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Study of the structure-activity relationship of polymyxin analogues

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ABSTRACT

A structure-activity relationship study on three classes of polymyxin analogues focusing on hydrophobicity was conducted.

Colistin (also known as polymyxin E, Fig. 1, **1**) and polymyxin B (**2**)[1,2] were clinically used old antibacterial dugs beginning in the 1940s. Because of their kidney toxicity, they have been withdrawn from clinical use. However, in an era of increasing rates of multidrug resistance in conjunction with a very limited number of new drugs as well as drug candidates, their use has seen a substantial resurgence for the treatment of infections caused by Gram-negative bacteria including as a last resort treatment against multidrug-resistant (MDR) *P. aeruginosa*, *A. baumannii,* and *K. pneumoniae*.[3–6] Bacteria can acquire resistance to any antibacterial drug, and colistin and polymyxin B are no exception; however, polymyxin resistance rates are known to be relatively low compared to those of other antibacterial drugs. In fact, colistin-resistant bacterial pathogens have recently been isolated from patients.[7] Therefore, the next generation of colistin derivatives effective to those resistant bacteria should be developed.

Unlike Gram positive bacteria, Gram negative bacteria have an extra outer membrane, which acts as a physical barrier against the permeation of antibacterial drugs.[8,9] This is one of the reasons why Gram negative bacterial pathogens tend to be less susceptible to antibacterial drugs. Lipopolysaccharides (LPS) such as lipid A are present in their outer membrane, and the LPS are tightly assembled via chelation of the phosphates in lipid A to divalent ions such as Mg^{2+} and Ca^{2+}. Polymyxins are amphiphilic molecules composed of a fatty acyl side chain a cyclic peptide containing five L-α,γ-diaminobutanoic acid (l-Dab) residues and hydrophobic amino acids (l-Leu or l-Phe). They are known to bind LPS[9–11] through electrostatic interactions between the highly basic l-Dab residues and the phosphodiesters of LPS as well as hydrophobic interactions between the 6-methyloctanoyl side chain and the lipid bilayer of the outer membrane or fatty acyl chain attached to the LPS.[12–17] Its binding weakens the LPS assembly ·disrupting the outer membrane and ultimately resulting in cytoplasmic leakage and cell death. The hydrophobic interaction is key to the antibacterial activity, and deacylated colistin has a drastically lower antibacterial activity compared to that of **1**.[18] Many polymyxin analogues have been synthesized, and their structure-activity relationships (SARs) have been investigated.[19] Cooper et al. reported that increasing the hydrophobicity of d-Leu at the 6-position and l-Leu at the 7-position increases the anti-Pseudomonas activity.[20] We focused on the lipophilicity of polymyxins, which are key factors in the interaction with the target LPS. Herein we describe a structure-activity relationship study of three classes of polymyxin analogues focusing on hydrophobicity.

To provide a systematic SAR comparison, a series of polymyxin analogues were proposed. First, analogues with a longer acyl side chain connected to the Dab residue at the 1-position were designed including known simple octanoyl analogue **3a** (Fig. 2). Lengthening the linear alkyl chain may cause micelle formation. Therefore, a *cis*-alkene and branching were incorporated into the

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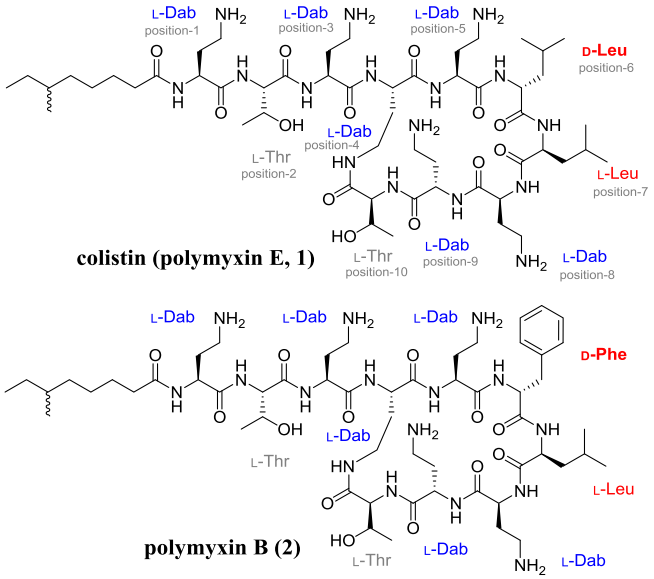


Fig. 1. Structures of Colistin and Polymyxin B.

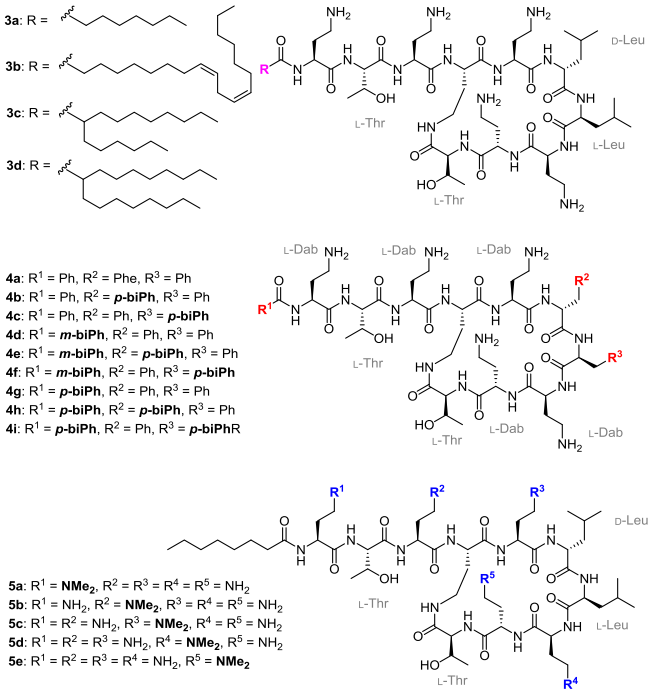
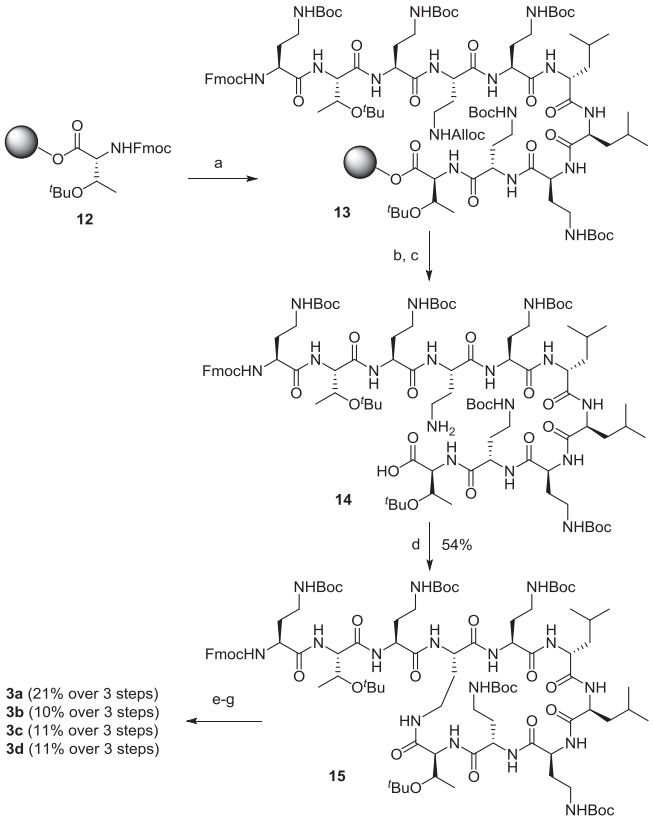


Fig. 2. Structures of Polymyxin analogues.

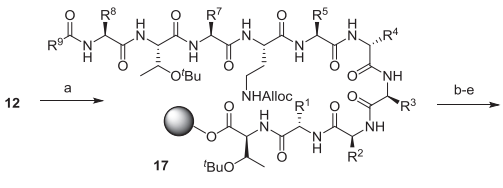
side chain to give **3b** as well as **3c** and **3d**, respectively. It is suggested that there is an intramolecular hydrophobic interaction among the hydrophobic amino acid residues in the 6- and 7-positions and the fatty acyl side chain.[21] Analogues **4a-i**, which included three aryl groups, were designed to systematically investigate the effect of increasing the hydrophobicity of these moieties. We also intended to investigate the effects of making each l-Dab residue more hydrophobic and more basic by replacing with an L-*N*,*N*-dimethylDab residue (**5a**–**e**).

To prepare the series of analogues, a solid-phase synthesis using an Fmoc strategy was applied according to a previously reported method with some modifications (Scheme 1). l-Threonine at the 10 position was cyclized on to the side chain of the Dab residue



Scheme 1. Synthesis of **3a–d**. Reagents and conditions. (a) solid-phase peptide synthesis; (b) $Pd(PPh_3)_4$, $MeNH{\cdot}BH_3$, CH_2Cl_2, rt.; (c) 1%TFA/CH_2Cl_2; (d) DPPA, $^iPr_2NE_t$, DMF, rt.; (e) Et_2NH, aq. MeCn, rt.; (f)carboxylic acid, EDCI, CH_2Cl_2, rt.; and (g) TFA/Et_3SiH/H_2O.

at the 4-position, the amino group of which was protected with an Alloc group; the cyclization was conducted in a manner similar to a synthesis previously reported.[22] Starting from Fmoc-l-Thr immobilized onto 2-chlorotrityl resin (2CT), decapeptide **13** was prepared by an Fmoc-strategy. Removal of the Alloc group by Pd $(PPh_3)_4$ and $Me_2NH{\cdot}BH_3$ in CH_2Cl_2 followed by cleavage from the 2CT resin upon treatment with 1% TFA/CH_2Cl_2 gave linear carboxylic acid **14**, which is the cyclization precursor. Linear peptide **14** was treated with diphenylphosphoryl azide (DPPA) and $^iPr_2NEt$ in DMF at room temperature for 24 h to afford cyclic peptide **15** in 54% yield. The Fmoc protecting group was removed with Et_2NH and the liberated amine was acylated with octanoic acid, linoleic acid, isopalmitic acid, and isostearic acid in the presence of **1-ethyl-3-(3-dimethylaminopropyl)carbodiimide** hydrochloride (EDCI) in CH_2Cl_2, respectively. Finally, removal of the Boc groups was conducted by a treatment with TFA to afford analogues **3a–d**. In a manner similar to the synthesis of **3**, analogues **4a–i** were synthesized via the preparation of the decapeptides on 2CT resin, deprotection of the Alloc group, cyclization, and Boc deprotection (Scheme 2).



Scheme 2. Synthesis of **4a–i**. Reagents and conditions. (a) solid-phase peptide synthesis; (b) $Pd(PPh_3)_4$, $MeNH{\cdot}BH_3$, CH_2Cl_2, rt.; (c) 1%TFA/CH_2Cl_2, rt.; (d) DPPA, $^iPr_2NE_t$, DMF, rt.; (e) TFA/Et_3SiH/H_2O.