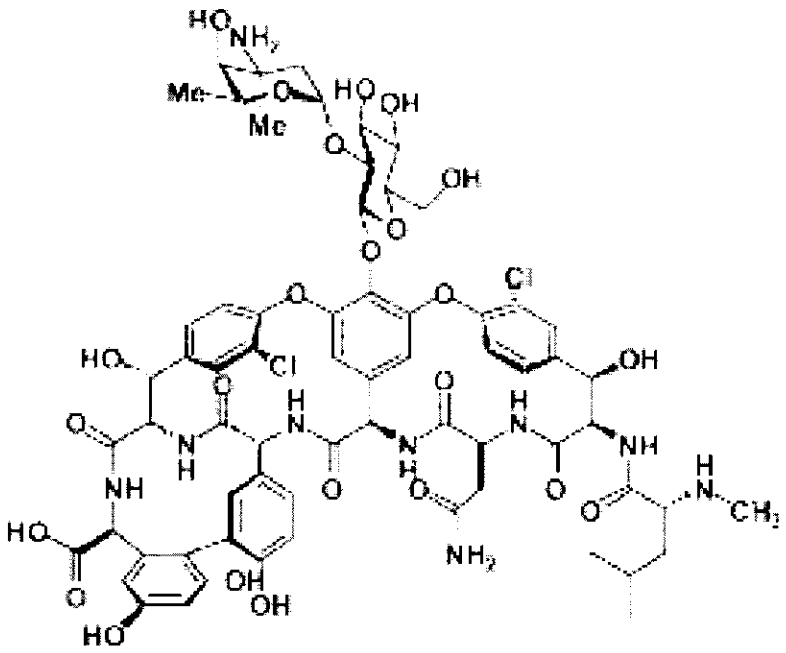
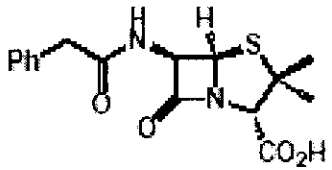
# Partitioning the Loss in Vancomycin Binding Affinity for D-Ala-D-Lac into Lost H-Bond and Repulsive Lone Pair Contributions



Casey C. McComas, Brendan M. Crowley, and Dale L. Boger

*J. Am. Chem. Soc.* **2003**, *125*, 9314

# *Antibiotics – Historical Perspective*




**Penicillin - 1929**

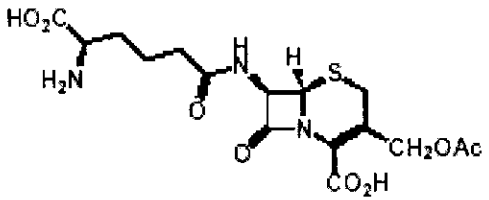
The Public Becomes Aware of Penicillin

Given the political climate under which it was rediscovered and produced, it is not surprising that initially penicillin was used almost exclusively to treat soldiers injured during the war. That would change, though, with one fateful disaster.

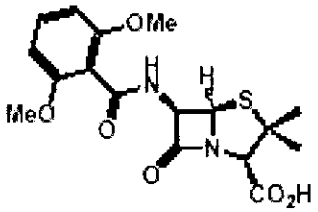
Perhaps penicillin's most important clinical trial occurred after a fire at a Boston club, which resulted in numerous burn victims being sent to Boston-area hospitals. At that time, it was common for severe burn victims to die of bacterial infections, such as those from *Staphylococcus*. In response to this crisis, Merck rushed a large supply of a "priceless" drug (penicillin) to the Massachusetts General Hospital. The success that physicians had in treating severely burned victims that night was largely attributed to the effects of penicillin. The fire—and the success of penicillin—made national headlines, vaulting the drug into the public spotlight.

By 1946, the drug had become widespread for clinical use.

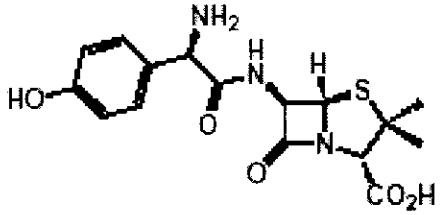
http://www.molbio.princeton.edu/courses/mb427/2001/projects/02/antibiotics.htm



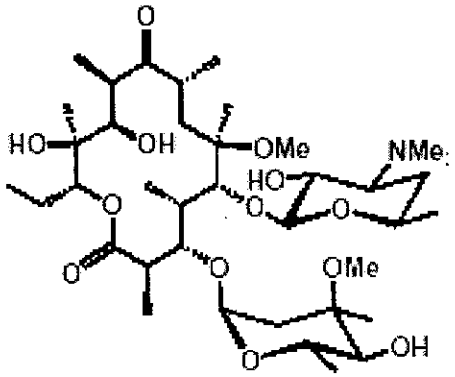
Cephalasporin - 1945



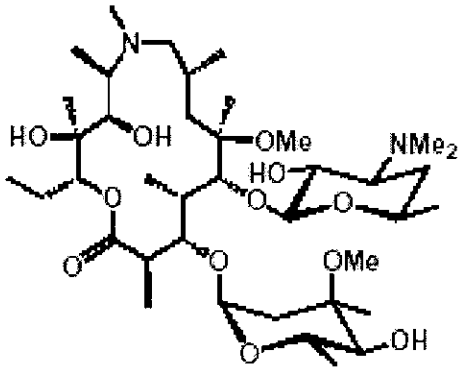
Methicillin - 1960



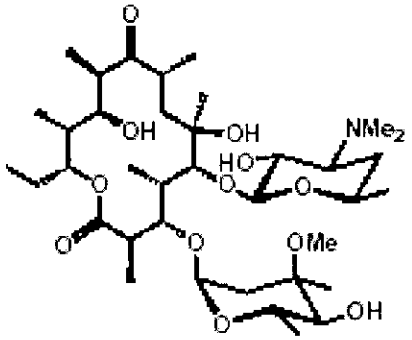
Amoxicillin - 1981



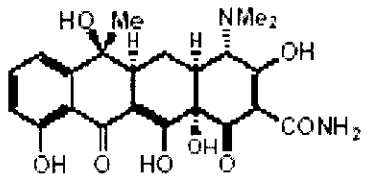
Erythromycin - 1952



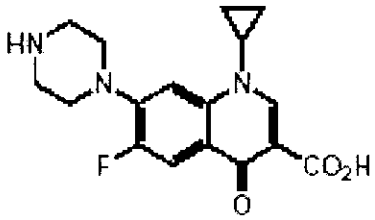
Azithromycin - 1988



Clarithromycin - 1990

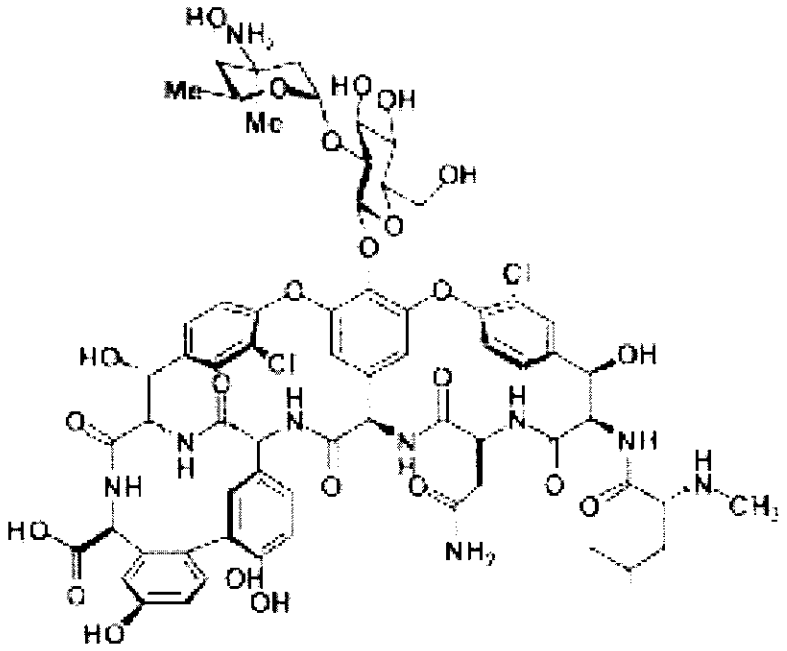


Tetracyclines - 1947

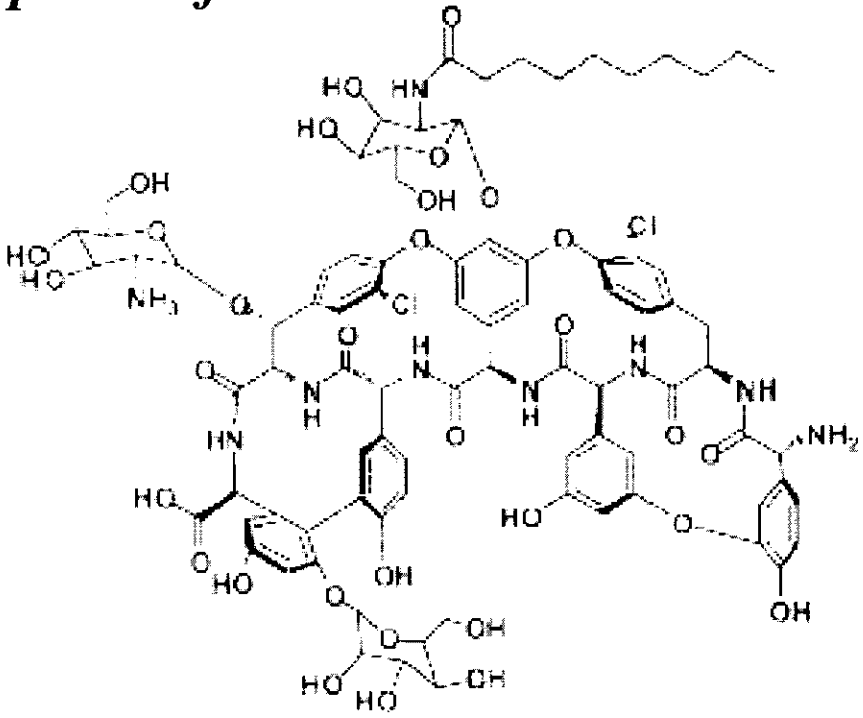


Ciprofloxacin

# *Glycopeptide Antibiotics Approved for Use in Humans*

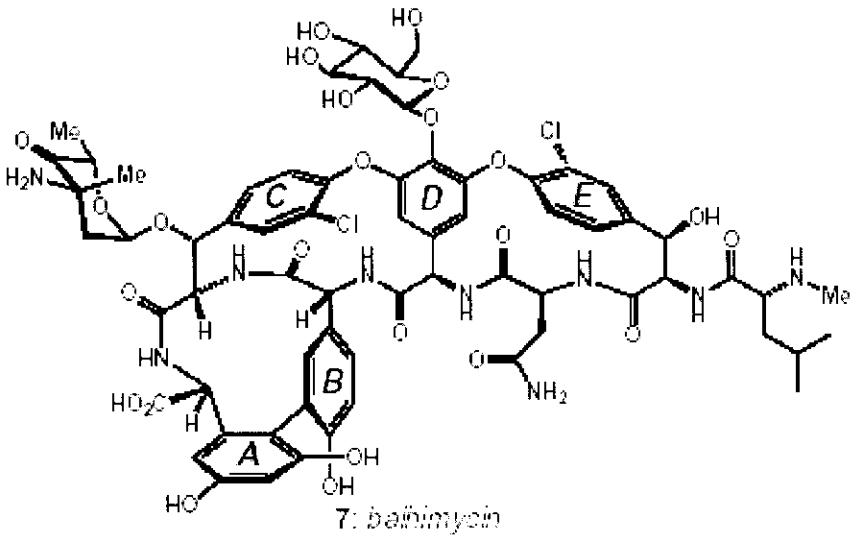


Vancomycin - 1956

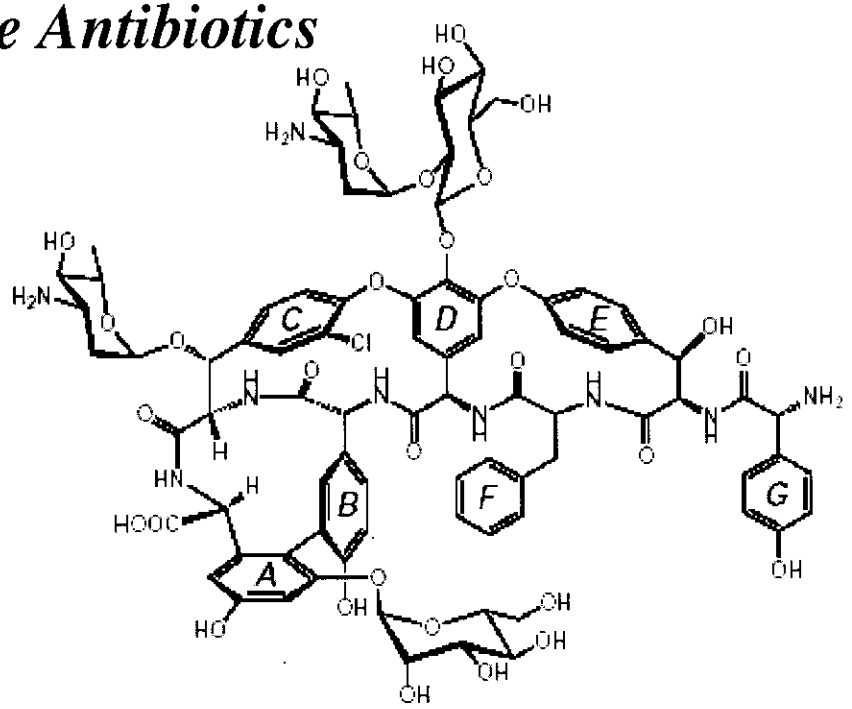


Teicoplanin - 1988

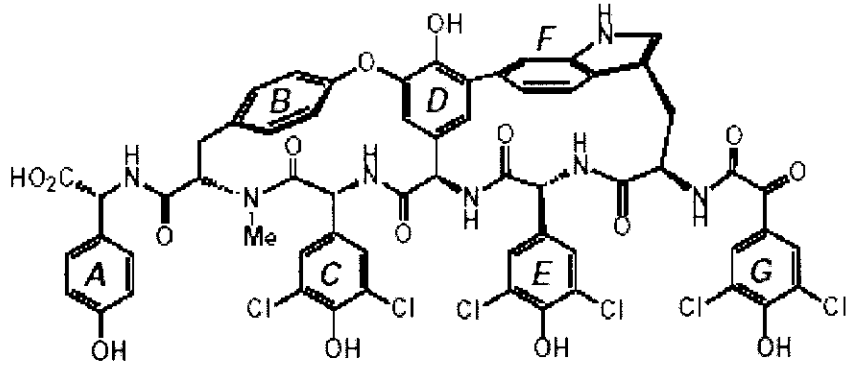
# *Other Glycopeptide Antibiotics*



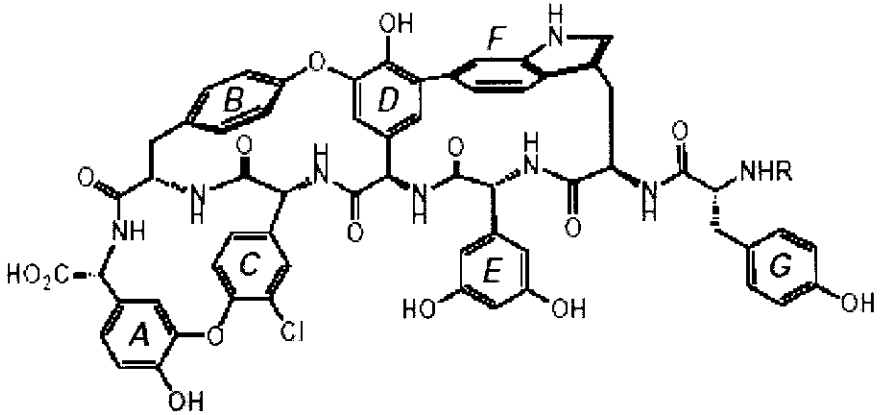
**7**: *balhimycin*
[***type I***: *contain aliphatic chains in AA-1 and AA-3*]



**8**: *actinoidin A*
[***type II***: *contain aromatic chains in AA-1 and AA-3*]



**11**: *complestatin*



**13a**: *kistamicin A (R = H)*
**13b**: *kistamicin B (R = CONHCH$_2$CH$_2$Ph)*
[***type V***: *contain a tryptophan linked to central AA*]

# *Sites of Action of Antimicrobial Agents*

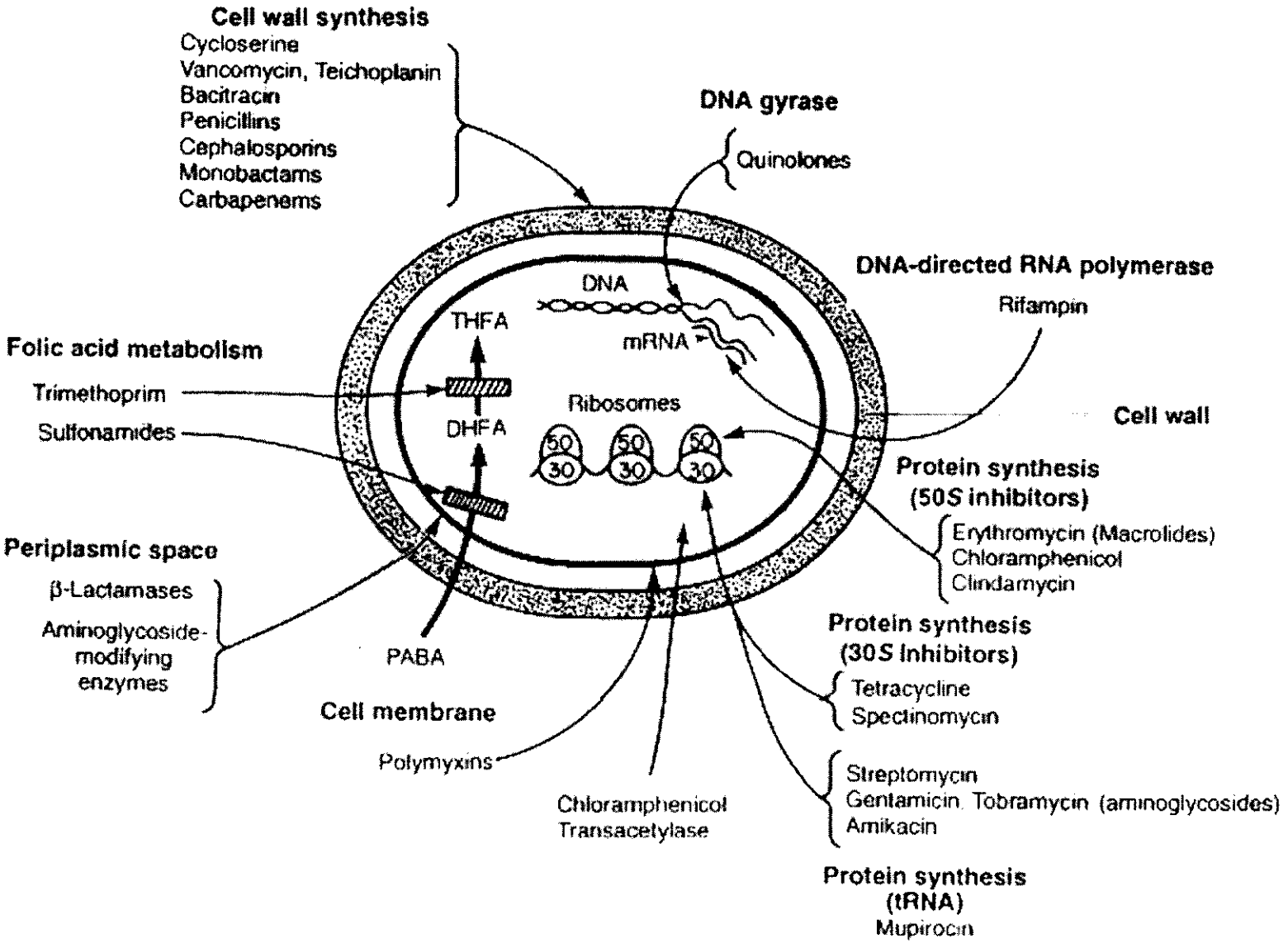



**Fig. 1.** Sites of action of various antimicrobial agents. mRNA, messenger RNA; tRNA, transfer RNA; PABA, *p*-aminobenzoic acid; DHFA, dihydrofolic acid; THFA, tetrahydrofolic acid.

# *Bacterial Targets and Resistance Mechanisms*

***Table 2:*** Antibiotic targets and resistance mechanisms.

| Antibiotic | Target | Resistance mechanism |
|---|---|---|
| *cell wall* | | |
| β-lactams | transpeptidase/transglycosylases (PBPs) | β-lactamases, PBP mutants |
| vancomycin | D-Ala-D-Ala termini of peptidoglycan and lipid II | reprogramming of D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser |
| teicoplanin | D-Ala-D-Ala termini of peptidoglycan and lipid II | reprogramming of D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser |
| *protein synthesis* | | |
| erythromycins | peptidyl transferase/ ribosome | rRNA methylation/efflux |
| tetracyclines | peptidyl transferase | drug efflux |
| aminoglycosides | peptidyl transferase | drug modification |
| oxazolidinones | peptidyl transferase | unknown |
| *DNA replication/ repair* | | |
| fluoroquinolones | DNA gyrase | gyrase mutations |

# *Modes of Transmission of Resistance*

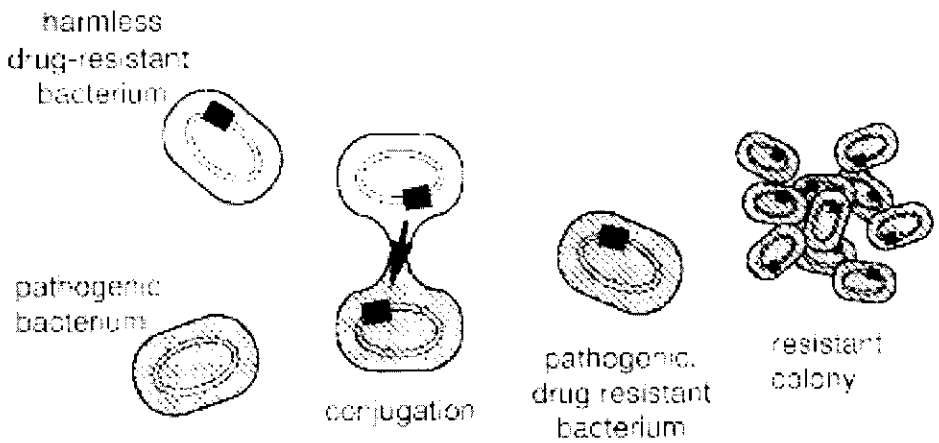



Figure 1. Drug resistance can be passed horizontally between species of bacteria. For example, a nonpathogenic, but antibiotic-resistant bacterium can physically join with a disease-causing bacterium and pass on the genetic information required for antibiotic resistance through a process called conjugation. With the advantage of drug resistance, the pathogenic bacterium can then proliferate into an untreatable, disease-causing colony.

Williams, D. H.; Bardsley, B.
*Angew. Chem. Int. Ed. Engl.*, **1999**, *38*, 1172

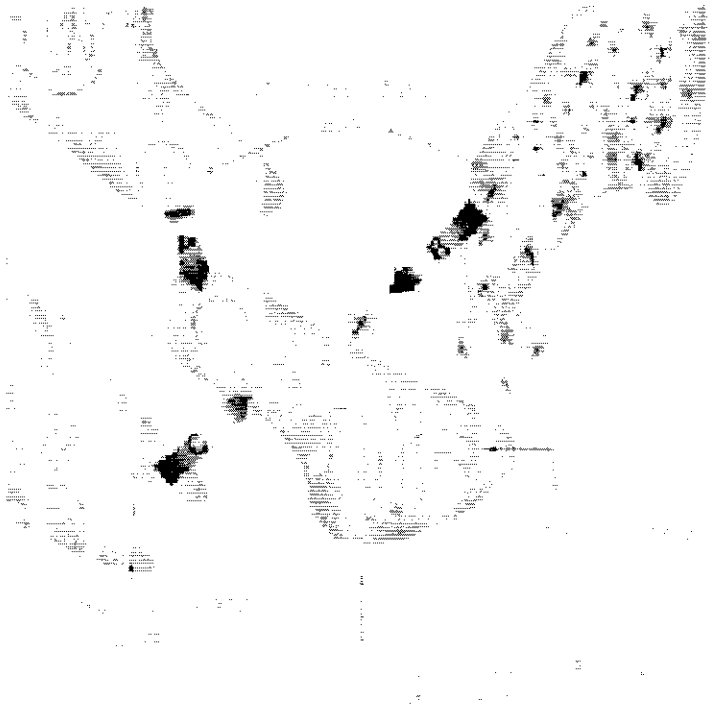
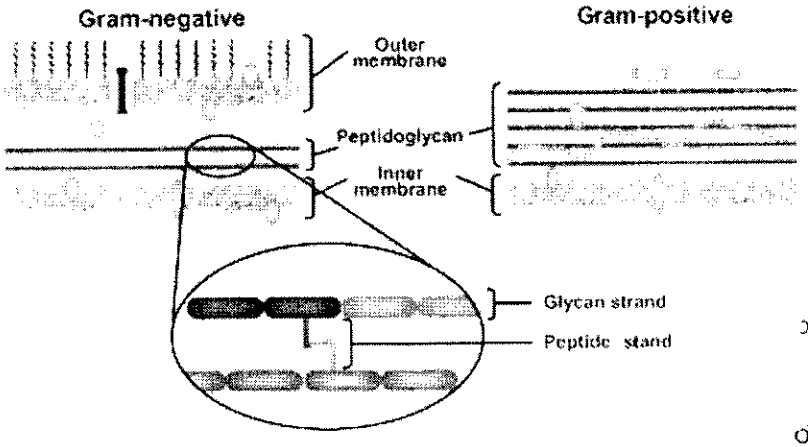


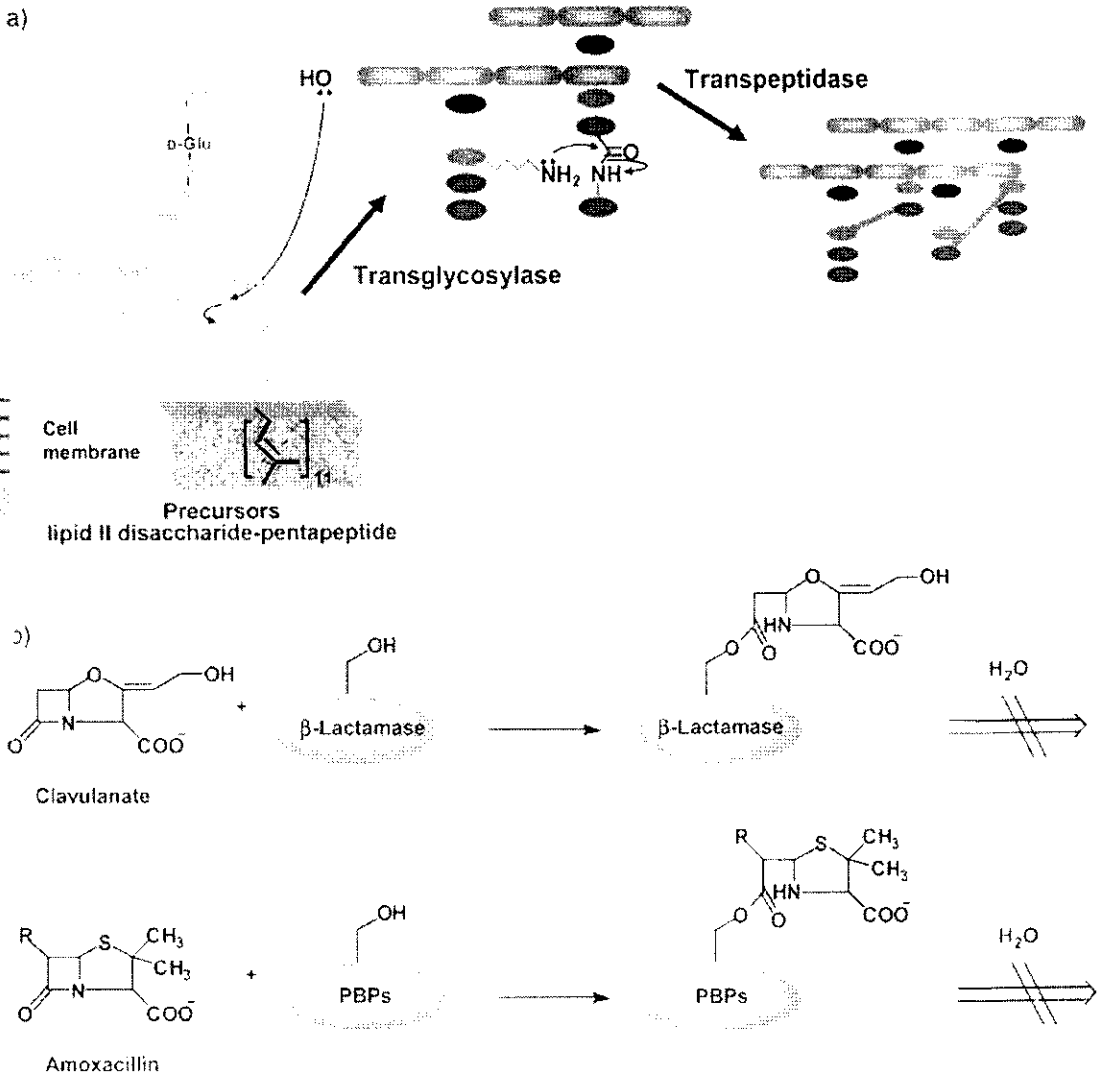
Figure 5. Three modes of resistance transfer between different bacteria: A) a plasmid containing the genes encoding for antibiotic resistance (yellow) is transferred from a donor bacterium to a new bacterium; B) bacterial DNA containing the genetic information for resistance is transferred through a virus to a new bacterium; C) incorporation of genes encoding for antibiotic resistance through DNA scavenged from dead cells.

Nicolaou *et al Angew. Chem. Int. Ed. Engl.*, **1999**, *38*, 2096

# *Bacterial Cell Walls*




# *Peptidiglycan Biosynthesis*



# *How did Bacteria Become Vancomycin Resistant?*

Vancomycin and other antimicrobial agents are themselves produced by bacteia. While evolving production of the anti-bacterials, these bacteria have also developed self-protection and immunity from these chemical weapons. The antibiotic production and antibiotic resistance genes are co-localized, enabling these genes to be turned on in concert. Often these genes code for transmembrane proteins that serve as efflux pumps keeping the intracellular concentration of the antibiotic below harmful levels. A more specialized resistance mechanism for two glycopeptide producing bacteria is shown below. This is believed to be the source of resistance for Vancomycin Resistant Enterococci (VRE).

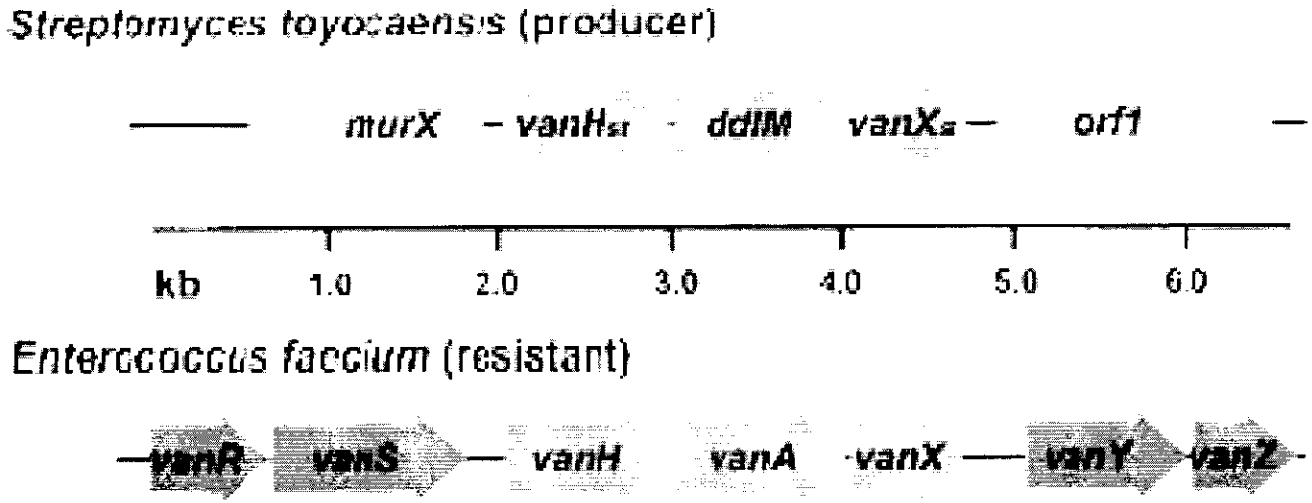
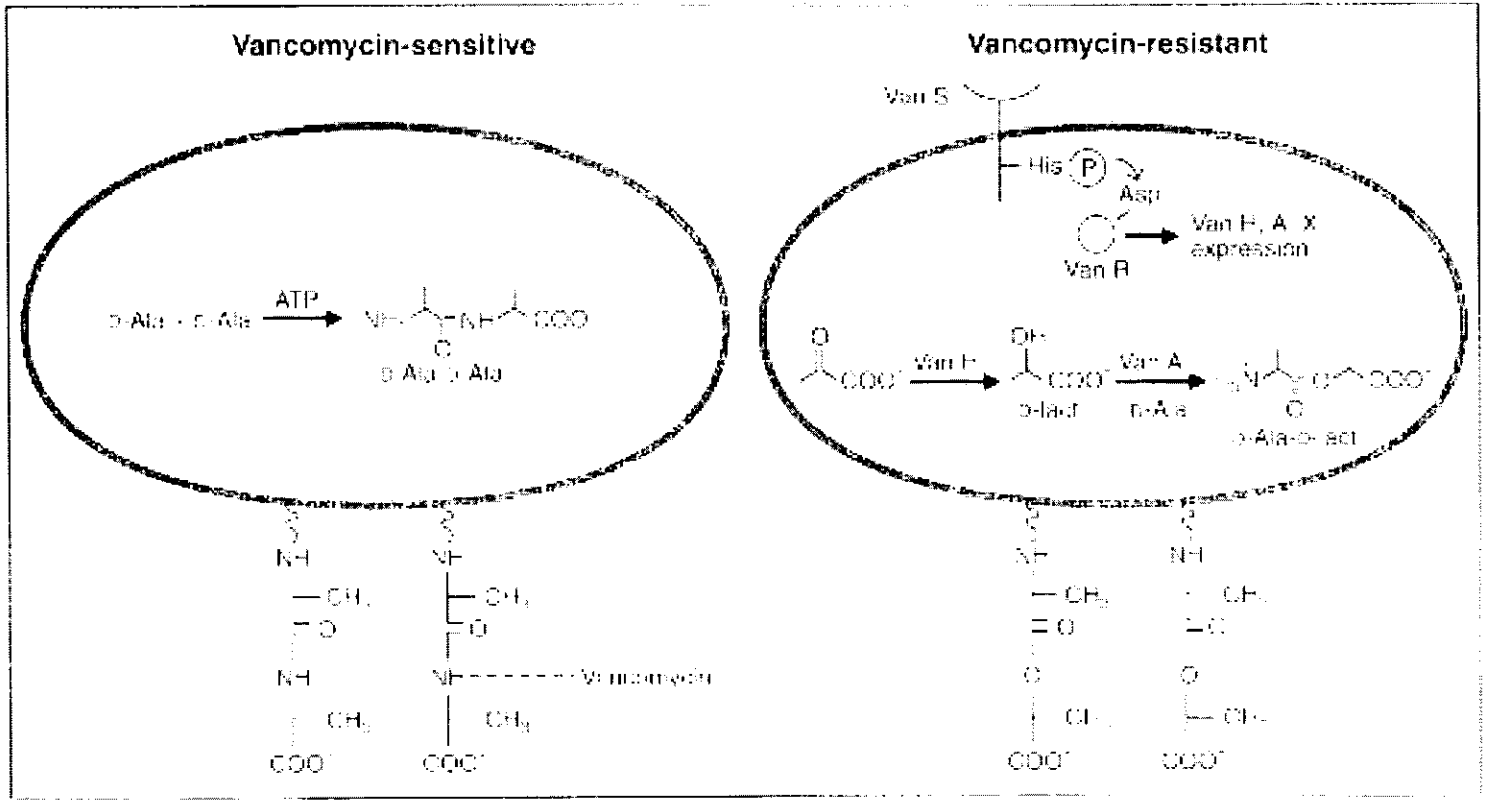



***Figure 5.*** Gene cassettes for vancomycin resistance in the producing organism and resistant strains.




**Molecular logic of vancomycin resistance.** Vancomycin-sensitive and -resistant bacteria differ in a critical component of their cell wall. Sensitive bacteria (**left**) synthesize PG strands that terminate in D-Ala-D-Ala. Antibiotic vancomycin binds avidly to these termini, thereby disrupting cell wall synthesis and leading to cell lysis. Resistant bacteria (**right**) harbor a transposable element encoding nine genes that contribute to the resistance phenotype. The gene products include a transmembrane protein (Van S) that senses the presence of the drug and transmits a signal by transfer of a phosphoryl group (P) to a response regulator protein (Van R) that activates transcription of the other resistance genes. The combined activities of Van H and Van A lead to the assembly of a depsipeptide, D-Ala-D-lactate, which can be incorporated into the PG strands of the cell wall. The altered PG termini do not affect the structural integrity of the cell wall, but substantially reduce its affinity for vancomycin, thereby rendering the bacteria resistant to the drug.

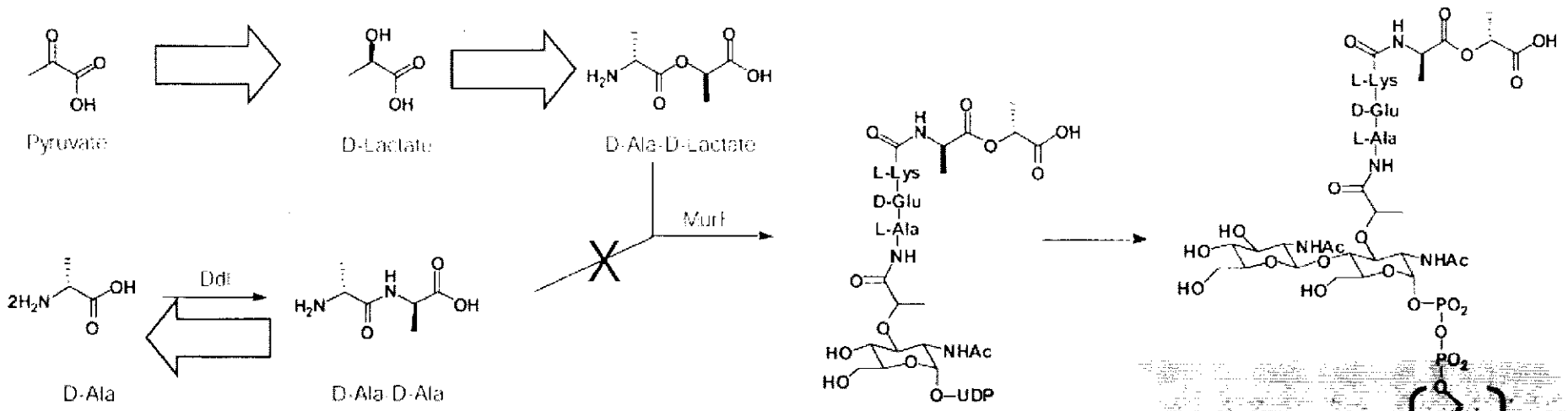
Walsh, C. T. *Science*, **1993**, *261*, 308

# *The Molecular Basis for Vancomycin Resistance*

Table V: Vancomycin Binding Experiments[a]

| compound | $K_d$ (mM) |
|---|---|
| NAc-D-Ala-D-Ala | 0.054 |
| NAc-D-Ala-D-Phe | >15[b] |
| NAc-D-Ala-D-ABut | >18[b] |
| NAc-D-Ala-D-Lac | >45[b] |
| NAc-D-Ala-D-HBut | >73[b] |
| *N,N'*-diacetyl-L-Lys-D-Ala-D-Ala | 0.021 |
| *N,N'*-diacetyl-L-Lys-D-Ala-D-Lac | >38[b] |

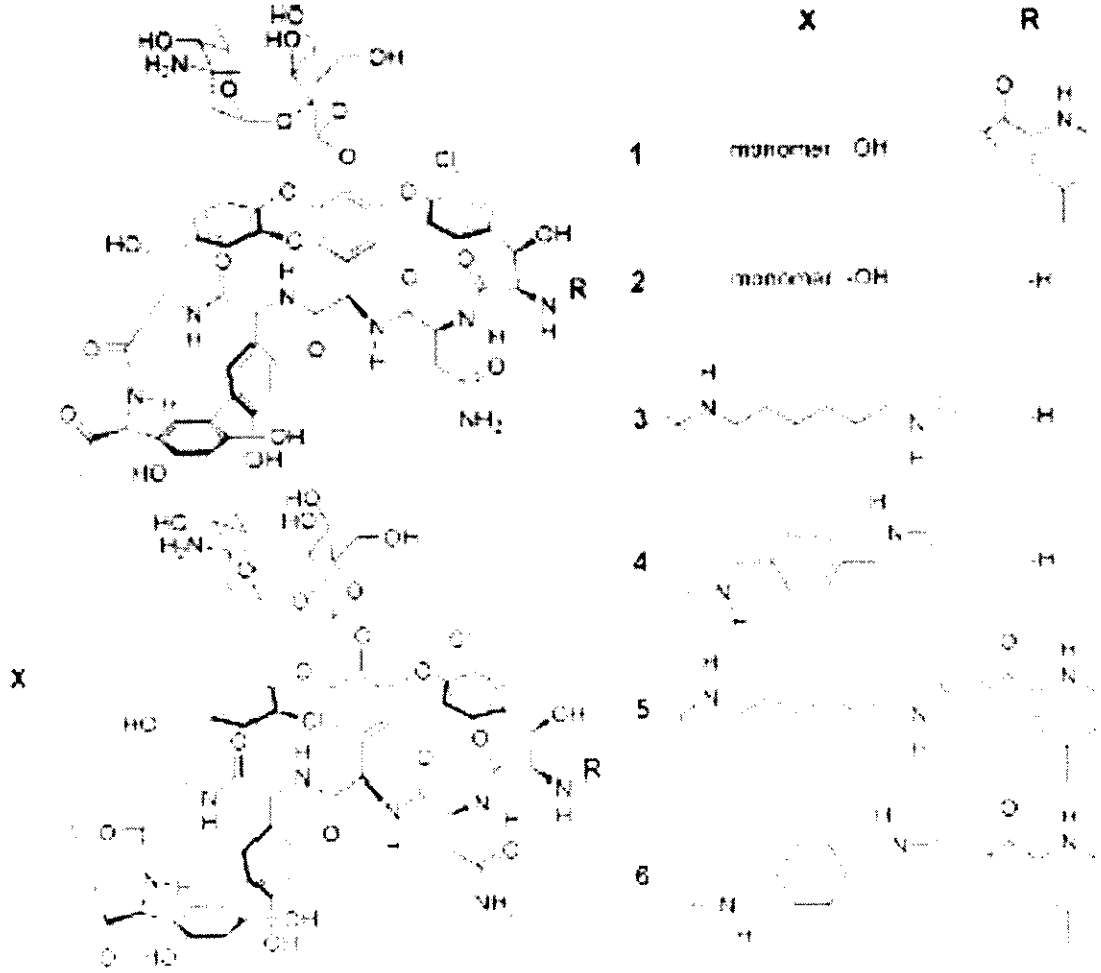
[a] Binding assays were carried out using the UV binding assay of Nieto and Perkins (1971). [b] Little or no binding was observed at the highest ligand concentration (experimental error 10%). The lower limit quoted for $K_d$ is five times the maximum concentration of ligand at which no binding is observed. Abbreviations: ABut, 2-aminobutyrate.




Bugg, T. D. H. *et al Biochemistry*, **1991**, *30*, 10408

# *Synthesis and Activity of Vancomycin Dimers*

Can pre-dimerization of Vancomycin lead to enhanced activity?




Jain, R. K.; Trias, J.; Ehmann, J. A. *J. Am. Chem. Soc.* **2003**, **125**, 8740

# *Glycopeptides Dimerize in the Presence of D-Ala-D-Ala*

***Table 1.*** Compound in Vitro Antibacterial Activity and Affinity for a Monomeric Model Peptide, dansyl-Lys(Ac)-D-Ala-D-Ala[13]

| compound | MIC ($\mu$g/mL)[a] | $K_d$ ($\mu$M)[b] |
|---|---|---|
| **1** | 1200 | 1.3 |
| **2** | > 1200 | > 60 |
| **3** | 12 | > 60 |
| **4** | 5.8 | > 60 |
| **5** | 1.5 | 1.1 |
| **6** | 1.5 | 2.1 |

[a] Measured against *E. faecium* (VanA phenotype).[12] [b] Determined at peptide = 1 $\mu$M, 25 °C, in 10 mM HEPES, 6 mM NaCl, pH = 7.0.

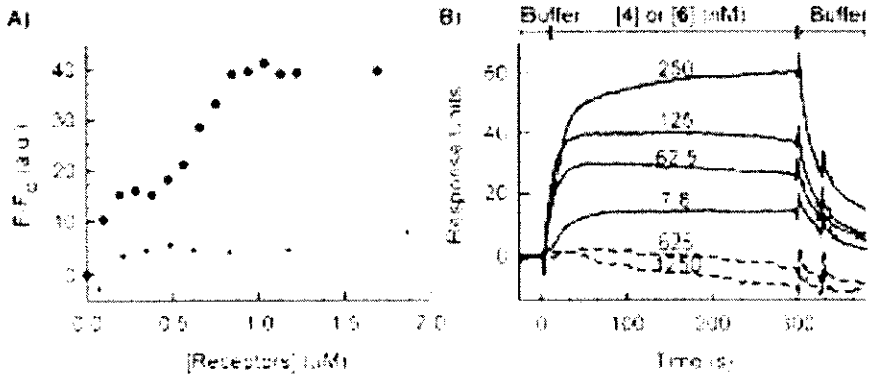
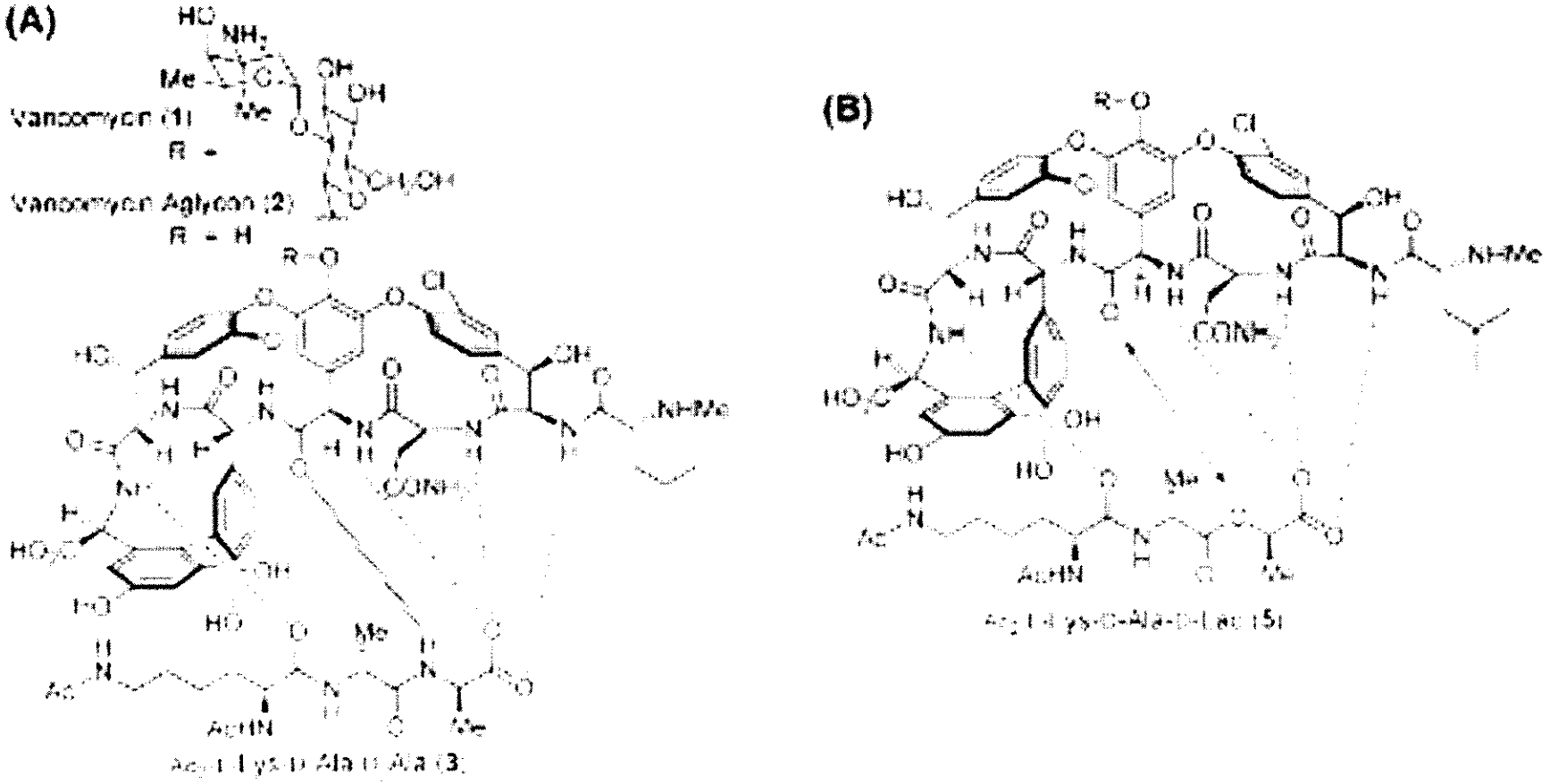



***Figure 1.*** (a) Fluorescence titration of dimeric model peptide $\epsilon$-*N*-succinyl-(dansyl-Lys-D-Ala-D-Ala)$_2$ with compounds **4** (+) and **6** (●). Determined at 25 °C, peptide = 1 $\mu$M, in 10 mM HEPES, 6 mM NaCl, pH = 7.0. (b) SPR sensorgrams of Ac-Lys-D-Ala-D-Ala immobilized surfaces eluted by indicated concentrations of **4** (dashed lines) or **6** (solid lines). Determined at 25 °C in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P-20.

The dimers prepared in this study show no binding to D-Ala-D-Lac, yet regain the activity of Vancomycin.

# *Partitioning the Loss in Vancomycin Binding Affinity for D-Ala-D-Lac into Lost H-Bond and Repulsive Lone Pair Contributions*



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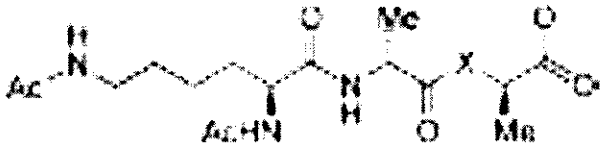

3 X = NH (Ac-Lys-D-Ala-D-Ala)
4 X = $CH_2$
5 X = O (Ac-Lys-D-Ala-D-Lac)

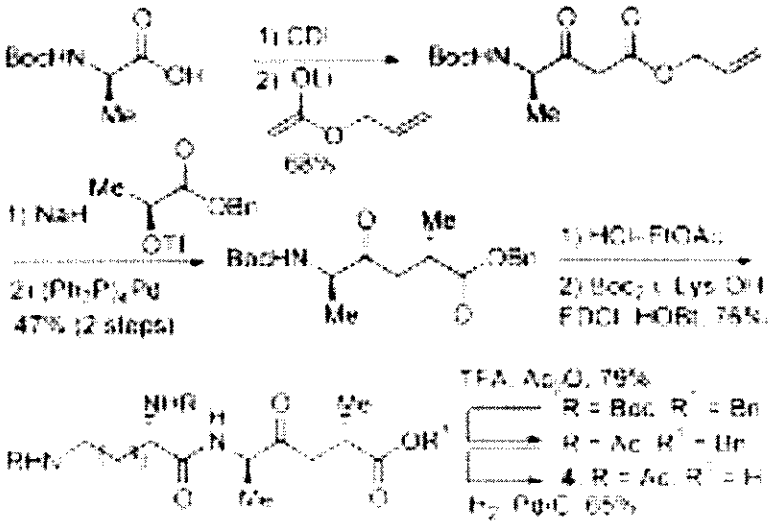


Table 1. Association Constants (K) and Binding Free Energy (−ΔG, 25 °C)[a]

| ligand | vancomycin (1) $K_a$, $M^{-1}$ (−ΔG°, kcal mol$^{-1}$) | vancomycin aglycon (2) $K_a$, $M^{-1}$ (−ΔG°, kcal mol$^{-1}$) | |
|---|---|---|---|
| 3 (X = NH) | $4.4 \times 10^5$ (7.7) | $5.8 \times 10^4$ (7.8) | 1.5-1.8 kcal/mol |
| 4 (X = $CH_2$) | $3.3 \times 10^4$ (6.2) | $2.5 \times 10^4$ (6.0) | |
| 5 (X = O) | $4.3 \times 10^3$ (5.0) | $3.1 \times 10^3$ (4.8) | 2.6 kcal/mol |

[a] 25 °C, 0.00001 M vancomycin in 0.02 M sodium citrate, pH 6.1, observed at 279 nm, ref 12

# *Summary*

- A number of model peptides or depsipeptides were prepared and their binding constants to Vancomycin and its aglycon were measured.
- The free energies of binding of the model compounds delineated the effect of H-Bond loss and repulsive lone-pair interactions.
- The loss in H-Bonding capability from D-Ala-D-Ala to D-Ala-D-Lac was determined to account for ~1.5 kcal/mol of the 4.1 kcal/mol (~1000 fold decrease in binding) that is lost. The lone-pair repulsion was found to account for the majority of the loss in binding affinity at 2.6 kcal/mol.