Published in final edited form as:

Biopolymers. 2013 April ; 100(2): 160–166. doi:10.1002/bip.22186.

Solid-phase Synthesis of Fusaricidin/LI-F Class of Cyclic Lipopeptides: Guanidinylation of Resin-bound Peptidyl Amines

Nina Bionda^{1,2}, Jean-Philippe Pitteloud¹, and Predrag Cudic¹

¹Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, FL 34987, USA

²Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, FL 33431, USA

Abstract

Fusaricidins/LI-Fs and related cyclic lipopeptides represent an interesting new class of antibacterial peptides with the potential to meet the challenge of antibiotic resistance in bacteria. Our previous study (N. Bionda *et al. ChemMedChem* 2012, 7, 871-882) revealed the significance of the guanidinium group located at the termini of the lipidic tails of these cyclic lipopeptides for their antibacterial activities. Therefore, devising a synthetic strategy that will allow incorporation of guanidinium functionality into their structure is of particular practical importance. Since appropriately protected guanidino fatty acid building blocks are not commercially available, our strategy toward guanidinylated fusaricidin/LI-F analogs include solid-phase synthesis of a cyclic lipopeptide precursor possessing a lipidic tail with a terminal amino group followed by its conversion into corresponding guanidine. To find the optimal method for this conversion, we have examined commonly used guanidinylation reagents under the conditions compatible with standard solid-phase peptide synthesis. Described experimental results demonstrated superiority of *N,N*-di-Boc-*N*-triflylguanidine in solid-phase preparation of fusaricidin/LI-F class of cyclic lipopeptides. The triflylguanidine reagent gave a single monoguanidinylated product in excellent yield independently of the type of solid-support.

Keywords

Antibiotics; cyclic lipopeptides; fusaricidin/LI-F; solid-phase synthesis; guanidinylation; triflylguanidine; pyrazole; thiourea

Introduction

Bacterial infections are becoming increasingly difficult to treat due to the development and spread of antibiotic resistance.¹⁻³ Currently, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species (the ESKAPE pathogens)⁴ are causing serious concerns due to the rapid spread of multidrug resistant strains.^{4,5} Cautious use of existing antibiotics may slow further development of resistance; however, in order to provide effective treatment options for the future, innovative antibiotics are necessary, preferably with novel modes of action and/or belonging to novel classes of drugs.

Naturally occurring cyclic depsipeptides, microbial secondary metabolites that contain one or more ester bonds in addition to the amide bonds, have emerged as an important source of

N.B. and J.-P. P. contributed equally to this work

novel antimicrobial agents.^{6,7} Within this class of natural products, cyclic lipodepsipeptide daptomycin (Cubicin®, Cubist Pharmaceuticals, Inc.),^{6,8,9} already approved by the US FDA for the treatment of infections caused by Gram-positive bacteria, best illustrates the potential of cyclic lipodepsipeptides for reverting multidrug bacterial resistance. Daptomycin is the only approved antibiotic exhibiting *in vitro* activity against vancomycin-resistant *Enterococcus* spp. Although rare, the emergence of bacterial resistance to daptomycin has been reported,¹⁰⁻¹⁵ highlighting the need to discover and develop new antibiotics.

A unique structure, potent antimicrobial activity and low toxicity, make fusaricidin or the LI-F class of natural products a particularly attractive source of candidates for the development of new antibacterial agents. Fusaricidins/LI-Fs are positively charged cyclic lipodepsipeptide antifungal antibiotics isolated from *Paenibacillus* sp, Figure 1.¹⁶⁻¹⁸ These natural products represent a new class of antibiotics structurally distinct from typical cationic antimicrobial peptides (CAMPs). Whereas CAMPs have a net positive charge between +2 to +9 due to the presence of cationic amino acids such as Arg, Lys, and His,¹⁹⁻²¹ fusaricidins/LI-Fs have a single positive charge located at the termini of their lipidic tails.¹⁶⁻¹⁸ Among isolated fusaricidin/LI-F antibiotics, fusaricidin A/LI-F04a (Thr¹-D-Val¹-Val³-D- α Thr⁴-D-Asn⁵-D-Ala⁶, Figure 1), showed the most promising antimicrobial activity against a variety of fungi and Gram-positive bacteria such as *S. aureus* (MICs ranging from 0.78-3.12 µg/mL). However, full exploitation of this class of natural products as new antibacterial drugs strongly depends on synthetic access to their analogs.

We have previously reported Fmoc solid-phase synthesis (Fmoc SPPS) of a series of fusaricidin A/LI-F04a depsipeptide and amide analogs containing 12-guanidinododecanoic acid, and the structure-activity relationship studies revealing key structural requirements for antibacterial activity and decreasing nonselective cytotoxicity.^{22,23} In these analogs, the positively charged guanidinium group at the end of the 12-carbon-atom lipidic chain, and the presence of hydrophobic amino acids were shown to be crucial for antibacterial and hemolytic activities. Guanidinylated cyclic lipodepsipeptide **1**, Figure 2, showed potent activity against multidrug resistant Gram-positive bacteria and was also hemolytic.²³ On the other hand, removal of the guanidino group, analog **2**, led to a complete loss of both antibacterial and hemolytic activities.²³ Guanidinium group exhibits similar role in activity of fusaricidin A/LI-F04a amide analogs such as **3**, Figure 2. Therefore, incorporation of the guanidino functionality into fusaricidin synthetic analogs is of utmost importance.

Although a number of methods for solid-phase guanidinylation have been reported,^{24,25} few described guanidinylation reactions are fully compatible with the standard solid phase peptide chemistry.²⁶⁻³¹ The diprotected triflylguanidines **4**, reagents based on 1*H*-pyrazole-1-carboxamidine **5** and di-urethane-protected thiourea **6** are the most commonly used reagents for this purpose, Figure 3.^{22,23,26-32} However, these reagents are not without shortcomings. 1*H*-pyrazole-1-carboxamidine **5** failed to completely guanidylate resin-bound amines even after prolonged reaction time,^{22,27,28} and it is used in great excess. Self-condensation of **5** under guanidinylation reaction conditions has been reported as well.²⁹ In the case of guanidinylation of resin-bound amines using di-Boc-protected thiourea **6** under Mukaiyama's conditions, the reaction efficacy depends on the steric hindrance of the amino group and the solvent.^{27,32} In addition, reaction of resin-bound amine may react with Mukaiyama's reagent **7**,²⁸ or reaction with thiourea **6** *via* S-atom may result in corresponding *S*-aminoisothiourea.³³ Our experience concerning the solid-phase guanidinylation of amino-precursors for fusaricidin/LI-F analogs synthesis using **5** mirrors published results, whereas use of **6** under Mukaiyama's reaction conditions afforded inconsistent yields and purities of the desired products.²² Taking all these into consideration, we have decided to examine reaction requirements for efficient solid-phase guanidinylation of the fusaricidin/LI-F class of cyclic lipopeptides.

Herein, we describe the effect of the reagents and the resins on guanidinylation of the lipidic side chain of cyclic lipopeptides.

Materials and Methods

All materials and reagents are commercially available and were used as received. Solvents used (DCM, DMF, ACN, water) were obtained from Fisher Scientific (Atlanta, GA) and were high-performance liquid chromatography (HPLC) or peptide synthesis grade. TentaGel S RAM resin was obtained from Advanced ChemTech (Louisville, KY; substitution level: 0.25 mmol/g; mesh size: 90 μ m; swelling: 3.9 mL/g in DMF, > 5 mL/g in DCM). Rink amide MBHA resin was purchased from Novabiochem (Gibbstown, NJ; substitution level: 0.66 mmol/g; mesh size: 75-150 μ m; swelling: 3.5 mL/g in DMF, 5.2 mL/g in DCM). Reagents for Kaiser ninhydrin test were purchased from AnaSpec (Fremont, CA). Fmoc-protected amino acids and coupling reagents (HOBt, HBTU, PyBOP) were purchased from Chem-Impex (Wood Dale, IL, USA) or Novabiochem. Guanidinylation promoters 1-methyl-2-chloropyridinium iodide (Mukaiyama's reagent) and *N*-iodosuccinimide (NIS) were purchased from Alfa Aesar (Ward Hill, MA). DIEA, TEA, *N,N*-di-Boc-thiourea, 1*H*-pyrazole-1-carboxamide hydrochloride and *N,N*-di-Boc-*N*-(trifluoromethylsulfonyl)guanidine were purchased from Sigma-Aldrich (St. Louis, MO).

Synthesis of Model Cyclic Lipopeptide 3

Synthesis of the model cyclic lipopeptide **3** was performed as reported previously.²³ Upon cyclization of the linear precursor, the Fmoc protecting group was removed from the lipidic tail terminal amino group using standard 20% piperidine/DMF deprotection protocol³⁴ generating a free amine suitable for subsequent guanidinylation. Four different guanidinylation conditions were used for this purpose. The guanidinylation reactions were carried out under identical reaction conditions (described below) on TentaGel S RAM and Rink amide MBHA resins. Progress of the guanidinylation reaction was monitored every hour by Kaiser test.³⁵ Guanidinated cyclic peptide was cleaved from the resin using a TFA/thioanisole/H₂O (95:2.5:2.5 v/v) cleavage cocktail, and the crude peptide was analyzed by RP HPLC (Grace Vydac C₁₈ monomeric column 250 $\times$ 4.6 mm, 5 μ m, 120 Å, 1 mL/min flow rate, elution method with a linear gradient of 2-98% B over 30 min, where A was 0.1% TFA in H₂O and B was 0.08% TFA in ACN) and MALDI-TOF MS. Relative quantification is based on the integrated areas of the reaction products HPLC peaks.

Guanidinylation Using *N,N*-di-Boc-*N*-(triflyl)guanidine 4

The peptidyl-resin was swollen in DCM for 20 min followed by solvent removal. The solution of *N,N*-di-Boc-*N*-(triflyl)guanidine **4** (5 eq) and TEA (5 eq) in DCM (5 mL) was added to the peptidyl-resin, and the reaction mixture was allowed to agitate at room temperature. A Kaiser test indicated complete consumption of resin-bound amine within 5 h on both TentaGel S RAM and Rink amide MBHA resins. Cyclic lipopeptide **3** was cleaved from the solid support, and the crude product was analyzed as described above.

Guanidinylation Using 1*H*-pyrazole-1-carboxamide hydrochloride 5

Peptidyl-resin was swollen in DMF for 20 min followed by solvent removal. The solution of 1*H*-pyrazole-1-carboxamide hydrochloride **5** (3 eq) and DIEA (3 eq) in DMF (5 mL) was added to the peptidyl-resin and allowed to agitate at room temperature. Peptidyl-resin samples (cca 20 mg) were taken after 8 h and 18 h. Cyclic lipopeptide was cleaved from the solid support, and the crude product **3** was analyzed as described above.

Guanidinylation Using *N,N'*-di-Boc-thiourea **6** and Mukaiyama's Reagent **7** as an Activator

Peptidyl-resin was swollen in DCM for 20 min followed by solvent removal. The solution of *N,N*-di-Boc-thiourea **6** (3 eq) and TEA (4 eq) in DCM (4 mL) was added to the resin, and the reaction mixture was allowed to agitate at room temperature for 15 min. Solution of Mukaiyama's reagent **7** (3 eq) in DCM (1 mL) was then added, and agitation was continued at room temperature. A Kaiser test indicated complete consumption of free resin-bound amine within 3 h on both TentaGel S RAM and Rink amide MBHA resins. Cyclic lipopeptide was cleaved from the solid support, and the crude product **3** was analyzed as described above.

Guanidinylation with *N,N'*-di-Boc-thiourea **6** and *N*-iodosuccinimide (NIS) **8** as an Activator

Peptidyl-resin was swollen in DCM for 20 min followed by solvent removal. The solution of *N,N*-di-Boc-thiourea **6** (3 eq), NIS **8** (3 eq) and TEA (4 eq) in DCM (5 mL) was added to the peptidyl-resin and allowed to agitate at room temperature. A Kaiser test indicated complete consumption of resin-bound amine within 2 h on TentaGel S RAM, whereas on Rink amide MBHA resin, the guanidinylation reaction did not go to completion even after a prolonged reaction time of 8 h. In both cases, the peptidyl-resin samples (cca 20 mg) were taken after 2 h, cyclic lipopeptide was cleaved from the solid support, and the crude product **3** was analyzed as described above. The guanidinylation reaction was repeated with 1.1 eq of di-Boc-thiourea, 1.1 eq of NIS and 1.5 eq of TEA.

Results and Discussion

Taking into consideration that the Fmoc SPPS is the method of choice for peptide synthesis as well as the lack of commercially available appropriate guanidino fatty acids, our approach for cyclic lipopeptide guanidinylation is comprised of solid-phase conversion of the fatty acid's primary amine into a guanidine. To find an optimal method for this conversion, we have examined the most frequently described guanidinylation reactions in the literature.^{24,25,32} The amide analog **3** was used in this study because of its simplified Fmoc SPPS and superior biological activities in comparison to the parent depsipeptide **1**, Figure 2. The solid-phase synthesis of **3** was accomplished by attaching the *C*-terminal Fmoc-*D*-Asp-OAllyl *via* side chain to amide resin, followed by linear peptide precursor assembly and on-resin head-to-tail cyclization using standard Fmoc-chemistry.^{22,23} The lipidic tail possessing Fmoc-protected terminal amino group [12-(Fmoc-amino)dodecanoic acid] was successfully incorporated into the peptidyl-resin precursor using the HBTU/HOBt/NMM procedure prior to cyclization. Alternatively, 12-(Fmoc-amino)dodecanoic acid can be coupled to the peptidyl-resin precursor using the same reagents after on-resin cyclization without affecting the final product purity.

Upon removal of Fmoc-protection, the lipidic tail primary amino group was converted into the desired guanidino functionality, Figure 4. Two different guanidinylation strategies were tested (a) direct guanidinylation with *N,N*-di-Boc-*N*-triflylguanidine **4**,^{36,37} or 1*H*-pyrazole-1-carboxamide hydrochloride **5**,²⁹ and (b) guanidinylation using di-Boc thiourea **6** activated with the Mukaiyama's reagent^{27,28} **7** or NIS **8**,^{30,33} Figure 3. Since side reactions associated with some of these reagents have been previously reported, we paid special attention to the purity of the final product **3**.^{28,29,33} Furthermore, since it is well known that the efficiency of SPPS depends on the properties and quality of the solid-support,^{38,39} the effect of the resin on guanidinylation was assessed as well. For this purpose, polystyrene (PS) based Rink amide MBHA and polyethylene glycol (PEG) based TentaGel S RAM resins were employed. Both resins are commonly used in Fmoc SPPS; and according to manufacturer's specifications, they have similar physical properties (see Materials and Methods). However, as demonstrated recently by Krchnak *et al.*,³⁸ even the resins with

identical specifications from different or the same sources often behave differently in solid-phase synthesis. In all cases guanidinylation protocols were followed as described before. Peptides were cleaved with a TFA/thioanisole/H₂O (95:2.5:2.5 v/v) cleavage cocktail, and the progress of the reactions was monitored by RP HPLC and MALDI-TOF MS analysis, Figure 5.

As shown in Figure 5, the best results were obtained using *N,N*-di-Boc-*N*-triflylguanidine **4**. Diprotected triflylguanidines were for the first time described by Goodman *et al.*^{36,37} and used in direct guanidinylation of primary and secondary amines under mild conditions in solution and on solid support. In our case, the solid-phase guanidinylation of resin-bound peptidyl amine with **4** was completed within 5 hours on both resins, resulting in desired product **3** ($R_t = 15.1$ min, m/z calculated 809.5123, found $[M+H]^+$ 810.5260) with no or an insignificant amount of byproducts detected. On the other hand, guanidinylation reagents **5-8** gave unsatisfactory results on both resins. Guanidinylation with 1*H*-pyrazole-1-carboxamidine **5** did not go to completion even after prolonged reaction times. Within 8 h, roughly, 91% of non-guanidinated cyclic lipopeptide **9** was recovered on TentaGel S RAM resin and 82% was recovered using polystyrene-based Rink amide MBHA resin ($R_t = 14.2$ min, m/z calculated 767.4905, found $[M+H]^+$ 769.1107), Figure 5B. Further extension of the reaction time to 18 hours had a modest impact on the guanidinylation efficacy, improving the yield of the desired product **3** by 10-15% on both resins. No side products were detected using the 1*H*-pyrazole-1-carboxamidine reagent **5**.

The conversion of amines into guanidines with thioureas, such as **6**, requires initial activation.²⁵ Among various activators that can be used for this purpose,^{25,32} Mukaiyama's reagent **7** and NIS **8** are fully compatible with Fmoc SPPS. It has been suggested that both Mukaiyama's reagent **7** and NIS **8** work for activation of the sulfur-leaving group leading to a highly electrophilic carbodiimide intermediate,^{27,40} and consequently to the desired protected guanidine. In the case of activation with the Mukaiyama's reagent **7**, consumption of the starting resin-bound peptidyl-amine was completed within 3 h on both resins, resulting in multiple reaction products, Figure 5C. The byproduct **10** ($R_t = 15.9$ min, m/z found $[M+Na]^+$ 873.4537) was identified as the main product of this reaction. The increase in molecular weight in the case of **10** over the starting resin-bound peptidyl-amine is compatible with the addition of two formimidamide groups. Other reaction products include the desired guanidine **3**, and the Mukaiyama adduct **11** ($R_t = 16.2$ min, m/z calculated 859.5405, found $[M]^+$ 859.6324). However, as illustrated in Figure 5C, the relative distribution of products' abundance depends on the type of resin. TentaGel S RAM resin gave 39% of **3**, 54% of **10** and 7.3% of **11**, whereas use of Rink amide MBHA resin resulted in 14% of **3**, 79% of **10** and 8% of **11**. Somewhat better results were obtained using NIS **8** as an activator and TentaGel S RAM resins, Figure 5D. In this case, starting resin-bound peptidyl amine was completely consumed within 2 h, yielding almost identical amounts of guanidine **3** (51%) and byproduct **10** (49%). Guanidinylation of resin-bound peptidyl amine on Rink amide MBHA resin under the same reaction conditions was less efficient as evidenced by 14% recovery of non-guanidinated cyclic lipopeptide **9** in addition to formation of 39% of **3** and 48% of **10**. Although reported in the literature,³³ formation of *S*-aminoisothiourea byproduct was not observed under applied guanidinylation conditions.

Considering that thiourea activation with Mukaiyama's reagent **7** and NIS **8** proceeds through a common carbodiimide intermediate, formation of the byproduct **10** in both cases is not surprising. The excess (3 eq) of thiourea **6** and activators **7** or **8** in guanidinylation reactions is required due to carbodiimide intermediate low stability.²⁷ However, the excess of guanidinylation reagents may also facilitate conversion of mono- to diguanidinated product.²⁹ To assess the effect of reagent excess on guanidinylation, resin-bound peptidyl amine guanidinylation was performed in the presence of 1.1 equivalents of **6** and an

appropriate activator. In this model reaction, NIS **7** was chosen as an activator since activation of thiourea **6** with NIS resulted in better yields of monoguanidine product **3** compared to activation of **6** with Mukaiyama's reagent **7**. As shown in Figure 5D, lowering amounts of guanidinylation reagents to 1.1 eq resulted in incomplete consumption of starting resin-bound peptidyl amine, and formation of **3** and **10** within 2 h, with more **10** formed on Rink amide MBHA resin. The fact that lower amounts of the guanidinylation reagents did not suppress formation of the byproduct **10**, indicates that **10** is formed relatively fast and in parallel with formation of monoguanidine **3**. These results also suggest that guanidine dimerization did not occur under the applied experimental conditions, and indicate the possibility of carbodiimide intermediate reaction with other functional groups present in the cyclic lipopeptide structure. Taking into consideration the amino acid sequence of cyclic lipopeptide **3**, such possibility could include partial deprotection of Thr³ side-chain hydroxyl group under Mukaiyama's and NIS guanidinylation conditions, allowing therefore for a competitive nucleophilic attack of the hydroxyl group on the carbodiimide intermediate and formation of an urea adduct **10**.⁴¹⁻⁴³ The feasibility of forming byproduct **10** was assessed by guanidinylation of 12-aminododecanoic acid attached to Rink amide MBHA resin. Standard Fmoc SPPS chemistry was applied in preparation of this control compound. Guanidinylation of resin bound 12-aminododecanoic acid using Mukaiyama's reaction conditions resulted in exclusive formation of corresponding monoguanidine product as evidenced by MALDI-TOF MS analysis of the reaction mixture (12-aminododecanamide, *m/z* calculated 214.2045; 12-guanidinododecanamide, *m/z* expected 256.2263; found 257.3374 [M]⁺, 279.3320 [M+Na]⁺). Obtained results eliminate the possibility of guanidine group dimerization and strongly support our initial assumption of byproduct **10** formation in parallel with desired cyclic lipopeptide **3** under the experimental conditions required for guanidinylation using thiourea and activators **7** or **8**.

Conclusion

Synthetic access to the fusaricidin/LI-F class of cyclic lipopeptide structures represents the first step in full exploitation of their antibacterial potentials. Taking into consideration the importance of the guanidine group on the biological activities of this class of antibacterial peptides, we explored the effectiveness of the most commonly used guanidinylation reagents in solid-phase conversion of resin-bound peptidyl amine into corresponding guanidine. Guanidinylation reactions were carried out using four different reagents and conditions compatible with standard Fmoc-peptide chemistry on PEG and PS-based resins. Our experimental results demonstrated superiority of *N,N*-di-Boc-*N*-triflylguanidine **4** in solid-phase guanidinylation of fusaricidin's cyclic lipopeptide precursors. Guanidinylation with **4** resulted in a sole guanidinated product **3** regardless of the type of resin used. On the other hand, use of pyrazole-based reagent or activated thiourea gave unsatisfactory results. In both of these approaches, reaction efficiency and the product distribution depends on guanidinylation mechanism as well as on the type of resin. Selection of an appropriate method for guanidinylation of resin-bound amines is critical in order to minimize formation of undesired side-products and increase the efficacy of guanidinylation reagents.

Acknowledgments

We acknowledge support of the work described herein by the NIH (grant 1S06-GM073621-01) and AHA (grant 0630175N) to P.C. We also thank Ms. Karen Gottwald for editing the text.

References

1. Gold HS, Moellering RC. Antimicrobial-drug resistance. *New Engl J Med*. 1996; 335(19):1445–1453. [PubMed: 8875923]

2. Lipsitch M. The rise and fall of antimicrobial resistance. *Trends Microbiol.* 2011; 9:438–444. [PubMed: 11553456]
3. Fluit AC, Schmitz FJ, Verhoef J. Multi-resistance to antimicrobial agents for the ten most frequently isolated bacterial pathogens. *Int J Antimicrob Agents.* 2001; 18(2):147–160. [PubMed: 11516938]
4. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. *J Infect Dis.* 2008; 197(8):1079–1081. [PubMed: 18419525]
5. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. Bad Bugs, No Drugs: No ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis.* 2009; 48(1):1–12. [PubMed: 19035777]
6. Bionda N, Cudic P. Cyclic lipodepsipeptides in novel antimicrobial drug discovery. *Croat Chem Acta.* 2011; 84(2):315–329.
7. Roongsawang N, Washio K, Morikawa M. Diversity of nonribosomal peptide synthetases involved in the biosynthesis of lipopeptide biosurfactants. *Int J Mol Sci.* 2010; 12(1):141–172. [PubMed: 21339982]
8. Woodford N. Novel agents for the treatment of resistant Gram-positive infections. *Expert Opin Inv Drug.* 2003; 12(2):117–137.
9. Kern WV. Daptomycin: First in a new class of antibiotics for complicated skin and soft-tissue infections. *Int J Clin Pract.* 2006; 60(3):370–378. [PubMed: 16494659]
10. Patel D, Husain M, Vidaillac C, Steed ME, Rybak MJ, Seo SM, Kaatz GW. Mechanisms of in-vitro-selected daptomycin-non-susceptibility in *Staphylococcus aureus*. *Int J Antimicrob Agents.* 2011; 38(5):442–446. [PubMed: 21840181]
11. Steed ME, Vidaillac C, Rose WE, Winterfield P, Kaatz GW, Rybak MJ. Characterizing vancomycin-resistant *Enterococcus* strains with various mechanisms of daptomycin resistance developed in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother.* 2011; 55(10):4748–4754. [PubMed: 21788457]
12. Jones T, Yeaman MR, Sakoulas G, Yang SJ, Proctor RA, Sahl HG, Schrenzel J, Xiong YQ, Bayer AS. Failures in clinical treatment of *Staphylococcus aureus* infection with daptomycin are associated with alterations in surface charge, membrane phospholipid asymmetry, and drug binding. *Antimicrob Agents Chemother.* 2008; 52(1):269–278. [PubMed: 17954690]
13. Khan SA, Sung K, Layton S, Nawaz MS. Heteroresistance to vancomycin and novel point mutations in Tn1546 of *Enterococcus faecium* ATCC 51559. *Int J Antimicrob Agents.* 2008; 31(1):27–36. [PubMed: 17936593]
14. Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest.* 2003; 111(9):1265–1273. [PubMed: 12727914]
15. van Hal SJ, Paterson DL, Gosbell IB. Emergence of daptomycin resistance following vancomycin-unresponsive *Staphylococcus aureus* bacteraemia in a daptomycin-naïve patient—a review of the literature. *Eur J Clin Microbiol Infect Dis.* 2011; 30(5):603–610. [PubMed: 21191627]
16. Kurusu K, Ohba K. New peptide antibiotics LI-F03, F04, F05, F07, and F08, produced by *Bacillus polymyxa*. I. Isolation and characterization. *J Antibiot.* 1987; 40:1506–1514. [PubMed: 3693120]
17. Kajimura Y, Kaneda M. Fusaricidin A, a new depsipeptide antibiotic produced by *Bacillus polymyxa* KT-8. Taxonomy, fermentation, isolation, structure elucidation and biological activity. *J Antibiot.* 1996; 49:129–135. [PubMed: 8621351]
18. Kajimura Y, Kaneda M. Fusaricidins B, C and D, new depsipeptide antibiotics produced by *Bacillus polymyxa* KT-8: Isolation, structure elucidation and biological activity. *J Antibiot.* 1997; 50:220–228.
19. Wu Z, Li X, de Leeuw E, Ericksen B, Lu W. Why is the Arg⁵-Glu¹³ salt bridge conserved in mammalian alpha-defensins? *J Biol Chem.* 2005; 280:43039–43047. [PubMed: 16246847]
20. Hancock REW, Lehrer R. Cationic peptides: A new source of antibiotics. *Trends Biotechnol.* 1998; 16:82–88. [PubMed: 9487736]
21. Peschel A, Sahl HG. The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat Rev Micro.* 2006; 4(7):529–536.
22. Stawikowski M, Cudic P. A novel strategy for the solid-phase synthesis of cyclic lipodepsipeptides. *Tetrahedron Lett.* 2006; 47(48):8587–8590. [PubMed: 17440603]

23. Bionda N, Stawikowski M, Stawikowska R, Cudic M, Lopez-Vallejo F, Treitl D, Medina-Franco J, Cudic P. Effects of cyclic lipopeptide structural modulation on stability, antibacterial activity, and human cell toxicity. *ChemMedChem*. 2012; 7(5):871–82. [PubMed: 22392790]
24. Manimala JC, Anslyn EV. Solid-phase synthesis of guanidinium derivatives from thiourea and isothiurea functionalities. *Eur J Org Chem*. 2002; 23:3909–3922.
25. Katritzky AR, Rogovoy BV. Recent developments in guanylation agents. *ARKIVOC*. 2005; iv:49–87.
26. Kowalski JA, Lipton MA. Solid phase synthesis of a diketopiperazine catalyst containing the unnatural amino acid (S)-norarginine. *Tetrahedron Lett*. 1996; 37(33):5839–5840.
27. Yong YF, Kowalski JA, Lipton MA. Facile and efficient guanylation of amines using thioureas and Mukaiyama's reagent. *J Org Chem*. 1997; 62:1540–1542.
28. Yong YF, Kowalski JA, Thoen JC, Lipton MA. A new reagent for solid and solution phase synthesis of protected guanidines from amines. *Tetrahedron Lett*. 1999; 40(1):53–56.
29. Bernatowicz MS, Wu Y, Matsueda GR. 1*H*-Pyrazole-1-carboxamide hydrochloride an attractive reagent for guanylation of amines and its application to peptide synthesis. *J Org Chem*. 1992; 57(8):2497–2502.
30. Zakhariyev, S.; Szekely, Z.; Guarnaccia, C.; Antcheva, N.; Pongor, S. A highly effective method for synthesis of N -substituted arginines. Kluwer Academic Publisher; Dordrecht: 2000.
31. Thamm, P.; Kolbeck, W.; Musiol, HJ.; Moroder, L. Other side-chain protections, guanidino group. Vol. Houben-Weyl. Georg Thieme Verlag Stuttgart; New York: 2004. p. E22a
32. Schneider SE, Bishop PA, Salazar MA, Bishop OA, Anslyn EV. Solid phase synthesis of oligomeric guanidiniums. *Tetrahedron*. 1998; 54(50):15063–15086.
33. Ohara K, Vasseur JJ, Smietana M. NIS-promoted guanylation of amines. *Tetrahedron Lett*. 2009; 50(13):1463–1465.
34. Chan, WC.; White, PD. Fmoc Solid Phase Peptide Synthesis. Vol. 222. Oxford University Press; New York: 2003.
35. Kaiser E, Colescott RL, Bossinger CD, Cook PI. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal Biochem*. 1970; 34(2):595–598. [PubMed: 5443684]
36. Feichtinger K, Sings HL, Baker TJ, Matthews K, Goodman M. Triurethane-protected guanidines and triflyldiurethane-protected guanidines: New reagents for guanidinylation reactions. *J Org Chem*. 1998; 63(23):8432–8439.
37. Feichtinger K, Zapf C, Sings HL, Goodman M. Diprotected triflylguanidines: A new class of guanidinylation reagents. *J Org Chem*. 1998; 63(12):3804–3805.
38. Bouillon I, Soural M, Miller MJ, Krchnak V. Resins with identical specifications are not identical. Identifying a useful solid-phase resin. *J Comb Chem*. 2009; 11(2):213–215. [PubMed: 19125667]
39. Pugh KC, York EJ, Stewart JM. Effects of resin swelling and substitution on solid phase synthesis. *Int J Pept Protein Res*. 1992; 40(3-4):208–213. [PubMed: 1478778]
40. Kim KS, Qian L. Improved method for the preparation of guanidines. *Tetrahedron Lett*. 1993; 34(48):7677–7680.
41. Hook D, Riss B, Kaufmann D, Napp M, Bappert E, Polleux P, Medlock J, Zanotti-Gerosa A. New processes for -amino- -biphenyl- -methylalkanoic acid, or acid ester. Jan 16.2009
42. Kantlehner, W.; Bowers, A. Encyclopedia of Reagents for Organic Synthesis. John Wiley & Sons, Ltd; Chichester, UK: 2007. *tert*-Butoxybis(dimethylamino)methane.
43. Crosignani S, White PD, Linclau B. Polymer-supported *O*-methylisourea: A new reagent for the *O*-methylation of carboxylic acids. *Org Lett*. 2002; 4(6):1035–1037. [PubMed: 11893215]

Figure 1.
Structure of fusaricidin A/LI-F family of natural products.

Figure 2.
Structures of fusaricidin A/LI-F04a synthetic analogs **1-3**.

Figure 3.
Structures of guanidinylation agents used in the study.

Products	R	R'	Reaction Conditions			
			A	B	C	D
3		—OH	+	+	+	+
9		—OH	—	+	—	—
10			—	—	+	+
11		—OH	—	—	+	—

A: *N,N'*-di-Boc-*N''*-triflylguanidine (4), TEA, DCM, 5 h
 B: 1*H*-pyrazole-1-carboxamidine hydrochloride (5), DIEA, DMF, 8 h
 C: *N,N'*-di-Boc-thiourea (6), Mukaiyama's reagent (7), TEA, DCM, 3 h
 D: *N,N'*-di-Boc-thiourea (6), *N*-iodosuccinimide (8), TEA, DCM, 2-8 h

Figure 4.
Solid-phase guanidinylation.

Figure 5. RP HPLC data Guanylation using A) *N,N*-di-Boc-*N* -triflylguanidine **4**; B) 1*H*-pyrazole carboxamide **5**; C) *N,N*-di-Boc-thiourea **6** and Mukaiyama's reagent **7**; D) *N,N*-di-Boc-thiourea **6** and NIS **8**, reaction mixture analysis with 3 eq (solid line) and 1.1 eq (dashed line) of guanidinylation reagents.