

# تفريغ هيديسنال 2



المحاضرة: Cell wall inhibitor  
Penicillin Part 1

الصيدلاني/ة: Rahaf Zyoud



لجان الرفعات

# Antibacterial antibiotics

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لا تتسوا زصيدنا اريهم  
الله يرحمه من دعائكم

إذا تم تطبيقها في الشروط  
تقدر أسوية antibiotic

# 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 لازم خليه كائن حي تفرزه لأول مرة حتى لو تم تصنيعه لاحقاً
2. Synthetic analogues of naturally occurring antibiotics زبي ال Penicillin اجا من Fungi فلتا اصل analogus semi-synthetic " رح يفتال
3. It antagonizes growth or survival of one or more species of microorganisms (Either kills the microbe (microbiocidal) or prevent its growth (microbiostatic)). زبي ← bactericidal
4. It is effective at low concentrations

صاحبه اللشياء صحن شرط لل anti-bacterial ادلا antibiotic  
 بين لما بهي امضع بغير لما بهي اخطر ب analog جديد او  
 لما يجي هنتف جديد ويدي اعرف بخصو  
 صمير عن خبره

# Antibacterial agents:

- The accidental discovery of penicillin is by Fleming in 1928 is the main reason for the initiation of modern antibiotic era.

ليني ينزل على السوق  
 ويستخدمه الانسان

- Clinically useful antibiotics need to have the following criteria:

1. Combat infection or neoplastic disease →

تقتل كل التهاب  
 او ورم سرطاني  
 (بمصل انه يقتل)  
 تماما عليه

أكثر خلايا تتأثر با  
 anti-cancer من الخلايا  
 بسريره النمو زرع  
 الشعر

(Selective toxicity) ← أكثر إلتصاف بشونه بال anti-cancer ما عليها  
 حيثة تؤثر على كل خلايا الإنسان

3. Stable for a period of time inside the body.

Long-half life عشان  
 يحب يكون  
 المريفه ما ينزع من أخذه أكثر من مرة

← ينجح تحويل  
 ال Route للإسف  
 سهل التامه تحله

Ease of administration by oral or parenteral route

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

لازم يكون في توازن بينه انحلولا يضل الجسم نقطه 3  
 وبينه انه يطلع من الجسم نقطه 5

# Potential targets for antibacterial agents

يعني التأثير على الـ ribosom

- ❑ Protein synthesis → aminoglycosid + Tetracyclin + Macrolid → (دوني بخير)
- ❑ Nucleic acid synthesis
- ❑ Cell metabolism (e.g. folate synthesis) → sulfonamid
- ❑ Cytoplasmic membrane
- ❑ Bacterial cell wall synthesis → Penicillin + Cephalosporin

# Potential targets for antibacterial agents

Sulfonamides  
(on metabolic enzymes)



Quinolones  
Rifampicin



Penicillins  
Cephalosporins



Aminoglycosides  
Tetracycline  
Chloramphenicol

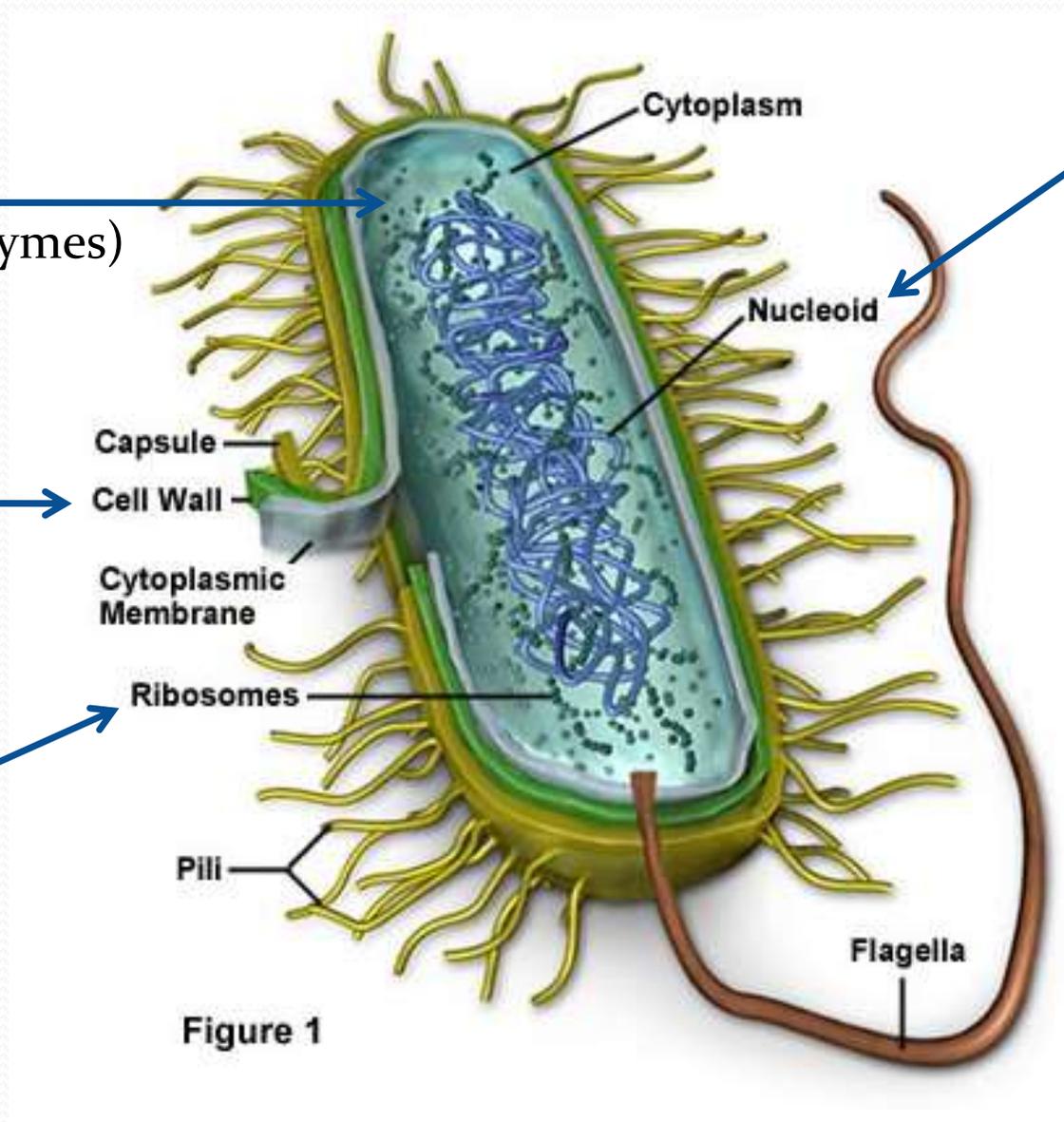


Figure 1



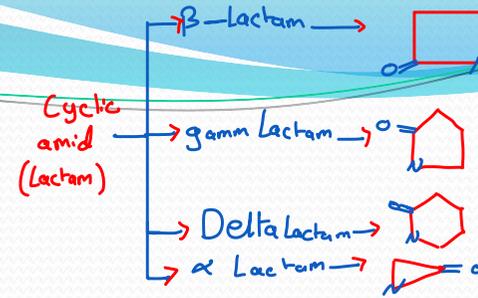
# Antibacterial agents acting on the cell wall biosynthesis

## Penicillins and Cephalosporins

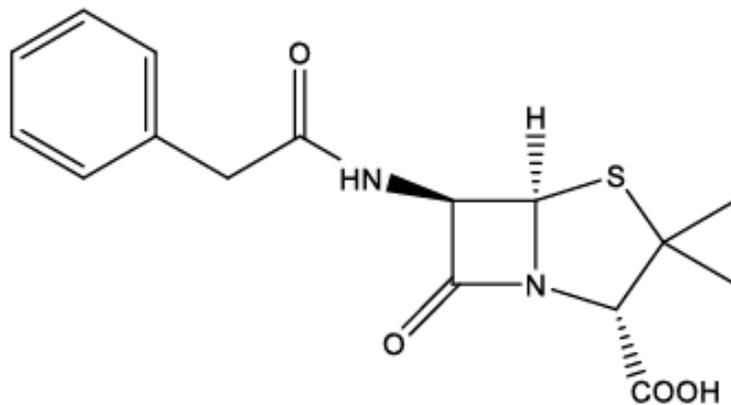
# $\beta$ -Lactam antibiotics

Cyclic amid تحتوي على

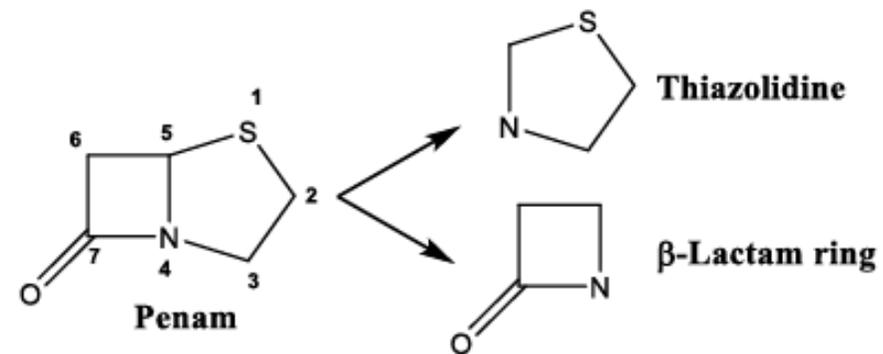
Cyclic ester  $\leftarrow$  Lacton  هي شكله موجود مكان واحد بالتملايد الجاي



- 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.



**Benzylpenicillin (Penicillin G)**

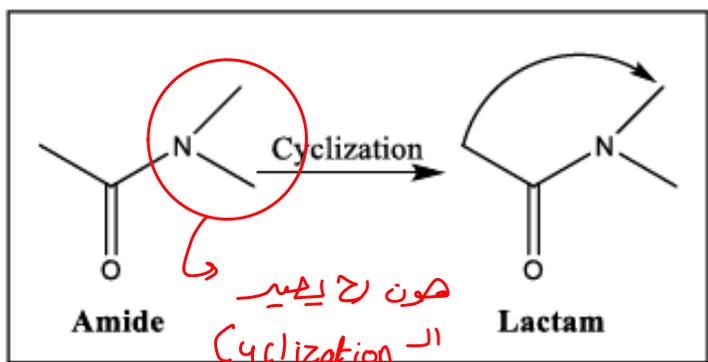


# $\beta$ -Lactam (the cyclic amide)

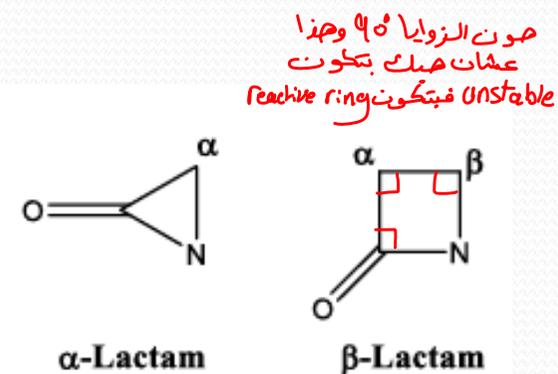
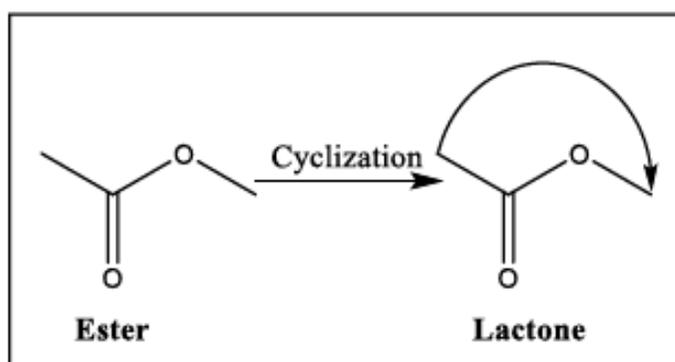
This ring is the result of cyclization of the amide group, as in case of Lactone which is the cyclic structure of ester.

Why it's called  $\beta$ -Lactam?

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

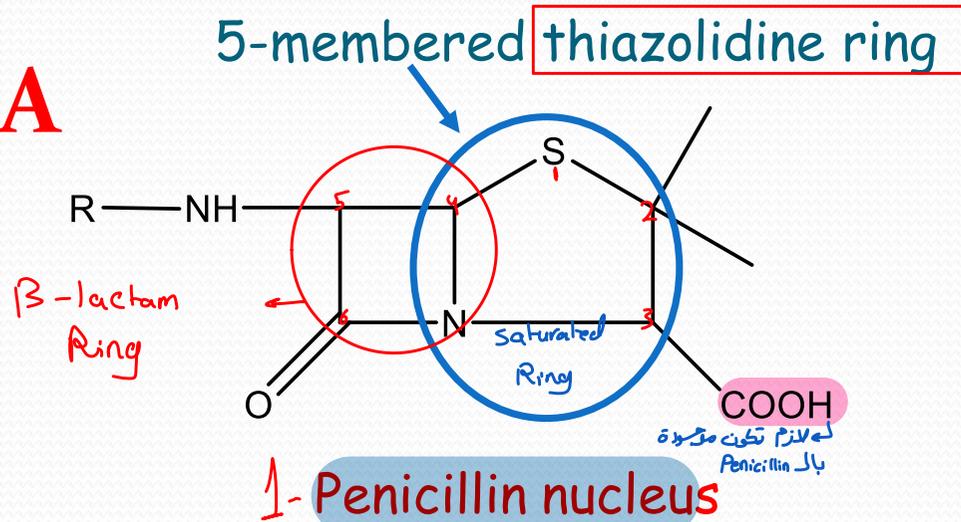


هون لا يغير  
Cyclization  
 $\beta$ -carbon

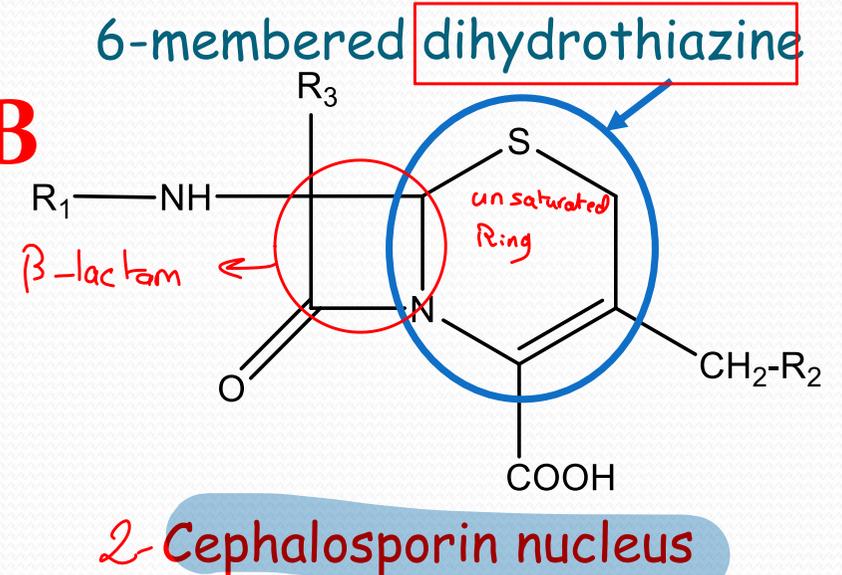


# $\beta$ -lactam antibiotics

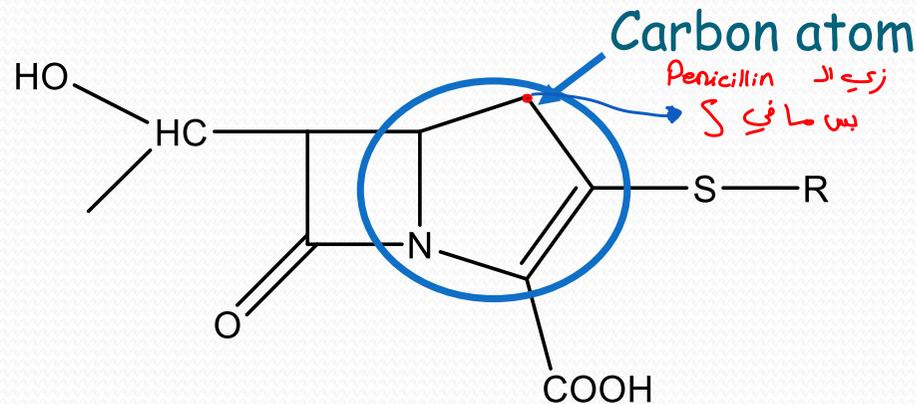
**A**



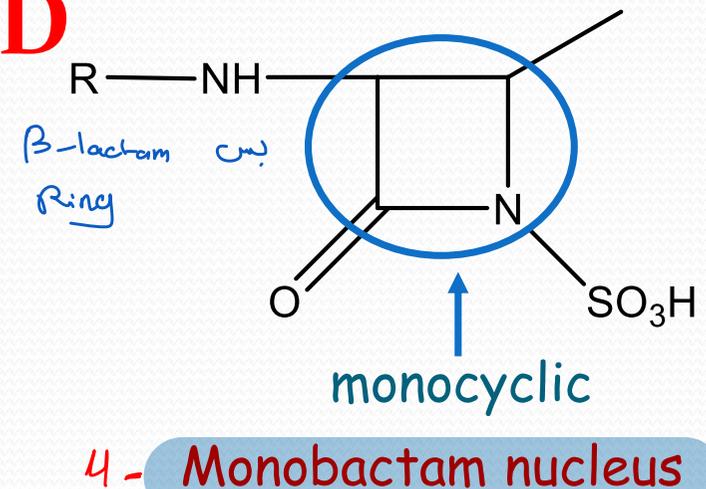
**B**



**C**



**D**

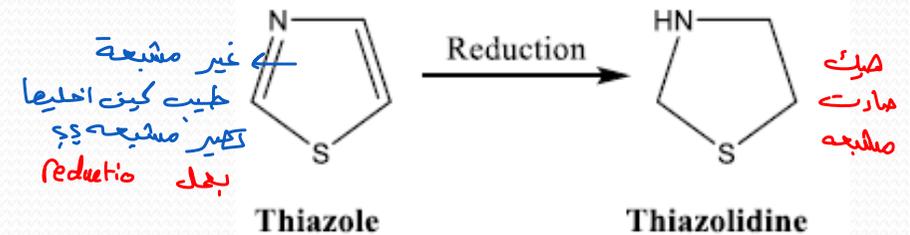
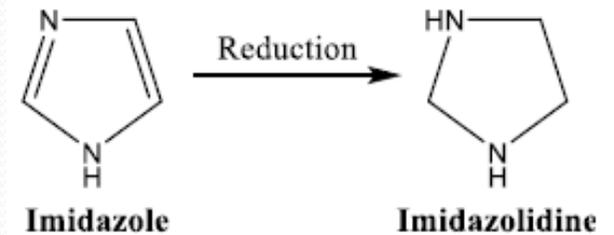
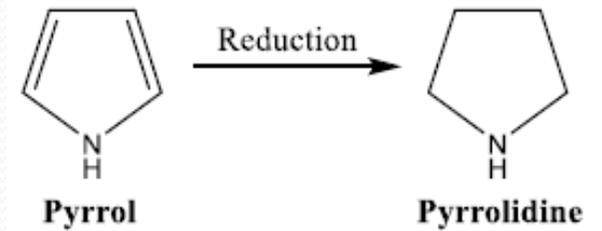


قبل ال reduction

بعد ال reduction

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

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

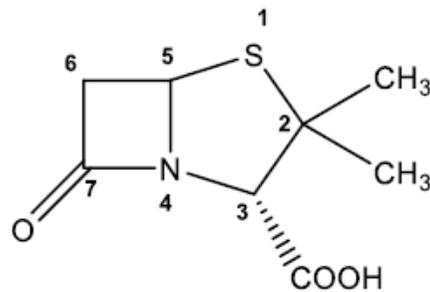


لاحظوا تغير الاسم بعد ما صار  
reduction الحلقه الخماسيه متحولت  
من غير مشبعة لمشبعة

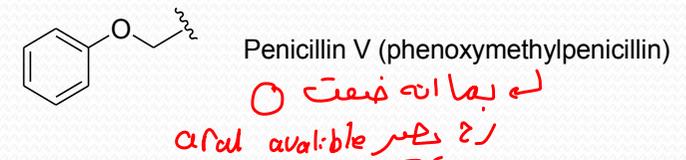
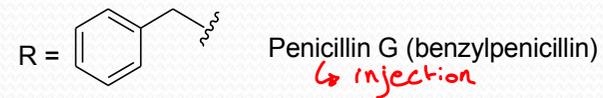
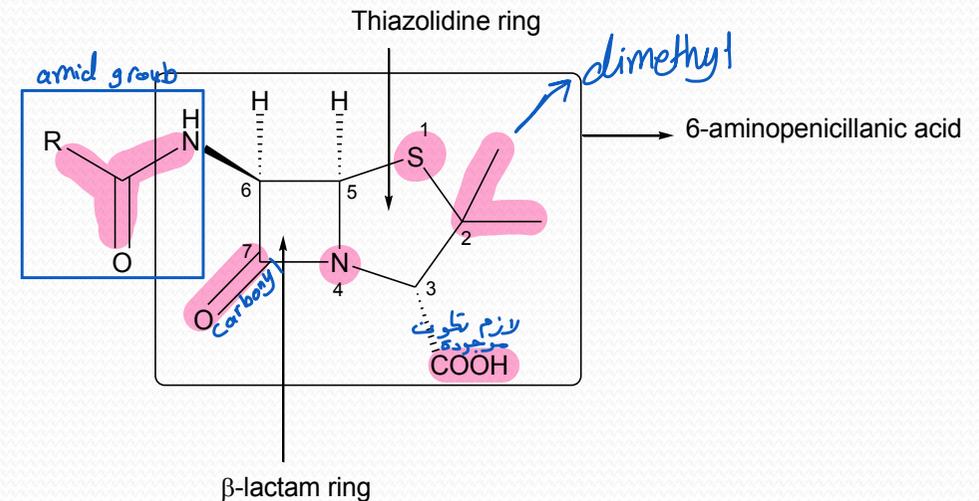
- To say that our compound is penicillin, firstly you have to look for the Penam system then the following substitutions also must present in all penicillins:

- ☐ Di-methyl group at the position 2.
- ☐ A carboxylic acid at position 3, below the plan, (S) oriented.

Until now, this compound is called "Penicillanic acid", a 2, 2-dimethyl-3-carboxy Penam.

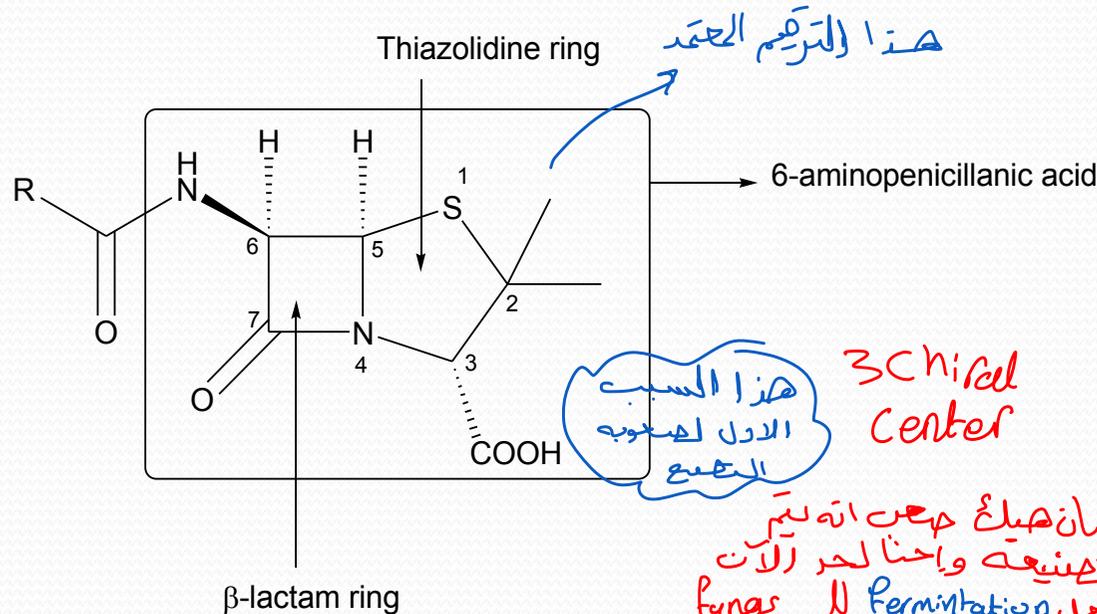


**Penicillanic acid**



# Penicillin's naming is problematic

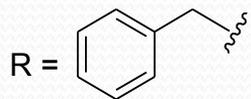
1<sup>st</sup> naming system related to the chemical abstracts



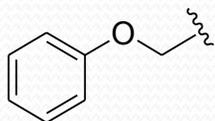
Fungus synthesizes penicillin using cysteine, valine and some of the fermentation products

Difficult to synthesize in the lab due to:

1. strained ring system.
2. The three chiral center it has which should be with certain



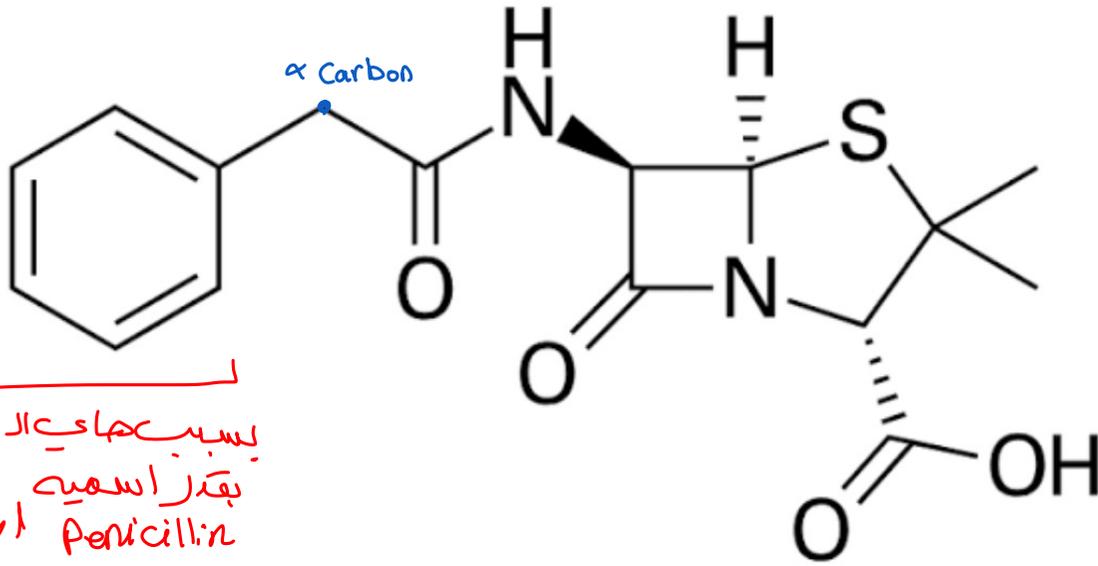
Penicillin G (benzylpenicillin)



Penicillin V (phenoxyethylpenicillin)

المركز الكيرالي (Chiral center) هو ذرة - وغالبًا تكون ذرة كربون - ترتبط بأربع مجموعات مختلفة.

بسبب هذا الاختلاف، لا يمكن أن تُطابق صورتها في المرآة، مثل اليد اليمنى واليسرى، ولهذا تُسمى الجزيئات التي تحتوي عليه جزيئات كيرالية (أي لها "يدوية").



هذا الـ Penicillin G وهو الـ Penicillin

عند عدة مشاكل وهمية

بسبب هائي الـ benzyl  
يقتر اسميه  
benzyl Penicillin

# Penicillin G

عشبات هيك يدي

اهل فاي الحشاكل

Stomach ← روعه روعه يكون unstable بالـ

Penicillinase ← بسبب الـ

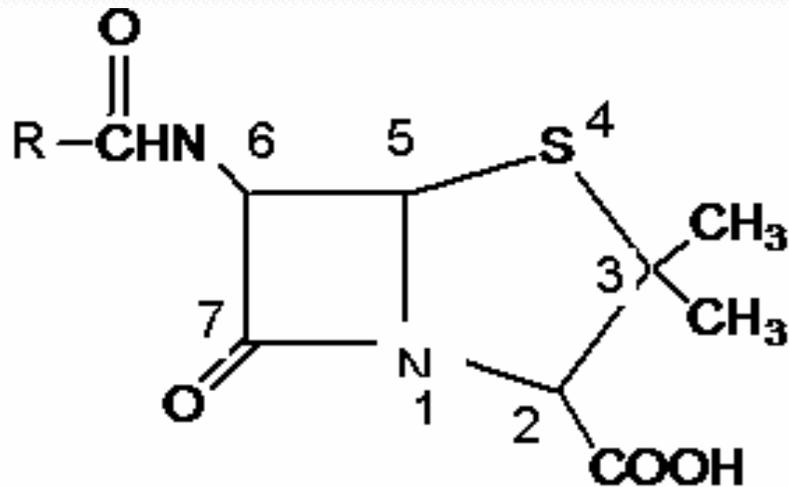
Gram + ← بسبب الـ

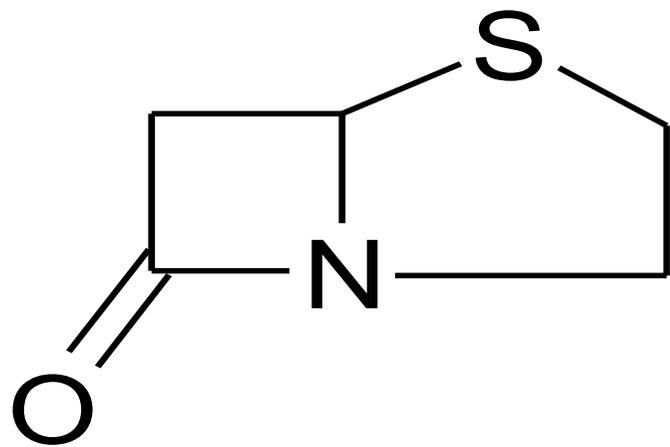
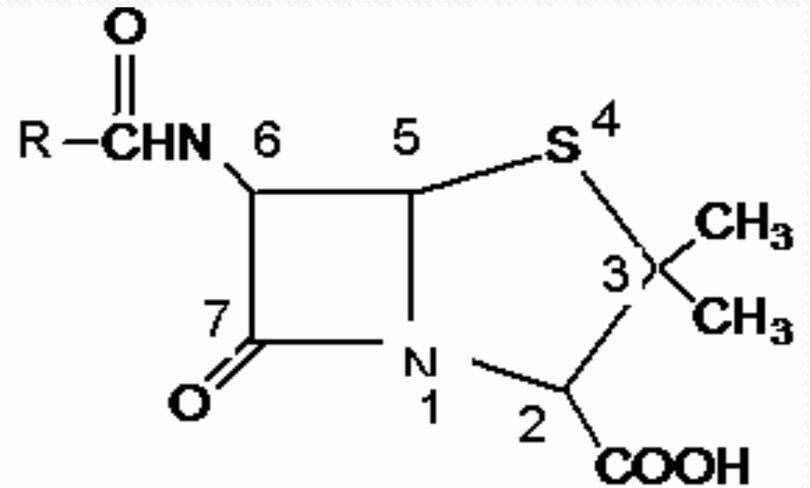
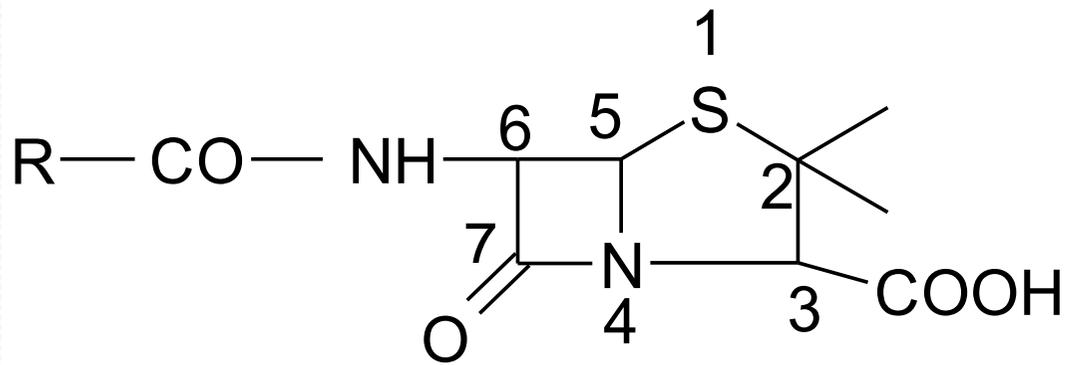
Penicillin G:

- 1) acid sensitivity (only I.V or IM admin)
- 2) Resistance due to lactamases
- 3) Narrow spectrum of activity

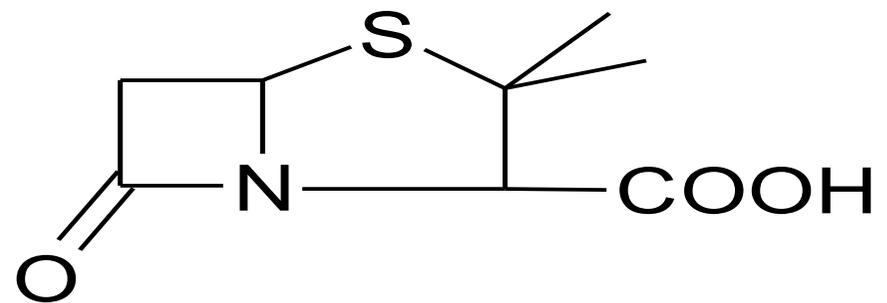
# Penicillin's naming is problematic

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





Penam

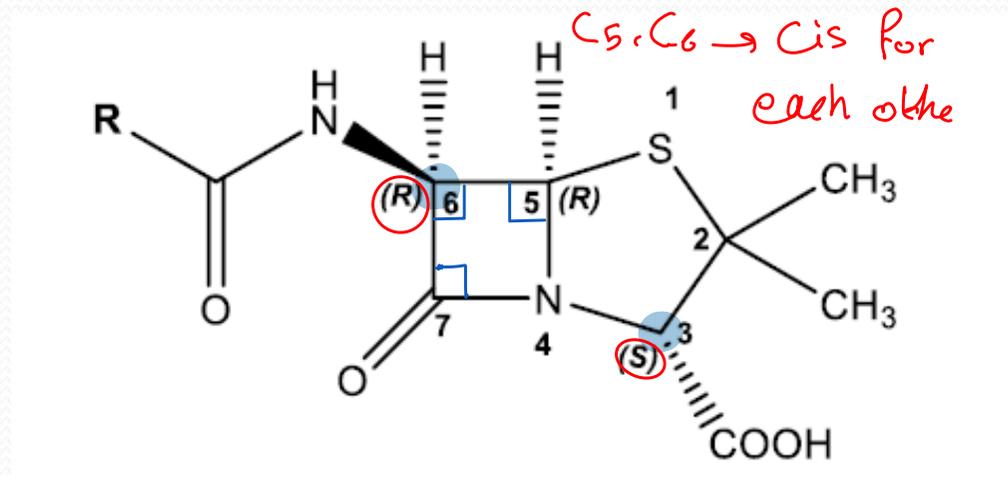


Penicillanic Acid

R → فوق  
S → تحت

# Stereochemistry

$C_3 / C_6 \rightarrow$  trans for each other



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.

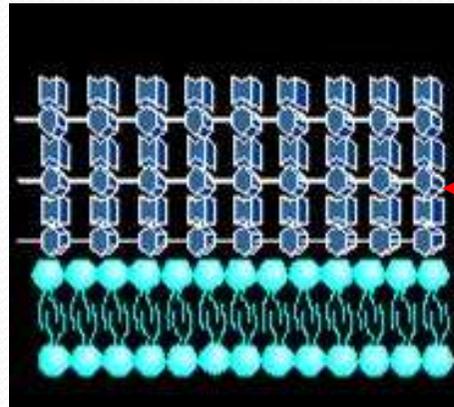
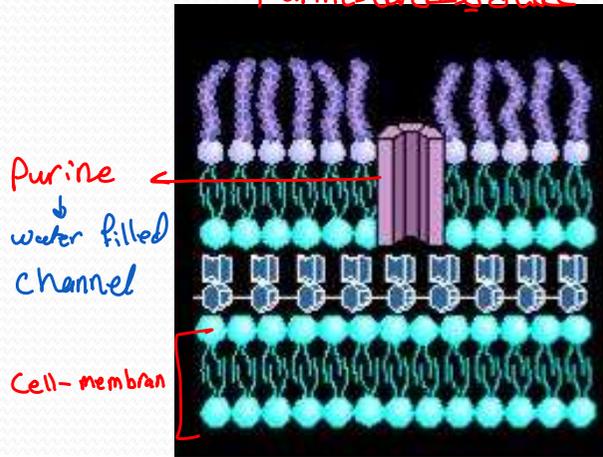
All synthetic and semi synthetic penicillin have same absolute configuration that of natural

3S:5R: 6R

# The bacterial cell wall

يحتاينعرف انه الخلايا  
 الحيوانية صافيا Cell  
 Wall  
 خلايا  
 الحيوان  
 خلايا  
 الانسان

الدرنا لازم يكون  
 water soluble  
 Purine يدخل في ال DNA



Peptidoglycan is Polymer of  
 sugar  
 Peptide

- N-acetylglucosamine (NAG)
- N-acetylmuramic acid (NAM)
- D-alanine (amino acid)

Gram -

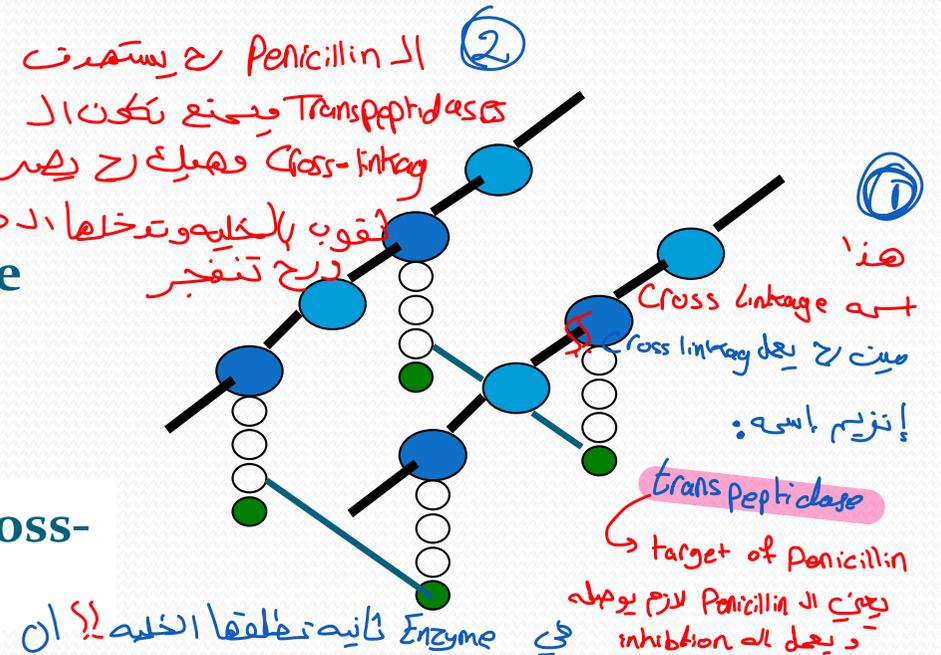
Only two layers of peptidoglycan

Gram +

Consists of 50-200 peptidoglycan layers

Transpeptidase

Involved in cross-linking



كيف ال Transpeptidase رح يعمل ال Cross linkage

عن طريق ال Data-Data

البكتيريا عندما enzyme اسمه  $\beta$ -lactamases او Penicillinases (نفس البنية) هنزل ال enzymes مرتبطون بال Resistance كيف رح يشتغلوا

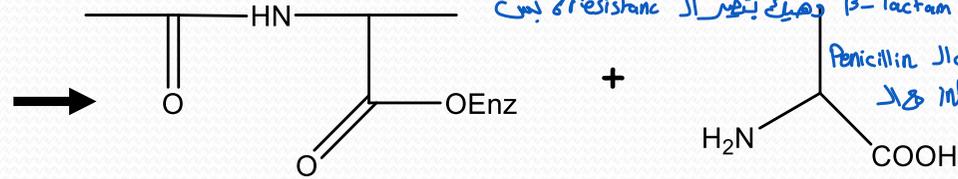
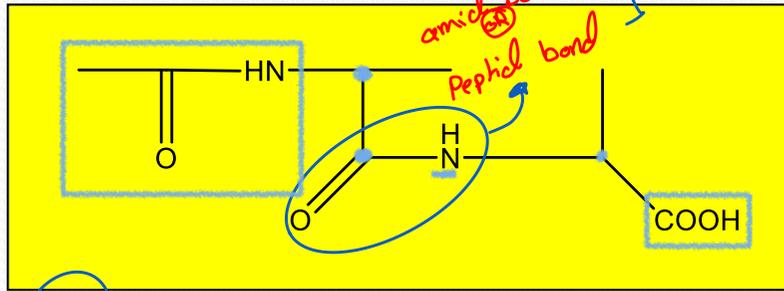
Penicillin لما يهاجم ال transpeptidase رح تكون السبب بروت

التيه ا بترية عدسات صلبة البكتيريا تطلت ال Penicillinases ( $\beta$ -lactamases)

هذا ال enzyme رح يوحد ال Penicillin قبل ما يهاجم ال Transpeptidase

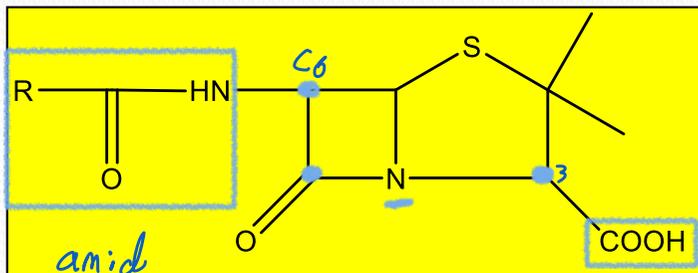
ورح يكسر ال  $\beta$ -lactam ring دهيك بتغير ال Resistance بوس

مادة اعلاية ال Cell wall

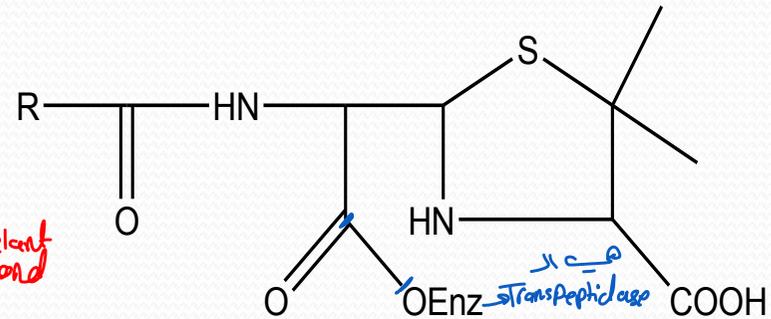


D-ala D-ala (natural substrate) for transpeptidase

Lala بالانسان عنا عشان هيك مافى toxicity



في تشابه بين ال Natural substrate ال Penicillin وبسبب هذا التشابه رح يعمل ال Transpeptidase enzyme



1 Penicillin-enzyme complex

هذا ال complex مارج يرتبط ال Cross-linkage

دهيك بكون غير فعال

2 cross-linking inhibited

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

بسبب اختلاف ال الضغط ال اسموزية

Excellent selective toxicity

ال Penicillinases لما تطلت البكتيريا رح يرتبط مع ال Penicillin ويكون معه complex دهيك مارج يشتغل لولا هسان هيك كل ما تعرضت البكتيريا لل Penicillin

رح تغير ال DNA ال Penicillinase جدير مقاوم

مشتركة بينها وبين ال D-ala Data

Penicillins

رح ال لاحظ انه الحماض بين ال COOH وال amid

ال D-ala D-ala تساو في نفس الحماض بال Penicillin

4 Bacterial cell lysis

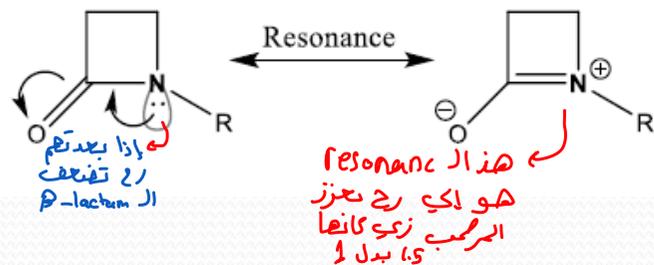
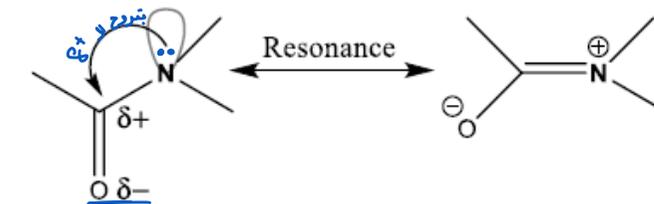
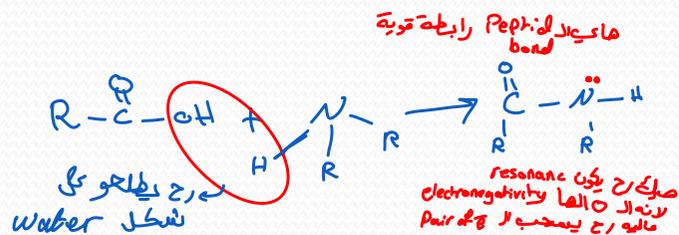
- Penicillin mimic the structure of D-ala-D-ala, because of that the transpeptidase mistakenly bind to it instead of D-ala-D-ala.
- Also this explains the lack of penicillin toxicity, since D-amido acids are not present in human, only the L-amino acids present. → ما ينفذ من الtoxicity ، عشان هيرى الفعلة الوحيدة اى بنفاز منها هي الحساسية
- Also targeting the cross linking in the peptidoglycan biosynthesis which is only present in bacteria explains the selective toxicity.

# chemical properties of $\beta$ -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  $\beta$ -Lactam ring (alone, no other substitutions) and the pair of electrons of N are not far from the carbonyl group



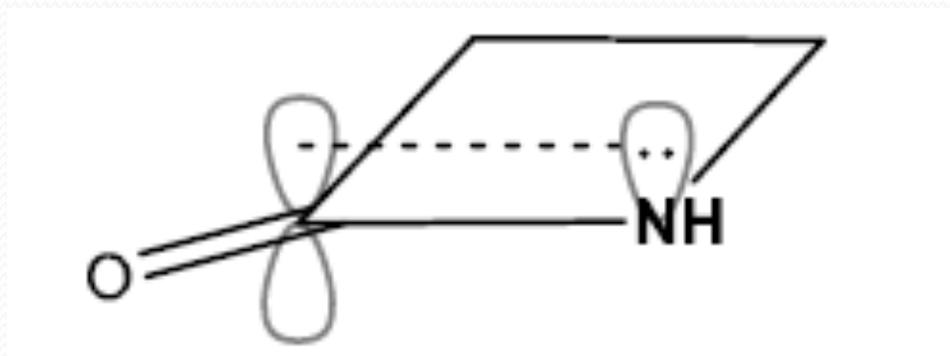
مخاف ضعفتها الحق  
 تغير سريع وانزوية  
 تغير 90 ويرفض هنا  
 السبب انها unstable



electrophilicity increase				
→				
C	N	O	F	↑
Si	P	S	Cl	
			Br	
			I	

## chemical properties of $\beta$ -Lactam ring:

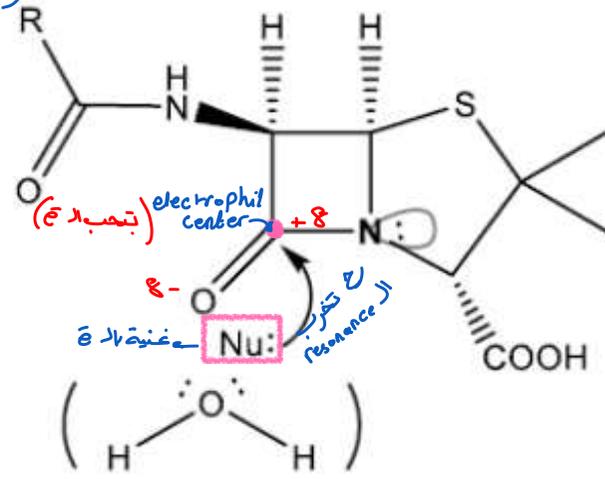
- Look at the pair of electrons of N in the  $\beta$ -Lactam ring alone
- (**NOTE** that the empty orbital of carbonyl group accepts the pair of electrons of N to make resonance)



- BUT when the  $\beta$ -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).

لأنه المركب معقد يتغير  
 لا يتغير عليه Nu attack  
 باون في شكل Powder ودي  
 احطها في طبق للبريفت ما يشربه  
 بعد ما يحطه يتسبح و يحطه  
 بالبالا جمعيات باخرات

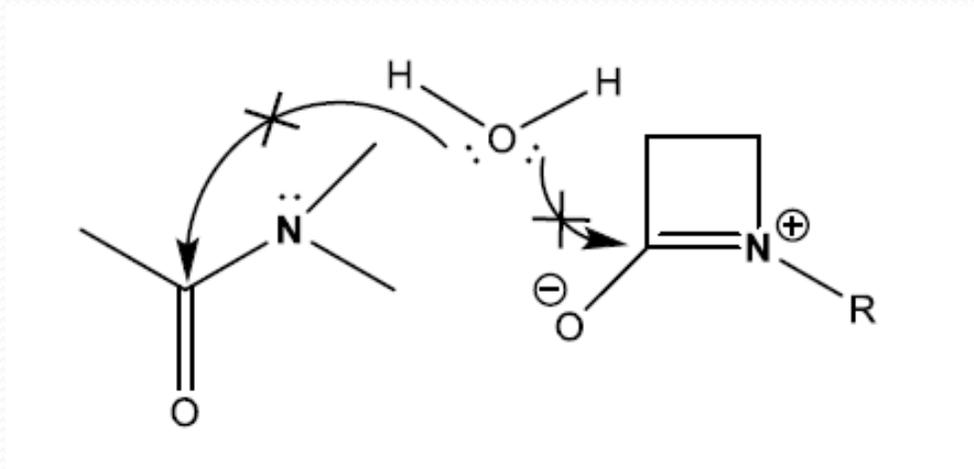
مشتقات الـ Penicillin  
 زي الـ Amoxicillin



مع Thiazolidine Ring الـ Nu الـ  $\beta$ -lactam  
 الـ e نتيجة الـ 3D (الـ optical) رح تنزلج وهنا  
 الـ الشيء ابي بخلي الـ resonance جمع وهيك رح  
 الـ رح تفعل صحبه لة الـ electrophil عثبات  
 هيك رح يحاول ياخذ e من البيئة المحيطة  
 ميتة ابي رح يهبط؟؟ مركبات اسرها nucleophile  
 وبكون عندها e unshared وتنجذب لة electrophile

- امثلة على الـ electrophil
- $H_2O$  - RNA<sub>2</sub>
  - $NH_3$  - Enzyme Protein  
 لأنه كلما  $\alpha$ - $\alpha$  عليه
  - $ROH$  -  $NH_2 / COOH$

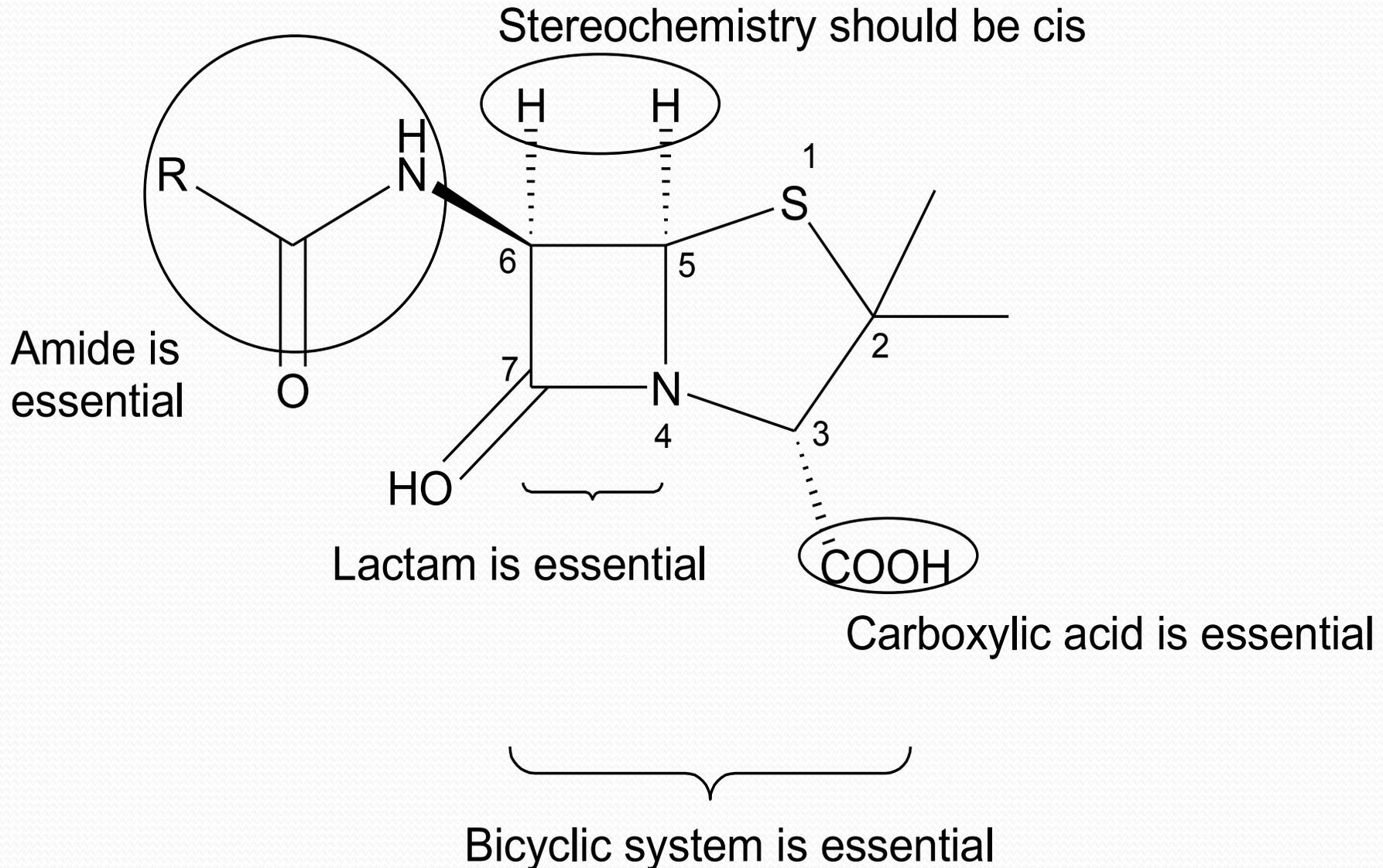
- **NOTE:** the  $\beta$ -Lactam alone is also resistant to hydrolysis as the amides due to resonance but easier than amides (the Nucleophilic attack is more easy).
- For that reason, when one of the penicillins is given orally as solution, it can be used only for one week after dissolving in water due 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  $\beta$ -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



لا تبلى حل مشكلات  
اي حكيما عنها روح  
تبلى بال acid sensitivity

# 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.  $\rightarrow$   $\beta$ -lactam ring

electrophilic  
Center  
اي حكيما عنه قبل  
شوي هو السبب

A highly reactive  $\beta$ -lactam carbonyl group:  $\rightarrow$  ح تتراح ليا تفتح

- 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

الزاوية  $90^\circ$  يعني  
انها مشغولة  
وبدها تفتح

يعني انه لازم يلف حول ال single bond  
بين الحلقة الرباعية تمنع هذا اللف

# 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 حفظها فوق
- Unpleasant taste لحظها مثل زانجى
- Strongly dextrorotay إذا يحطها بالـ polarimeter رح تلف نحو الـ dextrose
- Most penicillins are acidic  $Pka=2.5-3.0$  some are amphoteric زعيه  $\rightarrow$  Amoxicillin
- The free acids are not suitable for parenteral or oral administration. املاح تنزير الذاتية [Na and K salts are suitable to allow both oral and parenteral administration] تقلل الذاتية  $\rightarrow$  increases solubility املاح غير زانجيه زي ابرة الـ اروماتريم عشان
- 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 صيك يحطها لـ I.D ونكون Sustained release

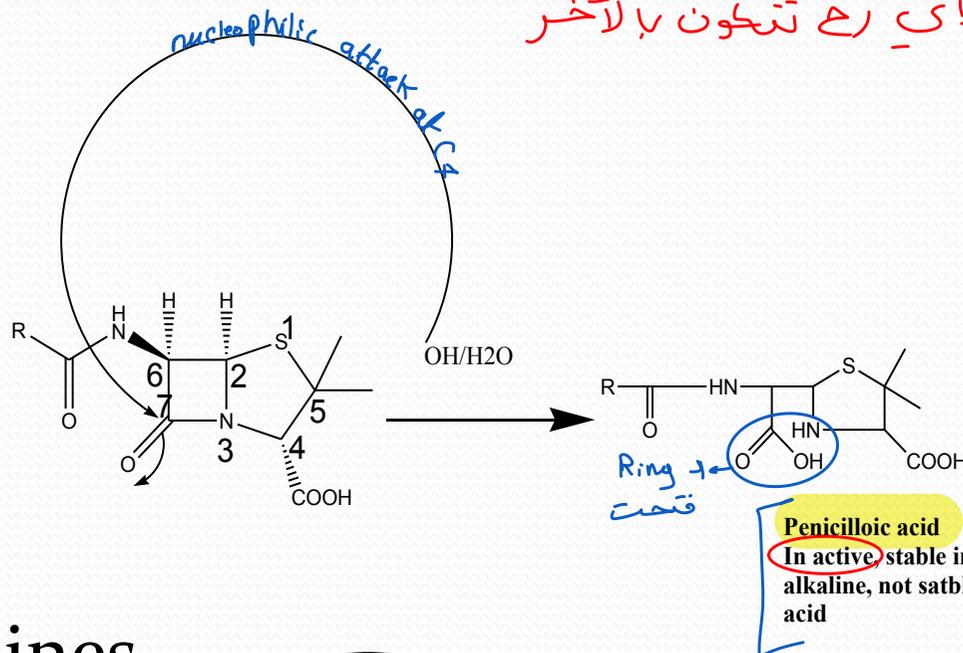
# Chemical reactivity

## 1. reactions with nucleophiles -OH, H<sub>2</sub>O, NH<sub>2</sub>-OH, R-NH<sub>2</sub>, R-OH, and body proteins

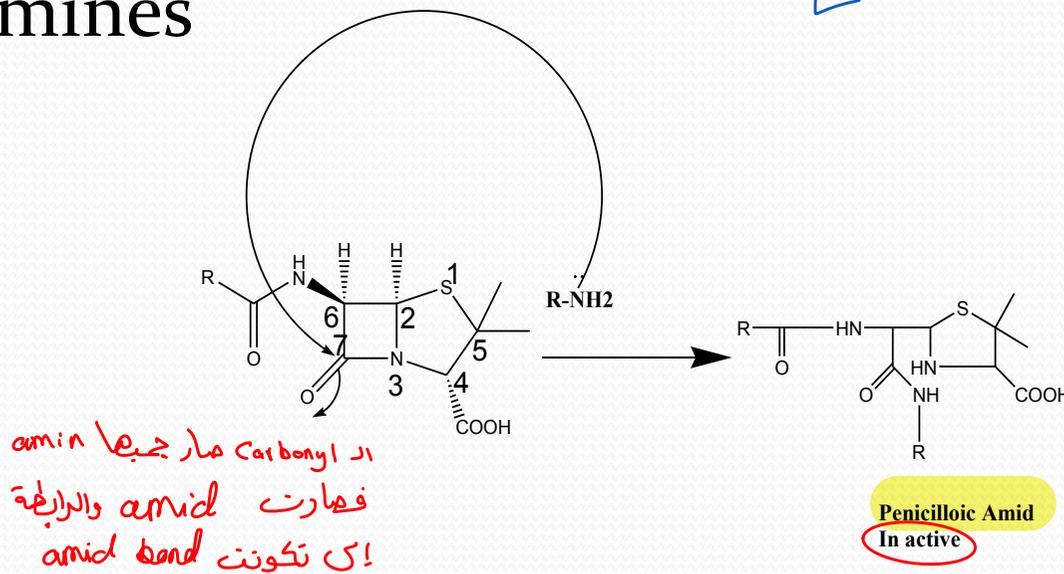
اد Nucleophile هو اي رح بحدود الحارة  
اي رح تتكون بالآخر

وسط قاعدي

### 1. reaction with -OH



### 2. Reaction with alkyl amines



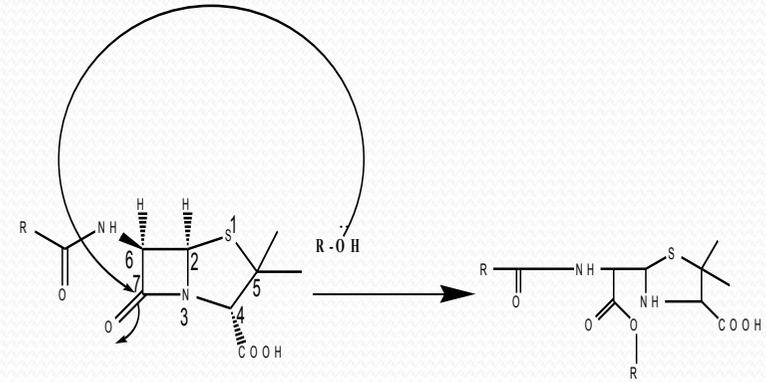
# Chemical reactivity

1. reactions with nucleophiles  $\text{-OH}$ ,  $\text{H}_2\text{O}$ ,  $\text{NH}_2\text{-OH}$ ,  $\text{R-NH}_2$ ,  $\text{R-OH}$ , and body proteins

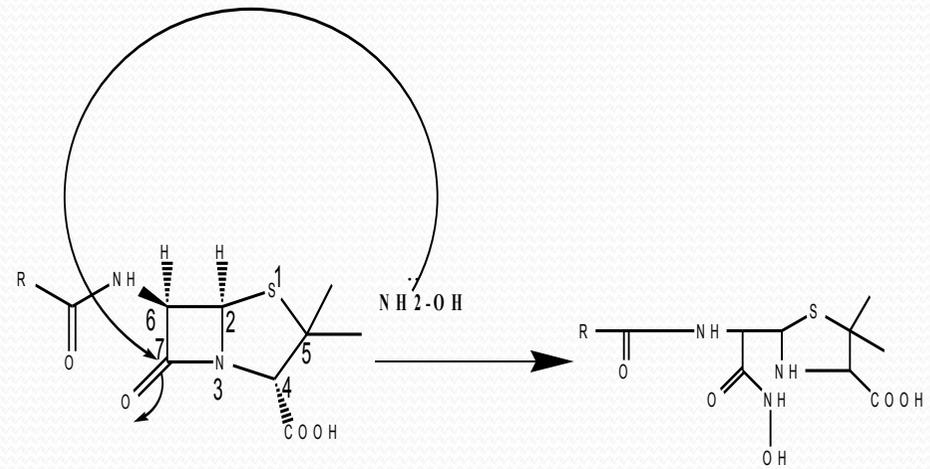
• 3. reaction with Alcohol

يخف جدا باله  
 $\text{NH}_2\text{-OH}$

4. Reaction with hydroxyl amine



Penicilloic Esters  
In active



Penicilloic hydroxamic acid  
In active

# Chemical reactivity

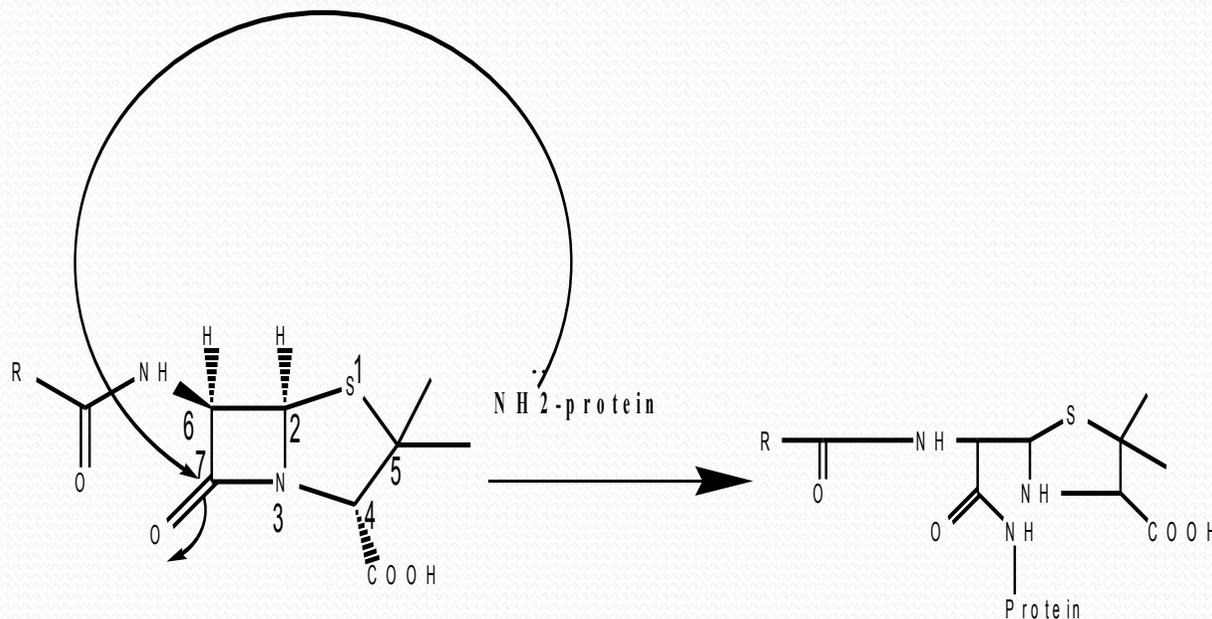
1. reactions with nucleophiles –OH, H<sub>2</sub>O, NH<sub>2</sub>-OH, R-NH<sub>2</sub>, R-OH, and body proteins

حساسية  
الـ Penicillins  
تتطور فجأة

لما جهاز المناعة يعرف كمال molecule في انة جسم غريب وبجهد immun rxn ولازم تعالجها و اذا البركيب دخل الجسم وجهاز المناعة ما

بشافته ما في مشكلة بين! اذا كان كبير رح يشوفه زي كاي ترتيبه و اذا Protein و كذا ما يشوفه الجسم رح يهاجمه

- 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

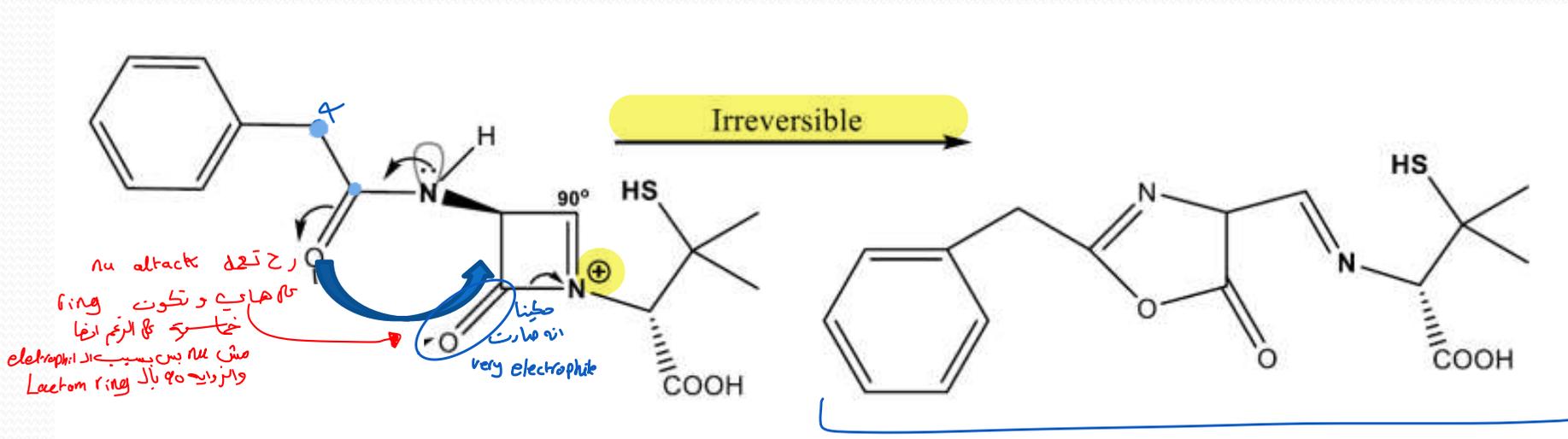




# Chemical reactivity

## 2. reactions with Acids

- Second step (irreversible)



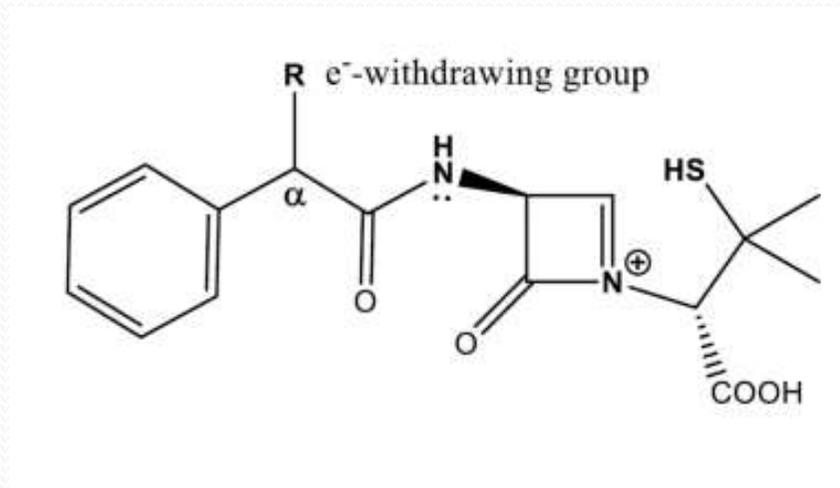
the oxygen becomes a strong nucleophile

هیک رهقی انه ما  
اح استید منه

کیرف اخل المسکة؟! بحط ال Carbon 4

withdrawing group وهیک بسحب ال  
من ال ال کرتبطه بال Carbon 4

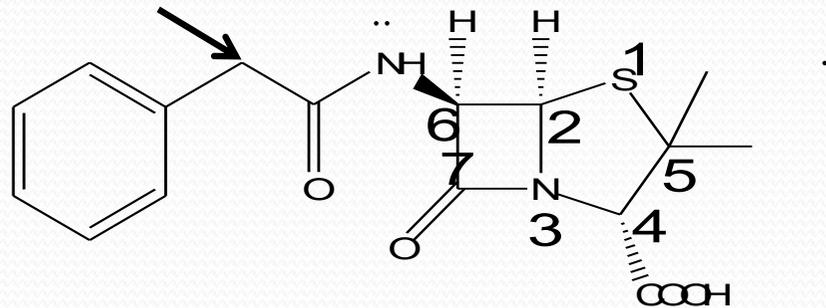
- And by knowing this mechanism, we can actually make "**Orally active penicillins**" **How?**
- if we take the electrons from this oxygen and prevent this attack we will prevent this step, then you have to add an electron withdrawing group at the  $\alpha$  carbon to the amidic carbonyl ( oxygen, amine).



# Acid sensitivity of penicillins

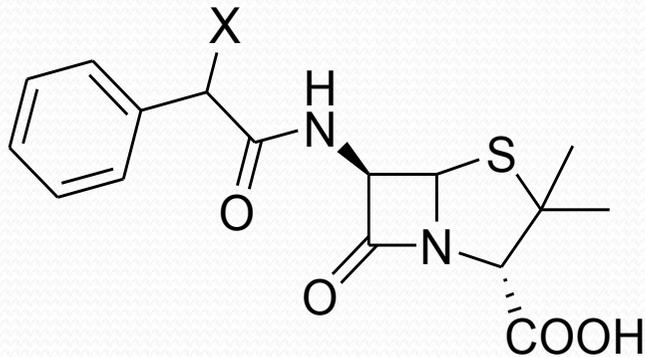
- Electron withdrawing groups in the  $\alpha$ -position of Benzyl penicillin will improve acid stability clearly

$\alpha$ -position

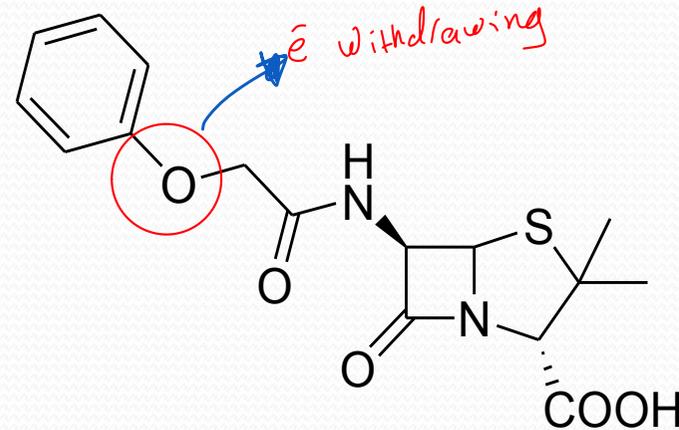


# Acid sensitivity of penicillins

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

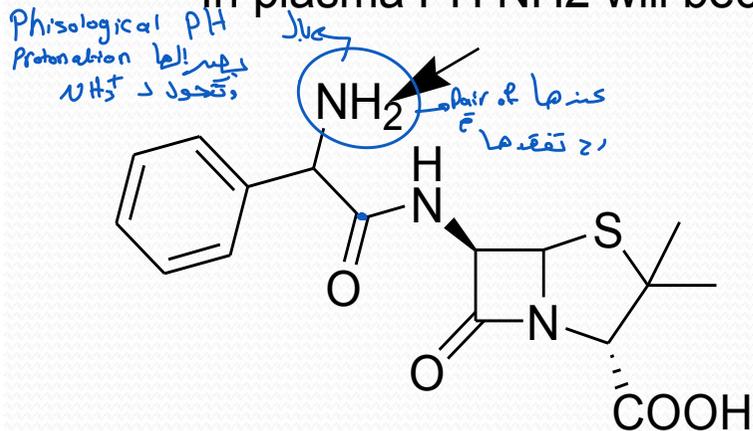


alpha - Halo benzyl penicillin (X=Cl, Br, I)

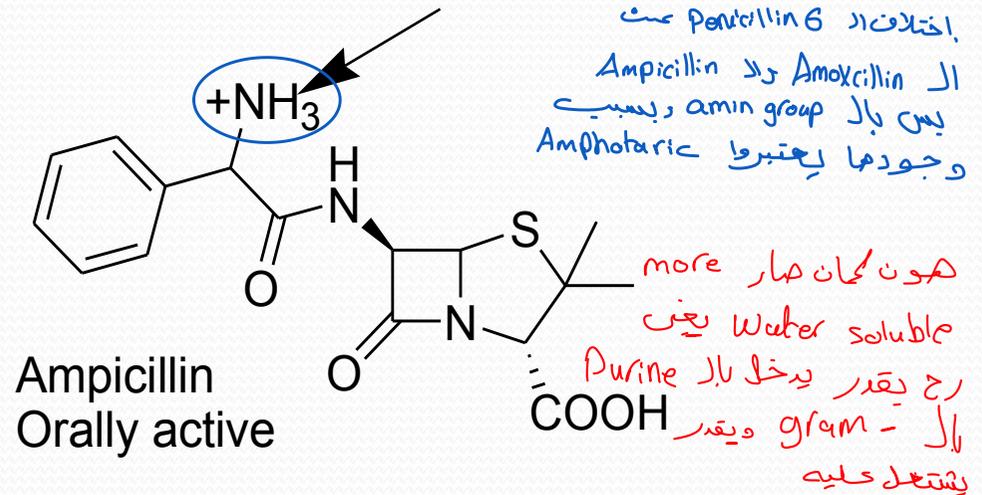


Phenoxy methyl penicillin: Penicillin V  
Acid resistant  
can be given orally

In plasma PH  $\text{NH}_2$  will become ionized to  $\text{NH}_3^+$  so it will become electron withdrawing

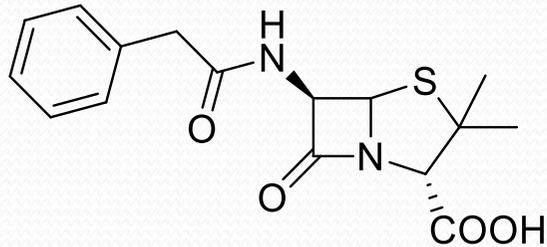


Plasma PH

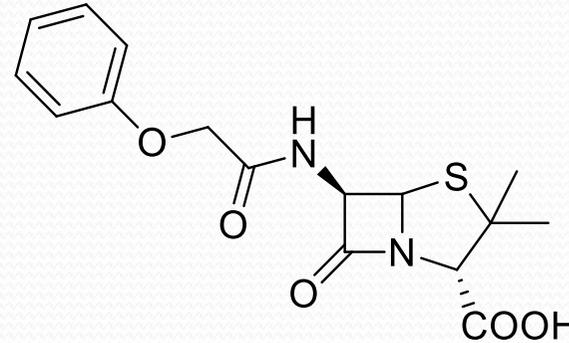


Ampicillin  
Orally active

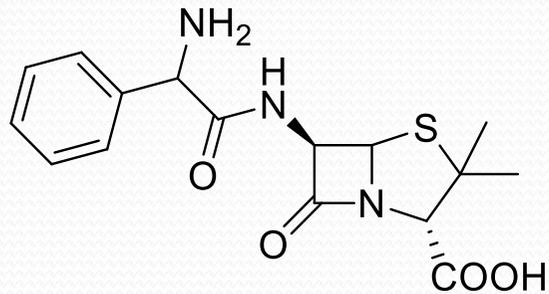
# Acid resistant Penicillins



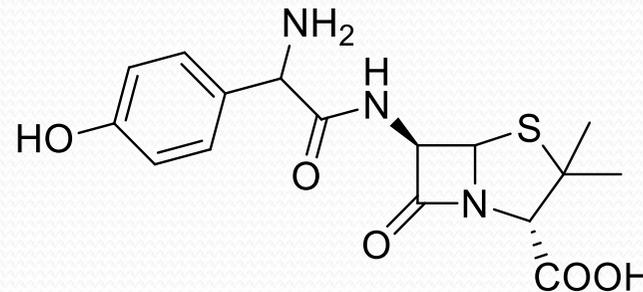
Penicillin G  
Acid labile  
Can not be given orally



Penicillin 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%