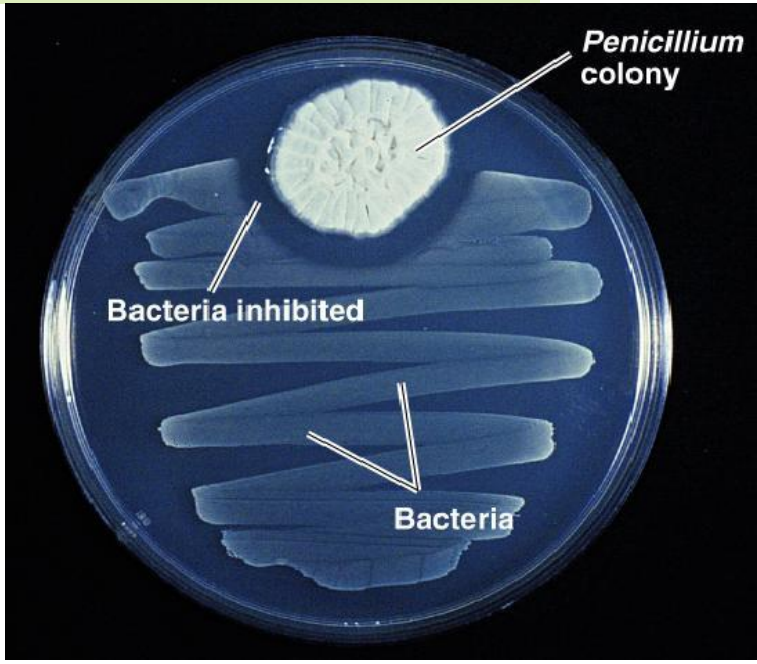
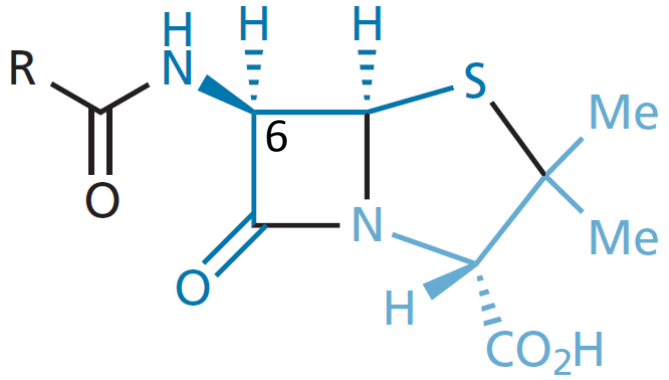
B-lactam antibiotics

PENICILLINS

Brief history

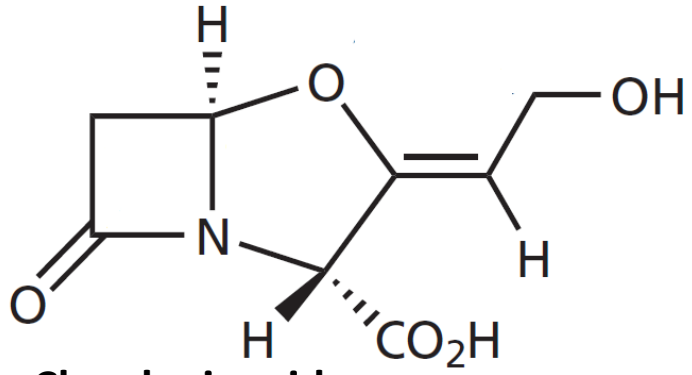
- **1928**: Alexander Fleming noted that growth of bacterial colonies is inhibited by co-existence of fungal colonies. Fleming concluded that the material produced by the fungus is not worth to be used clinically because it is difficult to be isolated.
- **1930**: Florey et al did isolate a compound by freeze drying from the fungus and named it penicillin that has antibiotic effect.
- **1945**: D. Hodgkins illustrated the chemical structure of penicillin and gave the excuse for Fleming's failure in isolating penicillin (Why ?).
- **1957**: Sheehan develops synthetic route for production of penicillin
- **1958**: Beechams isolates 6-aminopenicillanic acid (6-APA) to used as intermediate for semi-synthetic pencillin derivatives



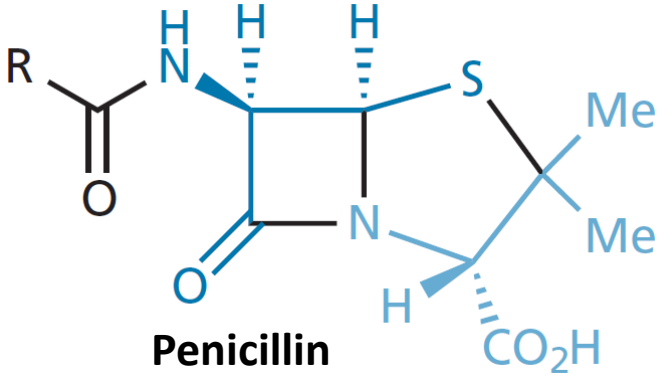


Brief history

- The careless use of penicillin led to the immergence of bacterial resistance. In **1976**, Beechams isolates natural product called clavulanic acid that is effective in preventing enzymatic digestion of pencillin in resistant-strains of bacteria.

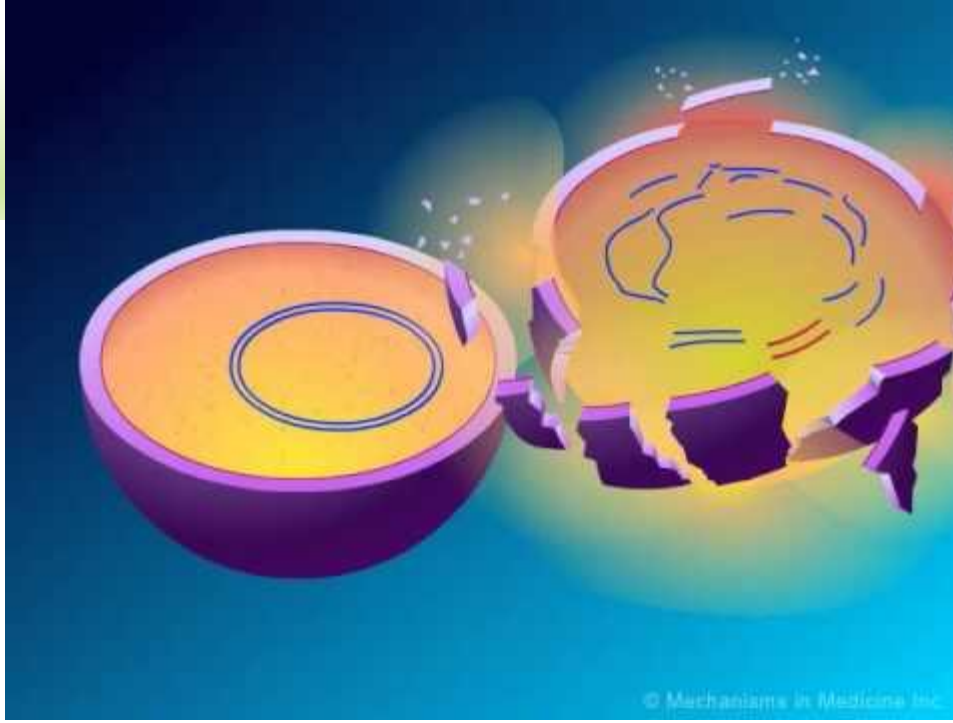


Clavulanic acid

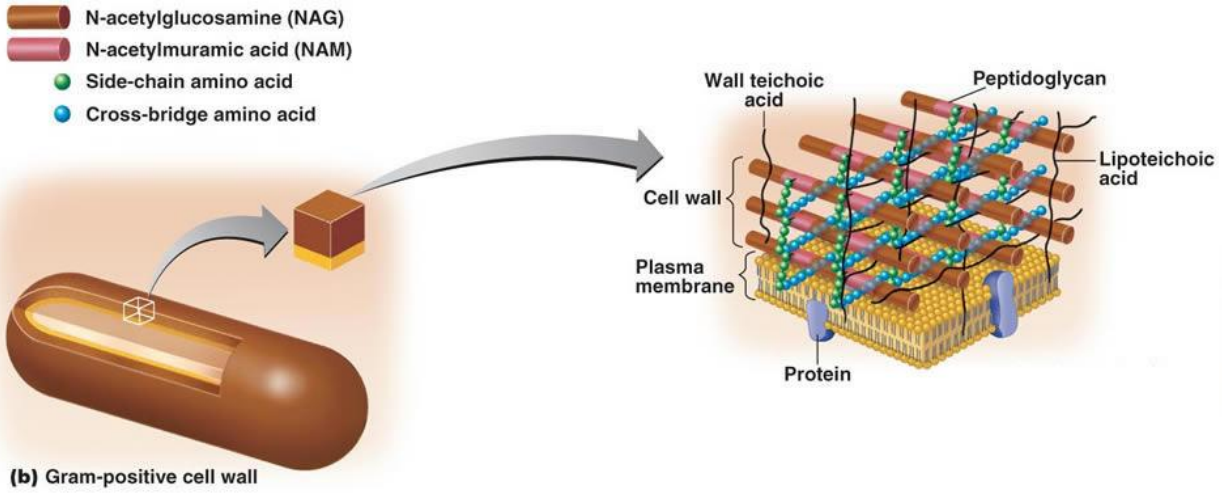


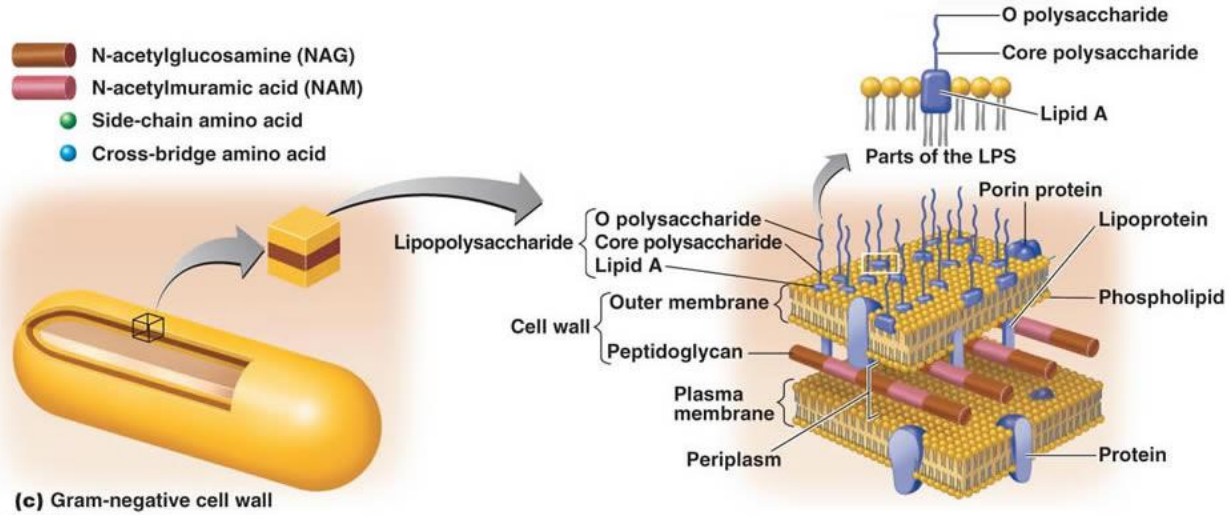
Penicillin

Bacterial cell-wall

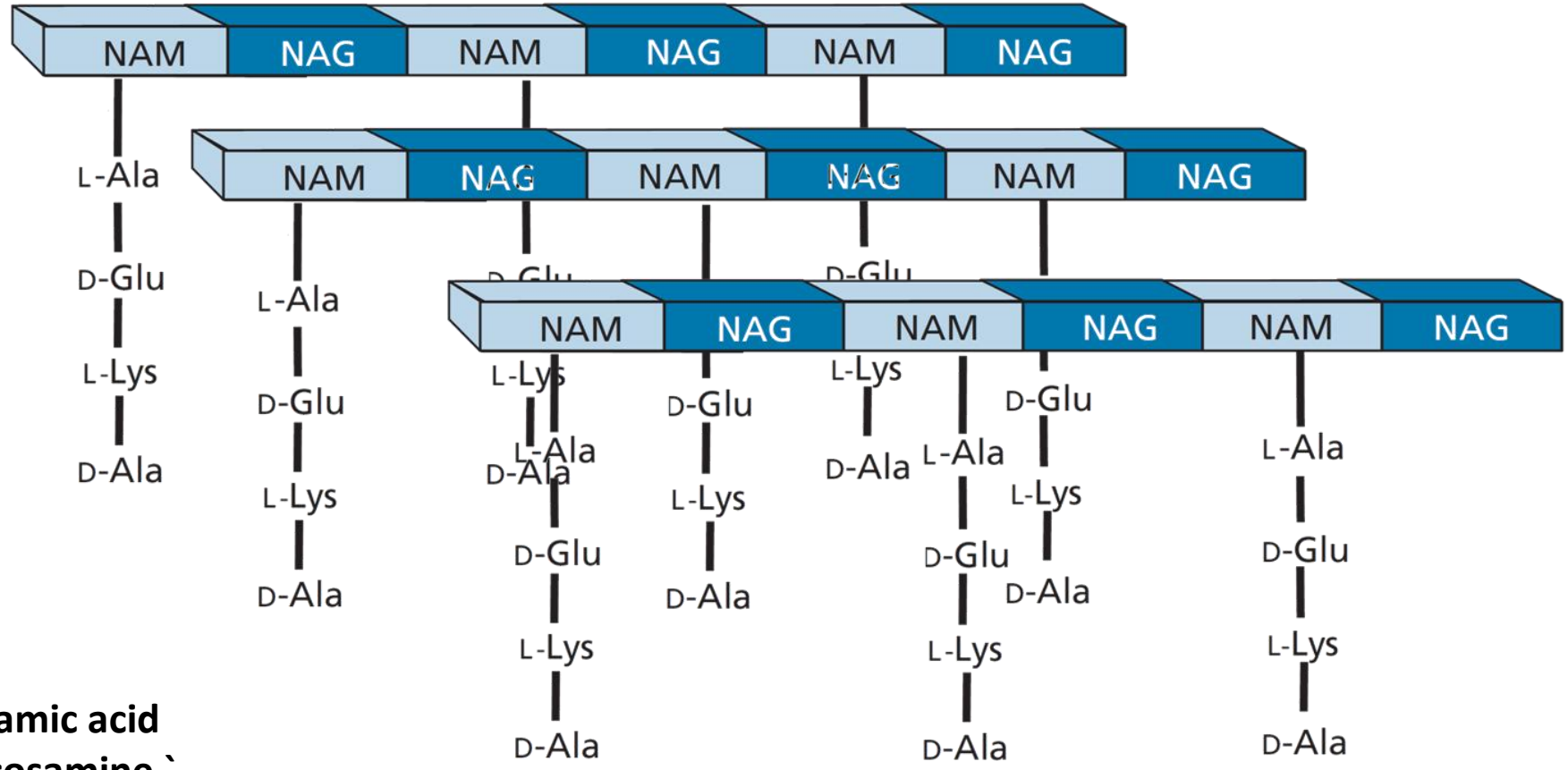


- Bacteria have cell walls in order to survive a large range of environmental conditions, such as varying pH, temperature, and osmotic pressure.
- Human and animal cells have no cell wall, which makes it perfect target for internally-used antibiotics.
- The structure of the wall consists of a parallel series of sugar backbones containing two types of sugar [*N* **-acetylmuramic acid** (**NAM**) and *N* **– acetylglucosamine** (**NAG**)]





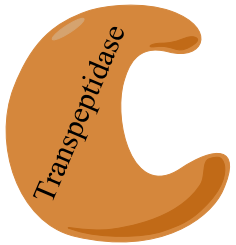
Cell-wall cross-linking

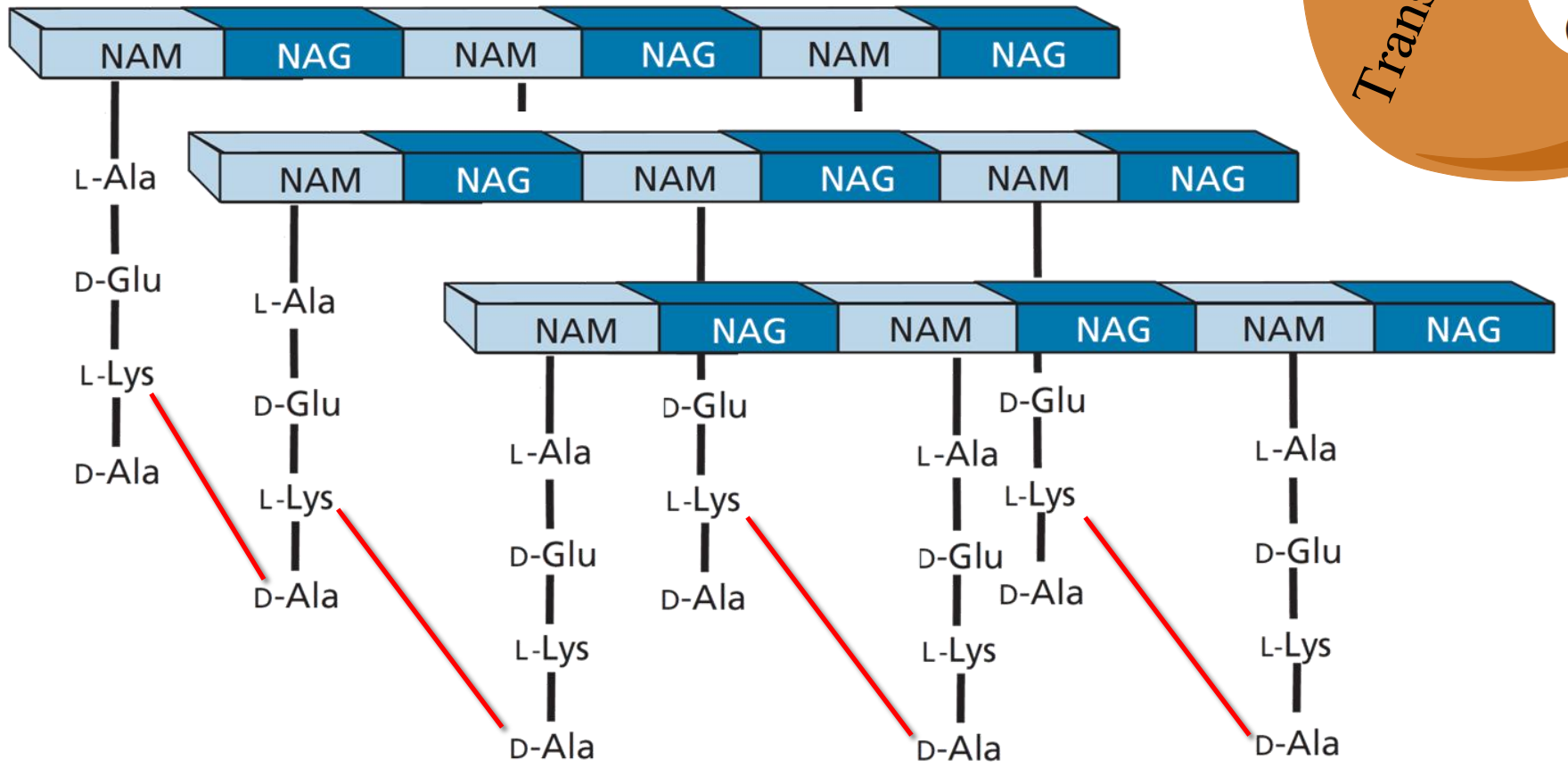


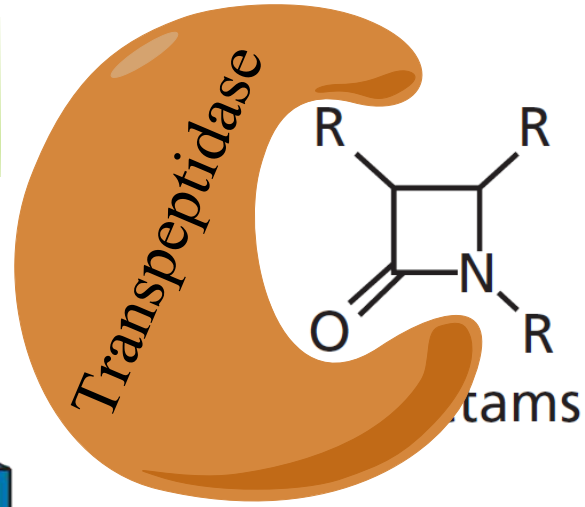
NAM *N* **-acetylmuramic acid**

NAG *N* **– acetylglucosamine `**

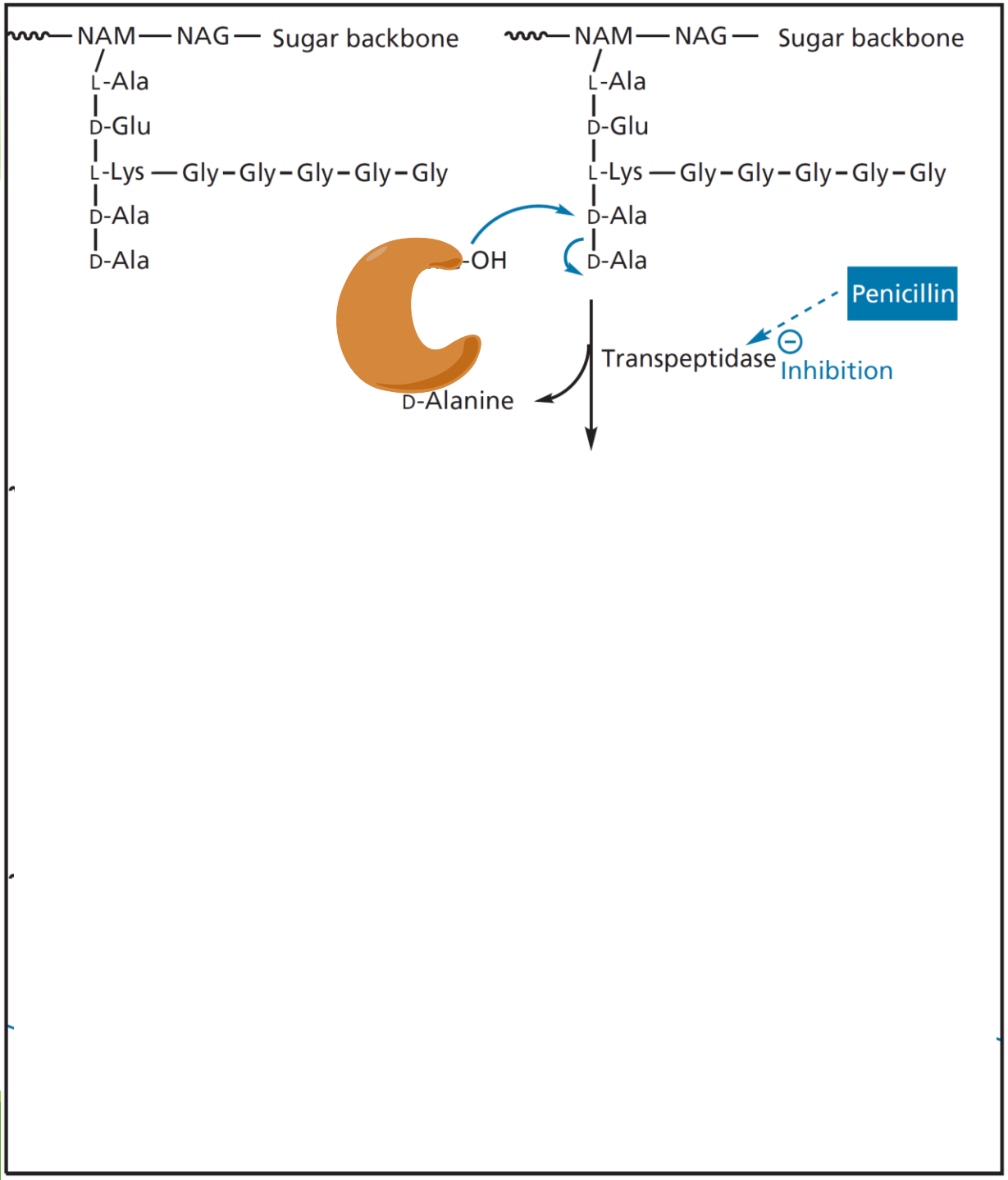
Cell-wall cross linking (cont.)





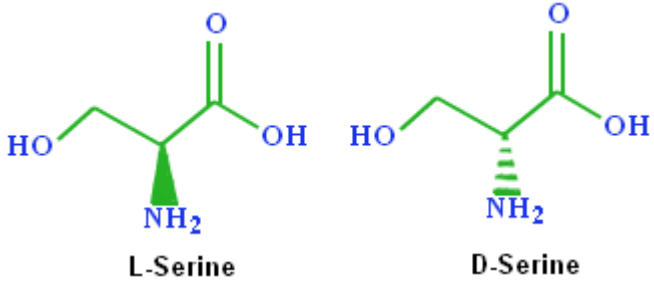


Cell-wall cross linking (cont.)

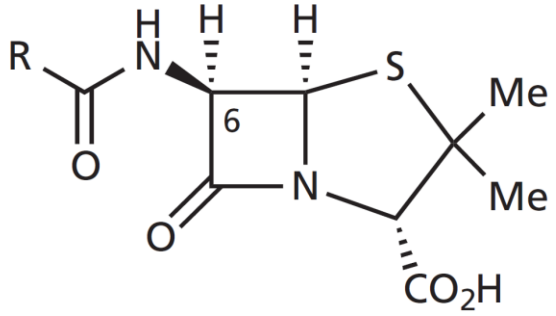


Bacterial cell-wall (cont.)

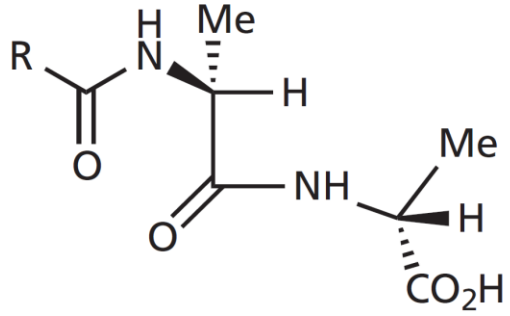
- Bacteria transpeptidase recognizes D-amino acids, while human transpeptidase recognizes only L-amino acids.



- Penicillin is selectively inhibit bacteria transpeptidase since it mimic D-Ala-D-Ala segment of cell-wall peptidoglycan.
- Each type of bacteria has different structure of transpeptidase, therefore being inhibited by penicillin differently.
- 6-methylpenicillin is **inactive** although being very similar to D-Ala-D-Ala



Penicillin



Acyl-D-Ala-D-Ala

FIGURE 19.17 Comparison of penicillin, 6-substituted penicillins, and acyl-D-Ala-D-Ala.

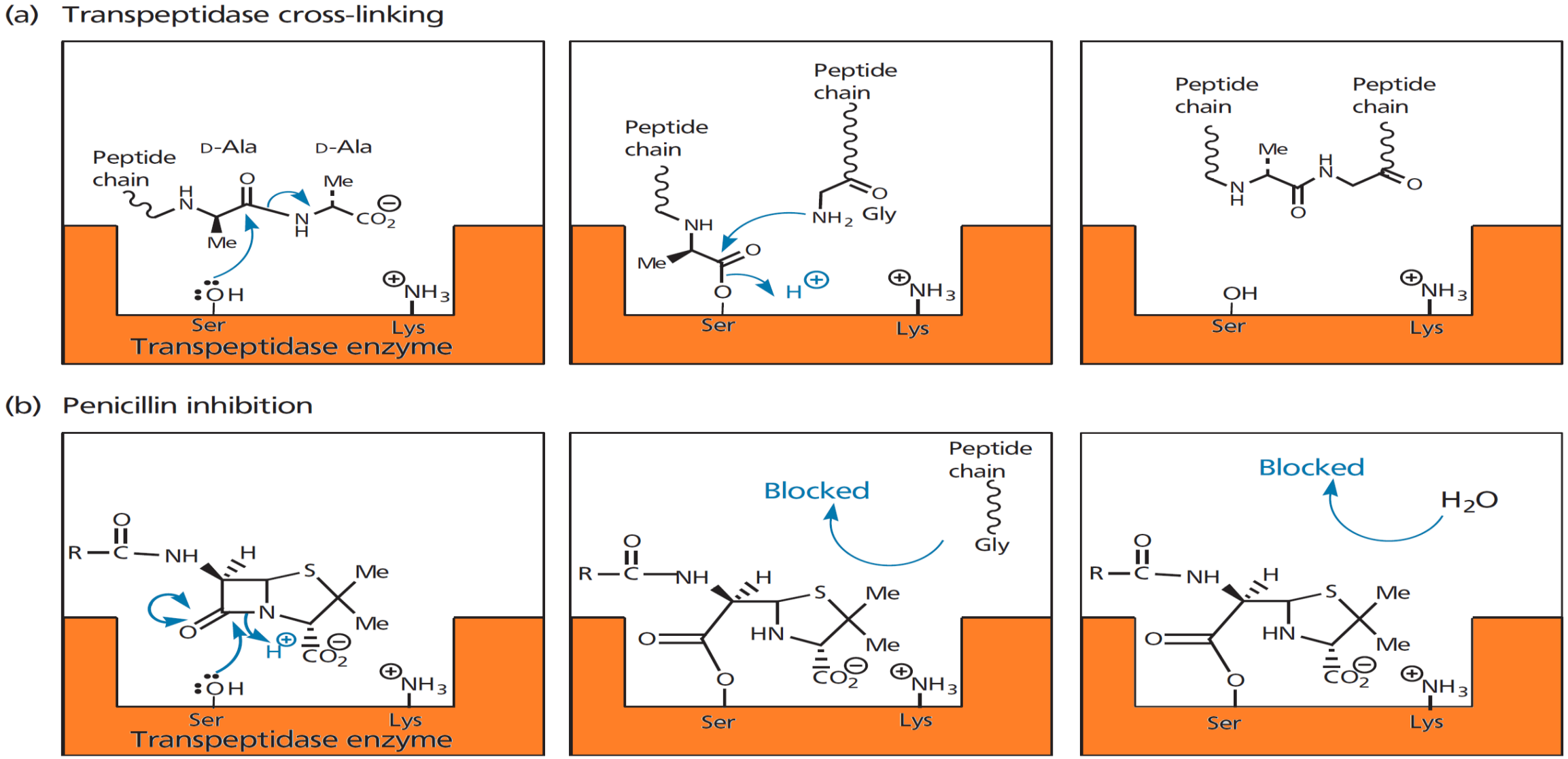
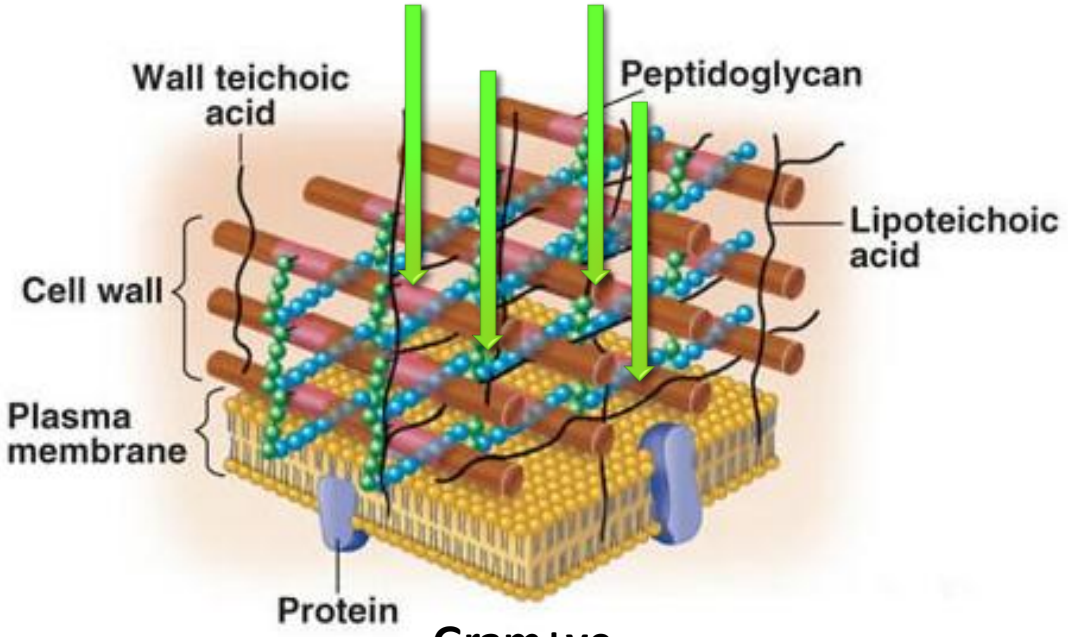


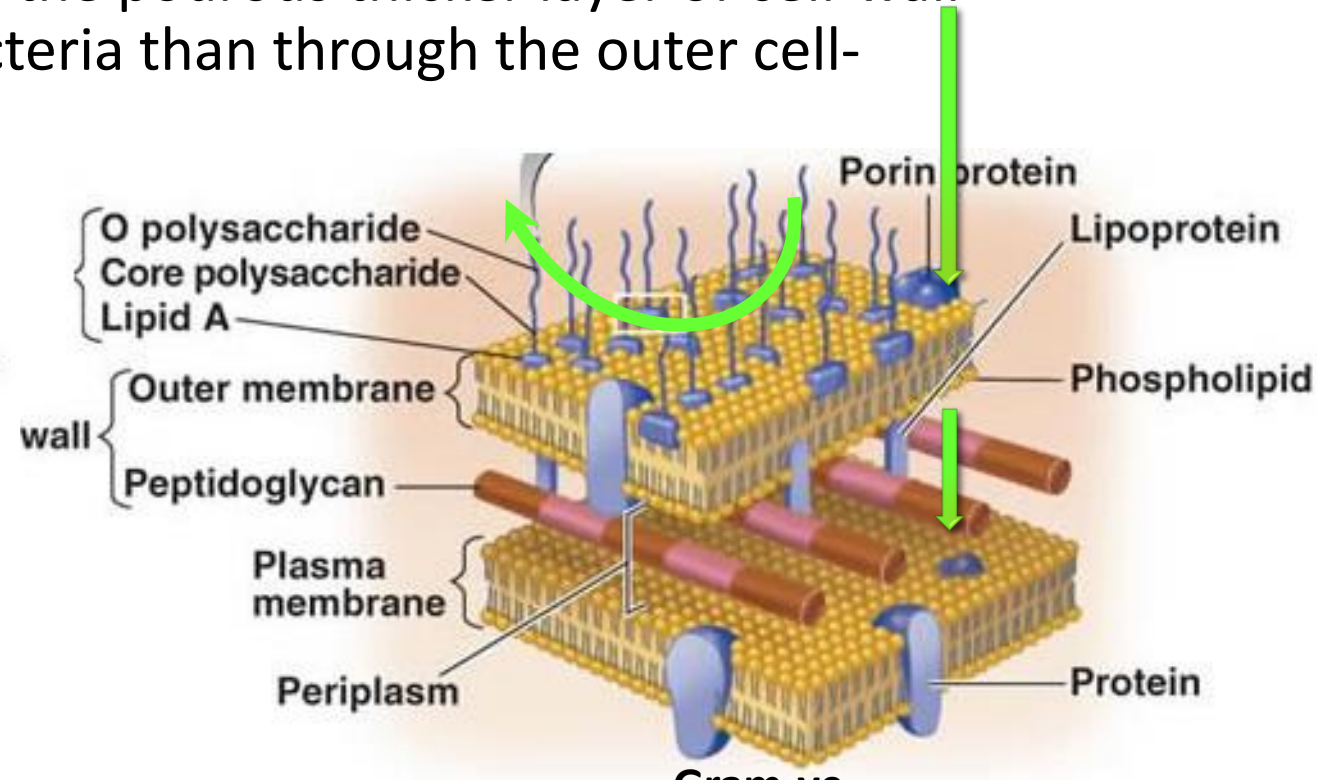
FIGURE 19.16 Mechanisms of transpeptidase cross-linking and penicillin inhibition.

Bacterial cell-wall (cont.)

- Bacterial cell-wall (peptidoglycan) is more porous than the cell-membrane (lipopolysaccharides).
- Penicillin penetrates easily through the pourous thicker layer of cell-wall (peptidoglycan layer) in Gram+ve bacteria than through the outer cell-membtane of Gram-ve bacteria.



Gram+ve



Gram-ve

Bacterial cell-wall (cont.)

Factors affect drug penetration through porins of Gram-ve bacteria include:

1) type of porin
2) Characteristic of penicillin (size, structure and charge)

Not favored molecules: are large, hydrophobic and negatively charged

Favored molecules: are small, hydrophilic and exist as zwitterion.

Resistance due to β-lactamases

- β-lactamases are enzymes which are similar to transpeptidases in having the ability to bind β-lactams but are able to release the opened β-lactams by hydrolyzing the ester bond.
- Some β-lactamases are specific to penicillin (penicillinases), cephalosporins (cephalosporinases), or to both.
- β-lactamase is continuously produce in Gram+ve bacteria, while it is stored in periplasmic space in Gram-ve, therefore, the latter is more resistant

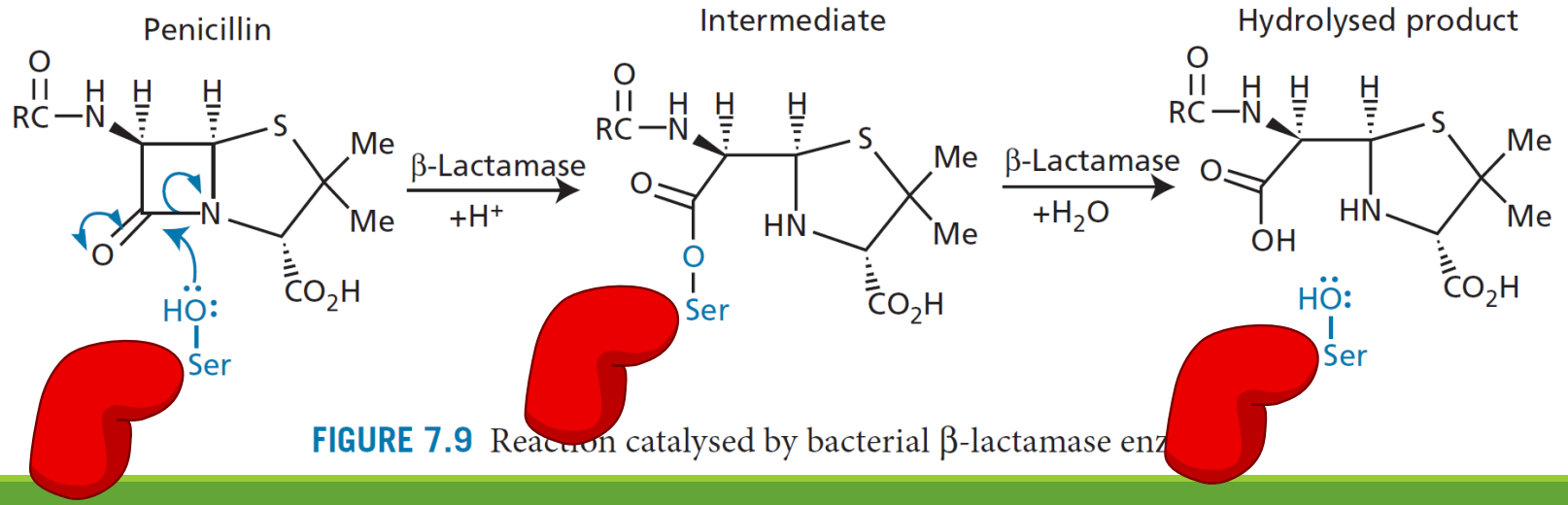


FIGURE 7.9 Reaction catalysed by bacterial β-lactamase enz

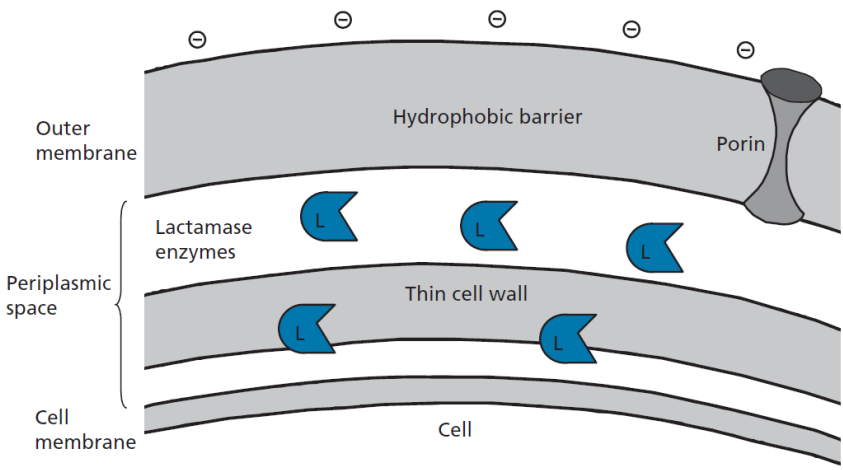
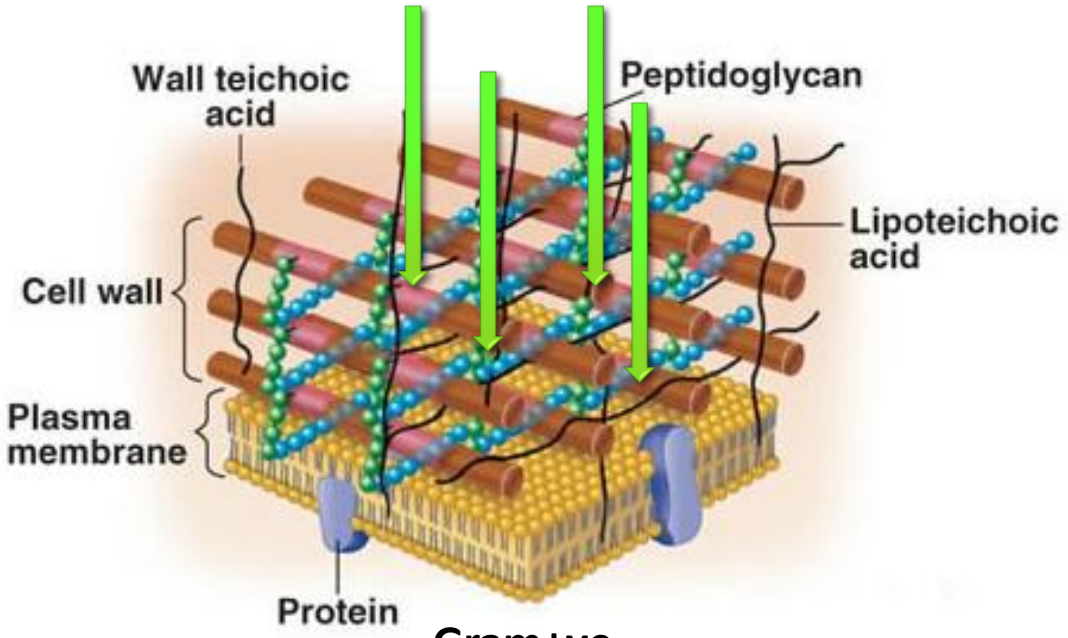


FIGURE 19.18 Outer surface of a Gram-negative bacterial cell.

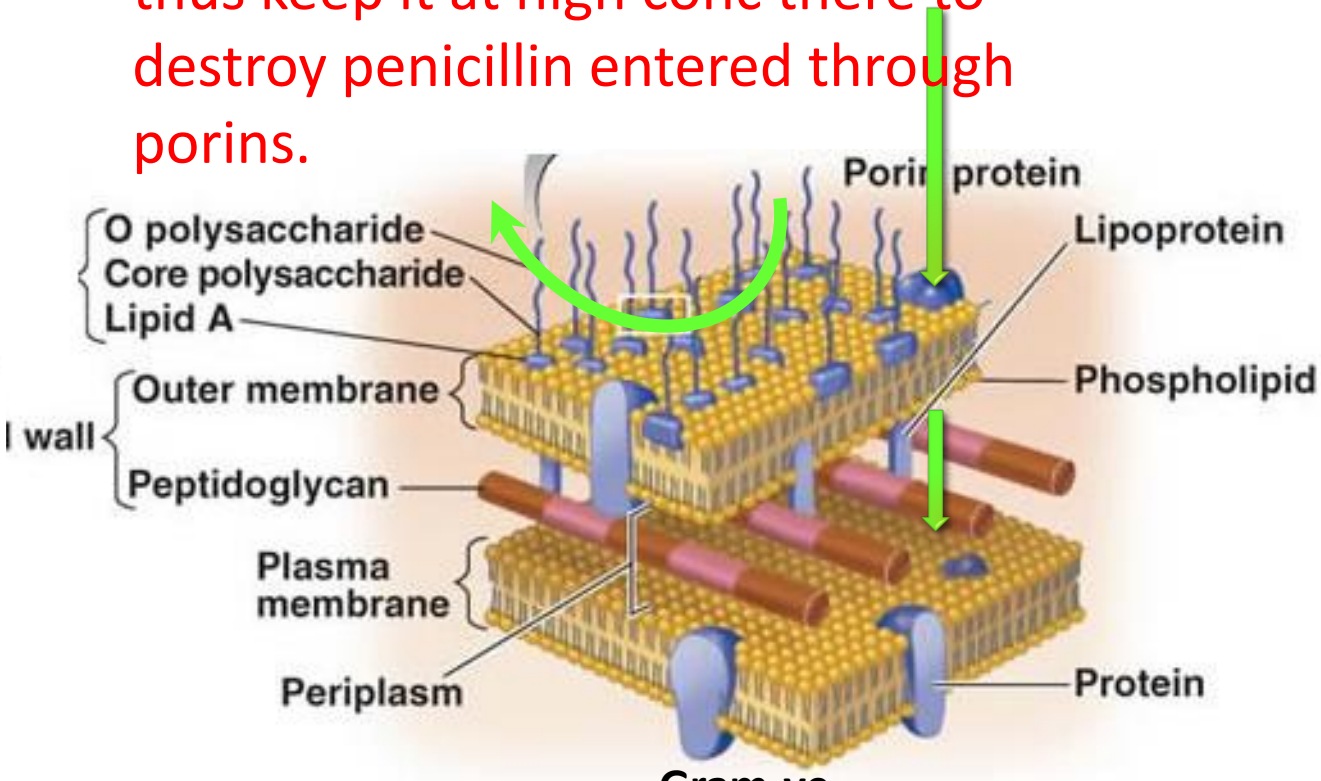
Resistance due to β-lactamases (cont.)

Some Gram+ve bacteria release β-lactamase to environment to destroy penicillin before coming close to cell wall (e.g. Staph aureus)



Gram+ve

Some Gram-ve bacteria release β-lactamase at the periplasmic space, thus keep it at high conc there to destroy penicillin entered through porins.



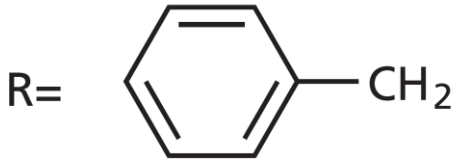
Gram-ve

Biosynthesis of penicillin

- it is synthesized within the penicillium by fusing two amino acids (L-cysteine and L-valine).

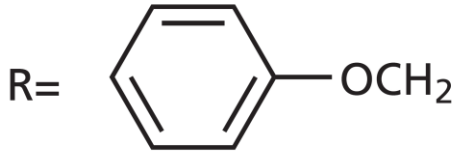
The acyl side chain (R) varies, depending on the components of the fermentation medium.

Corn steep liquor (contains high levels of phenylacetic acid ($PhCH_2CO_2H$)



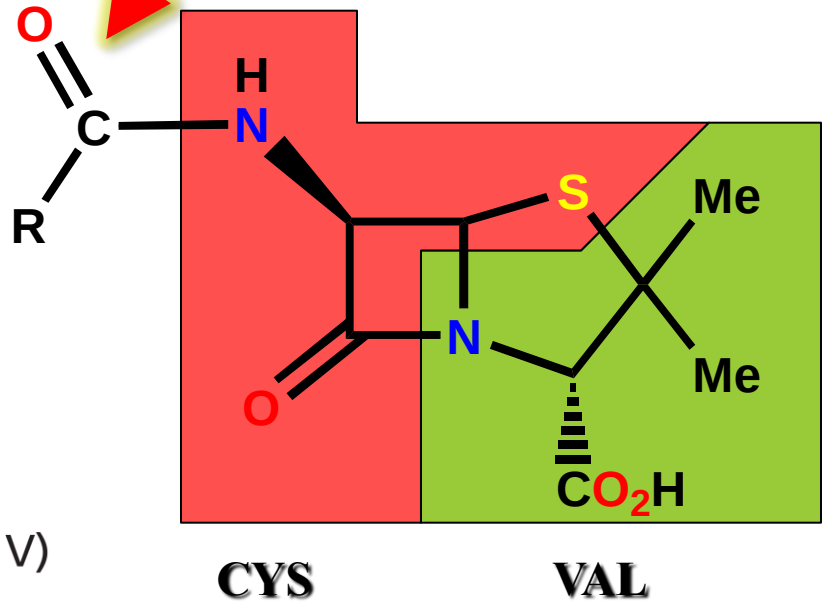
Benzylpenicillin (penicillin G)

Addition of phenoxyacetic acid ($PhOCH_2CO_2H$) to fermentation medium



Phenoxymethylpenicillin (penicillin V)

This group is variable according to compounds present in fermentation medium



Synthesis of penicillin analogues

1) **Fermentation:**
   - Addition of different carboxylic acids to fermentation medium to produce penicillins with different acyl side chains
   - Only suitable for unbranched carboxylic acids
   - Tedious and time-consuming

2) **Complete synthesis:**
   Long processes and low yielding (1%)

3) **Semi-synthesis:**
   - Use carboxylic acid deficient fermentation medium to generate 6-aminopenicillanic acid.
   - 6-APA is (very weak antibiotic) and reacted with different acyl chlorides to synthesize penicillin analogues

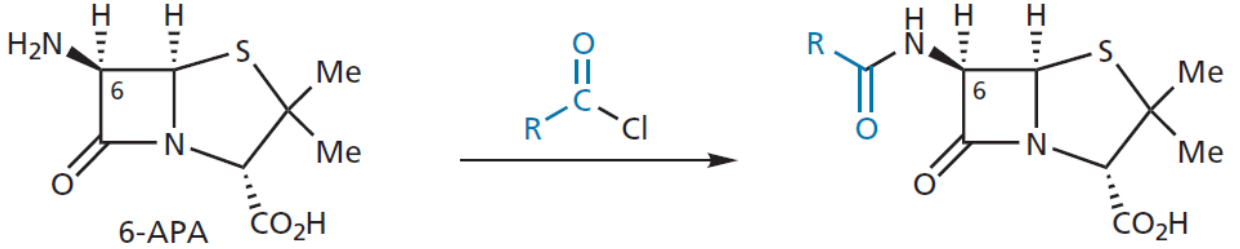


FIGURE 19.20 Penicillin analogues synthesized by acylating 6-APA.

Synthesis of penicillin analogues (cont.)

The intermediate of 6-APA can also be obtained by hydrolysis of penicillin G (or penicillin V) either by

- ✓ The enzyme penicillin acylase
- ✓ Or by some mild chemical methods that not affect the integrity of β-lactam ring

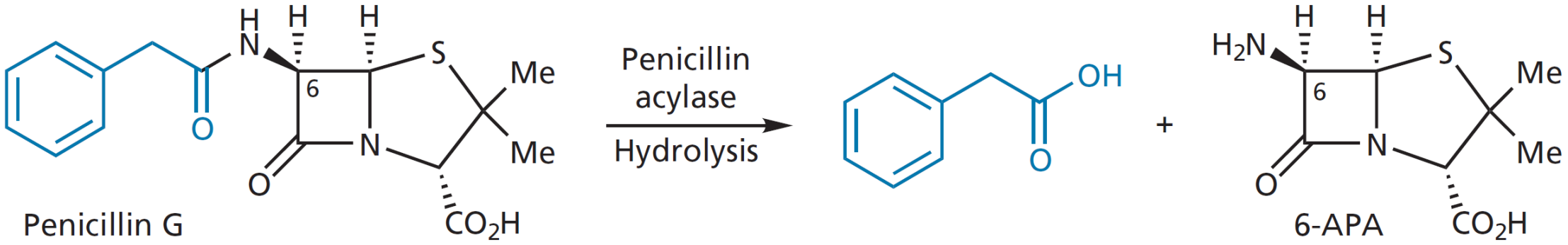
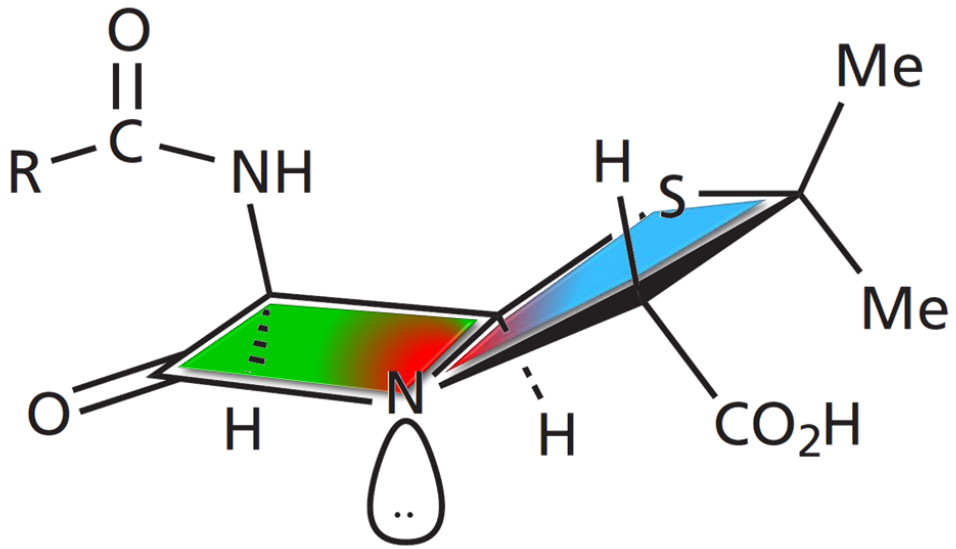
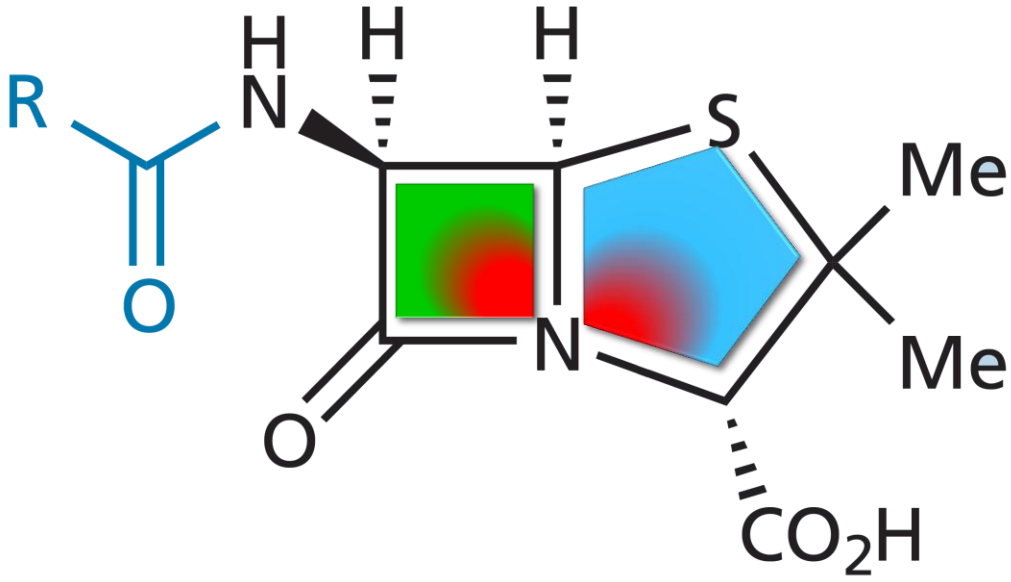


FIGURE 19.21 Synthesis of 6-APA from penicillin G.

Structural properties of penicillin

- Peniclillin contains highly unstable bicyclic system (**4-membered β-lactam** fused to **5-membered thiazolidine**)
- Has three chiral carbon atoms (C-3, C-5, and C-6)





Problem of acid-sensitivity for penicillin

The main deterioration of penicillin is the reactivity of the strained β-lactam ring to hydrolysis. The hydrolysis is effected by pH. There are three main reasons for acid sensitivity of penicillin G:

1) **Ring strain** : due to the fusion of β-lactam ring to thiazolidine ring. The strain is relieved by breaking the β-lactam ring which either started by nucleophilic attack at carbonyl using water (or OH- ions) or started with protonation of N which eventually lead to formation of penicilloic acid

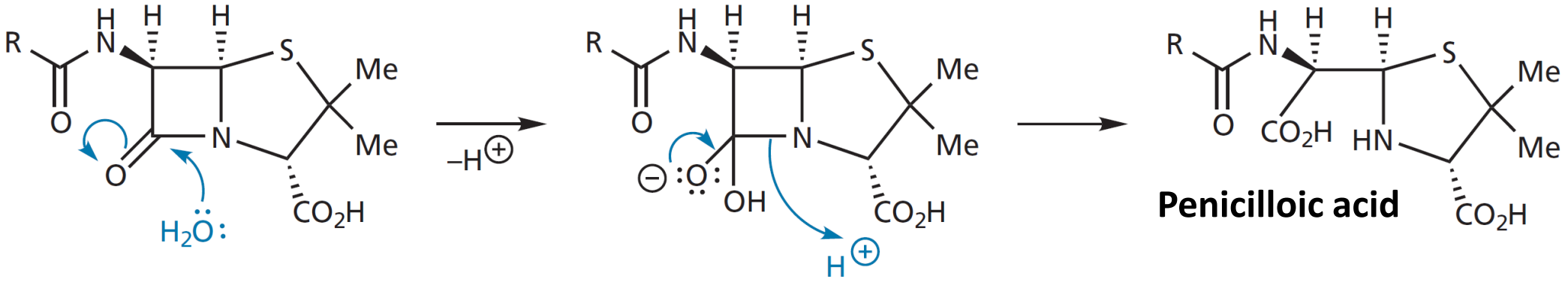


FIGURE 19.23 Ring-opening of the β-lactam ring under acidic conditions.

Problem of acid-sensitivity for penicillin (cont.)

There are three main reasons for acid sensitivity of penicillin G:

2) **Highly reactive β-lactam carbonyl group:** the group (=O) is deprived from electrons (electrophile) due to inability to form resonance with neighboring N because of unusual geometry (90° instead of 120°)

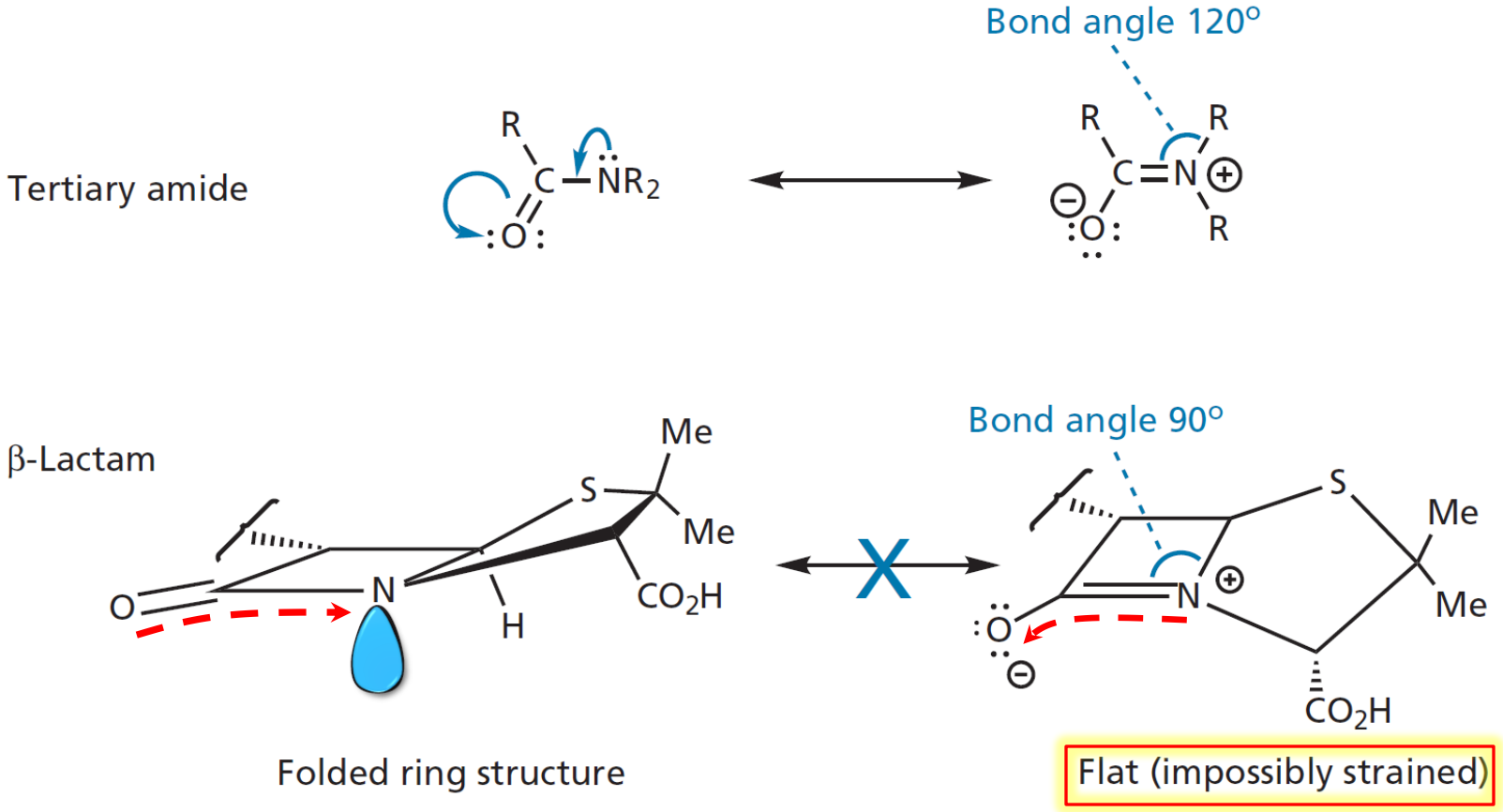


FIGURE 19.24 Comparison of *tertiary* amide and β-lactam carbonyl groups.

Problem of acid-sensitivity for penicillin (cont.)

There are three main reasons for acid sensitivity of penicillin G:

3) **Effect of R group at acylamido side chain:** the acyl group carbonyl (=O) is rich in electron (nucleophilic O) and able to attack the neighboring carbon of β-lactam (electrophilic C). Therefore, penicillin has self-destruction property which can be reduced by using electron withdrawing R group.

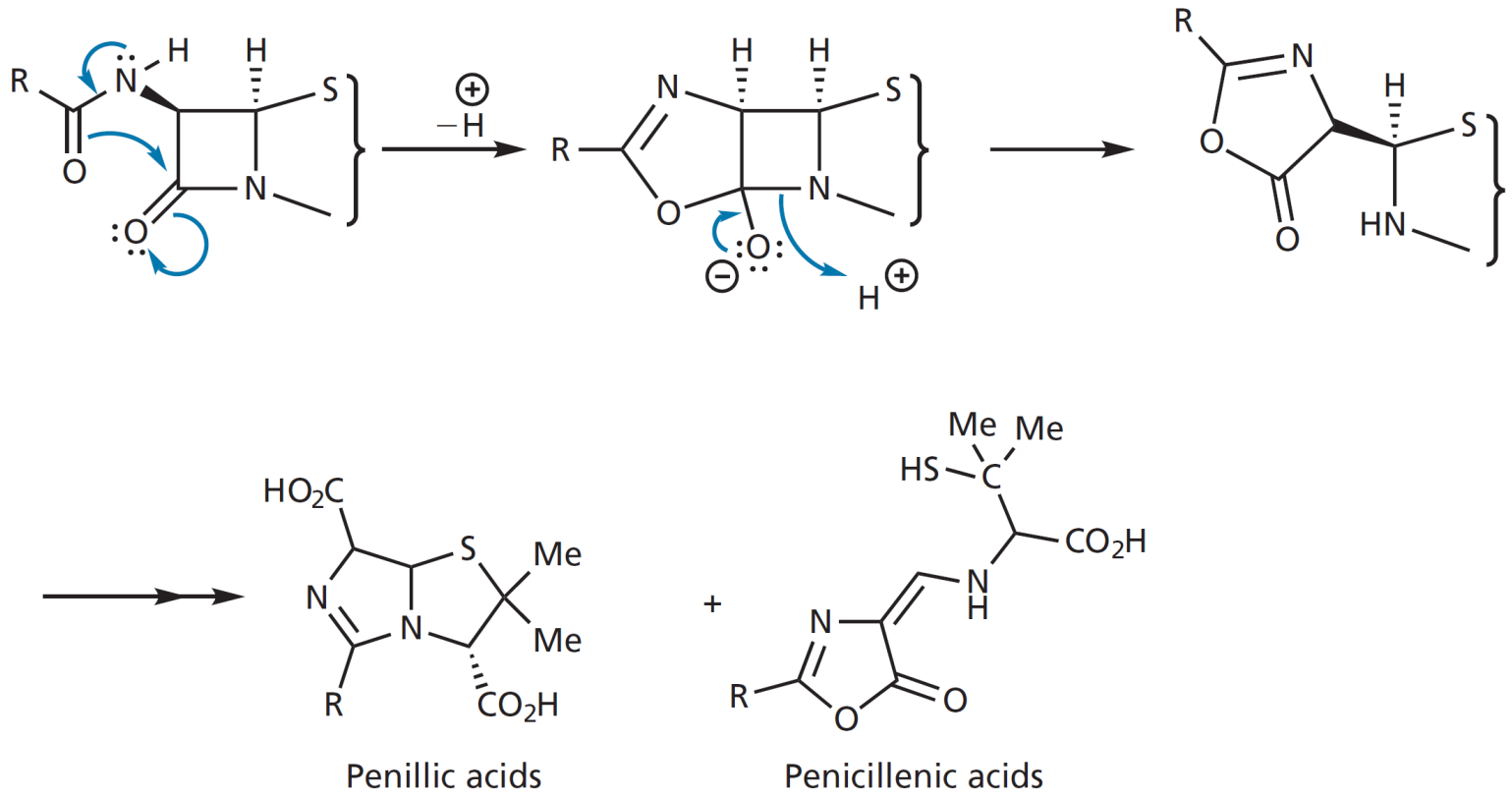


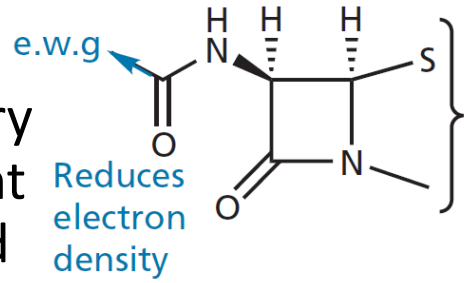
FIGURE 19.25 Influence of the acyl side chain on the acid sensitivity of penicillins.

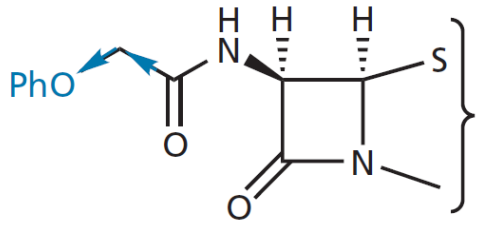
Solving problem of acid-sensitivity for penicillins

Treatment of acid sensitivity of penicillin G:

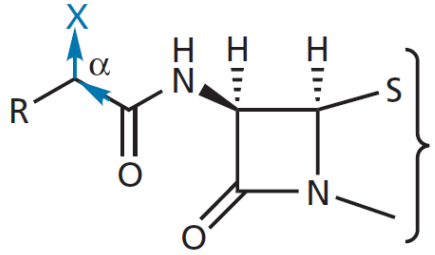
1) No change to β-lactam is allowed

2) No change to geometry of N at the fusion point between β-lactam and thiazolidine rings is allowed





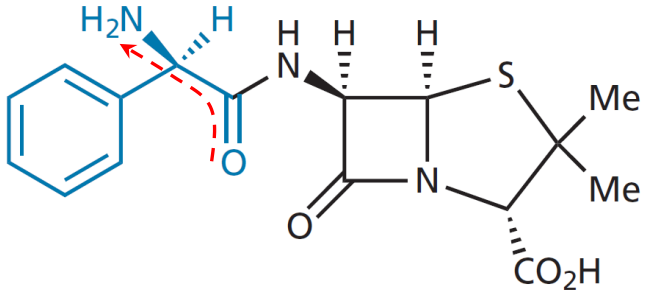
Penicillin V



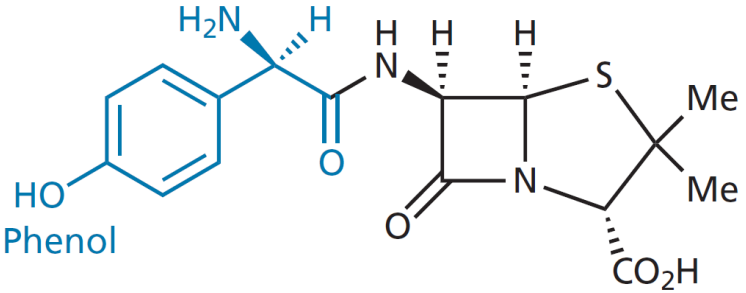
X = NH_2, Cl, PhOCONH, Heterocycles

FIGURE 19.26 Reduction of neighbouring group participation with an electron-withdrawing group (e.w.g.).

3) Can use electron withdrawing R group at acylamido side chain to reduce nucleophilicity of (=O). Examples of acid-stable penicillins are ampicillin and amoxicillin



Ampicillin (Penbritin)

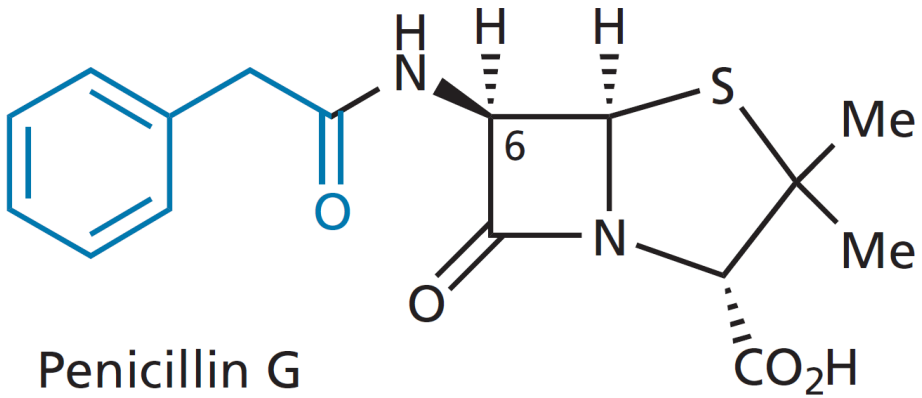


Amoxicillin (Amoxil)

NH2 is ionized in acidic medium to $NH3^+$ which is a strong electron withdrawing group

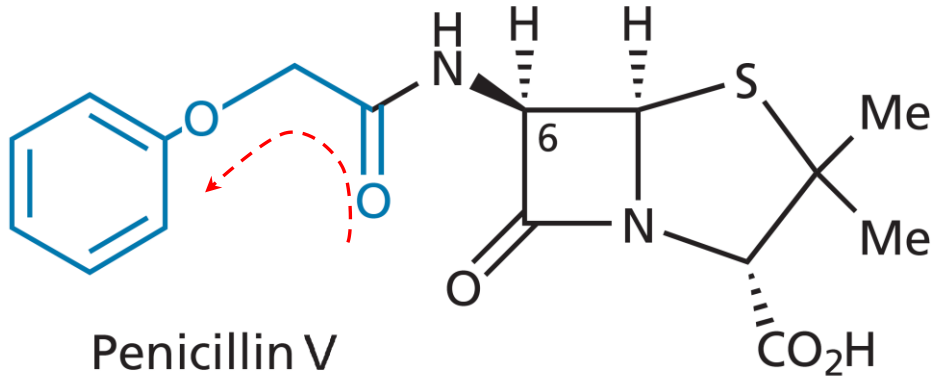
Benzylpenicillin (penicillin G)

- Has **narrow spectrum of activity**: active against Gram+ve bacilli (not producing β-lactamase) and Gram-ve cocci.
- No serious side effects
- **Acid-sensitive** (cannot be taken orally)
- **Sensitive to β-lactamase**
- May cause allergy to some patients.
- All *Psudomonas aeruginosa* strains (Gram-ve) and some *Staphyllococcus aureus* (Gram+ve) strains are resistant.



Penicillin G

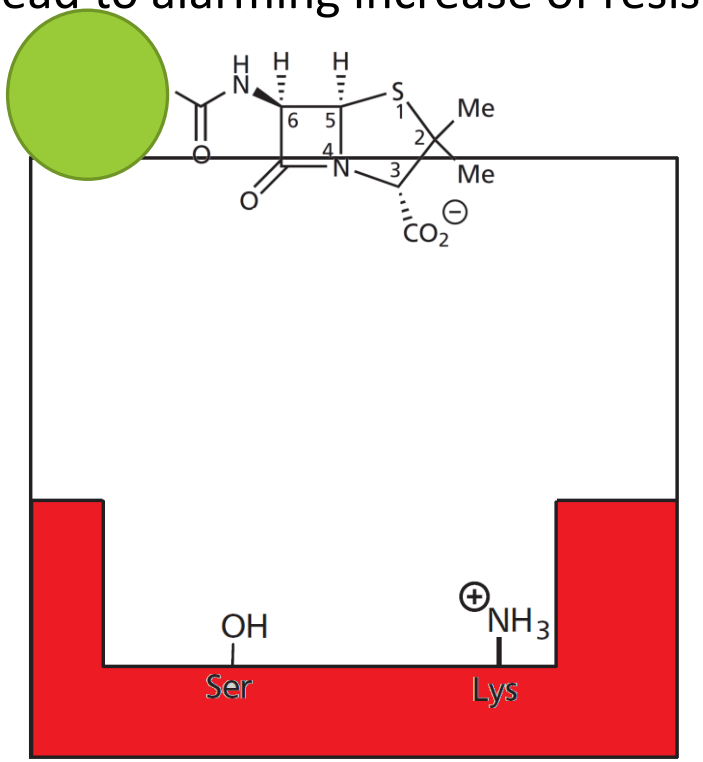
- Less active than Penicillin G.
- No serious side effects
- **Acid-stable**
- **Sensitive to β-lactamase**
- May cause allergy to some patients.

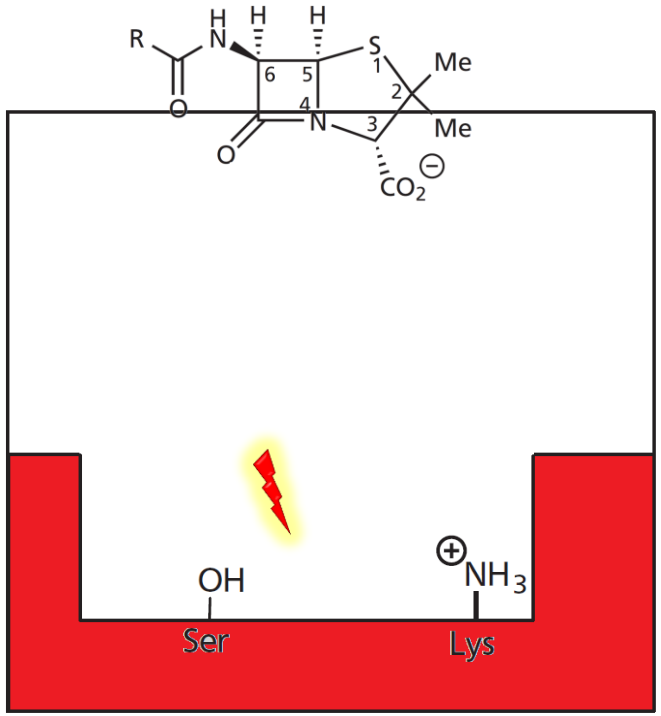


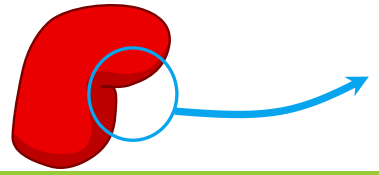
Penicillin V

Problem of β-lactamase sensitivity for penicillins

- The careless use of penicillin G lead to alarming increase of resistant strains of bacteria, mainly *Staphylococcus aureus*.
- ↑steric hindrance at α-carbon of acylg group >> ↑ resistance to staph β-lacatamase
- Substitutions at R ring close to α-carbon *ortho* of phenyl in methicillin (2,6-dimethoxy) or 2-position of a1-naphthyl in nafcillin (2-ethoxyl) increase steric hindrance of the acyl group
- **Suitably bulky R group** will block penicillin binding to β-lactamase but preserve its binding to transpeptidase





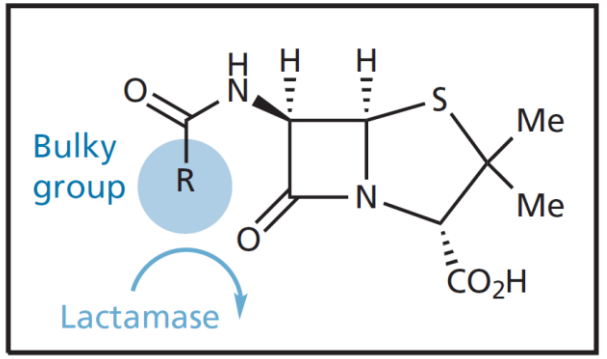


β-lactamase

Solving problem of β-lactamase sensitivity for penicillins

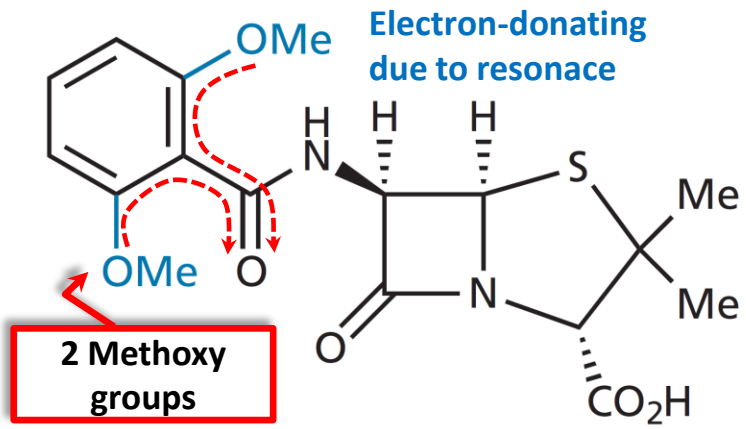
❖ **Addition of two *ortho*-methoxy groups to penicillin G provides:**

- β-lactamase resistant methicillin
- **More acid-sensitive** than penicillin G(only inj.)
- low affinity to several types of transpeptidases (↓ strep. &Gram-ve).

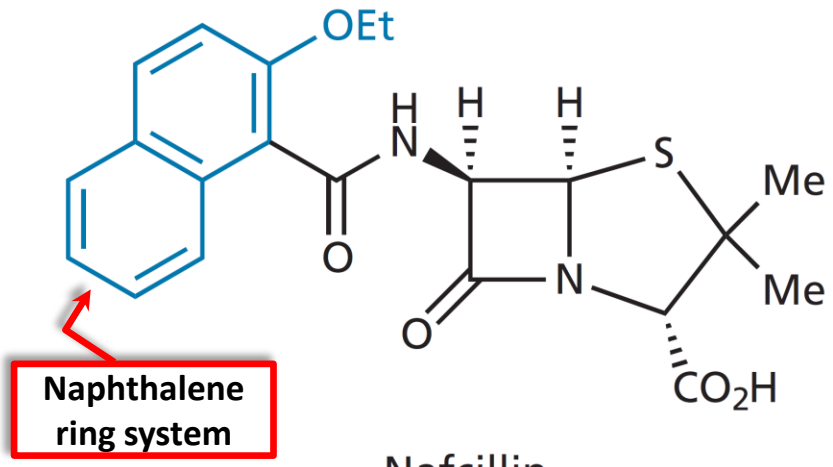


❖ **The bulky R group:**

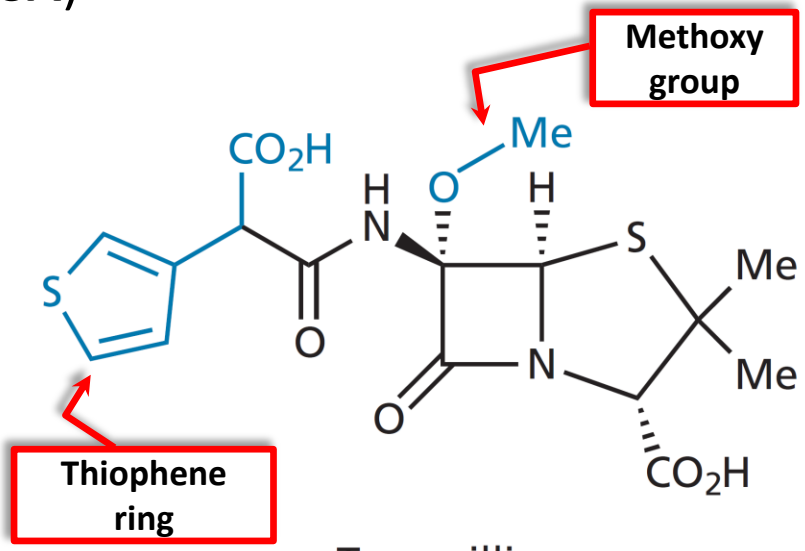
- ↑ hydrophobicity → ↓ penetration to Gram-ve bacteria
- reduces the similarity of penicillin to D-Ala-D-Ala ➔ ↓ potency, ↓spectrum
- some S. aureus transpeptidases were mutated to prevent binding of Methicillin (called Methicillin Resistant *Staphylococcus aureus* **MRSA**)



Methicillin



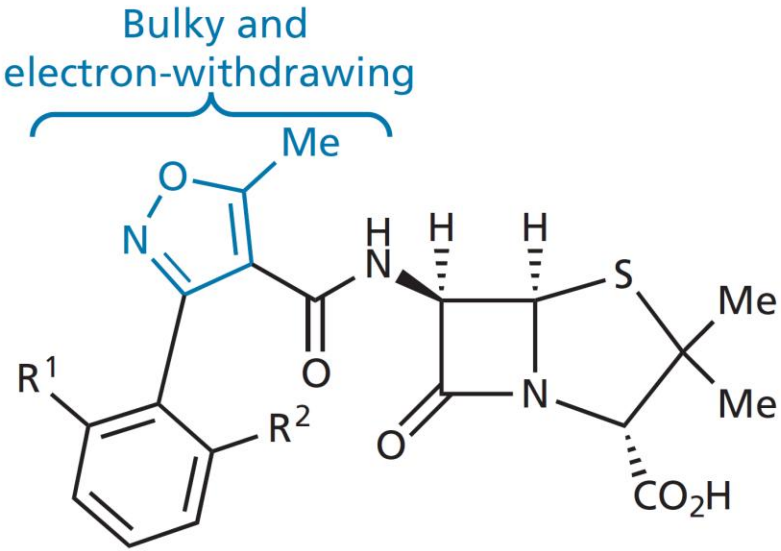
Nafcillin



Temocillin

Solving problem of **β-lactamase sensitivity** and **acid-sensitivity** for penicillins

- **Bulky + Electron withdrawing**
- Addition of electron withdrawing (isoxazole) group to the bulky R group of penicillin improves acid-stability
- The compounds are β-lactamase resistant and acid-resistant (can be taken orally)
- The compounds are active against resistant strains of *S. aureus*.
- Less active than penicilins (inactive vs Gram-ve)
- The compounds have different type and number of halogen atoms which affect the pharmacokinetic properties such as
  absorption (Cloxacillin > Oxacillin) and
  protein binding (Cloxacillin > Flucloxacillin)



Oxacillin	$R^1 = R^2 = H$
Cloxacillin	$R^1 = Cl$, $R^2 = H$
Flucloxacillin	$R^1 = Cl$, $R^2 = F$
Dicloxacillin	$R^1 = Cl$, $R^2 = Cl$

Incorporation of a five-membered heterocycle into a penicillin side chain.

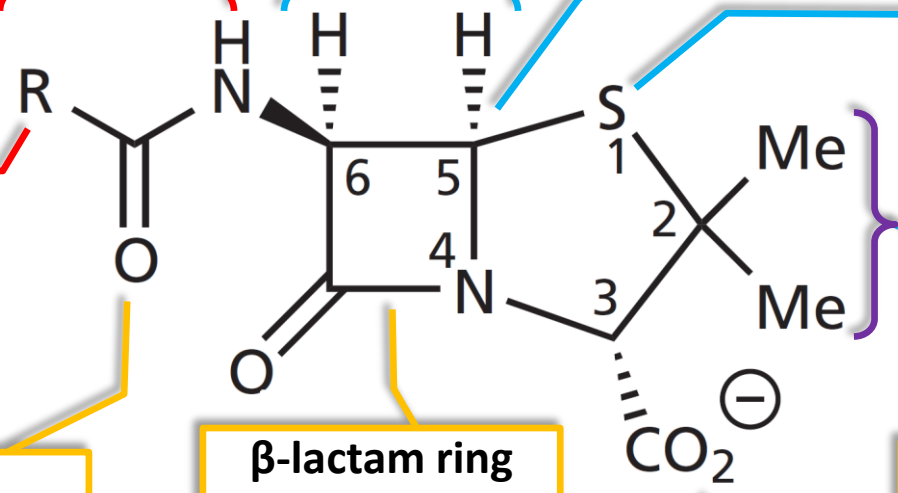
Structure-activity relationship (SAR)

Acylamido side chain is essential

Cis-stereochemistry is essential

No substitution allowed

R group:
1. Electron withdrawing groups →↓nucleophilicity of carbonyl oxygen → ↑stability
2. Bulky groups provides resistance to β-lactamase
3. Polar groups make structure more hydrophilic



Sulfur
is usual but not essential.

Thiazolidine
5-membered saturated ring contains nitrogen. The geminal dimethyl group at C-2 position is a characteristic of the penicillin

Carbonyl oxygen:
Is electrophilic because the lone pair electrons on N is not provided for resonance.
Thus =O is ready for nucleophilic attack

β-lactam ring strain is essential

Bicyclic system confers further strain to β-lactam ring
↑strain →↑activity→↑instability

Carboxylic group
1. Is usually ionized to form sodium of potassium salts.
2. Bind amino group of Lys at binding site
3. Is important for activity which is reduced if modified to alcohol or ester

Spectrum of activity for penicillins

Several factors affect the bacterial susceptibility to penicillins such as:

1) Structure
2) Ability to cross cell membrane of Gram-ve bacteria
3) Affinity to transpeptidases
4) Susceptibility to β-lactamases
5) Rate of pump out of the cell of Gram-ve bacteria

Spectrum of activity for penicillins (cont.)

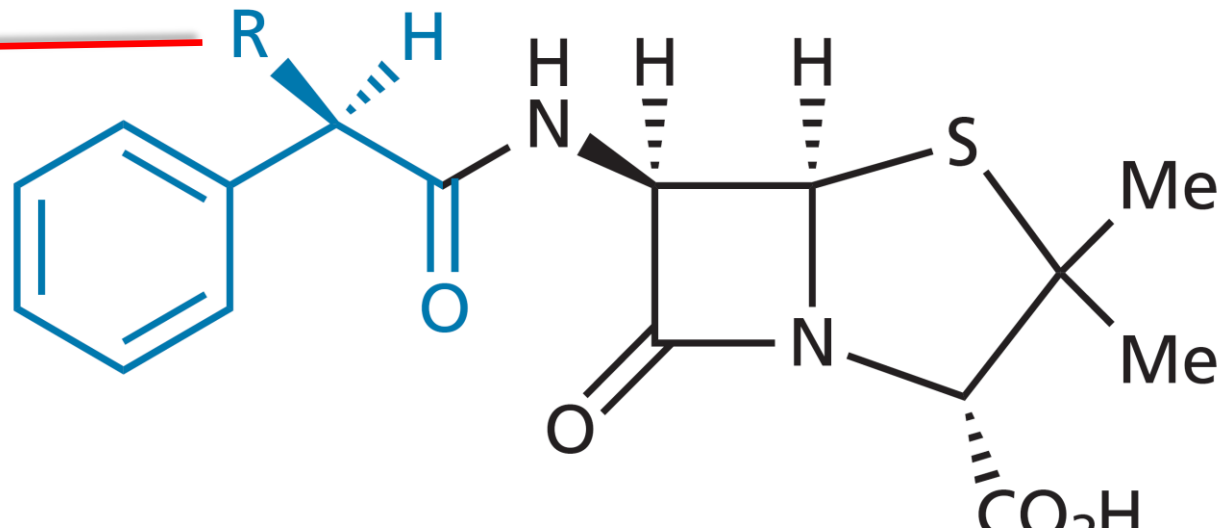
Several points to remember:

- Expansion of spectrum of activity for penicillins is not usually related to β-lactamase inhibition (i.e. ampicillin and amoxicillin are more degradable by β-lactamases than penicillin G).
- Expansion of spectrum of activity for penicillins is related to improved permeability through gram-ve cell membrane by addition of hydrophilic groups (i.e. through porins)

Effect of acylamino side chain on spectrum of activity

R effects on spectrum of activity

- ↑ Hydrophilic group → ↑ spectrum of activity
- Activity of α-OH < α-NH_2 penicillins
  (α-hydroxybenzylpenicillins < α-aminobenzylpenicillins)
- Acid-resistance of α-OH < α-NH2 penicillins
- α-COOH penicillins are too hydrophilic →↓ activity vs Gram+ve (e.g. carbenicillin)
- NH2 penicillins are zwitterionic → ↓ polarity → ↑ activity vs Gram+ve.
- Affects protein binding



Spectrum of activity for penicillins (cont.)

The spectrum of activity is related to the type of side chain variation (R)

Hydrophilic groups

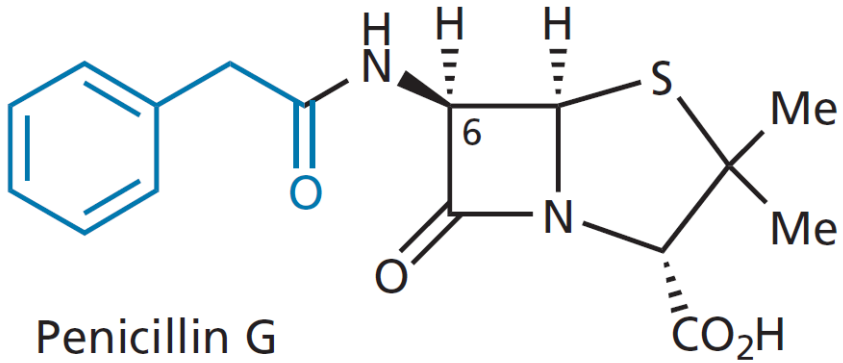
Activity = Gram+ve

Activity ↑ Gram-ve

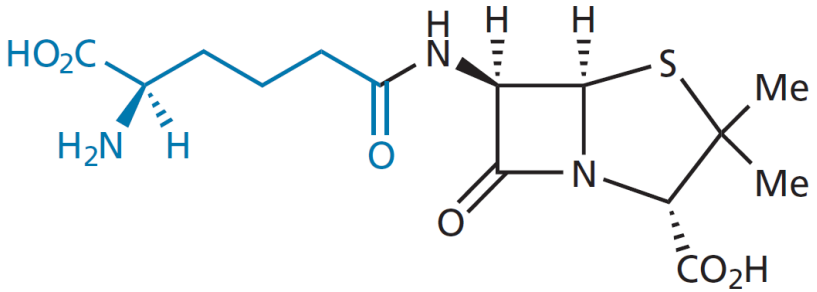
Hydrophobic groups

Activity ↑Gram+ve

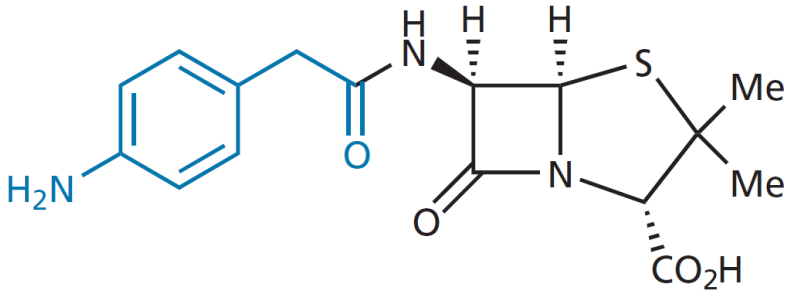
Activity ↓Gram-ve



Penicillin G



Penicillin N



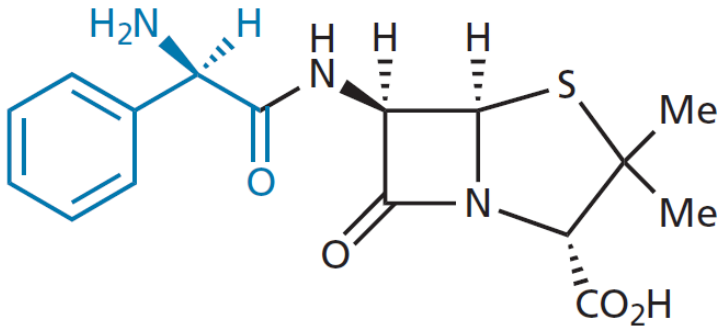
Penicillin T

Activity ↑ Gram-ve the hydrophilic ionizable group (e.g. NH_2, OH, COOH) is attached to the carbon that *is α to the carbonyl (C=O) group on the side chain* (e.g. ampicillin and carbenicillin).

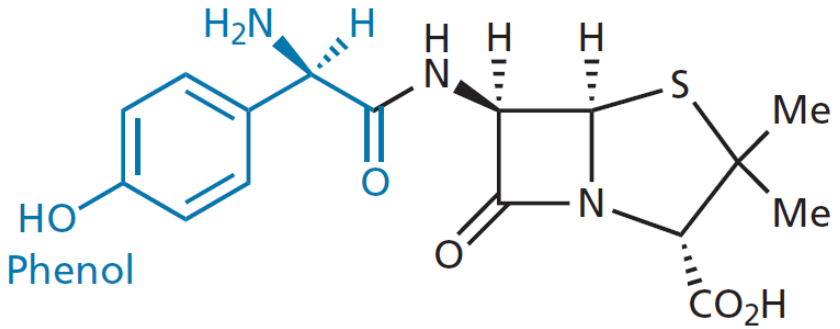
Broad-spectrum penicillins: 1. aminopenicillins

The group have the following properties:

1. Hydrophilic NH_2 group attached to C that is α to C=O of the acyl side chain.
2. Electron withdrawing effect of NH_2 → more acid stable
3. No bulky groups at acyl side chain → more sensitive to β-lactamase
4. NH_2 and COOH groups are ionized → poor absorption from gut
5. The ionizable groups can be masked to form prodrugs with better absorption.
6. The α-carbon becomes chiral (activity of D-isomer > L-isomer & penicillin G)



Ampicillin (Penbritin)



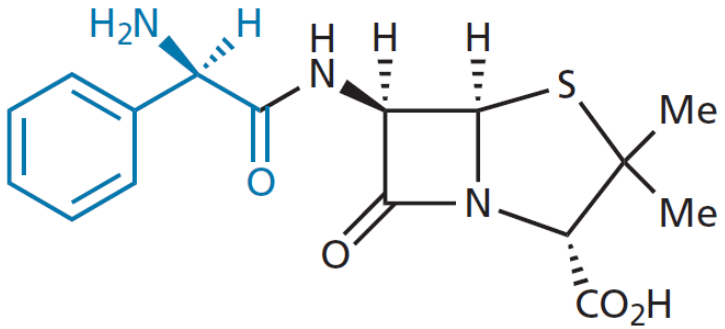
Amoxicillin (Amoxil)

FIGURE 19.29 Broad-spectrum penicillins—the aminopenicillins.

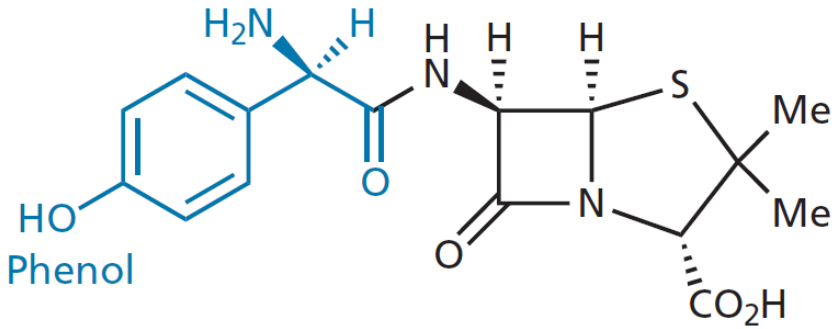
Broad-spectrum penicillins: 1. aminopenicillins

Ampicillin and amoxicillin have

1) Similar spectrum to Penicillin G but more active against Gram-ve cocci and enterobacteria
2) Inactive against *P. aeruginosa*
3) Non-toxic and can be taken orally
4) High doses → change gut flora → problems such as diarrhoea



Ampicillin (Penbritin)

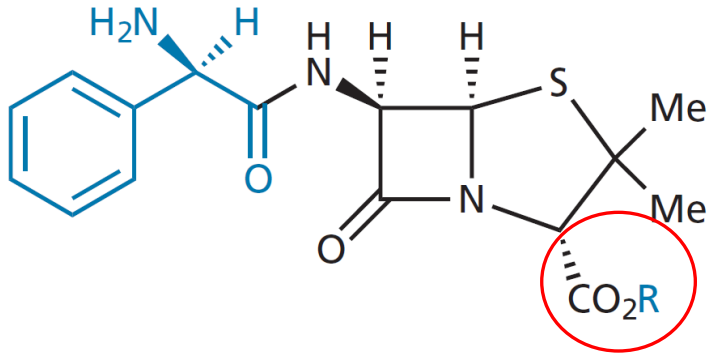


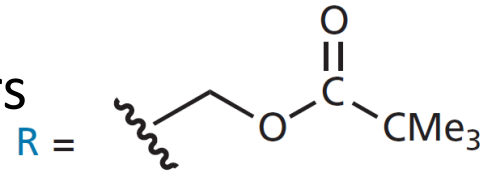
Amoxicillin (Amoxil)

FIGURE 19.29 Broad-spectrum penicillins—the aminopenicillins.

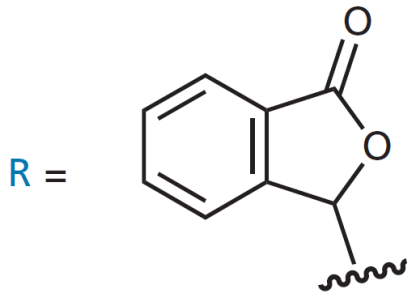
Broad-spectrum penicillins: 1. aminopenicillins (cont.)

1. Prodrugs of ampicillin have carboxylic group (COOH) changed to ester (COOR)
2. The prodrugs have better cell membrane penetration (absorption)
3. The COOR group is metabolized by estrases back to COOH ($COOCH_3$ can not be used because it is inaccessible to estrases).
4. The prodrugs are prepared as acyloxymethyl esters

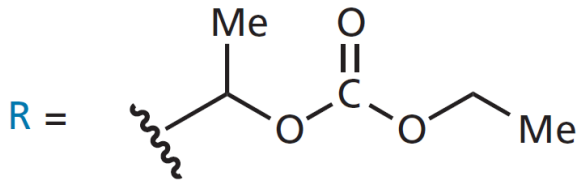




Pivampicillin



Talampicillin



Bacampicillin

FIGURE 1 Prodrugs used to aid absorption of ampicillin through the gut wall.

Broad-spectrum penicillins: 1. aminopenicillins (cont.)

4. Acyloxymethyl esters contain two sequential esters:
   The **outer ester** is not shielded by β-thiazolidine ring thus accessible to hydrolysis by esterase then the **inner ester** undergoes self-hydrolysis

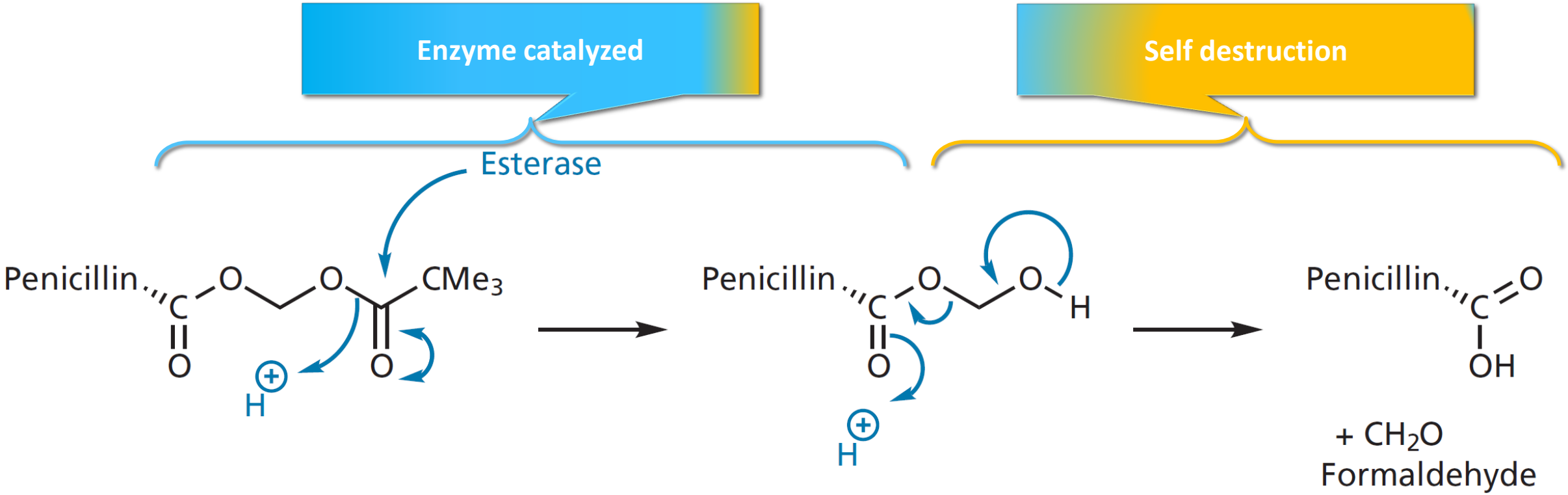
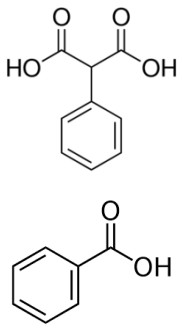


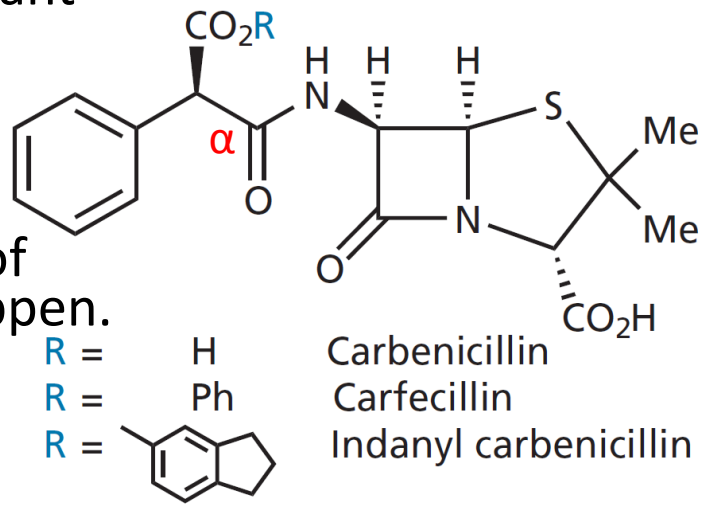
FIGURE 2 Mechanism by which acyloxymethyl esters are hydrolysed.

Broad-spectrum penicillins: 2. carboxypenicillins

The group have the following properties:

1. Hydrophilic COOH group attached to C that is α to C=O of the acyl side chain (→ ↓ activity vs Gram+ve , ↑activity vs Gram-ve including *P. aeruginosa* expt carb.)
2. Carfecillin and Indanyl carbenicillin (aryl esters) are prodrugs to Carbenicillin.
3. Hydrolysis to –COOH for aryl ester **>** alkyl ester due to electron withdrawing of aryl
4. The COOH group is ionized at pH 7
5. Stereochemistry of COOR at α carbon is not important for carb. **WHY?**
6. β-lactamase resistant is low for carb. **WHY?**
7. Acid-sensitivity for Pen. of phenylmalonic acid (Carbpen. >> Benzoic acid (Pen. G) due to electron donating effect of COOH





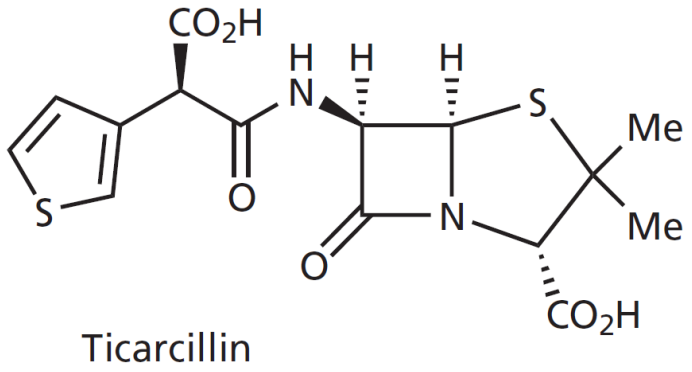


FIGURE 19.30 Carboxypenicillins.

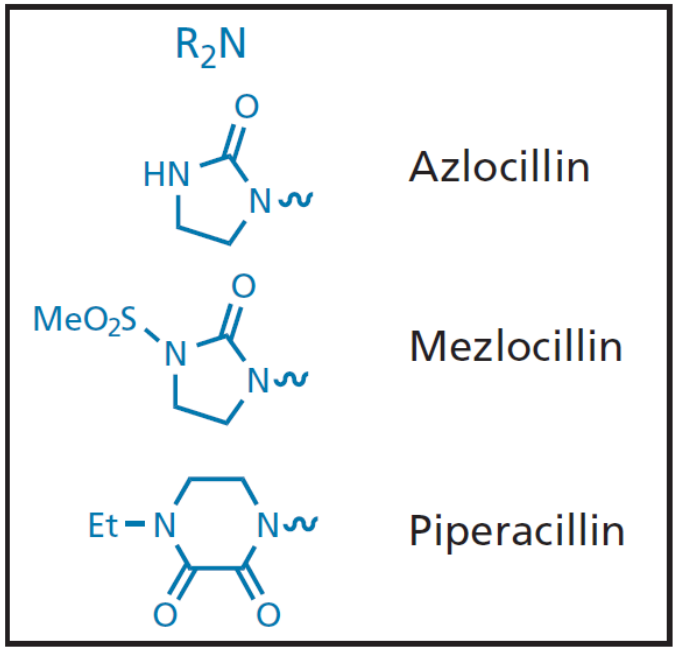
Broad-spectrum penicillins: 3. Ureidopenicillins

The group have the following properties:

1. Urea functional group attached to C that is α to C=O of the acyl side chain.
2. More active vs. Gram-ve than carbencillin (more cell-mem permeability).
3. Higher activity against *P. aeruginosa*
4. β-lactamase sensitive.
5. Acid-sensitive.

↑ affinity to transpeptidase
↑ cross outer membrane

Active against
anaerobic cocci and bacilli



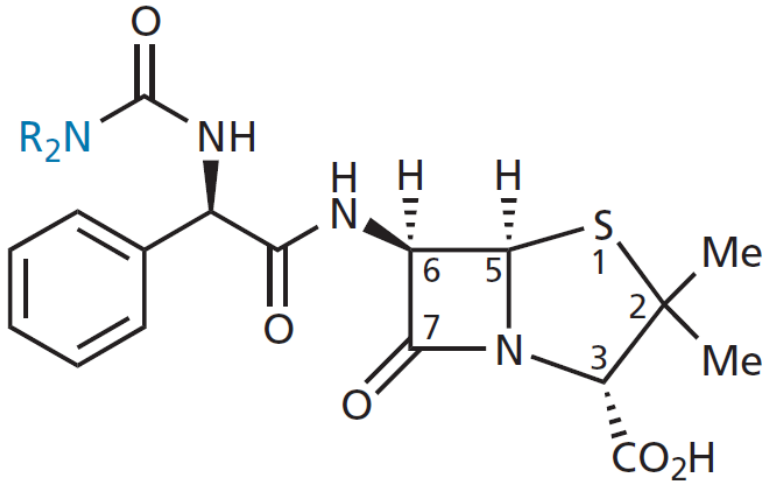


FIGURE 19.31 Ureidopenicillins.

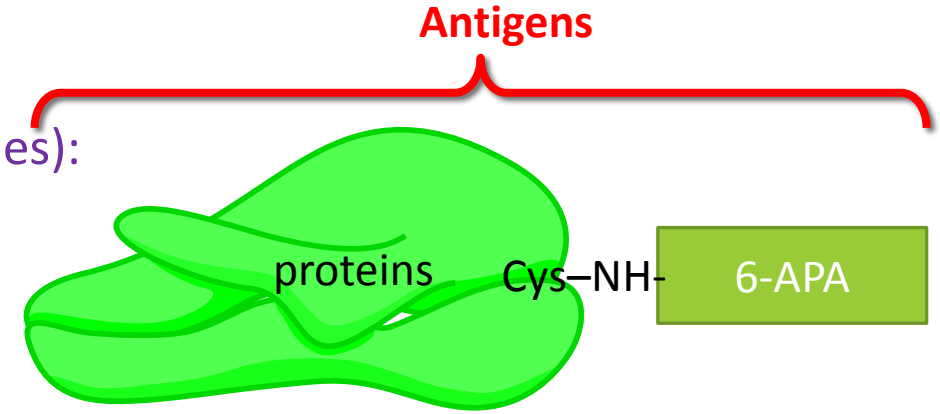
Protein-binding

- **The nature of acylamino side chain affects protein binding**
- ↑ hydrophobic group → ↑ protein binding
- ↑ hydrophilic group → ↓ protein binding
- Pr. Binding Carbenicilin 45%, Ticarcillin 55% **>** Ampicillin 25%, Amoxicillin 30%
- Pr. Binding nonpolar and lipophilic groups (nafcillin, isoxzole) about 90%
- ↑ Pr. Binding → ↓ tissue distribution
- Pr. Binding has little effect on plasma half-life.

Allergy to penicillins

- Major problem for penicillins
- Mainly caused by penicillin G and ampicillin. The allergy is cross-sensitive among the group
- Range from skin and mucous memb rxn to anaphylaxis.
- The allergy is due to formation of antigens (macromolecules):

- Rxn of 6-APA with Ser of proteins.

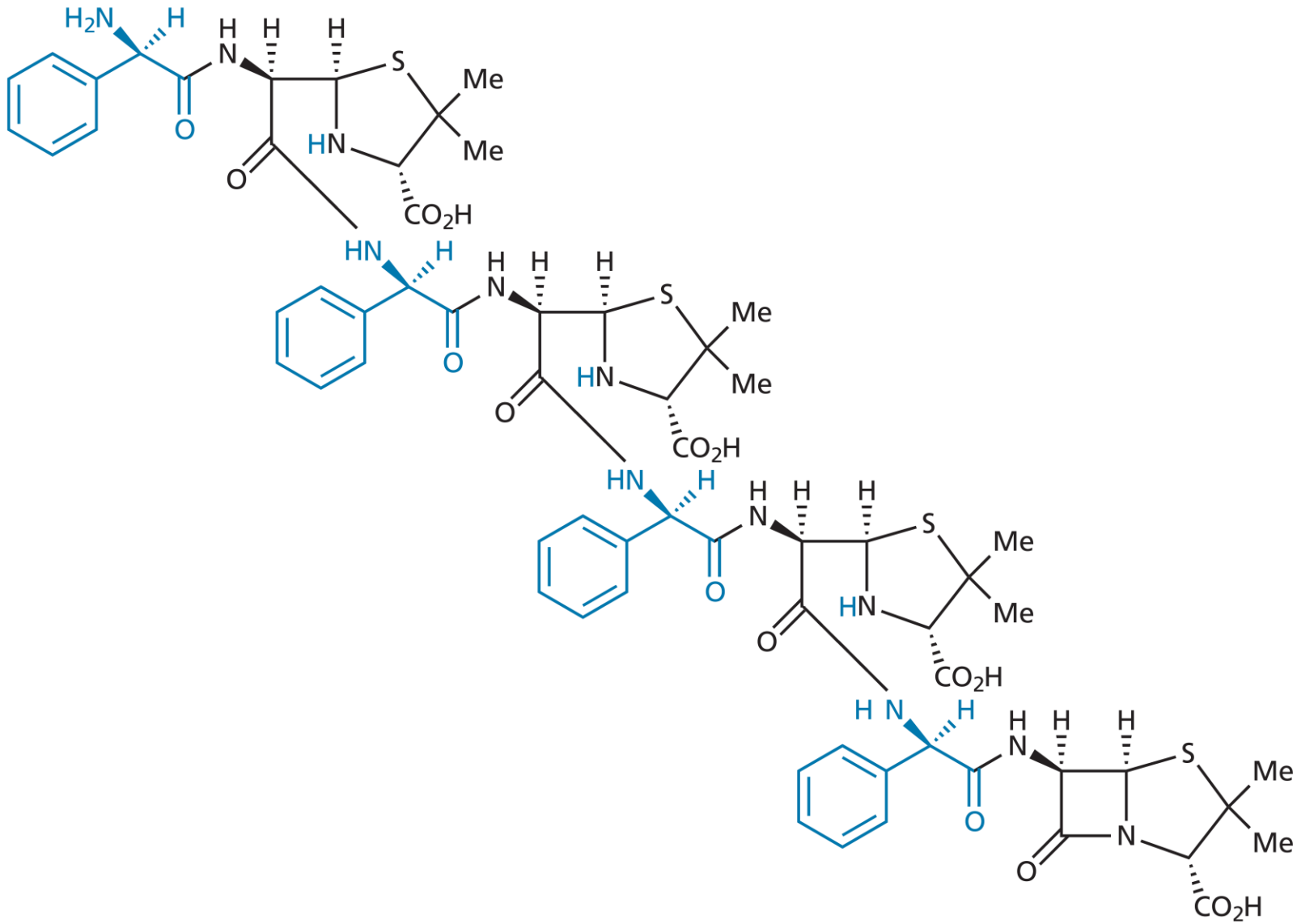


- Polymerization of ampicillin (pH dependent process)

- Animal products (e.g. chickens) need to be free from penicillins before being slaughtered to avoid future allergy to penicillin products among consumers.

Allergy to penicillins (Cont.)

pH-dependent
Ampicillin
Polymerization



Physicochemical properties of penicillins

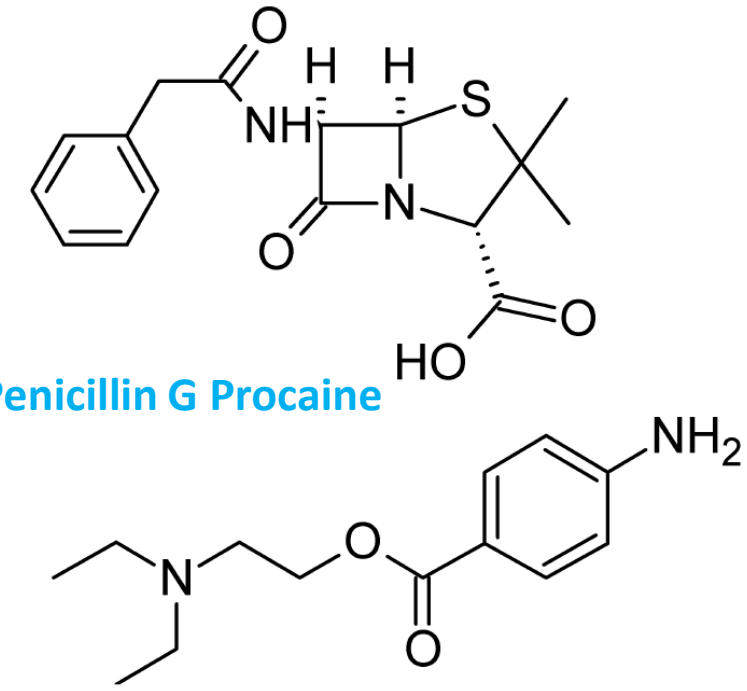
Have the following

- The purified form is white crystalline
- Unstable to moisture but if converted to salts can be stable for years
- unpleasant taste
- The solubility and other physicochemical properties depends on the nature of acyl R group and type of cation used to make the salt
- Most penicillins are acids with pKa 2.5-3.0 and thus not suitable for oral or parenteral use
- Sodium salts are soluble in water and ready for oral and parenteral use
- Salts with organic bases (e.g. benzathine, procaine and hydrabamine) have low water solubility and intended for depot forms

Examples of penicillins in use (Cont.)

- Metal salts (Na^+, K^+ or Ca^{+2}) are soluble in water and ready for oral and parenteral use
- Salts with organic bases (e.g. procaine and benzathine) are less soluble in water than metal salts and intended for depot forms (some times sesame and peanut oils are added)

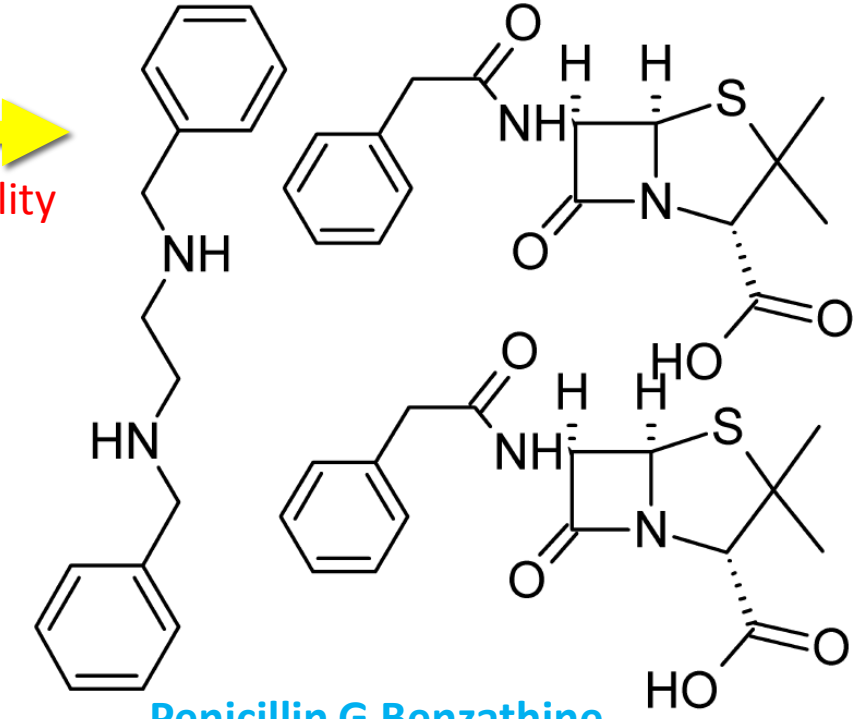
1 moles of penicillin in each molecule



Penicillin G Procaine

Lower solubility

2 moles of penicillin in each molecule

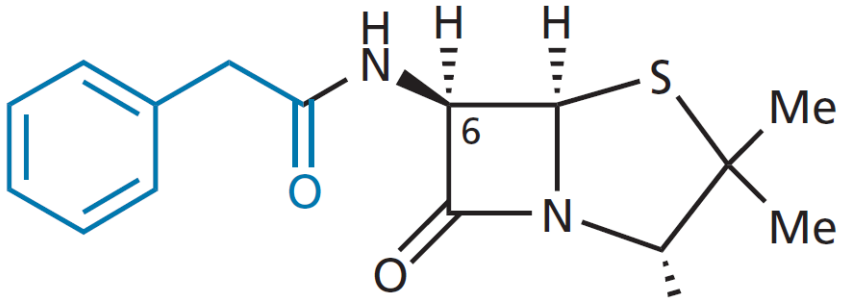


Penicillin G Benzathine

Examples of penicillins in use

Penicillin G

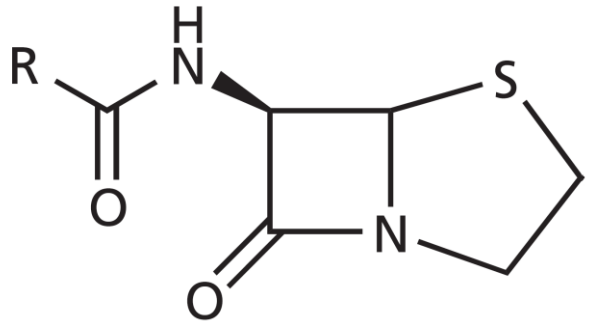
- The main penicillin used in the past
- Prepared as salts of Na^{+}, K^{+} or Ca^{+2}
- co-administered with antacids or buffers to reduce degradation at stomach
- poorly absorbed from GIT (up to 5X parenteral dose is taken).
- Prepared with peanut or sesame oil as depot dosage forms for inj.



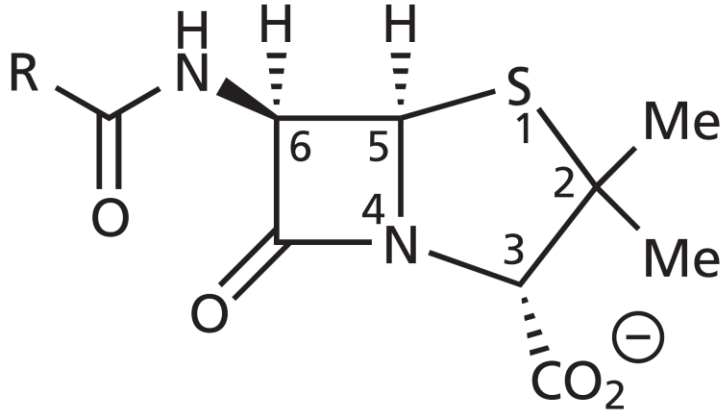
Penicillin G

Nomenclature of penicillins

- There are two types of numbering for the fused bicylic ring system of penicillin: whether which atom is number one Sulfur or Nitrogen.
- **Penam** nucleus is used in naming which comprise bicyclic system with the amide carbonyl group. Penicillin is named as 6-acylamino-2,2-dimethylpenam-3-carboxylic acid.
- **Penicillanic acid** nucleus: which includes the 2,2-dimethyl and 3-carboxyl groups. Penicillin is named as 6-carbonylaminopenicillanic acid.
- Penicillin nucleus: which includes 6-carbonylaminopenicillanic acid. So Penicillin G is named benzylpenicillin if R is benzene ring

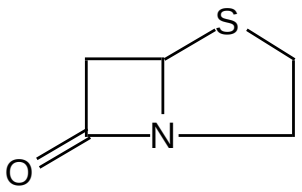


Penam

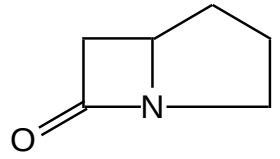


Pencillanic acid

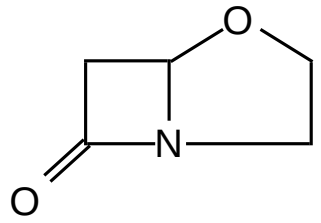
Nomenclature of penicillins (cont.)



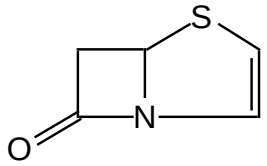
1. **Penam**



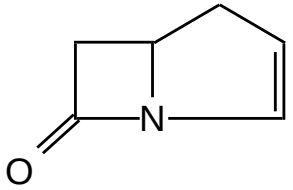
2. Carbapenam



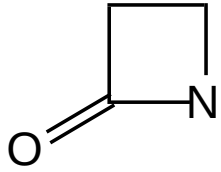
3. **Oxapenam**



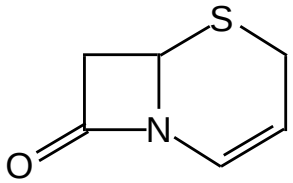
4. Penem



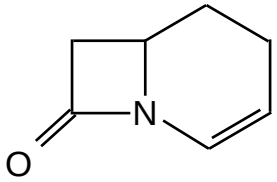
5. **Carbapenem**



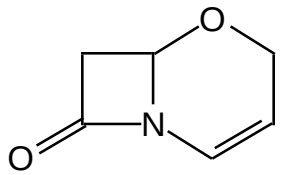
6. **Monobactam**



7. **Cephem**



8. Carbacephem

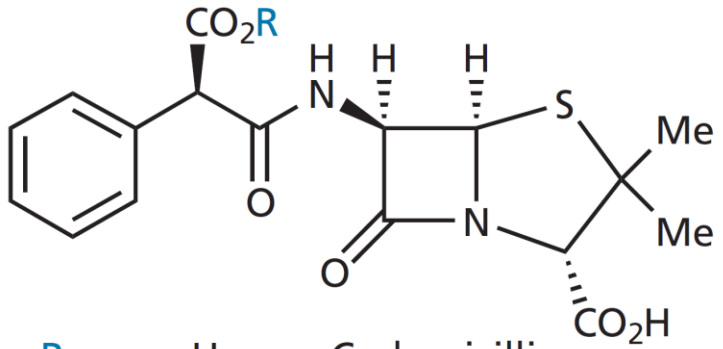


9. Oxacephem

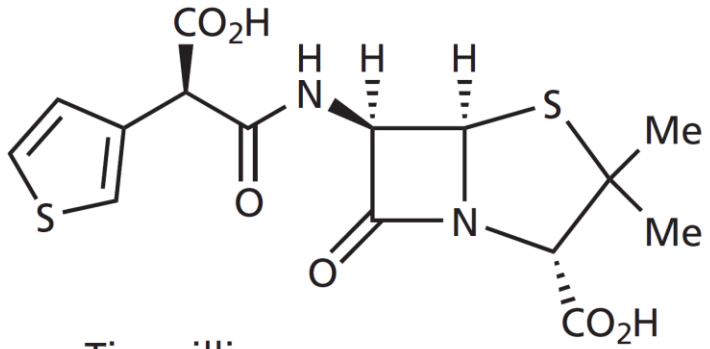
Broad-spectrum penicillins: 2. carboxypenicillins

Carbenicillin is

1) Active against *P. aeruginosa* (Ticarcillin is more active)
2) More active against Gram-ve bacteria than ampicillin
3) Less active against other bacteria than ampicillin
4) Toxic side effects, acid-sensitive, β-lactamase sensitive



R = H Carbenicillin
R = Ph Carfecillin
R = Indanyl carbenicillin



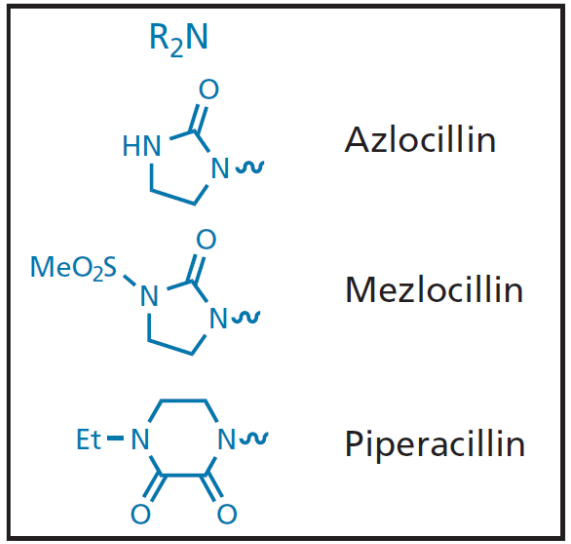
Ticarcillin

FIGURE 19.30 Carboxypenicillins.

Broad-spectrum penicillins: 3. Ureidopenicillins

Ureidopenicillins compared to carboxypenicillins ares:

1. More active against streptococci and Haemophillus species
2. Similar activity against Gram-ve aerobic rods such as P. aeruginosa
3. More active against Gram-ve bacteria.
4. Acid-sensitive, β-lactamase sensitive Urea functional group attached to C that is α to C=O of the acyl side chain.



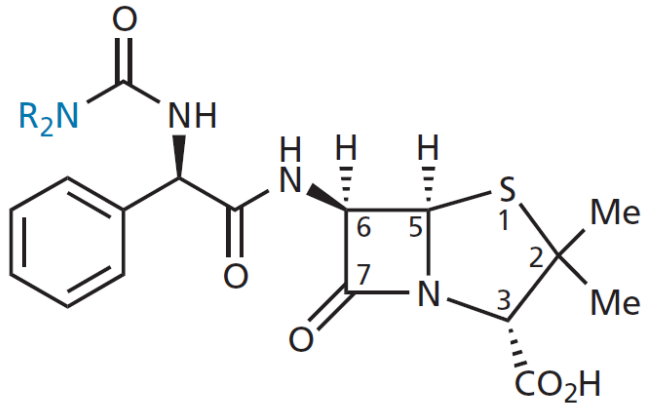


FIGURE 19.31 Ureidopenicillins.