Antibacterial antibiotics

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Antibacterial antibiotics

- A substance is classified as antibiotic if the following conditions are met:

1. It is a product of metabolism even if it was later duplicated by synthesis
2. Synthetic analogues of naturally occurring antibiotics
3. It antagonizes growth or survival of one or more species of microoganisms (Either kills the microbe (microbiocidal) or prevent its growth (microbiostatic)).
4. It is effective at low concentrations

Antibacterial agents:

- The accidental discovery of penicillin is by Fleming in 1928 is the main reason for the initiation of modern antibiotic era.
- Clinically useful antibiotics need to have the following criteria:

1. Combat infection or neoplastic disease
2. Selective toxicity
3. Stable for a period of time inside the body.
4. Ease of administration by oral or parenteral route
5. Rates of biotransformation and urinary elimination are slow enough to allow convenient dosing schedule and rapid enough to remove the drug and its metabolites after discontinuation

Potential targets for antibacterial agents

- Protein synthesis
- Nucleic acid synthesis
- Cell metabolism (e.g. folate synthesis)
- Cytoplasmic membrane
- Bacterial cell wall synthesis

Potential targets for antibacterial agents

Sulfonamides
(on metabolic enzymes)

Penicillins
Cephalosporins

Aminoglycosides
Tetracycline
Chloramphenicol

Quinolones
Rifampicin

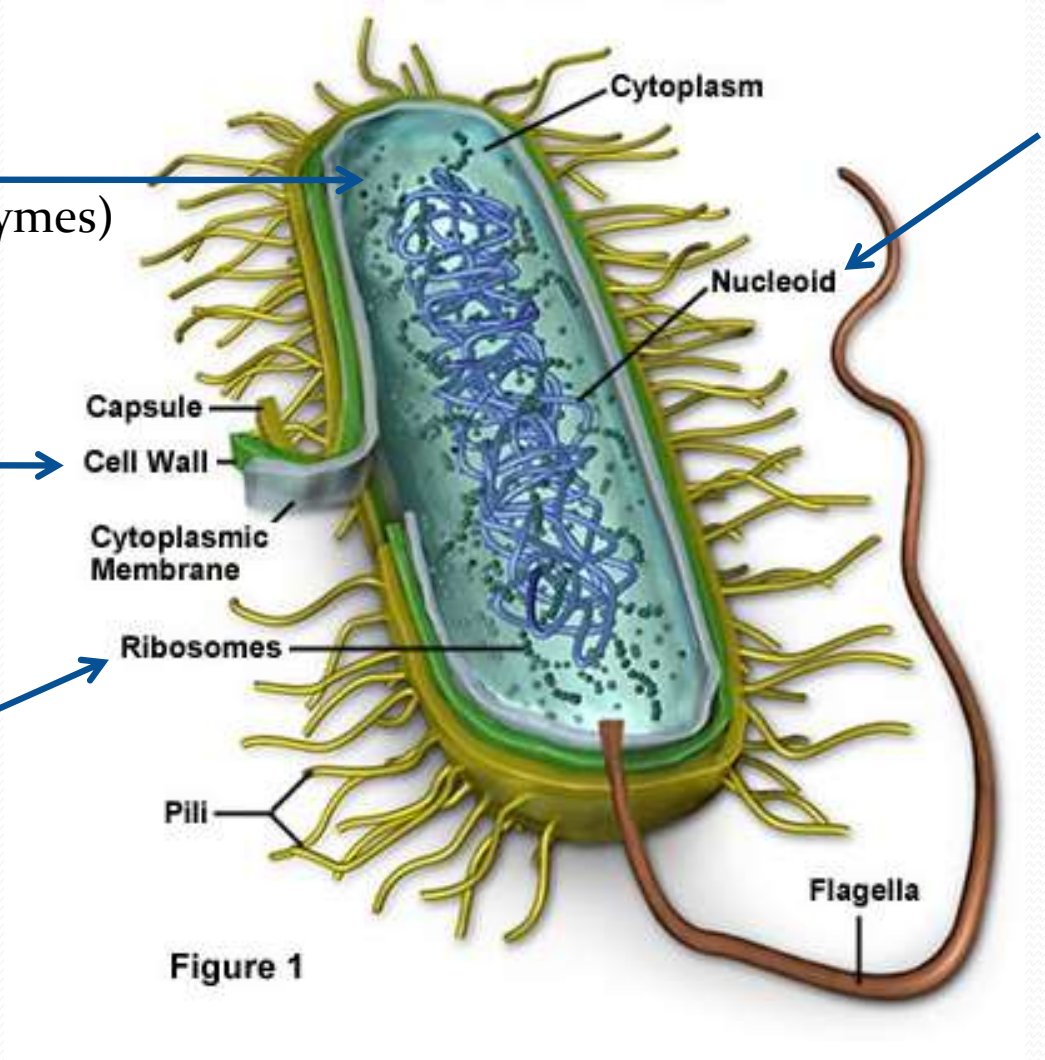


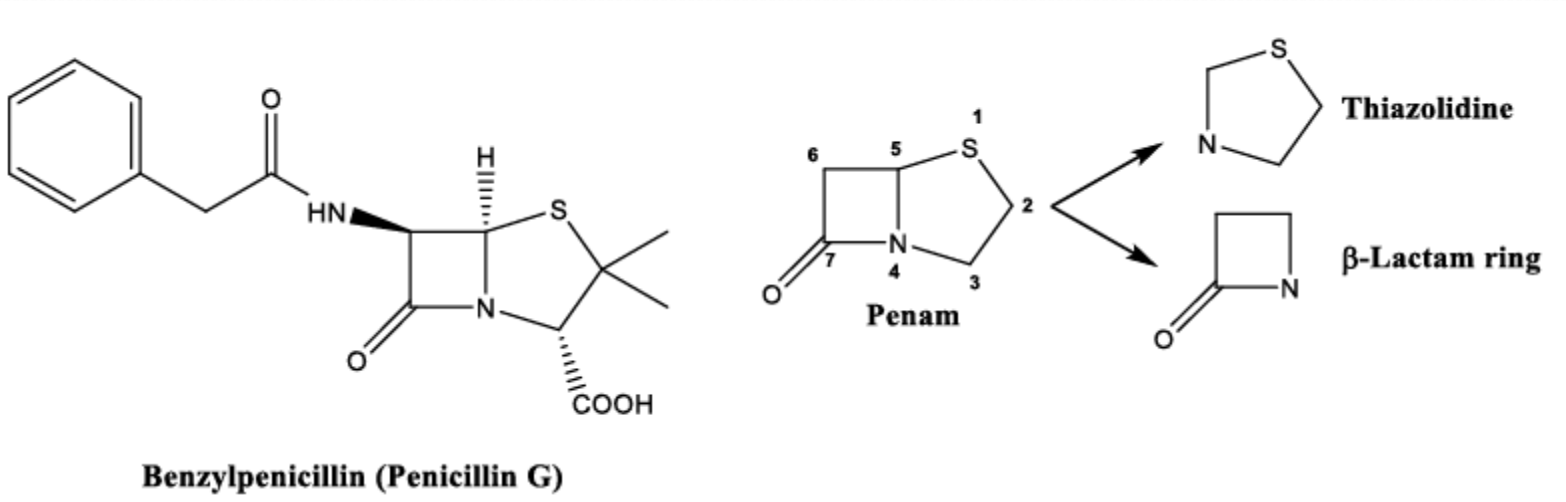
Figure 1

Antibacterial agents acting on the cell wall biosynthesis

Penicillins and Cephalosporins

β- Lactam antibiotics

- These includes both penicillins and cephalosporins.
- The name “lactam” is given to cyclic amides and is analog to the name “lactone”, which is given to cyclic esters.
- This ring ultimately proven to be the main component of pharmacophore.
- This ring is more reactive and sensitive to nucleophilic attack when compared to normal planar amides.

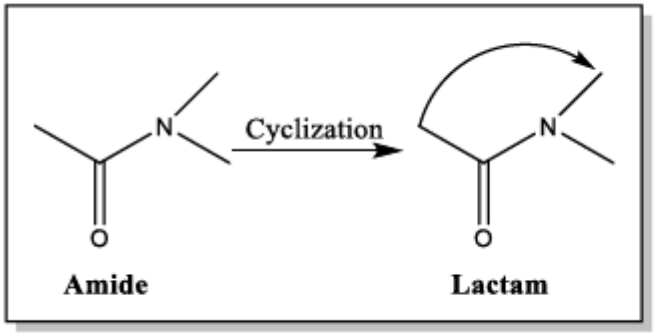


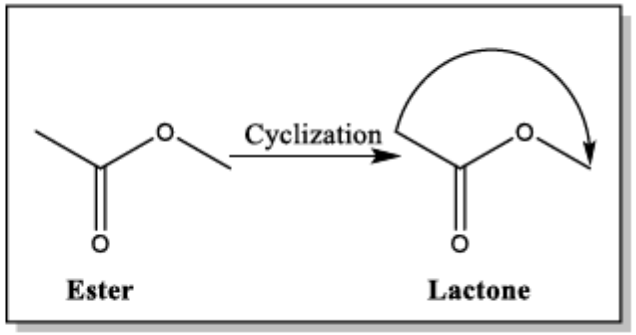
β-Lactam (the cyclic amide)

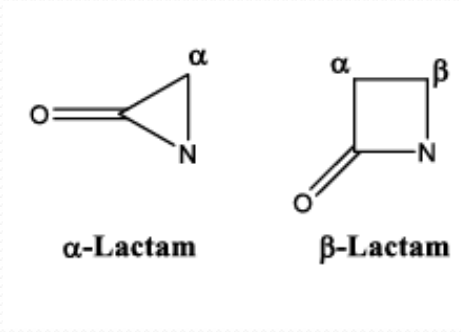
This ring is the result of cyclization of the amide group, as in case of Lactone which is the cyclic structure of ester.

Why it's called β-Lactam?

Because the first carbon beside the carbonyl group is α carbon and beside this α carbon is β carbon, so when the nitrogen atom substituting the β carbon we call it Lactam ring (β-Lactam), also there is α-Lactam rings

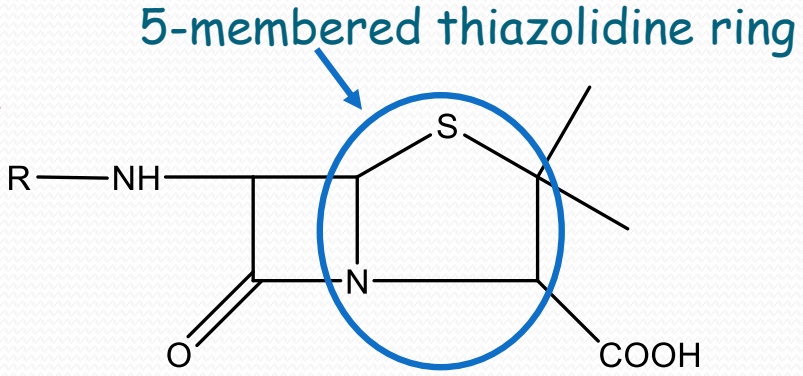






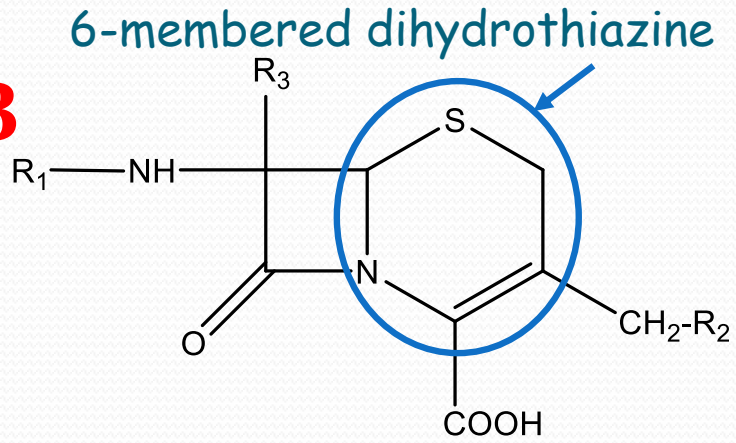
β-lactam antibiotics

A



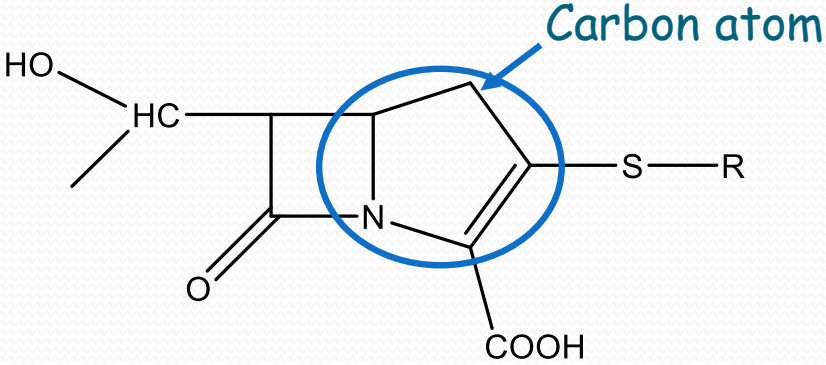
Penicillin nucleus

B



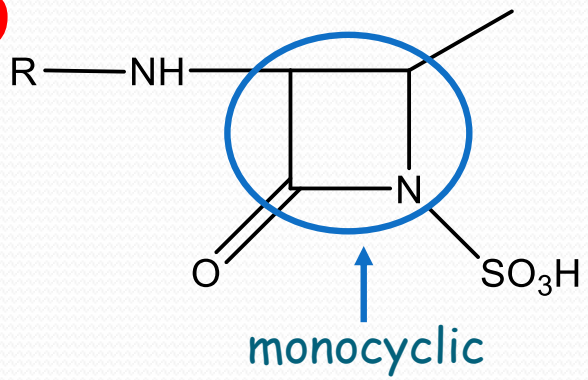
Cephalosporin nucleus

C



Carbapenem nucleus

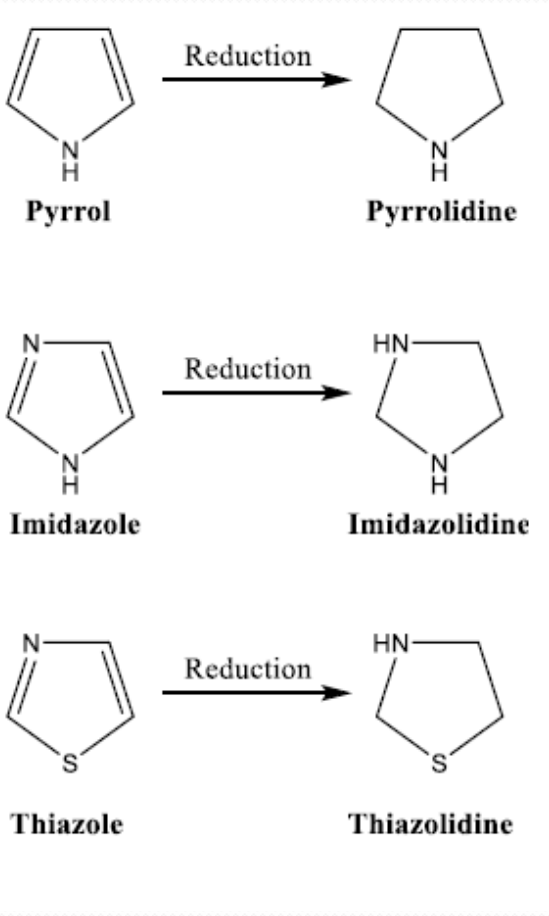
D



Monobactam nucleus

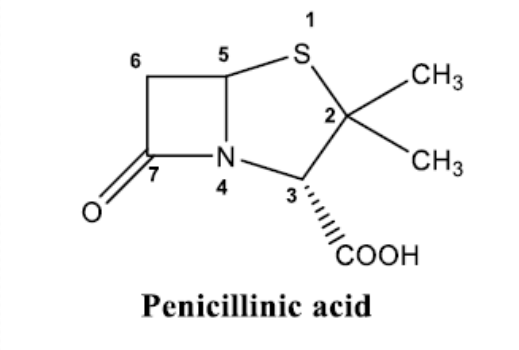
Thiazolidine ring: (S-containing N-containing reduced heterocyclic ring)

- Any **heterocyclic compo**und with **5-membered ring** ends with "**ole**", so we have for examples Pyrrole, Imidazole, and thiazole.
- Upon reduction these compounds will be Pyrrolidine, Imidazolidine, and Thiazolidine (thia=sulphur, aza= nitrogen) respectively.

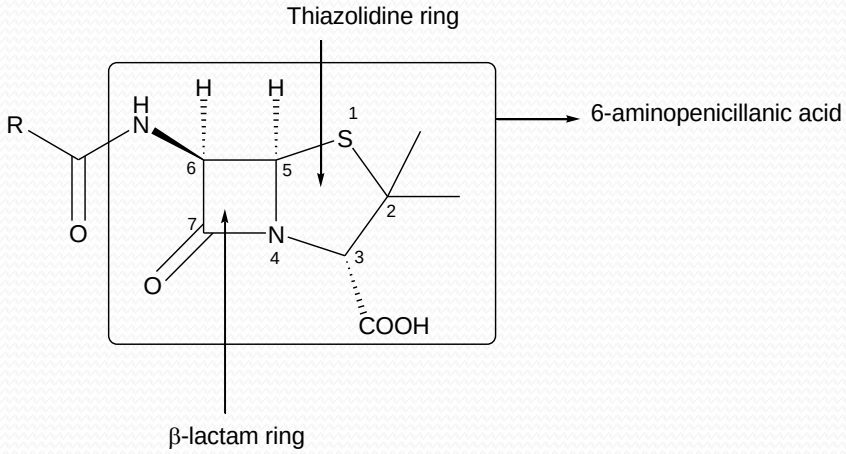


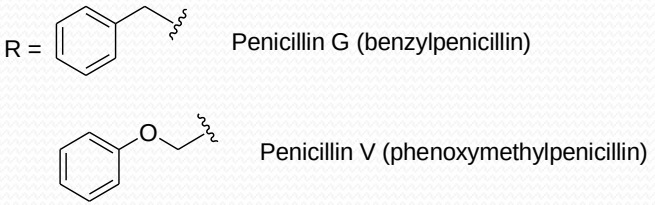
- To say that our compound is penicillin, firstly you have

to look for the Penam system then the following
substitutions also must present in all penicillins:
 Di-methyl group at the position 2.
 A carboxylic acid at position 3, below the plan, (S)
oriented.
Until now, this compound is called "Penicillinic acid",
a 2, 2-dimethyl-3-carboxy Penam.



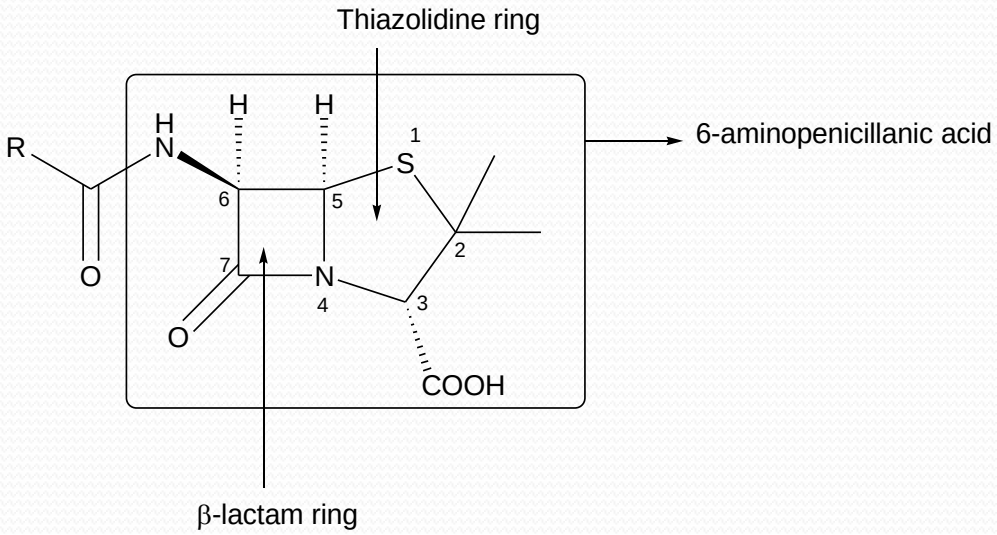
Penicillinic acid

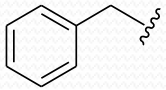




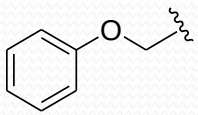
Penicillin's naming is problematic

1st naming system related to the chemical abstracts



R = 

Penicillin G (benzylpenicillin)



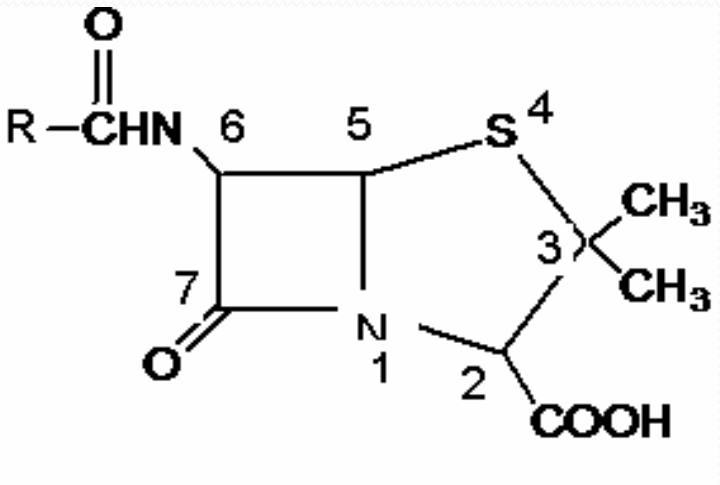
Penicillin V (phenoxymethylpenicillin)

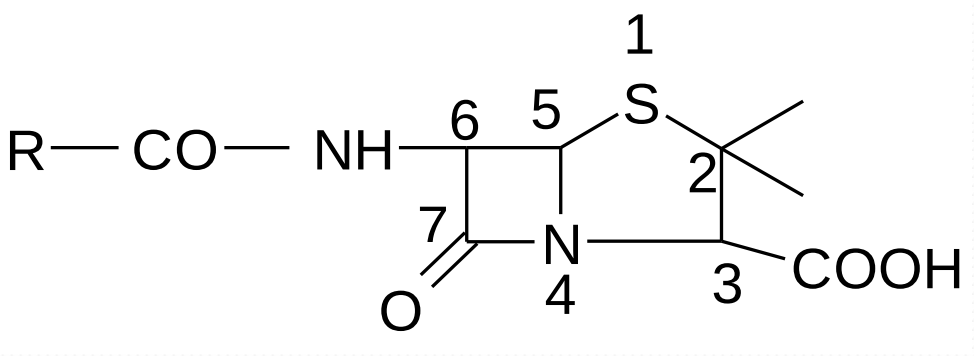
Fungus synthesizes penicillin using cysteine, valine and some of the fermentation products

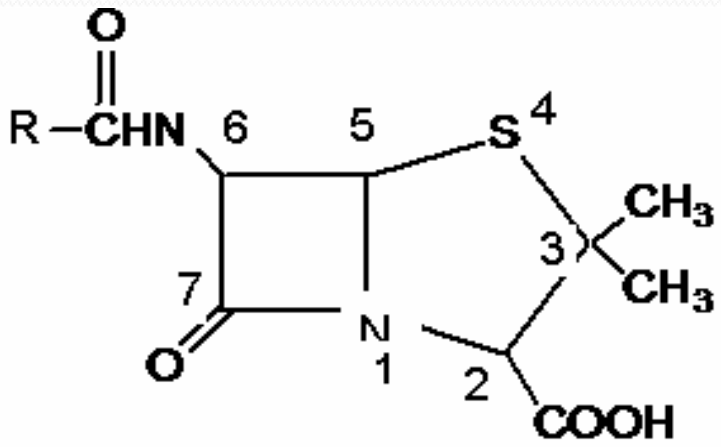
Difficult to synthesize in the lab due to:
The unstable highly
1. strained ring system.
2. The three chiral center it has which should be with certain stereochemistry

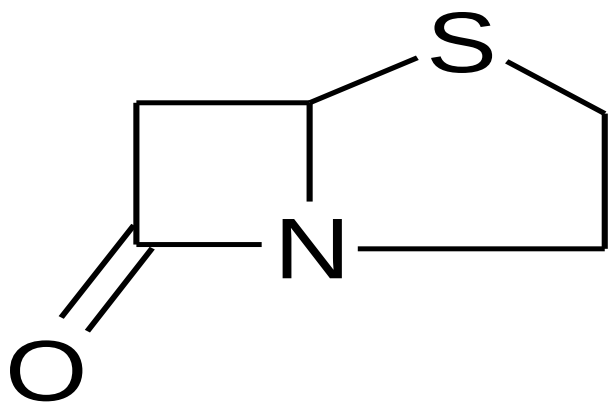
Penicillin's naming is problematic

- 1st naming system related to the USP:
- The correct IUPAC name of penicillin is 4-Thia-1-azabicyclo[3.2.0] heptanes

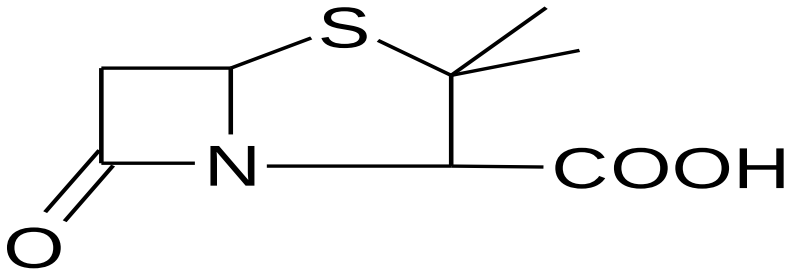






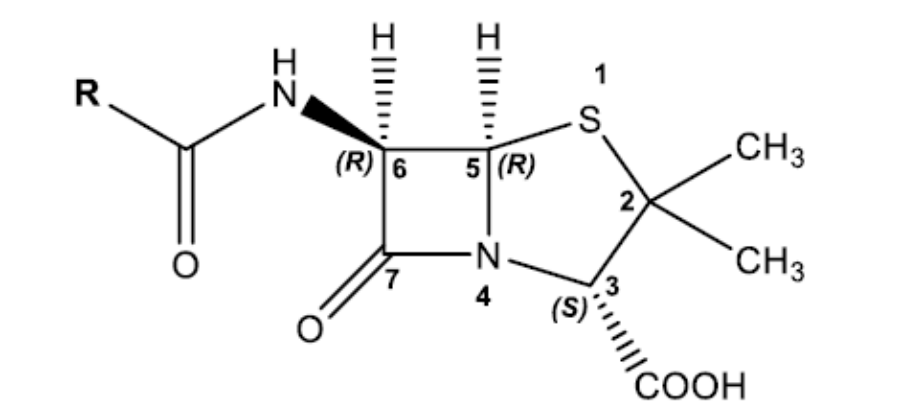


Penam



Penicillanic Acid

Stereochemistry



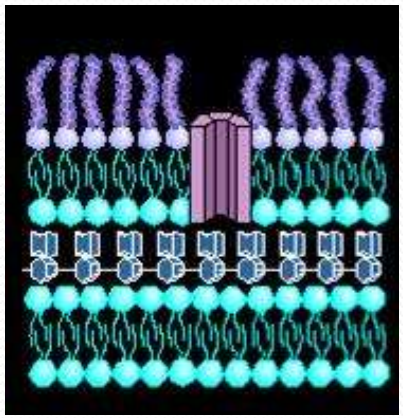
It contains three chiral carbon atoms at C_3, C_5 and C_6.

C_6-L configuration, C_3 and C_6 chiral centers are trans to each other.

All synthetic and semi synthetic penicillin have same absolute configuration that of natural

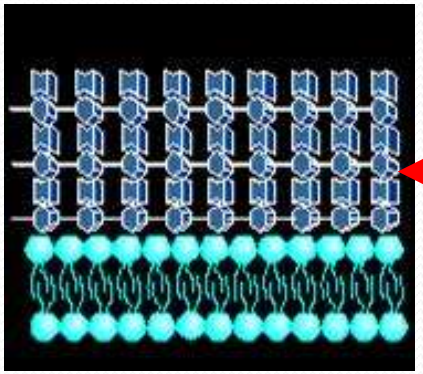
3S:5R: 6R

The bacterial cell wall



Gram –

Only two layers of peptidoglycan



Gram +

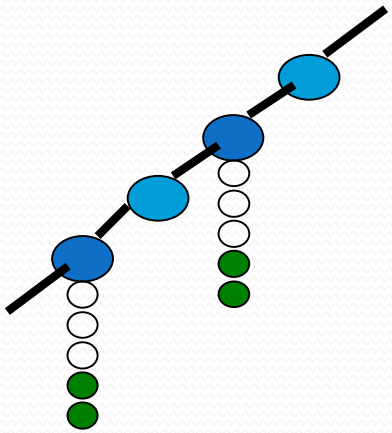
Consists of 50-200 peptidoglycan layers

Peptidoglycan

N-acetylglucosamine (NAG)

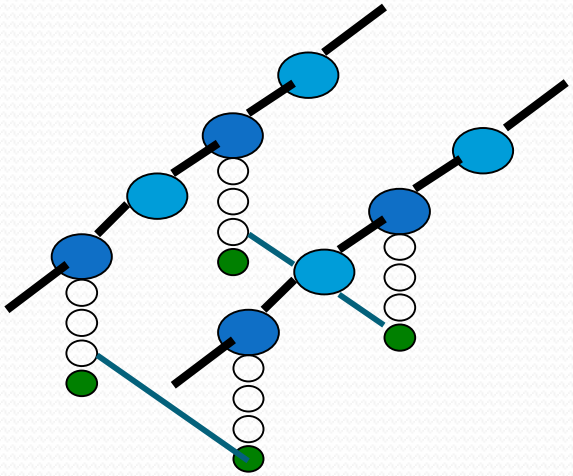
N-acetylmuramic acid (NAM)

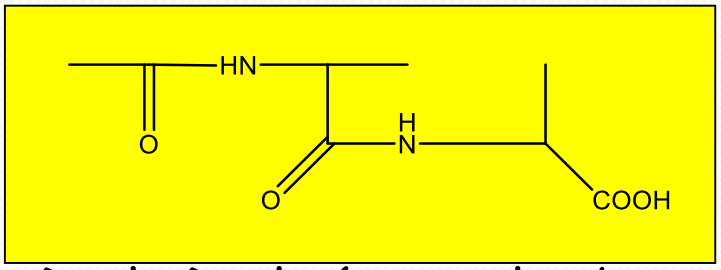
D-alanine



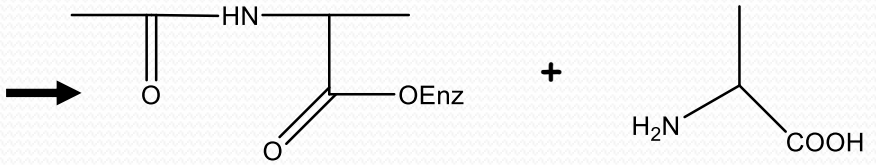
Transpeptidase

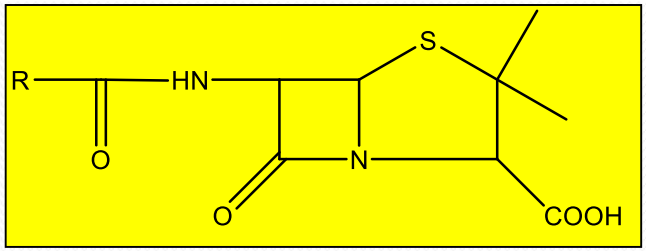
Involved in cross-linking



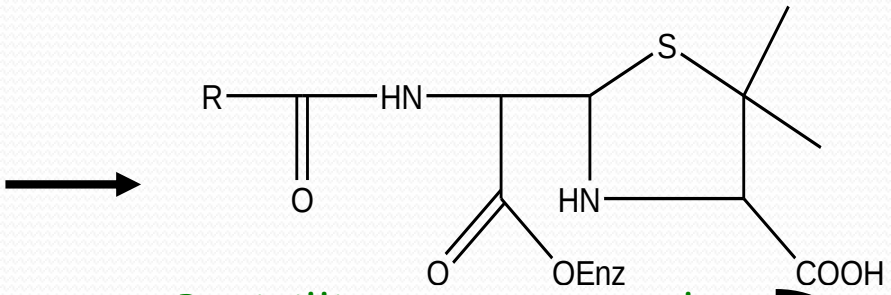


D-ala D-ala (natural substrate)





Penicillins



Penicillin-enzyme complex

cross-linking inhibited

The wall become fragile and can no longer prevent the cell from swelling and bursting

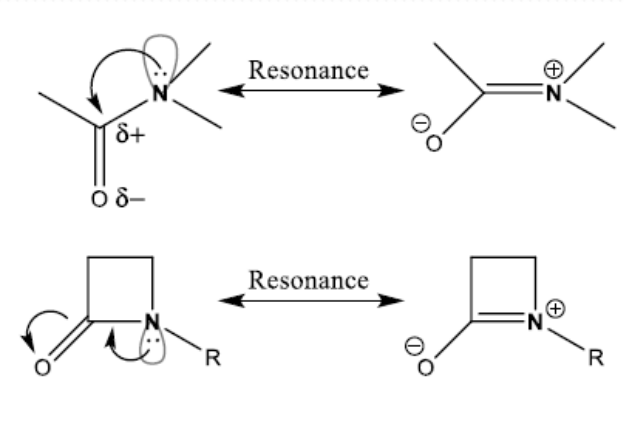
Bacterial cell lysis

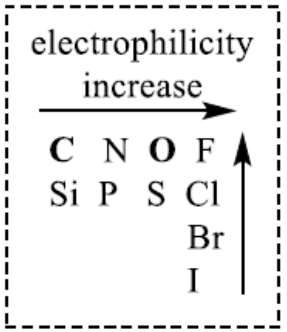
Excellent selective toxicity

- Penicillin mimic the structure of D-ala-D-ala, because of that the transpeptidase mistakenly bind to it instead of D-ala-D-ala.
- Also this explains the lack of penicillin toxicity, since D-amido acids are not present in human, only the L-amino acids present.
- Also targeting the cross linking in the peptidoglycan biosynthesis which is only present in bacteria explains the selective toxicity.

chemical properties of β-Lactam ring:

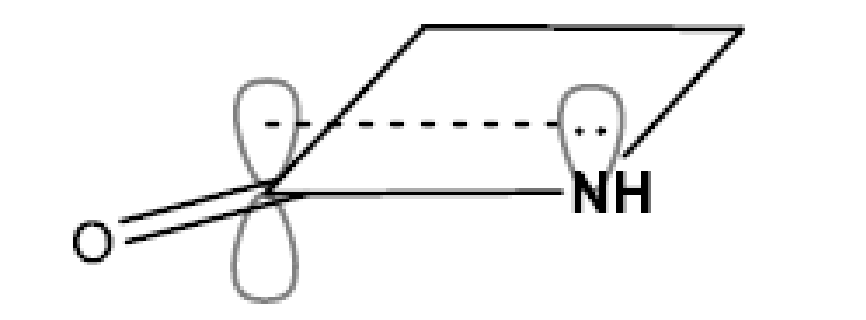
- In the original amide group (Linear amide), the Nitrogen atom that is beside the carbonyl group has a pair of unshared electrons which causes the Resonance between the N and the Carbonyl group (as we see in the periodic table, the O is more electronegative/electrophilic than C so the O in the carbonyl group withdraws electrons to have partial negative charge and C partial positive charge) So this carbonyl system is "Di-polar" and withdraws electrons from N.
- this Resonance is also applied for isolated β-Lactam ring (alone, no other substitutions) and the pair of electrons of N are not far from the carbonyl group



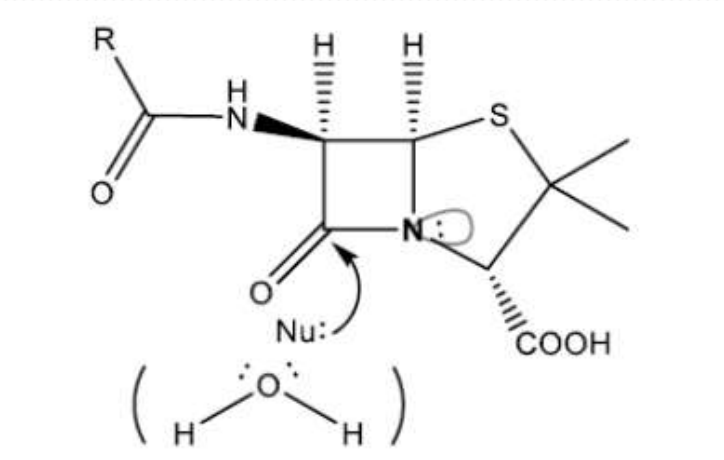


chemical properties of β-Lactam ring:

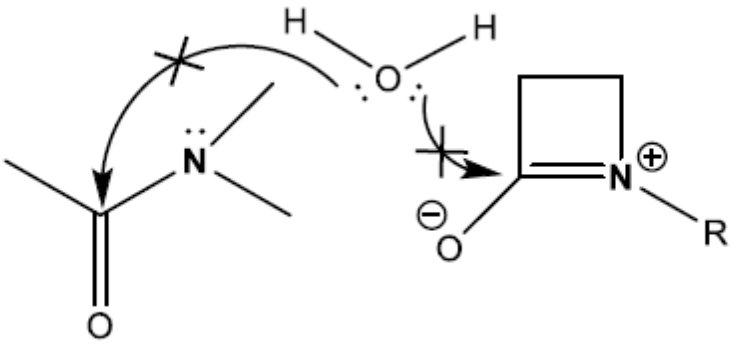
- Look at the pair of electrons of N in the β-Lactam ring alone
- (**NOTE** that the empty orbital of carbonyl group accepts the pair of electrons of N to make resonance)



- BUT when the β-Lactam is involved in the Penam system, and look at it in 3D view,
- the pair of electrons of nitrogen is far from the carbonyl group because the
- Thiazolidine ring bends this pair of electron (**Off-setting**) and the N becomes
- pyramidal (very close to the amines).
- So the Penam is completely different from amide, the carbonyl group is very electrondeficient and easily attacked by a nucleophile such as water (**No resonance** to make this carbonyl rich in electrons and resistant to hydrolysis as in amides that need enzymes to be hydrolyzed).



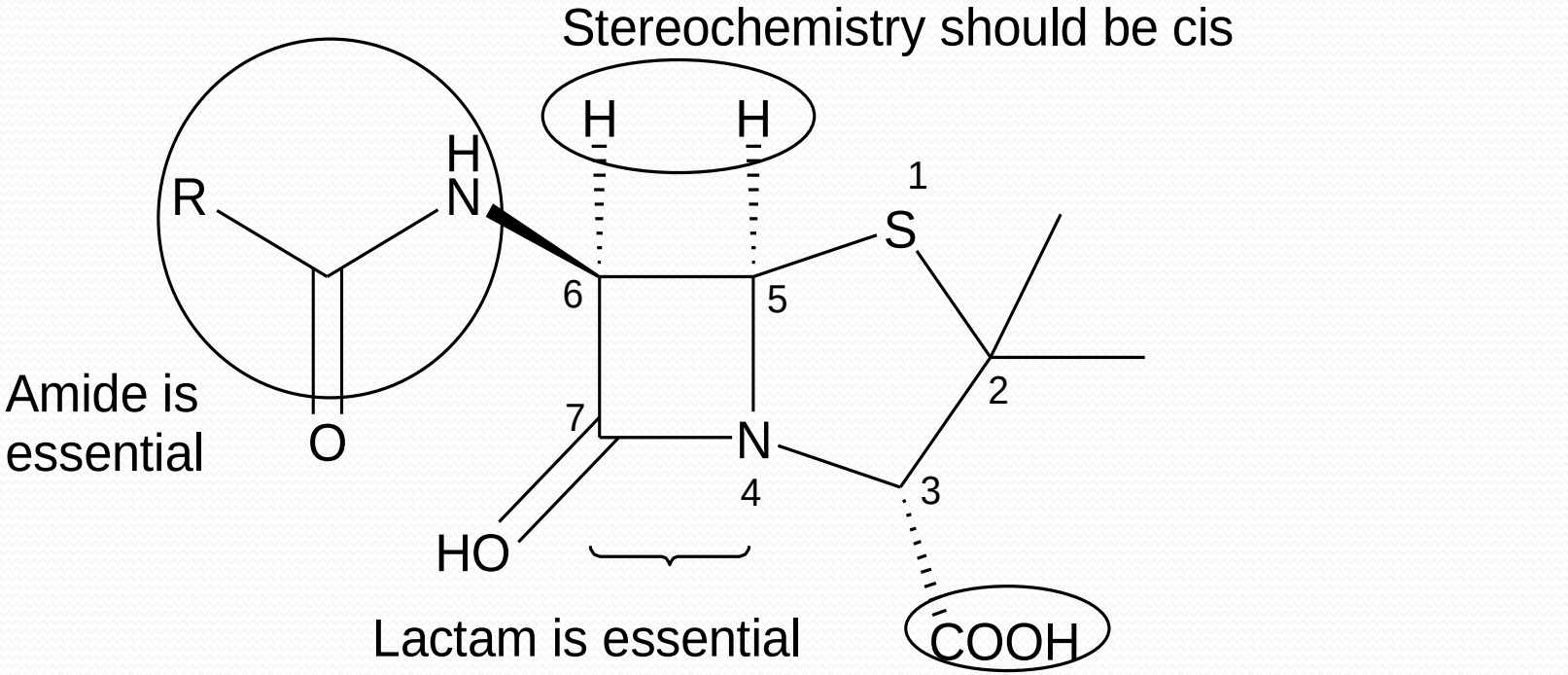
- **NOTE**: the β-Lactam alone is also resistant to hydrolysis as the amides due to
- resonance but easier than amides (the Nucleophilic attack is more easy).
- For that reason, when one of the penicillins is given orally as solution, it can be used only for one week after dissolving in water due to hydrolysis and must be stored in the fridge to slow down the hydrolysis rate by water (which is a nucleophile, contains two pairs of electrons).
- So all penicillins are unstable in water and tend to breakdown quickly in the aqueous conditions, and for that reason mostly these preparations are made as suspensions not solutions to reduce the water activity. This is also applied for parental penicillins that are given as re-constitutable powders.



Structure-activity relationships of penicillins (SAR)

- The strained β-lactam ring is essential.
- The free carboxylic acid is essential (the carboxylate ion binds to the charged nitrogen of the lysine at the active site.
- The bicyclic system is essential.
- The acylamino side chain is essential.
- Sulfur is not essential.
- The stereochemistry of the bicyclic ring with respect to the acylamino side chain is important.

Structure-activity relationships of penicillins



Bicyclic system is essential

Reasons for the acid sensitivity of penicillin G:

- Ring strain: due to the large angle and torsional strain exist, acid catalyzed ring opening will relief these strains.
- A highly reactive β-lactam carbonyl group:
- This amide bond is exceptionally unstable compared to the normal amide, because it is a 4-membered ring this will increase the angular and torsional strain

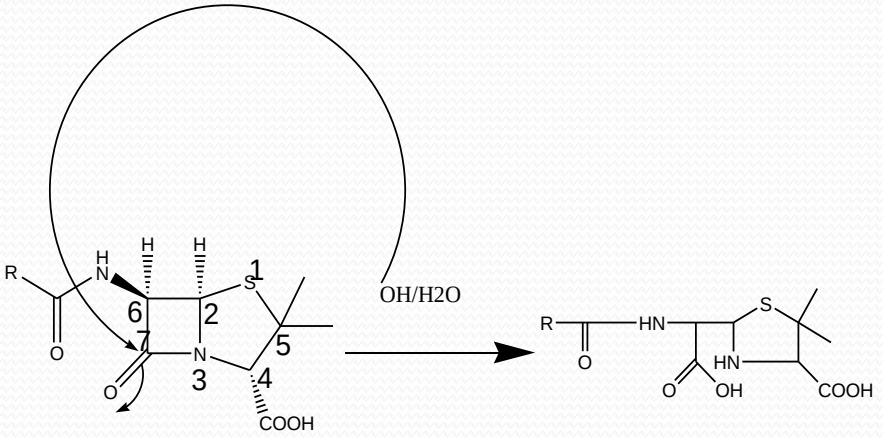
Chemical degradation and properties

- The early penicillins were yellow to brown in color and very unstable so refrigeration was required to maintain activity for short time
- Unpleasant taste
- Strongly dextrorotay
- Most penicillins are acidic Pka=2.5-3.0 some are amphoteric
- The free acids are not suitable for parentral or oral administration. Na and K salts are suitable to allow both oral and parentral administration.
- Some salts of penicillins with organic bases such as Benzathine, Procaine and Hydrabamine have limited water solubility and so they are suitable as depot to provide effective conc. Of penicillin for long time to treat chronic infections

Chemical reactivity

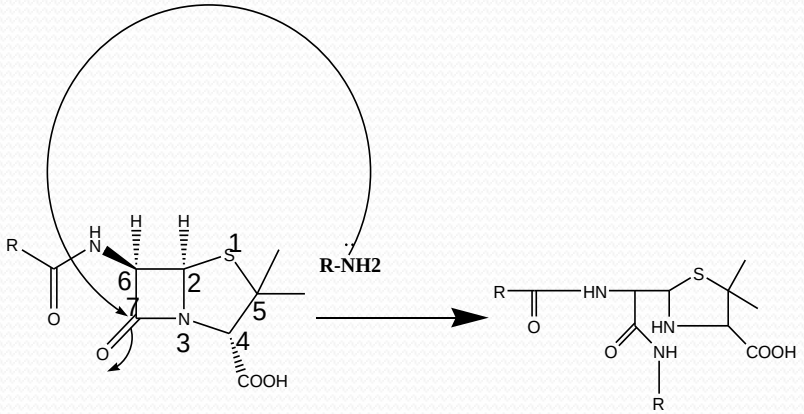
1. reactions with nucleophiles –OH, H_2O, NH_2-OH, R-NH_2, R-OH, and body proteins

- 1. reaction with –OH



Penicilloic acid
In active, stable in alkaline, not satble in acid

2. Reaction with alkyl amines

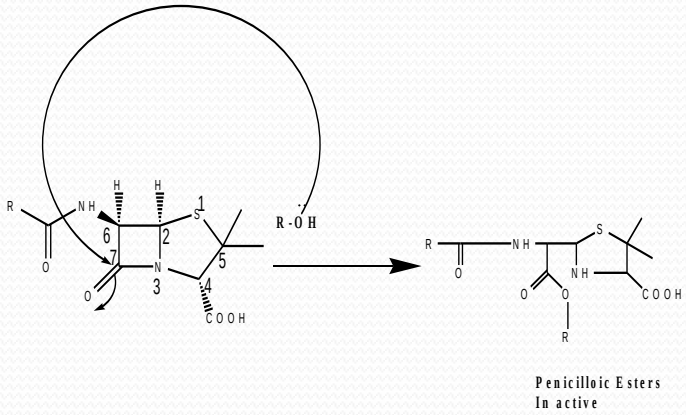


Penicilloic Amid
In active

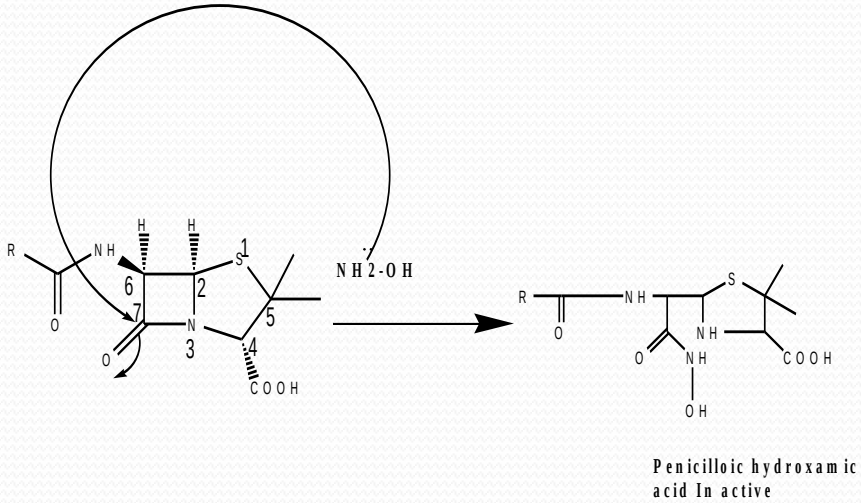
Chemical reactivity

1. reactions with nucleophiles –OH, H_2O, NH_2-OH, R-NH_2, R-OH, and body proteins

- 3. reaction with Alcohol



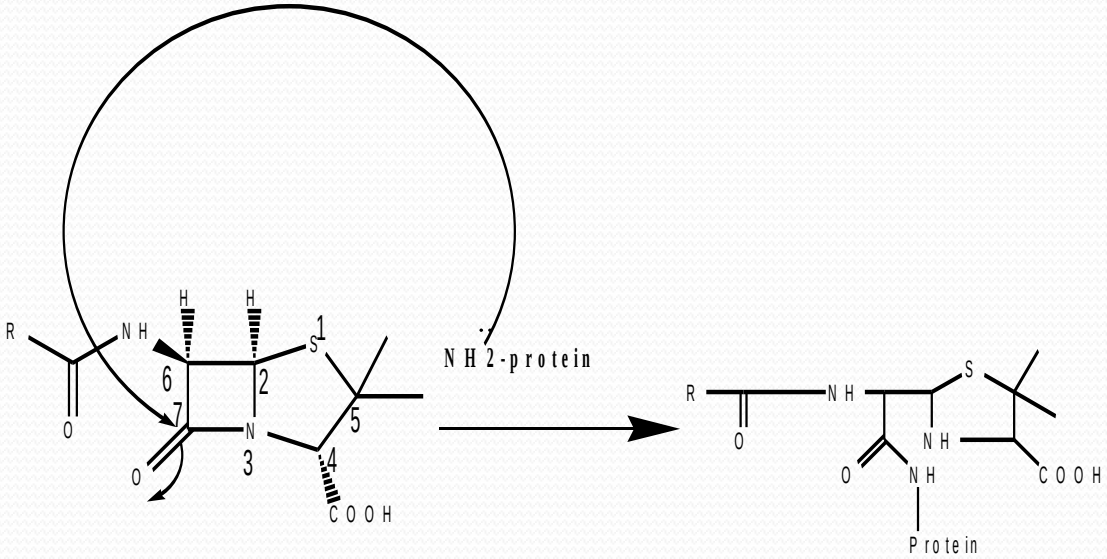
4. Reaction with hydroxyl amine



Chemical reactivity

1. reactions with nucleophiles –OH, H_2O, NH_2-OH, R-NH_2, R-OH, and body proteins

- 5. reaction with body proteins: the nucleophilic attack on B-lactam rings by body proteins (specific) generate penicilloyl proteins that are suspected to be the reason for the allergic reactions to penicillins

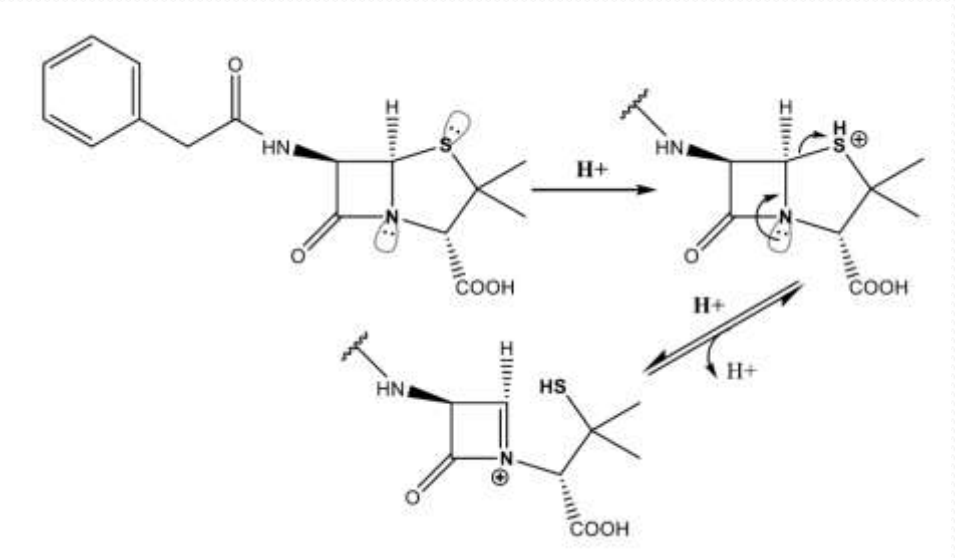


Chemical reactivity

2. reactions with Acids

- In strongly acidic media (PH<3), penicillins undergo complex series of reactions as in the following:
- First step (reversible)

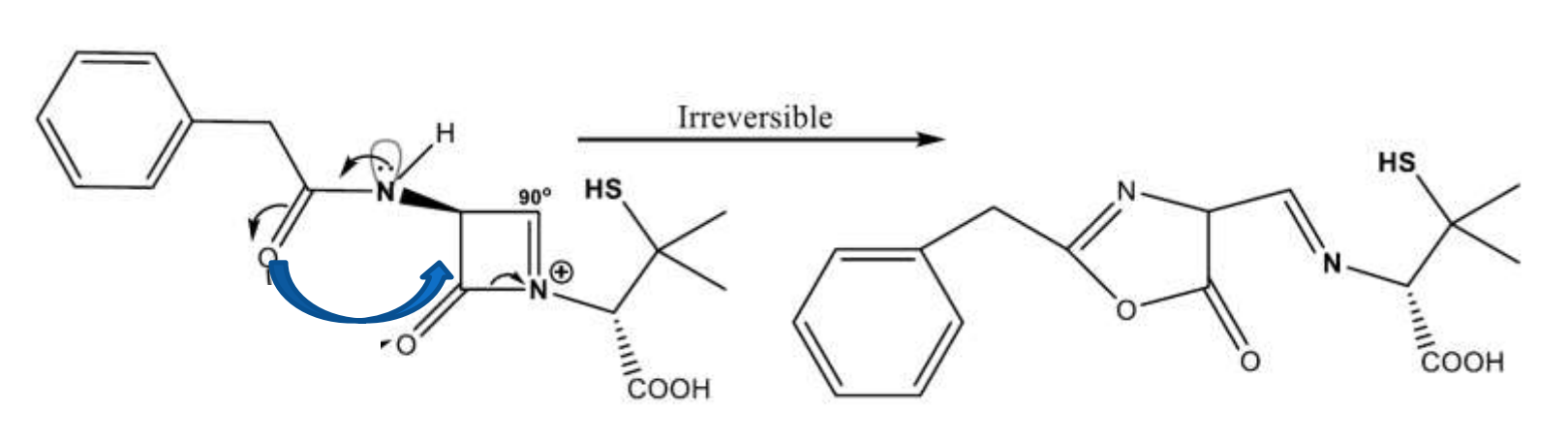
protination of the sulfur atom will occur then a partial positive charge will be formed on S and becomes a good leaving group, the pair of electrons of N will move causing the structure to become with a thiol group and a double bond, and the N becomes quaternary with a positive charge.



Chemical reactivity

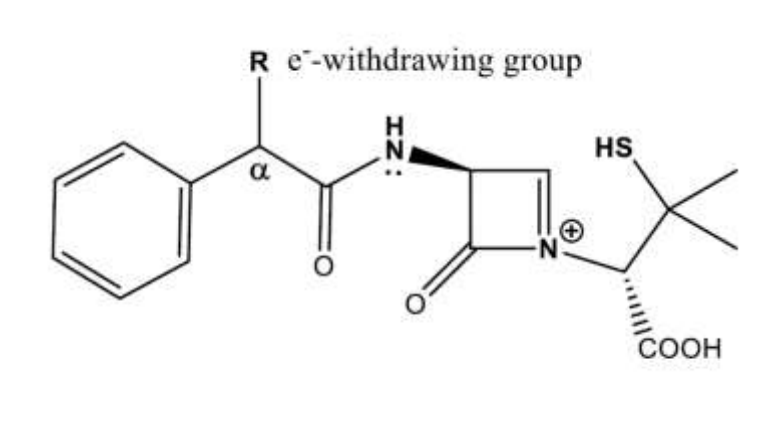
2. reactions with Acids

- Second step (irreversible)



the oxygen becomes a strong nucleophile

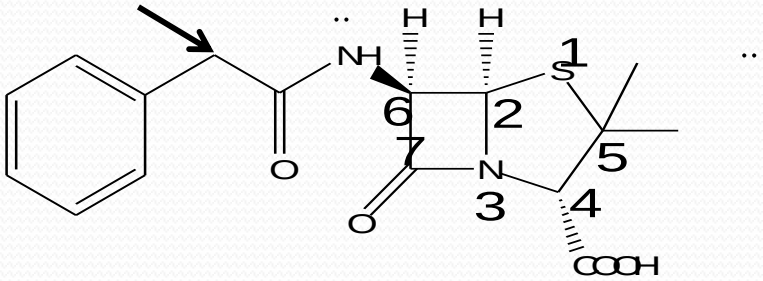
- And by knowing this mechanism, we can actually make "**Orally active penicillins“ How?**
- if we take the electrons from this oxygen and prevent this attack we will prevent this step, then you have to add an electron withdrawing group at the α carbon to the amidic carbonyl (oxygen, amine).



Acid sensitivity of penicillins

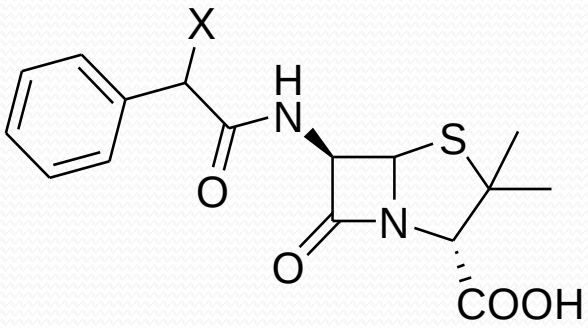
- Electron withdrawing groups in the α-position of Benzyl penicillin will improve acid stability clearly

α-position

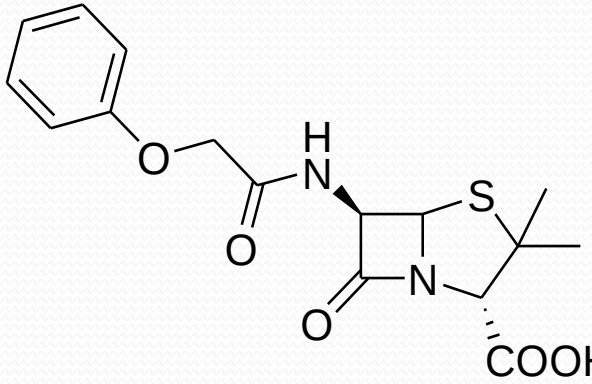


Acid sensitivity of penicillins

Accordingly the following compounds are significantly more stable than Benzyl penicillin:

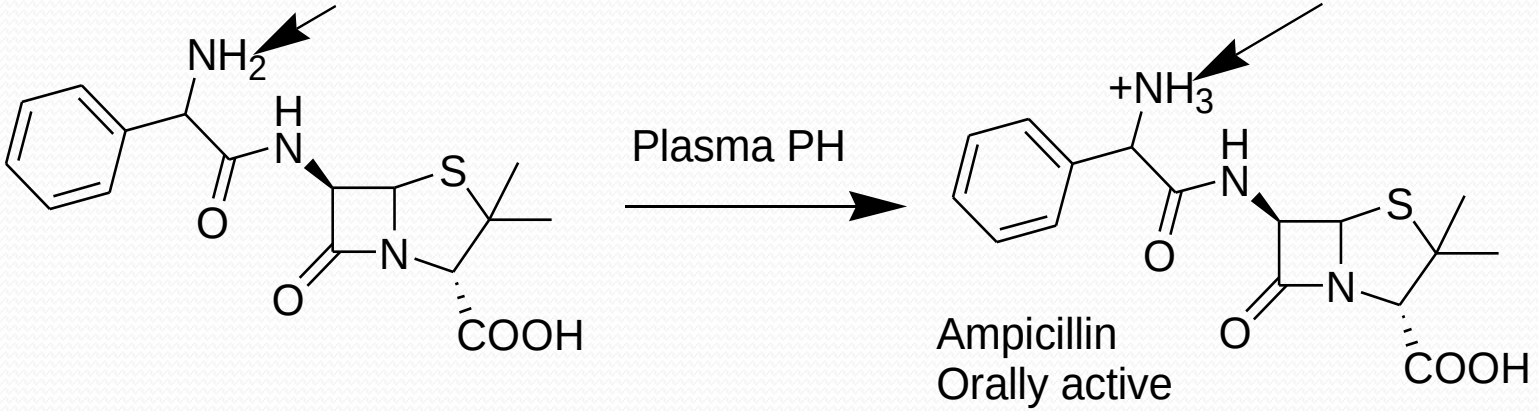


alpha - Halo benzyl penicillin (X=Cl, Br, I)



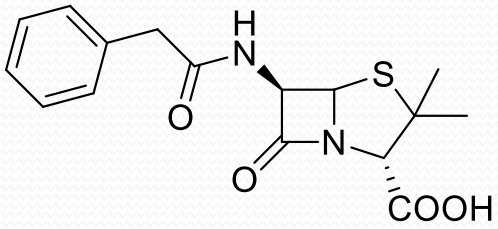
Phenoxy methyl penicillin: Penicllin V
Acid resistant
can be given orally

In plasma PH NH2 will become ionized to NH3+ so it will become electron withdrawing

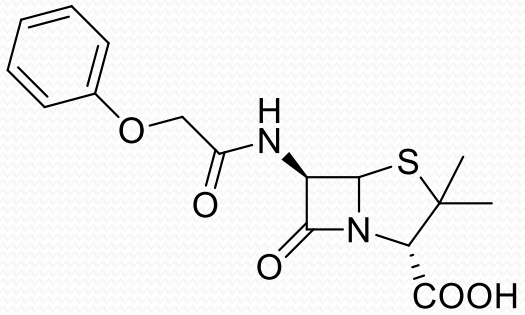


Ampicillin
Orally active

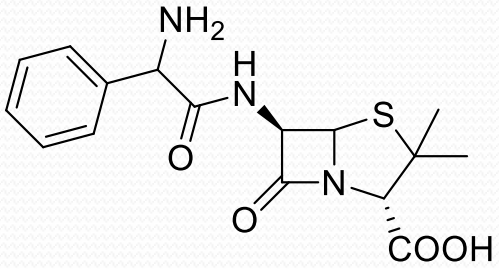
Acid resistant Penicillins



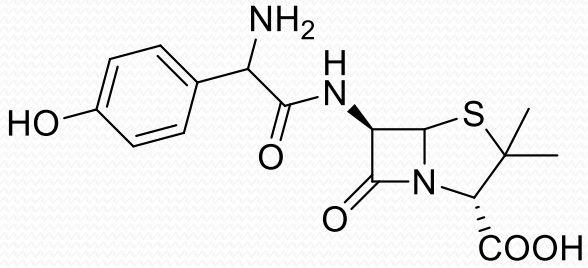
Penicllin G
Acid labile
Can not be given orally



Penicllin V
Acid resistant
can be given orally



Ampicillin
Acid resistant
Orally active



Amoxicillin
Acid stable
Orally active

Amoxicillin: given once each 8 hours (longer half life).
Ampicillin: given once each 6 hours

- The half-life of amoxicillin is 61.3 minutes. Approximately 60% of an orally administered dose of amoxicillin is excreted in the urine within 6 to 8 hours. Detectable serum levels are observed up to 8 hours after an orally administered dose of amoxicillin. Since most of the amoxicillin is excreted unchanged in the urine, its excretion can be delayed by concurrent administration of probenecid
- The half-life of amoxicillin is almost one hour (60 minutes)
- But the % absorption of intact drug of Ampicillin is 30-50% meanwhile The % absorption of Amoxicillin is 75-90%

Bacterial Resistance:

- Two types:
- 1. Natural (innate) resistance, this is particularly important in gram negative (G-) bacteria mediated by the reduced permeability of the outer cell envelope of Gram negative bacilli which is linked to the cell wall via the peptidoglycan, such cell envelope is not present is gram positive bacteria
- 2. Other normally resistant bacteria can develop resistance by generating resistance enzymes by mutation or natural selection

Bacterial Resistance:

- The second type of enzymatic resistance is the most common resistance mechanism.
- The resistant enzymes are collectively known as “ penicillinases” and are of two general types:
- 1. B-lactamases (most important)
- 2. Acylases

β-lactamase (Penicillinase):

- β-lactamase break the β-lactam ring, they are either chromosomal or plasmid, constitutive or inducible depending on particular species:
- Gram +ve *S.aureus: 1. inducible* β-lactamase

2. synthesized at cell wall and released extracellularly

95% of *S.aureus* became resistant to penicillins

- Gram –ve bacillic 1. constitutive R-factor

2. Cytoplasmic enzymes

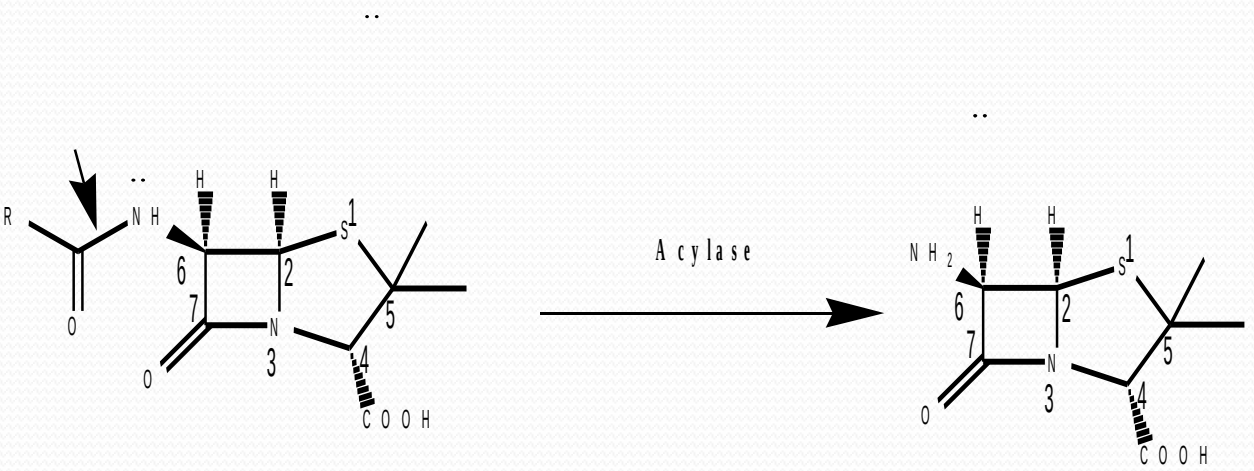
Again β-lactamase from different species are different in structure and properties.

β-lactamase (Penicillinase):

- Gram +ve bacteria normally release β-lactamase to outside of the cell that will cleave penicillin before reaching the bacteria.
- Gram –ve bacteria release β-lactamase into the periplasmic space, which again will cleave penicillin before reaching the plasma membrane.
- Penicillin has to reach the plasma membrane where the transpeptidase present to do its antibacterial action.
- Most of gram –ve bacteria are β-lactamase producing bacteria

2. Acylases

- These enzymes can hydrolyze the acylamino-side chain of penicillins

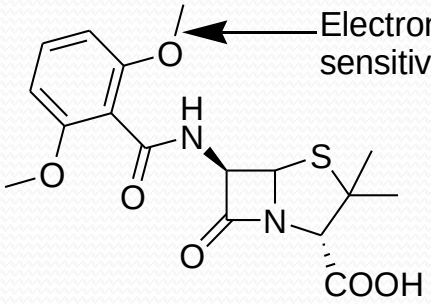


6-Amino penicillanic Acid (6-APA)

Rules to create Pinicillinase resistant Penicillins

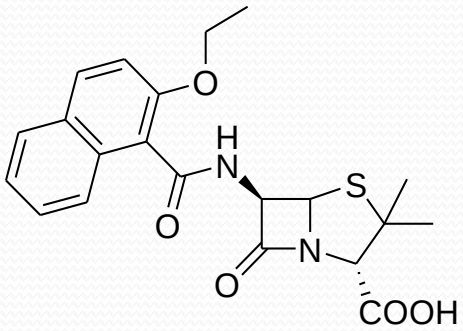
- Increase the steric bulk at the α-carbon on the acyl-amino group enhances good penicillins activity
- Antibacterial activity is enhanced when the α-carbon is part of aromatic ring, so based on these points we can conclude that Ortho substituted aromatic ring should produce excellent β-lactamase resistance
- See the following slide for examples.

β-lactamase resistant Penicillins



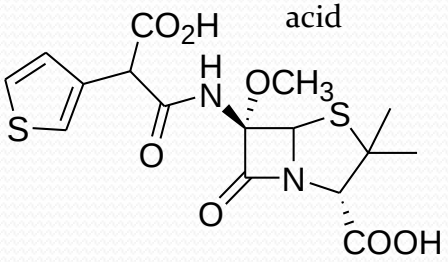
Electron donating groups (methoxy) so more sensitive to acid than penicillin G

Methicillin
β-lactamase stable
Acid sensitive (why?)



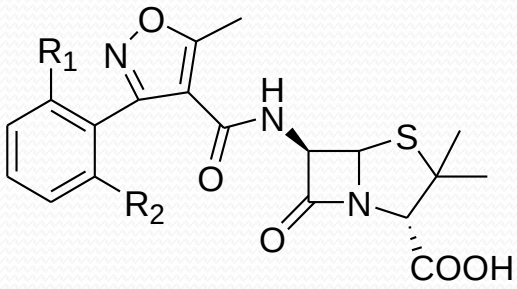
Nafcillin
β-lactamase stable
Acid sensitive

Ticarcillin Orally inactive, Broad spectrum and β-lactamase sensitive given with clavulinic acid



Temocillin

Isoxazoyl Penicillins



R_1, R_2 = H	Oxacillin
R_1 = Cl, R_2 = H	Cloxacillin
R_1 = Cl, R_2 = F	Flucloxacillin
R_1, R_2 = Cl	Dicloxacillin

Flucloxacillin: (how to think and analyze)

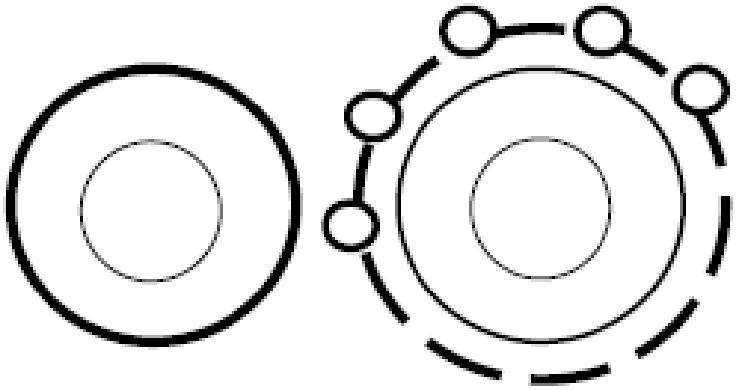
- Bulky which means β-lactamase resistant
- E-withdrawing which means acid stable
- α-carbon is part of an aromatic ring which means good activity

- Bulkier substituents are required for small sized heterocycles to give good anti-β-lactamase activity
- Acid stable due to the electron withdrawing effect of the isoxazole ring.
- Used against *S.aureus* resistant bacteria.
- Most penicillinase-resistant penicillins are less active than Penicillin G or Phenoxymethyl penicillin (V) against most non-β-lactamase procedures that are normally sensitive to penicillins, increasing α carbon bulkiness is with price
- Penicillinase-resistant penicillins tend to be bulky and lipophilic with poor penetration into Gram negative bacteria cell envelope

G+ve

-Thick cell wall

-Cell Membrane



G-ve

-Thinner cell wall

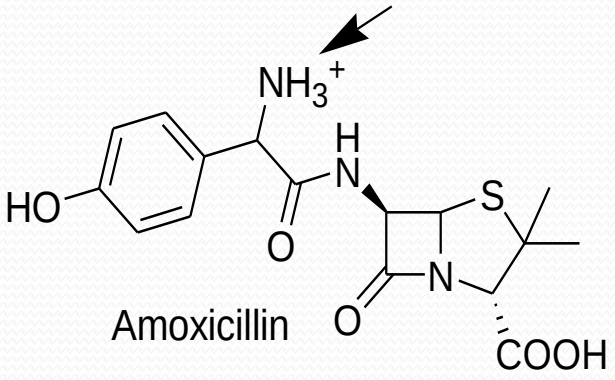
-Inner Cell Membrane

-Outer membrane with porins

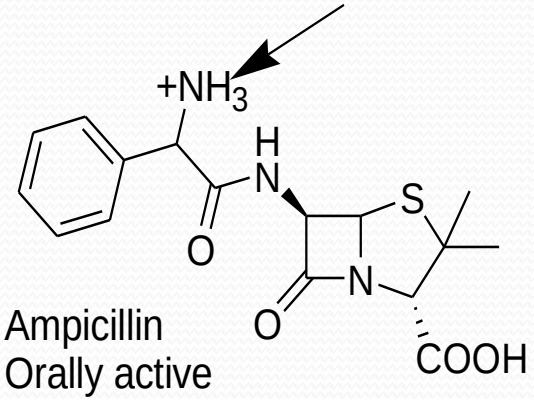
Broad Spectrum Penicillins

- Very important finding is the fact that substitution of α-carbon with polar or ionized group will produce wide spectrum of activity including Gram negative bacteria It works against (Pseudomonas , E.coli , Haemophilus influenza , …)

In plasma PH NH2 will become ionized to NH3+



Amoxicillin



Ampicillin
Orally active

Broad Spectrum Penicillins

Ampicillin and Amoxicillin are effective against Gram negative genera E.Coli, Klebsiella, Haemophila Salmonella, Shiegella and some proteus

Ampicillin and Amoxicillin largely retain Gram positive activity

D-isomer is more active than the L-isomer

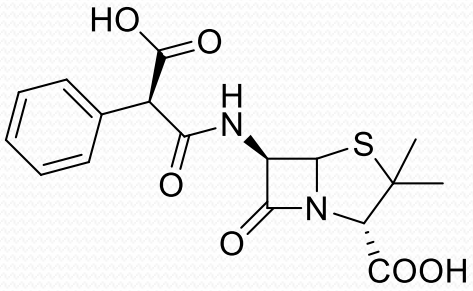
The extended activity of α-amino-benzyl-penicillin is not due to the anti-β-lactamase resistant activity rather it is due to the hydrophillic nature of the molecule which enables it to penetrate the outer cell-envelope through the porin channels

Broad Spectrum Penicillins

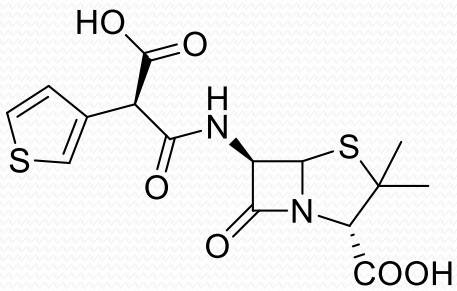
- α-OH also expand the activity but they are less active than the α-NH_2 derivatives and less acid-stable than the α-amino group
- α-COOH has wide spectrum activity including all the bacteria that are α-NH_2 sensitive as well as gram negative bacilli of the genera "**Pseudomonas,** Klebsiella, Enterobacter and Proteus" however its potency against Ampicillin-Sensitive bacteria is lower than Ampicillin

Carboxypenicillins

- They have a carboxylic acid at the α-carbon of the acyl side chain.
- They have broad spectrum activity.



Carbenicillin

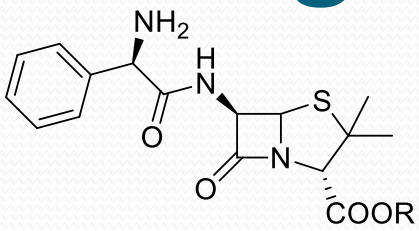


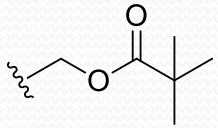
Ticarcillin

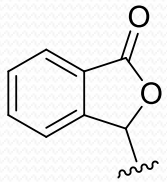
Ampicillin and amoxicillin prodrugs

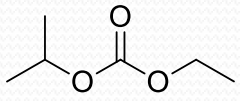
- Ampicillin and amoxicillin are Poorly absorbed through the mucus membrane, this is due to the fact that they formed a zwitter ionic molecule at physiological pH (they contain a carboxylic acid and an amino group in their structure.
  - The oral bioavailability can be improved by masking one of them, mainly the carboxylic acid…. By preparing a prodrug esters.

Ampicillin prodrugs

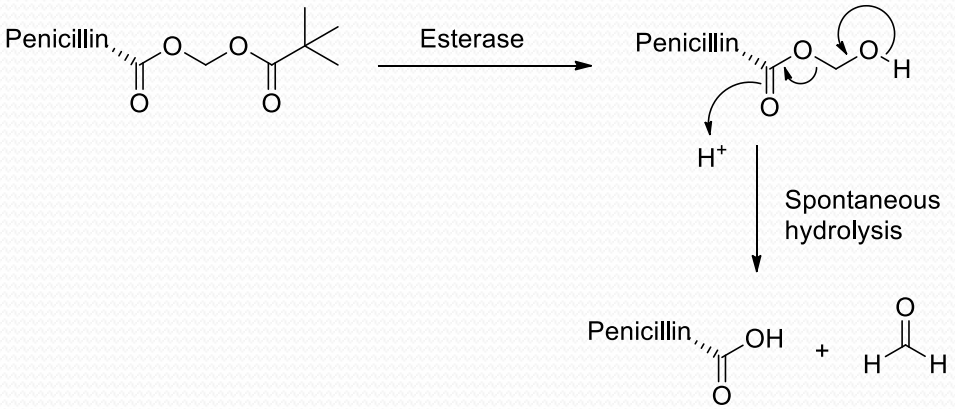


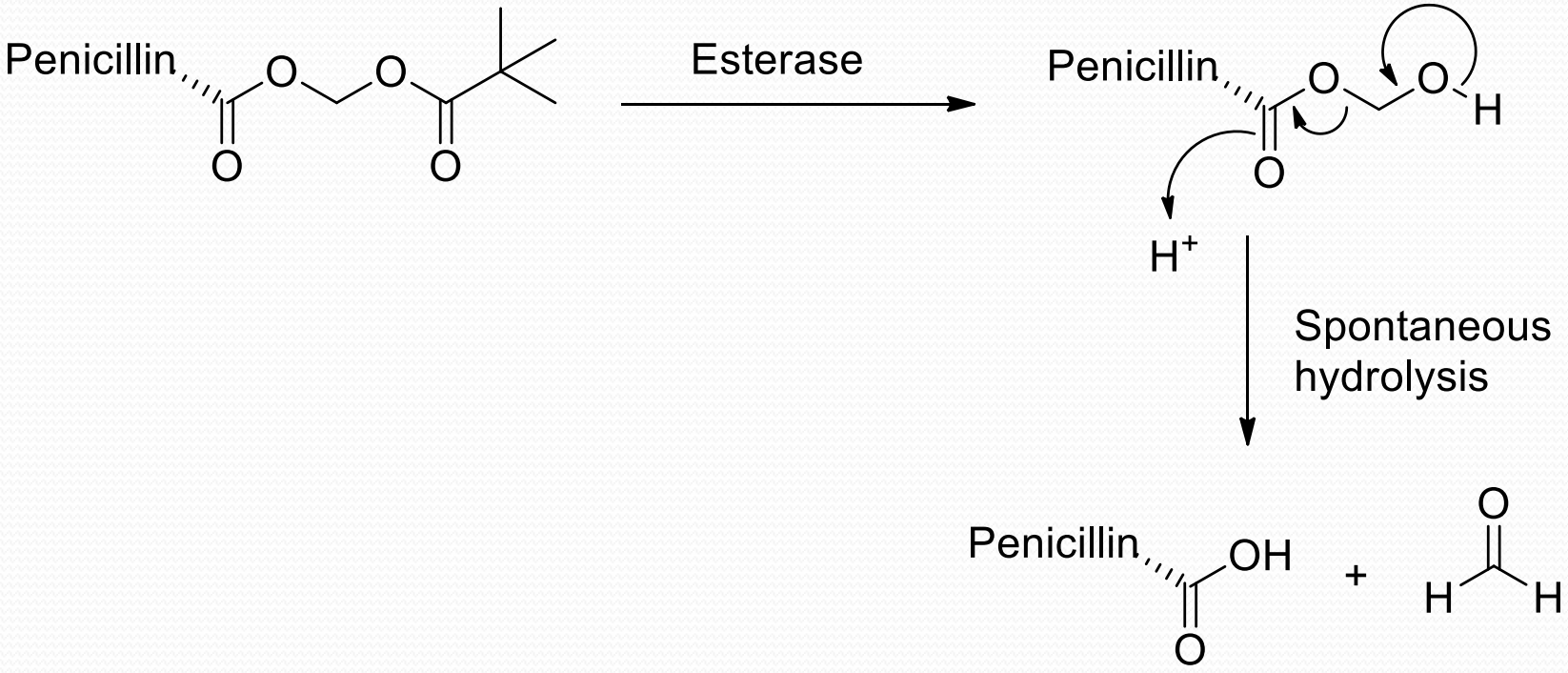
R =  Pivampicillin

R =  Talampicillin

R =  Bacampicillin

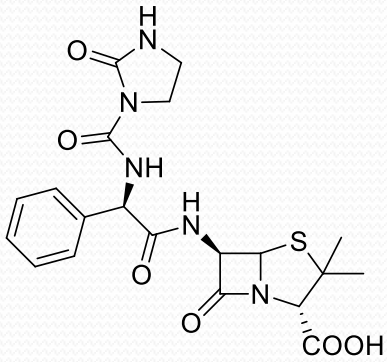
- The methyl ester did not give the same improvement in absorption and activity (why?).



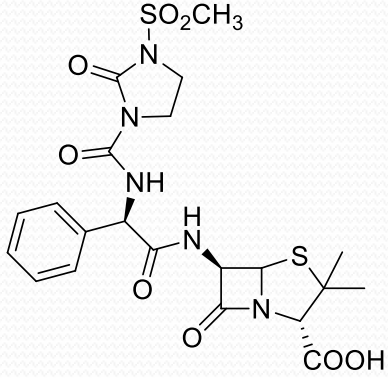

Penicillin
O
O
O
O
Esterase
Penicillin
O
O
H
O
H+
Spontaneous
hydrolysis
Penicillin
OH
O
+
O
H
H

Ureidopenicillins

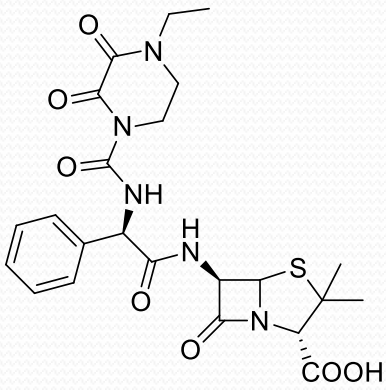
- They all have a urea group at the α-carbon in the acyl side chain.
- They have better activity compared to amoxicillin and they are more resistant to β-lactamase.
- Used parenterally for gram –ve infections especially *P.aeruginosa*.
- the ureido group though to mimic Some of the peptidoglycan structure, which means that it can bind to penicillin-binding protein



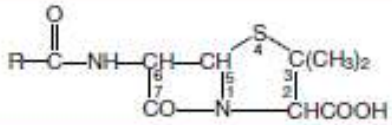
Azlocillin

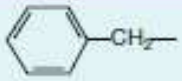
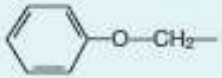
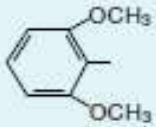
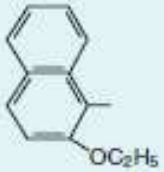
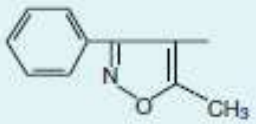
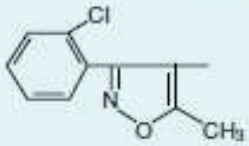
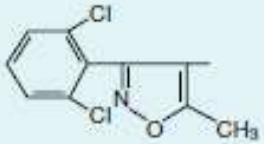
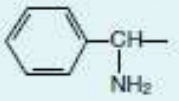


Mezlocillin



Piperacillin



Generic Name	Chemical Name	R Group
Penicillin G	Benzylpenicillin	
Penicillin V	Phenoxymethylpenicillin	
Methicillin	2,6-Dimethoxyphenyl-penicillin	
Nafcillin	2-Ethoxy-1-naphthyl-penicillin	
Oxacillin	5-Methyl-3-phenyl-4-isoxazolylpenicillin	
Cloxacillin	5-Methyl-3-(2-chlorophenyl)-4-isoxazolylpenicillin	
Dicloxacillin	5-Methyl-3-(2,6-dichlorophenyl)-4-isoxazolylpenicillin	
Ampicillin	D-α-Aminobenzyl-penicillin	

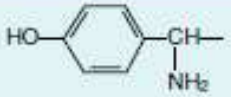
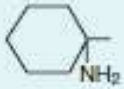
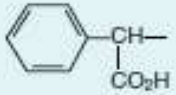
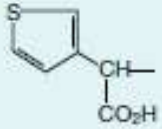
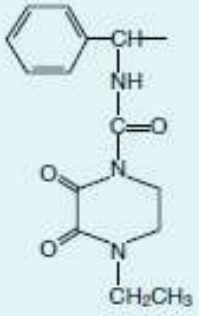
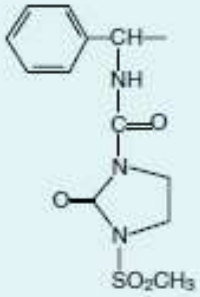
Generic Name	Chemical Name	R Group
Amoxicillin	D-α-Amino-*p*-hydroxybenzylpenicillin	
Cyclacillin	1-Aminocyclohexyl-penicillin	
Carbenicillin	α-Carboxybenzyl-penicillin	
Ticarcillin	α-Carboxy-3-thienyl-penicillin	
Piperacillin	α-(4-Ethyl-2,3-dioxo-1-piperazinylcarbonyl-amino)benzylpenicillin	
Mezlocillin	α-(1-Methanesulfonyl-2-oxoimidazolidino-carbonylamino)benzyl-penicillin	

TABLE 8.3 Classification and Properties of Penicillins

Penicillin	Source	Acid Resistance	Oral Absorption (%)	Plasma Protein Binding (%)	β-Lactamase Resistance (*S. aureus*)	Spectrum of Activity	Clinical Use
Benzylpenicillin	Biosynthetic	Poor	Poor (20)	50–60	No	Intermediate	Multipurpose
Penicillin V	Biosynthetic	Good	Good (60)	55–80	No	Intermediate	Multipurpose
Methicillin	Semisynthetic	Poor	None	30–40	Yes	Narrow	Limited use
Nafcillin	Semisynthetic	Fair	Variable	90	Yes	Narrow	Limited use
Oxacillin	Semisynthetic	Good	Fair (30)	85–94	Yes	Narrow	Limited use
Cloxacillin	Semisynthetic	Good	Good (50)	88–96	Yes	Narrow	Limited use
Dicloxacillin	Semisynthetic	Good	Good (50)	95–98	Yes	Narrow	Limited use
Ampicillin	Semisynthetic	Good	Fair (40)	20–25	No	Broad	Multipurpose
Amoxicillin	Semisynthetic	Good	Good (75)	20–25	No	Broad	Multipurpose
Carbenicillin	Semisynthetic	Poor	None	50–60	No	Extended	Limited use
Ticarcillin	Semisynthetic	Poor	None	45	No	Extended	Limited use
Mezlocillin	Semisynthetic	Poor	Nil	50	No	Extended	Limited use
Piperacillin	Semisynthetic	Poor	Nil	50	No	Extended	Limited use