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Article

The Total Chemical Synthesis and Biological Evaluation of the Cationic Antimicrobial Peptides, Laterocidine and Brevicidine

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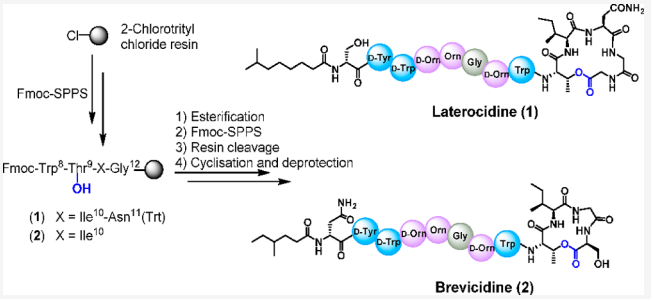
ABSTRACT: Antimicrobial resistance is a significant threat to public health systems worldwide, prompting immediate attention to develop new therapeutic agents with novel mechanisms of action. Recently, two new cationic non-ribosomal peptides (CNRPs), laterocidine and brevicidine, were discovered from *Brevibacillus laterosporus* through a global genome-mining approach. Both laterocidine and brevicidine exhibit potent antimicrobial activity toward Gram-negative bacteria, including difficult-to-treat *Pseudonomas aeruginosa* and colistin-resistant *Escherichia coli*, and a low risk of resistance development. Herein, we report the first total syntheses of laterocidine and brevicidine via an efficient and high-yielding combination of solid-phase synthesis and solution-phase macrolactamization. The crucial depsipeptide bond of the macrolactone rings of laterocidine and brevicidine was established on-resin between the side-chain hydroxy group of Thr^9 with Alloc-Gly-OH or Alloc-Ser(*t*Bu)−OH, respectively. A conserved glycine residue within the lactone macrocycle is exploited for the initial immobilization onto the hyper acid-labile 2-chlorotrityl chloride resin, subsequently enabling an efficient solution-phase macrocyclization to yield laterocidine and brevicidine in 36% and 10% overall yields, respectively (with respect to resin loading). A biological evaluation against both Gram-positive and Gram-negative bacteria demonstrated that synthetic laterocidine and brevicidine possessed a potent and selective antimicrobial activity toward Gram-negative bacteria, in accordance with the isolated compounds.



The emergence and rapid spread of AntiMicrobial Resistance (AMR) has significantly threatened public health worldwide.[1] In 2018, the World Health Organization (WHO) declared AMR to be one of the biggest threats to global health, food security, and development.[2] Globally, at least 700 000 people die each year from AMR-related infections and diseases, including infections caused by multidrug-resistant (MDR) organisms (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecium*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*).[3,4] High rates of morbidity and mortality (in both humans and animals) due to AMR are a burden to society and the economy. It is predicted that AMR infections will result in nearly 10 million deaths per year by 2050 and a total gross domestic product (GDP) loss of US$100.2 trillion by 2050 based on current actions.[5,6] In particular, there is a critical urgency for the development of new antibiotics targeting Gram-negative bacteria (e.g., *E. coli* and *P. aeruginosa*), given they possess an outer membrane of lipopolysaccharide (LPS), which forms a permeability barrier to prevent the penetration of antibiotics.[7]

Naturally occurring Anti-Microbial Peptides (AMPs) have been recognized as potentially useful therapeutic agents against AMR.[8−11] They exhibit rapid, broad-spectrum and potent antimicrobial activities against both Gram-positive and Gram-negative bacteria (sometimes selectively), including antibiotic-resistant microbes, such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), MDR *P. aeruginosa* and MDR *M. tuberculosis*.[12−14] A notable example is teixobactin, a recently discovered macrocyclic Non-Ribosomal Peptide (NRP) that displays excellent activity against Gram-positive bacterial pathogens, including MDR strains, such as MRSA, VRE, and *M. tuberculosis*.[15] Teixobactin proved refractory toward bacterial resistance development, thought to be a result of its ability to simultaneously target a range of highly conserved critical components of the bacterial cell wall via unique modes of action.[15] Numerous research groups, including our own, have pursued antibiotic development programs based upon this promising scaffold.[16−23] Cationic Non-Ribosomal Peptides (CNRPs) are a large and structurally

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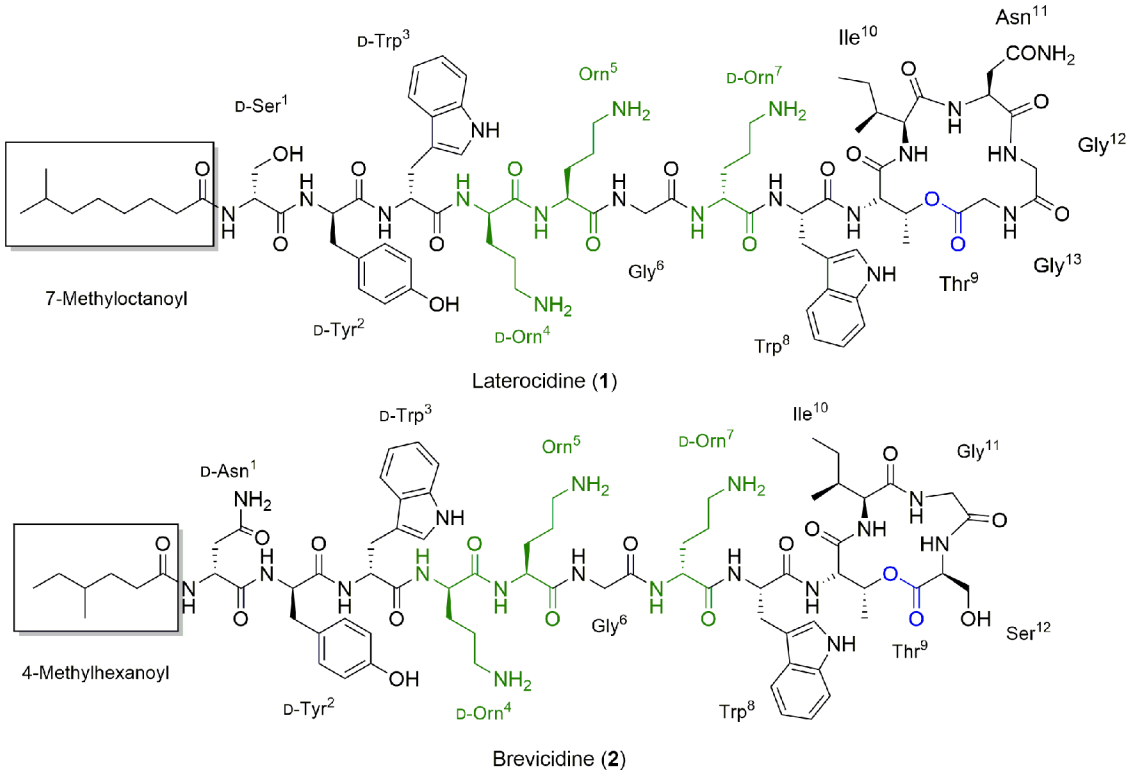

Figure 1. Structure of laterocidine (**1**) and brevicidine (**2**). The N-terminal lipid of each compound is shown in a box. The positively charged ornithine residues are shown in green. The ester bond of the lactone ring is highlighted in blue.

diverse family of AMPs produced by nonribosomal peptide synthetases in soil-dwelling or plant-associated bacteria, such as *Pseudomonas*, *Bacillus*, and *Streptomyces* spp.[24−27] Naturally occurring CNRPs are generally amphipathic, small (12−50 amino acids), carry an overall net positive charge, and possess a significant proportion of cationic and hydrophobic residues.[11,12,28] Possessing these unique chemical and structural properties enables them to selectively target highly impermeable anionic components of the bacterial envelope surface, such as phosphate groups in LPS of Gram-negative bacteria or lipoteichoic acid in Gram-positive bacteria, through a combination of electrostatic and hydrophobic interactions.[26,28−30] As such, resistance to CNRPs is often minimized, as it would require multiple mutational events for a bacteria to significantly alter the composition or organization of its cell membrane or cell wall components to reduce these interactions.[28,29,31] Additionally, CNRPS have demonstrated multifaceted mechanisms, and their highly cationic nature can promote interactions with secondary anionic intracellular targets, impairing processes such as DNA and protein synthesis, protein folding, enzymatic activity, and cell wall synthesis, further mitigating the development of resistance.[32−36] Furthermore, CNRPs often possess macrocyclic rings and contain nonproteinogenic and D-amino acids, conferring a large structural diversity and an enhanced resistance to proteolytic cleavage.[12,29,31] Currently there is a small number of CNRPs on the market for the treatment of Gram-negative infections, including polymyxin B (Poly-Rx) and polymyxin E/colistin (Xylistin).[37,38] Unfortunately, the use of polymyxins as effective antimicrobial agents against Gram-negative bacteria declined in the 1970s due to their nephrotoxic and neurotoxic side effects.[38] More recently, however, with the emergence of Gram-negative bacteria resistant to nearly all other classes of antimicrobial agents, polymyxins are becoming increasingly relied upon as treatments of last resort.[38,39] This highlights the urgency for development of new antibiotics with novel mechanisms of action and improved safety and efficacy profiles.

In 2018, Li et al.[40] developed a global genome-mining approach based on antibiotics & Secondary Metabolite Analysis Shell (antiSMASH) for the discovery of CNRPs.[40,41] Through the analysis of 7395 bacterial genomes, the authors identified two new CNRPs with an antimicrobial activity against clinically relevant Gram-negative bacterial strains, coined laterocidine (**1**) and brevicidine (**2**), from *Brevibacillus laterosporus* strains ATCC 9141 and DSM 25, respectively (Figure 1).[40] The structure elucidation of laterocidine (**1**) and brevicidine (**2**) by nuclear magnetic resonance (NMR), Marfey's, and tandem mass spectrometry (MS/MS) analyses revealed a new class of CNRPs presenting N-terminal acylation, an eight-residue exocyclic cationic segment with three positively charged ornithine residues, and a C-terminal cyclic depsipeptide. Laterocidine (**1**) consists of 13 amino acids, a five-membered cyclic core cyclized via a lactone formation between the hydroxy group of Thr^9 and the C-terminal Gly^{13}, and an isononanoyl (7-methyloctanoyl) lipid tail (Figure 1). Brevicidine (**2**) has 12 amino acids, four of which form a cyclic core cyclized via a lactone bond between the α-carboxyl of Ser^{12} and the hydroxy group of Thr^9, and a configurationally undefined 4-methylhexanoyl lipid tail (Figure 1).[40]

To avoid the bottleneck of engineering and expressing large biosynthetic complexes required to prepare nonribosomally produced peptides, Kuipers and co-workers[42] recently designed a strategy to prepare simplified brevicidine (**2**) analogues by a ribosomal synthesis and post-translational

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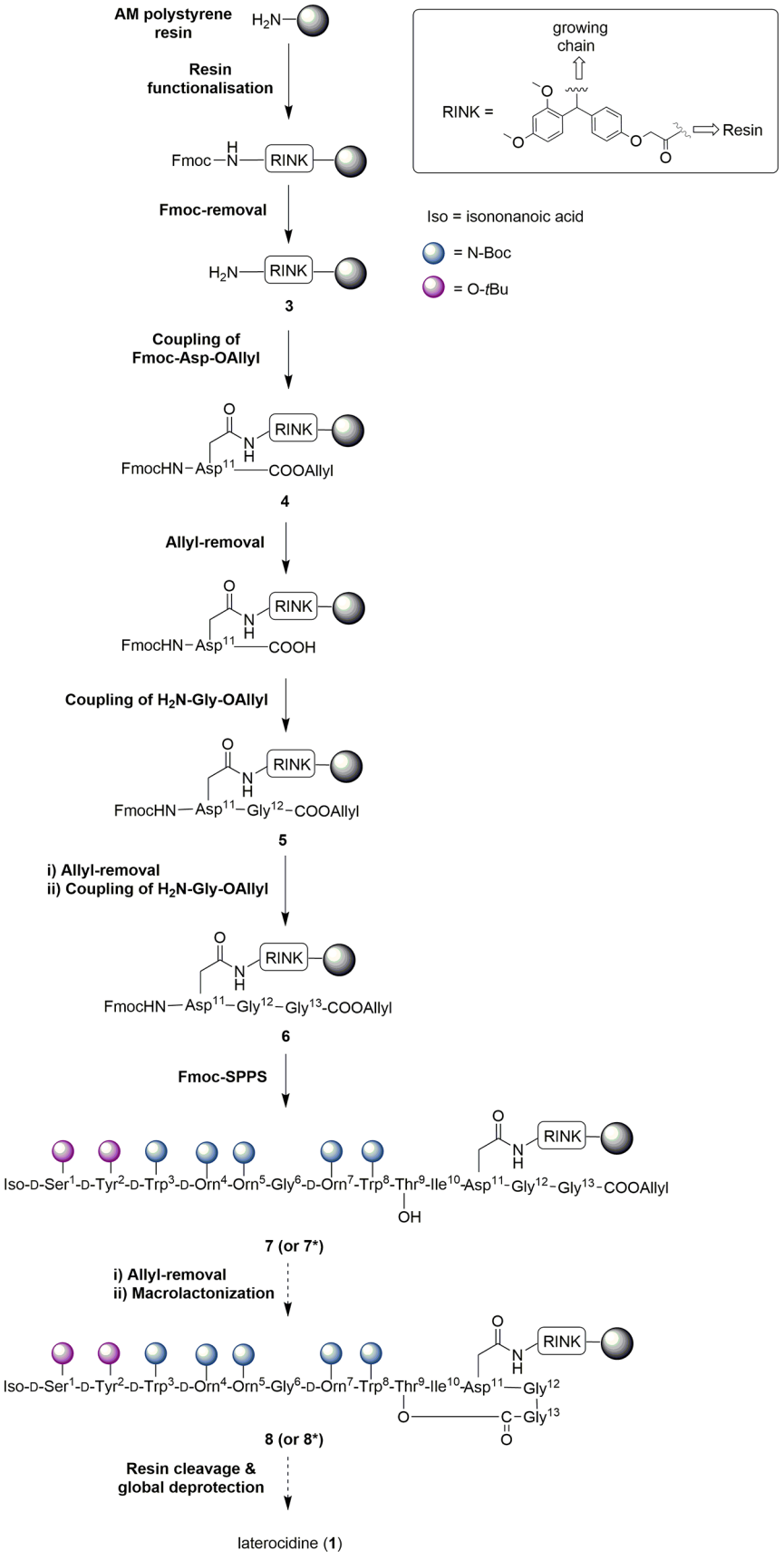
Scheme 1. Initially Proposed Synthetic Strategy toward Laterocidine (1)[a]



[a]7*, 8* = simplified analogue where all D-AAs are replaced with L-AAs, and the N-terminal fatty acid is *n*-octanoyl.

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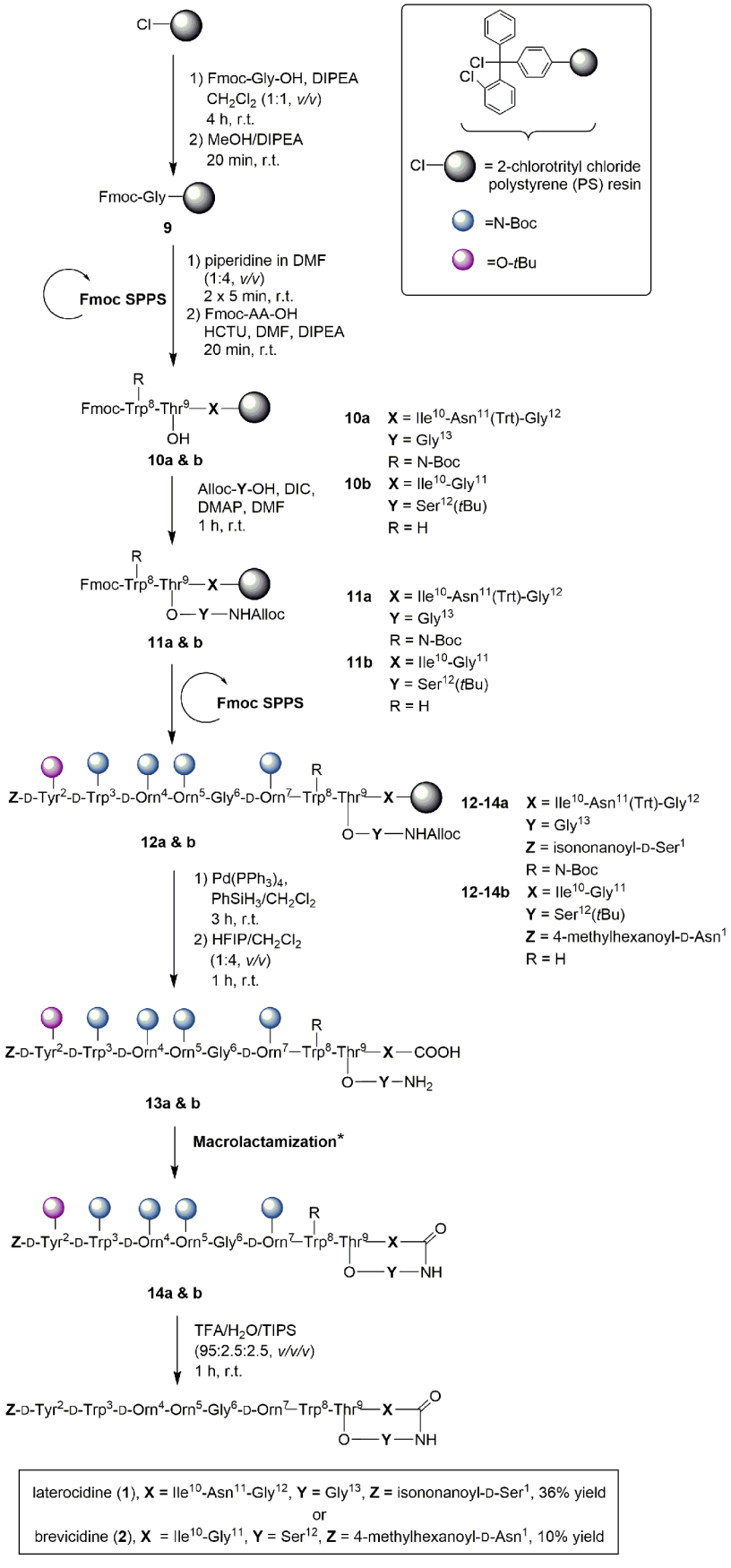
Scheme 2. Succesful Synthetic Route to Prepare Laterocidine (1) and Brevicidine (2)[a]



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Scheme 2. continued

[a]Throughout the scheme the intermediates depicted "a" refer to laterocidine (**1**), while "b" refers to brevicidine (**2**). *Macrolactamization reagents and conditions; (**13a**) peptide (10 mM in DMF), BOP (5 equiv), HOAt (5 equiv), and DIPEA (10 equiv); (**13b**) peptide (10 mM in DMF), PyBOP (5 equiv), and DIPEA (5 equiv).

modification. Despite the inability to prepare the key lactone ring, they were able modify the peptide sequence and prepare mimics of the lactone ring (thioethers). However, the prepared analogues were unable to replicate the activity of the native peptide, and their best candidate was 4- to 16-fold less active across the test strains. This highlights the importance of developing efficient chemical syntheses of these new antibiotics, allowing atomic precision in the structure modification, which is key to developing clinical drug leads.

Herein, we report an optimized and successful strategy to prepare both laterocidine (**1**) and brevicidine (**2**) in multimilligram amounts, exploiting both solid-phase and solution-phase techniques. The primary structures of both depsipeptides, laterocidine (**1**) and brevicidine (**2**), were confirmed by an extensive NMR analysis, and both displayed a potent activity against *E. coli*, with laterocidine (**1**) possessing an activity equipotent to clinically relevant polymyxin B.

RESULTS AND DISCUSSION

Initial Synthetic Strategy Toward Laterocidine. Several strategies have been employed for the synthesis of cyclic depsipeptides, which can prove troublesome due to the lability of the ester.[43−47] An on-resin macrocyclization approach that involves linking the side chain of reactive amino acids, such as Ser/Thr/Lys/Asp/Glu, to a solid-phase resin, and orthogonal protection strategies, has proven to be a successful approach.[48] For the total syntheses of laterocidine (**1**) and brevicidine (**2**), we initially envisioned the preparation of the linear precursor via a 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide synthesis (SPPS) followed by a late-stage on-resin macrocyclization. In contrast to brevicidine (**2**), laterocidine (**1**) comprises an Asn residue in the C-terminal macrolactone. We anticipated that anchoring the Asp side chain to a Rink amide linker would provide a suitable handle for SPPS. Following an acid-mediated detachment of the peptide from the amide-liberating linker, the native Asn[11] would be generated (Scheme 1).

This synthetic strategy relies on an efficient macrolactonization between the hydroxy group of Thr[9] and the α-carboxylic acid of Gly[13], and noteworthy, no further postcleavage modifications of the synthetic peptide are required. Furthermore, despite the demanding reaction conditions often required for the macrolactonization, Gly[13] is achiral, thus avoiding any chance of epimerization upon its activation. The anchoring of Fmoc-Asp-OAllyl through its side-chain carboxyl to Rink amide linker **3** would afford resin **4**. The subsequent deprotection of the α-carboxylic acid of resin **4** followed by the coupling of H_2N-Gly-OAllyl would yield peptidyl-resin **5**. A second round of allyl-deprotection and coupling of H_2N-Gly-OAllyl would subsequently yield peptidyl-resin **6**. Following an iterative Fmoc-SPPS, coupling of the N-terminal isononanoyl fatty acid, allyl ester deprotection and on-resin macrolactonization would afford resin-bound laterocidine (**8**). A resin cleavage and global deprotection would then deliver laterocidine (**1**) directly. This strategy, employing a late-stage esterification, would circumvent the risk of premature aminolysis of the ester bond during Fmoc-SPPS, as observed in our previous synthesis of the cyclic lipodepsipeptide daptomycin.[49]

To establish this synthetic route, we sought to prepare a simplified analogue of laterocidine, where all D-amino acid residues were substituted with their L-isomers and the N-terminal fatty acid was replaced by the linear *n*-octanoyl group. The assembly of the linear sequence **7*** (Scheme 1) and the removal of the allyl ester protection proceeded smoothly as confirmed by a reversed-phase high-performance liquid chromatography mass spectrometry (RP-HPLC/MS) analysis upon resin mini-cleavage. However, a subsequent ring closure to form the lactone between a Thr[9] side-chain hydroxy group and Gly[13] was unsuccessful, and only the linear precursor corresponding to peptidyl-resin **7*** was recovered after cleavage from the resin. A prolonged reaction time and an increasing excess of the coupling reagents had no effect, and no macrocyclization was observed.

Second & Third Synthetic Strategies Toward Laterocidine. Upon failing to prepare resin **8*** by lactonization, we redesigned our synthesis (Supporting Information, Scheme S1), instead harnessing the anticipated ease of lactamization to afford the macrocycle. The amended strategy also maintained the late-stage introduction of the ester bond, thus avoiding an undesired ester aminolysis during repeated piperidine-mediated Fmoc removals.

However, the esterification with Alloc-Gly-OH was sluggish, providing a very poor conversion (ca. 25%) to the desired product (Figure S22), leading us to abandon the synthetic route at this stage (as indicated by the red cross in Scheme S1). We postulated that the presence of the N-terminal fatty acid might hinder the esterification by forming hydrophobic interactions with the resin or neighboring peptide chains, reducing the accessibility of the esterification site. Therefore, we modified our synthetic strategy accordingly (Scheme S2).

In this instance, the esterification was undertaken on a truncated sequence. Unfortunately, little improvement in the esterification was observed under a range of conditions (Table S1), and only a maximum of 60% conversion to the esterified resin (Figure S23) was obtained using long reaction times or elevated temperatures.

Successful Synthetic Strategy toward Laterocidine and Brevicidine. The poor conversion to the esterified Thr resin in our third strategy suggested that anchoring the peptide sequence through the side chain of Asn[11] may hinder the esterification. We therefore devised a new site for resin anchoring and instead performed a solution-phase macrolactamization of the linear lipodepsipeptide precursor (Scheme 2).

The revised synthesis of laterocidine (**1**) began by loading Fmoc-Gly-OH onto the hyper acid-labile 2-chlorotrityl chloride resin (2-CTC) using *N,N*-diisopropylethylamine (DIPEA) in CH_2Cl_2, followed by the capping of any unreacted sites with a CH_2Cl_2 solution of MeOH and DIPEA (17:2:1, v/v/v), for 20 min. Resin **9** was then elongated from the C- to N-terminus until the inclusion of the Fmoc-Trp[8](Boc)−OH residue with repeated cycles of N^{α}-Fmoc removal with 20% piperidine in *N,N′*-dimethylformamide (DMF) (v/v, 2 × 5

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min, room temperature (rt)) and coupling of amino acids with 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) as the coupling reagent, and DIPEA in DMF for 20 min at rt. During this process, the Thr[9] residue was introduced without side-chain protection to facilitate the subsequent esterification.

The resin-bound linear precursor **10a** was then O-acylated at the side chain of Thr[9] with Alloc-Gly-OH (Supporting Information) using *N,N′*-diisopropylcarbodiimide (DIC) in the presence of catalytic 4-dimethylaminopyridine (DMAP) in DMF to yield depsipeptidyl resin **11a**. Gratifyingly, the esterification proceeded to completion after 1 h at rt (ca. 76% purity, Figure S24); no detectable starting material was observed, and there was no aminolysis of the ester bond during subsequent removals of the N^{α}-Fmoc protection. The completion of the amino acid sequence by Fmoc-SPPS to yield depsipeptidyl resin **12a** (ca. 80% purity, Figure S25) was followed by a selective removal of the Alloc protection via a treatment with tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) and phenylsilane ($PhSiH_3$) in CH_2Cl_2 for 3 h at rt. Upon resin cleavage effected with 20% hexafluoroisopropanol (HFIP) in CH_2Cl_2 (v/v), the resulting side-chain protected peptide **13a** was subjected to macrolactamization in the presence of benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP), 1-hydroxy-7-aza-benzotriazole (HOAt), and DIPEA for 5 h at rt in DMF (10 mM). Finally, crude laterocidine (**1**) was obtained following a global side-chain deprotection using a cleavage cocktail solution of trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/H_2O (95:2.5:2.5, v/v/v, 1 h, rt) (ca. 77% purity, Figure S7). Following purification by RP-HPLC, laterocidine (**1**) was afforded in an excellent overall yield (36%, based on the initial resin loading capacity, ca. greater than 98% purity, Figure S8).

Brevicidine (**2**) was synthesized following the same protocol with minor modifications. In this case, the side chain of Thr[9] was esterified with Alloc-Ser(O*t*Bu)−OH (Supporting Information). To facilitate esterification with the more hindered Alloc-Ser(O*t*Bu)−OH, we made a minor adaption to our SPPS strategy (with respect to the synthesis of laterocidine). Thus, we introduced Trp[8] without side-chain protection to reduce potential hindrance at the site of esterification. The esterification proceeded in 88% conversion following 2 × 1 h treatments using DIC/catalyst DMAP to yield resin **11b** (ca. 70% purity, Figure S26). After the coupling of the N-terminal Asn[1] residue and acylation with (*R,S*)-4-methylhexanoic acid, the Alloc group was removed from depsipeptidyl resin **12b**, and the resin was cleaved with 20% HFIP in CH_2Cl_2 (v/v) to yield the side-chain protected peptide **13b**. We opted to substitute the coupling reagent BOP for benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) when effecting the solution-phase cyclization for brevicidine (**2**), due to its improved safety profile.[50] Cyclization was therefore effected with PyBOP and DIPEA in DMF (10 mM) to yield the side-chain protected peptide **14b**. Global deprotection and resin cleavage provided crude brevicidine (**2**) (ca. 54% purity, Figure S9), and gratifyingly, complete consumption of the starting material was observed for the cyclization step. However, byproducts resulting from a common residual contamination of the PyBOP reagent with pyrrolidine[51] were frequently observed and may have consequently reduced the overall yield. A purification by RP-HPLC yielded brevicidine (**2**) in an acceptable overall yield (10%, based on the initial resin loading capacity, ca. greater than 96% purity, Figure S10).

^{1}H and ^{13}C NMR, MS/MS, and HRMS analyses of synthetic laterocidine (**1**) and brevicidine (**2**) were performed. Pleasingly, the spectroscopic data of the synthetic compounds were in good agreement with those of the natural compounds reported by Li et al.[40] (Tables S2 & S3, Figures S11−S21), thus confirming the correct structural assignment and total syntheses of laterocidine (**1**) and brevicidine (**2**). As the configuration of the lipid tail of brevicidine was not resolved by Li et al., the commercially available racemic (*R,S*)-4-methylhexanoyl group was installed in the synthetic material. The resulting epimer mixture was not separable by achiral RP-HPLC (Figure S10). However, a subsequent biological evaluation of synthetic (**2**) did not reveal any difference in potency compared to the putative configurationally pure natural compound (vide infra).

Synthetic laterocidine (**1**) and brevicidine (**2**) were tested for antimicrobial activity against the Gram-positive pathogen *S. aureus* and Gram-negative pathogen *E. coli* by a minimum inhibitory concentration (MIC) assay (Table 1). Toward *E. coli* (ATCC 25922), the synthetic laterocidine (**1**) and brevicidine (**2**) displayed a potent antimicrobial activity (0.25 μg/mL and 2 μg/mL, respectively), in good agreement with the report by Li et al.,[40] who assayed the same strain. Although a different strain of *S. aureus* was used in the current study, little or no activity was observed toward this Gram-positive pathogen (>64 μg/mL), also in agreement with the original report.[40] As such, the potency of the synthetic laterocidine (**1**) was eightfold greater toward *E. coli* (ATCC 25922) than was reported for the isolated compound, while the synthetic brevicidine (**2**) was equally potent to the isolated compound. The observed increase in potency for synthetic laterocidine (**1**) is likely a result of the testing conditions. Whereas Li et al.[40] performed their MIC of the isolated material in tissue culture-treated plates, which are known to bind strongly to cationic AMPs (thus reducing their observed potency), our assays were instead performed in polypropylene plates.[52] Furthermore, our assay conditions also employed cation-adjusted Mueller-Hinton broth (MHB), which can antagonize the activity of some cationic AMPs.[53] Pleasingly, however, both compounds retained potent activity under physiologically relevant divalent cation concentrations, although the MIC of brevicidine (**2**) did not improve despite the use of polypropylene plates, possibly suggesting laterocidine (**1**) to possess greater salt resistance. Alternatively, the

Table 1. MIC Assay of Laterocidine (1) and Brevicidine (2)

	MIC (μg/mL)		Lit. MIC (μg/mL)[40]	
compound	*E. coli*(ATCC 25922)	*S. aureus* (ATCC 29213)	*E. coli*(ATCC 25922)	*S. aureus* MRSA
Brevicidine (synthetic)	2	>64		
Brevicidine (isolated)[40]			2	>64
Laterocidine (synthetic)	0.25	>64		
Laterocidine (isolated)[40]			2	>64
Amoxicillin	6	3		
Polymyxin B	0.3	>64	1	32

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absence of improvement in the activity of brevicidine might be the consequence of the presence of two epimers in the synthetic sample, resulting from the use of racemic (*R,S*)-4-methylhexanoic acid for the installation of the lipid tail. Assuming that the antimicrobial activity is entirely attributed to one isomer, the measured MIC for an equimolar mixture would be increased twofold. However, the lipid configuration in brevicidine was not resolved in the original report, and such a drastic effect on the antimicrobial activity is purely hypothetical.

CONCLUSIONS

In summary, we have successsfully executed the first total syntheses[54] of the cationic antimicrobial peptides laterocidine (**1**) and brevicidine (**2**), using a solid-phase synthesis of the linear lipodepsipeptide and a solution-phase macrolactamization. The crucial depsipeptide bond in the macrolactone ring of laterocidine (**1**) and brevicidine (**2**) was established by an on-resin esterification between the side chain of Thr^9 with Alloc-Gly-OH or Alloc-Ser(*t*Bu)−OH, respectively. Anchoring the peptides onto the hyper acid-labile 2-chlorotrityl chloride resin enabled an off-resin solution-phase macrocyclization to be performed. A biological evaluation of laterocidine (**1**) and brevicidine (**2**) confirmed their potent activity against a Gram-negative bacterium, while little to no activity was observed toward the tested Gram-positive bacterium. Given their potent and selective activity toward Gram-negative pathogens, these lipopeptides are promising candidates for further research and development, and the preparation of analogues of laterocidine (**1**) and brevicidine (**2**) to establish structure−activity-relationships (SARs) is currently underway and will be reported in due course.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with an Autopol IV automatic polarimeter using the sodium-D line (589 nm), with the concentration measured in grams per 100 mL. UV irradiation was performed using a hand-held Spectroline UV lamp at a peak wavelength of 365 nm. IR spectra were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer using a diamond attenuated total reflectance (ATR) sampling accessory. NMR experiments were recorded at rt in $CDCl_3$ or deuterated dimethyl sulfoxide (DMSO-d_6) on either a Bruker AVANCE 400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei or a Bruker AVANCE 500 spectrometer operating at 500 MHz for ^{1}H nuclei and 125 MHz for ^{13}C nuclei. Chemical shifts were reported on the δ scale from tetramethylsilane (TMS) relative to the solvent in which the sample was analyzed ($CDCl_3$: δ_H 0.00 (TMS), δ_C 77.16; DMSO-d_6: δ_H 2.50, δ_C 39.52). Structural assignments were achieved with the aid of correlated spectroscopy (COSY) and heteronuclear single quantum correlation (HSQC).

High-resolution mass spectra (HRMS) were obtained using a Bruker micrOTOF-QII mass spectrometer. Tandem mass spectrometry (MS/MS) was recorded on a Waters QSTAR XL Quadrupole-Time-of-Flight instrument with capillary scale RP-HPLC columns for chromatographic separations.

Analytical thin-layer chromatography (TLC) was performed on Kieselgel F254 200 μm (Merck) silica plates and visualized using UV or by staining with potassium permanganate or vanillin followed by a heating of the plate. Column chromatography was performed using Grace Davison Discovery Sciences, Davisil LC60A 40−63 Micron Chromatographic Silica Media, with the indicated eluent.

Analytical liquid chromatography with tandem mass-spectrometry (LC-MS) was performed on an Agilent Technologies 1260 infinity LC connected to an Agilent Technologies 6120 quadrupole MSD spectrophotometer. Data processing was performed on OpenLAB software. An Agilent Zorbax 300SB-C3; 3.0 × 150 mm; 3.5 μm (0.3 mL/min) column and a linear gradient was used as specified. For the LC-MS analysis, solvent A was H_2O containing 0.1% formic acid, and solvent B was MeCN containing 0.1% formic acid.

For purifications, semipreparative RP-HPLC was performed on a Dionex UltiMate 3000 with a Phenomenex Gemini C18 110 Å, 10 × 250 mm, 5 μm column at a flow rate of 4 mL/min. A gradient was used as specified, where solvent A was 0.1% TFA in H_2O, and B was 0.1% TFA in MeCN and using UV−vis detection at 210 nm. Gradient systems used for semipreparative RP-HPLC were altered according to the elution time and peak profiles obtained from the analytical RP-HPLC chromatograms. Analytical RP-HPLC was performed on a Dionex UltiMate 3000 with Phenomenex Gemini C18 110 Å, 4.6 × 150 mm, 5 μm (1 mL/min) as the column and using a linear gradient as specified, where solvent A was 0.1% TFA in H_2O and B was 0.1% TFA in MeCN.

All reagents and solvents for peptide synthesis and RP-HPLC were purchased as synthesis and HPLC grade, respectively, and used without further purification. Ethyl acetate (EtOAc) and petroleum ether (PET) were purchased from Burdick & Jackson. 2-Chlorotrityl chloride (2-CTC) polystyrene resin, Fmoc-Ile-OH, Fmoc-Thr-OH, Fmoc-Trp-OH, Fmoc-Trp(Boc)−OH, Fmoc-Asn(Trt)−OH, Fmoc-Gly-OH, and Fmoc-D-Tyr(*t*Bu)−OH were purchased from Chempep Inc. Benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), Fmoc-D-Trp(Boc)−OH, Fmoc-D-Orn(Boc)−OH, and Fmoc-D-Asn(Trt)−OH were purchased from Aapptec. Fmoc-Orn(Boc)−OH, Fmoc-D-Ser(tBu)−OH, isononanoic acid, tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$), and hexafluoroisopropanol (HFIP) were purchased from AK Scientific. CH_2Cl_2, sodium hydroxide (NaOH) (AR grade), hydrochloric acid (HCl), calcium chloride anhydrous (LR), magnesium chloride (AR), and anhydrous sodium sulfate (Na_2SO_4) were purchased from ECP Limited. 4-Dimethylaminopyridine (DMAP) was purchased from Novabiochem. H-Ser(*t*Bu)-OMe·HCl, 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), *N,N*-diisopropylethylamine (DIPEA), piperidine, *N,N*′-diisopropylcarbodiimide (DIC), triisopropylsilane (TIPS), phenylsilane, sodium carbonate (Na_2CO_3), (*R,S*)-4-methylhexanoic acid, allyl chloroformate, and sodium diethyldithiocarbamate trihydrate were purchased from Sigma-Aldrich. Trifluoroacetic acid was purchased from Oakwood Chemicals. Octanoic acid was purchased from Honeywell Fluka. Formic acid, tetrahydrofuran (THF), and diethyl ether (Et_2O) were purchased from Macron Fine Chemicals. $CDCl_3$ and DMSO-d_6 were purchased from Cambridge Isotopes. Milli-Q high-purity deionized water (MQ H_2O) was available from a Sartorius Arium Pro Ultrapure Water System from Sartorius Stedim Biotech. DMF (synthesis grade), MeCN (HPLC grade), and Oxoid Mueller Hinton Broth (MHB) were purchased from Thermo Fisher Scientific. Polypropylene 96-well flat bottom plates were purchased from Greiner Bio-One.

General Methods for Peptide Synthesis. Peptides were synthesized manually by a standard 9-fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis (SPPS) and solution-phase peptide synthesis at rt.

General Method 1: Loading of the First Amino Acid to the 2-CTC Resin. Polystyrene 2-CTC resin (118 mg, 0.85 mmol·g^{-1}, 0.1 mmol) was swollen in CH_2Cl_2 (5 mL) for 10 min. The solvent was drained, and a solution of Fmoc-Gly-OH (89.2 mg, 0.3 mmol) and DIPEA (105 μL, 0.6 mmol) in CH_2Cl_2/DMF (1:1, v/v) was added. The resin was agitated for 4 h at rt, after which time unreacted sites were capped by an addition of a mixture of CH_2Cl_2, MeOH, and DIPEA (17:2:1, v/v/v) and agitation for 20 min at rt. The resin was filtered and washed with CH_2Cl_2 (3 × 5 mL) and DMF (3 × 5 mL). A sample of dried resin (~2 mg) was treated with a solution of 20% piperidine in DMF (v/v, 1 mL) and agitated at rt for 10 min. After centrifugation, 200 μL of the supernatant was diluted with 1.8 mL of DMF, and the absorbance of dibenzofulvene was measured at 290 nm to determine the experimental loading.

General Method 2: Removal of N^{α}-Fmoc Protecting Group. To the swelled N^{α}-Fmoc-protected peptidyl resin was added a

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solution of 20% piperidine in DMF (v/v, 3 mL) followed by agitation for 2 × 5 min at rt. The resin was filtered, drained, and washed with DMF (3 × 5 mL). After the incorporation of the ester bond (**General method 4**), 5% formic acid was added to 20% piperidine in DMF, (v/v/v, 3 mL), and the reaction time was modified to 2 × 1 min at rt.

General Method 3: Coupling of Amino Acids. The N^{α}-Fmoc amino acid (0.4 mmol) and HCTU (157 mg, 0.38 mmol) were dissolved in DMF (3 mL), DIPEA (174 μL, 1 mmol) was added, and the solution was transferred to the swelled peptidyl resin (0.1 mmol). Coupling was performed for 20 min at rt before the resin was washed with DMF (3 × 5 mL). The reaction completion was monitored by the Kaiser test.[55]

General Method 4: On-Resin Esterification. To the swelled peptidyl resin (0.1 mmol) was added a solution of N^{α}-Alloc-protected amino acid (0.3 mmol), DIC (40 μL, 0.3 mmol), and DMAP (1.2 mg, 0.01 mmol) in DMF (3 mL). The reaction was performed for 1 h at rt, and the resin was washed with DMF (3 × 5 mL). The reaction completion was monitored by RP-HPLC/MS analysis on a small sample of cleaved peptide.

General Method 5: Coupling of The Lipid Tail. To the peptidyl resin (0.1 mmol) was added a solution of fatty acid (0.15 mmol), HATU (46 mg, 0.12 mmol), and DIPEA (105 μL, 0.6 mmol) in DMF (3 mL). The resin was agitated for 1 h at rt and drained before being washed with DMF (3 × 5 mL). The coupling completion was confirmed by the Kaiser test.[55]

General Method 6: Removal of Allyl Ester and Allyloxycarbonyl (Alloc) Protecting Groups. The peptidyl resin (0.1 mmol) was swelled in CH_2Cl_2 before the addition of a solution of $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) and $PhSiH_3$ (123 μL, 1 mmol) in CH_2Cl_2 (3 mL). The resin was shaken at rt for 3 h and filtered before being washed with CH_2Cl_2 (3 × 5 mL), a solution of 0.5% sodium diethyldithiocarbamate in DMF (m/v, 5 mL, 3 × 1 min), and DMF (3 × 5 mL).

General Method 7: Cleavage. The resin-bound peptide was washed with DMF (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL) before it was dried in vacuo. A solution of 20% HFIP in CH_2Cl_2 (v/v, 10 mL) was added, and cleavage was performed for 1 h at rt. After the filtration, the resin was washed with CH_2Cl_2 (3 × 5 mL), and the combined filtrates were concentrated under a stream of N_2. The crude protected peptide was dissolved in *tert*-butanol (10 mL) and lyophilized.

General Method 8: Global Deprotection. The cyclized peptide was treated with a solution of TFA/H_2O/TIPS (95:2.5:2.5, v/v/v, 10 mL) for 1 h at rt and concentrated under a stream of N_2. After a precipitation by the addition of cold Et_2O (45 mL), the crude peptide was recovered by centrifugation and washed with cold Et_2O (2 × 45 mL) before lyophilization.

General Method 9: Resin Mini Cleavage. The peptidyl resin (1−5 mg) was treated with a solution of TFA/H_2O/TIPS (95:2.5:2.5, v/v/v, 1 mL) for 30 min at rt and concentrated under a stream of N_2. After a precipitation by the addition of cold Et_2O (10 mL), the crude peptide was recovered by centrifugation and washed with cold Et_2O (2 × 10 mL) before lyophilization. The resulting peptide was obtained as a white powder and used to analyze the progress of on-resin reactions.

Synthesis of Laterocidine (1). Fmoc-Gly-OH (89 mg, 0.3 mmol) was loaded onto 2-chlorotrityl chloride polystyrene resin (118 mg, 0.85 mmol.g^{-1}, 0.1 mmol) following **General method 1**. The loading efficiency was 89% as determined by Fmoc release.[56] The removal of the N^{α}-Fmoc protecting group was performed using **General method 2**. A solid-phase peptide synthesis was performed by coupling Fmoc-Asn(Trt)−OH, Fmoc-Ile-OH, Fmoc-Thr-OH, and Fmoc-Trp(Boc)−OH following **General method 3**. Alloc-Gly-OH (48 mg, 0.3 mmol) was coupled to the Thr side-chain hydroxyl group using **General method 4** and repeated twice. Subsequent Fmoc-removals were performed using 5% formic acid and 20% piperidine in DMF (v/v/v) as described in **General method 2**. A peptide elongation was continued using Fmoc-D-Orn(Boc)−OH, Fmoc-Gly-OH, Fmoc-Orn(Boc)−OH, Fmoc-D-Orn(Boc)−OH, Fmoc-D-Trp-(Boc)−OH, Fmoc-D-Tyr(tBu)−OH, and Fmoc-D-Ser(tBu)−OH. Isononanoic acid (26 μL, 0.15 mmol) was coupled to the N-terminal amino group following **General method 5**. The removal of the Alloc protecting group was performed by **General method 6**. The protected peptide was cleaved from the resin using **General method 7** to afford the crude product **13a** as an amorphous off-white solid (152 mg, 76% based on resin loading). The crude side-chain protected peptide **13a** (152 mg, 0.068 mmol) was dissolved in DMF (6.8 mL) together with BOP (150 mg, 0.34 mmol), HOAt (58 mg, 0.34 mmol), and DIPEA (118 μL, 0.68 mmol) to reach a final peptide concentration of 10 mM. The reaction mixture was shaken at room temperature for 5 h. The crude cyclized peptide was precipitated by an addition of H_2O (40 mL) and recovered by centrifugation. The resulting material underwent a global deprotection using **General method 8**. Crude laterocidine (**1**) was purified by semipreparative RP-HPLC using Dionex UltiMate 3000 on a Phenomenex Gemini C18 110 Å, 10 × 250 mm, 5 μm column using a gradient of 15−65% B at 1% B·min^{-1} over 40 min, 4 mL/min at rt. Fractions were collected and analyzed by electrospray ionization mass spectrometry (ESI-MS) and analytical RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilized to afford **1** as a white amorphous solid (59.6 mg, 36% overall yield based on initial 0.1 mmol scale), >98% purity); t_R 29.9 min; **NMR**, Table S2; **HRMS (ESI+)** *m*/*z* 535.6249 (calcd for $[C_{78}H_{113}N_{19}O_{18}+3H]^{3+}$, 535.6243).; $[\alpha]_D^{20.5}$ −14.9 (*c* 0.067, MeOH) (a literature value was not reported).

Synthesis of Brevicidine (2). Fmoc-Gly-OH (89 mg, 0.3 mmol) was loaded onto 2-chlorotrityl chloride polystyrene resin (112 mg, 0.89 mmol·g^{-1}, 0.1 mmol) following **General method 1**. The loading efficiency was 67% as determined by an Fmoc release.[56] The removal of the N^{α}-Fmoc protecting group was performed using **General method 2**. A solid-phase peptide synthesis was performed by coupling Fmoc-Ile-OH, Fmoc-Thr-OH, and Fmoc-Trp-OH following **General method 3**. Alloc-Ser(*t*Bu)−OH (73.5 mg, 0.3 mmol) was coupled to the Thr side-chain hydroxyl group using **General method 4**. Subsequent Fmoc-removals were performed using 5% formic acid and 20% piperidine in DMF (v/v/v) as described in **General method 2**. A peptide elongation was continued using Fmoc-D-Orn(Boc)−OH, Fmoc-Orn(Boc)−OH, Fmoc-D-Trp(Boc)−OH, Fmoc-D-Tyr(*t*Bu)−OH, and Fmoc-D-Asn(Trt)−OH. (*R*,*S*)-4-Methylhexanoic acid (26 μL, 0.15 mmol) was coupled to the N-terminal amino group following **General method 5**. The removal of the Alloc protecting group was performed by **General method 6**. The resin was then split into four portions, and the following steps were performed taking 0.017 mmol of the peptidyl resin. The protected peptide was cleaved from the resin using **General method 7** to afford the crude product **13b**, which was dissolved in DMF (1.7 mL) to achieve the final concentration of 10 mM. PyBOP (8.8 mg, 0.085 mmol) and DIPEA (14.8 μL, 0.085 mmol) were added to effect cyclization, and the reaction was stirred for 5 h at rt. To the crude reaction mixture, water was added such that, for every 1 mL of the reaction mixture, 39 mL of H_2O was added, and the resulting supernatant centrifuged. The water layer was decanted, and the obtained precipitate was redissolved in H_2O/MeCN (1:1) + 0.1% TFA and lyophilized. The resulting material underwent a global deprotection using **General method 8**. Crude brevicidine (**2**) was purified by semipreparative RP-HPLC using Dionex UltiMate 3000 on a Phenomenex Gemini C18 110 Å, 10 × 250 mm, 5 μm column (3 mL/min) using a gradient of 5−95% B at 3% B/min over 65 min, 4 mL/min at rt. Fractions were collected and analyzed by ESI-MS and analytical RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilized to afford **2** as a white solid (2.6 mg, 10% overall yield (based on resin split at 0.017 mmol scale), >96% purity); t_R 34.7 min; **NMR**, Table S3; **HRMS (ESI+)** *m*/*z* 760.4072 (calcd for $[C_{74}H_{106}N_{18}O_{17}+2H]^{2+}$, 760.4064); $[\alpha]_D^{20.1}$ + 6.9 (*c* 0.058, MeOH) (a literature value was not reported).

Minimum Inhibitory Concentration (MIC) Assay. *Staphylococcus aureus* ATCC 29213 and *E. coli* ATCC 25922 were grown in a cation-adjusted Mueller Hinton (MH) broth at 37 °C with shaking (200 rpm). MIC assays were performed in accordance with the CLSI recommended protocol.[57] Briefly, a twofold dilution series of the test compounds (from 64 to 0.25 μM, final) was prepared in triplicate in polypropylene 96-well plates, using cation-adjusted MH media. Cultures of bacteria grown for 8 h were diluted accordingly in

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fresh media before 50 μL of inoculum was added to each well of the MIC plate, to achieve a final volume of 100 μL with a uniform CFU/ml of ~5 × 10^5 in each well. A growth control (untreated) and sterility control (noninoculated) well was included for each test compound replicate. Plates were incubated at 37 °C with shaking for 18 h before the MIC was determined. MIC values were determined as the lowest concentration at which no growth was observed consistently across all three biological replicates of the assay and within triplicates of each test compound.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.1c00222.

Unsuccessful synthetic strategies toward laterocidine. Supplementary figures and schemes. Synthetic procedures and characterization of synthetic compounds. RP-HPLC profiles, spectroscopic data, and NMR assignments of synthetic laterocidine and brevicidine (PDF)

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Prestinaci, F.; Pezzotti, P.; Pantosti, A. *Pathog. Global Health* **2015**, *109* (7), 309−318.

(2) WHO. WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health https://www.who.int/news/item/30-04-2014-who-s-first-global-report-on-antibiotic-resistance-reveals-serious-worldwide-threat-to-public-health (accessed 2020-10-29).

(3) New report calls for urgent action to avert antimicrobial resistance crisis https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis (accessed 2020-10-29).

(4) Toner, E.; Adalja, A.; Gronvall, G. K.; Cicero, A.; Inglesby, T. V. *Health Secur.* **2015**, *13* (3), 153−155.

(5) Chokshi, A.; Sifri, Z.; Cennimo, D.; Horng, H. *J. Glob. Infect. Dis.* **2019**, *11* (1), 36.

(6) Dadgostar, P. *Infect. Drug Resist.* **2019**, *12*, 3903−3910.

(7) Laxminarayan, R.; Duse, A.; Wattal, C.; Zaidi, A. K. M.; Wertheim, H. F. L.; Sumpradit, N.; Vlieghe, E.; Hara, G. L.; Gould, I. M.; Goossens, H.; Greko, C.; So, A. D.; Bigdeli, M.; Tomson, G.; Woodhouse, W.; Ombaka, E.; Peralta, A. Q.; Qamar, F. N.; Mir, F.; Kariuki, S.; Bhutta, Z. A.; Coates, A.; Bergstrom, R.; Wright, G. D.; Brown, E. D.; Cars, O. *Lancet Infect. Dis.* **2013**, *13* (12), 1057−1098.

(8) Pushpanathan, M.; Gunasekaran, P.; Rajendhran, J. Antimicrobial Peptides: Versatile Biological Properties https://www.hindawi.com/journals/ijpep/2013/675391/ DOI: 10.1155/2013/675391 (accessed Apr 27, 2020).

(9) Kruse, T.; Kristensen, H.-H. *Expert Rev. Anti-Infect. Ther.* **2008**, *6* (6), 887−895.

(10) Magana, M.; Pushpanathan, M.; Santos, A. L.; Leanse, L.; Fernandez, M.; Ioannidis, A.; Giulianotti, M. A.; Apidianakis, Y.; Bradfute, S.; Ferguson, A. L.; Cherkasov, A.; Seleem, M. N.; Pinilla, C.; de la Fuente-Nunez, C.; Lazaridis, T.; Dai, T.; Houghten, R. A.; Hancock, R. E. W.; Tegos, G. P. *Lancet Infect. Dis.* **2020**, *20* (9), e216−e230.

(11) Lei, J.; Sun, L.; Huang, S.; Zhu, C.; Li, P.; He, J.; Mackey, V.; Coy, D. H.; He, Q. *Am. J. Transl. Res.* **2019**, *11* (7), 3919−3931.

(12) Hancock, R. E. *Expert Opin. Invest. Drugs* **2000**, *9* (8), 1723−1729.

(13) Nguyen, L. T.; Haney, E. F.; Vogel, H. J. *Trends Biotechnol.* **2011**, *29* (9), 464−472.

(14) Yeaman, M. R.; Yount, N. Y. *Pharmacol. Rev.* **2003**, *55* (1), 27−55.

(15) Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schaberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K. *Nature* **2015**, *517* (7535), 455−459.

(16) Yim, V. V.; Cameron, A. J.; Kavianinia, I.; Harris, P. W. R.; Brimble, M. A. Thiol-Ene Enabled Chemical Synthesis of Truncated S-Lipidated Teixobactin Analogs. *Front. Chem.* **2020**, *8*. DOI: 10.3389/fchem.2020.00568

(17) Schumacher, C. E.; Harris, P. W. R.; Ding, X.-B.; Krause, B.; Wright, T. H.; Cook, G. M.; Furkert, D. P.; Brimble, M. A. *Org. Biomol. Chem.* **2017**, *15* (41), 8755−8760.

(18) Jin, K.; Sam, I. H.; Po, K. H. L.; Lin, D.; Ghazvini Zadeh, E. H.; Chen, S.; Yuan, Y.; Li, X. *Nat. Commun.* **2016**, *7* (1), 12394.

(19) Gunjal, V. B.; Reddy, D. S. *Tetrahedron Lett.* **2019**, *60* (29), 1909−1912.

(20) Giltrap, A. M.; Dowman, L. J.; Nagalingam, G.; Ochoa, J. L.; Linington, R. G.; Britton, W. J.; Payne, R. J. *Org. Lett.* **2016**, *18* (11), 2788−2791.

(21) Yang, H.; Chen, K. H.; Nowick, J. S. *ACS Chem. Biol.* **2016**, *11* (7), 1823−1826.

(22) Yang, S.-H.; Hermant, Y. O. J.; Harris, P. W. R.; Brimble, M. A. *Eur. J. Org. Chem.* **2020**, *2020* (8), 944−947.

(23) Jad, Y. E.; Acosta, G. A.; Naicker, T.; Ramtahal, M.; El-Faham, A.; Govender, T.; Kruger, H. G.; de la Torre, B. G.; Albericio, F. *Org. Lett.* **2015**, *17* (24), 6182−6185.
(24) Jahanshah, G.; Yan, Q.; Gerhardt, H.; Pataj, Z.; Lämmerhofer, M.; Pianet, I.; Josten, M.; Sahl, H.-G.; Silby, M. W.; Loper, J. E.; Gross, H. *J. Nat. Prod.* **2019**, *82* (2), 301−308.
(25) Loper, J. E.; Hassan, K. A.; Mavrodi, D. V.; Davis, E. W.; Lim, C. K.; Shaffer, B. T.; Elbourne, L. D. H.; Stockwell, V. O.; Hartney, S. L.; Breakwell, K.; Henkels, M. D.; Tetu, S. G.; Rangel, L. I.; Kidarsa, T. A.; Wilson, N. L.; van de Mortel, J. E.; Song, C.; Blumhagen, R.; Radune, D.; Hostetler, J. B.; Brinkac, L. M.; Durkin, A. S.; Kluepfel, D. A.; Wechter, W. P.; Anderson, A. J.; Kim, Y. C.; Pierson, L. S.; Pierson, E. A.; Lindow, S. E.; Kobayashi, D. Y.; Raaijmakers, J. M.; Weller, D. M.; Thomashow, L. S.; Allen, A. E.; Paulsen, I. T. *PLoS Genet.* **2012**, *8* (7), e1002784.
(26) Tajbakhsh, M.; Karimi, A.; Fallah, F.; Akhavan, M. M. *Cell. Mol. Biol.* **2017**, *63* (10), 20−32.
(27) Payne, J. A. E.; Schoppet, M.; Hansen, M. H.; Cryle, M. J. *Mol. BioSyst.* **2017**, *13* (1), 9−22.
(28) Hancock, R. E. W.; Lehrer, R. *Trends Biotechnol.* **1998**, *16* (2), 82−88.
(29) Hancock, R.; Rozek, A. *FEMS Microbiol. Lett.* **2002**, *206*, 143.
(30) Bahar, A. A.; Ren, D. *Pharmaceuticals* **2013**, *6* (12), 1543−1575.
(31) Straus, S. K.; Hancock, R. E. W. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758* (9), 1215−1223.
(32) Le, C.-F.; Fang, C.-M.; Sekaran, S. D. Intracellular Targeting Mechanisms by Antimicrobial Peptides. *Antimicrob. Agents Chemother.* **2017**, *61* (4). DOI: 10.1128/AAC.02340-16
(33) Powers, J. H. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **2004**, *4*, 23−31.
(34) Kragol, G.; Lovas, S.; Varadi, G.; Condie, B. A.; Hoffmann, R.; Otvos, L. *Biochemistry* **2001**, *40* (10), 3016−3026.
(35) Patrzykat, A.; Friedrich, C. L.; Zhang, L.; Mendoza, V.; Hancock, R. E. W. *Antimicrob. Agents Chemother.* **2002**, *46* (3), 605−614.
(36) Wenzel, M.; Rautenbach, M.; Vosloo, J. A.; Siersma, T.; Aisenbrey, C. H. M.; Zaitseva, E.; Laubscher, W. E.; van Rensburg, W.; Behrends, J. C.; Bechinger, B.; Hamoen, L. W. The Multifaceted Antibacterial Mechanisms of the Pioneering Peptide Antibiotics Tyrocidine and Gramicidin S. *mBio* **2018**, *9* (5). DOI: 10.1128/mBio.00802-18
(37) Tally, F. P.; DeBruin, M. F. *J. Antimicrob. Chemother.* **2000**, *46* (4), 523−526.
(38) Shatri, G.; Tadi, P. Polymyxin. In *StatPearls;* StatPearls Publishing: Treasure Island, FL, 2020.
(39) Mogi, T.; Kita, K. *Cell. Mol. Life Sci.* **2009**, *66* (23), 3821.
(40) Li, Y.-X.; Zhong, Z.; Zhang, W.-P.; Qian, P.-Y. *Nat. Commun.* **2018**, *9* (1), 1−9.
(41) Medema, M. H.; Blin, K.; Cimermancic, P.; de Jager, V.; Zakrzewski, P.; Fischbach, M. A.; Weber, T.; Takano, E.; Breitling, R. *Nucleic Acids Res.* **2011**, *39*, W339−346.
(42) Zhao, X.; Li, Z.; Kuipers, O. P. *Cell Chem. Biol.* **2020**, *27* (10), 1262−1271e4.
(43) Pelay-Gimeno, M.; Albericio, F.; Tulla-Puche, J. *Nat. Protoc.* **2016**, *11* (10), 1924−1947.
(44) Kjeldsen, F.; Zubarev, R. A. *J. Am. Soc. Mass Spectrom.* **2011**, *22* (8), 1441−1452.
(45) Choudhary, A.; Raines, R. T. *ChemBioChem* **2011**, *12* (12), 1801−1807.
(46) Alcaro, M. C.; Sabatino, G.; Uziel, J.; Chelli, M.; Ginanneschi, M.; Rovero, P.; Papini, A. M. *J. Pept. Sci.* **2004**, *10* (4), 218−228.
(47) Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2002**, *4* (19), 3219−3221.
(48) Davies, J. S. *J. Pept. Sci.* **2003**, *9* (8), 471−501.
(49) Xu, B.; Hermant, Y.; Yang, S.-H.; Harris, P. W. R.; Brimble, M. A. *Chem. - Eur. J.* **2019**, *25* (62), 14101−14107.
(50) Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31* (2), 205−208.
(51) Alsina, J.; Barany, G.; Albericio, F.; Kates, S. A. *Lett. Pept. Sci.* **1999**, *6* (4), 243−245.
(52) Wiegand, I.; Hilpert, K.; Hancock, R. E. W. *Nat. Protoc.* **2008**, *3* (2), 163−175.
(53) Bowdish, D. M. E.; Hancock, D. J. D. and R. E. W. A Re-evaluation of the Role of Host Defence Peptides in Mammalian Immunity https://www.eurekaselect.com/79060/article DOI: 10.2174/1389203053027494 (accessed 2021-03-01).
(54) Al-Ayed, K.; Ballantine, R. D.; Zhong, Z.; Li, Y.; Cochrane, S.; Martin, N. Total Synthesis of the Brevicidine and Laterocidine Family of Lipopeptide Antibiotics. *ChemRxiv*. Cambridge, Cambridge Open Engage; 2021; DOI: 10.26434/chemrxiv.13660949.v1.
(55) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34* (2), 595−598.
(56) Eissler, S.; Kley, M.; Bächle, D.; Loidl, G.; Meier, T.; Samson, D. *J. Pept. Sci.* **2017**, *23* (10), 757−762.
(57) M100Ed30 | Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition https://clsi.org/standards/products/microbiology/documents/m100/ (accessed 2020-12-20).

https://doi.org/10.1021/acs.jnatprod.1c00222
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