Chemistry 259

Medicinal Chemistry of Modern Antibiotics

Spring 2012

Lecture 6: *Antibiotics Classes & Targets*

Part I: Drugs Targeting Bacterial Cell Wall & Membrane

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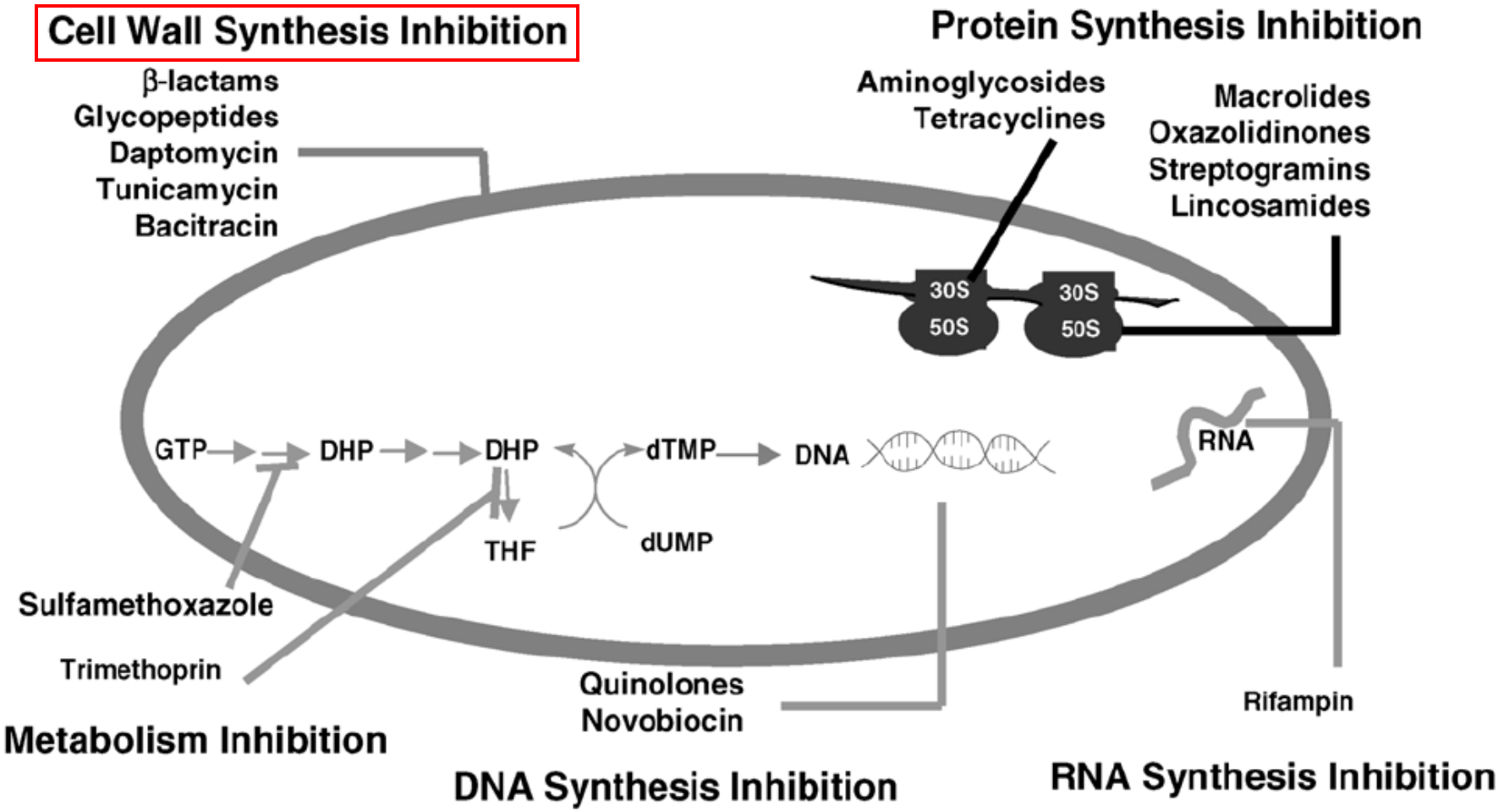
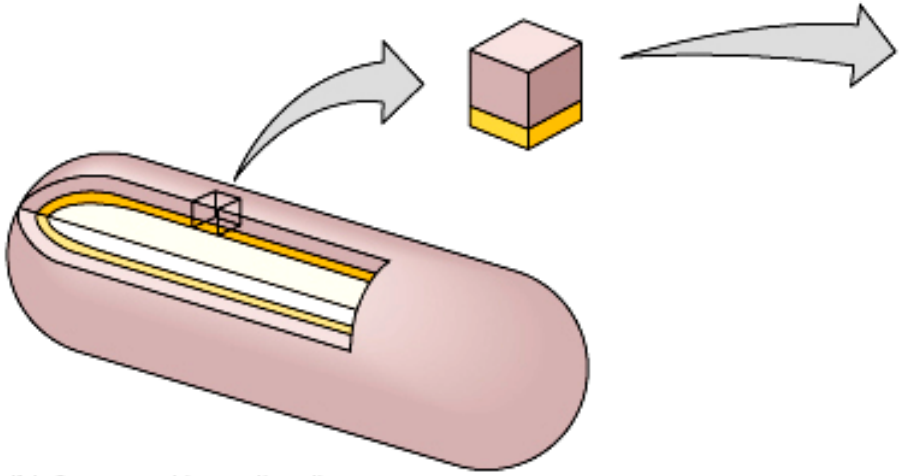
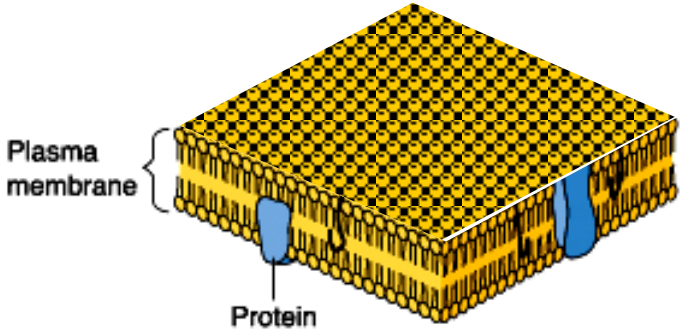
Antibacterial Targets: Overview

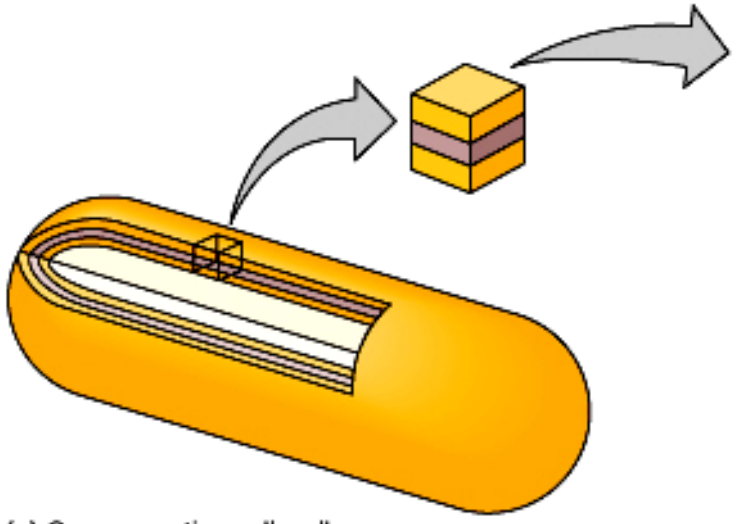


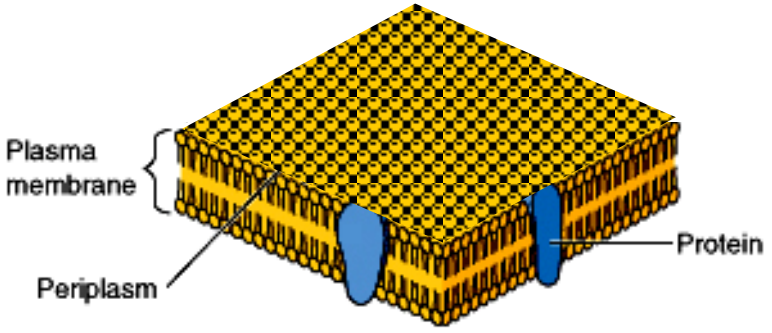
Bacterial Cell Wall: Gram Positive & Gram Negative Architecture





(b) Gram-positive cell wall





(c) Gram-negative cell wall

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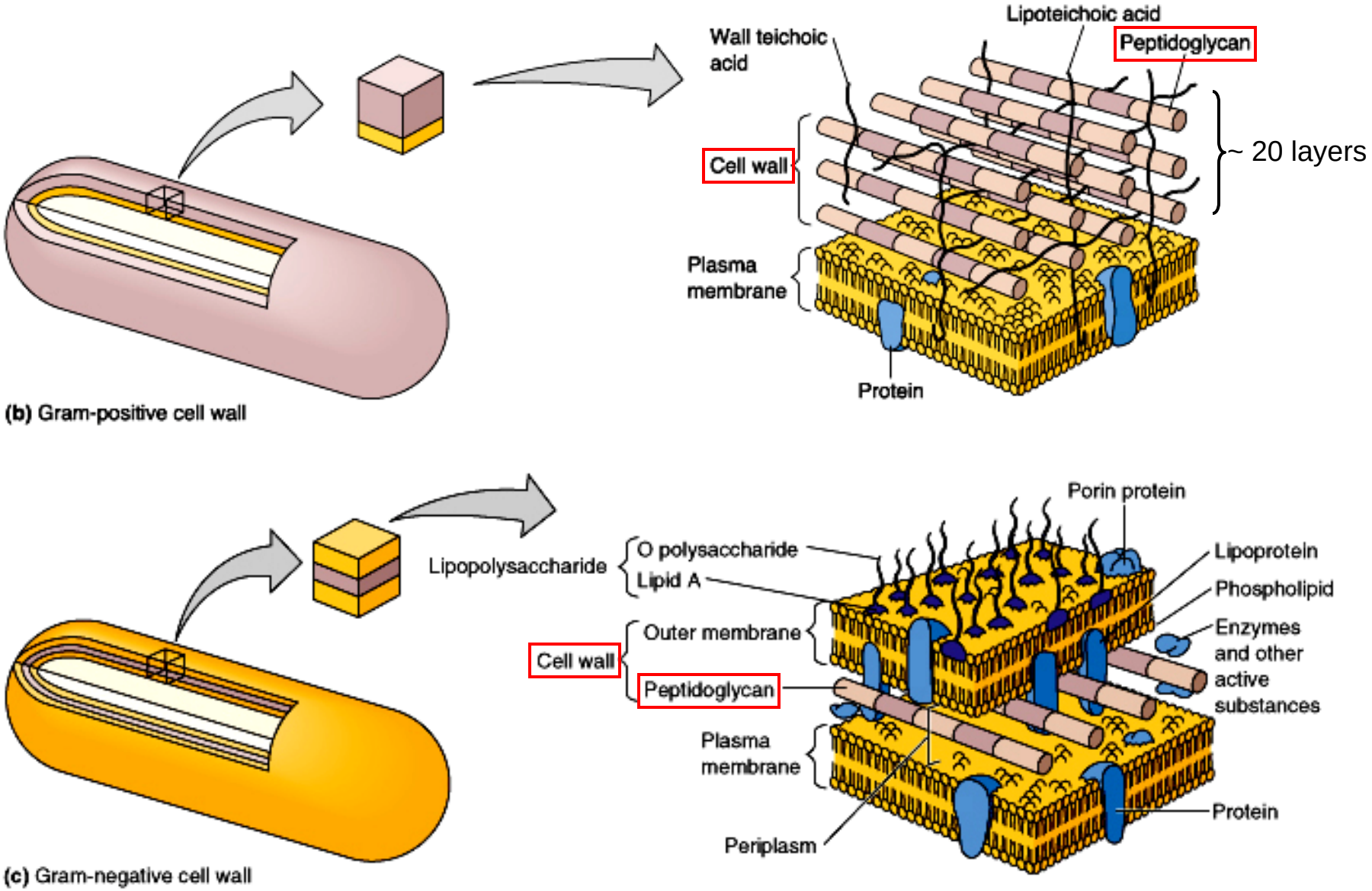
Bacterial Cell Wall: Gram Positive & Gram Negative Architecture



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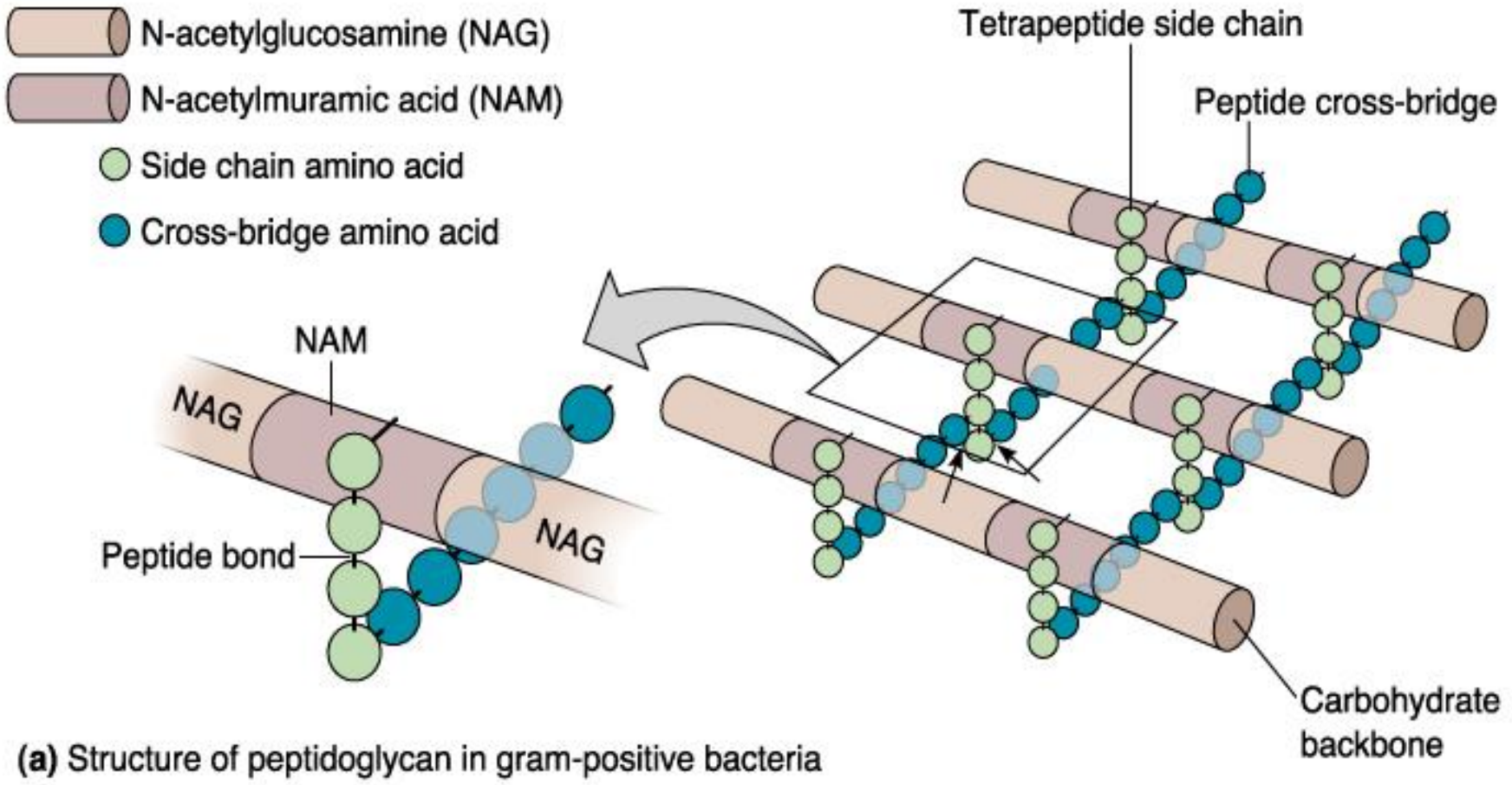
Bacterial Cell Wall: Peptidoglycan



(a) Structure of peptidoglycan in gram-positive bacteria

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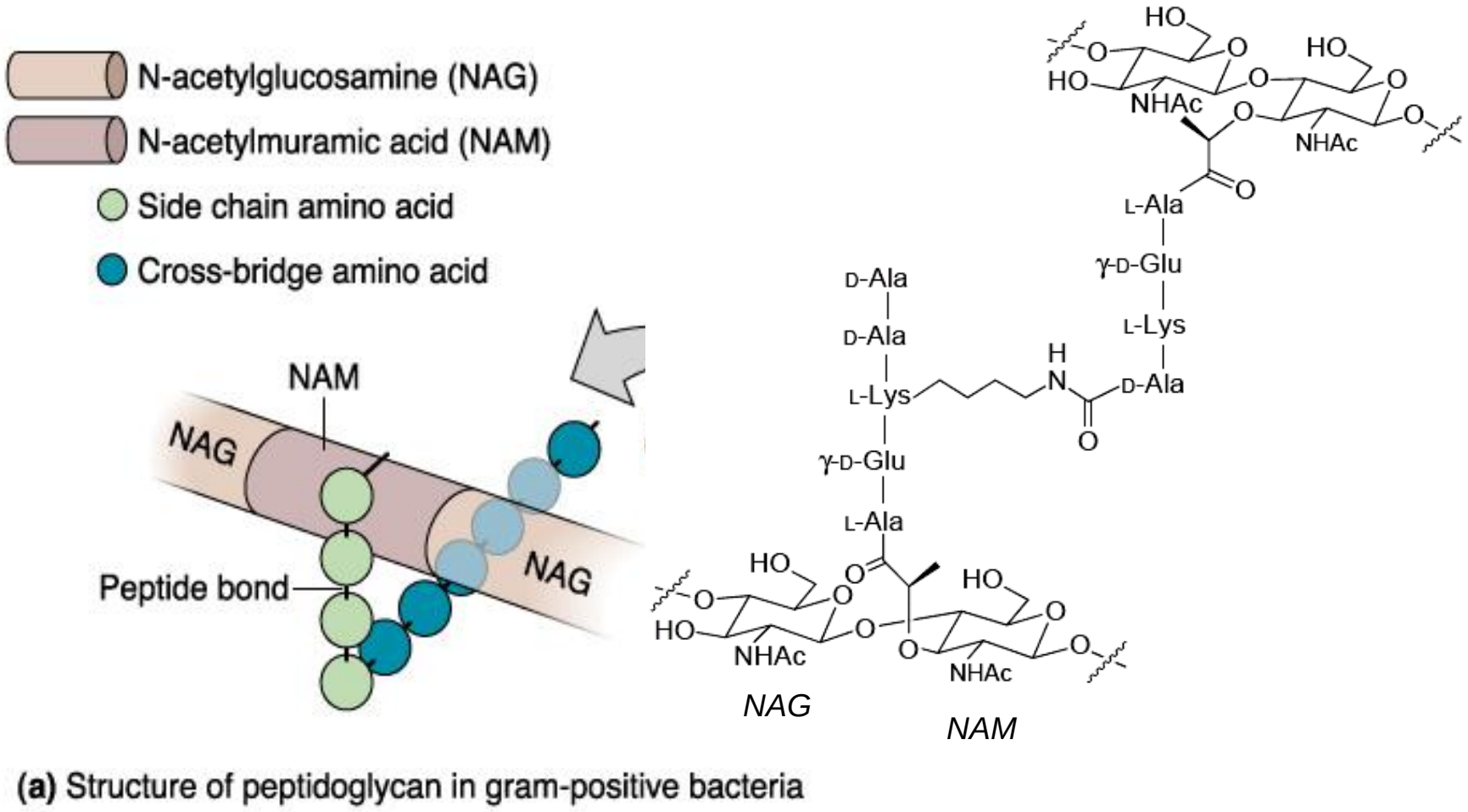
Cell wall composition:
Gram + : 95% peptidoglycan
Gram - : ~ 20% peptidoglycan

Bacterial Cell Wall: Peptidoglycan



(a) Structure of peptidoglycan in gram-positive bacteria

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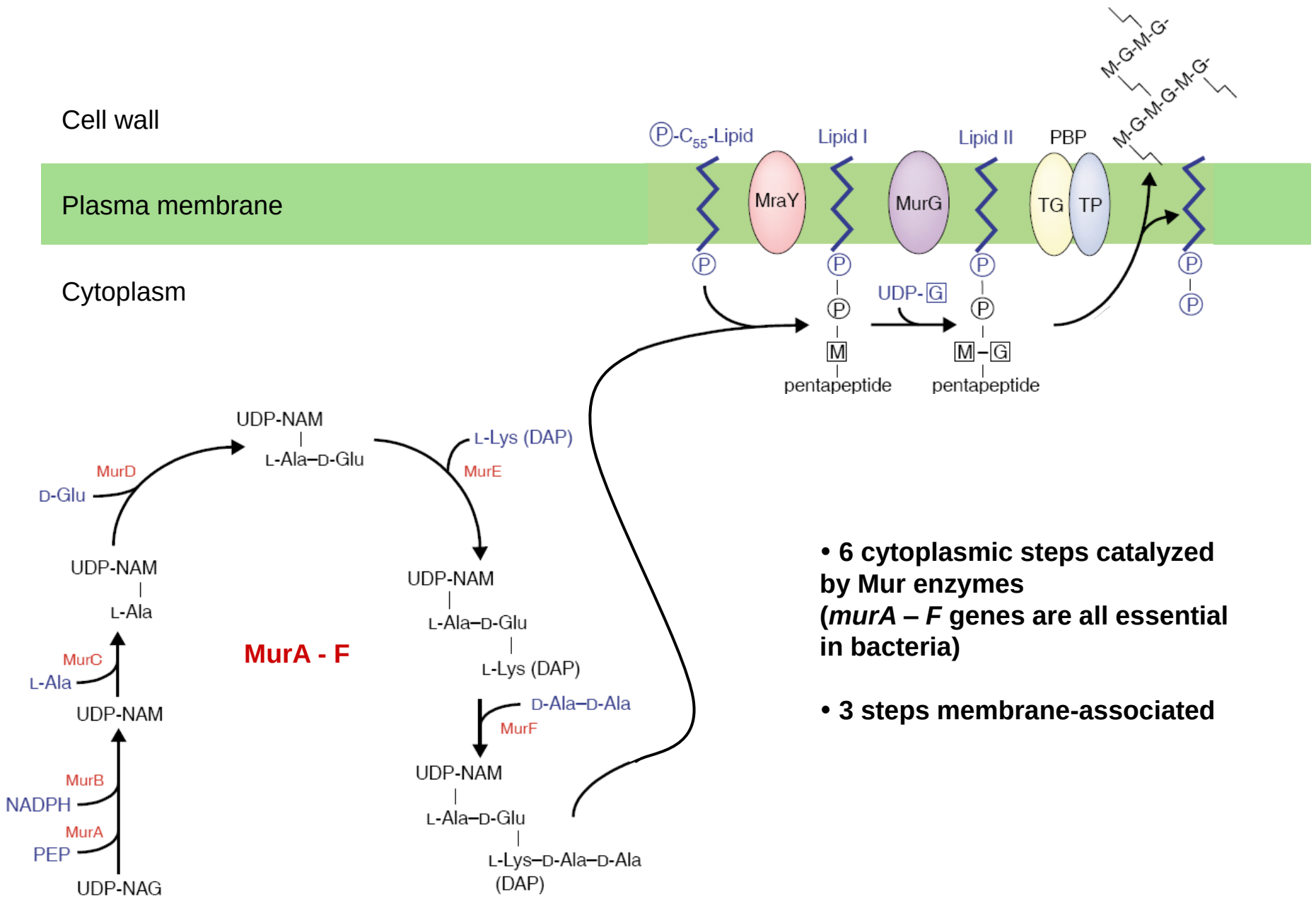
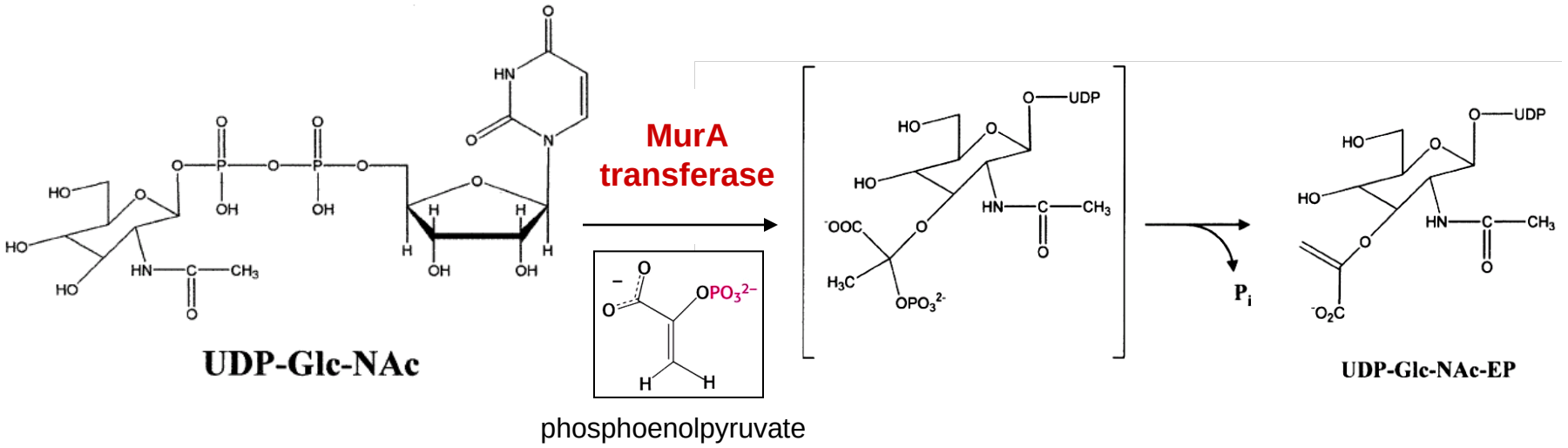
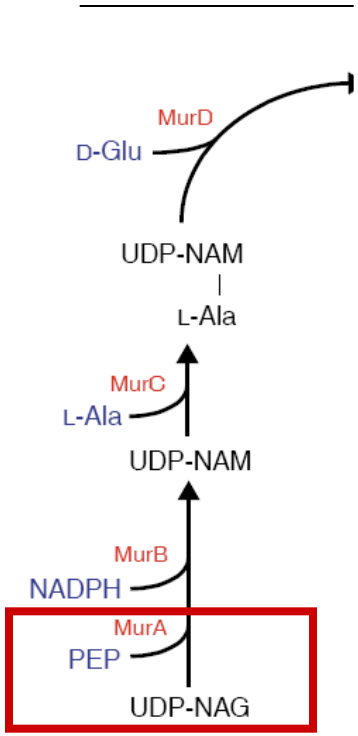
Bacterial Cell Wall: Peptidoglycan Biosynthesis

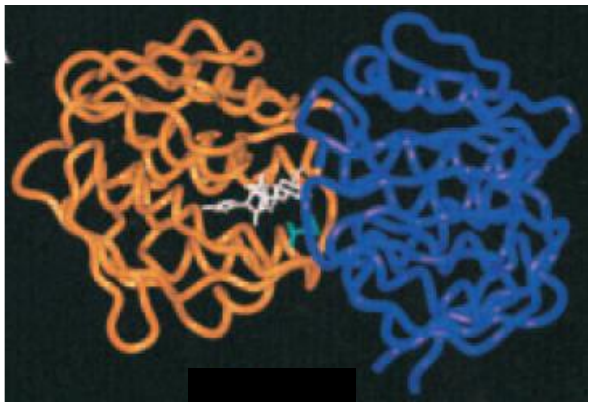


- **6 cytoplasmic steps catalyzed by Mur enzymes (*murA – F* genes are all essential in bacteria)**
- **3 steps membrane-associated**

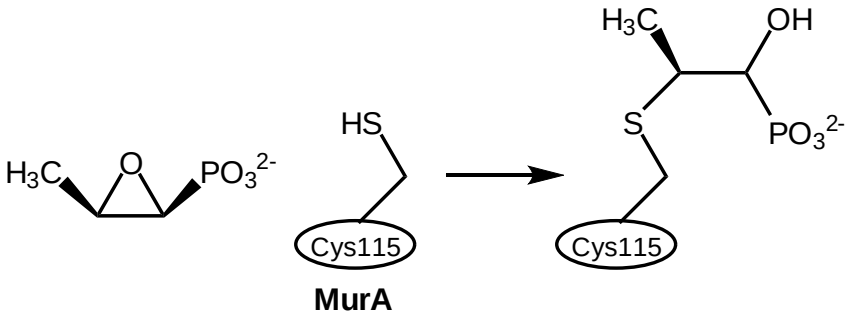
Bacterial Cell Wall: Peptidoglycan Biosynthesis – MurA and Fosfomycin







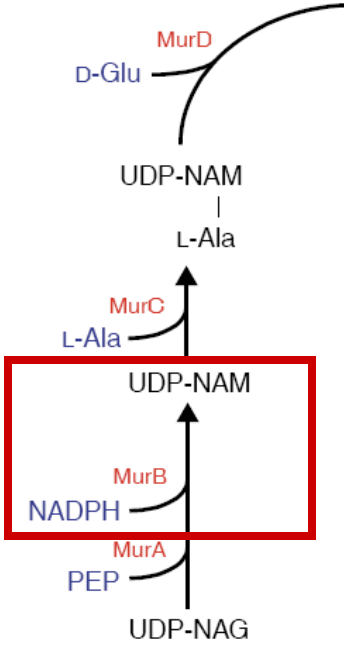
MurA:
UDP-N-acetylglucosamine enolpyruvyltransferase
44 kDa (419 aa)

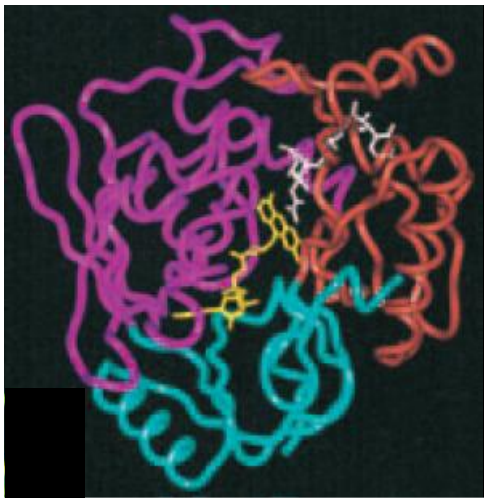


Fosfomycin:

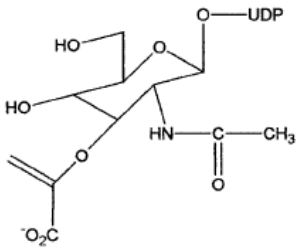
- naturally occuring PEP analog (*Streptomyces*)
- broad spectrum antibiotic used against gastrointestinal infections (Shiga-like toxin-producing *E. coli*, STEC) and bacterial infections of the urinary tract.
- enters bacterial cells by active transport.
- resistance by uptake impairment and enzymatic modification.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – MurB





MurB:
UDP-N-acetylenolpyruvyl-glucosamine reductase
38 kDa (342 aa)

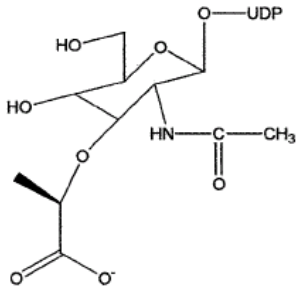


UDP-Glc-NAc-EP

MurB reductase

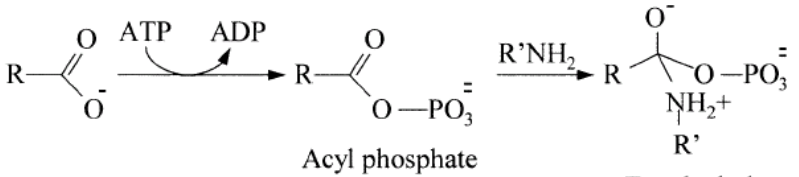
$NADPH + H^+$

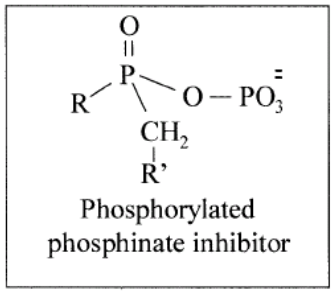
$NADP^+$



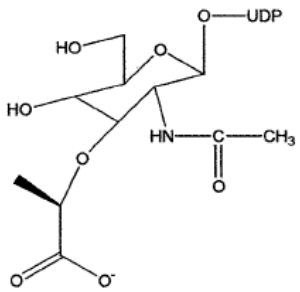
UDP-Mur-NAc

Bacterial Cell Wall: Peptidoglycan Biosynthesis – MurC, MurD, MurE, MurF

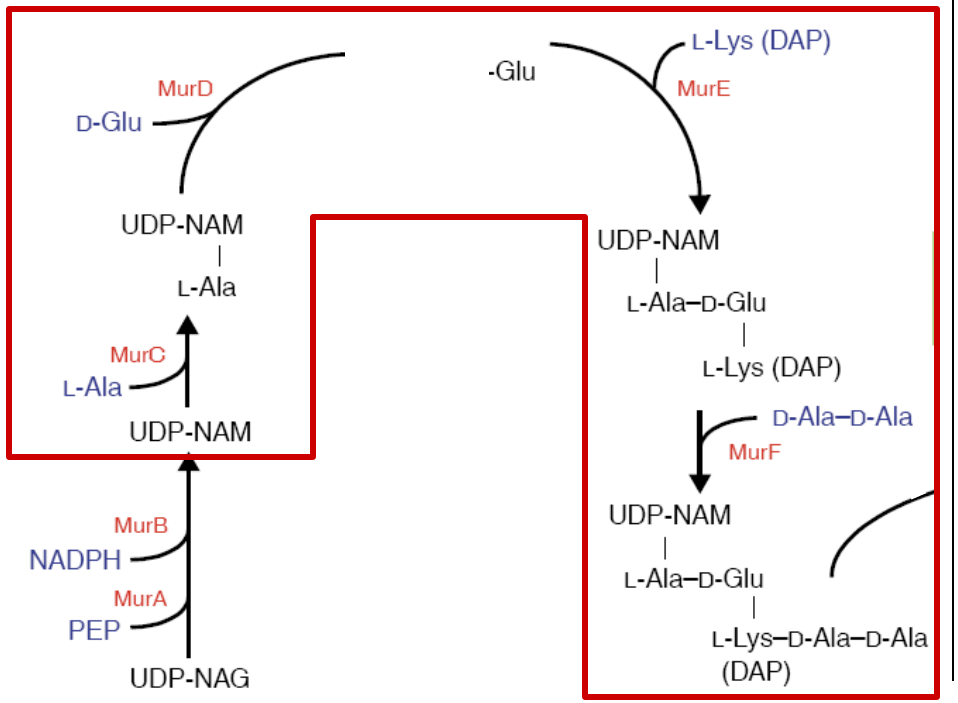


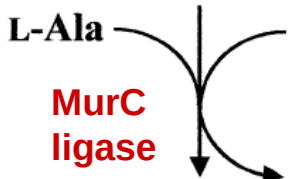


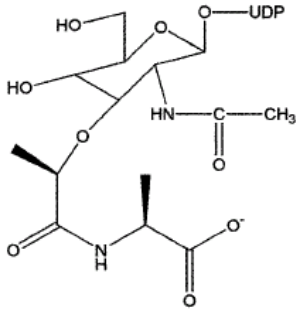
<: Mechanism of ATP-dependent amino acid ligases MurC, D, E, and F



UDP-Mur-NAc



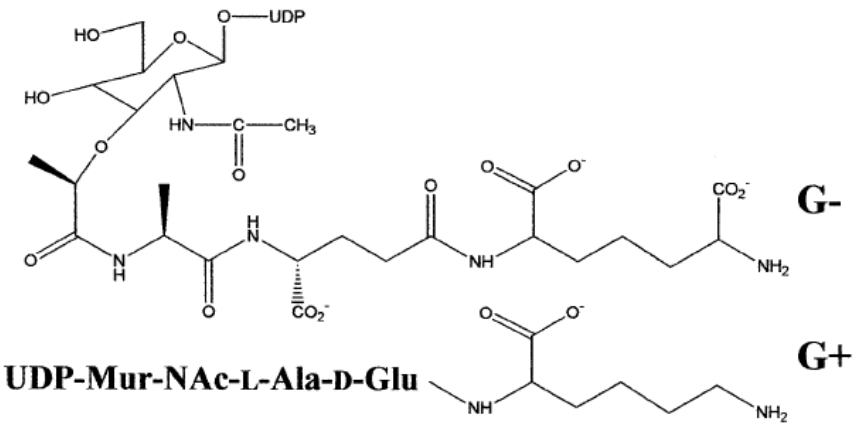




UDP-Mur-NAc-L-Ala

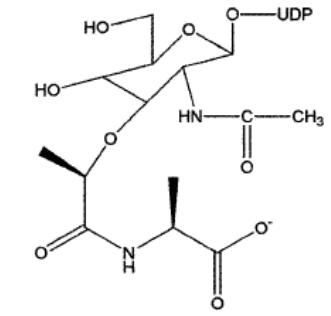
MurC: UDP-N-acetylmuramate L-alanine ligase
50 kDa (478 aa)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – MurD & MurE



UDP-Mur-NAc-L-Ala-D-Glu

UDP-Mur-NAc-Tripeptide



UDP-Mur-NAc-L-Ala

D-Glu + ATP

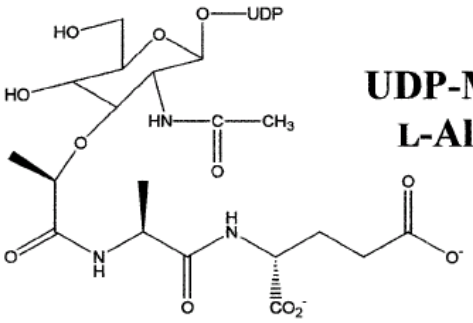
MurD ligase

ADP + P_i

ADP + P_i

ATP

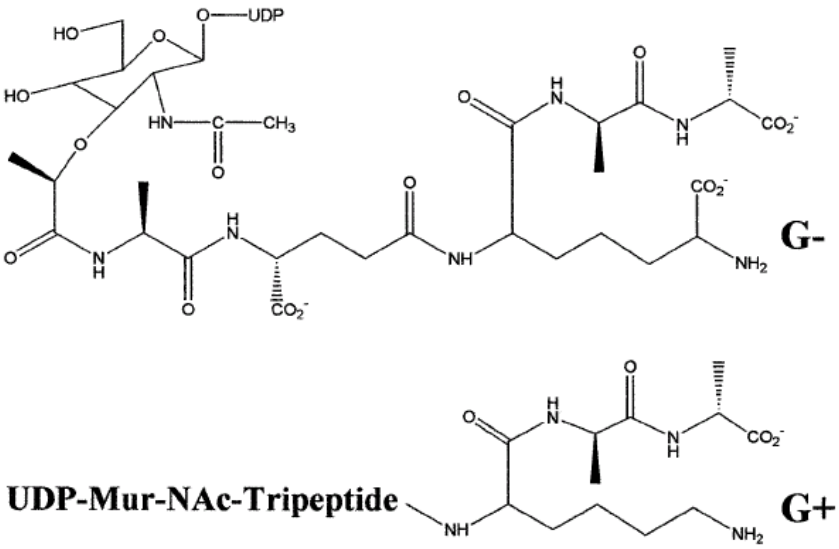
MurE ligase



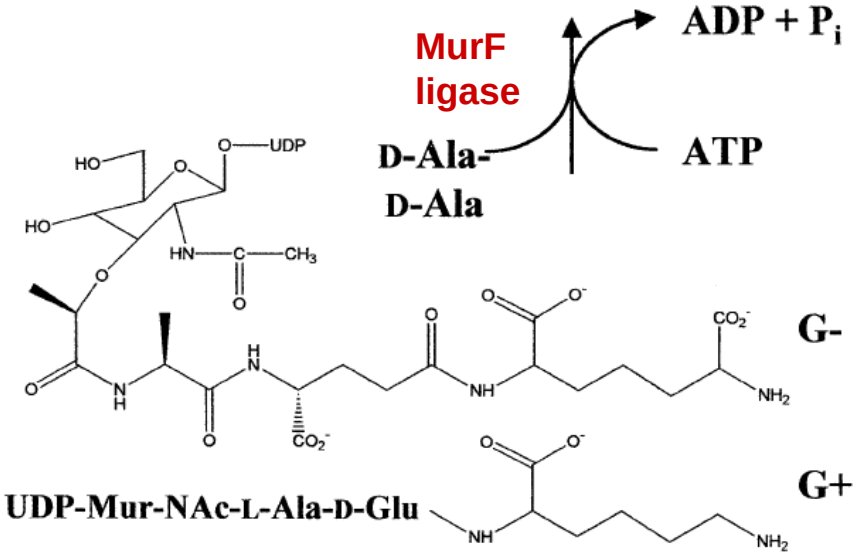
UDP-Mur-NAc-L-Ala-D-Glu

Gram+: *meso*-diaminopimelic acid (mDAP)
Gram-: L-lysine

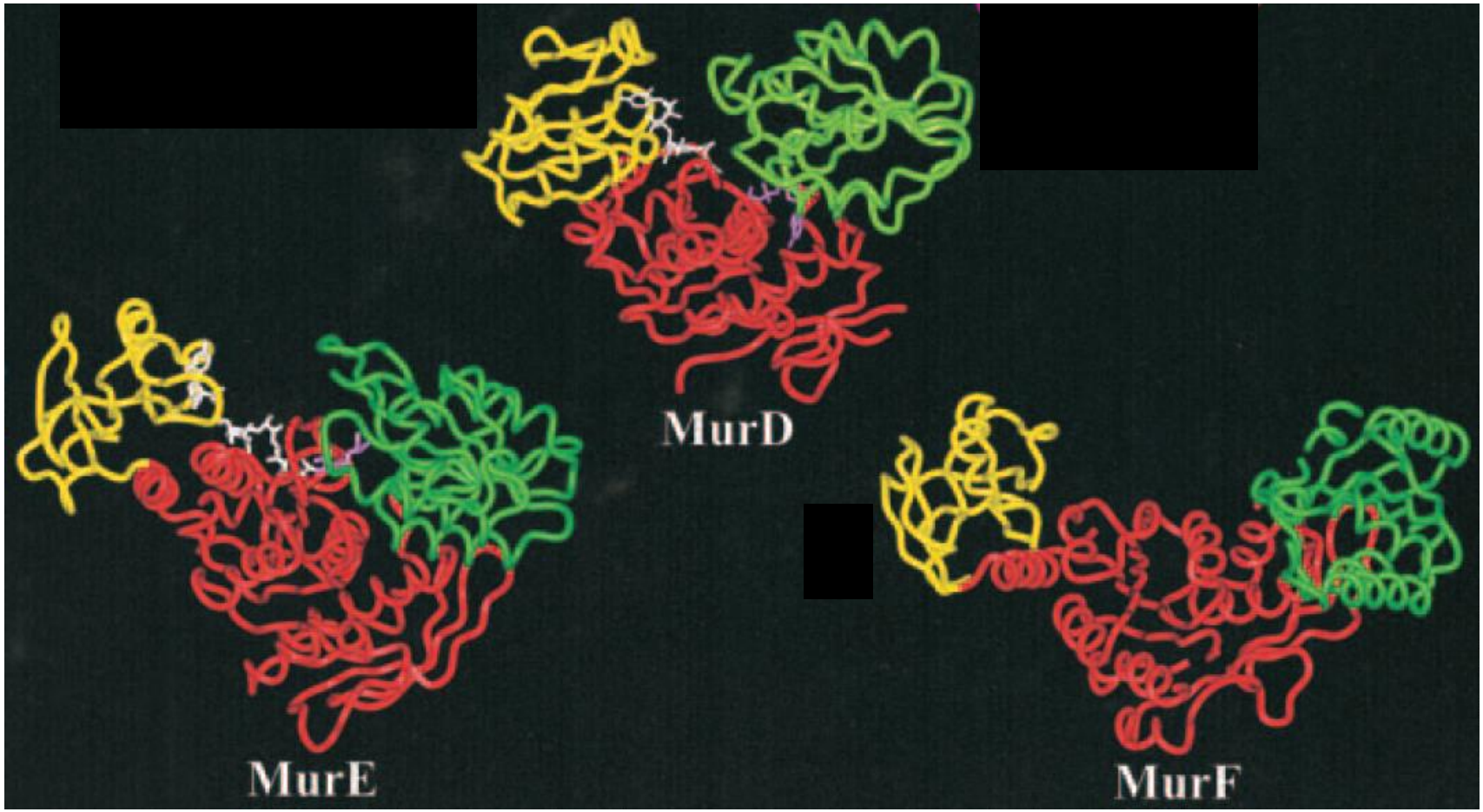
Bacterial Cell Wall: Peptidoglycan Biosynthesis – MurF



UDP-Mur-NAc-Pentapeptide (Park Nucleotide)



Bacterial Cell Wall: Peptidoglycan Biosynthesis – MurD, MurE, MurF



MurD: UDP-N-acetylmuramoyl-L-alanine D-glutamate ligase; 47 kDa (437 aa)
MurE: UDP-N-acetylmuramoyl-L-alanyl-D-glutamate *meso*-diaminopimelate ligase; 52 kDa (497 aa)
MurF: UDP-N-acetylmuramoyl-L-alanyl-D-glutamyl-*meso*-diaminopimelate D-alanyl-D-alanine ligase; 46 kDa (452 aa)

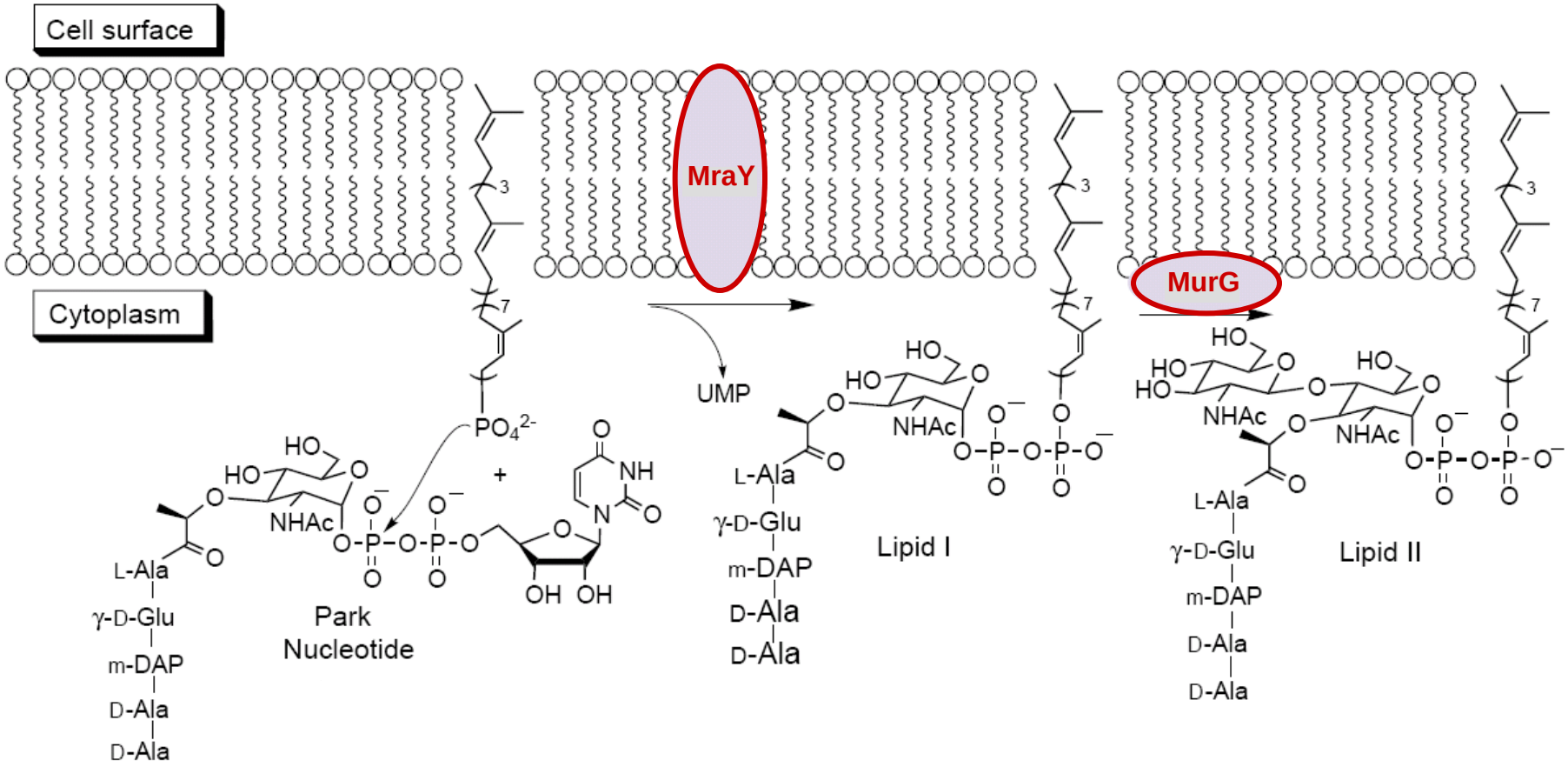
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Inhibitors of MurA - F

Enzyme	Inhibitor	Extent of inhibition	Reference
MurA	Fosfomycin	$IC_{50} = 8.8\ \mu M$	Baum *et al.* (2001)
	Cyclic disulphide	$IC_{50} = 0.2\ \mu M$	Baum *et al.* (2001)
	Purine analogue	$IC_{50} = 0.9\ \mu M$	Baum *et al.* (2001)
	Pyrazolopyrimidine	$IC_{50} = 0.3\ \mu M$	Baum *et al.* (2001)
MurB	4-Thiazolidinones	IC_{50}s 7.7–28.4 μM	Andres *et al.* (2000)
MurC	Phosphinate	$IC_{50} = 49\ \mu M$	Reck *et al.* (2001)
	L-Alanine analogues:		
	β-Alanine	$K_{is} = 110$ mM	Emanuele *et al.* (1996)
	β-CN-L-Alanine	$K_{is} = 3.3$ mM	Emanuele *et al.* (1996)
	L-Vinylglycine	$K_{is} = 5.8$ mM	Emanuele *et al.* (1996)
	β-Chloro-L-alanine	32.8%	Gubler *et al.* (1996)
	L-Cysteine	19%	Liger *et al.* (1995)
	β-Chloro-L-alanine	76%	Liger *et al.* (1995)
	β-Cyano-L-alanine	88%	Liger *et al.* (1995)
	β-Fluoro-L-alanine	94%	Liger *et al.* (1995)
MurD	Phosphinate	$IC_{50} = 680$ nM	Tanner *et al.* (1996)
	Phosphinate	$IC_{50} < 1$ nM	Gegnas *et al.* (1998)
	D-Glutamic acid analogues:		
	DL-Homocysteic acid	58%	Pratviel-Sosa *et al.* (1994)
	D-*erythro*-3-Methylglutamic acid	47%	Pratviel-Sosa *et al.* (1994)
	D-*erythro*-4-Methylglutamic acid	26%	Pratviel-Sosa *et al.* (1994)
MurE	Phosphinate	$IC_{50} = 1.1\ \mu M$	Zeng *et al.* (1998)
	A_2pm[a] analogues:		
	(2*S*,3*R*,6*S*)-3-Fluoro-A_2pm	$IC_{50} = 2.3$ mM	Auger *et al.* (1996)
	N-Hydroxy-A_2pm	$IC_{50} = 0.56$ mM	Auger *et al.* (1996)
MurF	Aminoalkyl phosphinates:		
	N-Acyl phosphinate	$K_i = 700\ \mu M$	Miller *et al.* (1998)
	N-Glutaryl phosphinate	$K_i = 200\ \mu M$	Miller *et al.* (1998)
	Pseudo-tetrapeptide phosphinate	$K_i = 200\ \mu M$	Miller *et al.* (1998)
	ATP analogue	$K_{is} = 33.6\ \mu M$	Anderson *et al.* (1996)

a. A_2pm, meso-diaminopimelic acid.

(El Zoeiby *et al.*, *Mol. Microbiol.* 2003, 47, 1)

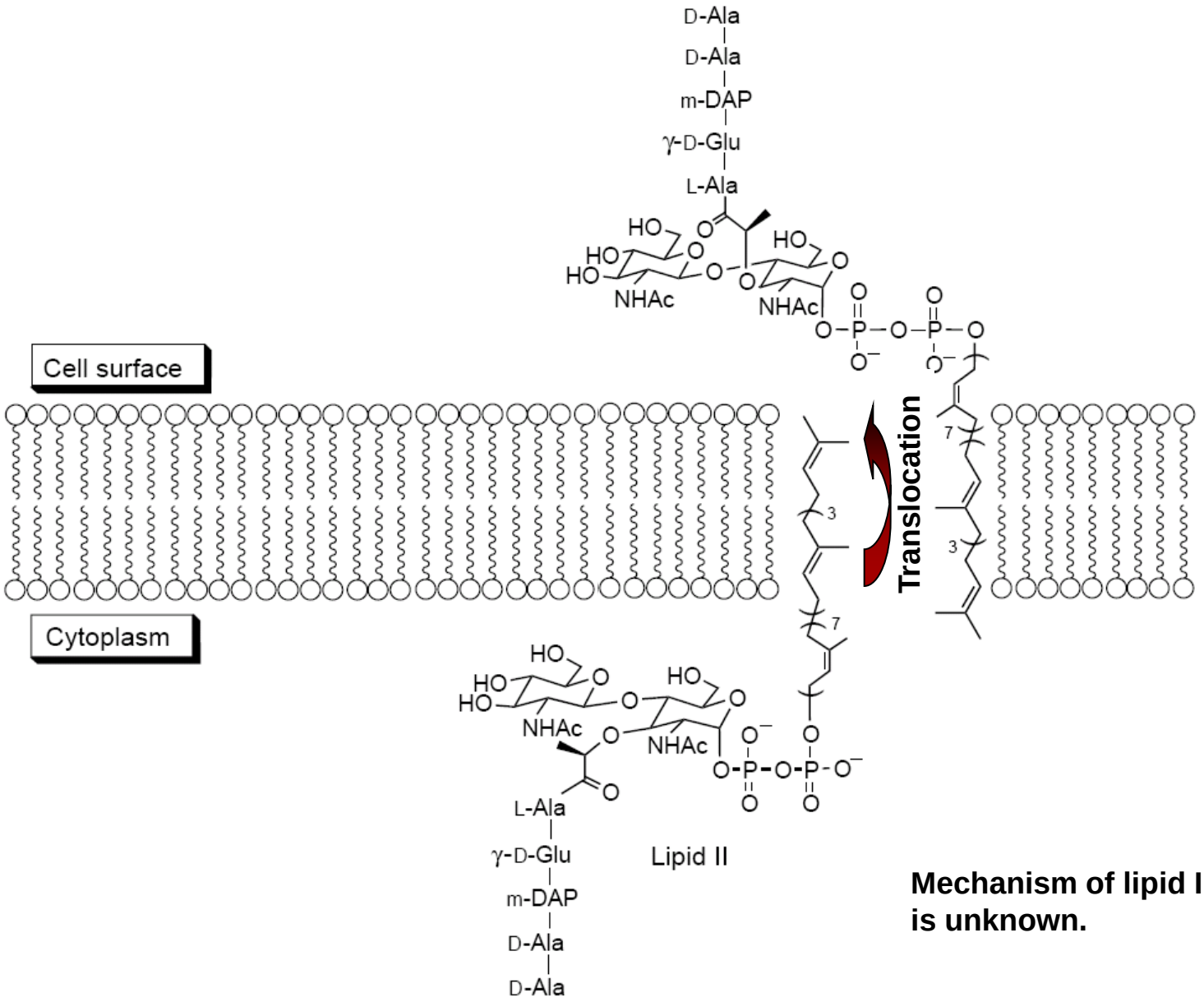
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Lipid Attachment



MraY transfers muramyl pentapeptide from UDP to a C55 undecaprenyl phosphate membrane anchor to form **Lipid I**.

MurG couples N-acetylglucosamine to the 4-OH group of the muramyl moiety to form **Lipid II**.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Translocation



Mechanism of lipid II translocation is unknown.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Polymerization

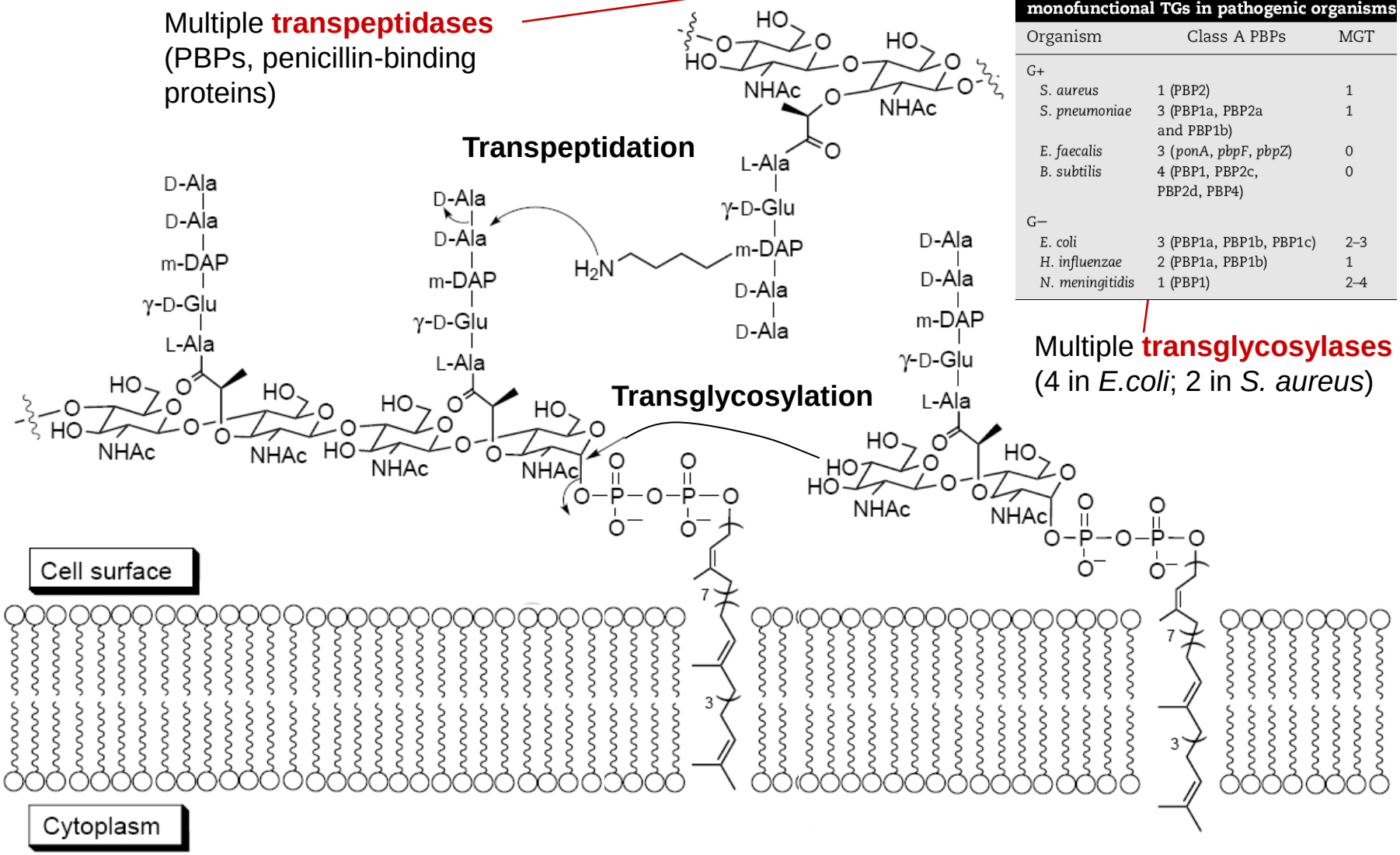
also: Bifunctional TG/TPases

Multiple **transpeptidases** (PBPs, penicillin-binding proteins)

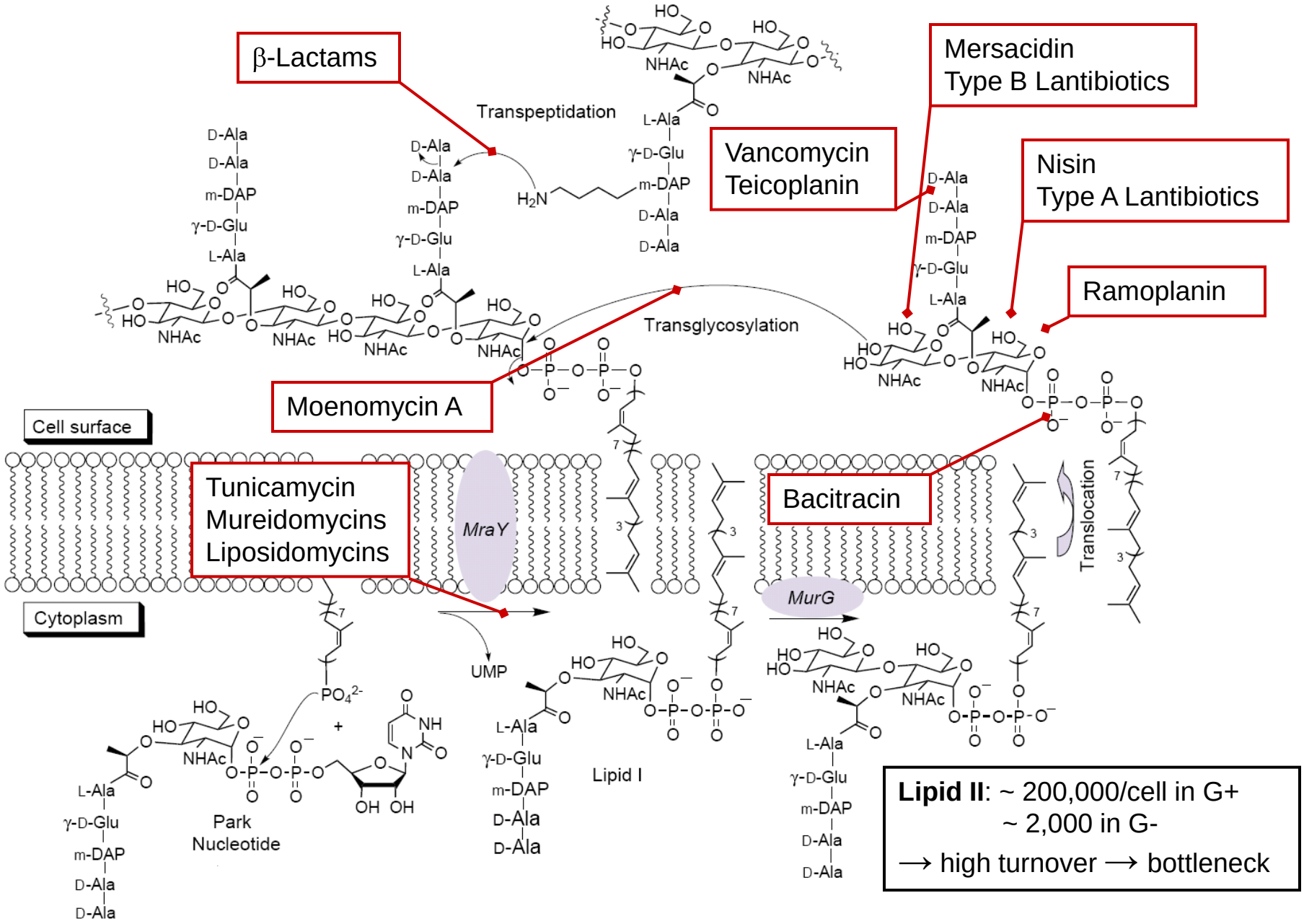
Table 1 – Putative class A multimodular PBPs monofunctional TGs in pathogenic organisms

Organism	Class A PBPs	MGT
G+		
S. aureus	1 (PBP2)	1
S. pneumoniae	3 (PBP1a, PBP2a and PBP1b)	1
E. faecalis	3 (*ponA*, *pbpF*, *pbpZ*)	0
B. subtilis	4 (PBP1, PBP2c, PBP2d, PBP4)	0
G–		
E. coli	3 (PBP1a, PBP1b, PBP1c)	2–3
H. influenzae	2 (PBP1a, PBP1b)	1
N. meningitidis	1 (PBP1)	2–4

Multiple **transglycosylases** (4 in *E.coli*; 2 in *S. aureus*)



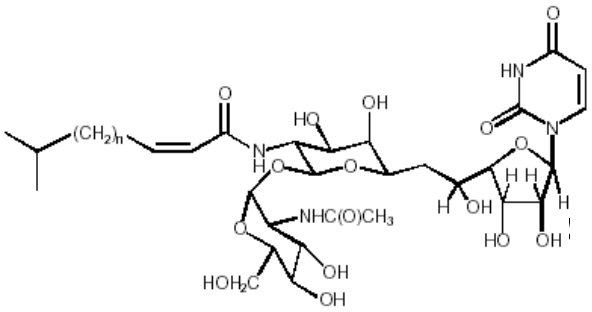
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Antibiotics



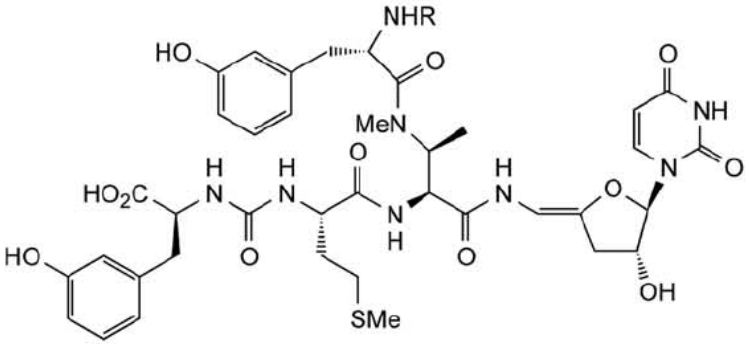
Lipid II: ~ 200,000/cell in G+
~ 2,000 in G-

⟶ high turnover ⟶ bottleneck

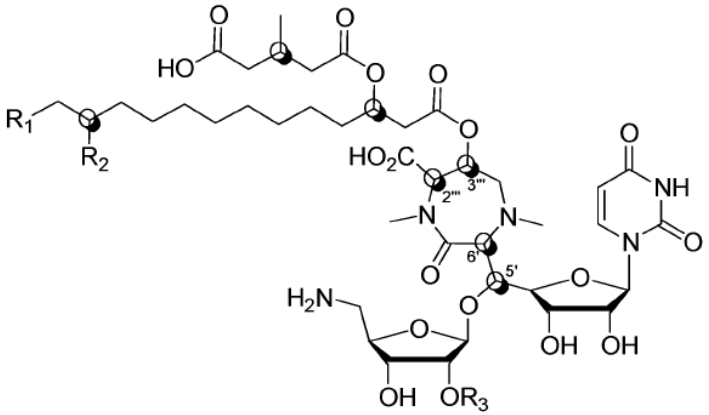
Bacterial Cell Wall: Peptidoglycan Biosynthesis – MraY as a Target



Tunicamycin (n=8-11)
(*inhibits also eukaryotic protein glycosylation*)



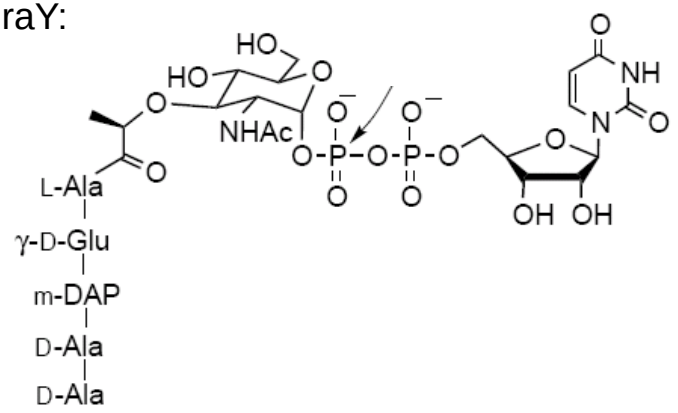
Mureidomycins
(*MraY selective*)
(**1**) MrdA (R = H, uracil)
(**2**) MrdB (R = H, dihydrouracil)
(**3**) MrdC (R = Gly, uracil)
(**4**) MrdD (R = Gly, dihydrouracil)



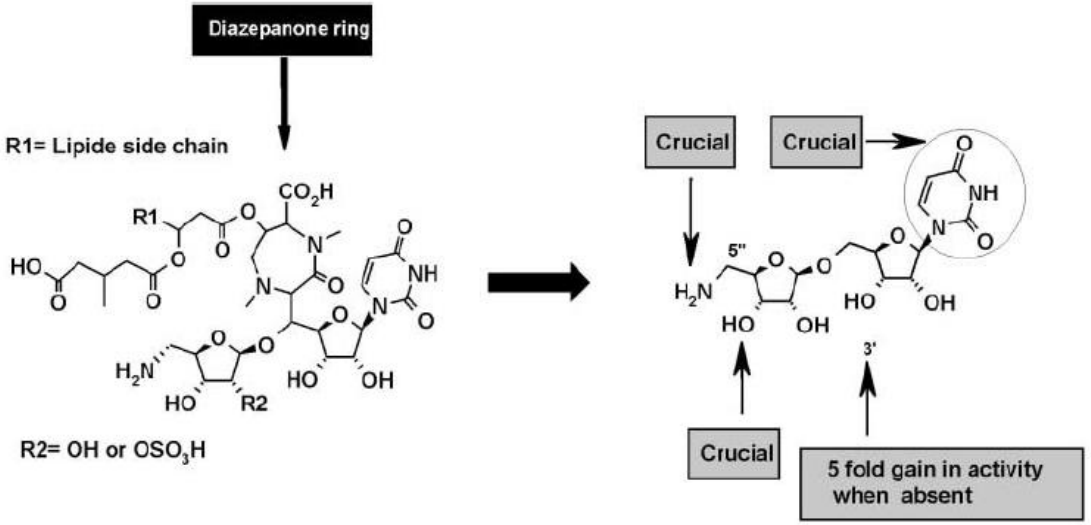
Liposidomycins (*MraY selective*)
(A-III, **1**): R_1 = Me; R_2 = Me; R_3 = H
(B, **2**): R_1 = H; R_2 = Me; R_3 = SO_3H
(C, **3**): R_1 = Me; R_2 = H; R_3 = SO_3H

◔ = *Unnassigned Configurations*

Natural **uridyl peptide antibiotics** compete with UDP-muramyl pentapeptide as substrate for MraY:

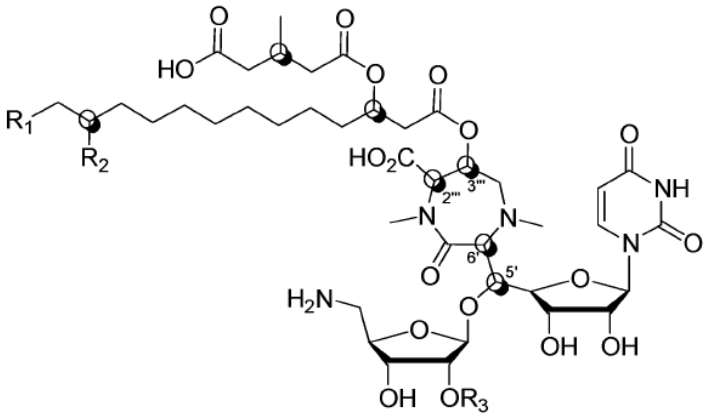


Bacterial Cell Wall: Peptidoglycan Biosynthesis – MraY as a Target



Simplified liposidomycin analogues

(Dini *et al.*, *BMCL* 2002, 12, 1209)



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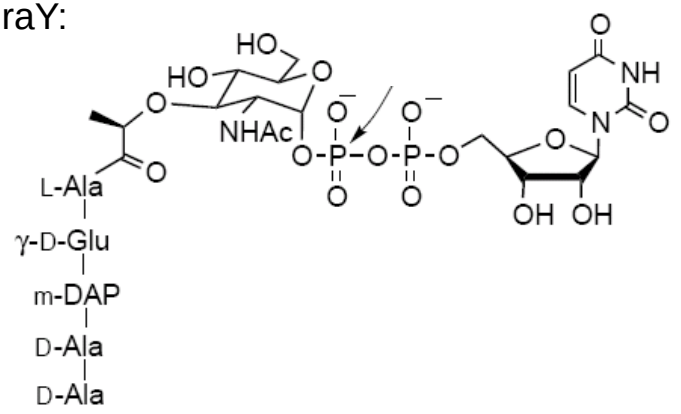
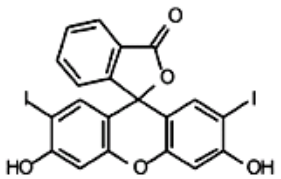
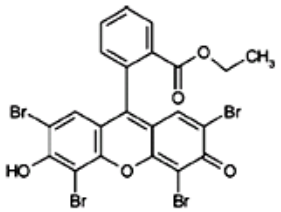
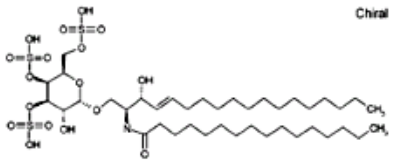
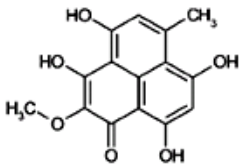


Bacterial Cell Wall: Peptidoglycan Biosynthesis – MraY & MurG as Targets

Structural information and inhibition data for MraY + MurG screen isolates

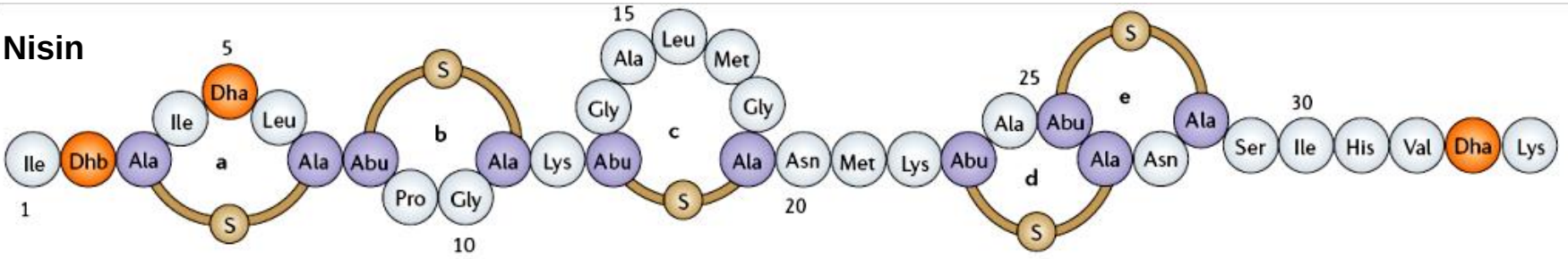
BMS No.	Structure	FW	MW	IC_{50} (μM)	MIC (μg/mL)	CC_{50} (μM)
BMS-185937		584.1	584.1	16.2	>128	166
BMS-187979		714.1	676.0	7.1	16	18.4
BMS-190134	 Chiral	1006.2	940.2	17.5	>128	108.6
BMS-304245		288.3	288.3	25.5	64	54.0

IC_{50}s reported are from the MraY + MurG membrane plate assay described under Materials and methods. Minimum inhibitory concentration (MIC) values are against *Staphylococcus aureus* (A15090) as determined by the same method described in [25]. The cell cytotoxicity values at 50% (CC_{50}) represent cytotoxicity against HEp-2 cells as measured in [25].

(Zawadzke *et al.*, *Anal. Biochem.* 2003, 314, 243)

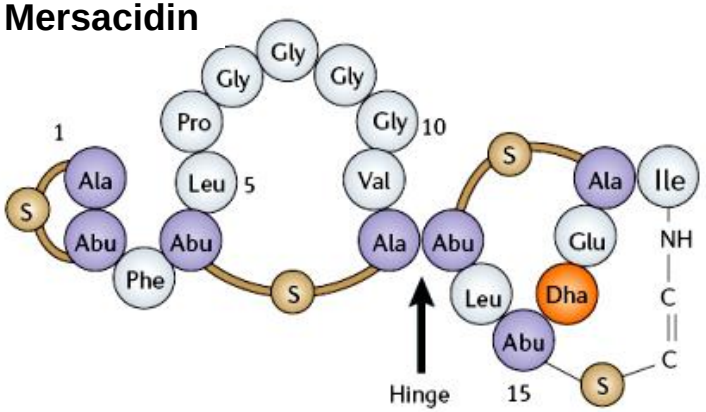
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Lantibiotics & Lipid II

Nisin



Nisin is produced by *Lactococcus lactis* and is used as a food preservative.

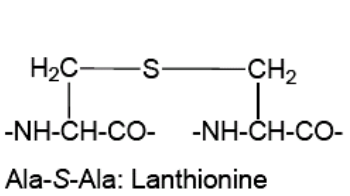
Mersacidin



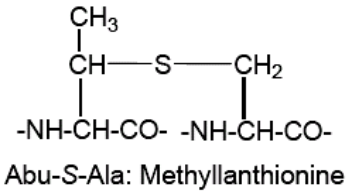
Mersacidin (from *Bacillus sp.*) **and other type B lantibiotics** bind to **lipid II** involving both Glc-NAc and Mur-NAc and prevent incorporation into peptidoglycan.

Mersacidin is active against MRSA (methicillin-resistant *S. aureus;* G+) and is currently in preclinical development.

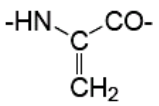
Lantibiotics: lanthionine-containing antibiotics



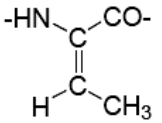
Ala-*S*-Ala: Lanthionine



Abu-*S*-Ala: Methyllanthionine



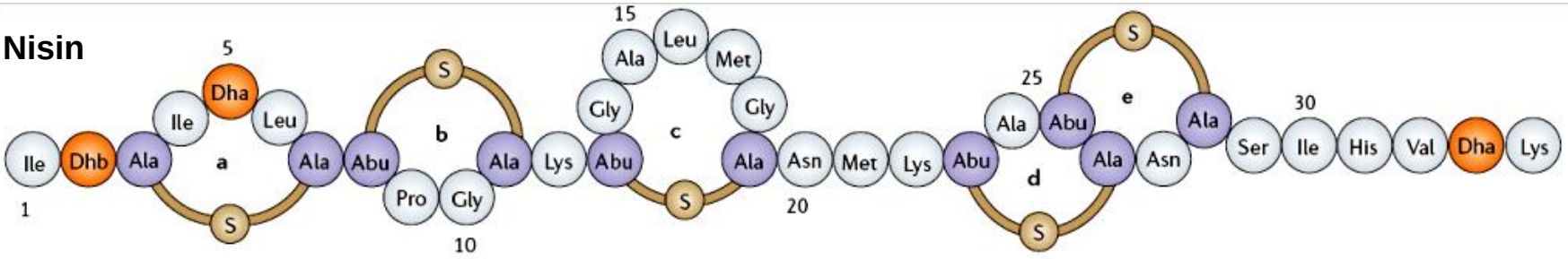
Dha: Dehydroalanine



Dhb: Dehydrobutyrine

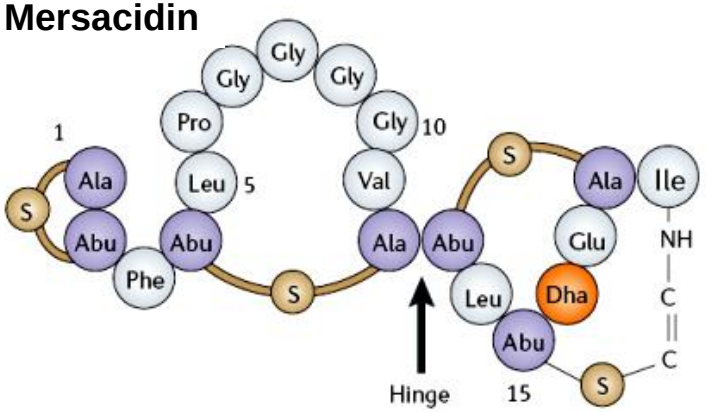
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Lantibiotics & Lipid II

Nisin



Nisin is produced by *Lactococcus lactis* and is used as a food preservative.

Mersacidin



Mersacidin (from *Bacillus sp.*) **and other type B lantibiotics** bind to **lipid II** involving both Glc-NAc and Mur-NAc and prevent incorporation into peptidoglycan.

Mersacidin is active against MRSA (methicillin-resistant *S. aureus;* G+) and is currently in preclinical development.

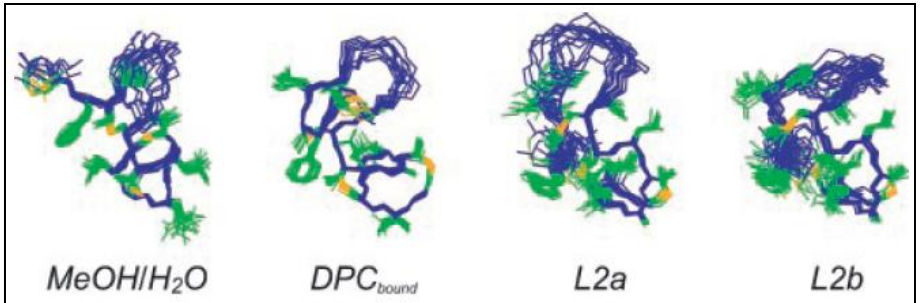
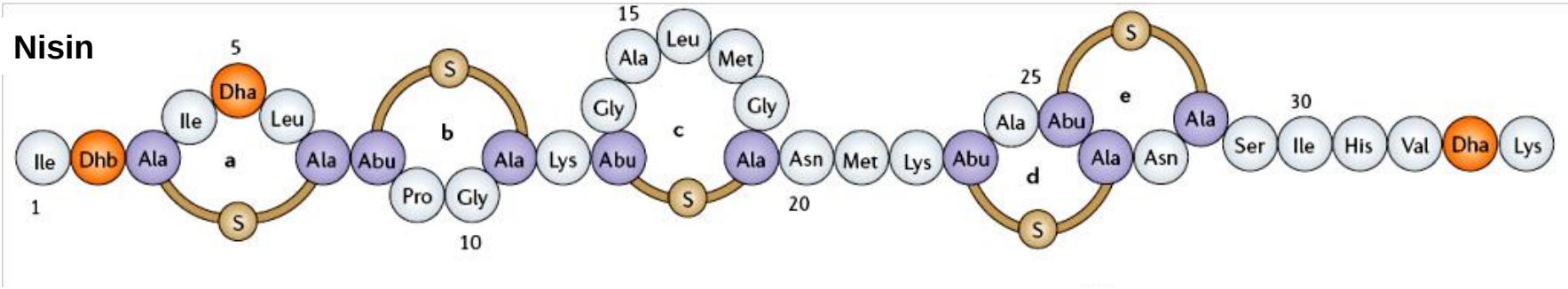


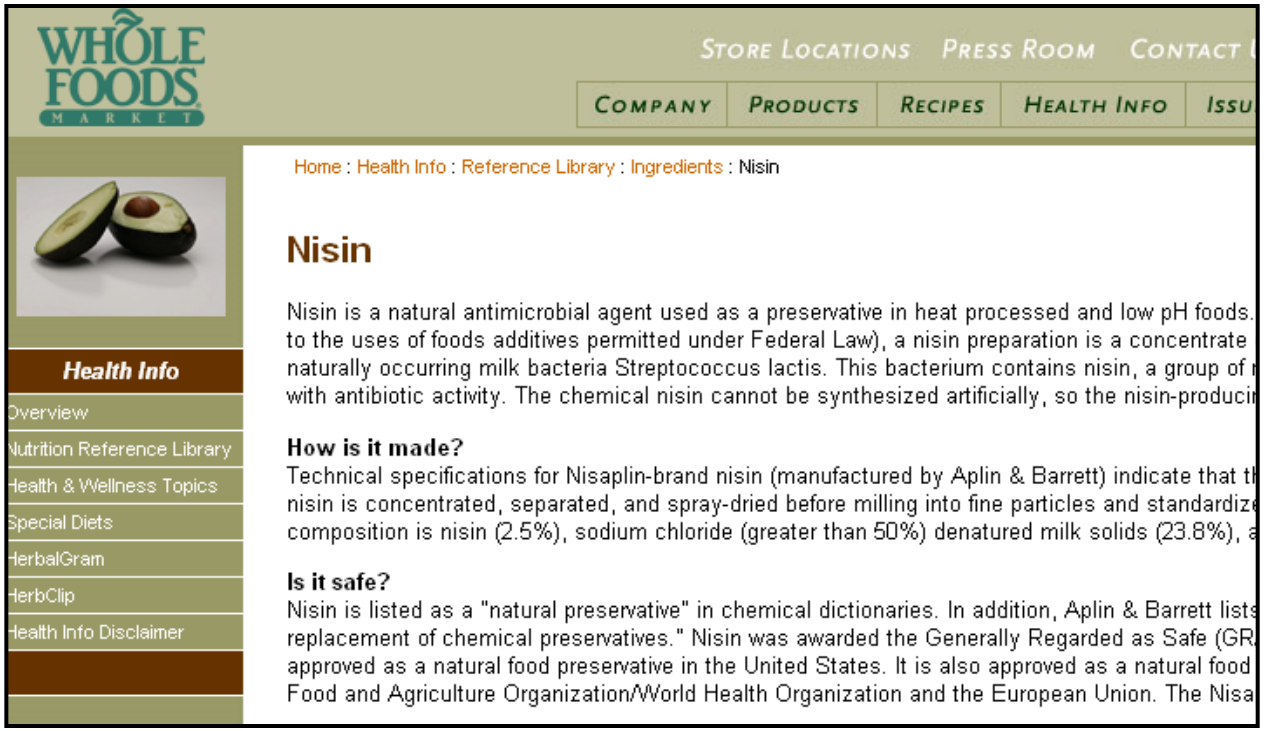
FIG. 6. **Solution NMR structures of mersacidin in various sample conditions.** Only heavy atoms are shown for clarity. Backbone and side chains are shown in *blue* and *green*, respectively; sulfur atoms of the lanthionine rings are shown in *yellow*. MeOH/H_2O, free mersacidin in methanol/water mixture; DPC_{bound}, DPC micelle-bound form; L2a and L2b, the two conformations of the lipid II-bound form differing only in the glycine-rich loop (see text for detail).

(Hsu *et al.*, *JBC* 2003, 278, 13110)

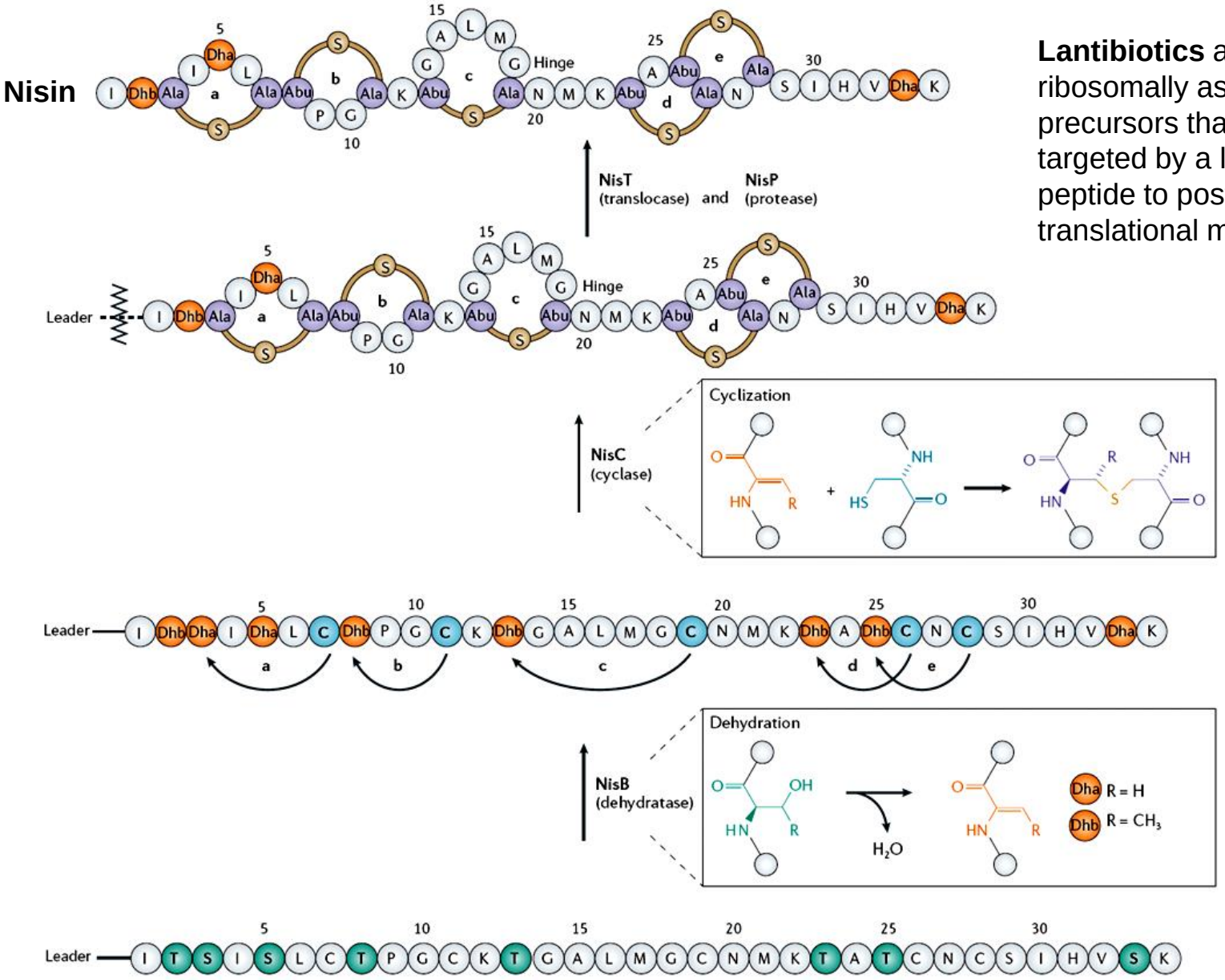
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Lantibiotics & Lipid II



Nisin is produced by *Lactococcus lactis* and is used as a food preservative.



Bacterial Cell Wall: Peptidoglycan Biosynthesis – Nisin Biosynthesis



Lantibiotics are produced ribosomally as inactive precursors that are targeted by a leader peptide to post-translational modification.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Nisin & Type A Lantibiotics

Nisin and type A lantibiotics kill bacteria by lipid II-targeted pore formation and permeabilization of the membrane.

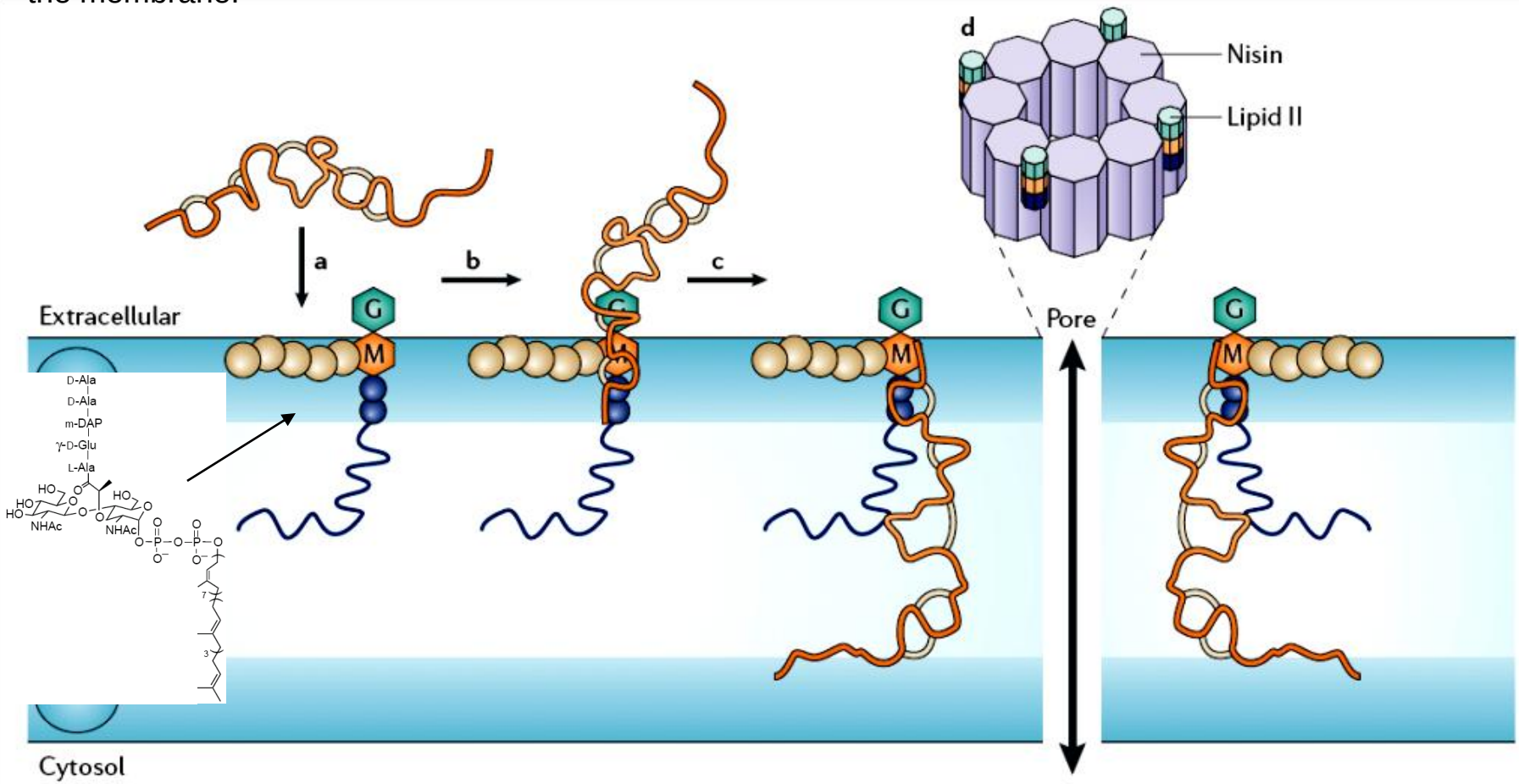
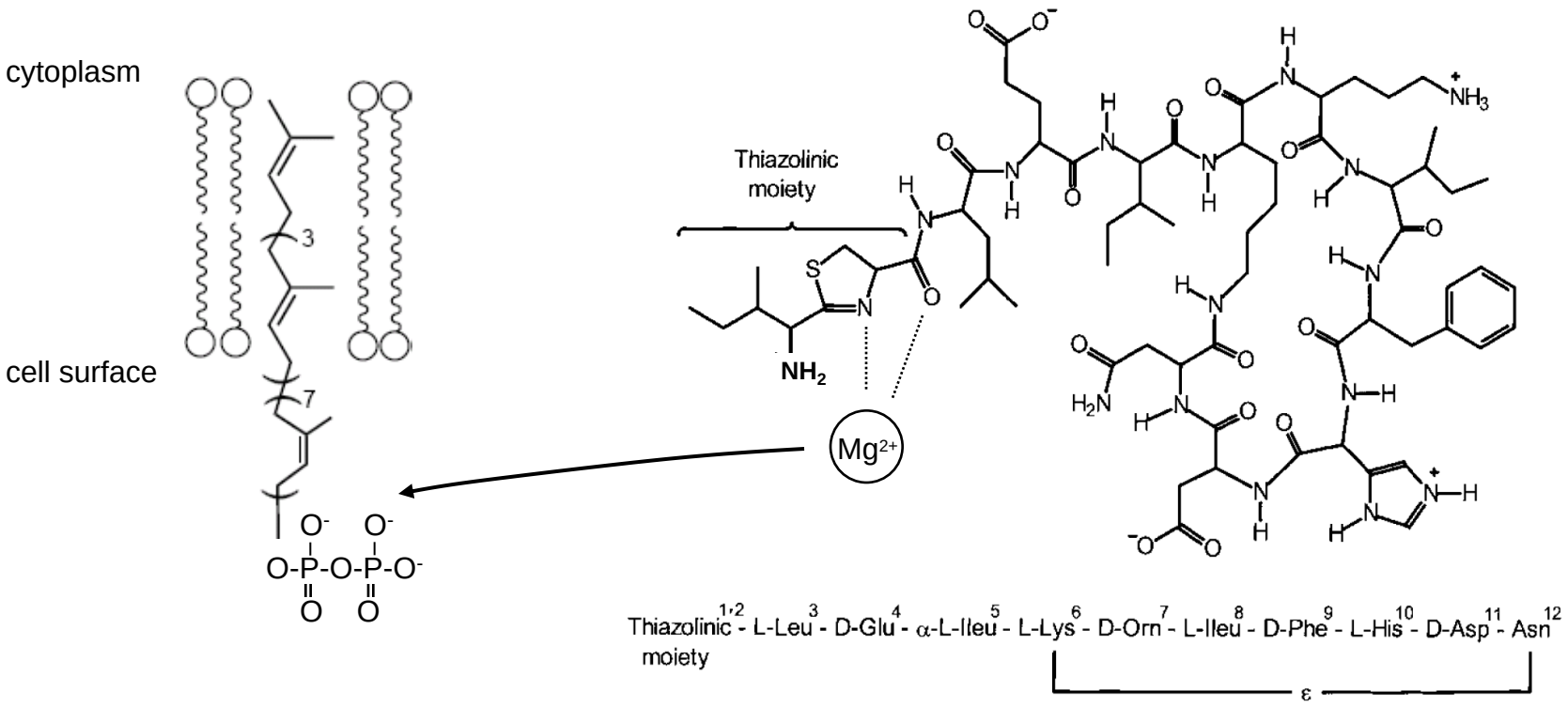


Figure 4 | **Model for the target-directed pore-formation mechanism of nisin.** First, nisin reaches the bacterial plasma membrane (**a**), where it binds to Lipid II via two of its amino-terminal rings (**b**). This is then followed by pore formation (**c**), which involves a stable transmembrane orientation of nisin. During or after assembly of four 1:1 (nisin: Lipid II) complexes, four additional nisin molecules are recruited to form the pore complex (**d**).

(Breukink & De Kruijff, *Nature Rev. Drug Discov.* 2006, in press)

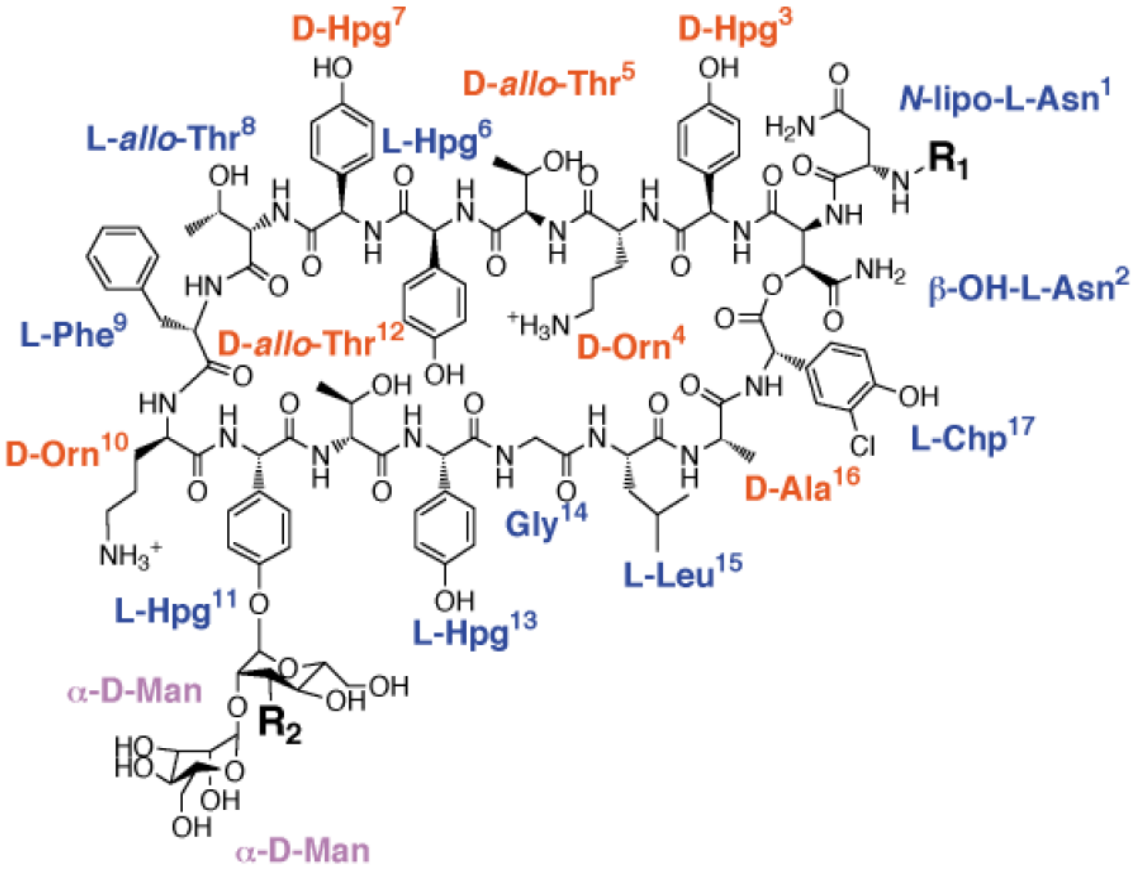
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Bacitracin

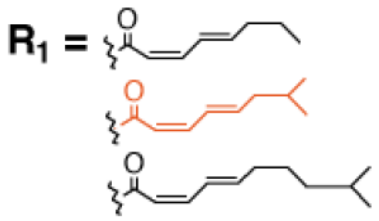


Bacitracin (isolated first 1945 from *B. subtilis*) is a nonribosomally produced cyclic decapeptide that sequesters the C55 undecaprenyl pyrophosphate membrane anchor, likely by binding in a cation-dependent fashion to the PP group.

Bacitracin is used as a topical antibiotic for skin and eye infections and via intramuscular injection for severe staphylococcal (G+) pneumonia in children.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Ramoplanin

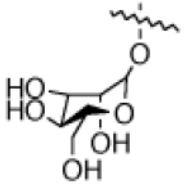




1: Ramoplanin A1
2: Ramoplanin A2,
3: Ramoplanose
4: Ramoplanin A3

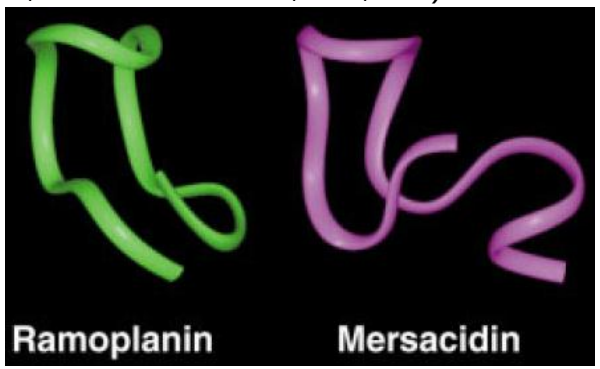
Ramoplanins A1-3
R_2 = OH

Ramoplanose
R_2 =



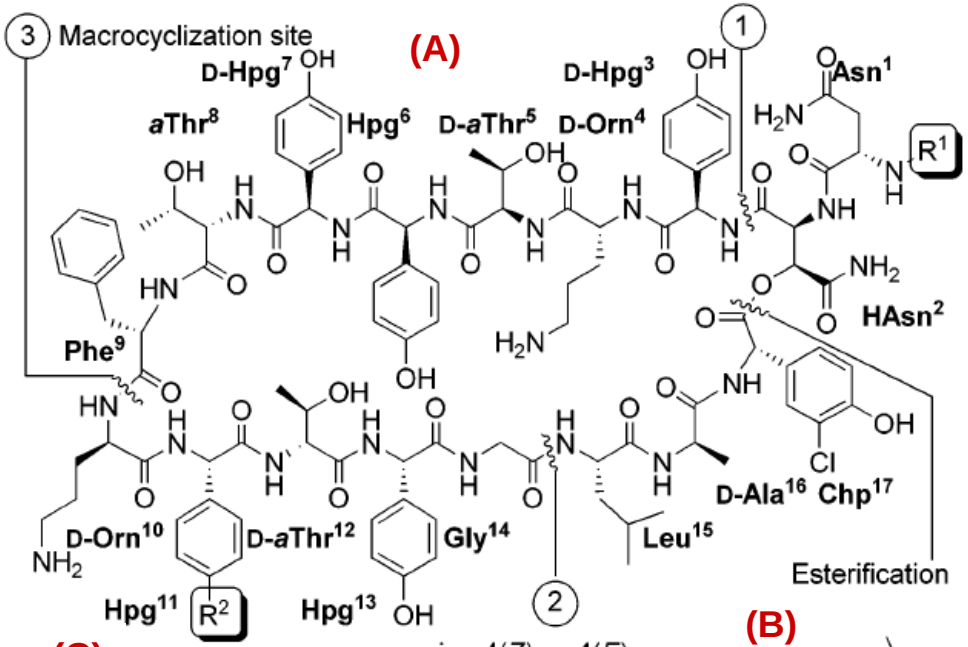
Ramoplanin (isolated first 1984 from *Actinoplanes* actinomycetes) is a nonribosomally produced cyclic peptide that sequesters lipid II at the cell membrane surface and thereby blocks transglycosylation.

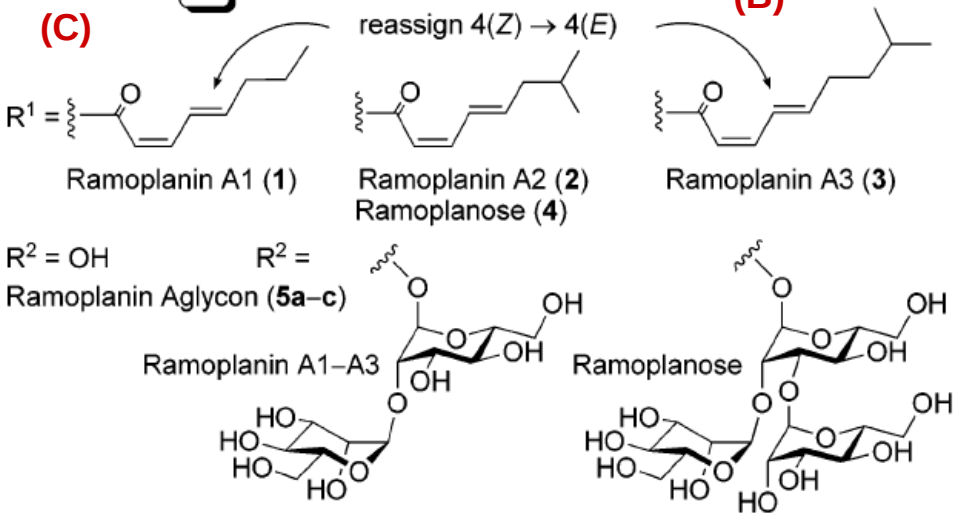
Its mode of action has been controversial (MurG has been proposed as a target before it was known that MurG is located at the cytosolic membrane side).
Its main target is lipid II. (Walker *et al.*, *Chem. Rev.* 2005, 105, 449)

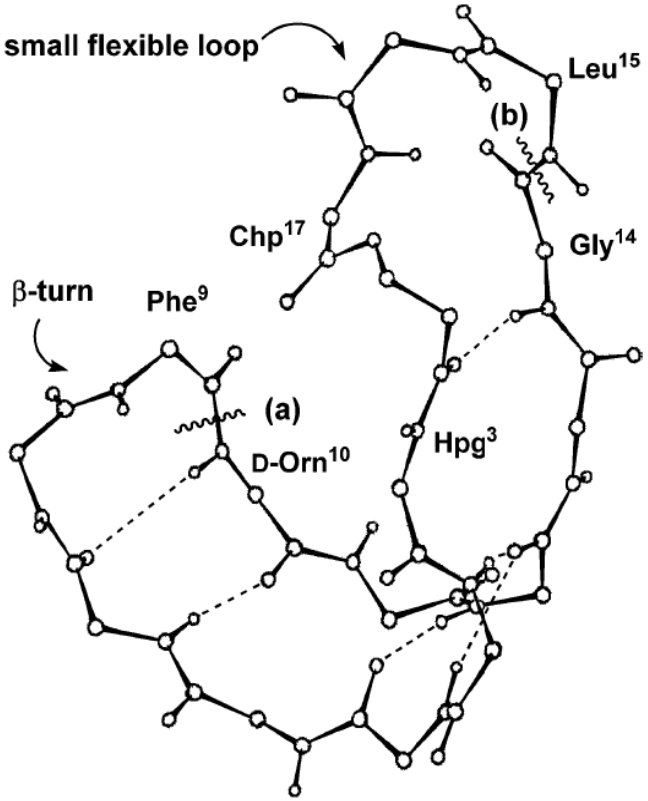


Currently in Phase III clinical trials for i.v. treatment of vancomycin-resistant enterococci (VRE).

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Ramoplanin Synthesis



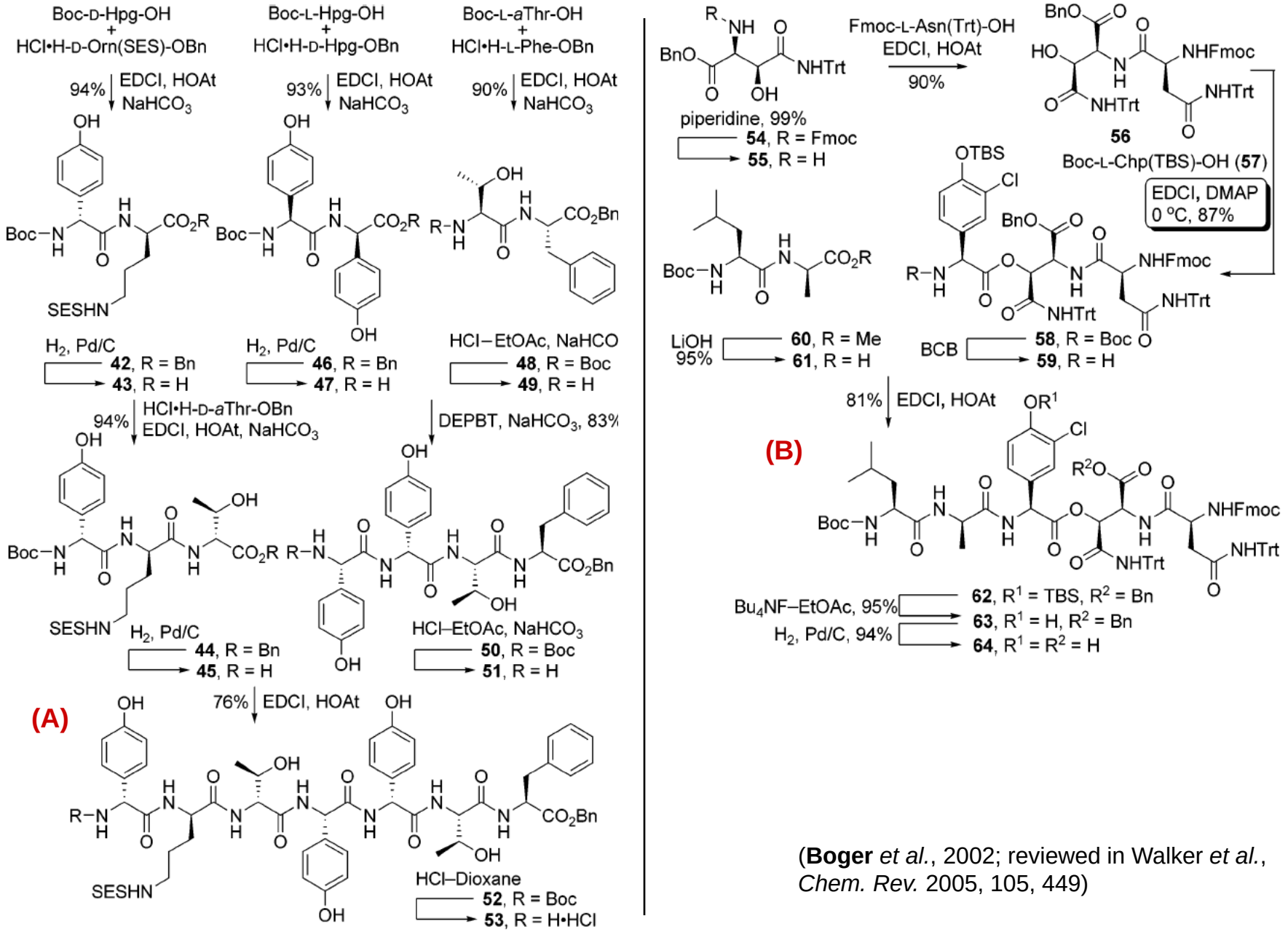




- Rigid β-Sheet conformation stabilized by intramolecular H-bonding and a cluster of aromatic side chains
- Macrolactamizations that may benefit from β-sheet preorganization of substrates
  (a) Cyclization at L-Phe^{9}–D-Orn^{10}:
  - closure at the corner of β-turn with a D-amine
  (b) Cyclization at Gly^{14}–Leu^{15}:
  - No racemization with glycine activation

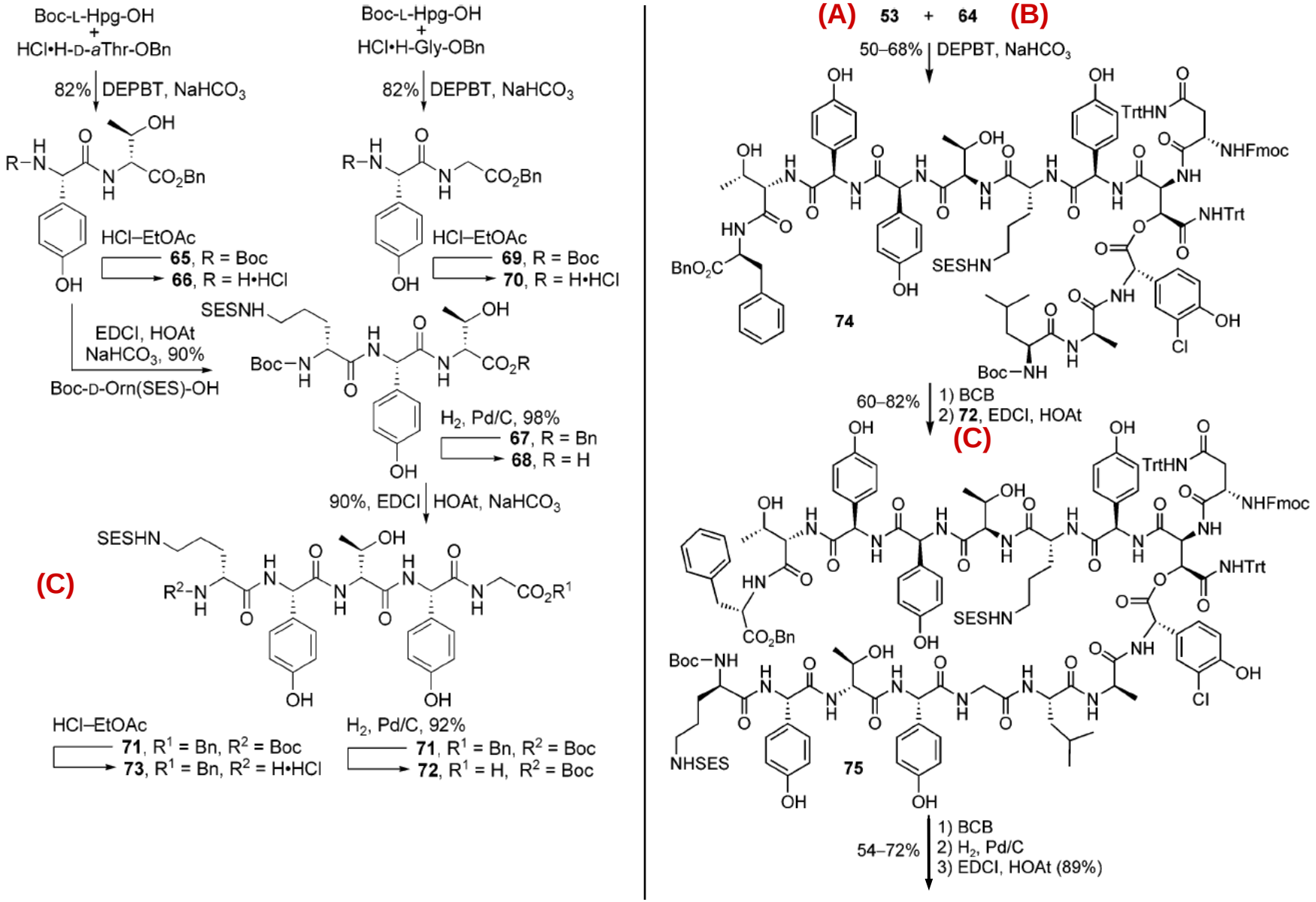
(**Boger** *et al.*, 2002; reviewed in Walker *et al.*, *Chem. Rev.* 2005, 105, 449)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Ramoplanin Synthesis



(**Boger** *et al.*, 2002; reviewed in Walker *et al.*, *Chem. Rev.* 2005, 105, 449)

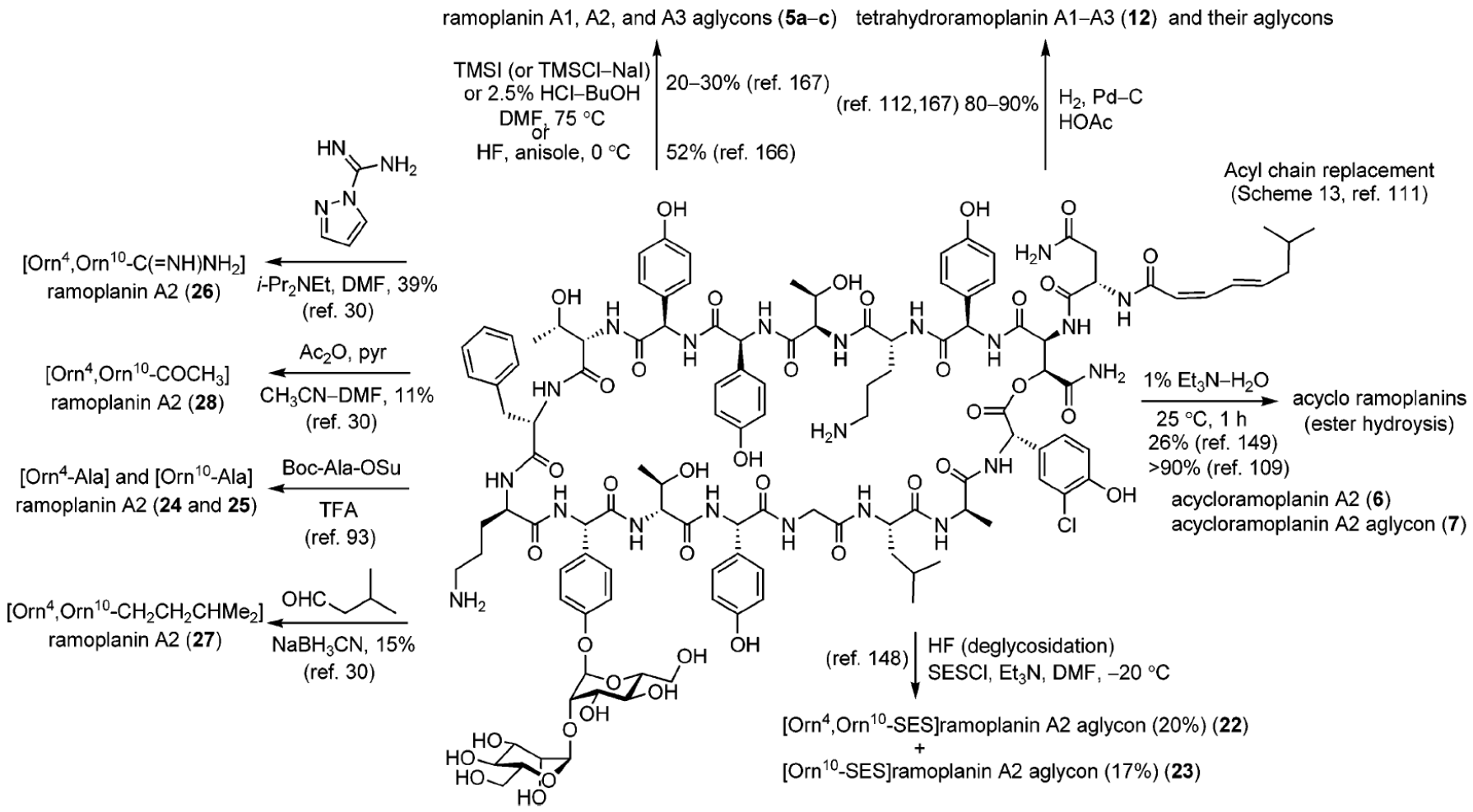
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Ramoplanin Synthesis



(**Boger** *et al.*, 2002; reviewed in Walker *et al.*, *Chem. Rev.* 2005, 105, 449)

etc.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Ramoplanin Derivatives



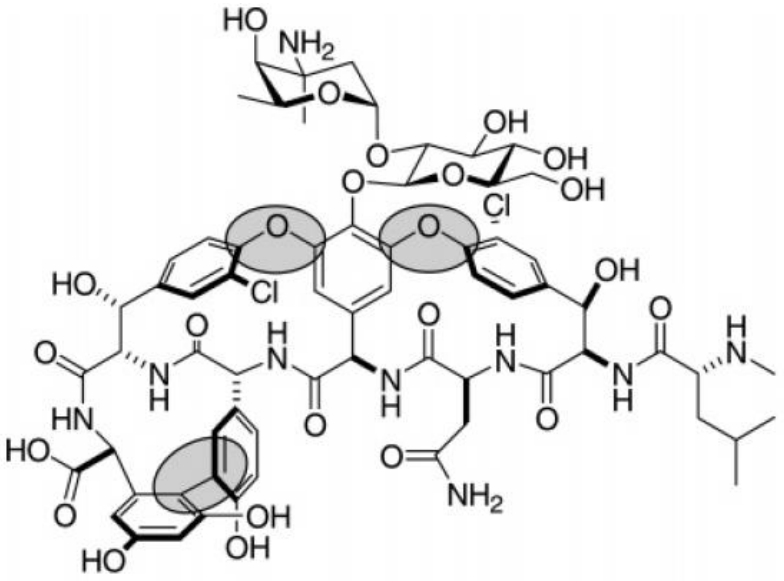
(Walker *et al.*, *Chem. Rev.* 2005, 105, 449)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Lipid II-Targeted Comp.

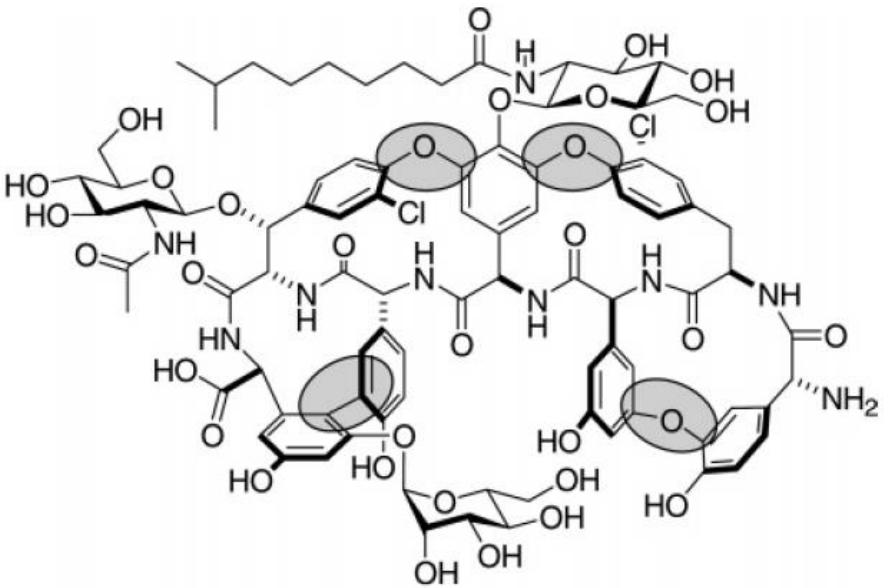
Antibiotic	Important strains	MIC (mg l^{-1})	Development stage
Nisin	*Staphylococcus aureus* *Enterococcus faecalis/faecium* Vancomycin-resistant *Enterococci* (VRE) *Streptococcus pneumoniae*	1.5–83.6 8.4–33.4 1.5–16 0.03–0.25	Preclinical
Mutacin	*S. aureus* *E. faecalis/faecium* VRE *S. pneumoniae*	0.1–18.1 1.6–25.6 6.4 0.03–6.4	Preclinical
Mersacidin	*S. aureus* *E. faecalis/faecium* VRE *S. pneumoniae*	0.78–32 32–64 Not published 2–4	Preclinical
Ramoplanin	*S. aureus* *E. faecalis/faecium* VRE *S. pneumoniae*	0.03–1.5 0.06–1 0.1–1.5 0.03–0.12	Phase III
Mannopeptimycin (AC98-6446)	*S. aureus* *E. faecalis/faecium* VRE *S. pneumoniae*	0.03–0.06 0.06–0.25 0.06–0.12 ≤0.008	Preclinical
Katanosin B	*S. aureus* *E. faecalis/faecium* VRE *S. pneumoniae*	0.39 0.78 0.78 Not published	Preclinical
Plusbacin A_3	*S. aureus* *E. faecalis/faecium* VRE *S. pneumoniae*	0.78–1.56 3.13 1.56–3.13	Preclinical

(Breukink & De Kruijff, *Nature Rev. Drug Discov.* 2006, in press)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Glycopeptides



Vancomycin



Teicoplanin

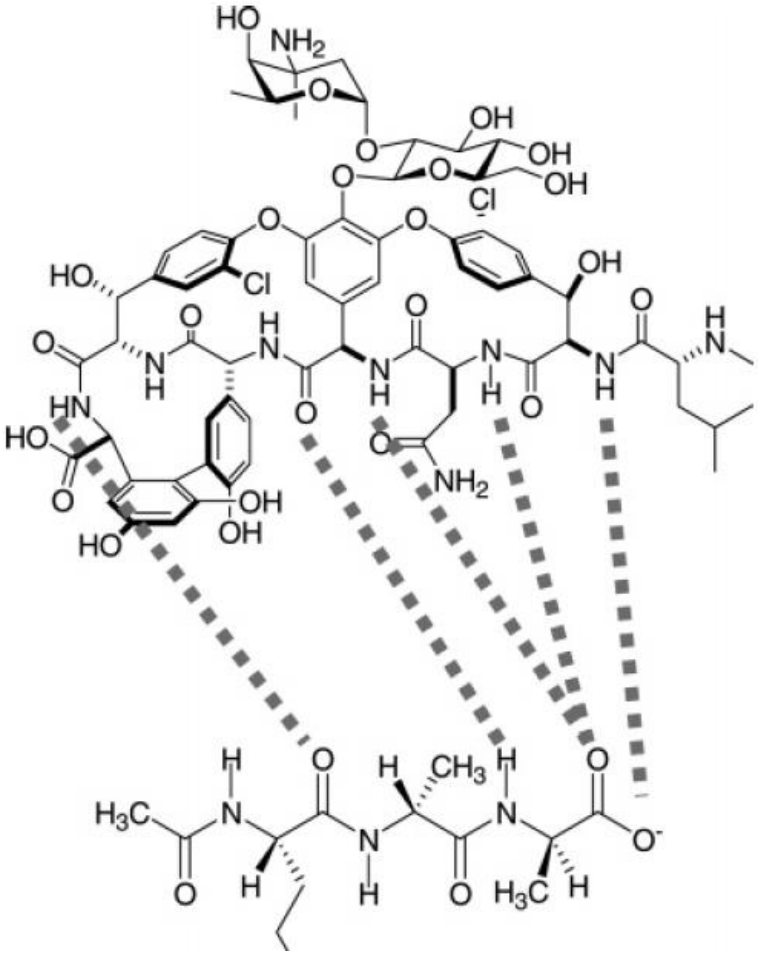
Vancomycin (isolated first ~1954 from *Amycolatopsis* actinomycetes) **and teicoplanin** (isolated first ~1978 from *Actinomplanes* actinomycetes) are nonribosomally produced cyclic peptides that contain biphenyl and biphenylether moieties produced by oxidative crosslinking.

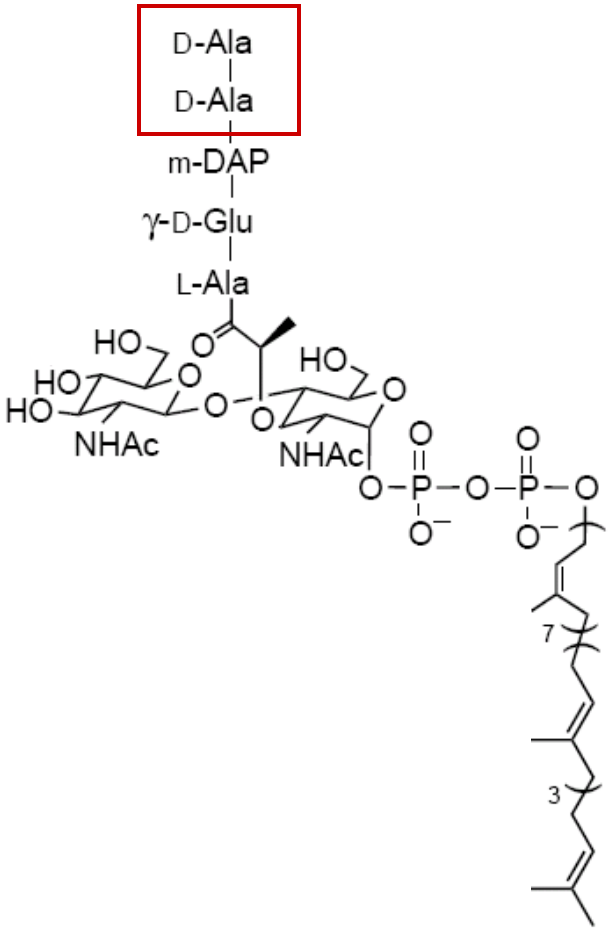
In clinical use as injectable antibiotics for serious G+ infections (*Staphylococcus*, *Enterococcus*, *Streptococcus*), specifically methicillin-resistant *S. aureus* (MRSA).

Do not penetrate the pores of the G- outer cell membrane.

Since 1986: vancomycin-resistant enterococci (VRE).

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Glycopeptides

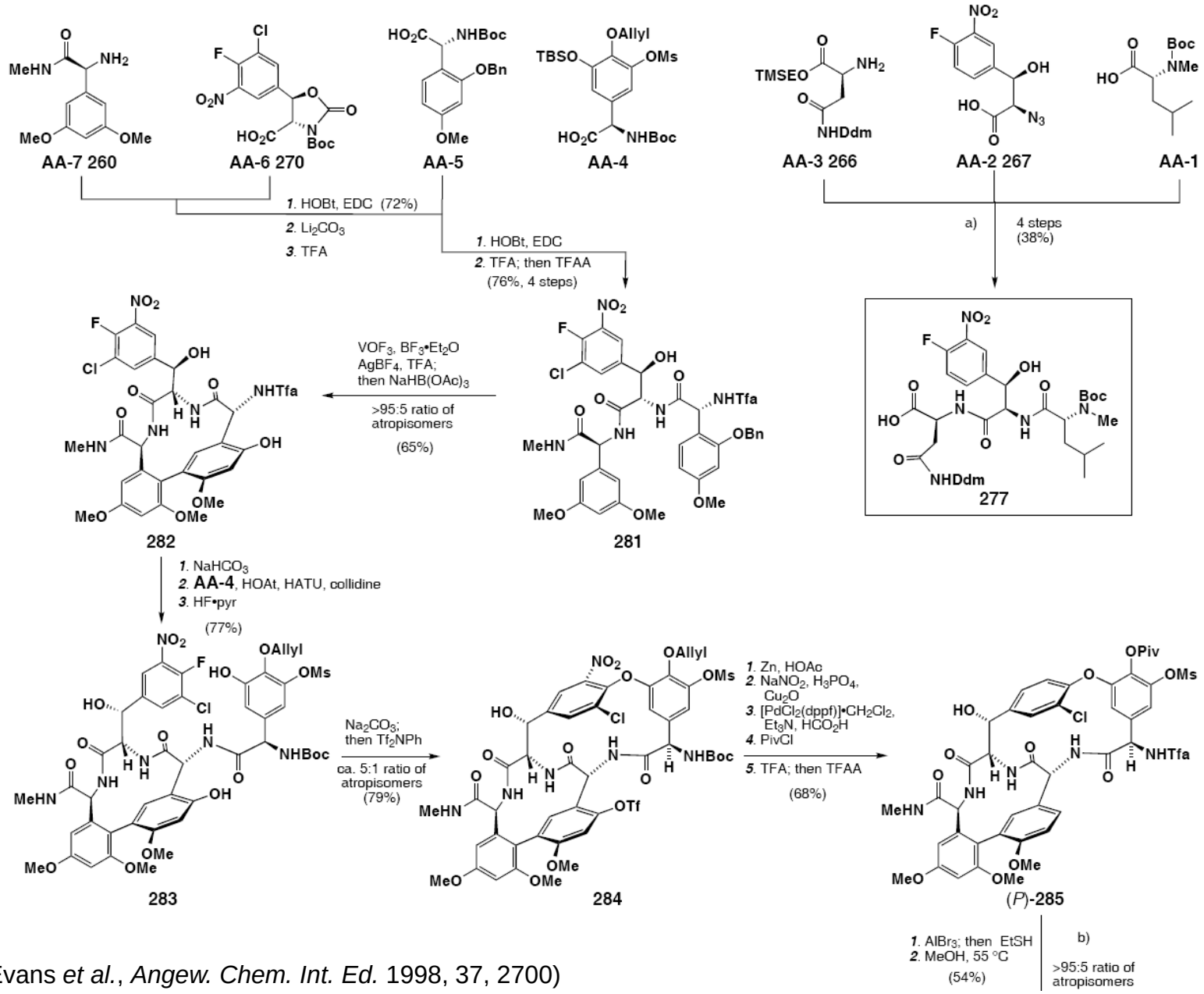




The glycopeptides block transglycosylation by binding to the D-Ala-D-Ala dipeptide module in lipid II or the growing peptidoglycan at the cell membrane surface.

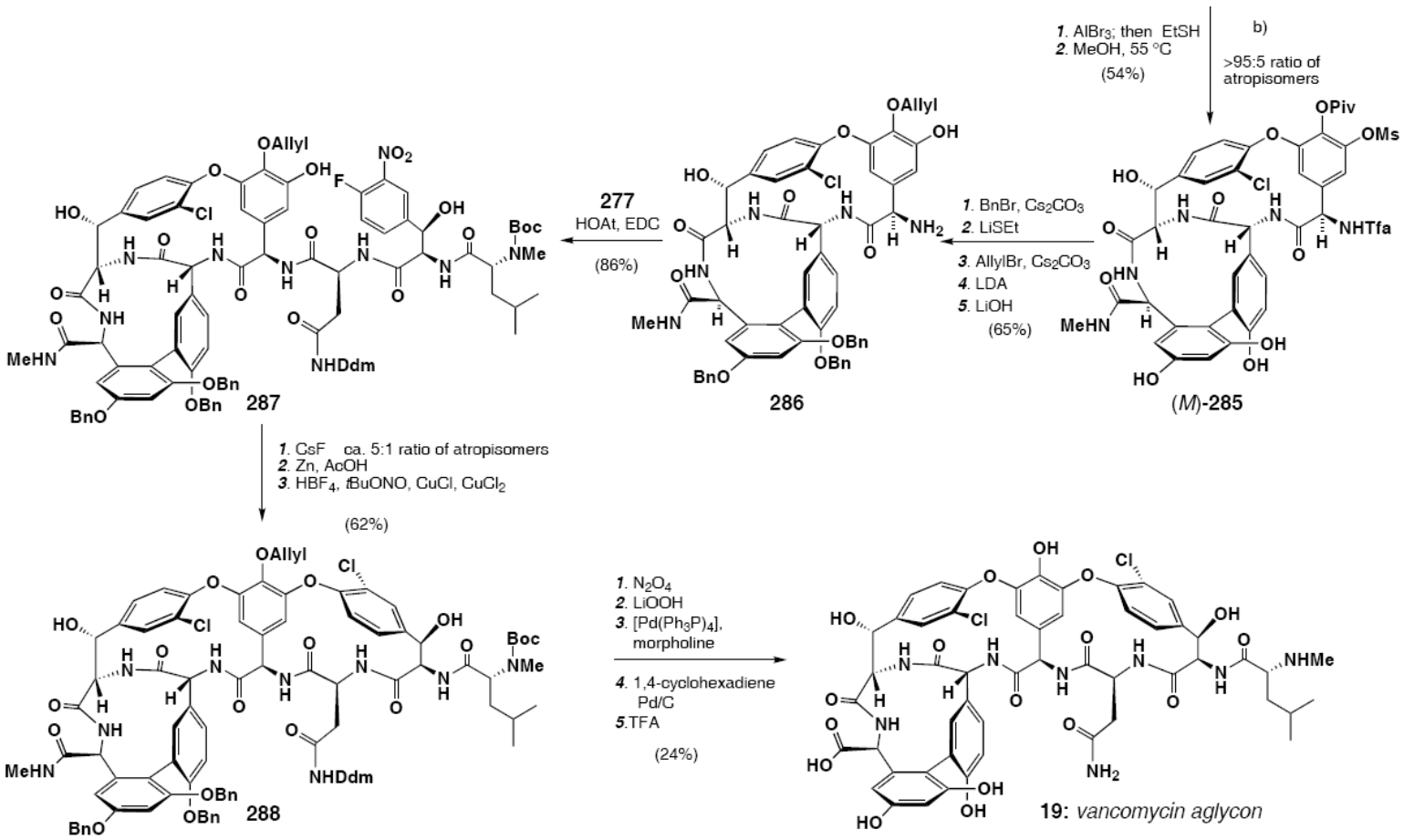
(Kahne *et al.*, *Chem. Rev.* 2005, 105, 425)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Vancomycin Synthesis



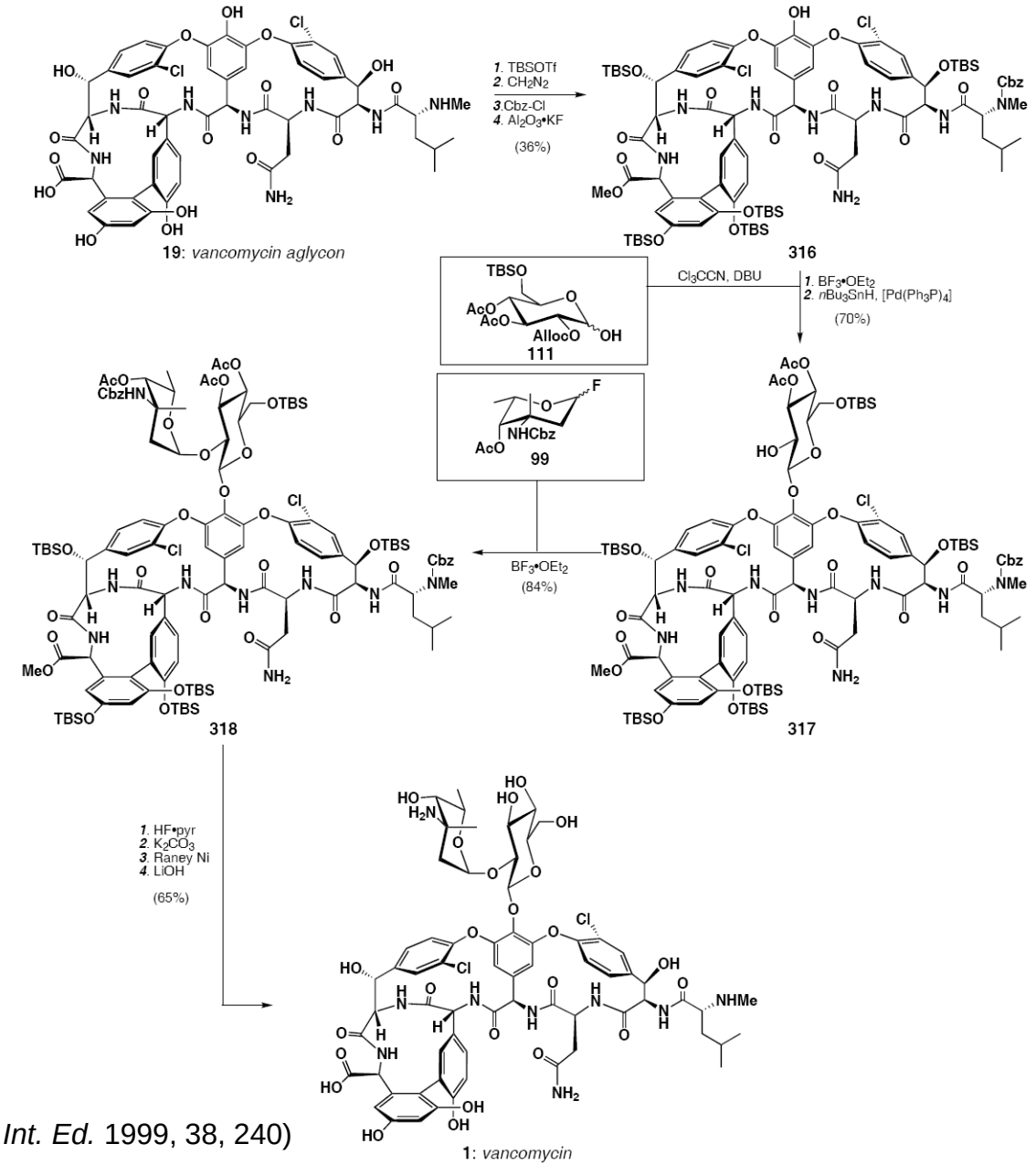
(Evans *et al.*, *Angew. Chem. Int. Ed.* 1998, 37, 2700)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Vancomycin Synthesis



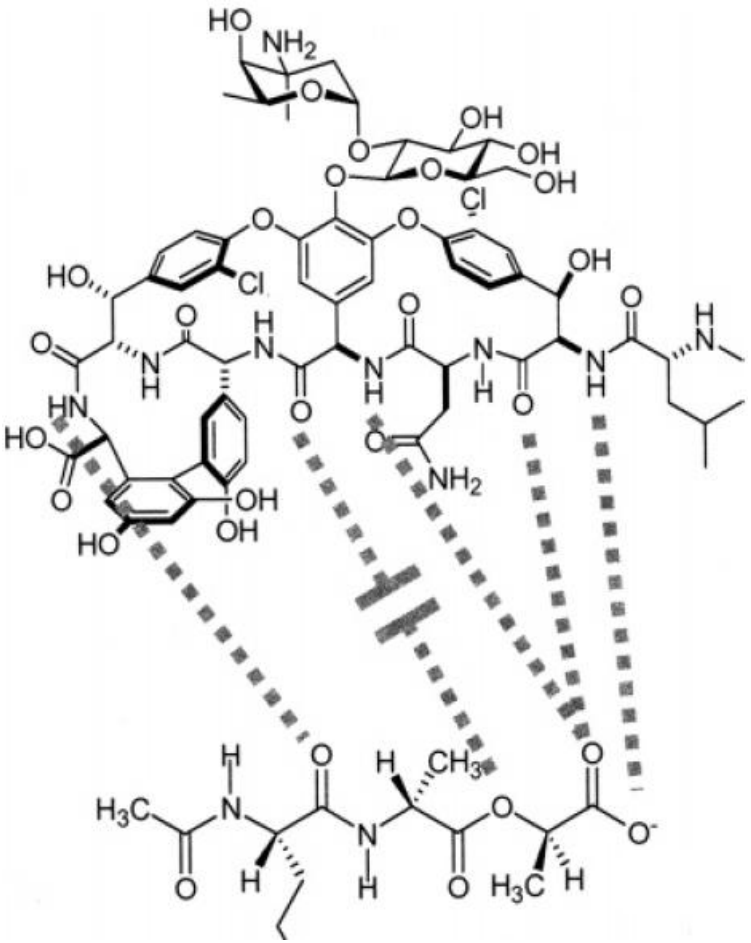
(Evans *et al.*, *Angew. Chem. Int. Ed.* 1998, 37, 2700)

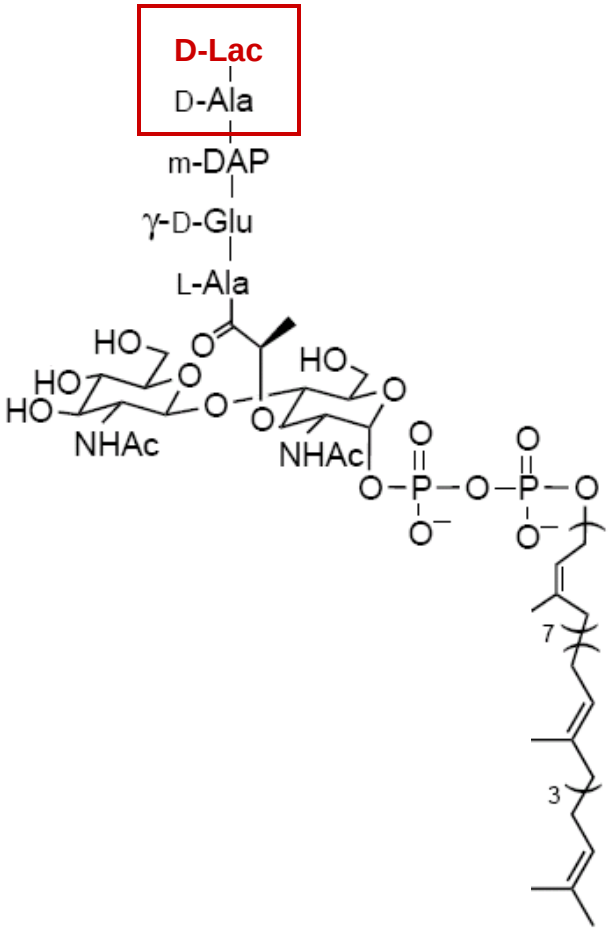
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Vancomycin Synthesis



(Nicolaou *et al.*, *Angew. Chem. Int. Ed.* 1999, 38, 240)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Glycopeptides Resistance

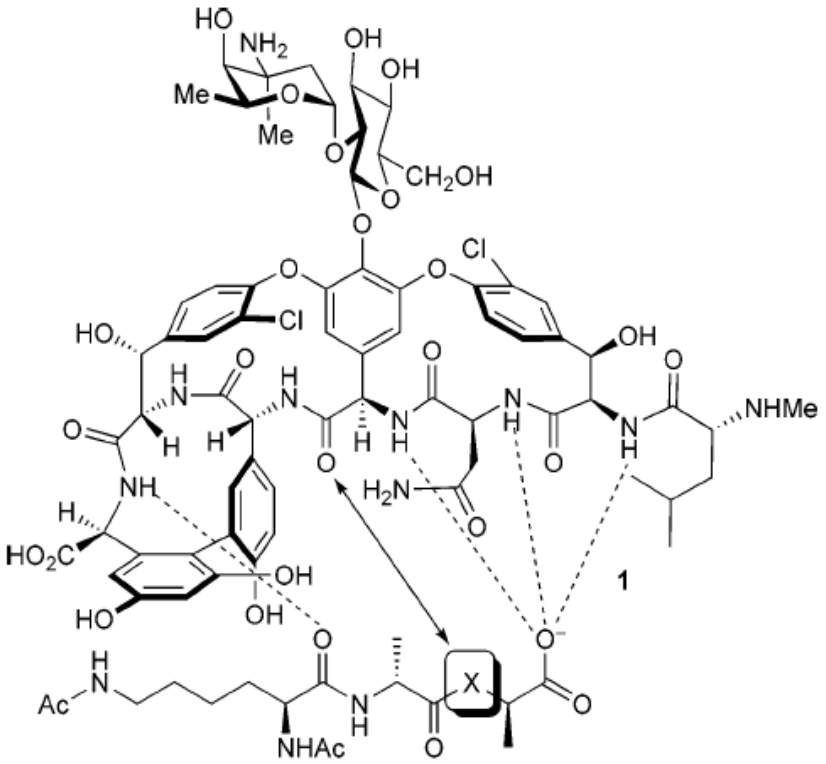


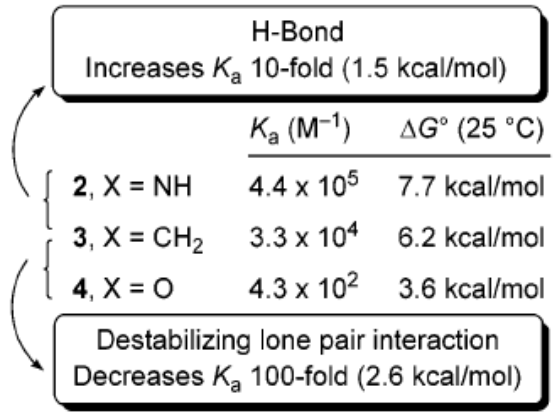


Resistance against glycopeptides develops by replacement of the terminal D-Ala by D-Lac which reduces vancomycin binding affinity ~ 1000-fold (VanA, VanB).

(Kahne *et al.*, *Chem. Rev.* 2005, 105, 425)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Glycopeptides Resistance





Reduced vancomycin binding to D-Ala-D-Lac is caused by loss of H-bond and repulsive lone-pair contributions.

(McComas *et al.*, *JACS* 2003, 125, 9314)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Vancomycin Reengineered

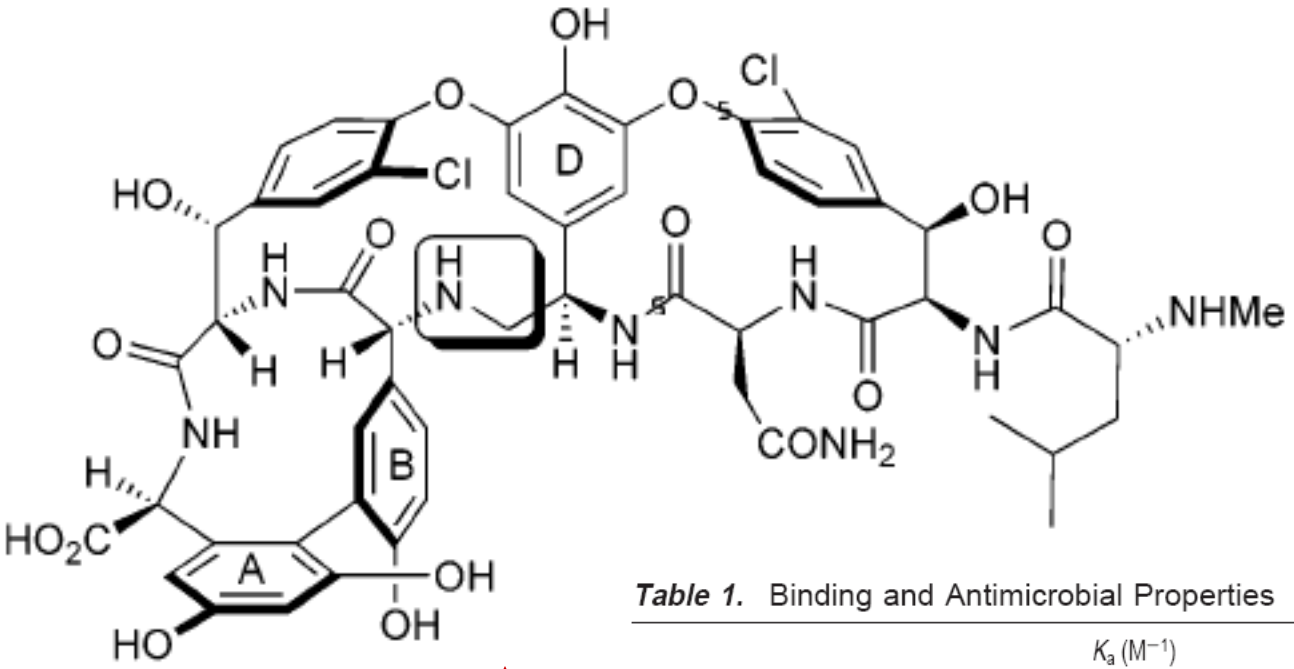


Table 1. Binding and Antimicrobial Properties

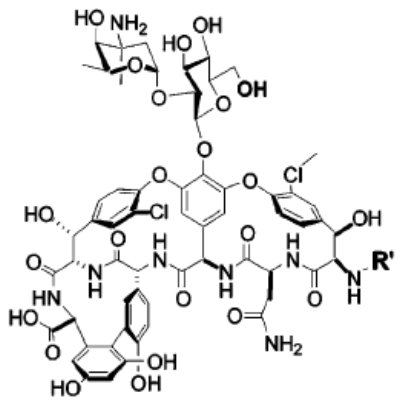
compound	K_a (M^{-1})		K_a**2**/K_a**4**	VanA,[c] MIC (μg/mL)[d]
	2[a]	**4**[b]		
1, vancomycin	2.0×10^5	1.8×10^2	1100	>500 (2000)[e]
38, vancomycin aglycon	1.7×10^5	1.2×10^2	1400	>500 (640)[e]
5	4.8×10^3	5.2×10^3	0.92	31
41	1.6×10^3	4.1×10^3	0.40	31

[a] Ac_2-L-Lys-D-Ala-D-Ala. [b] Ac_2-L-Lys-D-Ala-D-Lac. [c] *Enterococcus faecalis* (VanA, BM4166). [d] Vancomycin and vancomycin aglycon exhibit MICs of 1−2.5 μg/mL against wild-type *E. faecalis*. [e] Taken from ref 25.

(Crowley & Boger, *JACS* 2006, 128, 2885)

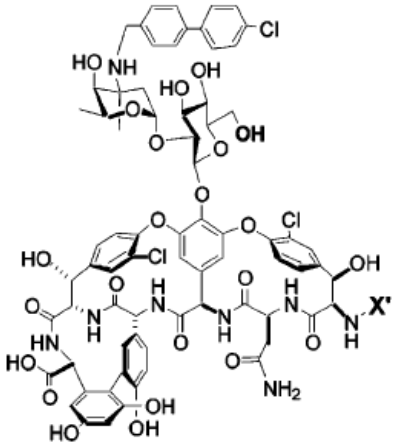
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Glycopeptides

Table 2. MICs for a Series of Glycopeptides Derivatives against Sensitive and Resistant Strains[a]



Vancomycin (R' = *N*-Me-D-Leu)

Damaged Vancomycin (R' = H)



Chlorobiphenyl Vancomycin (X' = N-Me-D-Leu)

Damaged Chloro-biphenly Vancomycin (X' = H)

glycopeptide	MIC (μg/mL) sensitive *E. faecium*	MIC (μg/mL) resistant *E. faecium*
vancomycin	1	2048
chlorobiphenyl vancomycin	0.03	16
damaged vancomycin	no activity	no activity
damaged chlorobiphenyl vancomycin	10	40

[a] Compounds lacking the *N*-terminal methylleucine amino acid were used to evaluate which component of the activity derives from a peptide-binding-independent mechanism.

Second generation vancomycin derivatives, such as chlorobiphenyl vancomycin, can overcome some vancomycin-resistance mechanisms.

There is no evidence that these derivatives can bind to the D-Ala-D-Lac peptide.

(Kahne *et al.*, *Chem. Rev.* 2005, 105, 425)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Glycopeptides

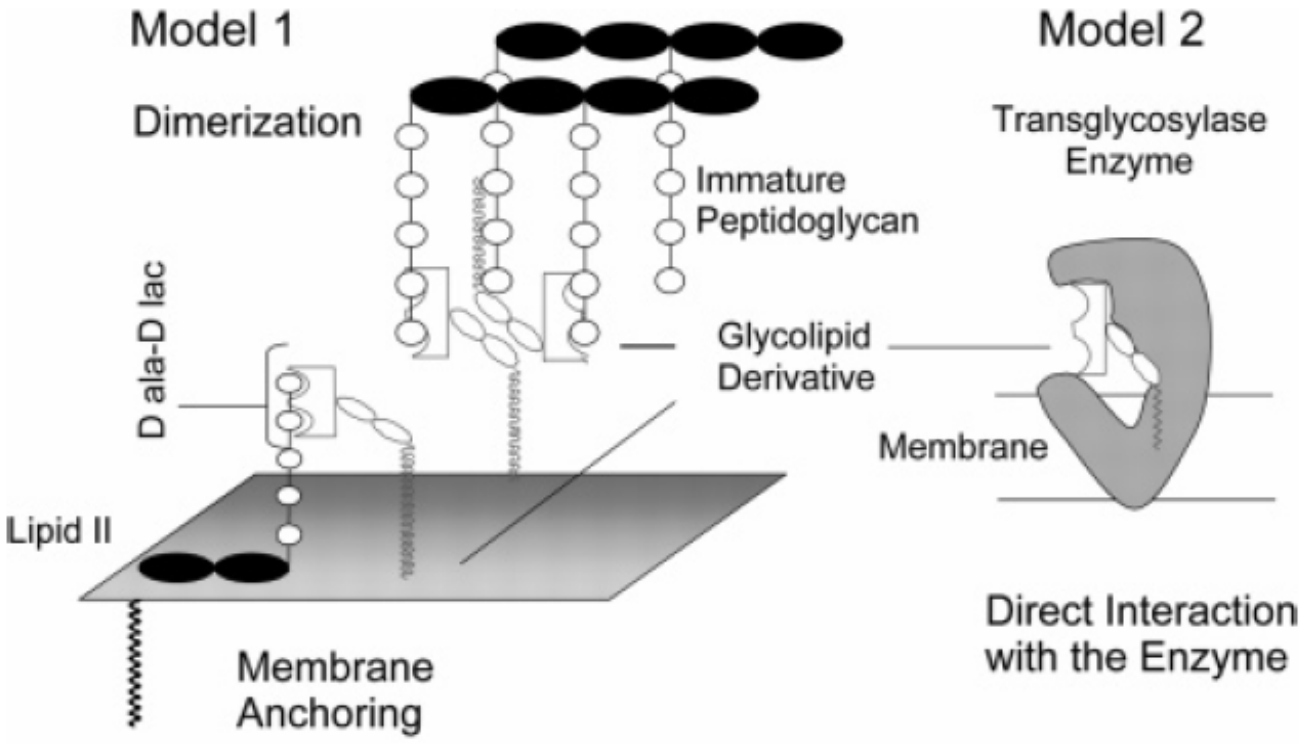
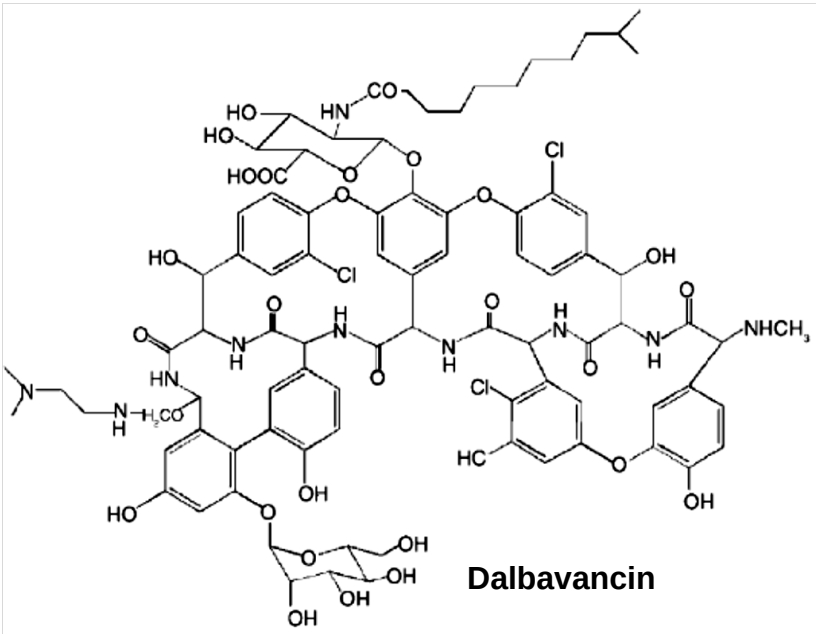


Figure 22. Two mechanisms of action for glycopeptides: (a) inhibition of the transpeptidase step by binding to the D-Ala-D-Ala terminus and (b) direct inhibition of the transglycosylases.

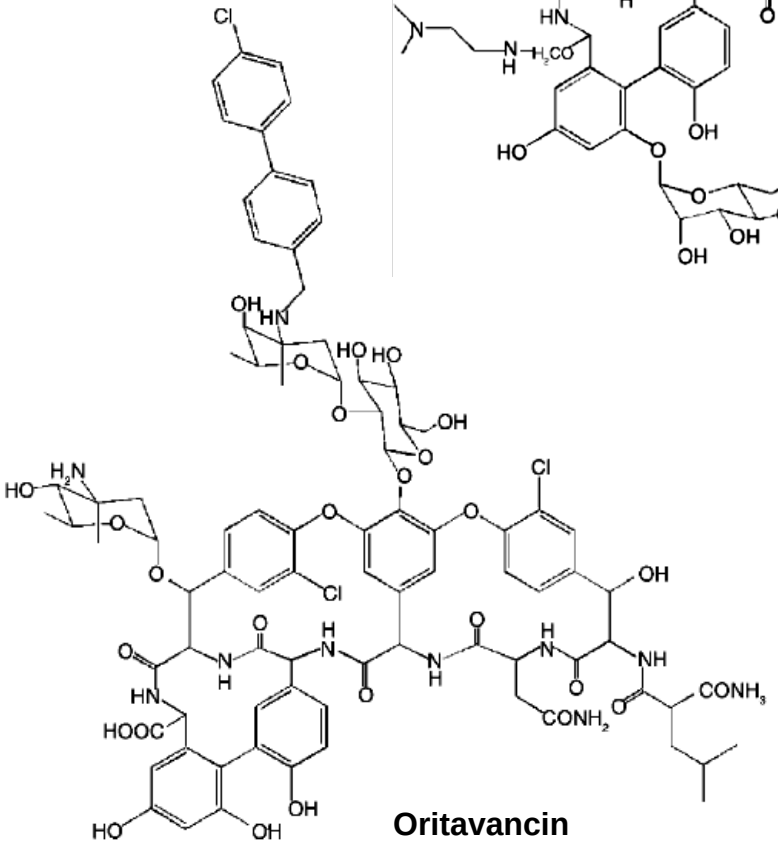
The increased potency of chlorobiphenyl vancomycin and related derivatives may be based on dimerization, or membrane anchoring, or direct inhibition of transglycosylase.

(Kahne *et al.*, *Chem. Rev.* 2005, 105, 425)

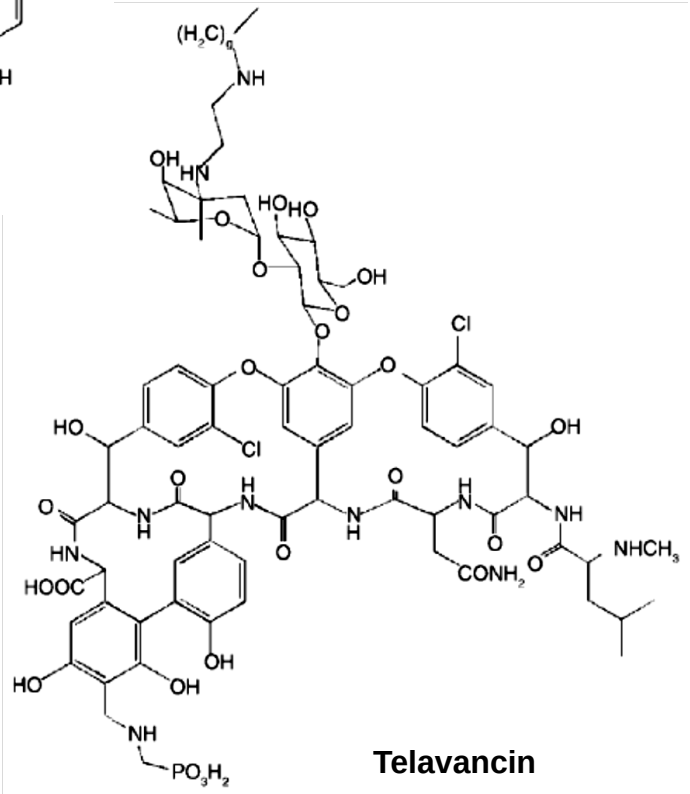
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Glycopeptides 2nd Gen.



Dalbavancin

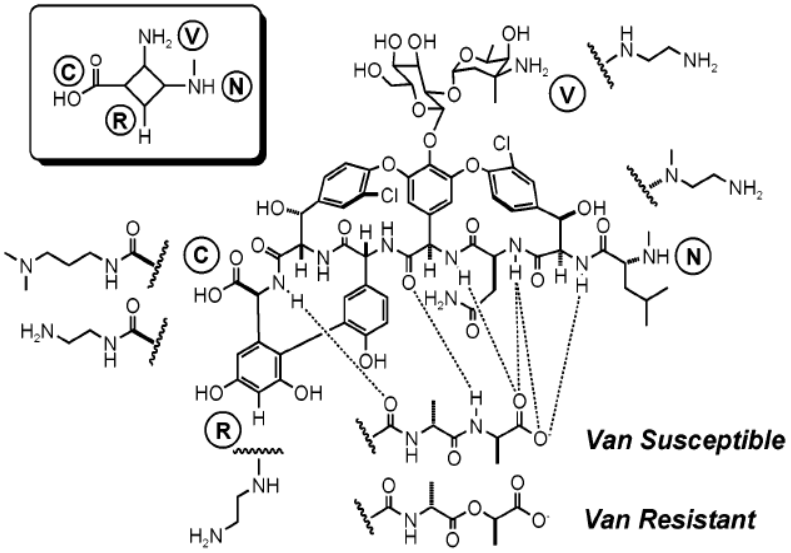


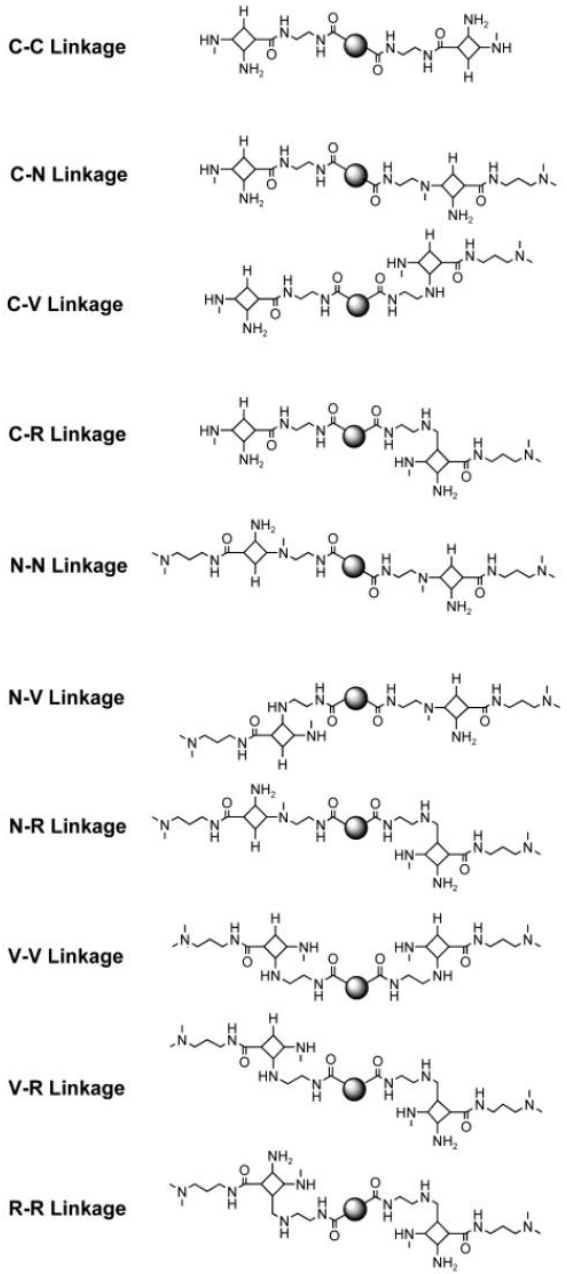
Oritavancin



Telavancin

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Vancomycin Dimers



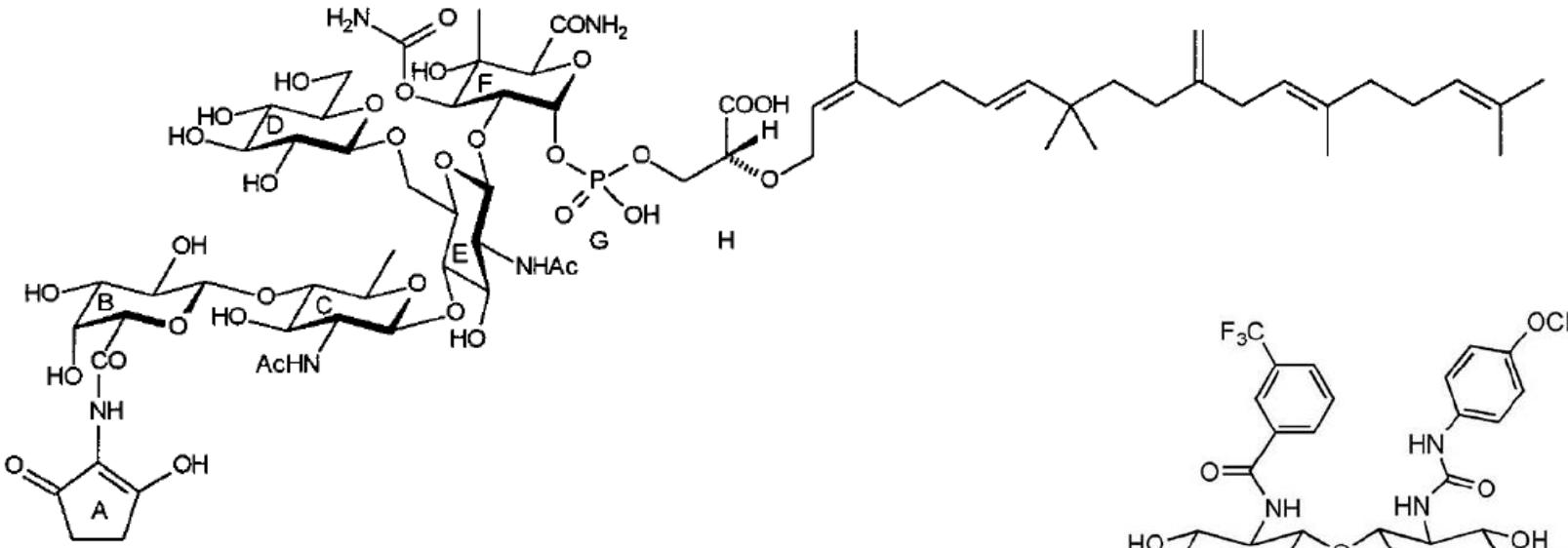


Covalently linked dimers have been systematically prepared.

These compounds do exhibit greater target affinity and antibacterial activity, and in some cases recovered activity against vancomycin-resistant bacteria

(Griffin *et al.*, *JACS* 2003, 125, 6517)

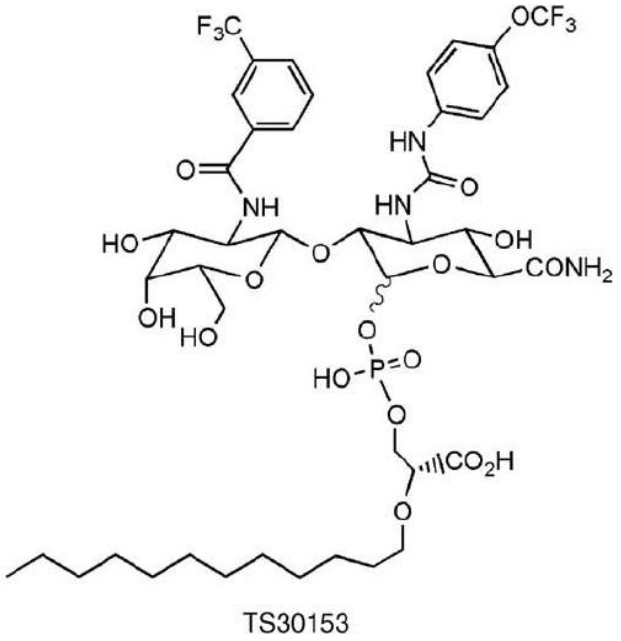
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Transglycosylation



Moenomycin A (isolated first ~1965 from *Streptomyces ghanaensis*), an antibiotic lipid-saccharide that is used in veterinary medicine (active only against G+ bacteria).

Inhibits directly the transglycosylase activity of penicillin-binding proteins (PBP1b and 1 transglycosylases in *E. coli*) by competing with the growing peptidoglycan chain and perhaps with lipid II binding.

(Halliday *et al.*, *Biochem. Pharmacol.* 2006, 71, 957)



TS30153

Moenomycin-like disaccharide inhibitor of TG. (discovered out of a combinatorial library of ~ 1300 compounds)

(Sofia *et al.*, *J. Med. Chem.* 1999, 42, 3193)

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Transglycosylation

Table 2 – Summary of inhibitors of the transglycosylation process

Compound	Class	Proposed mode of action	*S. aureus*			*E. faecium*		*E. faecalis*		*S. pneum*
			MSSA	MRSA	VISA	VSE	VRE	VSE	VRE	PR
Vancomycin	Glycopeptide	Binds to lipid II	0.13–1.0	0.5–4.0	8.0	0.25–4.0	>128[a]	0.25–4.0	>128	0.25–2.0
Oritavancin	Semi-synthetic glycopeptide	Dimerization, hydrophobic anchoring and direct TG binding	0.13–1.0	0.13–4.0	1.0–8.0	0.06–0.25	0.06–1.0[a]	0.06–0.25	0.06–1.0	0.002–0.06
Dalbavancin	Semi-synthetic glycopeptide	Dimerization, hydrophobic anchoring and direct TG binding	0.03–0.5	0.06–1.0	2.0	0.06–0.13	0.5–128[a]	0.06–0.13	0.5–128[a]	0.008–0.13
Telavancin	Semi-synthetic glycopeptide	Multiple. Lipid II binding and membrane depolarisation	0.5	0.5–1.0	2.0	0.5	4–8[a]	0.5	4–8[a]	–
Ramoplanin	Glycolipodepsi-peptide	Binds lipids I and II. Fibril formation and TG inhibition	0.5–1.56	<2.0	–	0.1	<1.0	0.1	<1.0	<2.0
AC98-6446	Cyclic glycopeptide	Binds lipid II but not at vancomycin region	0.03–0.06	0.015–0.06	0.015–0.06	0.06–0.25	0.06–0.12	0.06–0.25	0.06–0.12	0.008
Mersacidin	Lantibiotic (type B)	Binds to lipid II	12.5	12.5	–	25	25	–	–	–
Moenomycin A	Natural product glycolipid	Direct TG enzyme binding	0.05	0.062	–	>200	0.39–1.56	0.078	0.062	–
TS3O153 (23)	Disaccharide	Direct TG enzyme binding	6.25	6.25	–	6.25	6.25–12.5	6.25	6.25–25.0	–
ACL 19273	Disaccharide	Direct TG enzyme binding	1.0	4.0[b]	–	4.0[b]	4.0[b]	4.0[b]	4.0[b]	–

MIC's are in μg/mL. MSSA/MRSA are methicillin-susceptible and methicillin-resistant *S. aureus*, respectively, and VISA is vancomycin intermediate-resistant *S. aureus*. *S. pneum* PR resistant *S. pneumoniae*. VSE/VRE are vancomycin-susceptible/resistant *enterococci*. Telavancin figures are MIC_{90} all others MIC range.

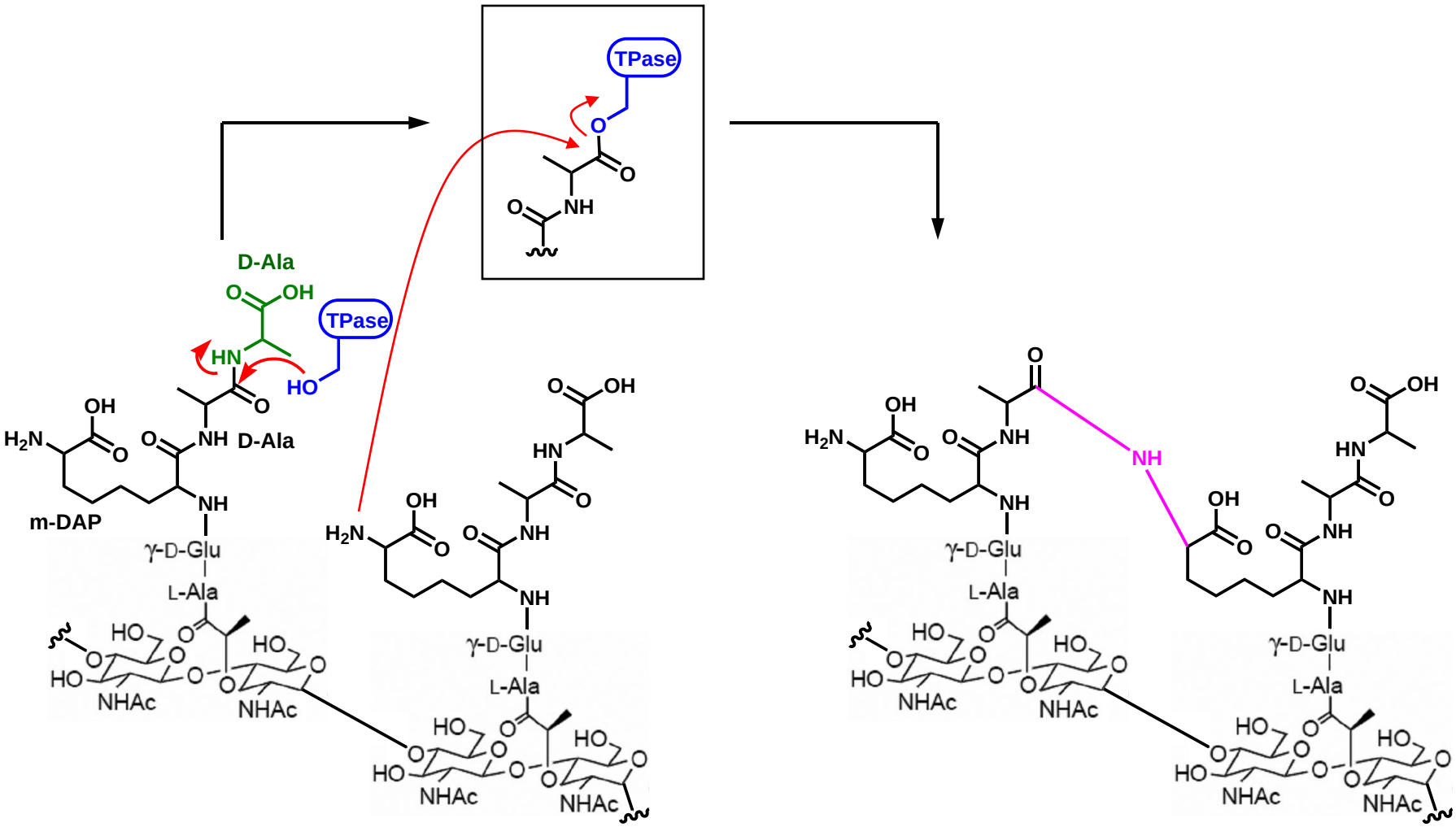
[a] VanA-resistant species.

[b] Based on complete inhibition of bacterial growth for a broad range of Gram-positive organisms at single concentration on agar plate.

(Halliday *et al.*, *Biochem. Pharmacol.* 2006, 71, 957)

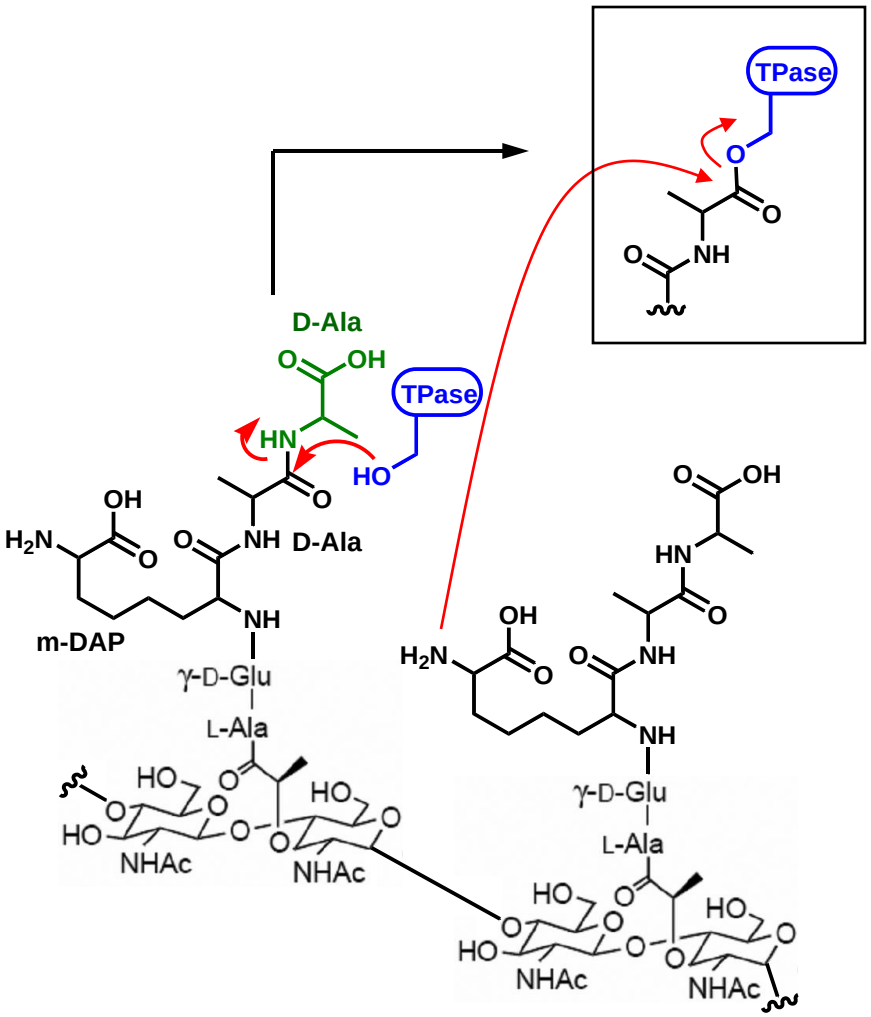
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Transpeptidation

Peptidoglycan crosslinking:

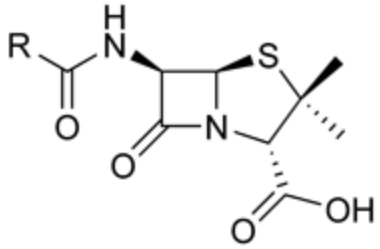


Bacterial Cell Wall: Peptidoglycan Biosynthesis – β-Lactam Antibiotics

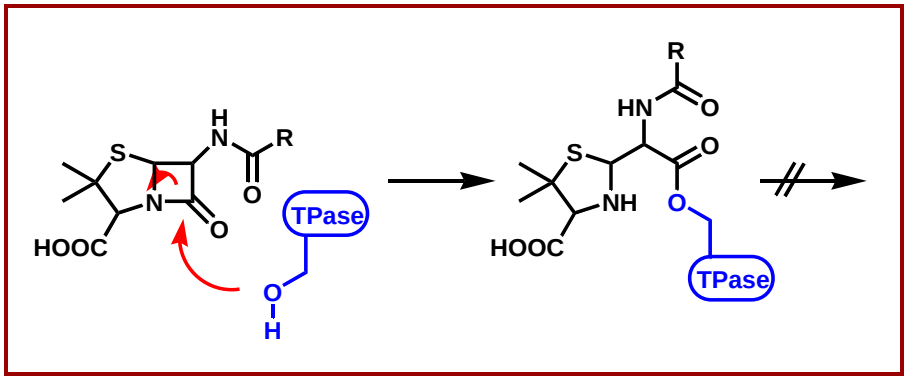
Peptidoglycan crosslinking:



β-Lactam antibiotics are suicide substrates of PG transpeptidases:



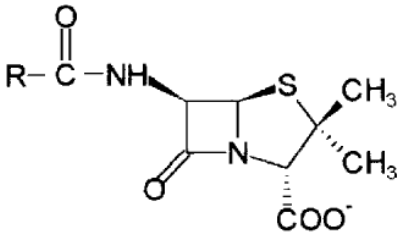
Penicillin



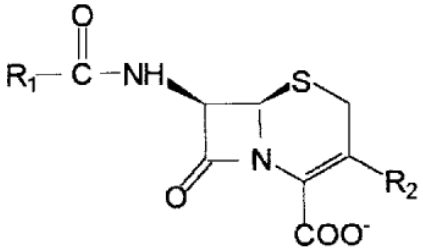
β-Lactamases can reduce the efficacy of β-lactam antibiotics, leading to drug resistance.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – β-Lactam Antibiotics

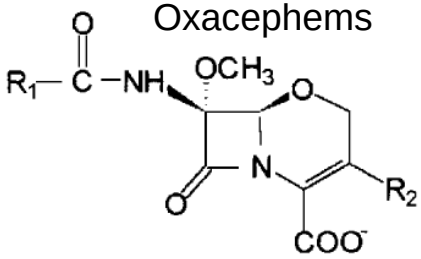
Penicillins (Penams)



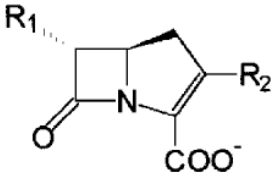
Cephalosporins (Cephems)



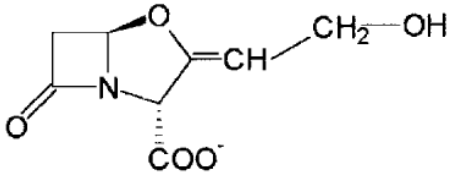
Oxacephems



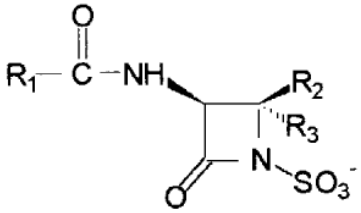
Carbapenems

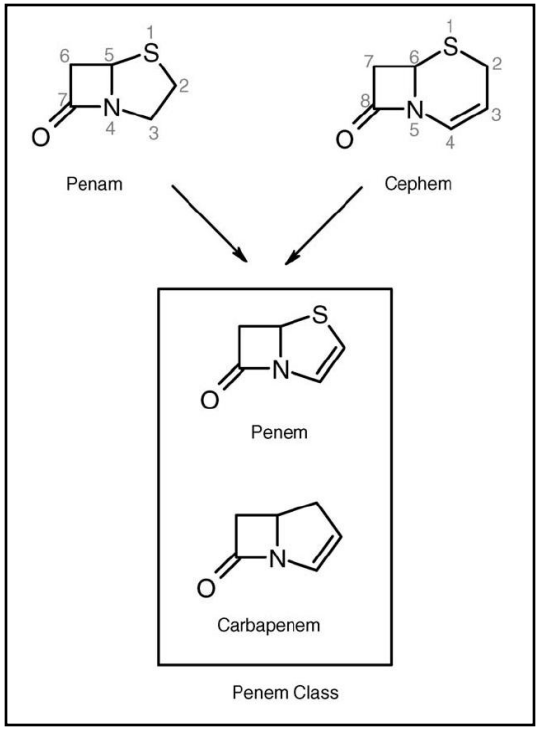


Clavulanate



Monobactams





β-Lactam Antibiotics Biosynthesis

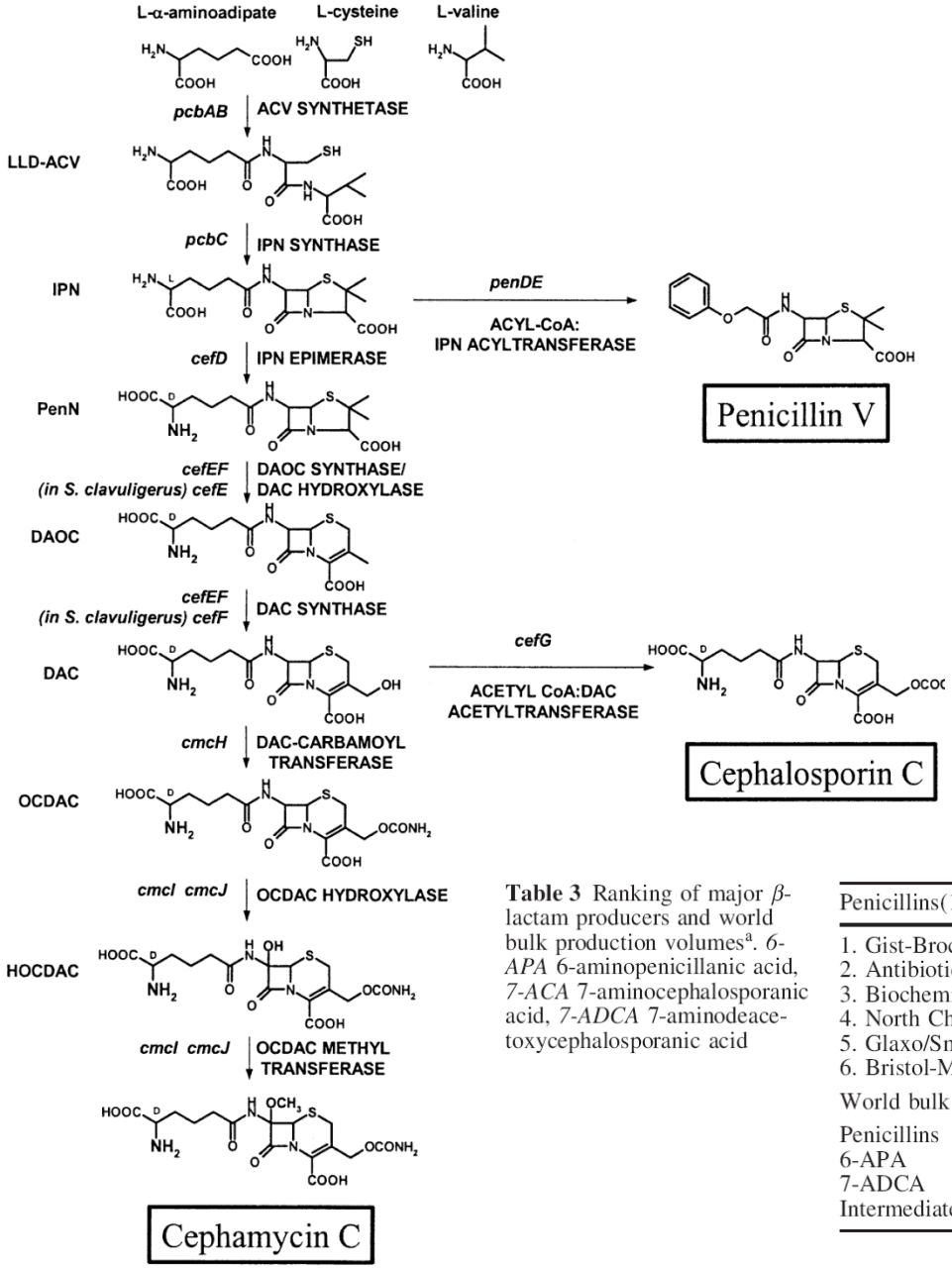
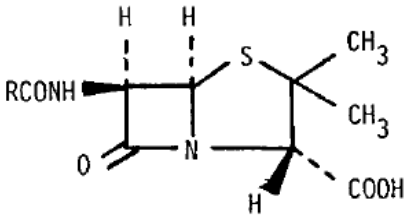


Table 3 Ranking of major β-lactam producers and world bulk production volumes[a]. *6-APA* 6-aminopenicillanic acid, *7-ACA* 7-aminocephalosporanic acid, *7-ADCA* 7-aminodeacetoxycephalosporanic acid

Penicillins(1995)		Cephalosporins (1999)	
1. Gist-Brocades		1. Antibioticos SpA	
2. Antibioticos SpA		2. Biochemie/Hoechst	
3. Biochemie		3. Glaxo/Wellcome	
4. North China Pharma Works		4. Fujisawa	
5. Glaxo/SmithKline		5. Cheil Jedang (Korea)	
6. Bristol-Myers Squibb		6. Bristol-Myers Squibb	
World bulk production volumes (metric tons)			
Penicillins	~33,000	Cephalosporin C	~4,300
6-APA	~8,800	7-ACA	~2,140
7-ADCA	~1,950		
Intermediates	~2,130		

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Natural Penicillins (PCN)



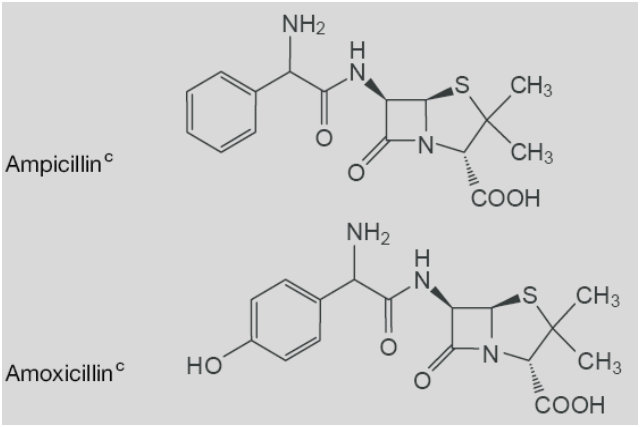
Generic Name	R (N-Acyl side chain)
Penicillin G	$C_6H_5CH_2$-
Penicillin F	$CH_3CH_2CH{=}CHCH_2$-
Dihydropenicillin F	$CH_3(CH_2)_4$-
Penicillin K	$CH_3(CH_2)_6$-
Penicillin X	p-OH-C_6H_4-CH_2-
Penicillin V	$C_6H_5OCH_2$-
Penicillin N	HO_2C-$CH(NH_2)(CH_2)_3$- (D)
Isopenicillin N	HO_2C-$CH(NH_2)(CH_2)_3$- (L)
KPN	HO-$(CH_2)_3$-

- PCN G (IV/IM; $12/day)
- PCN V (Oral; $0.50/day)
- Active against *Streptococcus*, *peptostreptococcus, Bacillus anthracis*, *Actinomycosis*, *Corynebacterium*, *Listeria*, *Neisseria* & *Treponema*.
- Used for common oral infections.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Semisynthetic Penicillins

Aminopenicillins

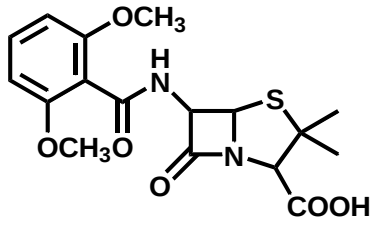
- Ampicillin (IV; $2/day)
- Ampicillin/sulbactam (Unasyn; IV; $30/day)
- Amoxicillin (Oral; $0.30/day).
- Amoxicillin/clavulanate (Augmentin; $7/day)
- Sulbactam and clavulanic acid are β-lactamase inhibitors and increase activity against lactamase-producing organisms.
- Extended antimicrobial spectrum.
  - Gram negatives: *E. coli*, *Proteus*, *Salmonella*, *Haemophilus, M. catarrhalis, Klebsiella, Neisseria, Enterobacter, Bactoroides*.
- Used as first line therapy for acute otitis media and sinusitis.



Bacterial Cell Wall: Peptidoglycan Biosynthesis – Semisynthetic Penicillins

Anti-staphylococcal penicillins

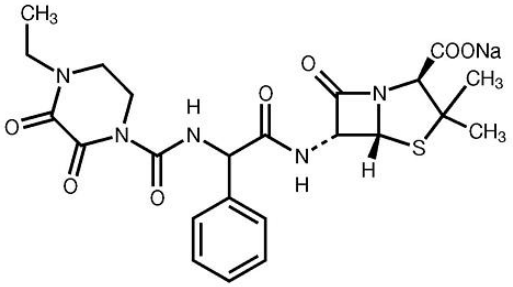
- Methicillin, nafcillin, oxacillin, cloxacillin and dicloxacillin.
- Resist degradation by penicillinase.
- Useful for treating *S. aureus.*
  - No added benefit in treating *Strep*. species.
- Methicillin is rarely used due to toxicity.
- Dicloxacillin ($0.9/day) - highest serum levels orally.
- Nafcillin ($15/day) - preferred parenteral drug.



Methicillin

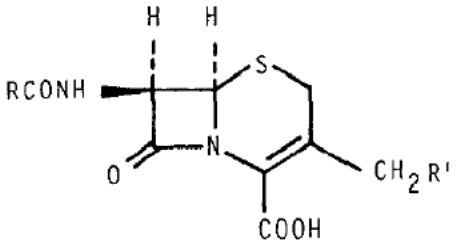
Anti-pseudomonal penicillins

- Ticarcillin, Piperacillin ($49/day), Mezlocillin.
- Piperacillin/tazobactam (Zosyn; IV; $53/day)
  - Tazobactam (β-lactamase inhibitor)
- Ticarcillin/clavulanate (Timentin; IV; $39/day)
- Active against *Pseudomonas*, *E. coli, Klebsiella, Enterobacter, Serratia and Bacillus fragilis*.
- Lower activity against gram positives
- Often used with aminoglycosides when treating pseudomonal infections.



Piperacillin

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Cephalosporins



Generic Name	R	R'	Refere
Cephalosporin C	$HO_2C-CH(NH_2)(CH_2)_3-$	$-OCOCH_3$	5
Deacetyl-cephalosporin C	$HO_2C-CH(NH_2)(CH_2)_3-$	$-OH$	42
Deacetoxy-cephalosporin C	$HO_2C-CH(NH_2)(CH_2)_3-$	$-H$	52
A 16886 A	$HO_2C-CH(NH_2)(CH_2)_3-$	$-OCONH_2$	93
C 43219	$HO_2C-CH(NH_2)(CH_2)_3-$	$-SC(CH_3)_2CH(NH_2)CO_2H$	68
F-1	$HO_2C-CH(NH_2)(CH_2)_3-$	$-SCH_3$	66
N-Acetyl-deacetoxy-cephalosporin C	$HO_2C-CH(NH-COCH_3)-(CH_2)_3-$	$-H$	121
Compound a	$HO_2C-(CH_2)_3-$	$-H$	75
b		$-OH$	
c		$-OCOCH_3$	

- Semisynthetic β-lactams derived from chemical side chains added to 7-aminocephalosporanic acid.
- Generally more resistant to β-lactamases.
- 4 Generations developed so far.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – Cephalosporins

1st Generation

- Cefazolin (Ancef; IV; $10/day), Cephalexin (Keflex; Oral; $0.8/day)
- Spectrum: Most gram positive cocci (*Streptococcus*, *S. aureus*), *E. coli, Proteus, Klebsiella*.
- Used against *S. aureus*, for surgical prophylaxis.

2nd Generation

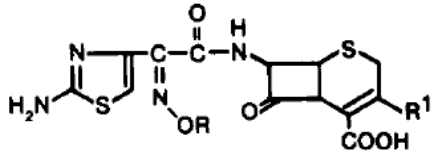
- Cefuroxime (Ceftin; IV $8/day; Oral $14/day)
- Increased activity against *Haemophilus influenzae, Enterobacter, Neisseria, Proteus, E. coli, Klebsiella, M. catarrhalis, B. fragilis*.
- Not as effective against *S. aureus* as the 1st generation.

3rd Generation

- Spectrum: gram negative > gram positive.
- Ceftriaxone (Rocephin; IM/IV; $24/day), Cefotaxime ($12/day).
  - Useful for meningitis.
  - Ceftriaxone used for highly resistant and multi drug resistant streptococcal pneumonia along with vancomycin.

4th Generation

- Cefepime (IV; $22/day)
- Active against *Streptococcus, Staphylococcus*, aerobic gram negatives (*Enterobacter, E. coli, Klebsiella, Proteus* and *Pseudomonas*).

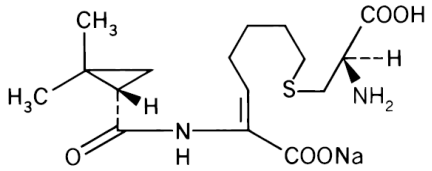


R	R^1	COMPOUND
CH_3	$-CH_2-O-C(=O)-CH_3$	Cefotaxime
CH_3	$-H$	Ceftizoxime
CH_3	$-CH_2-S-$ (thiadiazole) $-CH_2-COOH$, CH_3	Cefodizime
CH_3	$-CH_2-S-$ (triazine) OH, $=O$	Ceftriaxone
CH_3	$-CH_2-S-$ (tetrazole) CH_3	Cefmenoxime
$C(CH_3)_2COOH$	$-CH_2-N^+$ (pyridinium)	Ceftazidime
CH_3	$-CH_2-N^+$ (pyrrolidinium) CH_3	SMY 28142
CH_3	$-CH_2-N^+$ (bicyclic)	HR 810

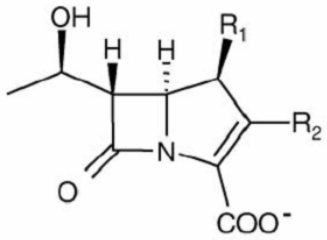
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Carbapenems

Carbapenems

- Imipenem-Cilastatin (Primaxin; IV; $85/day).
- Cilastatin = dehydropeptidase inhibitor that inhibits degradation into a nephrotoxic metabolite.
- Broadest spectrum β-lactams.
  - *Staphylococcus* (not MRSA), *Streptococcus* (highly resistant), *Neisseria, Haemophilus, Proteus, Pseudomonas, Klebsiella, Bacteroides*, anaerobes



Cilastatin

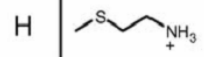
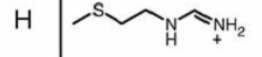
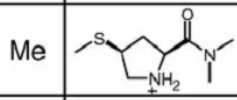
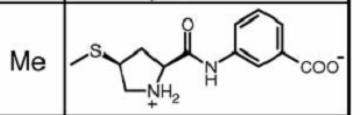
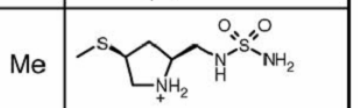


Carbapenem

Table 1 – In vitro activities of carbapenems against selected key pathogens[a]

	Dori	Erta	Imi	Mero
E. coli, ESBL−	≤0.015/≤0.015	≤0.015/≤0.015	0.12/0.25	≤0.06/≤0.06
E. coli, ESBL+	0.03/0.06	0.03/0.05	0.25/0.5	≤0.015/0.06
K. pneumoniae ESBL−	0.03/0.03	≤0.015/0.03	≤0.06/≤0.06	≤0.12/≤0.12
K. pneumoniae ESBL+	0.06/0.12	0.06/0.25	0.5/1	0.03/0.06
P. aeruginosa	0.25/0.5	4/16	1/2	0.25/1
H. influenzae	0.12/1	0.06/0.25	0.5/1	–
M. catarrhalis	≤0.015/0.03	≤0.015/≤0.015	0.06/0.12	≤0.015/≤0.015
S. pneumoniae (penS)	≤0.015/≤0.015	≤0.015/0.03	≤0.06/≤0.06	≤0.015/≤0.015
S. pyogenes (penS)	≤0.015/≤0.015	≤0.015/≤0.015	≤0.015/≤0.015	≤0.015/≤0.015
S. aureus (MSSA)	0.06/0.06	0.25/0.25	≤0.06/≤0.06	0.06/0.25
S. aureus (MRSA)	1/4	2/16	0.12/2	1/8
E. faecalis	2/>32	8/>32	1/>8	8/16
B. fragillis	0.25/0.5	0.5/1	0.25/1	0.12/1
Prevotella spp.	0.12/0.25	0.25/1	0.25/1	0.12/0.25

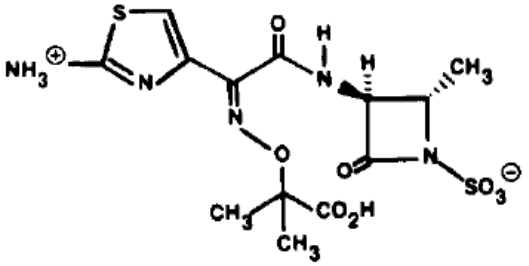
[a] Data represent MIC_{50}/MIC_{90} values in μg/mL [28–35].

	R_1	R_2
Thienamycin	H	
Imipenem	H	
Meropenem	Me	
Ertapenem	Me	
Doripenem	Me	

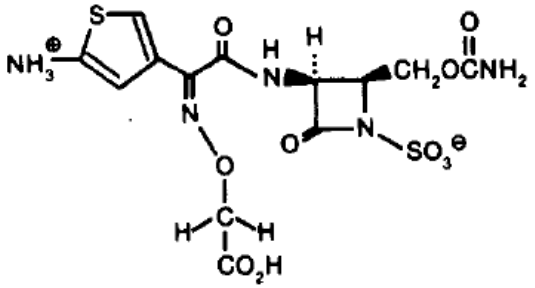
Bacterial Cell Wall: Peptidoglycan Biosynthesis – Monobactams

Monobactams

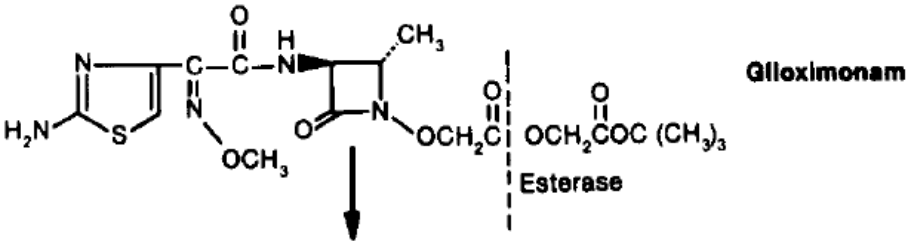
- Aztreonam (Azactam; IM/IV; $53/day)
- β-lactamase resistant.
- Narrow antibacterial spectrum.
  - Aerobic gram negative rods (*Haemophilus influenzae, Neisseria gonorrhea* (penicillinase producers), *E. coli, Klebsiella, Proteus, Pseudomonas*.
  - Ineffective against gram positive and anaerobic organisms.



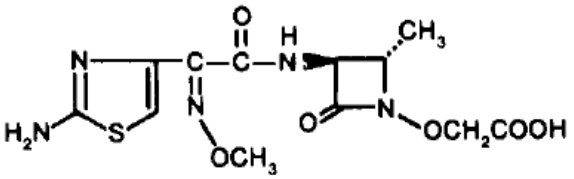
Aztreonam



AMA-1080



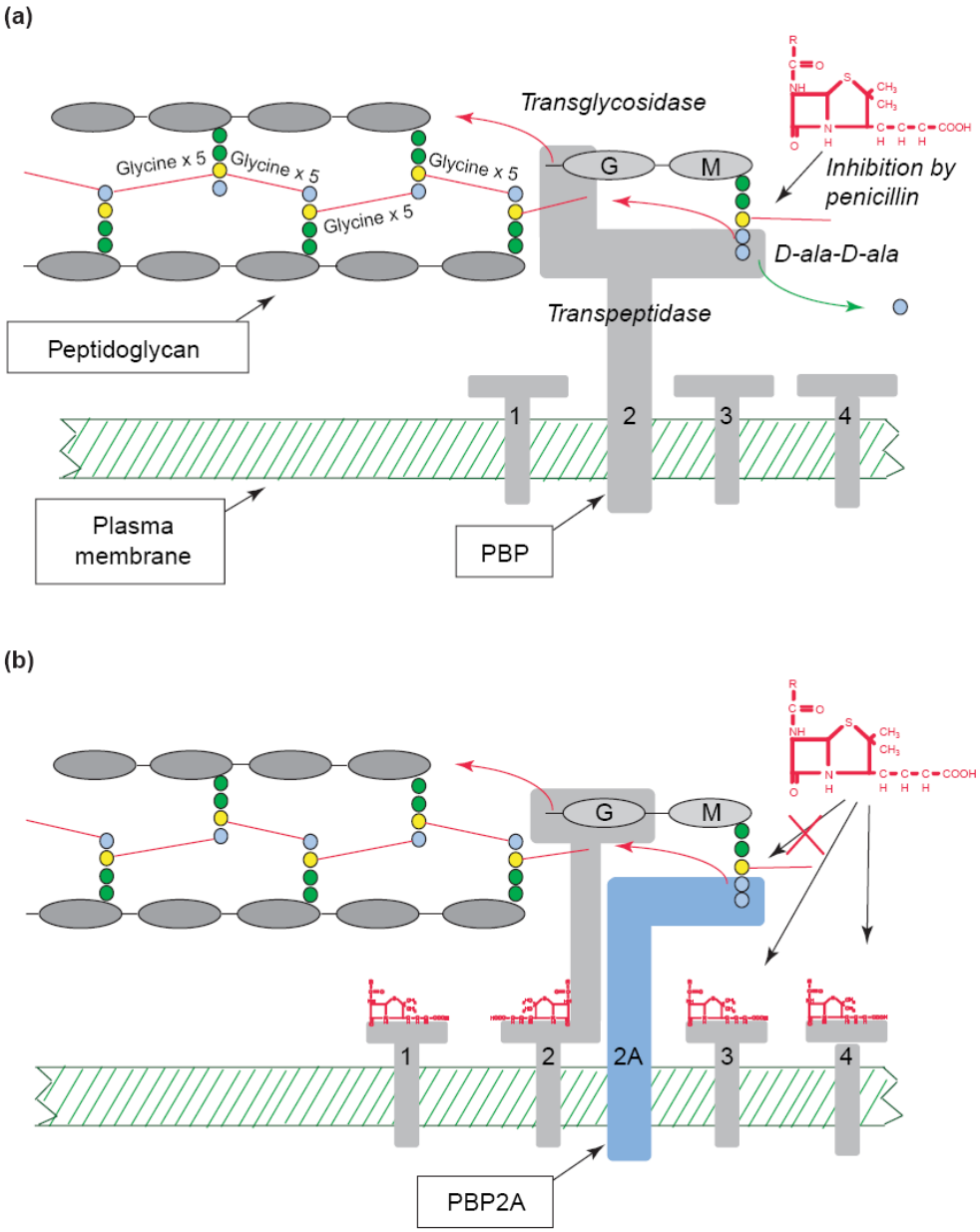
Gloximonam



Oximonam

Bacterial Cell Wall: Peptidoglycan Biosynthesis – β-Lactam Resistance

- β-lactamases – hydrolyze the β-lactam ring.
- Penicillinase (first β-lactamase to emerge)
- Alteration of penicillin-binding protein (PBP) affinity. (*Streptococcus,* MRSA)



Peptidoglycan assembly in wild-type Staphyloccocus aureus and in MRSA. >

(a) After membrane translocation, the cell wall precursors are processed by membrane PBPs. High molecular weight PBPs are bifunctional enzymes that perform both the transglycosidase and transpeptidase steps. Penicillin is a mechanism-based inhibitor of the transpeptidase domain of PBPs.

(b) MRSA carry an additional PBP called PBP2A, which has very low affinity for most available β-lactam drugs. Therefore, when β-lactams are present, they block the normal PBPs, but not PBP2A. PBP2A has only a transpeptidase domain, and must 'hijack' the transglycosydase domain of normal PBP2 to be active.

Bacterial Cell Wall: Peptidoglycan Biosynthesis – β-Lactamases

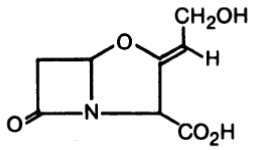
Table 3 – Substrate preferences of various β-lactamases[a]

Molecular classes (Ambler)	Functional group (Bush)	Activity[b]				Inhibition by clavulanate
		Penicillin	Cephaloridine	Imipenem	Faropenem	
Serine β-lactamases						
A	2a	+++	±	–	–	++
	2b	+++	++	–	–	++
	2c	++	+	–	?	+
	2e	++	++	–	–	++
	2f	++	+	++	?	+
C	1	++	+++	–	–	–
D	2d	++	+	–	Inhibitor	±
Metallo β-lactamases						
B	3	++	++	++	++	–

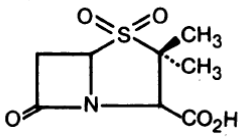
[a] Modified according to Refs. [40,88].

[b] +++, preferred substrate (highest V_{max}); ++, good substrate; +, hydrolyzed; ±, barely hydrolyzed; –, stable;?, not known as not tested.

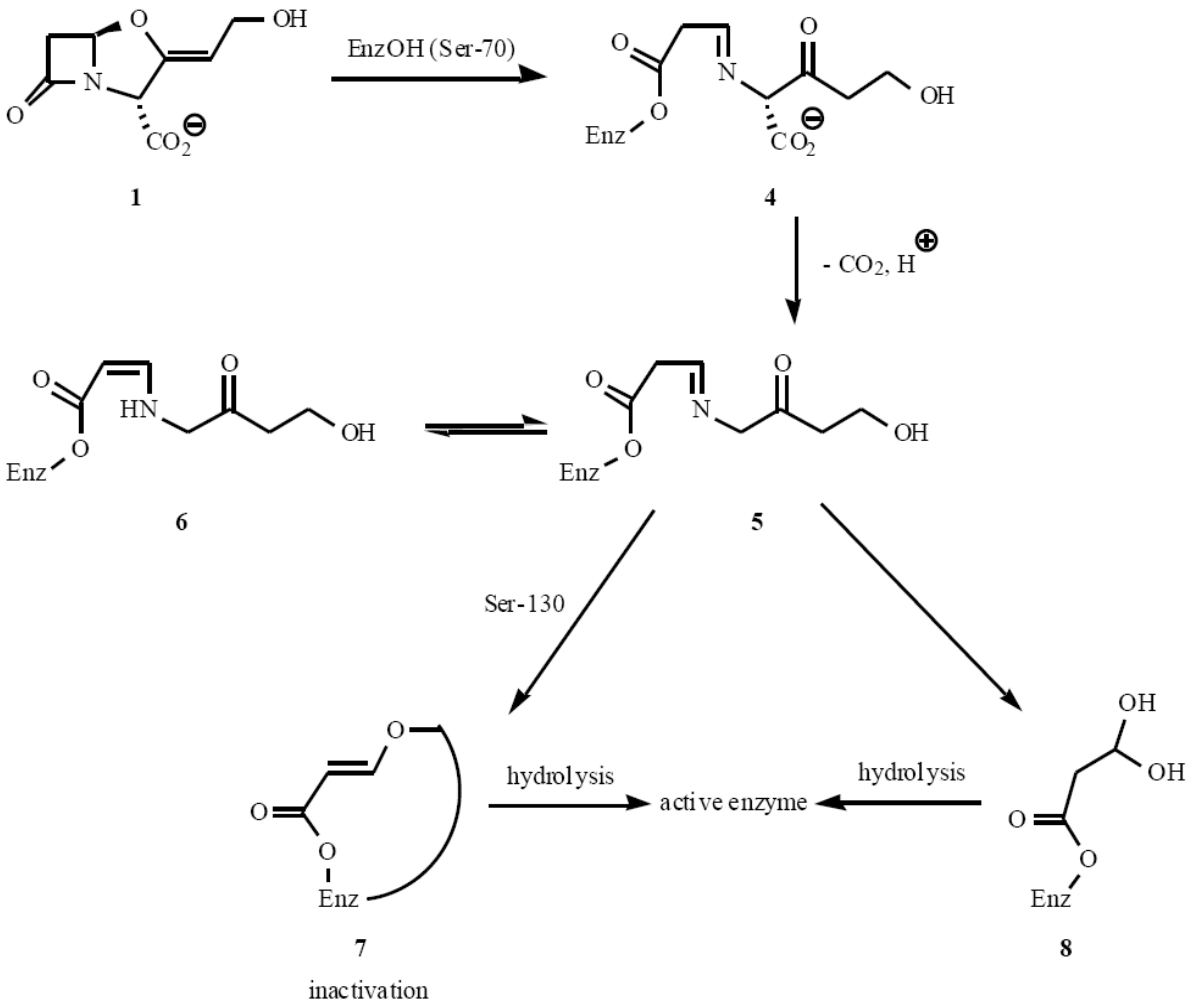
Bacterial Cell Wall: Peptidoglycan Biosynthesis – β-Lactamase Inhibitors



Clavulanic acid



Sulbactam



Bacterial Cell Wall: Peptidoglycan Biosynthesis – β-Lactamase Inhibitors

TABLE 8. Protection of β-lactam antibiotics by clavulanic acid (CA) in β-lactamase-producing *S. aureus* (methicillin susceptible)

Antibiotic	*n*	MIC (μg/ml)		Reference
		β-Lactam	β-Lactam/CA	
Amoxicillin	29	8.0[a]	0.5/8.0[a]	11
Ampicillin	1	500	0.02/5.0	120
Mezlocillin	8	256	4.0/1.0	145
Cephaloridine	1	0.6	0.06/5.0	120
Ticarcillin	1	200	25/1.7	82
	1	128	8.0/5.0	72
Piperacillin	1	>200	1.6/0.4	108

[a] MIC for 90% inhibition.

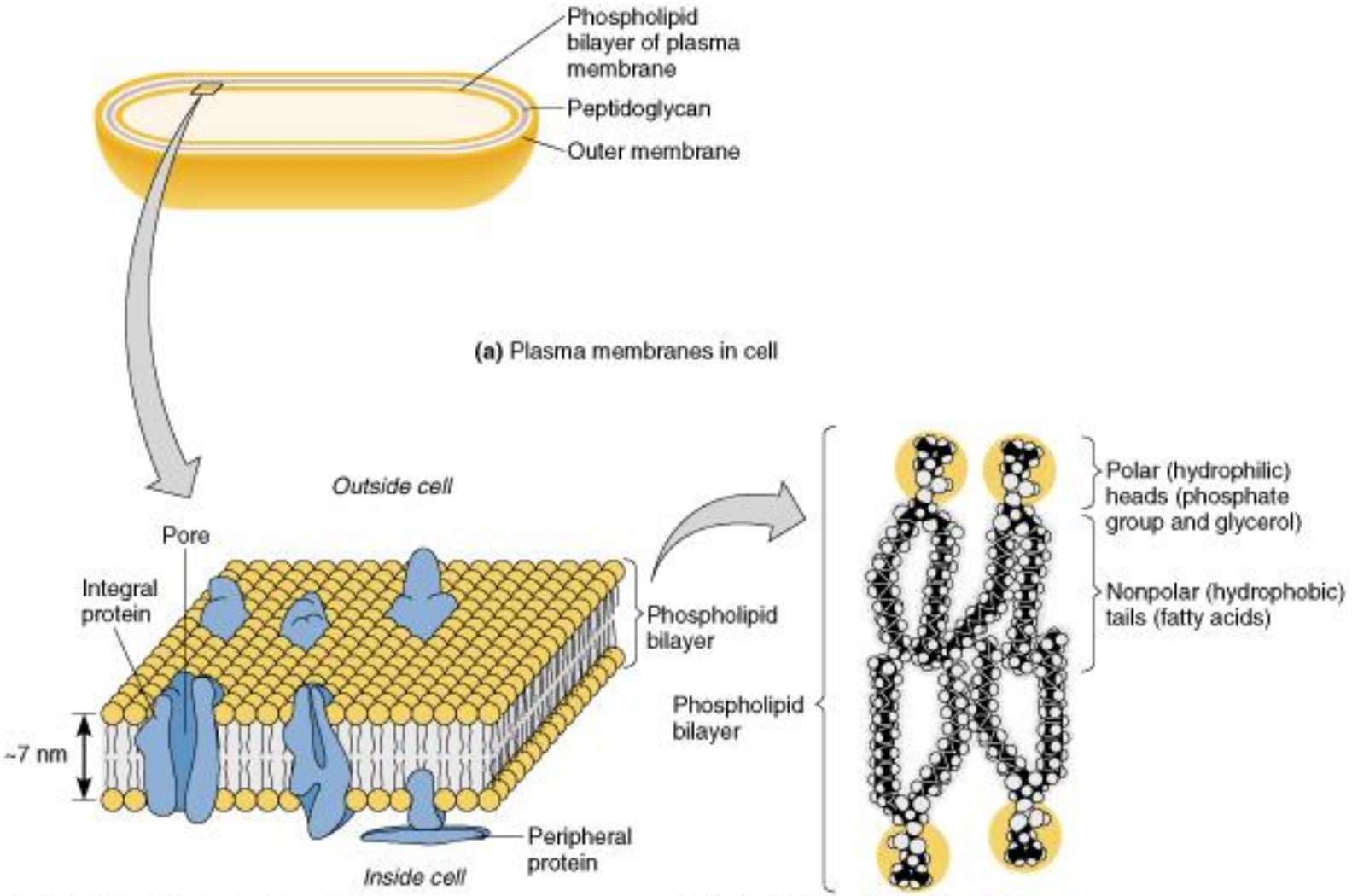
Augmentin = amoxicillin/clavulanic acid
- first β-lactamase inhibitor approved worldwide
- selected based on similar pharmacokinetics of the two components (similar $t_{1/2}$)

TABLE 9. Protection of amoxicillin (AMX) by clavulanic acid (CA) in β-lactamase-producing organisms

Organism	*n*	MIC_{90} (μg/ml)[a]	
		AMX	AMX/CA
Bacteroides fragilis	28	33[b]	0.48/1.0[b]
Branhamella catarrhalis	35	2.0	0.125/0.062
	53	8.0	0.25/ND[c]
Citrobacter diversus	8	128	2.0/8.0
Citrobacter freundii	12	>128	>128/8.0
Escherichia coli (R^+)	100	>5,000[b]	3.0/10[b]
	21	>128	8.0/8.0
Enterobacter spp.	25	>128	>128/8.0
Haemophilus spp.	132	>32	2.0/1.0
	15	150[b]	1.1/0.5[b]
Enterobacter aerogenes	45	315[b]	1.75/1.0[b]
Mycobacterium tuberculosis	13	>32	4.0/4.0
Neisseria gonorrhoeae	6	>40[b]	0.44/0.5[b]
Proteus spp.	23	433[b]	5.0/2.5[b]
Serratia spp.	20	>128	>128/8.0
Staphylococcus aureus	35	197[b]	1.1/0.5[b]

[a] MIC for 90% inhibition.

Bacterial Cell Membrane



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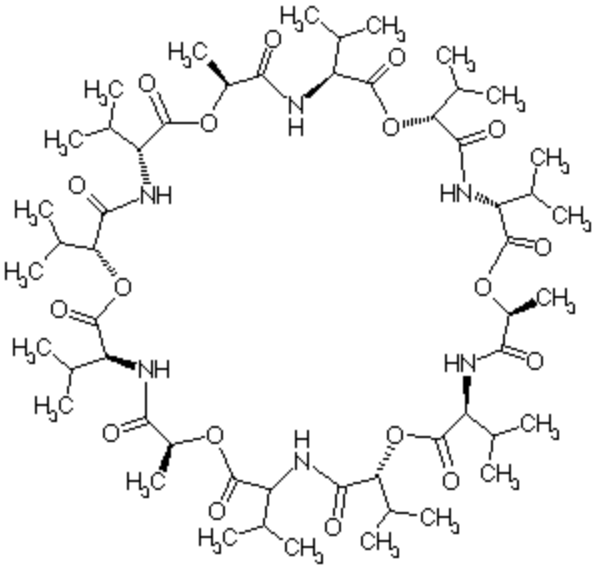
Bacterial Cell Membrane: Permeabilizing Antibiotics - Valinomycin

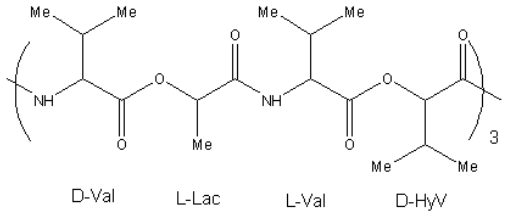
Valinomycin, Gramicidin S, Polymyxin B

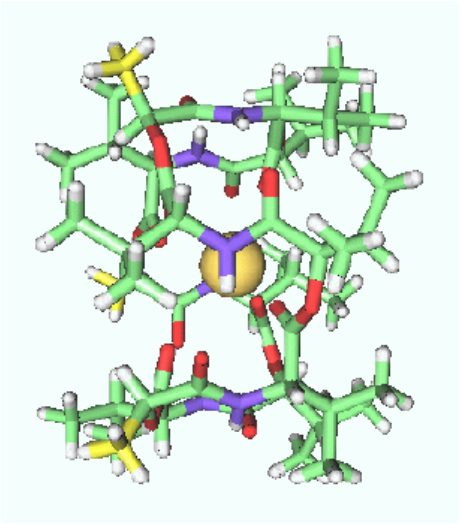
- nonribosomal cationic peptides
- insert into bacterial membrane and cause permeabilization
- toxic side effects prevent systemic application; used as topical antibiotics

- Valinomycin carries K+ ions across the membrane



Valinomycin





- In hydrophobic conditions, the methyl and isopropyl groups face out rendering the molecule hydrophobic

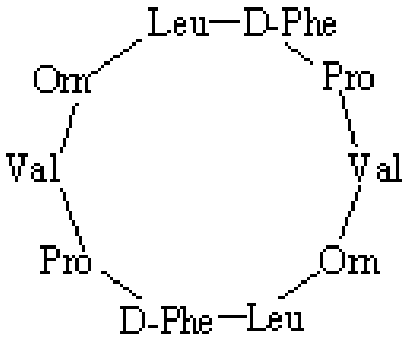
- In hydrophilic conditions, the carbonyl groups face outwards. Molecule adopts a hydrophilic conformation.

Bacterial Cell Membrane: Permeabilizing Antibiotics - Gramicidin

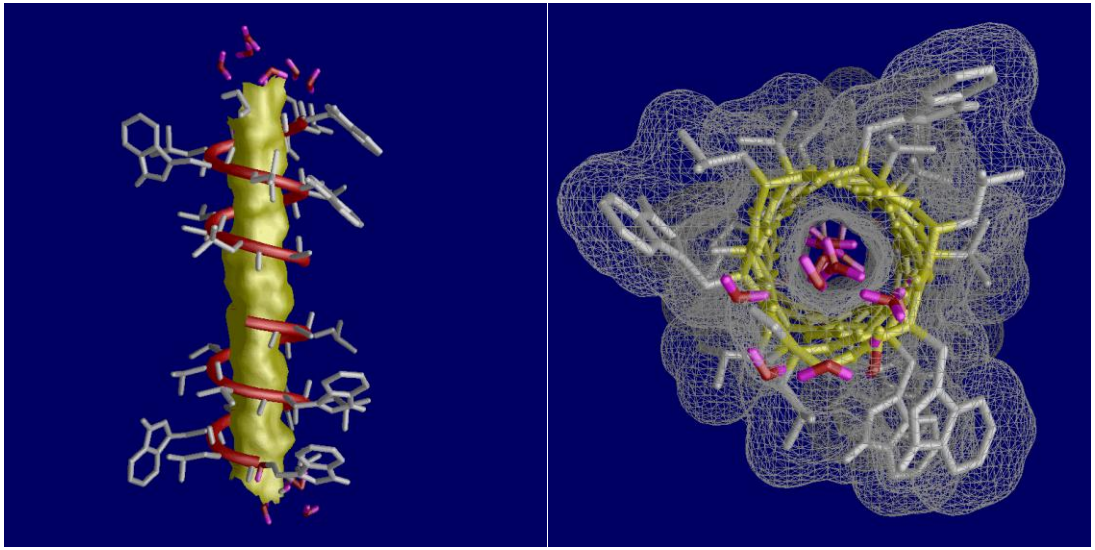
Valinomycin, Gramicidin S, Polymyxin B

- nonribosomal cationic peptides
- insert into bacterial membrane and cause permeabilization
- toxic side effects prevent systemic application; used as topical antibiotics

- Gramicidin dimerizes reversibly and forms a membrane-spanning channel.



Gramicidin

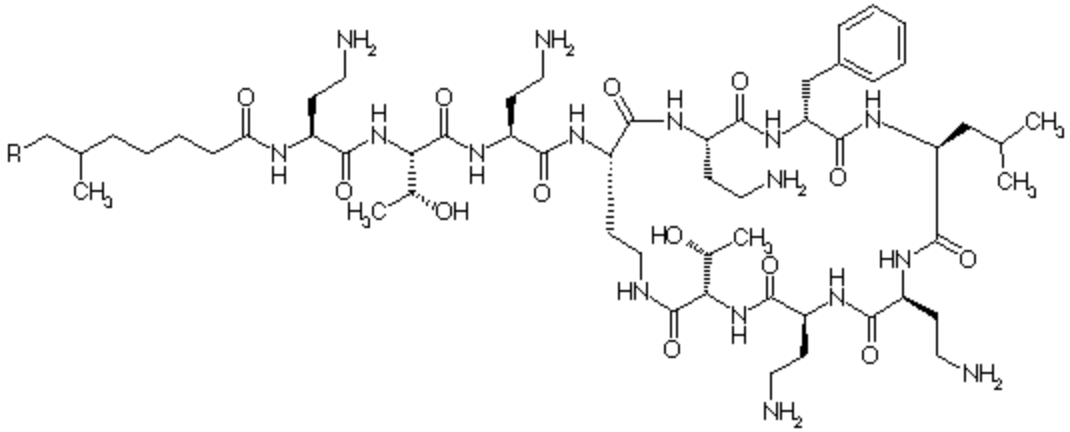


Bacterial Cell Membrane: Permeabilizing Antibiotics - Polymyxin

Valinomycin, Gramicidin S, Polymyxin B

- nonribosomal cationic peptides
- insert into bacterial membrane and cause permeabilization
- toxic side effects prevent systemic application; used as topical antibiotics

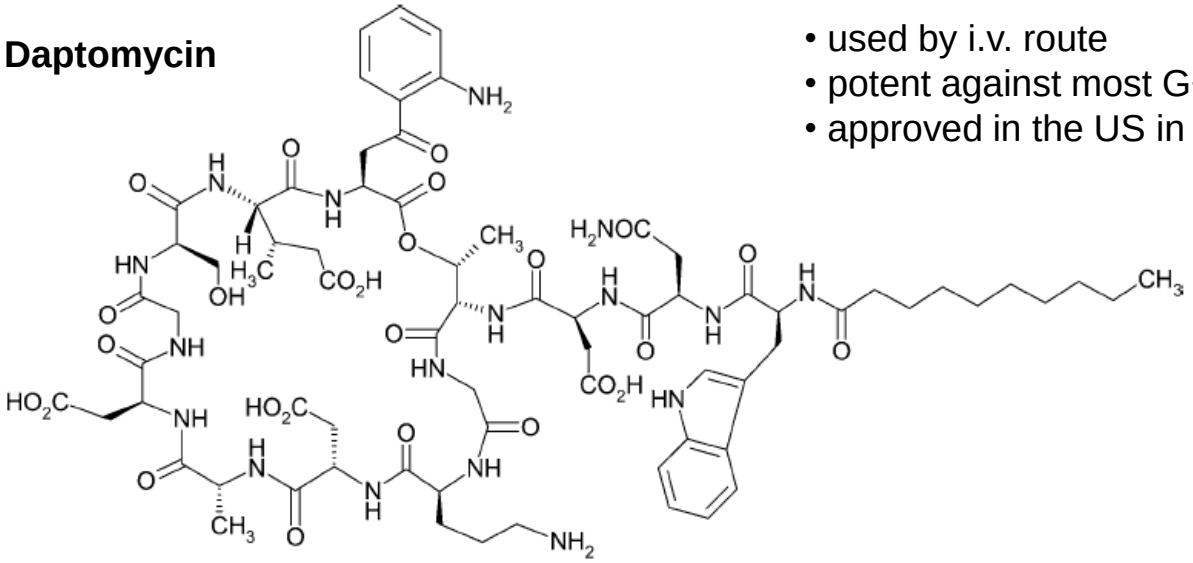
- Polymyxin binds to the bacterial membrane and disrupts the phospholipid bilayer.



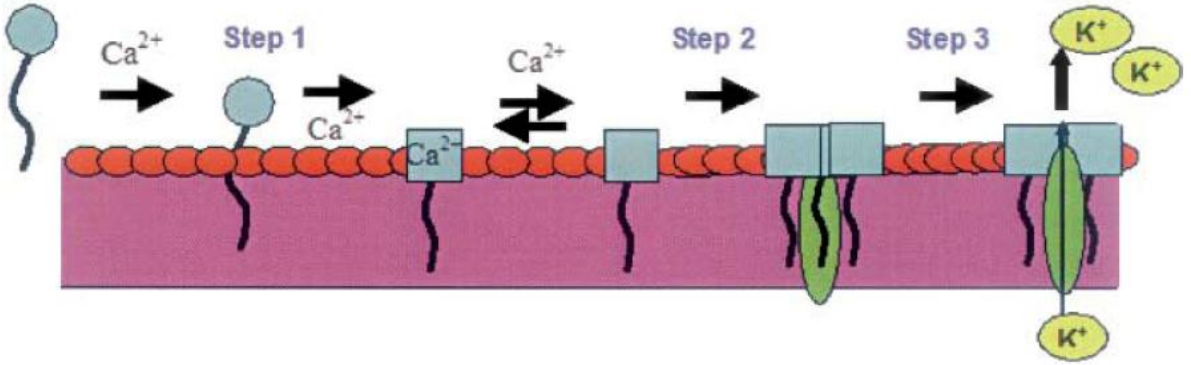
Polymyxin B

Bacterial Cell Membrane: Permeabilizing Antibiotics – Lipopeptides

Daptomycin



- used by i.v. route
- potent against most G+ bacteria including MRSA
- approved in the US in 2004



Daptomycin mechanism of action:

•Step 1, daptomycin binds to the cytoplasmic membrane in a calcium-dependent manner;

•Step 2, daptomycin oligomerizes, disrupting the membrane

•Step 3, release of intracellular ions and rapid cell death.