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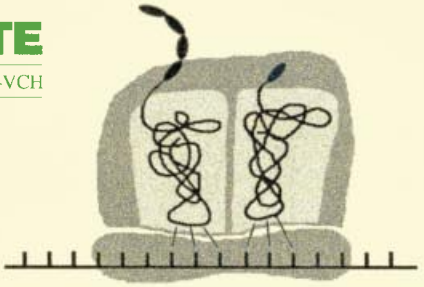

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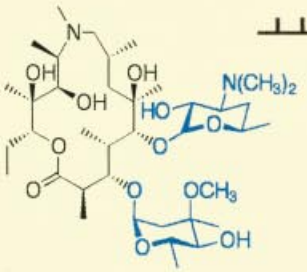

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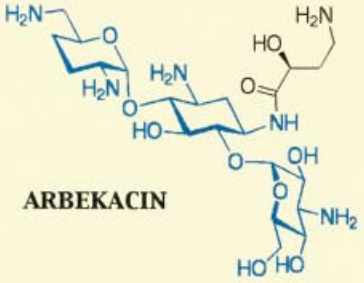

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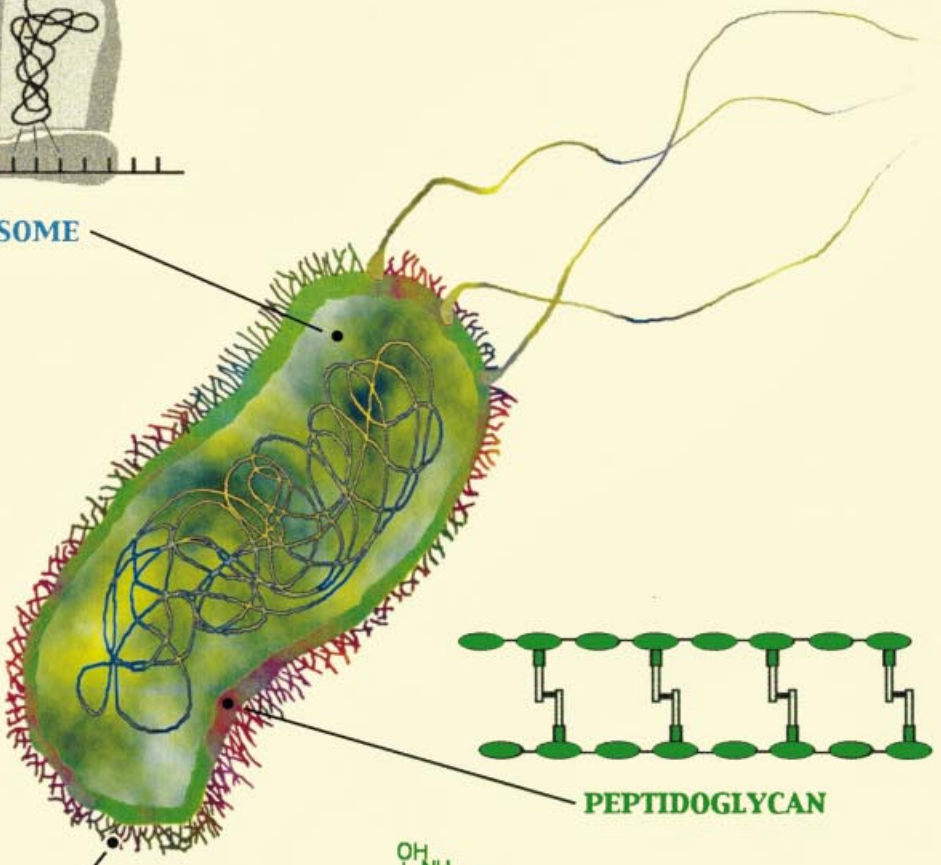

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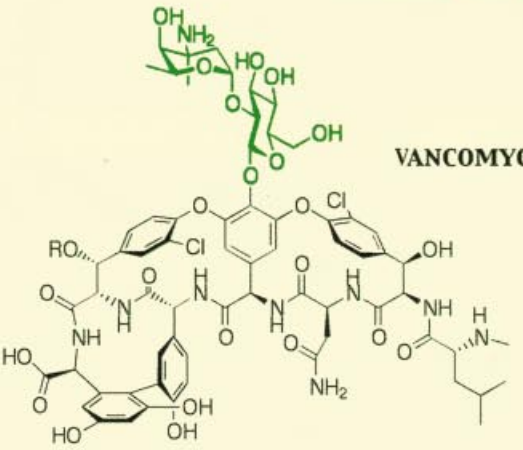

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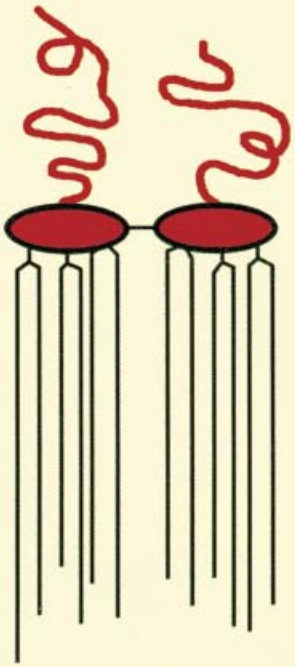

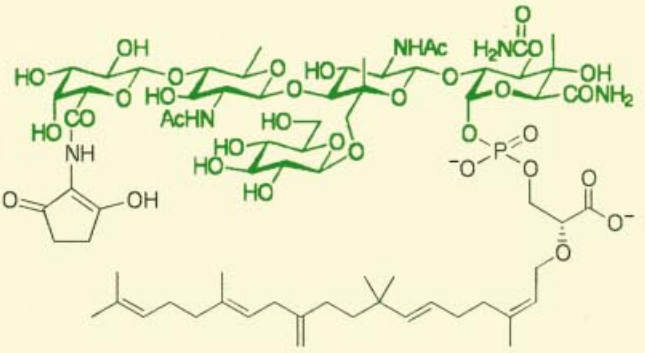

MOENOMYCIN

Carbohydrate-Based Antibiotics: A New Approach to Tackling the Problem of Resistance

Thomas K. Ritter and Chi-Huey Wong*

Recent interest in the problem of antibiotic resistance has led to the identification of new targets and strategies for antibiotic discovery. Among these efforts, the development of small molecules as antibiotics to target carbohydrate receptors or carbohydrate-modifying enzymes represents a new direction. This review covers recent work in this regard and discusses the impact of each strategy on the development of drug resistance. Particularly interesting targets include unique cell-surface carbohydrates, the transglycosylase involved in peptidoglycan biosynthesis, and bacterial RNA. With a greater understanding of the genome of different bacteria as well as advances in functional genomics and proteomics, we can expect the discovery of a variety of targets for the development of novel antibiotics.

Keywords: antibiotics · carbohydrates · enzyme inhibitors · peptidoglycan · RNA

1. Introduction

Antibiotics are bacterial or fungal products that inhibit the growth of other organisms. To date, the term antibiotics often includes synthetically produced antibacterial substances. Penicillins, the first major class of antibiotics, were introduced in the 1940s and 1950s. They were hailed as miracle drugs that eliminate bacteria without doing much harm to the cells of the treated individuals. Nevertheless, as a result of the saturation of the market, many large pharmaceutical companies lost interest in the development of antibiotic drugs and instead focused on chronic diseases. Through mutation and exchange of genes, however, bacteria have continued to develop resistance not only to single but also to multiple antibiotics.[1, 2] Strains of at least three bacterial species that cause life-threatening diseases (*Enterococcus faecalis*, *Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa*) have developed resistance to all available antibiotics. Enterococci, which are known to exchange resistance genes quite easily, have been able to transfer their resistance to *Staphylococcus aureus* in the laboratory. *S. aureus* is a particularly virulent organism that causes a wide range of diseases, and the fear is that they will eventually do so in nature as well.[3] Two major factors are known to aggravate the problem of antibiotic resistance: overuse of antibiotics in humans and animals, and noncompliance to the course of treatment by patients. Both the long-term exposure to low doses and the failure to finish a prescription encourage more resistant bacterial strains to flourish. Bacterial resistance to an antibiotic drug is mediated by one or more of the following processes:[4, 5]

1. Prevention of the drug from reaching its target either by active efflux from or by reduced uptake into the cell, as well as by sequestration of the antibiotic by protein binding.
2. Deactivation of the antibiotic by enzymatic modification.
3. Modification of the drug's target, thereby eliminating or reducing the binding of the antibiotic.
4. Metabolic bypass of the inhibited reaction.
5. Overproduction of the antibiotic target.

Of these mechanisms, the first three represent the most common processes that enhance bacterial resistance to antibiotics (Figure 1). The widespread occurrence of such resistance mechanisms has renewed industrial interest in this field. Currently available antibacterial drugs act on only a small number of targets (see Section 2), and all experience resistance problems. To overcome these resistance problems efficiently, the identification of new targets as well as the improvement of existing drugs will be necessary.

Carbohydrates are found ubiquitously in nature as building blocks of the structural framework of cells, as sources of metabolic energy, and as key components of various intercellular recognition processes.[6, 7] It is therefore not surprising that various existing antibiotics are based on carbohydrates, that is, they contain a glycan portion in their structure or they

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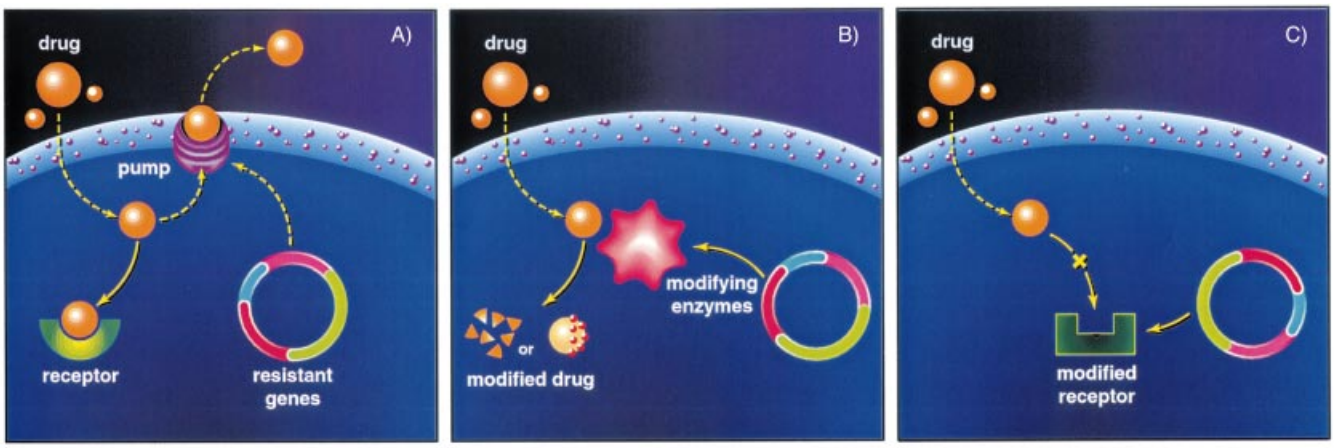

Figure 1. Mechanisms of bacterial drug resistance: A) active efflux; B) enzymatic modification of the drug; C) modification of target receptor or enzyme.

are non-carbohydrate molecules that target an enzyme or receptor associated with carbohydrate synthesis, metabolism, or recognition. Most of these carbohydrate-based antibiotics occur naturally or are semisynthetic. Although the development of carbohydrate-based therapeutics appears to have great potential, it has been hampered by a series of obstacles.[8] Carbohydrates are densely functionalized with hydroxy groups of similar reactivity, leading to difficulties in their synthesis. They often bind their targets with a relatively low affinity. Furthermore, medicinal chemists consider complex carbohydrates as an uninteresting class of molecules for drug development, since carbohydrates are usually too complex for process development and their bioavailability is hampered as they are too hydrophilic. They are generally not stable enough to allow oral administration. One way to overcome these problems is to design mimics of carbohydrates that have improved properties with regard to stability, specificity, affinity, and synthetic availability.[9, 10] These carbohydrate mimics potentially allow the targeting of the numerous natural processes in which glycans are involved, without causing the problems that arise from the undesirable properties of carbohydrates.

2. Site of Action of Antibiotics

Antibiotics are most commonly classified by target and mechanism of action. In this respect, antibiotics that interfere with the biosynthesis and function of the bacterial cell envelope represent a primary target for drug development. Historically, bacteria have been classified as Gram-positive or Gram-negative, based on the cell envelope structure (Figure 2). The cytoplasm of Gram-positive bacteria is encapsulated by the plasma membrane. This phospholipid bilayer is surrounded by the cell wall, which consists of a polysaccharide/polypeptide framework, known as peptidoglycan or murein. The surface of Gram-positive bacteria is studded with teichoic acids (phosphodiester polymers) that often

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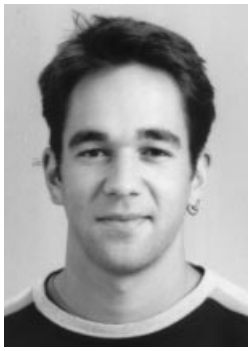
C.-H. Wong



T. K. Ritter

Thomas K. Ritter was born in Bühl (Germany) in 1972. He first studied at the University of Constance, before moving to Portland State University (Oregon), where he carried out research under the guidance of D. H. Peyton and received his M.S. in 1998 for his work on paramagnetic NMR spectroscopic studies of metalloproteins. Since then he has been working in the group of C.-H. Wong at The Scripps Research Institute in La Jolla (California) using synthetic organic chemistry to study biological systems, in particular the biosynthesis of bacterial cell walls.

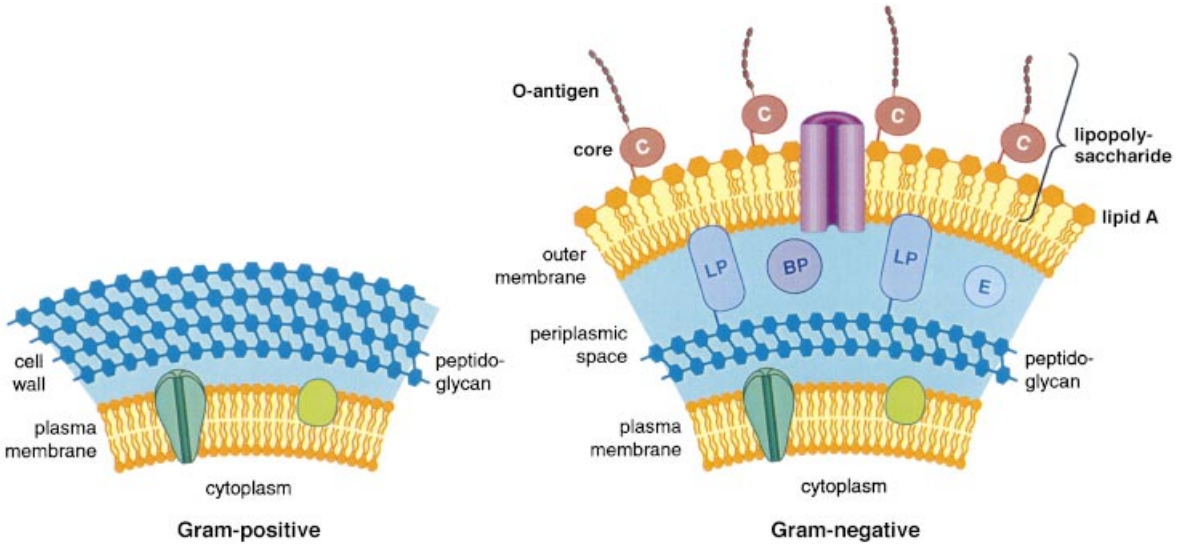


Figure 2. The cell envelope of Gram-positive and Gram-negative bacteria. Gram-positive bacteria have a thicker peptidoglycan layer than that of Gram-negative bacteria, but lack the outer membrane at the cell surface.

terminate in lipopolysaccharides (LPS). In contrast, Gram-negative bacteria are surrounded by two phospholipid bilayers (inner and outer membrane), which are separated by an aqueous compartment (periplasmic space). LPS represent the major component of the outer leaflet of the Gram-negative bacterial outer membrane. The periplasmic space contains a peptidoglycan layer as well as proteins that transport sugar and other nutrients. As a result of the importance of the cell envelope for the viability of bacteria, antibiotics that disrupt its integrity have been employed clinically for decades. Numerous mechanisms toward this end exist, with the inhibition of peptidoglycan biosynthesis perhaps being one of the most effective. The peptidoglycan layer provides the strength and rigidity of the cell envelope of bacteria, which allows them to live in a hypotonic environment and gives them their characteristic shape. Antibiotic-induced defects lead to cell lysis as a result of the inability of the bacteria to cope with the internal osmotic pressure. The biosynthetic pathway of peptidoglycan provides an attractive target for antibiotics, as it has no counterpart in mammalian cells. Other mechanisms geared towards the disruption of the cell envelope include the inhibition of LPS biosynthesis and pore formation in the bacterial membranes.

The prokaryotic translational machinery is the target of the majority of known antibiotics, probably because of the complexity of the ribosome, which makes it vulnerable to disruption in many ways. The crystal structure of the prokaryotic ribosome has only recently been solved.[11–13] It is composed of two unequal subunits: the large (50S) subunit consists of a 5S and a 23S rRNA molecule and 31 polypeptides, whereas the small (30S) subunit contains a 16S rRNA and 21 different polypeptides. The translation of mRNA into a protein commences with the formation of the initiation complex: in prokaryotes, the 30S and 50S ribosomal subunits and formylmethionine-tRNA assemble on a mRNA molecule. The ribosome then elongates the polypeptide in a multistep reaction cycle (Figure 3). Aminoacyl-tRNA binds to the A site; subsequently the growing polypeptide chain is transferred from the P-site tRNA to the aminoacyl-tRNA in the A site. Transfer of the resulting uncharged P-site tRNA out of the ribosome, and translocation (movement of the peptidyl tRNA from the A site to the P site) then complete the cycle. Finally, binding of two release factors to the termination codon induces transfer of the peptidyl group to

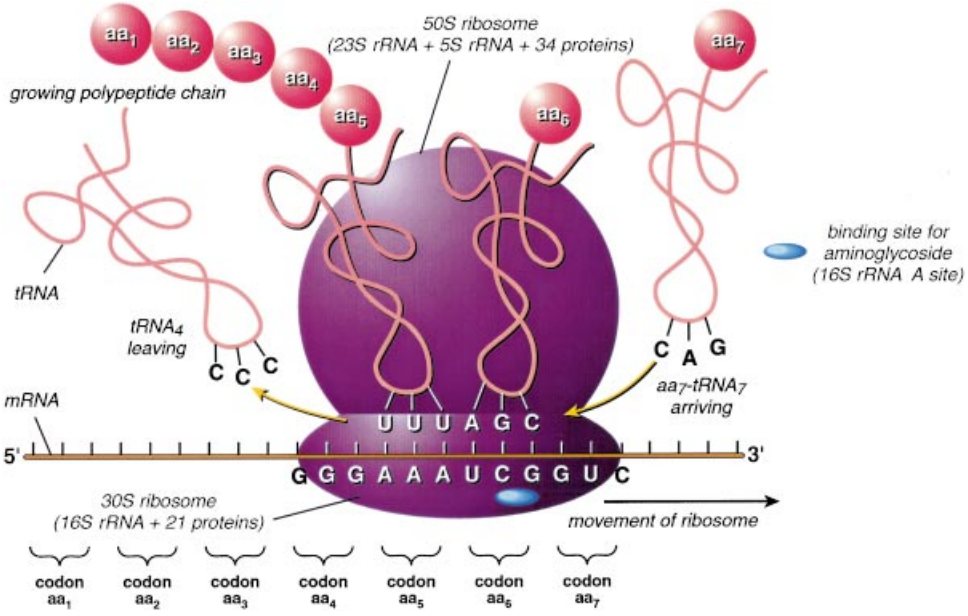


Figure 3. Ribosomal protein synthesis and the 16S rRNA A site for the binding of aminoglycosides.

water rather than to an aminoacyl-tRNA. The aminoglycoside and tetracycline antibiotics inhibit translation by binding to the small ribosomal subunit, whereas the macrolide antibiotics, chloramphenicol, and the oxazolidinones, a relatively new class of synthetic antibiotics, target the large subunit. In addition to the two major classes, there are antibiotics that affect RNA synthesis (rifamycins), DNA topoisomerase inhibitors (quinolones, 2-pyridones),[14, 15] and the so-called antimetabolites (sulfonamides, trimethoprim) that interfere with folic acid metabolism.

3. Antibiotics That Target the Cell Envelope

3.1. LPS

In higher animals and humans, the release of LPS, also known as endotoxins, upon treatment with antibacterial agents may invoke septic shock. This further leads to an inflammatory response, resulting in numerous deaths in intensive-care units worldwide. A late treatment with conventional antibiotics will lead to acceleration of LPS release and therefore aggravation of the disease. Strategies for the control of LPS-mediated pathophysiological disorders include methods that interfere with LPS/serum or LPS/cell receptor interactions (e.g. LPS antibodies and LPS antagonists) or with the production and secretion of inflammatory mediators.[16] Additionally, agents that interfere with the biosynthesis of LPS are bacteriocidal or at least inhibit bacterial growth, thus resulting in higher susceptibility to the action of other antibiotics. Moreover, such agents might lower the risk of Gram-negative sepsis by reducing the amount of endotoxins released when other antibiotics are administered simultaneously.

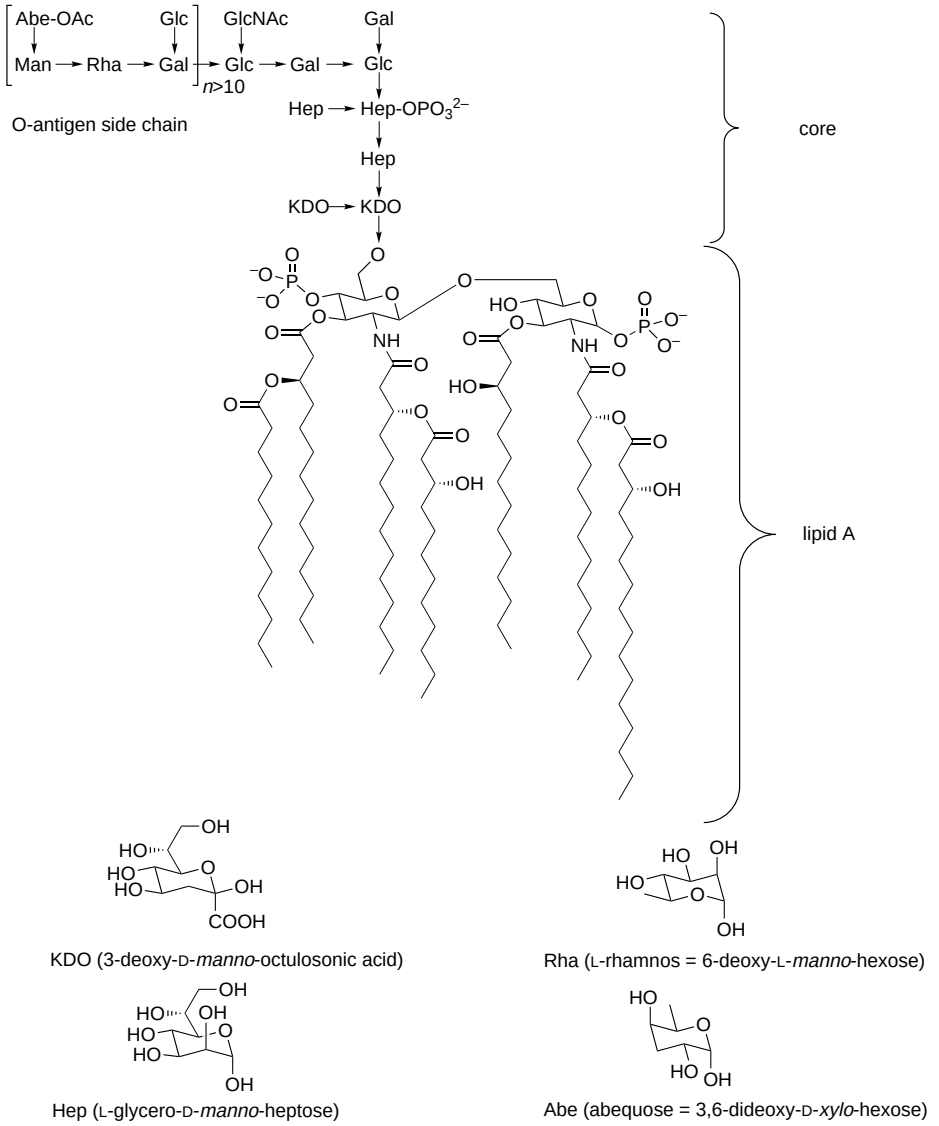
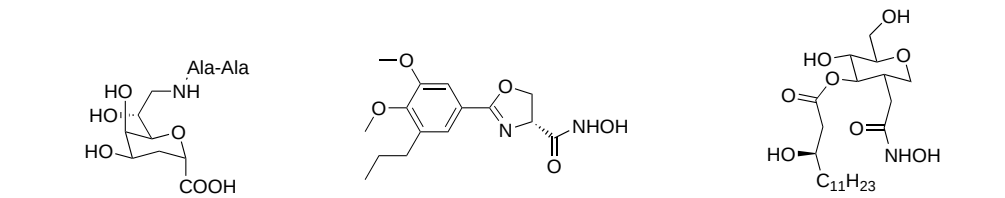


Figure 4. Lipopolysaccharide (LPS) of *Salmonella typhimurium* (GlcNAc = *N*-acetylglucosamine).

The structure of endotoxins consists of three regions (Figure 4). The lipid region (lipid A) consists of a GlcNAc–GlcNAc disaccharide that is modified by phosphates at the 1- and 4′-positions as well as by a variable number of fatty acids. This phosphoglycolipid is linked to a 3-deoxy-D-*manno*-octulosonic acid (KDO) unit of the core oligosaccharide, which varies from species to species. A repeating oligosaccharide polymer, the O-antigen, extends from the core oligosaccharide. The smallest LPS structure that is required for continued bacterial growth consists of lipid A and two KDO moieties. The pathological effects seen upon the release of LPS can be induced by its lipid A region alone.

One of the strategies that has been investigated towards the inhibition of LPS biosynthesis involves targeting the unique enzyme CMP-KDO synthetase, which activates KDO as cytidyl nucleotide prior to incorporation into the LPS layer. Although KDO exists as four different tautomers in solution, only the β-pyranose form can act as a substrate for CMP-KDO synthetase.[17] The 2-deoxy analogue of KDO is locked in the β-pyranose conformation and acts as a competitive inhibitor of the synthetase. 2,8-Dideoxy-8-amino KDO was found to be an even more potent inhibitor in vitro; therefore dipeptide conjugates were synthesized through the 8-amino group to facilitate transport across the membrane by peptide permeases. The 2,8-dideoxy-8-amino KDO dipeptides (Scheme 1) exhibited minimum inhibitory concentrations (MICs) of $5-100\,\mu g\,mL^{-1}$, depending on the species and agent tested.[17] The requirement for active aminopeptidases for activity suggests that these conjugates act as prodrugs that release the KDO analogue inhibitor once inside the cell. In an



2-amino-2,8-dideoxy KDO-dipeptide (CMP-KDO synthase) | L-161,240 (UDP-3-*O*-acyl-GlcNAc deacetylase) | TU-517 (UDP-3-*O*-acyl-GlcNAc deacetylase)

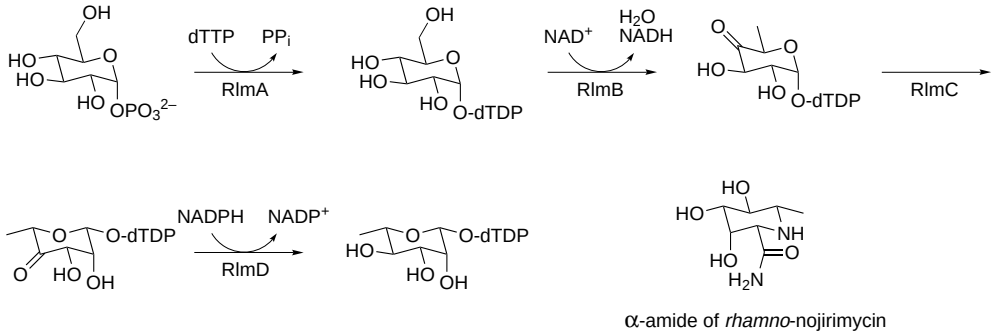
Scheme 1. Inhibitors of LPS biosynthesis. The targets are indicated in parentheses.

extension of this study, C-glycosyl derivatives of 2-deoxy KDO were synthesized.[18] However, the introduction of any substituent at the C2 position of KDO resulted in almost complete loss of inhibitory activity against CMP-KDO synthetase. No further development was reported with regard to these approaches.

L-*glycero*-D-*manno*-Heptose (Hep) is the second sugar of the core oligosaccharide that is highly conserved among several genera of bacteria, but not found in mammalian cells. The recent structural determination of ADP-Hep 6-epimerase, an enzyme of the Hep biosynthetic pathway, along with the mechanistic conclusions drawn from its structure may allow the design of mechanism- and structure-based inhibitors that have antibiotic activity.[19] Although these approaches, that is, the inhibition of the enzymes involved in the synthesis, activation, and transfer of KDO and Hep (the unique components of the core oligosaccharide) are interesting and certainly a new direction, they have so far not resulted in any new drugs.

A different strategy towards the inhibition of LPS production is to target the biosynthesis of lipid A, and some inhibitors have been reported (Scheme 1). Screening and subsequent optimization led to the discovery of hydroxamic acid derivatives that inhibit UDP-3-*O*-acyl-GlcNAc deacetylase (LpxC).[20] The hydroxamic acid moiety is thought to inhibit the deacetylase by chelating a metal ion in the active site, thereby mimicking a pyrophosphate group. The most active compound, L-161,240, had an MIC of about 1 $\mu g\,mL^{-1}$ against *E. coli* and rescued mice from an *E. coli* infection that was 100% fatal without treatment. In a more rational approach, hydroxamic acid moieties were attached to the 2-amino group of monosaccharides that resemble a portion of the LpxC substrate.[21] Compounds that feature a long alkyl chain, such as TU-517, exhibited a wider spectrum of antibacterial activity than the hydroxamic acids obtained by simple screening.

A more specific strategy than targeting the biosynthesis of core oligosaccharide and lipid A is the inhibition of enzymes involved in the biosynthesis of the O-antigens, as these vary from species to species. Deoxysugars are frequently found in O-antigens and L-rhamnose is one of the most common components. Incorporation of L-rhamnose into the cell wall occurs through its activated form, dTDP-L-rhamnose. This precursor is synthesized in four steps (Scheme 2):[22] coupling of glucose-1-phosphate with dTTP (deoxythymidine triphosphate) is followed by the action of an NAD^+-dependent 4,6-dehydratase, epimerization at the 3- and 5-positions, and reduction. The enzymes that catalyze this pathway represent an appealing target, since humans do not synthesize dTDP-L-rhamnose. Micromolar concentrations of the analogues of *rhamno*-nojirimycin and

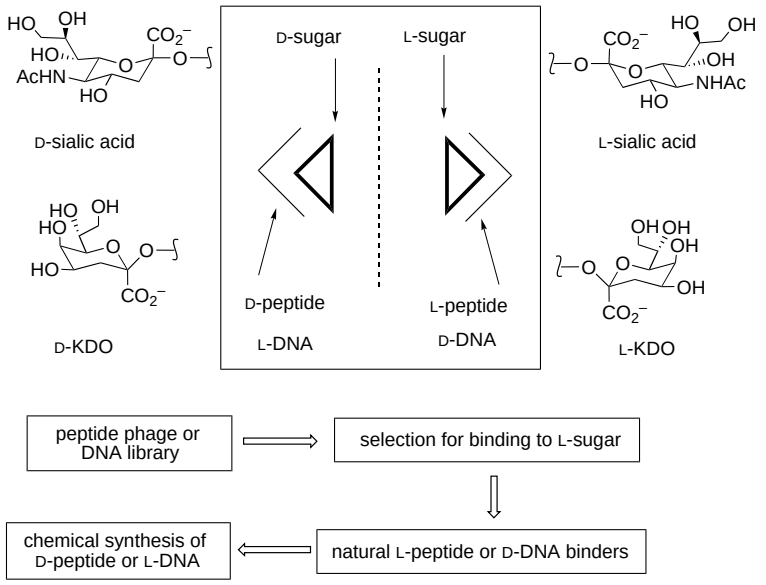


α-amide of *rhamno*-nojirimycin

Scheme 2. The dTDP-L-rhamnose biosynthetic pathway and one of its inhibitors (RlmA = glucose-1-phosphate thymidyl transferase, RlmB = dTDP-D-glucose 4,6-dehydratase, RlmC = 6-deoxy-D-xylo-4-hexulose 3,5-epimerase, RlmD = dTDP-6-deoxy-L-xylo-4-hexulose reductase).

related trihydroxypipecolic acid derivatives were shown to inhibit the biosynthesis of dTDP-L-rhamnose.[23] The recent structural work on the second, third, and fourth enzyme of the dTDP-L-rhamnose pathway could soon make a structure-based approach towards the inhibition of these enzymes possible.[24–26]

A new approach to the development of antibacterial agents that also makes use of the unique cell-surface carbohydrates, is the design of D-peptides, L-DNA, or L-RNA as synthetic receptors (Figure 5). These enantiomers of the natural L-peptides and D-nucleic acids are resistant to enzymatic degradation and are thus more stable in the circulation. To discover such enantiomeric oligomers, one can take advantage of biological replication and evolution methods by using phage display and DNA or RNA evolution.[27] Phage display has been used to select short peptides that bind small molecules such as dioxin[28] and biotin[29] with moderate affinities, and it may be possible to obtain similar binding to sugars. In a recent demonstration of this strategy, a phage peptide display was used to identify L-peptides that bind to L-sialic acid or L-KDO. The corresponding D-peptides can be synthesized chemically and are expected to bind the natural D-sugars. Initial studies have identified peptides on phage that binds sialic acid with a dissociation constant in the nanomolar range.[30] This strategy is complementary to the traditional carbohydrate-based vaccine approach.[31]



The negative charge of the phosphate substituents of lipid A provides the initial site of interaction of some cationic peptide antibiotics (e.g. defensins, magainins, cecropins).[32] These cationic peptides are amphipathic, that is, they have both a hydrophobic and a hydrophilic part. The presence of positively charged residues gives them an affinity for LPS that is higher than that of the metal ions that normally bind the LPS phosphate groups. Thus, the hydrophobic face of the antibiotic is positioned to disturb the integrity of the outer membrane, thus allowing insertion of the antibiotic into the membrane and formation of channels ("self-promoted uptake"). These pores promote leakage from the periplasm and increased uptake of antimicrobial compounds that act in synergy with the perturbing peptide. Additionally, the ability to bind to LPS gives these peptide antibiotics anti-endotoxic properties.

3.2. Peptidoglycan

Peptidoglycan biosynthesis[33] (Figure 6) commences with the transfer of an enolpyruvyl group from phosphoenolpyruvate (PEP) to UDP-*N*-acetylglucosamine (UDP-GlcNAc). The NADPH-dependent reduction of the resulting unsaturated acid gives UDP-*N*-acetylmuramic acid (UDP-MurNAc). Three amino acid residues, L-Ala, D-Glu, and L-Lys (in Gram-positive bacteria) or *meso*-diaminopimelic acid (*m*DAP in Gram-negative bacteria) are then attached sequentially in ATP-dependent reactions to the lactic acid residue of UDP-MurNAc. This is followed by the coupling of a preformed dipeptide, usually D-Ala-D-Ala, to the peptide terminus. The D-amino acids are synthesized either from L-amino acids under racemase catalysis, or from α-ketoglutarate in a reaction catalyzed by a D-amino acid aminotransferase. In the next step, MurNAc-pentapeptide phosphate is transferred from its UDP derivative to undecaprenyl phosphate, a carrier molecule embedded in the cytoplasmic membrane. A second GlcNAc residue is then coupled at the 4-position of MurNAc-pentapeptide. In many Gram-positive bacteria, the formation of the disaccharide is followed by the addition of several amino acids (usually glycines) to the ε-amino group of Lys. At this point, the GlcNAc-MurNAc peptide is translocated across the cytoplasmic membrane to the cell surface by an unknown mechanism. Here, the disaccharide units are polymerized by the action of several transglycosylases. Finally, transpeptidases catalyze the attack of the terminal side-chain amino group of *m*DAP (Gram-negative bacteria) or the amino terminus of the oligo-Gly linker (Gram-positive bacteria) at the peptide bond between the two D-Ala residues in a neighboring peptide chain, resulting in crosslinking with a concomitant loss of D-Ala.

Each of these enzymes represents a target for antibiotic development. The most commonly used antibiotics (the

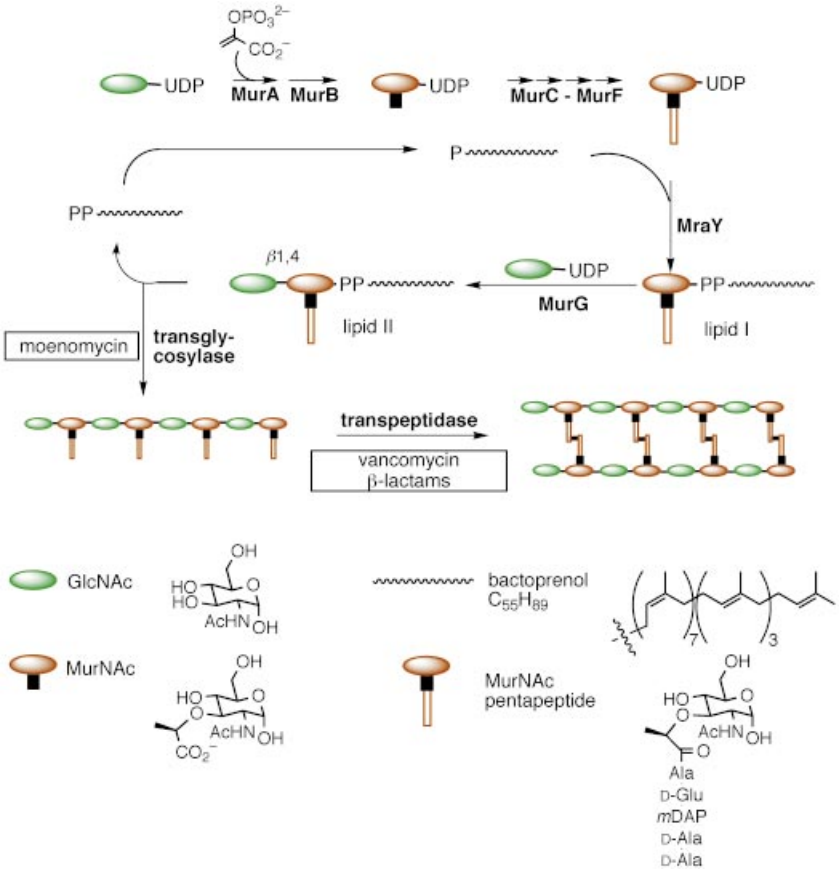
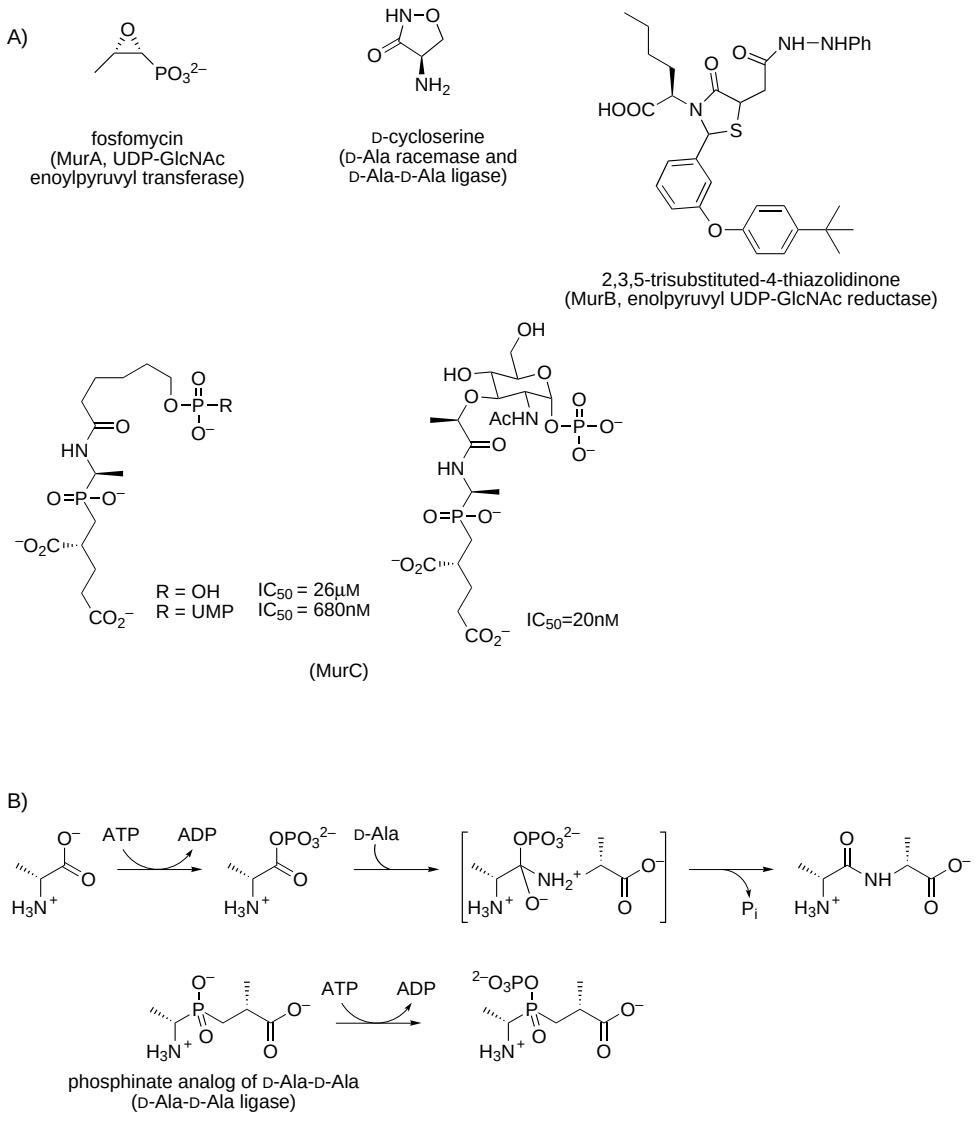


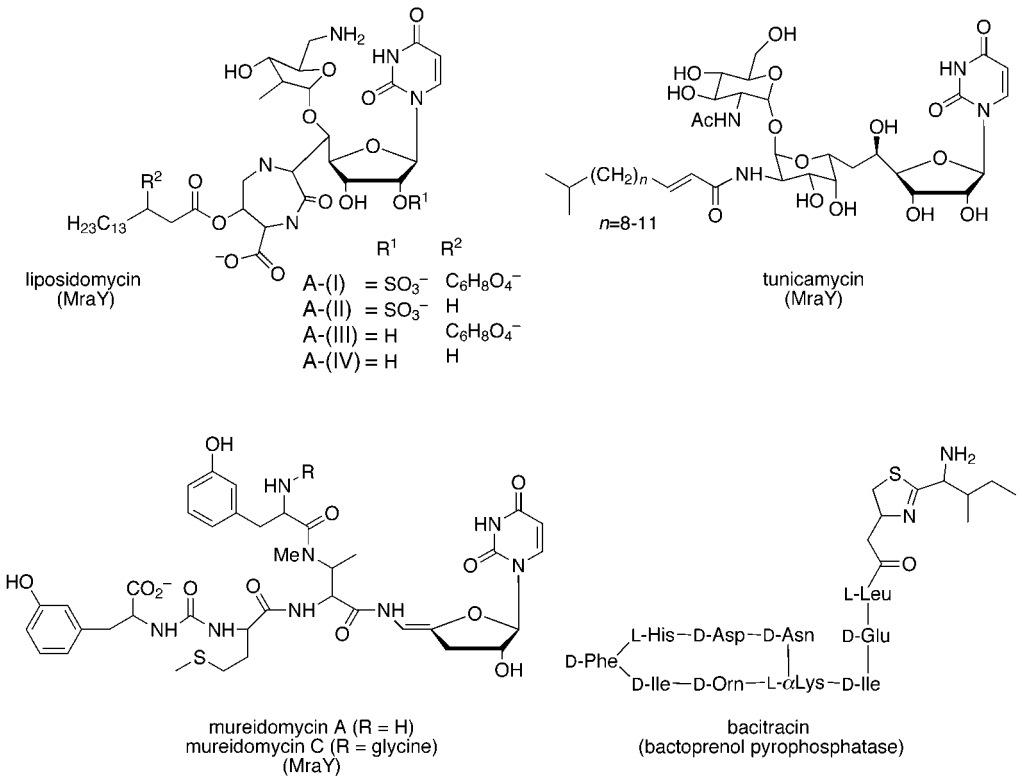
Figure 6. Biosynthesis of peptidoglycan (UDP = uridine diphosphate, Mur = muraminic acid, MurNAc = *N*-acetylmuraminic acid, P = phosphate substituent, MraY = translocase, *m*DAP = *meso*-diaminopimelic acid).

penicillins and cephalosporins) target the enzyme transpeptidase that catalyzes the crosslinking step. However, the widespread occurrence of resistance against these antibiotics as a result of the induction of β-lactamases, which catalyze the hydrolysis of the β-lactam moiety, has increased the significance of antibiotics that target the earlier steps in the peptidoglycan biosynthetic pathway (Scheme 3). Fosfomycin, an analogue of PEP, is an irreversible inhibitor of UDP-GlcNAc enolpyruvyl transferase (MurA). The crystal structure of the MurA/fosfomycin complex revealed that the antibiotic was covalently bound to a cysteine residue in the active site. This indicates that inactivation arises from the attack of the cysteine thiol at the epoxide moiety of fosfomycin.[34] The enolpyruvyl-UDP-GlcNAc reductase (MurB) has received attention only recently, and a first series of inhibitors has been found.[35] Diastereomeric mixtures of the reported 2,3,5-trisubstituted 4-thiazolidinones were designed to mimic the pyrophosphate moiety of the MurB substrate. The enzymes MurC-F, which are responsible for the sequential addition of amino acid residues to the lactic acid of MurNAc, as well as the D-Ala-D-Ala ligase (Ddl) all catalyze the formation of peptide bonds. Inhibitors of these enzymes mimic the tetrahedral intermediates of the ligase reaction by incorporation of a phosphorus-containing group in place of the peptide bond.[36] Recent structural and mechanistic studies of Ddl have revealed the mechanistic aspect of a phosphinate analogue that acts as a transition-state mimic of the reaction.[37] D-Ala-D-Ala racemase and Ddl are also targeted by the antibiotic D-cycloserine.[38] This antibiotic shows competitive inhibition of both enzymes and is thought to be a structural analogue of D-Ala. First generation inhibitors against MurD and MurE consisted of a phosphate group or UDP connected through a simple alkyl linker to the peptide group that mimicked the transition state.[39, 40] Substitution of the alkyl linker with the native MurNAc residue increased the potency of the inhibitors by several orders of magnitude.[41] Bacitracin, a cyclic peptide, inhibits the recycling of the carrier lipid by complexation to its pyrophosphate moiety in the presence of magnesium ions.[42]

Several classes of nucleoside antibiotics inhibit the phospho-MurNAc-pentapeptide translocase (MraY).[43] These include tunicamycin, the liposidomycins, and the mureidomycins (Scheme 4). Although tunicamycin is a fairly potent inhibitor, its selectivity for MraY is low. Tunicamycin also inhibits N-linked glycan formation of the precursors of mammalian glycoproteins, thus causing toxicity problems. In contrast, mureidomycins and liposidomycins exhibit excellent selectivity for MraY.[44, 45] In spite of their potent in vitro activity against MraY, liposidomycins showed only weak activity against bacteria. Transportation into cells might be limited by the hydrophilic sulfate moiety. Different medium components allowed the isolation of new liposidomycin analogues that lack the sulfate and/or 3-methylglutamic acid moiety.[46, 47] Not only did these new analogues selectively inhibit MraY, but they were also more potent than their natural, more hydrophilic counterparts.[46] An initial study towards the identification of the pharmacophore of the liposidomycins has been undertaken by superimposing their lowest energy conformation on the nucleoside substrate, UDP-MurNAc-pentapeptide.[48] Mo-



Scheme 3. A) Inhibitors of the early stages of peptidoglycan biosynthesis (targets are indicated in parentheses); B) the D-Ala-D-Ala ligase reaction.



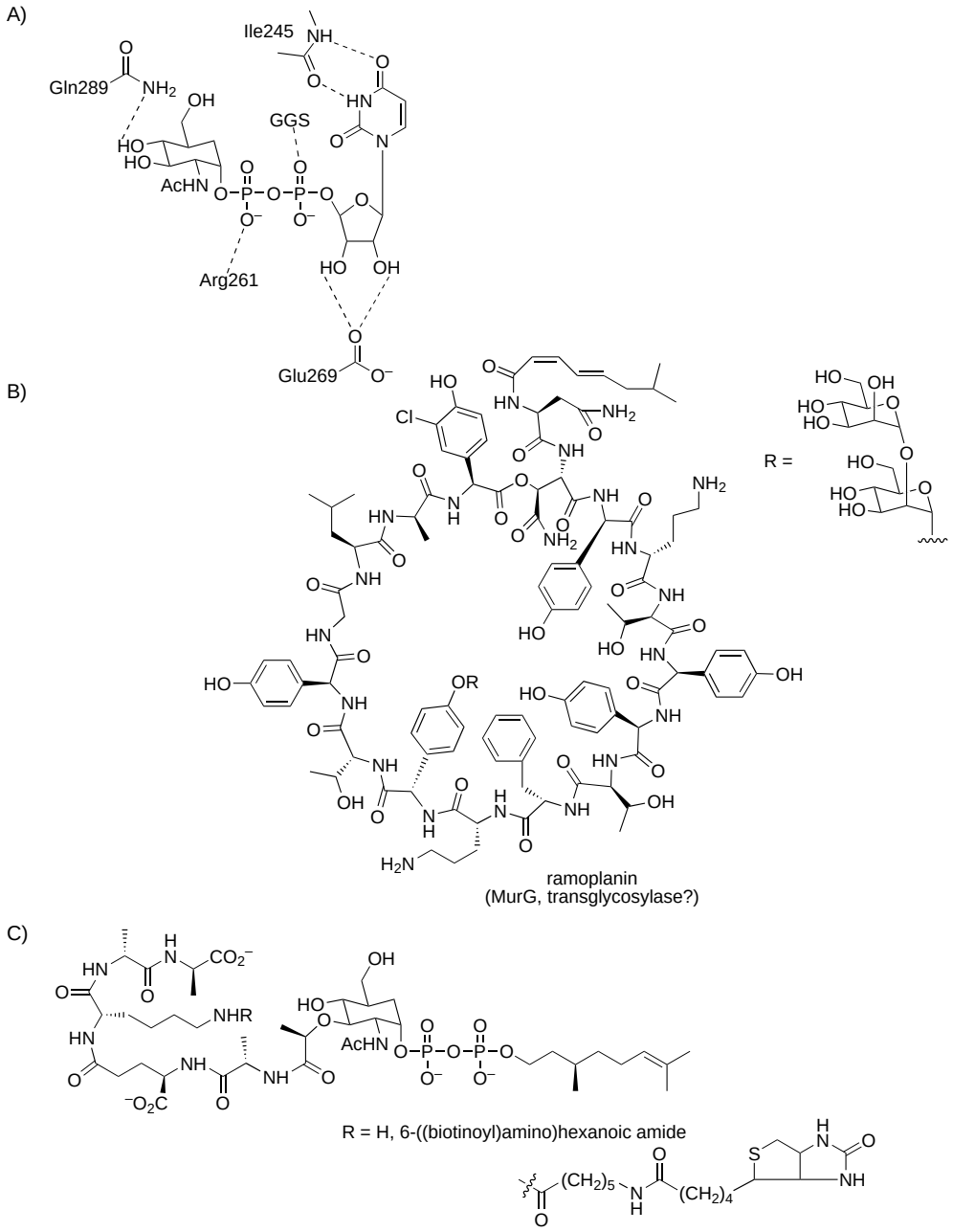
Scheme 4. Inhibitors of MraY (translocase) and of bactoprenol pyrophosphatase.

lecular overlap analysis indicated that the ribosamine sugar of the liposidomycins mimics the pyrophosphate group of UDP-MurNAc-pentapeptide. The nucleoside ribosamine core of the liposidomycins was synthesized and showed fair inhibitory activity. For a more complete structure – activity relationship (SAR) study, the elucidation of the role of each functional group present in the liposidomycins is required. Furthermore, analogues of the mureidomycins were synthesized in an attempt to identify their mechanism of inhibition.[49] A structurally unique feature of the mureidomycins is the central enamide functionality that connects the nucleoside moiety to the peptide portion of the molecule. Enamides are known to tautomerize under acidic conditions to reactive *N*-acylimminium ions. It has been hypothesized that a nucleophilic attack at this reactive species in the active site leads to the covalent modification of the enzyme. However, the lack of reactivity of simple nucleoside structures that contain the unusual mureidomycin enamide functionality indicates that this moiety may not be involved in the slow binding inhibition of MraY by the mureidomycins.

Ramoplanin, a glycodepsipeptide, is the only known natural occurring inhibitor of the GlcNAc transferase (MurG) (Scheme 5).[50] The development of synthetic inhibitors of MurG has been hampered by difficulties in obtaining the enzyme and its substrate, which are present in cells in only small quantities. Recently, however, MurG has been successfully isolated[51] and its crystal structure has been solved.[52] Furthermore, a simplified analogue of lipid I has been shown to act as a substrate, thus greatly improving the feasibility of a MurG assay.[53] The synthetic substrate differs from lipid I only in that the 55-carbon undecaprenol chain has been replaced by the ten-carbon chain of citronellol. Additionally, a biotin-labeled derivative was synthesized to simplify the assaying procedure, thus paving the way for the design and evaluation of new synthetic MurG inhibitors. With this new methodology available, MurG was kinetically characterized,[51] and the site of inhibition of ramoplanin was reinvestigated.[54] Results from this study indicated that ramoplanin's main mode of action may be the inhibition of transglycosylase, instead of the inhibition of MurG which had been postulated earlier.

Among the enzymes involved in the biosynthesis of peptidoglycan, the transglycosylase, which is responsible for the polymerization of disaccharide units, is perhaps the most interesting target for several reasons. The enzyme is located on the cell surface, thus making it easily accessible to small molecular drugs. In addition, the polysaccharide backbone always remains intact in wild-type and resistant strains. New antibiotics that target the transglycosylation step may therefore be less prone to resistance development. Transglycosylase activity cannot be assigned to a single enzyme. Within the family of the penicillin-binding proteins (PBPs) of *E. coli*, several members with transglycosylase activity have been identified. Some of the PBPs are bifunctional and also catalyze transpeptidation. A systematic study of mutants that lacked all possible combinations of eight PBPs confirmed the absolute requirement of at least PBP1A or 1B for the viability of the bacteria.[55] In addition to the bifunctional PBPs, monofunctional transglycosylases exist in bacteria,[56] but their role in cellular peptidoglycan synthesis is not known. The same difficulties that were encountered in the development of MurG inhibitors are also prominent in studies of transglycosylation.[57] Activity against transglycosylases is generally detected in a cumbersome and somewhat ambiguous assay format.[58] There is a high-throughput screen available for transglycosylase; however, it can only detect compounds with high affinity to PBPs, as it relies on competition with the high-affinity phosphoglycolipid moenomycin.[59]

Natural products that inhibit transglycosylation (Scheme 6) can be categorized into two classes: 1) those that directly inhibit the enzymes (e.g. moenomycin), and 2) those that bind lipid II, the substrate of transglycosylation (e.g. type B lantibiotics, glycopeptide antibiotics). The three-dimensional structure of the phosphoglycolipid moenomycin, which is employed as a growth promoter in animal nutrition, has been

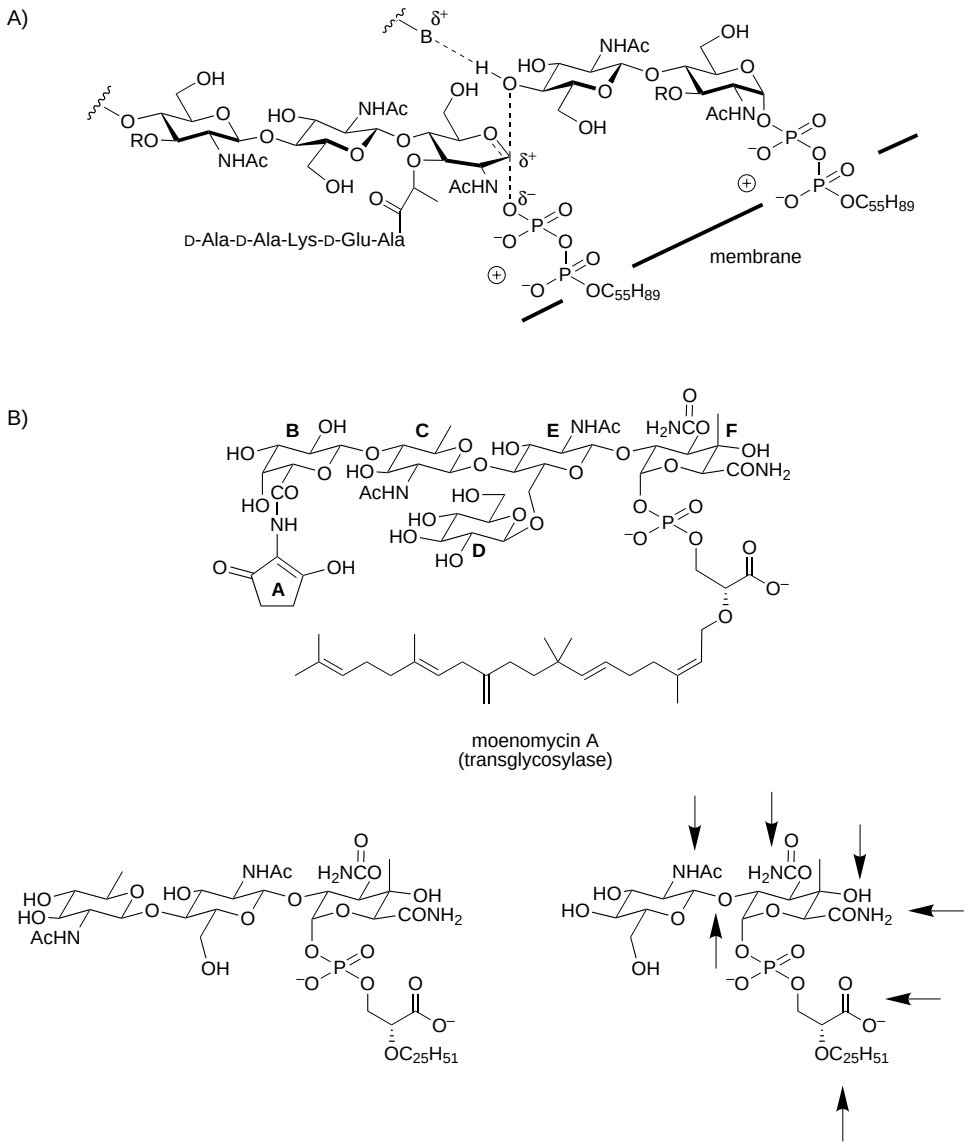


Scheme 5. GlcNAc (MurG): A) interactions between the donor substrate and the enzyme (determined by X-ray crystal structure analysis), B) a MurG inhibitor, C) structure of acceptor derivatives used in a MurG assay.

determined[60] and extensive SAR studies have been carried out.[61, 62] The moenomycins and analogues with at least three sugar units (C, E, F) are active in vivo against Gram-positive bacteria. Derivatives composed of only two sugars (E, F) are not active against antibiotics, but do inhibit transglycosylase in vitro, provided they have the correct substitution pattern. All functional groups on the F ring, the acetamide functionality of the E ring, and the lipid chain carboxylate and phosphate moiety are crucial for the activity. Unfortunately, the mechanism by which moenomycin mimics the natural substrates of transglycosylation is not clear upon examination of its structure. Rational design of more effective antibiotics based on the moenomycin core structure is therefore not trivial. However, a combinatorial library of novel disaccharides based on moenomycin has been synthesized, in which four key positions, the 3- and 4-substituents of the F ring, the 2-substituent of the E-ring, and the lipid side chain, were structurally explored (Scheme 7).[63] This study led to the discovery of a class of disaccharides with two aromatic substituents which inhibits peptidoglycan biosynthesis in vitro, are active as antibiotics, and exhibits an activity profile that is distinct from moenomycin.[64] Several inhibitors of transglycosylation that incorporate long alkyl chains to mimic the membrane anchor of lipid II and moenomycin were rationally designed. Phosphate,[65, 66] phosphonate,[66, 67] and thiazoline derivatives[68] of MurNAc and GlcNAc-MurNAc disaccharides have been pursued, but none of these molecules have efficiently inhibited transglycosylation. This result, in combination with the SAR studies on moenomycin, as well as the combinatorial moenomycin disaccharide library, indicates that small mono- and disaccharides probably do not provide a sufficient number of interaction sites for tight binding to the enzymes that catalyze transglycosylation.

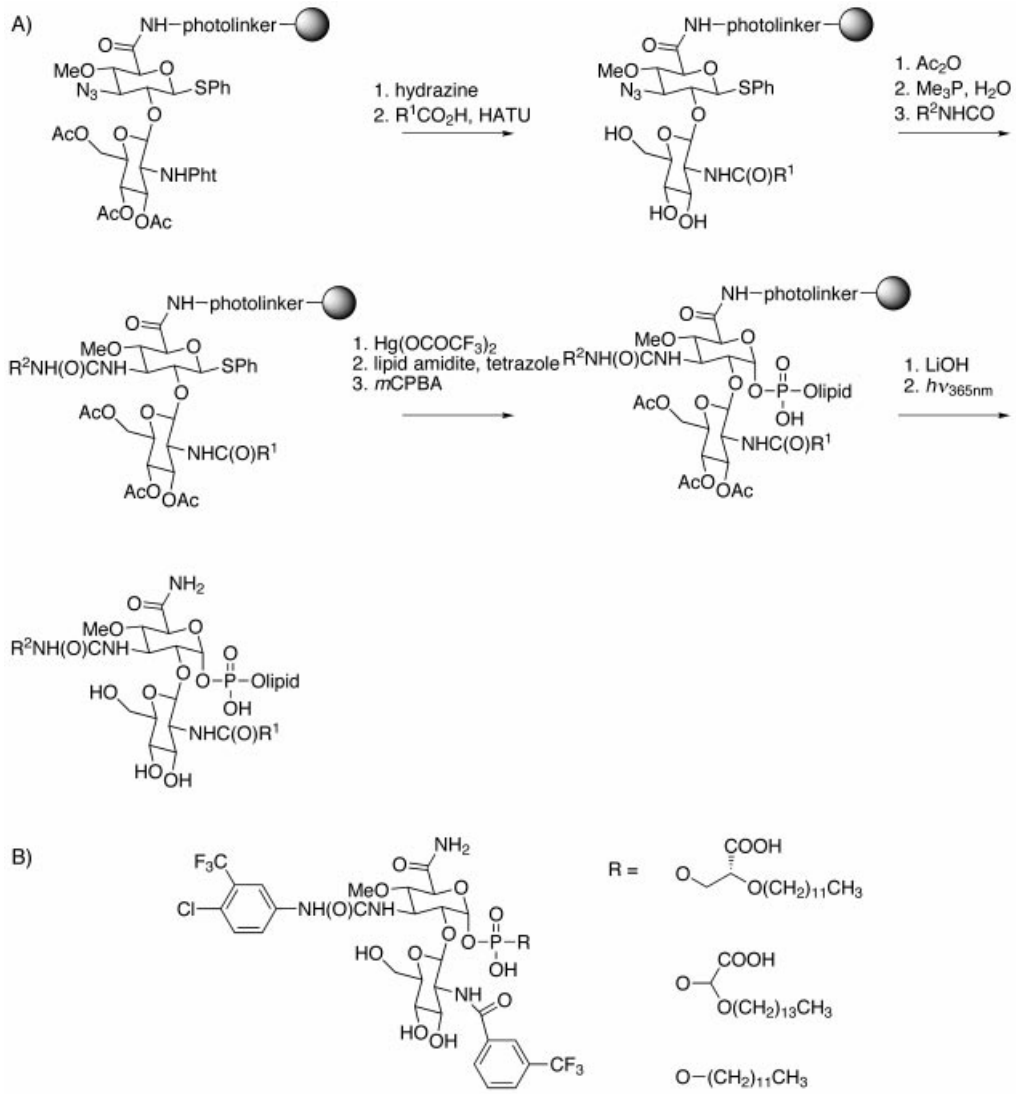
Lantibiotics are a class of peptide antibiotics that contain the unusual amino acids lanthionine and methyllanthionine and form intramolecular thioether linkages (Figure 7).[69, 70] They interact with docking molecules in the cytoplasmic membrane, for example, lipid II.[71, 72] The binding was not antagonized by D-Ala-D-Ala, which indicates an alternative binding mode to that of vancomycin (see Scheme 8). The lantibiotics can be subdivided into two classes, according to their mode of action. Type A lantibiotics (e.g. nisin) form pores in the cytoplasmic membrane upon binding to the docking molecules,[73] whereas type B lantibiotics (e.g. mersacidin, actagardine) directly interfere with peptidoglycan biosynthesis by inhibiting transglycosylation.[74]

During the last decade, the glycopeptide antibiotics have been intensively investigated and several reviews on this class of molecules have been published.[75, 76] Two glycopeptide



Scheme 6. The transglycosylase reaction: A) proposed transition state, B) moenomycin A and two simplified derivatives. The arrows indicate structural elements that were identified from SAR studies as essential for activity.

antibiotics, vancomycin and teicoplanin, are in clinical use and are often referred to as last-resort drugs, because of their utility in treatment of infections by bacteria that are resistant to many other classes of antibiotics, in particular methicillin-resistant *S. aureus* (MRSA) (Scheme 8). The mechanism of the glycopeptide antibiotics is well understood: the cyclic peptide portion binds noncovalently to the D-Ala-D-Ala termini of lipid II and immature peptidoglycan, thereby preventing transglycosylation and cross-linking of the peptide side chains (Scheme 9).[77] Resistance against vancomycin occurs when bacteria change the composition of the peptide portion of peptidoglycan.[78] Resistant strains that feature D-lactate or D-serine as the terminal amino acid have been isolated. In both cases, the hydrogen-bonding pattern between the cyclic peptide of the drug and the peptide termini of peptidoglycan is disturbed, leading to lower affinity binding, and therefore resistance. Even though the SAR of the glycopeptide antibiotics has been studied extensively,[79] the modifications necessary to improve the affinity for the peptide termini of vancomycin-resistant bacteria are not clear. Moreover, such altered antibiotics could be rendered useless in a short amount of time as a result of a further change in the peptide portion of peptidoglycan by the evolving bacteria. In contrast to the well-established binding mechanism of aglycon–peptidoglycan interaction, the role of the carbohydrate portion of glycopeptide antibiotics remains unclear. In vitro activity of vancomycin is affected minimally by removal of the sugar substituents; in vivo activity, however, is significantly reduced, which indicates the importance of the glycan for a favorable pharmacokinetic profile.[79] A comparative NMR spectroscopic study of vancomycin and its aglycon has demonstrated that the vancomycin disaccharide influences the conformation of the cyclic peptide. In particular, the alignment of the amide protons, which participate in the hydrogen-bonding network with the cell-wall precursors, differs between the two structures, thus indicating the possible role of glycosylation in maintaining the bioactive conformation.[80] A further task of the carbohydrate in dimerization has been suggested. Glycopeptide antibiotics are known to dimerize, thus leading to an increased activity as a result of multivalent effects. The tendency to dimerize and the antibiotic activity are stronger in A82846B (vancomycin plus one additional carbohydrate substituent) than in vancomycin itself, which indicates that the carbohydrate substituent may be involved in noncovalent dimer formation.[81] The concept of multivalency as a mechanism of glycopeptide antibiotic action has been investigated in more detail with synthetic covalent dimers of vancomycin (Scheme 10).[82, 83] These dimers not only bind to short peptides that terminate in D-Ala or D-lactate more strongly than to the corresponding monomers, they also showed increased activity against vancomycin-resistant enterococci (VRE). A trimeric vancomycin analogue that features a trifunctional amine as core, was found to bind a synthetic trimeric D-Ala-D-Ala peptide with a dissociation constant of 4×10^{-17} M, the tightest association known among low molecular weight organic species.[84] A vancomycin polymer has been synthesized by the introduction of an olefin-containing substituent, followed by polymerization with Grubbs catalyst.[85] This polymer also



Scheme 7. A combinatorial library based on moenomycin. a) Synthetic scheme (HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate, *m*CPBA = *meta*-chloroperbenzoic acid), b) some of the most active library members.

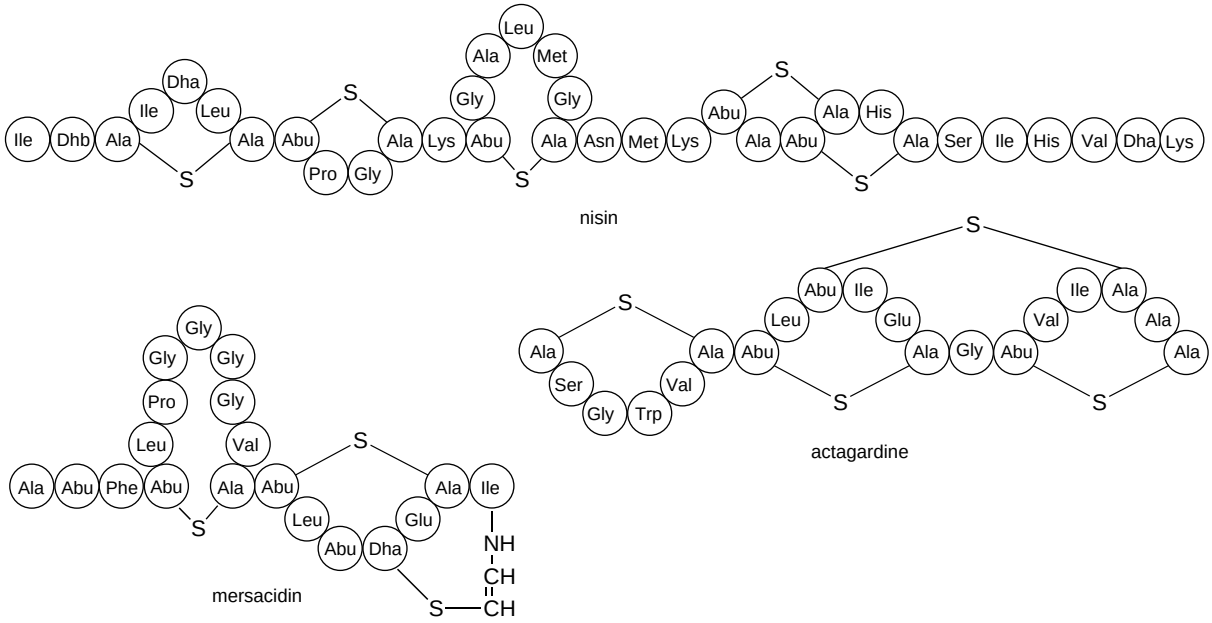
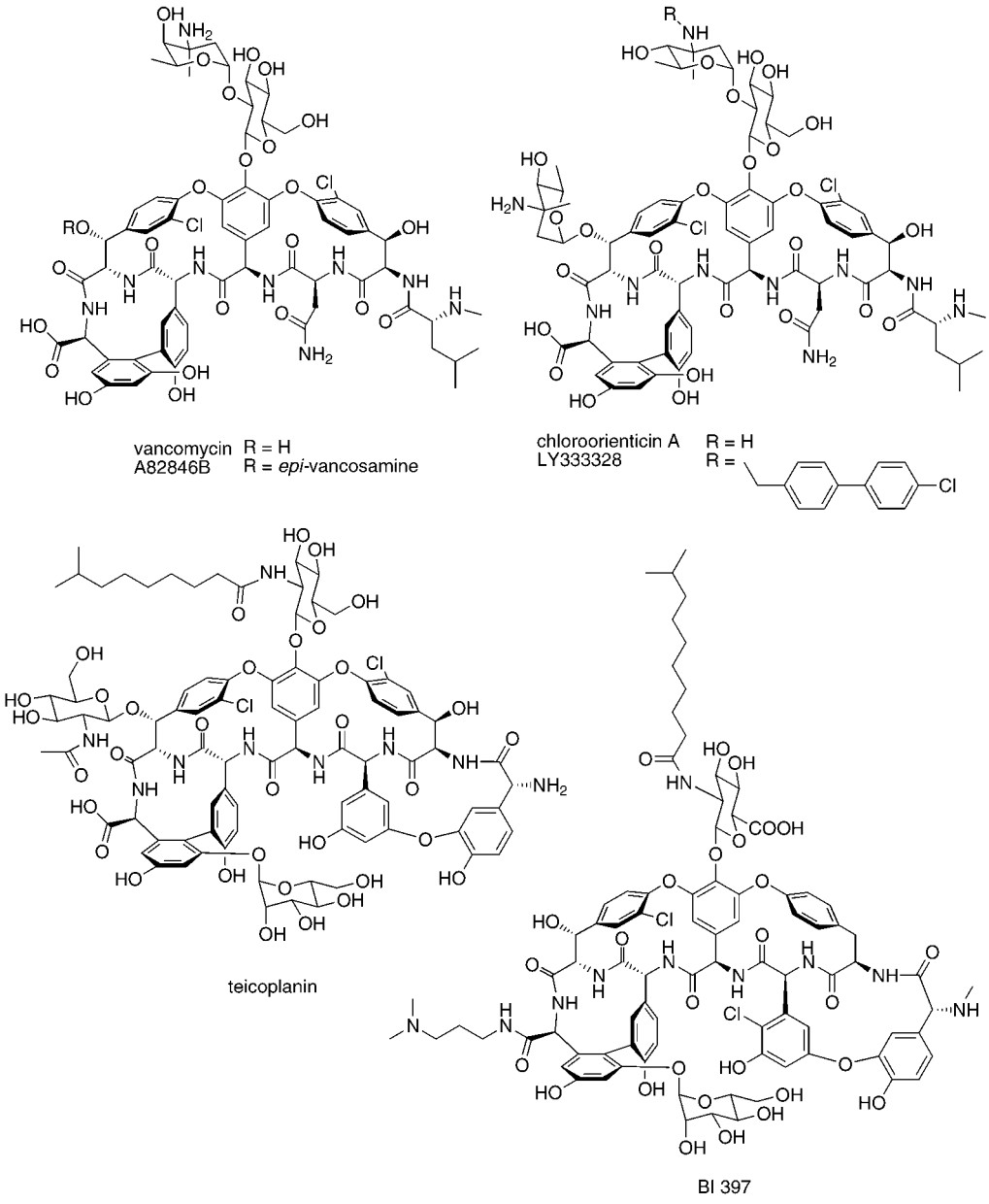


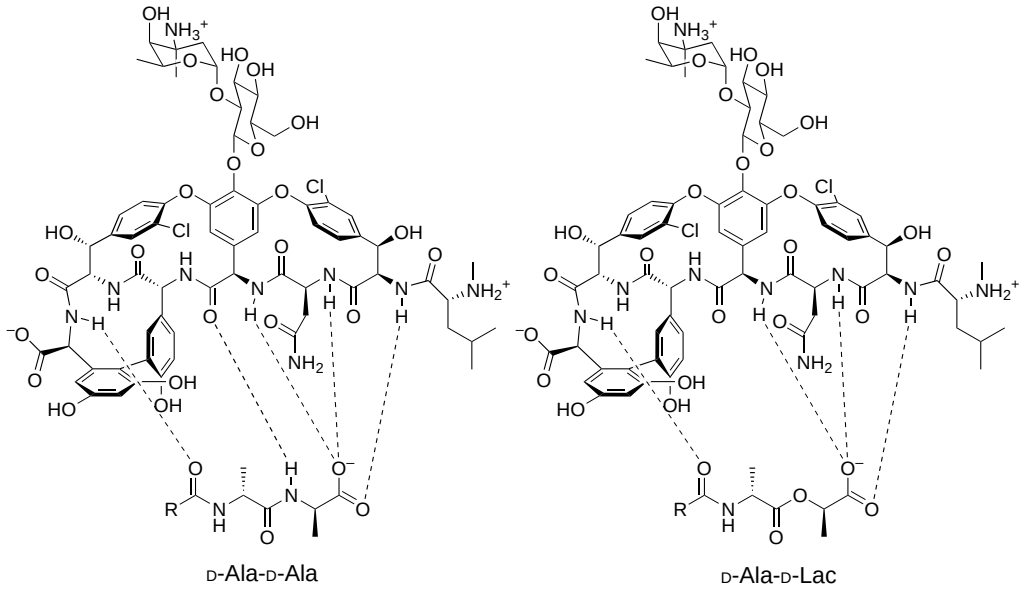
Figure 7. Lantibiotics (Dha = 2,3-didehydroalanine, Dhb = 2,3-didehydrobutyrine, Abu = α-aminobutyric acid, Ala-S-Ala = lanthionine, Abu-S-Ala = 3-methyllanthionine).



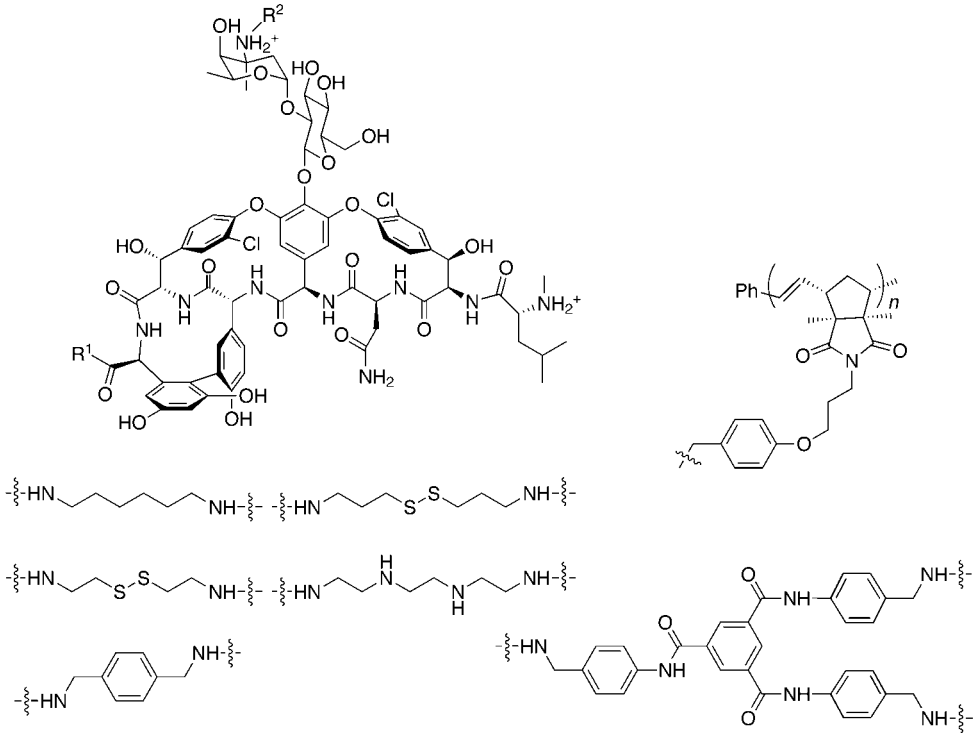
Scheme 8. Important glycopeptide antibiotics.

showed a significantly increased activity against VRE. In a "dynamic target-driven combinatorial synthesis,"[86] the greater formation constant of vancomycin dimers in the presence of its target was exploited to find a potent vancomycin derivative dimer with the optimal linker length (Scheme 11).[87] To this end, eight compounds (vancomycin, desleucyl vancomycin, β-alanine desleucyl vancomycin, and asparagine desleucyl vancomycin, each with a C_4 or a C_6 olefin tether attached to their terminal sugar substituent) were dimerized with Grubbs catalyst in the presence of a Lys-D-Ala-D-Ala tripeptide. Preferential formation of dimers that contained the native vancomycin aglycon structure and short tether lengths was observed, and these compounds were also most active against a range of enterococci and staphylococci. Indeed, the most active dimers had drastically improved activities against an intermediate vancomycin-resistant strain of *S. aureus* and a VRE strain. The correlation between dimerization rate enhancement and antibiotic activity demonstrates the usefulness of the target-accelerated combinatorial synthesis concept in predicting biological properties.

Several studies have been focused on modifying the carbohydrate portion of glycopeptide antibiotics. Hybrids between the aglycon part of vancomycin and various sugars were produced in vitro and in vivo by using cloned glycosyltransferases.[88] Chemical modifications of the carbohydrate portion were mainly carried out on the terminal sugar substituent of vancomycin, referred to as vancosamine.[89] The vancosamine amino functionality can be modified selectively without the protection of the other vancomycin functional groups. Although N-acylation led to only slight improvements in activity, N-alkylation had a more profound effect. The activity of vancomycin against VRE could be significantly improved by the incorporation of long-chain alkyl and aryl substituents. These hydrophobic moieties can act as anchors by inserting into the plasma membrane. This leads to an effective increase in the concentration of the antibiotic at the site of transglycosylation. The hydrophobic substitution also enhances dimerization, but it has not been demonstrated that an enhancement in dimerization has a direct correlation with an increase in antibiotic activity. The activity of one of the most active N-alkylated analogues (chlorobiphenyl desleucyl vancomycin) is independent of binding to the peptide terminus of peptidoglycan, thus identifying transglycosylase as its target.[90] This result implies that the modified saccharide is a key determinant for activity. Indeed, the disaccharide portion of chlorobiphenylvancomycin with a simple aryl substituent in place of the aglycon unit showed some inhibition of transglycosylase.[91] The glycopeptide antibiotic LY333328, a vancomycin derivative that features both the chlorobiphenyl side chain and the additional sugar substituent of A82836B, is highly efficient against MRSA and VRE and is now in clinical trials.[92, 93] BI 397, a new semisynthetic derivative of teicoplanin, is a complex of six related structures, which differ only in the structure of the N-acyl side chain of the *N*-acylaminoglu-



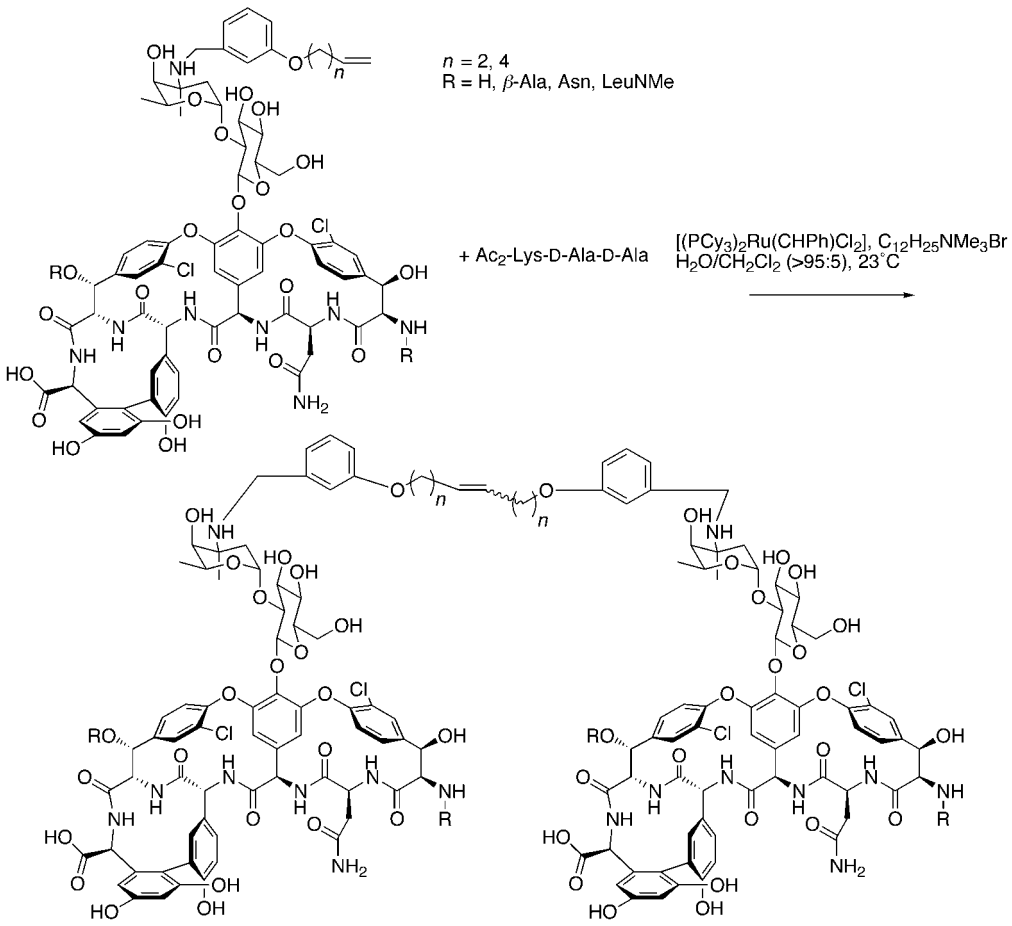
Scheme 9. The binding of vancomycin to the D-Ala-D-Ala termini of cell wall precursors is mediated through five hydrogen bonds. Substitution of the terminal D-Ala with D-Lac in vancomycin-resistant strains results in the loss of one hydrogen bond, and thus the binding affinity is decreased 1000-fold.



Scheme 10. Vancomycin dimers, trimer, and polymer (R^1 = linker attachment in the dimer and trimer; R^2 = linker attachment in polymer).

curonic acid substituent. It is more active in vitro against staphylococci than vancomycin and teicoplanin, including some teicoplanin-resistant strains.[94] Furthermore, lower dosages with a reduced number of daily treatments were necessary to reduce the bacterial loads in a model study of staphylococcal endocarditis in the heart of a rat.

Mycobacteria, a class of Gram-positive bacteria that include the causative agents of tuberculosis and leprosy, are known to be resistant to a large number of common antibiotics. This resistance is thought to be caused by the impermeability of their cell walls, which differ from those of other Gram-positive bacteria in several ways.[95] The peptidoglycan of mycobacteria is covalently linked to mycoylarabinogalactan (mAG) by means of a phosphodiester bond. mAG consists of D-galactofuranoses, D-arabinofuranoses, and mycolic acids, (≈C-90 branched fatty acids) which extend perpendicularly to the arabinogalactan/peptidoglycan layer. Surface glycolipids intercalate into the mycolic acid layer to form a pseudo lipid bilayer. Somewhat embedded into the mycolylarabinogalactan/peptidoglycan framework is the key surface molecule involved in the virulence and immunopathogenesis of mycobacterial diseases, lipoarabinomannan (LAM). Both mAG and LAM contain D-arabinofuranose residues, which are otherwise extremely rare in nature and thus good targets for antibiotics. Ethambutol, a naturally occurring inhibitor of arabinosyltransferase,[96] is used in the therapy of tuberculosis, and is specific in its antibiotic effects against mycobacteria (Scheme 12). Because of the relatively low activity and severe side-effects of ethambutol, synthetic inhibitors of arabinosyltransferase have been investigated, including ethambutol–sugar hybrids[97] and aza analogues of



dimers in order of abundance	MIC (μg/ml) against VRE
(LeuNMe)C_2-(LeuNMe)C_2	2
(LeuNMe)C_2-(β-Ala)C_2	8
(LeuNMe)C_2-(LeuNMe)C_4	4
(β-Ala)C_4-(β-Ala)C_4	8
(LeuNMe)C_4-(LeuNMe)C_4	>16
(β-Ala)C_2-(β-Ala)C_2	>16
vancomycin	>100

Scheme 11. Target-accelerated combinatorial synthesis of dimers of vancomycin analogues.

arabinofuranose.[98] Unfortunately, none of these molecules exhibited properties superior to those of ethambutol.

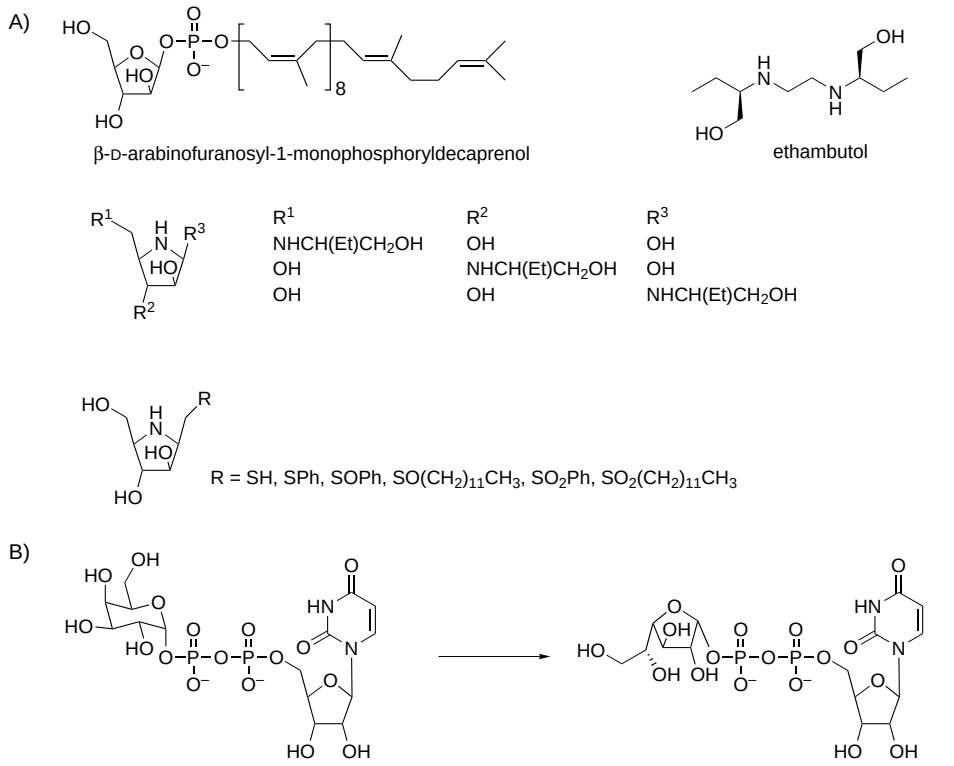
Galactofuranose, the second component of mAG, is also mostly restricted to mycobacteria. It is incorporated as the activated form, UDP-galactofuranose, which is synthesized by the unique enzyme UDP-galactopyranose mutase. The recent structural investigations of this enzyme may make its exploitation as an antibiotic target possible.[99]

4. Antibiotics That Target Prokaryotic Protein Biosynthesis

4.1. Macrolide Antibiotics

Macrolides are secondary metabolites that are structurally diverse, exhibit a variety of activities, and consist of a branched-chain fatty acid formed into a macrocyclic ring (Scheme 13). Typically one or more deoxysugars are attached to the macrolide core structure, and the sugar moiety is often essential for the activities. Erythromycin A, a 14-membered macrolide, has been known for almost fifty years and has provided an effective antibiotic therapy against Gram-positive pathogens much of the time, especially as an alternative for patients that are allergic to β-lactam antibiotics. A new interest in the macrolide antibiotics has been sparked by the introduction of semisynthetic erythromycin A derivatives. For example, 9-(*S*)-erythromycyl amines have potent in vitro activity against a variety of Gram-positive bacteria; however, they are only poorly absorbed by humans after oral administration. Dirithromycin, a 9-*N*-11-*O*-oxazine derivative, which is metabolized into erythromycylamine, is as active in vitro as the erythromycylamines but exhibits better pharmacokinetic properties.[100] Clarithromycin (6-OMe erythromycin)[101] and azithromycin,[102] the first member of the azalide family, are more stable than their parent compound in an acid medium and are therefore tolerant to oral administration. Furthermore, azithromycin has extended the spectrum of the macrolide antibiotics to Gram-negative species. All macrolide antibiotics are thought to function by binding to the 23S subunit of ribosomal RNA, where they interact with hairpin 35 in domain II and with the peptidyl transferase loop in domain V.[103] They stimulate the dissociation of peptidyl tRNA from the ribosome during translocation, thus resulting in the premature termination of the peptide chain.

The biosynthesis of macrolides and other polyketides resembles the synthesis of fatty acids in many ways.[104] Both are assembled from two-carbon acyl units by complex enzyme systems: polyketide synthetases (PKS) and fatty acid synthetases (FAS), respectively. Type I PKS are giant, multifunctional proteins that feature separate modules with FAS-like activities. Each of these modules consists of three to six catalytic domains. They are typically used in a noniterative way, that is, each module is responsible for the synthesis of one specific part of the polyketide, whereas the linear module



Scheme 12. A) The arabinosyltransferase donor, the naturally occurring inhibitor ethambutol, and some synthetic derivatives, B) the UDP-galactopyranose mutase reaction.

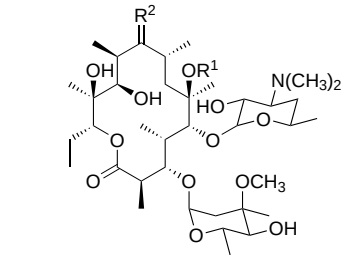
arrangement specifies the structure of its polyketide product. Type II PKS consist of several, largely monofunctional proteins, and each PKS complex is used iteratively for all synthetic steps. Type I and II PKS are generally involved in the synthesis of macrolides and fused aromatic ring systems (e.g. tetracyclines), respectively. However, exceptions are known: for example, fungal type I PKS also synthesize aromatic polyketides. Additionally, some PKS are responsible for the synthesis of polyethers and polyenes.

The biosynthesis of macrolides can be divided into four phases: priming, initiation, elongation, and termination.[105] In the priming phase, the acyl carrier proteins (ACP) of the individual modules are charged with prosthetic phosphopantothenoyl groups. These provide a flexible tether, which terminates in a thiol group and is the site of attachment of the monomer and growing chain intermediates. The chain growth is then initiated by the transfer of a monomer unit from acyl-CoA to the first ACP. Elongation occurs by means of Claisen-like condensation reactions of additional acyl building blocks with the growing chain. Each PKS module contains up to three additional FAS-like domains for the reduction of the ketone, dehydration, and reduction of the resulting conjugated double bond. The presence or absence of these domains, in addition to the variation in acyl building blocks accounts for the structural diversity of the macrolides. Finally, a thioesterase domain catalyzes an intramolecular attack, which results in the release of the cyclic macrolactone. This macrolide core structure is then typically altered by modifying enzymes such as hydroxylases, glycosyltransferases, and methyltransferases. The biosynthesis of erythromycin A is illustrated in Figure 8.

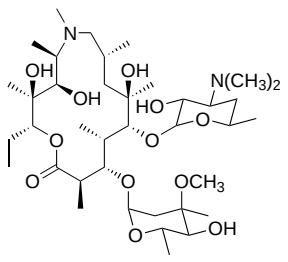
The linear, modular organization of the PKS has been exploited in the synthesis of designed macrolides and aromatic polyketides.[106, 107] Through the inactivation or substitution of certain catalytic domains, unnatural structures could be obtained as predicted by prior knowledge of domain function.[108, 109] Extension of this methodology to alter multiple carbon centers in a single molecule allowed the synthesis of a fifty-membered macrolide combinatorial library.[110] Such an undertaking would have been impractical by chemical synthetic methods alone.

Although the biosynthesis of polyketides can successfully provide variants of macrolide core structures, several major obstacles complicate the study of the deoxysugar components. Even though the genes that code for the biosynthetic proteins of deoxysugars are part of the overall macrolide gene cluster, their identification is a challenge as they are scattered at both ends of the PKS cluster, along with genes that encode for regulatory and aglycon-modifying enzymes. Furthermore, assignment of genes to a particular glycosylation enzyme is difficult in macrolides with more than one sugar substituent. Progress in the area of cloning, overexpression, and molecular assembly of the genes, and implications for the mechanism of the biosynthesis of deoxysugars have been extensively reviewed.[111–114]

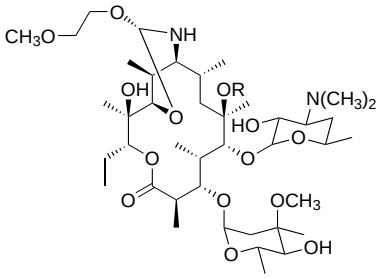
A major obstacle in the study of deoxysugars has been the complicated synthesis of activated nucleoside diphosphate deoxysugars that have been postulated as biosynthetic intermediates. Glycosyltransferases transfer a specific carbohydrate from its corresponding sugar nucleotide to the acceptor hydroxy group. The chemical synthesis of 2-deoxysugar nucleotides is particularly challenging, largely because the stereochemistry at the anomeric center cannot be controlled through the neighboring-group effect. Once synthesized, the activated sugar nucleotides are highly unstable as they are prone to hydrolysis and 1,2-elimination. However, enzymatic methods may make a wide variety of sugar nucleotides accessible. For example, the substrate specificity of an α-D-glucopyranosyl phosphate thymidylyltransferase was investigated.[115] Most of the twelve glycosylphosphates tested (all possible α-D-hexoses and monodeoxy-α-D-glucoses) provided an appreciable amount of the condensation product with both TTP and UTP. Interestingly, the substrate specificity seemed to be governed by the preferred conformation of the sugar substrate. The significance of these findings lies in the fact that they provide substrate sets for the further development of in vitro glycosylation systems. The general feasibility of incorporating unnatural sugar substitu-



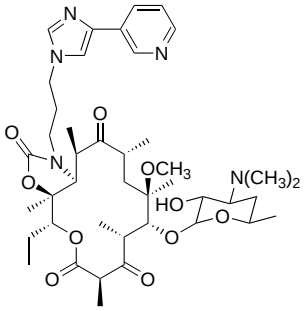
erythromycin A $R^1 = H$ $R^2 = O$
clarithromycin $R^1 = CH_3$ $R^2 = O$
9-*(S)*-erythromycylamine $R^1 = H$ $R^2 = (S)\text{-}NH_2$



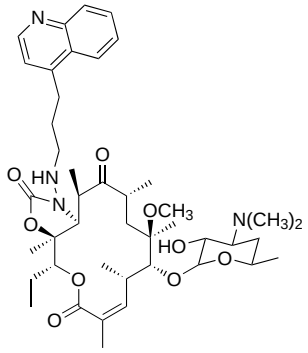
azithromycin, an azalide



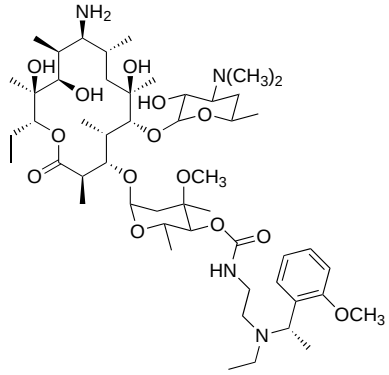
dirithromycin



telithromycin (HMR3647), a ketolide



A-179461, an anhydrolide



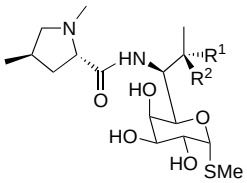
CP-544372, a 4'' carbamate macrolide

Scheme 13. The structures of some macrolide antibiotics.

ents into macrolide antibiotics had been demonstrated earlier for methymycin, a polyketide that bears a single sugar substituent, desosamine, as well as for other systems. In *streptomyces venzuelae*, desosamine is synthesized and attached to the methymycin aglycon by a series of eight gene products, DesI – VIII (Scheme 14). As predicted, the deletion of one of methymycin's *N*-methyltransferases (DesVI) led to an analogue with an amino instead of a dimethylamino substituent at the 3-position of the deoxysugar.[116] Transformation of an aminotransferase gene (calH) involved in the biosynthesis of calicheamicin into a methymycin-producing strain successfully provided a methymycin – calicheamicin hybrid,[117] whereas the transformation of two genes from the streptose pathway (strM and strL) resulted in a methymycin aglycon that bears an L-rhamnose substituent, thus demonstrating the promiscuity of the glycosyltransferase DesVII.[118] An extension of these methodologies may soon allow combinatorial biosynthetic approaches that give diverse macrolides that incorporate modified sugar substituents on various polyketide structures.

Polyketide deoxysugar substituents have traditionally been thought to affect pharmacokinetic properties, such as adsorption, distribution, metabolism, and excretion (ADME). More recently, additional roles of the sugar substituents have been considered. Deoxygenated sugars have a balance of hydrophilic and hydrophobic groups, which are preorganized on a rigid pyran ring scaffold and have the potential to participate in target recognition. The involvement of deoxysugars in the recognition of binding sites has primarily been studied for deoxysugar-containing antitumor drugs that target DNA. DNA molecules can easily be synthesized and are more stable than RNA. Additionally, the helical and relatively stable conformation of DNA in solution facilitates NMR spectroscopic studies. For example, it was shown that the oligosaccharide chain of calicheamicin is embedded in the minor groove of duplex DNA, thereby positioning the active aglycon enediyne unit for cleavage.[119] The participation of deoxysugar substituents in the recognition of RNA sequences by macrolide antibiotics remains to be demonstrated.

The lincosamides are another class of RNA-binding antibiotics that include a carbohydrate in their structure. The semisynthetic clindamycin



lincomycin A R = *i*Pr R^1 = OH R^2 = H
lincomycin B R = Et R^1 = OH R^2 = H
clindamycin R = *i*Pr R^1 = H R^2 = Cl

has replaced the naturally occurring lincomycins clinially because of its higher activity and reduced side-effects. Macrolides, lincosamides, and group B streptogramins have overlapping binding sites on the 50S ribosomal subunit. Full cross-resistance between the groups (MLS_B resistance), which is mediated by specific base methylation of the 23S RNA, is therefore observed for these three antibiotic families. This modification of ribosomal RNA is catalyzed by the Erm

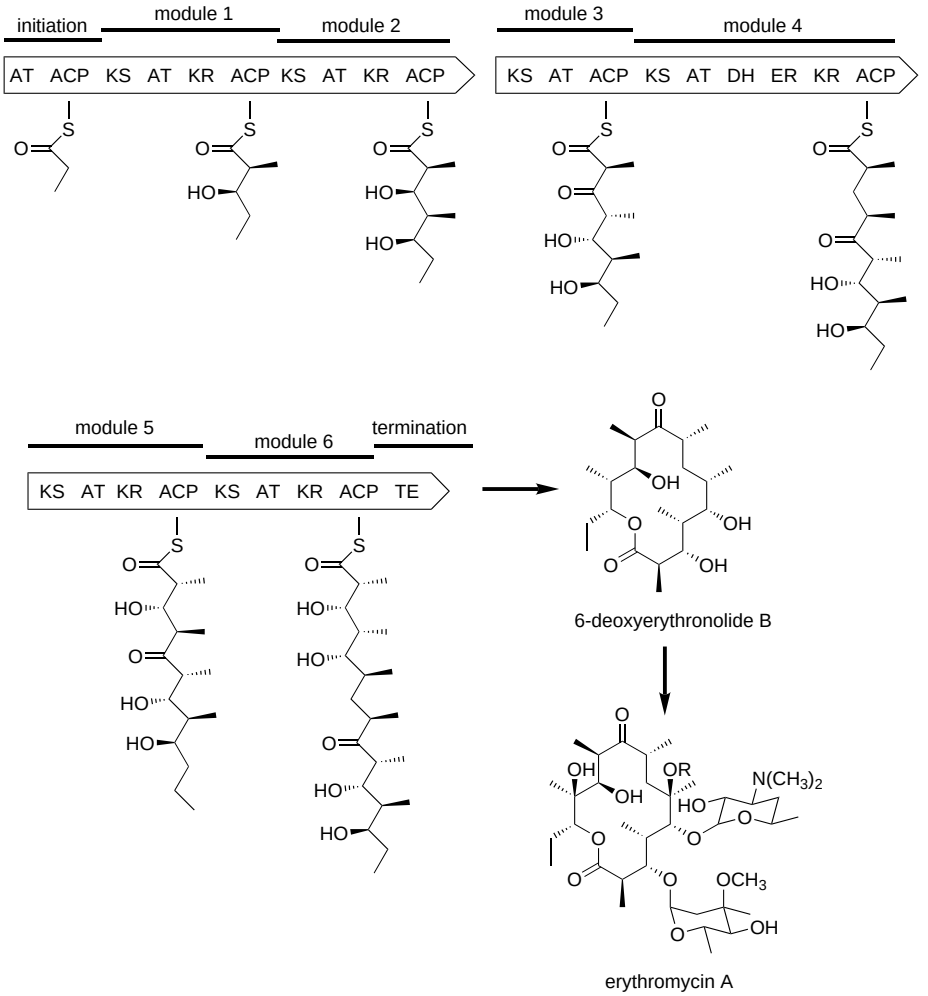


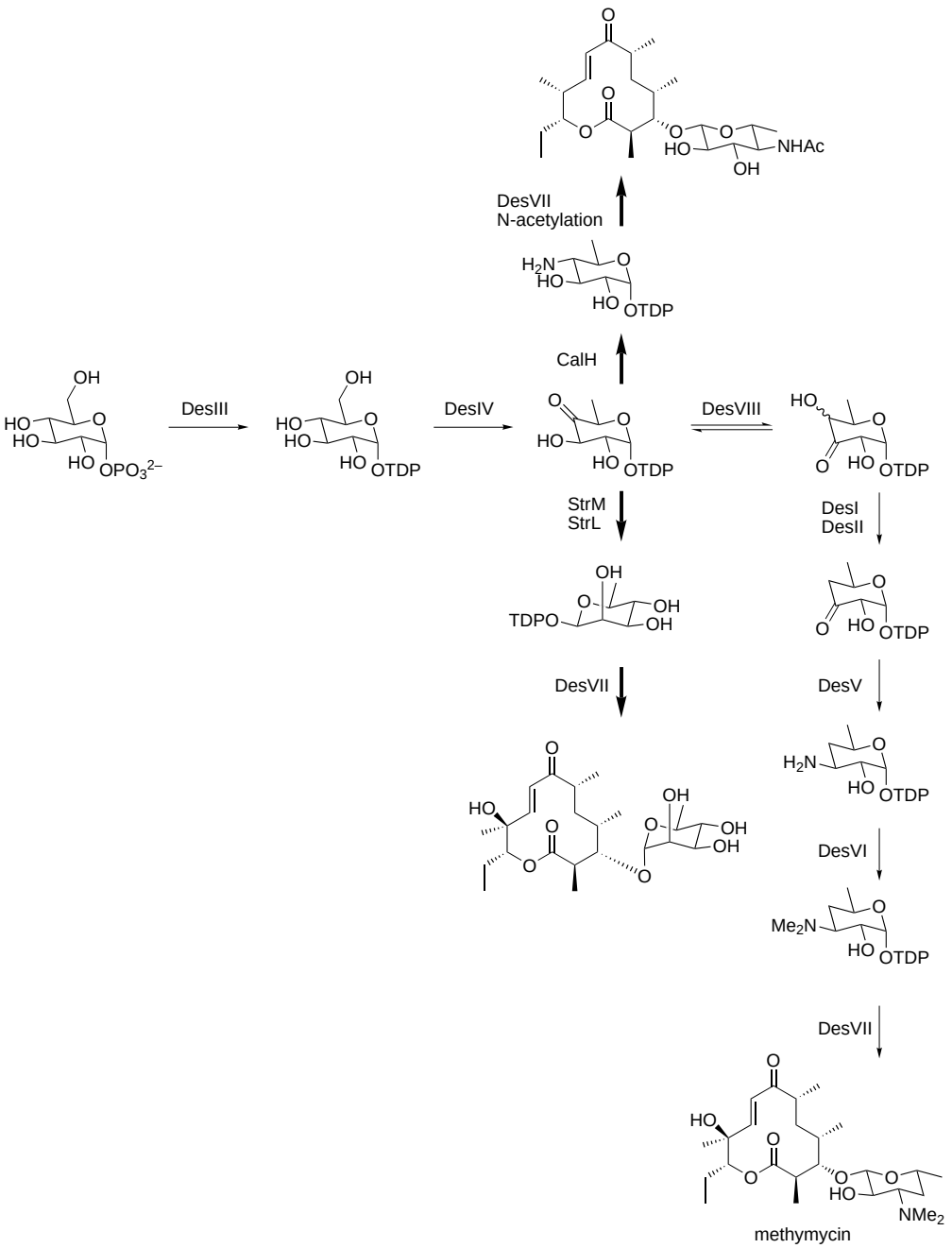
Figure 8. Illustration of the function of a modular polyketide synthetase, with the biosynthesis of erythromycin A shown as an example (AT = acyl transferase, ACP = acyl-carrier protein, KS = ketosynthase, KR = ketoreductase, DH = dehydratase, ER = enoyl reductase, TE = thioesterase).

(erythromycin ribosome methylation) family of methyltransferases.[120] A large number of compounds were screened for activity against these enzymes and a group of seemingly unrelated chemicals were found to selectively inhibit ErmC.[121] However, none of these inhibitors potentiated the ability of erythromycin to prevent ErmC-containing *S. aureus* from generating acute infections in mice. The interaction of small molecules with the Erm family of methyltransferases were probed in an NMR spectroscopic based screen, and a series of substituted triazines were identified as compounds that increase the activities of lincosamides and group B streptogramins.[122] The lead triazines were optimized by the parallel synthesis of a large number of analogues, and resulted in structures that inhibited the RNA methyltransferase at micromolar concentrations. Resistance to macrolide antibiotics can also result from two non-MLS_B resistance mechanisms: chemical modifications (phosphorylation, glycosylation, esterification) and active efflux systems across the membrane (associated with the *metE* determinant) (see Figure 1).

Ketolides are the 3-descladinosyl-3-oxocarbamate analogues of erythromycin or clarithromycin, many of which feature an additional 11,12-cyclic carbamate moiety.[123] The ketolide telithromycin (formerly known as HMR-3647) is not only less prone to induce resistance than other macrolide antibiotics, but also displays good pharmacokinetic parameters in vivo, as well as a high therapeutic efficacy in mice that were infected with respiratory pathogens.[124] Furthermore, anhydrolides, in which a carbon–carbon double bond was introduced at the C2–C3 position,[125] and 4″-carbamate macrolides[123] have similar in vitro activities as those of the ketolides.

4.2. Aminoglycosides

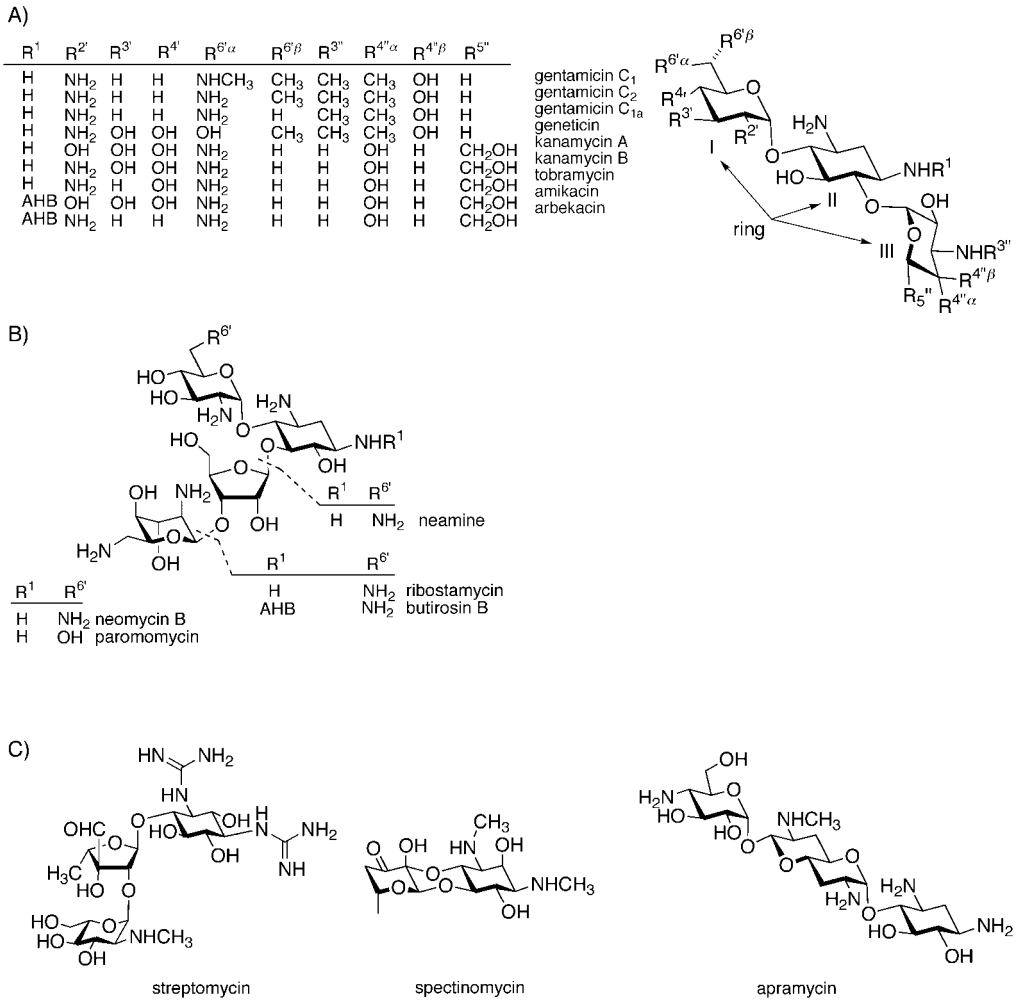
Aminoglycosides consist of a six-membered carbacyclic nucleus (aminocyclitol) with a varying number of sugar substituents (Scheme 15). This core is most commonly 2-deoxystreptamine, but other aminocyclitols such as streptidine (streptomycins), actinamine (spectinomycins), and fortamine (fortimicins) occur. The aminoglycosides are generally classified according to their attached amino sugar groups. For example, three amino sugars are attached to the central 2-deoxystreptamine in the neomycin family, whereas members of the kanamycin and gentamicin families bear only two sugar substituents. The aminoglycosides function as inhibitors of protein translation by binding to the highly conserved A-site sequence of the small 30S ribosomal subunit (Figures 9 and 10). The structure of paromomycin, a member of the neomycin group, complexed to the A site of a model oligonucleotide hairpin has been solved by using NMR spectroscopy.[126] The aminoglycosides provide hydrogen-bond donors that are preorganized on the sugar ring scaffold and interact with conserved nucleotides in the RNA. Aminoglycoside binding in the major groove within a pocket created by a mismatched A–A base pair and a single unpaired adenine induces a conformational change that stabilizes the tRNA–A site complex.[127] Prevention of efficient proofreading then results in erroneous proteins. The accumulation of mutant membrane proteins compromises the integrity of the bacterial envelope, which then allows the uptake of larger quantities of aminoglycosides and results in further misreading and eventually in cell lysis. A change in the RNA conformation is also crucial for the mode of action of the aminoglycoside spectinomycin.[128] The RNA conformational change induced by spectinomycin inhibits the translocation of tRNA from the A site to the P site, the same step that is targeted by the macrolide antibiotic erythromycin (see Section 4.1).



Scheme 14. The biosynthesis of methymycin in *streptomyces venzuelae*. The bioengineered synthesis of new macrolides that bear designed sugar moieties is indicated by the bold arrows.

Aminoglycoside derivatives have been synthesized to investigate the structural features that are responsible for their activity. Simple aminols were found to bind to miniature constructs of the A-site region, thus suggesting that the 1,3-aminol moiety represents the essential pharmacophore of aminoglycosides.[129, 130] Monosaccharides with the 1,3-hydroxyamine substitution pattern of aminoglycosides bind to phosphodiesters and the Hoogsten face of guanosine,[129] which is consistent with the results from the structural studies of the paromomycin—A site complex (Figure 10).[126] A library of 6-deoxy-6-amino GlcNAc derivatives was synthesized to further investigate the activity based largely on the 1,3-hydroxyamine motif.[131] Since the exact location of the idose ring IV in the paromomycin–RNA complex could not be defined by using NMR spectroscopy,[126] its role in the binding event was investigated with a series of neomycin B derivatives with modified at the idose ring.[132] Compounds in which ring IV was replaced with amines on flexible linkers were antibiotically as active as neomycin B. However they bind less specifically to an isolated A-site hairpin than a negative control, which indicates that the idose ring scaffold is necessary for the correct placement of the positive charge. The naturally occurring neamine is the simplest effective aminoglycoside antibiotic and has been explored as a nucleus for the synthesis of new aminoglycoside derivatives.[133] These analogues feature a variety of amino substituents attached to the 5-hydroxy group of 2-deoxystreptamine. Despite binding more strongly than neamine to an isolated A-site model hairpin, some of the analogues were weaker antibiotics. Their antibiotic activity correlated better with the in vitro inhibition of translation (assayed by means of a luciferase reporter gene) and antibiotic activity. The apparent differences in the binding affinity of the aminoglycoside for the A site, whether isolated in a model oligonucleotide or in the context of the ribosome, may be the result of a number of factors. For example, contacts to neighboring ribosomal proteins might stabilize aminoglycoside binding or the surrounding ribosome may have structural effects on the A site. During the development of a fluorescence assay of RNA binding, it was observed that aminoglycosides modified with a fluorescent reporter group bind to RNA A site constructs with a higher affinity than that of their parent molecules.[134] This increase in affinity was attributed to the intercalating properties of the fluorescent dye. A systematic study of this effect led to the discovery of a paromomycin–thiazole orange hybrid with a 41-fold higher affinity than paromomyin itself, while still exhibiting specificity for the A site.[135]

Resistance mechanisms against aminoglycosides include efflux-mediated processes and enzymatic modification of the



Scheme 15. Some natural and semisynthetic aminoglycoside antibiotics: A) Gentamicin and kanamycin aminoglycosides (AHB = aminohydroxybutane), B) neomycin aminoglycosides, C) other aminoglycosides.

drugs or the target site (RNA methylation). N-acetylation, O-phosphorylation, and O-nucleotidylation processes catalyzed by aminoglycoside acetyltransferases (AAC), phosphotransferases (APH), and adenyltransferases (AAD) are the clinically most important mechanisms.[136] A large variety of semisynthetic aminoglycoside antibiotics have been developed to overcome such resistance mechanisms.[137] Particularly problematic is the bifunctional AAC(6′)-APH(2″) present in multiple-drug-resistant *S. aureus* and *S. epidermis* strains, which has rendered most aminoglycosides useless against these pathogens. Arbekacin, a semisynthetic kanamycin derivative, is a notable exception; a small number of MRSA strains with a moderate level of arbekacin resistance have been clinically isolated, but no highly resistant strains have occurred.[138] The low susceptibility of arbekacin to modifying enzymes is not only a consequence of the 1-*N*-aminohydroxybutyro group and the absence of hydroxy groups at the 3′ and 4′ positions, but also of the fact that arbekacin retains its activity after acetylation by several AACs. In a mechanism-based approach to overcome inactivation by APH(3′), 2′-nitro derivatives of neamine and kanamycin were synthesized (Scheme 16).[139] The 3′-phosphorylated compounds that arise from the action of the modifying enzyme undergo elimination to generate nitroalkenes, which presumably undergo a Michael addition of the active-site nucleophiles. In spite of their APH(3′)-inhibiting properties, these compounds were not effective in reversing aminoglycoside resistance. The APH(3′) resistance enzyme was avoided by synthesizing a 3′-oxo-kanamycin derivative.[140] The hydrolyzed form of this ketone is a substrate for APH(3′). The resulting 3′-phospho derivative is unstable and expected to eliminate phosphate, thereby regenerating the antibiotic. This aminoglycoside modification lowered the MIC in the presence of the resistant enzyme APH(3′)-Ia by an order of magnitude.

In a surface plasmon resonance (SPR) spectroscopic study, neamine was found to bind the A-site sequence with a 2:1 stoichiometry, each with a dissociation constant of approximately 10 μM.[141] Various dimers were therefore constructed to identify a bivalent aminoglycoside that would bind to the A site with high affinity owing to the simultaneous ligation to both binding sites. Dimers coupled through a flexible hydrophilic linker possessed a significantly increased antibiotic activity against neamine. A nearly linear relationship between the IC_{50} of translation inhibition and the MIC values validated translation as the

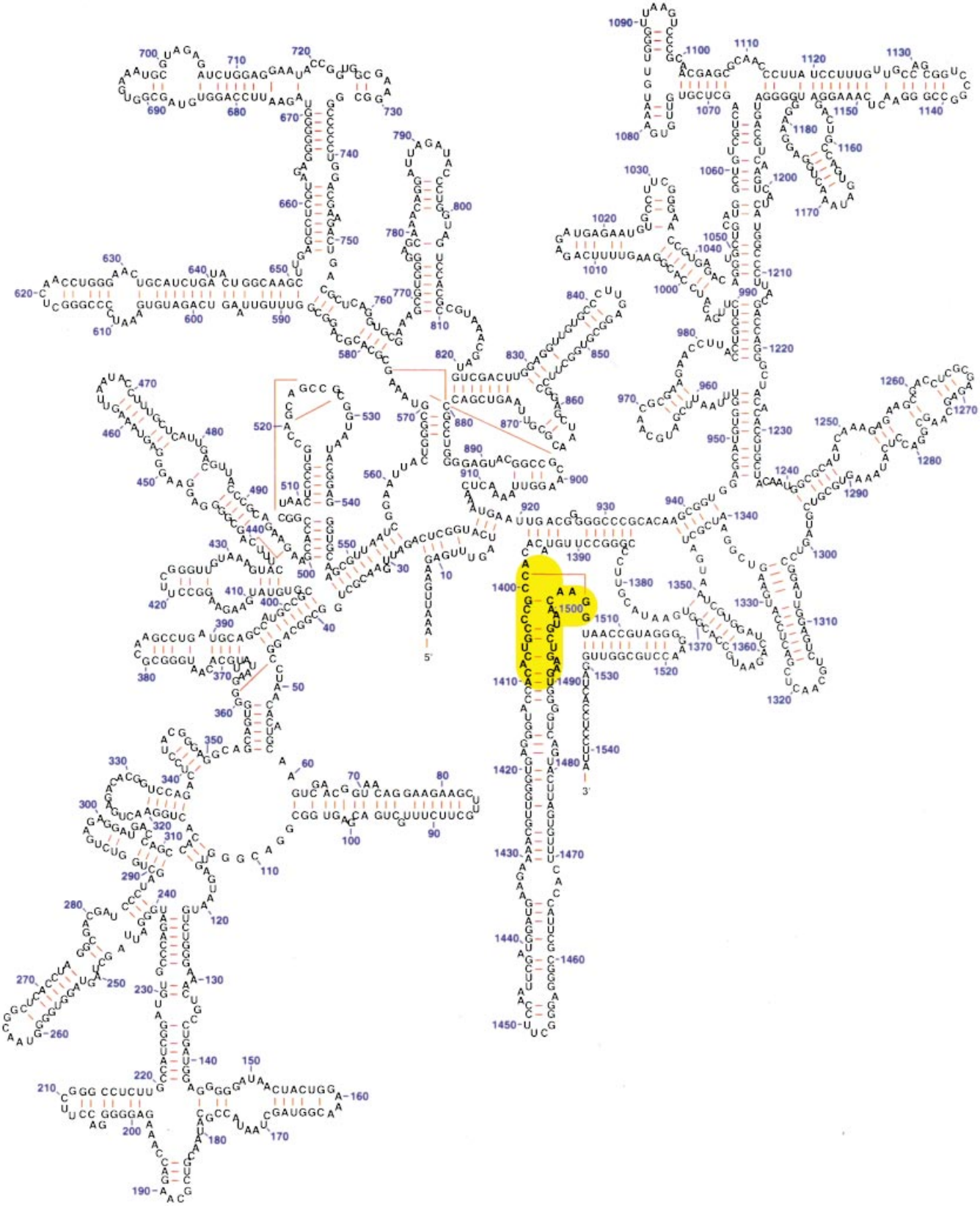


Figure 9. The ribosomal 16S RNA sequence and the A site (yellow) for aminoglycoside binding.

target of this new class of aminoglycoside antibiotics. Interestingly, several of these neamine dimers also were found to be inhibitors of the APH(2″) activity of the bifunctional APH(2″)-AAC(6′) aminoglycoside-modifying enzyme. These dimeric neamine constructs therefore represent a new strategy toward thwarting the resistance to aminoglycosides.

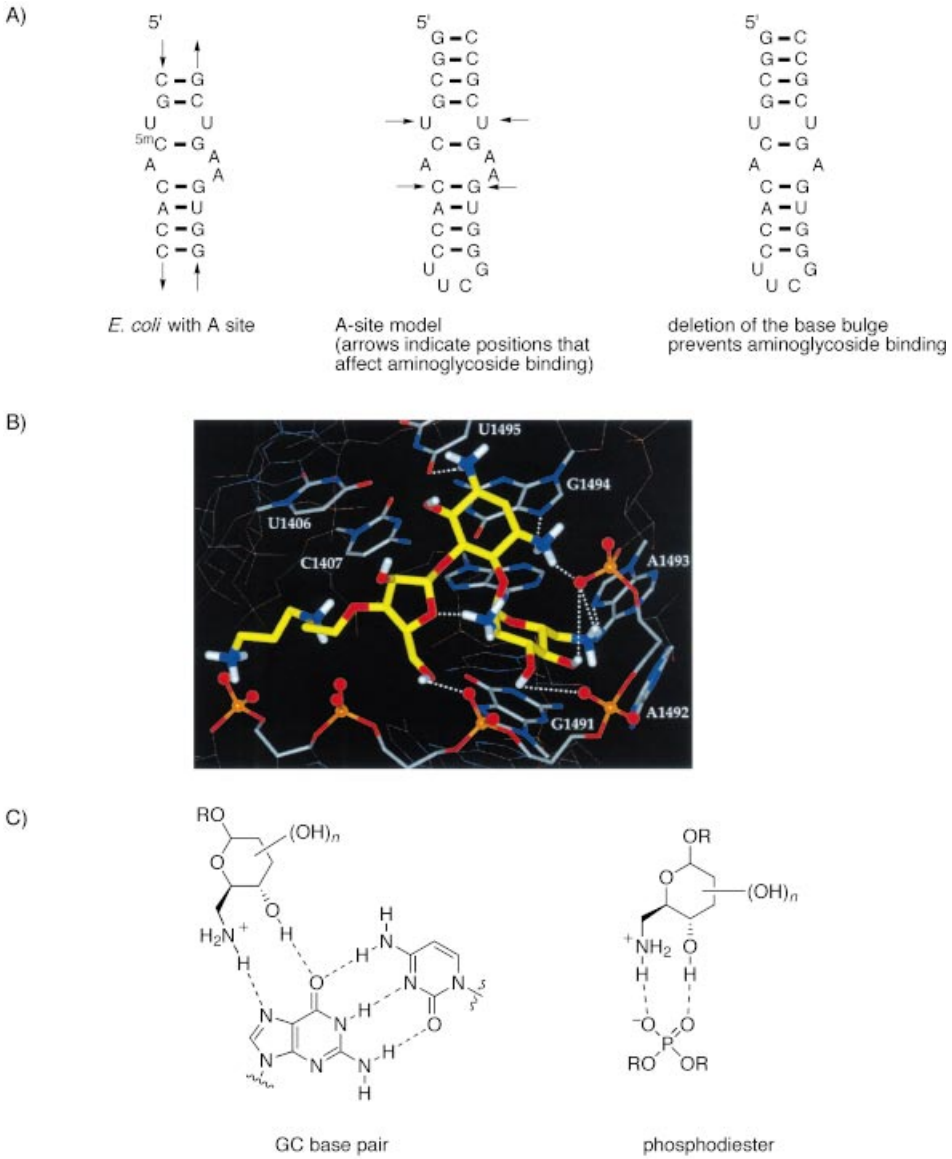
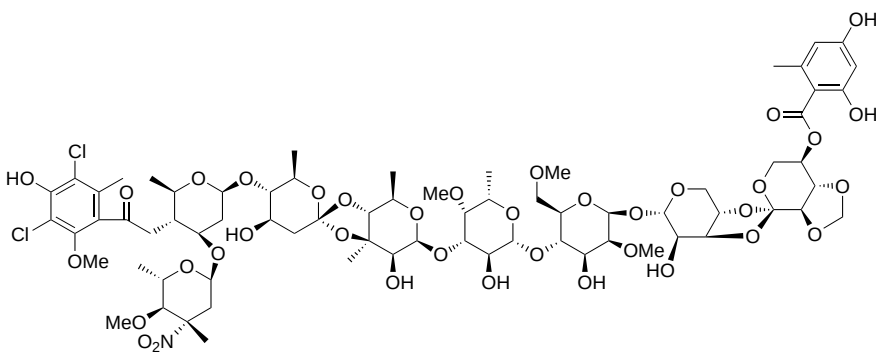


Figure 10. A) RNA sequences related to the ribosomal decoding A site, B) structure of paromomycin complexed to the A site, determined by using NMR spectroscopy, C) putative modes of the interactions of hydroxyamines with phosphodiesters and with the Hoogsten faces of the GC base pairs.

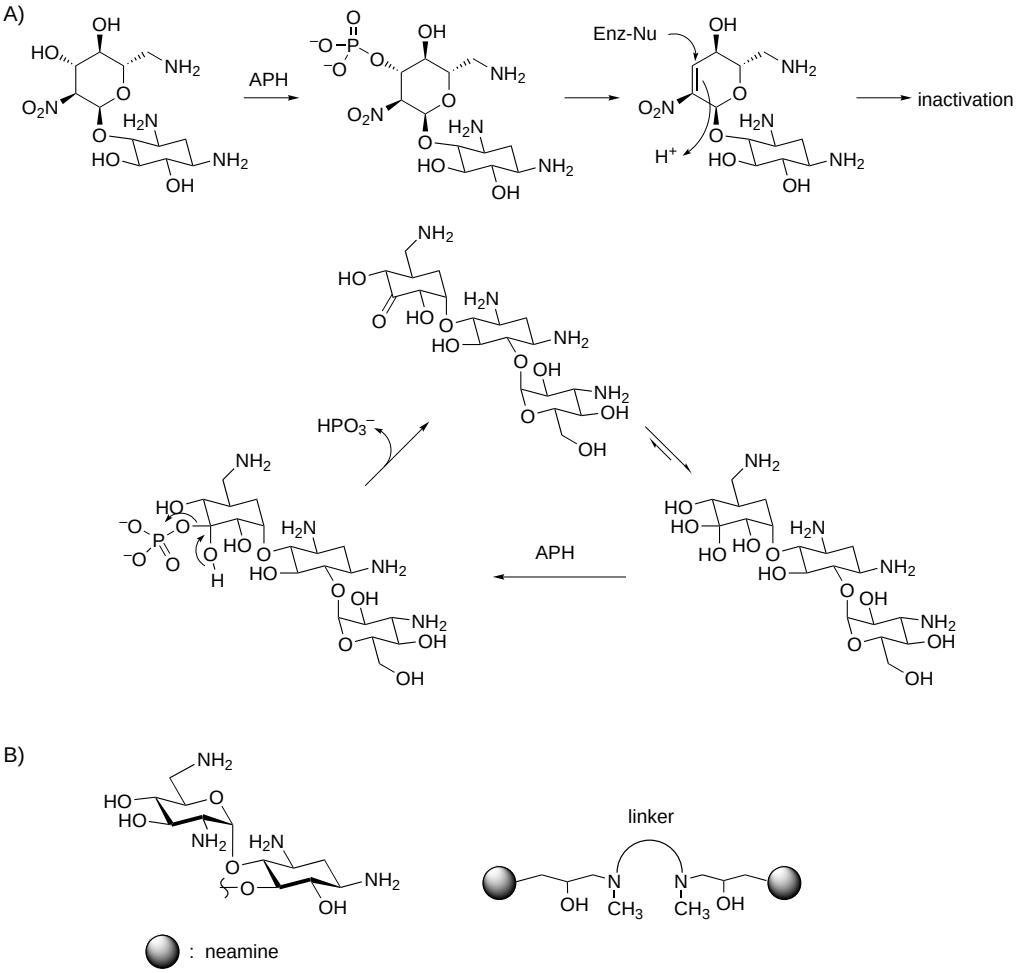
4.3. Everninomycins

The everninomycins, for example, everninomycin 13,384-1, are a class of complex oligosaccharide antibiotics. They are part of the orthosomycin family, which feature one or more orthoester linkages.[142] Their complex structure has attracted the interest of synthetic organic chemists and a total synthesis has been reported.[143] The everninomycin antibiotics are thought to inhibit RNA to protein translation by preventing the assembly of the initiation complex. Everninomycins are active both in vitro and in vivo against MRSA and VRE; however, the first members of this class were toxic. Ziracin, a cyclodextin complex of everninomycin 13,384-1 which was proven to be safe in vivo, and analogues thereof are being developed for clinical trials.[144]



4.4. RNA as a Drug Target

Recently, there has been an increased interest in the potential of RNA as a target for small-molecule drug discovery and aminoglycosides have served as a paradigm for this approach.[145, 146] Protein synthesis, mRNA maturation, and ribonucleoprotein–RNA interactions are generally considered as the most promising points of interaction. As RNA functional domains are more highly conserved than the shapes of enzyme-active sites, drug resistance is expected to develop at a slower pace. For example, type I human immunodeficiency virus (HIV-I) has rapidly developed resistance to enzyme inhibitors. RNA-based antiviral targets offer a potential solution to this problem. Aminoglycosides were shown to disrupt the interaction between a *trans*-activating region (TAR) and its cognate peptide (Tat),[147] as well as between a Rev-response element (RRE) and the viral transactivator protein (Rev).[148] These regulatory interactions are necessary for viral replication. The synthesis of a library of neamine-based derivatives by using four-component Ugi reactions resulted in the discovery of compounds that bind RRE more effectively than neamine.[149] Since binding of the Rev peptide to RRE induces a single base bulge, it was suggested that an aminoglycoside–intercalator hybrid might have a very high affinity for RRE.[150] Indeed, a neomycin–acridine conjugate is the strongest known competitive inhibitor of Rev–RRE binding with an affinity that is only two times lower than that of the Rev peptide. In cystic fibrosis, a point



Scheme 16. Strategies to overcome aminoglycoside resistance caused by modifying enzymes. A) Mechanism-based approaches, b) aminoglyoside dimers (APH = aminoglycoside phosphotransferase).

mutation introduces a stop codon into the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The addition of aminoglycosides to an in vitro protein expression system for mutant CFTR promoted the read-through of the stop codon.[151] Fusion genes that arise from chromosomal translocation are thought to be the cause of certain forms of cancer. Antisense agents to the breakpoints of these genes have been shown to inhibit cancer growth.[152, 153] A library of neamine derivatives was tested for their binding affinities to two of these fusion genes, namely PAX3-FKHR and Bcr-Abl.[154] Polyamine-substituted neamine derivatives were found to be the most efficient binders, with dissociation constants in the submicromolar range. Based on their affinities, it is reasonable to believe that aminoglycosides can potentially act as regulators of gene expression. Furthermore, the aminoglycoside antibiotics also act as inhibitors of catalytic RNAs, such as the group I intron,[155] the hammerhead ribozyme,[156] and the ribozymes of the hepatitis delta virus (HDV).[157]

With recent and future advances in functional genomics research and the revelation of the genomes of different species, unique RNA sequences will be identified as new drug targets. Aminoglycosides may serve as the primary source of small molecules for the identification of new leads to target RNA. The development of efficient combinatorial methods for the synthesis of aminoglycosides thus represents a new direction. The programmable one-pot oligosaccharide synthesis methodology,[158] which gives relatively easy access to oligosaccharides, should be applicable to the synthesis of aminoglycoside libraries. In combination with the tools used in genomic and proteomic research (Figure 11), this may allow the discovery of molecules that target RNA and the evaluation of their selectivity.

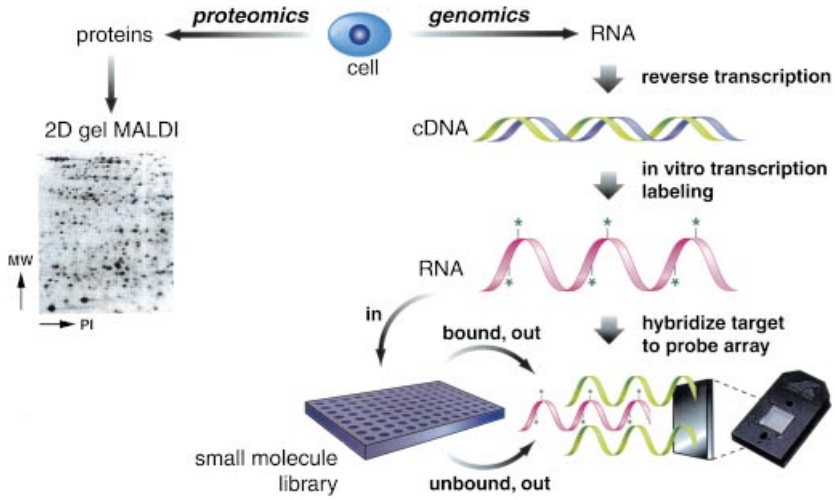


Figure 11. RNA binding analysis by using genomic and proteomic tools. Proteomics: Comparison of the proteome (visualized on a 2D gel, MW = molecular weight, PI = isoelectric point) before and after incubation of the cell with the molecule under investigation. A subsequent analysis of the affected proteins by MALDI (matrix-assisted laser-desorption ionization) mass spectrometry facilitates the identification of selective inhibitors of translation. Genomics: Labeling of the complete pool of RNA present in a cell (through reverse transcription and in vitro transcription labeling of the resulting cDNA) is used to detect the specific binding of small molecules to RNA.

5. Summary and Outlook

The development of new antibiotics to tackle the problem of antibiotic resistance has emerged as an important area of drug discovery. The carbohydrate-based approach described herein represents a relatively new strategy, and several targets appear to be interesting candidates for antibiotic discovery. New methods that recently emerged in carbohydrate research have not only advanced the field, but have also created a new direction in antibiotic development. With a greater understanding of the genome of different bacterial species and with advances in functional genomics and proteomics, additional targets for antibiotics are expected to be identified.

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