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Multiple Attack on Bacteria by the New Antibiotic Teixobactin**

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Dedicated to Dr. Paul Dieter Häbich on the occasion of his 65th birthday

The international press acknowledges original research contributions from natural sciences only in rare cases. However, the story behind a recent publication in *Nature* about the discovery of teixobactin (**1**)[1] experienced a worldwide euphoric echo in the media ("super-antibiotic"). How was this possible? In their contribution, Ling et al. not only reported on the discovery of a new lead structure that displays multiple modes of action, but also raised great hopes for the emergence of future antibiotics without the potential to develop resistance.

In the search for new antibacterial leads[2] against Gram-positive pathogens, the appreciation of teixobactin as a new milestone is very plausible. Based on a collaboration between research groups from academia and the biotech industry, a sophisticated cultivation technology allowed the discovery of a new producing strain and a novel depsipeptide class (German Centre for Infection Research at the University of Bonn, Northeastern University Massachusetts, and NovoBiotic).

A number of new drugs against problematic Gram-positive bacteria (daptomycin, oritavancin, telavancin) have been approved in the past years. As a consequence, the research focus is currently shifting toward Gram-negative pathogens. Nevertheless, there remains a clear medical need for new compound classes with new mechanisms of action against Gram-positive bacteria. Still, it is questionable whether antibiotics without the risk of emerging resistances are a realistic scenario. Experience gained over decades teaches us that antibiotics generally encounter bacterial resistance, both because of false usage but also because of their correct use. The emergence of resistance is unavoidable! This statement was valid for drugs with a single mode of action, for example, rifampicin, as well as for those with multiple modes of action, for example, glycopeptides. Even if there is no experimental evidence for resistances against teixobactin, there is a high probability that resistance will kick in sooner or later. Indeed, resistances have been observed earlier than initially anticipated for nearly all classes of antibiotics.[3]

The *Nature* publication reporting on the discovery of teixobactin traces the complete development cascade of an antibiotic from the isolation of the strain to initial pharmacological compound profiling in animal models. The cultivation of the bacterial producer, which originates from soil, was based on the sophisticated iChip technology dedicated to the investigation of microorganisms previously considered as "uncultivable". Bacterial cultures were contained in a "non-natural" diffusion chamber, but also had contact to their natural sediment environment through a semipermeable membrane. This membrane enabled the supply of natural nutrients and growth factors. A sufficiently high dilution at the beginning of this procedure secured the presence of only a single bacterial cell per compartment of the chip. The extract of the previously unknown Gram-negative β-proteobacterium *Eleftheria terrae* attracted attention, as it inhibited the growth of the problematic pathogen *Staphylococcus aureus*. The subsequent activity-based isolation led to the discovery of teixobactin, the structure of which was elucidated by mass spectrometry, amino acid analysis, and NMR-spectroscopy (Scheme 1).

Teixobactin (**1**) is an undecapeptide of moderate structural complexity. The peptide is characterized by a cyclotetradepsipeptide substructure formed by lactonization at the C terminus of Thr^{8}-Ile^{11}. Apart from D-amino acids and *N*-Me-Phe, the uncommon amino acid enduracididin (End) is present, which is also a constituent of the peptide antibiotic mannopeptimycin (**2**).[4] The analysis of sequence data allowed the assignment of a nonribosomal peptide synthesis to teixobactin.

The relatively high molecular mass of teixobactin (1242 $g\,mol^{-1}$) hints toward the cell-wall biosynthesis as a likely molecular target. Indeed, in addition to lipid III (**6**), a building block of the wall teichoic acid biosynthesis, namely lipid II (**5**), seems to be the main target of teixobactin (Scheme 2). Remarkably, in this case it is not an enzyme that

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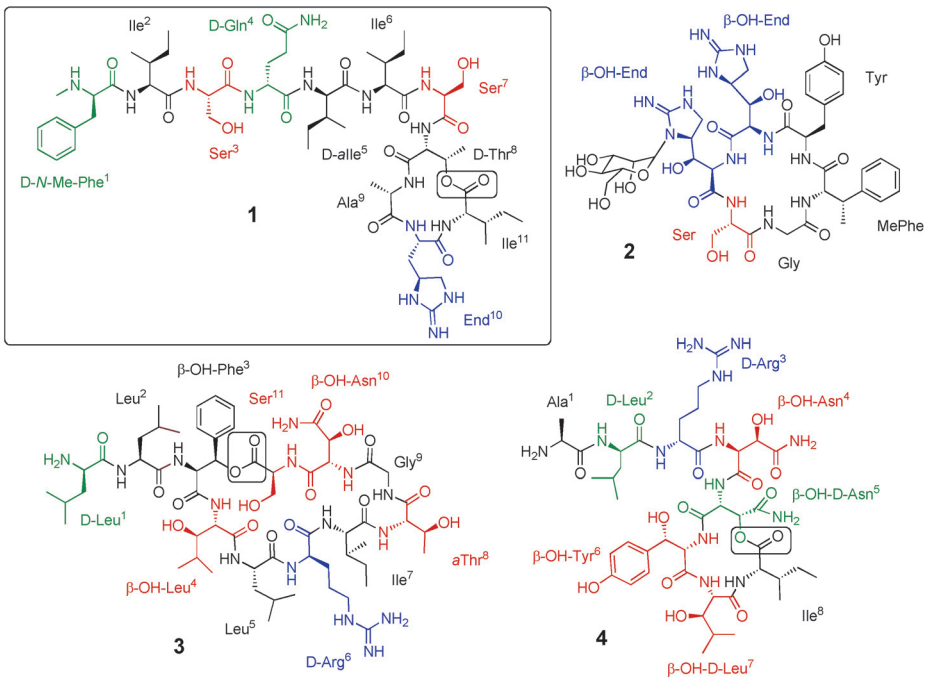
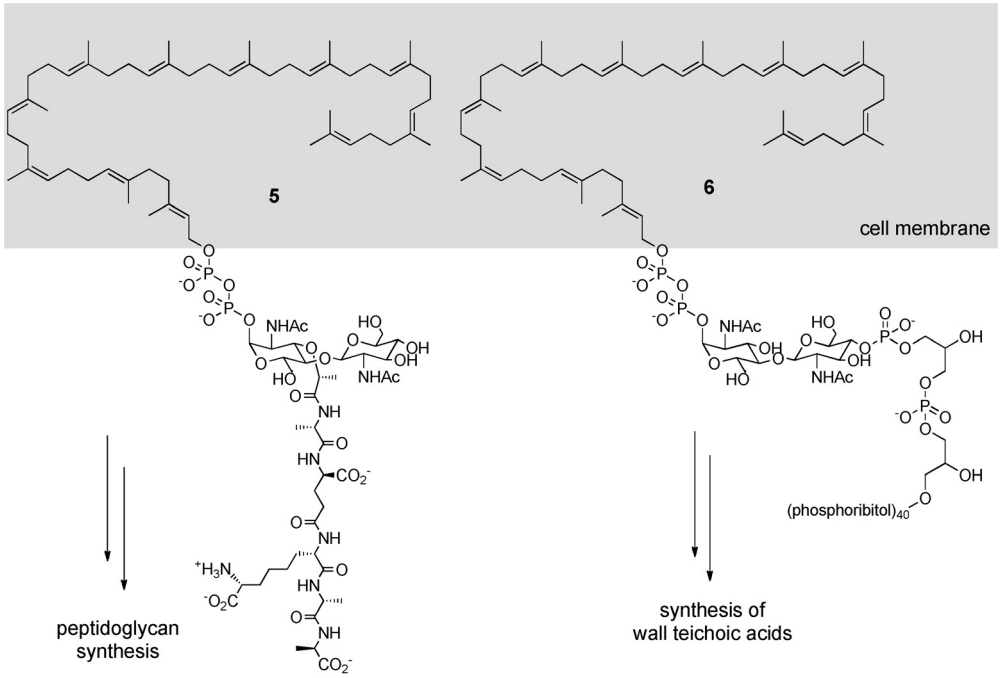


Scheme 1. Structural analogy between the natural lipid II binders teixobactin (**1**), mannopeptimycin (**2**), and katanosin B (lysobactin, **3**). Also hypeptin (**4**) shows analogous structural motifs (target unknown).[10] While **1**, **3**, and **4** are cyclodepsipeptides, **2** is a cyclopeptide. Obviously, nature has developed a certain structural archetype to address precursors of the bacterial cell wall biosynthesis: all structures are large ring systems carrying a characteristic pattern of guanidine amino acids (blue), β-hydroxy amino acids (red), D-amino acids (green), and branched lipophilic amino acids (Ile, Leu, Val, MePhe).



Scheme 2. The cell-wall precursor lipid II (**5**) and lipid III (**6**), a precursor of the wall teichoic acid synthesis, are molecular targets of teixobactin. Apparently, lipid II is the biologically more relevant target.

is inhibited. Rather the biosynthetic precursor, lipid II, is blocked by teixobactin based on a molecule–molecule interaction (teixobactin/lipid II 2:1).

Lipid II consists of a polyprene membrane anchor, two central carbohydrate units (GlcNAc and MurNAc), and a linear pentapeptide chain. During the biosynthesis of the

bacterial cell wall, the disaccharide part undergoes polymerization, followed by the cross-linking of the peptide side chains to ensure stability of the exterior bacterial cell wall. As the reaction rate of cell-wall synthesis seems to be controlled by a continuously low lipid II concentration, lipid II has already been exploited various times during evolution as a valuable antibacterial target.[5] Hence, the antibacterial effects of various highly potent antibiotics are based on the interaction with lipid II, which apparently is a "landing platform"[6] for large (cyclic) structures on the outer surface of the bacterial cell wall for depsipeptide-like secondary metabolites of a certain structural archetype, such as katanosin B, plusbacin, enduracidin, and ramoplanin. Regardless of multiple investigations, the detailed nature of this interaction remains elusive. It has been speculated whether formation of pores ultimately leads to lethal lysis of the bacterial cell.[7]

It is not surprising that teixobactin displays a number of structural characteristics that, in a similar or altered manner, are also found in other lipid II-binding depsipeptides. In most of these depsipeptides, the guanidine side chain secures a positive charge and thus possibly plays an important role, while interacting with the phosphate moiety of lipid II. Further characteristic structural elements seem to be hydroxy amino acids (Ser, Thr, or other unusual β-hydroxy amino acids) and amino acids containing amide side chains, accompanied by aliphatic amino acids, mostly Val, Leu, and Ile. Negatively charged amino acid side chains, with the exception of plusbacin (compensated by Ca^{2+} binding), are fully absent. The above-mentioned structural preconditions are particularly noteworthy when comparing teixobactin and katanosin B (**3**):[8] with regard to their molecular formulae, **1** and **3** differ only to a small extend ($C_{58}H_{95}N_{15}O_{15}$ versus $C_{58}H_{97}N_{15}O_{17}$). However, teixobactin features a relatively small ring.[9] The structural resemblance to the antibiotic hypeptin (**4**; Scheme 1), which shows activity against *S. aureus*, is even more pronounced.[10] Hypeptin displays a somehow similar shape and the same ring size as teixobactin, associated with a comparable amino acid pattern, including β-hydroxy amino acids and the positively charged Arg (instead of End).

Teixobactin shows no cross-resistance with vancomycin, which is also a lipid II binder. Moreover, in a direct comparison with vancomycin in in vitro models, a faster killing of *S. aureus* cultures has been observed for teixobactin. Furthermore, in toxicological in vitro studies, teixobactin showed no adverse effects (cytotoxicity, hemolysis, hERG inhibition, genotoxicity). Initial pharmacokinetic in vitro studies displayed a good half-life in plasma (rodents, dogs, humans). These properties translated into in vivo efficacy in three rodent infection models with parenteral application. While in a mouse-MRSA-sepsis model, efficacy was observed at 0.5 mg kg^{-1}, in the more challenging MRSA thigh infection model with immunocompromised mice only higher doses of 2.5–5 mg kg^{-1} were efficacious. In both cases, vancomycin showed comparable efficacy at slightly higher doses. Likewise, teixobactin was efficacious also in a *Streptococcus pneumoniae* lung infection model at a dose comparable to that of a standard therapy (amoxicillin).

Will teixobactin now give rise to a new class of clinically relevant antibiotics? To answer this question, teixobactins would have to prove in vivo efficacy in animal models, in which standard therapies fail. Furthermore, teixobactin would have to be benchmarked with novel therapy options, such as daptomycin or telavancin. Foremost, toxicological in vivo studies have to be performed, which traditionally constitute a high barrier for antibiotics. It should be considered to further optimize the overall profile of teixobactin by semisynthesis or by de novo synthesis. Hence, the structure of teixobactin could become a rewarding working platform for synthetic chemists.

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[9] In the case of teixobactin, a 28-membered katanosin-like ring could also formally be constructed by lactonization of the C-terminal Ile^{11} with Ser^{2} instead of D-Thr^{8}.
[10] Based on the structural resemblance to teixobactin, hypeptin might also target lipid II. For the structure of hypeptin, see: J. Shoij, H. Hinoo, T. Hattori, K. Hirooka, Y. Kimura, T. J. Yoshida, *J. Antibiot.* **1989**, *42*, 1460–1464.

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