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Brief Article

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Total Synthesis of Laspartomycin C and Characterization of its Antibacterial Mechanism of Action

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ABSTRACT: Laspartomycin C is a lipopeptide antibiotic with activity against a range of Gram-positive bacteria including drug-resistant pathogens. We report the first total synthesis of laspartomycin C as well as a series of structural variants. Laspartomycin C was found to specifically bind undecaprenyl phosphate (C_{55}-P) and inhibit formation of the bacterial cell wall precursor lipid II. While several clinically-used antibiotics target the lipid II pathway, there are no approved drugs that act on its C_{55}-P precursor.

INTRODUCTION

Laspartomycin C is a cyclic lipopeptide that belongs to the family of calcium-dependent antibiotics (CDAs).[1] Originally isolated from *Streptomyces viridochromogenes*, laspartomycin C has been found to possesses antibacterial activity against a variety of Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA) and vancomycin-resistant *enterococci* (VRE).[2,3] The structure of laspartomycin C (**1**, Figure 1) was elucidated in 2003 showing it to be a member of the CDA family .[1,4] In the same year daptomycin (**2**) became the first CDA to receive FDA approval for clinical use and it is now a widely used antibiotic of last resort.[5] In addition, surotomycin, a semi-synthetic analog of daptomycin, is currently undergoing phase 3 clinical trials for the treatment of *Clostridium difficile* infections.[6] In light of emerging bacterial resistance to conventional antibiotics, interest in the CDAs has grown steadily over the past two decades. This increased interest is driven by findings which indicate that the various CDAs operate via modes of action unlike those of conventional antibiotics.[7,8]

Laspartomycin C, also known as glycinocin A, consists of a 10 amino acid cyclic core and an N-terminal exocyclic region.[4] The macrocycle contains a number of nonproteinogenic and D-amino acids including L-2,3-diaminopropionic acid (L-2,3-Dap), D-pipecolic acid (D-Pip), and D-*allo*-threonine, as well as the Asp-X-Asp-Gly motif, which is implicated in Ca^{2+} binding and conserved among all known CDAs. Laspartomycin C bears an unsaturated and branched C_{15} fatty acid tail linked to the exocyclic N-terminal aspartic acid residue. By comparison, daptomycin contains a larger exocyclic tripeptide unit terminated with a straight chain, fully saturated C_{10} lipid. In addition, while the 10 amino acid macrocycle in daptomycin is formed via an ester linkage between its C-terminal residue (L-kynurenine) and a threonine side chain, the laspartomycin C macrocycle is closed via an amide linkage between its C-terminal proline and the side chain of the L-2,3-Dap residue at position 2.

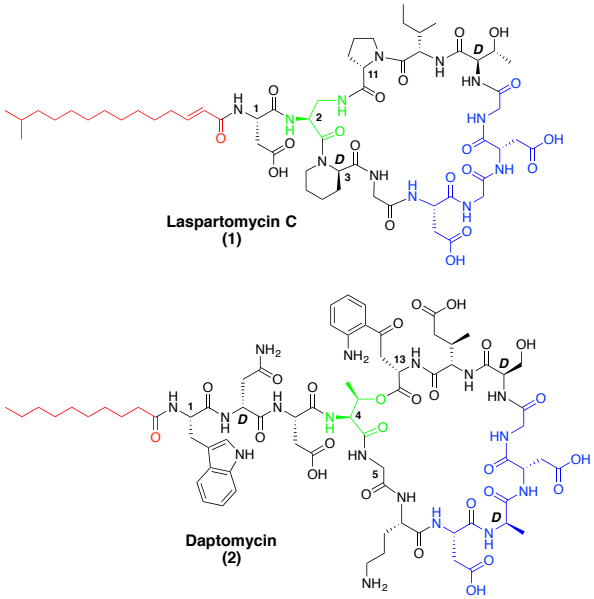


Figure 1. Structures of laspartomycin C (**1**) and daptomycin (**2**) indicating N-terminal lipids (red) and conserved Asp-X-Asp-Gly motif (blue). The peptide macrocycles are formed biosynthetically via cyclization of the C-terminal residue with the side chain of L-2,3-Dap2 in laspartomycin C or Thr4 in daptomycin (linkages shown in green).

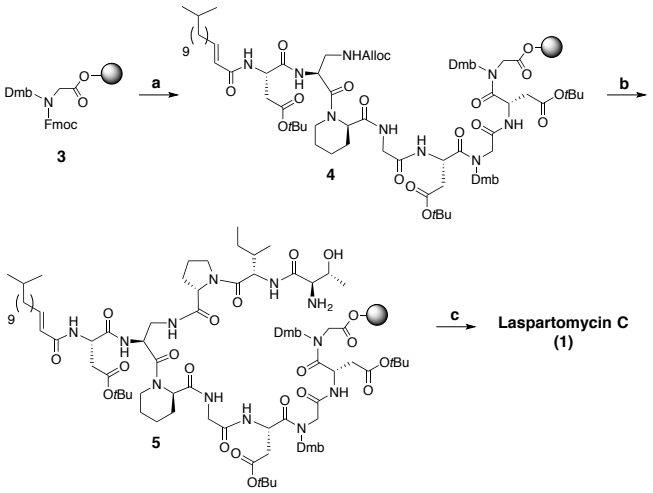
Interestingly, while all CDAs require the presence of Ca^{2+} to achieve their antimicrobial activity, they do not all act via the same antibacterial mechanism.[7] Daptomycin is the most potent of the known CDAs and acts on the bacterial membrane where the proposed formation of daptomycin oligomers is believed to induce membrane perturbation.[8-10] Alternatively, the CDAs amphomycin

and friulimicin B were recently shown to exert their bactericidal effect by inhibiting bacterial cell wall synthesis through complex formation with the essential cell wall precursor undecaprenyl-phosphate (C_{55}-P).[11,12] While laspartomycin C shares structural similarities with amphomycin and friulimicin B (all three contain amide-linked macrocycles flanked by D-Pip and Pro residues at positions 3 and 11), the mechanistic details of its antibiotic mode of action have not been reported. Furthermore, while multiple syntheses of daptomycin have been reported,[13,14] to date no other CDAs have been prepared in synthetic fashion. In this study we report the first total synthesis of laspartomycin C as well as the preparation and evaluation of a series of chimeric lipopeptides that also comprise elements of the daptomycin macrocycle. In addition, we here describe the application of a range of biochemical and biophysical approaches in characterizing the antibacterial mechanism of laspartomycin C.

RESULTS AND DISCUSSION

Our synthetic route to laspartomycin C involved a strategy wherein cyclization at Gly8 provided the laspartomycin C macrocycle (Scheme 1). Ring closure at glycine offers the inherent advantage of avoiding racemization upon activation and cyclization, an approach we previously employed in the preparation of various daptomycin analogues.[15] The solid phase portion of the laspartomycin C synthesis began with immobilization of Fmoc(Dmb)-Gly-OH on the 2-chlorotrityl resin (the Dmb protecting group was employed at Gly6 and Gly8 to prevent possible aspartamide formation). Interestingly, treatment of the resin-bound Fmoc(Dmb)-Gly with piperidine:DMF did not result in complete Fmoc deprotection nor did treatment with a more potent mixture of DBU:piperidine:DMF. However, treatment with ethanolamine:DMF did lead to full Fmoc removal indicating that deprotection of resin-bound Fmoc(Dmb)-Gly requires a less sterically-hindered base. After coupling of aspartic acid, the Fmoc loading of the resin was determined spectrophotochemically (0.52 mmol g^{-1}). Standard Fmoc SPPS was then employed to obtain intermediate **4** with N-terminal acylation achieved using (*E*)-13-methyltetradec-2-enoic acid (synthesized in 6 steps from methyl undec-10-enoate, see Supplemental Scheme S2). Removal of the side chain Alloc protecting group in resin-bound **4,** followed in turn by addition of the remaining three amino acids, then provided intermediate **5**. Cleavage from the resin using mild acidic conditions yielded the protected peptide, which was directly subjected to solution phase cyclization. Complete conversion from the linear to the cyclic peptide was achieved within 12 hours (as evidenced by HPLC analysis) followed by global deprotection. After purification by reverse phase HPLC, NMR analysis showed that the synthetic peptide was identical to natural laspartomycin C, confirming the previously assigned chemical structure.[1,4] Characteristic ^{13}C chemical shifts of Pro at positions β (29.1 ppm) and γ (24.2 ppm) confirm a *trans* orientation as was also reported for natural laspartomycin C.[16,17] Similarly, ^{1}H and ^{13}C chemical shifts at D-Pip positions α (^{1}H 4.80 ppm, ^{13}C 55.8 ppm) and ε (^{1}H 2.88/4.36 ppm, ^{13}C 39.5 ppm), along with strong NOESY correlations between the α proton of D-pip and Dap (^{1}H 4.66 ppm), confirm the *cis* conformation of the D-Pip residue.[16]

Previously in our group, daptomycin analogues were prepared in which the ester linkage of the macrocycle was exchanged for an amide as found in laspartomycin C (see analogues **6**, and **7** in Figure 2).[15] This modification led to a significant loss of antimicrobial activity, likely due to the different conformational restrictions associated with an amide linkage compared to an ester. In this regard it is interesting to note that in laspartomycin C and the structurally-related amphomycins and friulimicins, conformationally restricted cyclic amino acids are found on either side of ring-closing amide linkage. The presence of Pro and D-Pip residues likely play an important role in establishing the biologically relevant conformation of the peptide. The flexibility of our synthetic route to laspartomycin C provides convenient access to structurally related compounds. To this end we next prepared a series of analogues to specifically investigate whether incorporating conformationally restricting amino acids would improve the antibacterial activity of our previously prepared daptomycin "amide-analogues" **6** and **7**. As illustrated in Figure 2, analogs **8** and **9** contain a Gly to D-Pip mutation in comparison with compounds **6** and **7**. Analogue **10** bears a Pro in place of the Kyn normally present in daptomycin and in analogue **11** both Pro and D-Pip residues are introduced as in laspartomycin C. Analogue **12** represents a hybrid structure wherein the laspartomycin C macrocyle is augmented with the daptomycin exocyclic tripeptide unit and C_{10} lipid tail. The synthesis of analogues **8-12** proceeded without incident and with no detectable racemization to provide the target compounds in an average yield of 7.1% (30 reaction steps) based on initial resin loading.



Scheme 1. a) Fmoc SPPS; b) (i) $Pd[(C_6H_5)_3P]_4$, $C_6H_5SiH_3$, CH_2Cl_2, 1h (ii) Fmoc SPPS; c) (i) HFIP, CH_2Cl_2, 1h; (ii) BOP, DIPEA, CH_2Cl_2, 16h (iii) TFA, TIS, H_2O, 1h (Fmoc-D-Thr was employed without side chain protection and incorporated without incident).

The antibacterial activities of laspartomycin C, daptomycin, as well as the chimeric analogues were determined against *Staphylococcus aureus* and *Staphylococcus simulans* (Table 1, for activity of all analogues see Supplemental Table S1). The MICs were measured at various calcium concentrations to investigate the influence this has on antibiotic potency. The antibacterial activity of laspartomycin C and daptomycin increases significantly at higher calcium concentrations, an effect that was also reported by Taylor and coworkers who observed that elevated calcium concentrations were required to achieve full potency with their daptomycin analogues.[14] The influence of introducing conformationally restricted amino acids in the macrocycle was investigated with analogues **8-12**. In general, incorporation of D-Pip and Pro residues was found to be detrimental, resulting in large increases in

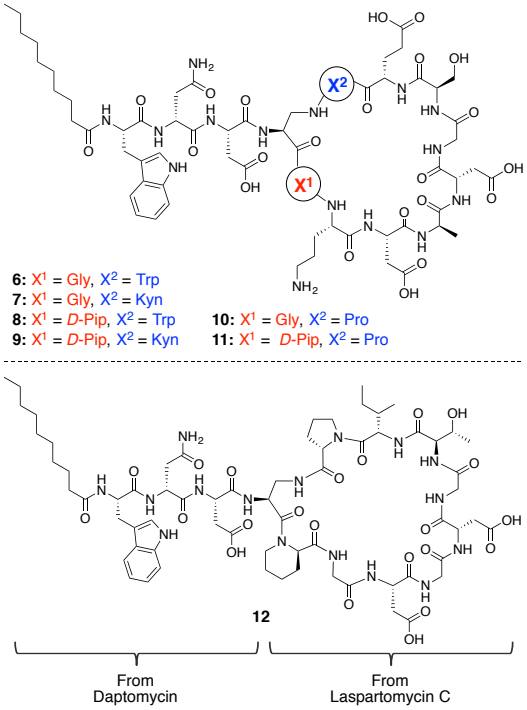


Figure 2. Structures of analogues **6**-**11** with variations at positions X^1 and X^2. Compound **12** is a hybrid comprised of the peptide macrocycle of laspartomycin C and the exocyclic tripeptide unit and C_{10} lipid of daptomycin.

MIC or complete loss of antibacterial activity (see Supplemental Table S1). Of particular note is the observation that compound **12**, a daptomycin-laspartomycin C hybrid, is completely inactive. The decreased activity of these analogues does not, however, appear to be due to an inability to bind to calcium. The circular dichroism spectra obtained (Supplemental Figure S1) indicate that all analogues undergo conformational changes upon mixing with calcium, as was previously observed for daptomycin and the amphomycin-derived MX-2401.[9,18] Particularly intriguing are D-Pip and Pro bearing analogues **11** and **12**, which, although devoid of activity, exhibit calcium-induced conformational changes very similar to that of laspartomycin C. These findings prompted us to further investigate the antibacterial mechanism of laspartomycin C.

We began by examining the effect of laspartomycin C on bacterial cell wall biosynthesis by specifically looking for accumulation of the cytoplasmic lipid II precursor UDP-MurNAc-pentapeptide in response to administration of antibiotic.[12] As is clearly seen in Figure 3, when *S. aureus* cells are treated with laspartomycin C there is a significant accumulation of UDP-MurNAc-pentapeptide, an effect not seen with daptomycin. These findings indicate that the target of laspartomycin C lies downstream of UDP-MurNAc-pentapeptide, implicating one of

Table 1. MICs[a] (μg mL^{-1}) measured against indicator strains *S. simulans* 22 and *S. aureus* 29213 supplemented with 5.0 and 10 mM Ca^{2+}

Compound	*S. aureus* 29213		*S. simulans* 22	
	5 mM Ca^{2+}	10 mM Ca^{2+}	5 mM Ca^{2+}	10 mM Ca^{2+}
Laspartomycin C (**1**)	4	2	4	≤1
Daptomycin (**2**)	0.25	0.125	0.031	≤0.031

[a]MIC = minimum inhibitory concentration

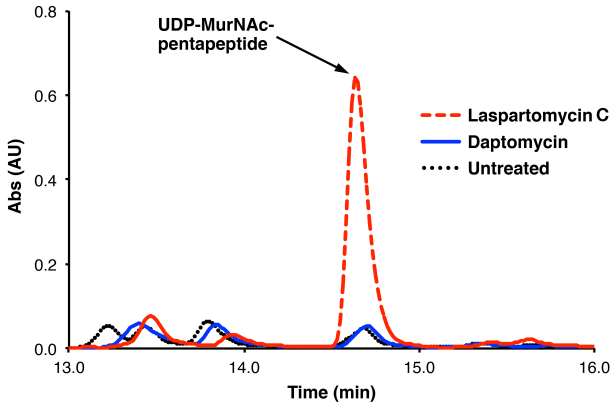


Figure 3. HPLC traces for UDP-MurNAc-pentapeptide accumulation assay: Treatment of *S. aureus* 29213 with laspartomycin C results in accumulation of UDP-MurNAc-pentapeptide, an effect not observed with daptomycin (full-length traces provided in the Supplemental Figure S2).

the subsequent membrane-associated steps involved in bacterial cell wall biosynthesis.

We then investigated whether the antimicrobial activities of laspartomycin C, daptomycin, and the active synthetic analogues were antagonized by various bacterial cell wall precursors including: lipid I, lipid II, UDP-MurNAc-pentapeptide, UDP-GlcNAc, undecaprenyl-pyrophosphate (C_{55}-PP), and undecaprenyl-phosphate (C_{55}-P). The antibiotics (as well as vancomycin as a positive control) were pre-incubated with the various bacterial cell wall precursors and administered to *S. simulans* 22, which was used as an indicator strain (Supplemental Table S2). As expected, the activity of vancomycin was fully antagonized by lipid I, lipid II, and UDP-MurNAc-pentapeptide, each of which contains the D-Ala-D-Ala motif recognized by vancomycin. In contrast, daptomycin and analogues **6**-**12** were not antagonized by any of the bacterial cell wall precursors. For laspartomycin C, however, both C_{55}-P and C_{55}-PP gave an indication of antagonism. Upon repeating the assay with the water soluble C_{15}-P and C_{15}-PP it became clear that only the monophosphate species is capable of antagonizing the activity of laspartomycin C.

Next, a TLC-based binding assay was employed to assess the binding of laspartomycin C to C_{55}-P. Mixing of C_{55}-P with laspartomycin C in Ca^{2+}-containing buffer led to formation of a surprisingly stable complex that could be clearly visualized by TLC (Figure 4). By comparison, there was no indication of C_{55}-P binding by daptomycin. When the same TLC experiment was performed with lipid II, no complex formation was observed (Supplemental Figure S4) further indicating that laspartomycin C selectively targets C_{55}-P. We next investigated whether laspartomycin C is capable of inhibiting lipid II synthesis by means of an *in vitro* assay. To do so, C_{55}-P was pre-treated with laspartomycin C at a variety of concentrations, followed by addition of UDP-GlcNAc, UDP-MurNAc-pentapeptide, and *M. flavus* membrane vesicles known to contain the lipid II-producing enzymes MraY and MurG. Under these conditions laspartomycin C blocked lipid II synthesis in a dose-dependent manner while incubation with daptomycin had no effect lipid II synthesis (Supplemental Figure S5). These findings support a mode of action for laspartomycin C wherein the sequestration of C_{55}-P leads to

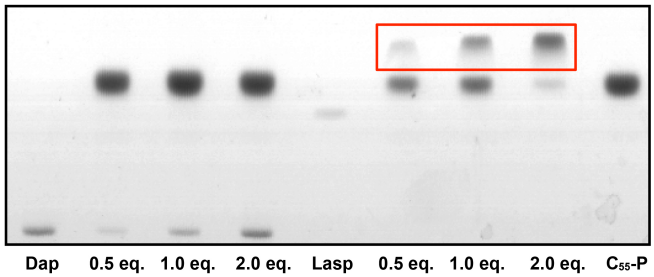


Figure 4. TLC analysis of C_{55}-P incubation with 0.5-2 eq. of laspartomycin C and daptomycin. Laspartomycin C forms a stable complex with C_{55}-P (red box) contrary to daptomycin. MS analysis of the laspartomycin C:C_{55}-P complex recovered from the TLC plate indicated the presence of intact laspartomycin C. The brightness and contrast of the figure has been adjusted to enhance visibility of the laspartomycin reference band (the original unadjusted figure is included in the supplemental information, see Figure S3).

blocked formation of lipid II and prevention of bacterial cell wall biosynthesis.

To obtain a more quantitative understanding of the binding of C_{55}-P by laspartomycin C we turned to isothermal titration calorimetry (ITC). ITC was used to study the interaction of laspartomycin C with large unilamellar vesicles (LUVs) comprised of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1.5 mol% C_{55}-P, a system mimicking a simple membrane environment. Titration of the C_{55}-P containing LUVs into a solution of laspartomycin C results in an isotherm that appears to be the combination of two distinct binding events (Figure 5). The initial stage of the isotherm (left side) shows an endothermic event resulting from the interaction of laspartomycin C with the DOPC membrane vesicles. Similarly, titration of "empty" DOPC vesicles, not containing C_{55}-P, into laspartomycin C solution resulted in an isotherm displaying an identical interaction (Supplemental Figure S6). However, as the quantity of C_{55}-P injected increases, a second binding event is apparent (Figure 5, enlarged). This is ascribed to the binding of C_{55}-P by laspartomycin C and indicates a remarkably high affinity interaction with a dissociation constant in the low nanomolar range (K_d = 7.3 ± 3.8 nM). The ITC approach employed further reveals that enthalpic (ΔH = -9.8 ± 0.6 kJ mol^{-1}) and entropic (ΔS = 122.9 ± 4.7 J mol^{-1} K^{-1}) factors both contribute to the tight binding of C_{55}-P by laspartomycin C.

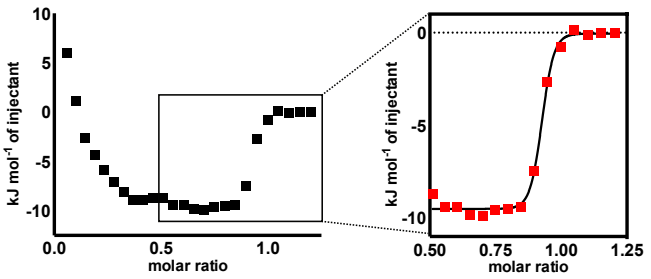


Figure 5. Isothermal titration calorimetry: DOPC vesicles containing 1.5 mol% C_{55}-P were titrated into a solution of laspartomycin C in HEPES buffer containing $CaCl_2$. Fitting of the second binding curve (red) provides a K_d value of 7.3 ± 3.8 nM. For a full summary of all associated thermodynamic parameters see Supplemental Table 3. Each point is the average of three independent experiments.

CONCLUSION

In summary, we here describe the total synthesis of laspartomycin C by means of a flexible route that also allows for the preparation of structural analogues. While the synthesis of daptomycin, the preeminent depsipeptide CDA, has been previously described,[13,14] our synthesis of laspartomycin C represents the first of its kind among the macrolactam sub-family of CDAs. Hybrid structures combining aspects of laspartomycin C and daptomycin were also prepared and evaluated for antibacterial activity. In all cases these variants were less active than either parent compound, suggesting a significant difference in the modes of action of laspartomycin C and daptomycin. Following up on these findings we established that unlike daptomycin, laspartomycin C exerts its antibiotic effect by tightly complexing C_{55}-P. In further assessing this interaction, we also report the first ITC-based characterization of a C_{55}-P-targeting CDA and determined the thermodynamic parameters governing the binding of laspartomycin C to C_{55}-P. Of particular note is the low nanomolar K_d value associated with laspartomycin C's binding to its structurally simple phospholipid target.

At present, daptomycin is the only clinically approved CDA and our findings show it to be a generally more potent antibiotic than laspartomycin and the structural analogue here investigated. That said, laspartomycin C's ability to kill a range of Gram-positive pathogens[2-3] via a mode of action different than that of daptomycin indicates that it may have potential for development. At present, no clinically used antibiotic acts via a C_{55}-P targeting mode of action. In this regard our synthetic route provides the means for future structure-activity relationship studies with this interesting class of CDAs. While the therapeutic potential of C_{55}-P targeting peptides requires further validation, such compounds may be of value in addressing the growing threat posed by antibiotic-resistant bacteria.

EXPERIMENTAL SECTION

General procedures

All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. Fmoc-(Dmb)Gly-OH and Fmoc-Kyn-OH were obtained via previously published procedures.[15,19] Fmoc-*L*-Dap(Aloc)-OH, Fmoc-*D*-allo-Thr and 2-chlorotrityl resin were obtained from Iris Biotech GmbH and the latter was used without protection of the side chain hydroxyl moiety. All known compounds prepared had NMR spectra, mass spectra, and optical rotation values consistent with the assigned structures.

Instrumentation for compound characterization

NMR spectra were recorded at 400 or 500 MHz with chemical shifts reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS). 2D NMR experiments (TOCSY, HSQC and NOESY) were performed on a 500 MHz instrument. High-resolution mass spectrometry (HRMS) analysis was performed using an ESI instrument. Circular Dichroism spectra were recorded on a Jasco J-810 CD-spectrometer and ITC experiments were carried out using a MicroCal Auto-ITC$_{200}$. Automated peptide synthesis was performed on a CS Bio CS336x peptide synthesizer.

Preparation of Laspartomycin C and analogues

Chlorotrityl resin (5.0 g, 1.60 mmol/g) was loaded with DMB-Fmoc-Gly-OH. Resin loading was determined after coupling of the second amino acid because complete Fmoc deprotection of resin bound DMB-Fmoc-Gly required nonstandard conditions: DMB-Fmoc-Gly 2-chlorotrityl resin (6.0 g) was thus treated with ethanolamine:DMF (1:4 v:v, 1x30 min, 1x90 min) followed by washing with DMF. Overnight coupling of Fmoc-Asp(^{t}Bu)-OH (3.7 g, 9.0 mmol), BOP (4.0 g, 9.0 mmol) and DiPEA (3.1 mL, 18.0 mmol) in DMF followed by end capping with Ac_2O:DiPEA:DMF (0.5:0.5:9 v:v:v, 20 mL) yielded Fmoc-Asp(tBu)-(DMB)-Gly 2-chlorotrityl resin (0.52 mmol.g^{-1} as determined spectrophotometrically).

Linear precursor peptides encompassing Gly_8 to Asp_1 were assembled via standard Fmoc solid-phase peptide synthesis (SPPS) either via manual synthesis (resin bound AA:Fmoc-AA:BOP:DiPEA, 1:4:4:8 molar eq.) or automated synthesis (resin bound AA:Fmoc-AA:HBTU:HOBt:DiPEA, 1:4:3.75:3.75:8 molar eq.) typically on 0.25 mmol scale. NMP or DMF was used as solvent and Fmoc deprotections were carried out with piperidine:DMF or piperidine:NMP (1:4 v:v). Amino acid side chains were protected as follows: Boc for Orn and Trp, Trt for *D*-Asn, Aloc for DAP, ^{t}Bu for Asp, Glu and *D*-Ser, DMB for Gly in Asp-Gly sequences. Kyn and *D-allo*-Thr were introduced without side chain protection. Following coupling and Fmoc deprotection of Asp_1, N-terminal acylation was achieved by coupling (*E*)-13-methyltetradec-2-enoic acid using the same coupling conditions used for the SPPS.

The resin-bound, Alloc protected intermediate was next washed with CH_2Cl_2 and treated with $Pd(PPh_3)_4$ (74 mg, 0.06 mmol) and $PhSiH_3$ (0.74 mL, 6.0 mmol) in CH_2Cl_2 (ca. 10 mL) under argon for 1 hour. The resin was subsequently washed with CH_2Cl_2 (5x10 mL), followed by a solution of diethyldithiocarbamic acid trihydrate sodium salt (5 mg mL^{-1} in DMF, 5x10 mL), and DMF (5x10 mL). The remaining three amino acids where added via standard Fmoc SPPS with removal of the final Fmoc protecting group to yield the complete linear resin-bound peptide with a free N-terminal amine. The resin was treated with $(CF_3)_2CHOH$:CH_2Cl_2 (1:4, 10 mL) for 1 hour and rinsed with additional $(CF_3)_2CHOH$:CH_2Cl_2 and CH_2Cl_2. The combined washings were then evaporated to yield the linear protected peptide with free C- and N-termini. The residue was dissolved in CH_2Cl_2 (250 mL) and treated with BOP (0.22 g, 0.5 mmol) and DiPEA (0.17 mL, 1.0 mmol) and the solution was stirred overnight after which TLC indicated complete cyclization. The reaction mixture was concentrated and directly treated with TFA:TIS:H_2O (95:2.5:2.5, 10 mL) for 60-90 minutes. The reaction mixture was added to Et_2O:hexanes (1:1) and the resulting precipitate washed once more with Et_2O:hexanes (1:1). The crude cyclic peptide was lyophilized from ^{t}BuOH:H_2O (1:1) and purified with reverse phase HPLC by applying a gradient of 25% to 65% buffer B (buffer A: H_2O:MeCN:TFA, 95:5:0.1 v:v:v; buffer B: H_2O:MeCN:TFA, 5:95:0.1 v:v:v) over 1 hour with a flow rate of 12 mL min^{-1} on a C_{18} Maisch 250x22 mm column. Pure fractions were pooled and lyophilized to yield the desired cyclic lipopeptide products in >95% purity (based on analytical HPLC analysis) as white powders, typically in 10-20 mg quantity (4.2-9.3 % yield based on resin loading).

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and analytical data for all new compounds including characterization data and MIC determinations. Supplemental figures and tables for: CD spectra, bacterial cell wall synthesis antagonization assays, lipid II binding assays, ITC binding studies, 2D-NMR spectra, and analytical RP-HPLC traces. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

Alloc, allyloxycarbonyl; CD, circular dichroism; CDA, calcium-dependent antibiotic; C_{15}-P, farnesyl phosphate; C_{15}-PP, farnesyl pyrophosphate; C_{55}-P, undecaprenyl phosphate; C_{55}-PP, undecaprenyl pyrophosphate; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; Dmb, 3,4-dimethoxybenzyl; DMF, dimethylformamide; DOPC, l-α-phosphatidylcholine; d-Pip, d-pipecholic acid; FDA, Food and Drug Administration; Fmoc, fluorenylmethyloxycarbonyl; HPLC, high performance liquid chromatography; GlcNAc, *N*-acetylglucosamine; K_d, dissociation constant; l-2,3-Dap, l-2,3-diaminopropionic acid; LUV, large unilamellar vesicle; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; MS, mass spectrometry; MurNAc, *N*-acetylmuramic acid; NMR, nuclear magnetic resonance; SPPS, solid-phase peptide synthesis; TLC, thin-layer chromatography; UDP, uridine diphosphate glucose; VISA, vancomycin-intermediate *S. aureus*; VRSA, vancomycin-resistant *S. aureus.*

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