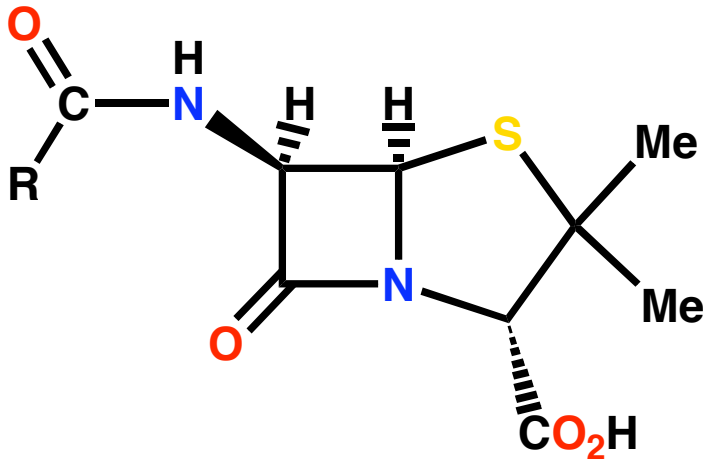
Topic 8-1 Antibacterial Agents

β-Lactam antibiotics-Chapter 16
Patrick

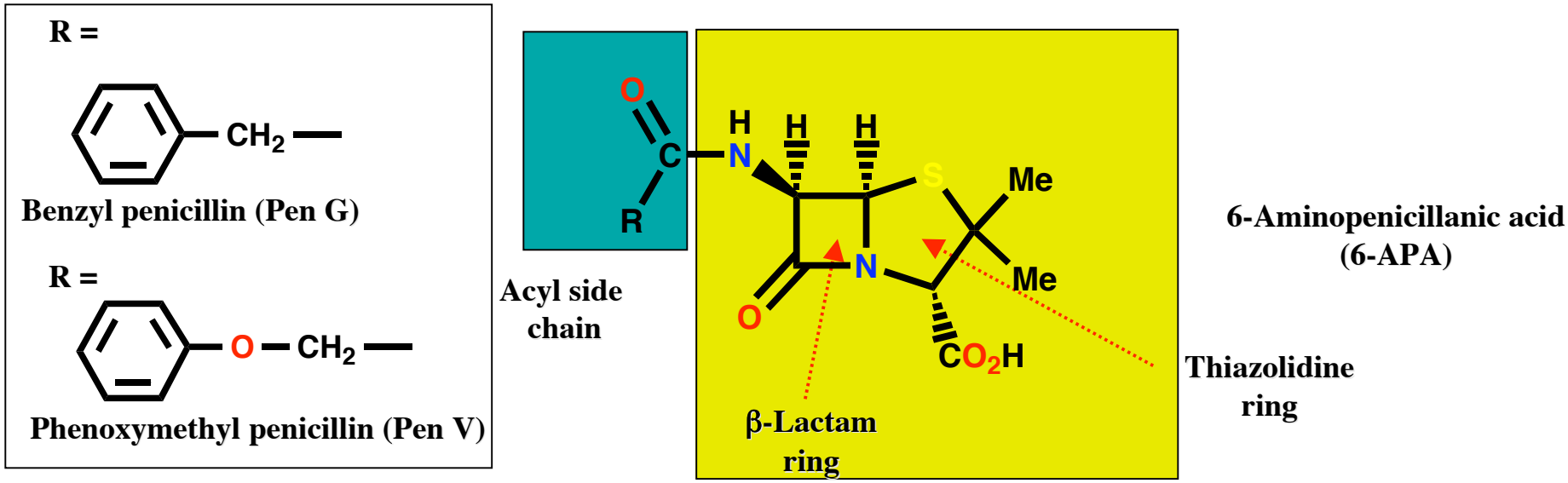
PENICILLINS



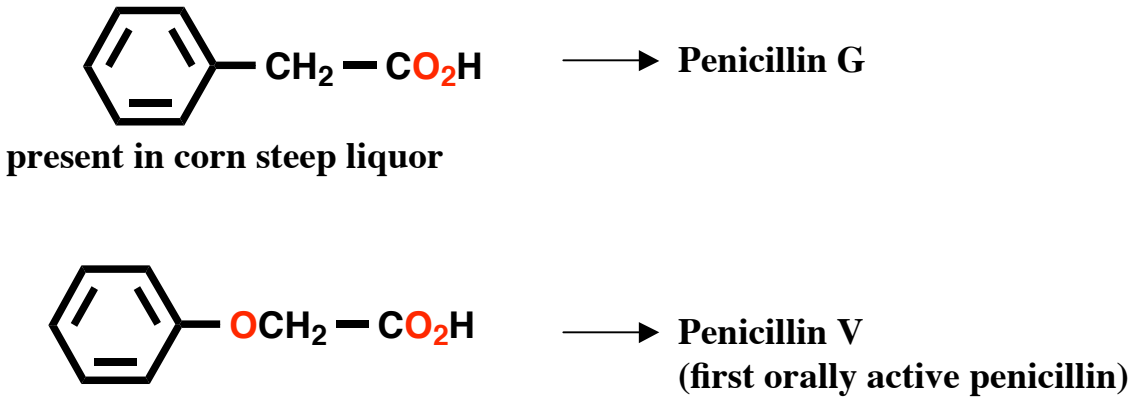
INTRODUCTION

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

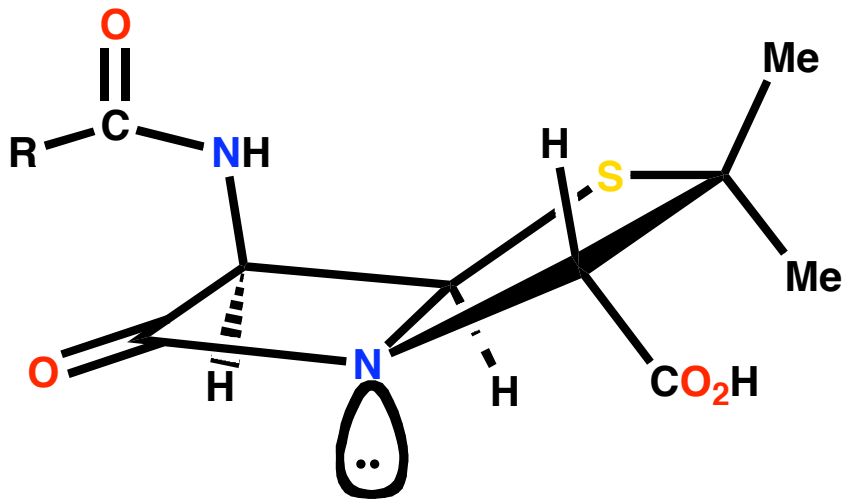
STRUCTURE



Side chain varies depending on carboxylic acid present in fermentation medium

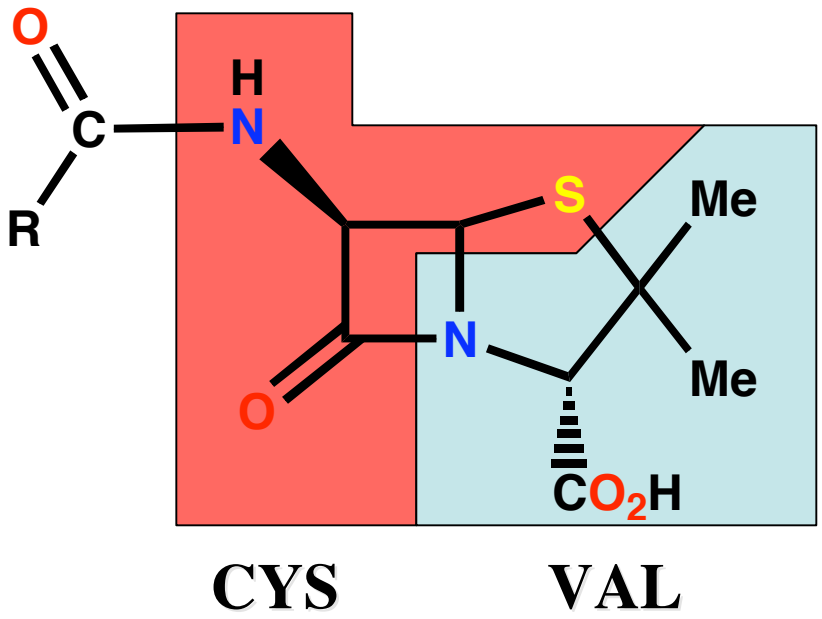


Shape of Penicillin G



Folded ‘envelope’ shape

Biosynthesis of Penicillins



Properties of Penicillin G

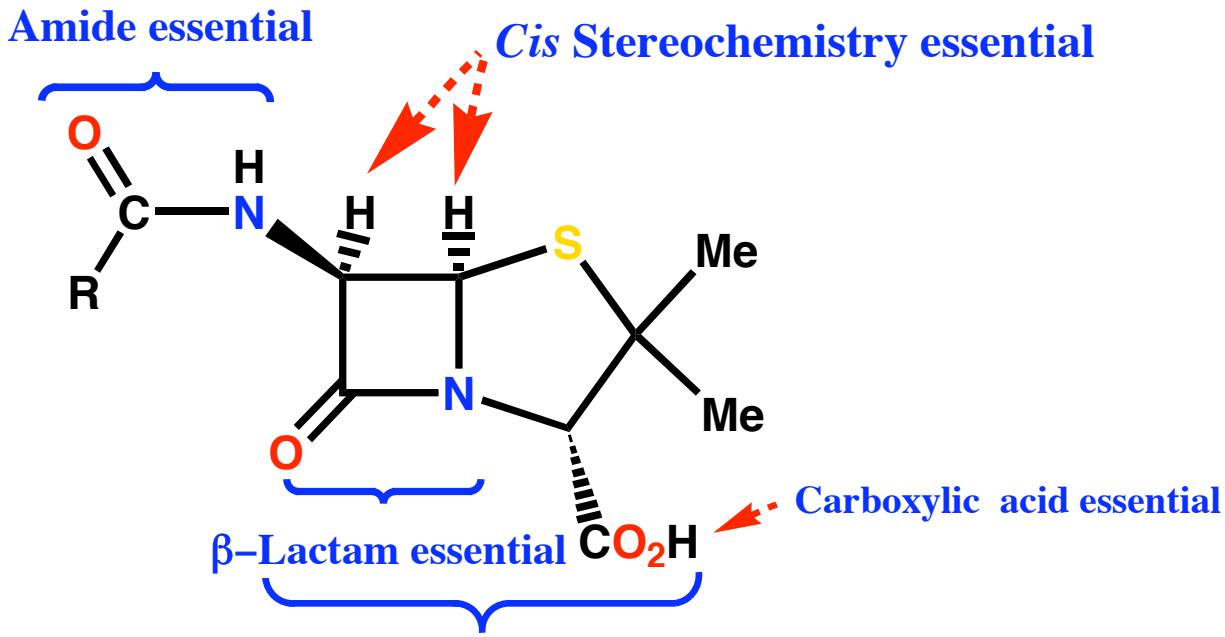
- Active vs. Gram + bacilli and some Gram - cocci
- Non toxic
- Limited range of activity
- Not orally active - must be injected
- Sensitive to β-lactamases
  (enzymes which hydrolyse the β-lactam ring)
- Some patients are allergic
- Inactive vs. *Staphylococci*

Drug Development

Aims

- To increase chemical stability for oral administration
- To increase resistance to β-lactamases
- To increase the range of activity

SAR

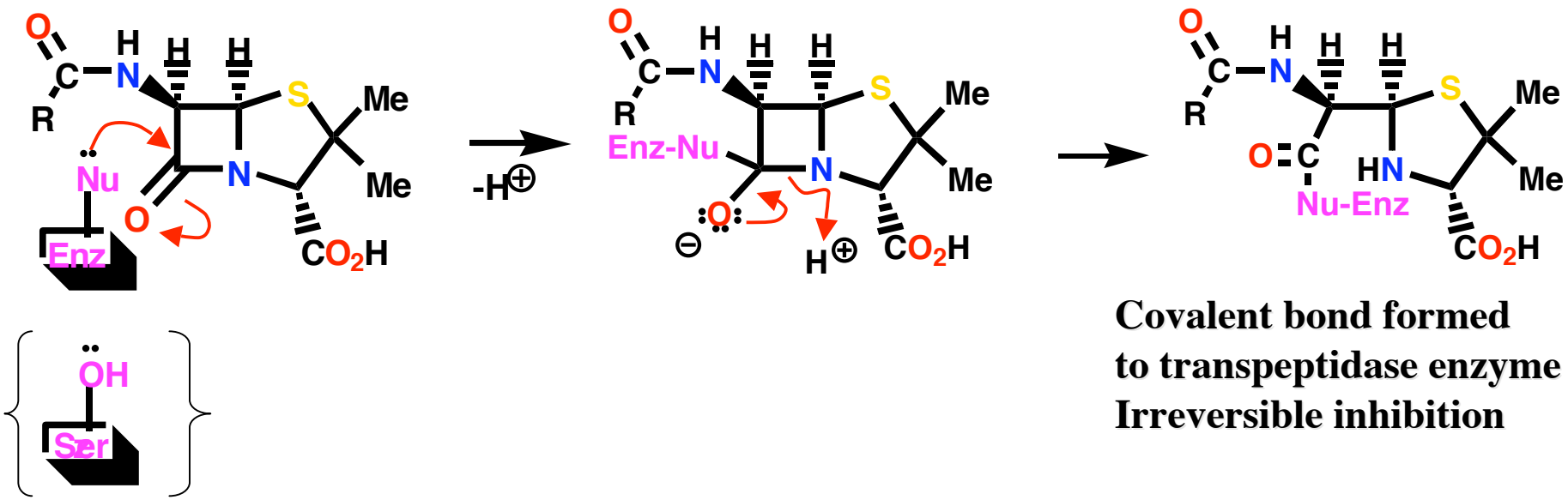


Conclusions

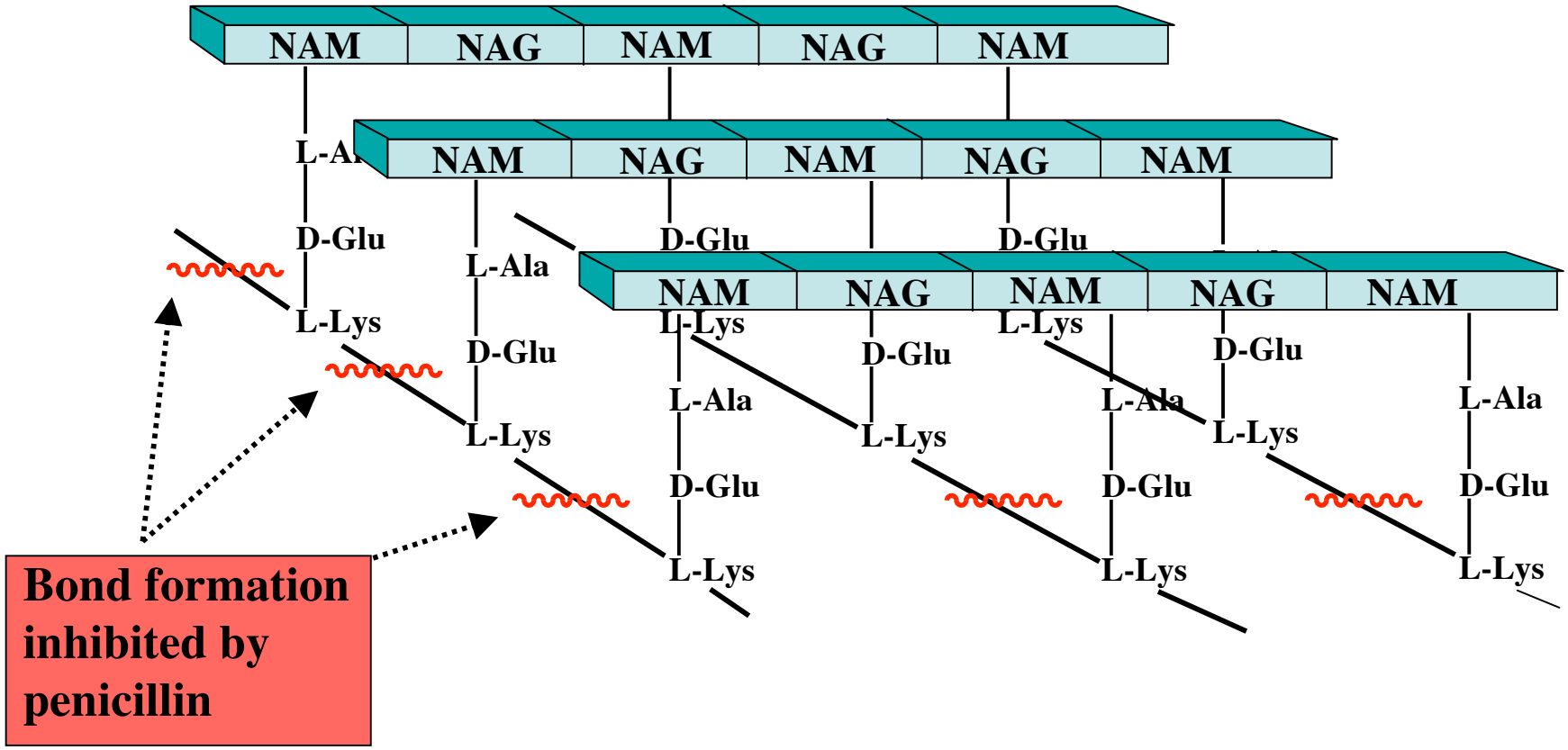
- Amide and carboxylic acid are involved in binding
- Carboxylic acid binds as the carboxylate ion
- Mechanism of action involves the β-lactam ring
- Activity related to β-lactam ring strain (subject to stability factors)
- Bicyclic system increases β-lactam ring strain
- Not much variation in structure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

Mechanism of action

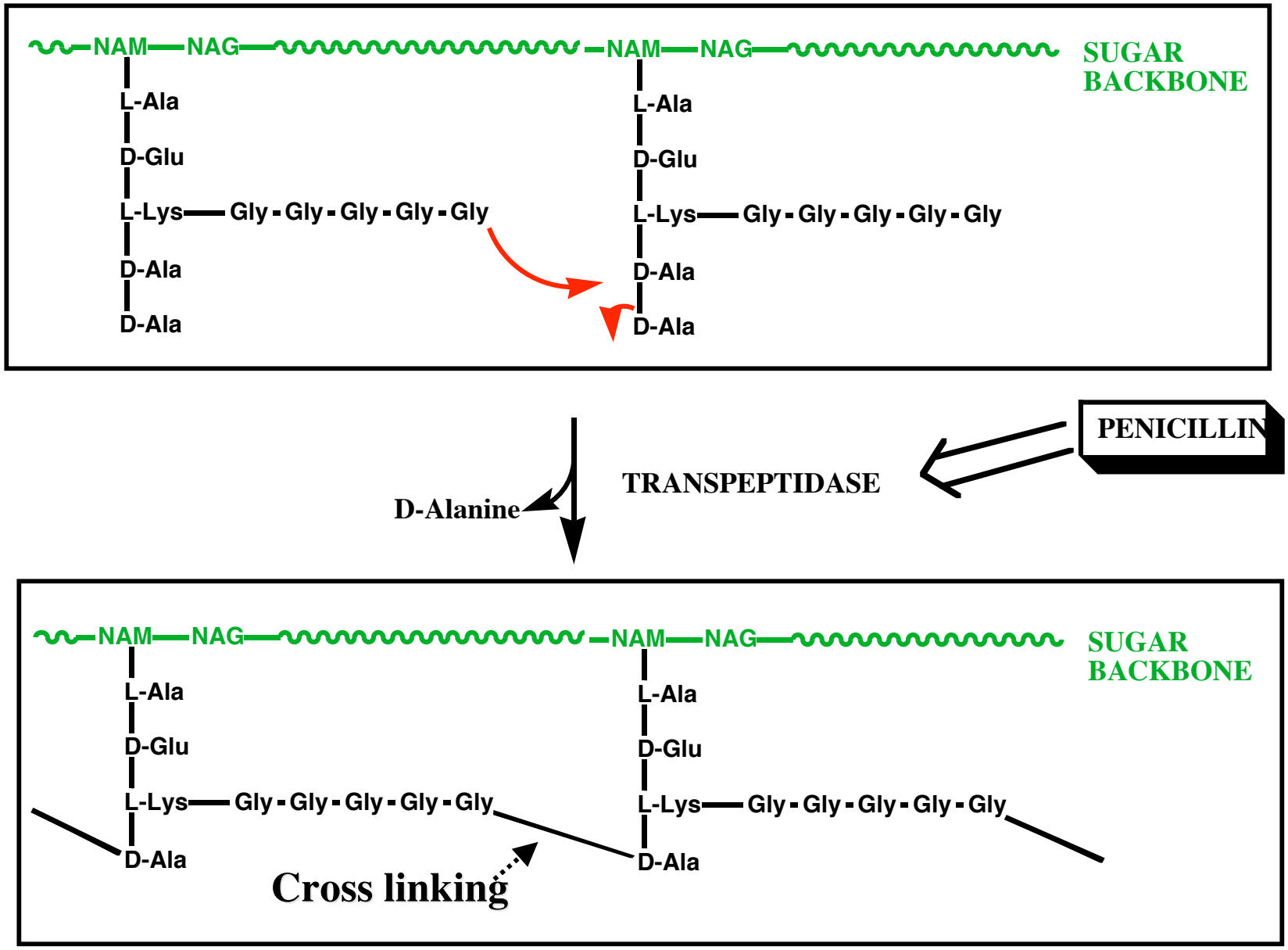
- Penicillins inhibit a bacterial enzyme called the transpeptidase enzyme which is involved in the synthesis of the bacterial cell wall
- The β-lactam ring is involved in the mechanism of inhibition
- Penicillin becomes covalently linked to the enzyme's active site leading to irreversible inhibition



Mechanism of action - bacterial cell wall synthesis



Mechanism of action - bacterial cell wall synthesis



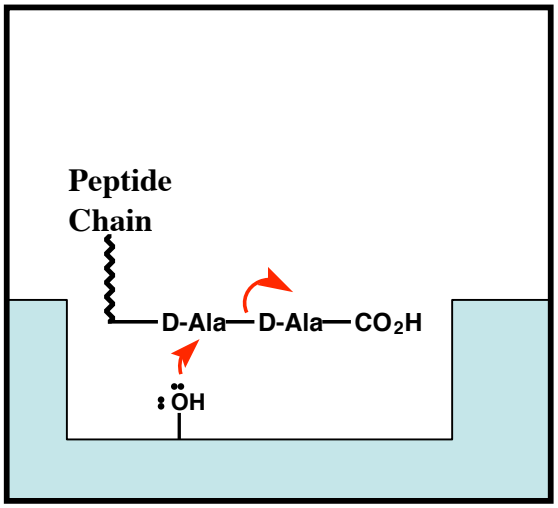
Mechanism of action - bacterial cell wall synthesis

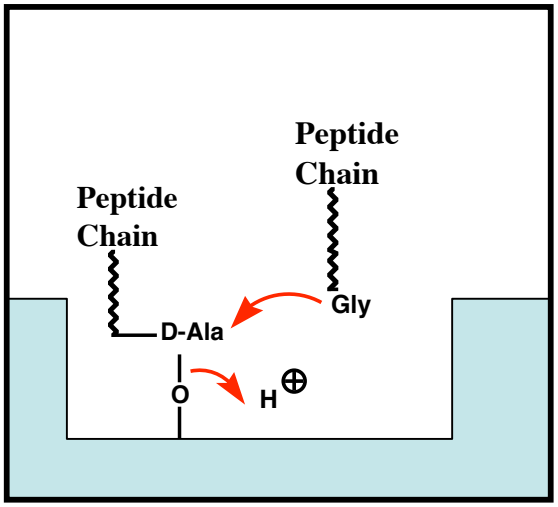
- Penicillin inhibits final crosslinking stage of cell wall synthesis
- It reacts with the transpeptidase enzyme to form an irreversible covalent bond
- Inhibition of transpeptidase leads to a weakened cell wall
- Cells swell due to water entering the cell, then burst (lysis)
- Penicillin possibly acts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

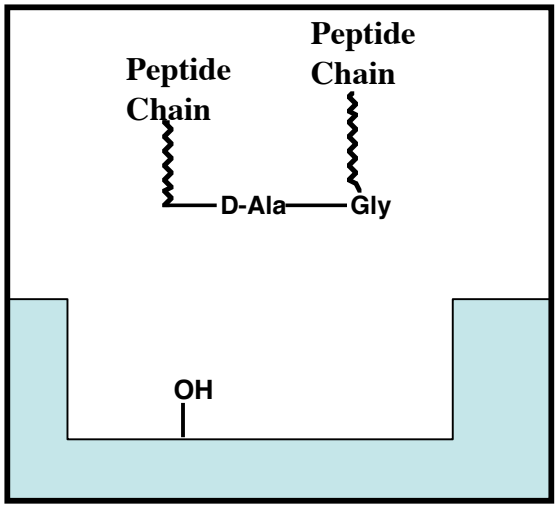
Mechanism of action - bacterial cell wall synthesis

Alternative theory- Pencillin mimics D-Ala-D-Ala.

Normal Mechanism



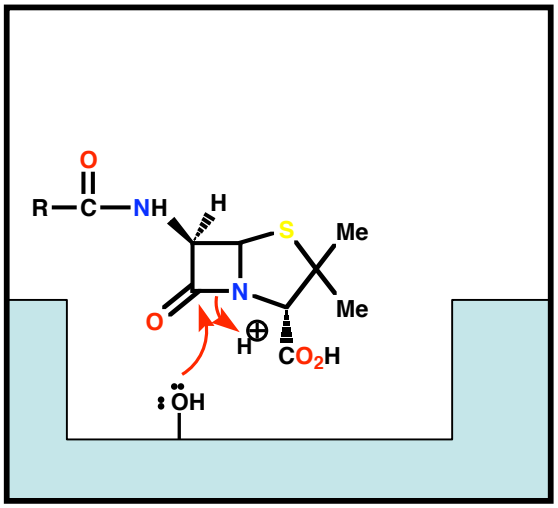


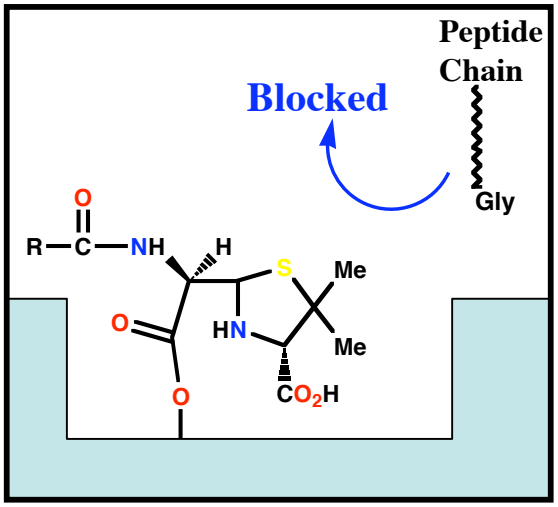


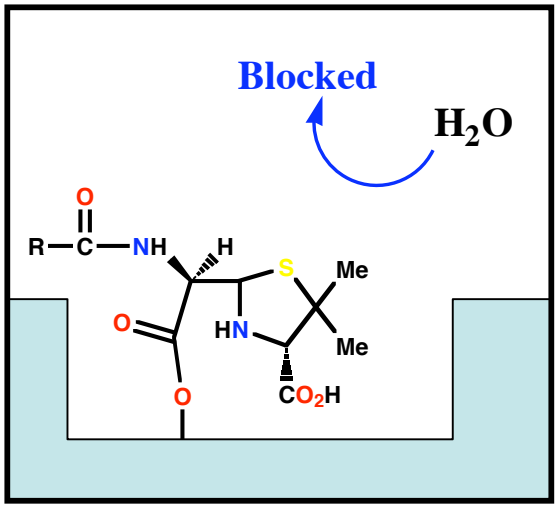
Mechanism of action - bacterial cell wall synthesis

Alternative theory- Pencillin mimics D-Ala-D-Ala.

Mechanism inhibited by penicillin

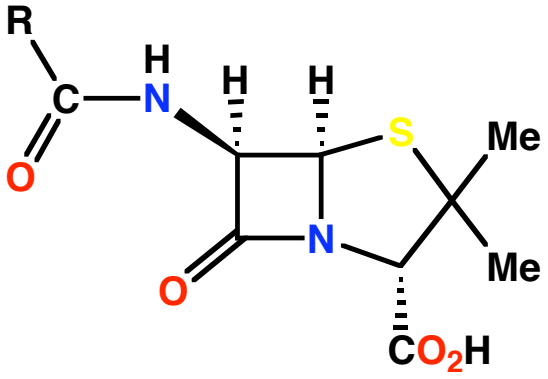




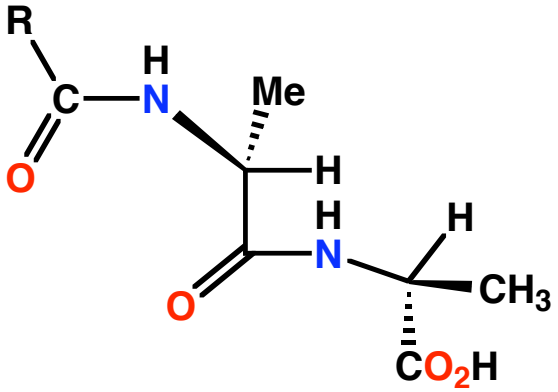


Mechanism of action - bacterial cell wall synthesis

Penicillin can be seen to mimic acyl-D-Ala-D-Ala



Penicillin



Acyl-D-Ala-D-Ala

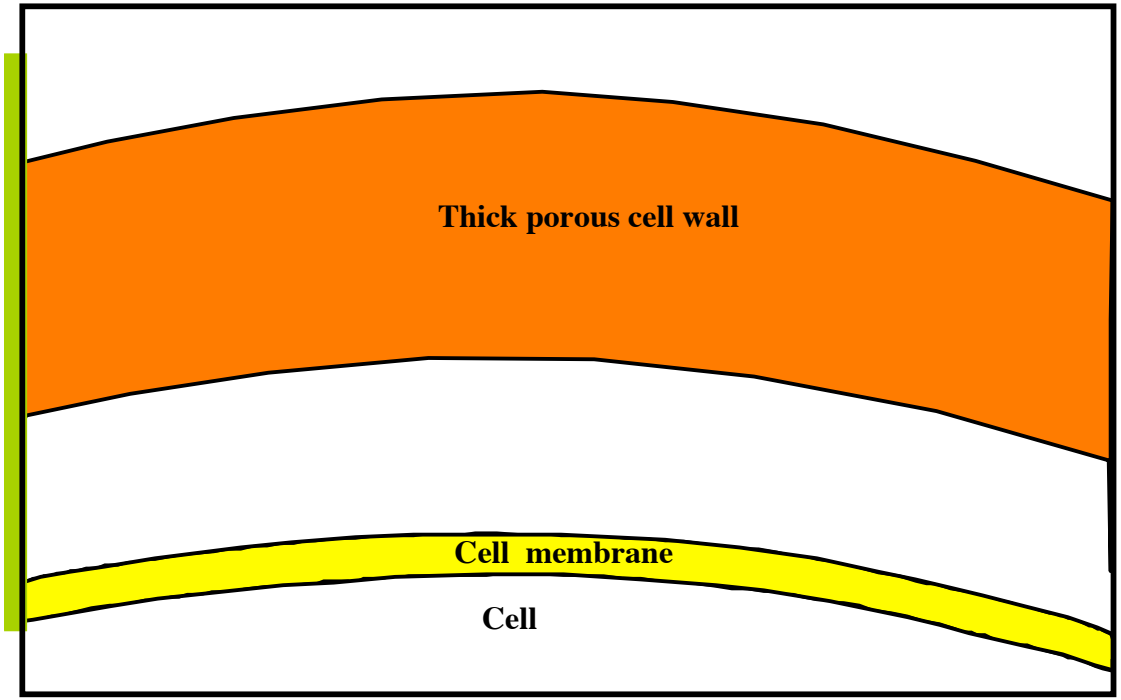
Resistance to Penicillins

- Penicillins have to cross the bacterial cell wall in order to reach their target enzyme
- But cell walls are porous and are not a barrier
- The cell walls of Gram + bacteria are thicker than Gram - cell walls, but the former are more susceptible to penicillins

Resistance to Penicillins

Gram + bacteria

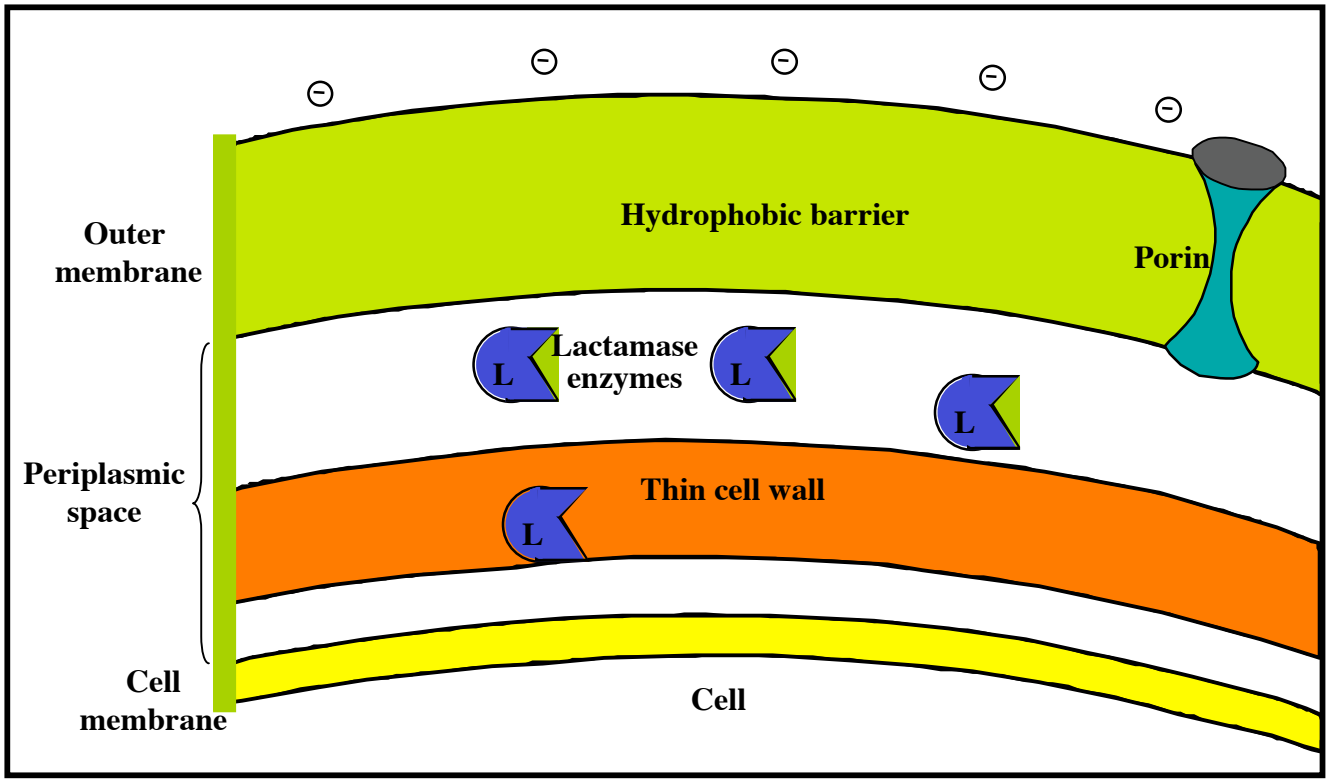
- Thick cell wall
- No outer membrane
- More susceptible to penicillins



Resistance to Penicillins

Gram - bacteria

- Thin cell wall
- Hydrophobic outer membrane
- More resistant to penicillins



Resistance to Penicillins

Factors

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

Penicillin Analogues - Preparation

1) By fermentation

- vary the carboxylic acid in the fermentation medium
- limited to unbranched acids at the α-position i.e. RCH_2CO_2H
- tedious and slow

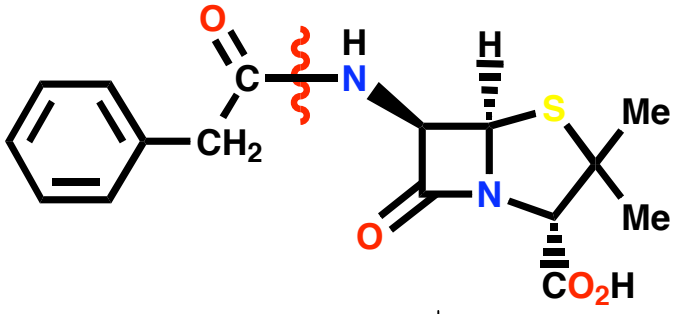
2) By total synthesis

- only 1% overall yield (impractical)

3) By semi-synthetic procedures

- Use a naturally occurring structure as the starting material for analogue synthesis

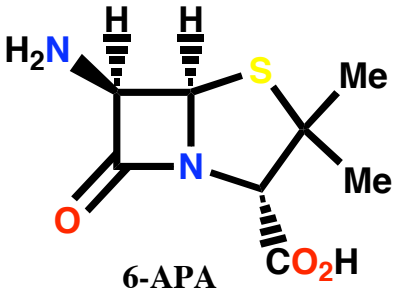
Penicillin Analogues - Preparation



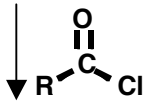
Penicillin G

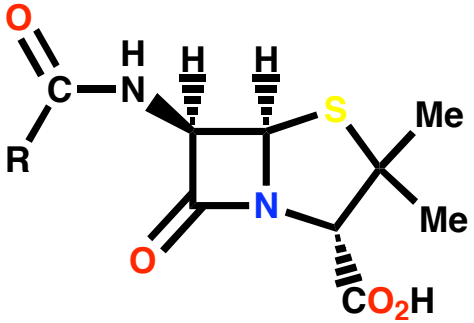
Penicillin acylase
or chemical hydrolysis

Fermentation →



6-APA

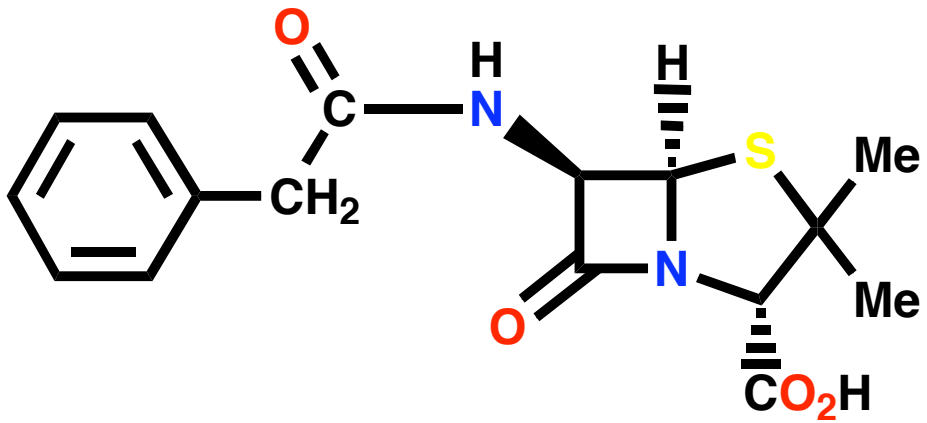




Semi-synthetic penicillins

Problems with Penicillin G

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

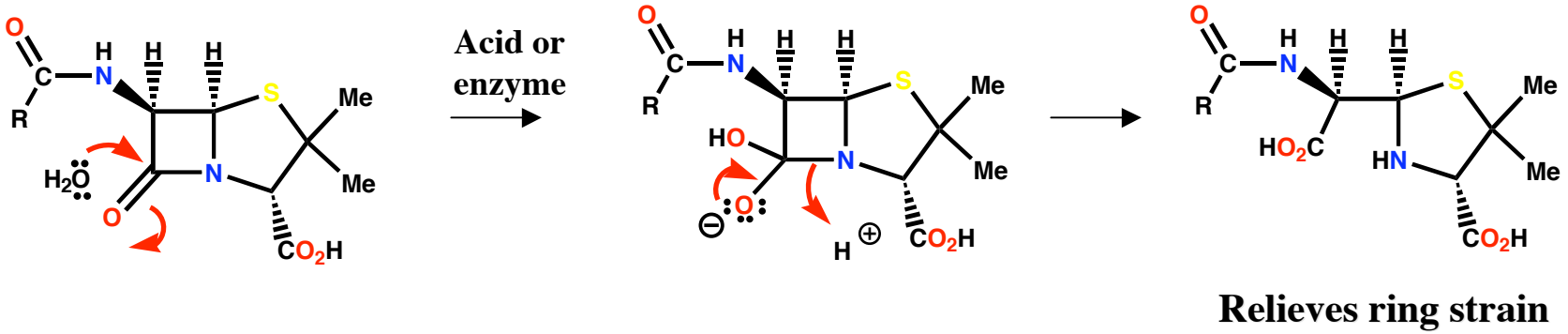


Penicillin G

Problem 1 - Acid Sensitivity

Reasons for sensitivity

Factor 1) Ring Strain



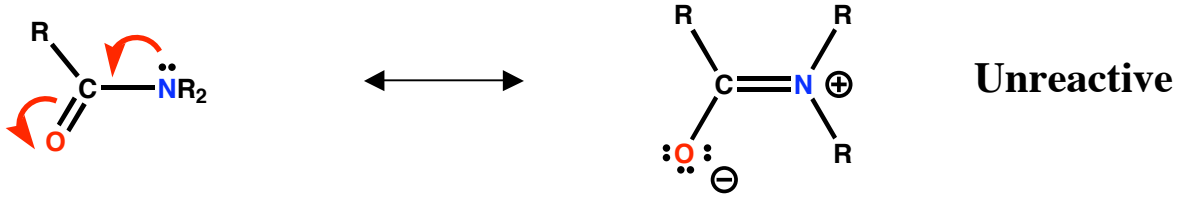
Problem 1 - Acid Sensitivity

Reasons for sensitivity

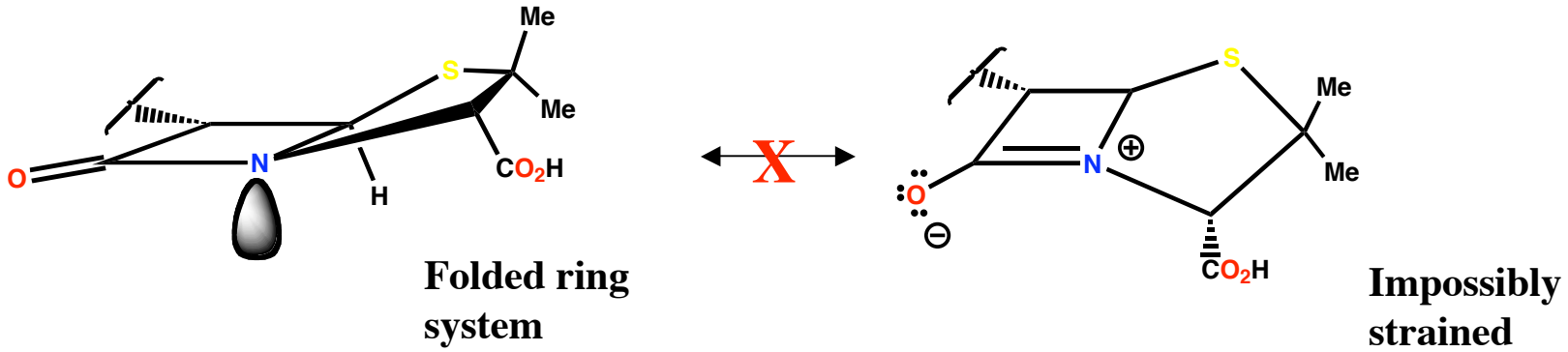
Factor 2) Reactive β-lactam carbonyl group

Does not behave like a tertiary amide

Tertiary amide



β-Lactam



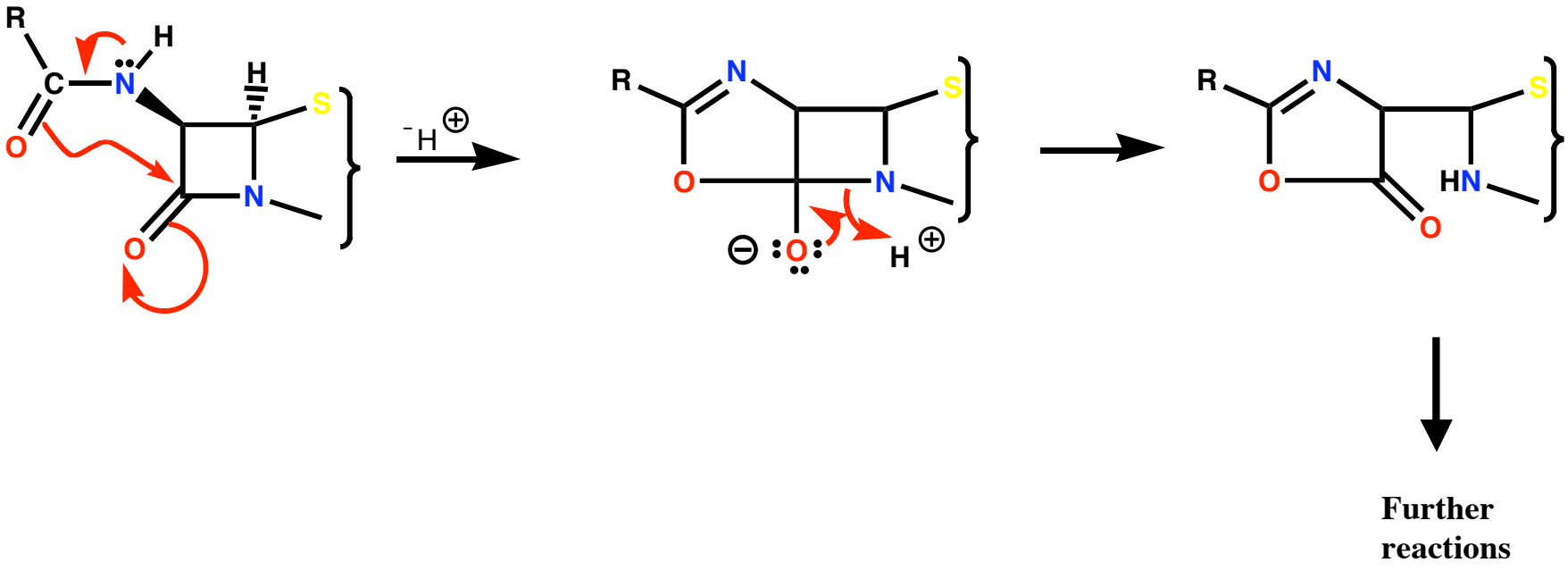
- Interaction of nitrogen's lone pair with the carbonyl group is not possible
- Results in a reactive carbonyl group

Problem 1 - Acid Sensitivity

Reasons for sensitivity

Factor 3) Acyl Side Chain

- neighbouring group participation in the hydrolysis mechanism



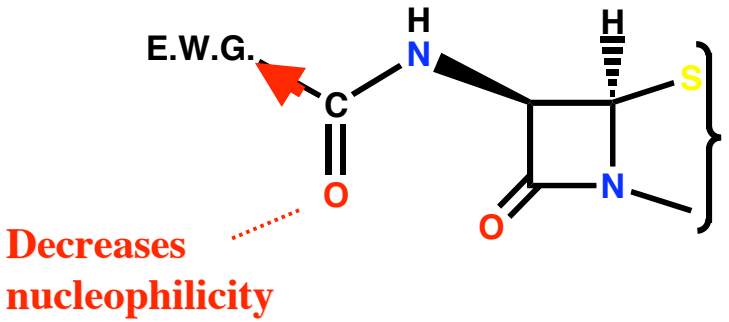
Problem 1 - Acid Sensitivity

Conclusions

- The β-lactam ring is essential for activity and must be retained
- Therefore, cannot tackle factors 1 and 2
- Can only tackle factor 3

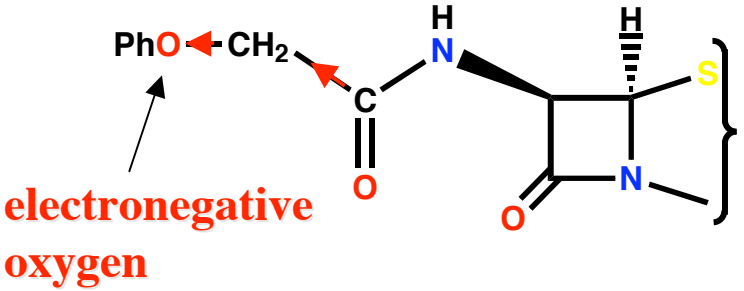
Strategy

Vary the acyl side group (R) to make it electron withdrawing to decrease the nucleophilicity of the carbonyl oxygen



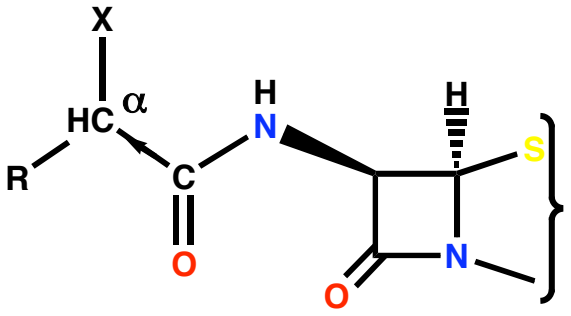
Problem 1 - Acid Sensitivity

Examples



Penicillin V
(orally active)

- Better acid stability and orally active
- But sensitive to β-lactamases
- Slightly less active than Penicillin G
- Allergy problems with some patients



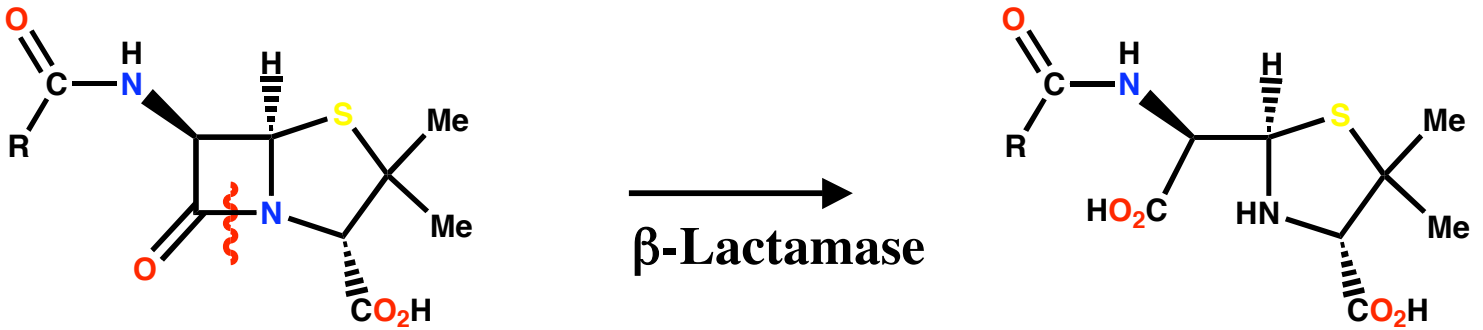
X = NH_2, Cl, PhOCONH, Heterocycles, CO_2H

- Very successful semi-synthetic penicillins
  e.g. ampicillin, oxacillin

Problem 2 - Sensitivity to b-Lactamases

Notes on β-Lactamases

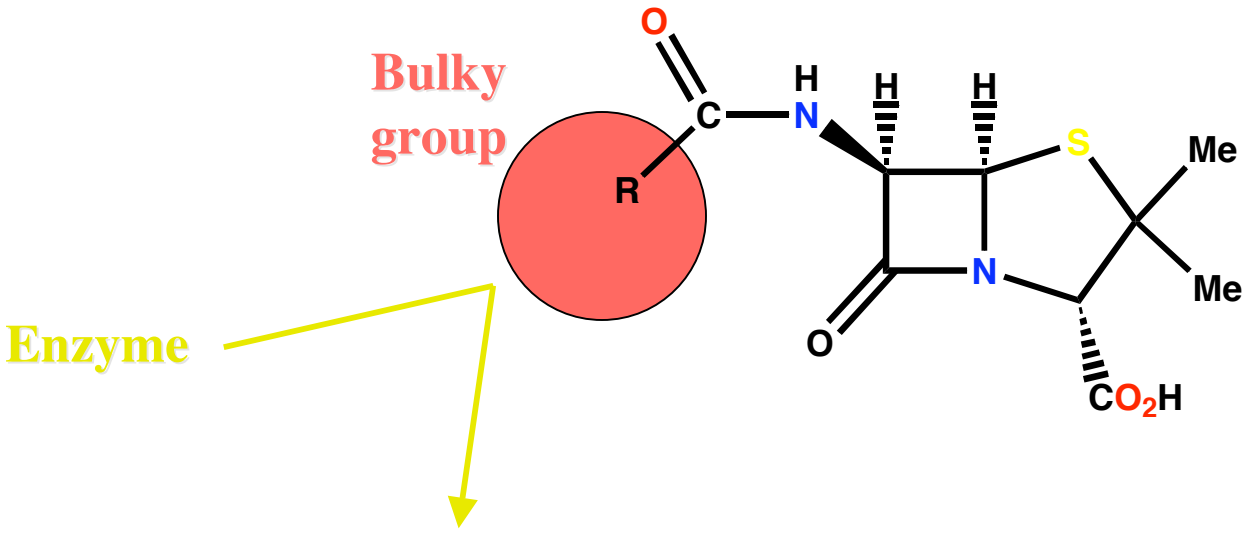
- Enzymes that inactivate penicillins by opening β-lactam rings
- Allow bacteria to be resistant to penicillin
- Transferable between bacterial strains (i.e. bacteria can acquire resistance)
- Important to *Staphylococcus aureus* infections in hospitals
- 80% *Staph*. infections in hospitals were resistant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



Problem 2 - Sensitivity to b-Lactamases

Strategy

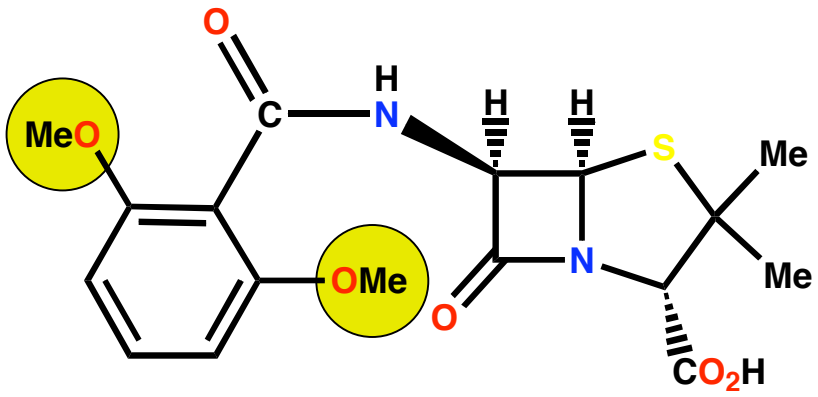
- Block access of penicillin to active site of enzyme by introducing bulky groups to the side chain to act as steric shields
- Size of shield is crucial to inhibit reaction of penicillins with β-lactamases but not with the target enzyme (transpeptidase)



Problem 2 - Sensitivity to b-Lactamases

Examples - Methicillin (Beechams - 1960)

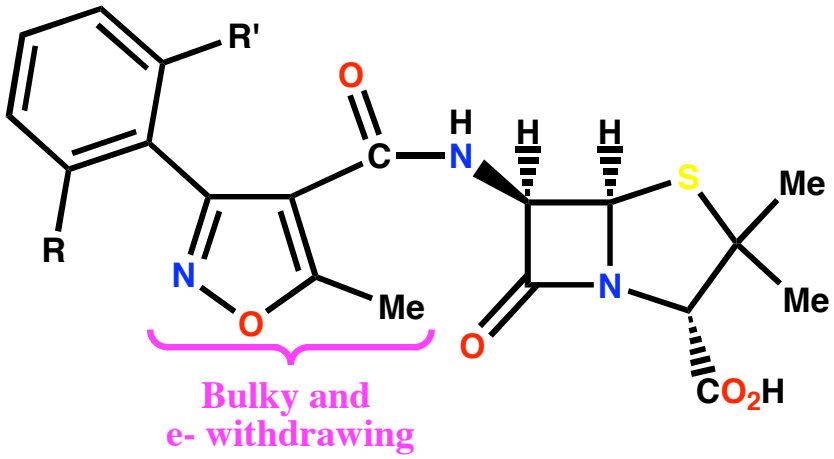
***ortho* groups important**



- Methoxy groups block access to β-lactamases but not to transpeptidases
- Active against some penicillin G resistant strains (e.g. *Staphylococcus*)
- Acid sensitive (no e-withdrawing group) and must be injected
- Lower activity w.r.t. Pen G vs. Pen G sensitive bacteria (reduced access to transpeptidase)
- Poorer range of activity
- Poor activity vs. some *streptococci*
- Inactive vs. Gram - bacteria

Problem 2 - Sensitivity to β-Lactamases

Examples - Oxacillin



Oxacillin R = R' = H
Cloxacillin R = Cl, R' = H
Flucloxacillin R = Cl, R' = F

- Orally active and acid resistant
- Resistant to β-lactamases
- Active vs. *Staphylococcus aureus*
- Less active than other penicillins
- Inactive vs. Gram -ve bacteria
- Nature of R & R' influences absorption and plasma protein binding
- Cloxacillin better absorbed than oxacillin
- Flucloxacillin less bound to plasma protein, leading to higher levels of free drug

Problem 3 - Range of Activity

Factors

1. Cell wall may have a coat preventing access to the cell
2. Excess transpeptidase enzyme may be present
3. Resistant transpeptidase enzyme (modified structure)
4. Presence of β-lactamases
5. Transfer of β-lactamases between strains
6. Efflux mechanisms

Strategy

- The number of factors involved make a single strategy impossible
- Use trial and error by varying R groups on the side chain
- Successful in producing broad spectrum antibiotics
- Results demonstrate general rules for broad spectrum activity.

Problem 3 - Range of Activity

Results of varying R in Pen G

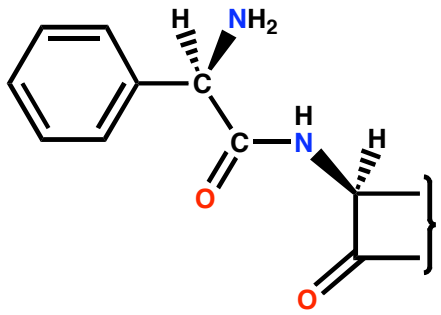
1. R= hydrophobic results in high activity vs. Gram + bacteria and poor activity vs. Gram -ve bacteria
2. Increasing hydrophobicity has little effect on Gram + activity but lowers Gram -ve activity
3. Increasing hydrophilic character has little effect on Gram + activity but increases Gram - activity
4. Hydrophilic groups at the α-position (e.g. NH_2, OH, CO_2H) increase activity vs Gram - bacteria

Problem 3 - Range of Activity

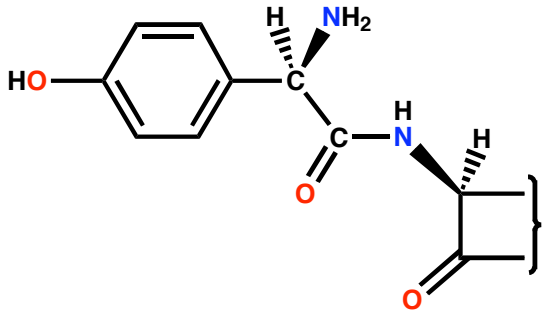
Examples of Broad Spectrum Penicillins

Class 1 - NH_2 at the α-position

Ampicillin and Amoxycillin (Beechams, 1964)



Ampicillin (Penbritin)
2nd most used penicillin



Amoxycillin (Amoxil)

Problem 3 - Range of Activity

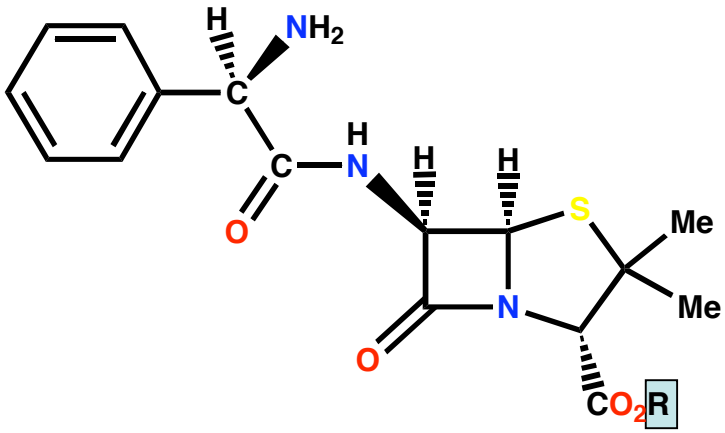
Examples of Broad Spectrum Penicillins

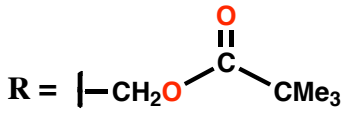
Properties

- Active vs Gram + bacteria and Gram - bacteria which do not produce β-lactamases
- Acid resistant and orally active
- Non toxic
- Still sensitive to β-lactamases
- Increased polarity due to extra amino group
- Poor absorption through the gut wall
- Disruption of gut flora leading to diarrhea
- Inactive vs. *Pseudomonas aeruginosa*

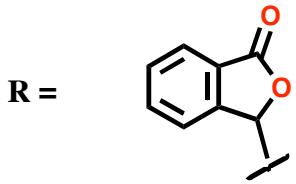
Problem 3 - Range of Activity

Prodrugs of Ampicillin (Leo Pharmaceuticals - 1969)

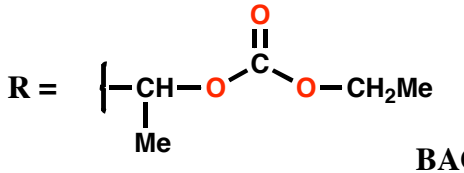




PIVAMPICILLIN



TALAMPICILLIN



BACAMPICILLIN

Properties

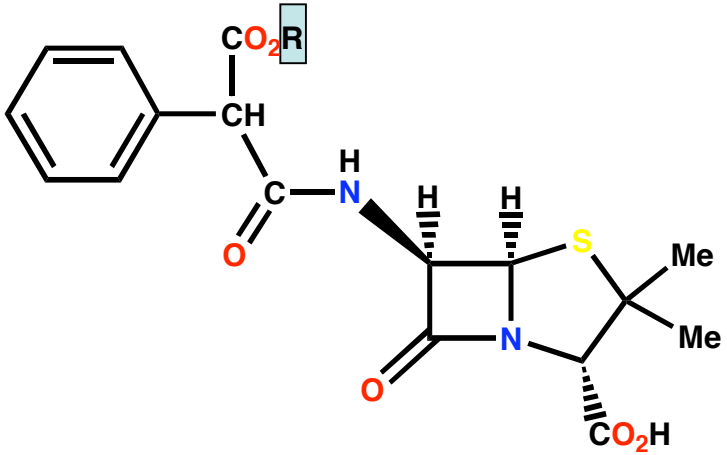
- Increased cell membrane permeability
- Polar carboxylic acid group is masked by the ester
- Ester is metabolised in the body by esterases to give the free drug

Problem 3 - Range of Activity

Examples of Broad Spectrum Penicillins

Class 2 - CO_2H at the α-position (carboxypenicillins)

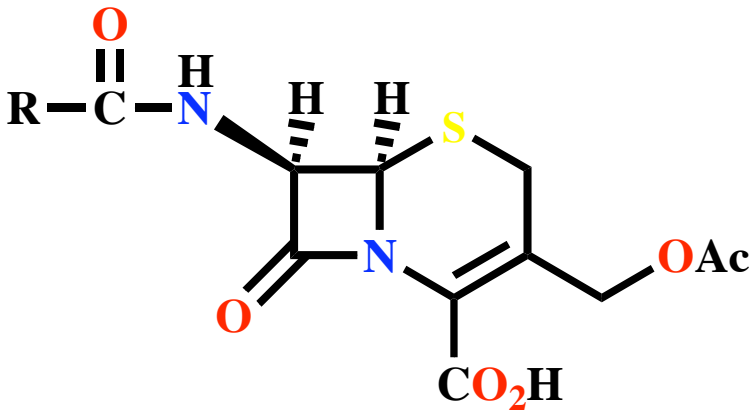
Examples



R = H CARBENICILLIN
R = Ph CARFECILLIN

- Carfecillin = prodrug for carbenicillin
- Active over a wider range of Gram -ve bacteria than ampicillin
- Active vs. *Pseudomonas aeruginosa*
- Resistant to most β-lactamases
- Less active vs Gram + bacteria (note the hydrophilic group)
- Acid sensitive and must be injected
- Stereochemistry at the α-position is important
- CO_2H at the α-position is ionized at blood pH

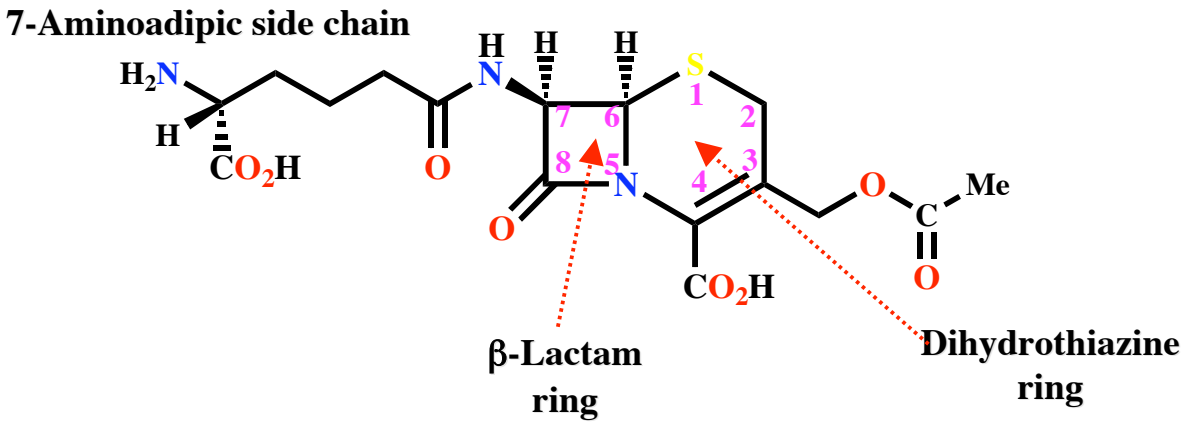
CEPHALOSPORINS

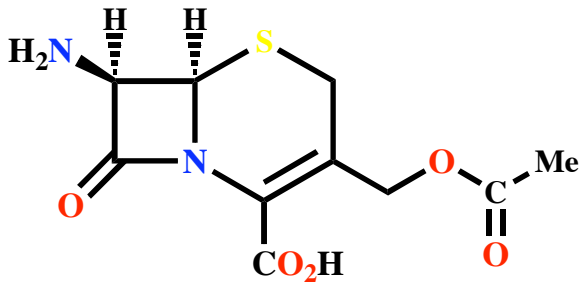


1. Introduction

- Antibacterial agents which inhibit bacterial cell wall synthesis
- Discovered from a fungal colony in Sardinian sewer water (1948)
- Cephalosporin C identified in 1961

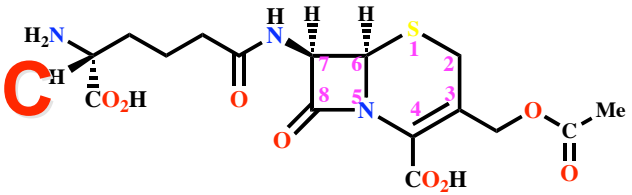
2. Structure of Cephalosporin C





7-Aminocephalosporinic acid (7-ACA)

3. Properties of Cephalosporin C



Disadvantages

- Polar due to the side chain - difficult to isolate and purify
- Low potency - limited to the treatment of urinary tract infections where it is concentrated in the urine
- Not absorbed orally

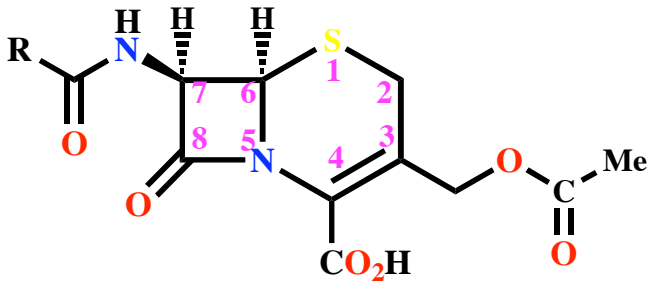
Advantages

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

Conclusion

- Useful as a lead compound

4. SAR of Cephalosporins



Similar to penicillins

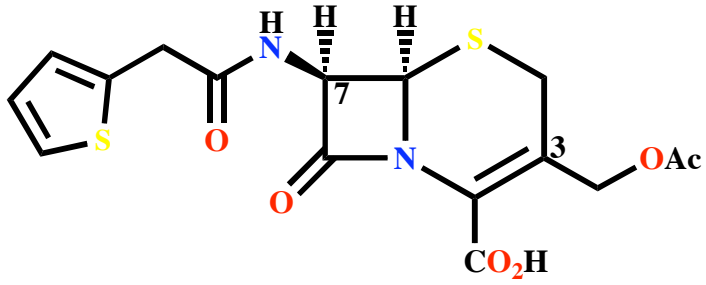
- The β-lactam ring is crucial to the mechanism
- The carboxylic acid at position 4 is important to binding
- The bicyclic system is important in increasing ring strain
- Stereochemistry is important
- The acetoxy substituent is important to the mechanism

Possible modifications

- 7-Acylamino side chain
- 3-Acetoxymethyl side chain
- Substitution at C-7

5. First Generation Cephalosporins

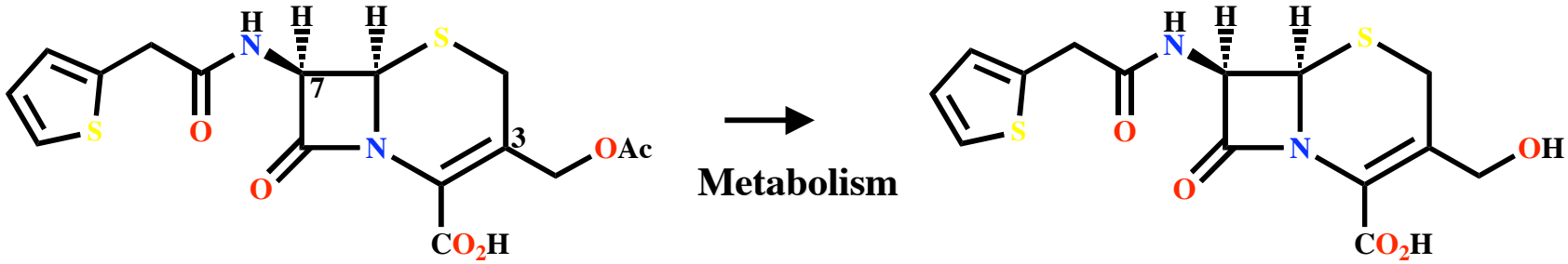
Cephalothin



- First generation cephalosporin
- More active than penicillin G vs. some Gram - bacteria
- Less likely to cause allergic reactions
- Useful vs. penicillinase producing strains of *S. aureus*
- Not active vs. *Pseudonomas aeruginosa*
- Poorly absorbed from gut
- Administered by injection
- Metabolised to give a free 3-hydroxymethyl group (deacetylation)
- Metabolite is less active

5. First Generation Cephalosporins

Cephalothin - drug metabolism



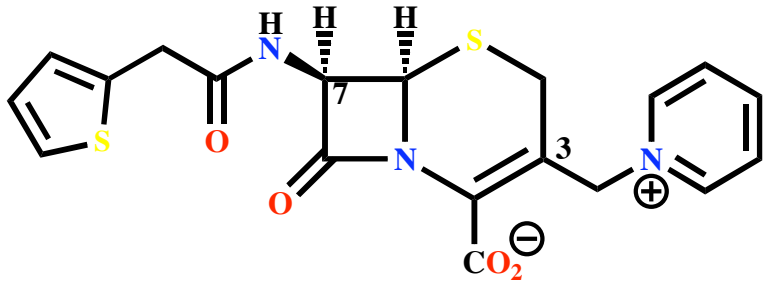
Less active
OH is a poorer leaving group

Strategy

- Replace the acetoxy group with a metabolically stable leaving group

5. First Generation Cephalosporins

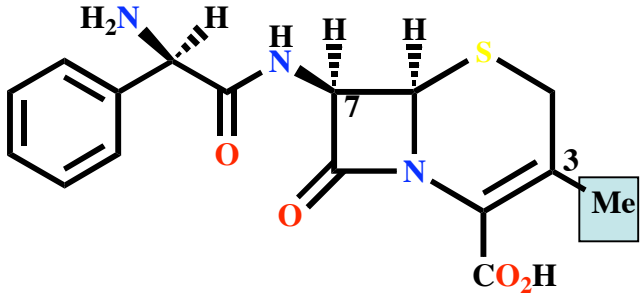
Cephaloridine



- The pyridine ring is stable to metabolism
- The pyridine ring is a good leaving group (neutralisation of charge)
- Exists as a zwitterion and is soluble in water
- Poorly absorbed through the gut wall
- Administered by injection

5. First Generation Cephalosporins

Cefalexin



- The methyl group at position 3 is not a good leaving group
- The methyl group is bad for activity but aids oral absorption - mechanism unknown
- Cefalexin can be administered orally
- A hydrophilic amino group at the α-carbon of the side chain helps to compensate for the loss of activity due to the methyl group

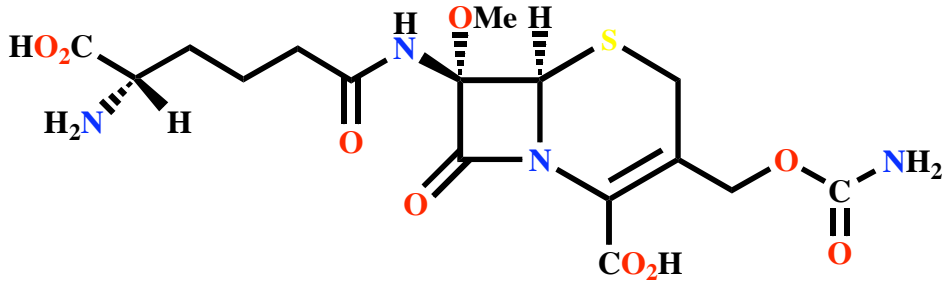
6. First Generation Cephalosporins

Summary

- Generally lower activity than comparable penicillins
- Better range of activity than comparable penicillins
- Best activity is against Gram-positive cocci
- Useful against some Gram negative infections
- Useful against *S. aureus* and streptococcal infections when penicillins have to be avoided
- Poorly absorbed across the gut wall (except for 3-methyl substituted cephalosporins)
- Most are administered by injection
- Resistance has appeared amongst Gram negative bacteria (presence of more effective β-lactamases)

6. Second Generation Cephalosporins

Cephamycins

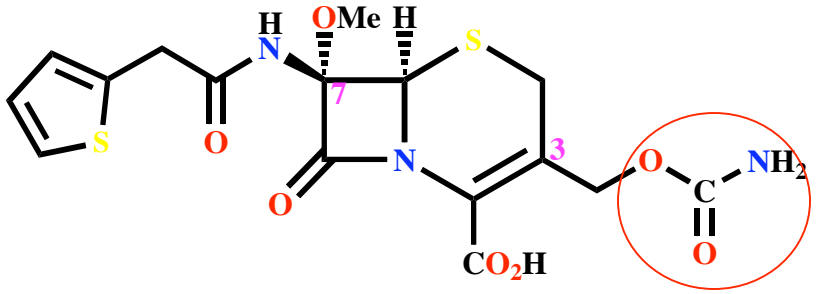


Cephamycin C

- Isolated from a culture of *Streptomyces clavuligerus*
- First β-lactam to be isolated from a bacterial source
- Modifications carried out on the 7-acylamino side chain

6. Second Generation Cephalosporins

6 Cephamycins

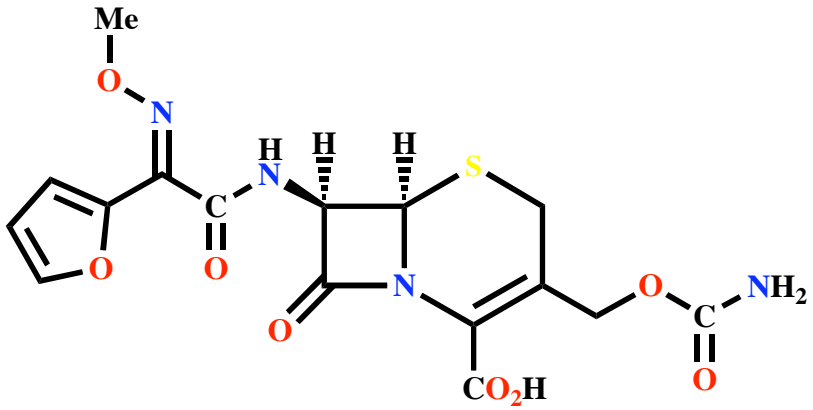


Cefoxitin

- Broader spectrum of activity than most first generation cephalosporins
- Greater resistance to β-lactamase enzymes
- The 7-methoxy group may act as a steric shield
- The urethane group is stable to metabolism compared to the ester
- Introducing a methoxy group to the equivalent position of penicillins (position 6) eliminates activity.

6. Second Generation Cephalosporins

Oximinocephalosporins

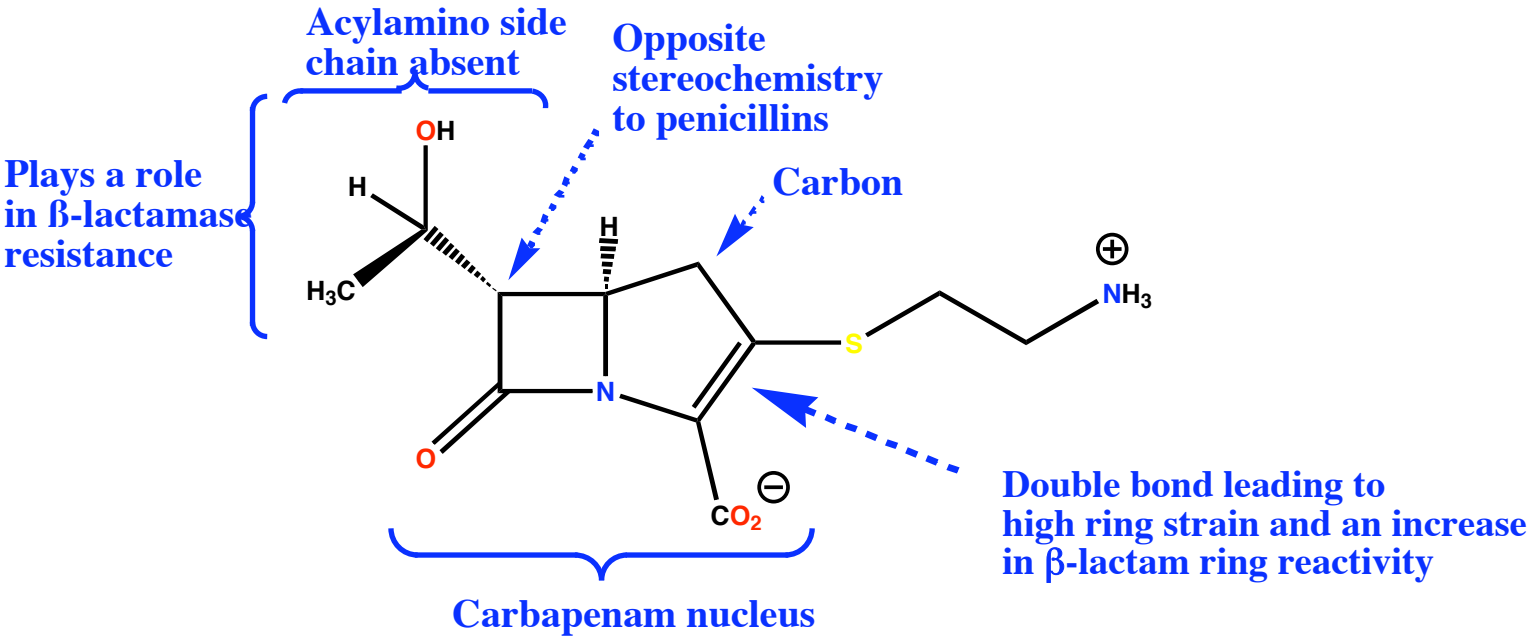


Cefuroxime

- Much greater stability against some β-lactamases
- Resistant to esterases due to the urethane group
- Wide spectrum of activity
- Useful against organisms that have gained resistance to penicillin
- Not active against *P. aeruginosa*
- Used clinically against respiratory infections

Newer β-Lactam Antibiotics

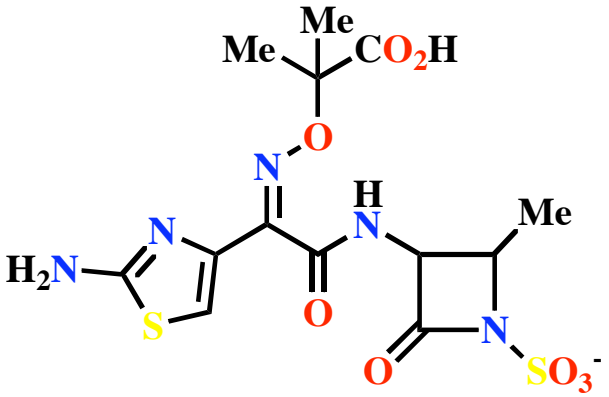
Thienamycin (Merck 1976)(from Streptomyces cattleya)



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

Newer β-Lactam Antibiotics

Clinically useful monobactam

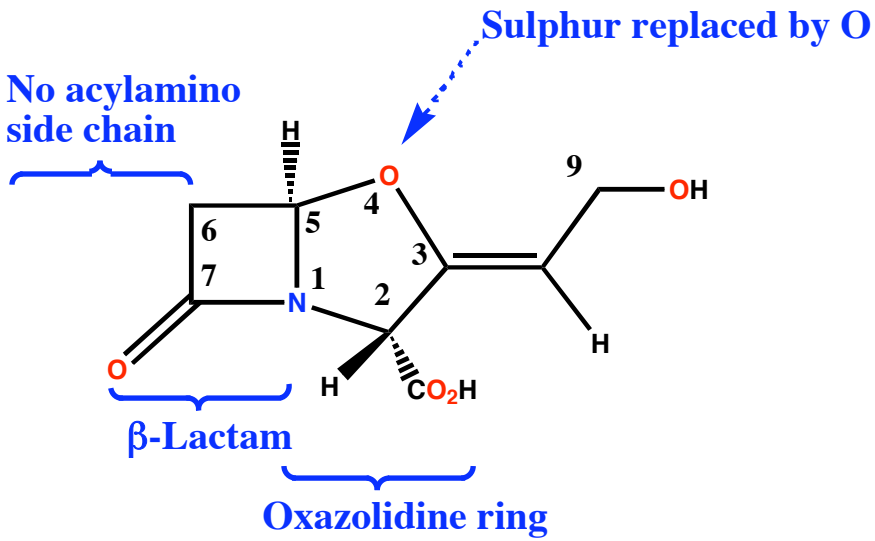


Aztreonam

- Administered by intravenous injection
- Can be used for patients with allergies to penicillins and cephalosporins
- No activity vs. Gram + or anaerobic bacteria
- Active vs. Gram - aerobic bacteria

β-Lactamase Inhibitors

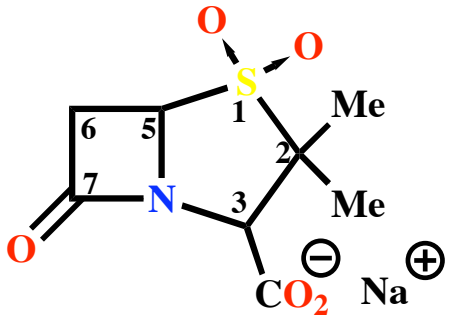
Clavulanic acid (Beechams 1976)(from *Streptomyces clavuligerus*)



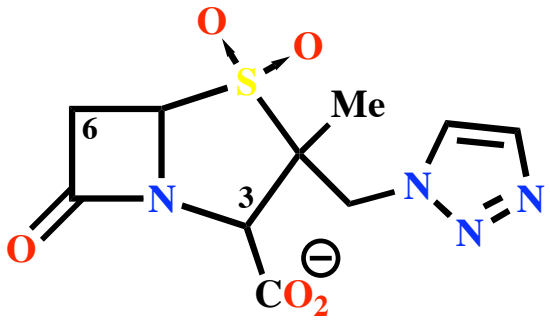
- Weak, unimportant antibacterial activity
- Powerful irreversible inhibitor of β-lactamases - suicide substrate
- Used as a sentry drug for ampicillin
- Augmentin = ampicillin + clavulanic acid
- Allows less ampicillin per dose and an increased activity spectrum
- Timentin = ticarcillin + clavulanic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

β-Lactamase Inhibitors

Penicillanic acid sulfone derivatives



Sulbactam



Tazobactam

- Suicide substrates for β-lactamase enzymes
- Sulbactam has a broader spectrum of activity vs β-lactamases than clavulanic acid, but is less potent
- Unasyn = ampicillin + sulbactam
- Tazobactam has a broader spectrum of activity vs β-lactamases than clavulanic acid, and has similar potency
- Tazocin or Zosyn = piperacillin + tazobactam