ANTIBIOTICI

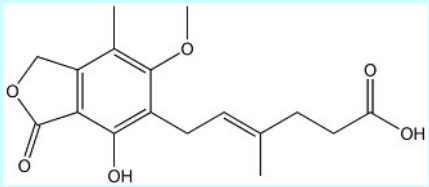
DEFINIZIONE DI ANTIBIOTICI:

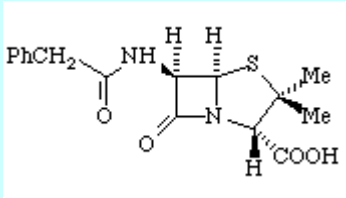
Sostanze, prodotte da microrganismi, capaci di inibire a basse dosi la crescita ed eventualmente distruggere altri microrganismi.

Selman Waksman (1941)

ANTIBIOTICI

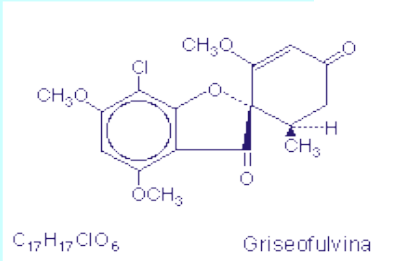
1893 - Acido Micofenolico dal *Penicillum brevicompactum* (Gosio)





1929 - Penicillina dal *Penicillum notatum* (Fleming)

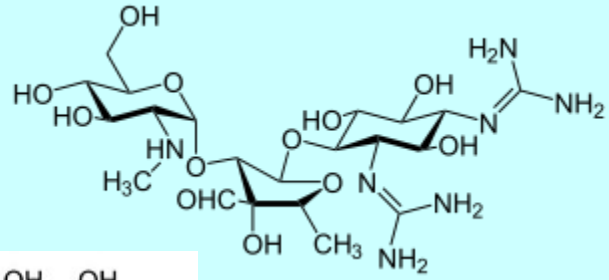
1939 – Tirotricina (tiroidina + gramicidina) dal *Bacillus brevis* (Dubos), solo per uso topico.



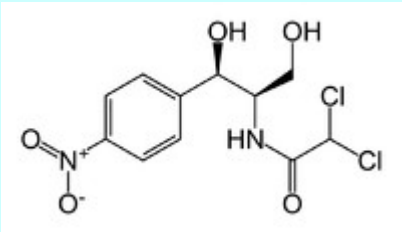
1939-1947 – Griseofulvina dal *Penicillum griseofulvum* (Oxford, Grove e McGowan)

1940-1941 – Produzione Penicillina da *Penicillum notatum* (Florey e Chain)

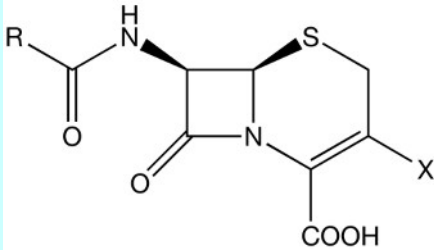
1943 - Produzione Penicillina da *Penicillum chrysogenum*



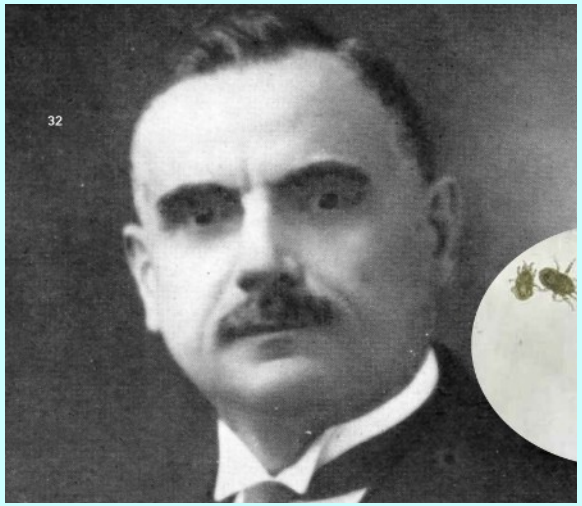
1944 – Streptomicina da *Streptomyces* (Waksman), tubercolostatico



1947 – Cloramfenicolo da *Streptomyces venezuelae* (Gottlieb), largo spettro



1947 – Cefalosporine (Brotzu)



Bartolomeo Gosio (1863 – 1944)

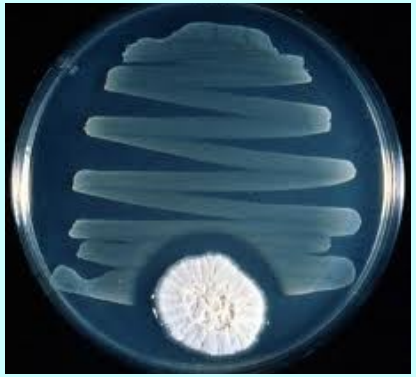
Medico, microbiologo e biochimico italiano.
In una specie di muffa, scoprì un metabolita con delle proprietà antibiotiche, e la purificò. L'acido micofenolico (MPA) è stato il primo antibiotico della storia a essere stato ben caratterizzato.
L'acido micofenolico inibisce l'IMP deidrogenasi, l'enzima che controlla la velocità di sintesi della guanina monofosfato per la via di sintesi de novo delle purine, usata in particolare dai linfociti B e T in fase proliferativa.
Il micofenolato è un farmaco potente e può essere utilizzato al posto di altre terapie anti-proliferative, come l'azatioprina. Di solito viene usato come parte di un regime composto da tre immunosoppressori, tra cui anche un inibitore della calcineurina (ciclosporina o tacrolimus) e prednisolone.

Vincenzo Tiberio (1869 – 1915)

Ricercatore e ufficiale medico del Corpo Sanitario della Marina Militare italiana.
Una quarantina di anni prima che Fleming riscontrasse la colonia di Penicillium notatum in una coltura di stafilococchi, vale a dire nel 1893, un medico della Marina militare italiana, Vincenzo Tiberio, aveva già formulato l'ipotesi, avvalorata poi dai risultati di ricerche sperimentali da lui condotte e documentata in uno studio, del potere di distruzione sui batteri di certe muffe.

Tutto ebbe inizio nella casa di via Zanardelli ad Arzano, un paese vicino Napoli, dove Tiberio viveva e dove vi era un pozzo per la raccolta dell'acqua piovana utilizzata per bere. L'umidità del luogo faceva sì che sul bordo della cisterna crescesse spesso un denso strato di muffa che veniva periodicamente rimosso. Tiberio notò che ogni qual volta il pozzo veniva ripulito, gli abitanti della casa andavano incontro a gastroenteriti, mentre ciò non accadeva nei periodi in cui le muffe erano presenti. Egli intuì che tali muffe fossero dotate di un'azione battericida, pertanto, le isolò, ne testò l'effetto benefico, sia in vitro, sia in vivo su varie cavie, e preparò un farmaco sperimentale con effetti antibiotici. Nel gennaio del 1895 Tiberio pubblicò sulla rivista universitaria napoletana Annali di Igiene Sperimentale i risultati di queste ricerche in un articolo dal titolo *Sugli estratti di alcune muffe*. Purtroppo per Tiberio, l'articolo era in italiano ed ebbe una pressoché nulla diffusione all'estero, e comunque i colleghi italiani ritennero i suoi risultati il frutto di fortuite coincidenze e di nessun interesse scientifico.

ANTIBIOTICI



Il batteriologo **Alexander Fleming**
(St. Mary's Hospital di Londra nel 1928)

Howard Florey **Ernst Chain** **Edward Abraham**
(Oxford University 1939)

Andrew J. Moyer
(Peoria Lab, Illinois 1941)

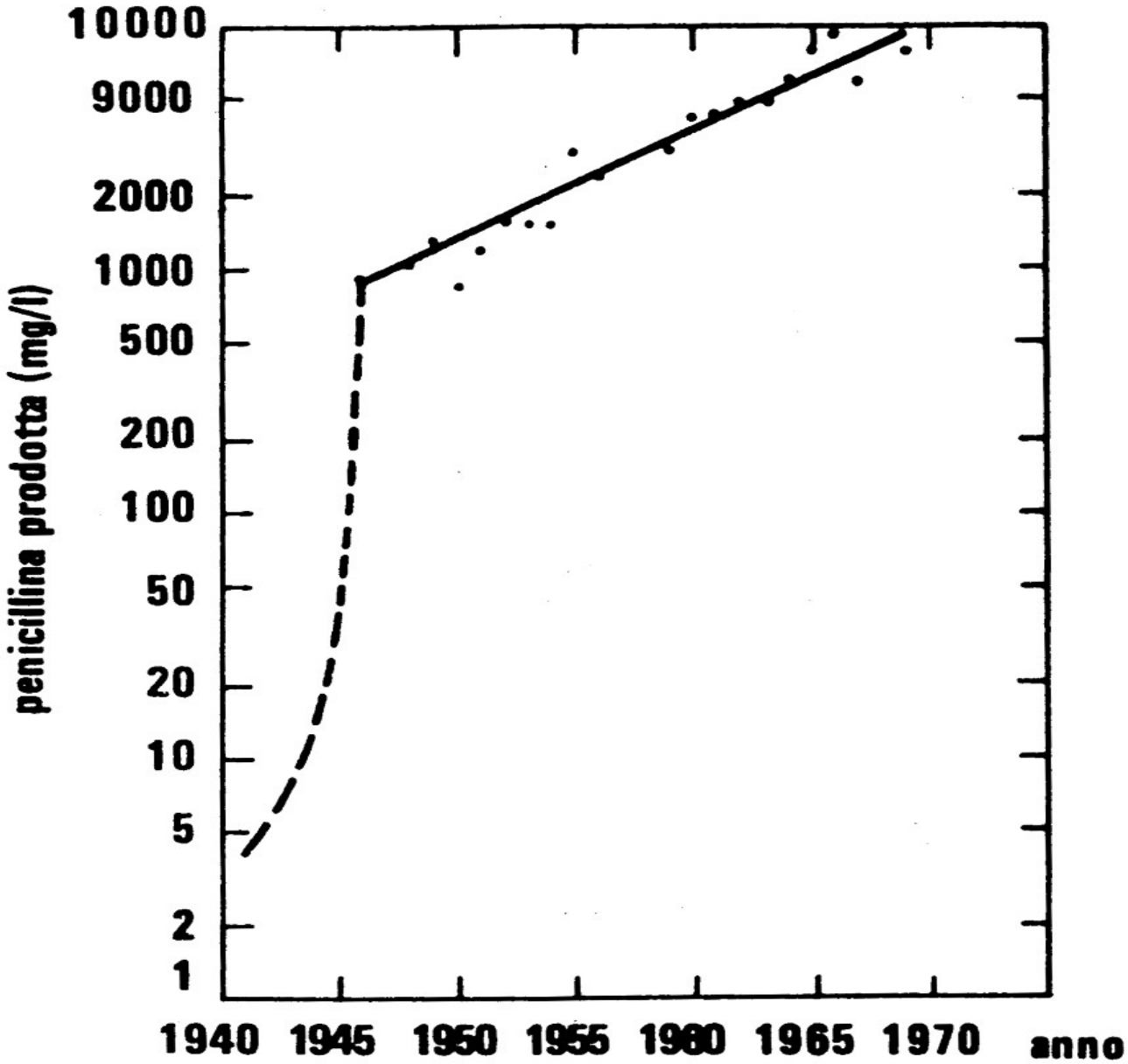


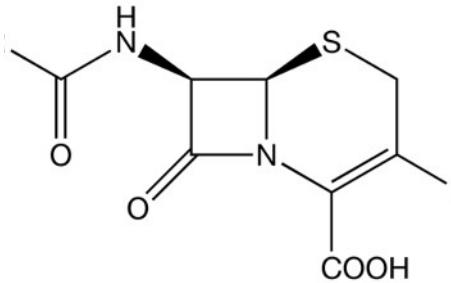
Fig. 10.1 – Aumento della quantità di penicillina ottenuta per litro di colture negli anni dal 1941 al 1969

Giuseppe Brotzu (1895 –1976)

LAVORI DELL'ISTITUTO DI IGIENE DI CAGLIARI

Ricerche su di un nuovo antibiotico

CAGLIARI - Tip. C. E. L. 1948



Prof. GIUSEPPE BROTZU

Isolation of Antibiotics from a Species of *Cephalosporium*. Cephalosporins P_1, P_2, P_3, P_4 and P_5

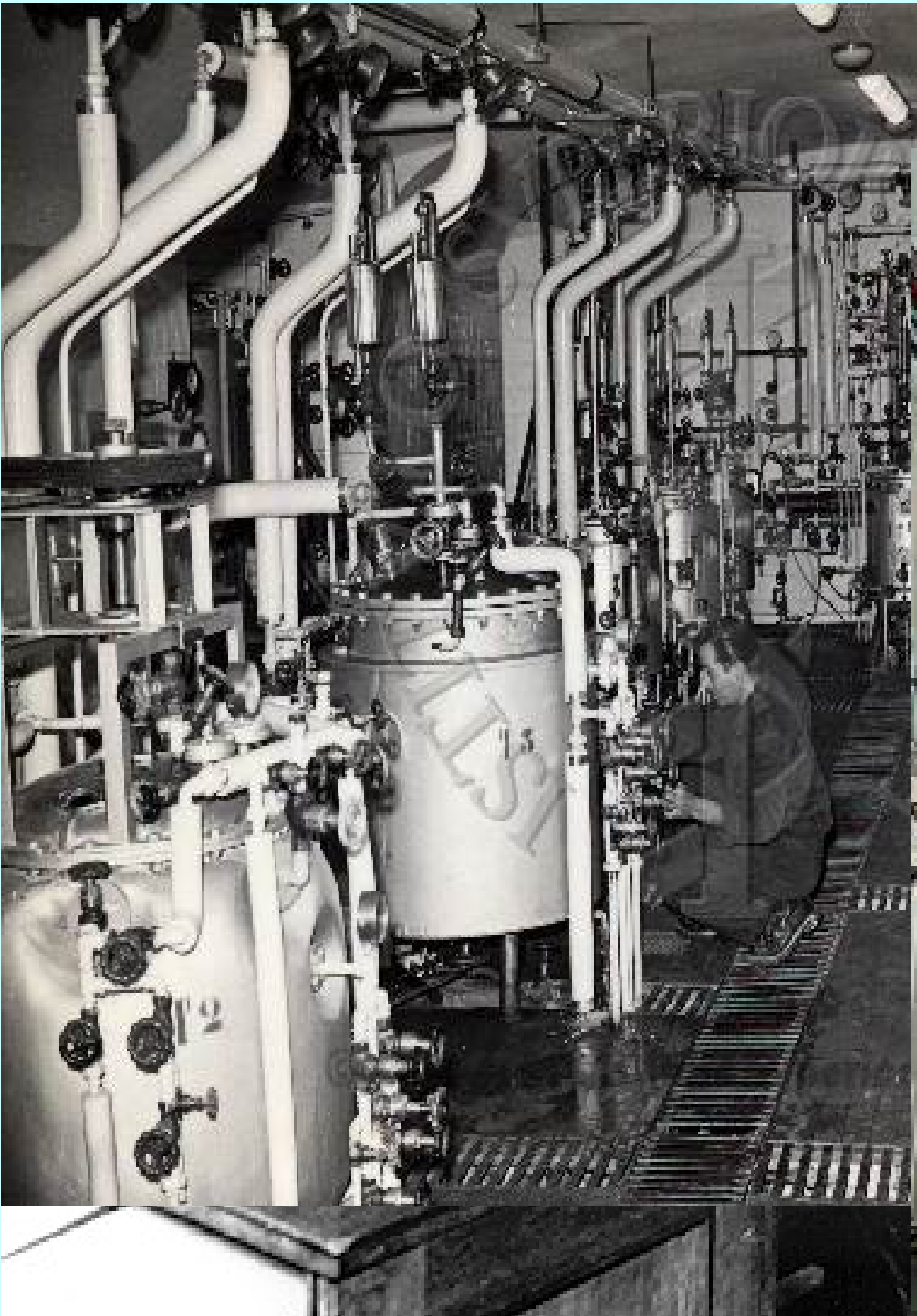
By H. S. BURTON and E. P. ABRAHAM
Sir William Dunn School of Pathology, University of Oxford

(*Received* 2 *May* 1950) Biochem. J. 50:168-174;1951.

Brotzu (1948) reported that a species of *Cephalosporium* isolated from the sea near Sardinia produced a substance that inhibited the growth of a number of Gram-positive and Gram-negative bacteria. The

in crystalline form. All five substances have been found to have some properties in common with helvolic acid, an antibiotic produced by various strains of *Aspergillus fumigatus* (Chain, Florey,

ANTIBIOTICI



ANTIBIOTICI

QUALI SONO I VANTAGGI E GLI SVANTAGGI DI UN BIOPROCESSO RISPETTO AD UN PROCESSO CHIMICO?

Vantaggi	Svantaggi
Produzione di molecole complesse	Facile contaminazione
Rese superiori	Necessità di processi di separazione a valle
Condizioni di lavorazione più blande (temp. più basse, pH quasi neutro)	Approvvigionamento, gestione e scarico di ingenti volumi d'acqua
Catalisi più specifica	Lentezza del processo
Ottenimento di un composto isomerico	

MODALITÀ CON CUI SI COLTIVANO I MICRORGANISMI ALL'INTERNO DI UN BIOREATTORE

colture in batch (a sistema chiuso) : Si inoculano microrganismi in un volume fisso di terreno liquido; mentre si sviluppa la crescita microbica, le sostanze nutritive si consumano e i prodotti della crescita (biomassa, metaboliti) si accumulano; perciò, l'ambiente nutritivo all'interno del bioreattore è soggetto a continue variazioni, che a loro volta provocano cambiamenti nel metabolismo cellulare. Infine le cellule cessano di moltiplicarsi, in seguito all'esaurimento o alla scarsità del nutriente o dei nutrienti e all'accumulo delle sostanze tossiche di rifiuto, escrete dagli organismi stessi.

colture in fed batch: per fare aumentare la fase stazionaria si addiziona gradualmente il terreno, così da aumentare il volume della coltura

Le fasi del processo di fermentazione

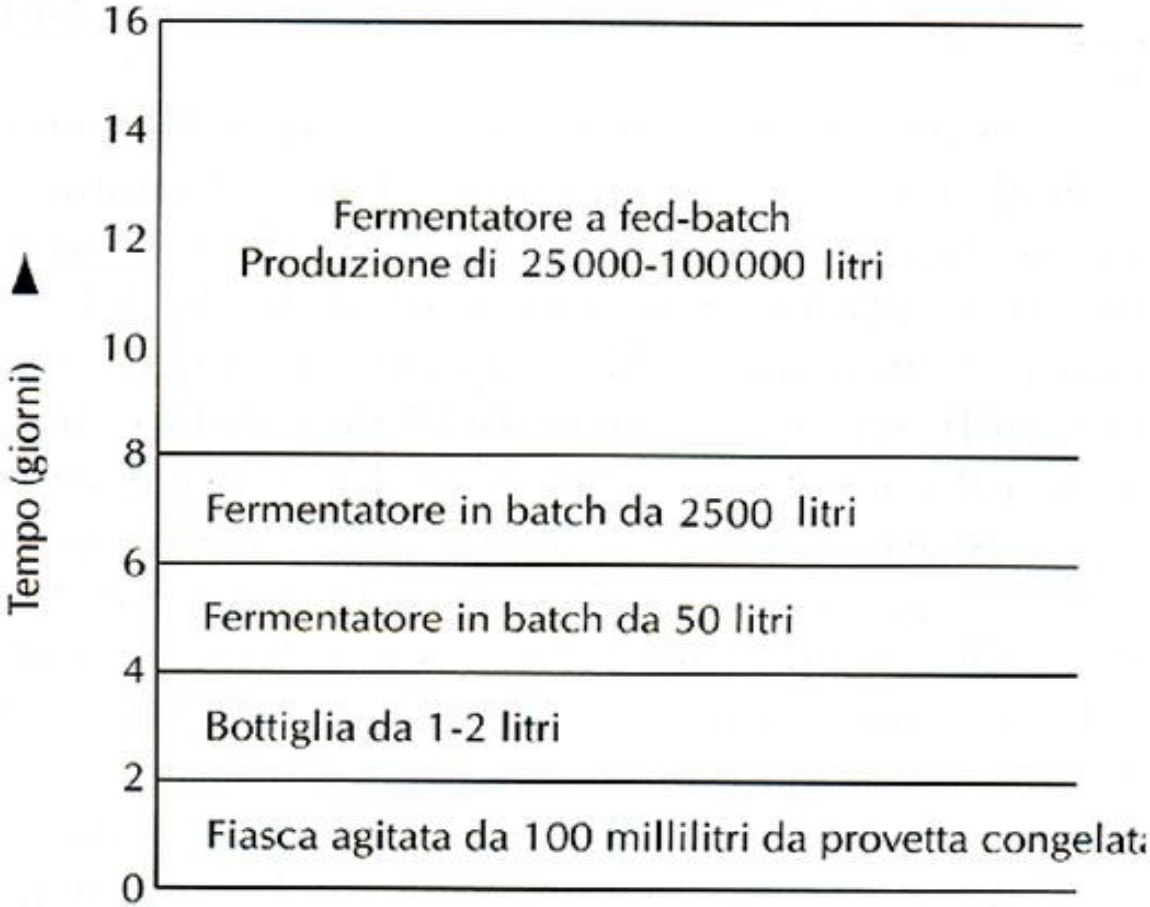
Massima produttività volumetrica
Minore tempo di *turn around* (tempi di fermata) per i fermentatori di produzione

Produttività = R/ t + tempo di fermata

Uso di treni di fermentatori di volume crescente per un veloce aumento della biomassa
(1-5% volume successivo)

Terreni di coltura semplici nelle fasi di amplificazione
Carboidrati facilmente assimilabili

Ottimizzazione del processo fermentativo con fed-batch



ANTIBIOTICI

CARATTERISTICHE DEL CEPPO DA USARE NEL PROCEDIMENTO INDUSTRIALE DI FERMENTAZIONE

- Il ceppo deve essere geneticamente stabile
- Deve riprodursi rapidamente nelle condizioni usate
- Devono essere assenti contaminanti (altri microrganismi, virus)
- La fermentazione deve essere veloce
- Il ceppo non deve produrre altre sostanze tossiche e/o difficilmente separabili
- Le condizioni di crescita dovrebbero ostacolare la crescita di eventuali microrganismi concorrenti
- Il ceppo deve poter essere conservato per periodi di tempi ragionevolmente lunghi

Mutazioni genetiche (con agenti mutageni chimici e fisici)

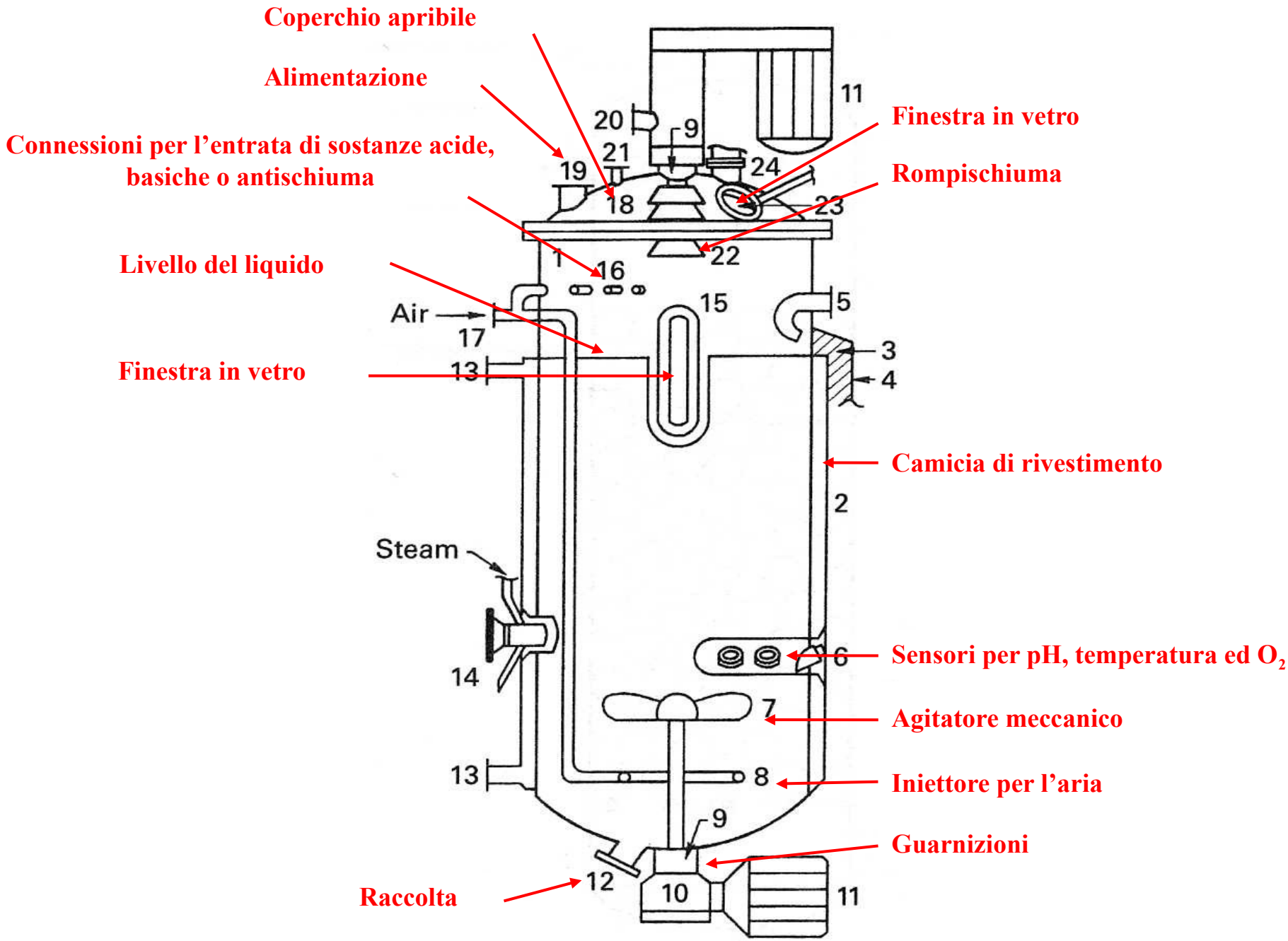
Selezione dei mutanti (screening)

Incroci e ingegneria genetica (coniugazione e trasferimento di geni con enzimi di restrizione)

Funzioni principali di un bioreattore

- **Mantenere la sterilità**
  - si deve fare attenzione alle **aperture del reattore** utilizzate per introdurre le sonde di controllo, per l'agitazione, per l'immissione ed il prelievo di substrati e prodotti
  - in particolare devono essere sempre efficienti **valvole**, **giunti** e **guarnizioni**
  - è opportuno mantenere una piccola **sovrapressione** di 0.2 - 0.4 bar
- **Mantenere l'omogeneità del mezzo (agitazione)**
- **Permettere gli scambi gassosi, soprattutto O_2**
- **Smaltimento del calore, termostatazione**
- **Controllo del processo (reattore solitamente chiuso e controllato da varie "sonde")**

Bioreattori: caratteristiche generali



Bioreattori: caratteristiche generali

- **I più utilizzati sono quelli a batch**
- **Capienza: da 20 l a 250-300 m^3**
- **La maggior parte dei processi utilizzano colture sommerse**
- **Materiali**
  - **Acciaio inox**
  - **Vetro (per reattori da laboratorio fino a 40 l circa)**
  - **Rame**, **alluminio**, **cemento, legno**
  - **Rivestiti di materiale polimerico** (per produzioni "speciali")
- **Devono resistere alla corrosione;** pericolosa non solo dal punto di vista strutturale ma anche come fonte di inquinamento del processo
- **Forma cilindrica;** permette il miglior scambio di materia e sono più facili da sterilizzare

Bioreattori: caratteristiche generali

- **Il reattore è riempito per 3/4**; nella testa tende a formarsi schiuma controllata tramite un agitatore rompischiuma o con l'uso di agenti antischiuma.
- **Massima pressione tollerata: 3.8 – 4.1 bar**
- **Massima temperatura tollerata 150 – 180 °C (si sterilizza solitamente a 121 °C)**
- **Deve tenere il vuoto per evitare "implosioni" (depressione nella fase di raffreddamento dopo sterilizzazione)**
- **Non ci devono essere zone interne difficili da raggiungere (anfratti, cavità) nelle fasi di pulizia e sterilizzazione**
- **Di solito il reattore è rivestito con una camicia isolante esterna (controllo temperatura, smaltimento calore ...)**

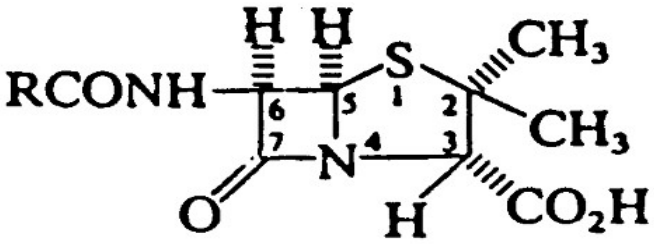
ANTIBIOTICI

Table 1.3 General medium composition for industrial batch and fed-batch cultivation in penicillin V production [210].

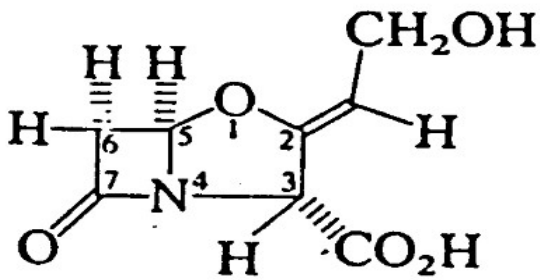
Constituent	Batch	Fed-batch
Corn Steep Liquor	50 g/L	50-200 g/L
Glucose/Sucrose	30 g/L	3 g/L
Phenoxyacetic acid*	–	5 g/L
$(NH_4)_2SO_4$	10 g/L	12 g/L
KH_2PO_4	2 g/L	1 g/L
$CaCl_2.2H_2O$	60 mg/L	60 mg/L

*In penicillin G production phenylacetic acid is used instead of phenoxyacetic acid. Due to the higher toxicity phenylacetic acid concentrations are in general regulated at a lower level than phenoxyacetic acid.

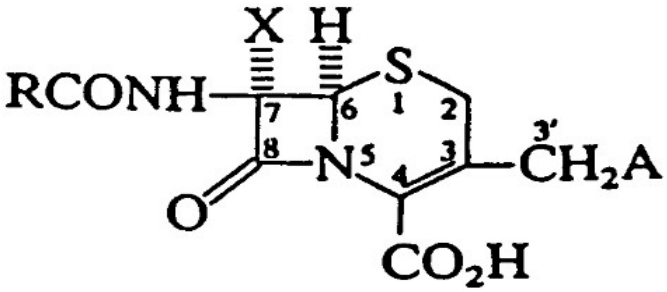
ANTIBIOTICI β-LATTAMICI



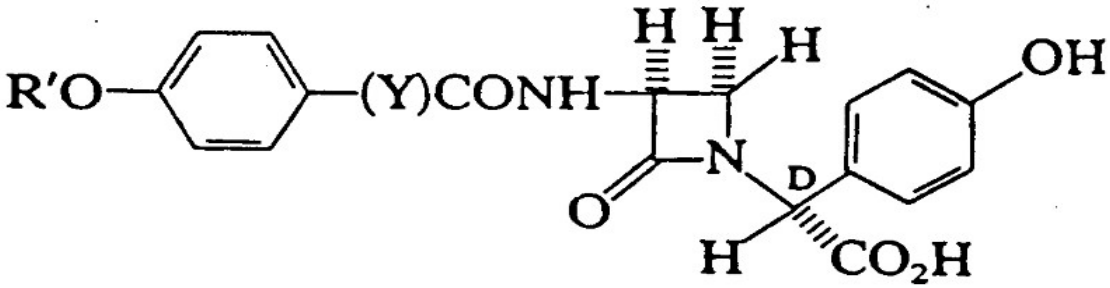
Penicillins



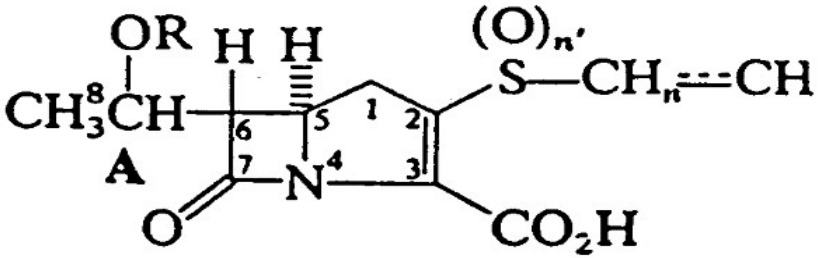
Clavulanic Acid



Cephalosporins (X = H)
Cephamycins (X = OCH_3)



D
R′ = H, $HO_2CCH(NH_2)CH_2CH_2$-;
Y C=NOH (syn, anti), $CHNH_2$, C=O
Nocardicins A–G



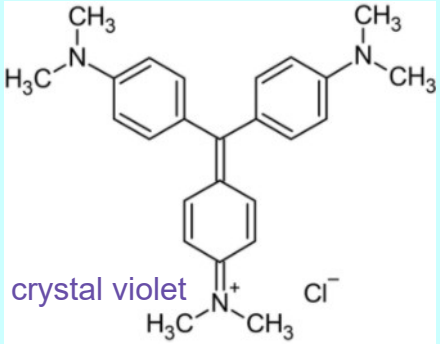
(Epi) Thienamycins
6α-CH_3CHOH
6β-CH_3CHOH
A = R, S; R = H, SO_3H; R¹ = H, $COCH_3$
$n = 1, 2$; $n' = 0, 1$

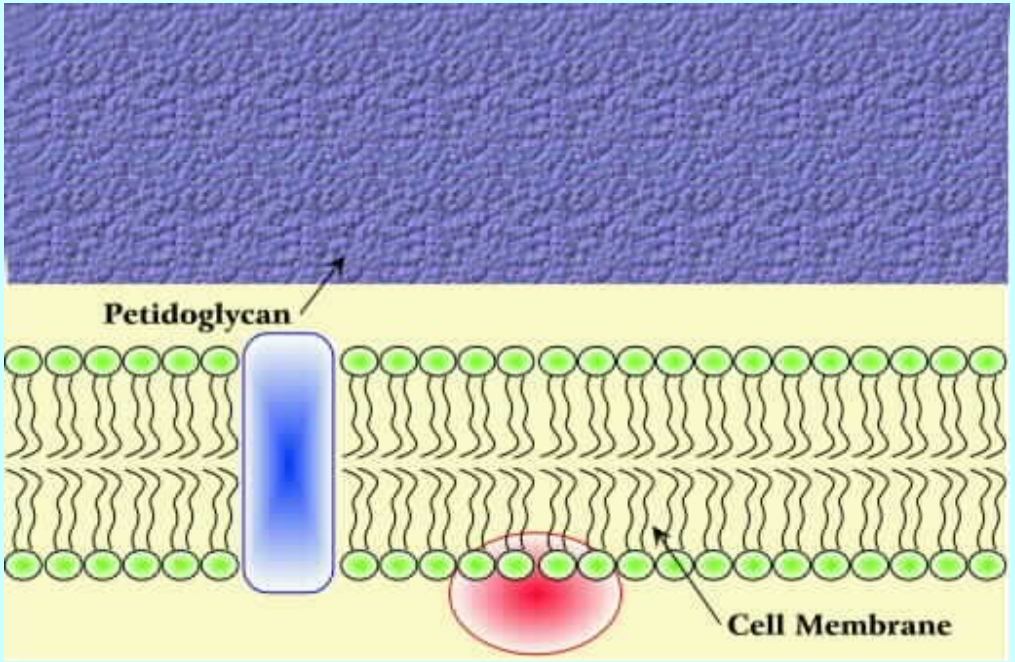
BATTERI GRAM POSITIVI E GRAM NEGATIVI

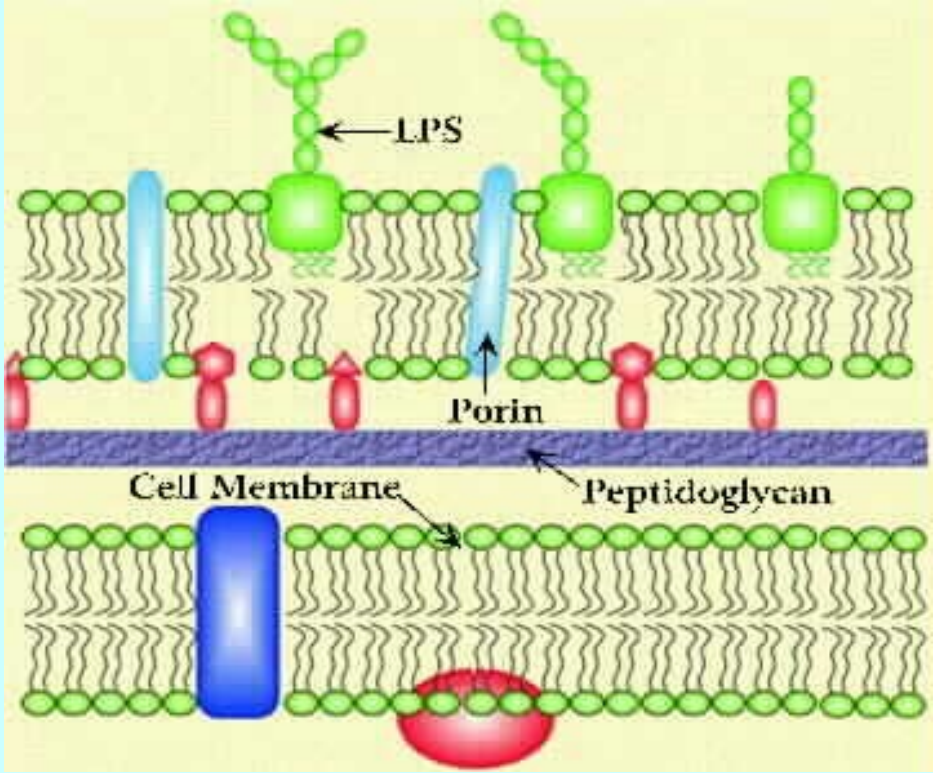
La denominazione dipende dalla capacità di trattenere il crystal violet (blue = **Gram** positivo) contenuto nel colorante di **Gram** dopo decolorazione con alcohol. Il controcolorante (safranina o fucsina) rende i batteri **Gram** negativi rossi. Entrambi hanno un reticolo di peptidoglicano (acido N-acetilmuramico e N-acetilglucosamine).

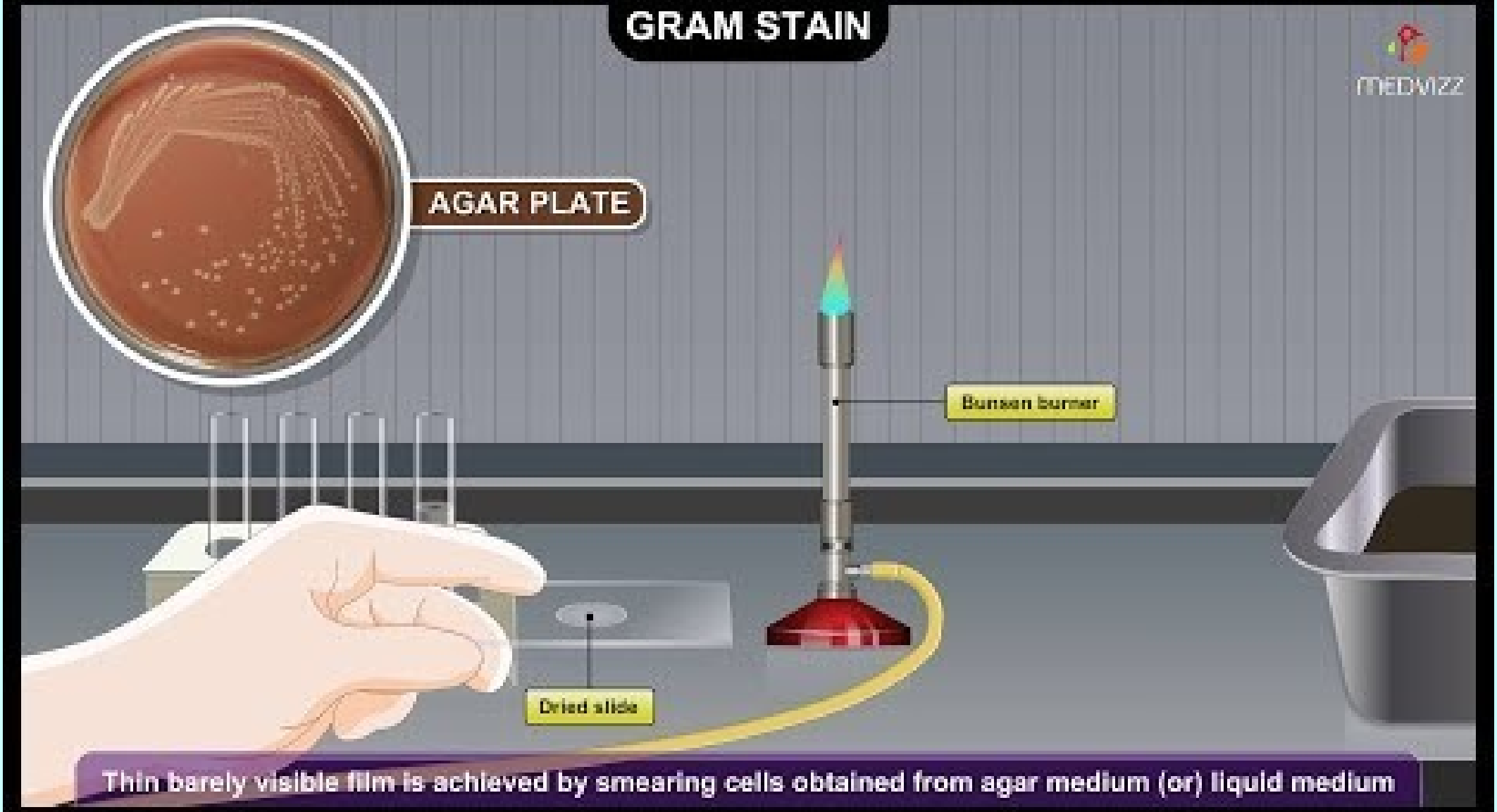
Confronto tra pareti cellulari batteriche di Gram + e Gram -

	Gram +	**Gram -**
Peptidoglicano	spesso	sottile
lipopolisaccaridi	no	si
lipoproteine	no	si
lysozyme sensibile	si	+/-
polisaccaridi	si	si

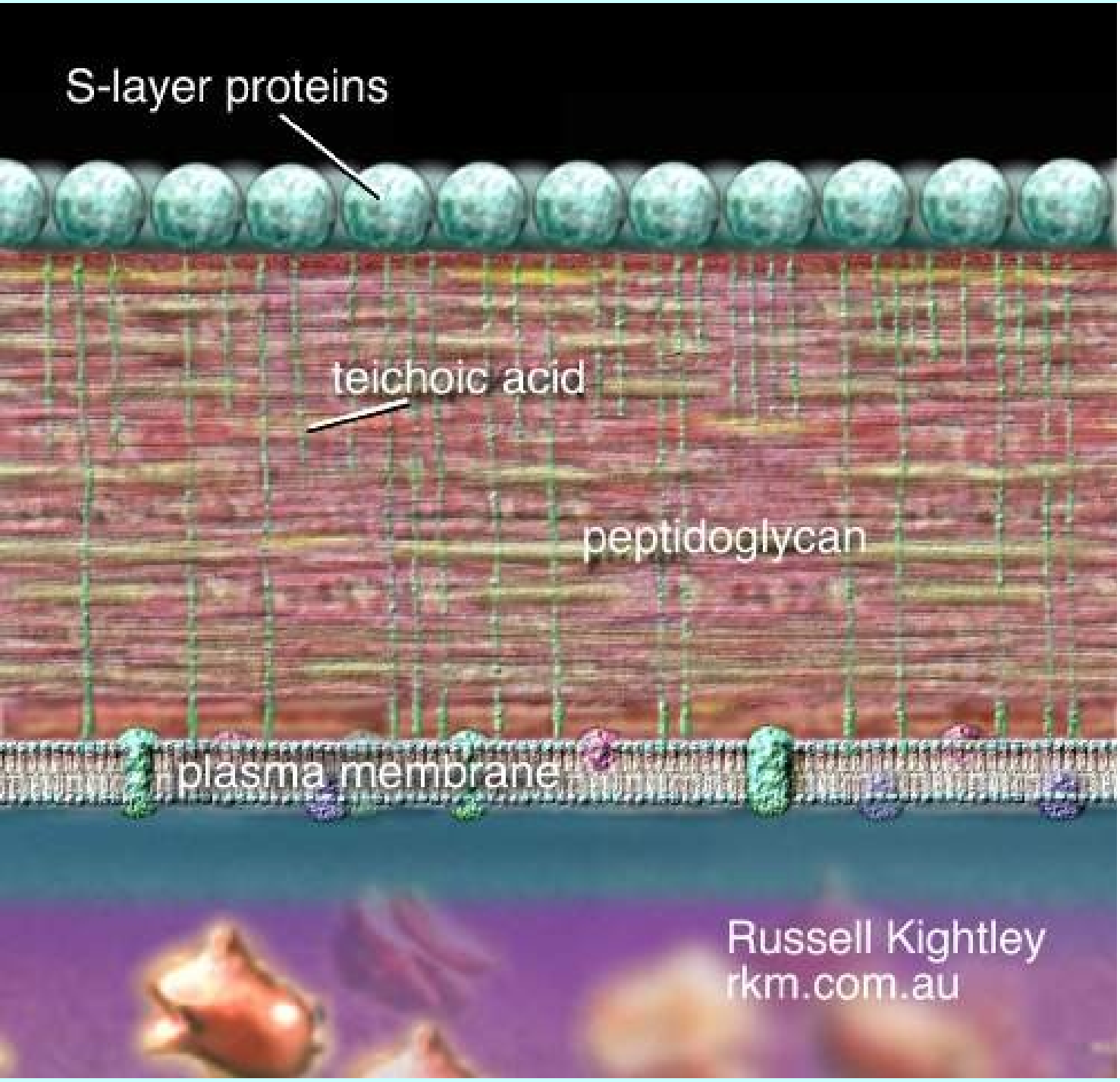


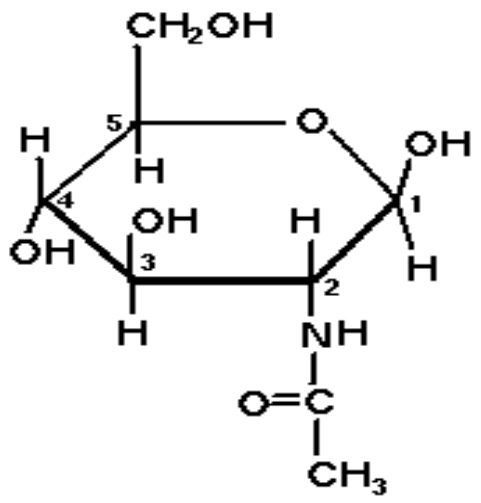




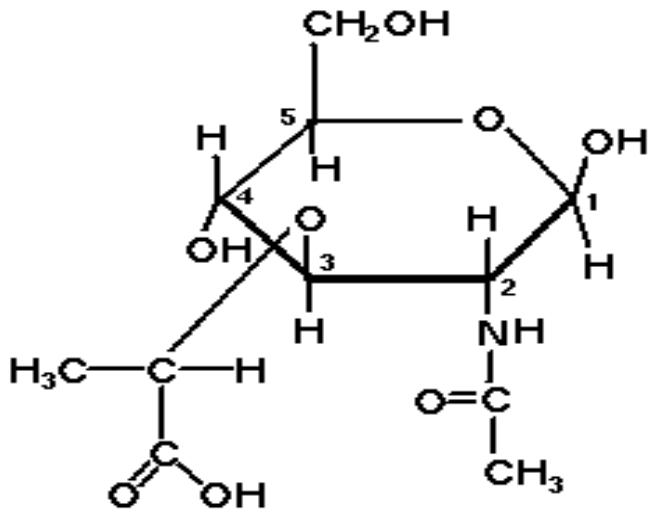

GRAM STAIN
MEDVIZZ
AGAR PLATE
Bunsen burner
Dried slide
Thin barely visible film is achieved by smearing cells obtained from agar medium (or) liquid medium

PARETE DEI BATTERI GRAM POSITIVI



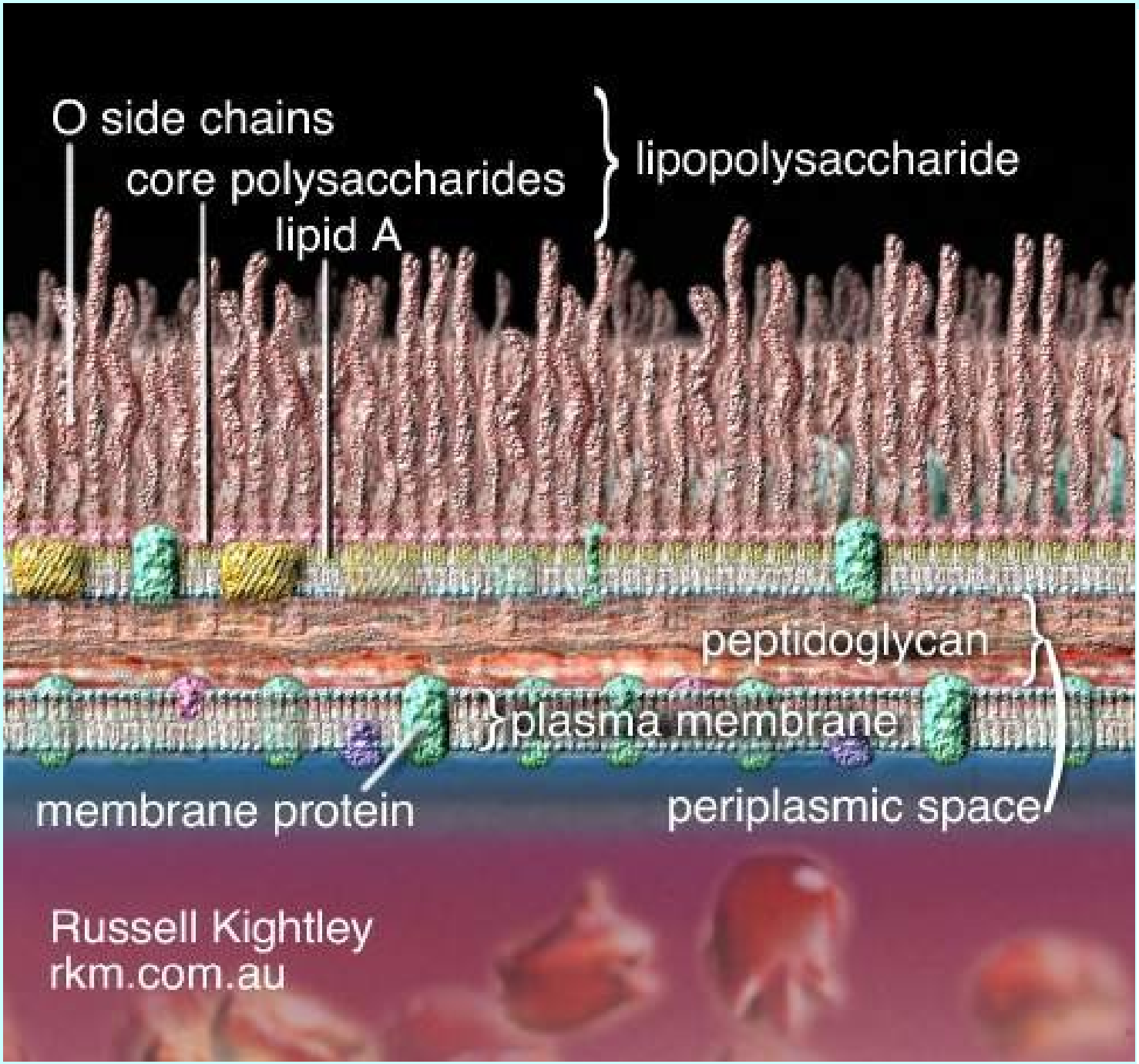


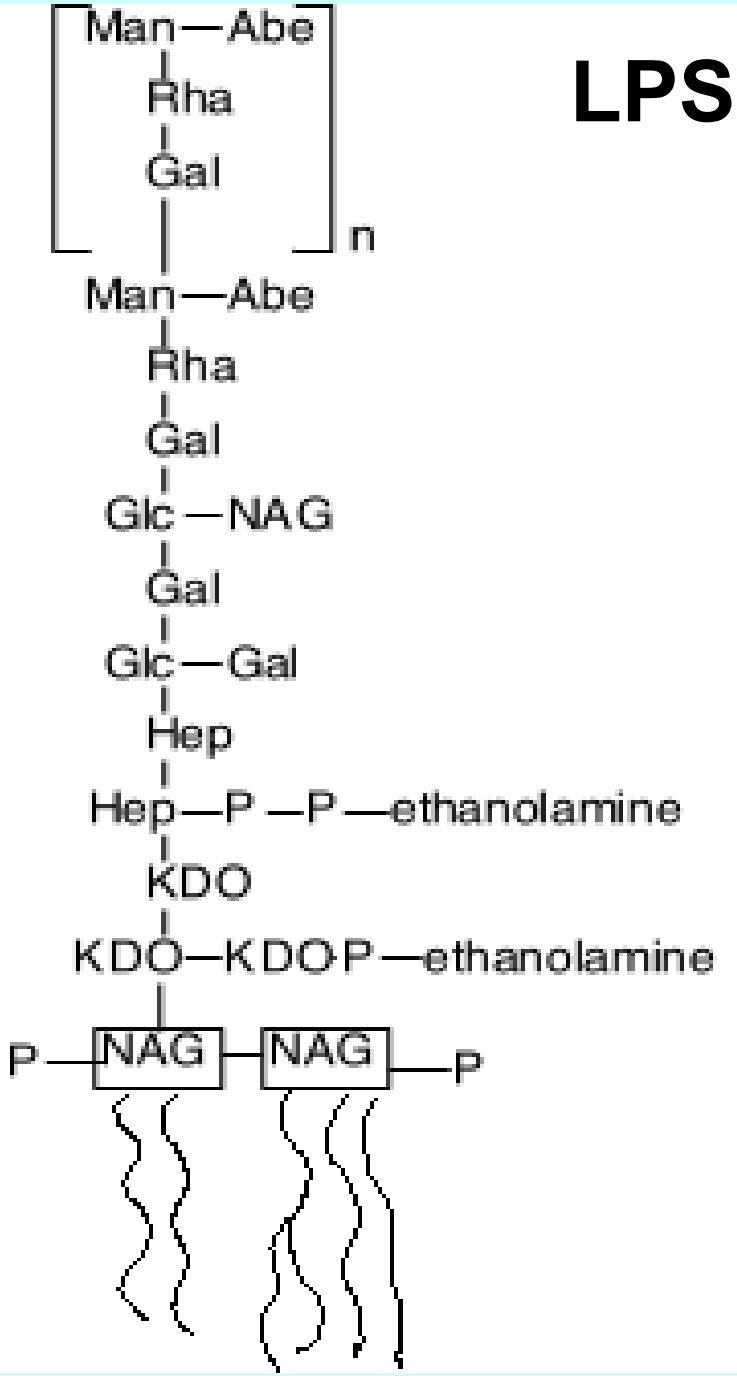
N–acetylglucosamine (NAG)

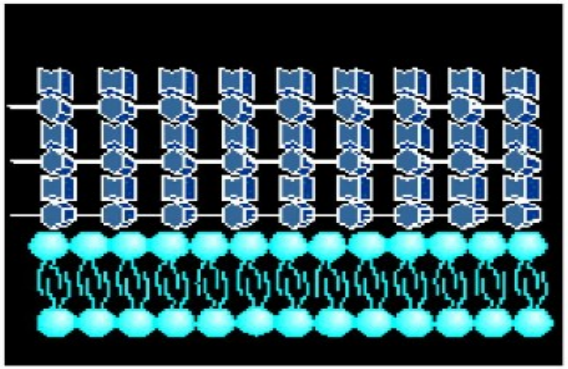
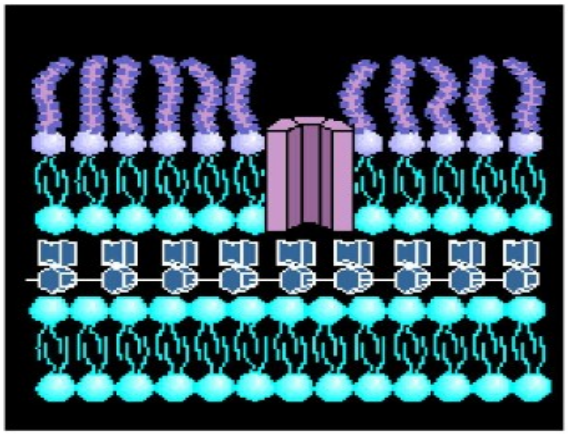
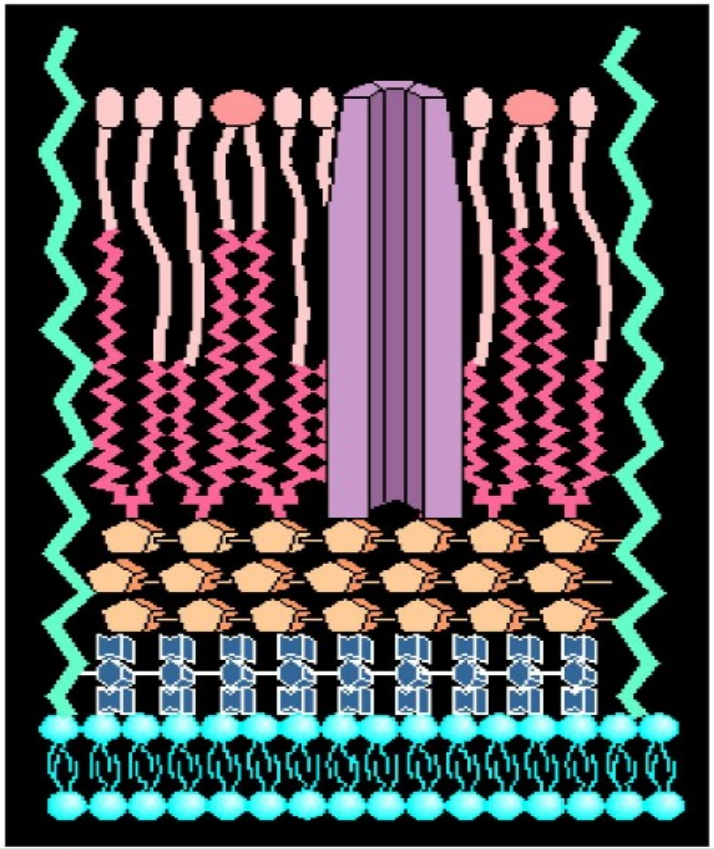


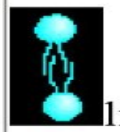
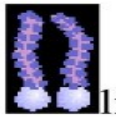
N–acetylmuramic acid (NAM)

PARETE DEI BATTERI GRAM NEGATIVI

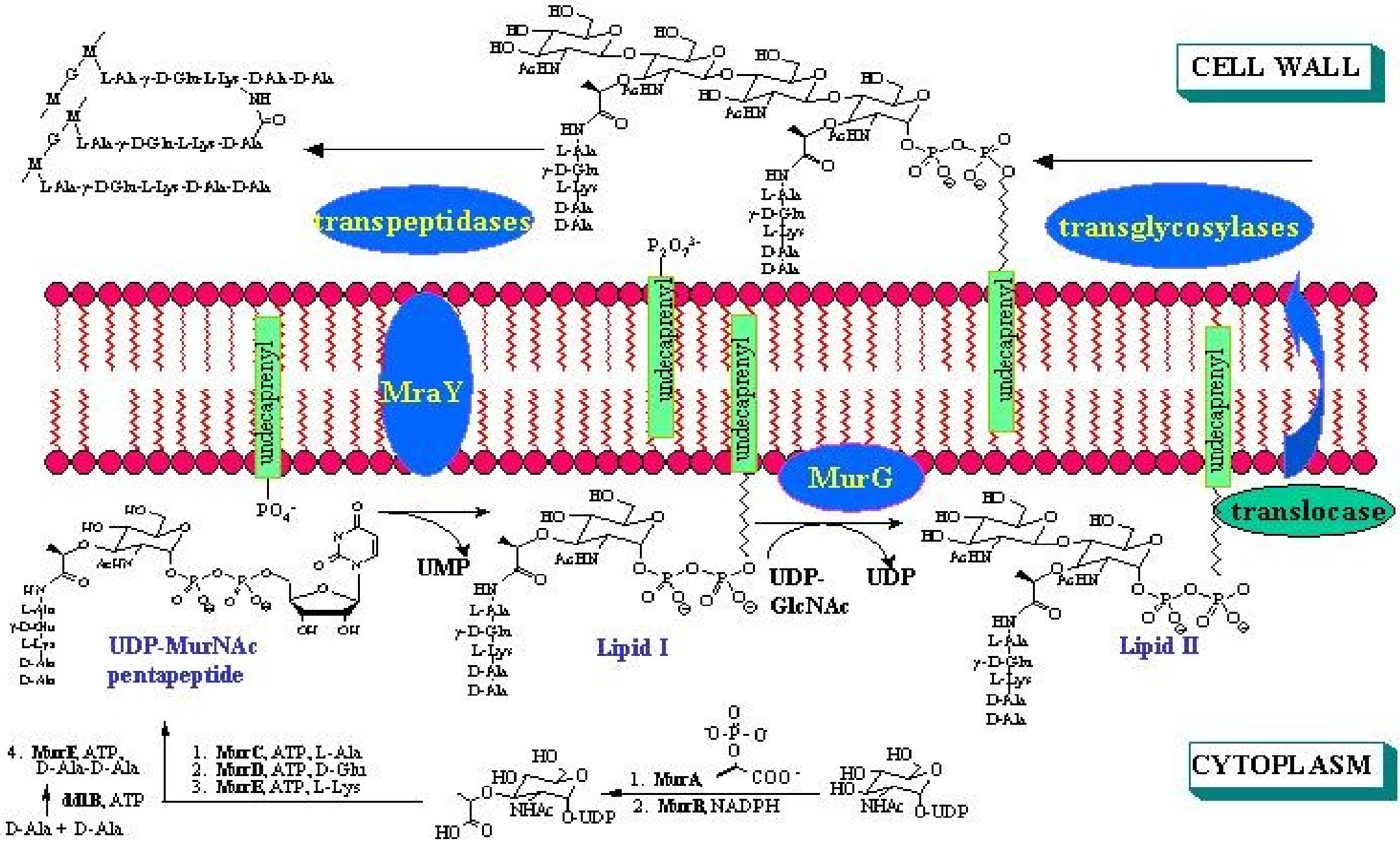




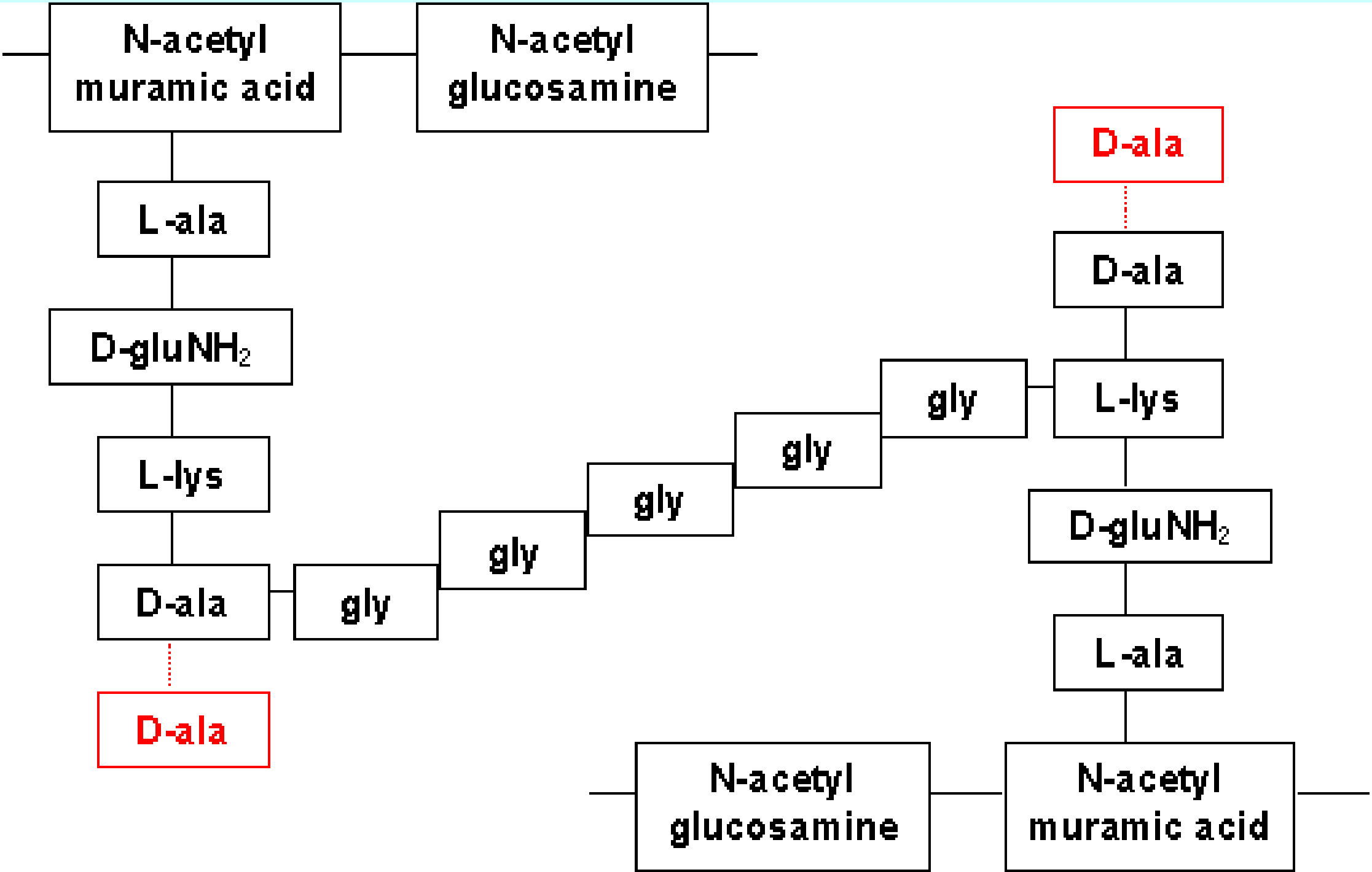
Gram-positive organisms	Gram-negative organisms	Mycobacteria
The lipid bilayer cell membrane of most of the Gram-positive bacteria is covered by a porous **peptidoglycan layer** which does not exclude most antimicrobial agents. 	Gram-negative bacteria are surrounded by two membranes. The outer membrane functions as an efficient permeability barrier because it contains lipopolysaccharides **(LPS)** and porins. 	Mycobacteria produce a thick **mycolate-rich** outer covering which functions as an exceptionally efficient barrier. 

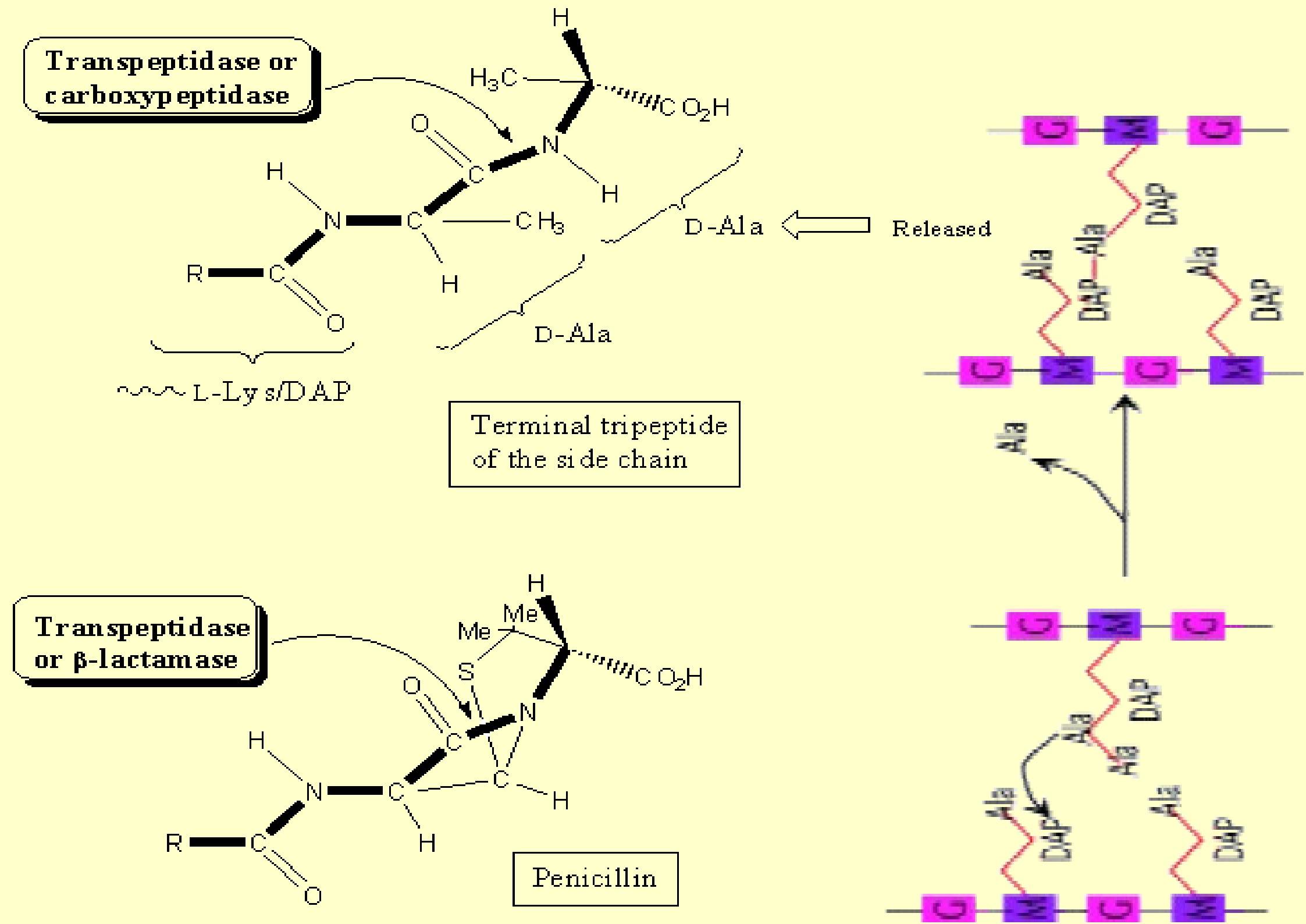
 lipid bilayer peptidoglycan  lipid + LPS porins MYCOLATE acyl lipids LAM arabinogalactan

Peptidoglycan Biosynthesis

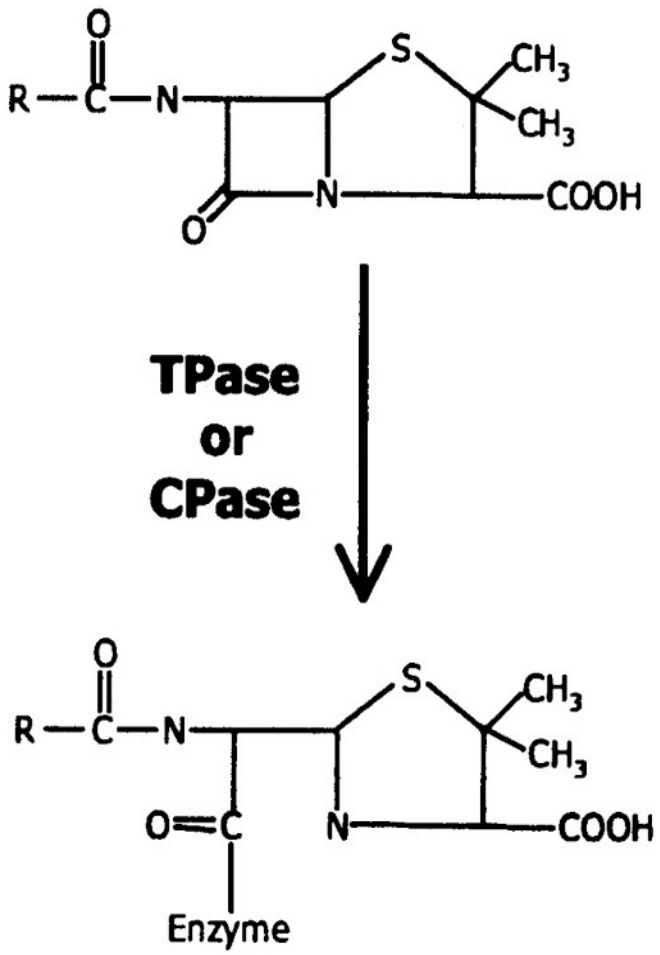


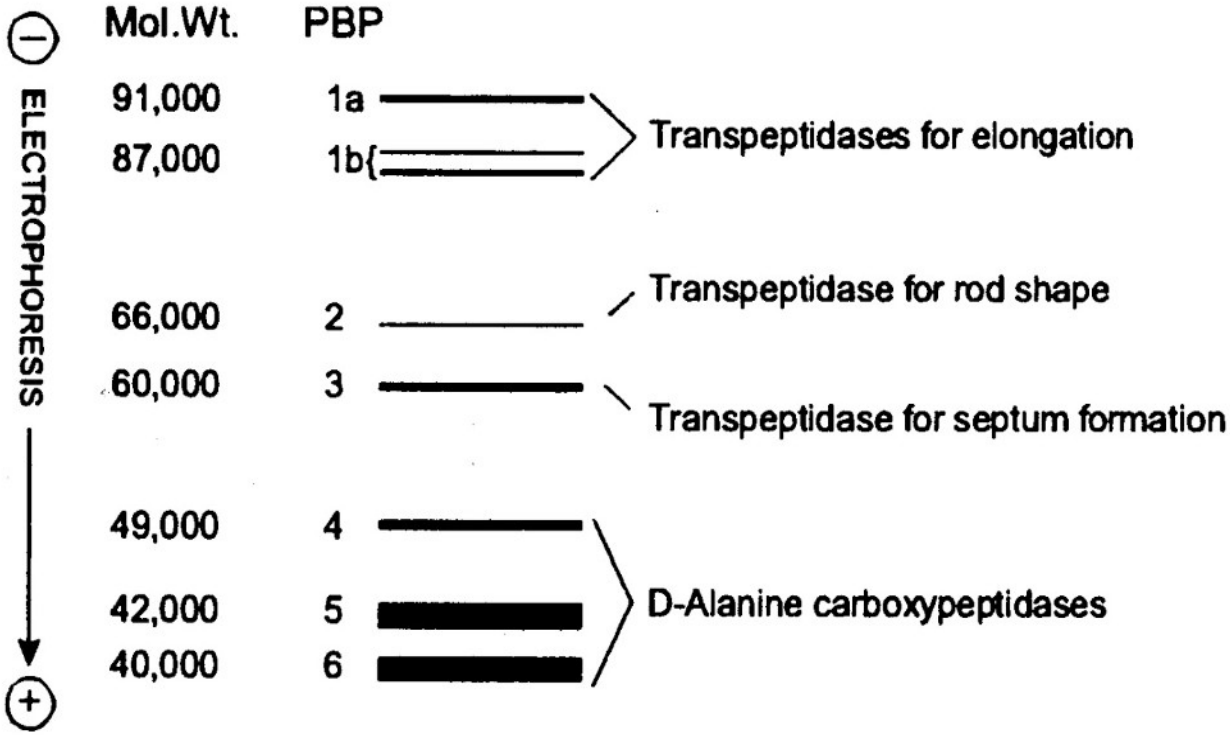
SINTESI DEL PEPTIDOGLICANO




Transpeptidase or carboxypeptidase
H
H3C
CO2H
O
N
C
H
H
N
C
CH3
R
C
H
O
D-Ala
Released
D-Ala
L-Lys/DAP
Terminal tripeptide of the side chain
Transpeptidase or β-lactamase
H
Me
Me
S
CO2H
O
N
C
H
N
C
C
H
R
C
H
O
Penicillin
Ala
DAP
Ala
DAP
Ala
DAP
Ala
DAP
Ala
DAP
Ala
DAP
Ala
DAP

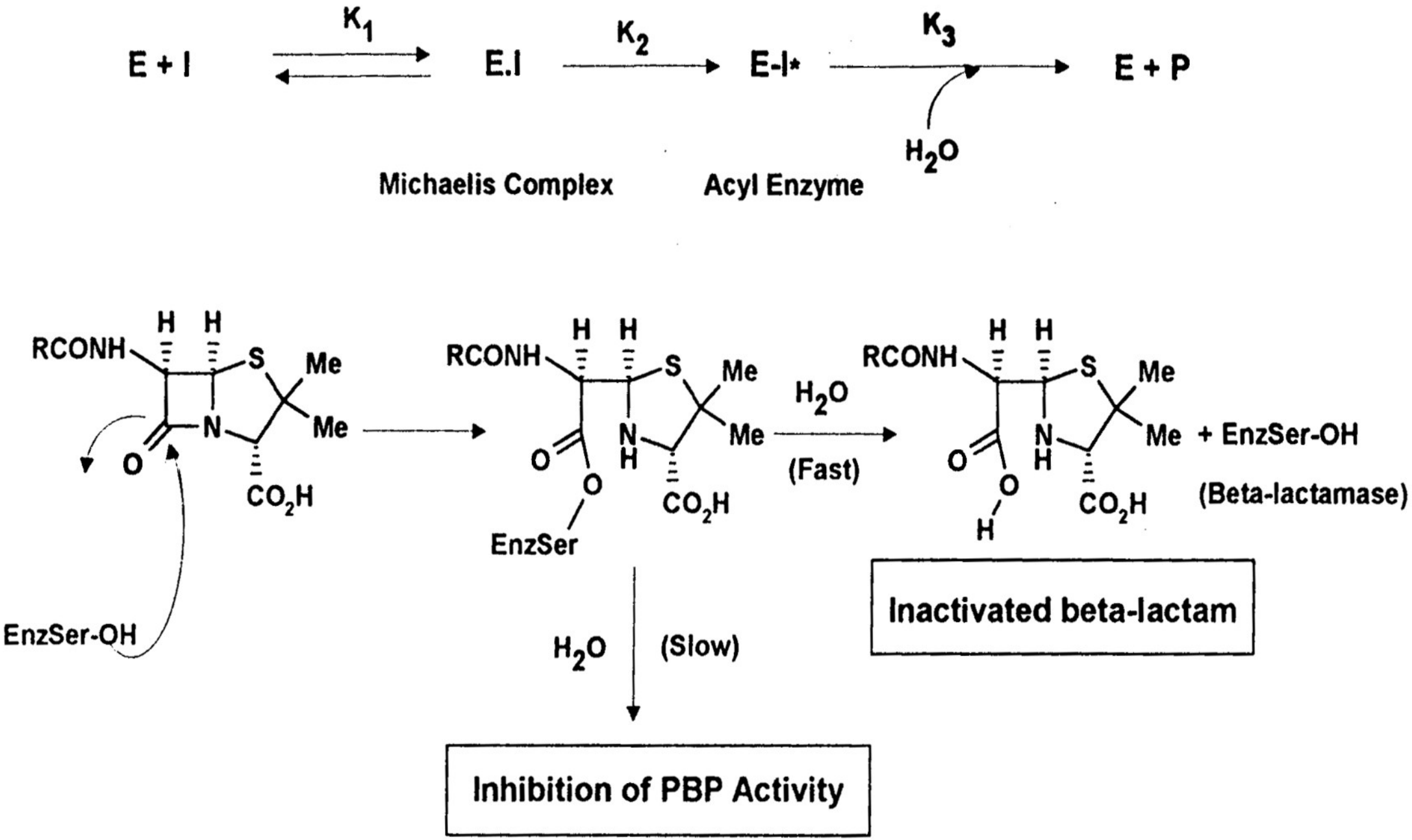
ENZIMI INIBITI DAGLI ANTIBIOTICI β-LATTAMICI (PBP)



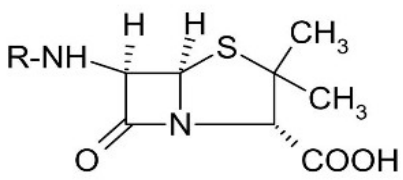


Autoradiogram of SDS-polyacrylamide electrophoresis gel separating *E. coli* proteins incubated with [^{14}C]benzylpenicillin.

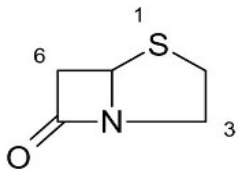
INIBIZIONE DI SERINA PEPTIDASI CON PENICILLINA



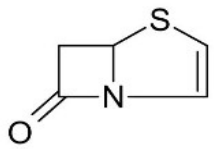
ANTIBIOTICI β-LATTAMICI



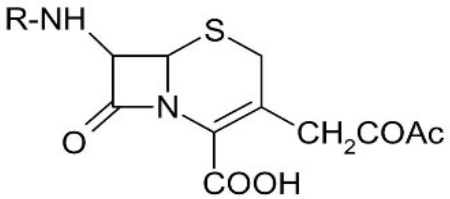
PENICILLINA



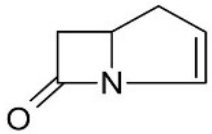
PENAM



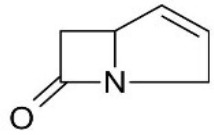
Δ²-PENEM



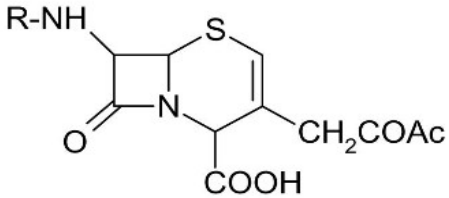
Δ³-CEFALOSPORINE



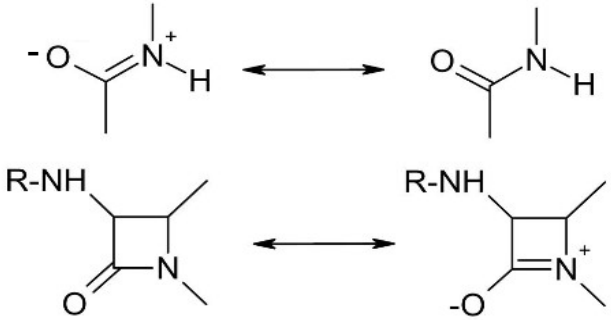
Δ²-CARBAPENEM



Δ¹-CARBAPENEM



Δ²-CEFALOSPORINE



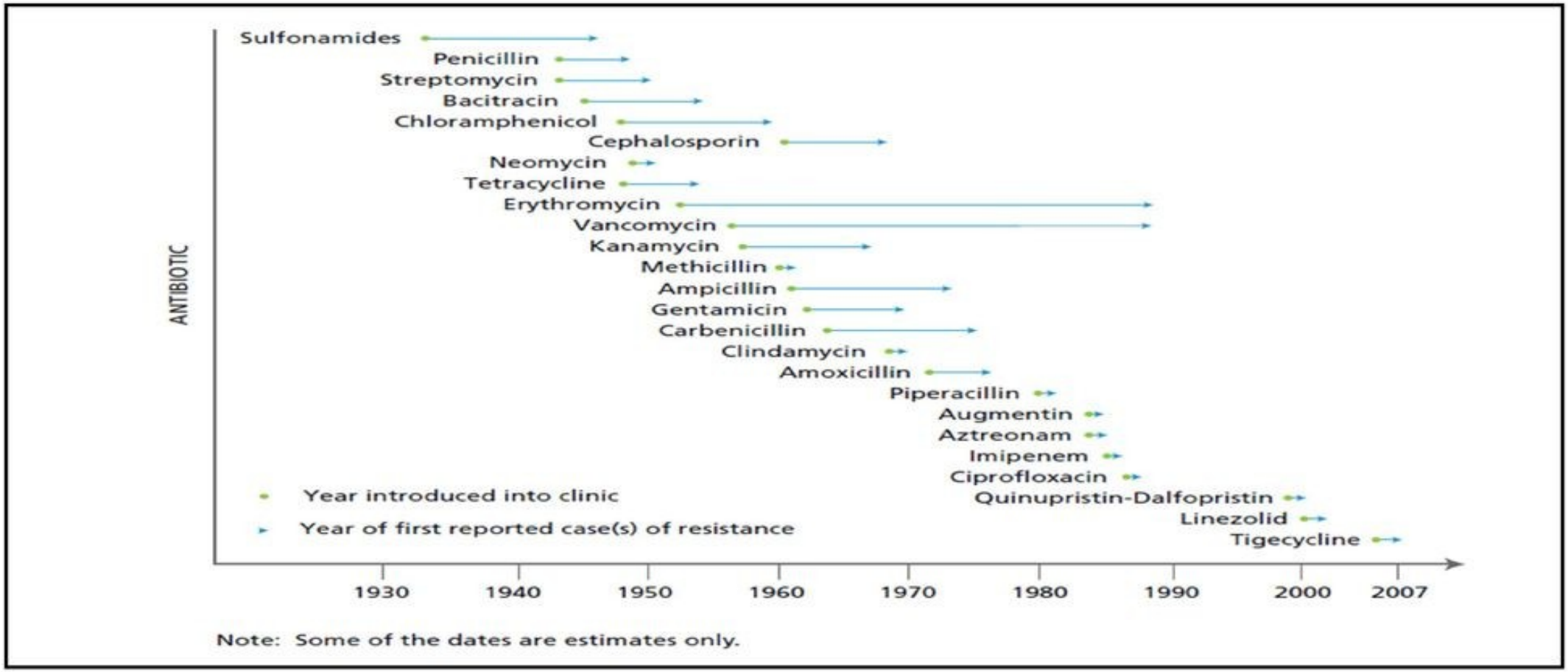
Distanza del N dal piano della molecola	
Ammidi normali	0
Δ²-cefalosporine	0.06
Δ³-cefalosporine	0.24-0.32
Penam	0.38-0.40
Δ²-penem	0.43-0.44
Δ²-carbapenem	0.49-0.50
Δ¹- carbapenem	0.54

	C=O Streching (cm^{-1})	Distanza C-N (Å)	Distanza C=O (Å)
Ammidi normali	1730-1760	1.325	1.240
Δ²-cefalosporine	1756-1760	1.353	1.207
Δ³-cefalosporine	1764-1776	1.374	1.205
Penam	1770-1790	1.383	1.188

OBIETTIVI DELLA RICERCA

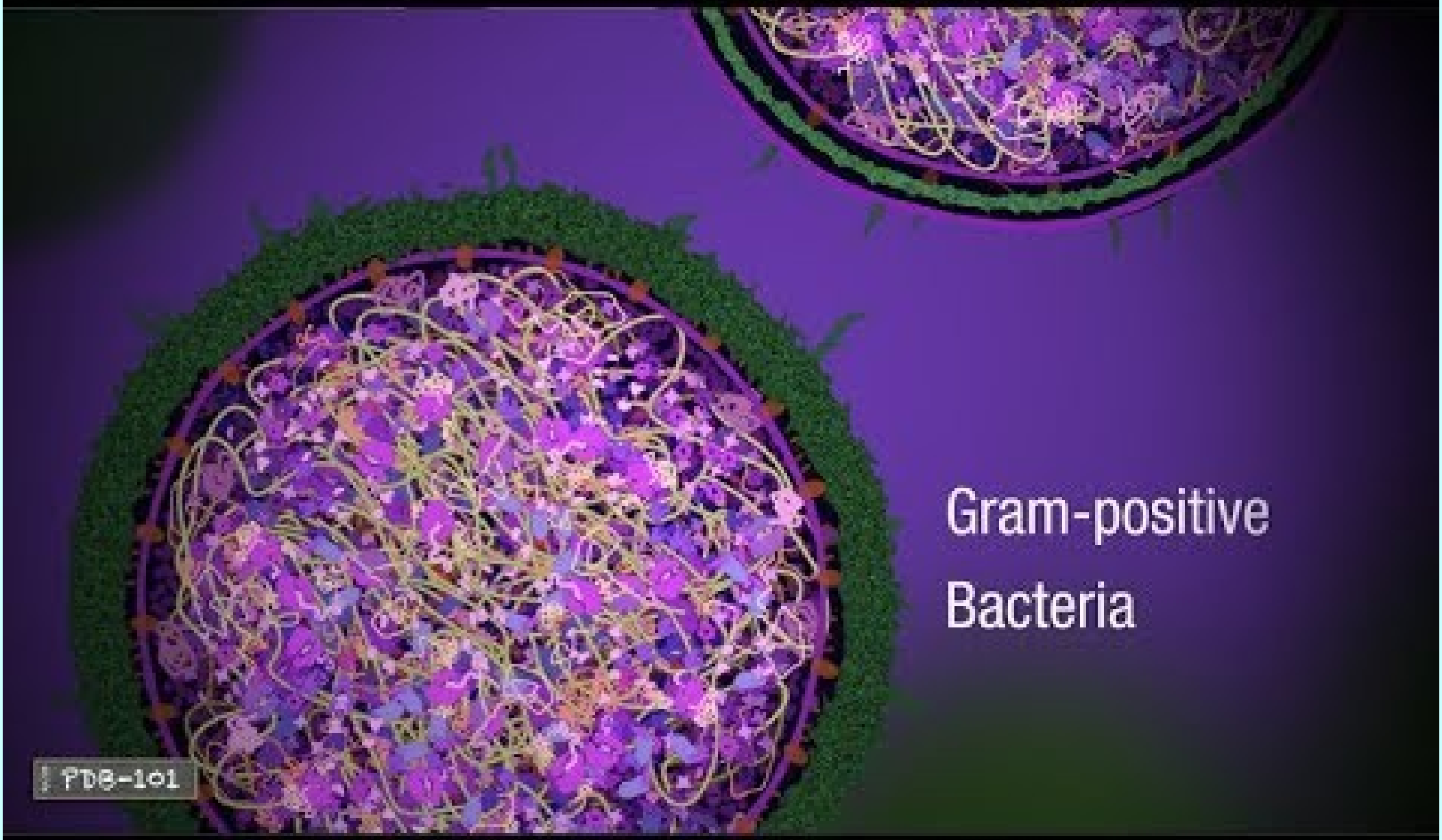
- Aumentare la stabilità chimica all'idrolisi acida
- Aumentare la durata dell'azione (emivita)
- Eliminare (diminuire) le reazioni allergiche
- Aumentare lo spettro d'azione
- Superare la resistenza batterica

Comparsa delle antibiotico-resistenze



Efficacia clinica

Prescrizioni ed uso



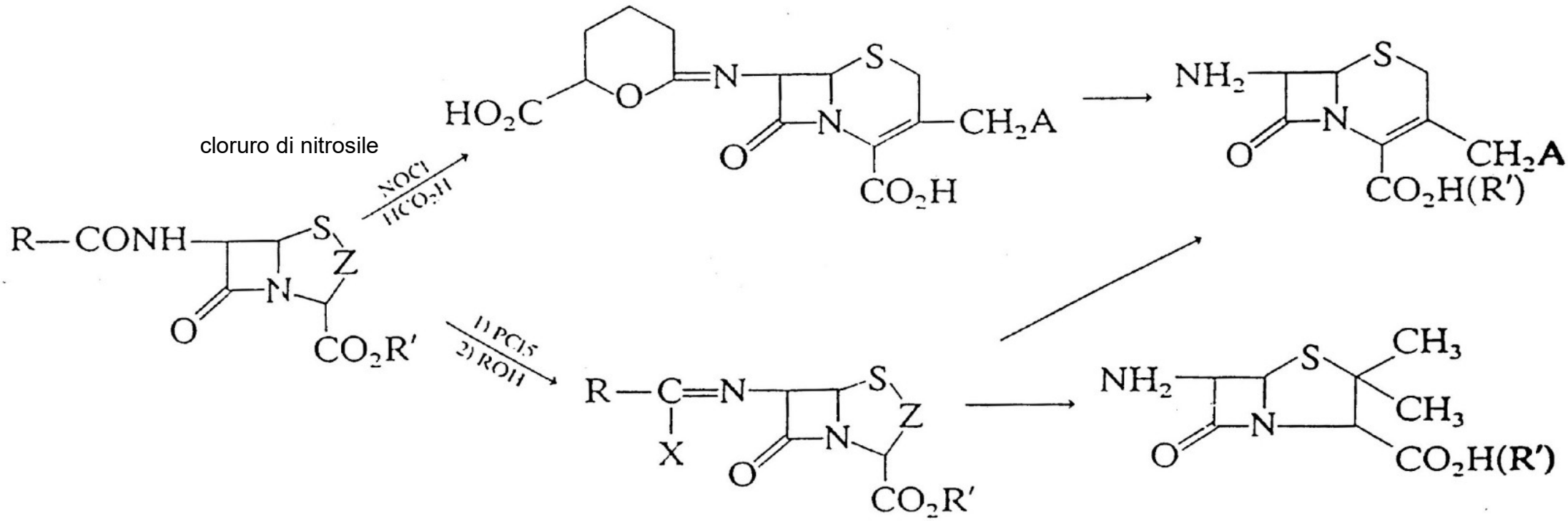
Penicillin and Antibiotic Resistance (from PDB-101)

RESISTENZA AGLI ANTIBIOTICI β-LATTAMICI

- Modificazione delle proteine PBP (PBP a bassa affinità per i β-lattamici, mutazioni di PBP)
- β-Lattamasi (serina proteasi o metallo proteasi)
- Variazioni della permeabilità della membrana esterna (Gram -)
- Pompe di efflusso (più probabili per tetracicline, eritromicina e cloramfenicolo)
- Disattivazione dei meccanismi di autolisi

DEACILAZIONE DI PENICILLINE E CEFALOSPORINE

Basic Structural Variations and Interconversions



Penicillins: R = $C_6H_5CH_2$, $C_6H_5OCH_2$; Z = —$C(CH_3)_2$
Cephalosporins: R = $R'O_2CCH(NH_2)(CH_2)_3$; Z = —$C(CH_2A)CH_2$—
R′ = $Si(CH_3)_3$, $CH_2C_6H_4NO_2$, CH_2CCl_3; A = H, OAc, S-Het, etc.
X = Cl, OCH_3

Chemical cleavage of 6(7)-acyl groups from penicillins and cephalosporins.

DEACILAZIONE DI PENICILLINE E CEFALOSPORINE

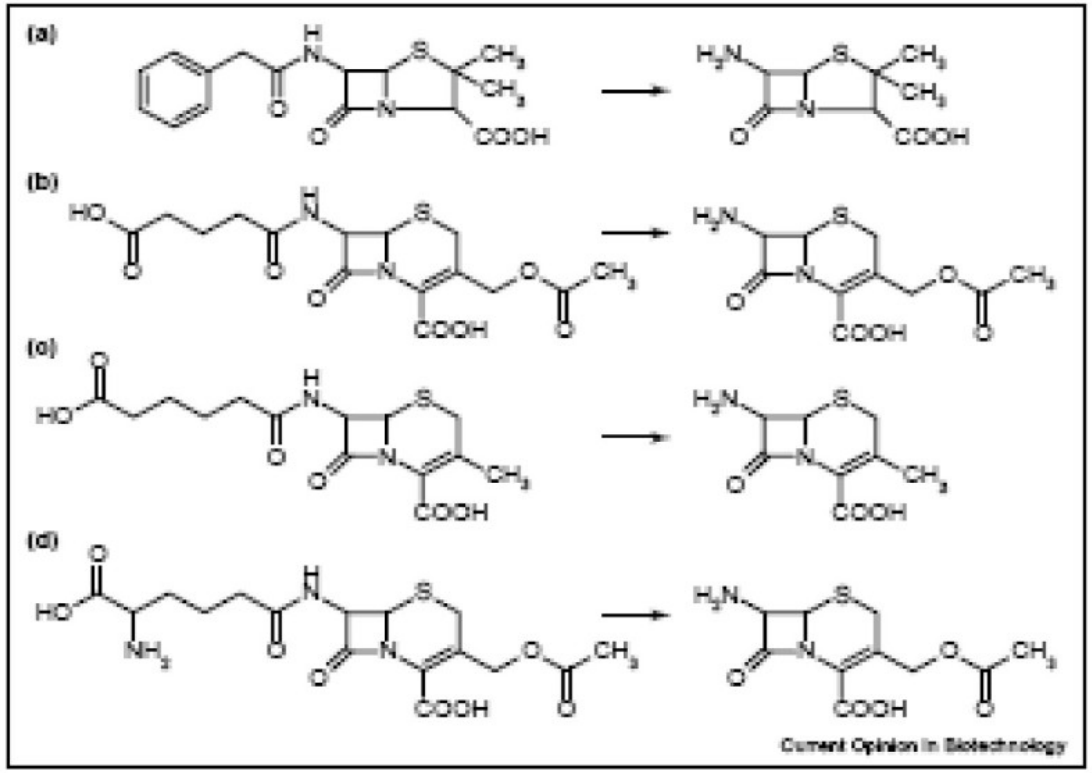
Reazioni di idrolisi catalizzate dalle β-lattam-acilasi: rimozione della catena laterale acilica

La penicillina acilasi idrolizza la penicillina G ad acido 6-aminopenicillanico (a).

La cefalosporina acilasi idrolizza il glutaril-7-ACA a 7-ACA (b).

Mutanti della cefalosporina acilasi idrolizzano l'adipil-7-ADCA a 7-ADCA (c) e la cefalosporina C a 7-ACA (d).

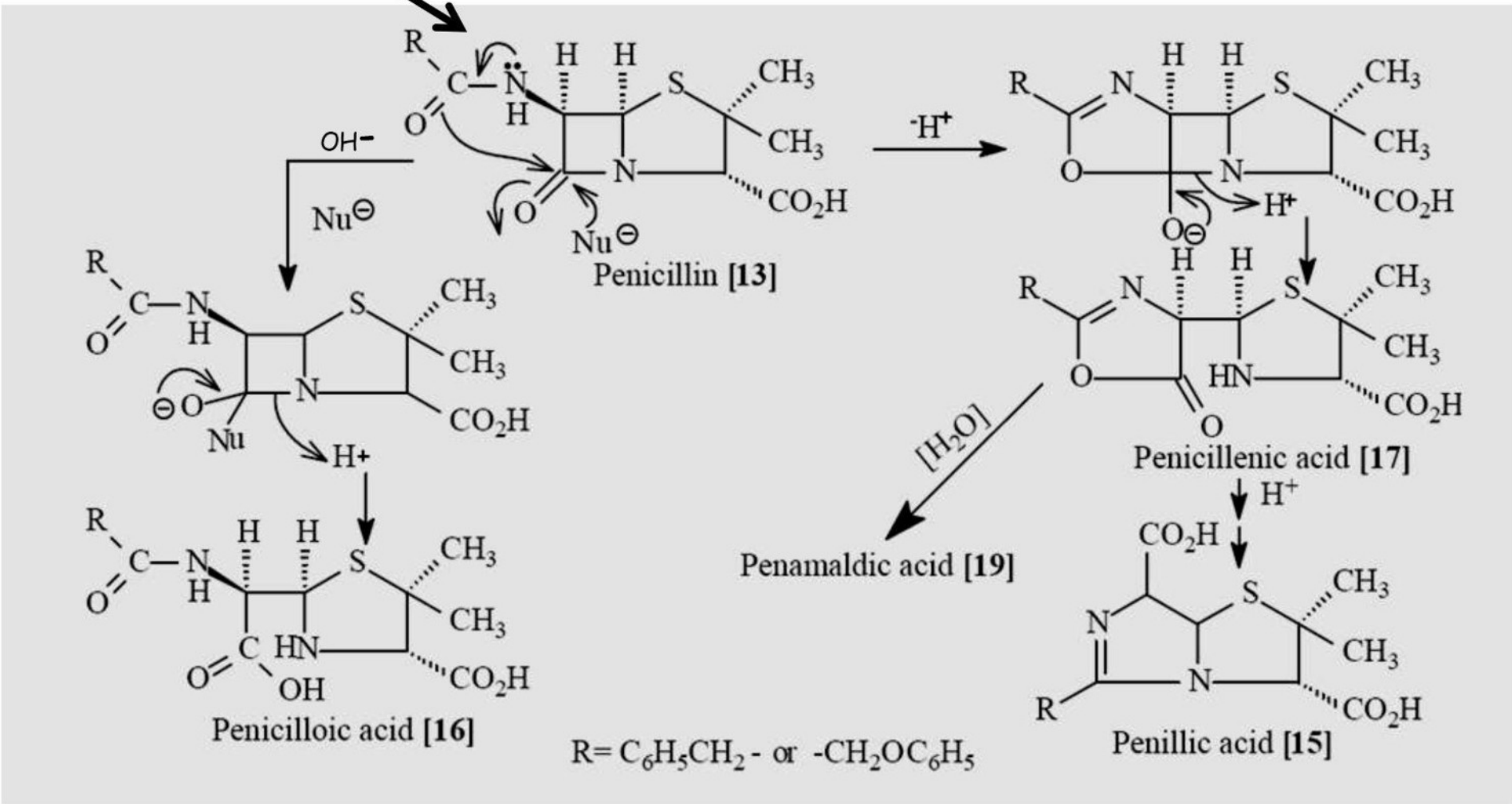
Il 6-APA e il 7-ACA sono i precursori delle penicilline e delle cefalosporine semi-sintetiche



INSTABILITA' IN AMBIENTI ACIDI/BASICI

(Assistenza Anchimerica)

Reattività vs nucleofili



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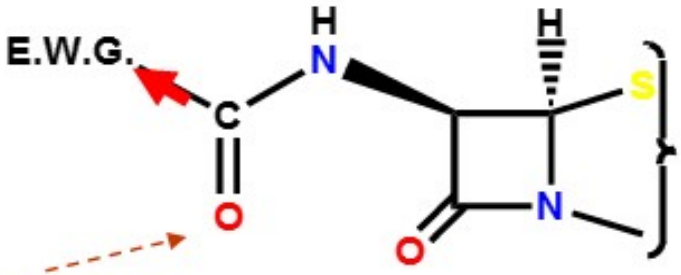
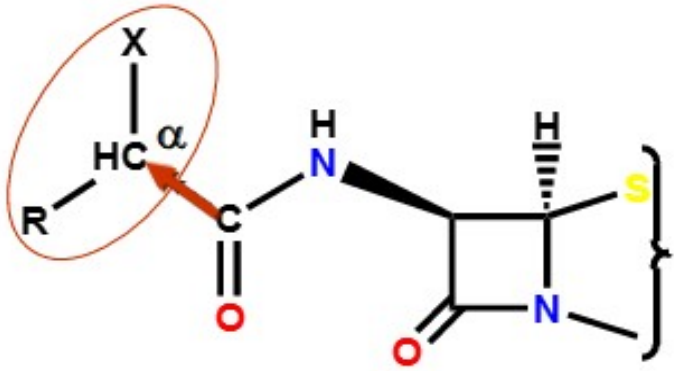
Penicilline stabili in ambiente acido (o.s.=)

Strategia

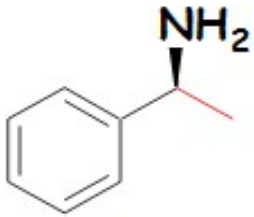
Introdurre sostituenti elettronattrattori nel gruppo 6-β-acilammidico per ridurre la nucleofilia dell'ossigeno carbonilico



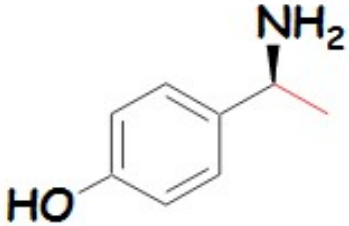
Riduzione della nucleofilia/assist. anchimerica

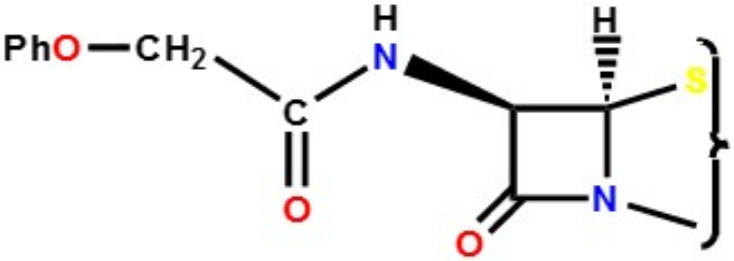


Amoxicillina

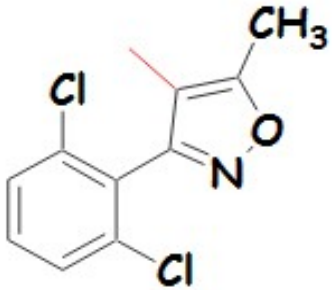


Ampicillina

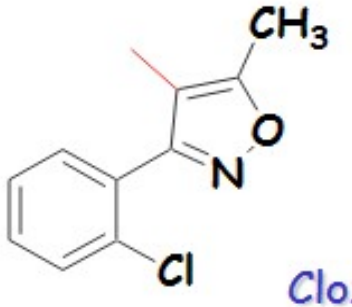




Penicillina V (attiva per o.s.)

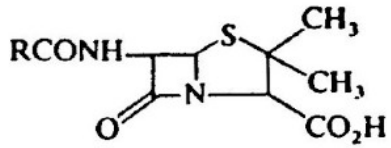


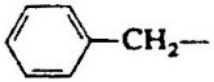
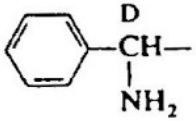
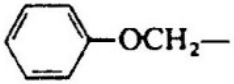
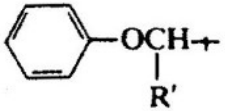
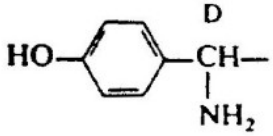
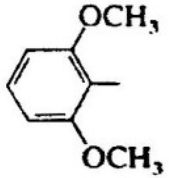
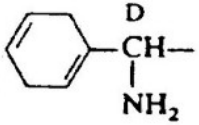
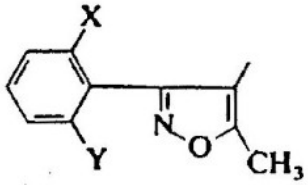
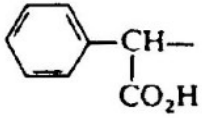
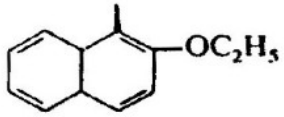
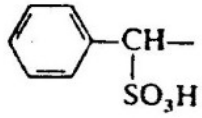
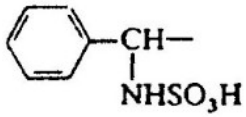
Dicloxacillina



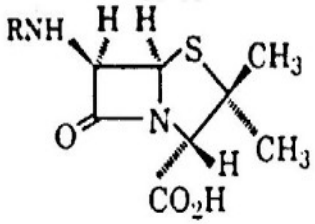
Cloxacillina

Penicillins for Clinical Use

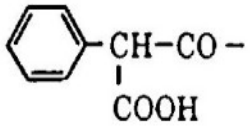
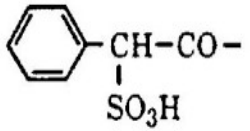
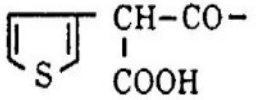
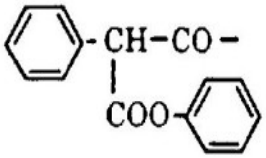


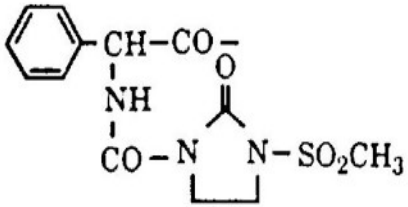
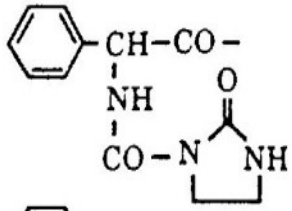
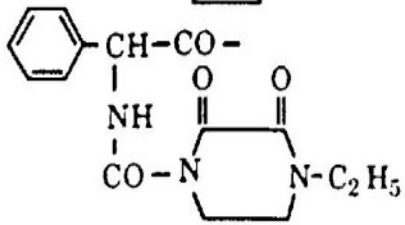
Generic Name	R	Generic Name	R
Narrow spectrum Benzylpenicillin		Broad spectrum Ampicillin	
Phenoxymethylpenicillin		(Hetacillin, metampicillin) (Pivampicillin, talampicillin, bacampicillin)	(Side chain amine adducts) (Esters)
R′ Phenethicillin CH_3 Propicillin C_2H_5 Phenbenicillin C_6H_5		Amoxycillin	
β-Lactamase resistant Methicillin		Epicillin	
X Y Oxacillin H H Cloxacillin H Cl Dicloxacillin Cl Cl Flucloxacillin F Cl		Broad spectrum—Narrow use Carbenicillin (and side chain esters)	
		Ticarcillin	
Nafcillin		Sulbenicillin	
		Suncillin	

β-LATTAMICI E GRAM NEGATIVI



MIC (μg/mL)

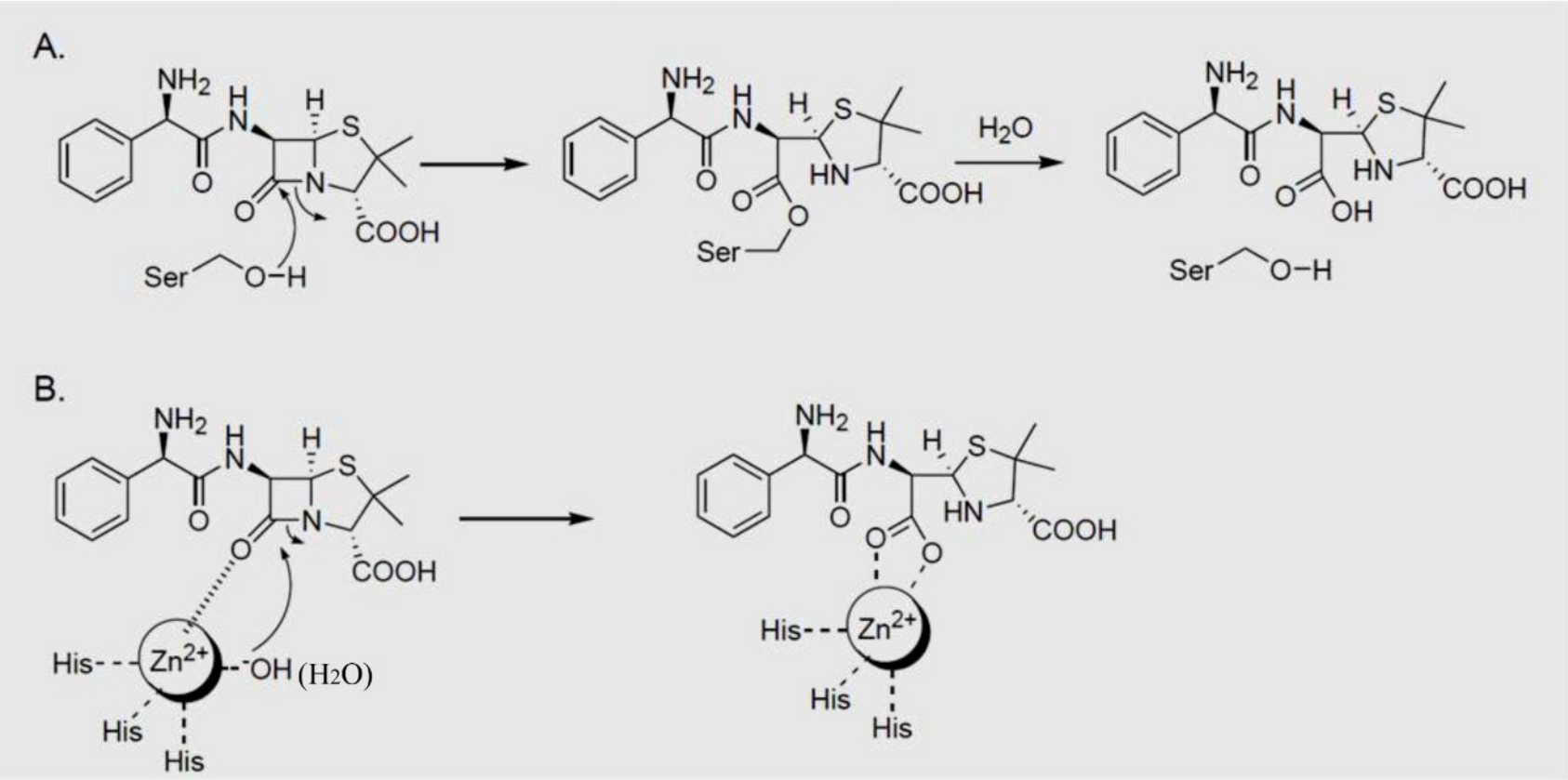
R	Nome	*E. coli*	*P. vulgaris*	*P. mirabilis*	*P. aeruginosa*
	Carbenicillina	8	2	1	62
	Sulbenicillina	12.5	25	-	31
	Ticarcillina	4	4	2	31
	Carfecillina	idrolizzata a carbenicillina			

		E. coli	*P. vulgaris*	*P. mirabilis*	*P. aeruginosa*
	Mezlocillina	2	8	1	16
	Azlocillina	8	16	2	1
	Piperacillina	1	4	0.5	4

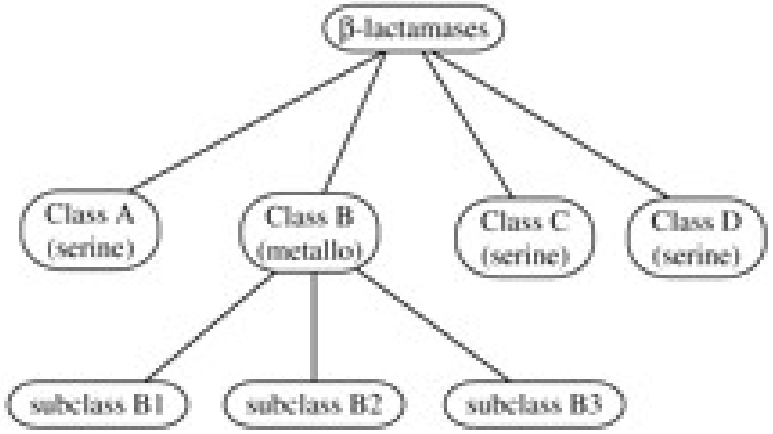
Penicilline attive contro infezioni da *Pseudomonas*.

Meccanismo di azione di due classi di Beta-lattamasi

β-LATTAMASI



Current Ambler classification and implied relationships



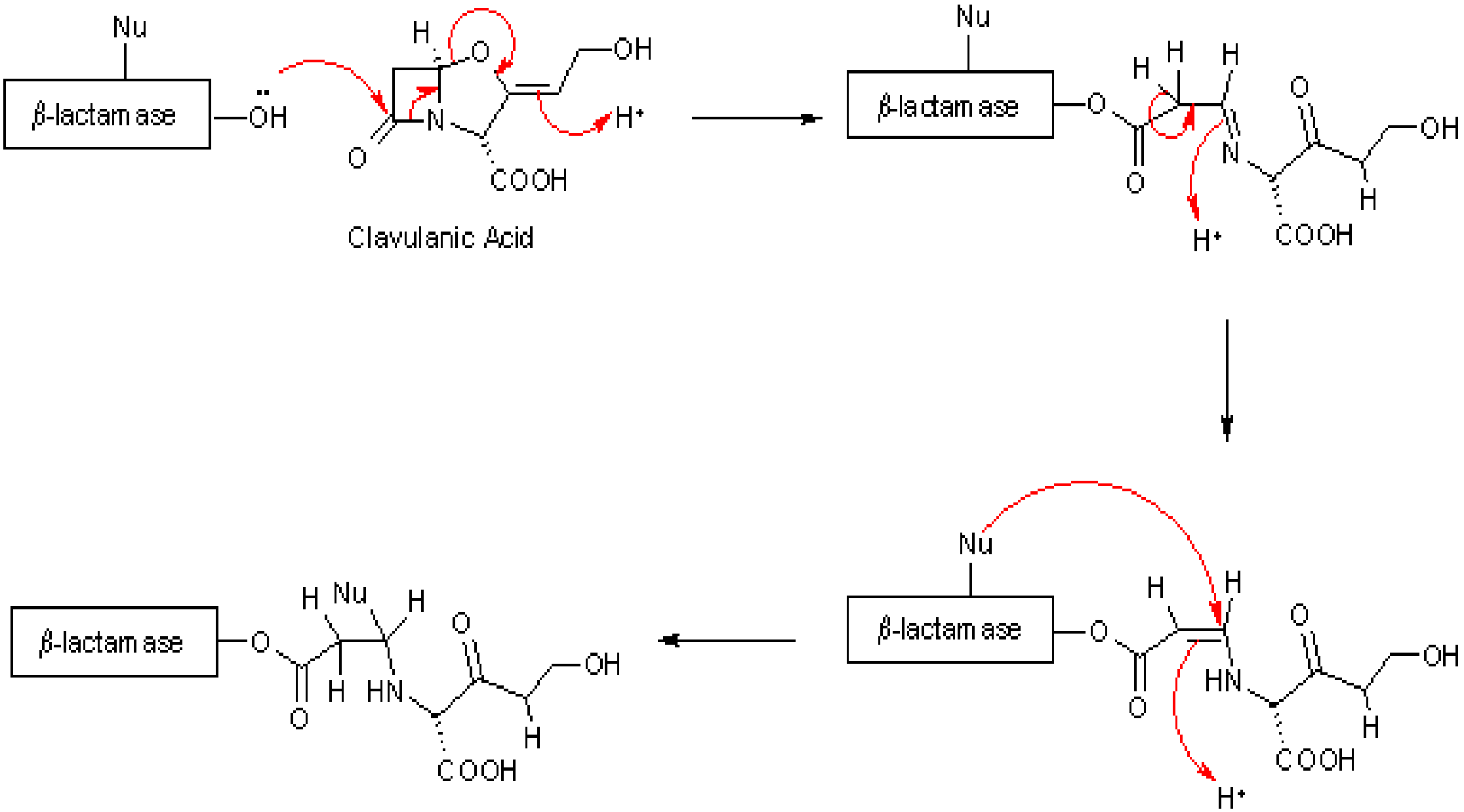
Hydrolyse β-lactam bond in structurally related antibiotics

No consistent relationship

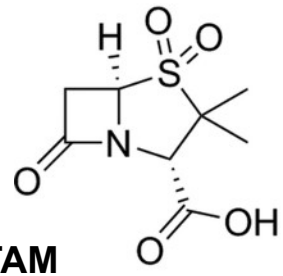
No consistent relationship

J Antimicrob Chemother, Volume 55, Issue 6, June 2005, Pages 1050–1051, https://doi.org/10.1093/jac/dki130

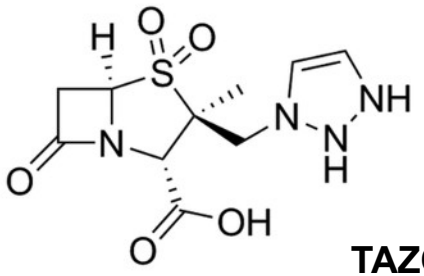
ACIDO CLAVULANICO



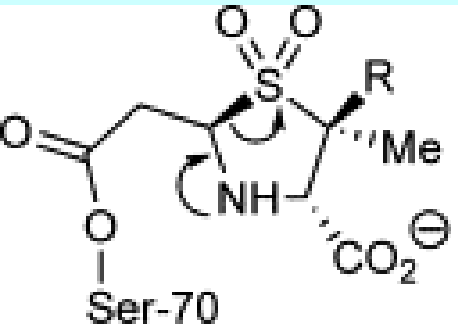
SULBACTAM e derivati



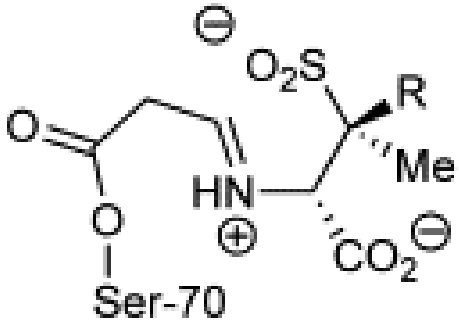
SULBACTAM



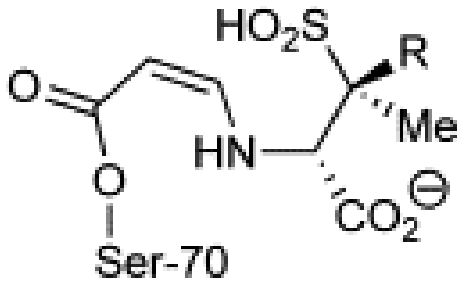
TAZOBACTAM



(E–I)



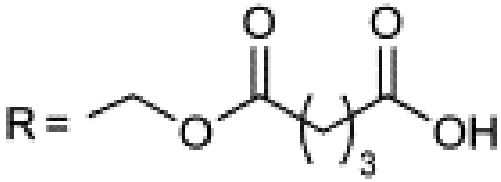
(E–I′)



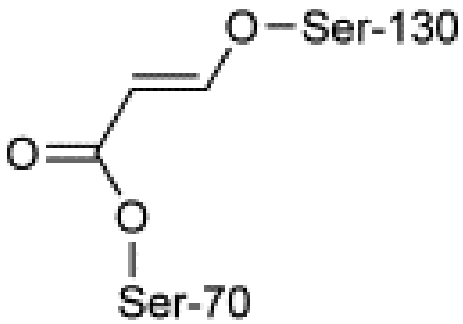
(*cis*-Enamine)

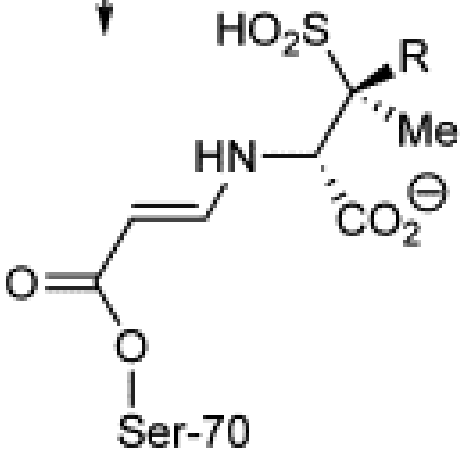
slow hydrolysis

H_2O



(SA2–13)



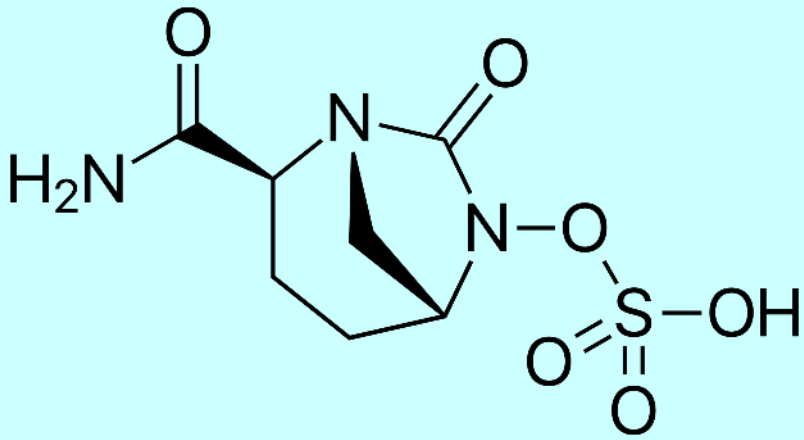


(*trans*-Enamine)

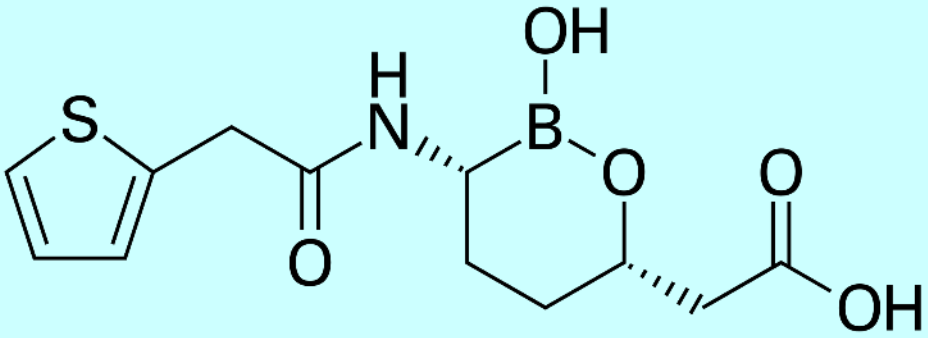
very slow hydrolysis

H_2O

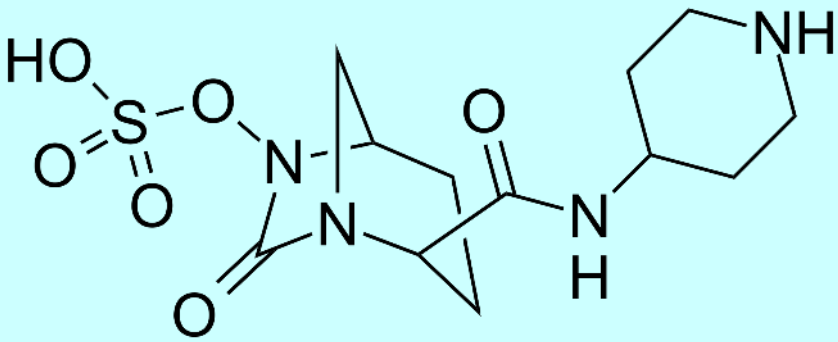
NON-β-LACTAM β-LACTAMASE INHIBITOR



Avibactam
approvato in associazione con ceftazidime da FDA nel 2015

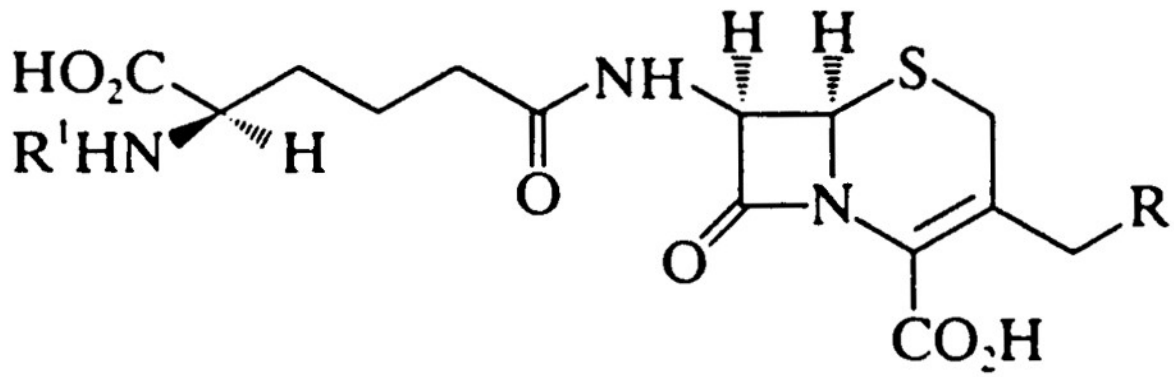


Vaborbactam
approvato in associazione con meropenem da FDA



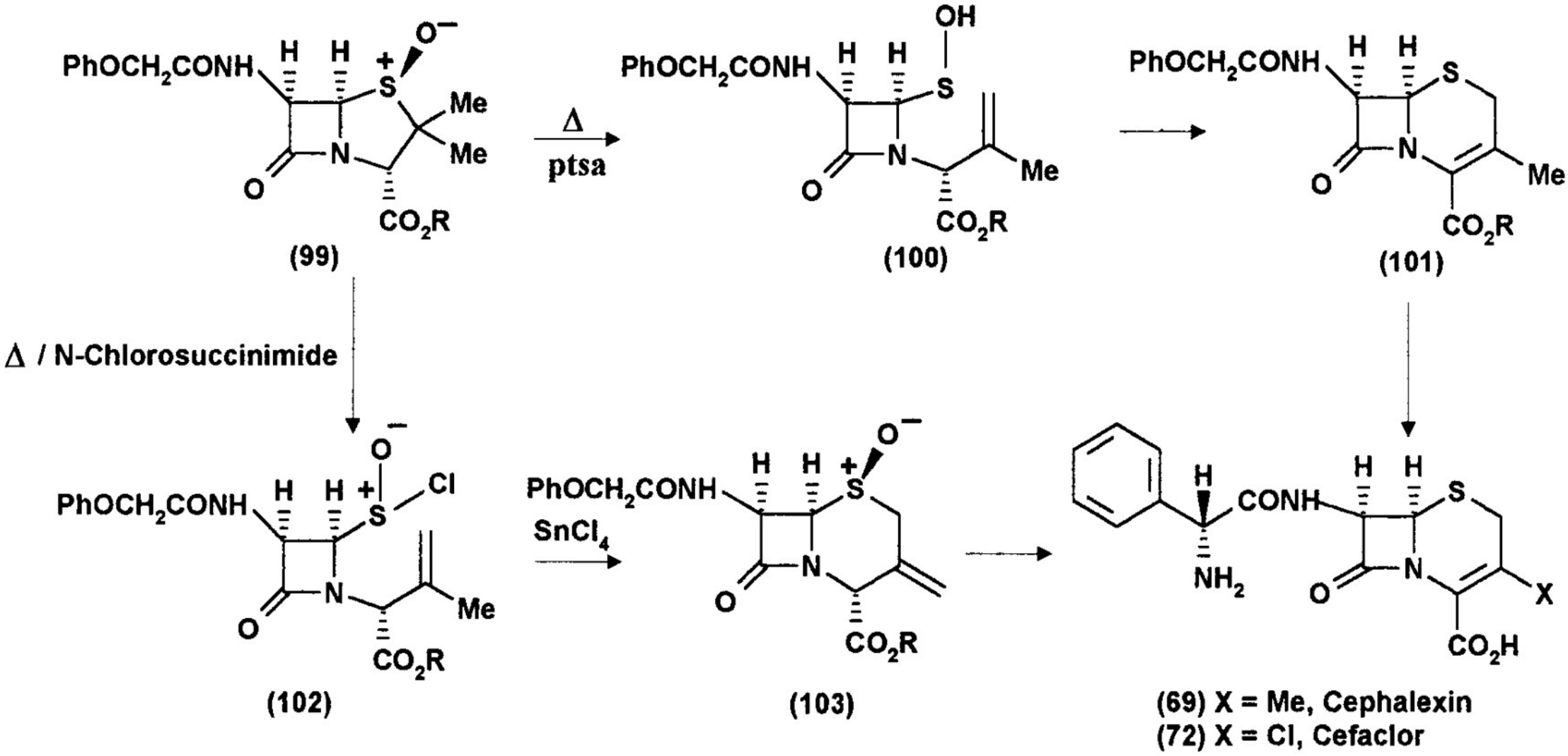
Relebactam
approvato da FDA in associazione con imipenem/cilastatin nel 2019

Naturally Occurring C(7)-H Cephalosporins with the α-Aminoadipic Acid Side-Chain



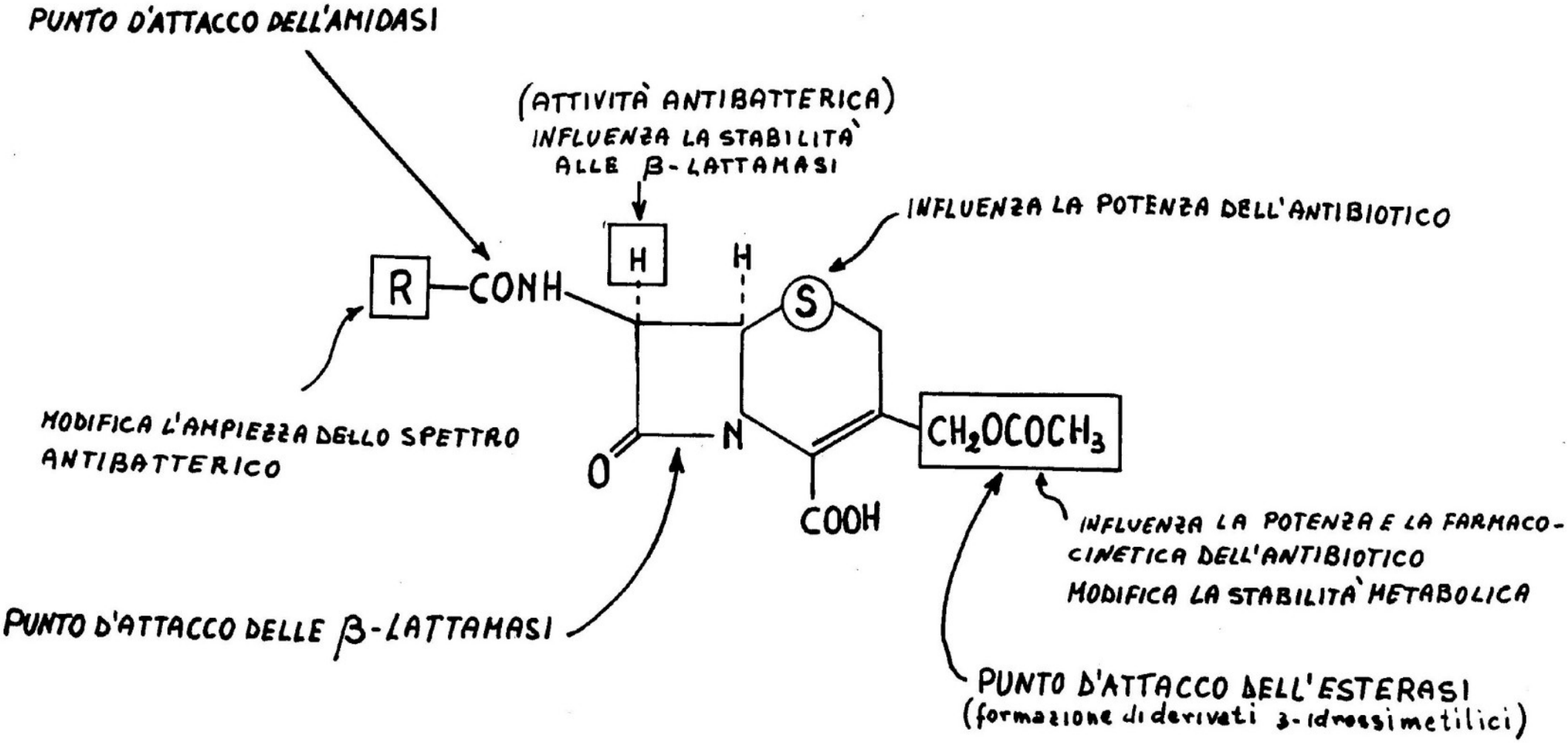
Cephalosporin	R	R[1]	Reference
Cephalosporin C	OCOMe	H	136, 137
Deacetylcephalosporin C	OH	H	139
Deacetoxycephalosporin C	H	H	140
N-Acetyldeacetoxycephalosporin C	H	COMe	141
O-Carbamoyldeacetylcephalosporin C	$OCONH_2$	H	4
F1	SMe	H	142
C 43-219	$SCMe_2CH(CO_2H)NH_2$	H	143

TRASFORMAZIONE DI PENICILLINE IN CEFALOSPORINE



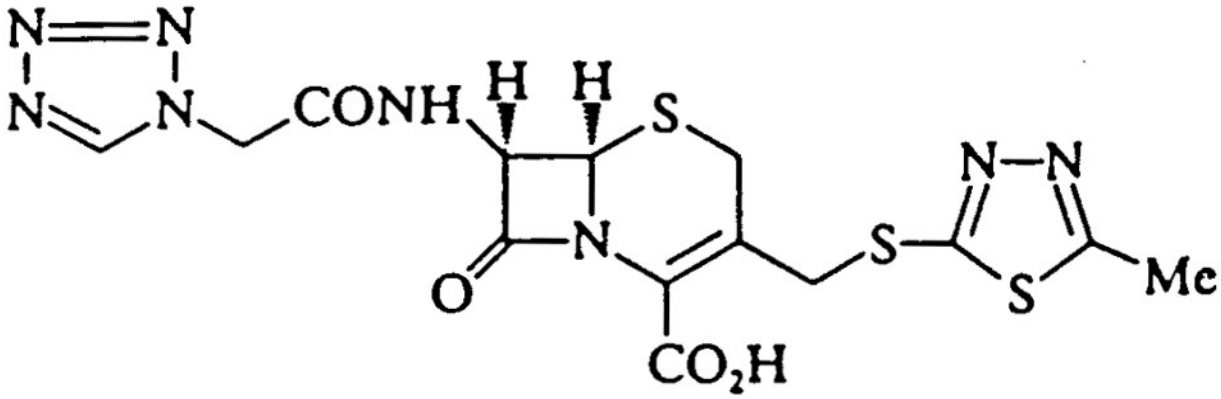
R= CH_2CCl_3 or p-Nitrobenzyl

RELAZIONI TRA STRUTTURA ED ATTIVITÀ



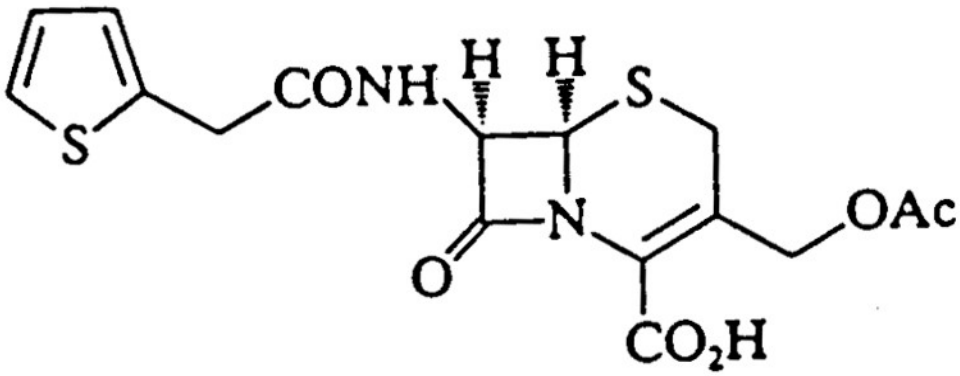
I RADICALI SOSTITUITI POSSONO CONFERIRE ALTRE CARATTERISTICHE ALLA MOLECOLA ATTRAVERSO UNA INTERAZIONE TRA DI ESSI

CEFALOSPORINE DI PRIMA GENERAZIONE

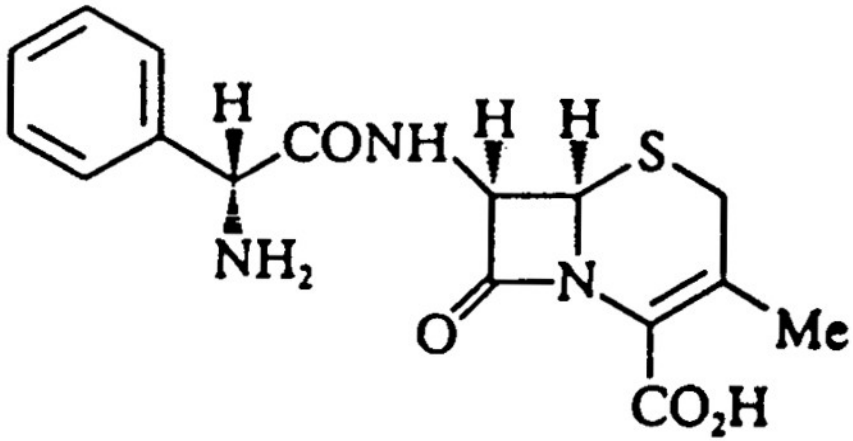


Cefazolin

Poco efficaci contro Gram-
Resistenza da β-lattamase



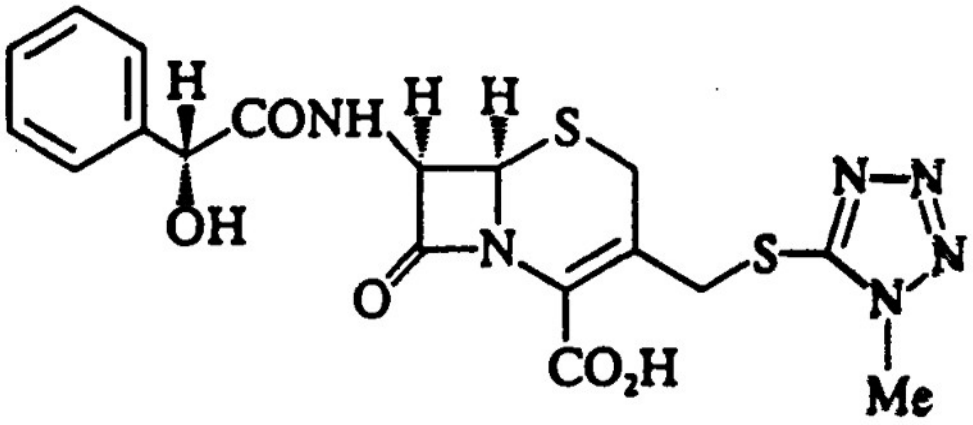
Cephalothin



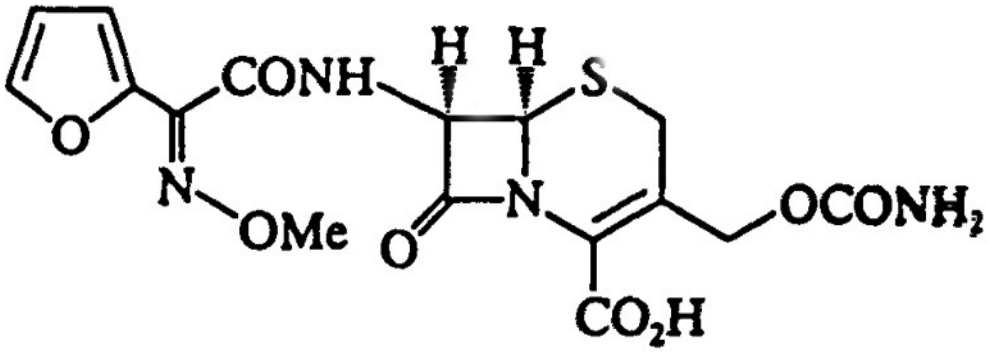
Cephalexin

Via orale

CEFALOSPORINE DI SECONDA GENERAZIONE

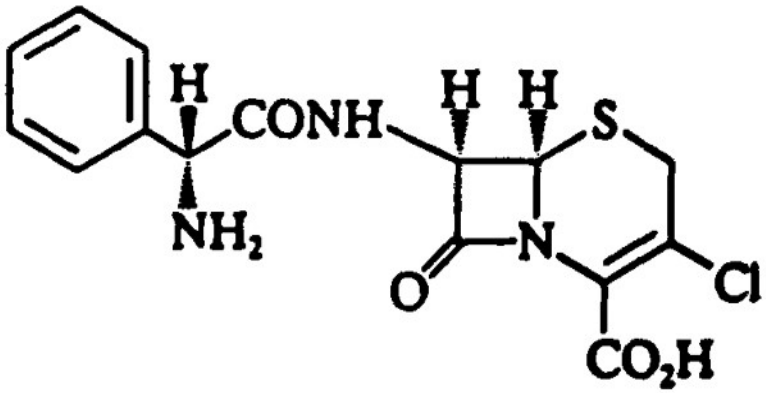


Cefamandole



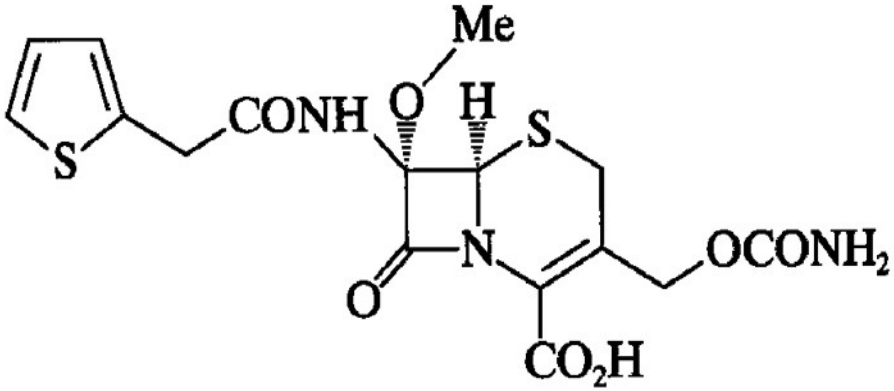
Cefuroxime

Efficaci contro Gram-
Stabili alla β-lattamase



Cefaclor

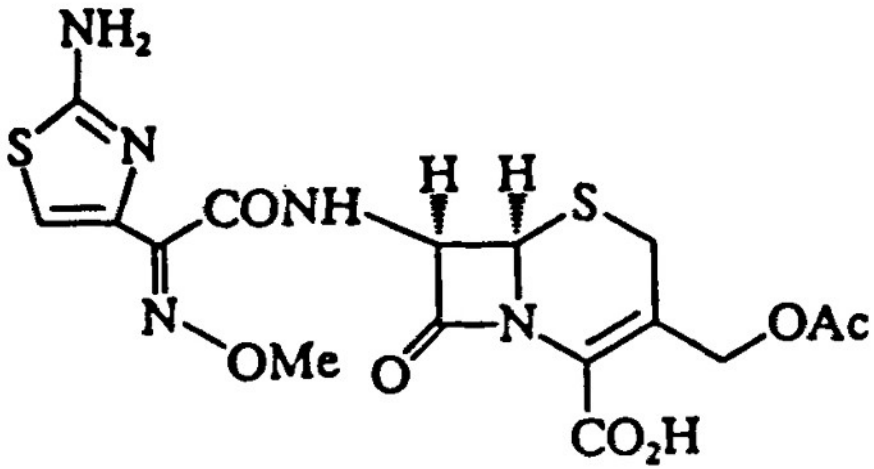
Via orale



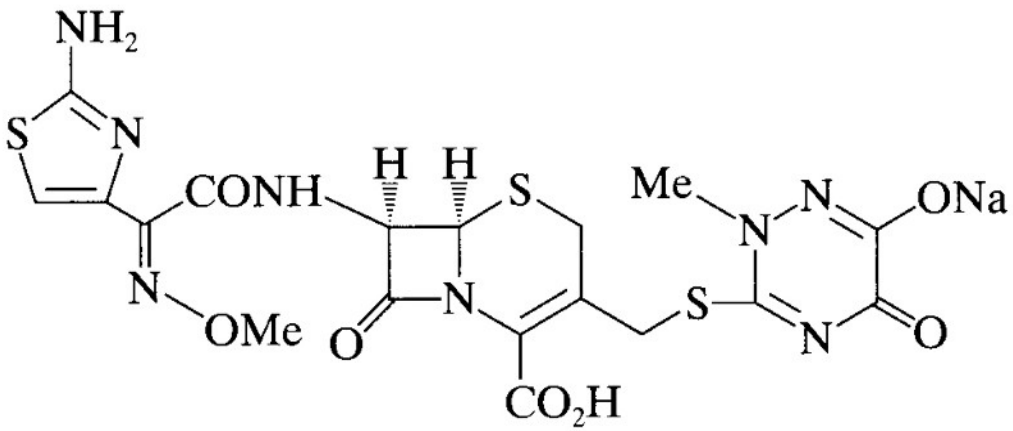
Cefoxitin

Cefamicine

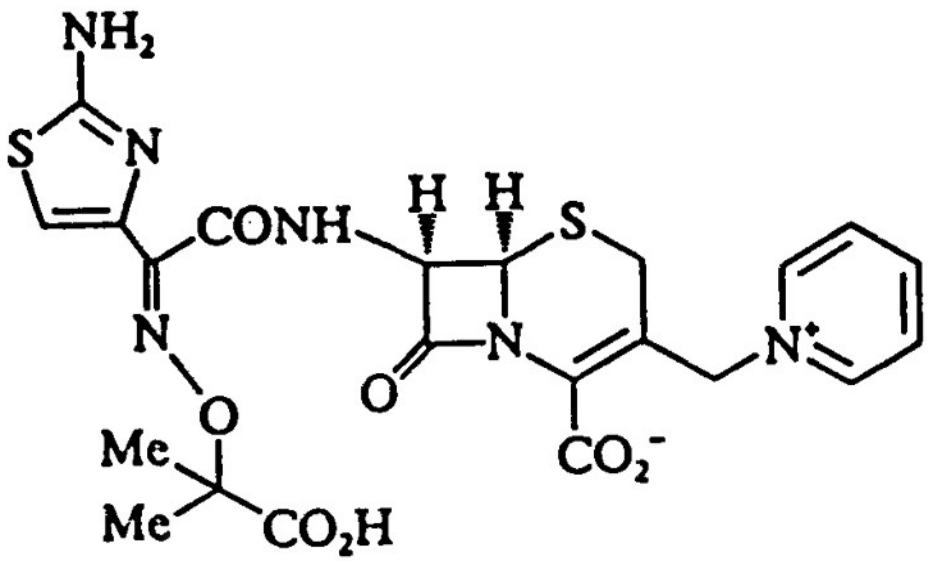
CEFALOSPORINE DI TERZA GENERAZIONE



Cefotaxime



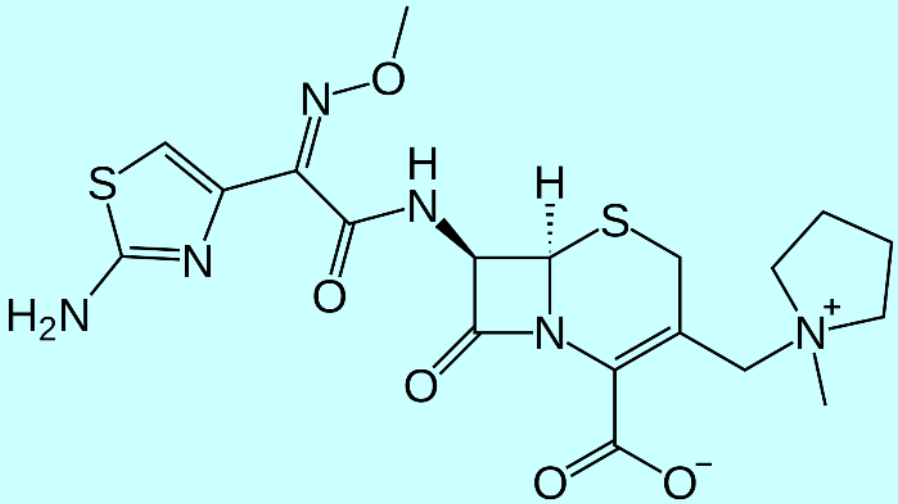
Ceftriaxone



Ceftazidime

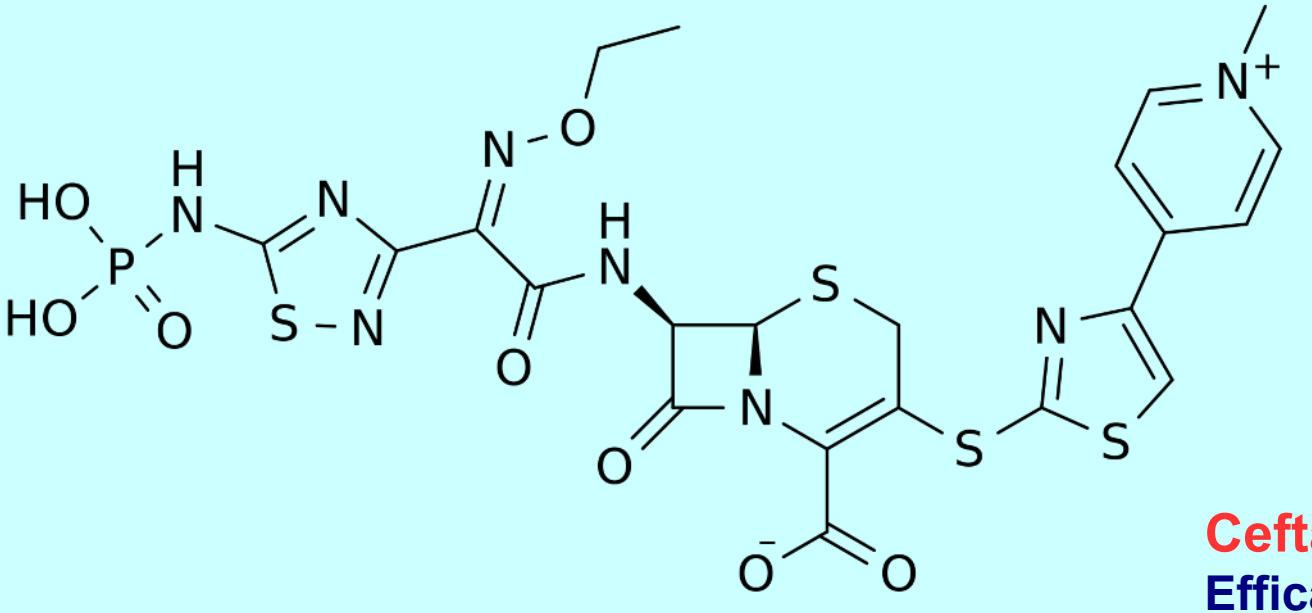
Efficaci anche contro Pseudomonas
Aumentata stabilità alla β-lattamase (anche plasmidica)
Elevata emivita

CEFALOSPORINE DI IV° e V° GENERAZIONE



Cefepima (Maxipime)

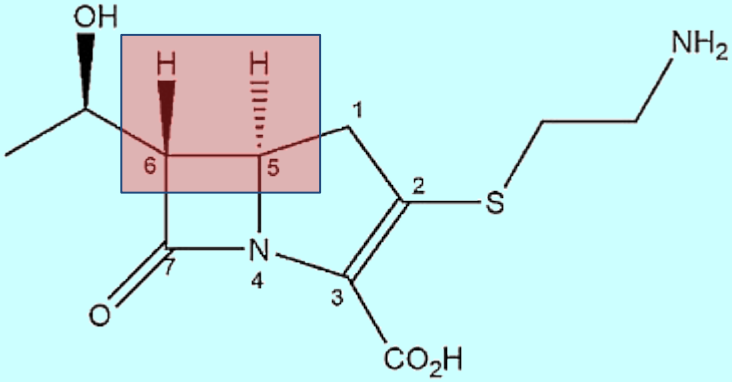
Sintetizzato nel 1994 efficace contro enterobatteri, Escherichia coli, Proteus spp., Klebsiella, Streptococcus pneumoniae e Staphylococcus aureus meticillino sensibile



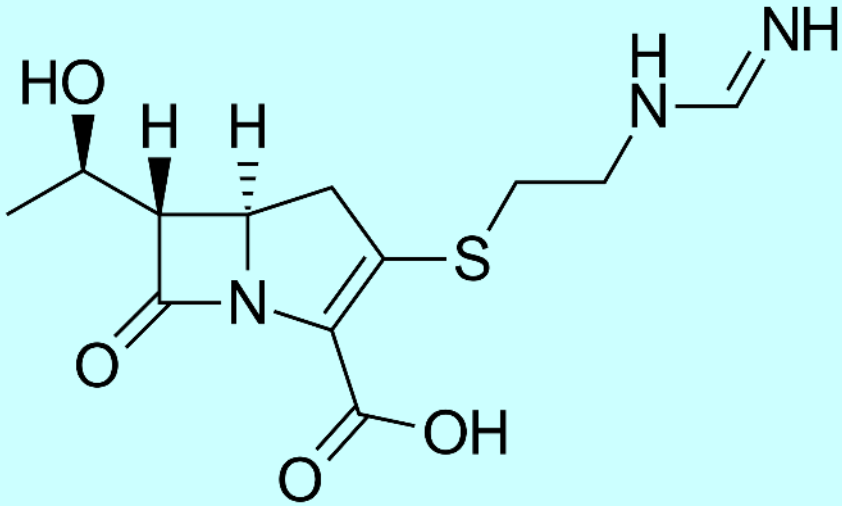
Ceftarolina (Zinforo)

Efficace contro Staphylococcus aureus resistente alla meticillina

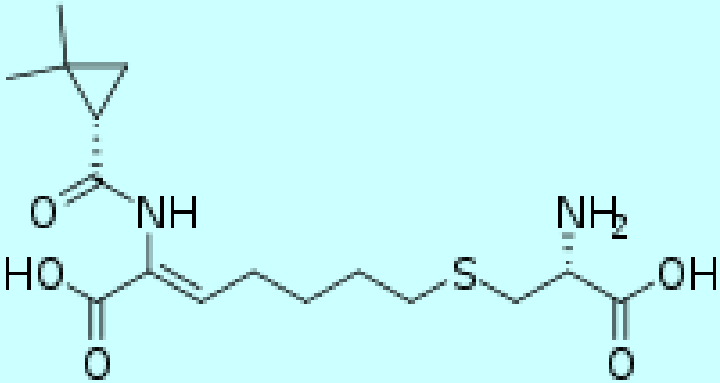
TIENAMICINE (CARBAPENEM)

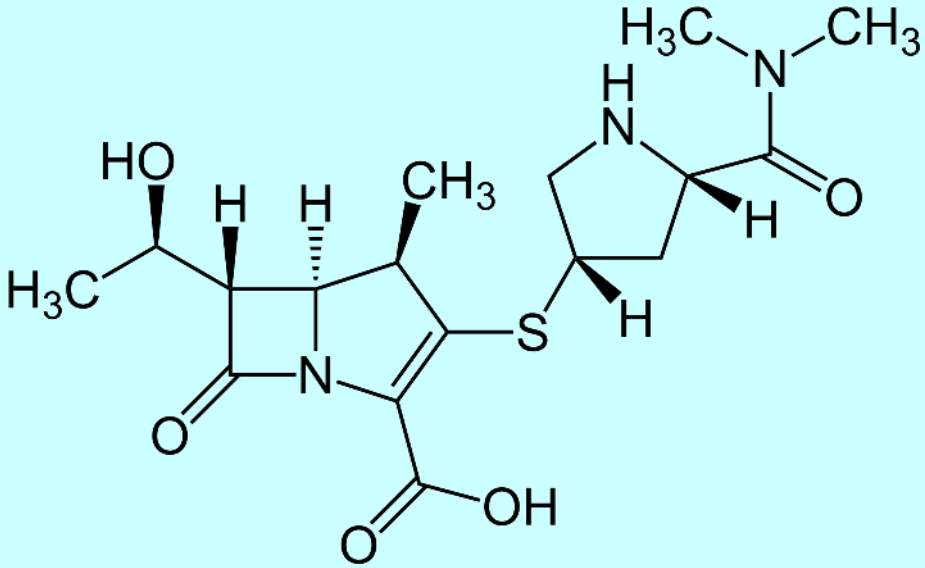


Thienamycin isolata ne 1976 da *Streptomyces cattleya*

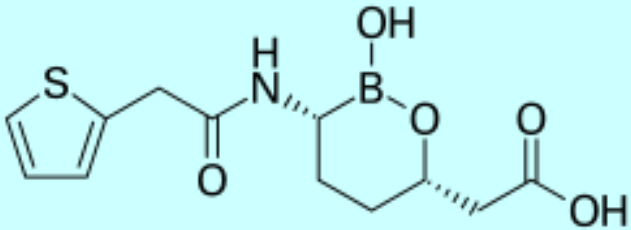


Imipenem brevettato nel 1975 e approvato nel 1985
Cosomministrato con **Cilastatina** (inbitore della Deidropeptidase 1)

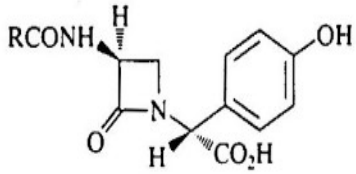


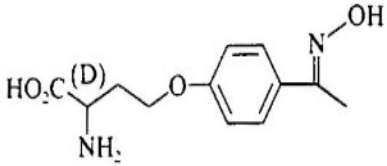
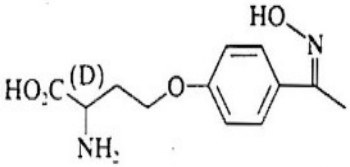
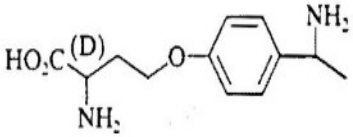
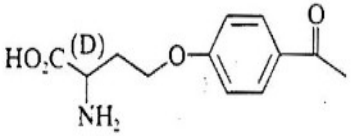


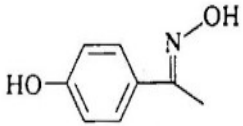
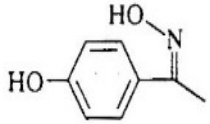
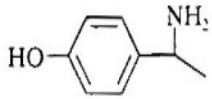
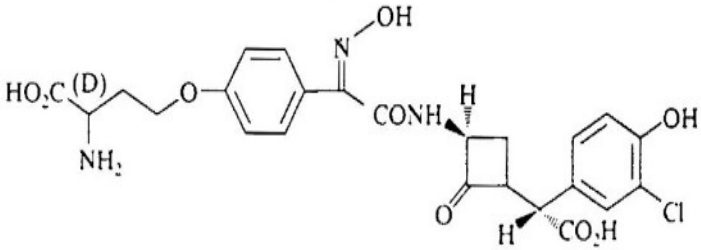
Meropenem brevettato nel 1983 e approvato nel 1996
Approvato in associazione con **Vaborbactam** (inbitore di β-lattamasi) nel 2017



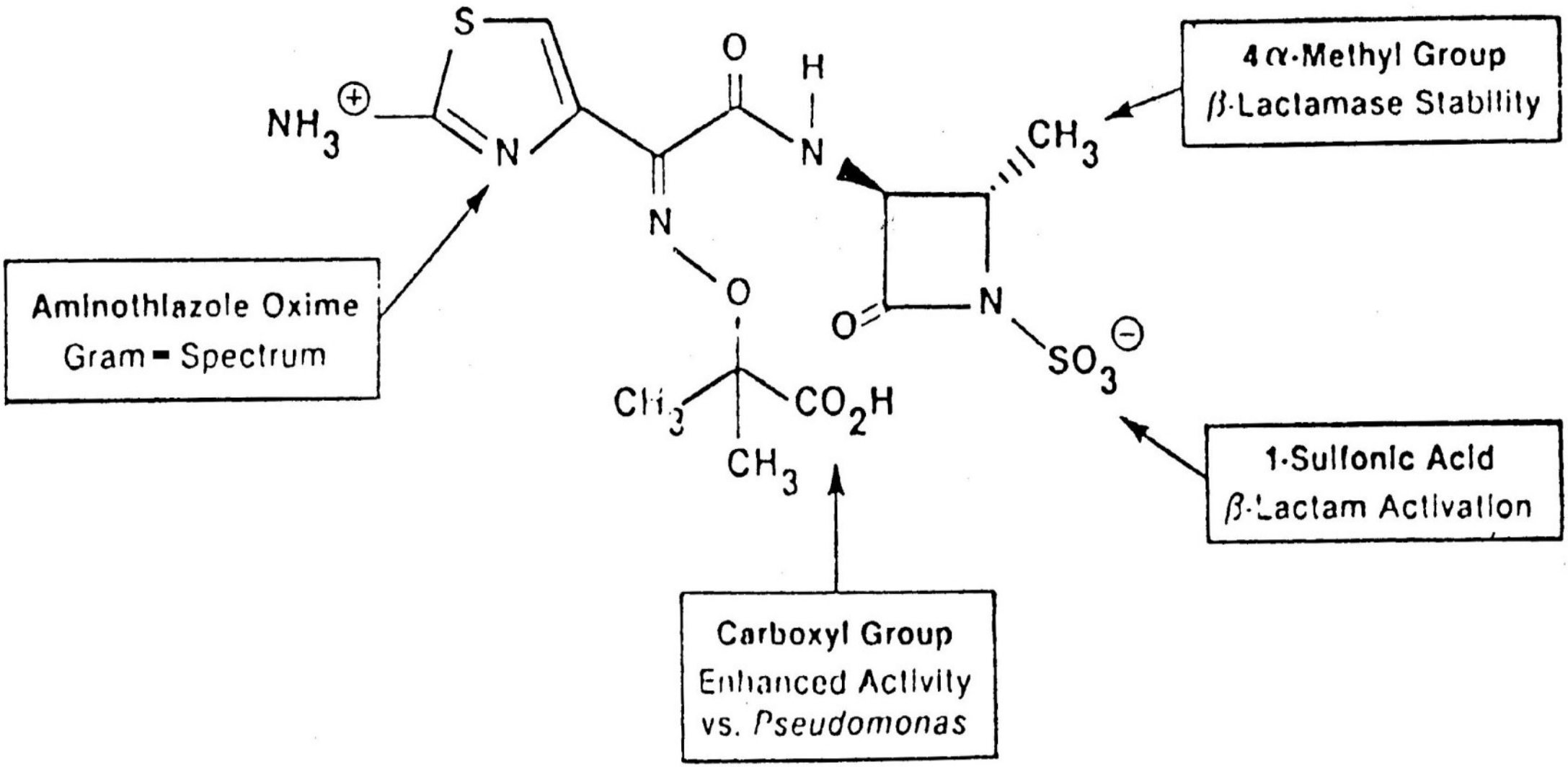
NOCARDICINE NATURALI



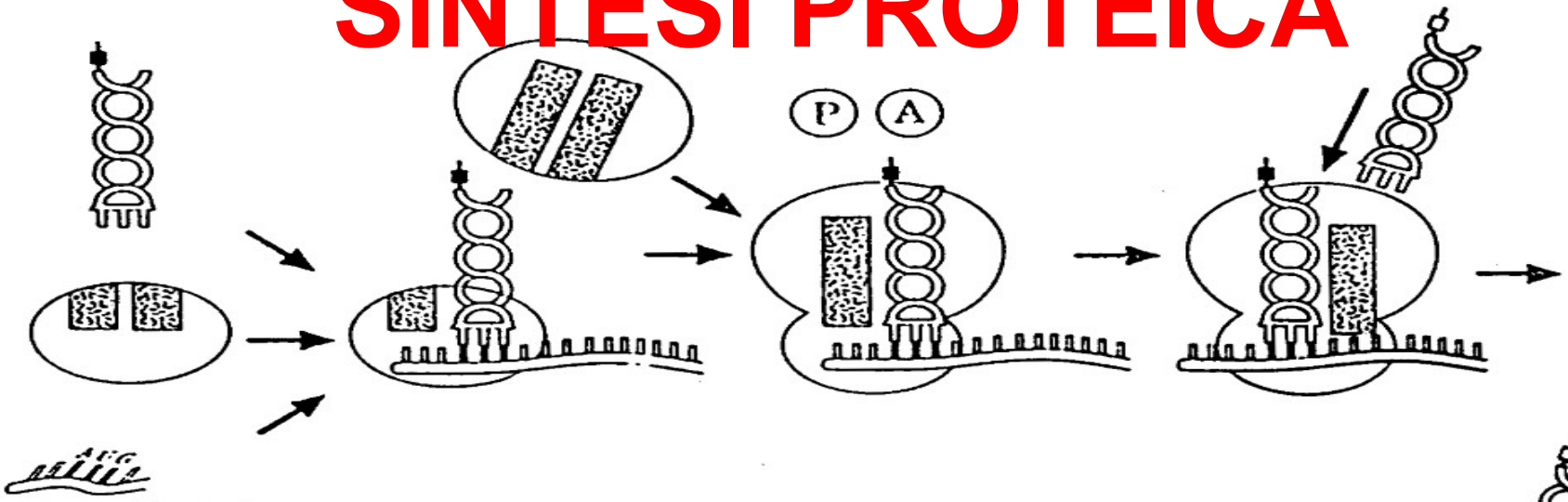
Nocardicin	R	Reference
A		229, 230, 232
B		229–232
C		229, 232
D		229, 232

Nocardicin	R	Reference
E		229, 232
F		229, 232
G		229, 232
Chlorocardicin		233

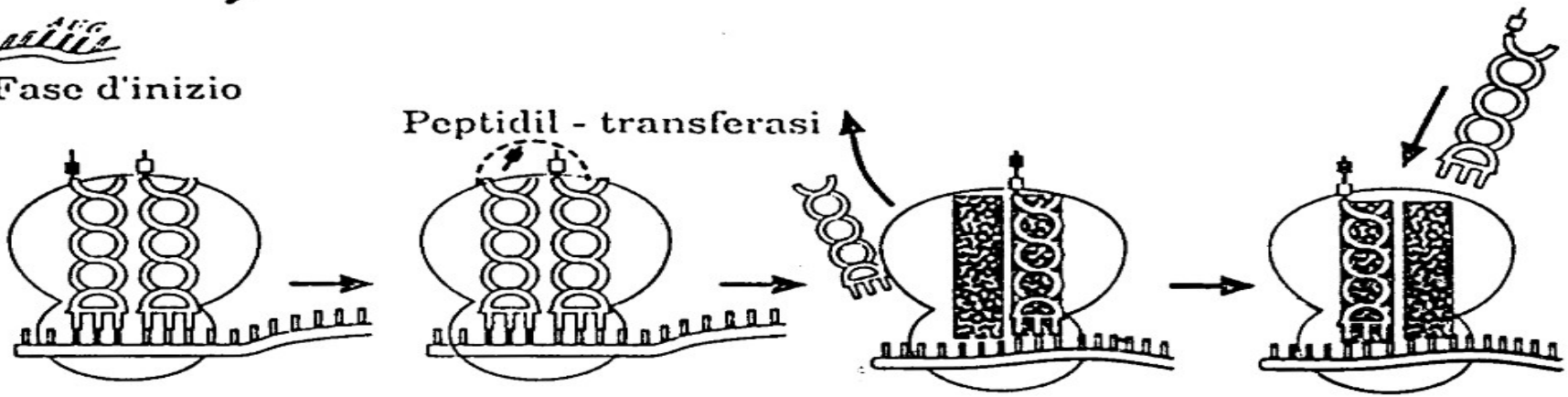
STRUCTURE-ACTIVITY RELATIONSHIPS IN THE AZTREONAM MOLECULE



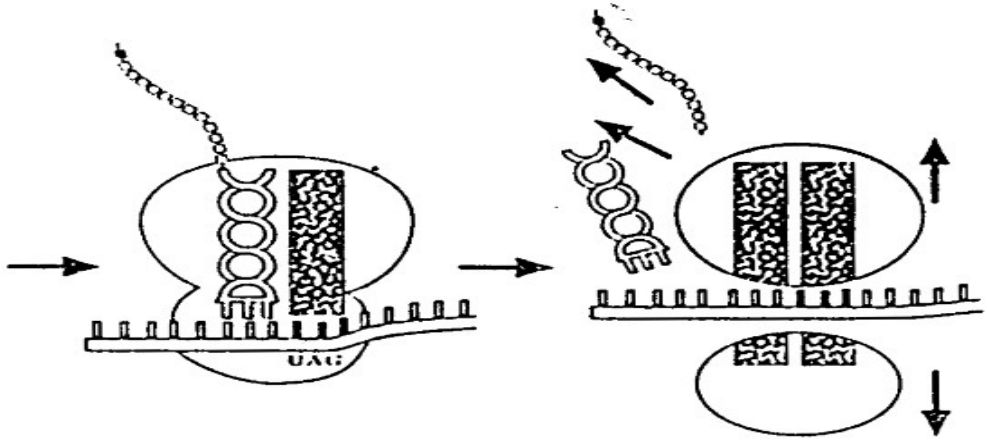
SINTESI PROTEICA



(A) Fase d'inizio

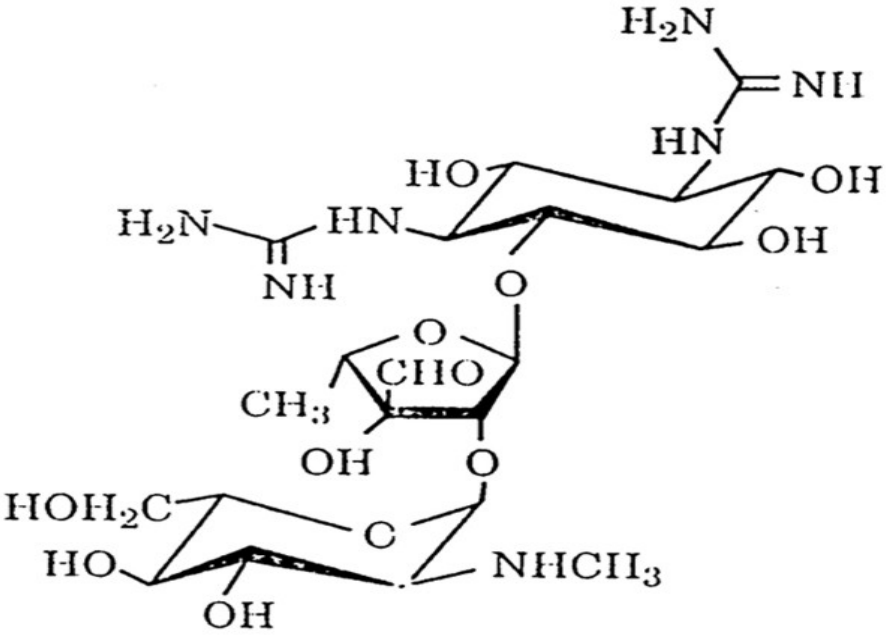


(B) Fase di elongazione



(C) Terminazione

ANTIBIOTICI - SINTESI PROTEICA

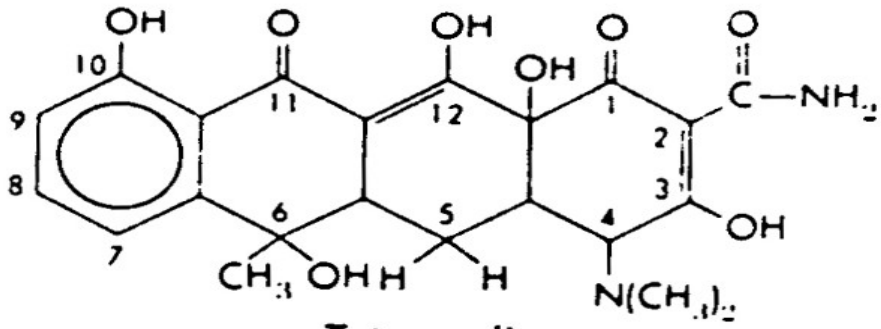


MIC (μg/mL)

S. aureus	1
S. pneumoniae	0.2
E. faecalis	2
E. coli	1
P. mirabilis	4
P. aeruginosa	20
M. tuberculosis	0.5

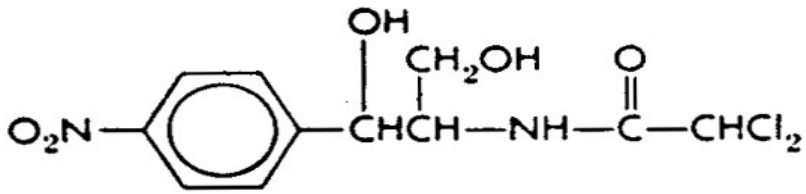
STREPTOMICINA

STRUCTURAL FORMULAS OF THE TETRACYCLINES



Tetracycline

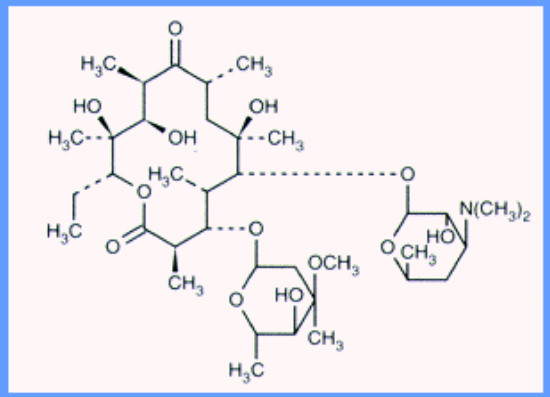
CONGENER	SUBSTITUENT(S)	POSITION(S)
Chlortetracycline	—Cl	(7)
Oxytetracycline	—OH,—H	(5)
Demeclocycline	—OH,—H; —Cl	(6: 7)
Methacycline	—OH,—H; =CH_2	(5: 6)
Doxycycline	—OH,—H; —CH_3,—H	(5: 6)
Minocycline	—H,—H; —$N(CH_3)_2$	(6: 7)



Chloramphenicol

ANTIBIOTICI - SINTESI PROTEICA

Erythromycin



Growing peptide

50S portion

t-RNA

r-RNA

m-RNA

30S portion

Chloramphenicol binds to 50S r-RNA and inhibits formation of peptide bond

Erythromycin binds to 50S r-RNA and prevents movement along m-RNA

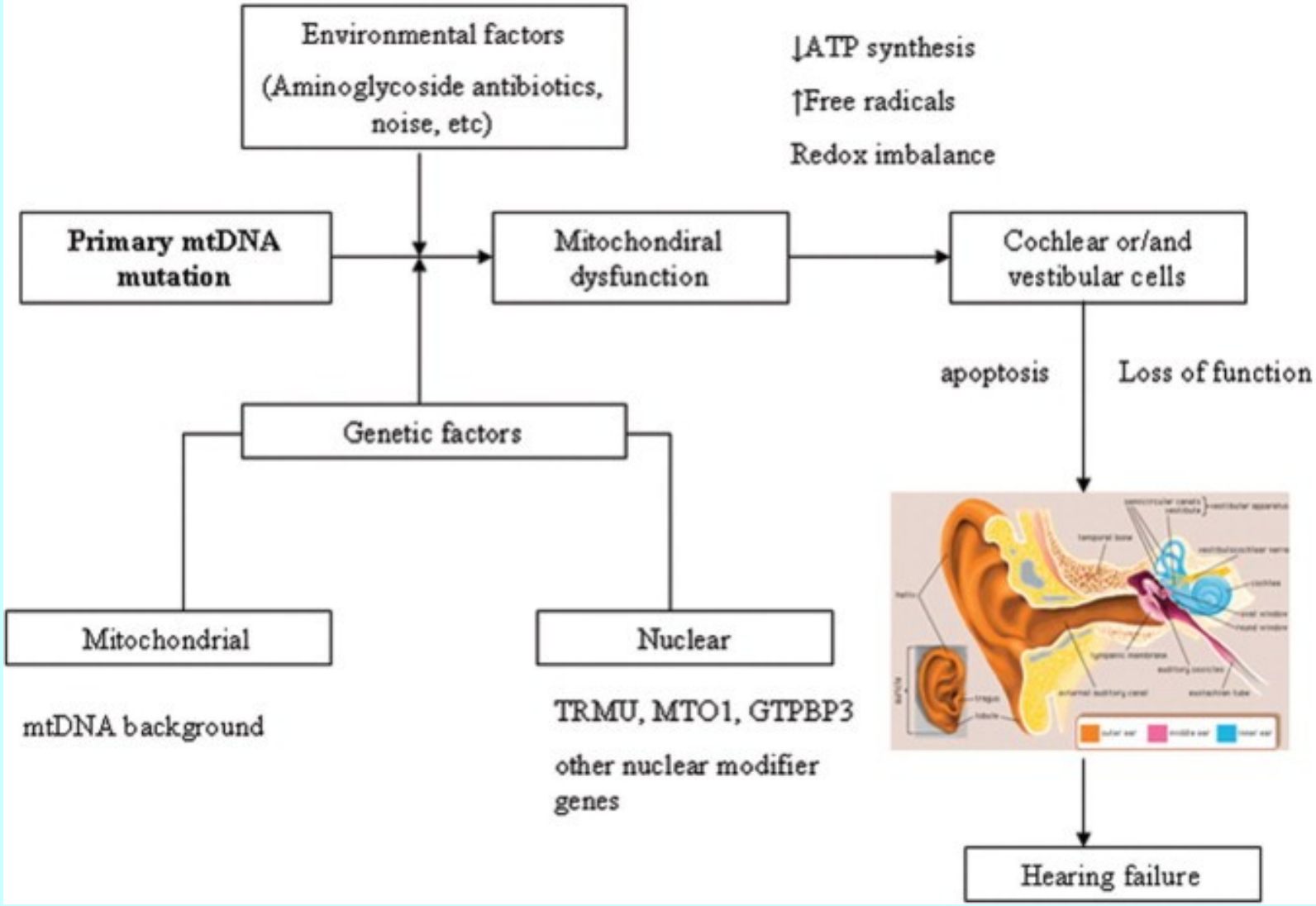
Streptomycin changes shape of 30S r-RNA and causes m-RNA to be read incorrectly

Tetracycline interfers with the t-RNA anticodon reading of m-RNA codon

C. Ophardt, c. 2003

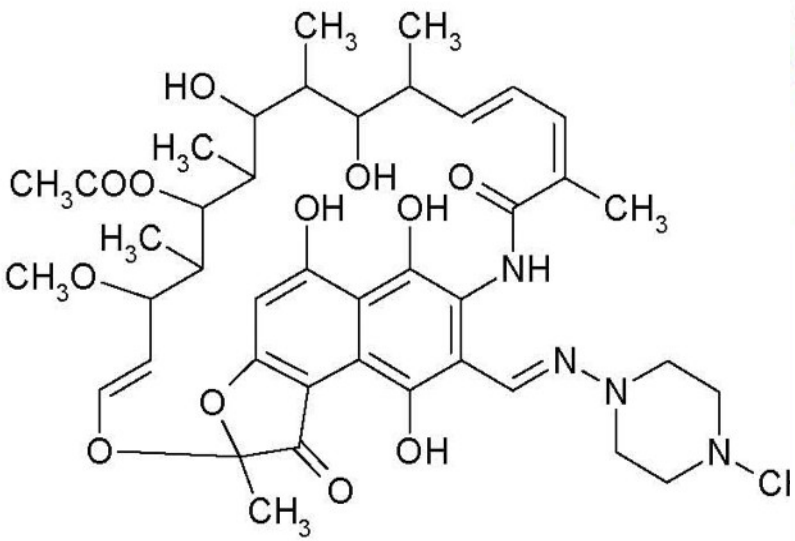
Macrolides: Mechanisms of Action and

OTOTOSSICITÀ DELLE STREPTOMICINE

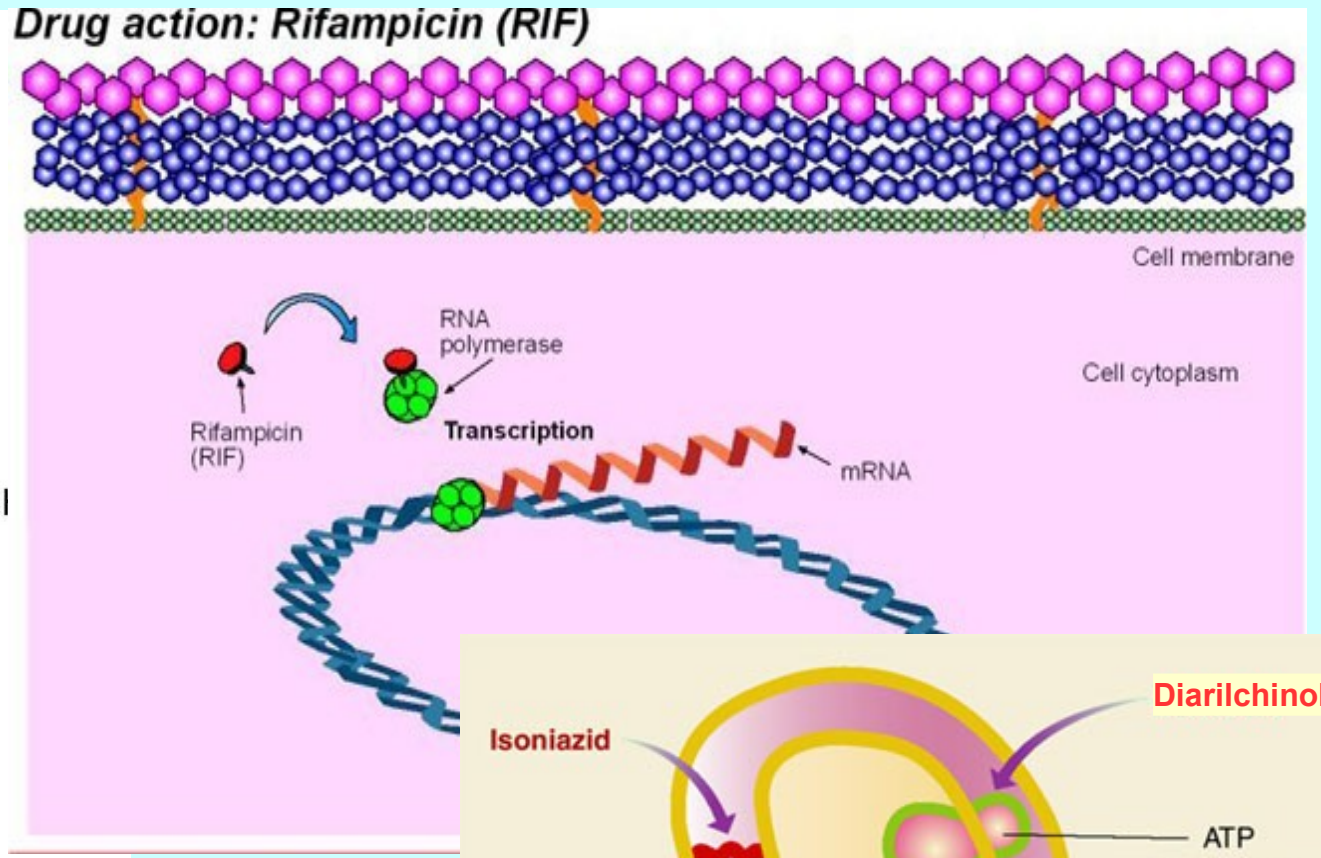


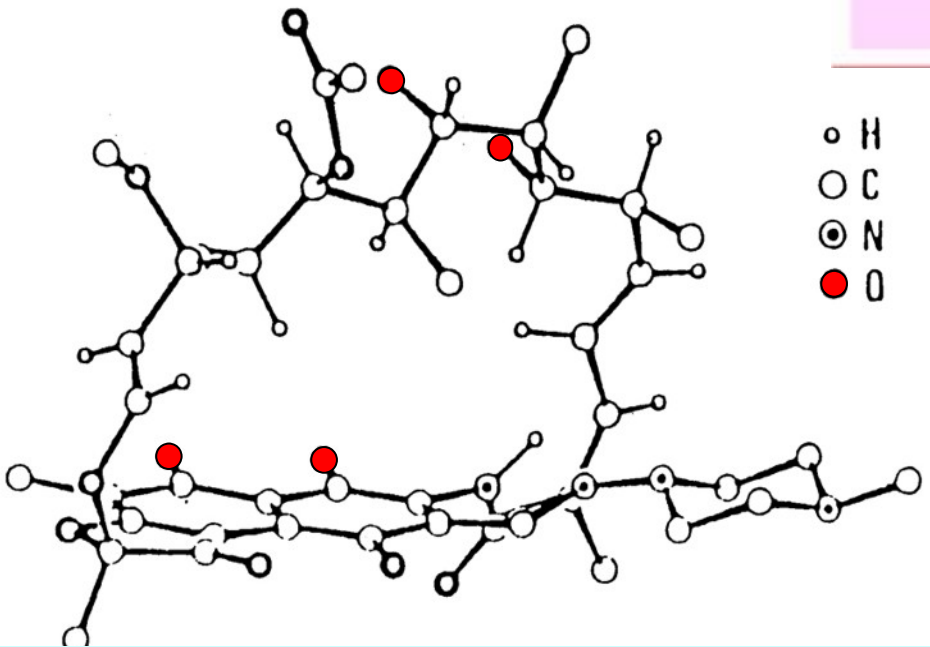
I ribosomi mitocondriali, soprattutto se portatori di una specifica mutazione, sono sensibili agli amminoglicosidi, che penetrano e si accumulano nei mitocondri della coclea e del vestibolo causando difetti di translazione nella sintesi proteica che portano a riduzione della sintesi di ATP e accumulo di ROS, i neuroni cocleari sono particolarmente sensibili alla presenza di radicali liberi.

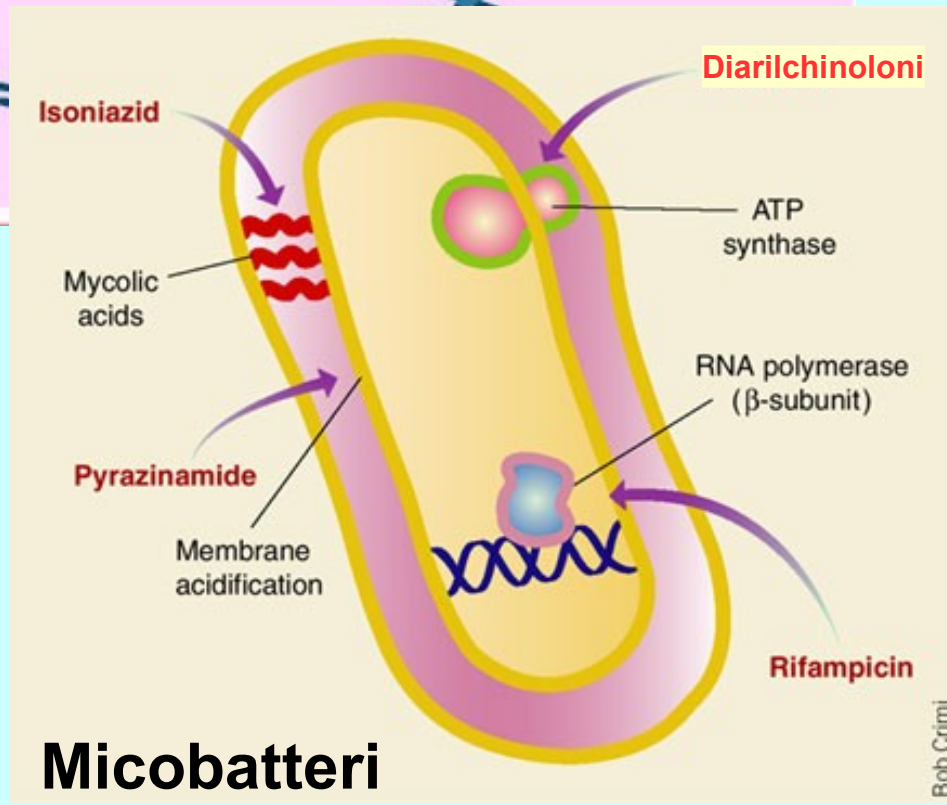
RIFAMICINE



RIFAMPICINA

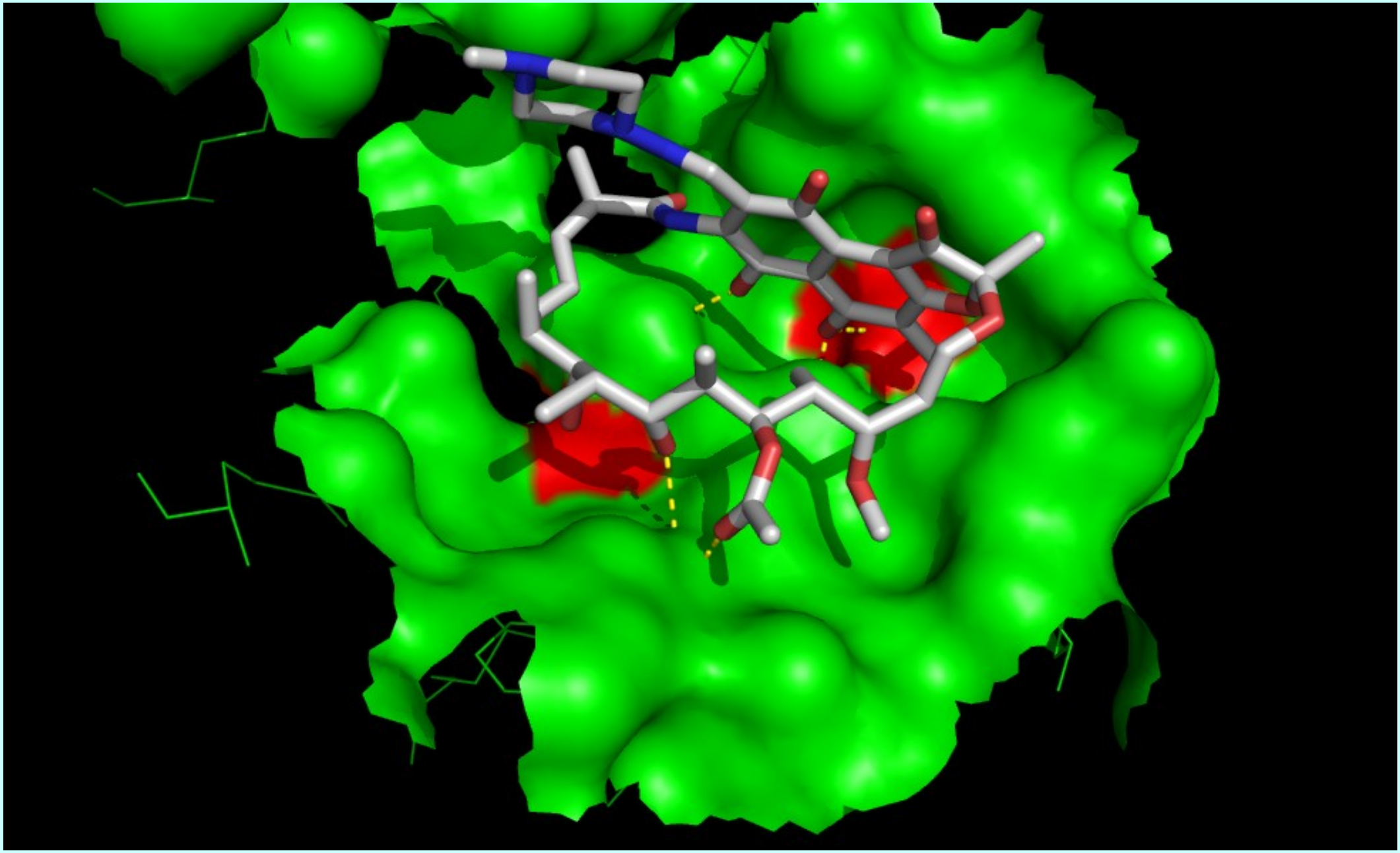






Micobatteri

RIFAMICINE



ISONIAZIDE

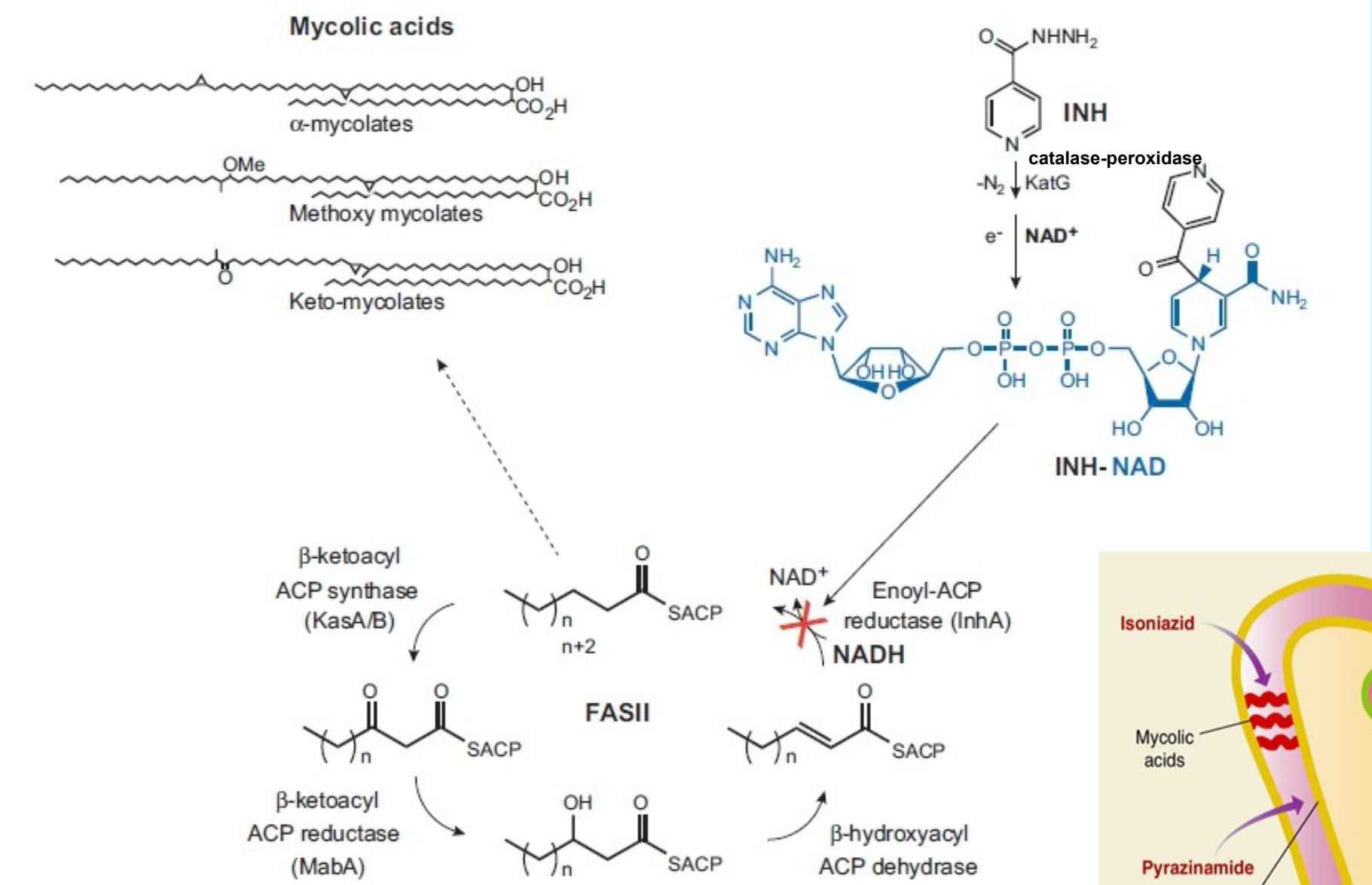
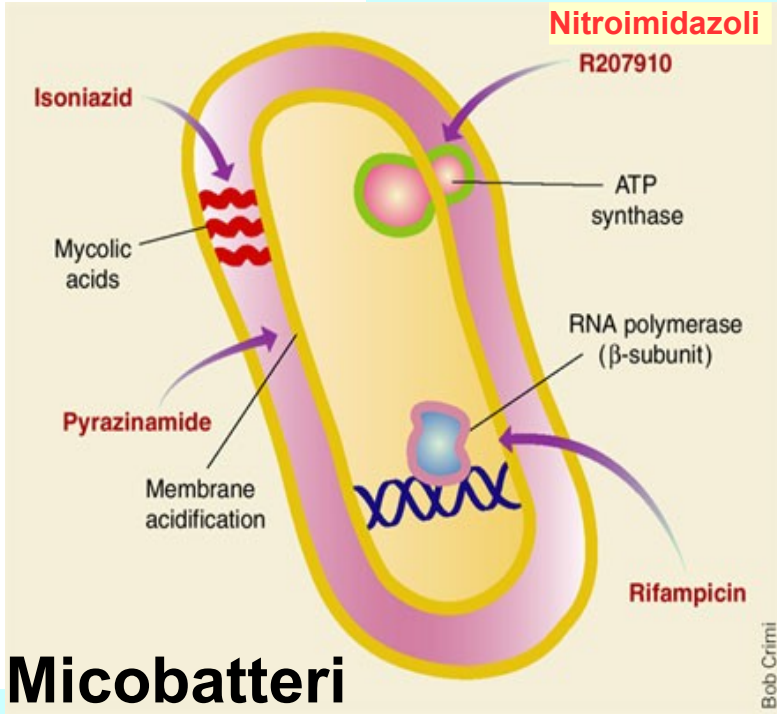
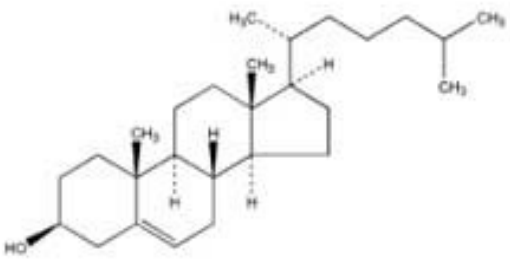


Figure 2

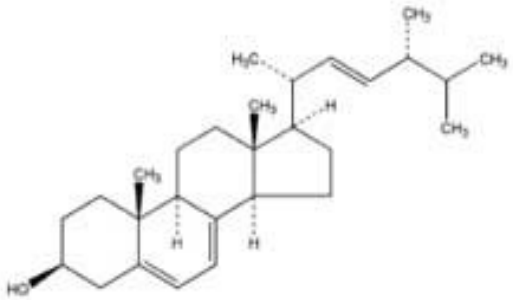
Mechanism of action of INH in *M. tuberculosis*. INH is activated by KatG to form the INH-NAD adduct. The adduct inhibits InhA, the enoyl-ACP reductase of the fatty acid synthase type II system (FASII), which synthesizes mycolic acids (representation of three mycolic acid classes) (50). This inhibition results in the inhibition of mycolic acid biosynthesis and ultimately cell death.



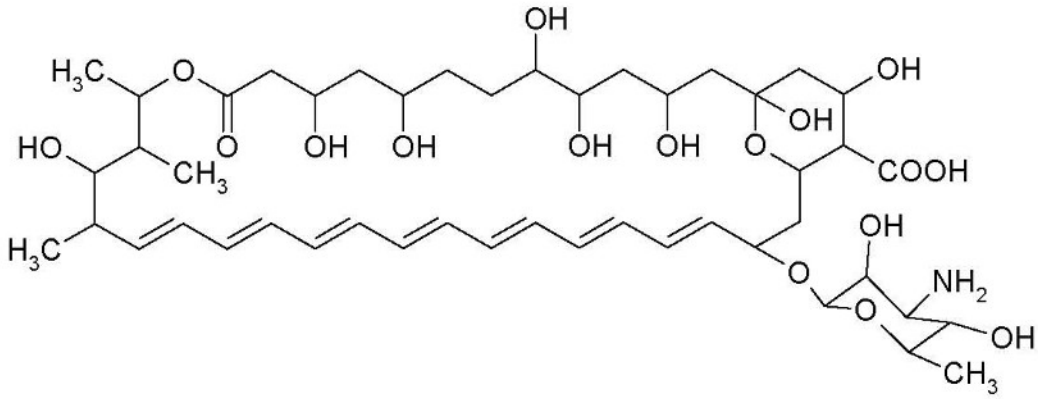
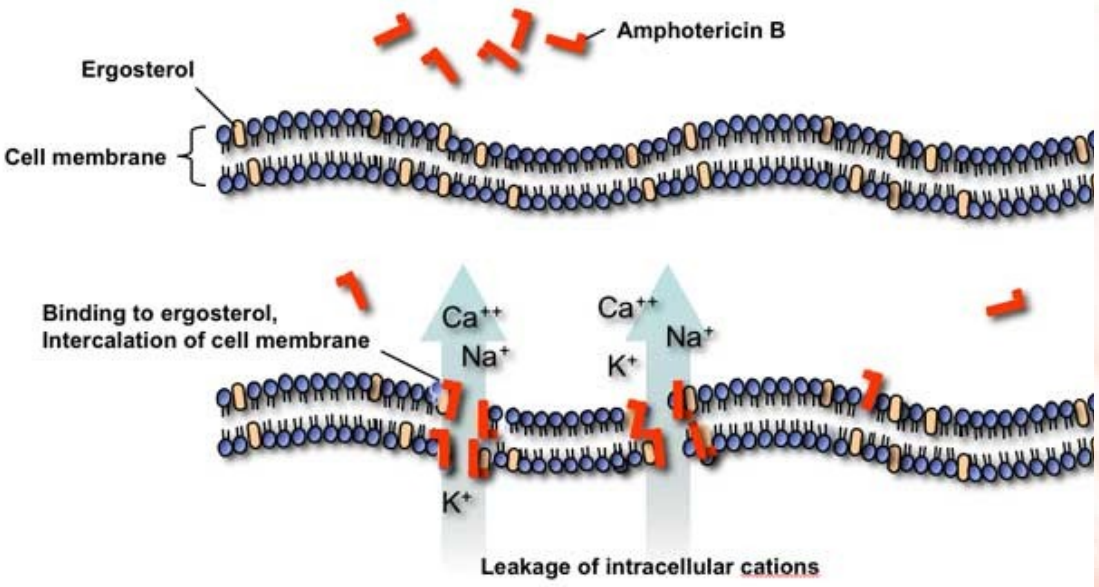
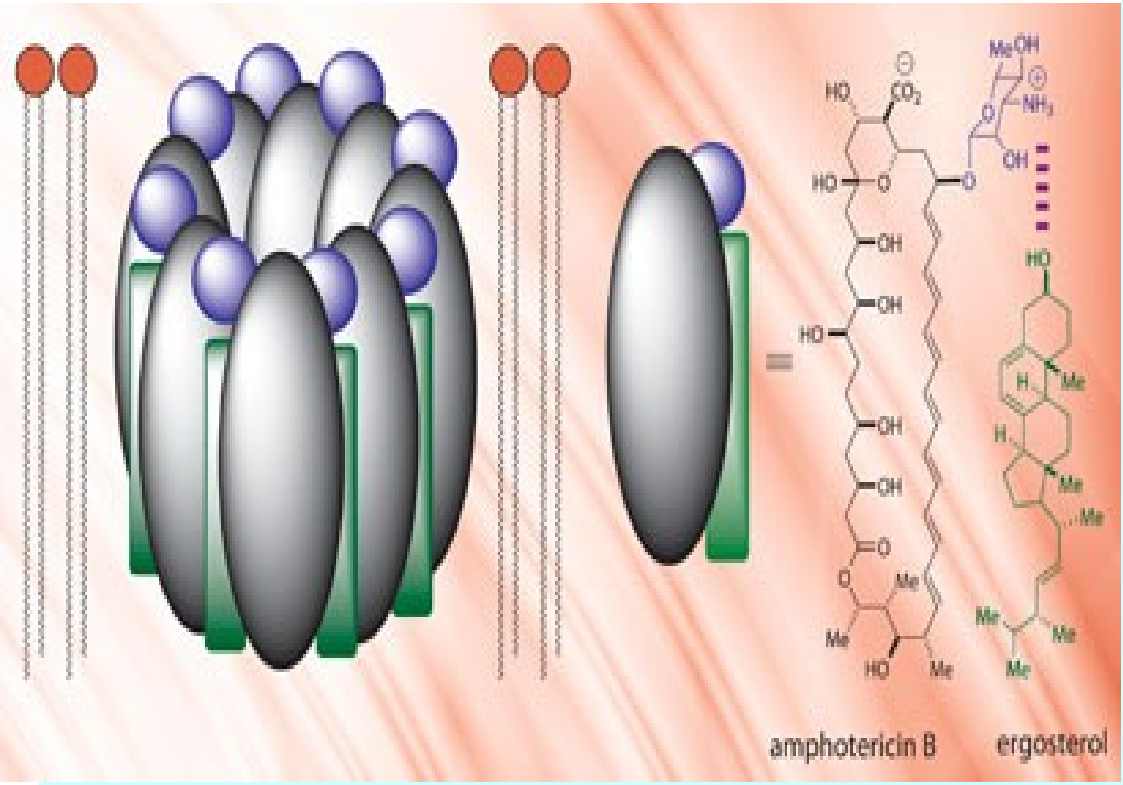
AMFOTERICINE (Antimicotici)



Cholesterol



Ergosterol



AMFOTERICINA B