Antibiotics

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Antibacterial Natural Products in Medicinal Chemistry—Exodus or Revival?

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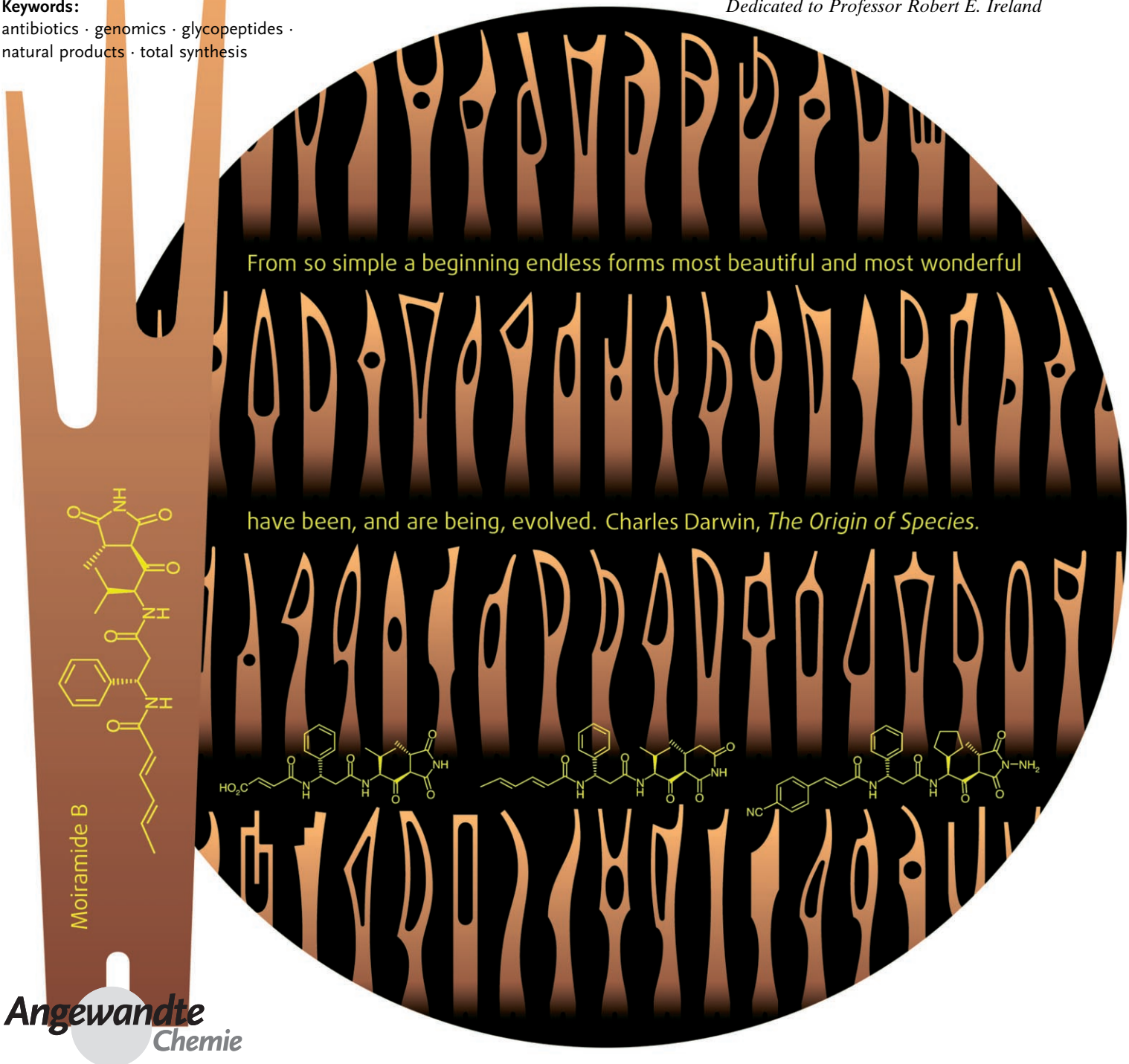
Keywords:
antibiotics · genomics · glycopeptides · natural products · total synthesis

Dedicated to Professor Robert E. Ireland



To create a drug, nature's blueprints often have to be improved through semisynthesis or total synthesis (chemical postevolution). Selected contributions from industrial and academic groups highlight the arduous but rewarding path from natural products to drugs. Principle modification types for natural products are discussed herein, such as decoration, substitution, and degradation. The biological, chemical, and socioeconomic environments of antibacterial research are dealt with in context. Natural products, many from soil organisms, have provided the majority of lead structures for marketed anti-infectives. Surprisingly, numerous "old" classes of antibacterial natural products have never been intensively explored by medicinal chemists. Nevertheless, research on antibacterial natural products is flagging. Apparently, the "old fashioned" natural products no longer fit into modern drug discovery. The handling of natural products is cumbersome, requiring nonstandardized workflows and extended timelines. Revisiting natural products with modern chemistry and target-finding tools from biology (reversed genomics) is one option for their revival.

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1. Introduction

All industrialized societies rely on the pharmaceutical industry's ability to address their inherent medical needs. With infectious diseases, this trust is nourished by the ready supply of a plethora of marketed antibiotics for the immediate treatment of routine or life-threatening bacterial infections.[1] Indeed, the research and development of antibacterial agents during the last century has been a chronicle of success, whereby all parties thrived. Since the introduction of the first sulfonamides and penicillins in 1935 and 1940 (Figure 1), the once marked mortality rate associated with bacterial infec-

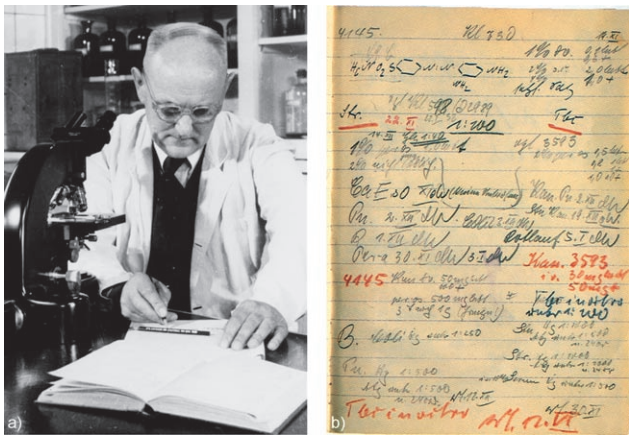

Figure 1. a) Nobel laureate, pioneer of antibacterial therapy, and inventor of the sulfonamides, Gerhard Domagk (1895–1964), with his most important working tool. Under the microscope he studied the effect of different chemicals on bacteria. b) Page from Domagk's laboratory notebook describing the exceptional activity of azo dye D 4145 on streptococci. D 4145, launched in 1935 as "prontosil", was a diazo prodrug of the active component 4-aminophenylsulfonamide that was marketed in 1936 under the name "prontalbin". At the outset, antibacterial chemotherapy stemmed from synthetic dyes rather than natural products. Essential principles of chemotherapy have been worked out with the sulfonamides.

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tions experienced a remarkable downturn.[2] Antibiotics[3] have saved millions of lives and eased patients' suffering.

Many of the antibacterial agents were natural products or potent semisynthetic variations thereof (Table 1).[4–7] Improved subclasses followed, such as the cephalosporins and carbapenems or, recently, the ketolides and glycylcyclines. As a whole, they have served as structural scaffolds for extensive medicinal-chemistry programs in virtually every major pharmaceutical company.

By the early 1970's, the existing therapies were seen as adequate and the need for new antibiotics began to be questioned.[8] Waning public interest and health measures as well as—with a time lag—declining industrial support for antibacterial research were the consequences of that fallacious consensus. After an innovation gap of several decades, an oxazolidinone (linezolid)[9] and a lipopeptide (daptomycin)[10,11] were the first truly new chemical entities (NCEs) to be launched.

Today, infectious diseases are the second major cause of death worldwide and the third leading cause of death in developed countries (Table 2).[12] In the US, bacteria are the most common cause of infection-related death.[13] Antibiotics are no longer effective in all cases, and treatment options for certain microorganisms have become increasingly scarce.[14] Former last-resort drugs have become the first-line therapy. "Resistance threatens to turn back the clock" and is rapidly

From left to right: B. Hinzen, F. von Nussbaum, D. Häbich, M. Brands, S. Weigand (Photograph P. Voits).

Franz von Nussbaum, born in Frankfurt/Main, studied chemistry at the University of Munich, where he received his PhD in the research group of Prof. W. Steglich (1998). After a postdoctoral Feodor-Lynen fellowship with Prof. S. D. Danishefsky at Columbia University (1999–2000), he joined Central Research at Bayer AG, Leverkusen. Since 2002 he has been working as a medicinal chemist for Bayer HealthCare AG in Wuppertal. The optimization of bioactive natural products is a central theme of his research interest.

Michael Brands, born in Duisburg in 1966, studied chemistry at the Universities of Münster and Bochum/MPI für Kohlenforschung, where he received his PhD in 1993 in the group of Prof. H. Butenschön. After a postdoctoral stay in the group of Prof. W. Oppolzer at the University of Geneva (1993–1995), he joined the pharmaceutical division of Bayer AG as a medicinal chemist. Since April 2006 he has been a Director of Medicinal Chemistry, responsible for the chemical drug-discovery activities in the therapeutic areas of diabetes and heart failure.

Berthold Hinzen was born in Cologne, Germany, in 1967. He studied Chemistry in Basel and obtained his PhD with Prof. F. Diederich at the ETH in Zürich, Switzerland (1996). After a postdoctoral fellowship in Prof. Steven Ley's group in Cambridge, UK, he joined Bayer AG in Wuppertal in 1998 as a medicinal chemist. Since 2004 he has been a Director of Medicinal Chemistry, responsible for the chemical drug-discovery activities in the therapeutic area thrombosis.

Stefan Weigand, born in 1969, studied chemistry at the Universities of Würzburg and Göttingen, where he received his PhD in 1997 in the group of Prof. R. Brückner. After a postdoctoral Feodor-Lynen fellowship under the guidance of Prof. B. M. Trost at Stanford University (1997–1998), he joined the pharmaceutical division of Bayer AG as a medicinal chemist. Since May 2005 he has been working as a group leader in cancer research at Roche Diagnostics GmbH in Penzberg.

Dieter Häbich, born in Stuttgart, studied chemistry at the University of Stuttgart, where he received his PhD in the group of Prof. F. X. Effenberger in 1977. After a postdoctoral NATO-research fellowship with Prof. R. E. Ireland at California Institute of Technology in Pasadena, USA (1979–1980), he joined Bayer AG, Wuppertal as a medicinal chemist. In 1991 he became a Director of Medicinal Chemistry and has been responsible for chemical anti-infective research in the areas of antibacterials, antimycotics, and antivirals.

Table 1: Introduction of new antibacterial classes for human therapy.

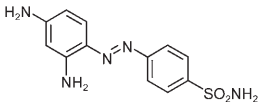
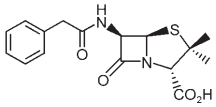
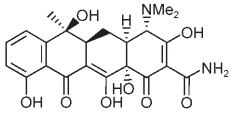
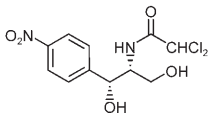
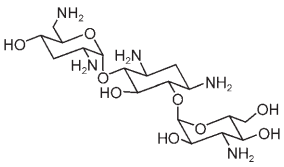
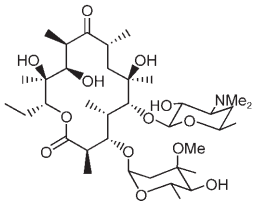
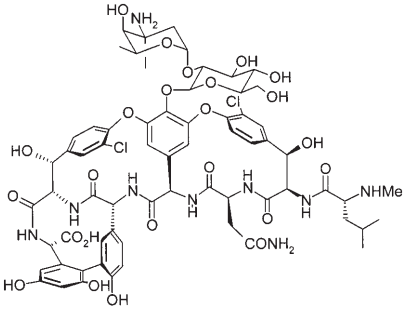
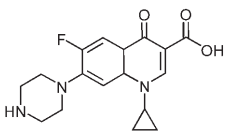
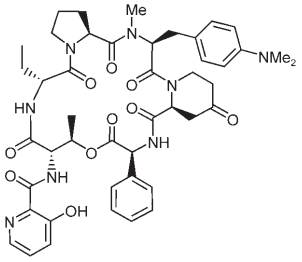
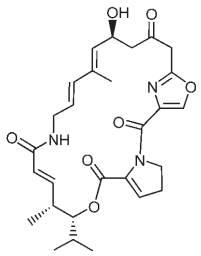
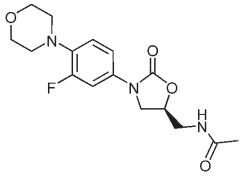
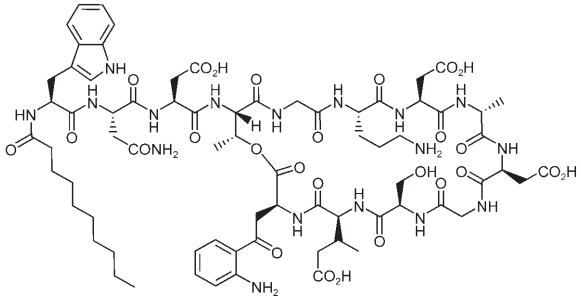
Year	Class	Target	Example	Structure
1935	sulfonamides (synthetic)	folate pathway	prontosil	 **1**
1940	β-lactams	cell wall	penicillin G	 **2**
1949	polyketides	protein biosynthesis	tetracycline	 **3**
1949	phenylpropanoids	protein biosynthesis	chloramphenicol	 **4**
1950	aminoglycosides	protein biosynthesis	tobramycin	 **5**
1952	macrolides	protein biosynthesis	erythromycin A	 **6**
1958	glycopeptides	cell wall	vancomycin	 **7**
1962	quinolones (synthetic)	DNA replication	ciprofloxacin	 **8**

Table 1: (Continued)

Year	Class	Target	Example	Structure
1962	streptogramins	protein biosynthesis	pristinamycin ($I_A + II_A$)	 **9**  **10**
...				
2000	oxazolidinones (synthetic)	protein biosynthesis	linezolid	 **11**
2003	lipopeptides	bacterial membrane	daptomycin	 **12**

spreading, particularly in hospitals, where antibiotics are heavily used.[15] The ability of bacteria to evade any form of established therapy has become apparent and pathogens resistant to one or more antibiotics are emerging and spreading worldwide.[16] Development of resistance is inevitably the result of antibiotic use and ultimately limits the efficacy and life span of every antibiotic. Although correct antibiotic use will slow down resistance, it will not prevent it: only the persistent discovery and development of new drugs can keep this problem in check. Nonetheless, more and more pharmaceutical companies are curtailing or exiting the antibacterial field and the pipeline for new antibiotics is running dry. "Bad Bugs, No Drugs", the call for action by the Infectious Disease Society of America (IDSA), places this desperate situation in the limelight.[17] The evolving crisis[18] results from a combination of scientific, economic, and regulatory causes. Despite tremendous technological advances in combinatorial chemistry, genomics, and high-throughput screening (HTS), companies large and small have been unsuccessful in identifying new and valid antibacterial agents. Furthermore, the pharmaceutical industry faces considerable fiscal and political challenges and its traditional virtues of individuality, commitment to science, and cultural and ethical standards are thought to be in danger, in particular with regard to the antibacterial field.[19] The dilapidation of antibacterial drug discovery and development in combination with a growing proportion of elderly and immuno-compromised patients and an increasing risk of acquiring infections caused by resistant bacteria, could culminate in a global public-health crisis and catapult physicians back to the pre-antibiotic days.[17]

2. Scope and Focus of this Article

This review focuses on selected contributions by medicinal chemistry to synthethic modifications of antibiotics through de novo or semisynthesis, rather than on unaltered pure natural-product drugs. The Sections were selected according to structural characteristics and encompass established classes such as the β-lactam antibiotics, novel candidates in clinical development, as well as the prospects of antibacterial natural products as lead structures. Many aspects of the traditional classes have been thoroughly studied and competently reviewed in numerous articles and books, and even attempting to compile a comprehensive

Table 2: Key bacterial pathogens and associated infectious diseases.

Pathogen	Infectious Disease
Staphylococcus aureus	skin and wound infection, abscess, bacteremia, nosocomial pneumonia, endocarditis, toxic shock syndrome
Streptococcus pneumoniae	upper respiratory infection, pneumonia, otitis, sinusitis, meningitis
Streptococcus pyogenes	pharyngitis, tonsillitis, skin and soft-tissue infection, scarlet fever
Enterococcus faecalis	bacteremia, endocarditis, urinary-tract infection, peritonitis
Enterococcus faecium	bacteremia, endocarditis, peritonitis
Escherichia coli	bacteremia, urinary-tract and gastrointestinal infection
Klebsiella pneumoniae	hospital-acquired pneumonia, bacteremia
Proteus spp.	urinary-tract infection
Haemophilus influenzae	respiratory infection, otitis, sinusitis, meningitis
Moraxella catarrhalis	respiratory infection
Pseudomonas aeruginosa	nosocomial pneumonia, burn infection, bacteremia
Acinetobacter spp.	pneumonia in immuno-compromised patients
Mycobacterium tuberculosis	tuberculosis

documentation would far exceed the scope of this review. Thus, there is no claim to completeness and some important innovations such as the aminoglycosides, the tetracyclines[20] and glycylcyclines,[21] or the peptide deformylase inhibitors[22] were deliberately omitted. The intention of the present article is to call attention to the promising prospects of antibacterial natural products as up-to-date lead structures for medicinal chemistry and guideposts for novel targets, pioneering the way to future therapies. We wish to alert researchers and decision makers to realistically assess society's urgent medical need for a sustainable supply of effective and safe antibiotics. New ideas and solutions are needed that encourage, facilitate, and support this endeavor. There is no long-term alternative to antibacterial research.

3. Novel, Effective, and Safe Antibiotics are Urgently Needed

More and more bacterial infections evade standard treatment and are difficult if not impossible to treat. Resistance to multiple antibiotics[23] is spreading throughout the world and the number of reports on therapy failures and rising treatment costs is growing, especially in the hospital environment[11,24] (Table 3). Extensive, sometimes inappropriate use of antibiotics, inadequate hygiene (even in modern hospitals), cosmopolitan travel, the increasing aging and immunocompromised population, and the lack of rapid diagnostics have fueled this problem.

Table 3: Prevalence of resistance in hospital-acquired infections, US 2004.[42]

Antibiotic	Pathogen	Resistance [%]
methicillin	*S. aureus*	59.5
	coagulase-negative staphylococci	89.1
vancomycin	enterococci	28.5
cephalosporins 3rd generation	*Enterobacter* spp.	31.1
	P. aeruginosa	31.9
	E. coli	5.8
	K. pneumoniae	20.6
imipenem	*P. aeruginosa*	21.4
quinolones	*P. aeruginosa*	29.5

The presence of an antibiotic exerts an evolutionary pressure on the microbial population and selects resistant organisms. Bacteria can evade the lethal effects of antibiotics through several mechanisms,[25,26] for example, by alteration of the target (proteins),[27] enzymatic deactivation of the antibacterial drug,[28] restricted antibiotic penetration,[29] and increased efflux.[30] Mobile genetic elements that might accelerate the spread of resistance have become a serious threat as well (plasmides).[31] Resistance among Gram-positive bacteria has been primarily encountered in nosocomial infections in intensive care units (ICU), but is now also observed in community-acquired infections[32] that eventually require hospitalization. Gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA),[33,34] vancomycin-resistant enterococci (VRE),[35] and penicillin-resistant *Streptococcus pneumoniae* (PRSP),[36–38] are the most prominent pathogens in this respect. Methicillin-resistant *Staphylococcus epidermidis* (MRSE) is advancing quickly.[39] Regardless of their historic titles, MRSA, VRE, etc., they have all acquired resistance to multiple antibiotic classes, often already during the 1990's.[40] This development, in tandem with the emergence of new bacterial pathogens, such as *Acinetobacter* and *Legionella* species or coagulase negative staphylococci,[41] clearly calls for new antibiotics without cross-resistance to currently used drugs.

In many cases vancomycin, quinupristin/dalfopristin, or linezolid provide the last option for responsive therapy and determine if a patient lives or dies. Therefore, the observation of clinical strains with reduced vancomycin susceptibility (VISA),[43] increased glycopeptide resistance in enterococci, and the first reports on cases of high-level vancomycin resistance in *S. aureus* (VRSA)[44] are of considerable

concern.[45] Staphylococci resistant to quinupristin/dalfopristin and linezolid are emerging.[46] In July 2004, the Infectious Disease Society of America (IDSA) reported[17] that "about two million people acquire bacterial infections in US hospitals each year, and 90000 die as a result. About 70% of those infections are resistant to at least one drug. The trends toward increasing numbers of infections and increasing drug resistance show no sign of abating. Resistant pathogens lead to higher health-care costs because they often require more-expensive drugs and extended hospital stays. The total cost to US society is nearly $5 billion annually."

The evolution of resistance in ICU patients in US hospitals (but also in Europe) is alarming.[47] Seven years ago, only half of the *S. aureus* isolated from ICU patients in US hospitals were multiresistant (MRSA). Driven by prevailing use, the corresponding MRSA proportion in Japan has been even higher.[48] Meanwhile, physicians also face significant worldwide resistance problems with Gram-negative pathogens,[30e,49] in particular with *Pseudomonas aeruginosa* (HAP, cSSTI), *Escherichia coli* (cUTI, IAI), enterobacteriaceae with extended spectrum β-lactamases, and some *Klebsiella* species.[50] Furthermore, multiresistant variants of *Mycobacterium tuberculosis* demand new and more-effective drugs against tuberculosis, the most widespread and persistent infection worldwide.[51–55] About 1.9 billion people are estimated to carry *M. tuberculosis*! At the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC),[56,57] the pre-eminent forum for current issues on antibiotic resistance, clinical development, and antibacterial discovery programs, the infectious disease community has expressed serious concerns that more untreatable pathogens may develop. Antimicrobial resistance is contributing to detrimental clinical results and increased health-care expenditures.[47] It has become evident that there is an urgent need for novel antibacterial drugs without cross-resistance to antibiotics in use, more initiatives to foster the responsible and appropriate use of antibiotics,[58] and better infection control measures. As "dead bugs don't mutate", bactericidal rather than bacteriostatic agents have been recommended as resistance breakers.[59] Frequently, high-dose therapy with antibacterial drug combinations is the method of choice to suppress breakthroughs of resistance. In addition, the use of pharmacodynamic models[60] can help to minimize resistance for the patients benefit.

4. Antibacterials, an Arduous Marketplace

With over 200 marketed drugs, antibacterials represent a well-developed area with a high therapeutic standard. Currently it costs about US$ 800 million and often takes more than a decade to bring a new drug to the market.[61] These tremendous expenditures and the increasing complexity of drug discovery and development have caused an industry-wide research and development shift away from acute infectious diseases (short-course therapy) towards more-profitable chronic illnesses (long-term treatment). Without doubt, fast and effective antibacterials represent one of medicine's major achievements! However, from a marketing standpoint, efficient antibiotics are the worst sort of drug as they cure the disease usually within a few days and thus eliminate their own need (auto-obsolence). Thus, overall treatment costs are often low. This results in less revenue per patient and is commercially less attractive than some of the chronic disease states in which products are prescribed over years or lifelong once an initial diagnosis is made. The commercial success of "chronic drugs" along with the "auto-obsolence of antibacterials"[62] has tempted many companies to preferentially invest into areas such as hyperlipidaemia, hypertension, dementia, mood disorders, pain, asthma, rheumatoid arthritis, or obesity.[63]

Investment in antibacterial discovery and development is flagging in big pharmaceutical companies. Abbott, Aventis, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Proctor & Gamble, Hoffmann-La Roche, and Wyeth have left the field or are downsizing, while Bayer and others are about to follow. Several adverse factors have played a role in weakening this essential therapeutic area:[15,18,64] resistance limits the life span of every antibiotic, thereby fundamentally threatening the investment. However, resistance alone is not the only reason why interest in antibacterials has waned; the dynamics of the pharmaceutical market are also a contributory factor. Increasing fragmentation of the market, recent patent expiry of blockbuster drugs, and the growing regulatory hurdles for the clinical evaluation of new antibiotics are also to be held responsible.[65] The demand for blockbuster drugs[63] pressures large companies to focus on safe broad-spectrum antibacterials for extensive use. At the same time, the call for more-limited use of broad-spectrum agents discourages the first-line treatment with new antibiotics and reduces their expected sales.[66]

In all, activities have shifted away from big pharmaceutical companies to smaller biotechnology or specialty firms. These biotech companies concentrate more on novel small-spectrum or niche products that are commercially less viable for big pharmaceutical companies. In addition, biotech has taken up and successfully progressed products such as daptomycin,[10,11] which was initially put aside by their more powerful partners. Yet, most of the biotech companies cannot bear the significant costs of large-scale clinical trials on their own.[67]

Still, antibacterials have an advantage. The development of antibiotics has been benefited from highly predictive animal models, with above-average clinical success rate of about 17% (average 11%). In contrast to other therapeutic areas in which lack of efficacy often is the major cause of attrition in the clinic, the toxicology, clinical safety, and commercial aspects are most critical for an antibiotic.[68]

The number of antibacterials approved by the US Food and Drug Administration (FDA) decreased by more than half over the last two decades (Figure 2) and only 6 of 506 "new molecular entities" in development were antibacterial agents.[62] In 2002, out of 89 new drugs, no new antibiotics were approved.

According to commercial analysts,[70] antibacterial companies are currently facing significant hurdles in maintaining product revenue levels and, in particular, large companies face the prospect of leaner times. Some of their most

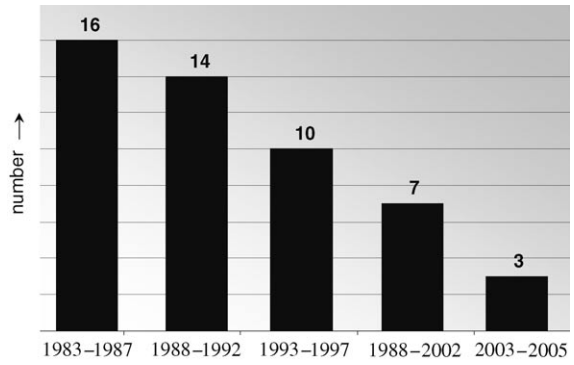


Figure 2. New antibacterial agents approved in the US.[62]

profitable drugs such as amoxycillin/clavulanic acid and ciprofloxacin have gone off-patent, allowing other companies to sell cheaper generic versions of them. Generic competition, drug resistance, and increased regulatory scrutiny have placed great pressure on antibacterial profit margins. Shareholder value interests increasingly frame research and development investment strategies and stagnating growth induces lower commercial attention. As a result, many analysts and managers cultivate a wary view of the prospects of the antibacterial market.[63] Indeed, in comparison to other areas, the antibacterial market remained relatively flat in recent times and Datamonitor[70] predicts an average growth rate of merely 1.4% for the year 2010. The number of antibacterial blockbusters is expected to decrease and the hospital sector will be dominated by specialist products. Activity against resistant isolates, shorter treatment courses, the possibility to switch from i.v. to oral application, cost, and improved side-effect profile are driving the hospital sector in which the fastest growing indications are expected to be respiratory tract infections (RTI; 6.2% of 2002 sales), urinary tract infections (UTI; 4.9%), and skin and soft tissue infections (SSTI; 4.9%).[70] On the other hand, antibiotics represent the third largest pharmaceutical drug market segment with global sales of US$ 25782 million (Table 4), a third of which is spent on parenteral (i.v.) hospital antibiotics. Despite the poor growth rates, the overall antibacterial market is still attractive.

Table 4: Top 10 antibacterial companies by global sales of antibiotics in 2004 (Source: Wood Mackenzie[69]).

Rank	Company	US$ million
1	Pfizer	2938
2	GlaxoSmithKline	2425
3	Abbott	1657
4	Bayer	1346
5	Johnson & Johnson	1295
6	Hoffmann-La Roche	1142
7	Wyeth	846
8	Merck & Co.	704
9	Daiichi	687
10	Shionogi	678
Others		12064
Total		**25782**

5. Biological Targets and Chemical Leads

5.1. Natural Products as Antibacterial Lead Structures[71]—Coevolution of Targets and Inhibitors

Less than 1% of all known organic compounds are natural products; 99% are synthetica. Despite this extreme situation, more than a third of all drug sales (1981–2004) were based on natural products or the "intellectual DNA" behind them.[4] Natural products did not only contribute to drug discovery as chemical lead structures but also served as guideposts to—pharmaceutically unexplored but evolutionary validated—targets and modes of action that could subsequently be explored with synthetic mimetics.[72]

Structural diversity and complexity within natural products is unique and easily dwarfs synthetica. The functional complexity found in natural products will never be invented de novo in a chemistry laboratory. Natural products present unique ring architectures (vancomycin, stephacidin B) and complex molecular pharmacophores (erythromycin, epothilone). Their sphere of action ranges from small-molecule binding (cycloserine, salicylic acid) to quasi supramolecular interactions (ramoplanin, palytoxin). Many natural pharmacophores are more like molecular machines (β-lactams, mitomycin C) than static binders and are often flanked with recognition domains that can selectively trigger a "warhead" within target proximity (calicheamicin). Binding to the molecular target may even craft a higher-order interaction, as found for the pore-forming nisin/lipid II complex. Many natural products do not attack a single enzyme, but rather a whole enzyme family (β-lactams/penicillin-binding proteins (PBPs)), and can even have multiple modes of action (vancomycin, inhibition of transpeptidases and transglycosylation). Interestingly, this natural multiple-target approach contrasts with the HTS-governed "single-target dogma" of the current drug-discovery processes.

The extraordinary success of natural products as guideposts to new drugs is most obvious in antibacterials.[6] Here, more than other indications, the reliance on natural-product lead structures has remained the most-successful route to discovering clinically relevant therapeutics.[73–75] Over 75% of new chemical entities (NCEs) submitted between 1984 and 2004 were based on natural-product lead structures.[4] Only 21% of antibacterial NCEs had a pure synthetic history,[4] for example, the oxazolidinones, which originally stemmed from a Du Pont company screening hit.

From an evolutionary point of view, it is not surprising that natural products are promising lead structures especially for antibacterial drugs.[76] Microorganisms, in particular fungi, but also bacteria (actinomycetes), successfully fostered their armamentarium against bacterial competitors for millions of years. Antibiotics were vital weapons in the persistent fight for space and resources. Microorganisms evolved sustainable strategies to modulate competitors and aggressors, based on the coevolution of their secondary metabolites with the corresponding targets in bacteria. Molecules that kill their bacterial neighbors or prohibit their replication have coevolved with their microbial producers and their defense strategies (resistance). In vitro potency, self-tolerability, and

biosynthetic effort have been key selectors within the evolution of antibacterials. To exert an effect, most of these antibiotics must penetrate bacterial membranes[77] and attack at least one adversarial molecular target. Clearly, controlling bacterial resistance was a major challenge for microbial producers of antibacterial metabolites. In general, antibiotics are the products of nonessential secondary metabolic pathways that are switched on when required. Structural adaptation within bioactive secondary metabolites relies on the evolution of biosynthetic pathways, that is, the evolution of the biosynthesis machinery on a genetic level (gene duplication, gene shuffling). A strong interest in these evolutionary processes has evolved recently:[78,79] the modification of known biosynthetic pathways (combinatorial biosynthesis, metabolic engineering) or the discovery of silent biosynthetic gene clusters, which possibly hold the, genetically encrypted,[80] instructions for the synthesis of unexplored natural products (metabolome mining) that can't be produced from classical fermentation approaches.[81]

The concept of "privileged structures"[82] is another way to explain the high success rate of natural products within diverse therapeutic areas, not only within antibiotics. Structural analogy between enzymes of utterly different biological function and divergent peptide sequence is based on folding types and domain families that are common in the whole proteome and seems to explain the "cross-talk" of natural-product leads in "uncommon" therapeutic areas. This concept is especially useful for rationalizing the behavior of natural products within therapeutic areas that are not correlated to the original ecological purposes of secondary metabolites.[83] Therefore, it does indeed make sense to screen antibacterial natural products against a central-nervous-system target

Statistical investigations also have been employed to help explain the high success rate[4] of natural products versus synthetica in drug discovery. So far, no clear rules could be extracted from this work. It seems that the structural secret to success, incorporated in natural products, cannot be easily disclosed by statistical means, that is, by simply counting chemical functionalities, rings, and chiral centers or looking at physicochemical parameters such as membrane affinity, polar surface area, pK_a, etc. The biological relevance of natural products in medicinal chemistry is apparently not based on a general physicochemical or structural master plan, but on the fact that every single secondary metabolite has been evolutionary engineered within a complex network of biological interdependencies. However, modern search methods (data mining) have extracted some trends that distinguish synthetica from natural products and indicate a certain structural complementarity in both substance pools.[84,85] Statistically, natural products have higher molecular weights, higher counts of hydroxy groups, and higher polarity than "average" synthetica.

5.2. The "Classical" Approach—From Bug to Drug

Antibacterial activity is assessed in MIC tests that determine the lowest concentration of a substance required to completely inhibit bacterial growth over an 18–24 h period. MIC testing and other microbiological in vitro procedures[86] have been enormously helpful in the search for promising structural scaffolds to initiate programs.[87] Medicinal chemists optimized compound stability, potency, antibacterial spectrum, and selectivity along with pharmacological properties. To date, all established antibacterial classes have been identified by using MIC whole-cell assays.[88] Animal models[89,90] that reflect antibacterial activity and are predictive for clinical situations have traditionally complemented this "classical approach", whereas microbial pharmacodynamics,[60] which link drug exposure and potency to microbiological or clinical effect for a specific pathogen, have increasingly gained importance (see Section 6.1). Although, cytotoxicity is easily monitored with standard in vitro models, setting up animal models for the assessment of systemic toxicity is elaborate and expensive. In general, the throughput of animal models is low and thus only selected lead candidates are examined.

Extensive use of antibiotics exerts evolutionary pressure on microbial populations and selects resistant bacteria. To keep pace with microbial resistance, the classical approach of pharmaceutical companies has, for decades, been to investigate incremental structural variations of established antibacterial classes. In this way, many classes have been advanced towards their third- and fourth-generation forms, and it is an open question as to how many more generations can follow before a class will loose its efficiency.

The majority of marketed antibiotics inhibit or deregulate the biosynthesis of bacterial macromolecules through a mere handful of clinically validated modes of action, that is, target areas (Table 5). For example, β-lactam antibiotics, glycopeptides, and fosphomycin inhibit bacterial cell-wall biosynthesis (Figure 3), whereas lipopeptides and cationic peptides, such as polymyxins, disrupt membrane integrity. The folate coenzyme biosynthetic pathway is blocked by trimethoprim and sulfonamides, novobiocin and the quinolones inhibit bacterial DNA replication, and rifampicin obstructs transcription (RNA-synthesis).[91] Inhibition of bacterial protein synthesis (translation) by selectively blocking the ribosome[92] is a very common target area.[93–95] Many structurally diverse classes of antibiotics, such as the streptogramins,[96] tetracyclines,[20]

Table 5: Estimated number of essential broad-spectrum genes/targets.

Target area	Essential genes	Established antibiotic classes
DNA replication	20	3
divisome	8	0
transcription	6	1
translation	58	8
fatty acid biosynthesis	12	0
cell-wall biosynthesis	17	3
nucleotide biosynthesis	9	0
coenzyme biosynthesis	6	2
secretion	5	0
Total	**141**	**17**

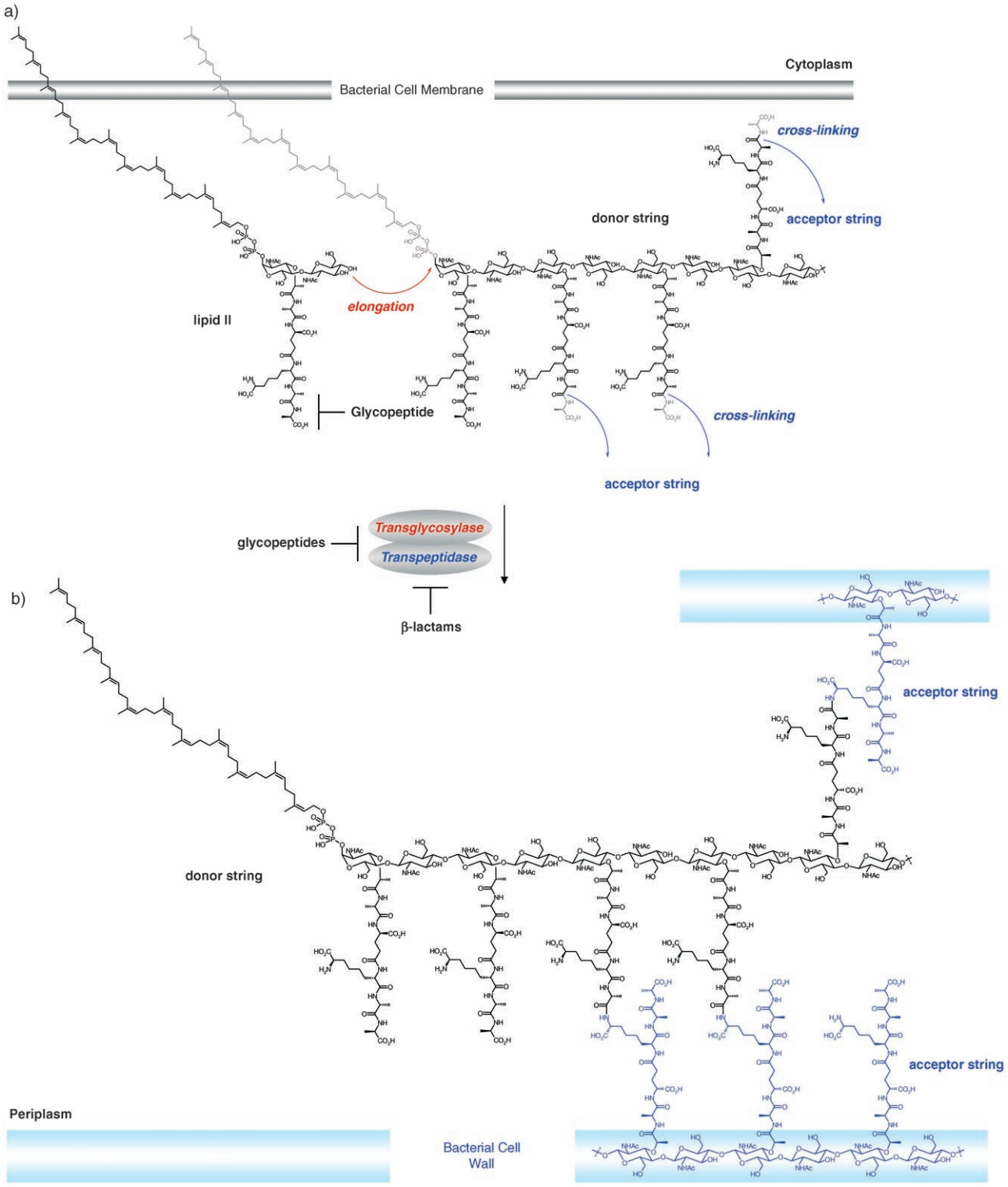


Figure 3. Peptidoglycan biosynthesis from a chemists point of view. An idealized scheme for the Gram-negative bacterium *E. coli* is depicted—the chemical structure of peptidoglycan varies amongst different bacteria. a) Lipid II (the next monomer) is attached to a nascent, growing peptidoglycan string in the elongating transglycosylation step (red). Both, the monomer and the growing chain navigate in the bacterial membrane with their C_{55} lipid tail. In the cross-linking transpeptidation step, the donor peptidoglycan string grasps for available acceptor stings (blue). b) The resulting peptidoglycan (or murein) sacculus is a flexible and enormously stable macromolecular meshwork that defines the shape of the bacterial cell and protects the microorganism against its high internal osmotic pressure. Different antibiotics interfere with these concluding steps of the bacterial cell-wall synthesis: glycopeptide antibiotics bind to the D-Ala-D-Ala terminus of Lipid II and barricade the action of bacterial transglycosylases and transpeptidases. β-Lactams inhibit transpeptidases.

macrolides, lincosamides, aminoglycosides,[97] and chloramphenicol all work through this mode of action, albeit by interacting with distinct subunits[98] or at different steps of this complex process (Figure 4). Traditionally, the mode of action of an antibacterial agent was investigated and elucidated after its discovery and sometimes long after its introduction into clinical therapy. With penicillin, it took almost half a century for its molecular targets, the penicillin-binding proteins (PBPs), to be identified.[99] Drug discovery processes in the pharmaceutical industry underwent dramatic changes (genomics revolution)[100] thanks to new computational methods and the remarkable advances in high-throughput technologies in screening, combinatorial chemistry, and genomics. Turning away from traditional approaches and established antibacterial classes, scientists and executives swiftly envisaged a revolutionary potential in the identification and pursuance of novel biological targets.

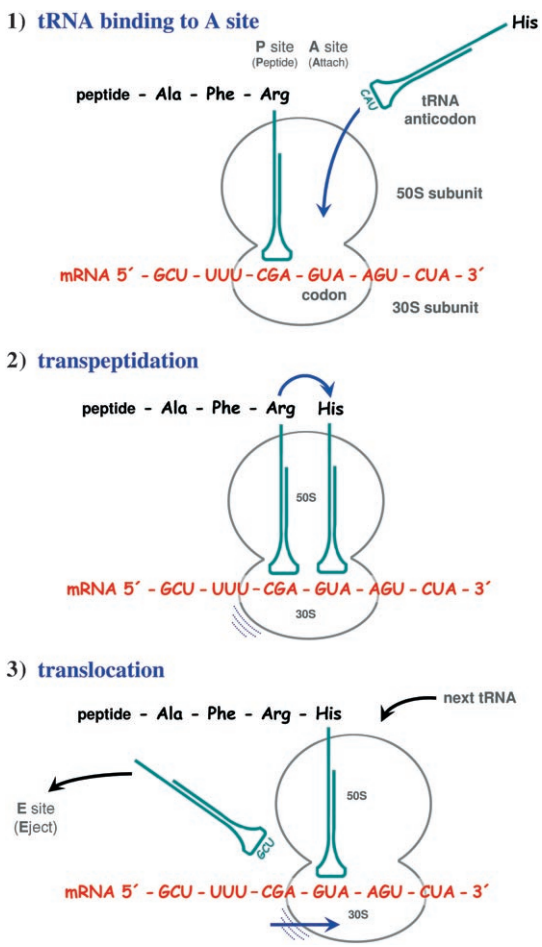


Figure 4. The bacterial ribosome, composed of a 30S and a 50S subunit, is the apparatus for protein synthesis. The ribosome moves along a messenger RNA (mRNA) template and "translates" the successive codons (triplets of nucleotides code for specific amino acids) into a growing peptide chain in three schematic steps: 1) Binding: A transfer RNA (tRNA) loaded with an amino acid (His) binds to the acceptor (**A**) site by complementary base pairing (anticodon). 2) Transpeptidation: The tRNA in the peptidyl (**P**) site is discharged by transfering the growing peptide chain to the His on the tRNA in the **A** site. 3) Translocation: The ribosome advances to the next codon on the mRNA. The new **A** site is, therefore, free for the next tRNA and the empty tRNA is ejected (**E** site). Different inhibitors of bacterial protein synthesis interact at different steps or with distinct subunits of this complex process: tetracyclines compete with the tRNA for **A** site binding, aminoglycosides cause misreading of the mRNA code and incorporation of incorrect amino acids into the peptide, chloramphenicol and tetrahydropyrimidinone antibiotics block transpeptidation, and macrolides/ketolides inhibit translocation. Many antibiotics act by binding into and physically obstructing the peptide exit tunnel of the ribosome.

5.3. The "Target-Based" Approach—From Target to Lead

The growing information on bacterial genetics, initiated by the first report on the complete bacterial genome sequence of *Haemophilus influenzae* in 1995[101] along with novel tools for gene-expression profiling[102] and proteomics,[103] boosted the new paradigm of functional genomics not only in antibacterial drug discovery.[104,105] The exponentially growing delivery of further prokaryotic whole-genome sequences[106] all of a sudden revealed a plethora of so far unexploited bacterial targets waiting to be mined.[107] However, identifying valid targets from genomic sequences is not trivial, and the selection of the right targets for screening compound libraries has become a vital decision in all areas of pharmaceutical research.[108–111] Today, knowing the mode of action and the molecular target of a novel antibacterial is considered a prerequisite for a project. Only with this information can a good in vitro/in vivo screening workflow be established in the interest of a rational structure–activity relationship (SAR; the medicanal chemist's roadmap for optimizing potency and selectivity).

The target-based approach has offered new opportunities, but the process of target validation is multifaceted and often associated with uncertainty. A valid antibacterial target[107,112] must be conserved across a broad range of medically relevant pathogens (spectrum) and either absent or sufficiently different in mammals (selectivity).[113] It must be essential,[112,114,115] screenable,[116] and druggable[117,118] and must avoid potential cross-resistance to marketed antibiotics. Any new antibacterial must produce a low frequency of resistance. Approximately 10% of all microbial genes are thought to be essential for bacterial growth in vitro (Table 5),[119,120] however, proving a target's in vivo essentiality can be quite complex.[121] After passing all these requirements, a fair number of broad-spectrum antibacterial targets remain that exceed the number of targets addressed by established antibiotics.

Proteins, derived from gene sequences that fulfill these criteria, are selected as targets and used for establishing HTS[122,123] to scan compound libraries. In a HTS, the effect of a great number of compounds on the enzymatic activity[105] or on genetically engineered whole cells[124] is monitored. Active compounds, identified as "hits" are rescreened to afford a set of "confirmed hits" which is investigated in secondary assays, for example, to confirm the mode of action and to perform resistance analyses. Confirmed hits are clustered, prioritized, and preliminary SARs are extracted from the screening data. A clear SAR is the medicinal chemist's roadmap for optimizing potency and selectivity on the basis of target activity (IC_{50}), MIC, and, if available, target protein struc-

ture.[125,126] At the same time, the chemical purity of the hits is checked and often they are resynthesized and their eligibility is discussed before they qualify as lead structures for focused chemistry programs that aim at improving not just the effect, but also the pharmacological profile (e.g. serum half-life, tissue distribution, and solubility).

In principle, the exact target of a drug candidate, stemming from a single-target screen, is known. However, for many successful antibacterial classes such as the β-lactams, glycopeptides, or quinolones, the real mode of action is multifaceted and cannot be reduced to the simple interaction with a single target.[127] Indeed, the "one-target-one-disease" philosophy and the scope of "monotarget medicine" clearly has its limitations.[128] Despite sophisticated genetic strategies and techniques, no new clinical antibacterials have been identified through these HTS-based processes.[129,100,130] Past HTS approaches across industry have suffered from target and assay diversity and low hit-to-lead success rates.[107] In the hope of developing more "first-in-class drugs", research activities were shifted from classical targets and "priviledged" structural scaffolds to new target and non-natural product leads.

Yet, drugs that target novel mechanisms generally have a significantly higher risk of failure and have added little value when compared with drugs that are based on known targets.[131] In their critical analysis: "Antibiotics: where did we go wrong?" Overby and Barrett speak of the "seduction of genomics and forgetting how to make a drug".[18b] Indeed, target-based approaches have primarily concentrated on target activity and have produced potent inhibitors that were often unable to penetrate bacterial cell walls, thus being devoid of antibacterial activity. Yet, attaining and enhancing antibacterial activity requires an enormous medicinal-chemistry effort and, even for novel structures with MIC, it is extremely difficult to immediately match activity, spectrum, and tolerability of the established classes' advanced-generation agents. Consequently, companies, large and small, have been unsuccessful in identifying new and valid antibacterial agents through target-based approaches. The shift to target-based drug discovery could be a contributing factor to the decline in productivity of the pharmaceutical industry.[132] For antibacterial drug discovery, however, this approach has ultimately failed and needs to be modified or replaced to achieve a closing of the productivity gap.[133] A risk-balanced portfolio requires that this process be supplemented with precedented targets, me-toos, or the reversed-genomics approach.

5.4. The "Reversed-Genomics" Approach—From Active Structure to Lead

Identifying and selecting a viable lead structure is the key to success. Inadequate lead quality was a prominent reason for the failure of many previous discovery programs. For the generation of a lead structure, the reversed-genomics (RG) approach provides a powerful and efficient complementary alternative to the target-based approach (Figure 5). It starts with an active antibacterial compound that exhibits MIC and for which the target and mode of action are not yet known. Modern molecular biology offers excellent techniques to rapidly identify and validate unknown target areas and molecular targets.[86,100,134,135] Bacteria respond to adverse environmental conditions such as heat, starvation, or the presence of an antibiotic by activating stress-dependent regulatory networks. The stress response triggered by an antibiotic relates to its mode of action and can be monitored by various RG techniques, such as: 1) the incorporation of specific radio-labeled precursor molecules, 2) transcriptome analysis, 3) proteome analysis, and 4) FTIR spectroscopy. Such standard tools can be complemented with specific assays for metabolic pathways, enzyme tests, and resistant mutants. Nonpathogenic *Bacillus subtilis* has served as an ideal model organism for proteomics and expression profiling.[135,136] It is one of the best-studied bacteria and is closely related to clinically relevant Gram-positive pathogens.[137–140]

1) Precursor incorporation: A fast method for identifying the mode of action of an antibacterial compound is to examine its effect on the key bacterial macromolecular biosyntheses in the presence of specific radio-labeled precursor molecules in whole cells (Figure 6). Radiolabel incorporation indicates which of the four key processes, protein biosynthesis or the synthesis of DNA, RNA, or cell wall, shows the greatest reaction to the agent, whereas nonselective compounds tend to block several pathways simultaneously.[142,143] This assay serves as a guidepost for more detailed studies.

2) Transcriptome analysis: Inhibition of a specific target by an antibiotic induces characteristic changes in the microorganism's global expression profile, resulting in up- or down-regulation of certain genes. The transcriptional activity of all genes encoded in a bacterial genome can be monitored at the same time and studied by expression profiling techniques (transcriptome analysis) that are based on DNA microarrays. Such profiles, when compared to the reference profiles of established antibiotics, are characteristic for the mode of

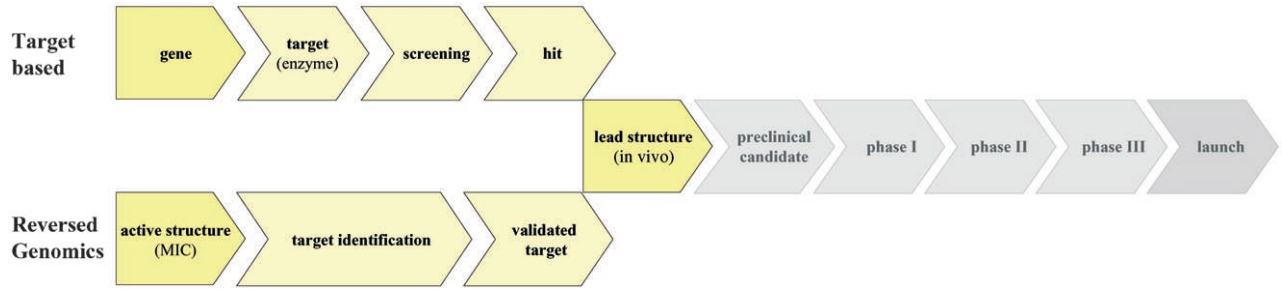


Figure 5. Target-based and reversed-genomics approaches are complementary drug-discovery processes for antibacterial lead-structure generation.

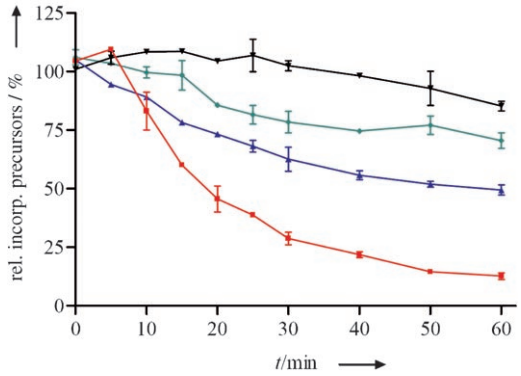


Figure 6. Precursor incorporation: The effect of tetrahydropyrimidinone antibiotic **13** on the principal bacterial biosyntheses pathways of *S. aureus* in the presence of specific radiolabeled precursor molecules was examined. Radiolabel incorporation kinetics indicated which of the four key processes—protein biosynthesis/[^{14}C]leucine (red), synthesis of DNA/[^{14}C]thymidine (blue), RNA/[^{14}C]uridine (black), or cell wall/*N*-acetyl [^{14}C]glucosamine (green)—shows the greatest reaction to the agent. For example, **13** preferentially inhibited protein biosynthesis.[141]

action of an antibacterial agent. Transcriptome analysis has proven to be an efficient tool for tracking a novel agent's mode of action and to elucidate its target.[142,138,144] With an appropriate set of reference antibiotics at hand, compounds with an analogous mode of action can rapidly be identified.

3) Proteome analysis: Changes in gene expression of bacterial pathogens after treatment with antibiotics can also be studied by quantifying proteins, typically by separating cellular proteins by using two-dimensional gel electrophoresis (Figure 7).[136] This method allows the detection of changes at the translation level, that is, protein variants and modifications. Proteome analysis has successfully been used in classifying the mode of action of novel antibacterials[136] as well as for target validation.[145]

4) FTIR spectroscopy: Infrared spectra (IR) of entire bacteria can be used to obtain further insight. When bacteria are treated with antibiotics, characteristic changes in absorption occur. Monitoring these changes by FTIR reveals the principal modes of action.[146,147]

These RG techniques complement each other and all together provide a powerful and efficient platform for lead structure identification and validation. In our experience over the last decade with both the target-based HTS and the RG approaches, the latter, in particular when used to examine natural products, had a significantly higher success rate in generating novel and valid antibacterial leads. Nevertheless, we expect that a well-balanced combination of all these technologies, along with new ideas and efforts, will eventually illuminate the way to valid lead structures and novel antibacterial agents.

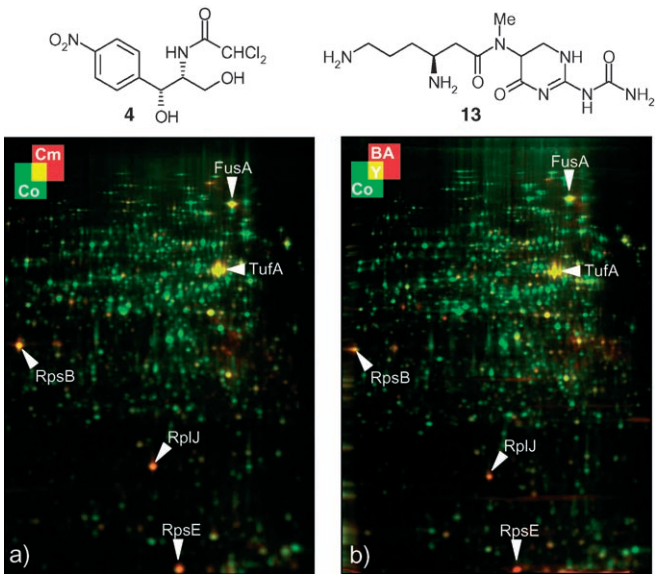


Figure 7. Proteome analysis is a valuable tool for classifying the mode of action of novel antibacterials in the pathogen. Changes in gene expression can be studied by separating and quantifying cellular proteins by using two-dimensional gel electrophoresis. Although each antibiotic shows an individual protein expression profile, overlaps in the expression of marker proteins (arrows) indicate a similar mode of action. By comparing its profile with the profile of chloramphenicol (**4**), **13** was found to be a peptidyl-transferase inhibitor.[136]

6. Chemical Postevolution of Antibacterial Natural Products

In the current environment of the pharmaceutical industry, natural-product chemists often feel like Neanderthal men,[148] not fitting into the paradigm of modern drug discovery. Natural-product chemistry is stigmatized as being old fashioned, expensive, ineffective, and incompatible with modern drug-discovery processes.

6.1. Natural-Product-Derived Antibacterials are Different from Other Drugs

Antibacterial drugs differ from "other drugs" in many aspects (Table 6). Empirical rules (Lipinski et al.),[149] found in medicinal chemistry, are often irrelevant for antibacterials, in particular for those derived from natural products (macrolides, glycopeptides). Many antibacterial drugs have to satisfy the requirements for parenteral and oral administration, a highly demanding task for any drug.

Antimicrobial "chemotherapy" is based on Paul Ehrlich's principle of "selective toxicity". Antibacterial drugs inhibit the growth of bacteria (bacteriostatic) or even better kill them (bactericidal). The treatment goal is the fast eradication of bacteria in cooperation with the immune system. The fact that antibacterial therapy is a "killing discipline" has many implications for the physicochemical and pharmacological profile of antibacterial drugs.

Targets: In many therapeutic areas, the partial modulation of a single target is sufficient. Yet, for antibacterial therapy, complete inhibition of multiple targets is normal. Essential bacterial biosynthetic pathways have to be blocked completely. Inhibition of multiple targets will increase the in vitro potency and minimize the development of resistance. A variety of large natural products exerts multiple modes of action (vancomycin). They act like "pharmacophore chame-

Table 6: Natural-product-derived antibacterials versus "other" drugs. Empirical rules of thumb.

	Topic	Antibacterials	Other drugs *(Lipinski etc.)*
Target/ Mode of Action		multiple targets; multivalent; block target	single target; selective; modulate target
Structure		complex; multiple pharmacophores	simple; single pharmacophore
Physicochemistry	M_w [g mol^{-1}]	> 500	< 500
	log MA (pH 7.5)	< 3 (parenteral) < 5 (oral)	< 5
	rotatable bonds	> 10	< 10
	H donors	> 5 (parenteral)	< 5
	H acceptors	> 10	< 10
	solubility [g L^{-1}]	> 0.5–2	< 1
Pharmacokinetics	dose	often high	often low
	protein binding [%]	critical	less critical

leons". Indeed, antibacterial drugs tend to have high molecular weights and "repellently" complex structures.

Physicochemistry: The polarity window of antibacterials is defined by solubility requirements and penetration phenomena. In Gram-negative bacteria, porins are essential for uptake and often represent an entry barrier for too-lipophilic molecules.[77] For parenteral drugs, a high solubility in aqueous media is a must (0.5–2 g L^{-1} or even higher). Consequently, parenteral antibacterials have to be polar (log MA < 3). On the other hand, too-polar molecules do not sufficiently penetrate through the cytoplasmic membrane of bacteria.[150] Yet, polar drugs may be concentrated in bacteria through specific transport mechanisms. Passive diffusion through the membrane is not mandatory in this case. Efflux and influx phenomena also have to do with polarity. Bacterial multidrug transporters pump out amphiphilic and lipophilic compounds to a higher degree than hydrophilic molecules. To achieve efficacy in vivo, a compound needs to show substantial passive absorption from the gut into the blood stream, which is determined by adequate aqueous solubility and moderate lipophilicity.[151] Owing to these polarity requirements, many combinatorial libraries are not "antibiotic-like". In this respect, free OH and NH groups are "endangered species" as they entice combinatorial chemists to derivatize them. Indeed, standard combinatorial libraries preferentially were built within the lipophilic rather than in the polar range.

Pharmacokinetics/Pharmacodynamics: Bacterial pathogens can colonize every part of the human body. However, many infections are localized (blood stream, skin, respiratory or urinary tract). The time course of free-drug concentration at the site of infection (time versus concentration) is determined by pharmacokinetics (V_{SS}, $t_{1/2}$, AUC, f_U). Protein binding of antibacterials should not be too high (< 90 %) as only the free drug can act on the target (fraction unbound, f_U > 10 %).[152]

The killing effect of a specific drug concentration depends on its pharmacodynamics (concentration versus antibacterial effect). Only an appropriate time course of drug concentration leads to clinical efficacy. This special time course of drug concentration is the "driver of efficacy" and is further described by the pharmacokinetic/pharmacodynamic index (PK/PD; time versus antibacterial effect)[153,60] that may differ significantly between antibacterial drugs. For example, the PK/PD index of most β-lactams is "time over MIC". Sufficient killing of bacteria is only achieved if the β-lactam concentration remains above the MIC at the site of infection for ≈ 40 % of the treatment time (≈ 9 h day^{-1}).

Dose: In contrast to most other therapeutic areas, very high doses are necessary to attain sufficient drug concentrations in the infected tissue. For example, up to 36 g of penicillin G or 4 g of erythromycin may be given daily to patients with severe infections.[154] Only excellent tolerability will allow for such dosage regimens. As expected, drugs addressing bacterial targets that are absent in mammals (peptidoglycan) have a higher chance to exhibit an outstanding therapeutic index. These drugs will not show a mechanism-based toxicity. However, they may have other toxicity issues (nephro- and ototoxicity of aminoglycosides, QT interval prolongation of the heart, or phototoxicity of quinolones).

6.2. Are Natural Products Good Drugs?

Many natural products are a good starting point for medicinal chemistry. Yet, only a few "pure" natural products fulfill the complex property profile of a pharmaceutical drug (daptomycin, erythromycin, penicillin G, tetracycline, vancomycin).[73,74] Though the medicinal chemist and the "antibacterial microbe" both have the common goal to control bacterial pathogens, a drug has to match additional physicochemical, pharmacological, toxicological, and technical requirements that have not been selectors within the evolution of antibacterial secondary metabolites.

Typical limitations of natural-product leads are limited chemical stability or low solubility, which especially hamper the development of parenteral drugs. In addition, the intellectual property situation is often less clear in unmodified natural products. Many natural products are complex structures with a high molecular weight. "Heavy" structures break Lipinski's rules[149] and will most likely exhibit no absorption from the gut into the blood, therefore impeding oral formulation. Furthermore, complex structures look expensive for technical development. A narrow antibacterial spectrum of the natural-product lead might be the result of low target affinity but often has to do with physicochemical limitations (membrane affinity window) or efflux phenomena that affect transmembrane transport in certain pathogens (spectrum gap). Some natural antibiotics do not show sufficient in vitro potency in standard models (MIC) owing to high protein binding (low F_U) or adhesion phenomena within the test system. Unfavorable pharmacokinetic parameters in animals or humans, such as low half-life in the body ($t_{1/2}$), high clearance of the drug (C_L), low metabolic stability, low

exposure (AUC), or insufficient distribution into infected tissue (V_{SS}), might result in a total lack of in vivo efficacy. Tolerability is one of the big issues of natural antibacterial lead structures. For example, tetracyclines exhibit pronounced phototoxicity, and the use of aminoglycosides is complicated by nephrotoxicity as well as irreversible ototoxicity.

A serious chemistry program will only be initiated if the natural screening hit can be "validated" with further SAR data points. Only a validated hit has the potential to fulfill the criteria for a new lead structure. Combinatorial libraries often yield hit clusters including preliminary SAR data, whereas, natural products only yield solitary hits (singletons), thereby providing no further information. At this point, the synthesis of new congeners is a prerequisite for preliminary SAR information. Usually, chemists will decide to evaluate a hit cluster (synthetica) rather than a singleton (natural-product hit).

6.3. Natural Evolution versus Chemical Postevolution—Orthogonality in Substructure Space

Natural products arise from natural evolutionary processes. In a similar way, a medicinal chemist also shapes the structure of natural templates in an evolutionary optimization process (chemical postevolution). Within learning cycles, successive generations of lead-structure variants of the original natural products evolve. Favorable attributes are passed on to the next generation, unfavorable properties are discarded. Individual mutations are mixed in each generation and tested for their compatibility. Permutation of properties from male and female individuals (sex) is a key strategy in natural evolution, whereas the structural combination of distinct chemical leads is crucial for success in medicinal chemistry (additive SAR).

Obviously, natural selection and chemical postevolution do not have exactly the same objectives. However, many properties of a molecule play an important role during both the process of natural evolution and the process of chemical optimization. In both processes, individual parameters such as target affinity, solubility, and cytotoxicity variably contribute to the "fitness" of a specific molecule. Nature continues to design highly complex pharmacophores from scratch by the everlasting interplay of mutation and selection over vast periods of time. With a rather limited palette of building blocks—acetate, propionate, mevalonate, shikimate, amino acids—nature creates surprisingly manifold structures. Evolutionary change in natural products stems from mutations in the corresponding biosynthesis genes. Structural diversity is driven by the rearrangement of modular biosynthesis genes (polyketides) and a refinement toolbox that is typically applied within the late stage biogenesis (oxidation, methylation, condensation).

Rolling out of new starting materials was a rare event within evolution. Truly novel starting materials required a fundamental changeover of the biosynthetic machinery that has evolved over millions of years. Presumably, the coevolution of proteins and natural products was another reason for nature's evolutionary focus on a few mainstream biosynthetic pathways to primary and secondary metabolites. Hence, a remarkable substructural conservatism results. In most natural products, the skilled eye can discover the same substructural motives time and time again. Indeed, the impressive macroscopic diversity of natural products is composed from a fairly narrow set of substructural motives.

In general, a medicinal chemist can easily evade nature's rigid biosynthetic pathways. Ordinary structural elements are virtually inaccessible for natural producers (*tert*-butyloxy, fluoroaryl, most heterocycles), and switching starting materials is a common method towards diversity. Complementary to nature, the medicinal chemist can explore additional substructural space: in a sense, chemical postevolution of natural products is orthogonal to natural structural evolution. Within a short time, medicinal chemistry can explore white spots in structural space (and biological activity) that have never been touched by microorganisms, fungi, plants, and animals over the entire period of evolution.

6.4. Chemical Postevolution—Improving Nature's Blueprints

Medicinal chemistry has the challenging task of simultaneously optimizing the natural-product lead structure for in vitro potency, in vivo efficacy, low toxicity, druglike physicochemical properties, and good pharmacokinetics (chemical postevolution). This multidisciplinary effort intertwines chemistry, microbiology, pharmacology, and toxicology. SAR, structure–toxicity relationships (STRs), and a basic understanding of pharmacokinetics are established in learning cycles (test sequences) and are based on biological data from the individually designed "screening cascade". Most natural hits in antibacterials have been picked because they show satisfactory in vitro potency (MIC). Now, medicinal chemistry is used to induce into the structure additional properties, such as solubility or tolerability, without losing its intrinsic antibacterial activity.

In the industrial research environment—governed by project timelines—it is especially difficult to work with complex natural products. Only a real commitment to the lead will prevent a medicinal chemist from sidestepping towards "easier" small molecules that are amenable to efficient parallel synthesis and guarantee the production of more test compounds. Sometimes natural-product leads are hastily judged to be not optimizable owing to a steep SAR (few potent derivatives) or a SAR that parallels STR (potent derivatives are toxic). Often this has to do with limited diversity of the available derivatives rather than with insufficient lead potential. In these particular cases, the chemical methodology does not really capture the lead structure (core variations), but plasters the pharmacophores with lipophilic residues (limitations of the template concept).

Despite a common prejudice, it is possible to increase the innate antibacterial activity of natural products in the laboratory even though the natural product has been evolutionarily optimized for antibacterial activity[155] over millions of years.

6.5. How to Get Starting Material?

A natural-product chemist working with natural products often works with very limited amounts of material. Substance supply is the key challenge for most natural-product chemistry programs in the pharmaceutical industry. Typically, the natural-product screening hit is only available in minor amounts (mg range) in the initial project phase. Only few projects can afford to bring up further "starting material" (the natural product) through total synthesis without losing too much time and resources (see below). Cooperation with dedicated academic groups may help to assure material supply in time (de novo synthesis).

Regularly, fermentation is the most efficient source of the natural-product starting material. Still, not every natural-product screening hit can be easily obtained through biotechnological means. Many producer strains are not available or cannot be grown in culture (mycorrhiza fungi). Other organisms grow well, but the biosynthesis of antibiotic secondary metabolites cannot be elicidated under culture conditions. Only dedicated expert groups guarantee reproducible fermentation conditions that are a prerequisite for technical development and production on a large scale.

6.6. The Modest Life of De Novo Synthesis in Industry

Only de novo synthesis—and not semisynthesis—provides the most manifold options for broad structural variation that can fully assess a lead structure's potential (Table 7). Various natural products have been explored through total synthesis, and peptidic structures and structures of intermediate complexity seem to be especially suitable for this approach in industry. For example, in the carbapenem area, de novo synthesis has played a key role in chemical optimization and technical development. However, for complex structures with high molecular weights, de novo synthesis tends to be expensive and slow. The highest hurdle for the de novo synthesis of complex molecules is the phase of technical development. A "good" drug candidate may be found in research by means of de novo synthesis, but if there is no practical semisynthetic entry for development, this compound might never be produced on a technical scale. Therefore, not many medicinal-chemistry programs in antibacterials dealing with complex natural-product leads can afford the "luxury" of establishing SARs based on de novo synthesis within the exploratory project phase. Indeed, the medicinal potential of many "old", but complex, classes has not yet been fully assessed by means of de novo synthesis. Not a single purely synthetic glycopeptide, tetracycline, aminoglycoside, or rifampicin has ever reached the clinic. Although total syntheses have been described for these molecules, no comprehensive SAR/STR has been established to date. From a chemist's structural perspective these classes are not "old" at all! Serious de novo synthetic explorations would most likely yield novel drug candidates.

De novo synthesis has demonstrated its impact in some therapeutic areas (antitumor, antiviral). The "cost-effective" de novo solid-phase synthesis of enfuvirtide, an anti-HIV peptide composed of 36 amino acids, in 106 steps is most impressive.[156] Recent masterpieces in the chemistry of epothilones,[157] ecteinascidin 743, and discodermolide demonstrated that it is indeed possible, when academia and industry cooperate, to employ complex de novo syntheses in the development of new drugs.

Table 7: Perception of de novo synthesis and semisynthesis of antibacterial natural products.

	De novo synthesis	Semisynthesis
perception	total synthesis, art in organic chemistry—"create"	derivatization, tool for organic chemistry—"fix problems"
literature	uncountable	underrepresented (scattered in patents)
timelines	slow	fast
mandatory supply with natural-product lead compound	only small amounts of reference material needed	larger quantities of "starting material" crucial (fermentation and isolation)
structure–activity relationship	global and comprehensive	locally restricted
biology of products	bioactivity "guaranteed"	mostly inactive congeners
disadvantage	long synthetic routes	selectivity issues (handle a minefield of functionality)
advantage	controlled selectivity (orthogonal protecting groups)	short synthetic routes
experience with functional natural product	in the last synthetic step	all the time
technical development	"cost of goods" critical	proven economic significance

6.7. Semisynthesis is Key in Industry

Semisynthesis can only address a limited chemical space of the natural-product lead structure. But it is fast and apparently straightforward. In practice, semisynthesis is the method of choice for lead optimization in the exploratory project phase with complex natural products. Experience and today's analytical techniques (HPLC, LC-ESI-MS, NMR spectroscopy) allow for reactions in the 100-μg scale for initial SAR studies even with complex molecules (microderivatization). Preliminary in vitro testing can be done with less than 1 mg of material (MIC, cytotoxicity). Also in late-stage industrial development (kg scale), semisynthesis is often the common logical consequence of structural complexity, timelines, and "cost of goods" estimates. Many marketed antibacterial drugs are semisynthetic congeners of natural products, and are obtained from the chemical refinement of

fermentation products (oritavancin, tigecyclin,[21] telithromycin, rifampicin).[4]

6.8. The Modest Life of Semisynthesis in Academia

Derivatization chemistry is still under-represented in chemical literature despite its unrivalled importance for the drug discovery process. Although the chemical literature thrives on total syntheses, only few organic chemists from academia seem to be attracted by the semisynthetic elaboration of complex natural products. In the early 20th century, pioneers like Willstätter and Robinson groped their way through the constitution of complex alkaloids by doing decoration and degradation reactions.[158] Today, derivatization is regarded to be a simple tool for fixing problems in structure elucidation when spectroscopy alone cannot do the job (Mosher ester for NMR spectroscopy, persilylation for GC analysis, and heavy atoms for X-ray analysis).

Only total synthesis, which creates complex matter from scratch or thought, is cherished as "art in chemistry". Yet, derivatization of complex natural products is a highly demanding scientific task that can only be accomplished with state-of-the-art chemistry and analytics. When looking at the target molecule, the derivatization chemist is confronted with a minefield of unprotected functionality at a stage of high complexity. Every chemical operation planned may be spoiled by selectivity issues. Conversely, in total synthesis, troublesome functionalities are blocked with orthogonal protecting groups from the very beginning when it is still at a stage of low complexity. Typically, de novo synthesis deals with the real natural product only in the last, often redeeming, step. Throughout total synthesis, protecting groups block the natural chemical functionality that is important for biological activity. In contrast, during semisynthesis, most chemical experience is gained with the total and accordingly functional structure, as it has been invented by nature for target interaction. In this way, the medicinal chemist learns about the architecture and intrinsic reactivities of a natural-product lead that cannot be deduced by just looking at the Lewis structure or by working with (protected) fragments.

When starting the total synthesis of a natural antibiotic, bioactivity is often guaranteed for target molecules that come from literature. Instead, many semisynthetic analogues of natural products are inactive and not appreciated. In general, total syntheses towards natural products are published in high-impact journals, whereas semisynthesized natural-product analogues and mimetics face higher hurdles. The risk to explore the unpublishable is high in semisynthesis and the exploration of natural-product congeners.

6.9. Principle Types of Structural Modification—What is the best strategy?

Natural products may be tailored in a direct fashion by chemical derivatization (structural modification). Furthermore, formal products of structural modification may be obtained through de novo synthesis. The success of a natural-product chemistry program strongly depends on the chosen concept of modification. We differentiate three principle types of structural modification: 1) decorating modification (attach), 2) degrading modification (cut out), and 3) substituting modification (cut out and reattach). Various synthetic congeners of natural products are intermediate cases that have been obtained by combinations of several modification types. For example, telithromycin is obtained by degrading and decorating modification of the natural product erythromycin. There is no defined border between modification products and mimetics of natural-product lead structures (see Table 8).

Decorating modification is the most popular form of structural modification in medicinal chemistry, especially in semisynthesis. Functional groups presented by the "natural-product template" are used as anchors to attach additional, non-natural residues. Reductive alkylations, acylations, PEGylations,[159] and hydrogenations are "classical" reaction types of decorating derivatization. For example, various glycopeptides from the vancomycin series have been decorated with lipophilic biphenyl residues (oritavancin) through reductive alkylation, leading to a modified mode of action. Solubility may be increased by adding charged residues. Usually, regio- and chemoselectivity is a serious issue when decorating complex structures as they present a whole ensemble of, naturally free, functional groups. In these cases, the laborious handling of protecting groups is a prerequisite for sufficient chemical selectivity and acceptable yields. With smaller natural products, decorating derivatization often fails to yield bioactive congeners for a different reason:[160] the few functional groups present are important for target interaction and decorating the molecule merely results in the futile masking of pharmacophores. This kind of chemical "baroquization" has stigmatized many natural products as being nonoptimizable.

Substituting modification allows a deeper exploration of the natural-product lead. This is an especially powerful methodology in semisynthesis. In a first move, parts of the molecule are cut out by chemical means. Then, the gap is filled by reattachment of bioisosteric building blocks, mimetic structures, or abiotic constructs. This strategy has several major advantages: not only are peripheral functional groups of the chemical lead addressed, but even core modifications of the carbon skeleton are possible. Whether critical biological parameters can be tuned is assessed on the basis of a complete, but locally restricted, SAR map. Substituting derivatization is more tempting than decoration as a thorough understanding of reactivity within the natural-product lead is crucial. When selective degradation reactions are unavailable, de novo synthesis is the way to advance. For decades, penicillins have proven the high potential of substituting derivatization.

Degrading modification is successful when structurally defined toxophores or labile groups that are not simultaneously pharmacophores can be cut out of the molecule by chemical means. Unfavorable parts of the natural-product lead, not important for target interaction, may be degraded to obtain a drug of higher stability (ramoplanin aglycon) or lower structural complexity. However, chemoselectivity prob-

Table 8: Modification of natural-product lead structures through semisynthesis and de novo synthesis. Type and site of modification (blue) and progress achieved.

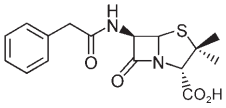
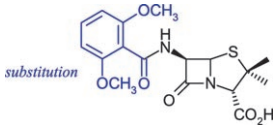
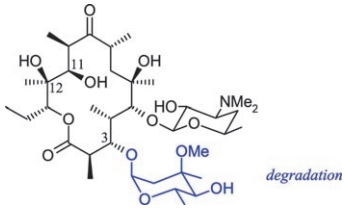
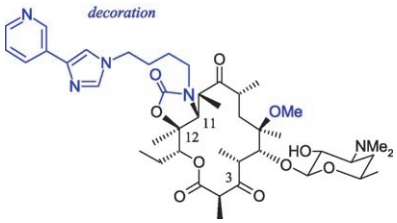
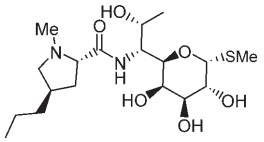
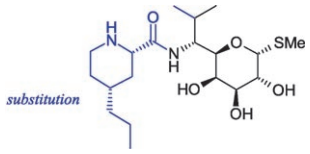
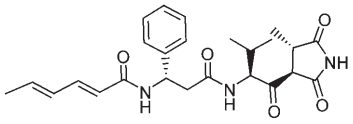
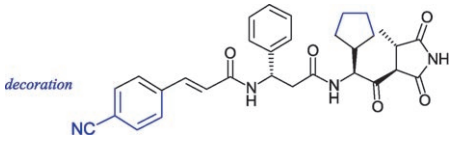
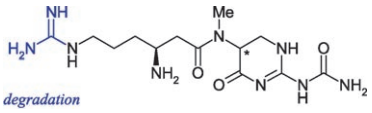
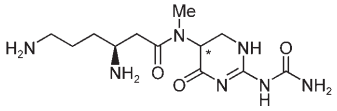
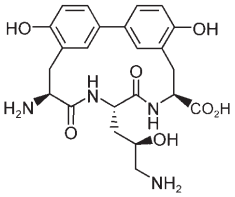
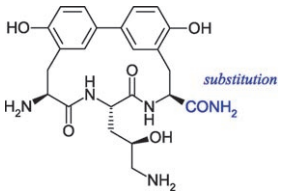
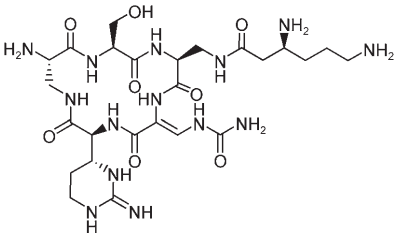
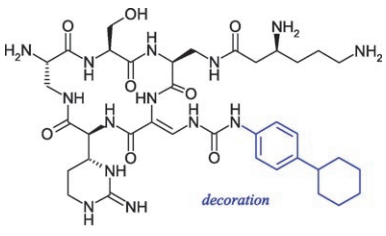
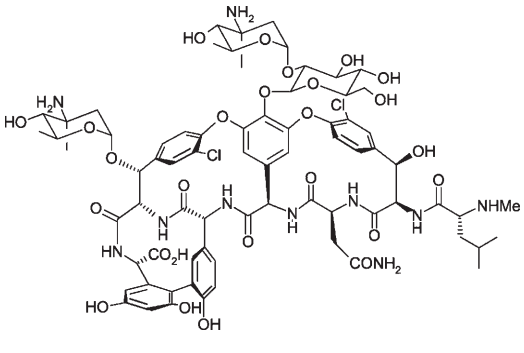
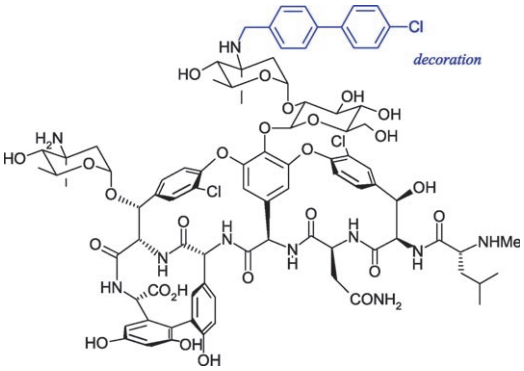
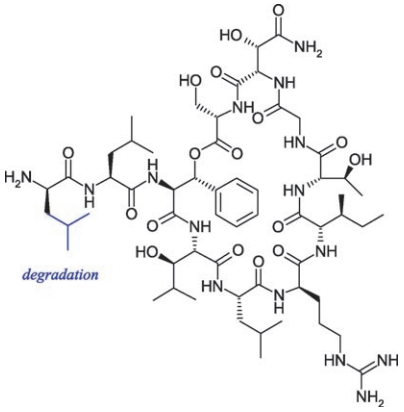
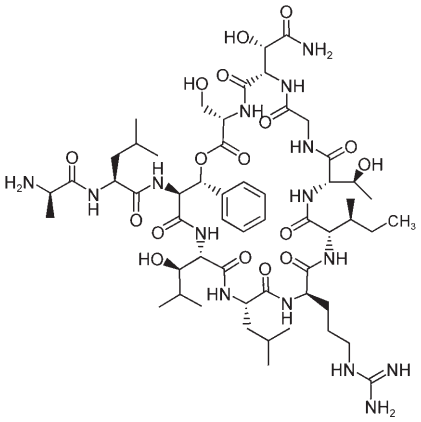
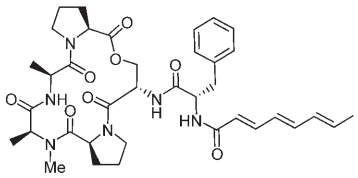
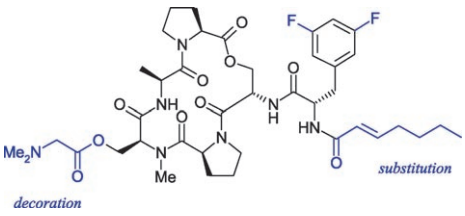
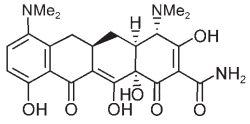
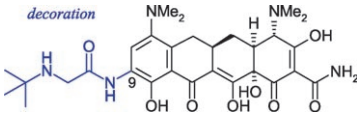
Natural-product lead structure	Synthetic congener	Type	Progress
 penicillin G (**2**)	 methicillin (**14**)	substituting modification through semisynthesis, mutasynthesis, and de novo synthesis	increased stability
 erythromycin A (**6**)	 telithromycin (**15**)	degrading and decorating modification; degradation of cladinose; decoration of C11/C12 and C6 area	new binding mode, low cross-resistance with erythromycin, stability in acidic medium
 lincomycin (**16**)	 VIC-105555 (**17**)	substituting modification through semisynthesis	reduced resistance induction
 moiramide B (**18**)	 **19**	decorating modification via de novo synthesis	improved potency and stability
 TAN-1057A/B (**20**)	 **13**	degrading modification of the toxophore through de novo synthesis	reduced toxicity
 biphenomycin B (**21**)	 **22**	substituting modification through semisynthesis and de novo synthesis	improved antibacterial spectrum
 capreomycin 1 A (**23**)	 **24** ($R^1 = OH$)	decorating modification through semisynthesis	improved antibacterial spectrum and potency

Table 8: (Continued)

Natural-product lead structure	Synthetic congener	Type	Progress
 chloroeremomycin (**25**)	 oritavancin (**26**)	decorating modification through semi-synthesis	additional mode of action, extended antibacterial spectrum
 lysobactin (**27**)	 [D-Ala^1]lysobactin (**28**)	degrading modification within the linear segment through semi-synthesis; Edman degradation	preliminary SAR
 A 54556 B (**29**)	 **30**	decorating and substituting modification through de novo synthesis	improved stability, solubility, and potency
 minocycline (**31**)	 tigecycline (**32**)	decorating modification through semi-synthesis	beat resistance by better binding to ribosome

lems often thwart "direct" semisynthetic degradation. In many cases, de novo synthesis is the method of choice for truncated derivatives of natural products. For example, as a result of guanidine degradation, **13** showed significantly reduced toxicity with respect to the natural-product lead TAN-1057 (**20**, Section 12). Yet, **13** was obtained by de novo synthesis and is just a formal degradation product of **20**.

7. β-Lactam Antibiotics

The β-lactam group of antibiotics was the first class of antibacterial natural products introduced as a therapeutic treatment of bacterial infections. Today, more than 75 years after Fleming's discovery of penicillin from cultures of *Penicillium notatum*,[161] this group still includes the clinically most-widely-used agents and accounts for about half of all antibacterial drugs prescribed (Figure 8).

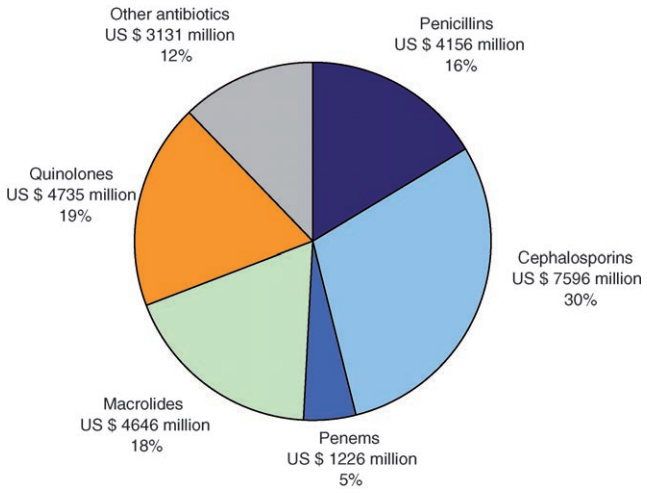
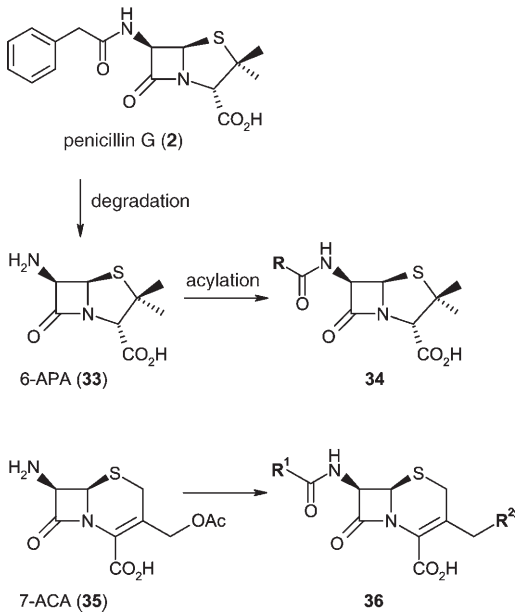


Figure 8. Global sales of the major antibacterial classes in 2004 (from Wood Mackenzie[69]).



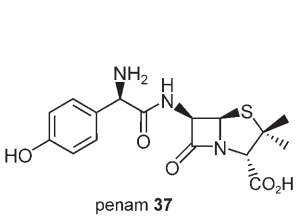
Scheme 1. Semisynthetic modifications of building blocks 6-APA (**33**) and 7-ACA (**35**) through a substituting-derivatization strategy.

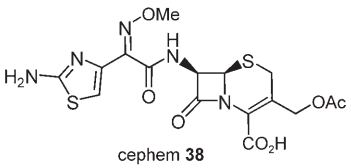
Owing to their broad antibacterial spectrum, their clinical efficacy, and their excellent safety profile, β-lactam antibiotics have been one of the preeminent areas of pharmaceutical drug discovery. Their compact structural density, along with their sensitivity, has provided a challenging "playground" for generations of medicinal chemists that has not lost its attractiveness even now. All β-lactam antibiotics share a common structural element, the four-membered azetidinone or β-lactam ring, their pivotal reference mark and center of action. In most antibiotics, this central β-lactam ring is fused to a second five- or six-membered ring system. In the biosynthetic multistep process to form penicillins and cephalosporins, nature employs L-valine, L-cysteine, and L-α-aminoadipic acid. With the the help of a modular nonribosomal peptide synthetase for the construction of an intermediate tripeptide that is oxidatively converted into the primary penicillin, isopenicillin N, by isopenicillin N synthetase.[162] Natural penicillin G (**2**), the first therapeutic antibiotic and lead structure of this class, still had a few critical features that needed improvement, for example, narrow antibacterial spectrum, instability in acidic (stomach) and basic (intestine) environments, limited solubility, pronounced sensitivity to hydrolysis by bacterial penicillase enzymes, and fast clearance from the body. Additional biosynthetic studies afforded the first orally applicable β-lactam antibiotic, penicillin V,[163] which has improved activity against staphylococci and better stability towards acids. However, not until the key building blocks 6-aminopenicillanic acid (6-APA, **33**)[164] and 7-amino cephalosporanic acid (7-ACA, **35**)[165] became readily available from high producing strains of *Penicillium chrysogenum*[164] by enzymatic cleavage of penicillin G (**2**) with penicillin acylase[166] or chemical synthesis, respectively, did medicinal chemists enter the infinite game of semisynthetic variations (Scheme 1).

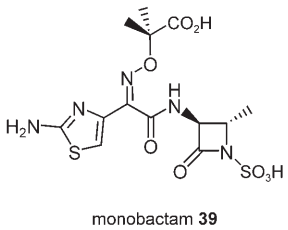
For about thirty years, penicillins (penams) **37**[167] and cephalosporins (cephems) **38**[167–169] remained the sole examples of β-lactam antibiotics. During a booming search and discovery period during the 1970s and 1980s, many related subgroups, such as the monobactams (aztreonam, **39**),[170] oxacephems (moxalactam, **40**),[167,171] carbacephems (loracarbef, **41**),[172] oxapenams (clavulanic acid, **42**),[173] penems (faropenem, **43**),[173a,174] carbapenems (imipenem, **44**),[170,175] and oxapenems (AM-112, **45**),[176] were discovered from microbes or were obtained by synthetic efforts. Endless structural variations of these β-lactam scaffolds provided derivatives with increased potency and improved physicochemical and pharmacokinetic profiles.[168] Many aspects and subgroups of this important research area have been extensively reviewed,[177] and therefore, only a few selected examples, issues, and references can be mentioned herein.

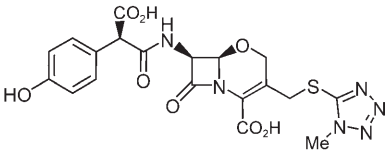
β-Lactam antibiotics inhibit bacterial growth by interacting with PBPs, enzymes that are normally involved in the terminal transpeptidation (cross-linking) steps of bacterial cell-wall biosynthesis.[178] The historic term penicillin-binding protein demonstrates how a class of antibiotics can serve to detect new targets (target fishing) and increase the understanding of biological processes. The enzymes mistake β-lactam antibiotics for the C-terminal L-Lys-D-Ala-D-Ala end of peptide chains yet to be cross-linked. Their active-site serine opens the β-lactam ring and blocks the PBP enzyme by forming an inert acyl enzyme intermediate.[179] As a result, well-defined peptidoglycan cross-linking damages occur that eventually kill susceptible microbes and in most cases lead to complete destruction of the bacterial cell by autolysins, bacterial cell-wall autolytic enzymes.[180]

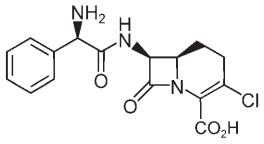
The peptidoglycan (or murein) sacculus is a flexible and enormously stable macromolecular meshwork that defines the shape of the bacterial cell and protects the microorganism against its high internal osmotic pressure.[181] The chemical structure of peptidoglycan varies among different bacteria. In most bacteria, peptidoglycan consists of strings of alternating β-1,4-linked *N*-acetyl-glucosamine and *N*-acetyl-muramic acids that are cross-linked by short peptide chains (Figure 3).[182] Peptidoglycan and related biosynthetic path-

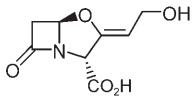

penam **37**

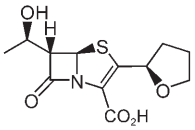

cephem **38**

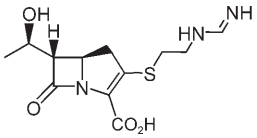

monobactam **39**

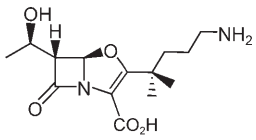

oxacephem **40**


carbacephem **41**


oxapenam **42**


penem **43**


carbapenem **44**

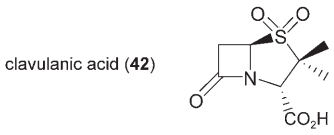

oxapenem **45**

marketed β-lactamase inhibitors, such as sulbactam (**46**)[177d] and tazobactam (**47**),[192] are not effective against class C enzymes. Most β-lactamase inhibitors are bad PBP binders and are themselves almost devoid of antibacterial activity.

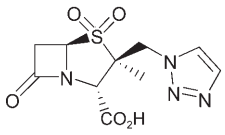
The stability of cephalosporins towards β-lactamases and the expansion of their antibacterial spectrum has been gradually improved during their chemical postevolution. Based on their efficacy and activity profile rather than on their structure, they have been grouped into four generations.[169] First-generation cephems, although active against Gram-positive staphylococci and streptococci, have only moderate activity against Gram-negative bacteria that do not produce β-lactamases. These Gram-negative limitations

ways do not exist in mammals. Therefore, β-lactam antibiotics, in general, have excellent safety profiles and often are the agents of choice for pediatric use. Furthermore, peptidoglycans or parts thereof warn our body about intruding bacteria and trigger an immune response.

The distinct activity of various β-lactam antibiotics arises from a combination of their different affinities to PBPs, their stability towards degrading β-lactamase enzymes, and differences in their physicochemical properties. These key features or combinations thereof also play a dominant role in the development of resistance. Resistance to β-lactam antibiotics can be caused by various events: An altered PBP, for example, in PRSP, attainment of an additional low-affinity PBP, such as PBP2a in methicillin-resistant *S. aureus* (MRSA),[183] diminished ability to penetrate bacterial cell walls, for example, in strains of *P. aeruginosa* lacking the outer membrane protein OprD (D2 porin),[184] activation of multiple-drug efflux systems, for example, MexAB-OprM in *P. aeruginosa*,[185] and production of β-lactamase enzymes, which inactivate the antibiotic by hydrolyzing its β-lactam ring.[186] Production of β-lactamases, in particular by Gram-negative bacteria is a significant cause of resistance to β-lactam antibiotics. At present, nearly 500 β-lactamases have been described and classified into four classes, A–D.[187] Classes A, C, and D are serine enzymes, whereas class B enzymes are zinc metallo-β-lactamases.[188] In particular, class A and also class C enzymes are of clinical relevance, but resistance in the other classes is on the rise. One successful therapeutic strategy has been to administer a β-lactamase inhibitor together with an antibiotic.[189] Augmentin, the combination of the β-lactamase inhibitor clavulanic acid (**42**) with amoxycillin (**37**), may serve as a representative and commercially unbeaten example.[190] Clavulanic acid (**42**), obtained from *Streptomyces clavuligerus*, remains one of the rare examples of an unaltered natural β-lactam that is used in therapy.[191] However, **42** and other

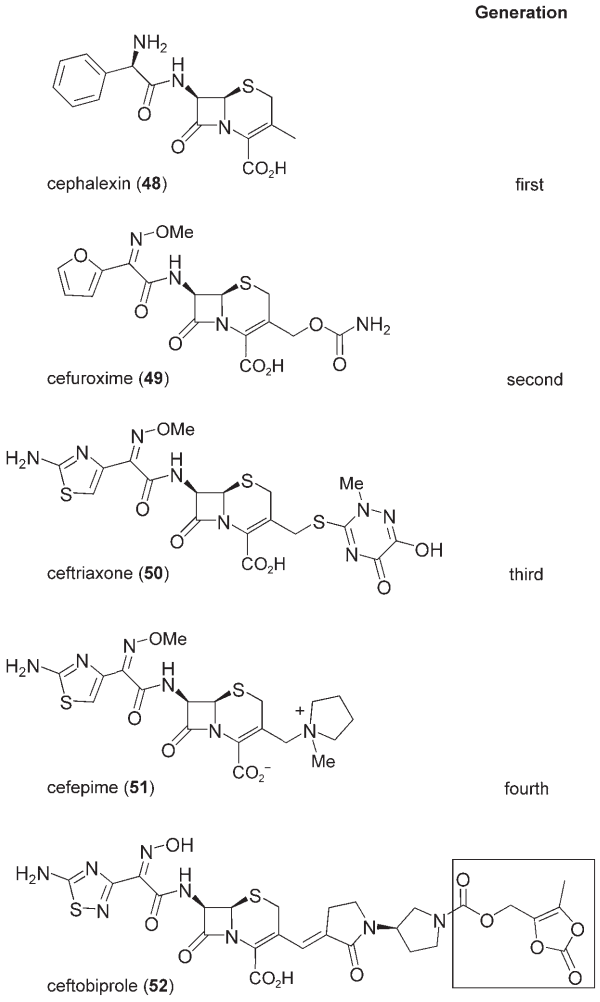

clavulanic acid (**42**)

sulbactam (**46**)


tazobactam (**47**)

have been improved in subsequent generations, albeit at the expense of their ability to cope with Gram-positive pathogens. In particular, the efficient and safe third-generation cephems, such as cefotaxime, cefixime, ceftibuten, and ceftriaxone (**50**), have been widely used in patients. Compared to earlier generations, fourth-generation cephalosporins, cefepime (**51**), and cefpirome, cover an extended Gram-negative antibacterial spectrum including multiple-drug-resistant *Enterobacter* and *Klebsiella* species.

Recently, a new group of so-called *anti*-MRSA-cephalosporins[193] has been added. In addition to the coverage of a Gram-negative spectrum comparable to third-generation drugs, the members of this group exhibit an extended Gram-positive spectrum, including, for the first time, useful activity against methicillin-resistant staphylococci. Ceftobiprole (BAL5788, **52**), the most-advanced representative, has recently completed phase-II clinical studies in patients with complicated skin and skin-structure infections (cSSSI).[169] Only cephalexin (**48**) is small and lipophilic enough to be orally available. All other examples are parenteral drugs. To achieve oral availability of large polar cephems, ester prodrugs, for example, cefuroxime axetil, have commonly been used.[194] After absorption from the gastrointestinal tract, the ester groups are rapidly cleaved and the active parent drug is released. In a different prodrug approach, the *N*-terminal carbamate function in **52** (see box) has been

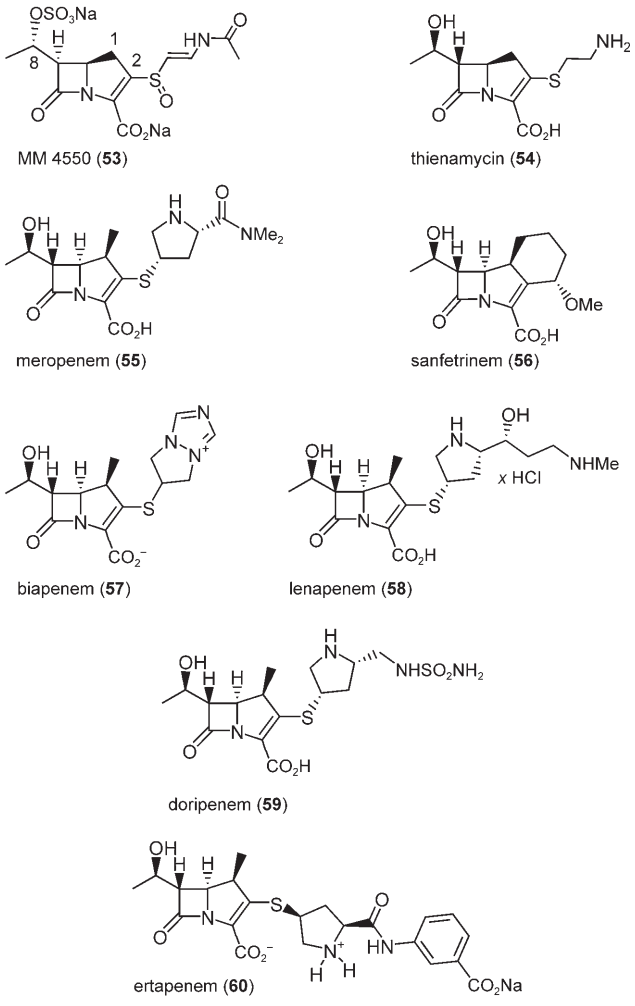


employed to achieve sufficient solubility for parenteral application of the active parent cephem BAL-9141.[169]

The carbapenems[175,177e] qualify as "first-line agents" for the treatment of severe nosocomial infections because of their excellent antibacterial activity, their very broad spectrum of activity, and their stability to most clinically relevant serine β-lactamases. Olivanic acids, such as MM 4550 (**53**)[195] and thienamycin (**54**)[196] were the first examples of this subgroup. The olivanic acids, discovered during a screening program for β-lactamase inhibitors from *Streptomyces olivaceus*, were *cis*-substited β-lactams that, in addition to their ability to inhibit β-lactamases, also exhibited broad-spectrum antibacterial activity.[197] However, they were too unstable for clinical application.

The first carbapenem introduced into clinical therapy was imipenem (**44**), the *N*-formimidoyl derivative of **54**, another highly unstable natural antibiotic with a free terminal amino group and a *trans*-substited β-lactam ring. Its *trans*-β-lactam substitution and 8*R* rather than 8*S* stereochemistry improved antibacterial activity without sacrificing the stability to β-lactamases. Thienamycin (**54**) was isolated from cultures of *Streptomyces cattleya* during a soil-screening program for inhibitors of peptidoglycan synthesis[198] and shortly after, its first total synthesis was reported.[199] Imipenem's basic amidino group is protonated at physiological pH, rendering it more stable than the natural antibiotic **54**. These pioneering works have served as a starting shot for numerous companies and academic groups to enter carbapenem research programs in the 1980s. In contrast to other β-lactams, carbapenems are stable to most clinically relevant β-lactamases.[200] In general, only class B and D enzymes are able to inactivate them.[201] However, besides creating stable and potent derivatives of sensitive natural products, a fair share of additional problems were waiting to be solved by medicinal chemists. As **44** was hydrolyzed in vivo by human renal dehydropeptidase I, it had to be administered as a 1:1 combination with the dehydropeptidase inhibitor cilastatin to prevent inactivation of the antibiotic.[202]

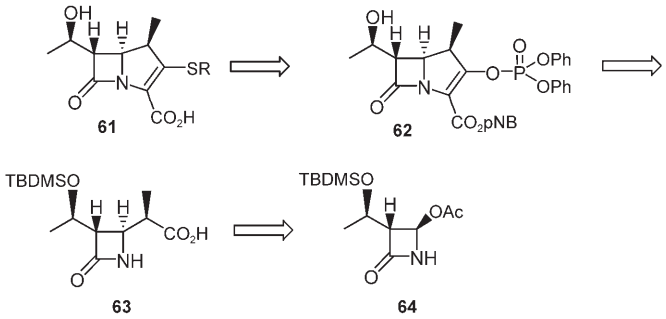
Panipenem, the next carbapenem to be approved in Japan, suffered from the same drawback, and likewise required coadministration of a dehydropeptidase I inhibitor, betamipron.[203] The medicinal chemists next goal was to combine excellent broad-spectrum activity with chemical and enzymatic stability in one single molecule. This was achieved with meropenem (**55**), the first clinical carbapenem with a β-methyl group in position 1.[204] 1-β-Methyl substitution rendered carbapenems stable to hydrolytic degradation by renal dehydropeptidase I, a discovery that was immediately taken



up and explored through related structural variations. For example, the 1-β-methyl group could also be part of a third fused ring, revealing novel tricyclic "tribactams" or "trinems" such as sanfetrinem (GV 104326, **56**).[205]

Nevertheless, most structural exploration efforts focused on modification of the 2-position. Further parenteral carbapenems, such as biapenem (L-627, **57**),[206] lenapenem (BO-2727, **58**),[207] doripenem (S-4661, **59**),[208] ertapenem (MK-826, **60**),[209] and others,[175] followed in a fascinating process of chemical postevolution. Ertapenem (**60**), a parenteral 1-β-methyl carbapenem with a longer serum half-life (once daily) than **44** and **55** may serve as an example for a drug with a distinct pharmacokinetic improvement.[209b]

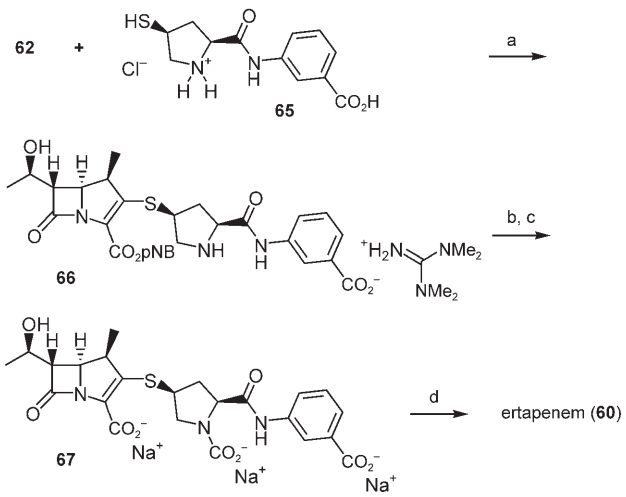
Technically, carbapenem antibiotics have been produced by de novo syntheses rather than by fermentation processes. The synthetic access to 1-β-methyl carbapenems was pioneered by chemists in the Merck company who, by their retrosynthetic concept, defined 4-nitrobenzyl-protected 1-β-methyl carbapenem enolphosphate **62**, carboxyethyl-azetidinone **63**, and acetoxy azetidinone **64** as key building blocks (Scheme 2).[210] Many synthetic pathways toward these crucial intermediates have been elaborated since.[211] Today, they are commercially available on a large scale, in particular from Japanese companies.



Scheme 2. Retrosynthesis of 1-β-methyl carbapenems.[210]

Ertapenem's chiral thiol side chain **65** has been obtained in an efficient one-pot synthesis amenable for large-scale production.[212] Coupling of enol phosphate **62** with unprotected thiol **65** to afford ertapenem sodium (**60**) proved to be delicate owing to the presence of acidic and basic functions. Nonetheless, Merck chemists devised an efficient reaction that minimized the use of protecting groups[213] and employed tetramethylguanidine as a base. The use of carbon dioxide during the hydrogenolysis of the 4-nitrobenzyl ester at pH 8 enabled transient protection of the pyrrolidine as sodium carbamate. Ion-pair extraction and crystallization under mild conditions concluded this challenging task (Scheme 3).

Sensitive β-lactam intermediates and products often require particularly mild synthetic methods.[214] As a "principle", reactions that work with β-lactams seem to work with almost any other structure, but not vice versa! Nevertheless, the very favorable clinical experiences with marketed carbapenems have rewarded medicinal chemists for many challenging syntheses and difficulties during development. β-Lactam antibiotics continue to be one of the most important



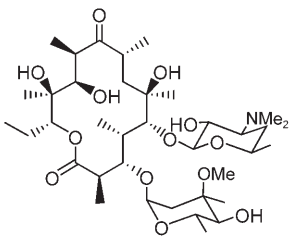
Scheme 3. The Merck synthesis of ertapenem (MK-0826, **60**), a novel carbapenem approved in 2001.[213] Reagents and conditions: a) tetramethylguanidine, *N*-ethylpyrrolidone, −40 °C, 3 h; b) H_2, 5% Pd/C, CO_2, pH 8, below 15 °C; c) $(PhO)_2P(O)OH$, 50% NaOH, isoamyl alcohol; d) 1-propanol, below −5 °C, pH 5.5.

classes in antibacterial research and development. Their efficacy and compatibility has allowed their broad therapeutic application in the community and in hospital environments including on children and elderly patients.

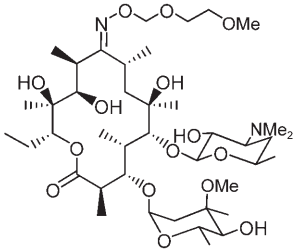
8. Macrolide and Ketolide Antibiotics

Macrolide antibiotics, a subgroup of the polyketide natural products, are an important class of therapeutic agents that act against community-acquired respiratory infections such as community-acquired pneumonia (CAP), acute bacterial exacerbations of chronic bronchitis, acute sinusitis, otitis media, and tonsillitis/pharyngitis.[215,216] *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are the predominant pathogens involved in this diseased state. Despite their bacteriostatic mode of action, macrolides account for about 20% of all the antibiotics prescribed. Macrolides have a unique distribution with very high concentrations in lung tissue. Many aspects of the macrolides have been thoroughly studied and competently reviewed in numerous articles[217] and books.[215,218] Herein, we intend to focus on a few selected contributions by medicinal chemistry.

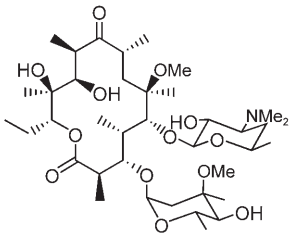
Erythromycin A (**6**), the prototype and ancestor of the macrolide family, was first isolated from *Streptomyces erythreus* discovered in a soil sample taken from the Philippine Archipelago by scientists from the company Lilly in 1952.[219] The structure of **6**, the major and most important component produced during fermentation along with its congeners erythromycin B and C, was subsequently elucidated[220,221] and its absolute configuration was established by NMR spectroscopy studies[222] and X-ray crystallographic analysis.[223] Immediately, its complex structure with two unusual sugars, L-cladinose and D-desosamine, attached to a 14-membered lactone ring with 10 asymmetric centers, attracted



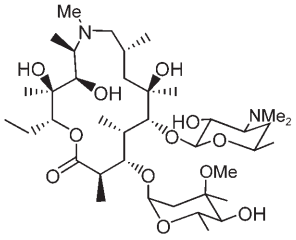
erythromycin A (**6**)



roxithromycin (**68**)



clarithromycin (**69**)



azithromycin (**70**)

the synthetic chemists' attention and resulted in one of the milestones in synthetic chemistry: Corey's total synthesis of erythronolide A in 1979[224] and Woodward's asymmetric total synthesis of **6** in 1981.[225]

Despite the creation of the principle tools for macrolide de novo synthesis, semisynthesis has been economically more viable and has remained the basis for all marketed macrolide (and ketolide) antibiotics to date.

In many respects, polyketide biosynthesis,[226] with erythromycin as the best-studied example,[227] resembles the biosynthesis of fatty acids. Macrolide antibiotics are derived from successive condensations of acetate, propionate, and butyrate building blocks put together with the help of modular polyketide synthases.[226,228] The resulting linear acyl chains cyclize through lactonization, and the final modification of the backbone proceeds in a highly controlled fashion. The modular organization of the underlying structural genes has facillitated the alteration of the structure of complex polyketides in a predicted manner and enabled an increase in molecular diversity through combinatorial biosynthesis.[229,78]

Macrolide antibiotics block bacterial protein biosynthesis by binding to the 23S ribosomal RNA of the 50S subunit and interfere with the elongation of nascent peptide chains during translation.[230] The crystal structure of the 50S ribosomal subunit of *Deinococcus radiodurans* alone and cocrystallized with erythromycin and other macrolides has been elucidated and revealed how these antibiotics work.[231] Located in domain V, near the peptidyl transferase site, macrolide antibiotics obstruct the peptide exit tunnel without affecting peptidyl transferase activity.

Erythromycin A (**6**), which helps against the major respiratory pathogens, is considered safe and is widely prescribed for children. However, it is stalled by a limited antibacterial spectrum and limited stability in acidic medium, therefore resulting in poor bioavailability and a number of side effects such as gastrointestinal motility, proarrhythmic action, and inhibition of drug metabolism.[232] Therefore, for decades enormous efforts have been made to obtain natural and semisynthetic erythromycin derivatives with more favorable profiles. The second-generation macrolide antibiotics, roxithromycin (**68**),[233] clarithromycin (**69**),[234] and azithromycin (**70**),[235] have gradually replaced **6** owing to their broader spectrum of activity, enhanced activity, improved physicochemical and pharmacokinetic profile, and attenuated side effects.[236]

However, similar to **6**, the second-generation variants also have poor activity against macrolide resistant pathogens. Macrolide resistance in Gram-positive bacteria[237] arises from several mechanisms: 1) decrease of intracellular macrolide concentration by efflux pumps such as *msr*(A) in staphylococci[238] and *mef*(A) in streptococci;[239] 2) methylation of the 23S ribosomal RNA at position A2058 by ribosomal methylases encoded by *erm* genes that obstructs binding of macrolides, lincosamides, and streptogramin B and causes MLS_B cross-resistance;[240] 3) esterase-mediated cleavage and inactivation of the macrocyclic lactone ring;[241] and 4) sporadic ribosome mutations.

Fueled by the prevalence of erythromycin resistance, the sustained efforts to discover new and potent structural subgroups within the macrolide family have led to the rediscovery of the ketolide antibiotics. Ketolides[242] are derived from 14-membered macrolides by removal of L-cladinose under acidic conditions and selective oxidation of the resulting 3-hydroxy group to the corresponding carbonyl group. Although the 3-keto motif has been known from weak natural antibiotics, such as picromycin,[243] for many years, the presence of L-cladinose was erroneously regarded as a crucial structural element for antibacterial activity. Thus, its far-reaching consequences only became apparent with the first semisynthetic ketolide RU-64004 (HMR 3004, **71**) synthesized by chemists at Roussel Uclaf.[244] Essentially, this prototype ketolide was stable in acidic media, showed good intracellular penetration, and demonstrated potent activity against erythromycin A resistant and penicillin-resistant streptococci and *H. influenzae*. Furthermore, it was found not to induce the MLS_B resistance phenotype.

Systematic exploration of the SAR options of the ketolide backbone unveiled various novel ketolide series with potent activity and an improved pharmacokinetic profile:[245–247] generally, the exchange of L-cladinose with a carbonyl function at C3 increased activity against resistant strains with Mef-mediated efflux and erm methyltransferase. Introduction of a C2 fluoro group substituent enhanced activity and improved the pharmacokinetics. A C11/12 cyclic carbamate group enhanced the activity against susceptible and resistant strains by stabilizing the ketolide conformation, and the hetero-aryl groups were responsible for improved activity against erm methylase mediated resistance owing to additional interaction and enhanced affinity for methylated ribosomes.[244] Structural modifications of the crucial heterocyclic moiety were systematically investigated and telithromycin (**15**, HMR 3647, Sanofi Aventis),[248] cethromycin (**72**, ABT-773, Abbott),[249] and subsequently the 6,11-*O*-bridged

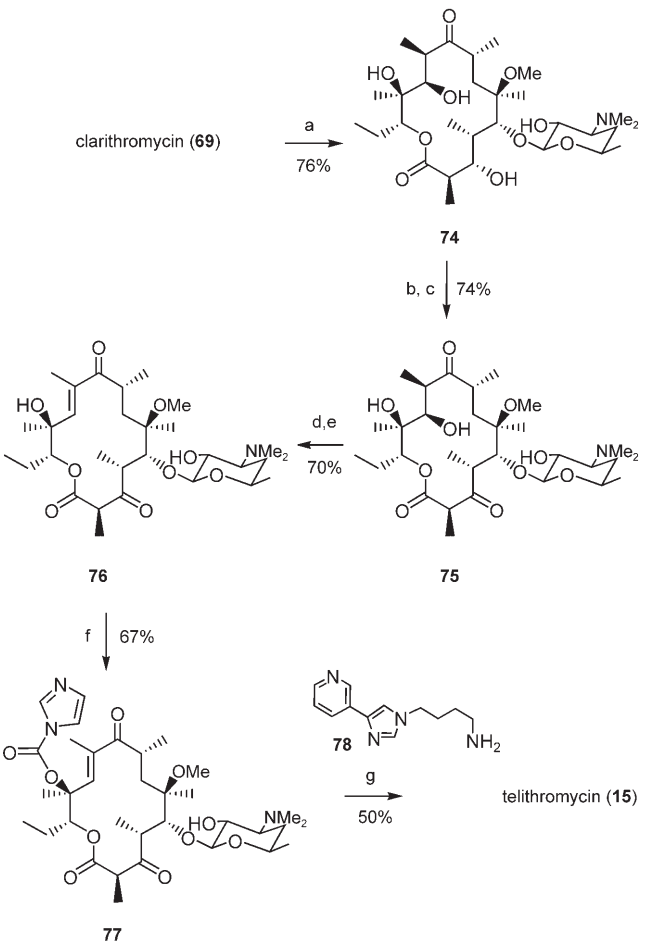
oxime ketolide EP-013420 (**73**, Enanta)[250] emerged as the leading compounds.

Typically, the ketolide telithromycin (**15**) was synthesized from 6-*O*-methylerythromycin (clarithromycin, **69**) in eight steps (Scheme 4):[244b,248,251] hydrolytic cleavage of L-cladinose under acidic conditions and modified Pfitzner-Moffat[252] oxidation of the resulting 3-hydroxy group afforded ketolide **75** that was selectively mesylated and eliminated to give enone **76**. Reaction of the corresponding acylimidazolyl derivative **77** with the primary amine **78** after stereoselective intramolecular Michael addition provided the cyclic carbamate **15**.

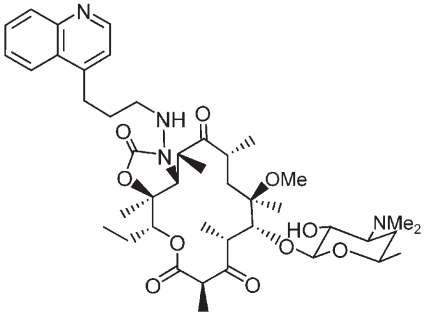
Telithromycin (**15**) was the first ketolide to be approved in Europe (2001), Japan (2003), and in the US (2004) for the once-daily oral treatment of respiratory tract infections such as acute exacerbation of chronic bronchitis due to *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*, acute bacterial sinusitis, and mild-to-moderate CAP, including infections caused by multidrug-resistant *S. pneumoniae*. The development of **72** was suspended during phase III clinical studies whereas **73** is currently in phase II trials. However, all ketolides lack activity against *E. coli* and constitutively MLS_B-resistant strains of *S. aureus* (Table 9).

The in vivo efficacy of **15** in acute murine infection models caused by erythromycin-sensitive Gram-positive cocci was comparable to **69**, but clearly superior against erythromycin-resistant strains (Table 10).

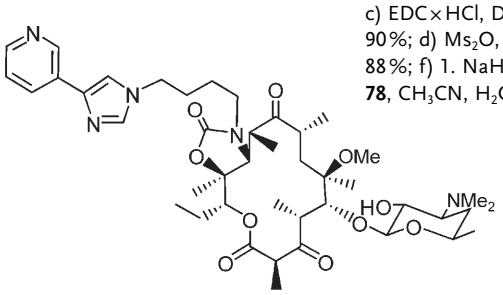
The main challenges for the uptake of a ketolide in the market will be its differentiation from other classes such as macrolides, fluoroquinolones, and β-lactam antibiotics that currently dominate the RTI segment. Approval for pediatrics and the development of a parenteral formulation could expand its promising market potential.[248c]



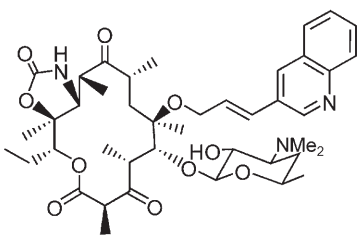
Scheme 4. Semisynthesis of telithromycin (**15**) from clarithromycin (**69**).[244b,248,251] Reagents and conditions: a) 1. 12 N HCl, H_2O, 2 h, RT; 2. NH_4OH pH 8, 76%; b) Ac_2O, K_2CO_3, acetone, 20 h, RT, 82%; c) EDC×HCl, DMSO, CH_2Cl_2, pyridinium trifluoroacetate, 4 h, RT, 90%; d) Ms_2O, pyridine, 5 h, RT, 79%; e) DBU, acetone, 20 h, RT, 88%; f) 1. NaH, DMF, CDI, −10°C, 1 h; 2. H_2O, 0°C, 67%; g) amine **78**, CH_3CN, H_2O, 60°C, 50%.



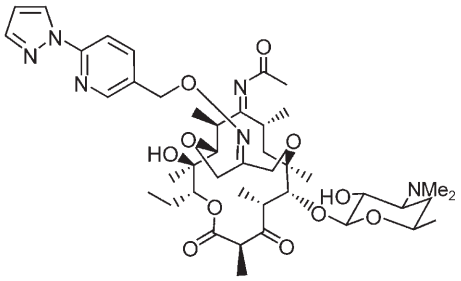
RU-64004 (**71**)



telithromycin (**15**)



cethromycin (**72**)



EP-013420 (**73**)

9. Lincosamides

Lincomycin (**16**) and and its semisynthetic congener clindamycin (**79**) were introduced into clinical medicine as oral

Table 9: Antibacterial activity in vitro of macrolide clarithromycin (**69**) versus ketolide telithromycin (**15**), MIC [$\mu g\,mL^{-1}$].[248]

	S. aureus[a]	*S. aureus*[b]	*S. aureus*[c]	*S. pneum.*[a]	*S. pneum.*[b]	*S. pneum.*[c]	*H. infl.*
69	0.3	40	40	0.04	40	40	5
15	0.04	0.08	40	0.02	0.02	0.15	1.2

[a] Erythromycin susceptible. [b] Inducibly erythromycin resistant. [c] Constitutively erythromycin resistant.

Table 10: Efficacy in vivo of macrolide clarithromycin (**69**) versus ketolide telithromycin (**15**), ED_{50} [$mg\,kg^{-1}$].[248]

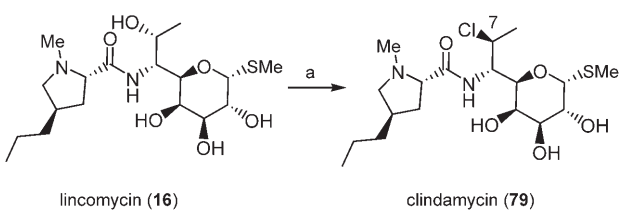
	S. aureus[a]	*S. aureus*[b]	*S. aureus*[c]	*S. pneum.*[a]	*S. pneum.*[b]	*S. pneum.*[c]	*H. infl.*
69	6	55	n.d.[d]	7.5	>50	>50	120
15	10	4.5	n.d.[d]	1	4	0.15	57

[a] Erythromycin susceptible. [b] Inducibly erythromycin resistant. [c] Constitutively erythromycin resistant. [d] n.d. = not done.

antibiotics in 1960 and 1969, respectively.[253] Extensions for parenteral applications followed shortly after. These low-molecular-weight antibacterials exhibit a spectrum similar to that of the macrolides, including activity against most Gram-positive organisms and the anaerobes, but not the Gram-negatives and enterococci.[254] The use of lincomycin **16** and clindamycin **79** strongly declined after a period of extensive application against severe staphylococcal sepsis, anaerobic infections and, in combination with other antibiotics, severe intra-abdominal or opportunistic sepsis.[255] Today, their use is mainly limited to topical applications. The relegation of both drugs from first-line antibiotics to niche products can be attributed to their limited antibacterial spectrum, the emergence of resistance, and mainly to a severe side effect of this class, the development of pseudomembraneous colitis in some patients.[256] This side effect is caused by *Clostridium difficile,* which is not covered by the antibacterial spectrum of lincosamides. Consequently, closure of this spectrum gap would be desirable.

Clindamycin (**79**)[257,258] is a semisynthetic derivative of the natural product lincomycin (**16**), which is produced by fermentation of *Streptomyces lincolnensis*,[259] (Scheme 5). This transformation is remarkable as one secondary alcohol is transformed selectively in the presence of three others. Selective transformations of polyfunctional natural products to generate SAR data are important tools for chemical postevolution. Maintaining a similar, favorable acute-toxicity profile, **79** is about two- to fourfold more potent than its parent **16** (Table 11).[261]

Both drugs, **16** and **79**, demonstrated favorable pharmacokinetics in laboratory animals and humans,[262,263] with excellent bioavailability and penetration into relevant tissues. Their solubility and stability in solution allowed for oral and parenteral application as well, but their relatively short half-life (2–4 h) required twice-daily (peroral) or even three-times-daily (i.v.) dosing. Thus, an improved pharmacokinetic profile, which would allow once-daily dosing, would be highly desirable for novel lincosamides.



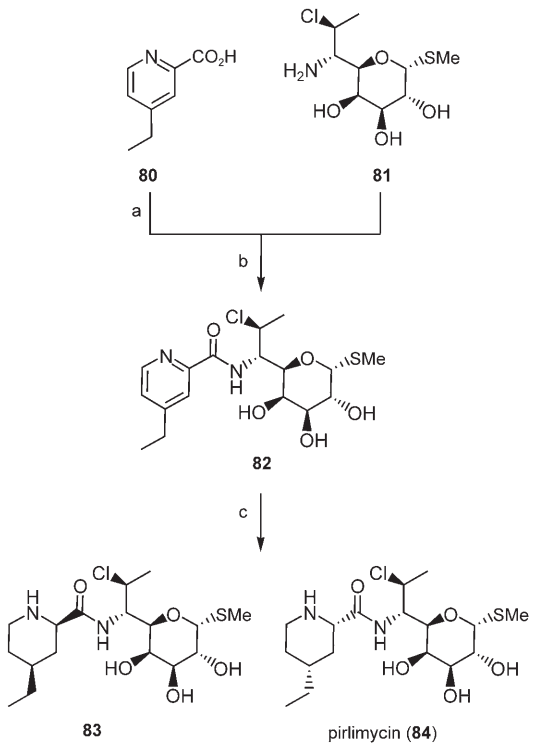
Scheme 5. Synthesis of clindamycin (**79**) from the natural product lincomycin (**16**).[260] Reagents and conditions: a) *N*-chlorosuccinimide, PPh_3, THF, reflux, 18 h, 84%.

Table 11: Antibacterial activity in vitro of lincomycin (**16**) and clindamycin (**79**), MIC [$\mu g\,mL^{-1}$].

Organism	Lincomycin (**16**)	Clindamycin (**79**)
S. aureus	0.5	0.125
S. hemolyticus	0.25	0.125
S. viridans	0.25	0.064
B. subtilis	32	1
E. coli	1000	64
K. pneumoniae	125	8

Lincosamides exert their antibacterial activity by binding to the ribosome and inhibiting bacterial protein synthesis. Specifically, macrolides, lincosamides, and streptogramin B type antibiotics bind to adjacent sites on the 50S ribosomal subunit. X-ray crystal structures of the complexes of the bacterial ribosome with these antibiotics have been solved.[264,265] Not surprisingly, resistance development of **79** is similar to the macrolides owing to a similar binding mode. Yet, the rate of resistance development for **79** is lower than that for erythromycin. Resistance occurs mainly by methylation of A2058 on 23S rRNA (erm methyltransferase), which reduces the binding affinity of lincosamides and macrolides to the ribosomal target. It may be speculated that resistance may be overcome, in analogy to the ketolides, with derivatives that bind slightly differently. Finding second-generation lincosamides with an extended antibacterial spectrum (including enterococci-, MRSA-, and clindamycin-resistant strains), an improved side-effect profile (coverage of *C. difficile* to prohibit pseudomembraneous colitis), and superior pharmacokinetics (once-daily dosing) remains a rewarding goal.

Semisynthetic pirlimycin (**84**, Scheme 6), prepared at Upjohn with a substituting derivatization strategy,[266] showed some of the desired characteristics. Dosed intraperitoneally, the 50% lethal dose (LD_{50}) for **84** in mice was 600 $mg\,kg^{-1}$, clearly superior to clindamycin (300 $mg\,kg^{-1}$). Furthermore, structural changes in **84**, lacking the metabolically labile *N*-methyl group of **79**, significantly improved the



Scheme 6. Upjohn's synthesis of pirlimycin (**84**) through substituting derivatization. Reagents and conditions: a) isobutyl chloroformate, Et_3N, CH_3CN, 10 °C, 1 h; b) **81**, acetone, H_2O, 25 °C, 18 h; c) MeOH, H_2O, 1 N HCl, PtO_2, H_2 (50 psi), 25 °C, 18 h; separation of diastereomers, 40% of **84**.

pharmacokinetic behavior resulting in higher plasma levels and a prolonged half-life. Although **79** and **84** covered an almost identical in vitro antibacterial spectrum, the latter was 2–20-times more potent in various animal infection models, most likely owing to an improved pharmacokinetic profile.[266] But, as with all lincosamides, pirlimycin (**84**) was still inactive against enterococci and no improvement in resistance development or activity against resistant strains could be achieved (Table 12).[267] Based on these limitations, the development of pirlimycin (**84**) was not warranted for use in humans—it has been marketed for veterinary use.

Several methods for semisynthetic modification of lincomycin (**16**) and clindamycin (**79**) have been reported[268] in addition to the methodology developed during total synthesis

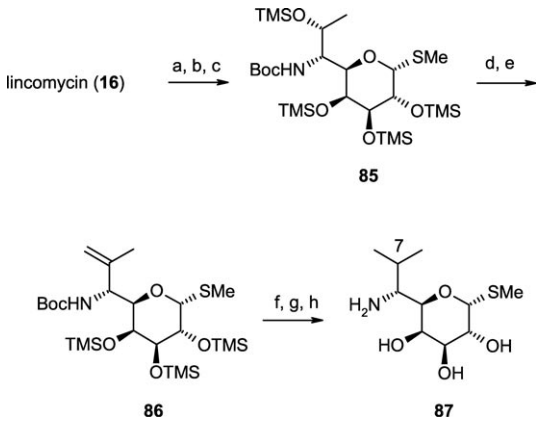
Table 12: Antibacterial activity in vitro of clindamycin (**79**) and pirlimycin (**84**), MIC_{90} [$\mu g\,mL^{-1}$].

Organism	Clindamycin (**79**)	Pirlimycin (**84**)
S. aureus[a]	0.125	0.5
S. aureus[b]	>16	>16
S. pyogenes	0.06	0.5
S. pneumoniae[c]	0.25	0.5
S. pneumoniae[d]	16	>16
S. viridans	0.06	0.5

[a] MSSA, methicillin-susceptible *S. aureus*. [b] MRSA, methicillin-resistant *S. aureus*. [c] PSSP, penicillin-susceptible *S. pneumoniae*. [d] PRSP, penicillin-resistant *S. pneumoniae*.

of the natural product.[269] However, none of these efforts led to significantly improved derivatives until Vicuron initiated a research program for second-generation lincosamides. Substitution of the 7-hydroxy group function by a methyl group in conjunction with novel amides resulted in the discovery of VIC-105404 (**90**)[270] and VIC-105555 (**17**).[271] The latter has been rapidly progressed into preclinical development.

Starting from **16**, 7-methylthiolincosamine (**87**) was obtained in eight steps (Scheme 7). Hydrazinolytic cleavage



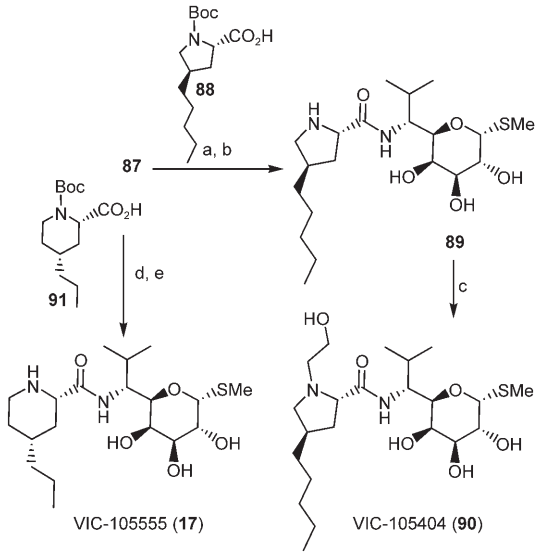
Scheme 7. Synthesis of 7-methylthiolincosamine (**87**). Reagents and conditions: a) $N_2H_4{\cdot}H_2O$; b) $(Boc)_2O$, Et_3N, MeOH; c) BSTFA, Et_3N, DMF; d) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, $-70\rightarrow-40$ °C; e) $PPh_3Me^+Br^-$, *t*BuOK; f) Dowex H^+, MeOH; g) H_2 (65 psi), Pd/C; h) TFA/H_2O (9:1).

of the amide followed by a selective Swern-type oxidation, Wittig olefination, and subsequent catalytic reduction of the methylene group were the key reactions in this transformation. Coupling of **87** with proline **88** followed by *tert*-butoxycarbonyl (Boc) deprotection and subsequent N-alkylation of **89** with oxirane gave VIC-105404 (**90**). Similar operations led to VIC-105555 (**17**, Scheme 8).

Compared with clindamycin (**79**), **17** displayed improved in vitro activity against enterococci and selected anaerobes, especially *C. difficile* (Table 13),[271,272] and showed superior efficacy in systemic-infection animal models.[273–275]

In rats, **17** had a longer half-life (3.72 versus 1.10 h), a larger volume of distribution (10.7 versus 4.54 $L\,kg^{-1}$), and a lower clearance (2.39 versus 4.46 $L\,h^{-1}\,kg^{-1}$)) than **79**. VIC-105555 (**17**) was stable in liver microsomes of all species and showed lower serum binding than **79**. Data from animals and allometric species scaling predicted a favorable human pharmacokinetic profile for **17**, which could eventually allow for once-daily dosing.[276] Despite the similar structure, comparable in vitro profile, and the same binding mode as clindamycin (**79**),[277] it did not seem possible to create mutants of *E. faecalis* resistant to **17** by serial passaging. However, mutants carrying an A2058 mutation by methylation on the 23S rRNA (erm methyltransferase) were still resistant to **17** ($MIC > 256\ \mu g\,mL^{-1}$).[278]

It will be interesting to see whether the in vitro and in vivo improvements achieved for **17** will translate into a clinical



Scheme 8. Vicuron's synthesis of VIC-105555 (**17**) and VIC-105404 (**90**) from 7-methylthiolincosamine (**87**). Reagents and conditions: a) **88**, HBTU, Et_3N; b) TFA/H_2O (9:1); c) oxirane, Et_3N; d) **91**, HBTU, Et_3N; e) TFA/H_2O (9:1).

Table 13: Antibacterial activity in vitro of clindamycin (**79**) and VIC-105555 (**17**), MIC [$\mu g\,mL^{-1}$].[272]

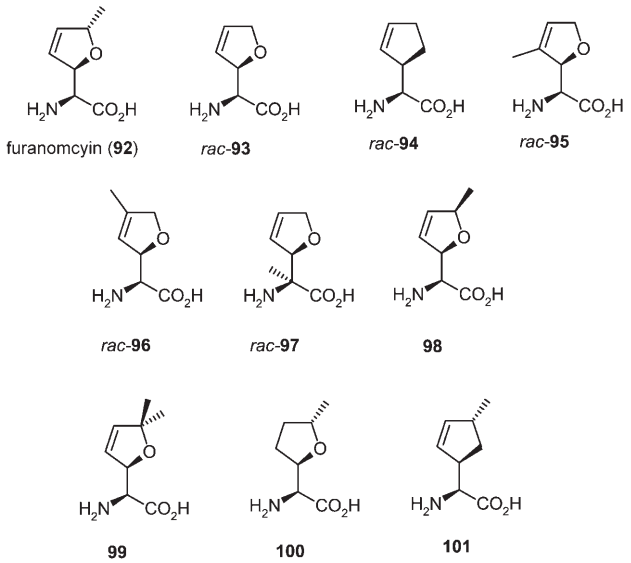
Organism	Clindamycin (**79**)	VIC-105555 (**17**)
S. pneumoniae	≤ 0.25	≤ 0.016–0.03
S. aureus	≤ 0.06–0.12	0.12–0.5
E. faecium	0.12 to >8	0.12 to >32
E. faecalis	8 to >8	0.5–1
B. fragilis	0.5–2	0.25–2
C. difficile	2–16	0.12–1

benefit. Second-generation lincosamides should allow successful treatment of infections by enterococci and offer a superior side-effect profile.

10. Furanomycin, a Lead with Insufficient Potential

With a molecular weight of 157 $g\,mol^{-1}$, L-(+)-furanomycin (**92**) is one of the smallest antibacterial natural products reported. This unusual α-amino acid was isolated by Katagiri et al. in 1967 from the fermentation broth of *Streptomyces threomyceticus L-803* (ATCC 15795)[279] and shown to inhibit the growth of bacteria such as *M. tuberculosis*, *E. coli*, *B. subtilis*, and some *Shigella*- and *Salmonella* species in the μM range. Its initially assigned absolute configuration was revised unambiguously to (+)-(α*S*,2*R*,5*S*) through syntheses starting from D-glucose,[280] and by an X-ray crystal-structure analysis of the *N*-acetyl derivative.[281]

Labeling experiments indicated that furanomycin biosynthesis proceeds through a polyketide pathway starting from two acetates and one propionate.[282] Analogous to the polyether antibiotics, its cyclic ether functionality stems from molecular oxygen that is introduced through epoxidation and intramolecular epoxide opening.[283] Furanomycin is accepted as a substrate by isoleucyl aminoacyl–tRNA synthetase and its antibacterial activity results from a substitution for isoleucine during the bacterial protein translation.[284] Therefore, the antibacterial activity of **92** is antagonized by isoleucine.[279] Furanomycin hampers the formation of isoleucyl–tRNA in *E. coli*, whereas other aminoacyl–tRNAs are not affected.[285] Aminoacyl–tRNA synthetases are essential in all living organisms[286] and have attracted considerable interest as novel targets in bacterial protein synthesis.[287] For **92**, no literature report on in vivo activity was found, but with pseudomonic acid,[288] a marketed antibiotic with a similar mode of action, a proof of concept existed. Besides antibacterial activity in vitro and in vivo,[289] **92** combined drug-like features,[290] such as polarity, sufficient solubility, and moderate structural complexity, which rendered it attractive to medicinal chemists. Several synthetic approaches towards **92** have been developed, some of which consist of up to 20 steps. Many syntheses have started from carbohydrates such as D-glucose,[280] D-ribose,[291] D-glucosamine,[292] L-xylose,[293] and D-mannitol.[294] Other approaches have used dimethyl L-tartrate,[295] serine,[296] glycine,[297] or furanes[280b,285b,298] as starting materials. In particular, approaches starting from amino acids were captivating as with six to seven steps they were short and flexible enough to rapidly access isomers and close congeners for evaluation of the SAR.

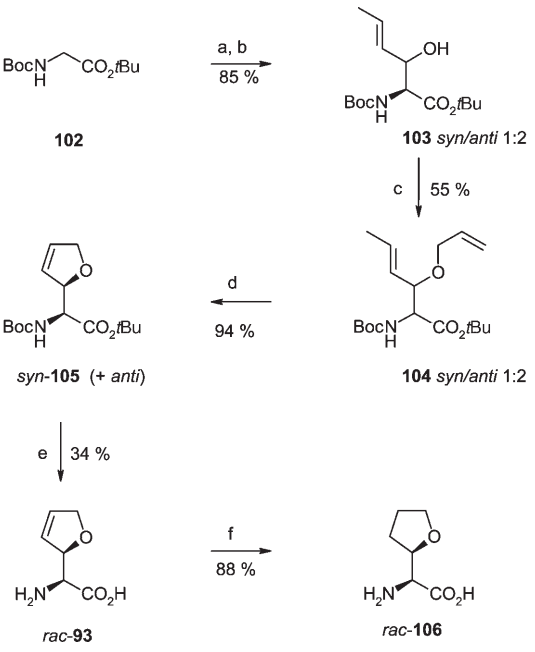


First of all, it was important to learn which structural parts in furanomycin were essential for activity and which parts could be removed or modified. Since norfuranomycin had been reported to exhibit antibacterial activity (MIC) against some Gram-negative bacteria,[298] straightforward protocols towards derivatives of **92** were envisaged. To gain rapid insight into SARs, control of diasteroselectivity was considered more important than controlling the absolute configuration. Ring-closing metathesis was used for the construction of the dihydrofurane ring. The required precursor **104**, accessible through *O*-allylation of β-hydroxyamino acid **103**,

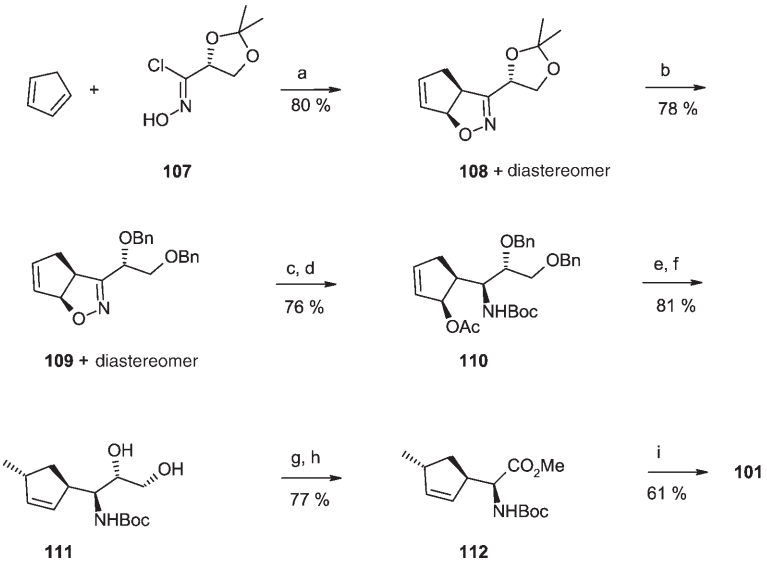
which was generated by aldol addition of an α,β-unsaturated aldehyde to the glycine ester **102** (Scheme 9).[297]

The effect of shifting the methyl group to various positions of the core structure was then investigated. The basic set of structures to probe the SAR was complemented by the α-*S*,2*R*,5*R* diastereomer 5′-*epi*-furanomycin (**98**), 5′-methylfuranomycin (**99**), and L-(+)-dihydrofuranomycin (**100**), as well as the carba analogue of the natural antibiotic **101**. These chiral congeners were prepared by 1,3-dipolar cycloaddition of glyceronitrile oxide as a chiral glycine equivalent[299] to either 2-methylfuran[294,300] or cyclopentadiene (Scheme 10).[301]

Unfortunately, the structural requirements of isoleucyl aminoacyl–tRNA synthetase for furanomycin-like substrates proved to be quite strict. A very tight SAR was observed and all of the synthetic isomers and derivatives of the natural antibiotic demonstrated no or clearly inferior MICs against a panel of selected Gram-positive and Gram-negative wild-type pathogens including *S. aureus* and *E. coli*. Only L-(+)-dihydrofuranomycin (**100**) showed borderline MIC (32–64 μg mL^{-1}) against *S. aureus* and the chiral carba analogue **101** exhibited weak antibacterial activity (4 μg mL^{-1}) against an efflux-pump-deficient *E. coli*. In summary, furanomycin harbored insufficient lead potential and was not a valid starting point for a drug-discovery program.



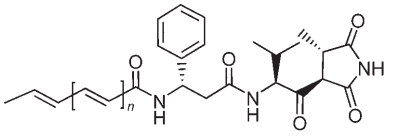
Scheme 9. Kazmaier's synthesis of norfuranomycin derivatives through ring-closing metathesis.[297] Reagents and conditions: a) 1. LDA (2.5 equiv), THF, −78 °C; 2. $TiCl(OiPr)_3$ (1.5 equiv); b) methacrolein (3 equiv), THF, −78 °C, 3 h, 85 %; c) allyl ethyl carbonate (1.5 equiv), $[\{(allyl)PdCl\}_2]$ (2.5 mol %), PPh_3 (11 mol %), THF, 50 °C, 3 d, 55 %; d) $[(Cy_3P)_2Cl_2Ru{=}CHPh]$ (2.5 mol %), CH_2Cl_2, 40 °C, 16 h, 94 %; e) 4 M HCl/dioxane, 4 h, 0 °C, 34 %; f) H_2, Pd/C, CH_3OH, 12 h, RT, 88 %.



Scheme 10. Jäger and co-workers's synthesis of the chiral carba analogoue **101** of natural L-(+)-furanomycin (**92**).[301] Reagents and conditions: a) NEt_3 (1.2. equiv), Et_2O; b) TFA (1. 3 %), MeOH, H_2O; 2. BnBr, NaH; c) 1. $LiAlH_4$, Et_2O; 2. Boc_2O, dioxane, H_2O; d) Ac_2O, DMAP, pyridine; e) MeMgBr, CuCN, Et_2O, −20 C; f) Na, NH_3(liquid), THF −78 °C; g) $Pb(OAc)_4$, CH_2Cl_2, −20 °C; h) 1. $NaClO_2$, CH_3CN, *t*BuOH, H_2O, 2-methyl-2-butene, NaH_2PO_4, −20 °C; 2. CH_2N_2, Et_2O; i) 1. 90 % TFA; 2. NaOH, $NaHCO_3$, H_2O, THF.

11. Pyrrolidinedione Antibacterials

The natural peptide antibiotic andrimid (**113**) was first isolated by Komura and co-workers in 1987 from cultures of a symbiont of the brown planthopper *Nilaparvata lugens*.[302] Some years later, **113** and related new metabolites, moiramides A–C, were also discovered in a marine isolate of *Pseudomonas fluorescens* obtained from a tunicate collected in Moira Sound at Prince of Wales Island, Alaska.[303]



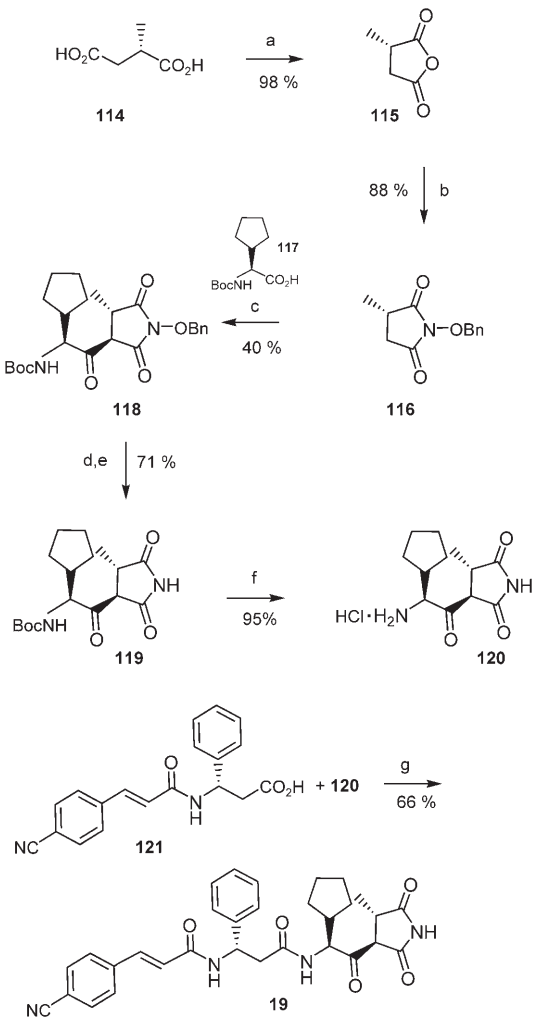
andrimid, *n* = 2 (**113**)
moiramide B, *n* = 1 (**18**)

The linear structure of these metabolites was elucidated by spectroscopic means[302,303] and was shown to contain four characteristic elements: a pyrrolidinedione head group, a valine derived β-ketoamide, a (*S*)-β-phenylalanine part, and a *N*-terminal polyunsaturated fatty acid. Isotope incorporation experiments revealed that nature constructs the essential right-hand part from valine, glycine, and acetate units.[303,304] Notably, metabolites **113** and **18** exhibited good in vitro antibacterial activity against MRSA. Various diastereoselective and asymmetric total syntheses of **113** and **18** have been described[304,305] and allowed for ready access to these antibiotics. Subsequently, these methods also served as a basis for

structural variation and evaluation of the potential of these lead structures in our laboratories.[306,307]

However, before starting a medicinal-chemistry program, it had to be clarified whether the pyrrolidinedione antibiotics act through an essential mode of action. In profiling **113** and **18**, their published biological activity could be endorsed, however, a reported effect on RNA synthesis[308] could not be confirmed by precursor incorporation studies.[309,310] Application of various RG techniques (see Section 5.4) led to the identification of the molecular target:[144,309,311] pyrrolidinedione antibiotics exert their effect by inhibiting the first committed step in bacterial fatty acid biosynthesis, a reaction catalyzed by the carboxyltransferase subunit of the multimeric bacterial enzyme acetyl-CoA carboxylase.[309] For most living organisms, fatty acid biosynthesis is a vital metabolic process, but the pathways in bacteria[312] and mammals[313] are different. Acetyl-CoA carboxylase is essential for microbial growth and is broadly conserved amongst bacteria.[310] No potent inhibitors of bacterial acetyl-CoA carboxylase were known and overall, this enzyme seemed to be an appealing target for future broad-spectrum antibacterials. Acting through an essential target, covering a broad spectrum of Gram-positive and Gram-negative bacteria, demonstrating low resistance induction, and showing a low rate of spontaneous development of resistance, natural pyrrolidinedione antibiotics **113** and **18** proved to be attractive lead structures but were far from being useful drugs. Several parameters, such as potency, chemical and metabolic stability, solubility, and drug–drug interaction needed to be improved by a medicinal-chemistry program.

Synthesis of novel pyrrolidinedione antibacterials was achieved by adapting pathways described in the literature (Scheme 11).[304,305] Dehydration of (*S*)-(−)-methylsuccinic acid (**114**) with acetyl chloride afforded methylsuccinic anhydride **115**, which was treated with *O*-benzyl hydroxylamine in the presence of *N*,*N*′-carbonyldiimidazole (CDI) to yield (*S*)-2-methyl-*N*-benzyloxy succinimide (**116**). *N*-Boc-protected (2*S*)-cyclopentyl glycine, prepared according to Andersson and co-workers,[314] was activated with CDI in THF, added to **116**, and the solution was slowly added to a solution of lithium hexamethyldisilazide (LiHMDS) in THF at low temperature to produce β-ketoamide **118**. Under the reaction conditions, *trans*-**118** was predominantly formed from an equilibrium mixture of the *cis*-, *trans*-, and enolic forms. Removal of the *N*-benzyloxy group to give acyl succinimide **119** was achieved by hydrogenolysis and subsequent treatment of the *N*-hydroxy intermediate with 2′-bromoacetophenone and triethylamine.[315] Deprotection of **119** under acidic conditions yielded the primary amine **120**, which was reacted with *N*-Acyl-(*S*)-β-phenylalanine (**121**) under standard peptide-coupling conditions to afford pyrrolidinedione antibacterial **19**, a formal double-decoration product of natural **18**. Alternatively, **19** and congeners could be obtained from **120** by a stepwise procedure in about 60% overall yield: coupling with *N*-Boc-(*S*)-β-phenylalanine, Boc removal under acidic conditions, and reaction of the resulting primary amine with the corresponding cinnamic acid under standard coupling conditions.[306]



Scheme 11. Synthetic route used to explore pyrrolidinedione structural variations and the SAR.[306] Reagents and conditions: a) CH_3COCl, 4 h, 60 °C, 98%; b) *O*-benzyl hydroxylamine, CDI, CH_2Cl_2, 12 h, RT, 88%; c) 1. *N*-Boc-(2*S*)-cyclopentyl glycine (**117**), CDI, THF; 2. LiHMDS, THF, 15 min, −65 °C; 3. conc. aqueous NH_4Cl, −65 °C→RT, 40%; d) H_2, Pd/C (10%), EtOH, 1 h, RT; e) 2′-bromoacetophenone, Et_3N, cat. DMAP, CH_3CN, 20 h, RT; f) 4 N HCl in 1,4-dioxane, 2 h, RT, 95%; g) HATU, iPr_2EtN, CH_2Cl_2, DMF, 10 h, 0 °C→RT, 66%.

Through this route, and also by solid-phase synthesis starting with polymer bound (*S*)-β-phenylalanine, a broad variety of pyrrolidinedione antibacterials became readily available.[306,307] Broad structural variations were tolerated at the fatty acid side chain without adversely affecting target activity. Inhibitory values (IC_{50}) remained in the nM range for the *E. coli* and *S. aureus* acetyl-CoA carboxylase enzymes with polar and lipophilic side chains as well (Table 14).

Apparently, the side chain was not involved in key interactions with the enzyme and could be used for tuning the physicochemical profile. On the other hand, the nature of the side chain had a significant influence on antibacterial activity (MIC). Comparing compounds **122** and **123** demonstrated that despite excellent target activity of **122** and the benefit of a polar substituent for other parameters, such as

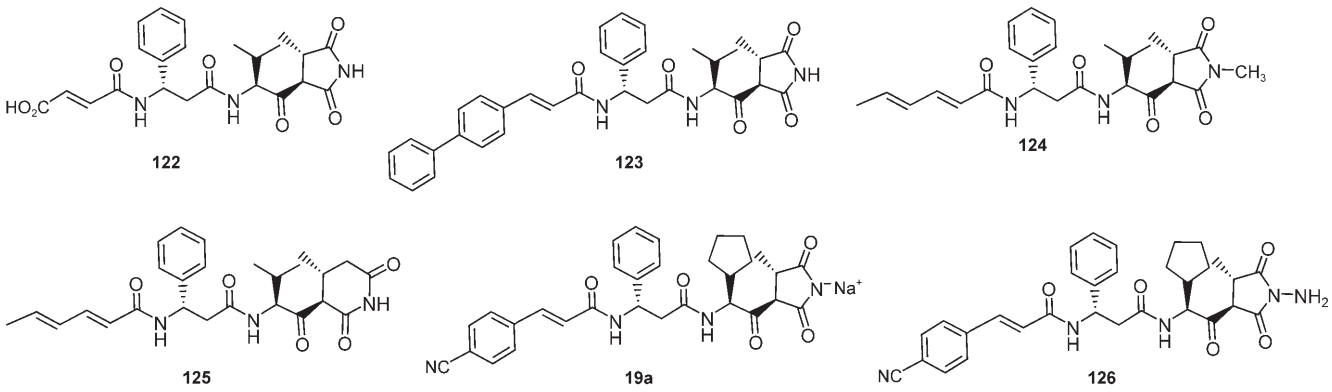


Table 14: Inhibitory values (IC_{50} [nM]) against carboxyltransferase (AccAD subunits), and in vitro antibacterial activity (MIC [$\mu g\,mL^{-1}$]) of pyrrolidinedione antibacterials.

	IC_{50}		MIC			
	E. coli	*S. aureus*	*E. coli*[a]	*E. coli*[b]	*S. aureus*	*S. pneum.*
113	13	305	32	>64	8	8
18	6	96	4	32	8	32
19	4	44	1	32	0.03	1
122	2	317	>64	>64	>64	>64
123	37	540	>64	>64	2	16
124	25	211	16	>64	16	>64
126	2	33	0.5	16	0.01	0.25

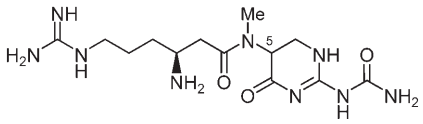
[a] Efflux-pump deletion strain. [b] Wild type.

solubility, reasonable lipophilicity was required for penetration into bacterial cells and for good MIC values. Replacing the (*S*)-β-phenylalanine by non-aromatic β-amino acids led to a loss in activity. On the other hand, significant improvement of antibacterial activity could be achieved by varying the lead's β-ketoamide part, for example, by replacing (*S*)-valine with (2*S*)-cyclopentyl glycine, whereas aromatic amino acids in this position rendered the molecule inactive.

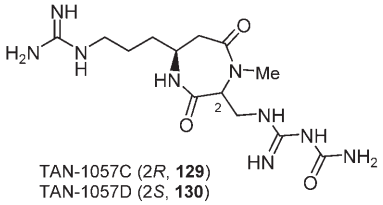
The pyrrolidinedione head group was thought to mimic the transition state of the carboxyltransferase reaction as it was most sensitive towards structural variations. In comparision to moiramide B (**18**), its *N*-methyl derivative **124** suffered from a two- to fourfold decrease in activity, yet the corresponding hydrazide was at least equipotent, whereas the piperidinedione congener **125** was inactive. These studies also confirmed the importance of the 4-(*S*)-methyl head-group substituent for efficient target interaction.[306] Sufficient water solubility for parenteral application ($>5\,g\,L^{-1}$) was achieved by preparing the corresponding pyrrolidinedione sodium salts, for example, **19a**, which were generally employed in murine infection models. Thus, parenteral treatment of lethal *S. aureus* infections in mice with single doses of **19a** ($50\,mg\,kg^{-1}$) or **126** ($25\,mg\,kg^{-1}$), resulted in 100% survival.[316] With the synthetic pyrrolidinedione antibacterials **19** and **126**, which are readily available in multi-gram scale, many critical properties of the natural antibiotics **113** and **18** could be ameliorated. Systematic exploration of the SAR will help to improve potency to evaluate the full potential of these attractive lead structures.

12. Tetrahydropyrimidinone Antibiotics

A structurally unique group of novel antibiotics was isolated from *Flexibacter* species found in soil samples collected in the Nachi mountain area in the Wakayama Prefecture in Japan. First published in a patent application,[317] scientists from Takeda disclosed the structure of these novel antibiotics in 1993 and denoted them as TAN-1057A–D (**127**–**130**).[318] The epimeric tetrahydropyrimidinone antibiotics TAN-1057A/B (**127**/**128**) were isolated from *Flexibacter* sp. PK-74, whereas the epimeric dioxo diazepans TAN 1057C/D (**129**/**130**) resulted from *Flexibacter* sp. PK-176.



TAN-1057A (5*S*, **127**)
TAN-1057B (5*R*, **128**)



TAN-1057C (2*R*, **129**)
TAN-1057D (2*S*, **130**)

The structure of **127**/**128** was elucidated through a combination of spectroscopic studies and degradation experiments yielding primarily (*S*)-β-homoarginine and α-*N*-methyl-2,3-diamino propionic acid. The constitution of TAN-1057C/D (**129**/**130**) was determined from their spectroscopic data as well as on the observation that **129**, upon treatment with base, was rapidly converted into a mixture of **127** and **128**. Owing to the instability of **129** and **130**, total synthetic endeavors and medicinal-chemistry optimization have concentrated on **127** and **128**.

The antibacterial activity, in particular of TAN1057A (**127**), was characterized in detail.[319] Although its in vitro MIC values against Gram-positive organisms such as *S. aureus* and *S. pneumoniae* were mediocre under standard conditions

(6.25–12.5 μg mL^{-1}), the compound was reported to exhibit in vivo efficacy superior to vancomycin and imipenem in a murine *S. aureus* sepsis model.

From incorporation studies with [^{14}C]leucine, it was concluded that TAN-1057A/B (**127**/**128**) acted by blocking bacterial protein biosynthesis,[319] and, in detailed studies, **127**/**128** was found to inhibit bacterial growth through binding to the 50S subunit of ribosomes.[320] Dissection of the translational apparatus revealed an effect on protein biosynthesis by inhibition of the peptidyl transferase step.[321] This target was independently confirmed by using proteomics technologies for **13**, a "degraded" analogue of TAN-1057A/B (**127**/**128**) with improved tolerability.[136] swelling and impairment of the cell wall was the macroscopic effect of **13** on the growth of *S. aureus* (Figure 9). Competition experiments with other antibiotics, inhibiting peptidyl transferase, revealed a unique binding site for **127**/**128**.[321] Consequently, *S. aureus* subtypes selected for **127**/**128** resistance did not show cross-resistance to a panel of known inhibitors of bacterial translation.[322] Natural **127**/**128** showed a comparable, nonspecific inhibitory activity in cell-free translation assays derived from prokaryotes and eukaryotes,[321] which could be the reason for the natural product's high acute toxicity in mice (LD_{50} 50 mg kg^{-1} i.v.).[318] Thus, **127**/**128** itself was far from being an appropriate clinical drug candidate, yet, its potent in vivo activity against *S. aureus* rendered it an attractive lead structure. Furthermore, its pronounced solubility was well suited for parenteral application.

A total synthesis of the natural product TAN-1057A/B (**127**/**128**) was thought to offer the structural flexibility needed for accessing analogues with improved properties. Despite manageable complexity, its structure and properties harbored some synthetic challenges: the peptide bond between the β-homoarginine side chain and the pyrimidinone heterocycle was difficult to form owing to steric hindrance and low nucleophilicity of the amino component **139**. Synthesis of the heterocycle itself was conceivable from a corresponding 2,3-diamino propionic acid precursor in which the congestion of functional groups rendered the orchestration of reaction centers and protective groups into a strategic exercise. The C5 stereogenic center of the heterocycle was prone to epimerization and it was known from the Takeda group that the heterocyclic component was sensitive towards strongly basic or acidic conditions. Consequently, harsh conditions, particularly during the final steps of the synthesis, had to be avoided. Three different total syntheses[323–325] of the diasteromeric mixture of TAN-1057A/B (**127**/**128**) and one stereoselective synthesis[326] resulting in the pure diastereomer **127** have been completed.

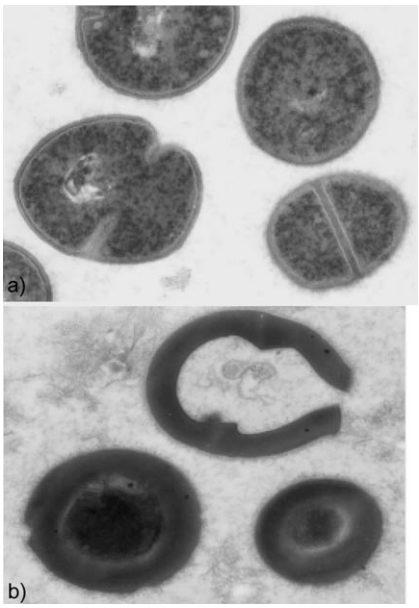
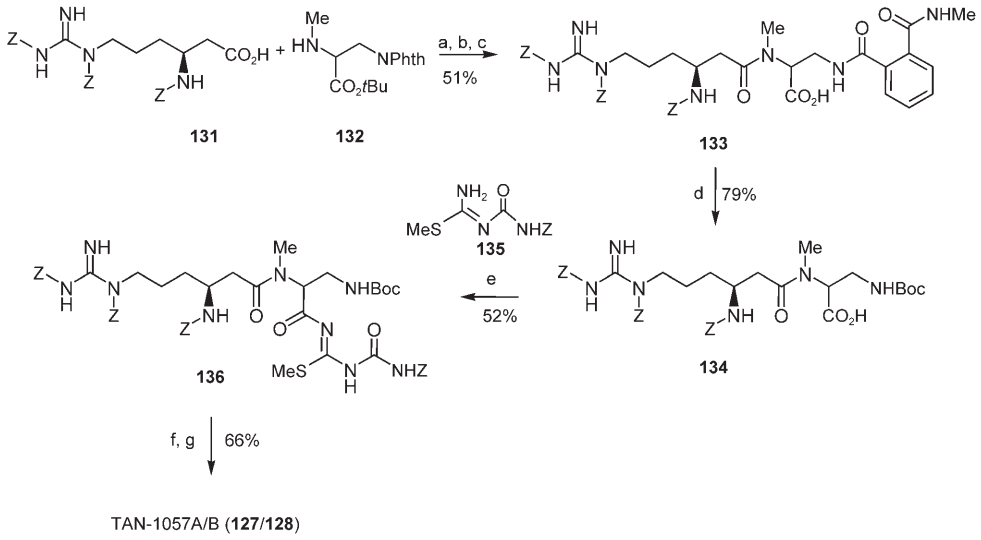


Figure 9. Action of **13** on *S. aureus*: Swelling and impairment of the bacterial cell wall. Electron micrograph a) before and b) after treatment with **13** (2 μg mL^{-1}) for 4 h.

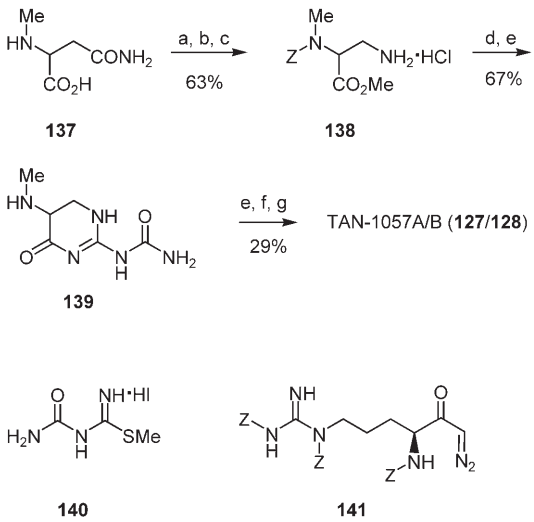
Yuan and Williams' synthesis of **127**/**128**[323] was published first and employed a rather linear sequence (Scheme 12). Triple-protected β-homoarginine **131** was coupled to the 2,3-diamino propionic acid derivative **132** as an open-chain precursor of the pyrimidinone heterocycle, thus avoiding difficulties with the peptide formation. After three protecting-group manipulations, isothiurea derivative **135** was coupled with the carboxylic acid moiety in **134**, setting the stage for ring closure after removal of the Boc group. In the final step, all four Z protecting groups were removed concomitantly through hydrogenation under mild conditions. The



Scheme 12. Yuan and Williams' synthesis of TAN-1057A/B (**127**/**128**).[323] Reagents and conditions: a) BOPCl, 16 h, 55%; b) CH_3NH_2, MeOH, 5 min.; c) TFA/anisole 25:1, 0°C→RT, 1 h, 93% (2 steps); d) Boc_2O, Et_3N, water/dioxane 1:1, 16 h; e) EDC, DMAP, CH_2Cl_2, 16 h, 52%; f) TFA/anisole 10:1, 15 min., evaporation; then NEt_3, THF, 10 min, 67%; g) $PdCl_2$, H_2, MeOH/CH_2Cl_2 2:1, 99%.

longest linear sequence comprised twelve steps, including five steps for the preparation of **132** starting from L-*N*-Z-asparagine. Originally, an enantioselective synthesis was planned by using **132** as a homochiral building block. However, epimerization under various conditions hampered this synthetic route. Yuan and Williams also completed the total synthesis of the seven-membered congeners TAN-1057C/D (**129**/**130**).[323]

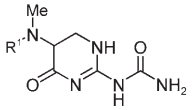
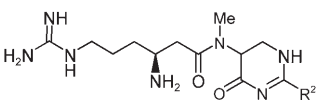
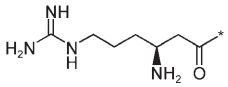
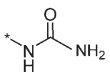
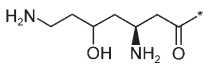
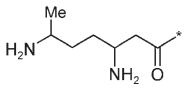
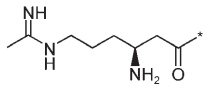
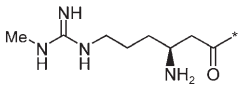
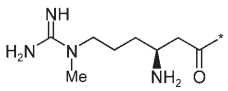
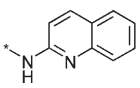
The more-convergent synthesis of de Meijere, Belov, and co-workers (Scheme 13) was published shortly after.[324] The racemic asparagine derivative **137** was prepared through a Michael addition of methylamine to maleamide. After protection, the 2,3-diamino propionic acid motif was included through a Hofmann rearrangement. Ring formation was successfully accomplished in a single step by employing the isothiurea building block **140**. The originally reported, moderate yield of 35% could be subsequently doubled by the reaction conditions (changing the solvent from isopropanol to acetonitrile, decreasing the temperature, and increasing the reaction time).[326] The crucial peptide bond formation was accomplished by irradiation of the β-homoarginine precursor **141** in the presence of the deprotected amino pyrimidinone **139**, which reacted with the ketene product of the Wolff rearrangement. Final triple deprotection gave TAN-1057A/B (**127**/**128**).



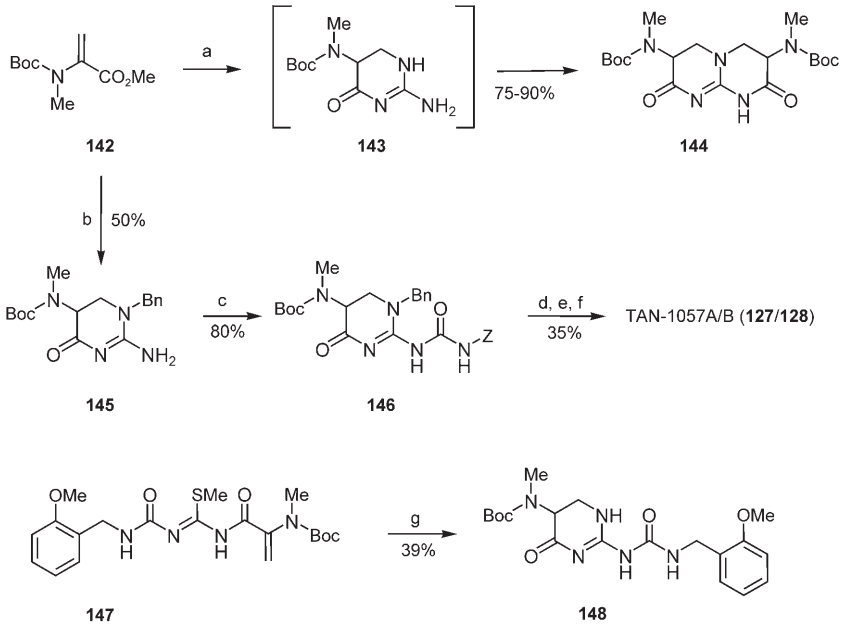
Scheme 13. De Meijere, Belov and co-workers's synthesis of TAN-1057A/B (**127**/**128**).[324] Reagents and conditions: a) $PhCH_2OCOCl$, aq. NaOH, 3 h, 88%; b) $PhI(OCOCF_3)_2$, pyridine, DMF/water 1:1, 4 h, 74%; c) MeOH, $SOCl_2$, −20 °C→RT, 24 h, 97%; d) **140**, NaOAc, MeCN, 55 °C, 70%;[326] e) H_2, 10% Pd/C, DMA, 24 h, 96%; f) **141**, *hν*, DMA, 2 h, 30%; g) $PdCl_2$, H_2, MeOH, 99%.

With eight steps, the synthesis by de Meijere, Belov, and co-workers was short and offered a practical access to **127**/**128**. The key building block **139** became available on a large scale in six steps and with a minimum of protecting group manipulation as the free amino group of **140** did not interfere with the cyclization reaction. This synthetic route was successfully employed to generate numerous different analogues (Table 15). Furthermore, careful optimization of the reaction conditions along this synthetic route resulted in the first enantioselective total synthesis of TAN-1057A (**127**).[326]

Table 15: Antibacterial activity in vitro of western derivatives with de novo synthesized β-amino acid side chains[330,334,336] and eastern variations of the urea,[325,328,334] MIC *S. aureus* [μg mL^{-1}].

		MIC			MIC
127/128		0.25	**127/128**		0.25
152		0.4	**157**		64
153		0.4	**158**		8
154		0.2	**159**		0.4
155		0.8	**160**		0.05
156		0.025	**161**		0.1

In the original approach, de Meijere, Belov, and co-workers envisaged the synthesis of **127**/**128** by addition of the guanidino moiety, hidden in the ureido pyrimidinone, to dehydro alanine **142** (Scheme 14).[324] Yet, addition did not stop after one Michael addition/ring closure, but rather triggered a tandem sequence forming the bicyclic derivative **144**. The second Michael addition could be suppressed by protecting one of the guanidino nitrogens as has been shown in different approaches: Ganesan and Lin[327] formed the guanidino function in situ from an elaborated dehydroalanine precursor **147** and scientists from Gilead used *N*-benzyl-protected guanidine in a Michael-addition approach yielding the intermediate **145**.[325] After incorporation of the urea moiety, the intermediate **146** was converted into the natural product **127**/**128** by using a modification of the original conditions by de Meijere, Belov, and co-workers [324] in 7 steps and 12% overall yield.

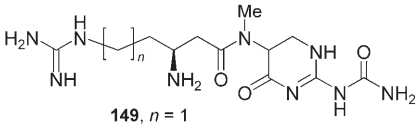


Scheme 14. Synthetic approaches to intermediates **144**,[324] **148**,[327] and natural product TAN-1057A/B (**127**/**128**)[327] by using dehydroalanine precursors. Reagents and conditions: a) Guanidine, *i*PrOH, 48 h; b) *N*-benzyl guanidine (TFA salt), K_2CO_3, *i*PrOH, RT, 16 h, then 50 °C, 4 h; c) Z-NCO, THF, 16 h; d) TFA, CH_2Cl_2; e) **131**, $AgClO_4$, NEt_3, DMF; f) $PdCl_2$, Pd/C, H_2, MeOH; g) 2 M NH_3 in MeOH, 20 h.

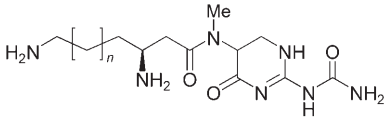
the formation of pyrrolidines and piperidines. By using β-amino acids synthesized de-novo,[330,334,335] further side-chain SAR trends were explored (Table 15). Incorporation of heterocycles, such as thiazoles or isoxazoles, deleted anti-staphylococcal activity.[334] Small groups, such as carbonyl, hydroxy (**152**), or methyl (**153**), were tolerated but the resulting antibiotics did not exhibit improved properties relative to **127**/**128**. Distinct variations of the original guanidine through an amidine group (**154**) or the methyl guanidines (**155** and **156**) led to compounds with high activity against stapylococci, which in the case of **156**, was accompanied with activity against pneumococci (Table 16).

The only variation so far reported for the dihydro pyrimidinone heterocycle was that of the N1-methylated derivative, which was inactive against staphyloccoci.[325] As methylation of the proximal NH function of the adjacent urea group led to inactive compounds (**157**, Table 15),[325] it seemed that the urea needed to be locked by a hydrogen bond (through the NH group of the ring) in a rigid conformation. Interestingly, the urea motif could be mimicked by various heterocycles such as pyrimidine **159**, pyridine **160**, and amino quinoline **161**.[334]

The different routes to **127**/**128** enabled structural variations in all parts of the molecule. One goal, pursued independently by scientists from the University of Colorado, Gilead, and Bayer, was to reduce the acute toxicity of the natural antibiotic while maintaining its excellent activity. The plan was to create a small-spectrum antibiotic that covered the spectrum gaps of established drugs such as imipenem. Both, the ω-guanidino group and the β-amino function were essential for activity against staphylococci.[328] A shift of the β-amino group to the α-position, maintaining the overall chain length, was not tolerated.[329] A systematic investigation revealed that reducing the distance between the amino and the guanidino group (**149**) was detrimental for activity, whereas the insertion of an additional methylene group (**150**) was tolerated.[330] The replacement of the ω-guanidino function by a ω-amino-group in **13** and **151** clearly improved tolerability. In this novel series, both, the *S* configuration and the β-amino position represented an optimum (Table 17).[330,331]



149, *n* = 1
150, *n* = 3



13, *n* = 1
151, *n* = 2

Table 16: Antibacterial activity in vitro of TAN-1057A/B (**127**/**128**) analogues, MIC [μg mL^{-1}].

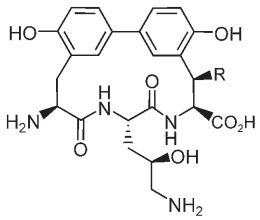
	S. aureus	*S. epidermidis*	*E. faecium*	*E. faecalis*	*S. pneumoniae*
127/128	0.25	0.25	2	>16	16
151	0.25	0.125	0.25	64	32
156	0.025	0.125	2	64	4
160	0.05	0.25	2	>64	2

The amino acid components for the optimization program were readily available, for example, by Arndt–Eistert chain elongation. To explore the SAR options in the side chain, for derivatives of **13** and **151**, the synthesis of novel β-lysine or β-homolysine derivatives became important.[332] Surprisingly little was known about this field.[333] A reason for the lack of de novo synthetic methods for β-lysine and β-homolysines might lie in the 1,4- and 1,5-relationship between the ω-amino center and the β-amino group, which is prone to side reactions, such as

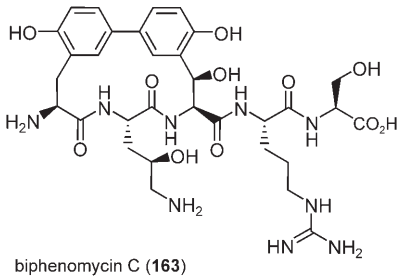
These optimization efforts resulted in compounds superior to the natural antibiotic **127**/**128** (Table 16, Table 17). Both, in vitro potency and in vivo efficacy of **151** were maintained, whereas it was more efficient in blocking protein biosynthesis in prokaryotes than in eukaryotes (Table 17).

This was reflected in an improved cytotoxicity profile and clearly improved tolerability. β-Homolysine **151** was tolerated in i.v doses of up to 150 mg kg^{-1} in mice and fulfilled the profile of a small-spectrum problem solver; a similar profile was achieved with β-lysine **13**. With **156** and **160**, the spectrum of in vitro antibacterial activity could be considerably broadened to include pneumococci, however, at the expense of the tolerability and therapeutic index. Thus, neither **156** nor **160** were suitable for clinical development.

The attractive antibacterial properties and the structure of natural antibiotics TAN-1057A/B (**127**/**128**) evoked the attention of several synthetic research groups. Systematic SAR exploration required novel routes to β-lysine and β-homolysine derivatives. The synthetic pyrimidinone antibiotics **13** and **151**, readily accessible on a large scale, exhibited improved cytotoxicity and tolerability while retaining eminent potency of the natural product. In this case, formal degradation of the natural product—through de novo synthesis—led to congeners with superior tolerability.



biphenomycin A, R = OH (**162**)
biphenomycin B, R = H (**21**)



biphenomycin C (**163**)

Table 17: Biological properties of TAN-1057A/B (**127**/**128**) analogues.

	S. aureus sepsis ED_{100} [mg kg^{-1}][a]	Translation prokaryotes IC_{50} [μM]	Translation eukaryotes IC_{50} [μM]	Cytotoxicity EC_{50} [μg mL^{-1}]
127/128	0.25	0.3	0.17	0.25
151	0.25	0.5	2.8	6
156	≤0.5	0.06	n.d.[b]	n.d.[b]
160	1	n.d.[b]	0.07	n.d.[b]

[a] 100% survival after parenteral administration of the given dose in mice. [b] n.d. = not done.

13. Biphenomycins

In 1967, a group from Lederle Laboratories reported the isolation of LL-AF283α, an antibiotic with unusual biological properties obtained from the fermentation of *S. filipinensis*.[337,338] Later, in 1991, Borders and co-workers[339] found that LL-AF283α was identical to the peptide antibiotic biphenomycin A (WS-43708A), which was reported by scientists from Fujisawa in 1984.[340,341,342] Biphenomycins displayed a structurally unique architecture with a cyclic tripeptide containing a biphenyl moiety in a 15-membered ring. The absolute stereochemistry of biphenomycin A (**162**) and its known congeners, biphenomycin B (**21**) and C (**163**),[343] was determined.[344,345]

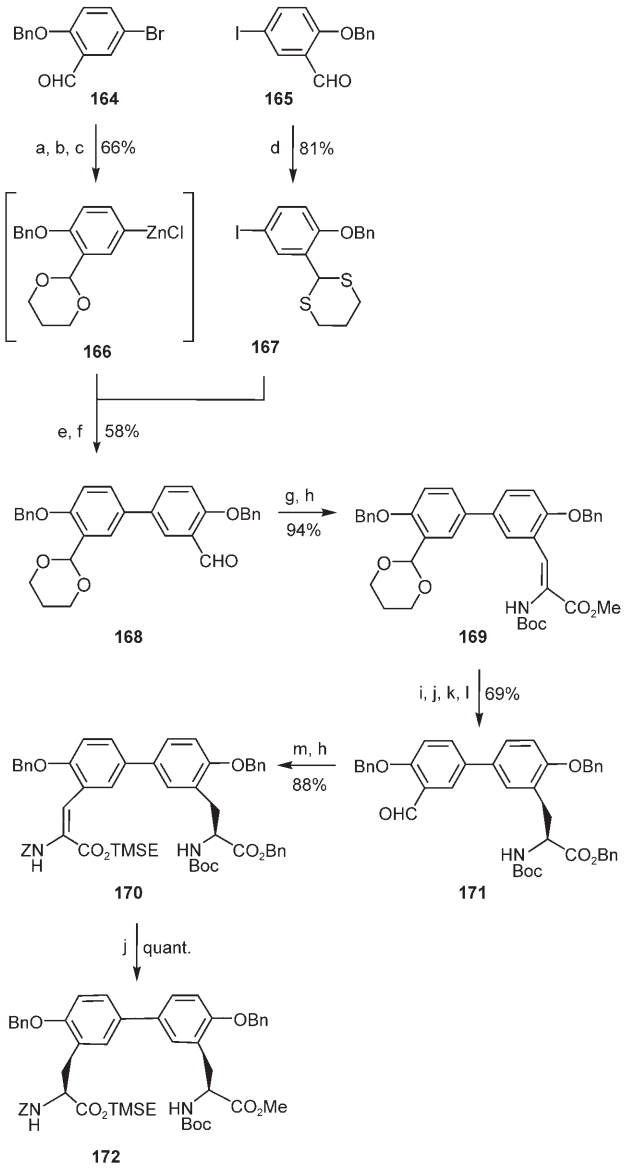
The in vitro activity of biphenomycin A (**162**) was virtually limited to *Cornybacterium xerosis*. Growth of other bacteria, such as *S. aureus*, *E. coli*, or *S. pyogenes*, was not affected up to 200 μg mL^{-1} using agar-well diffusion or agar-dilution MIC assays. However, **162** was highly effective in vivo in a murine sepsis model. Administered subcutaneously, **162** protected mice, from an otherwise lethal infection, against *S. aureus* Smith (ED_{90} 1 mg kg^{-1}) and was five-times more effective than vancomycin in the same experiment.[337] The reason for this discrepancy between in vivo and in vitro activity has remained unclear. MIC values seemed to be strongly influenced by the test conditions.[337,341] Any in vitro antibacterial activity was completely suppressed with complex media (e.g. Mueller–Hinton agar), yet, with semisynthetic media, MICs could be measured for Gram-positive bacteria, but not for their Gram-negative counterparts.[341] Furthermore, it appeared that highly resistant mutants were selected on first exposure of **162**, which pointed to an unfavorable resistance profile.[339] On the other hand, these mutants showed no cross-resistance to established antibiotics, such as vancomycin, tetracycline, ampicillin, or erythromycin. Biphenomycin A (**162**) was well tolerated after oral dosing of up to 640 mg kg^{-1}.[337]

With interesting in vivo efficacy, good tolerability, and no cross-resistance to marketed antibiotics, the biphenomycins represented an attractive starting point for an optimization program in medicinal chemistry. On the other hand, the lack of in vitro activity, the questionable resistance behavior, and the unknown target posed barriers which had to be overcome. Conditional to solving these issues was the availability of a total synthesis which would allow rapid structural modification of selected parts of the molecule.

The first total synthesis of biphenomycin B (**21**) was published by Schmidt et al.[346] in 1991. Its sequence consisted of 1) synthesis of (*S*,*S*)-isotyrosine (**172**), 2) formation of an *ansa*-tripeptide (**174**), 3) macrocyclization, and 4) removal of protecting groups (Scheme 15 and Scheme 16).

Key steps of the synthesis of orthogonally protected (*S*,*S*)-isotyrosine were the Pd-catalyzed coupling of the zinc compound **166** with aryl iodide **167** followed by the sequential introduction of the dehydroamino acids **169** and **170**. The initially formed *E*/*Z* mixtures of **169** and **170** were isomerized with triethylamine on carbon to the respective *Z* compounds. These *Z* alkenes set the stage for the enantioselective hydrogenation that proceeds with >99% *ee* to yield **171** and **172**, respectively.

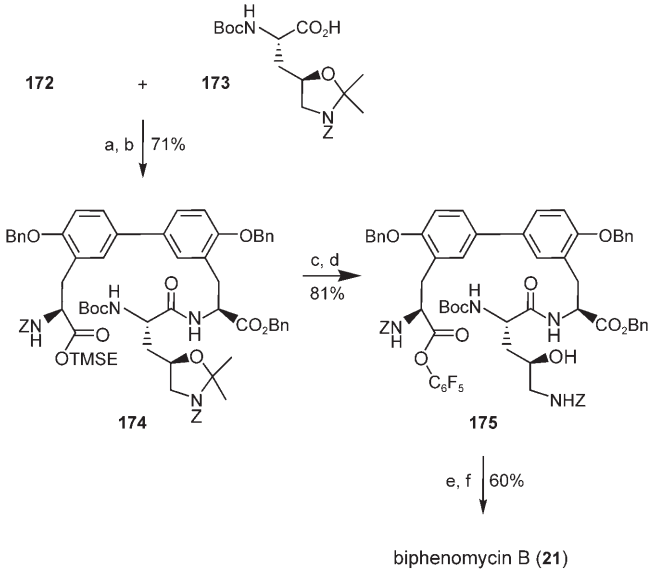
After Boc deprotection, **172** was coupled with (2*S*,4*R*)-hydroxyornithine (**173**), which was obtained from mannitol.[347] Alternative approaches to differently protected



Scheme 15. The synthesis by Schmidt et al. of (*S*,*S*)-isotyrosine (**172**).[346] Reagents and conditions: a) propane-1,3-diol, $BF_3{\cdot}Et_2O$, toluene, reflux, 4 h; b) Mg, THF, reflux, 3 h; c) $ZnCl_2$, THF, reflux, 1 h; d) propane-1,3-dithiol, $BF_3{\cdot}Et_2O$, toluene, reflux, 4 h; e) $[PdCl_2(PPh_3)_2]$, iBu_2AlH, RT, 3 h; f) NBS, 2,6-lutidine, CH_3CN, H_2O, 0 °C, 5 min; g) methyl *N*-*tert*-butoxycarbonyl(dimethoxyphosphoryl)glycinate, LiCl, DBU, CH_3CN, RT, 1 h; h) NEt_3, C, EtOH/$CHCl_3$ (1:1), RT, 2 d; i) LiOH, H_2O, dioxane, RT, 12 h; j) [Rh(cod)dipamp)]BF_4, H_2, MeOH, RT, 72 h; k) BnOH, DCC, DMAP, ethyl acetate, −15 to 20 °C, 12 h; l) PPTS, acetone, H_2O, reflux, 6 h; m) *N*-benzyloxycarbonyl(dimethoxyphosphoryl)glycine trimethylsilyl ester, LiCl, DBU, CH_3CN, RT, 2 h.

hydroxyornithine derivatives have been reported.[348] Manipulation of protecting groups and activation of the carboxy moiety as a pentafluorophenyl ester **175** (for the macrocylization) proceeded with excellent yields in a biphasic system under high-dilution conditions. Thus, **21** was obtained after simultaneous removal of five protecting groups in the final step (Scheme 16).

Overall, this route delivered the desired natural product in 22 steps with excellent optical purity in 9% overall yield

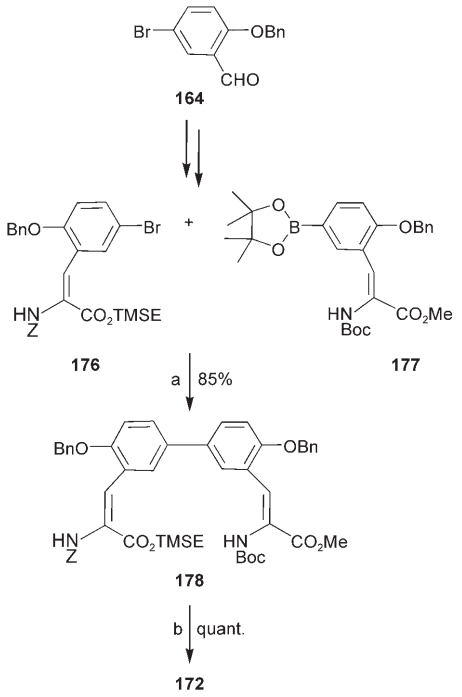


Scheme 16. Schmidt's total synthesis of biphenomycin B (**21**).[346] Reagents and conditions: a) HCl, dioxane, 20 °C, 2 h; b) EDC, HOBt, CH_2Cl_2, −15→20 °C, 14 h; c) AcOH/H_2O (9:1), 50 °C, 7 h; d) Bu_4NF, DMF, RT, 1 h, C_6F_5OH, EDC, CH_2Cl_2, −15→20 °C, 14 h; e) HCl, dioxane/CH_2Cl_2 (1:1), 0 °C, evaporation, $CHCl_3$, $NaHCO_3$, 20 °C, 5 min; f) trimethylsilyl trifluoromethanesulfonate, thioanisole, TFA, RT, 30 min.

starting from the appropriately substituted benzaldehyde derivatives (Scheme 15). Following his approach to biphenomycin B, Schmidt et al. also completed the synthesis of biphenomycin A (**162**) with some modifications in the preparation of the isotyrosine.[349,350] More-recent approaches by Paintner et al.[351] and others[352–354] were based on Schmidt's synthesis with modifications in the construction of the enantiopure isotyrosine. Thus, various methods for the construction of the biaryl have been successfully applied, including Stille, Suzuki, and oxidative cross-couplings with $VOCl_3$.

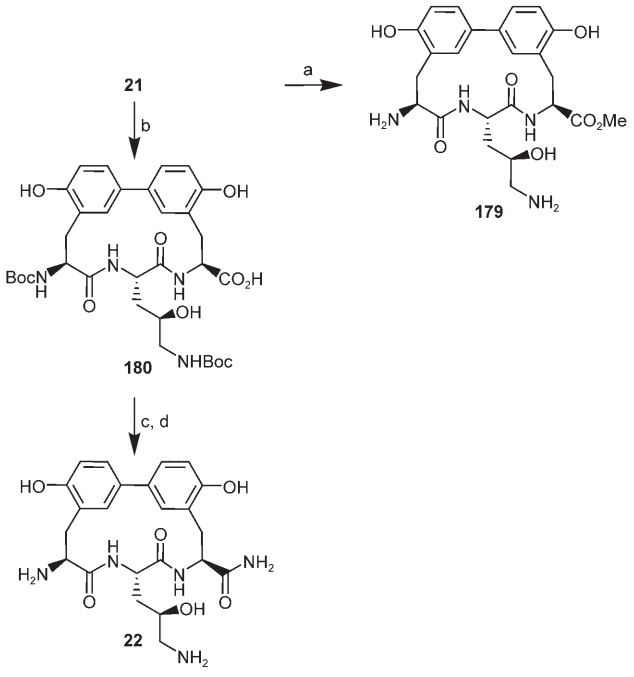
However, besides the total synthesis of natural biphenomycin A and B, neither derivatives nor close analogues thereof had been prepared. A first series of simplified amide and ester derivatives, including derivatizations at the peptide backbone have been reported recently.[355,356] The number of steps to the desired analogues of the natural product has been reduced significantly compared with the original sequence. Thus, biphenomycin B analogues have been prepared in 16 steps starting from benzaldehyde **164** through a Suzuki–Miyaura coupling[357] and subsequent double asymmetric hydrogenation (Scheme 17).[358,359] This route gave access to the same intermediate **172** as in the synthesis by Schmidt et al.. Intermediate **172** was obtained as a single diastereomer with complete stereocontrol (*ee* > 99%). Furthermore, the overall yield and feasibility of the synthesis has been improved by optimization of the macrocylization protocol and the final removal of the protecting groups.

The biphenomycin analogues obtained by this methodology could be selectively derivatized by either direct esterification or amide formation through 1) Boc protection



Scheme 17. Bayer's large-scale approach to hydroxydiisotyrosine **172**.[358] Reagents and conditions: a) Bis(diphenylphosphanyl)ferrocenpalladium(II)chloride, Cs_2CO_3, 1-methyl-2-pyrrolidon, H_2O, 50 °C, 10 h, 85 %; b) H_2 (3 bar), (*S,S*)-Et-DuPhos-Rh (1.5 %), EtOH/dioxane (1:1), RT, 3 d, quant., > 99 % *de*, > 99 % *ee*.

of the free amino groups, followed by 2) amide formation of the terminal carboxy group and, 3) acid-catalyzed Boc deprotection (Scheme 18). This approach led to novel con-



Scheme 18. Derivatization of biphenomycin B (**21**). Reagents and conditions: a) 4 M HCl in dioxane, MeOH, RT, 24 h, 97 %; b) di-(*tert*-butyl)-dicarbonate, H_2O, Na_2CO_3, MeOH, 0 °C→RT, 12 h, 83 %; c) $Na_2S_2O_4$, iPr_2NEt, NH_4Cl, HATU, DMF, 4 h, RT, 52 %; d) 4 M HCl in dioxane, RT, 30 min, 97 %.

Table 18: Antibacterial activity in vitro of **179** and **22** against Gram-positive pathogens, MIC [$\mu g\,mL^{-1}$].

	S. aureus	*S. aureus*	*E. faecalis*	*B. catarrhalis*
22	1.5	0.2	1	1
179	0.1	0.05	3	1

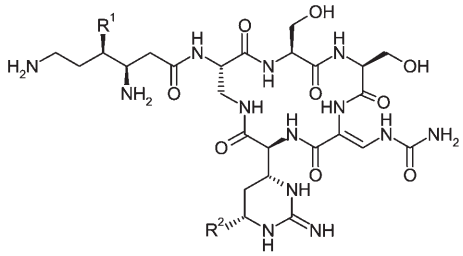
generers of biphenomycin B (**21**) that have improved in vitro activity (Table 18).[355,356,360]

Although both the biphenomycins and vancomycin possess a biphenyl group, no evidence for binding of **162** to the cell-wall analogue of *N*-Ac-D-Ala-D-Ala exists.[344] Instead, bacterial protein biosynthesis seemed to be the target of this novel class of antibacterials.[356]

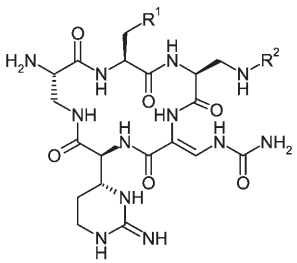
In summary, efficient pathways for the de novo synthesis of biphenomycins have been established and are paving the way to novel congeners (substituting derivatization) with improved in vitro activity and further insight into the molecular target of these novel antibacterials.

14. Tuberactinomycins and Capreomycins

The tuberactinomycin family of antibiotics consists of the closely related cyclic homopentapeptides tuberactinomycins and capreomycins. Viomycin (tuberactinomycin B, **182**), discovered in 1951,[361,362] was marketed by Ciba and Pfizer as a tuberculostatic agent in the 1960s. The capreomycins were isolated from the fermentation of *Streptomyces capreolus* as a four-component mixture, with **23** and **185** as the major and **186** and **187** as the minor compounds.[363] Both subclasses exhibit good activity against *Mycobacteria* including multidrug-resistant strains, but have only limited activity against other species.[364]

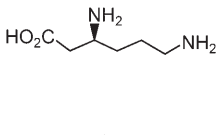


tuberactinomycin A, R^1 = OH, R^2 = OH (**181**)
tuberactinomycin B, R^1 = H, R^2 = OH (**182**, viomycin)
tuberactinomycin N, R^1 = OH, R^2 = H (**183**)
tuberactinomycin O, R^1 = H, R^2 = H (**184**)

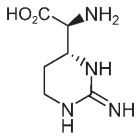


capreomycin IA, R^1 = OH, R^2 = β-lysyl (**23**)
capreomycin IB, R^1 = H, R^2 = β-lysyl (**185**)
capreomycin IIA, R^1 = OH, R^2 = H (**186**)
capreomycin IIB, R^1 = H, R^2 = H (**187**)

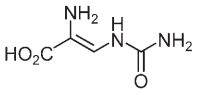
The nonproteinogenic amino acids, β-lysine (**188**), (2*S*,3*R*)-capreomycidine (**189**), and β-ureidodehydroalanine (**190**)—contained in the peptide backbone—complicated the structural elucidation of this class. The original structural



β-lysine (**188**)



(2*S*,3*R*)-capreomycidine (**189**)



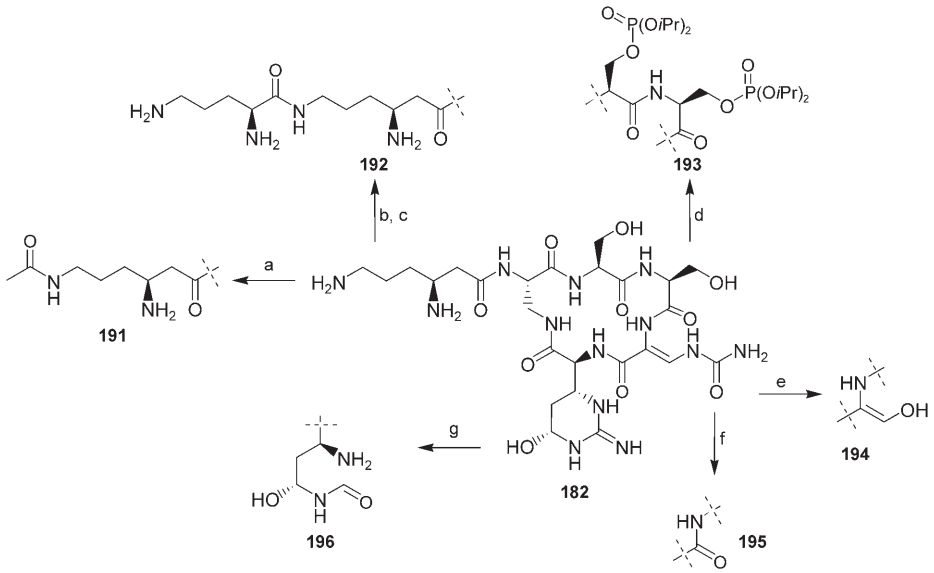
β-ureidodehydroalanine (**190**)

proposal[365] for capreomycin IB (**185**), was later revised by Shiba and co-workers.[366] Concurrently, several papers were published with proposed partial structures[367] or suggestions for the complete constitution of the tuberactinomycins (especially viomycin).[368–370] However, the correct structures were not determined until the X-ray crystal structure for tuberactinomycin O[371] (**184**) was resolved, and subsequently the structure of viomycin (**182**) was established.[372]

The activity of the tuberactinomycin family of antibiotics is limited mainly to Mycobacteria such as *Mycobacterium tuberculosis* or *Mycobacterium kansasii* (MIC 2–20 $\mu g\,mL^{-1}$).[373,374] They exert their antibiotic activity as potent inhibitors of the translation step of prokaryotic protein biosynthesis by inhibiting both the initiation and elongation steps. A detailed report on the interaction of tuberactinomycins at the target level illustrated how these compounds interacted with RNA.[375] When tested against a panel of *M. tuberculosis* strains in vitro, capreomycins compared favorably with streptomycin, cycloserine, and kanamycin.[376] No cross-resistance of tuberactinomycins with kanamycin, lividomycin, or paronomycin was observed.[377] Development of resistance in vitro seemed to be slow, especially when compared with kanamycin.[373] The in vivo efficacy of these compounds was low after oral dosing, but good after subcutaneous administration in experimental murine tuberculosis models.[373] Although tuberactinomycins were not devoid of toxicological problems, their toxicity profile after i.v. and peroral administration was quite favorable.[378] Overall, these biological features warranted further evaluation of this class for their clinical use as antituberculosis agents.

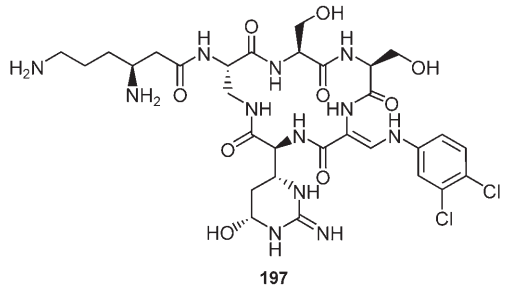
The early commercialization of viomycin (**182**) triggered intense research activities around these antimicrobials in academia and industry. Initial biological studies were often performed with mixtures of tuberactinomycins (**181**–**184**) or capreomycins (**23, 185**–**187**). Then, based on the studies of Gould and co-workers[379,380] on the biosynthesis of capreomycins, selective fermentation processes were developed.[381,382] Furthermore, several total syntheses enabled the selective access to viomycin (**182**),[383] tuberactinomycin N (**183**),[384] tuberactinomycin O (**184**),[385] and capreomycin.[366d] Despite the broad methodic arsenal to elucidate the SAR of tuberactinomycin-like antibiotics by total synthesis, most derivatives prepared for biological testing have been obtained through fermentation and semisynthesis. The first hints for the structural requirements necessary to produce activity against Mycobacteria were obtained from blocking specific functional groups in viomycin (**182**; Scheme 19).[386] Thus, the acetylation of the terminal amino group (**191**)[387] or both amino groups of β-lysine (not shown) led to a complete loss in activity (MIC *Mycobacterium* sp 607: 1.6 $\mu g\,mL^{-1}$ (**182**) versus $>400\ \mu g\,mL^{-1}$ (**191**)).[388] Acylation with uncharged or acidic amino acids at the same position also produced inactive compounds, whereas introduction of a basic amino acid (**192**) maintained the original MIC.[389] Similarly, blocking of the serine hydroxy groups, as in **193**, left the activity unchanged.[386] Surprisingly, hydrolysis of the urea functionality, which was regarded as one of the pharmacophores of this class, produced **194** with comparable in vitro activity as observed for viomycin (**182**; MIC *Mycobacterium* sp 607: 1.6 $\mu g\,mL^{-1}$ versus 3.1 $\mu g\,mL^{-1}$, for **182** and **194**, respectively).[386] Oxidation of **182** yielded the inactive bisamide **195**.[390] Finally, reductive opening of the capreomycidine ring of **182** led to a complete loss in activity (**196**).[391]

Similar modifications have been performed with tuberactinomycin N (**183**),[392–394] tuberactinomycin O (**184**),[395] and capreomycins.[396] However, none of these efforts resulted in a significantly improved activity against Mycobacteria or an extension of the antibacterial spectrum.



Scheme 19. Semisynthetic modification of viomycin (**182**). Reagents and conditions: a) *N*-acetoxy succinimide, Et_3N, carbonate buffer solution, dioxane, 1 h; b) *Z*-D-Orn(Z)-OSuc, Et_3N, carbonate buffer solution, THF, 0 °C, 12 h; c) H_2, Pd, DMF; e) hydrolysis; f) $KMnO_4$ (oxidation); g) $NaBH_4$ (reduction).

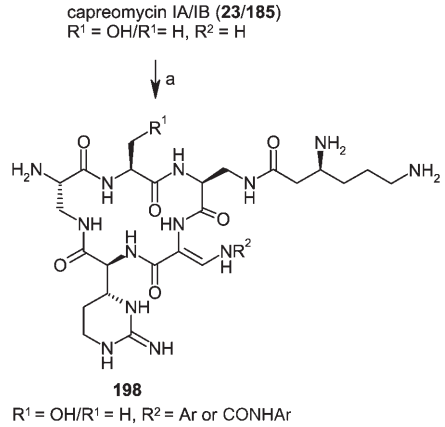
Driven by the widespread emergence of bacterial resistance, Pfizer initiated a HTS against *Pasteurella haemolytica* with the goal to discover novel leads for both animal and human infections.[397] The 3,4-dichlorophenylamino analogue **197** of viomycin (**182**), discovered by this approach, exhibited



197

good MICs against the animal pathogens *P. multocida* and *P. haemolytica*, but only mediocre activity against other bacteria (Table 19). However, further variation of the substituted ureido analogues of capreomycin IA/IB (mixture of **23** and **185**) yielded novel compounds with activities against several multidrug-resistant Gram-positive pathogens and Gram-negative *E. coli* (Scheme 20).[398]

Further to their in vitro potency, these compounds were also efficacious in murine infection models. For example, **201** exhibited an ED_{50} of 3 mg kg^{-1} (subcutaneous application) against MRSA and VRE. Starting from substituted urea **24**, guanidine **202** was obtained in 36% yield (Scheme 21).[399] Irrespective of this substantial change in basicity of the terminal amine in **202** compared with **24**, both compounds exhibited the same antibacterial spectrum against all tested Gram-positive strains.

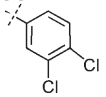
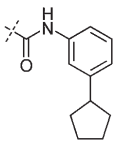
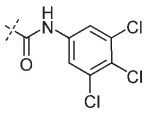
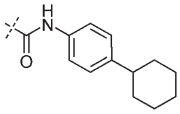


Scheme 20. Synthesis of the ureido derivatives of capreomycin IA/IB (**23**/**185**). Reagents and conditions: a) aniline or phenyl urea, 2 N HCl, dioxane, 65 °C, 4 h–12 h.

Treatment of viomycin (**182**) in trifluoroacetic acid with nucleophiles generated the respective Pictet–Spengler-like derivatives at the hydroxy group of viomycin with undetermined stereochemistry (Scheme 22).[400] Arylation or introduction of a sulfonamide group at C19 did not improve activity, whereas carbamate and urea substitution at this position were found to be more potent than the parent antibiotic (Table 20).

By using de novo synthesis, Pfizer scientists could significantly expand the antibacterial spectrum of tuberactinomycin-like antibiotics from *Mycobacterium*-only to Gram-positive pathogens, including multiresistant strains. Yet, despite proven in vitro and in vivo potency of the novel analogues, no further clinical development in this class has been reported to date.

Table 19: Antibacterial activity in vitro of ureido derivatives of capreomycin IA/IB (**23**/**185**), MIC [μg mL^{-1}].

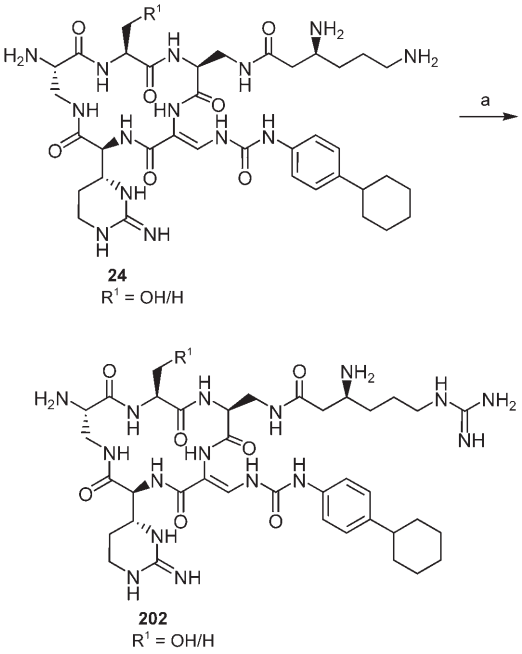
	R^2 (in **198**)	*P. multocida*	*E. coli*	MRSA	*E. faecium*	*E. faecalis*
23/**185**	$CONH_2$	50	200	100	>1000	>1000
197	[a]	0.78	12.5	12.5	50	25
199		0.39	6.25	0.78	25	12.5
200		0.39	12.5	3.12	3.12	3.12
201		0.2	6.25	0.78	3.12	1.56
24		n.d.[b]	n.d.[b]	1.56	3.12	3.12

[a] See formula in text. [b] n.d. = not done.

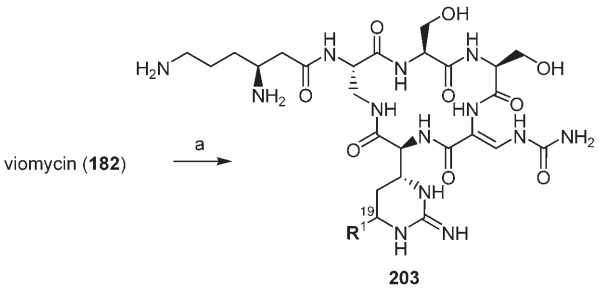
15. Glycopeptide Antibiotics

Owing to the lack of cross-resistance to other antibacterial drugs, the glycopeptide antibiotics[401] have become first-line drugs for the (parenteral) treatment of life-threatening multiresistant infections by Gram-positive bacteria in many hospitals. Vancomycin (**7**, Figure 10), the first glycopeptide antibiotic introduced into clinical practice in 1959,[402] was isolated from *Streptomyces orientalis* (now *Amycolatopsis orientalis*) from soil samples by Lilly scientists in the mid 1950s.[403] The elucidation of its complex structure took years and required several attempts and revisions until it could unequivocally be assigned in the early 1980s.[404,405]

Although related congeners have been used as growth promoters for livestock, teicoplanin (**208**) is the only additional member of this class that is available for human use, albeit not in the US. Both drugs

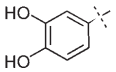
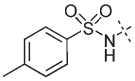
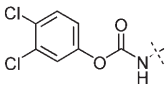
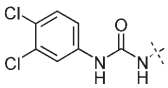


Scheme 21. N Terminal modification of capreomycin derivative **24**. Reagents and conditions: a) methyl imidothiocarbamate, H_2O, pH 10.7, RT, 7 d, 36%.



Scheme 22. C19 modifications of viomycin (**182**). Reagents and conditions: a) nucleophile (1,2-dihydroxybenzene, 4-methylsulfonamide, 3,4-dichlorophenylcarbamate, or 3,4-dichlorophenylurea), TFA, RT, 15 h.

Table 20: Antibacterial activity in vitro of C19 derivatives of viomycin (**182**), MIC [$\mu g\,mL^{-1}$].

	R^1	*P. multocida*	*E. coli*	MRSA
182	HO	200	200	n.d.[a]
204		200	200	n.d.[a]
205		200	200	n.d.[a]
206		6.25	25	3.12
207		1.56	6.25	25

[a] n.d. = not done.

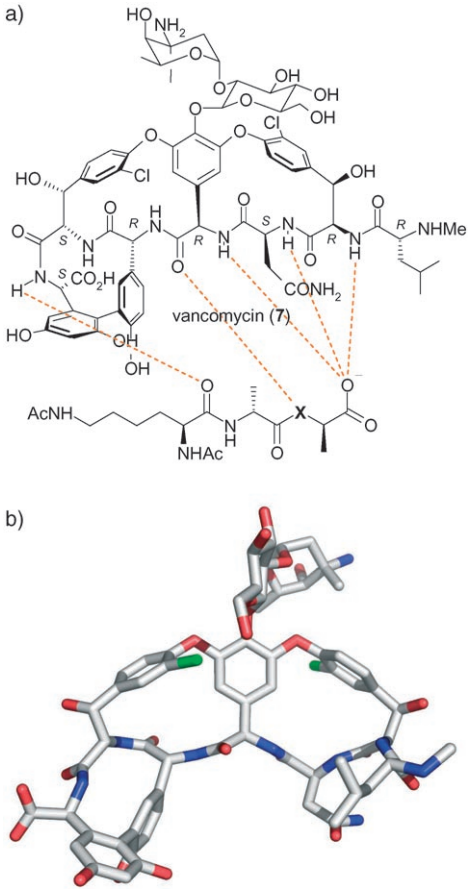


Figure 10. a) Hydrogen bonding between vancomycin (**7**) and the L-Lys-D-Ala-D-Ala (X = NH) peptidoglycan precursor terminus model peptide.[416b] In the resistance phenotypes VanA and VanB, the molecular recognition site D-Ala-D-Ala (X = NH) of the peptidoglycan precursor strands is replaced by a D-Ala-D-lactate terminus (X = O). b) The X-ray crystal structure of vancomycin illustrates its rigid concave shape[417a]

are unaltered natural antibiotics of the large dalbaheptide group that is produced by various actinomycetes.[406] Their common structural element is a linear heptapeptide backbone (configuration *R*,*R*,*S*,*R*,*R*,*S*,*S*) in which some aromatic amino acid residues are cross-linked (biphenyl and diphenylether motives) and build a rigid concave shape. For years, total syntheses of vancomycin's complex structure presented an enormous challenge for the foremost synthetic groups.[407] It is particularly informative to compare the successful synthetic strategies of Evans et al.,[408] Nicolaou et al.,[409] and Boger et al.[410] with nature's biosynthetic route to glycopeptides.[411,412] In contrast with the chemists, micorganisms assemble the complete linear heptapeptide (nonribosomal peptide synthetases) before oxidative (enzymatic) cross-linking of the side chains. With about 35 steps, the biosynthetic route to **7** remains considerably shorter than any de novo synthesis.

Glycopeptide antibiotics inhibit bacterial cell-wall biosynthesis by recognizing and strongly binding to the L-Lys-D-Ala-D-Ala termini of peptidoglycan precursor strands at the external side of the membrane. In this way, transpeptidases are prevented from executing their cross-linking job.[413–415]

NMR spectroscopic[416] and X-ray crystal[417] studies demonstrated that evolution has saliently shaped the rigid antibiotic cavity for tight binding to its target through five specific hydrogen bonds in a stoichiometric complex (Figure 10). The blocking of transpeptidases indirectly also affects transglycosylase action.

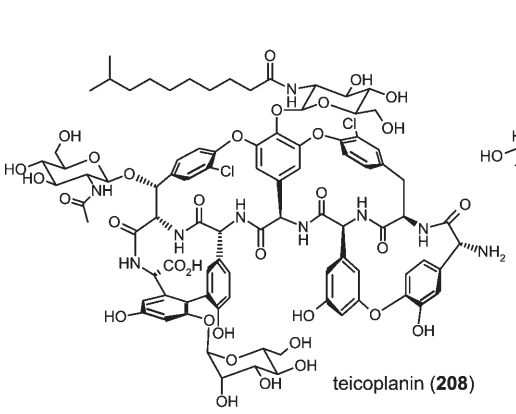
Glycopeptide antibiotics are restricted to treating Gram-positive infections as they cannot penetrate the outer membrane of Gram-negative bacteria. With the rise of MRSA infections in hospitals, vancomycin (**7**) became the antibiotic of last resort but, owing to its frequent use, resistant Gram-positive pathogens, in particular vancomycin-resistant enterococci (VRE) have emerged and worryingly spread.[418] By 2003, more than half of the clinical VRE isolates in the US had become resistant to glycopeptides.[35] In the main resistance phenotypes (VanA and VanB), the molecular recognition site D-Ala-D-Ala (X = NH) of the peptidoglycan precursor strands is replaced by a D-Ala-D-lactate terminus (X = O).[419] This "simple" amide to ester replacement at the peptidoglycan terminus, that is, the loss of a single hydrogen bond ($C^{1.4}$-carbonyl to X = O) and concomitant creation of a destabilizing lone pair/lone pair interaction between the ligand and antibiotic notably results in a 1000-fold decrease in binding affinity and a dramatic loss in antibacterial activity (MIC).[420]

In a spectacular example of chemical postevolution, Boger and co-workers have selectively omitted the "disturbing" $C^{1.4}$-carbonyl functionality (Figure 10). Their fully synthetic $C^{1.4}$-deoxo congener of vancomycin exhibited enhanced affinity for the D-Ala-D-lactate terminus (no repulsive interaction) leading to restored antibacterial activity against resistant pathogens.[421] Some glycopeptide antibiotics tend to form homodimers that bind much tighter to the peptidoglycan termini than the corresponding monomers.[413] The emergence of vancomycin-resistant enterococci and staphylococci[422] has encouraged the search for new (lipo)glycopeptides with improved pharmacokinetic and pharmacodynamic properties and activities towards resistant strains.[414,423,424]

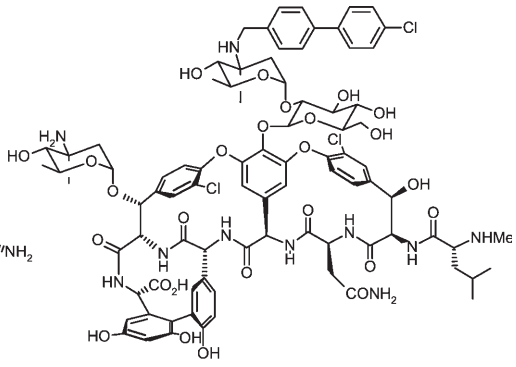
Despite successful total syntheses and available innovative synthetic methods, fermentation and subsequent semisynthetic variation of natural glycopeptide scaffolds has been the prevalent way to explore SARs[423] and the only practicable route to bulk production of clinical candidates. Novel, resistance-breaking glycopeptides possess structural elements that promote dimerization and membrane anchoring. Dimerization causes tighter binding of ligands terminating in D-Ala-D-lactate,[425] whereas lipophilic side chains endorse anchoring in the cytoplasmic membrane, thereby helping to position the antibiotic close to its target and eventually also disturb the bacterial-membrane integrity. These effects have stimulated chemists to prepare covalent vancomycin dimers.[426] Lipophilic side chains can restore activity against VRE while maintaining the effectiveness against MRSA.[427]

The presence of specific sugars is of vital importance for glycopeptide activity; aglycones are uniformly less active. An additional amino sugar at residue 6 and aromatic chlorine substituents promote favorable dimerization, and substitution of the free carboxylate function by basic carboxamides increases the activity against staphylococci. On the other hand, most efforts to change the natural heptapeptide backbones resulted in reduced activity.[406]

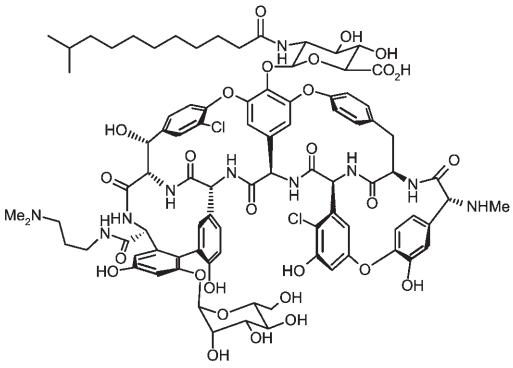
Three semisynthetic second-generation drugs, oritavancin (LY-333328, **26**),[428,415] dalbavancin (BI-397, **209**),[429] and telavancin (TD-6424, **210**),[430] have been advanced to clinical development[431] and have provided insight into how medicinal-chemistry programs work. Oritavancin (**26**) is the 4′-chlorobiphenylmethyl derivative of the natural vancomycin analogue chloroeremomycin (**25**). The spectrum of **26** covers VRE, MRSA, and to some extent also glycopeptide-intermediate *S. aureus* (GISA). Its bactericidal action and half-life allow for once-daily administration. Dalbavancin (**209**) has a



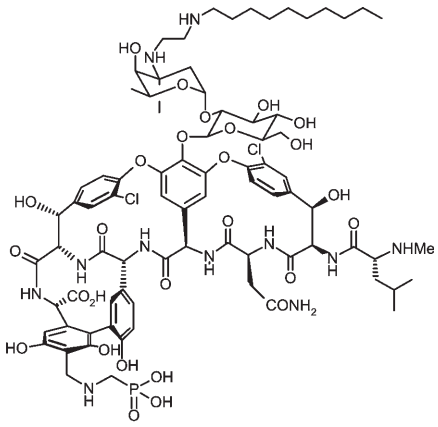
teicoplanin (**208**)



oritavancin (**26**)



dalbavancin (**209**)



telavancin (**210**)

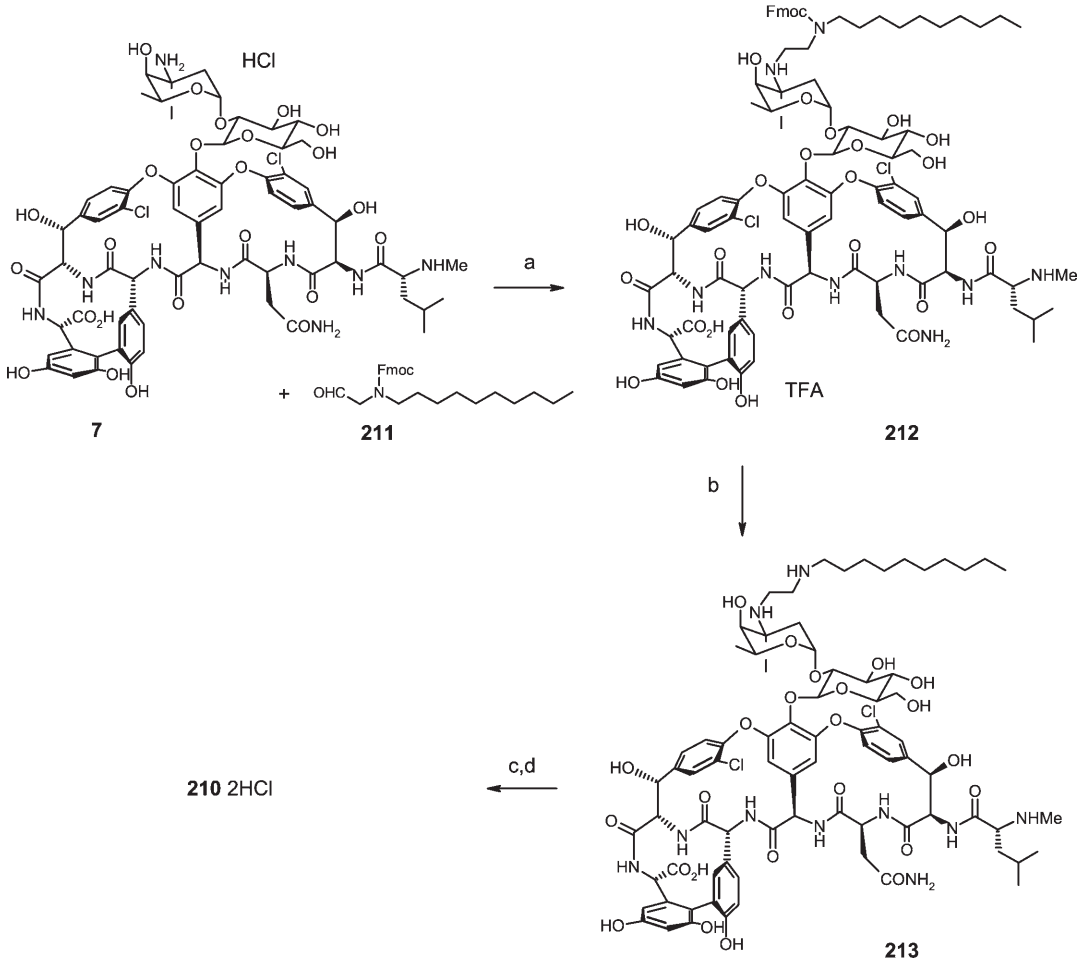
similar spectrum but is not active against VRE of the VanA-resistance genotype (Table 21). Owing to its prolonged retention in the body (half-life 174 h), one administration per week seems sufficient.

The discovery of telavancin (**210**),[432] the youngest and most promising congener, encapsulates the essential aspects of a successful glycopeptide optimization: in search of glycopeptides with improved in vitro activity, Theravance chemists prepared *N*-decylaminovancomycin (**213**, Scheme 23), the MIC of which was superior or at least equipotent to **7**. As expected, the introduction of the lipophilic substituent restored activity against enterococci regardless of their susceptibility while maintaining activity against MRSA and VISA. However, although increased lipophilicity improved in vitro activity, at the same time it negatively influenced physicochemicistry and absorption, distribution, metabolism, and excretion (ADME) properties: in contrast to **7**, **213** was poorly excreted from urine and showed an unfavorable tissue distribution in rats. To counterbalance lipophilicity and distribution properties, the supplementary addition of different hydrophilic groups was explored. Indeed, compounds with negatively charged polar groups exhibited increased urinary excretion and reduced accumulation in the liver and kidney while maintaining the improved in vitro profile. As a result of these studies, the lipoglycopeptide antibiotic **210** was selected for development.[430] It demonstrated potent in vitro activity against clinically relevant aerobic[433] and anaerobic[434] Gram-positive bacteria without cross-resistance to comparative drugs, showed rapid bactericidal action, and effected its activity through multiple modes of action (inhibition of peptidoglycan

Table 21: Antibacterial activity in vitro of glycopeptides vancomycin (**7**), oritavancin (**26**), dalbavancin (**209**), and telavancin (**210**), MIC_{90} [$\mu g\,mL^{-1}$].[430a]

	7	26	209	210
MSSA[a]	1	1	0.25	1
MRSA[b]	4	1	0.25	1
VRSA[c]	32	0.25	1	0.5
S. pneumoniae	0.5	0.008	0.06	0.008
Enterococcus spp. VanB	128	1	1	2
Enterococcus spp. VanA	>128	4	128	8

[a] Methicillin-susceptible *S. aureus*; [b] Methicillin-resistant *S. aureus;* [c] Vancomycin-resistant *S. aureus*.



Scheme 23. Theravance's semisynthesis of telavancin hydrochloride (**210**) from vancomycin hydrochloride.[430] Reagents and conditions: a) iPr_2NEt, DMF, 6–8 h, RT, MeOH, TFA, 15 min RT, borane-*tert*-butylamine complex, 2 h, RT; b) *tert*-butylamine, 40 °C, 7 h; c) (aminomethyl)phosphonic acid, CH_3CN, 20–30 °C, 15 min; iPr_2NEt, 1 h, RT, aqueous CH_2O, −7 °C, 12 h; 3 N HCl, pH 2.59, −5 °C; EtOH, 5 °C, 3 h; d) Amberlite XAD chromatography, CH_3CN, H_2O, HCl.

synthesis and perturbation of bacterial cell membrane potential and permeability).[435] In vivo pharmacodynamics in various animal models[436] as well as pharmacokinetics and tolerability in healthy subjects suggested an effective once-daily therapy.[437]

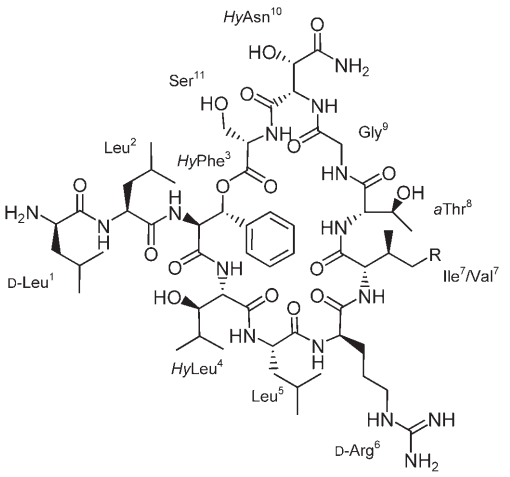
Telavancin (**210**) has been prepared from vancomycin hydrochloride and other commercially available starting materials through a three-stage process (Scheme 23):[438,439] selective reductive alkylation of **7** with *N*-Fmoc-*N*-decylaminoacetaldehyde (**211**) by means of borane-*tert*-butylamine complex followed by treatment with methanol and trifluoroacetic acid afforded the Fmoc-protected intermediate **212**. The Fmoc group of **212** was removed with *tert*-butylamine in DMF to yield *N*-decylaminovancomycin (**213**) in one pot. Mass spectrometric analysis confirmed that reductive alkylation selectively occurred at the vancosamine nitrogen and not at the N terminus of the peptide backbone. Mannich condensation with (aminomethyl)phosphonic acid and aqueous formaldehyde produced crude **210**, which was purified by Amberlite XAD chromatography to yield pure telavancin hydrochloride. The original procedure[432] has been continuously modified and optimized for production.[430]

Through chemical postevolution, the new, semisynthetic glycopeptide antibiotics, in particular oritavancin (**26**) and telavancin (**210**), have achieved significant technical progress over their established natural congeners in terms of activity against resistant strains and pharmacokinetic and pharmacodynamic properties. It will be interesting to learn to what extend these favorable profiles will be reflected in cure rates during advanced clinical trials. If approved, these compounds could become valuable drugs for the treatment of severe infections with multiresistant pathogens.

16. Lysobactins

The lysobactins are good examples of structurally exciting antibacterial natural products that are not the result of expeditions to remote tropical habitats, but were detected in urban soil organisms. Lysobactin (**27**) was isolated from the fermentation broth of *Lysobacter* sp. SC-14076 (ATCC 53042) by scientists from Squibb.[440,441]

The lysobactin strain was obtained from a leaf-litter sample found in the historic Washington Crossing State Park, US. Independently, scientists from Shionogi reported on katanosins A and B, which originate from a soil bacterium found in Katano City, Japan.[442] The producer strain PBJ-5356 was described as related to the genus *Cytophaga*, but not as *Lysobacter* sp.. Surprisingly, katanosin B later turned out to be identical to **27**.[443] Recently, katanosin A (**214**) has also been found to be a minor metabolite of the *Lysobacter* ATCC 53042 strain.[444] Most likely **27** and **214** belong to the armamentarium of their bacterial producers. Correspondingly, lytic attack of other microorganisms was the name-giving characteristic for the genus *Lysobacter*.[445] From a chemotaxonomic point of view it is surprising to find special secondary metabolites such as **27** and **214** being biosynthesized within two different phyla such as Proteobacteria (*Lysobacter*) and Bacteroidetes (*Cytophaga*).



lysobactin, R = CH_3 (**27**, ≡ katanosin B)

katanosin A, R = H (**214**)

The biosynthesis of bacterial cell walls appears to be the primary target area of lysobactin antibiotics.[441,442] Both **27** and **214** inhibited consumption of the cell-wall precursor [^{14}C]GlcNAc, a very good indicator for interference with the peptidoglycan biosynthesis (Figure 3). Most likely, inhibition of peptidoglycan formation is induced by the binding of lysobactin to lipid intermediates (not through binding to biosynthetic enzymes) that occur as biosynthetic precursors downstream of the muramyl pentapeptide. Lysobactin antibiotics seem to have a mode of action that is different from vancomycin (**7**).[446]

Lysobactin (**27**) and katanosin A (**214**) are highly active against Gram-positive bacteria, such as staphylococci or enterococci. In these pathogens submicromolar MIC values were obtained that were often superior to vancomycin.[440,442,447] The excellent antibacterial in vitro activity of **27** and **214** was maintained in vancomycin-resistant enterococci. Promising therapeutic in vivo efficacy could be demonstrated in a systemic murine *S. aureus* infection model (ED_{50} 1.8 mg kg^{-1} i.v., CFU ≈ 10^5).[442]

The primary structure of lysobactin (**27**) was elucidated in an elegant interplay of spectroscopic methodology and enzymatic degradation reactions.[448] Lysobactin has a "lariat structure" that consists of the dipeptidic "linear segment" (D-Leu^1-Leu^2) and the nonadepsipeptidic "cyclic segment" (*Hy*Phe^3-*Hy*Leu^4-Leu^5-D-Arg^6-Ile^7-*a*Thr^8-Gly^9-*Hy*Asn^{10}-Ser^{11}). So far, the secondary solution structure of **27** has not been fully elucidated.[449] The high content of nonproteinogenic amino acids[450] clearly hints at a nonribosomal biosynthesis.[451] The remarkable hydrolytic stability of all amide bonds towards proteases and peptidases may be regarded as the result of an evolutionary optimization that lead to a cyclic structure with a high content of β-hydroxylated as well as non-natural D-configurated amino acids. These structural motifs, cyclic, β-hydroxylated, and D-configurated, are often encountered in depsipeptidic and peptidic secondary metabolites. This opposes the common prejudice of the non-drugability of peptide structures owing to their low proteolytic stability.

However, in lysobactin (**27**) the lactone linkage is prone to hydrolysis.[448]

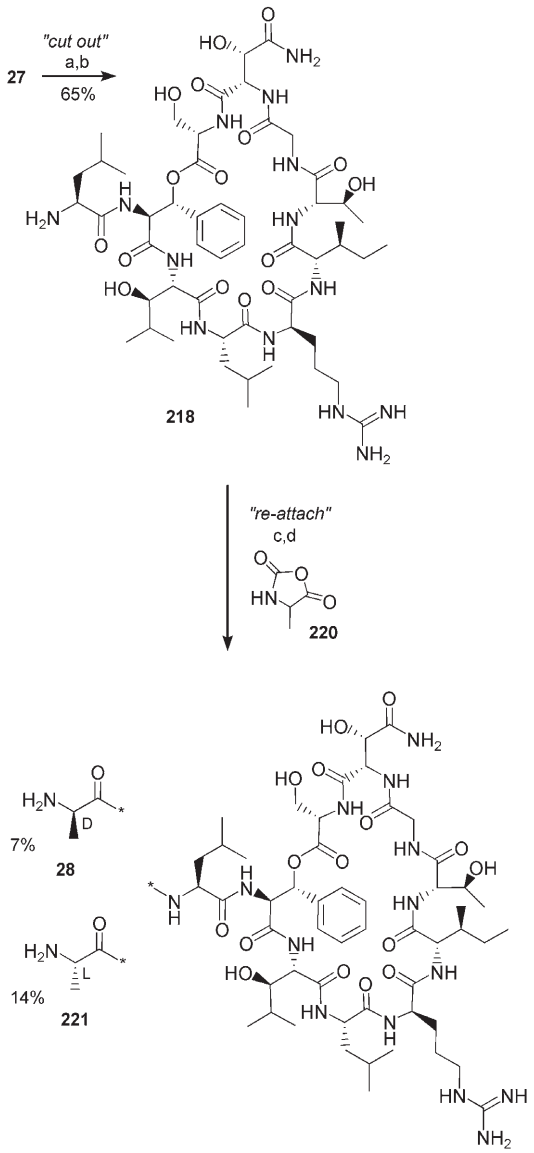
So far, a total synthesis of lysobactin (**27**) has not been published. Expected challenges are the synthesis of *threo*-β-hydroxyasparagine, the generation and conservation of the lactone function (in the presence of four hydroxy groups), and the crucial macrocyclization. Cardillo and co-workers,[452,453] Lectka and co-workers,[454] VanNieuwenhze and co-workers,[455] and Palomo et al.[456–458] started tackling the synthetic challenges of the lysobactin target molecule. Egner and Bradley[459] already reported on the solid-phase synthesis of a close lysobactin congener **217** (Scheme 24), however, no biological data have been published for **217**.

In the complex class of lysobactin antibiotics, semisynthesis was again the faster way to set up preliminary SARs. The Edman elaboration of the natural product allowed for selective modification of lysobactin at amino acid position 1 through a substituting-derivatization strategy (cut out and reattach; Scheme 25).[444,447] Reacylation of **218** showed that the N-terminal D-Leu[1] played a crucial role for the biological activity of lysobactin antibiotics. The N-terminal amino acid seems to be a prerequisite for activity, consequently, the Edman degradation product **218** turned out to be inactive. N Capping with a D-configured amino acid at position 1 (as in the natural product) appeared to be beneficial for in vitro activity (**28** versus **221**; Table 22).[447]

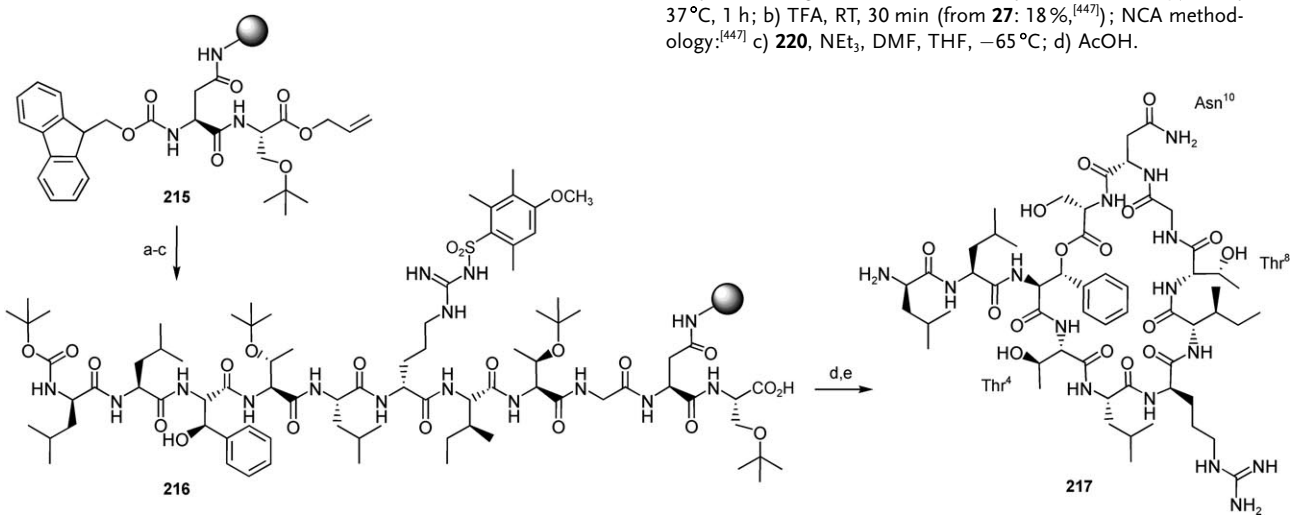
The lysobactins are interesting antibacterial lead structures with promising in vitro activity and in vivo efficacy. So far, knowledge about this class is based on the natural

Table 22: Antibacterial activity in vitro of lysobactin antibiotics against Gram-positive pathogens, MIC [$\mu g\,mL^{-1}$].[447]

	S. aureus	*E. faecalis*
27	0.2	0.8
218	100	50
28	0.4	1.6
221	3.1	6.3



Scheme 25. Squibb's single Edman degradation (substituting modification).[444,447] Reagents and conditions: a) excess PhNCS, pyridine/H_2O, 37 °C, 1 h; b) TFA, RT, 30 min (from **27**: 18%,[447]); NCA methodology:[447] c) **220**, NEt_3, DMF, THF, −65 °C; d) AcOH.

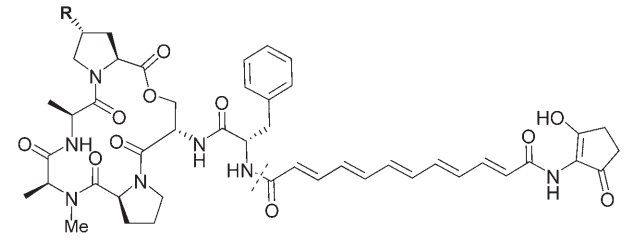


Scheme 24. Bradley's solid-phase synthesis of the lysobactin congener **217** through macrolactonization.[459] Reagents and conditions: a) Fmoc peptide synthesis, 6×DIC/HOBt couplings, CH_2Cl_2; b) Fmoc peptide synthesis, 3×EEDQ couplings, CH_2Cl_2; c) Pd^0; d) DIC, HOBt, DMAP, CH_2Cl_2, 37 °C, 12 h; e) TFA/iPr_3SiH/H_2O (95:2.5:2.5), 4 h (overall yield 15.6%).

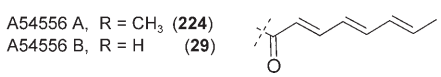
products **27** and **214** and some semisynthetic Edman derivatives. A preliminary SAR could be established for the amino acid position 1 within the linear segment. Further work has to be done for a real assessment of this interesting class.

17. Enopeptin Depsipeptide Antibiotics

Natural depsipeptides have a definite therapeutic potential as antibacterial agents and for various other indications.[460] Two depsipeptides from which the family name was derived, enopeptin A (**222**) and B (**223**), were isolated in 1991 from a culture broth of *Streptomyces* sp. RK-1051, found in a soil sample collected in Tsuruoka City, Japan.[461] Their structure, elucidated by chemical and spectroscopic means, consisted of a 16-membered lactone ring made up of five (*S*)-amino acids and a lipophilic polyene side chain attached to the serine N terminus.[462,463] Both their antimicrobial activity against Gram-positive bacteria and some Gram-negative membrane-defective mutants and their unique structural components evoked the chemists' interest in their total synthesis.[464] Almost a decade earlier, scientists from the company Eli Lilly described the isolation of a similar depsipeptide antibiotic A54556, a complex of eight depsipeptidic factors A–H, which was produced by aerobic fermentation of *Streptomyces hawaiiensis* (NRRL 15010).[465]



enopeptin A, R = CH_3 (**222**)
enopeptin B, R = H (**223**)



A54556 A, R = CH_3 (**224**)
A54556 B, R = H (**29**)

The complex A54556 and its separated factors exhibited interesting activity against penicillin-resistant staphylococci and streptococci inducing detailed investigations in our laboratories. As the original patent had been abandoned, producer strains and compounds were free for use and allowed repetition of the original experiments. Structural elucidation of the separated factors revealed that in constrast to reported results,[465] in our hands, the correct constitution of factor A corresponded to enopeptin depsipeptide **224**. Mode of action studies with *B. subtilis* demonstrated impaired bacterial cell division and induction of filamentation (Figure 11). By RG techniques, it could be shown that lead structures **224** and **29** inhibited bacterial growth by binding to ClpP (caseine lytic protease).[467,466] To protect the bacterial cell, ClpP is tightly regulated and requires a Clp–ATPase and accessory proteins for activation. Binding of **224** or **29** to ClpP eliminates the protection and triggers the ClpP core, which is

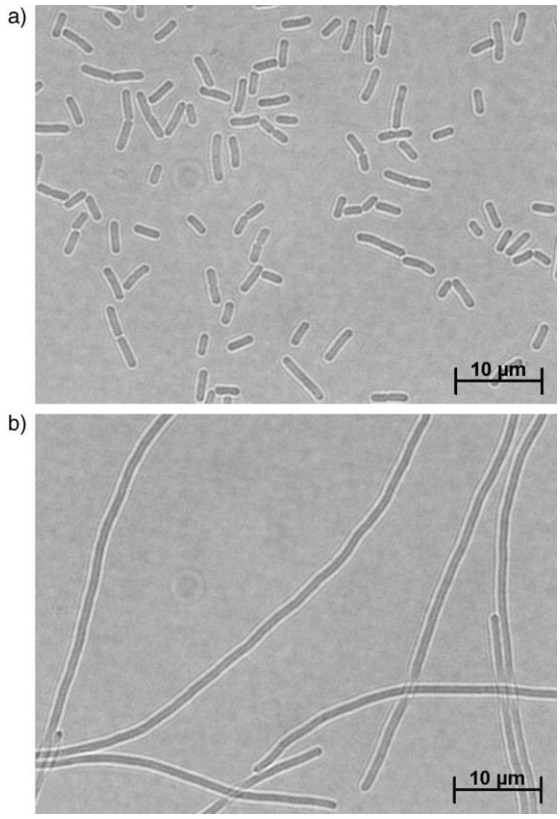


Figure 11. Action of **224** on *B. subtilis*: impaired cell division and induction of filamentation. Electron micrograph a) before and b) after treatment with **224** (0.4 μg mL^{-1}) for 5 h.[467]

then capable of proteolytic degradation in the absence of the regulatory Clp-ATPases. The consequence is uncontrolled proteolysis resulting in inhibition of bacterial cell division and finally cell death. Owing to this unprecedented mechanism of action, **224** and **29** were devoid of cross-resistance for all antibiotics on the market or in clinical development.[467]

On the other hand, the natural products suffered from various deficiencies that needed improval to assess the full potential of this class. Despite promising in vitro activity against enterococci and streptococci, natural enopeptin depsipeptide antibiotics **224** and **29** exhibited only moderate in vitro potency against staphylococci and were inactive against Gram-negative bacteria (Table 23). Both lead structures were not effective in vivo in standard lethal bacterial infection models in mice and their ADME profile was critical. Their chemical stability proved to be rather limited, their solubility was insufficient for parenteral application, and they were rapidly cleared from the body. Under these premises, an optimization program needed rational guidance and profited from a thorough understanding of the lead conformation based on X-ray crystal structure analysis of the synthetic congener **225** (Figure 12).[468]

Crystallization of **225** from aqueous acetonitrile gave solvent-free crystals. Acyldepsipeptide **225** adopted a conformation in which the lipophilic side chain was fixed by two hydrogen bonds to the top of the macrolactone ring. One NH···OC hydrogen bond (2.1 Å) was located between the NH

Table 23: Antibacterial activity in vitro of natural and synthetic enopeptin antibiotics against Gram-positive pathogens, MIC [$\mu g\,mL^{-1}$].

	S. aureus	*S. pneumoniae*	*E. faecium*	*E. faecalis*
224	8	0.5	1	1
29	16	1	2	2
231	>64	>64	>64	>64
232	1	0.25	≤0.125	0.125
233	0.5	≤0.125	≤0.125	0.125
234	8	2	2	1
235	1	≤0.125	≤0.125	≤0.125
236	<0.125	≤0.125	≤0.125	≤0.125
237	0.25	≤0.125	≤0.125	≤0.125
30	0.5	≤0.125	≤0.125	≤0.125

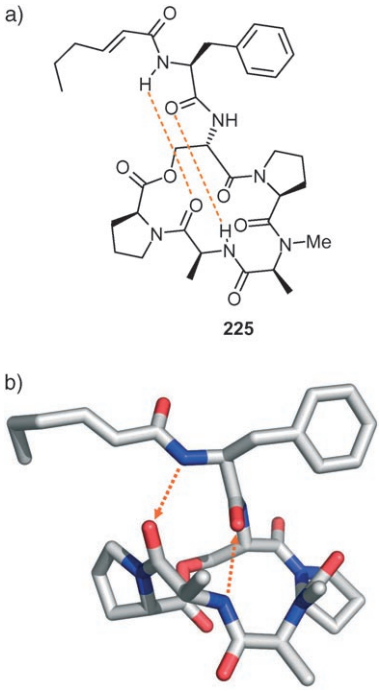
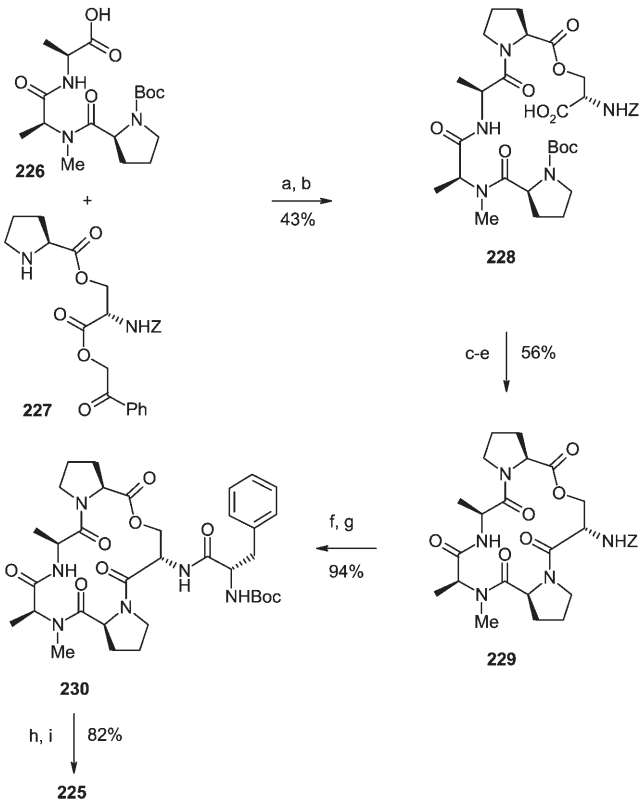


Figure 12. X-ray crystal structure of synthetic enopeptin **225**. The lipophilic side chain is fixed by two hydrogen bonds to the top of the macrocyclic core.[468]



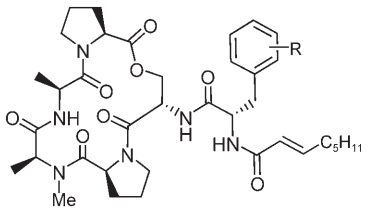
Scheme 26. Synthesis of novel enopeptin analogues.[469] Reagents and conditions: a) CH_2Cl_2, HOBt, TBTU, iPr_2EtN, 0°C→RT, 79%; b) $AcOH/H_2O$ (9:1), Zn, 2 h, RT, 54%; c) CH_2Cl_2, pentafluorophenol, EDC, 0°C→RT, 18 h; d) 4 N HCl in dioxane, 1 h, RT; e) CH_2Cl_2, H_2O, $NaHCO_3$, RT, 56%; f) MeOH, aqueous HCl, H_2 (1 bar), Pd/C, 97%; g) *N*-Boc-phenylalanine, DMF, HATU, iPr_2EtN, RT, 97%; h) CH_2Cl_2, TFA/H_2O (9:1), 45 min, RT, quant.; i) 2-hexenoic acid, DMF, HATU, iPr_2EtN, 82%.

group of phenylalanine and the carbonyl function of alanine, a second hydrogen bond (2.0 Å) was found between the alanine NH and the phenylalanine carbonyl. To preserve this active conformation, the medicinal chemists concentrated their structural variations on those parts of the molecule that were not involved in hydrogen bonding: *N*-methylalanine, proline in the upper portion of the structure, and the aryl and alkyl residues in the side chain (Scheme 26). The total synthesis by Schmidt et al. of enopeptin B[464] served as a basis for the synthesis of novel enopeptin analogues and for elaborating the enopeptin depsipeptide SAR.[469]

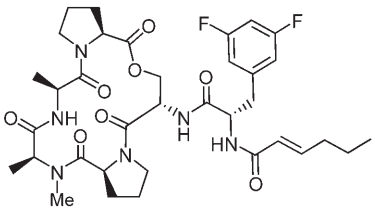
The synthesis proceeded by coupling tripeptide **226** with the ester **227** through standard peptide chemistry. Linear pentapeptolid **228** was cyclized to macrolactone **229** according to the pentafluorophenyl ester method by Schmidt et al..[470] The cyclization process may have thrived on a favored conformation of precursor **228**, which was preshaped by the hydrogen bond pattern that defined the ultimate conformation of the target macrocycle **229**. Removal of the Z protecting group and attachment of the phenylalanine side chain afforded the intermediate **230**. Acidic cleavage of the Boc group and coupling to hexenoic acid produced the desired enopeptin analogue **225**.

Starting from intermediate **230**, the side chain SAR (Table 23) was explored by substituting modification of the natural triene C8 acyl group. *E* configurated α,β-unsaturation proved to be crucial for biological activity, whereas additional double bonds were dispensable. Heptenoic acid side chains represented an optimum in length and lipophilicity and the corresponding analogues showed significantly improved chemical stability. To enhance solubility in water, polar functions were incorporated into the side chain, however, these efforts resulted in completely inactive congeners. Introduction of fluoro substituents in positions 3 and 5 of the side chain phenylalanine considerably improved activity against *S. aureus*, whereas an additional fluorine in position 4 was deleterious (**231**–**234**). Replacing the phenyl ring by a pyridyl or a cyclohexyl residue was not tolerated.

Substituting modifications in upper proline were demanding for chemistry. In this case, the natural 4-(*R*)-methyl



231 R = H **233** R = 3,5-F_2
232 R = 3-F **234** R = 3,4,5-F_3



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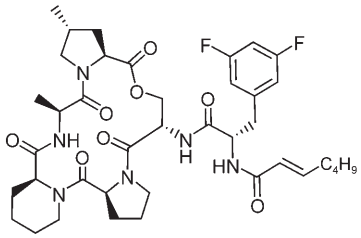

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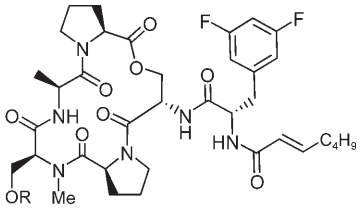

237 R = H
30 R = CO-CH_2NMe_2

substituent seemed to be the optimum. Increasing its size (ethyl, methoxy) or changing its position (4- to 3-substitution) clearly reduced antibacterial activity. For the natural *N*-methylalanine, *N*-alkyl substitution was indeed essential as removal of the *N*-methyl group yielded inactive depsipeptides. Replacing *N*-methylalanine by *N*-methylglycine was not tolerated, but incorporation of rigid cyclic amino acids afforded potent congeners: synthetic acyldepsipeptide **236** showed an impressive biological profile in vitro and in vivo. MIC values against Gram-positive pathogens were in the range of established antibiotics (Table 23), no cross-resistance was observed, and potency was retained with multiresistant clinical isolates. Intraperitoneal treatment of lethal *E. faecalis* infections in mice with a single doses of of **236** (0.5 mg kg^{-1}) resulted in 100% survival.[467] Finally, *N*-methylalanine in the variable lower part of the macrocycle was replaced with *N*-methylserine (compound **237**) to provide a handle for increased aqueous solubility. The corresponding exocyclic *N*,*N*-dimethylglycine ester **30** exhibited sufficient aqueous solubility to allow parenteral application while retaining excellent antibacterial activity. Indeed, medicinal chemists competently corrected inherent deficiencies (stability, solubility, potency) of natural enopeptin depsipeptide antibiotics (chemical postevolution). In this project, efficient de novo synthesis rather than a fermentative/semisynthetic approach has been used to systematically explore the potential of the natural-product lead structures **224** and **29**.

18. Summary and Conclusion

Accepting and defining the new socioeconomic environment for antibacterial research: Bacterial infections increasingly evade standard treatment as resistance to multiple antibiotics is spreading throughout the world. Reports on therapy failures and rising treatment costs are omnipresent, especially in the hospital environment. Resistant pathogens lead to higher health-care expenditures owing to extended hospital stays and expensive drugs. Yet, resistance is inevitably the result of antibiotic use and therefore limits the efficacy and life span of every antibiotic. There is an urgent medical need for a sustainable supply of new, effective, and safe antibacterial drugs without cross-resistance to currently used antibiotics. On the other hand, investment in antibacterial discovery and development is flagging and many big pharmaceutical companies have exited the field. Generic competition, drug resistance, and increased regulatory scrutiny have placed greater pressure on antibacterial profit margins. Indeed, efficient antibiotics eliminate their own need by rapidly curing the disease (auto-obsolence). As shareholder-value interests increasingly frame research and development investment strategies, the commercial success of drugs against chronic diseases has tempted many companies to preferentially invest into "chronic drugs" rather than into "short-term antibacterials". Moreover, industry has not delivered novel and valid antibacterial agents of late. Attempts to exploit novel antibacterial targets have been disappointing and the "one-target-one-disease" approach has been unsuccessful for this area. For antibacterial drug discovery, the high-throughput-screening approach has failed and needs to be modified or replaced to help close the productivity gap. Only the persistent discovery and development of novel resistance-breaking antibacterial lead structures will guarantee future therapy. New ideas and solutions are needed that facilitate and support this endeavor.

Reshaping of the research strategy and finding new ways of funding: A revision of the mainstream scientific approaches together with pharma-political consensus solutions to increase economic chances and commercial viability of novel antibacterials will help to avoid future "preantibiotic" scenarios. Public–private partnerships for antibacterial research and development (as for HIV, tuberculosis, and malaria) should be explored. Processes need to be improved by bringing key experts from academia, clinicians, regulators, and the pharmaceutical industry together. Some visionary biotech enterprises have already revitalized the field of antibacterial research and natural-product exploration with great success (AnalytiCon, Basilea, Cubist, Kosan, Replidyne, Vicuron, etc.). Incentives for antibiotics in relation to chronic and life-style drugs would help to convince decision makers to reinvest into antibacterial research and development: intellectual-property extension for priority antibiotics (wild card) that open up a new class (first in class), restoration of patent time lost during review, and streamlining clinical studies by allowing fast-track reviews, and the use of surrogate markers are some of the points for discussion. Increasing harmonization between regulatory authorities in different countries or even global approval criteria remain wishes for the future. Better infection control measures, monitoring of resistance and initiatives that foster the responsible and appropriate use of antibiotics will help to prolong the life span of established antibacterials and to retain public acceptance for antibiotic therapy.

Exploitation of contemporary scientific methodology for natural-product research: Medicinal chemistry provides the tools (semisynthesis, de novo synthesis) for the iterative optimization of natural products to obtain patentable drug molecules with improved pharmacokinetic, physicochemical, and toxicological properties (chemical postevolution). The diligent selection of natural antibiotic lead structures for medicinal-chemistry programs and guideposts for valid targets can reveal pathways to future therapies (reversed genomics). In silico modeling of the binding of these privileged structures to their targets allows the conception of less-complex molecules with improved properties that can serve as scaffolds for medicinal and combinatorial chemistry. With the exception of some β-lactam antibiotics, only the semisynthesis of congeners of antibacterial natural products has gained economic importance to date.

Revisiting "old" antibacterial classes and clinically validated targets: Even within known natural antibiotics, a dormant value of structural diversity has not been explored. Many "old" classes have never been thoroughly assessed by de novo synthesis and only partial SAR information is available on their backbone structures. Clearly, more could be done, in this case, to fully exploit the weapons against bacteria that are already in our possession. The clinically validated modes of action of the established antibacterial classes should not be neglected. New technologies, such as combinatorial biosynthesis, can create attractive novel natural products or provide (improved) congeners of known antibiotics. However, the benefit of these elaborate techniques for industrial antibacterial research has not yet been proven. It is currently not clear whether combinatorial approaches in biosynthesis will allow for a rationally directed and sufficiently diverse chemical postevolution that is needed to transform natural products into drugs.

Continuing natural-product research in the interest of patients: The demand on pharmaceutical companies to meet their business objectives and the demand for cost containment is forcing industry to think of efficient solutions. Under these premises, the last decade has been difficult for natural-product research. Although the idea that antibacterial research is feasible without natural products is widespread today, the past decades have taught us the opposite. As the resistance pendulum strikes back, there is no alternative to antibacterial research with natural products. Indeed, it might be hubris to assume that mankind can dispense with nature's universal knowledge on antibiotics. Natural scaffolds contain the key to bacterial vulnerability.

19. Abbreviations

ADME(T)	Absorption, Distribution, Metabolism, Excretion, (Toxicity)
AUC	area under the curve (pharmacokinetic parameter)
Boc	*tert*-butoxycarbonyl
BOPCl	(1-benzotriazolyl)oxy tris(dimethylamino)-phosphonium chloride
BSTFA	*N*,*O*-bis(trimethylsilyl)trifluoroacetamide
CAP	community-acquired pneumonia
CDI	*N*,*N*′-carbonyl diimidazole
CFU	colony-forming units
ClpP	caseine lytic protease
CNS	central nervous system
COD	1,5-cyclooctadiene
cSSSI	complicated skin and skin structure infections
cSSTI	complicated skin and soft tissue infections
cUTI	complicated urinary tract infection
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexyl carbodiimide
DIC	*N*,*N*-diisopropylcarbodiimide
DIPAMP	1,2-ethylene bis[(2-methoxyphenyl)phenyl-phosphane]
DMA	*N*,*N*-dimethylacetamide
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
ED	effective dose
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbo-diimide
EEDQ	2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydro-quinoline
(*S*,*S*)-Et-DuPhos-Rh	(+)-1,2-bis[(2*S*,5*S*)-2,5-diethylphospholano]benzene(cyclooctadiene)rhodium(I) trifluormethanesulfonate
FCS	fetal calf serum
FDA	US Food and Drug Administration
Fmoc	9-fluorenylmethoxycarbonyl
f_U	fraction unbound (pharmacokinetic parameter)
GISA	glycopeptide-intermediate *Staphylococcus aureus*
HAP	hospital-acquired pneumonia
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HBTU	benzotriazol-1-yl-*N*-tetramethyl-uronium hexafluorophosphate
HOBt	1-hydroxy 1*H*-benzotriazole
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HTS	high-throughput screen(ing)
IAI	intra-abdominal infections
ICAAC	interscience conference on antimicrobial agents and chemotherapy
ICU	intensive-care unit
IC_{50}	inhibitory concentration 50%
IDSA	infectious disease society of america
i.v.	intravenous (parenteral)
i.p.	intraperitoneal (application)
LDA	lithium diisopropylamide
MA	membrane affinity (descriptor for lipophilicity)
MDRSP	multidrug resistant *Streptococcus pneumoniae*
MIC	minimal inhibitory concentration
MLS_B resistance	macrolide, lincosamide, streptogramine B resistance

MRSA	methicillin-resistant *Staphylococcus aureus*
MRSE	methicillin-resistant *Staphylococcus epidermidis*
MSSA	methicillin-susceptible *S. aureus*
Ms	mesyl
NBS	*N*-bromosuccinimide
NCE	new chemical entity
PBP	penicillin-binding protein
Phth	phthaloyl
PK	pharmacokinetics
pNB	*p*-nitrobenzyl
p.o.	peroral (application)
PPTS	pyridinium *p*-toluene sulfonate
PRSP	penicillin-resistant *Streptococcus pneumoniae*
PSSP	penicillin-susceptible *S. pneumoniae*
QT	electrophysiological heart parameter
RG	reversed genomics
RTI	respiratory-tract infection
SAR	structure–activity relationship
s.c.	subcutaneous (application)
SSTI	skin and soft tissue infections
STR	structure–toxicity relationship
TBDMS	*tert*-butyldimethylsilyl
TBTU	benzotriazol-1-yl-*N*-tetramethyluronium tetrafluoroborate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMSE	trimethylsilylethyl
$t_{1/2}$	serum half-life (pharmacokinetic parameter)
UTI	urinary-tract infection
VISA	vancomycin-intermediate *Staphylococcus aureus*
VRSA	vancomycin-resistant *Staphylococcus aureus*
VRE	vancomycin-resistant *Enterococcus faecium*
V_{SS}	volume of distribution (pharmacokinetic parameter)
Z	benzyloxycarbonyl (protecting group)

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