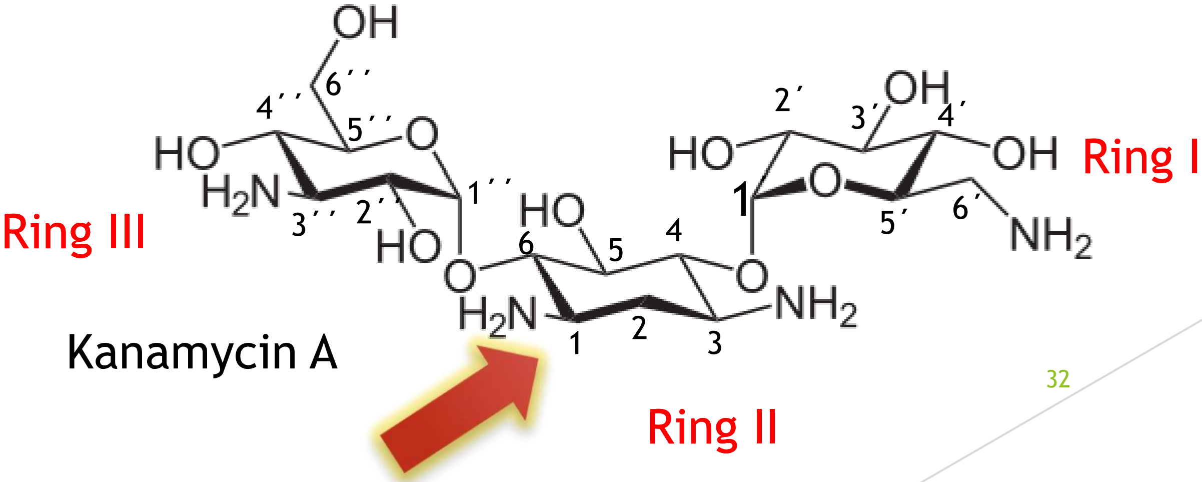
SAR for aminoglycosides (Ring II)

Ring II (deoxystreptamine ring) : Few modifications for functional groups are possible without loss of activity for example:

1. Acetylation of 1-amino group of kanamycin A to give amikacin)
2. Depending on type of aminoglycoside, the ring can be ribose, streptose (5 membered ring) or streptamine (6 membered ring)



Streptomycin



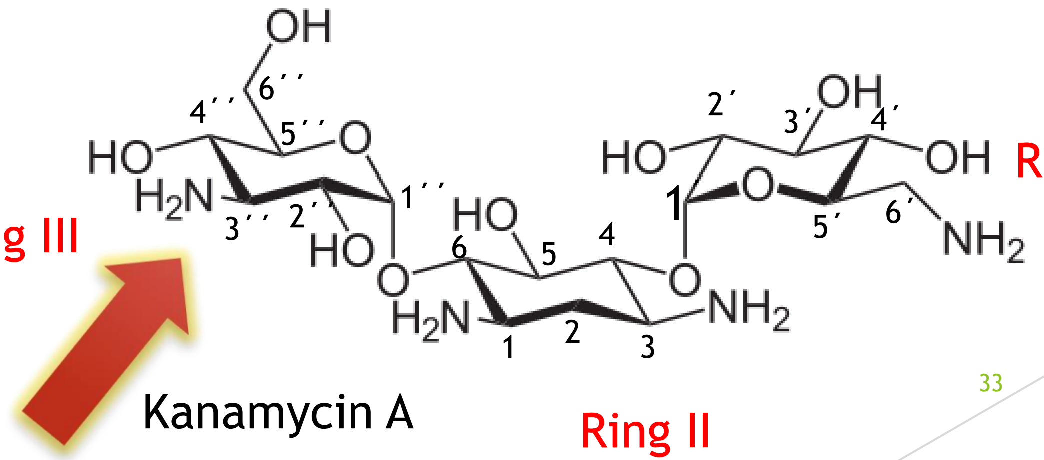
Kanamycin A

SAR for aminoglycosides (Ring III)

- **Ring III: Is less sensitive for changes in functional groups than Ring I and II**

1. The 2″ functional groups activity NH2 > OH > H
2. The 3″ amino group can be 1° or 2° amine which are active
3. The 4″ hydroxyl group can be axial or equatorial

- *Despite improvements in antibacterial potency and spectrum for semisynthetic aminoglycosides, efforts to find agents with improved margins of safety have been disappointing*

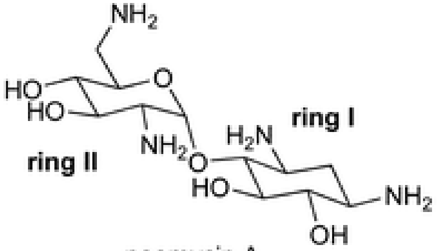


Ring III

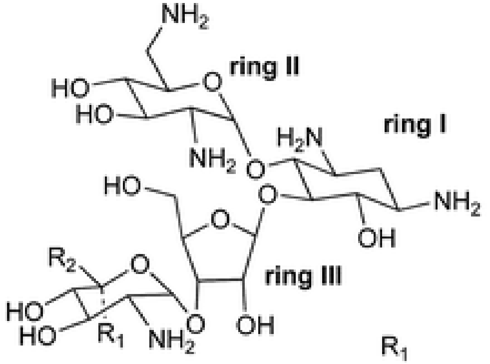
Ring I

Kanamycin A

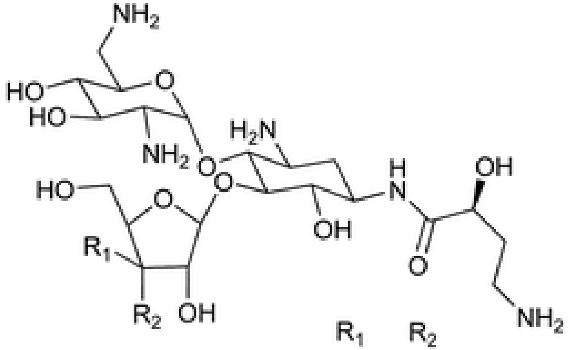
Ring II



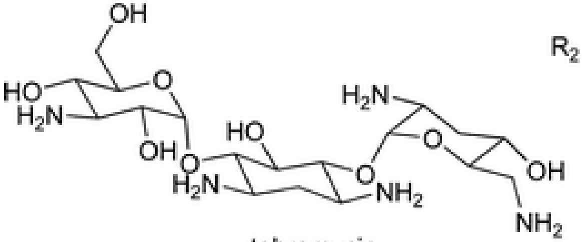
neomycin A
(neamine)



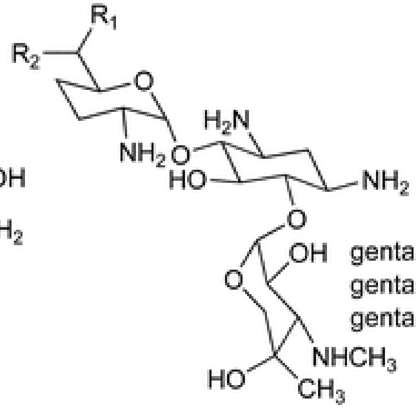
	R_1	R_2
neomycin B	CH_2NH_2	H
neomycin C	H	CH_2NH_2



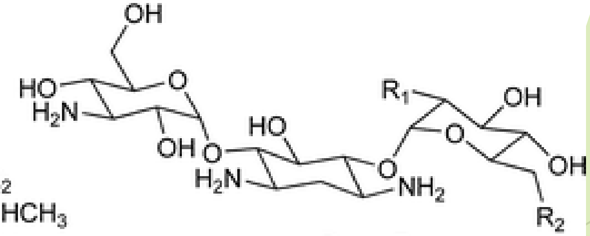
	R_1	R_2
butirosin A	OH	H
butirosin B	H	OH



tobramycin

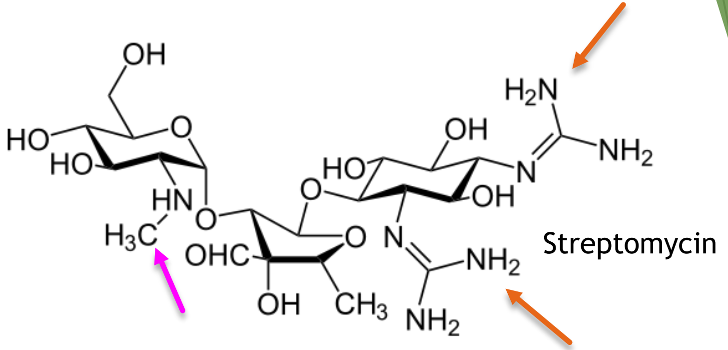


	R_1	R_2
gentamycin C_1	CH_3	$NHCH_3$
gentamycin C_{1A}	H	NH_2
gentamycin C_2	CH_3	NH_2



	R_1	R_2
kanamycin A	OH	NH_2
kanamycin B	NH_2	NH_2
kanamycin C	NH_2	OH

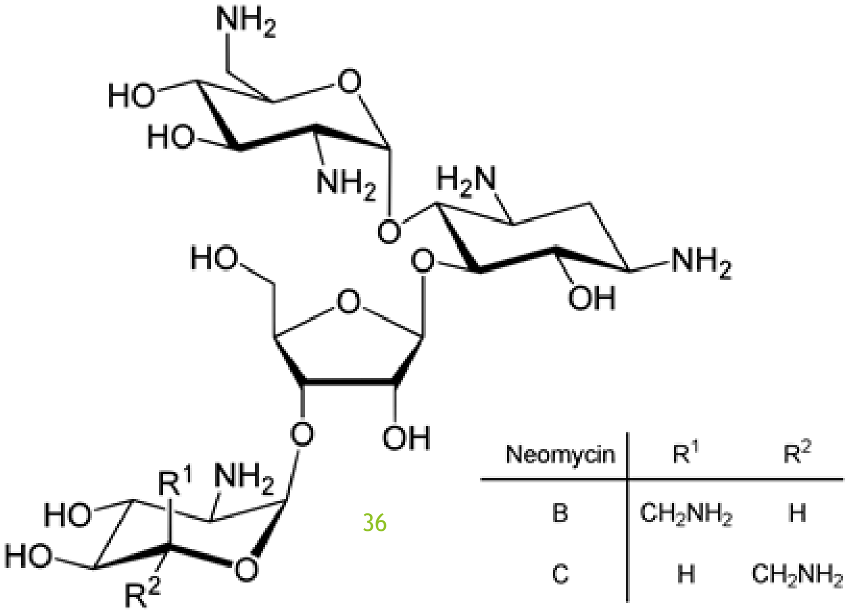
Streptomycin



- Isolated from *Streptomyces griseus* as mixture of streptomycins A, B & C
- Formulated as sulfate salt which is white, odorless, hydroscopic powder. It stable toward light and air but heat sensitive.
- Freely soluble in water and slightly soluble in alcohol and insoluble in organic solvents.
- Hydrolyzed by acids into streptadinine and streptobiosamine
- It has 2 strong **guanido groups** that give acidic character and **1 methylamino** group that gives basic character.
- It resist the N-acetylation at streptadinine
- Active vs Gram-ve and Gram+ve bacteria, and *M. tuberculosis*
- Cause neurotoxicity and occasional nephrotoxicity
- Bacterial resistance is rapidly acquired

Neomycin

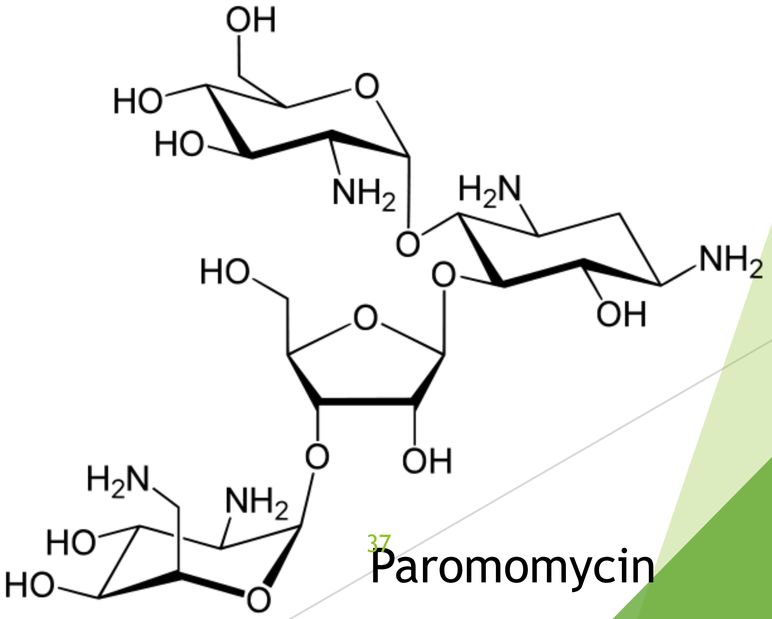
- Isolated from *Sterptomyces fradiae* as mixture of neomycin A (or called neamine) and neomycins B and C
- It is composed of 4 rings
- Discovered during the search for aminoglycoside less toxic than streptomycin.
- Formulated as sulfate salt which is hygroscopic photosensitive powder
- Used for GI, dermal and peritoneal infections
- Broad spectrum as streptomycin
- Less toxic than streptomycin
- Bacterial resistance is rarely acquired



Neomycin	R^1	R^2
B	CH_2NH_2	H
C	H	CH_2NH_2

Paromomycin

- Isloated from *Streptomyces rimosus* as mixture of Paromomycin I and II.
- It is composed of 4 rings
- The structure of paromomycin is similar to that of neomycin except that paromomycin contains D-glucosamine instead of the 6-amino-6-deoxy-D-glucosamine
- It has antibacterial activity similar to that of neomycin and streptomycin.

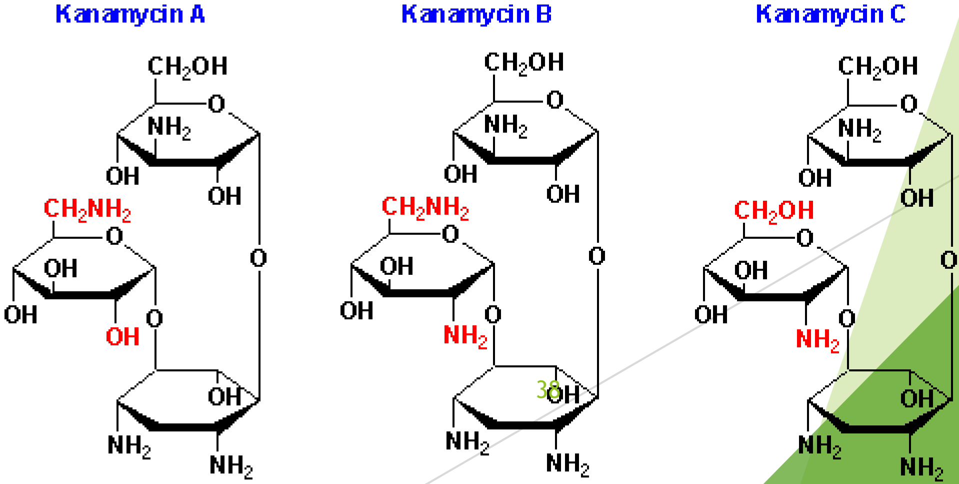


Paromomycin

Kanamycin

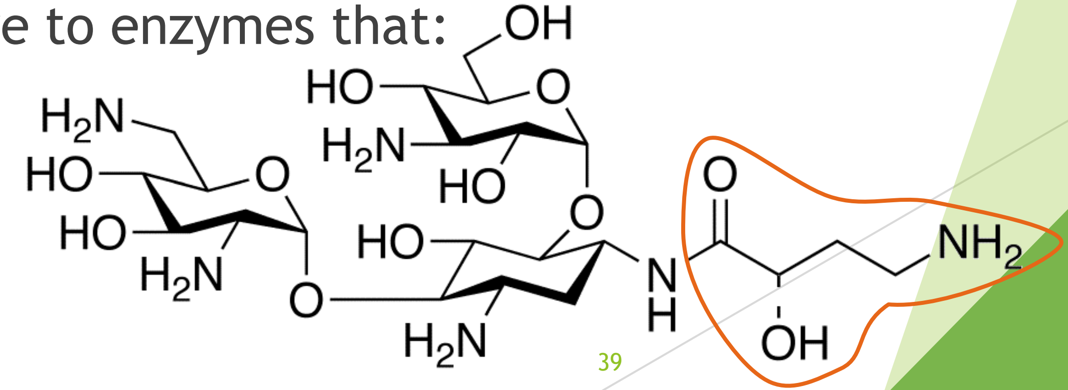
- Kanamycin is isolated from *Streptomyces kanamyceticus* as a mixture of at least 3 components (A,B and C)
- Kanamycin, Gentamicin, Neomycin and Paromomycin are the most chemically stable.
- *O*-phosphorylated on the C-3′ hydroxyl by enzymes APH and *N*-acetylated on the C-6′ amino group to form inactive metabolite
- *P. aeruginosa* and anaerobes are resistant
- Used in combination against Mycobacterium
- The kanamycins do not have the D-ribose molecule that is present in neomycins and paromomycins.

Kanamycins A, B and C are differ only in the sugar moieties attached to the glycosidic oxygen on the 4-position of the central deoxystreptamine



Amikacin

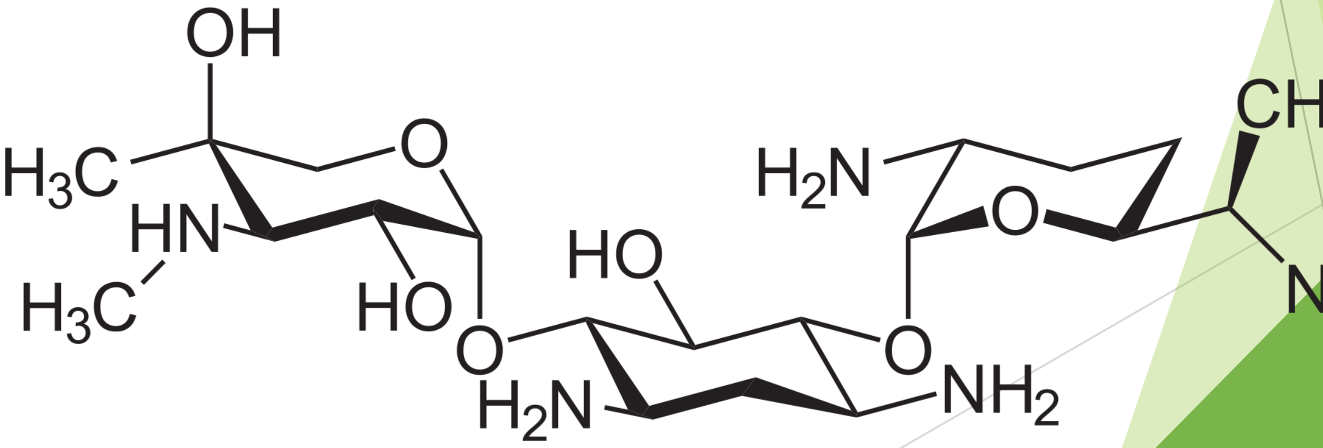
- Amikacin (or 1-*N*-amino-α-hydroxybutyrylkanamycin) is semisynthetic analogue of kanamycin prepared by acetylation of 1-amino.
- The acylamino substitution (L-HABA) retains about 50% of the original acivity of Kanamycin A against Gram-ve bacilli.
- Amikacin with L-HABA gives is more active than that with D-HABA.
- Amikacin resist most of the aminoglycoside inactivating enzymes that affect gentamicin and tobramycin
- Amikacin is ONLY sensitive to enzymes that:
  1. Acetylate 6′-NH2
  2. Phosphorylate 3′-OH
  3. Adenylate 4′-OH



Amikacin

Gentamicin

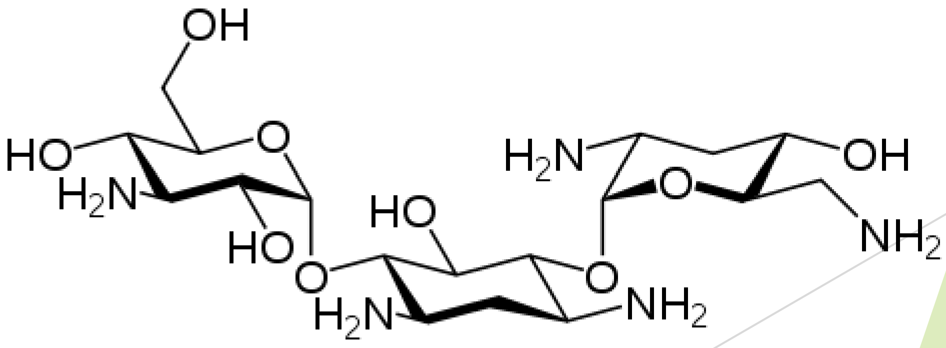
- Isolated from Micromonospora purpurea as Gentamycins C_1, C_2 and C_{1a}
- Active against Gram+ve and Gram-ve as well as *P. aeruginosa*.
- Used topically and internally
- Gentamycin C_1 has several properties render it stable toward inactivating enzymes (discussed previously)



Gentamicin C_2

Tobramycin

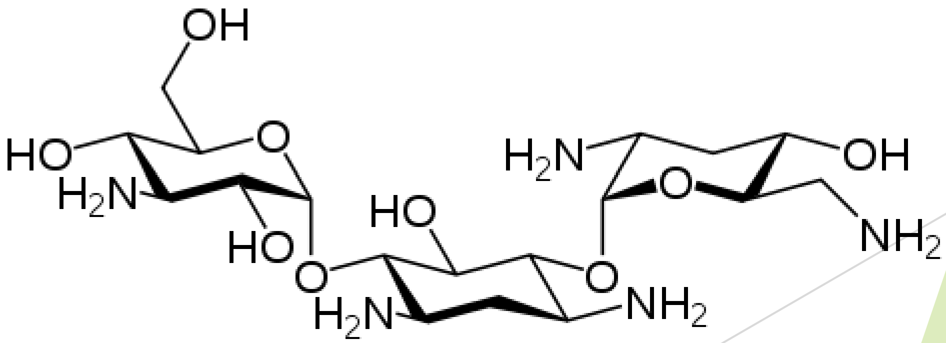
- Isolated from *Streptomyces tenebrarius* as three forms of tobramycins.
- Is the most active chemically-related aminoglycoside
- The most important feature is its high activity (2X or 4X) against
  P. aeruginosa compared to gentamicin



Tobramycin

Netilmicin

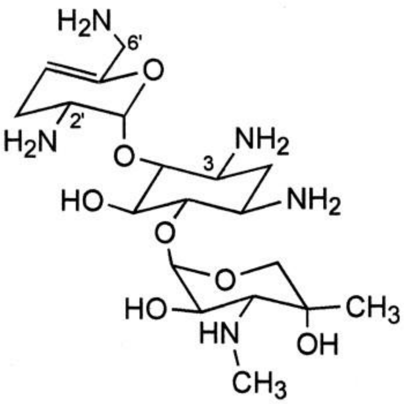
- Is semisynthetic derivative of sisomicin.
- Is the most active chemically-related aminoglycoside
- The most important feature is its high activity (2X or 4X) against *P. aeruginosa* compared to gentamicin



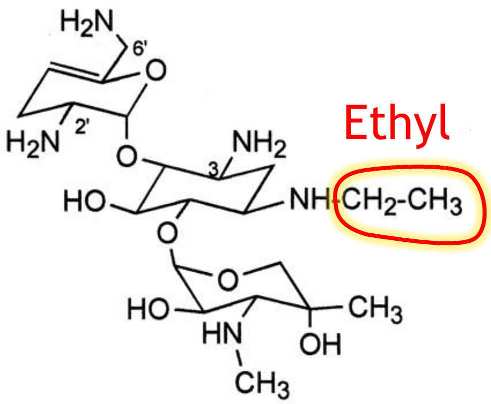
Tobramycin

Sisomicin and Netilmicin

- Sisomicin is isolated from *Micromonospora inyoensis*, while netilmicin is semisynthetic derivative of sisomicin.
- Both are structurally similar to gentamicin C_{1a}.
- Both have similar gentamicin in antibacterial spectrum and potency.
- Netilmicin is better than sisomicin in activity against some gentamicin-resistant strains, which may be attributed to netilmicin stability toward most inactivating enzymes (**except N-acetyltransferases**).
- The stability of netilmicin toward inactivating enzymes is due to 1-ethyl group (which act in a manner similar to L-HABA of amikacin)

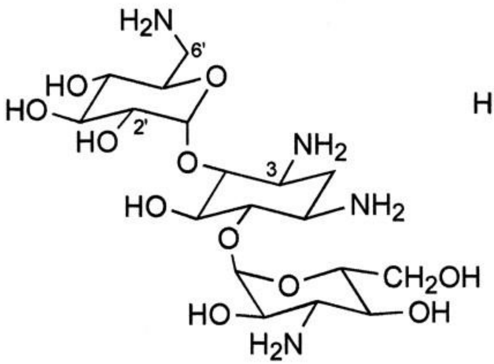


Sisomicin

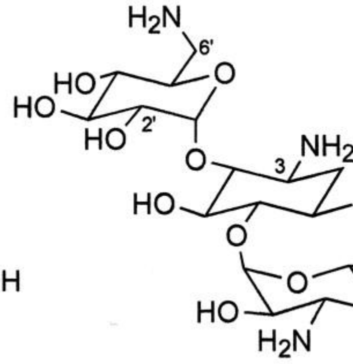


Ethyl

Netilmicin



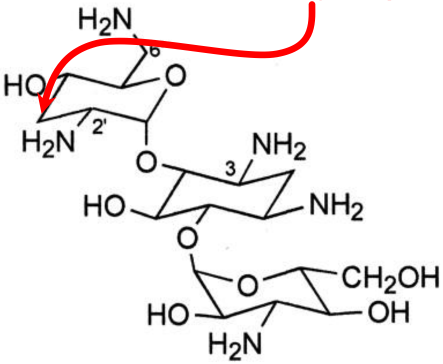
Kanamycin A



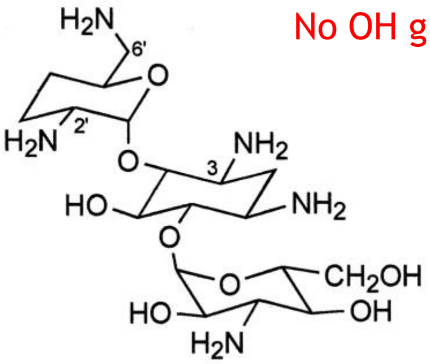
Amikacin

2° amine

No OH group

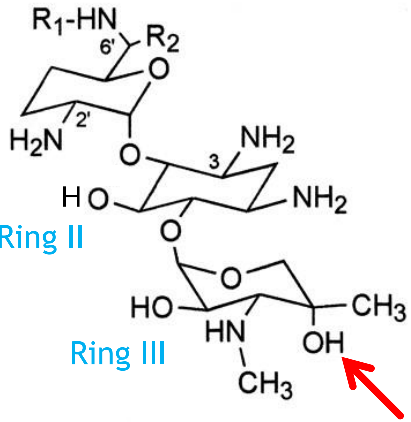


Tobramycin



Dibekacin

No OH group



Ring I

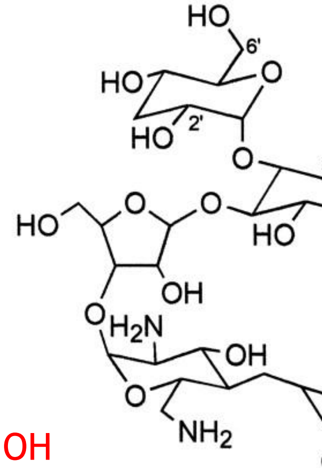
Ring II

Ring III

Axial OH

Gentamicin Complex

C_1: $R_1 = R_2 = CH_3$
C_{1a}: $R_1 = R_2 = H$
C_2: $R_1 = H, R_2 = CH_3$

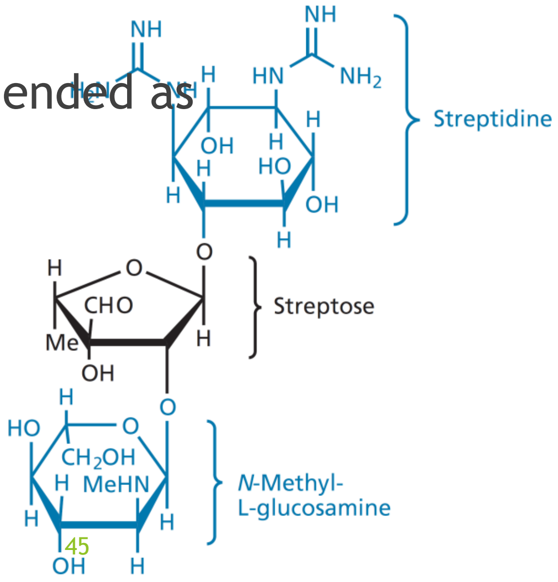


Lividomyc

Aminoglycosides

1. Streptomycin

- Isolated from Soil bacteria *Streptomyces griseus* in 1944.
- Was the first effective agent to treat tuberculosis, before using combination due to resitance development (Streptomycin + Isoniazid + *p*-aminosalicylic acid) unit 1970s
- Used for enterococcal endocarditis and plus doxycycline for TB
- Activity against Gram-ve > Gram+ve
- Resistance rapidly developed, therefore not recommended as monotherapy
- Not absorbed orally and acid-sensitive



Streptomycin (from *Streptomyces griseus*)

Aminoglycosides

1. Gentamycins

- Isolated from Gram+ve bacteria (*Micromonospora*)
- Activity Gram-ve > Gram+ve (active against *P. aeruginosa*)
- Used as IM or slow IV for septicemia, neonatal sepsis and meningitis, endocarditis, or topicaly as eye and ear drops.
- Poor oral bioavilability
- Systematic