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Semisynthetic Lipopeptides Derived from Nisin Display Antibacterial Activity and Lipid II Binding on Par with That of the Parent Compound

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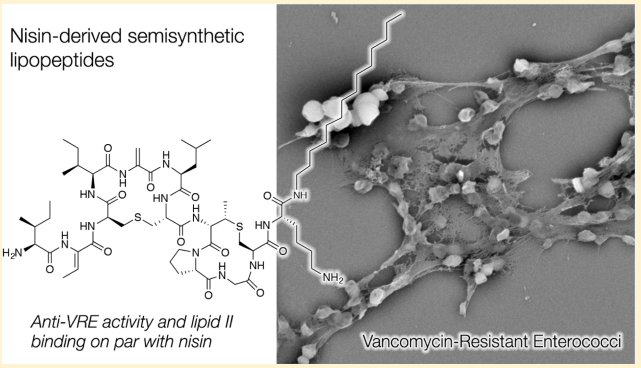
Supporting Information

ABSTRACT: The lipid II-binding N-terminus of nisin, comprising the so-called A/B ring system, was synthetically modified to provide antibacterially active and proteolytically stable derivatives. A variety of lipids were coupled to the C-terminus of the nisin A/B ring system to generate semisynthetic constructs that display potent inhibition of bacterial growth, with activities approaching that of nisin itself. Most notable was the activity observed against clinically relevant bacterial strains including MRSA and VRE. Experiments with membrane models indicate that these constructs operate via a lipid II-mediated mode of action without causing pore formation. A lipid II-dependent mechanism of action is further supported by antagonization assays wherein the addition of lipid II was found to effectively block the antibacterial activity of the nisin-derived lipopeptides.



INTRODUCTION

The increasing rates of morbidity and mortality caused by antibiotic-resistant bacteria present a growing threat to world health.[1] Recent projections suggest that, in the absence of dedicated intervention strategies, annual global deaths due to drug-resistant infections will reach 10 million by the year 2050.[2] In this light, there can be no mistaking the urgent need for new classes of antibiotics that operate via previously unexploited modes of action.[3,4] With high-throughput screening campaigns resulting in limited success,[5] the search for new antibiotics has shifted focus back to natural products and other compound classes that lie outside of the typical small molecule range. Bacterially produced antimicrobial peptides are one such class, encompassing clinically important "antibiotics of last resort" such as vancomycin and daptomycin. The challenges of modern antibiotic discovery have also given rise to new screening technologies[6,7] and the application of semisynthesis[8−13] as a means of identifying new agents capable of countering drug resistance. While penicillin and its many semisynthetic derivatives commenced and have largely sustained the antibiotic age, other naturally occurring antibacterial agents of similar vintage have not had the same clinical impact. One example is the lantibiotic nisin (Figure 1), which was described in the literature the same year that Fleming discovered penicillin.[14]

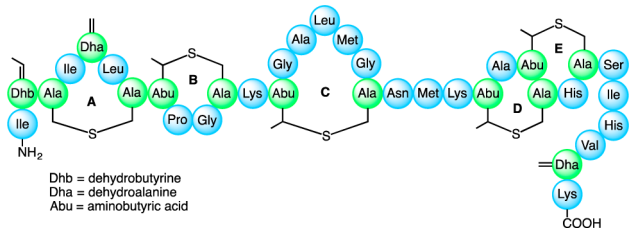


Figure 1. Nisin, the preeminent lantibiotic.

Produced by various strains of *Lactococcus lactis*, nisin is a potent antimicrobial and one of the most heavily studied members of the family of so-called lanthipeptides (lanthipeptides with antibacterial activity are commonly referred to as lantibiotics). Lanthipeptides are classified on the basis of the presences of lanthionine rings as well as a number of additional post-translational modifications.[15−18] The presence of five lanthionine rings (A−E) imparts a predefined conformation that is key for nisin's mode of action. Specifically, nisin's antibacterial activity stems from the ability of its N-terminal

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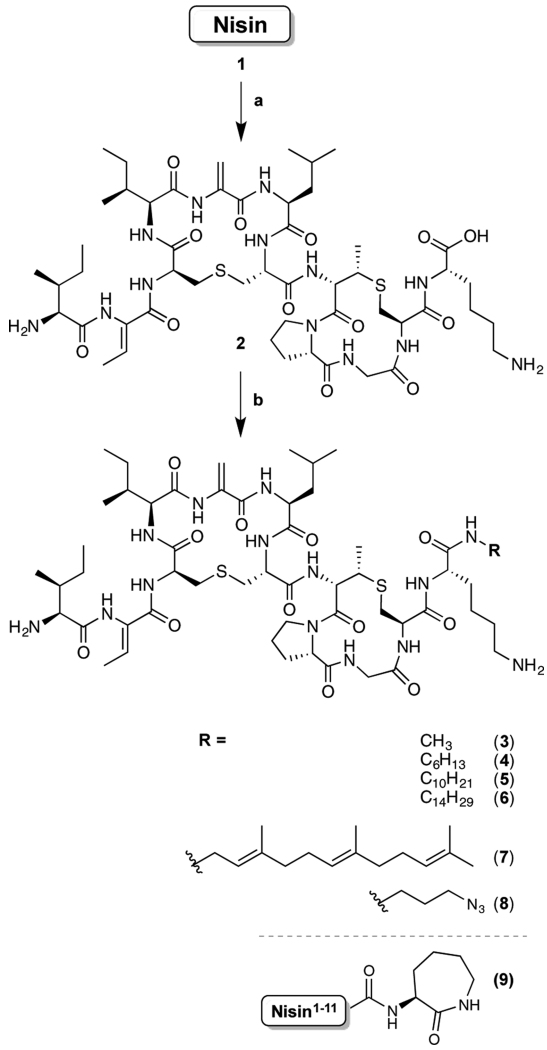
A/B ring system to bind lipid II, an essential cell wall precursor in bacteria. After lipid II binding on the outer surface of the cell, nisin's C-terminal region, containing rings C−E, then inserts into the bacterial membrane, leading to pore formation and bacterial cell death.[19−22] While it is not suited for use as a systemic antibiotic, nisin has been effectively applied as a safe food preservative for decades with little relevant resistance encountered.[23,24]

Nisin's unique mode of action and potent activity provide inspiration for the design of new antibiotics. However, nisin's peptidic nature presents certain drawbacks. Of particular limitation is its susceptibility to proteolytic degradation *in vivo*, toxicity and/or immunogenicity issues, and poor pharmacokinetics.[25] While chemical approaches have been applied toward the preparation of lantibiotics[26−33] and analogues with more desirable properties,[34−38] their complex structures make total synthesis challenging. An alternate strategy for producing lantibiotics with optimized properties is semisynthesis, whereby a naturally sourced compound is synthetically modified to yield the desired product. Most prominent in this regard is the recently reported C-terminal modification of deoxyactagardine B to yield NVB302, a lanthipeptide-based antibiotic with potent activity against *Clostridium difficile* and the first compound of its kind to enter clinical trials.[13] In the case of nisin, proteolytic degradation limits the possible therapeutic application of the full-length peptide. Roberts and co-workers previously demonstrated that nisin is cleaved at a number of sites by various proteases, leading, in all cases, to degradation products with diminished activity.[39] Of particular note was the finding that trypsinolysis of nisin effectively cleaves after Lys_{12} to yield a fragment containing the intact N-terminal A/B ring system that is stable to further proteolysis. While this fragment was found to be devoid of antimicrobial activity, it showed the ability to effectively antagonize the activity of full-length nisin, suggesting that it maintains an affinity for its bacterial biomolecular target, later identified as the pyrophosphate moiety of lipid II.[19,21] The presence of the protease-sensitive C-terminal region of nisin is required for full antimicrobial activity and is proposed to interact with bacterial membranes upon lipid II binding by the A/B ring moiety. With this in mind, we were prompted to investigate whether the C-terminus of nisin, comprising residues 13−34, could be substituted for a simple membrane-active lipid with the aim of maintaining the antimicrobial activity of the parent compound while imparting improved stability. In this article, we describe the optimized chemo-enzymatic degradation of nisin to generate the A/B ring fragment followed by C-terminal modification with a series of lipids. Among the semisynthetic analogues thus prepared, a subset of compounds was identified that possess antibiotic activity on par with that of nisin while displaying enhanced stability.

RESULTS AND DISCUSSION

Essential for the preparation of the semisynthetic nisin derivatives was access to suitable quantities of the nisin A/B ring fragment. We therefore optimized and scaled-up the previously reported trypsin digest procedure[39] to reliably yield multihundred milligram quantities of pure A/B ring fragment **2** following RP-HPLC purification (Scheme 1). Compound **2** was readily converted into lipidated constructs **3**−**8** by coupling with a large excess of the lipid-amine of choice in the presence of BOP/DIPEA for a short time, followed by immediate

Scheme 1. Nisin Digestion and Synthesis of Lipidated Analogues[a]



[a]Reagents and conditions: (a) trypsin, Tris·OAc buffer, pH 7.0, 30 °C, 48 h; (b) lipid-amine, BOP, DIPEA, DMF/THF, RT, 20 min.

isolation of the desired product by HPLC. Extended reaction times were found to lead to formation of unwanted adducts resulting from addition of the excess amine to the dehydro residues at positions 2 and 5. Failure to use an appropriate excess of lipid-amine (>50 equiv) invariably led to production of a side product, compound **9**, wherein the terminal lysine side chain amine cyclizes onto the C-terminal carboxylic acid. Compound **9** itself was produced, therefore, by simply treating compound **2** alone with BOP/DIPEA. A selection of straight-chain alkyl amines, as well as the unsaturated, branched farnesyl amine, was readily coupled to fragment **2** to yield compounds **3**−**7**. The known azide-modified construct **8**[40] was also prepared to provide a convenient handle for ligation to any number of alkyne-modified compounds via Cu(I)-catalyzed azide−alkyne click cycloaddition.[41,42] This made it possible to incorporate larger lipids, which, owing to solubility/miscibility limitations, could not be administered in the large excess required in the BOP/DIPEA coupling approach. Ligation of compound **8** with a single equivalent of the appropriate alkyne-modified lipid proceeded smoothly via the click reaction to yield compounds **10**−**15** (Scheme 2). The ligations were

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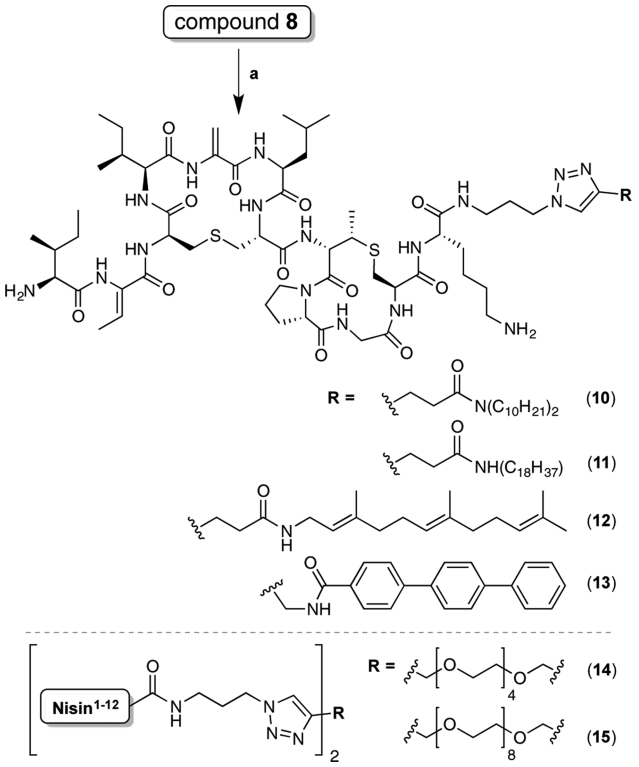
Scheme 2. Preparation of Triazole-Coupled Analogues[a]



[a]Reagents and conditions: (a) lipid-alkyne/glycol dialkyne, $CuSO_4$, sodium ascorbate, TBTA, DMF, microwave, 80 °C, 20 min.

carried out under microwave irradiation using TBTA as a ligand[43] to cleanly afford the expected products. Dimeric constructs **14** and **15** were prepared using bis-alkynes derived from tetraethylene glycol and octaethylene glycol, respectively. These dimeric analogues were specifically prepared in light of the proposed 2:1 stoichiometry of the nisin–lipid II complex.[44]

The antibacterial activities of compounds **2**–**15** were evaluated against a variety of Gram-positive and -negative organisms using an established broth microdilution assay to determine minimum inhibitory concentrations (MICs).[45] Nisin A/B fragment **2** was devoid of activity at the highest concentration tested (128 μg/mL), as was N-methyl amide analogue **3**. By comparison, many of the lipidated constructs did exhibit antibacterial activity (Supporting Information Table S1). Exceptions were compounds **10** and **11**, which, despite containing the largest lipid substituents, failed to show antibacterial effects at the highest concentrations tested. In addition, dimeric constructs **14** and **15** were found to be only weakly active (MIC > 16–32 μg/mL). Analogues **5**–**7**, containing the C_{10}, C_{14}, and farnesyl lipids, respectively, showed the most potent antibacterial activity. In this regard, it is interesting to consider the various clinically used lipopeptide antibiotics including telavancin, dalbavancin, and daptomycin that also contain lipids of similar size. Also of note is triazole-linked compound **13**, wherein incorporation of a terphenyl lipid produced an analogue with enhanced activity. Similar para-substituted polyaromatic lipids are also featured in the semisynthetic lipopeptide antibiotic oritavancin as well as the antifungal drug anidulafungin.

The enhanced antibacterial activities observed for constructs **5**–**7** and **13** prompted us to further evaluate their activity against clinically relevant and drug-resistant strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (Table 1). Compounds **5**–**7** and **13** effectively inhibited the growth of the MRSA and VRE strains tested with activities approaching that of nisin itself. In addition, these compounds were found to be bactericidal rather than bacteriostatic, effectively killing all strains tested at a concentration of ≤2× the MIC. The activity of **5**–**7** and **13** toward vancomycin-resistant enterococci is particularly noteworthy. The activity of these compounds against additional vancomycin-sensitive and -resistant enterococcus strains was therefore further investigated using a real-time BioScreen assay to monitor effects on *Enterococcus faecium* growth (Figure 2). In the BioScreen assays, each compound was administered at a fixed concentration of 5 μM. This corresponds to concentrations slightly below the MIC's measured for compounds **5**, **7**, and **13** and slightly above the MIC's measured for compound **6** and nisin against VRE E155 (Table 1). As seen in Figure 2, the differing effects of each compound on the bacterial growth curves correlate very well with the results of the MIC assay. When administered at a sub-MIC concentration of 5 μM, compound **5** (bearing the C_{10} lipid) has very little impact on the growth of the *E. faecium* strains tested. Likewise, compounds **7** and **13** (the farnesyl- and terphenyl-substituted analogues, respectively) also do not completely suppress cell growth at a 5 μM concentration. By comparison, the more active C_{14}-lipidated analogue **6** and nisin itself completely suppressed the growth of all strains tested when administered at a 5 μM concentration. To further establish the equivalency in anti-VRE activity of compound **6** and nisin, an extended panel of 30 different VRE strains was screened (Supporting Information Table S2). The MIC_{50} and MIC_{90} values thus obtained for compound **6** and nisin were the same (MIC_{50} = 4 μg/mL and MIC_{90} = 8 μg/mL).

Table 1. *In Vitro* Antibacterial Activity against Drug-Susceptible and -Resistant Gram-Positive Organisms[a]

compound	*B. subtilis*	*S. aureus*	MRSA USA300[b]	MRSA WKZ2[c]	VRE E155[d]
2	>128	>128	>128	>128	>128
5	4–8	16	16	16	8
6	4	16	64	32	4
7	4	8	16	16	8
13	4	8	8	8	8
nisin (**1**)	0.625	10	10	10	5
vancomycin	0.03–0.06	0.25–0.31	0.625	0.625	≥128

[a]MIC measured in μg/mL. All data stems from duplicate experiments. Where appropriate, values are represented as a range. [b]Community-associated methicillin-resistant *S. aureus*. [c]Methicillin-resistant *S. aureus* strain. Clinical isolate from Wilhemina Children's Hospital (Utrecht, NL). [d]Vancomycin-resistant *Enterococcus faecium* strain. Hospital outbreak isolate from Cook County Hospital (Chicago, IL, USA).

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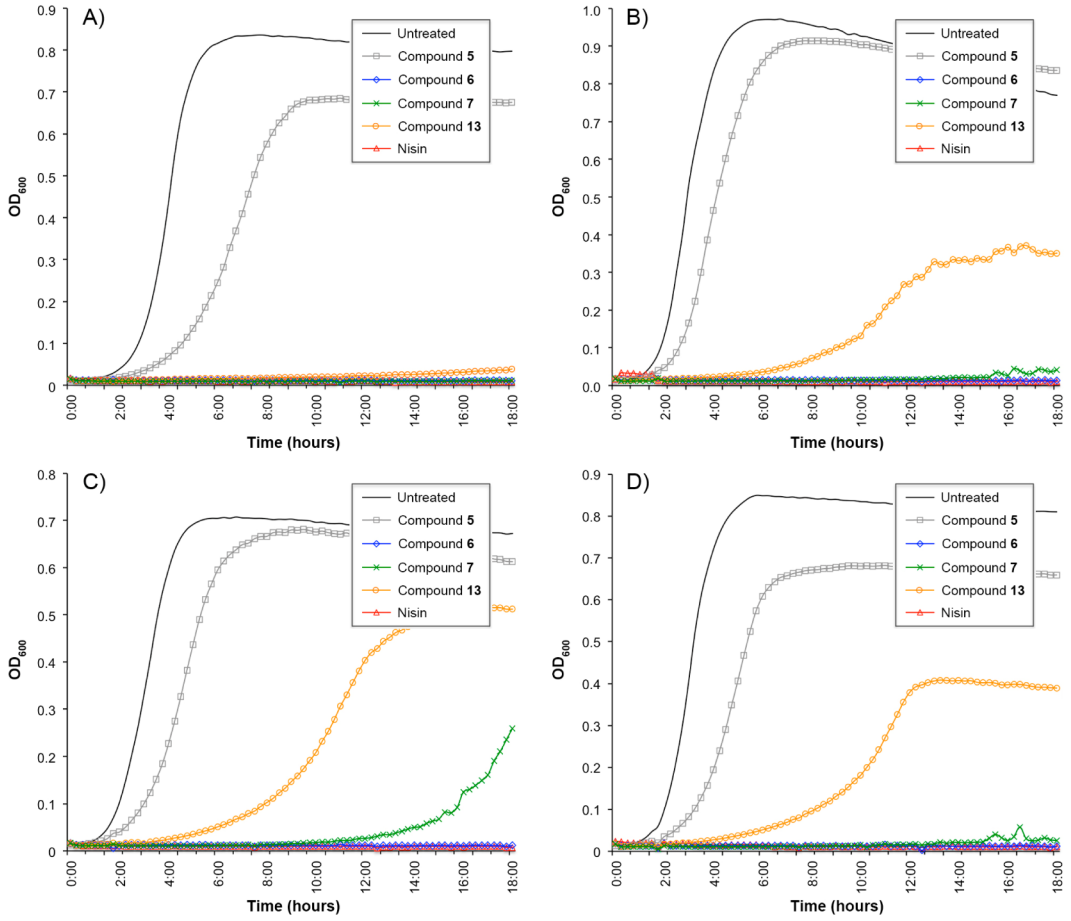

Figure 2. BioScreen growth curves for *E. faecium* strains grown in the absence or presence of 5 μM **5**−**7**, **13**, or nisin. (A) *E. faecium* E745 (vancomycin- and ampicillin-resistant hospital outbreak strain); (B) *E. faecium* E980 (vancomycin- and ampicillin-susceptible human commensal isolate); (C) *E. faecium* E1133 (vancomycin- and ampicillin-resistant hospital outbreak strain); (D) *E. faecium* E1162 (vancomycin-susceptible, ampicillin-resistant clinical isolate).

The effects of compound **6** and full-length nisin on the growth and cell morphology of VRE (strain E155) were examined using scanning electron microscopy (SEM; Figure 3). Treatment with both **6** and nisin leads to a dramatic phenotype compared to untreated VRE cultures. A notable reduction in the number of bacterial cells is observed along with a decrease in the size of the cell clusters observed. Most striking is the clear loss of structural integrity in cells exposed to either **6** or nisin with an indication of cell death via lysis. To further examine the membrane activity of the semisynthetic lipopeptides, a biophysical model of pore formation was employed. Specifically, the lipid II-mediated mode of action of compounds **5**−**7** and **13** was evaluated using a previously described dye-leakage assay.[38,46] In this assay, large unilamellar vesicles (LUVs) composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), spiked with 0.2% lipid II, are loaded with carboxyfluorescein (CF). As shown in Figure 4, treatment with nisin at a concentration of 5 nM results in leakage of ∼50% of the CF dye, as detected by fluorescence spectroscopy. By comparison, treatment with compounds **5**−**7** and **13** led to no detectable dye leakage (data not shown), suggesting that they have a distinctly different mechanism compared to that of nisin. A competition assay was next performed which demonstrated that each compound effectively antagonized nisin-induced membrane leakage when administered at a concentration 10-fold higher than nisin. Of particular note is the observation that compound **13** effectively inhibited dye leakage at equimolar concentrations, indicating an affinity for lipid II comparable to that of nisin.

The nisin A/B fragment has previously been described as an antagonist for nisin itself.[39] We therefore examined whether the A/B fragment was also capable of antagonizing the antibacterial activity of the nisin-derived lipopeptides, using compound **6** as a representative example. To perform this analysis, growth curve experiments similar to those illustrated in Figure 2 were performed for nisin and compound **6** using VRE E155 as an indicator strain. Working at a concentration slightly below the MIC value of nisin or compound **6** was necessary to be able to detect the impact of addition of the A/B fragment. For both nisin and compound **6**, addition of the A/B fragment was indeed found to antagonize antibacterial activity, as indicated by an increase in the rate of bacterial growth in response to addition of the A/B fragment at equimolar or greater concentrations (Supporting Information Figure S2). To further confirm that the activity of the nisin-derived lipopeptides is dependent on an interaction with lipid II, we examined the ability of lipid II and related lipids to antagonize the antibacterial activity of **5**−**7** and **13**. Again, using VRE E155 as an indicator strain, each lipopeptide was administered at a final concentration of 4× the MIC along with lipid II, lipid I, or C55-P (undecaprenyl monophosphate), which were added at 2-fold molar concentrations with respect to the concentration of

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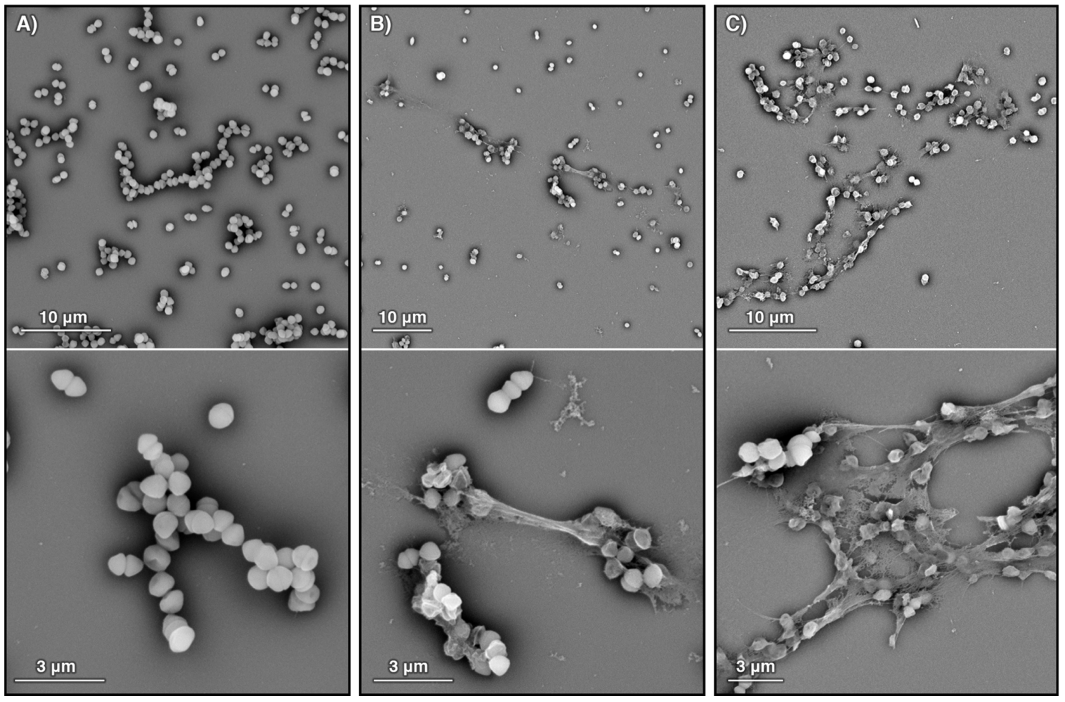

Figure 3. Structural changes in vancomycin-resistant *E. faecium* E155 upon treatment with nisin or compound **6**. Scanning electron micrographs of (A) untreated bacterial cultures scanned at 7200× (upper) and 24 000× (lower) magnification, (B) cultures treated with 5 μM nisin scanned at 4700× (upper) and 21 000× (lower) magnification, and (C) cultures treated with 5 μM **6** scanned at 6900× (upper) and 14 500× (lower) magnification.

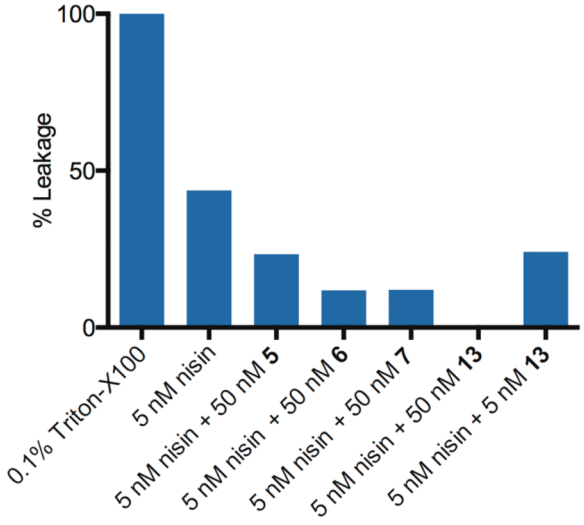

Figure 4. Inhibition of nisin-induced membrane leakage in large unilamellar vesicles (LUVs) containing lipid II. While compounds **5**–**7** and **13** do not cause dye leakage, each compound effectively antagonizes lipid II-mediated pore formation by nisin.

the antibiotic. In the presence of both lipid II and lipid I, the activity of all compounds was fully antagonized, whereas addition of C55-P had no effect on the antibiotic activity (Table 2).

Hemolysis assays were performed with compounds **5**–**7** and **13** to assess their lytic behavior toward human erythrocytes. At concentrations corresponding to the MIC of **5**–**7** and **13**, low hemolysis (1.7–6.9%) was detected after 1 h, with a slight increase in lysis (8.0–9.5%) after 16 h incubation (Supporting Information Figure S3). At concentrations corresponding to 2× MIC, the compounds exhibited levels of hemolysis ranging

Table 2. Antagonization of Antibiotic Activity by Bacterial Cell Wall Precursors Lipid II, Lipid I, and C55-P[a]

	result[b] with indicated antagonist		
antibiotic compound	lipid II	lipid I	C55-P
5	+	+	−
6	+	+	−
7	+	+	−
13	+	+	−
nisin (**1**)	+	+	−

[a]VRE E155 was incubated with a concentration corresponding to 4× the MIC for compounds **5**–**7**, **13**, and nisin (see Table 1); antagonists were added in 2-fold molar excess with respect to the concentration of antibiotics. [b]+, antibiotic activity antagonized; −, antibiotic activity unaffected.

from 1.3 to 10.9% after 1 h incubation, with a corresponding increase to 12.9–22.2% after 16 h. The stabilities of **5**–**7** and **13** in human serum were also investigated and compared with that of nisin. Notably, while only 33% of nisin remained intact after 24 h, compounds **5**–**7** and **13** showed enhanced stability, with up to 94% of the C_{10} species **5** remaining intact over the same time period (Supporting Information Figure S4).

CONCLUSIONS

In summary, the nisin A/B fragment, normally devoid of antibacterial activity, was synthetically modified to create antibacterially active and proteolytically stable derivatives. The approach here reported provides for a concise route to such compounds. Among the analogues prepared, compounds **5**–**7** and **13** possess the most potent antibacterial activity against drug-susceptible and -resistant strains of Gram-positive bacteria, including clinically relevant strains of MRSA and VRE. Taken together, their unique lipid II-mediated mode of action and

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superior stability compared to that of nisin, make these semisynthetic lipopeptides attractive candidates for further optimization and development as novel antibiotics.

EXPERIMENTAL SECTION

Full experimental details for the preparation of compounds **2**–**15**, and associated intermediates, are provided in the accompanying Supporting Information.

MIC Assays. Microorganisms to be tested were plated (from −80 °C glycerol stock) on blood agar and incubated at 37 °C for 24 h. Single colonies were selected and cultured in suspension for 16–20 h at 37 °C. Minimum inhibitory concentrations (MICs) were determined by broth microdilution according to CLSI guidelines.[45] The test medium used for all microorganisms was tryptic soy broth (TSB). DMSO was used to aid in solubilizing the compounds (1% final concentration). The overnight suspension cultures were diluted to 0.5×10^6 CFU/mL in TSB, transferred to test plates, and combined with the various antibiotic compounds (administered at 2-fold serially diluted concentration in each well). After incubation at 37 °C for 16–20 h, the MIC was assessed as the lowest concentration of antibiotic resulting in no visible growth. To determine the bactericidal concentration, 5 μL of each of the wells was plated on blood agar and incubated at 37 °C. The concentration at which no bacteria regrew was defined as the bactericidal concentration.

BioScreen Growth Assays with *E. faecium*. A BioScreen C instrument (Oy Growth Curves AB, Helsinki, Finland) was used to monitor effects of compounds **5**–**7**, **13**, and nisin on *E. faecium* growth. *E. faecium* strains were inoculated at an initial OD_{600} of 0.05 into 300 μL of TSB containing 1% DMSO and 1% glucose or into the same medium containing the antibiotic compounds at a final concentration of 5 μM. The cultures were incubated in the BioScreen C system at 37 °C with continuous shaking, and the absorbance at 600 nm (A_{600}) was recorded every 15 min for 15 h to determine growth/inhibitory effects. The nisin A/B antagonization experiments were performed using the same general growth assays described above with the following alterations: the antibiotic compound of interest was first mixed with 0, 1, 2, or 4 mol equiv of the nisin A/B fragment, after which it was added to the well plate followed by addition of the bacterial culture (VRE E155). Nisin was tested at 1 μM, and compound **6**, at 2 μM, corresponding to a concentration just below the MIC (Supporting Information Figure S2).

Scanning Electron Microscopy. *E. faecium* strains were fixed for 15 min with 1% (v/v) glutaraldehyde in phosphate buffered saline (PBS) at room temperature on poly-L-lysine covered glass slides (12 mm diameter). Samples were washed twice with PBS to remove excess fixative and were subsequently serially dehydrated by consecutive incubations in 1 mL of 25% (v/v) and 50% (v/v) ethanol–PBS, 75% (v/v) and 90% (v/v) ethanol–H_2O, and 100% ethanol (2×), followed by 50% ethanol–hexamethyldisilazane (HMDS) and 100% HMDS. The glass slides were removed from the 100% HMDS and air-dried overnight at room temperature. After overnight evaporation of HMDS, samples were mounted on 12 mm specimen stubs (12 mm, Agar Scientific) and bacteria were coated with gold to 1 nm using a Quorum Q150R sputter coater at 20 mA prior to examination with a Phenom PRO table-top scanning electron microscope (PhenomWorld).

Lipid II Binding in Model Membranes. Large unilamellar vesicles (LUVs) for carboxyfluorescein (CF) efflux were prepared as previously described.[46] The CF efflux was monitored by measuring the increase in fluorescence intensity at 515 nm, with excitation at 492 nm. In a cuvette, a solution (1 mL) of CF-loaded vesicles (20 μM final concentration) in buffer (50 mM Tris·HCl, pH 7.0, containing 100 mM NaCl) was prepared, the relevant final concentration of compounds **5**–**7** and **13** was added, and the mixture was stirred for 1 min, after which the fluorescence was recorded (A_0). After ∼10 s, nisin was added (5 nM final concentration), and the fluorescence was followed until it stabilized and then recorded (A_{stable}). Total membrane leakage was induced by the addition of Triton X-100 (final concentration 0.1%), and the fluorescence was recorded (A_{total}). The percentile values were calculated by $(A_{stable} - A_0)/(A_{total} - A_0) \times 100\%$

Antagonization Assays. VRE E155 was used as an indicator strain to assess the effect of lipid II, lipid I, and C55-P on the antibacterial activity of **5**–**7**, **13**, and nisin. Each lipopeptide was administered at a final concentration of 4× the MIC along with either lipid II, lipid I, or C55-P (undecaprenyl monophosphate), which were added at 2-fold molar concentrations with respect to the concentration of the antibiotic (1% DMSO in final solution). The appropriate quantity of each antagonist (used as a stock solution in methanol/chloroform 1:1) was transferred to a sterile glass tube, and the organic solvent was evaporated followed by redissolving in 50 μL of TSB. The antibiotic compound of interest was then added to the antagonist as a solution in 50 μL of TSB along with thorough mixing, after which the mixture was left to stand at room temperature for 30 min. Each 100 μL volume was then transferred to a well plate as two separate 50 μL aliquots to allow for duplicate measurement. Each well was inoculated with 50 μL of 1×10^6 CFU/mL VRE E155 in TSB. Well plates were incubated for 16 h at 37 °C at 350 rpm, and growth was determined by visual inspection. As controls, mixtures without the antibiotic or without antagonist were also prepared and tested under the same conditions.

Hemolysis Assay. Human whole blood was centrifuged at 600*g* for 15 min, and levels of plasma and hematocrit were marked on the tube. Plasma was removed, and the erythrocytes were washed 3× with PBS (centrifuging at 600*g* for 15 min). After discarding the supernatant, the packed cells were stored on ice. One-hundred microliters of the peptides (128 μg/mL in PBS, 2% DMSO) as well as a control solution composed of 2% DMSO in PBS was added to the top row of a polypropylene, round-bottomed 96-well plate, and 50 μL of PBS was added to the rest of the wells. The peptides and the DMSO control solutions were then diluted serially down the rows. Two-hundred microliters of the packed cells was added to PBS (10 mL), and 50 μL of this suspension was added to each well. A column with DI water containing 0.1% Triton X-100 was used as the 100% lysis control, and the column containing the serially diluted PBS (1.0% DMSO) control served as the 0% lysis reference. The cells were incubated at 37 °C for either 1 or 16 h. After incubation, the plates were centrifuged (800*g*, 5 min) and 25 μL of the supernatant was added to 100 μL of DI water in a flat-bottomed plate (polystyrene). The absorption at 414 nm was recorded to measure the amount of free hemoglobin.

Serum Stability. Peptide solutions of 2 mg/mL were prepared in 26% DMSO in Milli-Q. Duplicate samples were prepared with 42 μL of peptide solution and 518 μL of human serum, making the final DMSO concentration 2%. The samples were incubated at 37 °C, and samples were taken at t = 0, 1, 4, 8, and 24 h as follows: To 100 μL of serum solution was added 200 μL of MeOH (containing 0.075 mg/mL ethylparaben as an internal standard) to precipitate the proteins. The sample was vortexed briefly and allowed to stand for 10 min at RT. The samples were then centrifuged at 13 000 rpm for 5 min, and the supernatant was taken and stored at −20 °C until analysis. Each sample was analyzed by HPLC, on a C4 column. The peaks corresponding to intact peptide were integrated and normalized to the internal standard.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and analytical data for all new compounds. Table S1: MIC determinations against various Gram-positive and -negative strains. Table S2: MIC_{50} and MIC_{90} determination for compound **6** and nisin against VRE strains. Table S3: HPLC retention times and HRMS values for peptides **2**–**15**. Figure S1: Raw data for nisin dye leakage experiment in lipid II-containing LUVs. Figure S2: Nisin A/B fragment antagonization assay with nisin and compound **6**. Figure S3: Percent hemolysis of compounds **5**–**7** and **13** compared to nisin and vancomycin. Figure S4: Serum stability

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of compounds **5**−**7** and **13** and nisin. ^{1}H and ^{13}C NMR spectra for new compounds. Analytical RP-HPLC traces for compounds **2**−**15**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b04501.

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Notes

The authors declare no competing financial interest.

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