Depsipeptides

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Total Syntheses of Lysobactin (Katanosin B)

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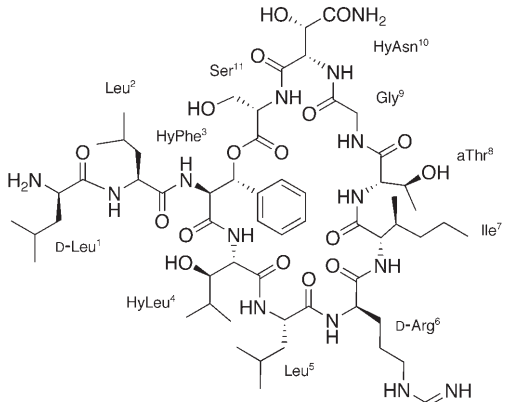
antibiotics · depsipeptides · peptides · total synthesis

Widespread overprescription and misuse of antibiotics have promoted new strains of harmful bacteria that resist traditional treatments. It has been recently estimated that 20% of hospital patients have or will develop an infection, and 70% of the bacteria that give rise to these infections are resistant to one or more of the main antibiotics.[1] Particularly, the emergence of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci is a major public health problem. Antibiotics possessing original structures and/or acting by new mechanisms are particularly attractive because they are less prone to show cross-resistance with known antibiotics.[2] However, only a few new antimicrobial agents have entered the market in the last 40 years.[3]

Lysobactin (**1**) and katanosin A (**2**) are highly active against Gram-positive bacteria, and they are particularly

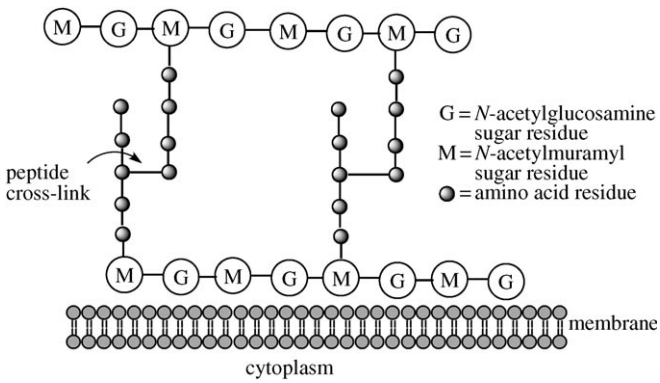


1 (R=CH_3) Lysobactin (Katanosin B)
2 (R=H) Katanosin A

strongly active against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Moreover, promising in vivo efficiency was also demonstrated in a systemic murine *S. aureus* infection model.[4] Lysobactin was first isolated by scientists from Squibb by fermentation of a species of *Lysobacter* (ATCC53042), obtained from a non-exotic leaf-litter sample found in the historic Washington Crossing State Park (US).[5,6] Independently, scientists from Shionogi isolated, from a soil bacterium (genus *Cytophaga*) found in Katano (Japan), two natural products referred to as katanosins A and B.[7–9] It was found later on that lysobactin and katanosin B exhibit the same structure.[10] More recently, katanosin A was also isolated, as a minor metabolite, from *Lysobacter* fermentation.[3] Lysobactin has a "lariat" structure with a linear part (a D-Leu-Leu dipeptide) and a cyclic part (a 28-membered cyclodepsipeptide). The macrocycle is made up of nine amino acids, five of which are non-proteinogenic (a D-Arg residue and β-hydroxylated HyAsn, HyPhe, aThr, and HyLeu residues), and the ester linkage is formed by the β-hydroxy function of HyPhe and the C terminus of Ser.[11] The primary structure was very recently confirmed by an X-ray analysis.[12] In the solid state, the structure is organized through four hydrogen bonds giving rise to a globular structure, in which hydrophobic and hydrophilic domains can be identified. This highly organized structure could be further confirmed by NMR solution-structure experiments.[12]

Although the mechanism of action of these antibiotics on a molecular basis is not clearly understood, a mode of action different from that of vancomycin is postulated.[4] Both vancomycin and lysobactin inhibit the peptidoglycan biosynthesis but at different sites. The walls of bacteria are made of complex polymeric material called peptidoglycan, which preserves cell integrity by withstanding internal osmotic pressure. Peptidoglycan is a complex structure constituted mainly of a polymer of β-1,4-linked *N*-acetylglucosaminyl-*N*-acetylmuramyl units cross-linked by short peptides (Scheme 1).

The biosynthesis of peptidoglycan takes place in three different stages (Scheme 2). From a very simplified chemical point of view,[13] phases 1 and 2 correspond to the formation of



Scheme 1. Simplified structure of peptidoglycan.

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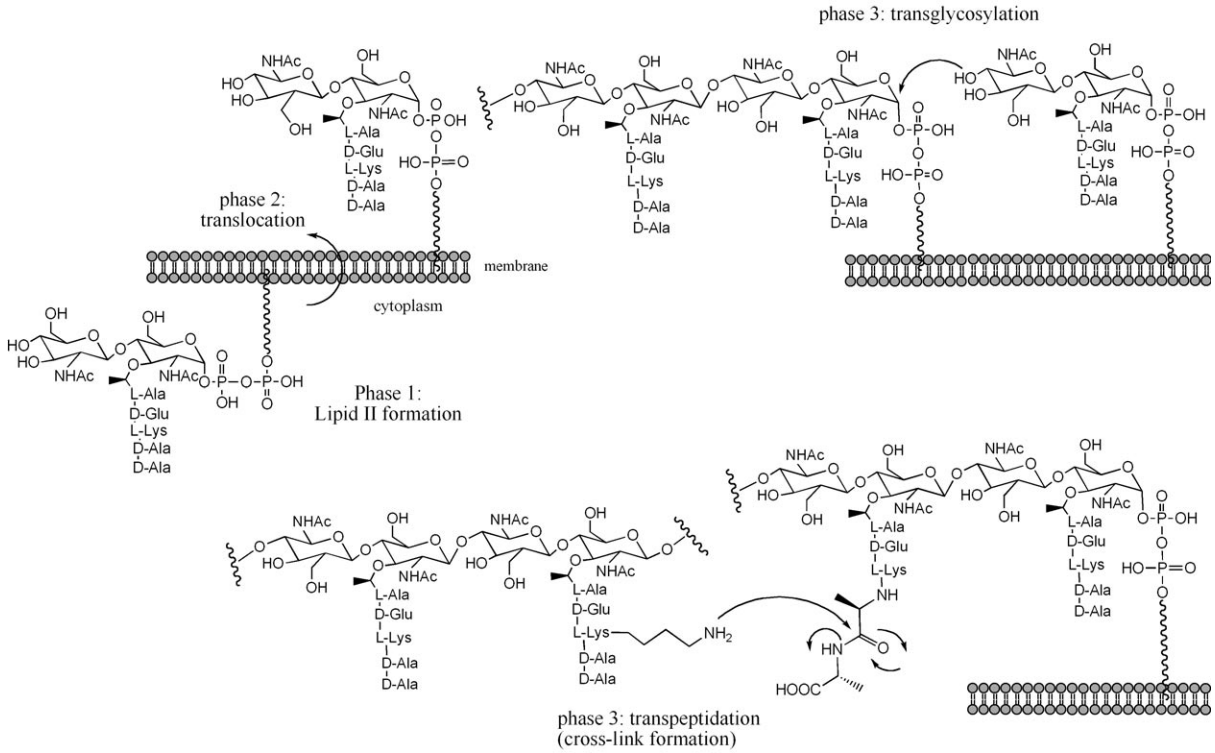
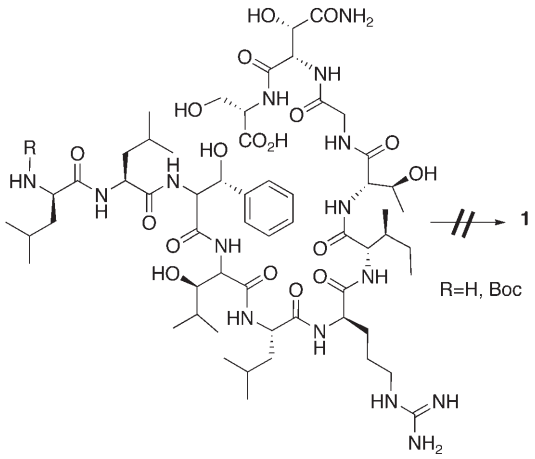


Scheme 2. A simplified overview of peptidoglycan biosynthesis.

so-called lipid II (the disaccharyl-pentapeptide "monomer") in the cytoplasmic phase, and the transfer of this monomer to the external surface of the bacterial membrane (the "translocation" process). Phase 3 then corresponds to the disaccharide polymerization (lipid II acts as a donor to the 4-OH group of GlcNAc at the ends of peptidoglycan chains undergoing elongation) catalyzed by transglycosylases;[14] and finally some cross-links are generated between the peptide chains. This transpeptidation occurs between the ε-NH_2 group of a Lys^3 residue with a neighboring D-Ala^4, liberating a D-Ala^5 amino acid. This "maturation" process confers to peptidoglycan its exceptional stability. Vancomycin inhibits the transglycosylation process by binding to the D-Ala-D-Ala terminus of the lipid II pentapeptide part. The addition of Ac-Lys-D-Ala-D-Ala suppresses the transglycolysation inhibition by vancomycin but does not suppress the lysobactin activity, clearly evidencing two different modes of action.[4] Although the precise mode of action of lysobactin is not yet clearly understood, its activity in bacteria is believed to stem from inhibition of transglycosylation inhibition and/or inhibition of lipid II formation. As a result, the inhibition of the peptidoglycan synthesis alters the mechanical properties of the bacterial cell wall and leaves the cell susceptible to lysis. It has been suggested that lysobactin might have a mode of action similar to that of other antibiotics targeting lipid II, such as ramoplanin (a cyclodepsipeptide with disaccharide and lipid appendages) and mannopeptimycins (a glycosylated cyclopeptide formed by six amino acids).[13a] An answer to this question is anticipated, as monomeric lipid II is now accessible by both synthetic and enzymatic methods.[13b,2b]

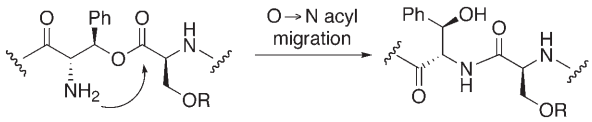
Owing to its original mode of action and submicromolar MICs (minimum inhibitory concentrations) which are 50-fold lower than those reported for vancomycin, lysobactin is a promising drug to fight infections due to vancomycin-resistant enterococci (VRE). Accordingly, several groups have been involved in the total synthesis of lysobactin,[15–20] and a solid-phase synthesis of a simplified analogue has been described by Egner and Bradley.[20] These synthetic efforts have recently culminated in total syntheses described by von Nussbaum et al. at Bayer AG[12] and by VanNieuwenhze et al. [21] The two syntheses are very different: the von Nussbaum approach was based mainly on considerations of the lysobactin 3D structure, whereas VanNieuwenhze approach is closer to the traditional route to peptide synthesis.

The von Nussbaum approach is based on the highly organized 3D structure, determined by the same group at Bayer AG. Accordingly, they planned to construct the macrocyclic core of lysobactin through a conformation-directed cyclization,[22] in which the unprotected hydroxy amino acids side chains should play a crucial role, through H-bonding, to favor the macrocyclization reaction. Consequently, only minimal protection of the hydroxy groups in the side chains was envisaged in the synthesis of the macrocyclization precursor. Whereas biosynthetic pathways usually involve macrolactonization processes,[23] cyclodepsipetides are usually chemically cyclized using macrolactamization reactions.[24] In the present case, owing to easy access to the seco acid from the natural product,[25] a semisynthesis through a macrolactonization was first investigated (Scheme 3). However, this approach was unsuccessful probably as a result of the presence of too many potentially reactive hydroxy functions.



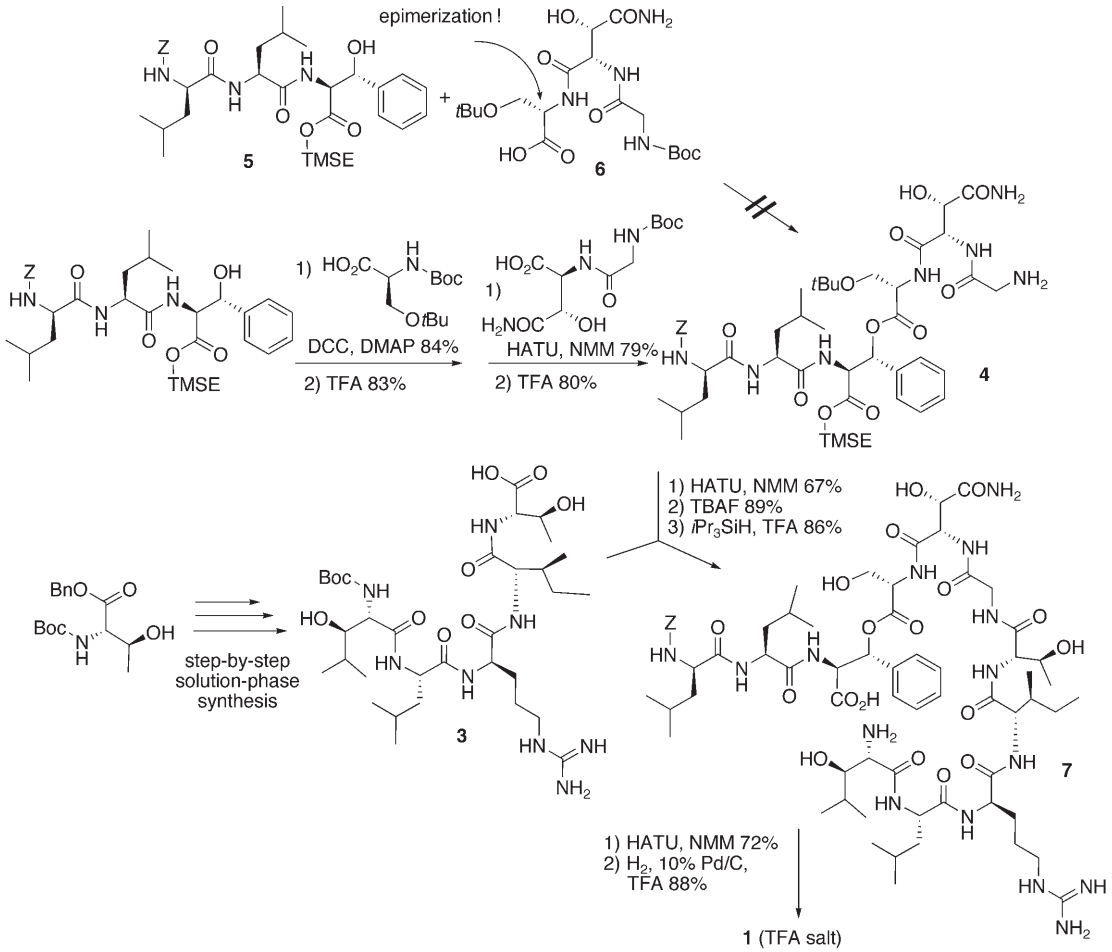
Scheme 3. Macrolactonization attempts.

In a macrolactamization approach, the peptide was divided, in a nonclassical way, into two fragments: an hexa(pseudo-peptide) **3** and a penta(pseudo-peptide) **4**. This strategy is somewhat surprising because it does not take advantage of the presence of a glycine residue (see below) that could allow a non-epimerizing coupling (inter- or intramolecular) of peptide fragments. Another important point is based on the necessity to introduce the dipeptide "linear" part of the lariat at the beginning of the synthesis. Indeed, the HyPhe nitrogen atom could not be deprotected without risking an O→N acyl migration, leading to the formation of the corresponding amide (Scheme 4).[26]



Scheme 4.

The pentapeptide **3** was obtained through a regular stepwise peptide synthesis in solution, using Boc as a temporary amino-protecting group, a benzyl ester at the C terminus, and HATU as the peptide-coupling reagent. It should be emphasized that none of the side chains (including the basic guanidinium group in D-Arg!) were protected. In the preparation of fragment **4**, for the sake of convergency, the coupling of **5** and **6** was first envisaged (Scheme 5). However, the esterification proceeds with high levels of

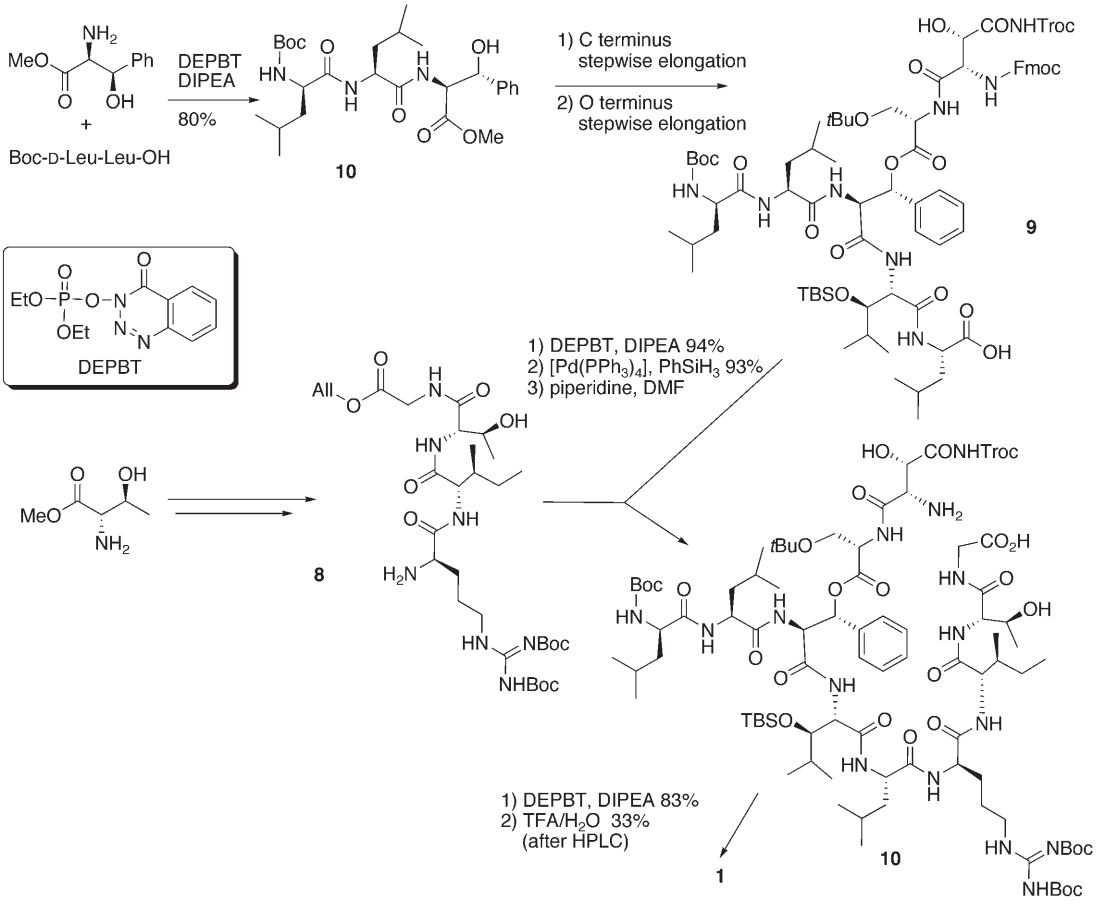


Scheme 5. Synthesis of lysobactin (**1**) by von Nussbaum et al. Bn = benzyl, Boc = *tert*-butyloxycarbonyl, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, NMM = *N*-methylmorpholine, TBAF = tetrabutylammonium fluoride, TFA = trifluoroacetic acid or trifluoroacetate, TMSE = 2-(trimethylsilyl)ethyl.

epimerization at the C_α serine residue. Thus, the serine residue was first introduced, and then the dipeptide Boc-Gly-HyAsn-OH could be coupled in good yield. This last peptide coupling leading to the northern fragment **4** is again quite impressive since neither epimerization nor dehydration of the asparagines residue occurs! With the two peptides **3** and **4** in hand, the von Nussbaum group carried out the first intermolecular peptide coupling at the least hindered site between the Gly and aThr residues, leading to the linear pseudo-peptide in 67% yield and no epimerization. After removal of the protecting groups on HyPhe[3], HyLeu[4], and Ser[11] residues, the conformation-directed macrolactamization could be carried out under classical conditions, and the macrolactam was obtained in an impressive 72% yield. Finally, after hydrogenolysis of the *Z* carbamate (in the presence of trifluoroacetic acid), lysobactin (**1**) was isolated as its trifluoroacetate salt in 88% yield.

The approach to lysobactin described by VanNieuwenhze et al. is more classical from a peptide-chemistry point of view (Scheme 6). The two main disconnections, leading to peptides **8** and **9**, were envisaged between Gly-HyAsn and between Leu-D-Arg: the achiral glycine allows epimerization-free peptide couplings, and peptide couplings between L and D residues are known to be more efficient than their D--D or L–L counterparts. Pseudo-peptide **8** was obtained, starting from D-*allo*-Thr-OMe, using standard stepwise peptide synthesis in solution. The synthesis of pseudo-peptide **9** is more tricky owing to the presence of the sensitive ester function and the possibility of O→N acyl migration (see Scheme 3). Compound **10** was thus synthesized by the coupling of Boc-D-Leu-Leu-OH and HyPhe-OMe. In this peptide coupling (and throughout the synthesis), the use of 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-3*H*)-one (DEPBT)[27,28] proved to be essential in minimizing the extent of epimerization during peptide-fragment couplings. Pseudo-peptide **9** was then obtained classically by first sequentially elongating the peptide chain at the C-terminus of **10**, and then by elongating the peptide starting from the unprotected hydroxy function of the HyPhe residue. Peptides **8** and **9** could then be joined in 94% yield and, thanks to DEPBT, without epimerization at the Cα leucine residue. After removal of the N- and C-terminal protecting groups, the macrolactamization was carried out, again using DEPBT, leading to the fully protected lysobactin **10** in 83% yield. The removal of all of the judiciously chosen protecting groups (Troc, TBS, *t*Bu and Boc) was finally carried out under acidic conditions leading (after HPLC purification) to lysobactin (**1**) in 33% yield.

In conclusion, two total syntheses of lysobactin, a promising natural product for the treatment of vancomycin-resistant bacteria, have been reported. A noteworthy point is the necessity of introducing the D-Leu-Leu fragment at the beginning of the synthesis (see above), thereby limiting the



Scheme 6. Synthesis of lysobactin (**1**) by VanNieuwenhze et al. DIPEA = diisopropylethylamine, TBS = *tert*-butyldimethylsilyl, Troc = trichloroethoxycarbonyl.

access to a large number of derivatives at the lariat extremity. The two approaches are very different from their conception. The more risky approach (peptide coupling of potentially epimerizable C-terminal amino acids, use of unprotected arginine and asparagine as well as hydroxylated amino acid residues) adopted by von Nussbaum is indeed very impressive, particularly the retroanalysis starting from the 3D structure of lysobactin. On the other hand, the very classical approach by VanNieuwenhze highlights the state of the art in peptide synthesis, particularly in the choice of the disconnection sites and the choice of a nice set of orthogonal protecting groups allowing a one-step full deprotection under acidic conditions. Although conceptually different, the two syntheses are both highly convergent and comparable in terms of number of steps (with nine and ten steps as the longest linear sequence, starting from a D-Leu-Leu-HyPhe tripeptide derivative). These two syntheses pave the way for further chemical modifications and should enable better understanding of biological mechanisms involved in the antibiotic activity of lysobactin.

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