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Natural product antibiotics: synthesis and next generation analogues

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Chapter 1

Introduction

The increasing threat of antimicrobial resistance

Antimicrobial resistance (AMR) is increasingly recognized as a global public health crisis that threatens the effectiveness of antibiotics and other antimicrobial agents, which have long been vital for treating infections, performing surgeries, and managing chronic diseases.[1-4] AMR occurs when microorganisms – such as bacteria, viruses, and fungi – evolve to resist the very drugs that once eradicated them or inhibited their growth.[5-8] This phenomenon has led to higher medical costs, prolonged hospital stays, and increased mortality, making AMR one of the leading causes of death worldwide, with the highest burden in low-resource settings. In 2019, it was estimated that 4.95 million deaths were associated with drug-resistant bacterial infections, while 1.27 million deaths could be directly attributed to them (**Figure 1**).[2,9] By 2050, these figures are projected to rise to 8.22 million deaths annually associated with bacterial AMR, indicating that AMR will become one of the most significant healthcare challenges of the 21st century. This alarming forecast implies that we are unlikely to achieve the proposed 10% reduction in AMR-related mortality by 2030, as outlined in the "10-20-30 by 2030" targets.[3] The diminishing availability of effective antibiotics presents a crisis wherein infections due to multidrug-resistant (MDR) pathogens continue to rise every year, underscoring the urgent need to explore novel compounds and alternative therapeutic strategies.[10,11]

Among Gram-positive pathogens, the mortality rate associated with methicillin-resistant *Staphylococcus aureus* (MRSA) has alarmingly doubled over the past three decades, and continues to exert a substantial burden on global health systems.[3,12] The treatment landscape for infections caused by Gram-positive bacteria has become increasingly complex, largely driven by the emergence of multidrug-resistant strains, such as MRSA, vancomycin-resistant *S. aureus,* and vancomycin-resistant *Enterococcus* (VRE).[13-17]

However, the most imminent threat, as recently established by the World Health Organization (WHO), is presented by carbapenem-resistant isolates of Gram-negative bacteria such as *Acinetobacter baumannii* or *Enterobacterales* species, including *Escherichia coli*.[18] The inherent structural and functional characteristics of this type of bacteria, particularly their thick outer membrane (OM) and efficient efflux pumps, have generally proven Gram-negative bacteria to be more resistant than Gram-positive pathogens.[19,20] This complexity poses significant challenges to the development of effective antimicrobials against Gram-negative pathogens and raises considerable concern regarding the global rise of multidrug-resistant Gram-negative species.[21,22]

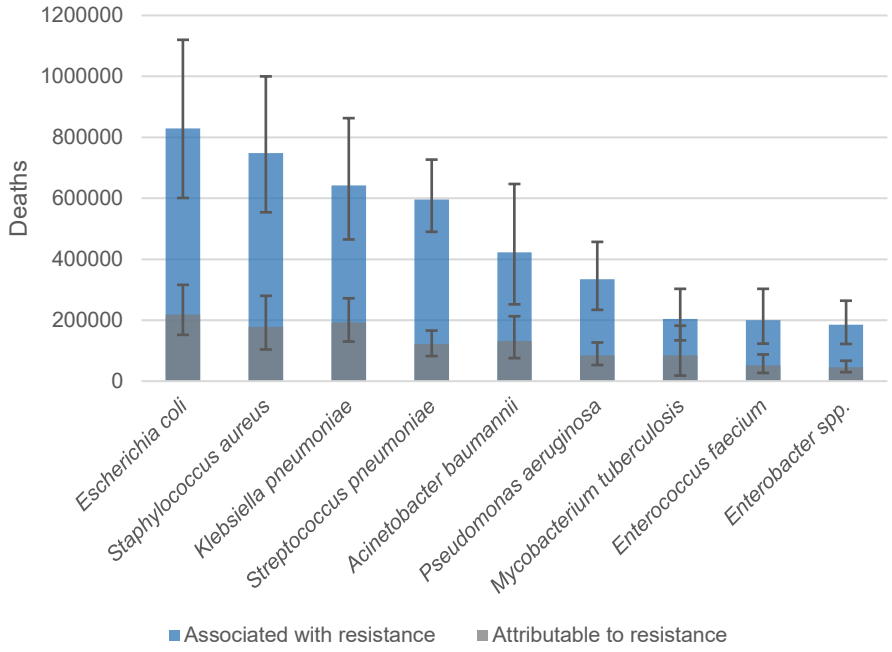

Figure 1. Global deaths attributable to and associated with bacterial antimicrobial resistance by pathogen, 2019.[2]

While the drug-resistant bacteria discussed above present a serious and growing problem, *Mycobacterium tuberculosis* remains the greatest global killer among infectious pathogens. *M. tuberculosis* is responsible for more than 10 million new cases of tuberculosis (TB) each year, with an associated >1 million deaths.[23] The conventional treatment regimen for TB typically consists of a multidrug approach administered daily over extended periods; however, this prolonged treatment duration often results in suboptimal patient adherence, leading to the emergence of resistant strains of the pathogen.[24,25] The escalating threat posed by extensively drug-resistant strains of TB amplifies the urgent need for innovative antibacterial therapies and the development of novel antimicrobials capable of effectively combating this formidable pathogen.[26-29]

Without the introduction of novel and efficacious antimicrobials targeting different types of bacteria, the capacity to manage acute infections, conduct routine surgical procedures, administer chemotherapy, and treat chronic illnesses will be severely compromised.[18,30] Therefore, there is an urgent need for action to discover and develop new antibiotics, which, according to current projections, could prevent over 10 million deaths attributable to AMR between 2025 and 2050.[3]

Natural products as antibiotics

The journey towards the discovery of the first natural antibiotic began in 1928, when Alexander Fleming made a fortuitous observation that would dramatically change the course of medicine. While conducting research at St. Mary's Hospital in London, he discovered that a mold called *Penicillium notatum* contaminated one of his petri dishes and, in the process, eradicated surrounding *S. aureus* bacteria. This pivotal moment led to the identification of penicillin (**Figure 2**), the first natural antibiotic, which demonstrated a potent ability to combat bacterial infections.[31] Despite its revolutionary potential, the therapeutic application of penicillin was fraught with challenges, particularly in the areas of purification and mass production. It wasn't until the early 1940s, amid the context of World War II, that these challenges were systematically addressed. Thanks to the collaborative efforts of scientists such as Howard Florey and Ernst Boris Chain, who refined the extraction and production processes, penicillin became widely available.[32-34]

The success of penicillin heralded the beginning of the "Golden Age of Antibiotics," a remarkable period that spanned the 1940s to the early 1960s. During this time, the discovery and widespread use of a myriad of other natural antibiotics further revolutionized clinical practice, highlighting the profound impact these medications had on public health.[35-38] The ability to effectively treat bacterial infections not only improved individual patient outcomes but also transformed public health metrics worldwide, contributing to longer life expectancies and a reduction in disease prevalence. The most notable antibiotics discovered and brought to the clinic in their unmodified forms during this time include: streptomycin, the first aminoglycoside; chlortetracycline, the first tetracycline; chloramphenicol, the first amphenicol; erythromycin, the first macrolide; vancomycin, the first glycopeptide; and colistin, the first polymyxin (**Figure 2**).[38,39]

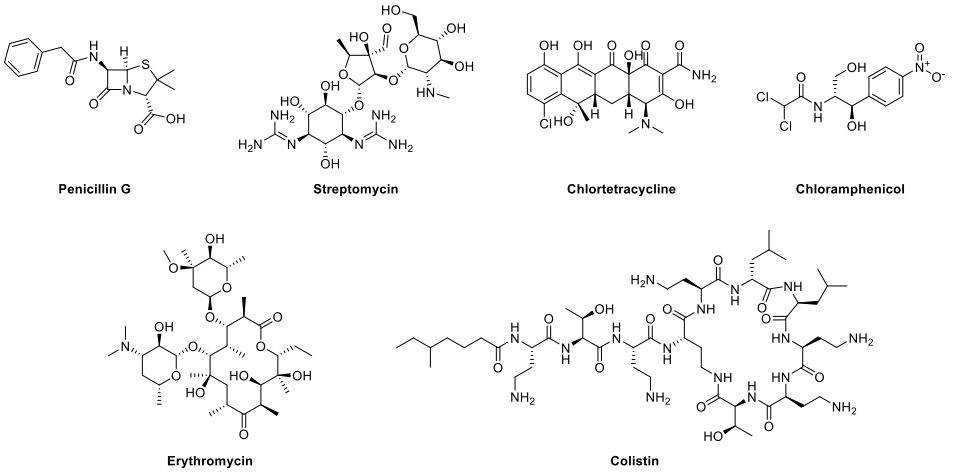

Figure 2. Structures of penicillin G and several other antibiotics discovered during the "Golden Age".[38,39]

Despite the remarkable advancements in antibiotic therapy during the "Golden Age", the emergence of antibiotic-resistant bacteria has significantly diminished the effectiveness of these agents, presenting a formidable challenge for contemporary medicine. In addition to this issue, the introduction of new antibiotics to the market has slowed, with only daptomycin (**Figure 3**) emerging as a novel class of natural product antimicrobials between 2000 and 2020.[40] Daptomycin is a lipopeptide antibiotic that was originally identified in the late 1980s, and its clinical application received regulatory approval in 2003. It is primarily employed for the treatment of serious infections caused by Gram-positive bacteria, including MRSA strains.[41-43] Daptomycin functions by disrupting the bacterial cell membrane potential and biosynthesis, ultimately leading to cell death.[44] This unique mechanism of action distinguishes it from other antibiotics, making it a crucial option against antibiotic-resistant infections.

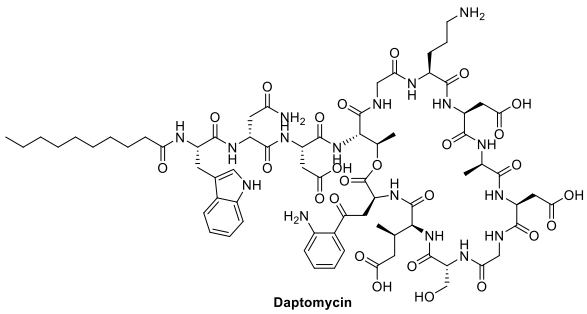

Figure 3. The structure of daptomycin.

When looking for new antibiotics in nature, the growing incidence of rediscovery of known compounds from established producers has made traditional screening methods less effective.[45,46] This trend has prompted the hypothesis that the biosynthetic capabilities of traditional producers have been thoroughly investigated and potentially exhausted.[35,47] However, recent advancements in sequencing technologies have provided a comprehensive repository of genomic data.[36,45] Once believed to be fully explored, these technologies have highlighted a wealth of undiscovered molecules hidden within biosynthetically capable bacterial genera.[48-51] In recent years, bioinformatics tools have made significant advancements in predicting natural product biosynthetic gene clusters (BGCs). Tools such as antiSMASH[52] and PRISM[53] have become pivotal in the analysis of BGCs, facilitating the identification of novel clusters and their annotation using reference data from curated databases, such as MIBiG.[54] Beyond mining individual genomes, large-scale comparative analyses are now possible through tools like BiG-SCAPE, which enables the grouping of BGCs into gene cluster families (GCFs) based on sequence similarity and domain architecture.[55,56] The combined utilization of these techniques allows for the rapid organization of vast collections of diverse sequences, enabling the identification of novel compounds. Such discoveries may lead to the

emergence of entirely new classes of natural products or contribute to the diversification of existing families of clinically relevant isolates.[57,58]

Notable examples of novel antibiotics discovered through genome mining methodologies include the glycopeptide corbomycin, the calcium-dependent antibiotic malacidin, as well as cationic lipopeptides such as brevicidine and laterocidine.[59-61] The integration of genome mining techniques with solid-phase peptide synthesis (SPPS) allows for the synthesis of peptides encoded within the bacterial genome that are not typically produced by the organism under standard conditions. A number of discoveries using this approach have been made by the Brady group and are highlighted by the following molecules: humimycin A and B, macolacin, and cilagicin, all of which are reported to have potent activity against MDR pathogens.[62-64]

Next-generation sequencing of microbial genomes has also rejuvenated the discovery and characterization of diverse natural products coming from known producers of antimicrobial compounds, pointing to the still largely untapped biosynthetic potential of bacteria.[50] A prominent example is the understudied genus *Paenibacillus*, renowned for producing polymyxins, which holds promise for antibiotic discovery through modern genome mining, metabolomics, and fermentation techniques.[65-67] In recent decades, several new classes of active antimicrobials have been identified from this genus, including the tridecaptins, paenibacterins, octapeptins, polypeptins, and pelgipeptins. The diversity of novel antimicrobials discovered so far highlights the remarkable biosynthetic potential of the *Paenibacillus* genus and supports the idea that many more active compounds remain to be found.[68,69] Therefore, we have also searched for new peptides produced by *Paenibacillus* spp., with the details of this research presented in **Chapter 2** of this thesis.

Another promising new approach to discovering natural products involves screening previously uncultured bacteria. Historically, the majority of natural antibiotics identified have originated from species that can be cultivated under controlled laboratory conditions. Exploring previously inaccessible, uncultured bacteria opens up new avenues in the search for unique antibiotics, as this technique can avoid the "rediscovery" problem that arises from screening already well-analyzed species.[46,70-72] Many of these bacteria rely on specific growth factors, such as siderophores or quinones, produced by neighboring species.[73,74] Cultivating them in their natural environments has led to the identification of several antimicrobials with unique mechanisms of action. Significant advancements in this field have emerged from the research conducted in the Lewis group, leading to the discovery of several antimicrobial natural products with diverse modes of action (**Figure 4**).[46,71] Among these are teixobactin and clovibactin, both of which target Gram-positive bacteria and demonstrate a unique ability to counter bacterial resistance mechanisms.[75,76] Another compound discovered by the Lewis group is darobactin A, which was identified as the first molecule specifically targeting BamA, a central component of the β-barrel assembly machinery in Gram-negative bacteria.[77] The most recent addition to Lewis group's impressive collection of antibiotics derived from

previously uncultured bacteria is evybactin, which selectively inhibits the growth of *M. tuberculosis*. The multifaceted mechanism underlying evybactin's selectivity is particularly intriguing: it exploits BacA, a vitamin B transporter unique to *M. tuberculosis*, to gain cell entry, after which it effectively inhibits DNA gyrase.[78] A comprehensive exploration of the synthesis and structure-activity relationship (SAR) of evybactin is detailed in **Chapters 3** and **4** of this thesis.

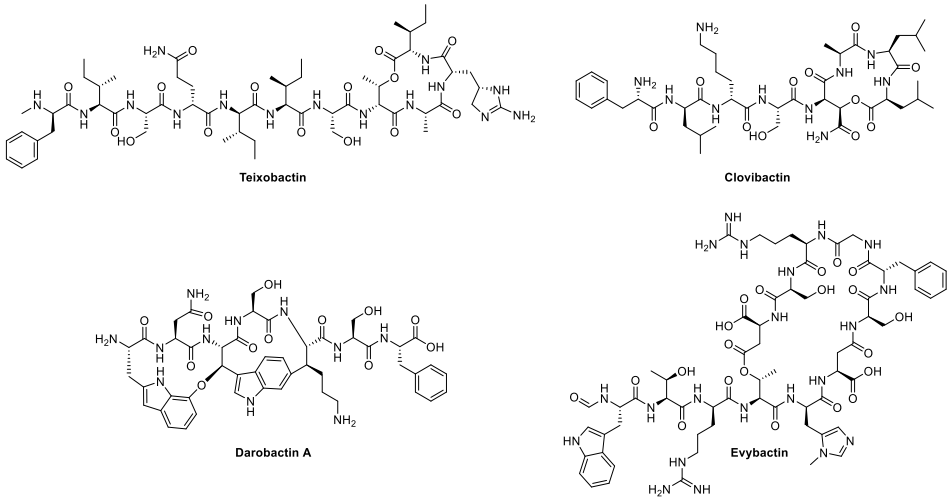

Figure 4. Structures of the compounds discovered from the isolates of the previously uncultured bacteria.[75-78]

The history of natural product antibiotics serves as a powerful testament to their transformative effect on public health. Notable milestones, such as the discovery of penicillin and the subsequent "Golden Age of Antibiotics", revolutionized the treatment of bacterial infections, significantly enhancing life expectancy and disease management. However, the emergence of antibiotic resistance highlights the pressing need for ongoing research and discovery. The introduction of advanced sequencing technologies and the investigation of previously uncultured bacteria present exciting new opportunities for identifying novel natural antibiotics to bolster our defenses against resistant infections. By leveraging such innovative strategies, the medical community can continue to develop new antimicrobial agents and effectively combat infectious diseases.

The use of synthetic methods in the natural product field

The total synthesis of complex natural products has long been one of the most challenging tasks in synthetic organic chemistry, driving the evolution of the field.[79] Representative publications in this field remain among the most highly read articles in chemistry-focused journals.[80] The realm of natural product antibiotics is no exception, with multiple total syntheses reported to date.[81] In many instances, the synthetic approach serves as the only viable means for structural identification and confirmation, making total synthesis essential for a comprehensive understanding of molecular structures, particularly when

spectroscopic and analytical methods fail to provide clear answers.[82] As a result, total synthesis has facilitated numerous structural reassessments of natural products across various fields.[83,84] Several antibiotic structures have also been revised following total synthesis investigations. For example, the structure of glabramycin B, which demonstrated moderate activity against *Streptococcus pneumoniae*, was recently corrected through total synthesis, leading to the accurate determination of the stereochemistries at two stereogenic centers (**Figure 5**).[85] Similarly, the synthesis of aspergillomarasmine A, an inhibitor of metallo-β-lactamases (MBLs), enzymes that contribute to bacterial antibiotic resistance, revealed that several essential stereocenters had initially been misassigned (**Figure 5**).[86,87] Additionally, extensive NMR analysis and total synthesis helped clarify uncertainties regarding the structure of kasarin, a marine antibiotic previously believed to be a β-lactam but was later reclassified as containing a pyrazin core instead.[88]

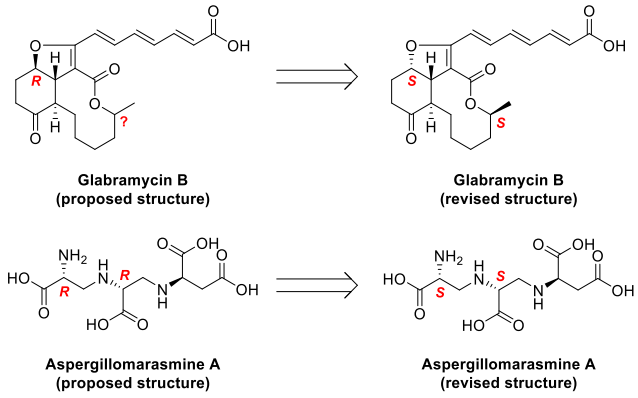

Figure 5. Recent stereochemical revisions of glabramycin B and aspergillomarasmine A achieved through a comprehensive total synthesis approach.[85-87]

A particularly notable achievement in the field of synthetic organic chemistry is the total synthesis of penicillin, first accomplished by John Sheehan.[89] While not useful for the industrial production of penicillin, this groundbreaking work demonstrated the feasibility of synthesizing complex natural products as well as the production of semisynthetic analogues starting from 6-aminopenicillanic acid, a readily available intermediate obtained by enzymatic hydrolysis of penicillin itself. This led to the development of a variety of derivatives such as ampicillin and amoxicillin, both of which exhibit broader activity and improved pharmacological properties than penicillin.[90]

In some cases, natural product antibiotics serve as a starting point for development rather than reaching the clinic in their original form. One such example is the naturally occurring cephalosporin C, a β-lactam antibiotic that was discovered in 1948 and then rediscovered again in 1955.[91,92] Its first total synthesis was achieved by Robert B. Woodward in 1965, and it was presented in his Nobel lecture before being published in 1966.[93] Although cephalosporin C did not become a clinical agent, its structure inspired numerous synthetic

efforts, resulting in the development of various analogues.[94,95] The majority of these derivatives were facilitated by the discovery of 7-aminocephalosporanic acid, a product of the hydrolysis of cephalosporin C.[96,97] This discovery enabled the creation of cephalosporin analogues through semisynthetic modifications, eliminating the need for total synthesis. This led to the emergence of potent antibacterial drugs, with cephalothin being the first cephalosporin antibiotic approved for clinical use (**Figure 6**).[98,99]

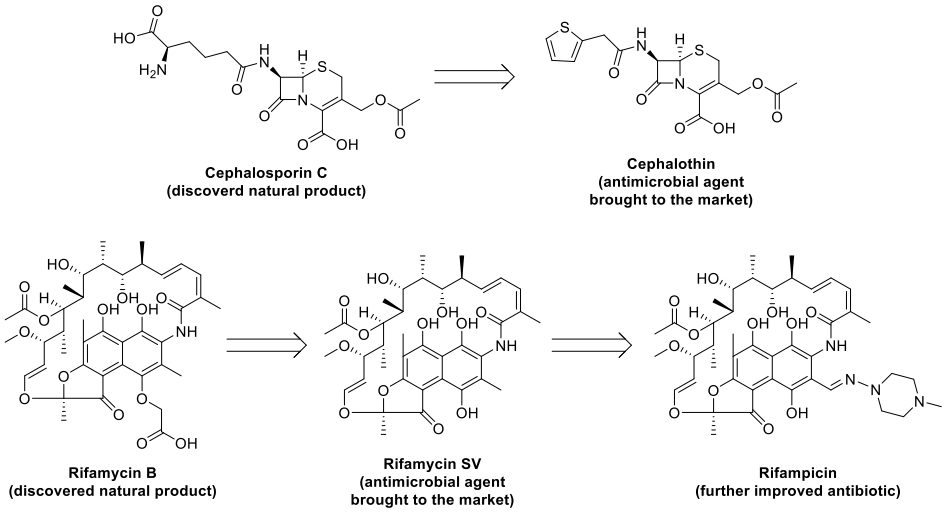

Figure 6. Examples of antibiotics that were initially discovered in natural sources but subsequently underwent synthetic modifications prior to their clinical application.[98,103]

A semisynthetic approach also paved the way for the development of rifampicin, a vital antibiotic used to treat infections caused by *M. tuberculosis* and other diseases caused by Gram-positive pathogens.[100,101] Rifampicin is part of the macrolactam antibiotic group known as rifamycins, with the first members reported in 1959, including the prominent example of rifamycin B.[102,103] The bactericidal effect of rifamycins stems from their ability to inhibit bacterial DNA-dependent RNA polymerase, thereby disrupting protein biosynthesis.[104,105] Although the naturally occurring rifamycin B did not advance to drug status, partially due to its low antimicrobial activity, a modified semisynthetic version, rifamycin SV, received approval as a clinical antibacterial agent and is particularly effective against TB.[103] Further research yielded improved and orally active derivatives, with rifampicin (**Figure 6**) emerging as a key component of the standard drug regimen for treating TB still used today.[100,106]

Another significant milestone in the field of organic chemistry is the total synthesis of vancomycin, a glycopeptide antibiotic that plays a crucial role in combating infections caused by Gram-positive bacteria. The first successful total synthesis of the aglycon of vancomycin was achieved simultaneously by the Evans and Nicolau groups in 1998, followed by the synthesis of vancomycin itself by Nicolau's group in 1999.[107-111]

Subsequently, numerous analogues of glycopeptide antibiotics with enhanced activity were generated using semisynthetic approaches, most notably telavancin, dalbavancin, and oritavancin, which were each approved for clinical use between 2009 and 2014.[112] Our research group has also contributed to the development of new vancomycin analogues with improved activity, among which **EVG7** (**Figure 7**) has emerged as particularly promising. This compound demonstrates superior efficacy compared to vancomycin against Gram-positive pathogens and can even inhibit the growth of vancomycin-resistant strains of *S. aureus* and *E. faecium*.[113]

In addition, our group has also developed several total syntheses of various peptidic natural product antibiotics and conducted SAR studies, which in many cases also enabled us to enhance their properties (**Figure 7**). A notable example of this approach is the work done on the natural antibiotic bacitracin. While the first total synthesis of this peptide was accomplished in 1996,[114] studies in our group led to the improved synthesis of this peptide, providing insights into the crucial role of the thiazoline group in bacitracin's activity.[115] Reliable synthesis, combined with SAR investigations, also led to the development of improved analogues featuring longer lipid chains at positions 3 and 8, demonstrating over 32-fold enhanced activity against *E. faecium* strains compared to bacitracin.[116]

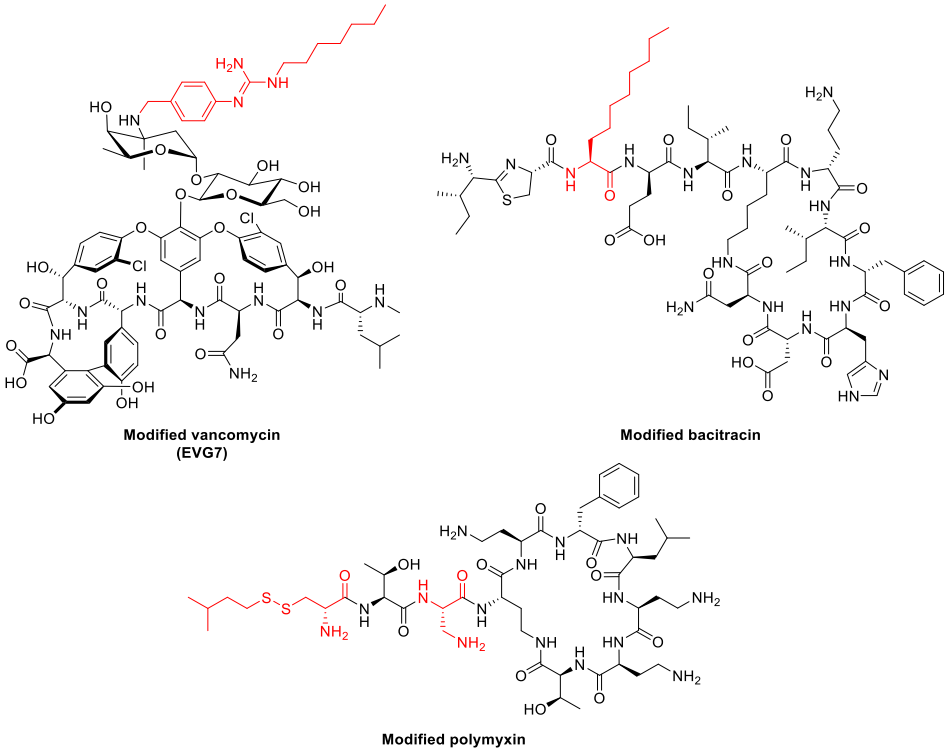

Figure 7. Some of the modified analogues of natural products developed in the Martin group; modifications are highlighted in red.[113,116,122]

In collaboration with the Cochrane group, we also synthesized two recently discovered antimicrobial nonribosomal lipopeptides, brevicidine and laterocidine. With a robust synthetic route established, we undertook extensive SAR studies that yielded several analogues with enhanced properties, with a macrolactam analogue of laterocidine standing out as the most prominent.[117-119] Subsequently, we carried out a similar synthesis of another antimicrobial peptide, relacidine, that shares structural similarities with laterocidine and brevicidine. Here, the amide version, relacidamide, preserved the activity of the natural product while exhibiting improved stability in serum.[120]

Our group has also made contributions in the area of polymyxin analogues, focusing primarily on strategies to mitigate nephrotoxicity without compromising antimicrobial activity. By employing a reductively labile disulfide bond, we demonstrated that such semi-synthetically modified polymyxin analogues remain active in vitro while exhibiting reduced toxicity toward kidney cells due to their ability to be reductively degraded within the cytoplasm.[121,122]

These and many more examples show that the landscape of natural antibiotics continues to evolve, driven by the application of advanced synthetic methodologies aimed at exploring and modifying these medically important compounds. From a practical standpoint, synthetic methods can help overcome challenges in producing natural products, in some cases ensuring sustainable and reliable access to them and their modified analogues. This progress highlights the creativity of modern chemistry and represents a crucial step in designing molecules capable of tackling antimicrobial resistance, one of the most pressing challenges in contemporary medicine.

“Trojan horse” antibiotics

As previously noted, infections caused by Gram-negative bacteria are particularly difficult to treat, primarily due to the complexity of their cell envelope.[19-22] Unlike Gram-positive bacteria, Gram-negative bacteria feature a hydrophobic outer membrane (OM) that encloses the peptidoglycan layer, along with an interstitial space known as the periplasm. The OM’s lipid bilayer consists of phospholipids on the inner leaflet and glycolipids, predominantly lipopolysaccharides, in the outer leaflet. This extra layer protects Gram-negative bacteria against potentially toxic compounds, including several antibiotics, rendering them ineffective.[123-125] However, the OM’s protective nature also affects crucial cellular functions, particularly nutrient uptake, which is facilitated by various embedded translocation systems that allow for selective entry. Such systems can be classified into outer membrane receptors, porins, specific diffusion channels, or energy-dependent transmembrane transport systems, including efflux pumps and TonB-dependent transporters.[126] Of particular interest are the TonB-dependent transporters, which are outer membrane proteins specific to bacteria that bind and transport a variety of compounds across the OM, including siderophores (**Figure 8A**) – small molecules that exhibit a high affinity for iron. Bacteria produce these compounds to secure iron from environments where this essential resource is scarce, such as in mammalian hosts.

Siderophores also play a role in the transport of non-iron metals, signaling, protection from oxidative stress, immunomodulation, biofilm formation, and virulence.[127-130] Among the more than 500 different siderophores identified and characterized to date, five primary iron-binding motifs have been recognized: catecholate, hydroxamate, phenolate, carboxylate, and α-hydroxy carboxylate. Those motifs are the fundamental components of natural siderophores (**Figure 8B**), exemplified by enterobactin and aerobactin produced by *E. coli* and *K. pneumoniae*, pyochelin generated by *P. aeruginosa*, and acinetobactin synthesized by *A. baumannii*.[131-134]

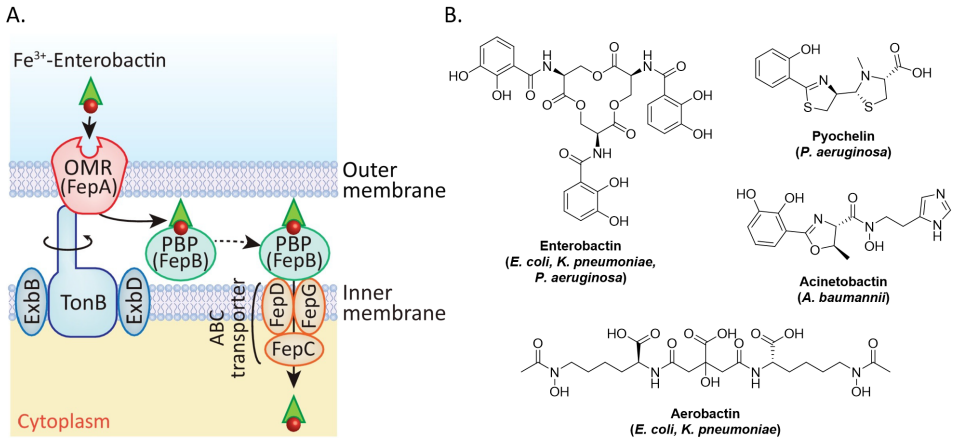

Figure 8. **A**. The mechanism of siderophore import in Gram-negative bacteria (*E. coli*) (adapted from [132]). **B**. The structural diversity of siderophores synthesized by Gram-negative bacteria.[131-134]

Certain microorganisms have evolved molecular defense strategies against bacteria wherein they synthesize and secrete compounds featuring siderophores that are covalently linked to a toxic moiety. This adaptation allows these molecules to be actively taken up by the target bacterial species, ultimately leading to the release of the toxic agent within the cell, resulting in the competitor's death. Hijacking bacteria's own iron-uptake mechanisms and utilizing them to deliver antibiotics that can act on intracellular targets is often referred to as a "Trojan horse" strategy (**Figure 9A**). Natural products that employ this strategy belong to the type of antibiotics known as sideromycins, with albomycin (**Figure 9B**) being a notable example.[130,135,136] Discovered in 1947 during a screening of a *Streptomyces griseus* strain collection for antibacterial substances, albomycin exhibits activity against a broad spectrum of Gram-positive and Gram-negative bacteria, including clinically significant staphylococci, streptococci, and various enterobacteria.[137,138] The iron-chelator component of the molecule serves as a vehicle for delivering the albomycin warhead into both Gram-positive and Gram-negative bacterial cells via the ferrichrome-specific transporter system. Once inside the bacterial cell, the siderophore part is cleaved, releasing the toxic nucleoside analogue payload.[139]

Inspired by the "Trojan horse" approach, researchers began to investigate different conjugation strategies that would facilitate the transport of previously membrane-

impermeable molecules into bacterial cells.[127-130,140] A notable success in this area is cefiderocol (**Figure 9B**), the first siderophore-antibiotic conjugate to receive FDA approval for clinical use.[141] Cefiderocol is a modified cephalosporin that shares similarities with both ceftazidime and cefepime, which are classified as third- and fourth-generation cephalosporins, respectively.[98,142] The key structural feature that distinguishes cefiderocol from other β-lactams is the presence of a chlorocatechol group, which promotes the formation of chelated complexes with ferric iron, thereby facilitating siderophore-like transport across the outer membrane of Gram-negative bacteria.[141-143] In contrast, most other β-lactams penetrate the outer membrane of Gram-negatives through passive diffusion via porin-mediated channels.[144] Cefiderocol's distinct mechanism of action arises from two key characteristics: 1) it utilizes a siderophore-like approach for cell entry, which leads to high drug concentrations at the target site, and 2) it shows remarkable stability against hydrolysis from nearly all β-lactamases.[142,143] These factors combined account for cefiderocol's superior antimicrobial efficacy when compared to carbapenems, β-lactam/β-lactamase inhibitor combinations, and advanced-generation cephalosporins, establishing it as one of the few viable options for treating multidrug-resistant Gram-negative pathogens.[145-147] Motivated by the success of cefiderocol, we explored designing antibiotic-siderophore conjugates using rifampicin as the core antimicrobial, which is discussed in **Chapter 5** of this thesis.

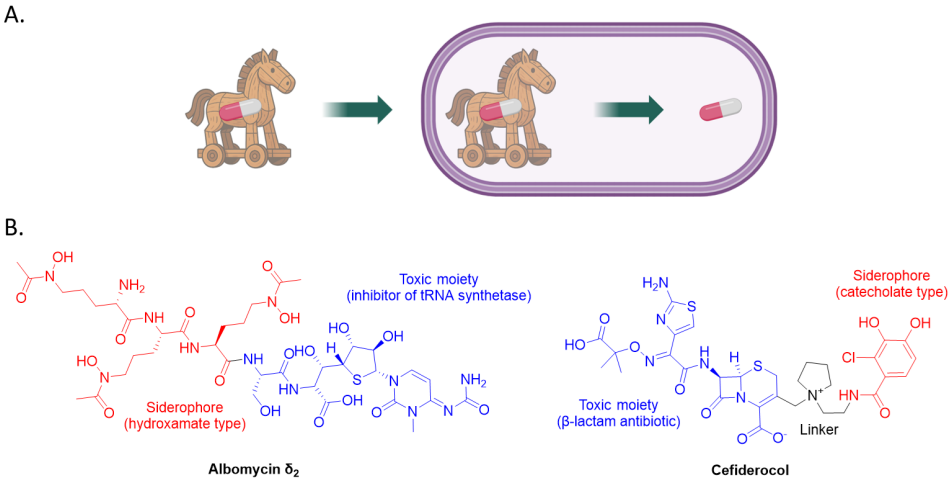

Figure 9. **A**. General idea of the "Trojan horse" strategy (generated using BioRender). **B**. Structures of the siderophore-containing natural antibiotic albomycin and the synthetically developed FDA-approved antimicrobial agent cefiderocol.[135,141]

Siderophore-antibiotic conjugates have been designed and developed as innovative antimicrobial therapeutics for decades, and with the recent success of cefiderocol, the field is gaining more traction. To date, most advanced work on such conjugates has focused on utilizing β-lactam antibiotics. However, there remains considerable potential in exploring alternative antibiotics and other generally toxic moieties as active

components. When combined with variations in linker strategies and types of siderophores, the breadth of variety embodied by this class of antimicrobial therapeutics becomes apparent, showing the infinite possibilities for designing novel antibiotics. In the future, the advancement in the development of multiple siderophore-antibiotic conjugates for clinical use may provide a critical piece of the puzzle in addressing the antimicrobial resistance crisis.

Outline of the thesis

The central theme of this thesis is the exploration of multiple approaches to address the urgent threat posed by antimicrobial resistance. This includes the discovery and synthesis of novel natural products, as well as the implementation of synthetic modifications and conjugation strategies to enhance the properties of previously identified natural product antibiotics. Through a comprehensive investigation of both natural and synthetically modified compounds, this research endeavors to contribute new insights and innovative solutions to the ongoing public health crisis posed by antimicrobial resistance.

Chapter 2 is organized into two sections, **2A** and **2B**. The first section focuses on the isolation and structural elucidation of the lipopeptides paenilipoheptin A and B, produced by *Paenibacillus* spp. Although one compound from this class, paenilipoheptin A, had been previously reported, it had never been isolated and fully characterized. Through our efforts to isolate a sufficient quantity of material for detailed NMR studies and Marfey's analysis, we successfully elucidated the structure of paenilipoheptin A, as well as reported the structure of a novel compound from the same class, paenilipoheptin B. In section **2B**, we further explored the stereochemistry of the unnatural β-amino acid and developed methods for its synthesis. With the protected amino acid in hand, we carried out the total synthesis of paenilipoheptin A, which enabled us to definitively establish its stereochemical framework and confirm the structure of this natural product. Additionally, the synthetic route developed allowed us to synthesize enough material for a comprehensive evaluation of the biological activity of paenilipoheptin A.

Chapter 3 focuses on the development of the total synthesis of evybactin, a cyclic depsipeptide natural product recognized for its selective activity against *M. tuberculosis*. This peptide has been shown to inhibit DNA gyrase, with its selectivity largely attributed to its ability to enter the cell through the unique mycobacterial transporter, BacA. During our synthetic studies, we uncovered a misassignment in the previously proposed structure. By collaborating with the Lewis group, where evybactin was initially discovered, we successfully revised the published structure. This advancement also enabled us to design a reliable synthetic route, with which we were able to produce the natural product in multi-hundred-milligram quantities.

In **Chapter 4**, we leverage our optimized synthesis of evybactin to create a series of analogues, thoroughly investigating the SAR of this promising natural product. To assess the contributions of individual amino acid residues to evybactin's antibacterial activity,

we conducted an alanine scan of the peptide. Additionally, we explored the significance of charged amino acids in greater detail and examined how modifications to the N-terminus and the cyclic scaffold impact the potency of evybactin.

Chapter 5 presents a detailed exploration of the design and synthesis of rifampicin-siderophore conjugates characterized by enhanced efficacy against Gram-negative bacteria. Rifampicin, a well-established therapeutic agent for treating tuberculosis and infections caused by Gram-positive bacteria, suffers from limited effectiveness against Gram-negative infections due to its inability to penetrate the outer membrane of these bacteria. To address this fundamental challenge of antibiotic resistance, we employed the "Trojan horse" strategy, leveraging siderophores to exploit bacterial iron transport systems as a means of delivering antibiotics intracellularly. To achieve this, we designed and synthesized various rifampicin-siderophore conjugates, focusing on the critical roles played by the linker and catechol moieties in modulating the activity of final compounds. Using the most active conjugate identified, we further investigated its mechanism of action and performed a comprehensive activity screen against an array of Gram-negative bacterial strains, including isolates exhibiting high levels of resistance to known antibiotics.

Finally, the major findings of this thesis are comprehensively summarized in **Chapter 6**, where we also discuss potential future developments and the implications of the results reported.

References

(1) Prestinaci, F.; Pezzotti, P.; Pantosti, A. Antimicrobial Resistance: A Global Multifaceted Phenomenon. *Pathog. Glob. Health* **2015**, *109* (7), 309–318.

(2) Antimicrobial Resistance Collaborators. Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *Lancet* **2022**, *399* (10325), 629−655.

(3) GBD 2021 Antimicrobial Resistance Collaborators. Global Burden of Bacterial Antimicrobial Resistance 1990–2021: A Systematic Analysis with Forecasts to 2050. *Lancet* **2024**, *404* (10459), 1199–1226.

(4) Salam, Md. A.; Al-Amin, Md. Y.; Salam, M. T.; Pawar, J. S.; Akhter, N.; Rabaan, A. A.; Alqumber, M. A. A. Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare* **2023**, *11* (13), 1946.

(5) Jian, Z.; Zeng, L.; Xu, T.; Sun, S.; Yan, S.; Yang, L.; Huang, Y.; Jia, J.; Dou, T. Antibiotic Resistance Genes in Bacteria: Occurrence, Spread, and Control. *J. Basic Microbiol.* **2021**, *61* (12), 1049–1070.

(6) Reygaert, W. C. An Overview of the Antimicrobial Resistance Mechanisms of Bacteria. *AIMS Microbiol.* **2018**, *4* (3), 482–501.

(7) Strasfeld, L.; Chou, S. Antiviral Drug Resistance: Mechanisms and Clinical Implications. *Infect. Dis. Clin. North Am* **2010**, *24* (2), 413–437. https://doi.org/10.1016/j.idc.2010.01.001.

(8) Lee, Y.; Robbins, N.; Cowen, L. E. Molecular Mechanisms Governing Antifungal Drug Resistance. *npj Antimicrob. Resist.* **2023**, *1* (1), 5.

(9) GBD 2019 Antimicrobial Resistance Collaborators. Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *Lancet* **2022**, *400* (10369), 2221–2248.

(10) Butler, M. S.; Henderson, I. R.; Capon, R. J.; Blaskovich, M. A. T. Antibiotics in the Clinical Pipeline as of December 2022. *J. Antibiot. (Tokyo)* **2023**, *76* (8), 431–473.

(11) Laxminarayan, R.; Duse, A.; Wattal, C.; Zaidi, A. K. M.; Wertheim, H. F. L.; Sumpradit, N.; Vlieghe, E.; Hara, G. L.; Gould, I. M.; Goossens, H.; Greko, C.; So, A. D.; Bigdeli, M.; Tomson, G.; Woodhouse, W.; Ombaka, E.; Peralta, A. Q.; Qamar, F. N.; Mir, F.; Kariuki, S.; Bhutta, Z. A.; Coates, A.; Bergstrom, R.; Wright, G. D.; Brown, E. D.; Cars, O. Antibiotic Resistance—the Need for Global Solutions. *Lancet Infect. Dis.* **2013**, *13* (12), 1057–1098.

(12) Gould, I. M.; David, M. Z.; Esposito, S.; Garau, J.; Lina, G.; Mazzei, T.; Peters, G. New Insights into Meticillin-Resistant Staphylococcus Aureus (MRSA) Pathogenesis, Treatment and Resistance. *Int. J. Antimicrob. Agents* **2012**, *39* (2), 96–104.

(13) Cassini, A.; Högberg, L. D.; Plachouras, D.; Quattrocchi, A.; Hoxha, A.; Simonsen, G. S.; Colomb-Cotinat, M.; Kretzschmar, M. E.; Devleesschauwer, B.; Cecchini, M.; Ouakrim, D. A.; Oliveira, T. C.; Struelens, M. J.; Suetens, C.; Monnet, D. L.; Strauss, R.; Mertens, K.; Struyf, T.; Catry, B.; Latour, K.; et al. Attributable Deaths and Disability-Adjusted Life-Years Caused by Infections with Antibiotic-Resistant Bacteria in the EU and the European Economic Area in 2015: A Population-Level Modelling Analysis. *Lancet Infect. Dis.* **2019**, *19* (1), 56–66.

(14) DiazGranados, C. A.; Zimmer, S. M.; Mitchel, K.; Jernigan, J. A. Comparison of Mortality Associated with Vancomycin-Resistant and Vancomycin-Susceptible Enterococcal Bloodstream Infections: A Meta-Analysis. *Clin. Infect. Dis.* **2005**, *41* (3), 327–333.

(15) Cetinkaya, Y.; Falk, P.; Mayhall, C. G. Vancomycin-Resistant Enterococci. *Clin. Microbiol. Rev.* **2000**, *13* (4), 686–707.

(16) McGuinness, W. A.; Malachowa, N.; DeLeo, F. R. Vancomycin Resistance in Staphylococcus Aureus. *Yale J. Biol. Med.* **2017**, *90* (2), 269–281.

(17) van Hal, S. J.; Fowler, V. G. Is It Time to Replace Vancomycin in the Treatment of Methicillin-Resistant Staphylococcus Aureus Infections? *Clin. Infect. Dis.* **2013**, *56* (12), 1779–1788.

(18) WHO Bacterial Priority Pathogens List, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: World Health Organization; **2024**. Licence: CC BY-NC-SA 3.0 IGO.

(19) Zgurskaya, H. I.; Rybenkov, V. V.; Krishnamoorthy, G.; Leus, I. V. Trans-Envelope Multidrug Efflux Pumps of Gram-Negative Bacteria and Their Synergism with the Outer Membrane Barrier. *Res. Microbiol.* **2018**, *169* (7–8), 351–356.

(20) Gauba, A.; Rahman, K. M. Evaluation of Antibiotic Resistance Mechanisms in Gram-Negative Bacteria. *Antibiotics* **2023**, *12* (11), 1590.

(21) Breijyeh, Z.; Jubeh, B.; Karaman, R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules* **2020**, *25* (6), 1340.

(22) Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2009**, *48* (1), 1–12.

(23) Global tuberculosis report 2024. Geneva: World Health Organization; **2024**. Licence: CC BY-NC-SA 3.0 IGO.

(24) Keshavjee, S.; Farmer, P. E. Tuberculosis, Drug Resistance, and the History of Modern Medicine. *N. Engl. J. Med.* **2012**, *367* (10), 931–936.

(25) Alsayed, S. S. R.; Gunosewoyo, H. Tuberculosis: Pathogenesis, Current Treatment Regimens and New Drug Targets. *Int. J. Mol. Sci.* **2023**, *24* (6), 5202.

(26) Knight, G. M.; McQuaid, C. F.; Dodd, P. J.; Houben, R. M. G. J. Global Burden of Latent Multidrug-Resistant Tuberculosis: Trends and Estimates Based on Mathematical Modelling. *Lancet Infect. Dis.* **2019**, *19* (8), 903–912.

(27) Orgeur, M.; Sous, C.; Madacki, J.; Brosch, R. Evolution and Emergence of *Mycobacterium Tuberculosis*. *FEMS Microbiol. Rev.* **2024**, *48* (2).

(28) Sacchettini, J. C.; Rubin, E. J.; Freundlich, J. S. Drugs versus Bugs: In Pursuit of the Persistent Predator *Mycobacterium Tuberculosis*. *Nat. Rev. Microbiol.* **2008**, *6* (41–52).

(29) Zumla, A.; Nahid, P.; Cole, S. T. Advances in the Development of New Tuberculosis Drugs and Treatment Regimens. *Nat. Rev. Drug Discov.* **2013**, *12*, 388–404

(30) Muteeb, G.; Rehman, M. T.; Shahwan, M.; Aatif, M. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals* **2023**, *16* (11), 1615.

(31) Fleming, A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to Their Use in the Isolation of B. Influenzae. *Clin. Infect. Dis.* **1980**, *2* (1), 129–139.

(32) Ligon, B. L. Penicillin: Its Discovery and Early Development. *Semin. Pediatr. Infect. Dis.* **2004**, *15* (1), 52–57.

(33) Gaynes, R. The Discovery of Penicillin—New Insights After More Than 75 Years of Clinical Use. *Emerg. Infect. Dis.* **2017**, *23* (5), 849–853.

(34) Bennett, J. W.; Chung, K.-T. Alexander Fleming and the Discovery of Penicillin. *Adv. Appl. Microbiol.* **2001**, *49*, 163-184.

(35) Hutchings, M. I.; Truman, A. W.; Wilkinson, B. Antibiotics: Past, Present and Future. *Curr. Opin. Microbiol.* **2019**, *51*, 72–80.

(36) Katz, L.; Baltz, R. H. Natural Product Discovery: Past, Present, and Future. *J. Ind. Microbiol. Biotechnol.* **2016**, *43* (2–3), 155–176.

(37) Gould, K. Antibiotics: From Prehistory to the Present Day. *J. Antimicrob. Chemother.* **2016**, *71* (3), 572–575.

(38) Nicolaou, K. C.; Rigol, S. A Brief History of Antibiotics and Select Advances in Their Synthesis. *J. Antibiot. (Tokyo)* **2018**, *71* (2), 153–184.

(39) Ribeiro da Cunha, B.; Fonseca, L. P.; Calado, C. R. C. Antibiotic Discovery: Where Have We Come from, Where Do We Go? *Antibiotics* **2019**, *8* (2), 45.

(40) Bernal, F. A.; Hammann, P.; Kloss, F. Natural Products in Antibiotic Development: Is the Success Story Over? *Curr. Opin. Biotechnol.* **2022**, *78*, 102783.

(41) Tally, F. P.; DeBruin, M. F. Development of Daptomycin for Gram-Positive Infections. *J. Antimicrob. Chemother.* **2000**, *46* (4), 523–526.

(42) Raja, A.; LaBonte, J.; Lebbos, J.; Kirkpatrick, P. Daptomycin. *Nat. Rev. Drug Discov.* **2003**, *2* (12), 943–944.

(43) Heidary, M.; Khosravi, A. D.; Khoshnood, S.; Nasiri, M. J.; Soleimani, S.; Goudarzi, M. Daptomycin. *J. Antimicrob. Chemother.* **2018**, *73* (1), 1–11.

(44) Grein, F.; Müller, A.; Scherer, K. M.; Liu, X.; Ludwig, K. C.; Klöckner, A.; Strach, M.; Sahl, H.-G.; Kubitscheck, U.; Schneider, T. Ca2+-Daptomycin Targets Cell Wall Biosynthesis by Forming a Tripartite Complex with Undecaprenyl-Coupled Intermediates and Membrane Lipids. *Nat. Commun.* **2020**, *11* (1), 1455.

(45) BALTZ, R. Renaissance in Antibacterial Discovery from Actinomycetes. *Curr. Opin. Pharmacol.* **2008**, *8* (5), 557–563.

(46) Lewis, K. The Science of Antibiotic Discovery. *Cell* **2020**, *181* (1), 29–45.

(47) Cooper, M. A.; Shlaes, D. Fix the Antibiotics Pipeline. *Nature* **2011**, *472* (7341), 32–32.

(48) Wipat, A.; Harwood, C. R. The Bacillus Subtilis Genome Sequence: The Molecular Blueprint of a Soil Bacterium. *FEMS Microbiol. Ecol.* **1999**, *28* (1), 1–9.

(49) Ikeda, H.; Ishikawa, J.; Hanamoto, A.; Shinose, M.; Kikuchi, H.; Shiba, T.; Sakaki, Y.; Hattori, M.; Ōmura, S. Complete Genome Sequence and Comparative Analysis of the Industrial Microorganism Streptomyces Avermitilis. *Nat. Biotechnol.* **2003**, *21* (5), 526–531.

(50) Bentley, S. D.; Chater, K. F.; Cerdeño-Tárraga, A.-M.; Challis, G. L.; Thomson, N. R.; James, K. D.; Harris, D. E.; Quail, M. A.; Kieser, H.; Harper, D.; Bateman, A.; Brown, S.; Chandra, G.; Chen, C. W.; Collins, M.; Cronin, A.; Fraser, A.; Goble, A.; Hidalgo, J.; Hornsby, T.; et al. Complete Genome Sequence of the Model Actinomycete Streptomyces Coelicolor A3(2). *Nature* **2002**, *417* (6885), 141–147.

(51) Clardy, J.; Fischbach, M. A.; Walsh, C. T. New Antibiotics from Bacterial Natural Products. *Nat. Biotechnol.* **2006**, *24* (12), 1541–1550.

(52) Blin, K.; Shaw, S.; Augustijn, H. E.; Reitz, Z. L.; Biermann, F.; Alanjary, M.; Fetter, A.; Terlouw, B. R.; Metcalf, W. W.; Helfrich, E. J. N.; van Wezel, G. P.; Medema, M. H.; Weber, T. AntiSMASH 7.0: New and Improved Predictions for Detection, Regulation, Chemical Structures and Visualisation. *Nucleic Acids Res.* **2023**, *51* (W1), W46–W50.

(53) Skinnider, M. A.; Dejong, C. A.; Rees, P. N.; Johnston, C. W.; Li, H.; Webster, A. L. H.; Wyatt, M. A.; Magarvey, N. A. Genomes to Natural Products PRediction Informatics for Secondary Metabolomes (PRISM). *Nucleic Acids Res.* **2015**, *43* (20), 9645–9662.

(54) Zdouc, M. M.; Blin, K.; Louwen, N. L. L.; Navarro, J.; Loureiro, C.; Bader, C. D.; Bailey, C. B.; Barra, L.; Booth, T. J.; Bozhüyük, K. A. J.; Cediel-Becerra, J. D. D.; Charlop-Powers, Z.; Chevrette, M. G.; Chooi, Y. H.; D'Agostino, P. M.; de Rond, T.; Del Pup, E.; Duncan, K. R.; Gu, W.; Hanif, N.; et al. MIBiG 4.0: Advancing Biosynthetic Gene Cluster Curation through Global Collaboration. *Nucleic Acids Res.* **2025**, *53* (D1), D678–D690.

(55) Navarro-Muñoz, J. C.; Selem-Mojica, N.; Mullowney, M. W.; Kautsar, S. A.; Tryon, J. H.; Parkinson, E. I.; De Los Santos, E. L. C.; Yeong, M.; Cruz-Morales, P.; Abubucker, S.; Roeters, A.; Lokhorst, W.; Fernandez-Guerra, A.; Cappelini, L. T. D.; Goering, A. W.; Thomson, R. J.; Metcalf, W. W.; Kelleher, N. L.; Barona-Gomez, F.; Medema, M. H. A Computational Framework to Explore Large-Scale Biosynthetic Diversity. *Nat. Chem. Biol.* **2020**, *16* (1), 60–68.

(56) Zhao, S.; Sakai, A.; Zhang, X.; Vetting, M. W.; Kumar, R.; Hillerich, B.; San Francisco, B.; Solbiati, J.; Steves, A.; Brown, S.; Akiva, E.; Barber, A.; Seidel, R. D.; Babbitt, P. C.; Almo, S. C.; Gerlt, J. A.; Jacobson, M. P. Prediction and Characterization of Enzymatic Activities Guided by Sequence Similarity and Genome Neighborhood Networks. *Elife* **2014**, *3*, e03275.

(57) Kalkreuter, E.; Pan, G.; Cepeda, A. J.; Shen, B. Targeting Bacterial Genomes for Natural Product Discovery. *Trends Pharmacol. Sci.* **2020**, *41* (1), 13–26.

(58) Foulston, L. Genome Mining and Prospects for Antibiotic Discovery. *Curr. Opin. Microbiol.* **2019**, *51*, 1–8.

(59) Culp, E. J.; Waglechner, N.; Wang, W.; Fiebig-Comyn, A. A.; Hsu, Y.-P.; Koteva, K.; Sychantha, D.; Coombes, B. K.; Van Nieuwenhze, M. S.; Brun, Y. V.; Wright, G. D. Evolution-Guided Discovery of Antibiotics That Inhibit Peptidoglycan Remodelling. *Nature* **2020**, *578* (7796), 582–587.

(60) Li, Y.-X.; Zhong, Z.; Zhang, W.-P.; Qian, P.-Y. Discovery of Cationic Nonribosomal Peptides as Gram-Negative Antibiotics through Global Genome Mining. *Nat. Commun.* **2018**, *9* (1), 3273.

(61) Hover, B. M.; Kim, S.-H.; Katz, M.; Charlop-Powers, Z.; Owen, J. G.; Ternei, M. A.; Maniko, J.; Estrela, A. B.; Molina, H.; Park, S.; Perlin, D. S.; Brady, S. F. Culture-Independent Discovery of the Malacidins as Calcium-Dependent Antibiotics with Activity against Multidrug-Resistant Gram-Positive Pathogens. *Nat. Microbiol.* **2018**, *3* (4), 415–422.

(62) Wang, Z.; Koirala, B.; Hernandez, Y.; Zimmerman, M.; Park, S.; Perlin, D. S.; Brady, S. F. A Naturally Inspired Antibiotic to Target Multidrug-Resistant Pathogens. *Nature* **2022**, *601* (7894), 606–611.

(63) Wang, Z.; Koirala, B.; Hernandez, Y.; Zimmerman, M.; Brady, S. F. Bioinformatic Prospecting and Synthesis of a Bifunctional Lipopeptide Antibiotic That Evades Resistance. *Science* **2022**, *376* (6596), 991–996. https://doi.org/10.1126/science.abn4213.

(64) Chu, J.; Vila-Farres, X.; Inoyama, D.; Ternei, M.; Cohen, L. J.; Gordon, E. A.; Reddy, B. V. B.; Charlop-Powers, Z.; Zebroski, H. A.; Gallardo-Macias, R.; Jaskowski, M.; Satish, S.; Park, S.; Perlin, D. S.; Freundlich, J. S.; Brady, S. F. Discovery of MRSA Active Antibiotics Using Primary Sequence from the Human Microbiome. *Nat. Chem. Biol.* **2016**, *12* (12), 1004–1006.

(65) Liu, Y.; Ding, S.; Shen, J.; Zhu, K. Nonribosomal Antibacterial Peptides That Target Multidrug-Resistant Bacteria. *Nat. Prod. Rep.* **2019**, *36* (4), 573–592.

(66) Singh, H.; Kaur, M.; Jangra, M.; Mishra, S.; Nandanwar, H.; Pinnaka, A. K. Antimicrobial Properties of the Novel Bacterial Isolate *Paenibacilllus* Sp. SMB1 from a Halo-Alkaline Lake in India. *Sci. Rep.* **2019**, *9* (1), 11561.

(67) Machushynets, N. V.; Al Ayed, K.; Terlouw, B. R.; Du, C.; Buijs, N. P.; Willemse, J.; Elsayed, S. S.; Schill, J.; Trebosc, V.; Pieren, M.; Alexander, F. M.; Cochrane, S. A.; Liles, M. R.; Medema, M. H.; Martin, N. I.; van Wezel, G. P. Discovery and Derivatization of Tridecaptin Antibiotics with Altered Host Specificity and Enhanced Bioactivity. *ACS Chem. Biol.* **2024**, *19* (5), 1106–1115.

(68) Olishevska, S.; Nickzad, A.; Déziel, E. *Bacillus* and *Paenibacillus* Secreted Polyketides and Peptides Involved in Controlling Human and Plant Pathogens. *Appl. Microbiol. Biotechnol.* **2019**, *103* (3), 1189–1215.

(69) Cochrane, S. A.; Vederas, J. C. Lipopeptides from Bacillus and Paenibacillus Spp.: A Gold Mine of Antibiotic Candidates. *Med. Res. Rev.* **2016**, *36* (1), 4–31.

(70) Wang, X.; Deng, Z.; Gao, J. Exploring the Antibiotic Potential of Cultured 'Unculturable' Bacteria. *Trends Microbiol.* **2024**, *32* (2), 124–127.

(71) Lewis, K.; Lee, R. E.; Brötz-Oesterhelt, H.; Hiller, S.; Rodnina, M. V.; Schneider, T.; Weingarth, M.; Wohlgemuth, I. Sophisticated Natural Products as Antibiotics. *Nature* **2024**, *632* (8023), 39–49.

(72) Lewis, K. Platforms for Antibiotic Discovery. *Nat. Rev. Drug Discov.* **2013**, *12* (5), 371–387.

(73) Fenn, K.; Strandwitz, P.; Stewart, E. J.; Dimise, E.; Rubin, S.; Gurubacharya, S.; Clardy, J.; Lewis, K. Quinones Are Growth Factors for the Human Gut Microbiota. *Microbiome* **2017**, *5* (1), 161.

(74) D'Onofrio, A.; Crawford, J. M.; Stewart, E. J.; Witt, K.; Gavrish, E.; Epstein, S.; Clardy, J.; Lewis, K. Siderophores from Neighboring Organisms Promote the Growth of Uncultured Bacteria. *Chem. Biol.* **2010**, *17* (3), 254–264.

(75) Shukla, R.; Peoples, A. J.; Ludwig, K. C.; Maity, S.; Derks, M. G. N.; De Benedetti, S.; Krueger, A. M.; Vermeulen, B. J. A.; Harbig, T.; Lavore, F.; Kumar, R.; Honorato, R. V.; Grein, F.; Nieselt, K.; Liu, Y.; Bonvin, A. M. J. J.; Baldus, M.; Kubitscheck, U.; Breukink, E.; Achorn, C.; Nitti, A.; Schwalen, C. J.; Spoering, A. L.; Ling, L. L.; Hughes, D.; Lelli, M.; Roos, W. H.; Lewis, K.; Schneider, T.; Weingarth, M. An Antibiotic from an Uncultured Bacterium Binds to an Immutable Target. *Cell* **2023**, *186* (19), 4059-4073.e27.

(76) Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schäberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K. A New Antibiotic Kills Pathogens without Detectable Resistance. *Nature* **2015**, *517* (7535), 455–459.

(77) Imai, Y.; Meyer, K. J.; Iinishi, A.; Favre-Godal, Q.; Green, R.; Manuse, S.; Caboni, M.; Mori, M.; Niles, S.; Ghiglieri, M.; Honrao, C.; Ma, X.; Guo, J. J.; Makriyannis, A.; Linares-Otoya, L.; Böhringer, N.; Wuisan, Z. G.; Kaur, H.; Wu, R.; Mateus, A.; Typas, A.; Savitski, M. M.; Espinoza, J. L.; O'Rourke, A.; Nelson, K. E.; Hiller, S.; Noinaj, N.; Schäberle, T. F.; D'Onofrio, A.; Lewis, K. A New Antibiotic Selectively Kills Gram-Negative Pathogens. *Nature* **2019**, *576* (7787), 459–464.

(78) Imai, Y.; Hauk, G.; Quigley, J.; Liang, L.; Son, S.; Ghiglieri, M.; Gates, M. F.; Morrissette, M.; Shahsavari, N.; Niles, S.; Baldisseri, D.; Honrao, C.; Ma, X.; Guo, J. J.; Berger, J. M.; Lewis, K. Evybactin Is a DNA Gyrase Inhibitor That Selectively Kills Mycobacterium Tuberculosis. *Nat. Chem. Biol.* **2022**, *18* (11), 1236–1244.

(79) Nicolaou, K. C.; Snyder, S. A. The Essence of Total Synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (33), 11929–11936.

(80) Baran, P. S. Natural Product Total Synthesis: As Exciting as Ever and Here To Stay. *J. Am. Chem. Soc.* **2018**, *140* (14), 4751–4755.

(81) Nicolaou, K. C.; Rigol, S. A Brief History of Antibiotics and Select Advances in Their Synthesis. *J Antibiot (Tokyo)* **2018**, *71* (2), 153–184. https://doi.org/10.1038/ja.2017.62.

(82) Paul, D.; Kundu, A.; Saha, S.; Goswami, R. K. Total Synthesis: The Structural Confirmation of Natural Products. *Chem. Commun.* **2021**, *57* (27), 3307–3322.

(83) Maier, M. E. Structural Revisions of Natural Products by Total Synthesis. *Nat. Prod. Rep.* **2009**, *26* (9), 1105.

(84) Nicolaou, K. C.; Snyder, S. A. Chasing Molecules That Were Never There: Misassigned Natural Products and the Role of Chemical Synthesis in Modern Structure Elucidation. *Ang. Chem. Int. Ed.* **2005**, *44* (7), 1012–1044.

(85) Yamamoto, M.; Ishigami, K.; Watanabe, H. First Total Synthesis of Glabramycin B and Revision of Its Relative Configuration. *Tetrahedron* **2017**, *73* (23), 3271–3280.

(86) Koteva, K.; King, A. M.; Capretta, A.; Wright, G. D. Total Synthesis and Activity of the Metallo-β-lactamase Inhibitor Aspergillomarasmine A. *Ang. Chem. Int. Ed.* **2016**, *55* (6), 2210–2212.

(87) Liao, D.; Yang, S.; Wang, J.; Zhang, J.; Hong, B.; Wu, F.; Lei, X. Total Synthesis and Structural Reassignment of Aspergillomarasmine A. *Ang. Chem. Int. Ed.* **2016**, *55* (13), 4291–4295.

(88) Kita, M.; Miwa, R.; Widianti, T.; Ozaki, Y.; Aoyama, S.; Yamada, K.; Uemura, D. Revised Structure of Kasarin, an Antibacterial Pyrazinone Compound from the Marine Microorganism Hyphomycetes Sp. *Tetrahedron Lett.* **2007**, *48* (49), 8628–8631.

(89) Sheehan, J. C.; Henery-Logan, K. R. The Total Synthesis of Penicillin V. *J. Am. Chem. Soc.* **1959**, *81* (12), 3089–3094.

(90) Geddes, A. M.; Klugman, K. P.; Rolinson, G. N. Introduction: Historical Perspective and Development of Amoxicillin/Clavulanate. *Int. J. Antimicrob. Agents* **2007**, *30*, 109–112.

(91) Brotzu, G.: Richerche su di un nuovo antibiotico. *Lavori dell'Istituto d'Igiene di Cagliari* **1948**.

(92) Newton, G. G. F.; Abraham, E. P. Cephalosporin C, a New Antibiotic Containing Sulphur and D-α-Aminoadipic Acid. *Nature* **1955**, *175* (4456), 548–548.

(93) Woodward, R. B.; Heusler, K.; Gosteli, J.; Naegeli, P.; Oppolzer, W.; Ramage, R.; Ranganathan, S.; Vorbrüggen, H. The Total Synthesis of Cephalosporin C [1]. *J. Am. Chem. Soc.* **1966**, *88* (4), 852–853.

(94) Singh, J.; Arrieta, A. C. New Cephalosporins. *Semin. Pediatr. Infect. Dis.* **1999**, *10* (1), 14–22.

(95) Abraham, E. P. Cephalosporins 1945-1986. *Drugs* **1987**, *34* (Supplement 2), 1–14.

(96) Loder, B.; Newton, G. G. F.; Abraham, E. P. The Cephalosporin C Nucleus (7-Aminocephalosporanic Acid) and Some of Its Derivatives. *Biochem. J.* **1961**, *79* (2), 408–416.

(97) Gröger, H.; Pieper, M.; König, B.; Bayer, T.; Schleich, H. Industrial Landmarks in the Development of Sustainable Production Processes for the β-Lactam Antibiotic Key Intermediate 7-Aminocephalosporanic Acid (7-ACA). *Sustain. Chem. Pharm.* **2017**, *5*, 72–79.

(98) Lin, X.; Kück, U. Cephalosporins as Key Lead Generation Beta-Lactam Antibiotics. *Appl. Microbiol. Biotechnol.* **2022**, *106* (24), 8007–8020.

(99) Godzeski, C. W.; Brier, G.; Pavey, D. E. Cephalothin, a New Cephalosporin with a Broad Antibacterial Spectrum. *Appl. Microbiol.* **1963**, *11* (2), 122–127.

(100) Grobbelaar, M.; Louw, G. E.; Sampson, S. L.; van Helden, P. D.; Donald, P. R.; Warren, R. M. Evolution of Rifampicin Treatment for Tuberculosis. *Infect. Genet. Evol.* **2019**, *74*, 103937.

(101) Lester, W. Rifampin: A Semisynthetic Derivative of Rifamycin-A Prototype for the Future. *Annu. Rev. Microbiol.* **1972**, *26* (1), 85–102.

(102) Sensi, P.; Margalith, P.; Timbal, M. T. Rifomycin, a New Antibiotic; Preliminary Report. *Farmaco Sci.* **1959**, *14* (2), 146–147.

(103) Sensi, P. History of the Development of Rifampin. *Clin. Infect. Dis.* **1983**, *5* (Supplement_3), S402–S406.

(104) Calvori, C.; Frontali, L.; Leoni, L.; Tecce, G. Effect of Rifamycin on Protein Synthesis. *Nature* **1965**, *207* (4995), 417–418.

(105) Campbell, E. A.; Korzheva, N.; Mustaev, A.; Murakami, K.; Nair, S.; Goldfarb, A.; Darst, S. A. Structural Mechanism for Rifampicin Inhibition of Bacterial RNA Polymerase. *Cell* **2001**, *104* (6), 901–912.

(106) Maggi, N.; Pasqualucci, C. R.; Ballotta, R.; Sensi, P. Rifampicin: A New Orally Active Rifamycin. *Chemotherapy* **1966**, *11* (5), 285–292.

(107) Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. Total Syntheses of Vancomycin and Eremomycin Aglycons. *Ang. Chem. Int. Ed.* **1998**, *37* (19), 2700–2704.

(108) Nicolaou, K. C.; Natarajan, S.; Li, H.; Jain, N. F.; Hughes, R.; Solomon, M. E.; Ramanjulu, J. M.; Boddy, C. N. C.; Takayanagi, M. Total Synthesis of Vancomycin Aglycon—Part 1: Synthesis of Amino Acids 4-7 and Construction of the AB-COD Ring Skeleton. *Ang. Chem. Int. Ed.* **1998**, *37* (19), 2708–2714.

(109) Nicolaou, K. C.; Jain, N. F.; Natarajan, S.; Hughes, R.; Solomon, M. E.; Li, H.; Ramanjulu, J. M.; Takayanagi, M.; Koumbis, A. E.; Bando, T. Total Synthesis of Vancomycin Aglycon-Part 2: Synthesis of Amino Acids 1-3 and Construction of the AB-COD-DOE Ring Skeleton. *Ang. Chem. Int. Ed.* **1998**, *37* (19), 2714–2716.

(110) Nicolaou, K. C.; Takayanagi, M.; Jain, N. F.; Natarajan, S.; Koumbis, A. E.; Bando, T.; Ramanjulu, J. M. Total Synthesis of Vancomycin Aglycon—Part 3: Final Stages. *Ang. Chem. Int. Ed.* **1998**, *37* (19), 2717–2719.

(111) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Winssinger, N.; Hughes, R.; Bando, T. Total Synthesis of Vancomycin. *Ang. Chem. Int. Ed.* **1999**, *38* (1–2), 240–244.

(112) Blaskovich, M. A. T.; Hansford, K. A.; Butler, M. S.; Jia, Z.; Mark, A. E.; Cooper, M. A. Developments in Glycopeptide Antibiotics. *ACS Infect. Dis.* **2018**, *4* (5), 715–735.

(113) van Groesen, E.; Mons, E.; Kotsogianni, I.; Arts, M.; Tehrani, K. H. M. E.; Wade, N.; Lysenko, V.; Stel, F. M.; Zwerus, J. T.; De Benedetti, S.; Bakker, A.; Chakraborty, P.; van der Stelt, M.; Scheffers, D.-J.; Gooskens, J.; Smits, W.

K.; Holden, K.; Gilmour, P. S.; Willemse, J.; Hitchcock, C. A.; van Hasselt, J. G. C.; Schneider, T.; Martin, N. I. Semisynthetic Guanidino Lipoglycopeptides with Potent in Vitro and in Vivo Antibacterial Activity. *Sci. Transl. Med.* **2024**, *16* (759).

(114) Lee, J.; Griffin, J. H.; Nicas, T. I. Solid-Phase Total Synthesis of Bacitracin A. *J. Org. Chem.* **1996**, *61* (12), 3983–3986.

(115) Buijs, N.; Vlaming, H. C.; van Haren, M. J.; Martin, N. I. Synthetic Studies with Bacitracin A and Preparation of Analogues Containing Alternative Zinc Binding Groups. *ChemBioChem* **2022**, *23* (24).

(116) Buijs, N. P.; Vlaming, H. C.; Kotsogianni, I.; Arts, M.; Willemse, J.; Duan, Y.; Alexander, F. M.; Cochrane, S. A.; Schneider, T.; Martin, N. I. A Classic Antibiotic Reimagined: Rationally Designed Bacitracin Variants Exhibit Potent Activity against Vancomycin-Resistant Pathogens. *Proc. Natl. Acad. Sci. U. S. A.* **2024**, *121* (29).

(117) Al Ayed, K.; Ballantine, R. D.; Hoekstra, M.; Bann, S. J.; Wesseling, C. M. J.; Bakker, A. T.; Zhong, Z.; Li, Y.-X.; Brüchle, N. C.; van der Stelt, M.; Cochrane, S. A.; Martin, N. I. Synthetic Studies with the Brevicidine and Laterocidine Lipopeptide Antibiotics Including Analogues with Enhanced Properties and *in Vivo* Efficacy. *Chem. Sci.* **2022**, *13* (12), 3563–3570.

(118) Ballantine, R. D.; Al Ayed, K.; Bann, S. J.; Hoekstra, M.; Martin, N. I.; Cochrane, S. A. Synthesis and Structure–Activity Relationship Studies of N-Terminal Analogues of the Lipopeptide Antibiotics Brevicidine and Laterocidine. *RSC Med. Chem.* **2022**, *13* (12), 1640–1643.

(119) Ballantine, R. D.; Al Ayed, K.; Bann, S. J.; Hoekstra, M.; Martin, N. I.; Cochrane, S. A. Linearization of the Brevicidine and Laterocidine Lipopeptides Yields Analogues That Retain Full Antibacterial Activity. *J. Med. Chem.* **2023**, *66* (8), 6002–6009.

(120) Al Ayed, K.; Zamarbide Losada, D.; Machushynets, N. V.; Terlouw, B.; Elsayed, S. S.; Schill, J.; Trebosc, V.; Pieren, M.; Medema, M. H.; van Wezel, G. P.; Martin, N. I. Total Synthesis and Structure Assignment of the Relacidine Lipopeptide Antibiotics and Preparation of Analogues with Enhanced Stability. *ACS Infect. Dis.* **2023**, *9* (4), 739–748.

(121) Slingerland, C. J.; Wesseling, C. M. J.; Innocenti, P.; Westphal, K. G. C.; Masereeuw, R.; Martin, N. I. Synthesis and Evaluation of Polymyxins Bearing Reductively Labile Disulfide-Linked Lipids. *J. Med. Chem.* **2022**, *65* (23), 15878–15892.

(122) Slingerland, C. J.; Lysenko, V.; Chaudhuri, S.; Wesseling, C. M. J.; Barnes, D.; Masereeuw, R.; Martin, N. I. Semisynthetic Polymyxins with Potent Antibacterial Activity and Reduced Kidney Cell Toxicity. *RSC Med. Chem.* **2023**, *14* (11), 2417–2425.

(123) Silhavy, T. J.; Kahne, D.; Walker, S. The Bacterial Cell Envelope. *Cold Spring Harb. Perspect. Biol.* **2010**, *2* (5), a000414–a000414.

(124) Rajagopal, M.; Walker, S. Envelope Structures of Gram-Positive Bacteria. *Curr. Top. Microbiol. Immunol.* **2017**, *404*, 1–44

(125) Combo, S.; Mendes, S.; Nielsen, K. M.; da Silva, G. J.; Domingues, S. The Discovery of the Role of Outer Membrane Vesicles against Bacteria. *Biomedicines* **2022**, *10* (10), 2399.

(126) Murdoch, C. C.; Skaar, E. P. Nutritional Immunity: The Battle for Nutrient Metals at the Host–Pathogen Interface. *Nat. Rev. Microbiol.* **2022**, *20* (11), 657–670.

(127) Schalk, I. J. Siderophore–Antibiotic Conjugates: Exploiting Iron Uptake to Deliver Drugs into Bacteria. *Clin. Microbiol. Infect.* **2018**, *24* (8), 801–802.

(128) Rayner, B.; Verderosa, A. D.; Ferro, V.; Blaskovich, M. A. T. Siderophore Conjugates to Combat Antibiotic-Resistant Bacteria. *RSC Med. Chem.* **2023**, *14* (5), 800–822.

(129) Górska, A.; Sloderbach, A.; Marszałł, M. P. Siderophore–Drug Complexes: Potential Medicinal Applications of the 'Trojan Horse' Strategy. *Trends Pharmacol. Sci.* **2014**, *35* (9), 442–449.

(130) Möllmann, U.; Heinisch, L.; Bauernfeind, A.; Köhler, T.; Ankel-Fuchs, D. Siderophores as Drug Delivery Agents: Application of the "Trojan Horse" Strategy. *BioMetals* **2009**, *22* (4), 615–624.

(131) Miethke, M.; Marahiel, M. A. Siderophore-Based Iron Acquisition and Pathogen Control. *Microbiol. Mol. Biol. Rev.* **2007**, *71* (3), 413–451.

(132) Wilson, B. R.; Bogdan, A. R.; Miyazawa, M.; Hashimoto, K.; Tsuji, Y. Siderophores in Iron Metabolism: From Mechanism to Therapy Potential. *Trends Mol. Med.* **2016**, *22* (12), 1077–1090.

(133) Schalk, I. J. Bacterial Siderophores: Diversity, Uptake Pathways and Applications. *Nat. Rev. Microbiol.* **2025**, *23* (1), 24–40.

(134) Khasheii, B.; Mahmoodi, P.; Mohammadzadeh, A. Siderophores: Importance in Bacterial Pathogenesis and Applications in Medicine and Industry. *Microbiol. Res.* **2021**, *250*, 126790.

(135) Braun, V.; Pramanik, A.; Gwinner, T.; Köberle, M.; Bohn, E. Sideromycins: Tools and Antibiotics. *BioMetals* **2009**, *22* (1), 3.

(136) Lin, Z.; Xu, X.; Zhao, S.; Yang, X.; Guo, J.; Zhang, Q.; Jing, C.; Chen, S.; He, Y. Total Synthesis and Antimicrobial Evaluation of Natural Albomycins against Clinical Pathogens. *Nat. Commun.* **2018**, *9* (1), 3445.

(137) Stapley, E. O.; Ormond, R. E. Similarity of Albomycin and Grisein. *Science* **1957**, *125* (3248), 587–589.

(138) Gause, G. F. Recent Studies on Albomycin, a New Antibiotic. *BMJ* **1955**, *2* (4949), 1177–1179.

(139) Travin, D. Y.; Severinov, K.; Dubiley, S. Natural Trojan Horse Inhibitors of Aminoacyl-TRNA Synthetases. *RSC Chem. Biol.* **2021**, *2* (2), 468–485.

(140) Tillotson, G. S. Trojan Horse Antibiotics-A Novel Way to Circumvent Gram-Negative Bacterial Resistance? *Infect. Dis.* **2016**, *9*, 45–52.

(141) Aoki, T.; Yoshizawa, H.; Yamawaki, K.; Yokoo, K.; Sato, J.; Hisakawa, S.; Hasegawa, Y.; Kusano, H.; Sano, M.; Sugimoto, H.; Nishitani, Y.; Sato, T.; Tsuji, M.; Nakamura, R.; Nishikawa, T.; Yamano, Y. Cefiderocol (S-649266), A New Siderophore Cephalosporin Exhibiting Potent Activities against Pseudomonas Aeruginosa and Other Gram-Negative Pathogens Including Multi-Drug Resistant Bacteria: Structure Activity Relationship. *Eur. J. Med. Chem.* **2018**, *155*, 847–868.

(142) Zhanel, G. G.; Golden, A. R.; Zelenitsky, S.; Wiebe, K.; Lawrence, C. K.; Adam, H. J.; Idowu, T.; Domalaon, R.; Schweizer, F.; Zhanel, M. A.; Lagacé-Wiens, P. R. S.; Walkty, A. J.; Noreddin, A.; Lynch III, J. P.; Karlowsky, J. A. Cefiderocol: A Siderophore Cephalosporin with Activity Against Carbapenem-Resistant and Multidrug-Resistant Gram-Negative Bacilli. *Drugs* **2019**, *79* (3), 271–289.

(143) Sato, T.; Yamawaki, K. Cefiderocol: Discovery, Chemistry, and In Vivo Profiles of a Novel Siderophore Cephalosporin. *Clin. Infect. Dis.* **2019**, *69* (Supplement_7), S538–S543.

(144) James, C. E.; Mahendran, K. R.; Molitor, A.; Bolla, J.-M.; Bessonov, A. N.; Winterhalter, M.; Pagès, J.-M. How β-Lactam Antibiotics Enter Bacteria: A Dialogue with the Porins. *PLoS One* **2009**, *4* (5), e5453.

(145) El Ghali, A.; Kunz Coyne, A. J.; Lucas, K.; Tieman, M.; Xhemali, X.; Lau, S.; Iturralde, G.; Purdy, A.; Holger, D. J.; Garcia, E.; Veve, M. P.; Rybak, M. J. Cefiderocol: Early Clinical Experience for Multi-Drug Resistant Gram-Negative Infections. *Microbiol. Spectr.* **2024**, *12* (2).

(146) Karruli, A.; Massa, A.; Andini, R.; Marrazzo, T.; Ruocco, G.; Zampino, R.; Durante-Mangoni, E. Clinical Efficacy and Safety of Cefiderocol for Resistant Gram-Negative Infections: A Real-Life, Single-Centre Experience. *Int. J. Antimicrob. Agents* **2023**, *61* (2), 106723.

(147) Kohira, N.; West, J.; Ito, A.; Ito-Horiyama, T.; Nakamura, R.; Sato, T.; Rittenhouse, S.; Tsuji, M.; Yamano, Y. *In Vitro* Antimicrobial Activity of a Siderophore Cephalosporin, S-649266, against Enterobacteriaceae Clinical Isolates, Including Carbapenem-Resistant Strains. *Antimicrob. Agents Chemother.* **2016**, *60* (2), 729–734.