Bachelor's project

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

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Combating Antibiotic Resistance by Chemical Synthesis:

Creation of new varieties of Glycopeptide Antiobiotics based on Vancomycin

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Supervisor: Odd Reidar Gautun
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Abstract

The emergence of multi-resistant bacteria has been described by WHO as the gravest disease threat we face, claiming up to 700,000 lives annually. Antibiotics of last-resort, such as the glycopeptide class antibiotic Vancomycin, play an integral role in the suppression of gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA). The emergence of both *Entercocci* and *Aureus* bacteria resistant to Vancomycin has recently been confirmed, leading to efforts to modify Vancomycin by chemical synthesis to maintain its efficacy. This review will describe the methods used to achieve an active 3 > 2 > 1 mechanism against resistant bacteria. This includes ligand substitution, as a single heavy atom exchange, as well as two peripheral modifications. The three mechanism are independent of each other, thus providing the antibiotic with a attack in three stages. Such modifications have been found to be not only effective, but are likely to remain so, as simple chemical substitution in the gram-positive bacteria's cell wall is unlikely to effectively block the pathways used by this modified class of Vancomycin-related antibiotics.

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1. Abbreviations

D-Ala – D-Alanine

D-Lac – D-Lactate

MRSA – Methicillin-resistant Staphylococcus aureus

NAG – N-acetylglucosamine

NAM – N-acetylmuramic acid

Single heavy atom – a singular atom or a known combination of atoms such as NH, H_2 or OH.

VRE – Vancomycin resistant Entrocicco

VRSA – Vancomycin resistant *S. aureus*

WHO – World Health Organization

2. Introduction

Antibiotic resistance is one of the major threats to modern medicine, and it continues increasing. The WHO has stated that "the biggest disease threat we face is not Ebola or AIDS, but multi-resistant bacteria", and assumes that by 2050, multi-resistant bacteria will take more lives than cancer (Myers, u.d.). It is, however, not necessary to travel in time to see the impact antibiotic resistance has on the world. It is estimated that approximately 700 000 human lives are lost yearly due to antimicrobial resistance (AMR).
In 2017 The World Health Organization publicized a global priority list of antibiotic-resistant bacteria to help guide research, discovery and development of new antibiotics. (WHO, u.d.) It was noted that vancomycin resistance, especially found in *Enterococcus faecium* organisms, is a danger to last-resort antibacterial medication.

The first member of the glycopeptide class of antibiotics, vancomycin, was discovered in the 1950s and still has an integral role today in the treatment of infections caused by Gram positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Mark S Butler, 2014). Vancomycin has long been considered the antibiotic of last resort against serious and multi-drug-resistant infections caused by gram-positive bacteria and is often used after other types of antibiotics have proven ineffective. Now, however, we have an increasing emergence of vancomycin resistance in not only enterococci, but more recently, in *Staphylococcus aureus*, as well (Ivo G Boneca, 2019).

This review will discuss how modifications to vancomycin can result in the elimination of bacterial resistance, as well as the effect of peripheral modifications of [¬[CH_2NH]Tpg^4] vancomycin.

A brief description will be given regarding properties of the bacterial cell wall of gram-positive bacteria, and regarding peptidoglycan synthesis, as well as the inhibiting mechanism of vancomycin on gram-positive bacteria, and present modifications to the vancomycin molecule that can minimize the threat of resistance. Vancomycin resistance will not be compared to other forms of antibiotics that are used in similar clinical cases, but rather, we will examine how modification to the vancomycin molecule compares to the unmodified version of the same antibiotic.

3. Theory

3.1 Gram positive bacteria

Bacteria are commonly divided into two categories: gram-positive and gram-negative bacteria, based on the composition of their cell wall and their reaction to the Gram stain test (Bailey, 2020). Gram-positive bacteria have a cell wall composed primarily of peptidoglycan, a substance unique to bacteria. Gram-negative bacteria, on the other hand, have a secondary level outside the cell wall, known as the outer membrane (W. Vollmer, 2008). Figure 3.1 shows a simplified version of the two types of bacteria.

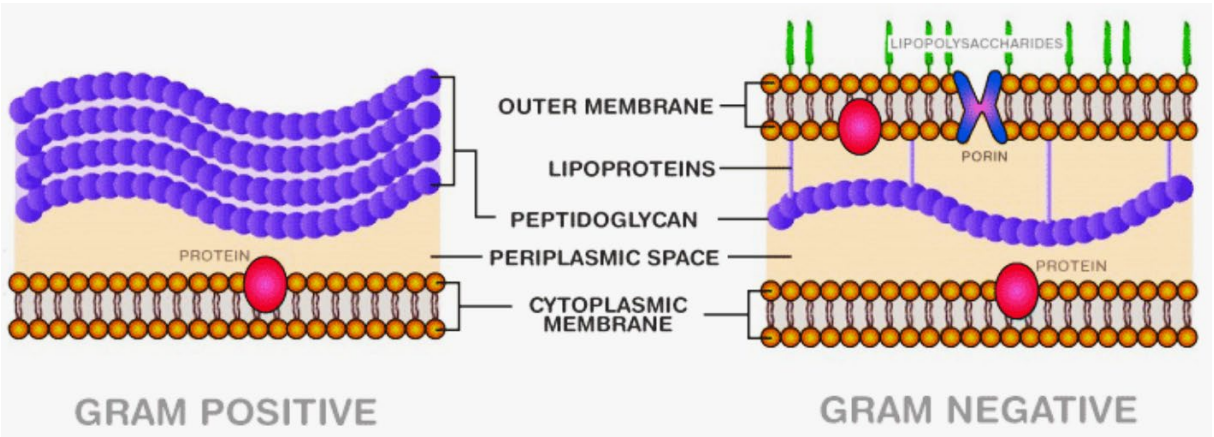


Figure 3.1: Simplified image of gram-positive and the gram-negative bacterial cell wall structure.

Peptidoglycan is a polymer composed of monosaccharides and amino acids resulting in a string of amino sugar component glycan(polysaccharide) chains. These are built up of alternating molecules of NAG, and NAM, which are crosslinked by flexible peptides, ref. Figure 3.2 (Bailey, 2020). The structure of the glycan chain of the peptidoglycan consists of the repeating unit of β-1,4 N-acetyl-glucosamine-N-acetyl-muramic acid (NAM-NAG) disaccharide (W. Vollmer, 2008).

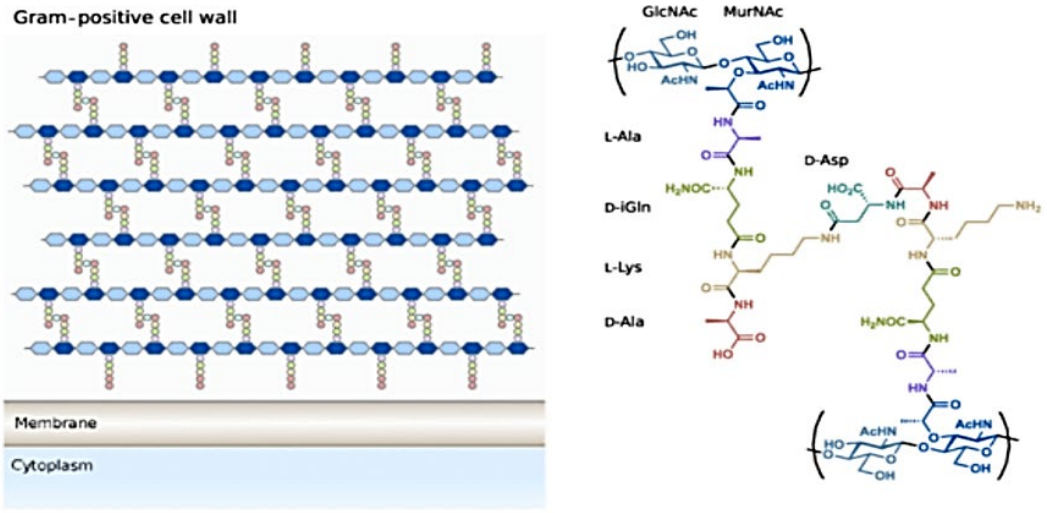


Figure 3.2: Structure of the cell wall of Gram-positive bacteria, as well as the chemical structure of the basic repeating unit of peptidoglycan in cross linkage. (Matthew E Griffin, 2019) The peptidoglycan is a disaccharide pentapeptide composed of NAG and NAM linked together by β-1,4 glycoside bonds. The crosslinking pentaglycine-pentapeptide extension is attached to the ε-amine on the L-Lys peptide strain. (W. Vollmer, 2008)

The peptide crosslink consists of a glycosidic bond between the D-ala-D-ala terminal, attached to the ε-amine on the L-Lys peptide strain, to the nearby NAM-NAG unit. This form of crosslinking gives peptidoglycan its strength and structure (R. Guan, 2019). Peptidoglycan is crucial to the viability and the synthesis of bacteria, as it protects the cytoplasm from leaking out as a result of the internal osmotic pressure. The inhibition of transglucosylase is therefore a target for antibiotics (Pasquina-Lemonche, 2020).

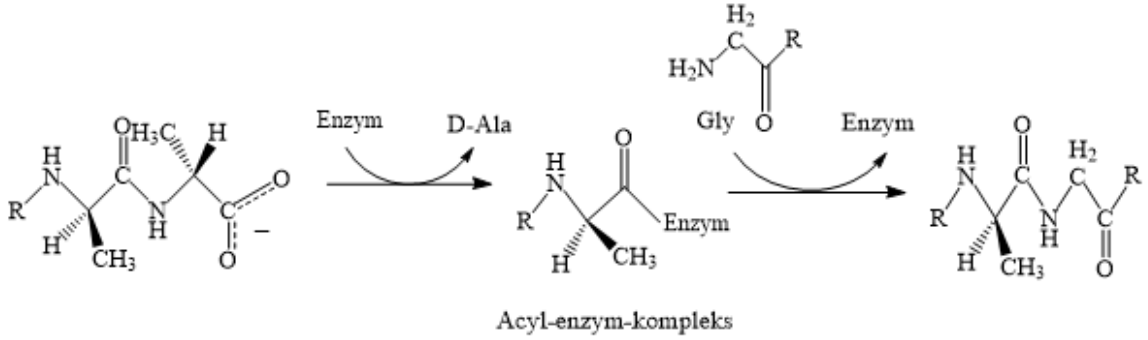


Figure 3.3: The mechanism of transglucosylase as it links together the NAM-NAG complex with the rest of the peptidoglycan. This is the last step in the cell wall biosynthesis, and it is here the glycopeptide antibiotics, such as vancomycin, inhibit the enzyme before the loss of the terminal D-Ala.

The specialized enzyme transglucosylase is used in the binding process of the peptide crosslink as shown in Figure 3.3 (Mark S Butler, 2014). The peptide bond is formed between two adjacent glycan chains by expression of D-Ala amino acid in an enzyme catalyzed reaction with PBP. The reaction consists of two steps; first the addition of the enzyme causes the terminal D-ala to loosen, and the formation of an acyl-enzyme complex occurs. And then, secondly, the formation of a β-1,4 glycoside bond between the glycan chains.

3.2 Antibiotics

Antibiotics is the collective term for medications used in antibacterial treatments and can be used to describe any substance that inhibits growth and replication of bacteria or kills the bacterium in or on the body. (Microbiology Society, 2021) To be used in a clinical setting, it is important that the antibiotic have a low toxicity towards the patient while still maintaining a strong effect on the bacteria, so that the body can get rid of the bacteria without major side effects (Sverre Dick Henriksen, 2018) .

There are two main methods for an antibiotic to target bacteria (Microbiology Society, 2021). They either prevent reproduction or kill the bacteria, usually by interfering with the formation of cell walls or by stopping protein synthesis.

3.2.1 Glycopeptide antibiotics

Glycopeptide antibiotics an important class of antibiotics. They are originally derived from actinomycete and consist of a unique cyclic or polycyclic heptapeptide core that is usually glycosylated and sometimes has additional lipophilic fatty acid side chains (Mark S Butler, 2014), ref. Figure 3.4.

Glycopeptide antibiotics act primarily by interfering in cell wall synthesis of gram-positive bacteria by inhibiting the enzyme transglucosylase (Tankeshwar, 2019). Because of the nonconformity of the outer membrane of gram-negative bacteria, the cell wall is impermeable to larger glycopeptide molecules such as glycopeptide antibiotics.

Glycopeptide antibiotics can be divided into four distinct structural subclasses I–IV according to the substituents and the type of residues at positions 1 and 3 of the heptapeptide. (Mark S Butler, 2014). Glycopeptides with valine-1 and asparagine-3/glutamine-3 residues such as vancomycin are classified as Type I, ref. Figure 3.4.

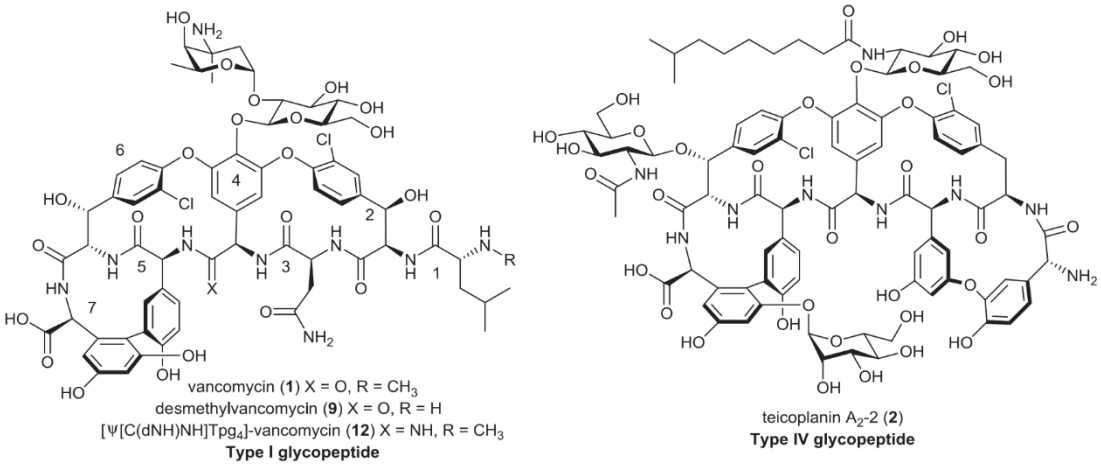


Figure 3.4: The structure of the natural occurring glycopeptide antibiotic vancomycin and its respective structural classes. (Lancini, 1989)

3.3 Vancomycin

Vancomycin is one of the most important glycopeptides, and alongside teicoplanin it is still used extensively as a last resort antibiotic against several gram-positive bacteria including MRSA (Williams, 2014). Vancomycin's spectra of activity tends to be limited to Gram-positive organisms both because of its large size and the mechanism of action targeting peptidoglycan in the cell wall (Tankeshwar, 2019). Gram-negative bacteria, as seen in Figure 3.1, only have a thin layer of peptidoglycan in the cell wall hidden behind an outer membrane impenetrable for larger molecules.

3.3.1 Mechanism of action

Gram positive bacteria have a cell wall consisting of peptidoglycan with the structural composition of the NAM-NAG unit. (Kaiser, 2021) These units are crosslinked with each other by a glycosidic bond between the D-ala-D-ala terminals to the NAM-NAG units. To

form this bond, the specialized enzyme transglucosylase captures and crosslinks the NAM-NAG units.

Vancomycin inhibits this bacterial cell wall synthesis by attaching itself to the D-ala-D-ala terminals of the NAM-NAG units, thus inhibiting attachment of the enzyme transglucosylase. By hindering the enzyme attachment, the antibiotic stops the peptidoglycan forming glycan chains (Silveira, 2017). This stops the reproduction of the cell wall and eventually leads to the cell wall being damaged and then destroyed. When the cell wall of a bacteria is sufficiently damaged the higher osmotic pressure inside the cell will lead to osmotic bust, killing the bacteria.

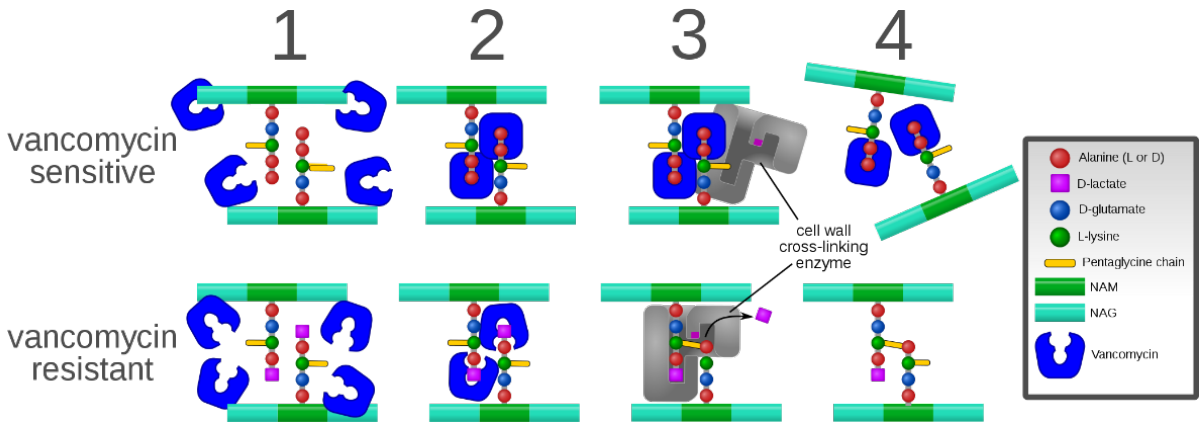


Figure 3.3: The mechanism of vancomycin appearing as an inhibitor in vancomycin sensitive bacteria such as MRSA, as well as the non-effective mechanism of vancomycin's inhibition in vancomycin resistant bacteria such as VRE.

3.4 Vancomycin resistant bacteria

Clinical resistance to vancomycin was first observed in vancomycin resistant Entrococci, VRE, approximately 30 years after start of clinical use, but now also includes vancomycin-resistant *S. aureus*, VRSA (Akinori Okano, 2017).

The bacterial evolution has led to antibiotic resistance, and bacterial infections that were highly susceptible to vancomycin, have evolved majorly by causing mutations in the transcriptase enzyme associated with peptidoglycan synthesis. (Zablon, u.d.) This is where the vancomycin mechanism is initiated, thus effectively hindering its mechanisms, and developing resistant to vancomycin.

3.4.1 Mechanism of resistance

As seen in section 3.3.1, vancomycin attaches itself to the alanine of D-ala-D-ala terminals of the growing peptidoglycan chain in order to inhibit the synthesis of the cell wall. (Kumar, 2017) In replacing the terminus alanine on the growing peptidoglycan chain with another amino acid the bacteria will gain resistance against vancomycin resulting in vancomycin resistant bacteria, ref. Figure 3.

As a result of multiple different genes there are six different possible resistance patterns designated as different phenotypes VanA through VanG (Tankeshwar, 2019). These phenotypes produce resistance by replacing alanine with the amino acid lactate or serine. The peptidoglycan intermediates are altered to either d-alanyl-d-lactate by the phenotypes VanA, VanB, and VanD, or d-alanyl-d-serine by the phenotypes VanC, VanE, and VanG. The modification seen in VanA from d-ala-d-ala to d-ala-d-lac, through an intricate late-stage remodeling of their peptidoglycan termini, is on a molecular level, an exchange of an ester oxygen for an amide NH (Boger, 2017). This modified terminus contains precursors having a low affinity for glycopeptides which results in a reduction in vancomycin binding affinity by 1000-fold, and thus leads to failure in preventing cell wall synthesis (Que Y-A, 2015). It also results in much higher MICs.

The primary mechanism of resistance to vancomycin, the VanA and VanB phenotypes, was transferred to pathogenic bacteria from nonpathogenic organisms that produce vancomycin and use the inducible resistance mechanism to protect themselves from their own vancomycin production (Akinori Okano, 2017). Pathogenic bacteria have not evolved effective resistance mechanisms themselves, and the elimination of VanA and VanB resistance may therefore provide antibiotics that have a long lasting clinical lifetime.

4. Discussion

4.1 Modifications to a single heavy atom

The change of one atom in a molecule can give extreme changes in the behavior of a molecule. The addition, removal, or exchange of a single heavy metal, which may entail more than one atom, in cases such as NH vs. O, can shift the properties of the compound to our preferred purpose (Boger, 2017).

4.1.1. The natural change in resistant bacteria

In exchanging D-ala-D-ala with D-ala-D-Lac vancomycin resistant bacteria make a single heavy atom change to the observed ligand X, from amide NH to an ester O, ref. Figure 4.1. This results in a loss of an H-bond, as well as introducing destabilizing electrostatic repulsion. (Boger, 2017) The change reduces the binding affinity of vancomycin with a 1000-fold. Through comparing the Ka for the bacteria with ligand NH, CH_2 and O, it is possible to see the effect of the loss of H-bond represented in both NH and CH_2 in comparison to the effect of electrostatic repulsion only present when the ligand is oxygen ie. the D-Ala-D-Lac modification of the resistant bacteria.

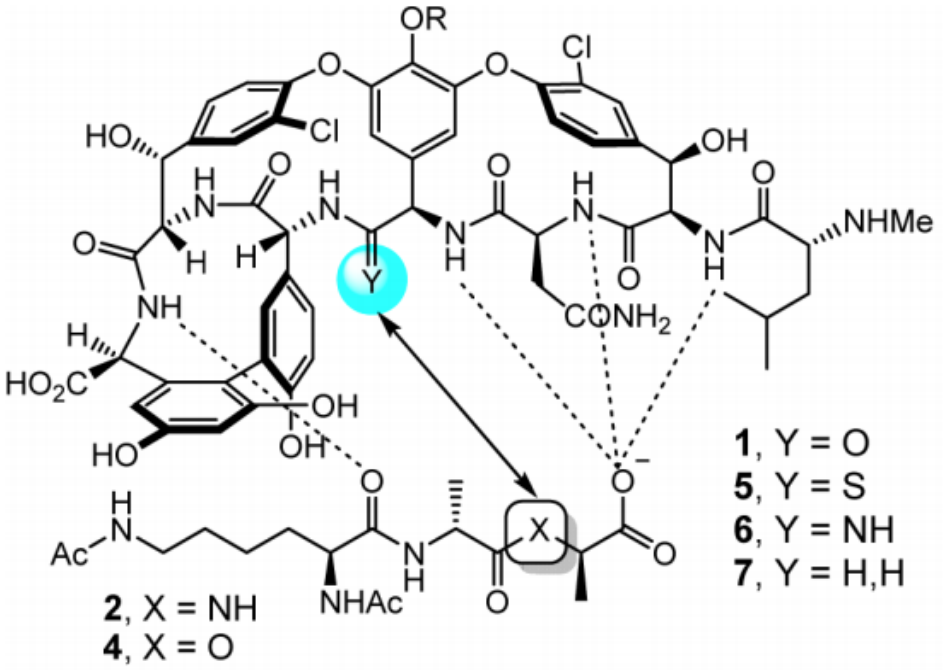


Figure 4.1: Vancomycin analogues that incorporate single heavy atom changes in the binding pocket (Y). The original single heavy atom in the binding pocket was the residue 4 amide carbonyl oxygen, and the replacements S, NH, H_2. The peptidoglycan termini D-Ala-D-Ala is represented by X = NH, and the resistant bacteria containing the D-Ala-D-Lac is represented by ligand X = O.

When the ligand amide NH is exchanged for CH_2 the difference is the loss of an H-bond, without other major properties that interfere with the potential binding of vancomycin. The change in binding affinity is shown by the change in the association constant Ka, a measure of affinity. From Table 3.1 it is observed that the change of binding affinity when an H-bond is lost is equivalent with a 10-fold decrease. When the ligand is exchanged with O, as seen in the VanA and VanB phenotypes of vancomycin resistance, the decrease amounts to a 1000-fold. It can therefore be concluded that the introduction of electrostatic repulsion between the ligand oxygen and the oxygen and the vancomycin molecule is related with a 100-fold decrease in binding affinity. The oxygen/oxygen interaction is in fact a lone pair/lone pair

repulsion (Younes Valadbeigi, 2018), and it is therefore only natural that the single heavy atom exchange resulted in this high decrease of bonding affinity.

Ligand	**Ka (M^{-1})**	**Δ G° (25 °C)**
X_1 = NH (alanine)	4.4×10^5	7.7 kcal/mol
X = CH_2	3.3×10^4	6.2 kcal/mol
X_2 = O (lactate)	4.3×10^2	3.6 kcal/mol

Table 4.1: The measured effect on the binding affinity of vancomycin through Ka and Δ G° at 25 °C for different model ligands that contain the single heavy atom exchange. (Boger, 2017)

4.1.2 The change in vancomycin

As the lone pair/lone pair repulsion is responsible for the majority of the decrease in binding affinity, as seen in Table 4.1, it indicates that by stabilizing this electrostatic repulsion, the bonding affinity would increase substantially (Boger, 2017).

VanA/VanB VRE has the D-Ala-D-Lac terminal, which means that the reacting single heavy atom is oxygen. To remove the lone pair/lone pair repulsion between the two oxygens the binding pocket in the core of the vancomycin must be replaced by a new ligand.

Removal of the amide carbonyl oxygen results in an antibiotic analogue with the capacity for dual ligand binding. This makes the antibiotic effective against vancomycin resistant bacteria, while simultaneously maintaining the ability to bind vancomycin sensitive bacteria. Even so, we still measure a 30-fold reduced affinity compared to the unaltered vancomycin against sensitive bacteria.

Compound	**Ligand, Ka (M^{-1})**		**Ka (X_1/X_2)**	**VanA MIC (µg/mL)**
	X_1 = NH	X_2 = O		
Y = O	1.7×10^5	1.2×10^2	1400	640
Y = S	1.7×10^2	1.1×10	-	> 640
Y = NH	7.3×10^4	6.9×10^4	1.05	0.5
Y = H, H	4.8×10^3	5.2×10^3	0.9	31

Table 4.2: The measured affinity of vancomycin analogues that incorporate single heavy atom changes in the binding pocket for the bacteria and its antibiotic resistant version. (Boger, 2017)

Table 4.2 list three different compounds, subsequently, O, S, NH and H_2 and their respective bond affinity with both the D-Ala-D-Ala and the D-Ala-D-Lac. The exchange of the vancomycin residue 4 amide carbonyl oxygen with an amide NH would could be viewed as a simple target exchange from the original non-resistant bacteria, however there are some fascinating consequences of the exchange of places (Boger, 2017). The place swap does not only reintroduce full affinity to the ligand, now D-Ala-D-Lac, but also maintain the full binding affinity to vancomycin sensitive bacteria. This means that the antibiotic now have a dual binding character with the amidine free base serving as the H-bond that was lost when the bacteria had a single heavy atom exchange. It is also possibly a weak reverse H-bond present as it is now a stabilized electrostatic interaction.
The H_2 also has an okay affinity, as well as the dual binding inhibits transpeptidase and cell wall biosynthesis. When introducing peripheral modifications the molecules most effective are those containing the ligands NH and H_2 in the binding pocket.
The sulfur ligand was not as fortunate, and the exchange resulted in a 1000-fold loss of affinity rather than increasing the affinity between the two compounds.

4.2 Peripheral modifications

Peripheral modifications to the vancomycin molecule are a result of a lengthy synthesis. The basis for the modifications is therefore the methylene pocket-modified vancomycin analog [Ψ[CH_2NH]Tpg^4]vancomycin, a result of section 4.1 modification of a single heavy atom (Akinori Okano, 2017).

4.2.1 CBP-modification

Peripheral functionalizations of the binding pocket modified vancomycin analogs with a remarkable specter of antimicrobial activity, producing more potent antibiotics. (Akinori Okano, 2017). The oritavancin (4-chlorobiphenyl)methyl (CBP) group, known to enhance antimicrobial potency, was then introduced to the pendant disaccharide. The new peripheral group improved the bonding affinity approximately with a 100-fold, and has a potency towards both vancomycin sensitive and resistant bacteria. The activity of the CBP- modified analogs use two synergic mechanisms of action, where only one of them is connected to the D-Ala-D-Ala/D-Ala-D-Lac binding.

4.2.3 C-terminal amide modification

The second peripheral modification seen in the modified vancomycin, Figure 4.2, is the C terminal amide functionalization with either a basic amine capable of protonation or a quaternary ammonium salt. This type of modification acts by disrupting the bacterial cell wall membrane integrity, increasing cell permeability and inducing membrane depolarization (Akinori Okano, 2017). Through testing the MIC of several versions of this modification, the addition of the tetradecyl (C14) was seen to be most effective, and thus ended up in the final vancomycin molecule.

4.3 Modified vancomycin

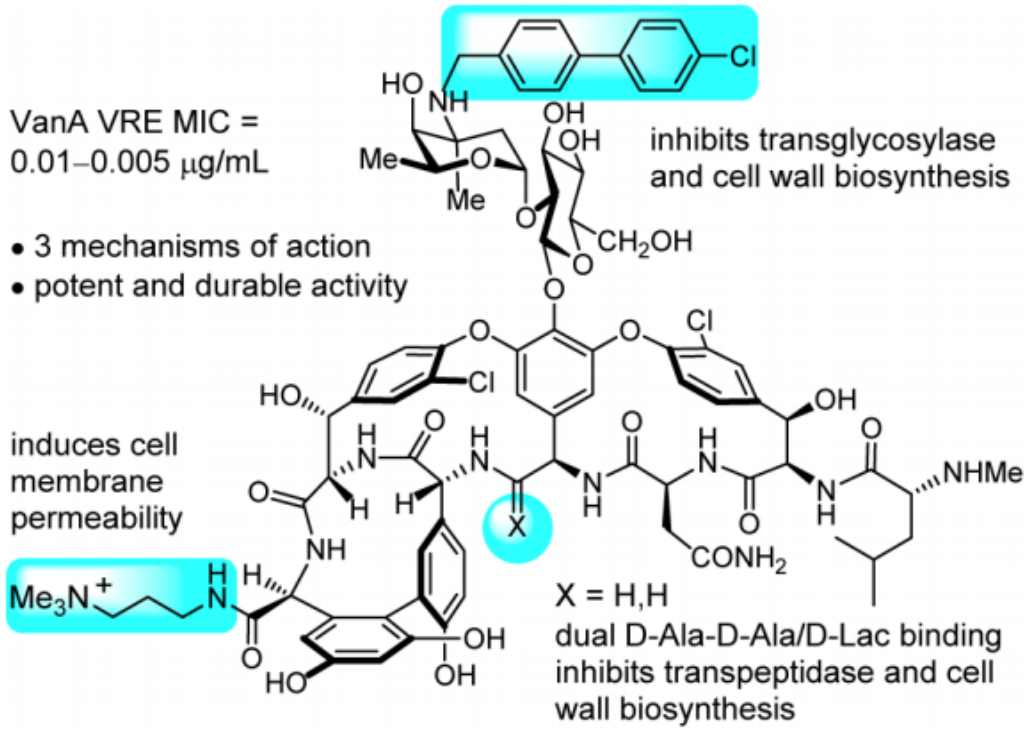


Figure 4.2: Vancomycin analogue that contains a single heavy atom change in the binding pocket, resulting in dual D-Ala-D-Ala/D-Ala-D-Lac binding and an affinity for most of the gram-positive bacteria. It also contains two peripheral modifications resulting in independent mechanisms of action against bacteria.

As seen in Figure 4.2 there are two major peripheral changes that are present in the final compound, named compound 18. From 4.1 it is evident that the ligand X would benefit from being either NH or H_2. Through various testing of bonding affinity the compound 18 with its X = H, H was more resilient and better then the equivalent compound 15 that housed X = NH.

The finished product therefore consist of the two peripheral compound (4-chlorobiphenyl)methyl (CBP) group, the most potent modification of a C-terminal amide, the

quaternary ammonium salts bearing a tetradecyl (C14), and the CH_2 in the binding pocket resulting in a ligand H.H.

These three modifications are independent of each other, and results in a more durable vancomycin analogue antibiotic. The CBP modification produced a 100-fold increase in antimicrobial activity, the secondary peripheral modification at the C terminus of the pocket modified analogs enhances the antimicrobial activity 200-fold against VanA VRE by inducing membrane permeability (Akinori Okano, 2017). When added to the already existing pocket modification , ref. 4.1.2, these independent mechanisms of action not only further increase the antimicrobial potency against VanA VRE with more than 6000-folds, but also reduce the possibility for the bacteria to gain resistance. The separate mechanisms of action were shown to follow a predictable 3 > 2 > 1 mechanism of action, which means the bacteria meats three lines of defense consecutively. The durability of this new vancomycin analogue is far exceeding the original vancomycin.

In the clinical testing of the antibiotics it was again notable that compound 18 was the best compound of the different versions synthesized. (Boger, Clinical test of Vancomycine. Appendix, 2017) Even with extended exposure to the blood, compound 18 displayed little hemolytic activity as well as behaving no different than the control linezolid which does not act on the bacterial cell membrane. These combined sets of studies indicate that compound 18, as well as 15, have less of an impact on mammalian red blood cell membranes than even the original vancomycin itself. Finally, the extraordinary potency of the key analog 18 would also be expected to minimize any nonselective toxicity because the amounts required for observation of antimicrobial activity are so low.

4.4 Other causes of resistance

To combat antibiotic resistance, it is important to review different causes of resistance from a broader perspective. There are several reasons why antimicrobial resistance has become widespread and it is necessary to also consider socioeconomic factors that contribute to this problem.

4.4.1 Prescription of medications

Improper prescription of antibiotics promotes the emergence of resistant bacteria. Studies have shown that the treatment, choice and duration of antibiotic treatment in 30% to 50% of cases are incorrect. While incorrect prescription of antibiotics may have some therapeutic benefits, the patient is exposed to possible complications during subsequent antibiotic treatments. Both subinhibitory and subtherapeutic antibiotic concentrations can promote the development of antibiotic resistance by supporting changes in genetic material; for example, these changes can occur in gene expression. Too low a concentration of antibiotic treatment has previously been shown to contribute to diversification in organisms thereby increasing their resistance.

4.4.2 Extensive use in agriculture

Antibiotics are widely used as growth supplements in pets. It is estimated that up to 85% of all antibiotics sold in the United States are used in animals, primarily to promote growth and prevent infection. Sixty different varieties of domestic animals are said to be treated with antimicrobial agents to improve their general health, obtain higher yields and a higher quality product. Humans end up consuming the antibiotics used by animals. The transmission of resistant bacteria through food was first detected 35 years ago, when high levels of resistance to antibiotics were found in the stomach flora of both farmers and their animals. New detection methods have shown that resistant bacteria in farm animals reach consumers through meat products. This often occurs in a causal chain where humans, as the ultimate consumer, end up with very high values of antimicrobial resistance. The use of antibiotics in agriculture further affects the environment in the microbiome. Up to 90% of all antibiotics administered to livestock are excreted in urine and feces and then spread through manure, groundwater, and surface flow.

The use of antibiotics in agriculture further affects the environment in the microbiome. Up to 90% of all antibiotics administered to livestock are excreted in urine and feces and then spread through manure, groundwater, and surface flow. In addition, tetracyclines and streptomycin are sprayed on fruit trees to act as pesticides in much of the world. Although this is not the largest contribution to total antibiotic consumption, it does lead to greater geographic spread. In addition, these practices contribute to a greater exposure of microorganisms in the environment to growth inhibitors and, therefore, to a greater risk of

changing the environmental ecology by becoming more resistant than sensitive microorganisms.

5. Conclusion

Modifications to the vancomycin molecule has resulted in a new durable antibiotic, with a lower chance of resistance then any other antibiotic. The compound 18 especially showed that it had a slower rate of resistance then the front liners linezolid and tigecycline know by today's standards of being extremely durable. This shows that the new vancomycin could hopefully be a new frontline antibiotic.

The three independent mechanisms work in a structural pattern and end up attacking the gram positive bacteria in a synergic 3 > 2 > 1 mechanism. By doing so it decreases the bacteria's surviving rate exponentially, as the three mechanisms doesn't rely on the same tactic, as only one of the mechanisms require the well-known D-Ala-D-Ala/D-Ala-D-Lac binding that is present in most vancomycin resistant bacteria.

The bonding affinity of the new antibiotic is also comparable to the original vancomycine. Due to the three mechanisms of the new vancomycin its potency against VanA VRE is over 6000-fold. This is a result of the increase in potency each of the modifications themselves brought, as well as the added effect of an repeated attack on a backteria.

The VanA/VanB phenotypes are the most common form of vancomycin resistant bacteria, but as seen in section 3.4.1 there are other phenotypes that can result in a different amino acid taking the place of D-Ala in the peptidoglycan chain. This however would most likely not be a problem due to the different types of ligand the binding pocket in the vancomycin core can exchange for the oxygen. It is therefore a possibility of synthesizing other similar vancomycin antibiotics based on the resistance that occurs in the vancomycin resistant bacteria. If however compound 18 is available as the main antibiotic, resistance would form quite slowly.

Antibiotic resistance is however a huge problem, and the ongoing buildup of resistant bacteria does not necessarily mean we should use more antibiotics. Actually maybe the amount of antibiotics given out should decrease, and the still potent antibiotics saved for a worse day. By introducing new types of antibiotics we also teach the bacteria the new mechanisms, this can

result in formerly harmless bacteria mutating into dangerous bacteria. This is because weaker antibiotics might then be useless before their time. As vancomycin is one of the last resort antibiotics it should be handled with care, and not be used recklessly so that we can minimize the spread of antibiotic resistant bacteria.

The downside of this synthesis is that it is a very long synthesis, with quite a lot of steps before the vancomycin is ready to be modified. The modifications in themselves on the other hand are not subjected to many steps but may be complicated to implement, but the proses of getting there is expensive and therefore not yet cost-efficient. In an ideal world the modifications could be done to the naturally grown vancomycin, but this is not yet been possible. The goal should therefore be to keep developing and finding ways to shorten down the steps in the synthesis to make a more cost-efficient antibiotic that could be commercially used to conquer the increasing number of vancomycin resistant gram positive bacterial, as vancomycin resistance is one of the major threats our future is facing.

BIBLIOGRAFI

(u.d.).

Akinori Okano, N. A. (2017). Peripheral modifications of [phi[CH_2NH]Tpg^4]vancomycin with added synergistic mechanisms of action provide durable and potent antibiotics. *PNAS: Proceedings of the National Academy of Science of the United States of America* .

Bailey, R. (2020). *thoughtco.co*. Hentet fra Gram positive vs. Gram negative bacteria : https://www.thoughtco.com/gram-positive-gram-negative-bacteria-4174239

Boger, D. L. (2017). Clinical test of Vancomycine. Appendix. *PNAS*.

Boger, D. L. (2017). The Difference a Single atom can make: synthesis and Design at the Chemistry-Biology interface. *The Journal of Organic Chemistry* .

Ivo G Boneca, G. C. (2019). *Vancomycin resistance: occurrence, mechanisms and strategies to combat it*. Hentet fra https://pubmed.ncbi.nlm.nih.gov/12783569/

Kaiser, G. (2021). *The Gram-Positive cell wall* . Hentet fra LibreTexts Cell Biology : https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_%28Kaiser%29/Unit_1%3A_Introduction_to_Microbiology_and_Prokaryotic_Cell_Anatomy/2%3A_The_Prokaryotic_Cell_-_Bacteria/2.3%3A_The_Peptidoglycan_Cell_Wall/2.3A%3A_The_Gram-Positive_Cel

Kumar, P. (2017). *Pharmacology and therapeutics for dentistry.*

Lancini, G. C. (1989). *Progress in Industrial Microbiology Vol. 27.* New York: Elsevier .

Mark S Butler, K. A. (2014). Glycopeptide antibiotics: Back to the future. *The Journal of Antibiotics* .

Matthew E Griffin, C. W.-C. (2019). Translation of peptidoglycan metabolites intoimmunotherapeutics. *Clinical & Translational Immunology*. doi:10.1002/cti2.1095

Microbiology Society. (2021). *microbiologysociety.org*. Hentet fra https://microbiologysociety.org/members-outreach-resources/outreach-resources/antibiotics-unearthed/antibiotics-and-antibiotic-resistance/what-are-antibiotics-and-how-do-they-work.html?fbclid=IwAR0mp8VqSc6u8l8PvH_gQ01SffmfvGGUQvS9WBPz37YzFECsXiJYyVIMjUQ

Myers, J. (u.d.). *This is how many people antibiotic resistance could kill every year by 2050 if nothing is done*. Hentet fra World Economic Forum: https://www.weforum.org/agenda/2016/09/this-is-how-many-people-will-die-from-antimicrobial-resistance-every-year-by-2050-if-nothing-is-done/

Pasquina-Lemonche, J. B. (2020). The architecture of the Gram-positive bacterial cell wall. *Nature*. doi:10.1038/s41586-020-2236-6

Que Y-A, M. P. (2015). *Principles and Practices of Infectious Diseases* .

R. Guan, R. A. (2019). *Trends in Microbiology.*

Silveira, D. (2017). *Enzyme Inhibation.*

Sverre Dick Henriksen, K. B. (2018). *snl.no*. Hentet fra Store medisinske leksikon: Antibiotika : sml.snl.no/antibiotika

Tankeshwar, A. (2019 , November 19). *Glycopeptides (Vancomycin, Teicoplanin): Mechanism of Action, Resistance and Uses*. Hentet fra The guide to Medical Microbiology : https://microbeonline.com/glycopeptides-vancomycin-teicoplanin/

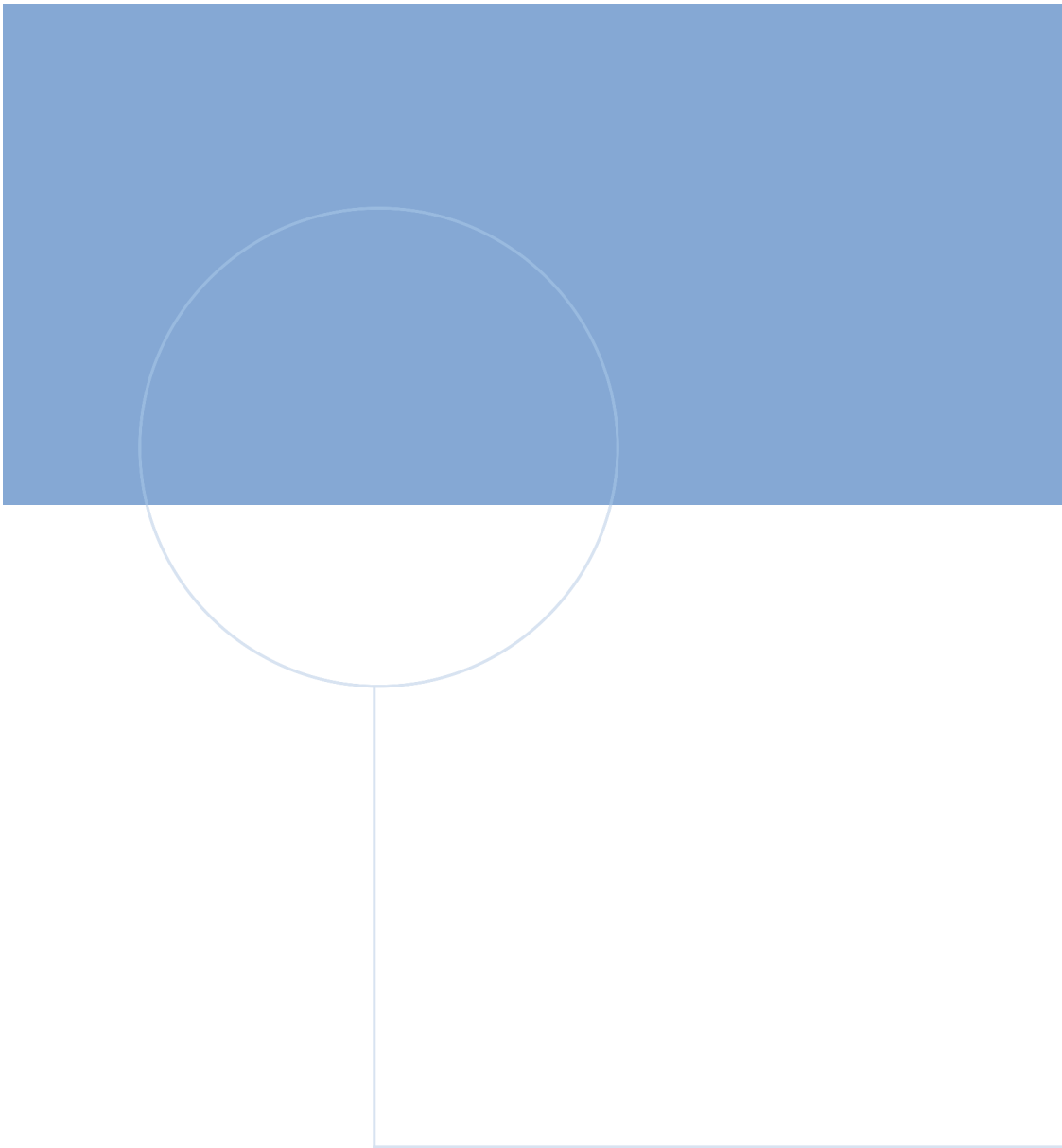
W. Vollmer, D. B. (2008). Peptide structure and architecture . *FEMS Microbiol*.

WHO. (u.d.). *A global priority list of antibiotic-resistant bacteria to help guide research, developement and discovery of new antibiotics*. Hentet fra World Health Organization : https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf

Williams, T. K. (2014). *Biofilms in Infection and Disease Control: A Healthcare Handbook.* Elsevier.

Younes Valadbeigi, Y. V. (2018). On the Significance of Lone Pair/Lone Pair and Lone Pair/Bond Pair Repulsions in the Cation Affinity and Lewis Acid/Lewis Base Interactions. *ACS Publications Omega*. doi:https://doi.org/10.1021/acsomega.8b01644

Zablon, F. M. (u.d.). *Vancomycin-resistant bacteria: VRSA and VRE*. Hentet fra Biology: https://thebiologynotes.com/vancomycin-resistant-bacteria-vrsa-and-vre/



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