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Solid-phase synthesis and in vitro biological activity of a Thr4→Ser4 analog of daptomycin

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ABSTRACT

Daptomycin is a Ca^{+2}-dependent cyclic lipodepsipeptide antibiotic used clinically to treat serious infections caused by Gram-positive bacteria. The recent appearance of daptomycin-resistant strains, daptomycin's lack of activity in the presence of lung surfactant, and its incompletely understood mechanism of action underscores the need for establishing detailed structure–activity relationships. Here we report a solid-phase synthesis of a daptomycin analog in which Thr4, 3-MeGlu12 and Kyn13 in daptomycin were replaced with Ser, Glu and Trp residues, respectively (Dap-S4-E12-W13). The Thr4 to Ser4 substitution was detrimental to activity, as Dap-S4-E12-W13 was at least 20-fold less active at physiological Ca^{+2} concentration than Dap-E12-W13. Much of its activity could be recovered at high (100 mM) Ca^{+2} concentration, suggesting that the residue at position 4 affects Ca^{+2} binding and, consequently, biological activity.

Daptomycin is an antibiotic first isolated from the soil bacterium *Streptomyces roseosporus* in the mid 1980s.[1a–c] Since its approval for clinical use in 2003, daptomycin has become a mainstay in the treatment of serious skin and skin structure infections caused by Gram-positive bacteria, and in particular, for the treatment of bacteremia and right-sided endocarditis caused by *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA).[1c,2] It is still the only member of a class of antibiotics known as cyclic lipodepsipeptides approved for clinical use.[3a,b]

Daptomycin (Fig. 1) consists of 13 amino acids, six of which are non-proteinogenic: (2*S*,3*R*)-methylglutamate ((2*S*,3*R*)-MeGlu12), kynurenine (Kyn13), Orn6, D-Asn2, D-Ala8, and D-Ser11. 10 of the amino acids are part of a macrocyclic core which contains a depsi (ester) bond between the side chain of Thr4 and the α-COOH of Kyn13. An exocyclic tripeptide is attached to the macrocyclic portion and decanoic acid is attached to the *N*-terminal tryptophan residue. While daptomycin's mechanism of action (MOA) is still uncertain, some studies suggest that daptomycin forms oligomeric pores in bacterial membranes, which results in dissipation of membrane potential and cell death.[4a,b]

While bacterial resistance to daptomycin itself is currently still uncommon, non-susceptible strains have been isolated from clinical samples and they are likely to become only more prevalent with continued widespread use of the drug.[5] This issue must be addressed in order to ensure that this important antibiotic remains effective in the future. Another constraint to the therapeutic use of daptomycin is its ineffectiveness for treating pneumococcal pneumonia which presumably is due to sequestration by lung surfactant.[6] These issues, along with daptomycin's incompletely characterized mechanism of action, emphasizes the need for establishing detailed structure–activity relationships (SARs) so that improved cyclic lipodepsipeptide antibiotics can be rationally designed and developed. With this in mind, we developed a solid-phase total synthesis of daptomycin and applied this methodology to the synthesis of several daptomycin analogs.[7] One of the daptomycin analogs we prepared, Dap-E12-W13 (Fig. 1), which does not contain Kyn13 or the synthetically challenging (2*S*,3*R*)-MeGlu12, exhibits in vitro biological activity approaching that of daptomycin, and it can therefore serve as a more accessible wild type analog, into which additional substitutions can be introduced in order to establish structure activity relationships.

Perhaps the most challenging aspect of solid-phase daptomycin and daptomycin analog synthesis is the formation of the depsi bond. In our first attempt to prepare daptomycin, we were unable to form the depsi bond in peptide **2** from peptide **1** which consisted of residues 1–10 and the decanoyl tail (Scheme 1).[7,8] Nevertheless, we found that the depsi bond could be formed in quantitative yield on peptide **4** which consists of residues 3–10 and bears an azido acid at the *N*-terminus (Scheme 2).[7] After formation of the depsi bond, the azido group in the resulting peptide **5** was reduced to

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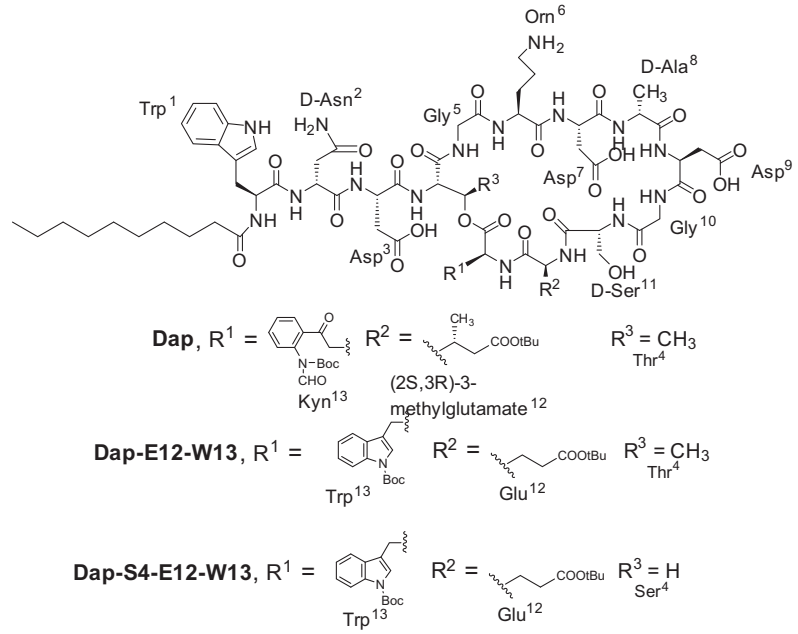
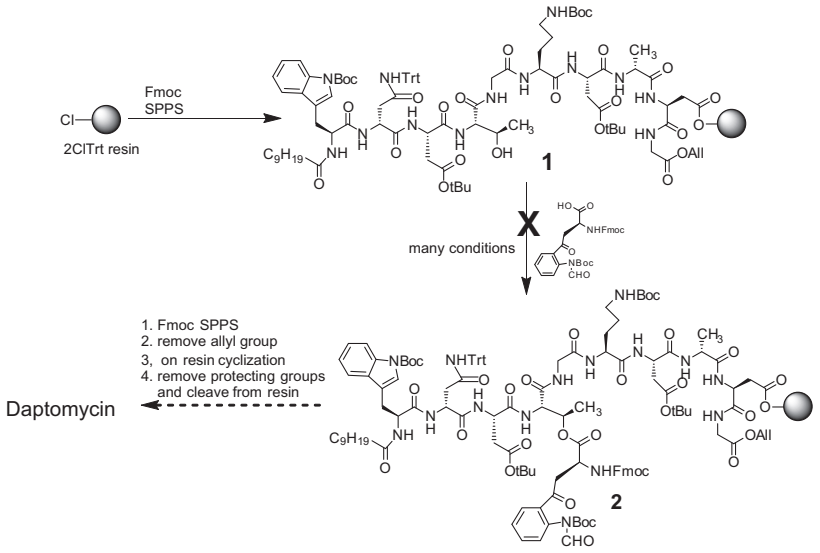


Figure 1. Structure of daptomycin, Dap-E12-W13 and Dap-S4-E12-W13.



Scheme 1. First attempted route to daptomycin.

an amine, residues 1 and 2 were introduced using N_3Asn(Trt)OH and N_3Trp(Boc)OH followed by attachment of the decanoyl (Dec) tail to give peptide **2**. The synthesis was completed using Fmoc SPPS and an on-resin cyclization. Although this approach to daptomycin and daptomycin analogs was successful, it would be preferable if the solid-phase synthesis of daptomycin analogs could be achieved entirely with Fmoc amino acids. This would be possible if the ester linkage found in the natural product was replaced with a more accessible linkage. Martin and coworkers reported that Dap-DAPA4-E12 and Dap-DAPA4-E12-W13, (DAPA = diaminopropionic acid), where the depsi bond was replaced with an amide linkage, were readily prepared in the solid phase.[9] Unfortunately, these analogs exhibited MICs that were 80–160-fold greater than that of native daptomycin.[9] These results suggest that replacing the ester linkage with an amide bond is highly detrimental to activity. However, as esters are usually easier to prepare from primary alcohols in comparison to secondary alcohols, we speculated that the route outlined in Scheme 1, which requires no azido acids, would be feasible if Thr4 was substituted with a Ser residue. If this derivative also retained wild type-like activity, it could serve as an even simpler template for SAR studies than the Dap-E12-W13 analog. Here we report the synthesis of a daptomycin analog in which Thr4 in Dap-E12-W13 is substituted with a Ser residue (daptomycin-S4-E12-W13, Figure 1). We also report the in vitro biological activity of this analog at various Ca^{+2} concentrations.

The synthesis of Dap-S4-E12-W13 began with attaching FmocAspGlyOAll to Wang resin via the side chain of the Asp residue using DIC/DMAP to give resin-bound dipeptide **6**. Fmoc SPPS using DIC/HOBt as coupling agents readily gave peptide **7**. The Trt group on Ser4 was selectively removed using 20% DCA in DCM to give peptide **8**. In our previous report on the synthesis of daptomycin, it was found that DIC/cat. DMAP were the most effective reagents