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Reduced pulmonary surfactant interaction of daptomycin analogs via tryptophan replacement with alternative amino acids

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ABSTRACT

Daptomycin was shown to interact in vitro with pulmonary surfactant leading to reduction of its antibacterial activity. We report herein the preparation and anti-staphylococcal activity of a series of daptomycin analogs with reduced pulmonary surfactant interaction by replacing tryptophan with various amino acids.
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Daptomycin, a fermentation product of *Streptomyces roseosporus,* is the first in a new class of antibiotics known as lipopeptides.[1] Daptomycin has a cyclic depsipeptide containing 10 amino acids and an exocyclic three-amino acid side chain with an *n*-decanoyl lipid tail attached to the *N*-terminus of tryptophan ([Trp^1]; Fig. 1). Daptomycin is rapidly bactericidal against a variety of Gram-positive bacteria, including antibiotic resistant isolates, such as MRSA, through a unique mechanism of action.[2] Daptomycin appears to bind to Gram-positive cell membrane by its lipid tail, and subsequent calcium dependent insertion and oligomerization cause disruption of bacterial membrane potential leading to rapid cell death. Daptomycin has been approved for the treatment of complicated skin and skin structure infections caused by *Staphylococcus aureus* including strains resistant to methicillin (MRSA)[3] and for the treatment of bacteremia and right-sided endocarditis.[4] Phase 3 clinical trials were also conducted for the treatment of hospitalized patients with community-acquired pneumonia (CAP). Despite potent in vitro activity (MIC_{90}, 0.06 μg/mL) against *Streptococcus pneumoniae*, the most common Gram-positive pathogen in CAP, daptomycin did not display adequate efficacy in these Phase 3 clinical studies. Subsequent research suggested that the lack of efficacy in lung tissues may be due to the binding of daptomycin to pulmonary surfactants leading to reduced antibacterial activity.[5]

To identify novel lipopeptides with activity in pulmonary infections while maintaining the favorable physiochemical and safety profiles of daptomycin, our efforts were focused on generating daptomycin analogs with reduced surfactant interactions. Synthetically, there are three positions in daptomycin that are readily accessible: [Orn^6], [Ser^{11}] and the lipid tail. Although some ornithine analogs[6] and serine analogs maintained daptomycin-like potency, none of them exhibited reduced pulmonary surfactant interaction in vitro. Similarly, lipid tail modifications resulted in a subset of analogs with improved antibacterial activity, but none of them showed reduced pulmonary surfactant interaction (data not shown). Our efforts were then directed towards the modification of synthetically less accessible [Trp^1] position. Herein we report syntheses and SAR studies of a novel series of daptomycin analogs with reduced pulmonary surfactant interactions.

The general synthesis of [Trp^1] analogs of daptomycin (**4a**–**4v**) is illustrated in Scheme 1.[7] The synthesis began with a Cbz protection of the [Orn^6] amine. Then the C10 lipid tail was removed via an enzymatic deacylation reaction.[7] The resulting amine **1** was converted to a thiourea which upon treatment with TFA led to formation of the Edman degradation product **2** in 58% yield. Compound **2** reacted readily with pentafluorophenyl esters of a variety of Boc protected amino acids and subsequent Boc-deprotection provided amine **3a**–**3v** in good yields. Acylation of **3a**–**3v** with decanoic acid pentafluorophenyl ester followed by final removal of Cbz group afforded the daptomycin analogs **4a**–**4v** (Table 1). All final products were purified by reverse phase prep-HPLC[8] and characterized by LC-MS and ^{1}H-NMR.

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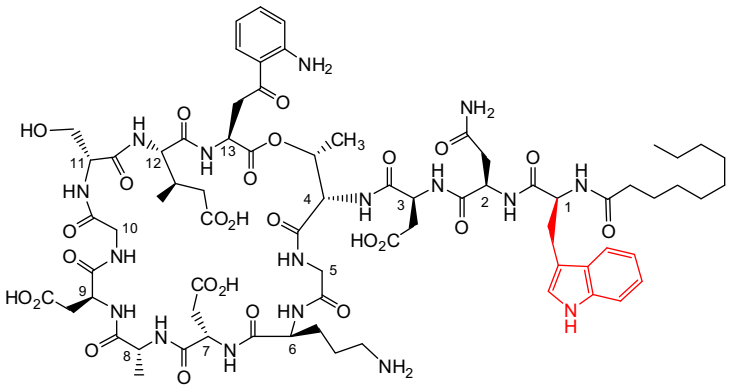
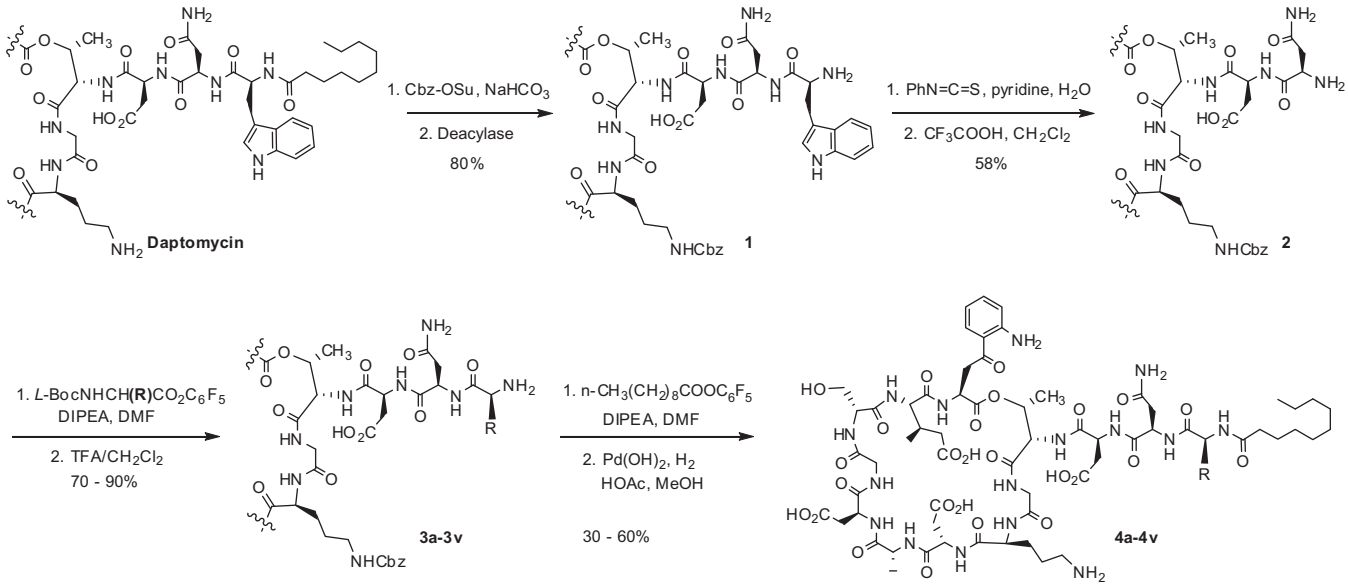


Figure 1. Structure of daptomycin.



Scheme 1. Synthesis of daptomycin analogs.

The antibacterial activity of the semi-synthetic [Trp[1]] analogs of daptomycin was determined by MIC testing against methicillin susceptible *S. aureus* (ATCC #29213) in the MHBc media.[9] Surfactant interactions were assessed by the MIC shift in the presence of various concentrations of surfactant.[10] The protein binding effect was also evaluated by measuring the MIC in the presence of 4% human serum albumin (HSA).[11]

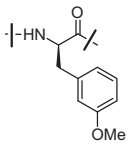
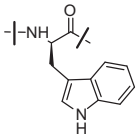
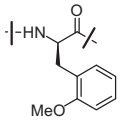
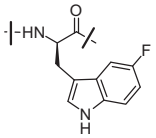
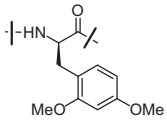
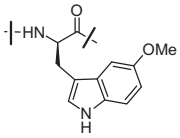
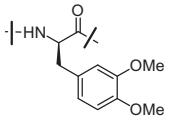
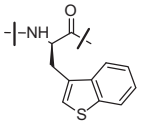
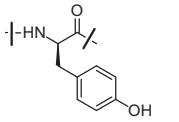
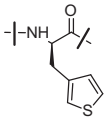
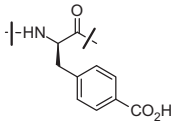
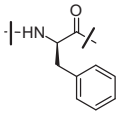
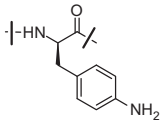
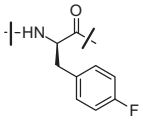
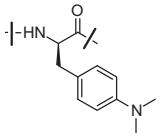
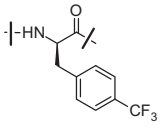
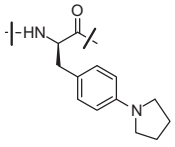
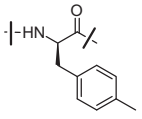
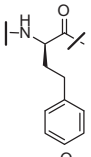
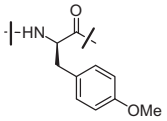
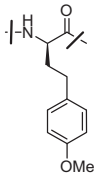
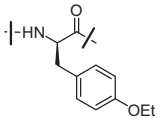
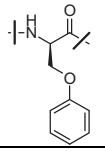
As shown in Table 1, the antibacterial activity of daptomycin was reduced by 32-fold with just 1% of surfactant, while Ceftriaxone's MIC was not affected by surfactant. Initial structure-activity relationship analysis at the [Trp[1]] position showed that the *L*-configuration of the amino acids is essential to the antibacterial activity. Analogs with *D*-Trp or other *D*-amino acids were less active with MIC against *S. aureus* greater than >8 μg/mL. Replacing [Trp[1]] with non-aromatic amino acids, such as Ala, Asn, Ser, etc., also resulted in loss of activity. Analogs with selected aromatic

L-amino acids as replacement to [Trp[1]] are shown in Table 1. In general, electron-withdrawing groups, such as –F, $–CF_3$, on the aromatic ring tend to maintain or improve potency (e.g., **4a** vs DAP) and increase surfactant effect, reflected by the larger MIC shift in the presence of 1% and 5% of surfactant (e.g., **4f, 4g** vs **4e**), whereas electron-donating groups, such as –OMe, –OEt, and $–NMe_2$, reduced surfactant MIC shifts, and in some cases reduced MIC potency as well (e.g., **4i**, **4m**, **4n** vs **4e**). Compounds with hydrogen-bond donors (–OH, –COOH, and $–NH_2$) on the aromatic ring were generally inactive (**4o**–**q**).

Among all the analogs synthesized, 4-MeOPhe analog **4i** and 4-Me_2NPhe analog **4r** not only maintained good antibacterial activities (2 μg/mL) against *S. auerus* in Mueller-Hinton Broth supplemented with 50 mg/L calcium (MHBc), but also showed no measureable MIC shifts in the presence of 1% and 5% surfactant, a significant improvement compared to daptomycin.

In summary, a variety of tryptophan analogs of daptomycin were synthesized. Some analogs showed improved in vitro MIC activity compared to daptomycin. A number of analogs showed reduced surfactant MIC shifts in the presence of 1% or 5% of surfactant, while maintaining good MIC potency. Results of further evaluation of these analogs in vivo will be reported in due course.

Table 1
In vitro antibacterial activities of daptomycin [Trp[1]] analogs (MICs; μg/mL).

Compd	–NHCH(**R**)CO–	*S. aureus* ATCC 29213				Cmpd	–NHCH(**R**)CO–	*S. aureus* ATCC 29213			
		MHBc	1% Surf	5% Surf	4% HSA			MHBc	1% Surf	5% Surf	4% HSA
Ceftri-axone	—	2	2	2	8	**4k**		2	2	4	4
DAP		1	16	>32	2	**4l**		2	2	4	8
4a		0.25	16	>32	1	**4m**		8	8	8	16
4b		4	16	32	8	**4n**		16	16	16	32
4c		2	8	16	4	**4o**		>32	>32	>32	>32
4d		4	8	16	32	**4p**		>32	>32	>32	>32
4e		2	4	8	4	**4q**		16	16	32	32
4f		2	8	16	8	**4r**		2	2	2	4
4g		2	32	>32	8	**4s**		1	4	4	32
4h		1	2	8	8	**4t**		1	8	32	4
4i		2	2	2	8	**4u**		2	8	32	8
4j		4	4	16	8	**4v**		4	32	>32	32

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7. Leese, R. A.; Curran, W. V.; Borders, D. B. U.S. Patent US 2003224475 A1, Appl. Publ. **2003**.
8. Prep-HPLC method: column, 250 × 21.2 mm SunFire® 5 μ C8 column; Solvents: A: water with 0.01% TFA; B: acetonitrile with 0.01% TFA. Gradient: 20–60% of B%. Purity and structural confirmation were obtained by LC-MS and NMR (600 MHz).
9. Minimum inhibitory concentration (MIC) was determined by broth microdilution according to Clinical and Laboratory Standards Institutes (CLSI) guidelines, except that Mueller-Hilton Broth was supplemented with 50 mg/L Ca^{2+} and all assays were performed at 37 °C as previously described.
10. For surfactant MIC testing, MHBc was supplemented with Survanta® to the indicated concentration and the assay followed as above. Survanta® contains 25 mg/mL phospholipids, including 11.0–15.5 mg/mL disaturated phosphatidylcholine, 0.5–1.75 mg/mL triglycerides, 1.4–3.5 mg/mL free fatty acids, and <1.0 mg/mL protein in 0.9% NaCl.
11. For human serum albumin (HSA) MIC testing, MHB was supplemented with 4% HSA and 75 mg/L Ca^{2+}. In the presence of serum or serum proteins (e.g., albumin), free calcium (Ca^{2+}) is added at 75 mg/L, roughly equivalent to physiological blood levels once chelation by albumin is factored in.