Chemistry 259

Medicinal Chemistry of Modern Antibiotics

Spring 2012

Lecture 8: *Antibiotics Classes & Targets*

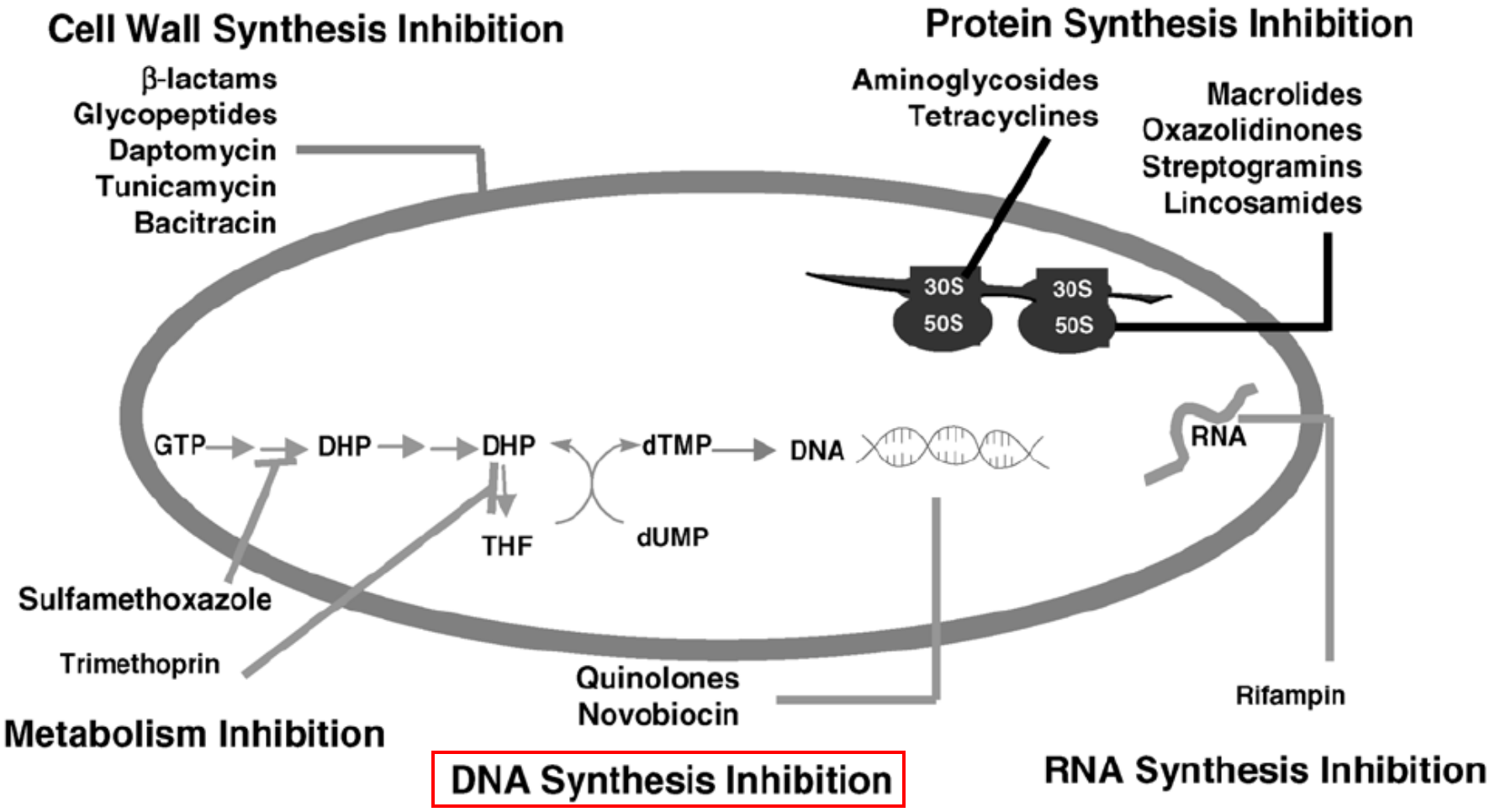
Part III: Drugs Targeting DNA & RNA Biosynthesis

Thomas Hermann

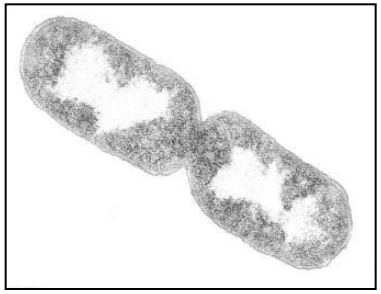
Department of Chemistry & Biochemistry

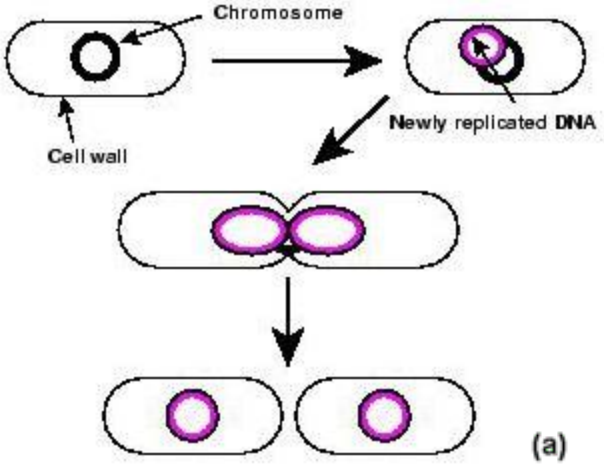
University of California, San Diego

Antibacterial Targets: Overview

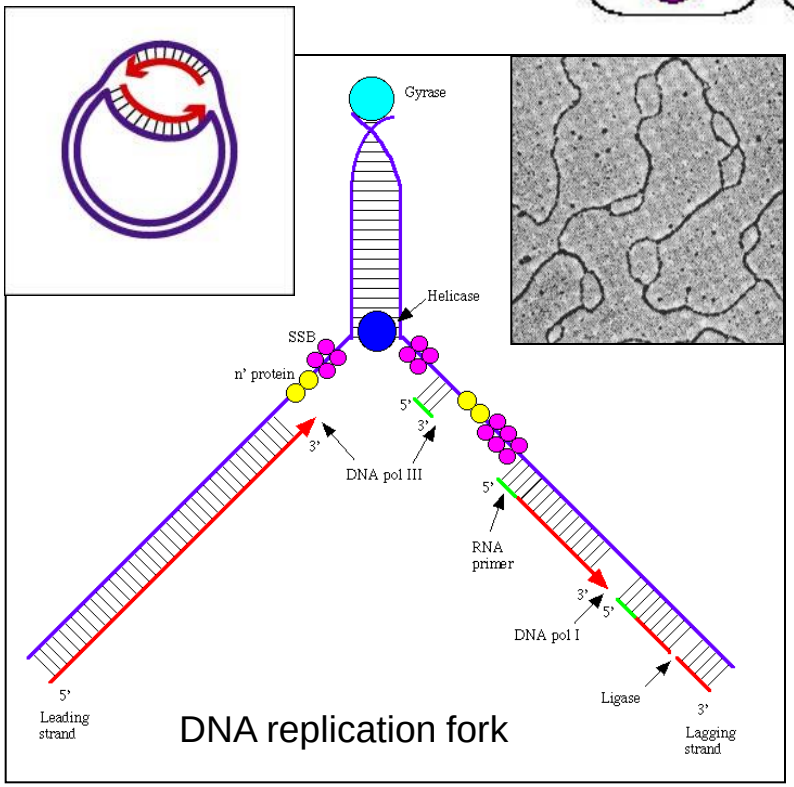


Bacterial DNA Synthesis

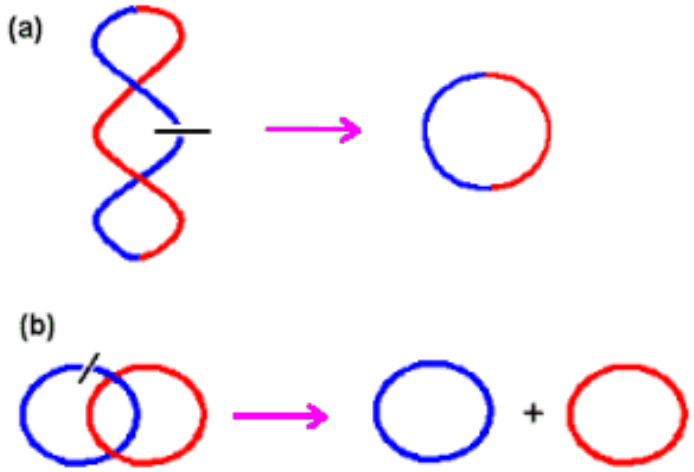




- DNA is synthesized at a rate of 50,000 bp/min/replication fork
- DNA strands must separate and tangles, crossovers and catenates must be rapidly removed
- DNA topoisomerases (gyrase, topoisomerase IV)

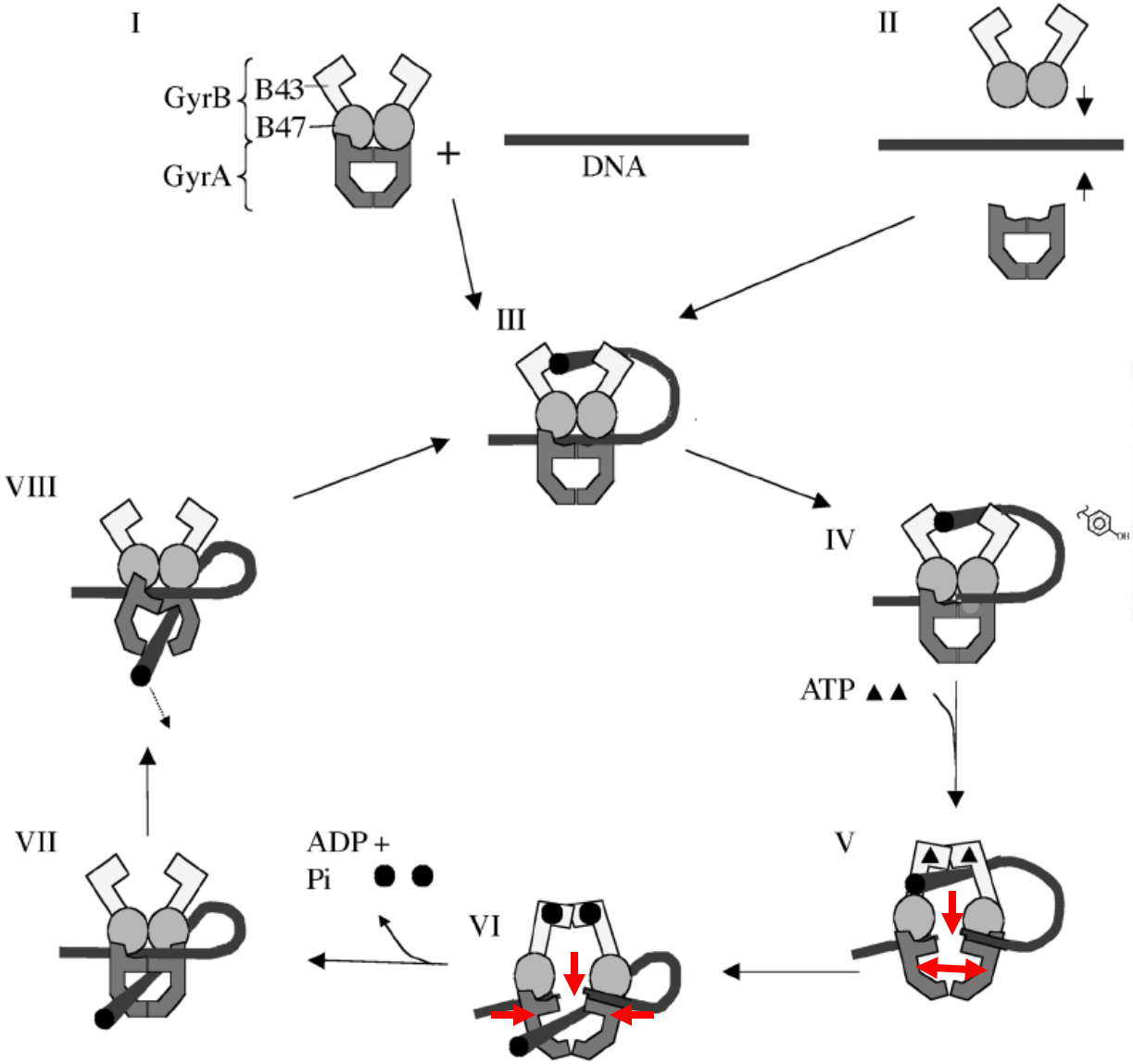


DNA replication fork

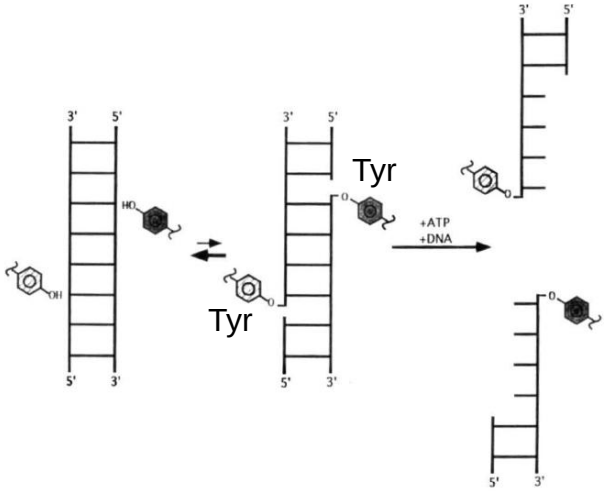


(a) DNA gyrase: removes **supercoils**. This involves a double-strand break, allowing the tangled segment to pass through. The break is then resealed.
(b) Topoisomerase IV: removes **catenanes**. Makes a double-strand break in one DNA molecule, allowing the other molecule to pass through. The break is then resealed.

Bacterial DNA Synthesis: Gyrase and Topo IV – Composition & Mechanism

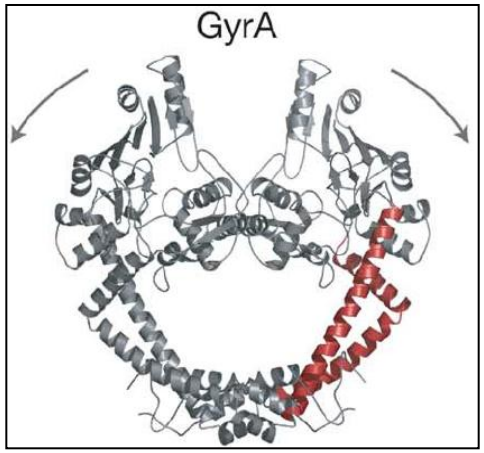


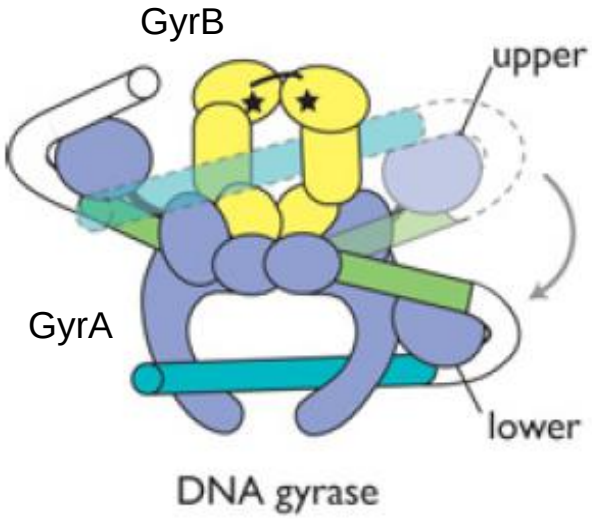
DNA gyrase is a heterotetramer (A2B2), composed of two A and two B subunits, products of *gyrA* and *gyrB* genes.



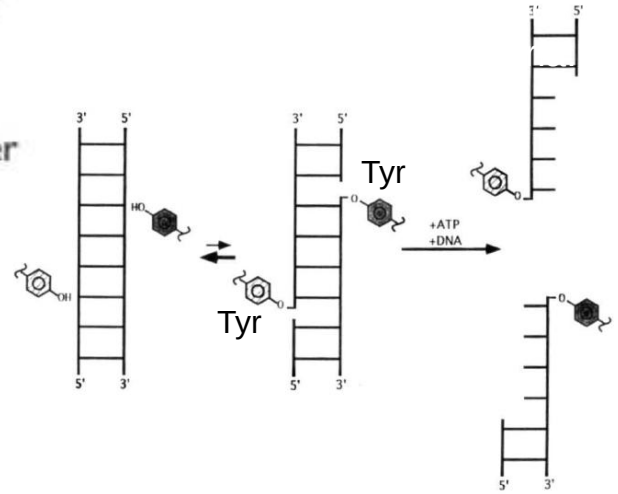
Topoisomerase IV comprises two subunits, ParC and ParE. The ParC protein is homologous to the gyrase A protein, while the ParE subunit is homologous to the gyrase B protein

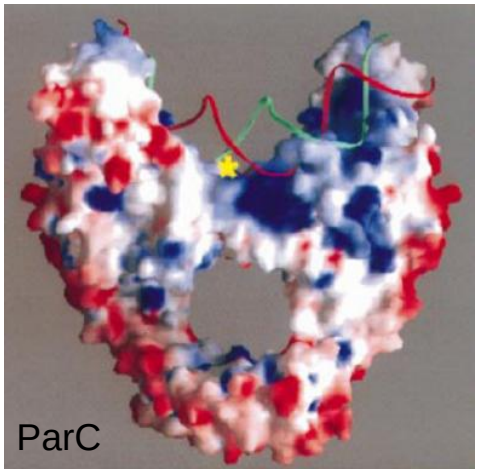
Bacterial DNA Synthesis: Gyrase and Topo IV – Composition & Mechanism

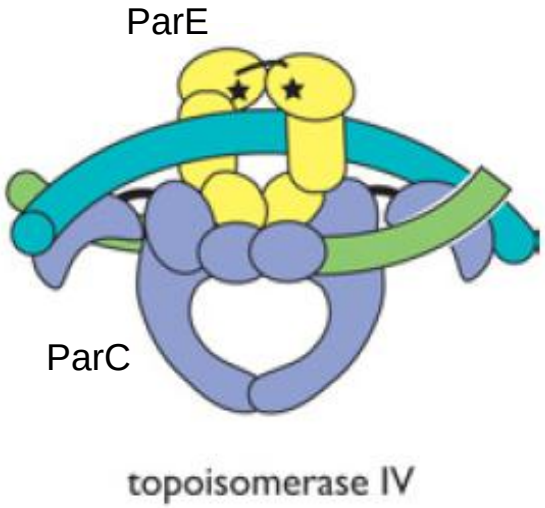




DNA gyrase is a heterotetramer (A2B2), composed of two A and two B subunits, products of *gyrA* and *gyrB* genes.

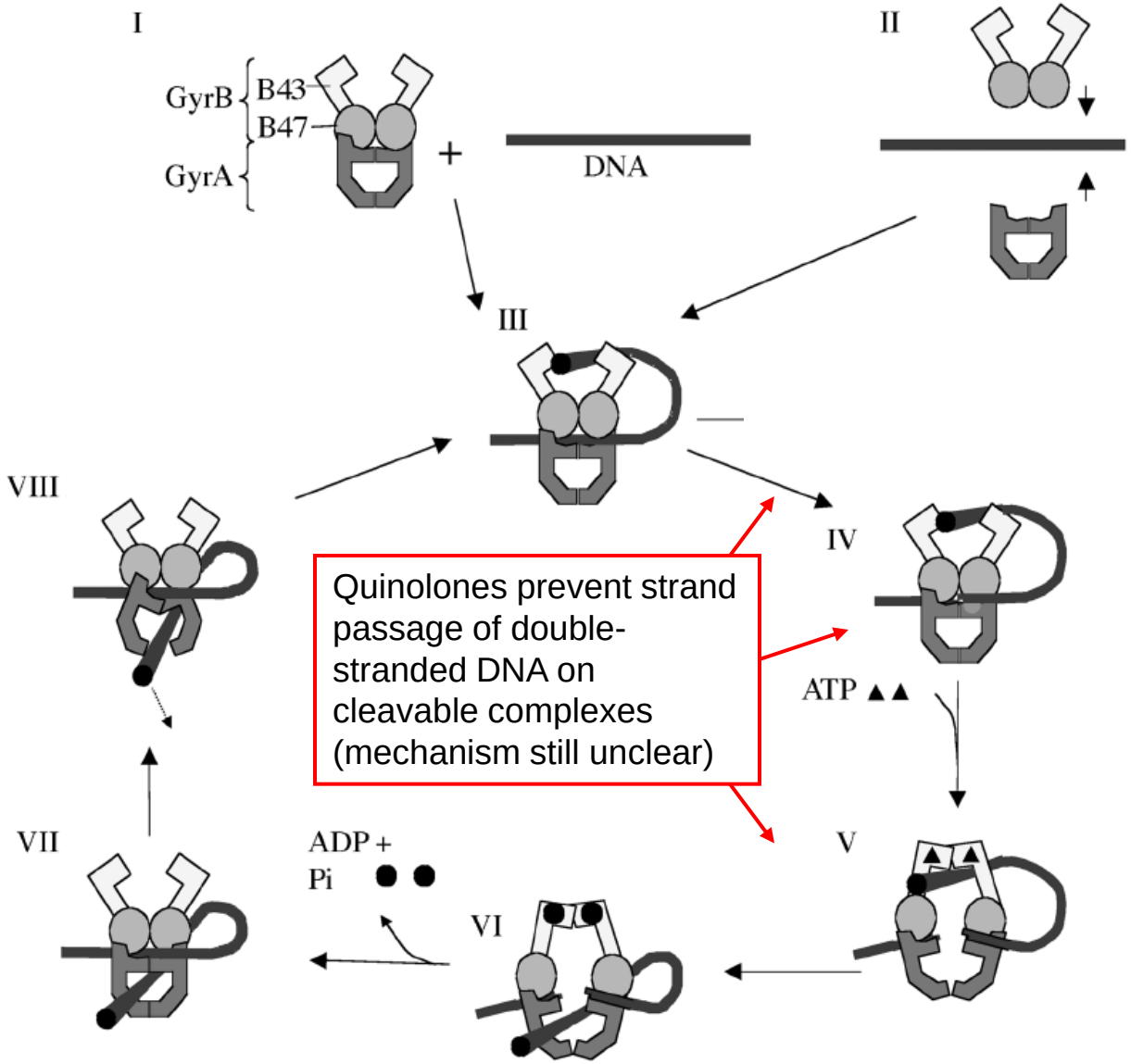






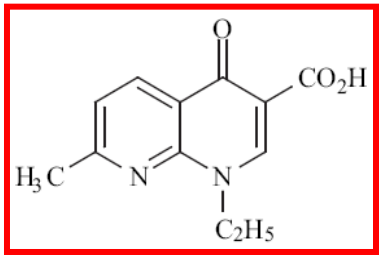
Topoisomerase IV comprises two subunits, ParC and ParE. The ParC protein is homologous to the gyrase A protein, while the ParE subunit is homologous to the gyrase B protein

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Inhibitors



DNA gyrase is a heterotetramer (A2B2), composed of two A and two B subunits, products of *gyrA* and *gyrB* genes.

Primary target of quinolones in Gram-negative bacteria.



Nalidixic acid

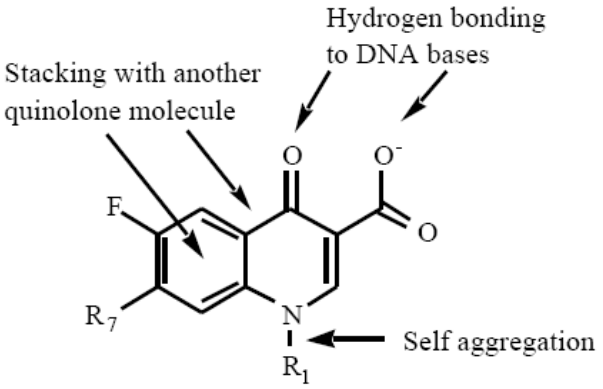
Topoisomerase IV comprises two subunits, ParC and ParE. The ParC protein is homologous to the gyrase A protein, while the ParE subunit is homologous to the gyrase B protein

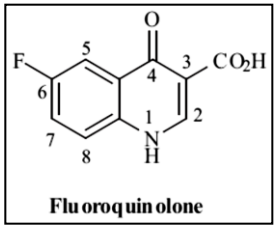
Primary target of quinolones in Gram-positive bacteria.

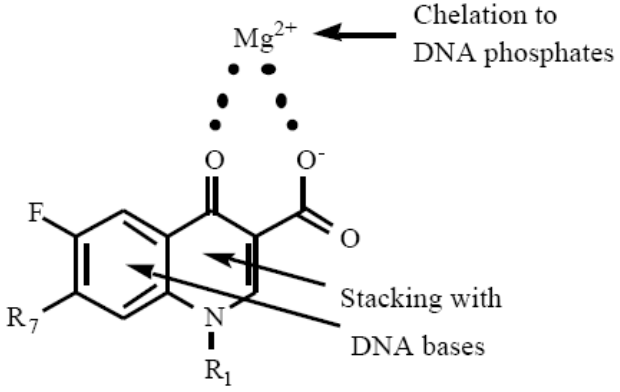
Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Mechanism

Cooperative Binding Model:
• drug binds to cleaved single-stranded DNA and thereby traps the enzyme
• four quinolone molecules bind cooperatively to DNA via H-bonds to DNA bases
• quinolones stack onto each other to form pairs
• quinolones aggregate via substituents at 1 and 8 position

Mg^{2+} Bridge Model:
• drug binds to DNA phosphates via chelation of Mg^{2+}
• quinolone ring stacks onto DNA bases
• quinolone binding induces a conformational change in the gyrase-DNA complex
• cleavage of DNA is not necessary for drug binding





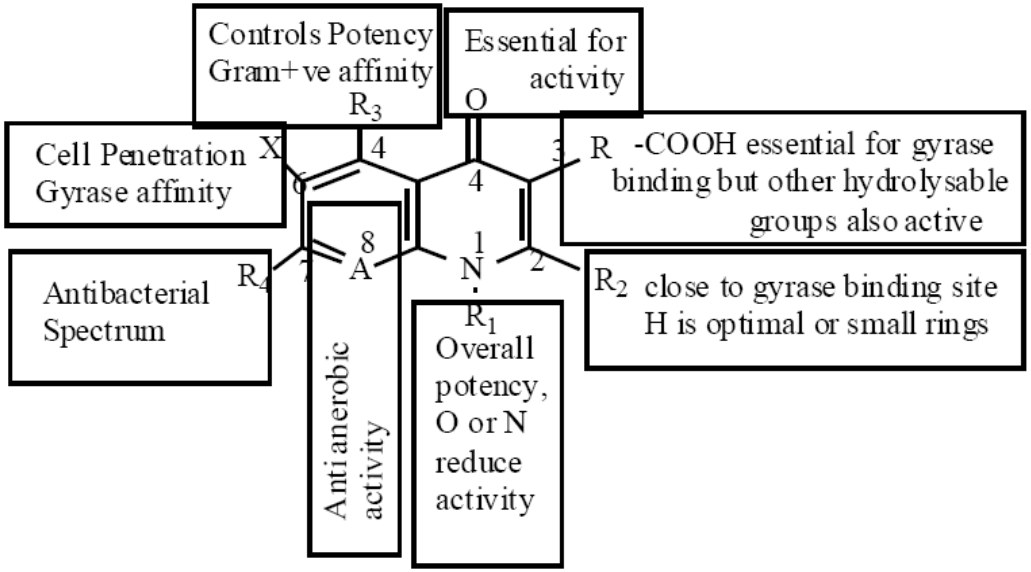


But:
• gyrase Tyr122 mutants which cannot cleave DNA can still bind quinolone
• -> single-stranded DNA not necessary as target for quinolones

(Berger, *Curr. Opin. Struct. Biol.* 1998, 8, 26)

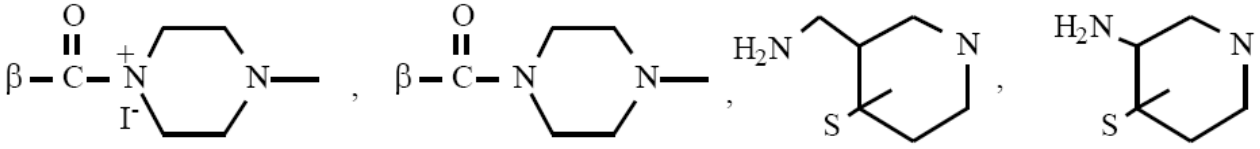
(Palumbo et al., *Trends Microbiol.* 1993, 1, 232)

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone SAR



- Synthetic compounds discovered 1962 as a by-product of an antimalarial program.
- Not used as antibiotics until discovery of improved 6-fluoroquinolones in 1980s (norfloxacin).
- Orally bioavailable; excellent distribution; active against G+/-
- Resistance development can be rapid by target modification (mutation in the quinolone resistance determining region QRDR of gyrase/topo IV) and by active efflux.

R; -COOH, -COOR'; Coumarin, penems, carbapenems or monobactam etc.
R_1; halo atom, halo substituted aromatic ring, hetro atom, (substituted) $C_{1\text{-}6}$ alkyl, (Substituted) $C_{3\text{-}6}$ cycloalkyl, (Substituted) aryl etc.
R_2; H, $-SCH_3$, $C_{1\text{-}6}$ alkyl thio, or R_2 & R_1 may join to form a ring.

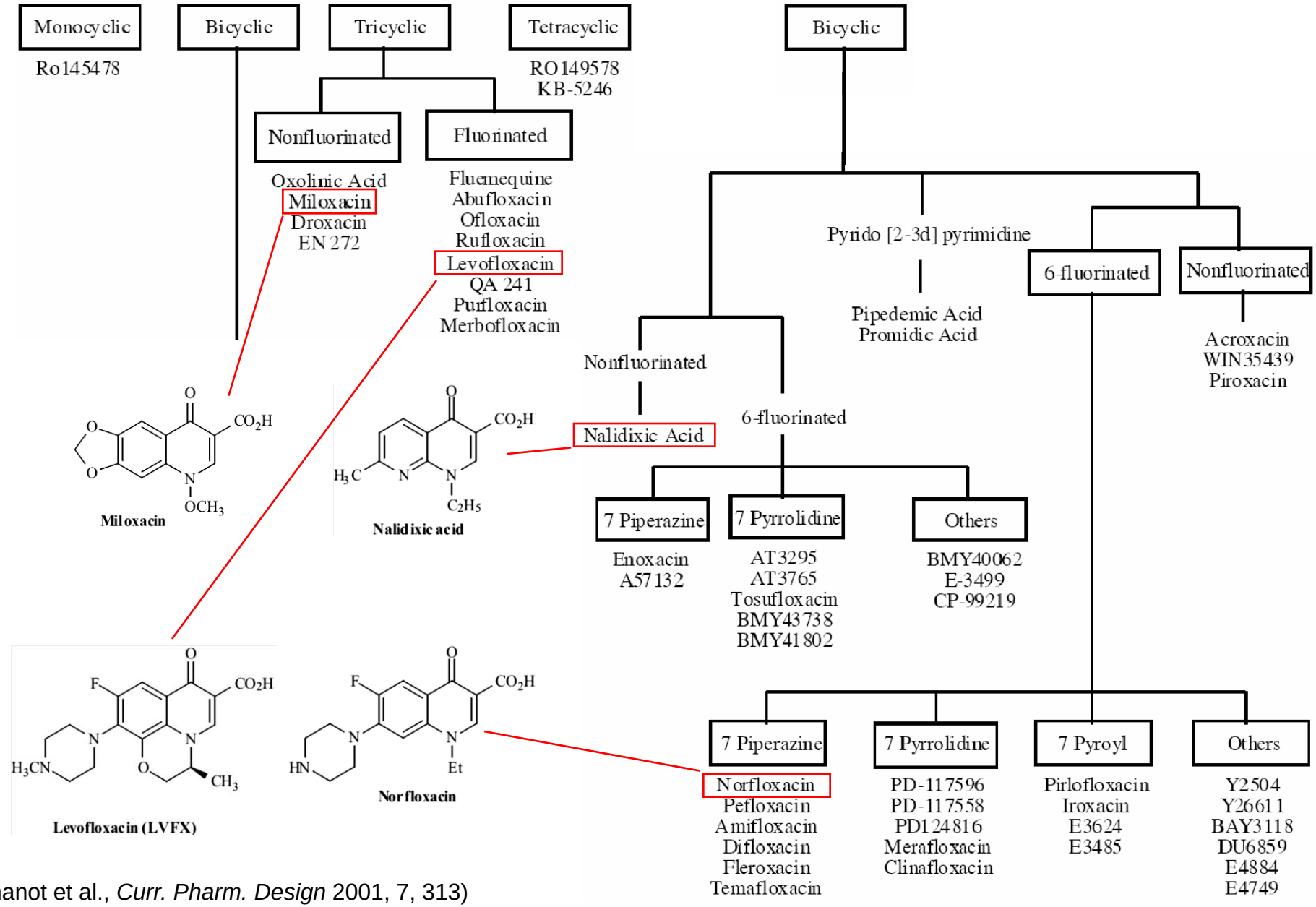
R_4; H halo atom, 

β = Penicillin or cephalosporin

(S = alkyl group)

X; H, $-CH_3$, $-NO_2$, F, $-OCH_3$
A; N, -CF, -CH, $-COCH_3$, C-halo, -CCN, -CSR, $-CNH_2$, COH, $-CCH_2OH$, or A and R_1 may join to form a ring.

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Inhibitors



(Bhanot et al., *Curr. Pharm. Design* 2001, 7, 313)

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Inhibitors

1) **First generation**: nalidixic acid, oxolinic acid, pipedinic acid, flumequine, ...

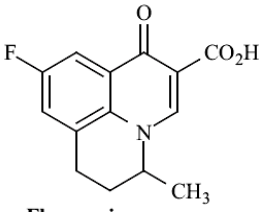
2) **Second generation** (most potent against *Pseudomonas*): norfloxacin, ciprofloxacin, enoxacin, fleroxacin, ofloxacin, levofloxacin, lomefloxacin, ...

3) **Third generation** (more potent against *Pneumococcus* and anaerobes): sparfloxacin, tosufloxacin, gatifloxacin, pazufloxacin, grepafloxacin, ...

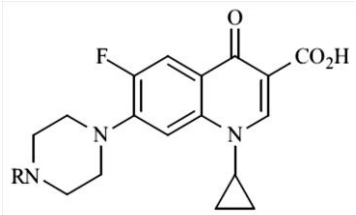
4) **Fourth generation** (most potent against *Pneumococcus* and anaerobes): trovafloxacin, clinafloxacin, sitafloxacin, moxifloxacin, gemifloxacin

Respiratory Q (active against *Streptococcus*, *Staphylococcus*, *Haemophilus*): levofloxacin, ...

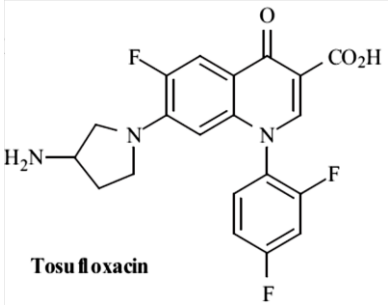
Antipseudomonas Q (active against *Pseudomonas*, *Haemophilus*): ciprofloxacin, ofloxacin, ...



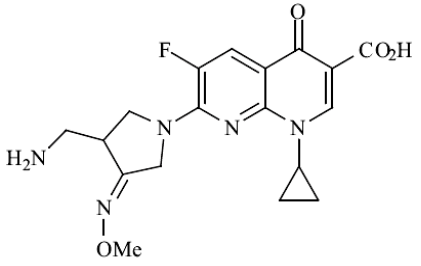
Flumequine



R = H - Ciprofloxacin
CH_3 - Danofloxacin
Et - Enrofloxacin



Tosufloxacin



Gemifloxacin (GMFX)

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Spectrum

Etiology	Ciprofloxacin	Norfloxacin	Ofloxacin	Enoxacin	Lomefloxacin	Fleroxacin	Tosufloxacin	Sparfloxacin
GI & bladder pathogens	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Pseudomonas	+ + + +	0	+ +	+ +	+ + + +	+ + + +	+ + + +	+ +
Staphylococci	+ + + +	+	+ +	0	0	+	+ + + +	+ + + +
Streptococcus pneumoniae	+ +	0	+ +	0	0	N/D	+ + + +	+ + + +
Anaerobes	0	0	0	0	0	0	+ +	+ +
Neisseria spp.	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Chlamydia	+ +	0	+ + +	0	+ + + +	+ + +	+ ++ +	+ + + +
Mycoplasma	+++	0	+++	0	N/D	N/D	N/D	++++
Legionella	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Mycobacteria	+ + +	N/D	+ + +	N/D	0	N/D	N/D	+ +

++++ MIC_{90} < 0,5 µg/mL; +++ MIC_{90} 0,51 – 1,0 µg/mL; ++ MIC_{90} 1,1 – 2,0 µg/mL; + MIC_{90} 2,1 – 4,0 µg/mL; 0 MIC_{90} > 4,0 µg/mL; N/D No Data Available

(Da Silva et al., *Curr. Med. Chem.* 2003, 10, 21)

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Pharmacology

Drug	Mol. Wt	pk_a	Dosage forms	Elimination half-life (h)	Protein binding (%)	*Metabolites*	Urinary excretion
Ciprofloxacin	331.3	6.0; 8.8	Lactate or hydrochloride	3-4	20-40	Sulpho, oxo, formyl, desethyl	50-70%, P 10% metabolites
Fleroxacin	369	5.5, 8.0		9-12	23	Desmethyl, N-oxide	>50%, with < 11% as metabolites
Levofloxacin	361.37	7.9	Hemihydrate	3-7	30	N-oxide, desmethyl	>75%, < 10% with metabolites
Lomefloxacin	351.37			7-8	15	Glucuronide	>60%, 5-10% as metabolites
Norfloxacin	319.3	6.2-6.4, 8.7-8.9		3-4	15	Formyl, oxo, desethyl, etc.	35%, with 10% as metabolites
Ofloxacin	361.37	7.9	Hydrochloride	3-7	30	N-oxide, desmethyl	>75%, <10% as metabolites
Pefloxacin	333.37		Mesylate dihydrate	8-13	20-30	As norfloxacin plus desmethyl	>60%, mostly as metabolite
Sparfloxacin	380.38			15-20	37	Glucuronide	<10%, mostly as metabolite

(Da Silva et al., *Curr. Med. Chem.* 2003, 10, 21)

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolones & Bioterrorism

Pathogen	*Postexposure prophylaxis*	*Treatment*
Bacillus anthracis (anthrax)	Agent of choice: ciprofloxacin (Cipro)* Alternative: doxycycline (Vibramycin)	Agents of choice: ciprofloxacin, doxycycline Alternative if organisms are penicillin sensitive: penicillin G
Vibrio cholerae (cholera)	Not available	Agents of choice: oral rehydration therapy, tetracycline, doxycycline, ciprofloxacin, norfloxacin (Noroxin)
Yersinia pestis (plague)	Agents of choice: doxycycline, ciprofloxacin Alternative: tetracycline	Agents of choice: streptomycin, gentamicin, ciprofloxacin Alternative: doxycycline
Brucella melitensis (brucellosis)	Agents of choice: doxycycline plus rifampin (Rifadin)	Agents of choice: doxycycline plus rifampin Alternative: ofloxacin (Floxin) plus rifampin
Francisella tularensis (tularemia)	Agent of choice: doxycycline Alternatives: tetracycline, ciprofloxacin	Agent of choice: streptomycin Alternatives: gentamicin, ciprofloxacin

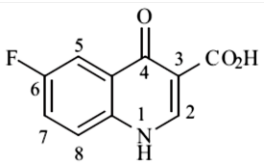
**—Levofloxacin (Levaquin) and ofloxacin are alternatives for postexposure prophylaxis in mass casualty settings.*

Adapted from Kortepeter M, et al., eds. USAMRIID's Medical management of biological casualties handbook. 4th ed. Frederick, Md.: U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, 2001. Retrieved November 2001 from http://www.usamriid.army.mil//education/bluebook.html.

(Oliphant & Green, 2002)

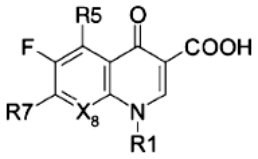
Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Toxicity

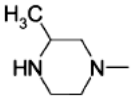
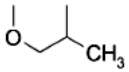
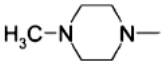
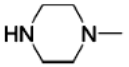
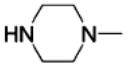
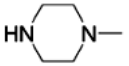
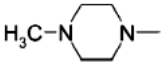
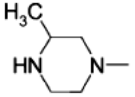
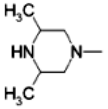
- Phototoxicity of fluoroquinolones with halogen substituents at 8-position



Fluoroquinolone

Structure–phototoxicity relationships in female Balb/c mice receiving a single intravenous administration of quinolones, and followed by UVA irradiation for 4 h



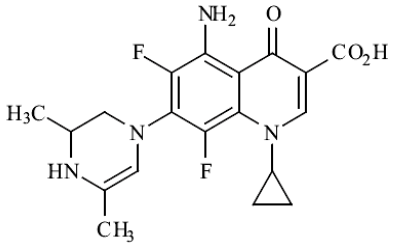
Drug	X8	R1	R5	R7	Phototoxicity
Gatifloxacin	$COCH_3$		H		–
Ofloxacin					–
Ciprofloxacin	CH		H		+
Norfloxacin	CH	C_2H_5	H		+
Enoxacin	N	C_2H_5	H		++
Fleroxacin	CF	CH_2CH_2F	H		++
Lomefloxacin	CF	C_2H_5	H		+++
Sparfloxacin	CF		NH_2		+++

Phototoxic potential was assessed by the results of the thickness and histopathological findings of the auricle at 96 h post-dose. The auricular thickness of lomefloxacin and sparfloxacin was estimated at 48 h post-dose because the auricles showed focal loss and could not be measured after this timepoint. (–) none, (+) mild, (++) moderate, (+++) severe.

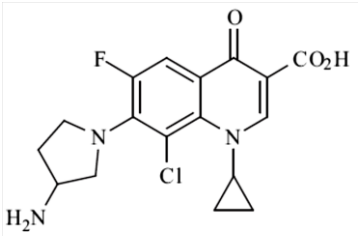
(Yabe et al., *Tox. Lett.* 2005, 157, 203)

Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Toxicity

- Phototoxicity of fluoroquinolones with halogen substituents at 8-position
  - Sparfloxacin (Zagam, Mylan/Aventis); approved 1996; withdrawn 1998 due to phototoxicity
  - Clinafloxacin (Warner Lambert/Pfizer); stopped clinical development due to phototoxicity

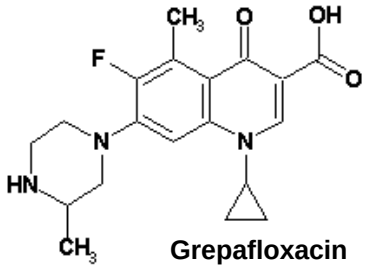


Sparfloxacin

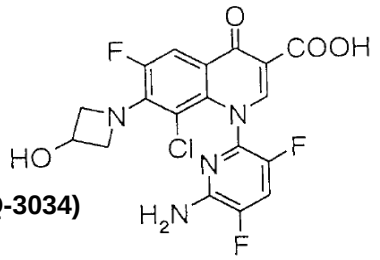


CI-960 (Clinafloxacin)

- Animal studies show joint & cartilage damage in weight-bearing joints of young animals (dogs; effect animal- & dose-dependent)
  - All fluoroquinolones have shown this toxicity
  - Mechanism unclear
  - Fluoroquinolones not approved for use in children (except in CF)
  - Compassionate use cases suggest that this toxicity is very rare in children
- QT interval prolongation (hERG) observed with some fluoroquinolones
  - Grepafloxacin (Raxar, Glaxo); approved 1997; voluntarily withdrawn 1999



Grepafloxacin

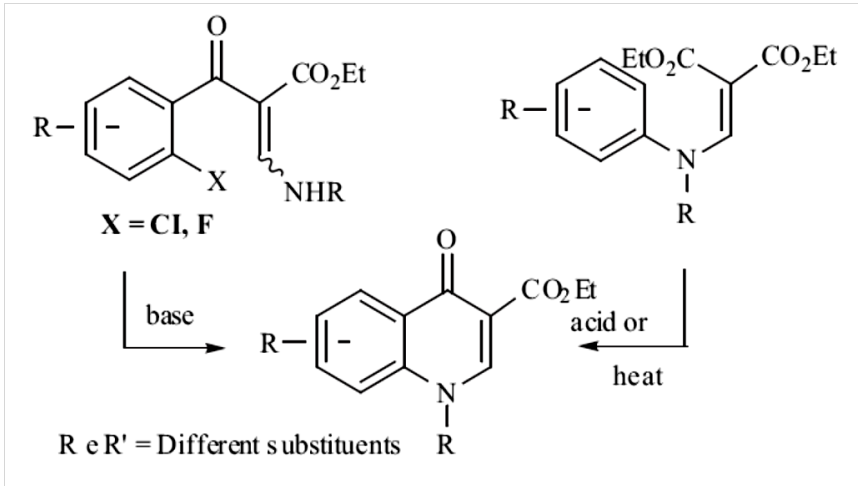


ABT-492 (WQ-3034)

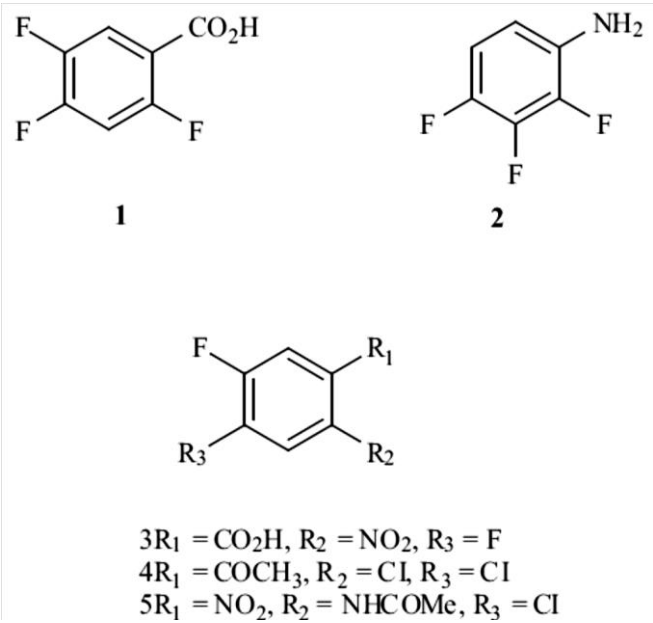
Bacterial DNA Synthesis: Gyrase and Topo IV: Fluoroquinolone Synthesis

Bayer AG Route:
base-induced ring closure of 2-(2-halobenzoyl-3-aminoacrylates)

Gould-Jacobs Route:
acid-induced ring closure of anilinomethylene malonates



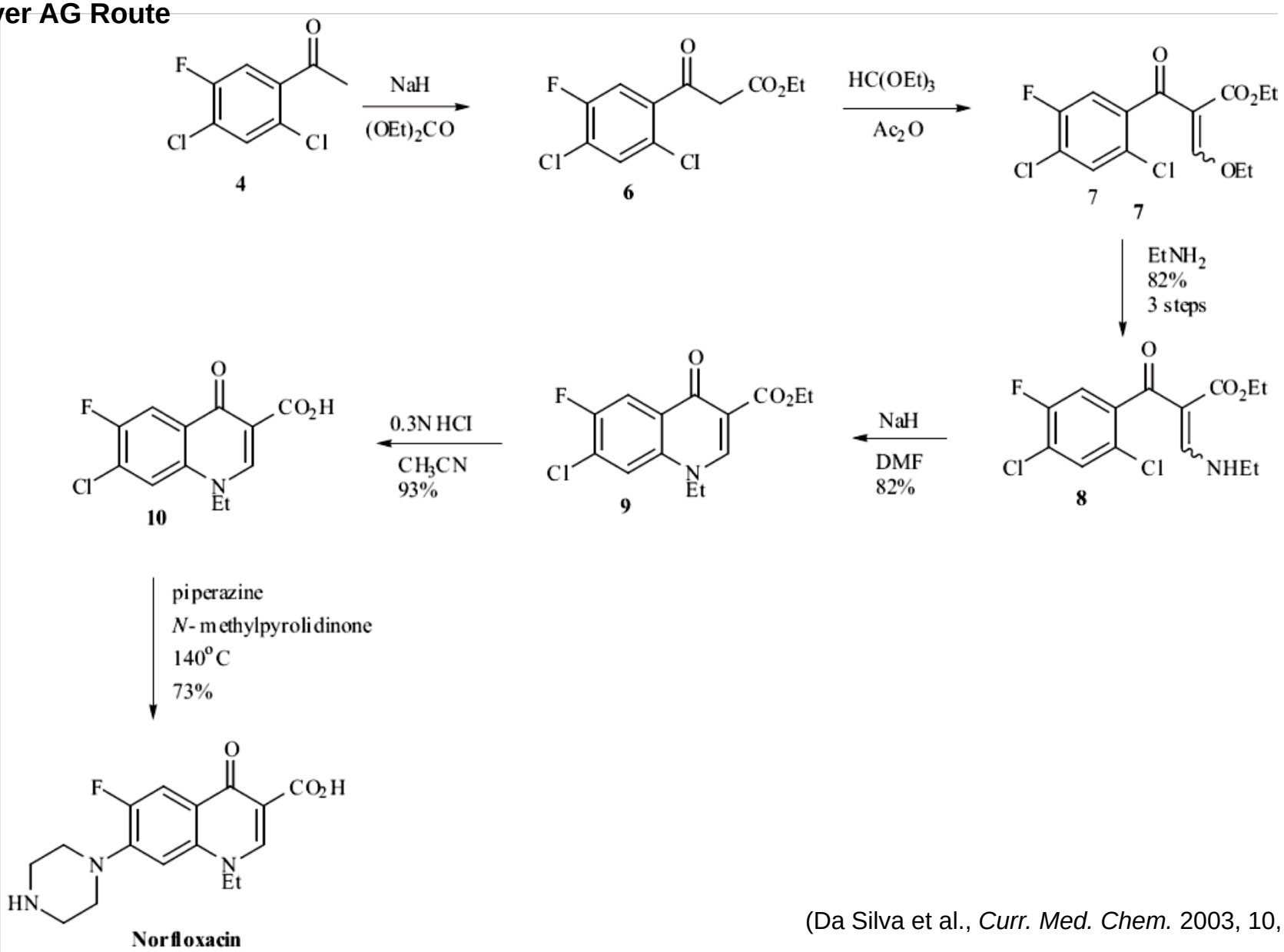
Typical Starting Materials:



(Da Silva et al., *Curr. Med. Chem.* 2003, 10, 21)

Bacterial DNA Synthesis: Gyrase and Topo IV: Norfloxacin Synthesis

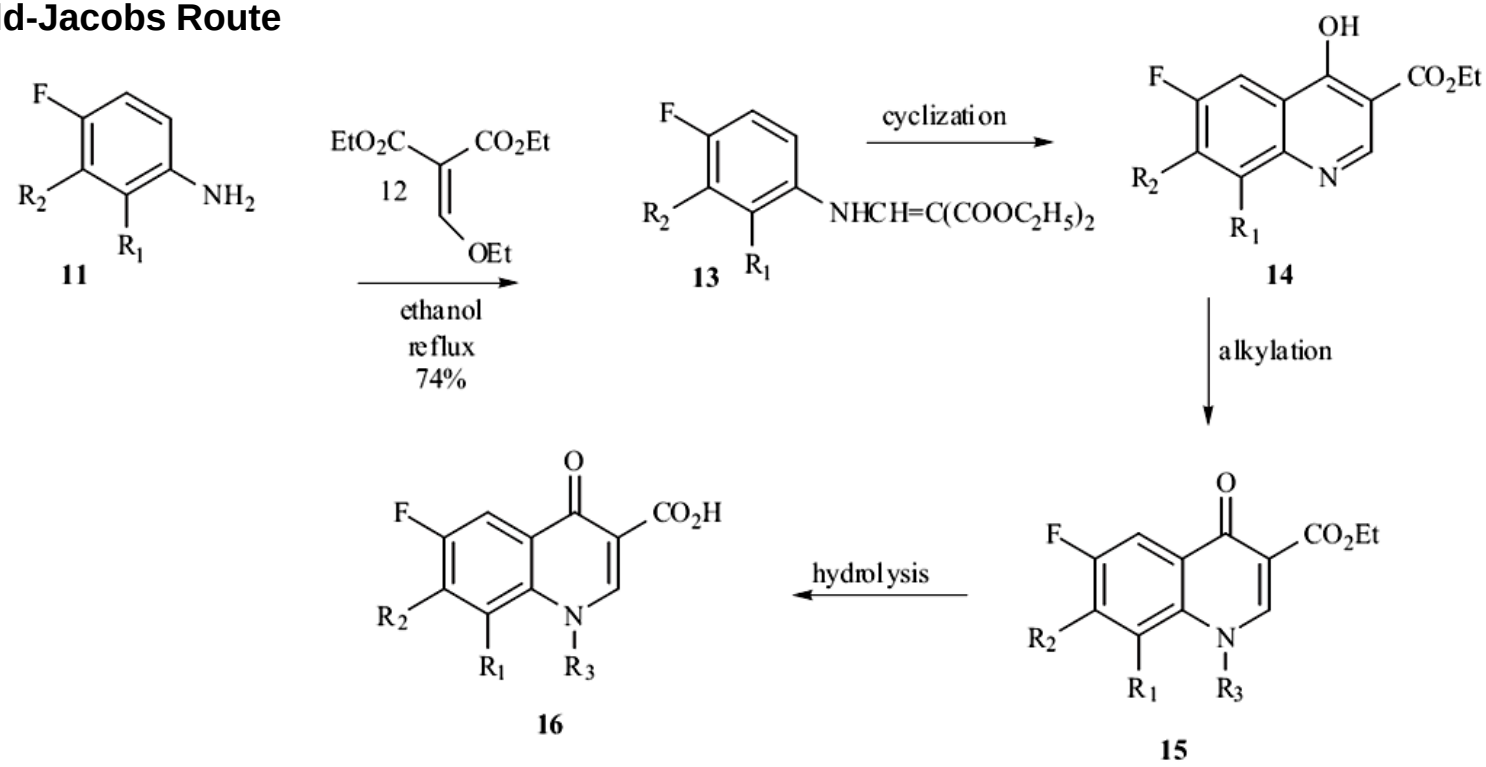
Bayer AG Route



(Da Silva et al., *Curr. Med. Chem.* 2003, 10, 21)

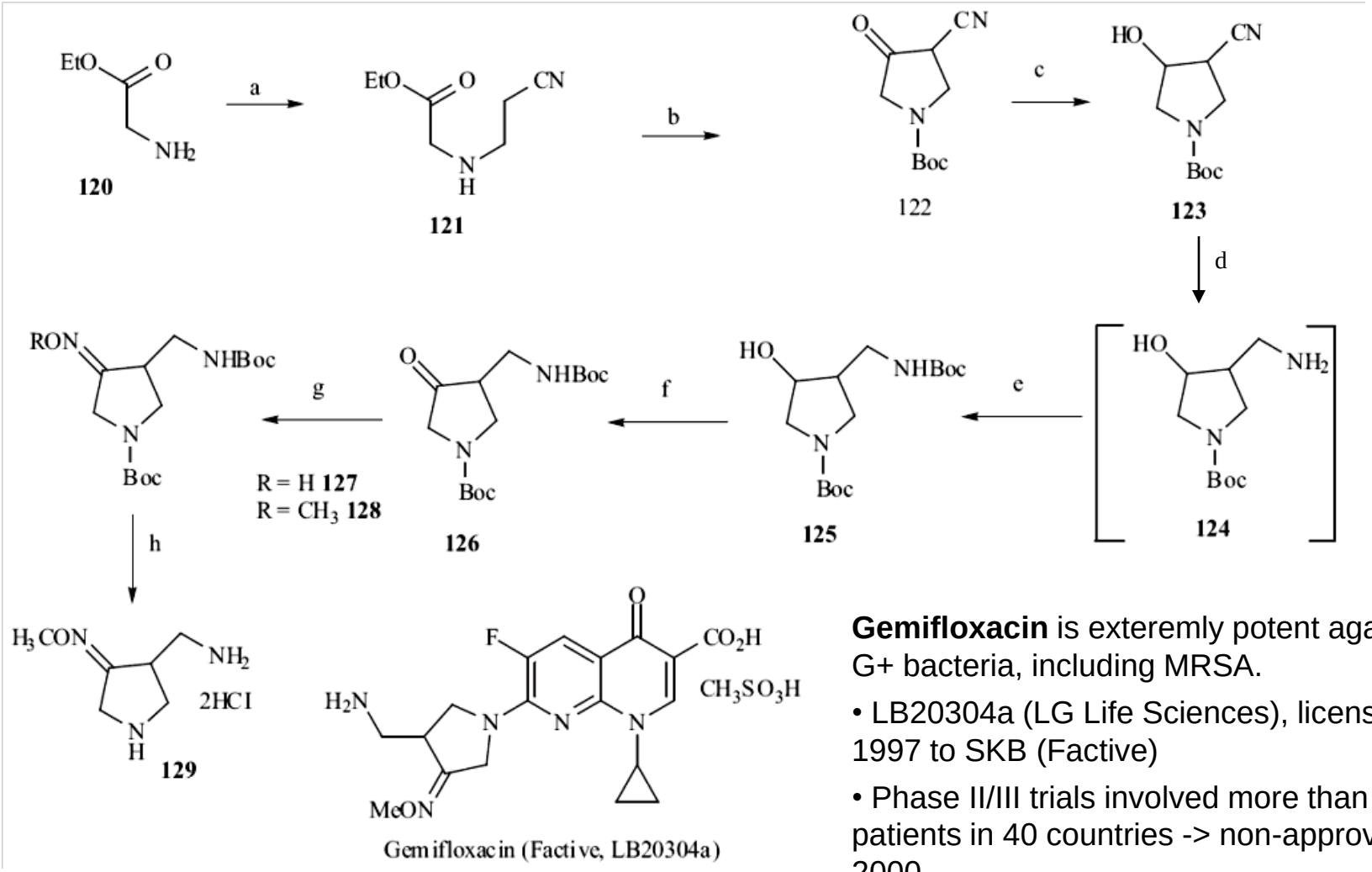
Bacterial DNA Synthesis: Gyrase and Topo IV: Quinolone Synthesis

Gould-Jacobs Route



(Da Silva et al., *Curr. Med. Chem.* 2003, 10, 21)

Bacterial DNA Synthesis: Gyrase and Topo IV: Gemifloxacin Synthesis



(a) CH_2CHCN, NaOH, 60°C; (b) $(t\text{-}BOC)_2O$, $CHCl_3$, then NaOEt, EtOH, reflux; (c) $NaBH_4$, EtOH, 0°C; (d) LAH, THF, -5°C; (e) $(t\text{-}BOC)_2O$, $NaHCO_3$, dioxane-H_2O; (f) pyridine-SO_3.Et_3N, DMSO, 5°C; (g) $RONH_2$.HCl, $NaHCO_3$, EtOH-THF; (h) Acetyl chloride, MeOH, 0°C.

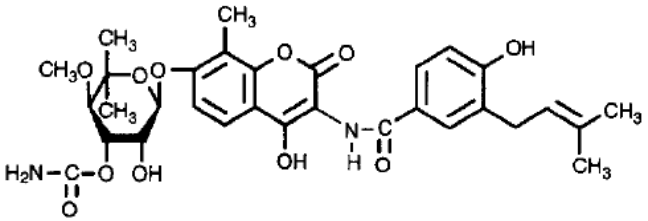
Gemifloxacin is exteremly potent against G+ bacteria, including MRSA.

- LB20304a (LG Life Sciences), licensed 1997 to SKB (Factive)
- Phase II/III trials involved more than 8000 patients in 40 countries -> non-approval 2000
- licensed 2000 to Oscient; approval 2003 (warning label required about QT interval prolongation etc.)

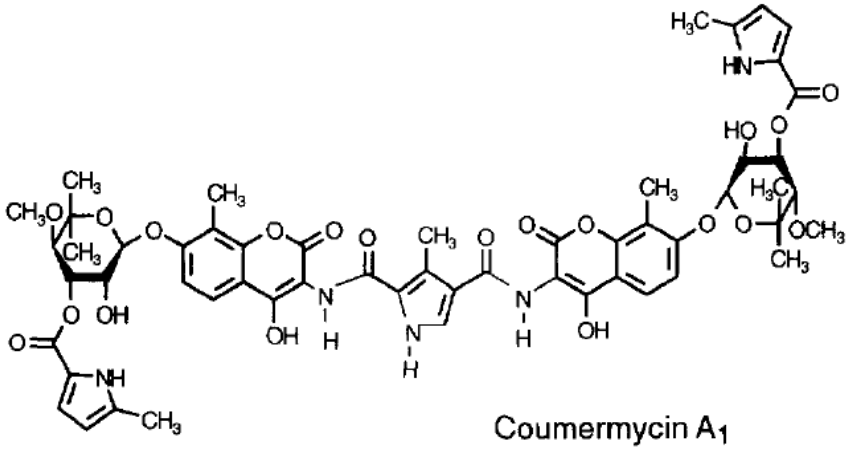
(Da Silva et al., *Curr. Med. Chem.* 2003, 10, 21)

Bacterial DNA Synthesis: Gyrase and Topo IV: Coumarine Inhibitors

Coumarins



Novobiocin

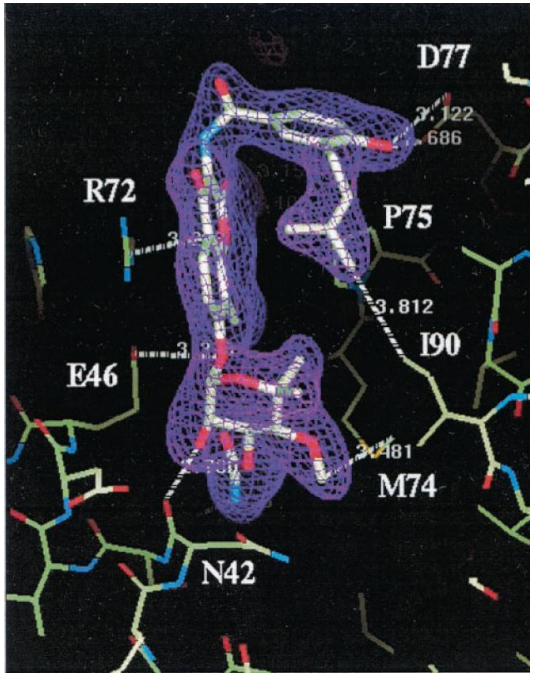


Coumermycin A_1

Coumarin antibiotics (from *Streptomyces*) have been discovered in the 1950s

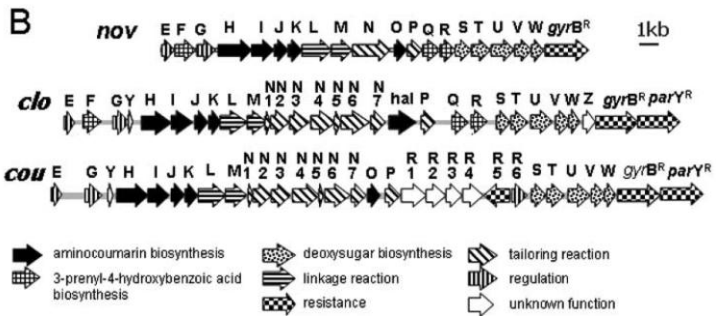
- inhibit gyrase/topo IV by competitive binding with ATP to GyrB/ParE subunit
- coumarin binding site overlaps partially with ATP binding site
- novobiocin binds to GyrB monomer
- coumermycin (resembling a novobiocin dimer) stabilizes GyrB dimer ("nocovalent crosslinker")
- low activity against Gram- resulting from poor permeability; toxicity in eukaryotes; low water solubility
- currently not used as drugs

Novobiocin bound to ParE

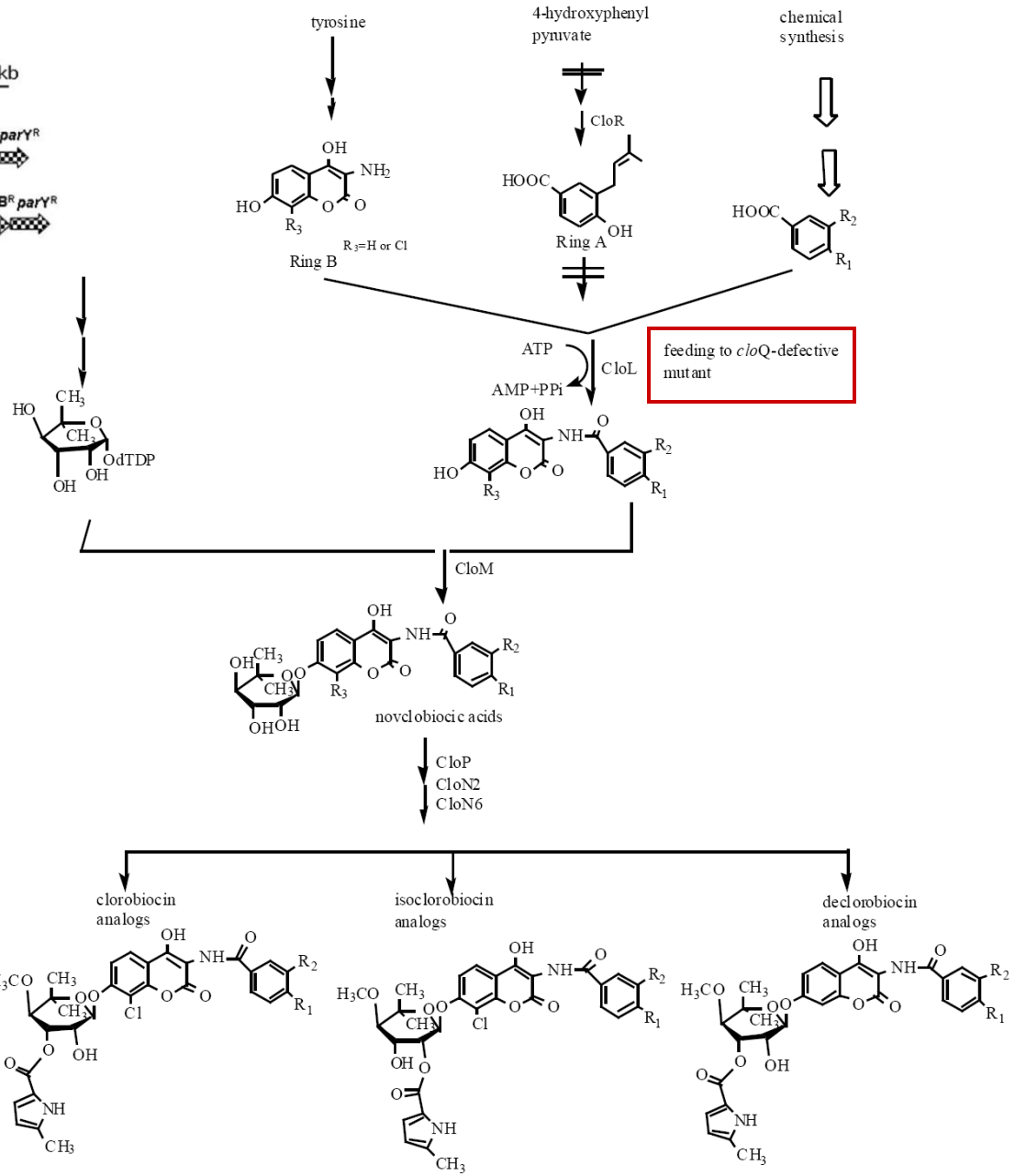


(Bellon et al., *AAC* 2004, 48, 1856)

Bacterial DNA Synthesis: Gyrase and Topo IV: Coumarine Mutasynthesis



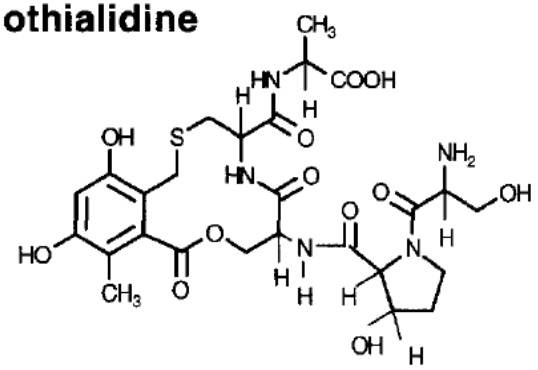
Biosynthetic gene clusters of novobiocin (top), clorobiocin (middle), and coumermycins A1 (bottom)

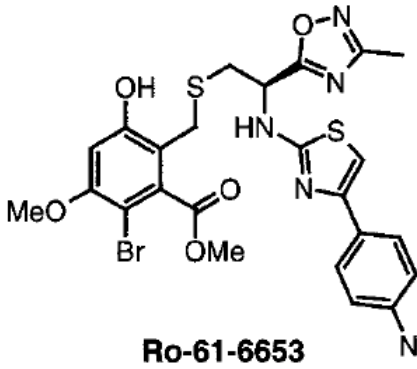


(Li & Heide, *Curr. Med. Chem.* 2005, 12, 419)

Bacterial DNA Synthesis: Gyrase and Topo IV: Cyclothialidine

Cyclothialidine





Ro-61-6653

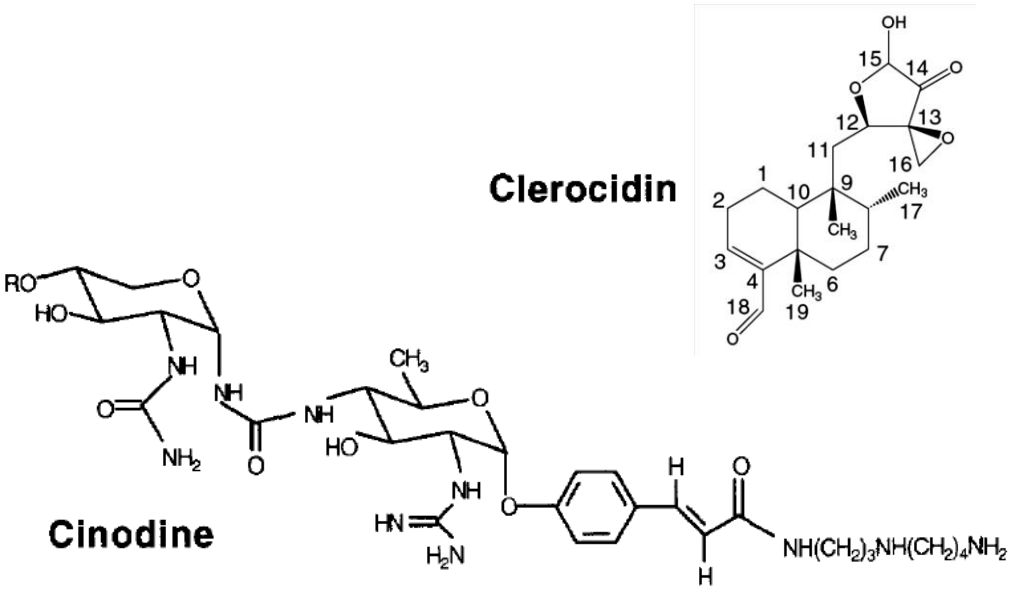
	MIC (μg/mL)
S. aureus 25923	0.12
S. aureus QR-54	0.25
S. epidermidis	0.12
S. pyogenes	0.12
E. faecalis	0.25

Cyclothialidine (from *Streptomyces*)

- inhibits gyrase/topo IV by competitive binding with ATP to GyrB/ParE subunit
- binding site overlaps partially with ATP and coumarin binding sites
- coumarin-resistant mutants are susceptible to cyclothialidine
- despite high activity at the target, low antibacterial activity due to poor membrane penetration
- lactone ring is not required for target activity
- open-chain analogues (*seco*-cyclothialidines) permeate membranes well

(Rudolph et al., *J. Med. Chem.* 2001, 44, 619)

Bacterial DNA Synthesis: Gyrase and Topo IV: Clerocidin & Cinodine



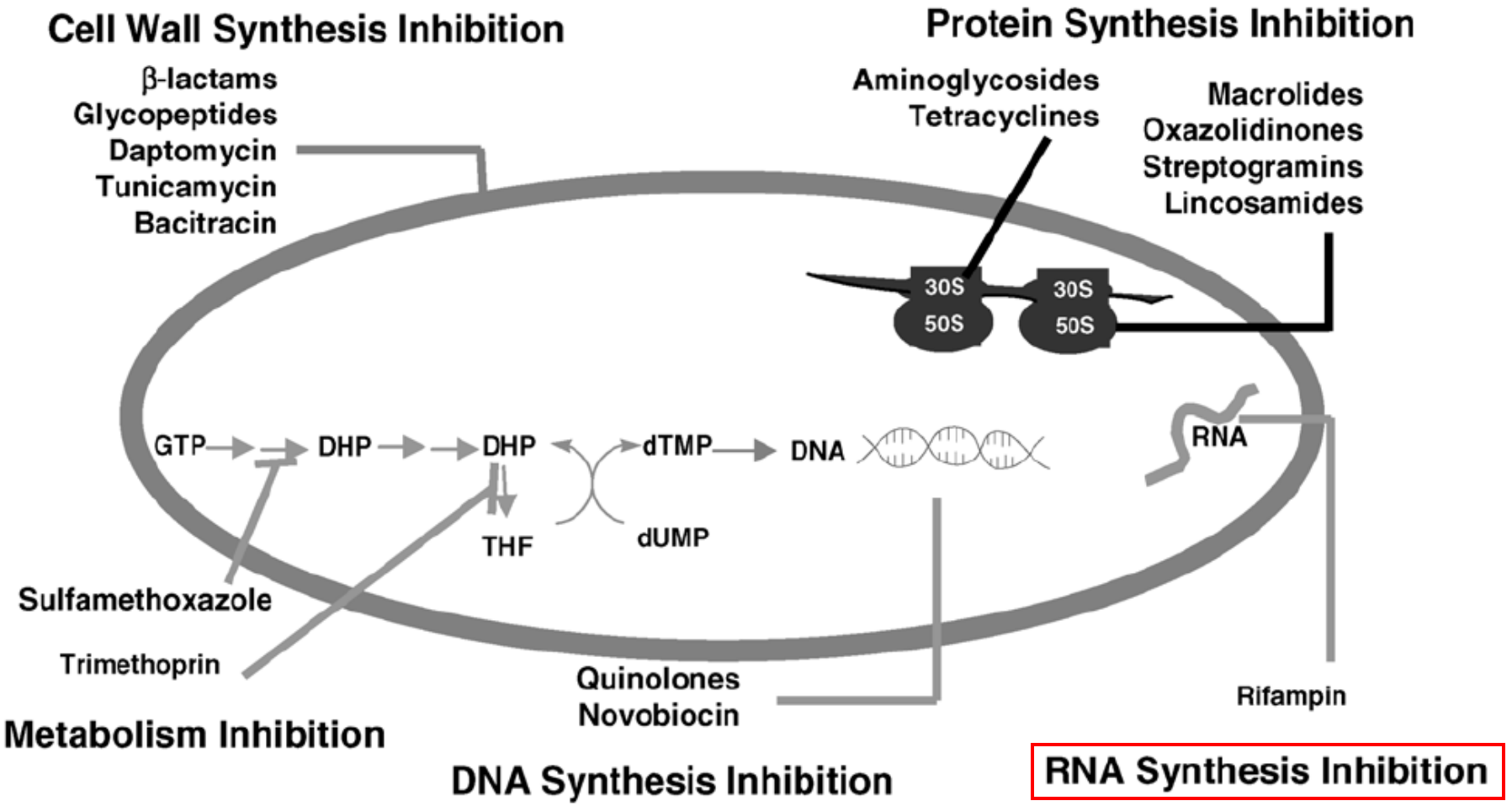
Clerocidin (from *Fusidium viridae*)

- diterpenoid
- alkylates single-stranded DNA via epoxide functionality
- alkylation requires ssDNA in complexes of gyrase/topo IV (i.e. post-cleavage complex <-> quinolones do not require ssDNA)
- resistant mutations suggest GyrA/ParC as a target
- cytotoxic due to action on human topo II

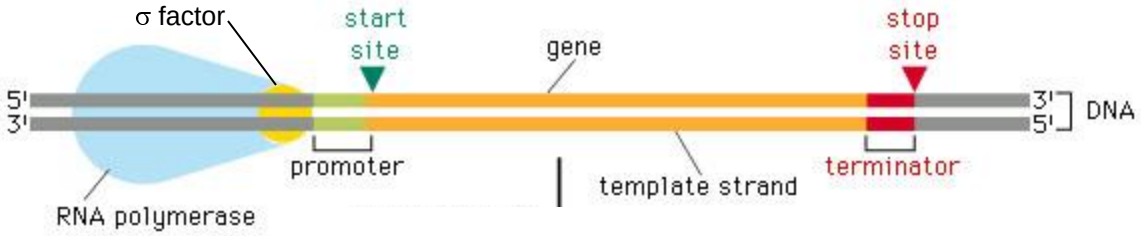
Cinodine (from *Nocardia spp.*)

- glycocinnamoylspermidine
- binds to DNA
- inhibits gyrase in vitro

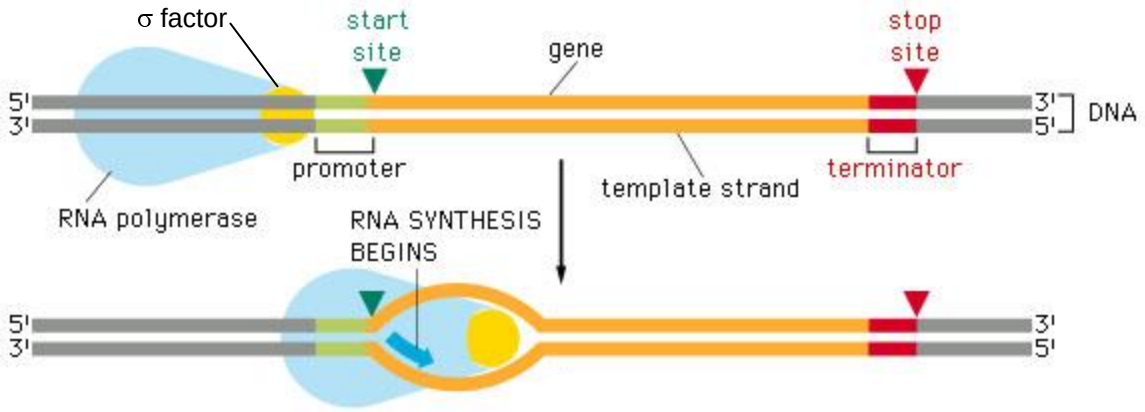
Antibacterial Targets: Overview



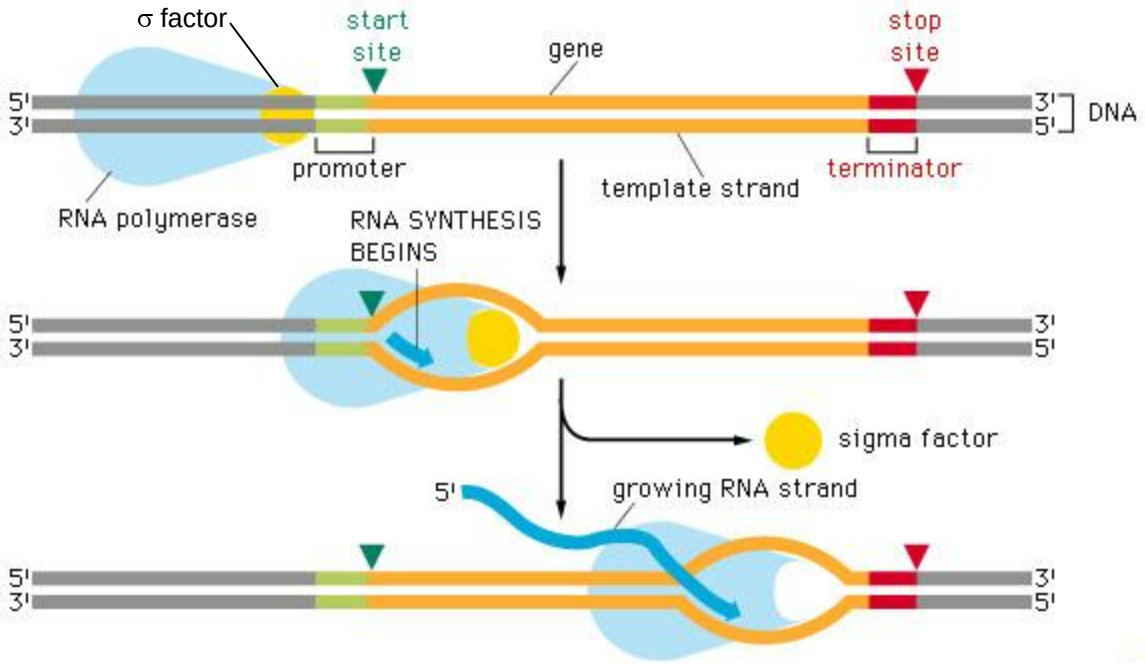
Bacterial RNA Synthesis (Transcription)



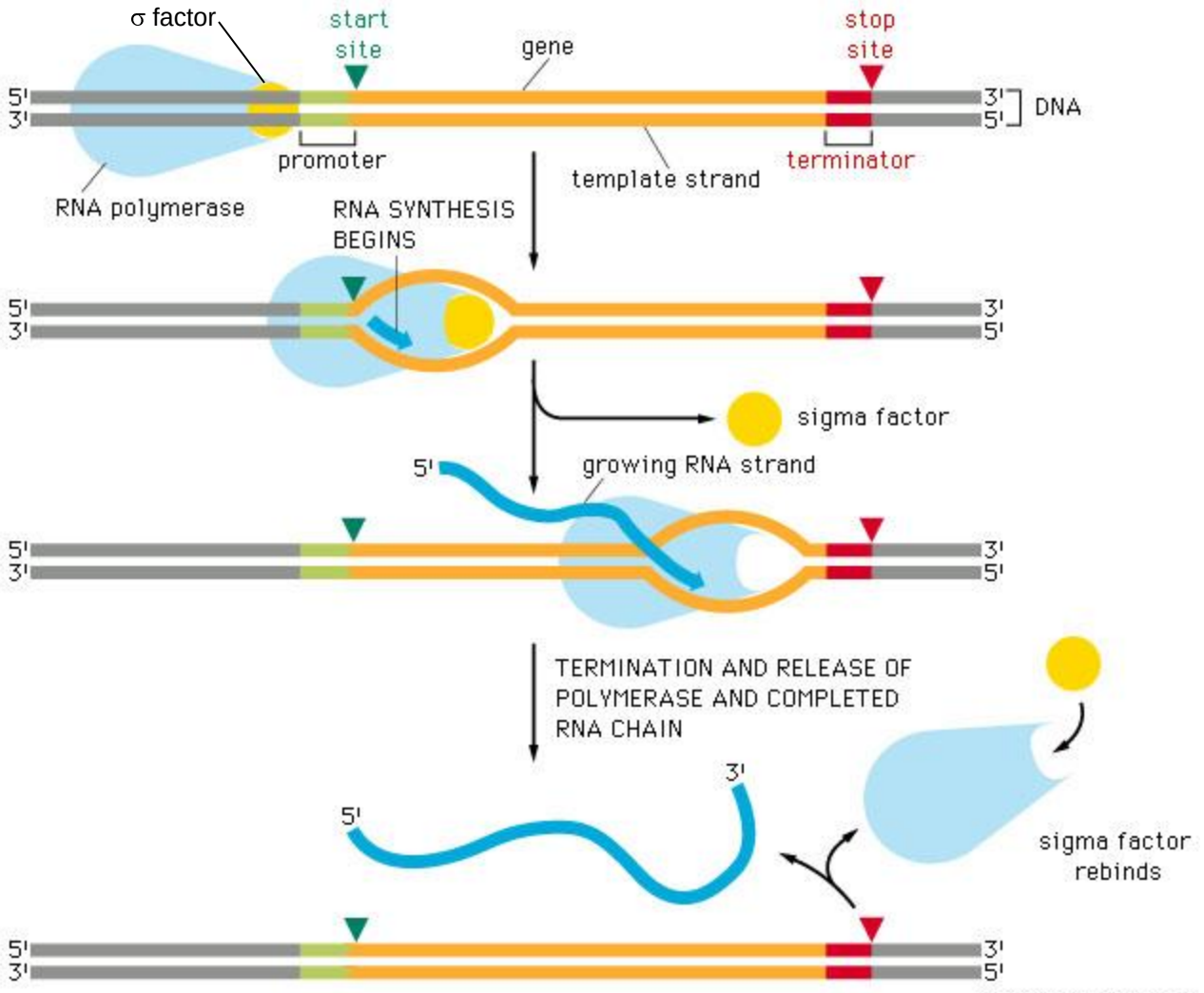
Bacterial RNA Synthesis (Transcription): Initiation



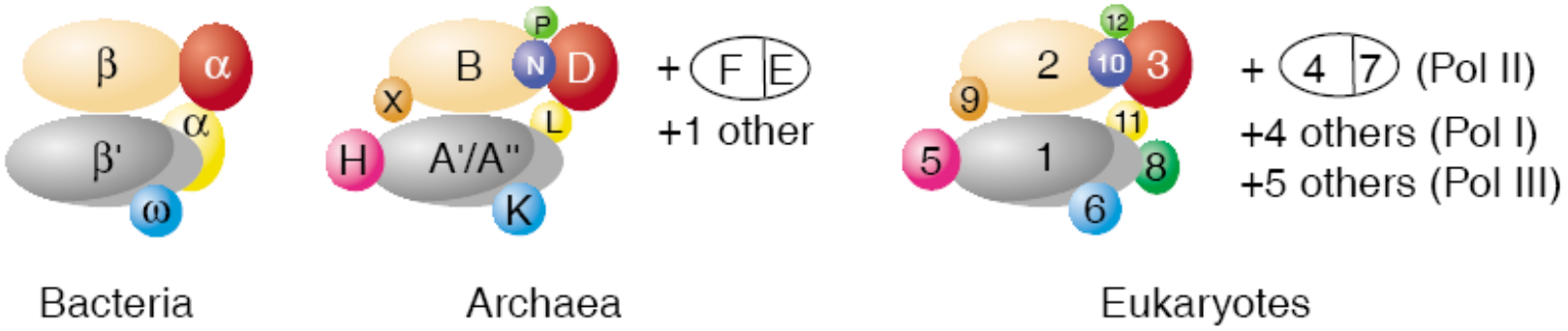
Bacterial RNA Synthesis (Transcription): Elongation



Bacterial RNA Synthesis (Transcription): Termination



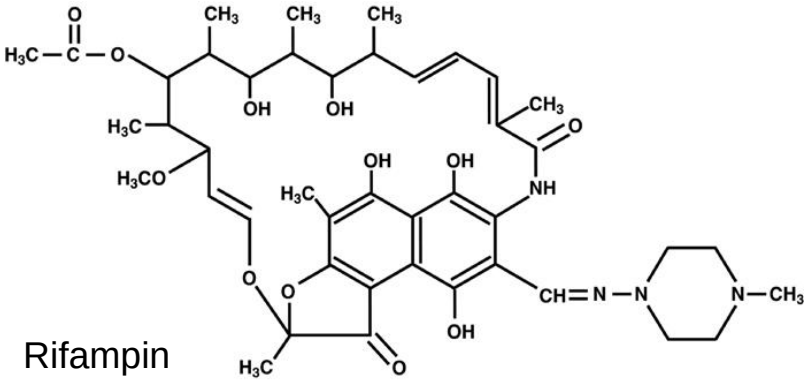
Bacterial Transcription: DNA-Dependent RNA Polymerase



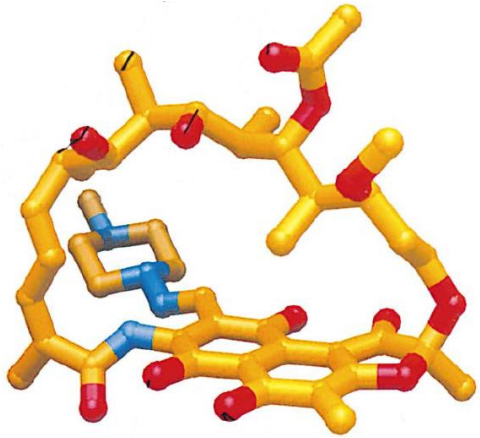
RNAP subunits.

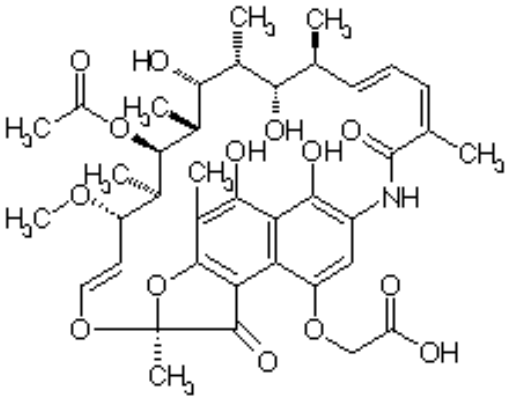
Eukaryotes Pol I	Pol II	Pol III	Archaea	Bacteria
A190	Rpb1	C160	A′+A″	β′
A135	Rpb2	C128	B (B′+B″)	β
AC40	Rpb3	AC40	D	α
AC19	Rpb11	AC19	L	α
Rpb6	Rpb6	Rpb6	K	ω
Rpb5	Rpb5	Rpb5	H	-
Rpb8	Rpb8	Rpb8	-	-
Rpb10	Rpb10	Rpb10	N	-
Rpb12	Rpb12	Rpb12	P	-
A12.2	Rpb9	C11	X	-
A14†	Rpb4‡	-	F	-
A43†	Rpb7‡	C25	E	-
+two others	-	+four others	+one other	-

Bacterial Transcription: RNA Pol Inhibitors - Rifamycins



Rifampin





Rifamycin B

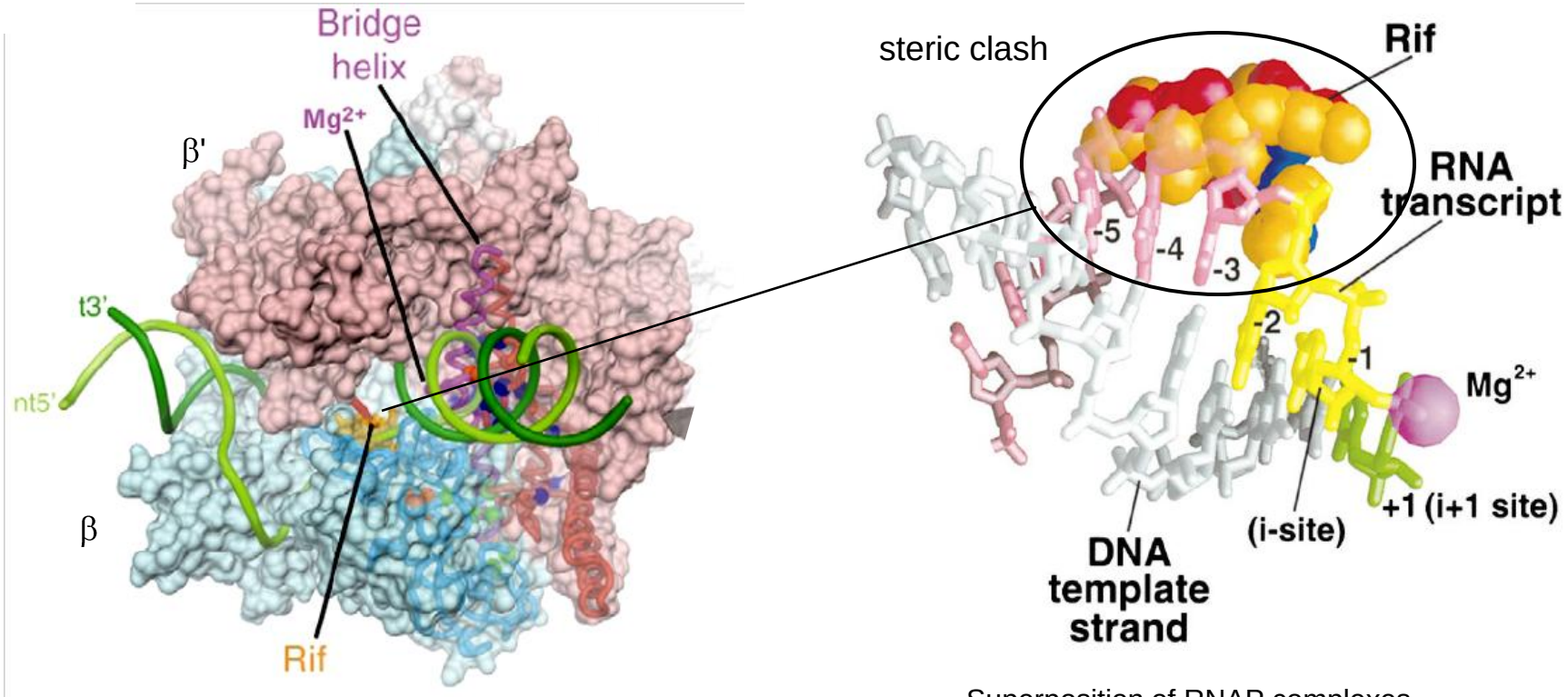
Rifampin

- ansamycin class antibiotic
- semisynthetic rifamycin derivative
- isolated 1957 from *Nocardia mediterranei*

- inhibits selectively bacterial RNA polymerase by binding to the β subunit, >12Å away from the active site
- binding site is highly conserved among bacteria but not in eukaryotic RNAPs
- blocks the exit path of elongating RNA when transcript is 2-3 nucleotides in length

- bacteriostatic
- active against G+/-, however MICs for G- are higher because of reduced outer membrane penetration
- primarily used against *Mycobacteria* (tuberculosis, leprosy) and *Meningococci*
- resistance develops easily by RNAP mutations that reduce target affinity
- often used in combination with other bactericidal antibiotics

Bacterial Transcription: RNA Pol Inhibitors - Rifamycins

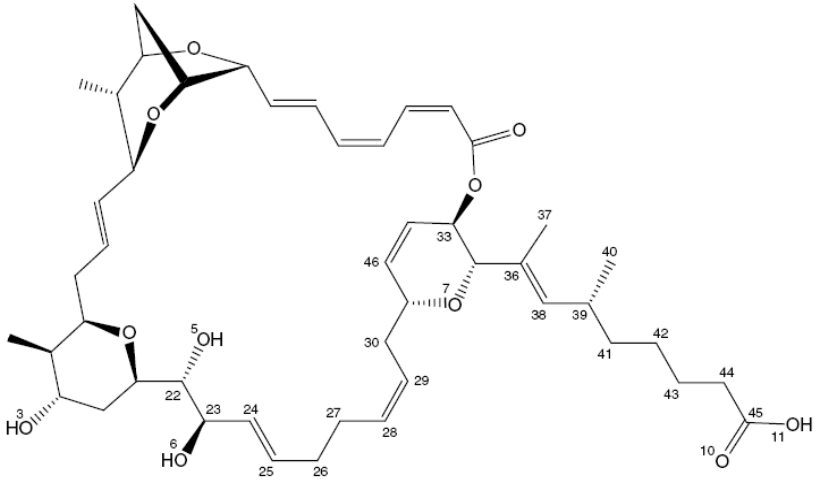


Superposition of RNAP complexes containing bound mRNA or rifampin.

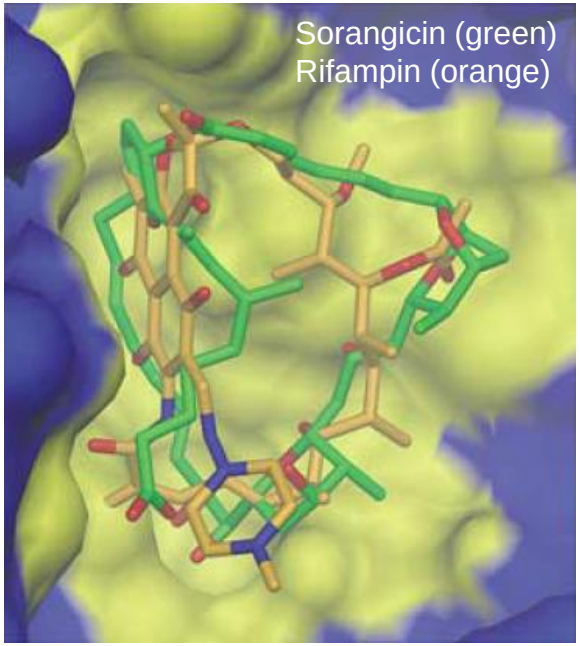
Rifampin blocks the exit pathway of mRNA such that RNAP is able to initiate RNA synthesis, but is unable to elongate mRNA beyond a length of 2-3 nucleotides.

(Darst, *TIBS* 2004, 29, 159; Campbell et al., *Cell* 2001, 104, 901)

Bacterial Transcription: RNA Pol Inhibitors - Sorangicin



Sorangicin A

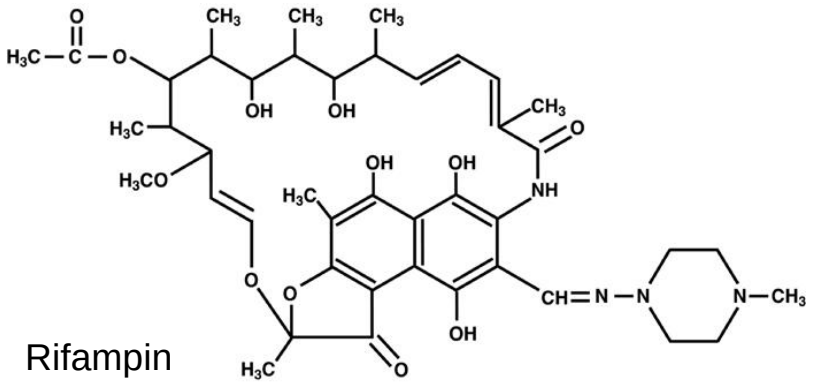


(Campbell et al., *EMBO J.* 2005, 24, 674)

Sorangicin A

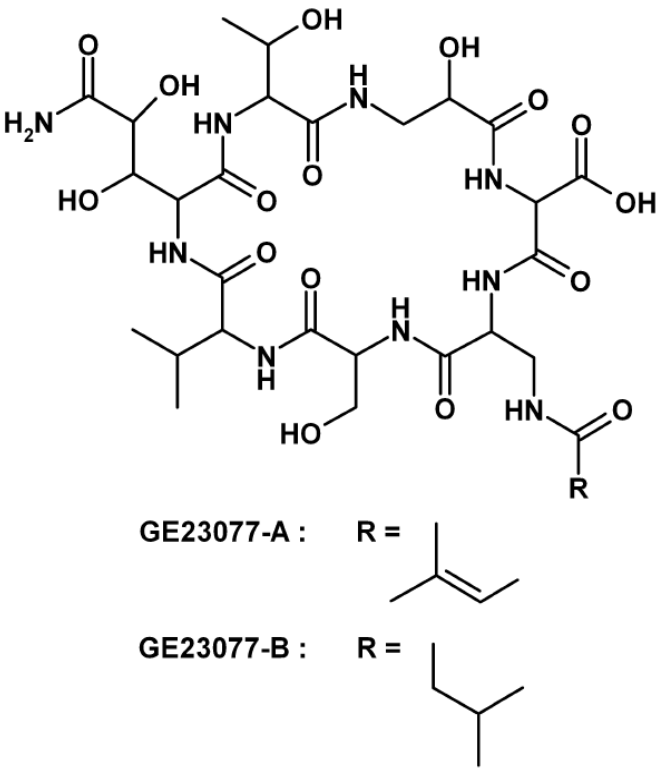
- macrolide polyether antibiotic
- isolated 1985 from *Sorangium cellulosum* (myxobacterium)

- inhibits selectively bacterial RNAP
- inhibition mechanism is identical to rifampin
- exactly occupies the rifampin binding site
- all residues that interact with rifampin also interact with sorangicin
- overall shape of the two antibiotics is very similar despite the lack of chemical similarity



Rifampin

Bacterial Transcription: RNA Pol Inhibitors – GE23077



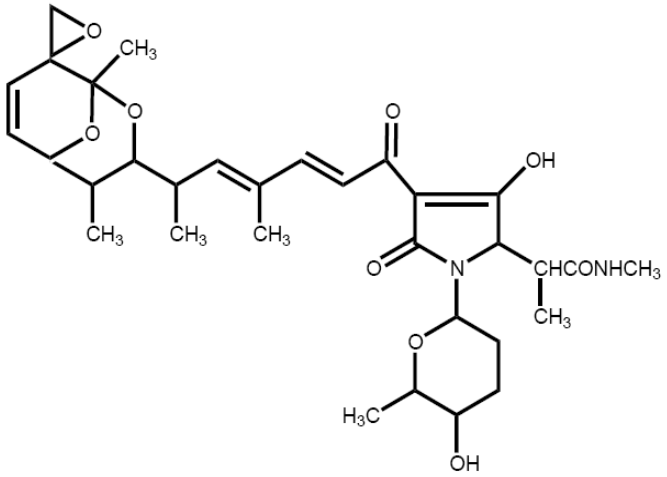
GE23077

- macrolide polyether antibiotic
- isolated 2004 from *Actinomadura spp.* (soil bacterium)

- inhibits selectively bacterial RNAP
- no cross-resistance with rifampin mutants
- good activity on RNAP in vitro but weak antibacterial activity, likely due to poor membrane permeation

(Sarubbi et al., *Eur. J. Biochem.* 2004, 271, 3146)

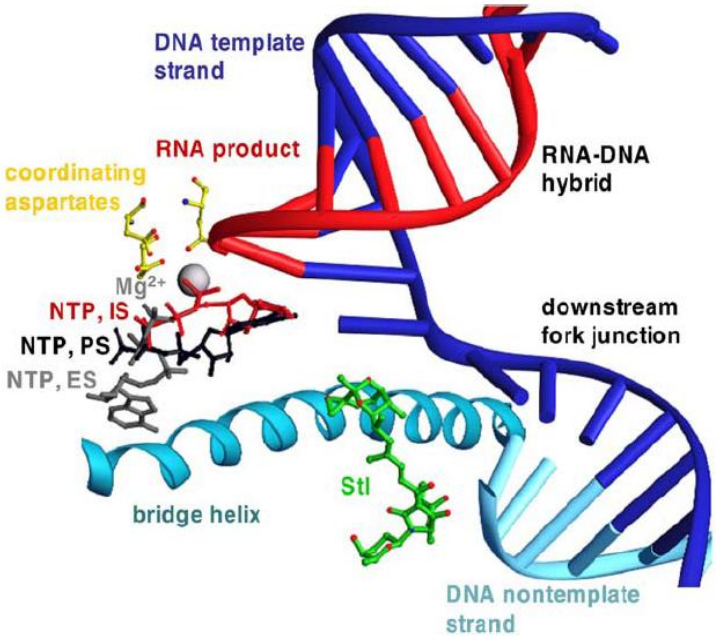
Bacterial Transcription: RNA Pol Inhibitors – Streptolydigin

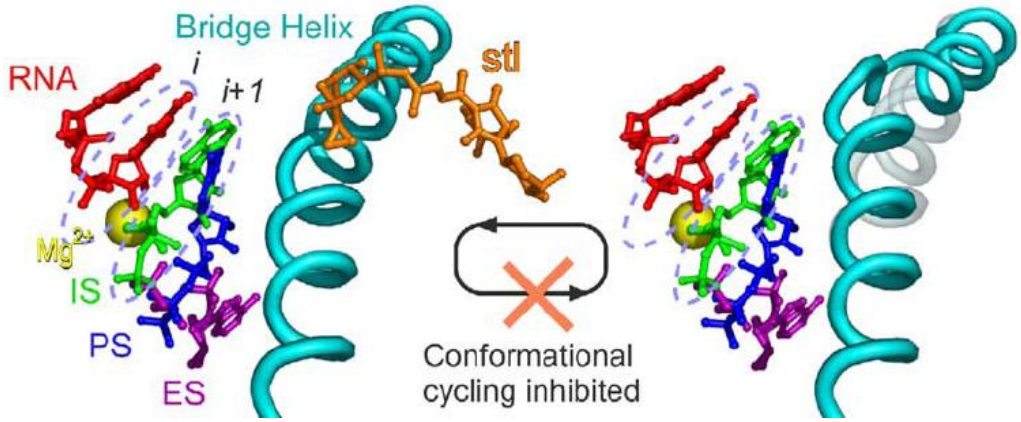


Streptolydigin

- acyl-tetramic acid antibiotic
- isolated 1955 from *Streptomyces lydigus*

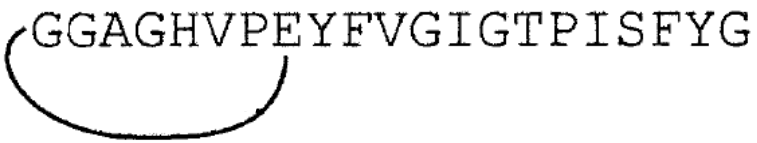
- inhibits selectively bacterial RNAP, however, with relatively low potency compared to rifampin
- binding site partially overlaps with rifampin binding site, but functions via an entirely different mechanism
- inhibits RNAP catalytic activity by preventing a reversible conformational change in an α-helix during the nucleotide addition cycle (related to the action of α-amanitin on eukaryotic RNAP II)

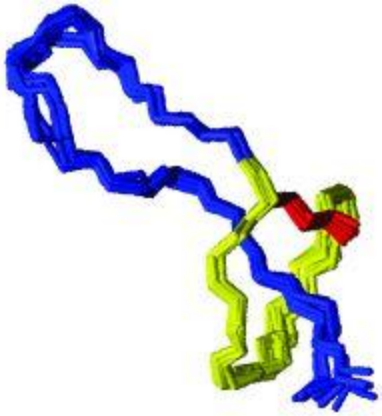


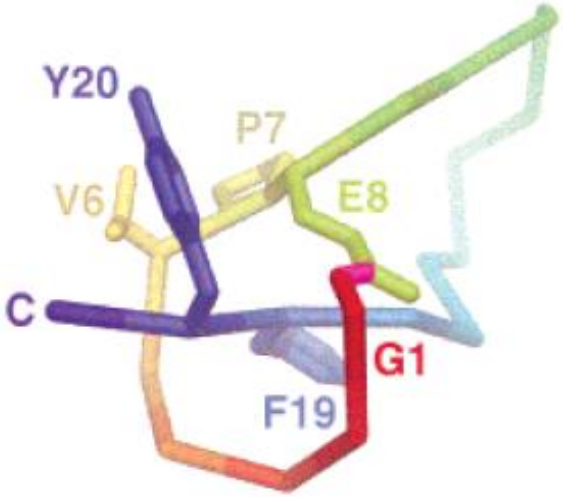


(Temiakov et al., *Mol. Cell.* 2005, 271, 3146)

Bacterial Transcription: RNA Pol Inhibitors – Microcin J25







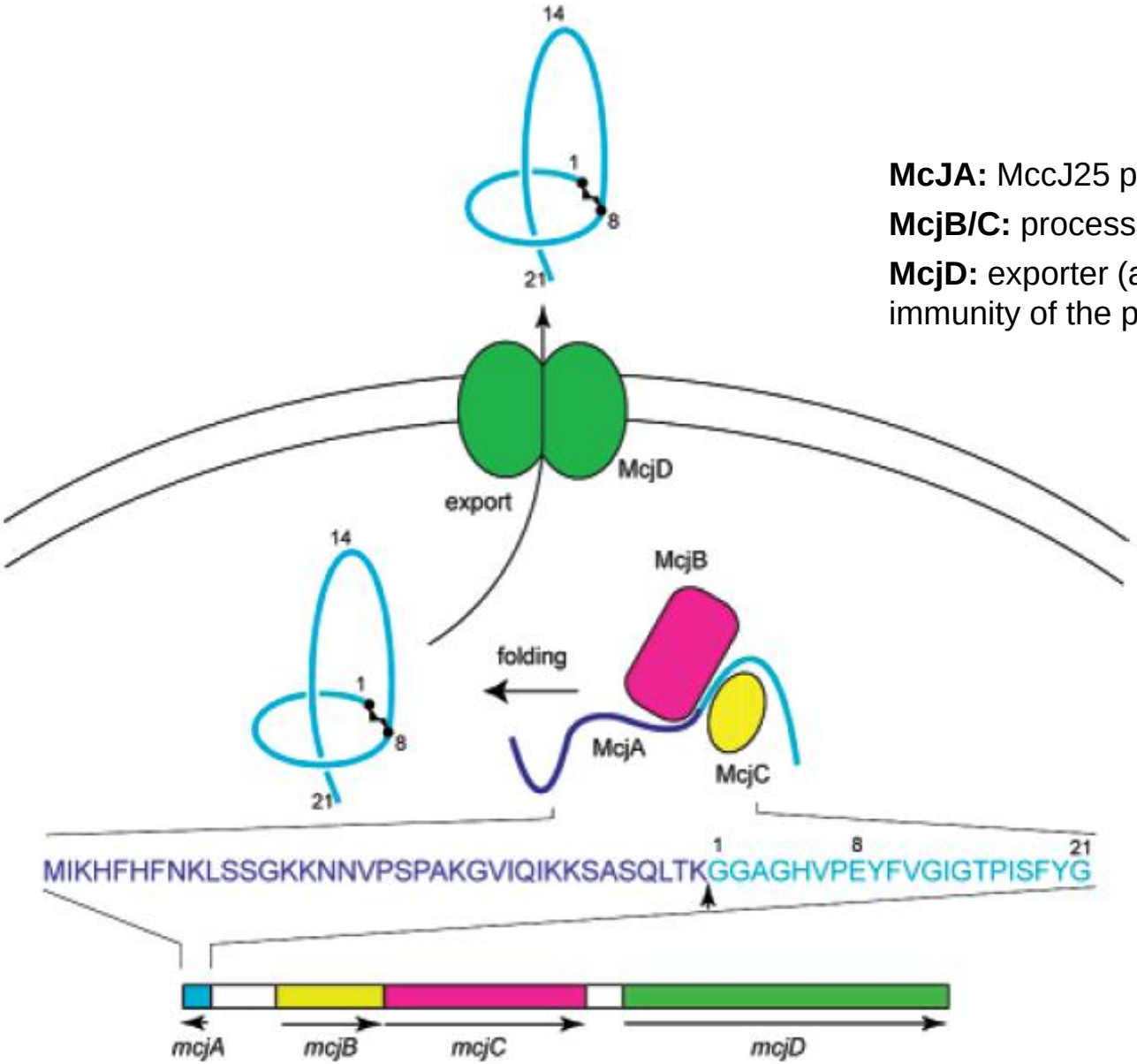
Microcin J25 (MccJ25)

- ribosomally synthesized cyclic peptide
- isolated from *E. coli* that contain a plasmid-located synthesis, maturation and export system

- inhibits bacterial RNAP
- resistance mutations overlap with those conferring resistance to streptolydigin
- acts through physical obstruction of the NTP-uptake channel in RNAP ("cork in a bottle"; Mukhopadhyay et al., *Cell* 2004, 14, 739)
- adopts an extraordinary lasso fold, with a cyclic segment and a 13-resiude linear tail that loops back and threads through the 8-residue ring
- two aromatic residues (F19 and Y20) lock the tail in a noncovalent rigid lasso conformation

(Wilson et al., *JACS* 2003, 125, 12475)

Bacterial Transcription: RNA Pol Inhibitors – Microcin J25 Biosynthesis

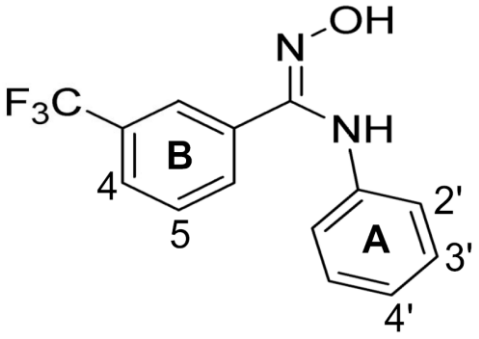


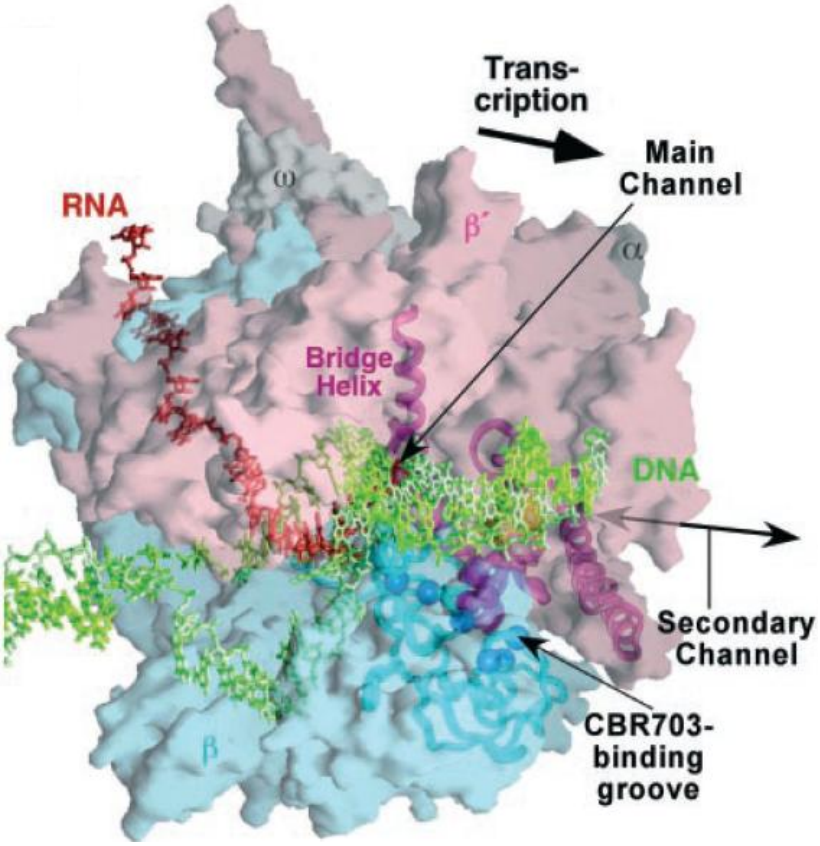
McJA: MccJ25 precursor peptide

McjB/C: processing/folding enzymes

McjD: exporter (also involved in self-immunity of the producing strain)

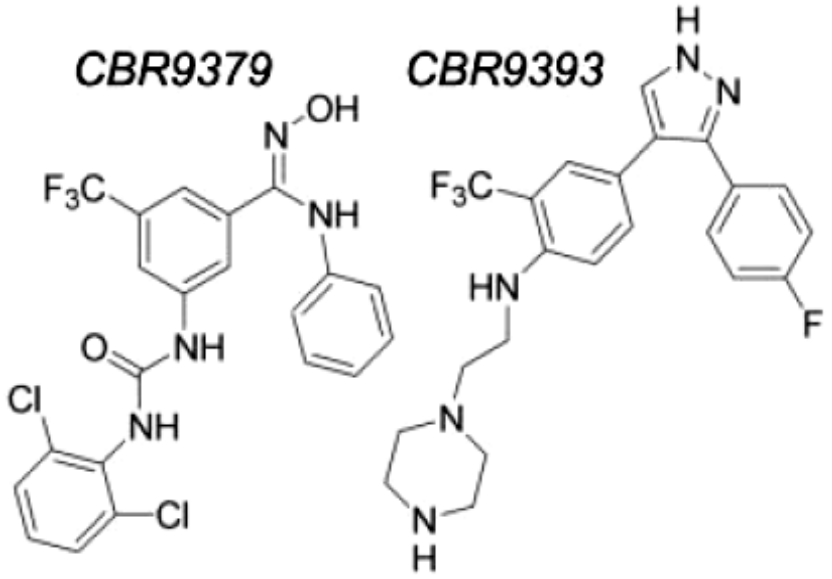
Bacterial Transcription: RNA Pol Inhibitors – N-Hydroxy-benzamidines





CBR703

- discovered in high-throughput screen of ~300,000 synthetic compounds for inhibitors of RNAP transcription
- activity at target but no effect on growth of wildtype *E.coli*
- inhibits growth of efflux-impaired tolC *E.coli* strain
- resistance mutations suggest binding to β subunit of RNAP, in proximity of "bridge helix"
- might act similarly as streptolydigin



(Artsimovitch et al., *Science* 2003, 302, 650)