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# Diversity-oriented synthesis of cyclic acyldepsipeptides leads to the discovery of a potent antibacterial agent

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# 1. Introduction

Infectious diseases are the third leading cause of death in developed countries and the second leading cause of death worldwide.<sup>1,2</sup> The efficacy of many antibacterial drugs has been compromised by the emergence of drug-resistant, pathogenic bacteria.<sup>3</sup> The Infectious Disease Society of America has recently outlined the deadly implications of a growing number of drugresistant pathogens.<sup>4</sup> Methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), and penicillinresistant Streptococcus epidermis are especially worrisome in clinical settings. Unfortunately, the prevalence of multidrug-resistant bacteria has made antibiotics of last resort, like vancomycin, the first-line of therapy. The capacity of bacteria to commonly develop resistance to virtually any antibacterial agent necessitates a continuous search for new drugs. There is much evidence in the literature that natural products derived from microorganisms will continue to be a source of novel antibacterial drugs.<sup>5</sup> Although natural products often have chemical properties that are incompatible with chemotherapy, it is possible to use medicinal chemistry as a

# ABSTRACT

A class of cyclic acyldepsipeptide antibiotics collectively known as the enopeptins has recently attracted much attention because of their activity against multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*. These antibiotics are further distinguished by their novel mechanism of action in which they bind and deregulate the tightly controlled activity of the cytoplasmic protease ClpP. Although the natural products have poor pharmacological properties, a synthetic derivative called acyldepsipeptide 4 (ADEP 4) showed remarkable antibacterial activity both in vitro and in mouse models of bacterial infections. A novel route to the ADEP 4 peptidolactone core structure, featuring the Joullié-Ugi three-component reaction, was developed. This multicomponent reaction and a related multicomponent reaction, the Ugi four-component reaction, were used to prepare analogs that were designed using the principles of conformational analysis. These cyclic acyldepsipeptides were tested for their activity against drug-resistant, clinical isolates of *Staphylococci* and *Enterococci*. One ADEP 4 analog in which the pipecolate was replaced by 4-methyl pipecolate exhibited in vitro antibacterial activity against *Enterococci* that was fourfold higher than the parent compound. © 2010 Elsevier Ltd. All rights reserved.

means to enhance their biological activity and/or pharmacological properties.  $^{\rm 6}$ 

A recent case wherein medicinal chemistry was used to improve the activity of a natural product was that of the enopeptins.<sup>7,8</sup> The parent compounds were isolated from the soil dwelling bacterium Streptomyces sp. RK-1051 and are defined by a 16-membered peptidolactone consisting of five L-amino acids to which a lipophilic polyene side chain is appended (Fig. 1).<sup>9</sup> A group of closely related compounds, called A54556 A and B, were isolated from Streptomyces hawaiienesis by a research group at Eli Lilly (Fig. 1).<sup>10</sup> The enopeptins attracted attention because of their potent activity against drug-resistant bacterial pathogens, including MRSA and VRE.<sup>11</sup> The apparent lack of cross-resistance for all antibacterial agents on the market or those in clinical development has been ascribed to a peculiar mechanism of action.<sup>11</sup> The enopeptin antibiotics inhibit cell division and cause cell death by binding and deregulating the activity of the casein lytic protease (ClpP).<sup>11</sup> Under normal conditions, this fourteen-subunit protease selectively degrades proteins through a physical and functional association with accessory ATPases that recognize and unfold its substrates.<sup>12-15</sup> In the presence of the enopeptins, ClpP indiscriminately degrades folded cytoplasmic proteins, which ultimately causes cell death.<sup>11</sup> Recent structural studies indicate that the enopeptins bind the ClpP core structure and cause it to undergo a conformational change that exposes the enzymatic active sites of its subunits.<sup>16</sup>





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Figure 1. Enopeptin natural products and the pharmacologically-enhanced derivative, ADEP 4.  $^{11}$ 

Although the enopeptin natural products have remarkable antibacterial activity in vitro, their chemical lability and poor solubility limit their efficacy in vivo.<sup>7</sup> In an effort to improve the pharmacological properties of the enopeptins, a research group at Bayer Pharmaceutical Research carried out an intensive structure-activity relationship (SAR) study of the enopeptins.<sup>7,8</sup> Their medicinal chemistry program was guided by the observation that the conformation of the peptidolactone core structure was constrained by two transannular hydrogen bonds to the phenylalanine moiety of the side chain. On this basis, it was anticipated that replacing the constituents of the peptidolactone with conformationally restricted amino acids would yield enopeptin derivatives with improved antibacterial activity. Although most changes in the peptidolactone decreased antibacterial activity, replacement of N-methylalanine with a rigid pipecolate moiety yielded a more potent analogue.<sup>7</sup> Presumably, rigidification of the peptidolactone lowers the entropic cost of binding to the ClpP core.<sup>7</sup> The stability and antibacterial activity of these rigidified enopeptin analogs were further enhanced by replacement of the exocyclic phenylalanine with 3,5-difluorophenylalanine and by substitution of the polyunsaturated side chain with a 2-heptenoyl moiety. The optimized enopeptin derivative, known as acyldepsipeptide 4 (ADEP 4, Fig. 1), exhibited remarkable activity both in vitro and in vivo.<sup>7</sup> As was the case for the parent enopeptins, no cross-resistance to ADEP 4 was observed and the compound was active against multidrug-resistant pathogens. The minimum inhibitory concentration (MIC) and in vivo antibacterial activity of ADEP 4 equaled or even surpassed that of established antibacterial drugs. Remarkably, mice infected with a lethal inoculum of *Enterococcus faecalis* had a 100% survival rate when treated with a single dose of ADEP 4 at 0.5 mg/kg.<sup>7</sup> The development of ADEP 4 clearly indicates the power of medicinal chemistry in enhancing the properties of natural products.

Given the promise of ADEP 4 as an antibacterial drug, we sought to develop an efficient synthetic route to the peptidolactone core of this molecule that also allowed for the preparation of other conformationally constricted enopeptin derivatives. In the interest of expediency, we planned to capitalize on the convergent scheme used in the syntheses of ADEP 4 (and of enopeptins A and B) in which a tripeptide fragment and an ester fragment are coupled, cyclized and acylated (Scheme 1).<sup>7,17</sup> Our objective was to develop an efficient and diversity-oriented synthesis of the tripeptide precursor of ADEP 4 (Ala-Pip-Pro). The published chemical synthesis of this tripeptide fragment uses standard peptide chemistry and as such is limited by three distinct steps for removal of protecting groups, a challenging acylation of a secondary amide (pipecolamide), and a dependence on commercially-available amino acids. An ideal synthesis of the tripeptide would be higher yielding, greater in atom economy, and enable facile exchange of ADEP 4's pipecolate with other conformationally rigid amino acids for SAR studies. We hypothesized that the tripeptide precursor of ADEP 4 and other conformationally restricted tripeptides could be prepared in a single step via isocyanide-based multicomponent reactions (Scheme 2).<sup>18,19</sup> Pipecolate containing tripeptides reminiscent of the ADEP 4 tripeptide precursor can be prepared from six-membered cyclic imines, N-Boc-proline, and an isocyanide derived from alanine methyl ester in a Joullié-Ugi three component reaction (Joullié-Ugi 3CR).<sup>20,21</sup> Similarly, peptides with conformationally restricted *N*-methyl.  $\alpha$ . $\alpha$ -disubstituted amino acids can be prepared with an Ugi four-component reaction (Ugi 4CR)<sup>22</sup> from methylamine, various ketones, N-Boc-proline, and an isocyanide derived from alanine methyl ester.

We developed a diversity-oriented synthesis of the tripeptide fragment featuring isocyanide-based multicomponent reactions that enabled the preparation of eight enopeptin derivatives. The antibacterial activity of these analogues against clinical isolates of methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant



Scheme 1. Retrosynthesis of simplified ADEP 4 analog 10a.



Scheme 2. The Ugi (top) and Joullié-Ugi (bottom) multicomponent reactions.

*S. aureus* (MRSA), vancomycin-susceptible *E. faecalis* and vancomycin-resistant *E. faecium* (VRE) was systematically assessed. Additionally, we report for the first time the minimum bactericidal concentration (MBC) of any enopeptin derivatives. It is noteworthy that a novel ADEP 4 derivative with 4-methyl pipecolate in place of pipecolate has considerably enhanced activity against pathogenic *Enterococci.* 

### 2. Results

# 2.1. Synthesis and elaboration of the ADEP 4 peptidolactone core structure

Our initial objective was to prepare **10a**, a more synthetically accessible analogue of ADEP 4 in which 4-methylproline is replaced by proline and 3,5-difluorophenylalanine is replaced by 3fluorophenylalanine. In analogy to the published synthesis of ADEP 4, our plan was to prepare 10a in a convergent fashion. We anticipated that the requisite tripeptide fragment could be synthesized via a Joullié-Ugi 3CR. The substrates of the reaction were N-Boc proline, dehydropiperidine, and an isocyanide derived from alanine methyl ester. While N-Boc proline was commercially available, the other two substrates had to be chemically synthesized. The isocyanide was prepared via the *N*-formylation and dehydration of alanine methyl ester under racemization-free conditions described in the literature.<sup>23</sup> N-formyl-L-alanine methyl ester was synthesized from L-alanine methyl ester hydrochloride according to a literature procedure for the preparation of amino acid *tert*-butyl esters.<sup>24</sup> Dicyclohexylcarbodiimide (DCC) activation of formic acid as the O-formylisourea intermediate followed by the addition of the amino acid ester provided the desired N-formyl product 1 in good yield. Dehydration of 1 to form the isocyanide (2) was effected by triphosgene in the presence of *N*-methylmorpholine (NMM).<sup>23</sup> Synthesis of the isocyanide was realized in 69% yield over two steps (Scheme 3). Chiral GC-MS analysis of the isocyanide product revealed that no racemization of the amino acid occurred during the formylation and dehydration.

We envisioned that the cyclic imine substrate of the Joullie-Ugi reaction could be prepared by halogenation and dehydrohalogenation of piperidine. N-chlorination of piperidine via in situ generation of tert-BuOCl gave the desired product in quantitative yield.<sup>24</sup> Dehydrohalogenation of the N-chloropiperidine was effected by sodium methoxide in methanol (Scheme 4).<sup>20</sup> Due to its instability,<sup>25,26</sup> the crude dehydropiperidine was directly used in a Joullié-Ugi 3CR with N-Boc-proline and the isocyanide derived from alanine methyl ester (2). After stirring for five days at room temperature, the expected tripeptide methyl ester, **3**, was isolated in 76% yield (Scheme 4). Analytical HPLC analysis of saponified 3 (LiOH, 1:1 THF/H<sub>2</sub>O, quantitative yield) revealed that its diastereomeric ratio was 70:30. Based on the retention time of an authentic standard [(S)-Boc-Pro-(S)-Pip-(S)-Ala-COOH], we determined that the desired tripeptide was the minor diastereomer. The basis of the diastereoselectivity of this reaction is not clear and is under investigation. In spite of the undesirable stereoselectivity, the yield and the atom economy of the one-pot Joullié-Ugi 3CR make it a viable alternative to standard peptide chemistry for synthesis of the ADEP 4 tripeptide precursor.

The diastereomeric mixture of tripeptide products were saponified and carried forward to prepare the desired peptidolactone using the convergent synthetic route described in the literature (Scheme 5).<sup>7.8</sup> In contrast to the reported synthesis of ADEP 4, the diastereomeric peptidolactones were chromatograpically separated and acylated with *N*-2-heptenoyl, 3-fluorophenylalanine to yield **10a** and its diastereomer **10b** (Scheme 5).

# 2.2. Diversity-oriented synthesis of cyclic acyldepsipeptides with conformationally constrained amino acids

In addition to the efficiency with which an isocyanide-based multicomponent reaction could be used to synthesize a tripeptide,



Scheme 3. Preparation of an enantiomerically-pure isocyanide from L-alanine methyl ester.



Scheme 4. Synthesis of dehydropiperidine and its use in the Joullié-Ugi three component reaction.



Scheme 5. Reagents and conditions: (a) TPTU, HOBt, DCM, DIEA, 0 °C to rt, 18 h; (b) Zn, 90% HOAc in H20, rt, 30 min; (c) pentafluorophenol, EDC, DCM, -78 °C to rt, 18 h; (d) 3 N HCl in EtOAc, 0 °C, 3.5 h; (e) DCM/10% NaHCO<sub>3</sub> (3:1) 0.1 mM, rt, 18 h; (f) NH<sub>4</sub>COOH, Pd/C, isopropanol, 85 °C microwave, 8 min; (g) N-2-heptenoyl-3-fluorophenylalanine (9), TPTU, HOBt, DIEA, DMF, rt, 18 h.

a major consideration in the selection of this reaction type was its applicability in the preparation of structurally diverse peptides. Rather than preparing a random collection of tripeptides, we sought to synthesize tripeptides that were more conformationally constrained than the tripeptide precursor of ADEP 4. Our utilization of conformationally constrained amino acids was an extension of the findings of the Bayer research group wherein replacement of the *N*-methylalanine moiety of the natural enopeptins with pipecolate yielded an enopeptin derivative with enhanced antibacterial activity.

Our first synthetic target was an analog of the ADEP 4 tripeptide fragment with a substituent on the pipecolate moiety. Based on the principles of conformational analysis, we predicted that strategic placement of a substituent on the ring would increase the inversion barrier of pipecolate.<sup>27</sup> In particular, a substituent on the 4-position of pipecolate would be especially rigidifying since ring inversion would result in a strongly disfavored 1,3-diaxial interaction between ring substituents. To prepare such a compound, we carried out a Joullié-Ugi 3CR with *N*-Boc-proline, the isocyanide derived from alanine methyl ester (**2**), and dehydro 4-methyl piperidine. The latter compound was prepared in an analogous manner to dehydropiperidine. Interestingly, the dehydro-4-methylpiperidine was a better substrate in the Joullié-Ugi 3CR, yielding the expected tripeptide methyl ester in 84% in a reaction time of only

2 days. Analytical HPLC analysis of the saponified product revealed that its diastereomeric ratio was 70:30. As the retention time of the minor diastereomer was very similar to that of the authentic ADEP 4 tripeptide precursor, we deduced that it was the desired diastereomer containing (2S,4R)-4-methylpipecolamide. The mixture of diastereomeric peptides was carried forward in the aforementioned scheme for convergent synthesis of the peptidolactone core structure. The diastereomeric peptidolactones were chromatograpically separated and acylated with *N*-2-heptenoyl, 3-fluorophenylalanine to yield the desired compound, **10c** (Fig. 2).

Our other synthetic targets were tripeptides containing  $\alpha, \alpha$ disubstituted amino acids. These amino acids, while of limited commercial availability, are known to limit the conformation of peptides.<sup>28,29</sup> We suspected that the desired tripeptides could be prepared from methylamine, *N*-Boc-proline, the isocyanide derived from L-alanine methyl ester (**2**), and various ketones in an Ugi 4CR. To avoid mixtures of diastereomeric products, we used symmetric ketones (i.e., 3-pentanone, cyclobutanone, cyclopentanone, and cyclohexanone) as substrates (Scheme 6). The reactants were stirred in MeOH for a period of 1–5 days to optimize reaction conditions. Products **5–8** were isolated in good to excellent yields. The results are summarized in Table 1. Good yields of the tripeptides were only observed with reaction times >4 days and reaction concentrations  $\ge 1.9$  M (Increasing the reaction temperature of a



Figure 2. Chemical structures of cyclic acyldepsipeptides prepared and evaluated in this study. Shown below are the yields for reactions yielding the protected pentapeptolide and the protected macrocycle precursors of compounds 10a-10h (see experimental Sections 5.2.6.1 and 5.2.6.2).



**Scheme 6.** Ugi reactions with symmetric ketones (R = H or  $CH_3$ , n = 0-3).

 Table 1

 Optimized Ugi 4CR conditions for symmetric ketones

Ugi product	R	n	Conc (M)	Temp (°C)	Time (d)	Yield (%)
5	CH₃	0	1.90	25	5	75
6	Н	1	1.90	25	5	93
7	Н	2	0.65	25	5	62
7	Н	2	1.90	25	5	78
8	Н	3	1.90	25	5	90

model reaction with cyclopentanone and methylamine to 50 °C did not significantly improve the reaction yield). Using four different ketones as substrates in the Ugi 4CR, we were able to prepare four different tripeptide methyl esters (**5–8**). Those products were saponified as previously described to yield the requisite tripeptides. The tripeptides prepared via the Ugi 4CR were used in the aforementioned convergent synthesis to yield enopeptin derivatives, **10e–h** (Fig. 2).

#### 2.3. Antibacterial assays of the synthetic acyldepsipeptides

The naturally occurring enopeptins and the synthetic derivative ADEP 4 are reported to have strong antibacterial activity.<sup>7</sup> Broth micro-dilution assays<sup>30,31</sup> were used to determine the MICs of the acyldepsipeptide derivatives **10a–h** against both ATCC strains and clinical isolates of *S. epidermis, S. aureus*, and *Enterococci* obtained from patients of the Veterans Affairs Medical Center in Providence, Rhode Island (Table 2). Clinical isolates were included to gain a better indication of the potential of these promising antibacterial agents. In the interest of assessing the cell-killing ability of these compounds, the minimal bactericidal concentrations (MBC) of the compounds was also determined against the aformentioned strains. The MIC was defined as the lowest concentration of an antimicrobial agent visually inhibiting more than 99% of the colonies. The MBC was defined as the lowest antibiotic concentration to show no growth (99.9% kill) after 24 h of incubation.

Compound **10a**, an analog of ADEP 4, exhibited the expected antibacterial activity against drug-resistant, pathogenic *S. aureus* strains. All synthetic acyldepsipeptides in which the pipecolate moiety was replaced by  $\alpha$ , $\alpha$ -disubstituted amino acids lacked antibacterial activity. In contrast, compound **10c**, which 4-methylpipe-

Table 2
Biological activity of synthetic acyldepsipeptides versus S. aureus (µg/mL)

	MSSA		MRS	Ā
	MIC	MBC	MIC	MBC
10a	1.25	1.25	1.25	1.25
10b	>312	>312	>312	>312
10c	0.6	0.6	0.6	2.4
10d	>156	>156	>156	.156
10e	78.1	>312	>312	>312
10f	312	>312	>312	>312
10g	39.1	>312	>312	>312
10h	39.1	>312	>312	>312

MSSA = Methicillin-susceptible S. aureus (ATCC 35556).

MRSA = Methicillin-resistant S. aureus (clinical isolate from blood).

 Table 3
 Biological activity of synthetic acyldepsipeptides versus Enterococci (µg/mL)

	EF <sup>a</sup>		EF <sup>b</sup>		VRE <sup>c</sup>		VRE <sup>d</sup>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
10a 10c	0.16 0.04	1.25 0.08	0.04 0.02	0.04 0.04	0.08 0.04	0.08 0.08	0.04 0.04	0.04 0.04

<sup>a</sup> Enterococcus faecalis (ATCC 29212).

Enterococcus faecalis (clinical isolate from rectal swab).

<sup>c</sup> Vancomycin-resistant *Enterococcus faecium* (clinical isolate obtained from tissue).

<sup>d</sup> Vancomycin-resistant *Enterococcus faecalis* (clinical isolate from rectal swab).

colate in place of pipecolate, had better activity than compound **10a** against MSSA. Compound **10a** was also evaluated against *Staphylococcus epidermis* (ATCC 25984) and exhibited an MIC of 1.25  $\mu$ g/mL and an MBC of 1.25  $\mu$ g/mL.

Based on the activities of compounds **10a** and **10c** against the *S. aureus* strains, they were selected for further evaluation against a panel of pathogenic *Enterococci* (Table 3). Remarkably, compound **10c** exhibited more potent activity against *E. faecalis* and vancomycin-resistant *E. faecium* than compound **10a**.

# 3. Discussion

The medicinal properties of natural products can be enhanced by medicinal chemistry.<sup>6</sup> Optimization of natural product structures can be facilitated through the design of diversity-oriented chemical syntheses.<sup>32</sup> In this regard, isocyanide-based multicomponent reactions are ideally suited for the preparation of peptide antibiotics and closely related analogs. Specifically, the Ugi 4CR and the related Joullié-Ugi 3CR can be used to construct peptides consisting of non-proteinogenic and other non-natural amino acids.<sup>18–21</sup> Here, we demonstrate the utility of these reactions in synthesizing a promising class of acyldepsipeptides known as the enopeptins. In terms of atom economy and versatility, multicomponent reactions offer advantages over standard peptide synthesis.<sup>18,19</sup>

A comparison of the in vitro activities of the acyldepsipeptide derivatives affords two major structure-activity relationships. First, placing an additional substituent on the  $\alpha$ -carbon of the Nmethylalanine moiety of the enopeptin peptidolactone core structure abolishes antibacterial activity. Indeed, derivatives containing  $\alpha, \alpha$ -disubstituted amino acids in the macrocycle were completely inactive. Second, the S-configuration of the pipecolate  $\alpha$ -carbon is essential for the bioactivity of ADEP 4 analogues. Specifically, the pipecolate derivatives containing (R)-pipecolic acid and (R)-4-methylpipecolic acid were inactive. Taken together, these observations suggest that the ClpP protease can only accommodate acyldepsipeptides with mono-substituted, L-amino acids in place of the N-methyl alanine moiety in the parent compound. In recently published crystal structures of acyldepsipeptides bound to ClpP, either the N-methyl alanine of enopeptin B or the pipecolate moiety of ADEP 4 are in close proximity to Tyr112 and Leu189 of ClpP.<sup>16</sup> It stands to reason that modification of the peptidolactone with bulkier amino acids at these positions would result in unfavorable steric interactions with the aforementioned residues

of ClpP. Alternatively, modification of the peptidolactone core structure could result in conformational constraints that preclude the formation of key hydrogen bonds to Ser60 and Tyr62 of ClpP.<sup>16</sup> It is not clear which of these two possibilities explain the decreased activity of our analogs (**10e**–**h**) with  $\alpha$ , $\alpha$ -disubstituted amino acids. In any case, the derivative of **10a** with 4-methylpipecolate in place of pipecolate was highly active. This compound, **10c**, had fourfold lower MIC values against *E. faecalis* than **10a**. This observation indicates that increasing the conformational rigidity of the macrocycle does indeed lead to improved antibacterial activity in vitro. It is highly likely that the replacement of the pipecolate of ADEP 4 with 4-methyl pipecolate will enhance its antibacterial activity. It remains to be seen if such changes will improve pharmacological activity in vivo.

The molecular mechanism of action of the enopeptins and derivatives thereof has been described in great detail.<sup>7,11,16</sup> From studies of *Bacillus subtilis* (a non-pathogenic model bacterium), it was evident that an early effect of these antibacterial compounds was inhibition of cell division. The nominal differences between the MICs and MBCs that we observed are indicative of bactericidal activity.<sup>33</sup> This is especially meaningful with regard to VRE, since current treatments involve mostly bacteriostatic drugs, and few compounds have been found that are bactericidal against VRE.<sup>34</sup> Given this observation, the enopeptin analogs hold great promise for use in the treatment of VRE infections.

### 4. Conclusion

A number of highly conformationally restricted enopeptin analogs were synthesized via isocyanide-based multicomponent reactions. Two strategies were explored: one involving the replacement of the *N*-methyl alanine moiety in the peptidolactone with  $\alpha, \alpha$ -disubstituted amino acids, and the other involving the replacement of this residue with a substituted pipecolic acid. The latter strategy was found to be successful, yielding a derivative that has better in vitro activity than the compound with pipecolic acid. This highlights the utility of conformational analysis in the medicinal chemistry optimization of the enopeptin antibiotics. The optimized compound shows especially promising activity against VRE in vitro, and additional assays are being carried out to further investigate this observation.

# 5. Experimental

# 5.1. General

All amino acids and peptide coupling reagents were purchased from NovaBiochem. Additional chemicals were purchased from Sigma-Aldrich unless otherwise stated. A Biotage Initiator microwave reactor was used. GC-MS analysis was performed on a Hewlett Packard 5971A GC-MS system using an HP5-MS column and helium as the carrier gas. Splitless injections of 1 µL were made using an initial temperature of 60 °C, holding 2 min, then 20 °C/ min ramp to 280 °C, and holding 2 min. Chiral GC-MS was performed on a Hewlett Packard G1800C instrument equipped with a Varian CP-Chirasil-DEX 25 m  $\times$  0.32 mm column. Low-resolution analytical LC-MS was performed in the positive ion mode on a Thermo LCQ Deca XP MAX high sensitivity MSn ion trap mass spectrometer with a Shimadzu HPLC system using a Waters X-Terra MS  $C_{18}$  column (2.5  $\mu m$ , 2.1  $\times$  50 mm). The method consisted of 5–95% MeCN in  $H_2O + 0.1\%$  TFA over 15 min. The flow rate was 0.2 mL/ min. High-resolution mass analyses were performed on a JOEL JMS-600H double focusing magnetic sector mass spectrometer using FAB ionization. NMR analyses were performed on a Bruker Avance Ultrashield spectrometer (400 MHz for <sup>1</sup>H, 100 MHz for <sup>13</sup>C). All spectra were referenced to residual solvent signals in CDCl<sub>3</sub> (7.24 ppm for <sup>1</sup>H, 77.0 ppm for <sup>13</sup>C.)

#### 5.2. Synthesis

5.2.1. Preparation of substrates for multicomponent reactions 5.2.1.1. N-Formyl-L-alanine methyl ester (1). Dicyclohexylcarbodiimide (DCC) (10 g, 48.5 mmol) was dissolved in 50 mL of DCM and cooled to 0 °C for 5 min. 96% formic acid (2.6 mL, 69 mmol) was added slowly. In a separate flask, L-alanine methyl ester HCl salt (5 g, 36 mmol) was dissolved in 25 mL dichloromethane (DCM) with 7 mL *N*-methylmorpholine (NMM) and 1 g of DMAP. The contents of this flask were added to the DCC-formic acid mixture at 0 °C and the reaction was allowed to warm to room temperature overnight with stirring. The majority of the solvent was removed in vacuo until a viscous slurry was obtained. This slurry was vacuum filtered through silica gel and the residue was washed with 25 mL DCM to ensure complete elution of product. The filtrate was concentrated in vacuo and loaded on a  $75 \times 50$  mm Si gel resin bed and eluted with 4:1 EtOAc/hexanes. Yield: 3.4 g (72.1%), clear oil,  $R_f = 0.6$  (4:1 EtOAc/hexanes), stains white with p-anisaldehyde. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.14 (1H, s), 6.43 (1H, br s), 4.54 (1H, m), 3.72 (3H, s), 1.39 (3H, d, J = 7 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 173.0, 160.5, 52.6, 46.7, 18.4. MS (EI, m/z): 131.0  $[M]^+$  for C<sub>4</sub>H<sub>9</sub>NO<sub>2</sub>.

5.2.1.2. Isocyanide derived from alanine methyl ester (2). N-formyl-L-alanine methyl ester (1) (2.2 g, 16.8 mmol) was dissolved in 50 mL anhydrous DCM and the solution was cooled to -78 °C. In a separate, dry, pear shaped flask was added triphosgene (1.7 g, 5.7 mmol) and 7 mL dry DCM. The triphosgene solution was added slowly to the N-formyl amino acid ester. Then, 3.6 mL (33 mmol) NMM added slowly over 10 min. The reaction was stirred for 1.5 h at -78 °C then guenched with 22 mL deionized water with a vent needle in the septum. The stirring solution was allowed to warm to room temperature. The resulting mixture was partitioned and the water laver washed with an additional 50 mL DCM. The organic fraction was collected, dried over Na<sub>2</sub>SO<sub>4</sub>, and solvent removed in vacuo. Crude product was loaded on a  $75 \times 50$  mm Si gel resin bed and eluted with 4:1 hexanes/EtOAc ( $R_f = 0.65$ , Iodine/ Si stain). Pale yellow oil, Yield: 1.8 g (95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 4.32 (1H, q), 3.81 (3H, s), 1.63 (3H, d, *J* = 8 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 167.3, 159.0, 60.0, 51.3, 19.3. MS (EI, *m/z*): 113.0 [M]<sup>+</sup> for C<sub>5</sub>H<sub>7</sub>NO<sub>2</sub>.

**5.2.1.3.** *N*-Chloropiperidine. 5.8 mmol (0.55 mL) of *tert*-BuOH, 11.7 mmol (1.39 mL) piperidine were added to 30 mL MTBE and stirred at 0 °C. 11.8 mmol (23.5 mL) cleaning bleach and 11.8 mmol (0.68 mL) of acetic acid were added together slowly and reaction mixture was stirred at 0 °C for 30 min. Reaction mixture was then quenched with 15 mL of water, extracted with 20 mL MTBE, and the organic layer was washed with brine and dried with Na<sub>2</sub>SO<sub>4</sub>. Evaporation of solvent under reduced pressure gave the desired product 1.4 g (10.5 mmol, 89% yield) as a pale yellow oil.  $R_f = 0.9$  (3:1 EtOAc/hexanes) MS (EI, *m/z*): 119.1 [M]<sup>+</sup> for C<sub>5</sub>H<sub>10</sub>NCl.

**5.2.1.4. Dehydropiperidine.** 10.5 mmol (1.4 g) of *N*-chloropiperidine was dissolved in 3.5 mL (10.91 mmol) of 25% by wt NaOMe in MeOH. The reaction was stirred for 10 min under N<sub>2</sub>, after which a white precipitate of NaCl was formed and the reaction was complete.

#### 5.2.2. General procedure for the Joullié-Ugi 3CR

The crude cyclic imines, 4 mL dry MeOH and *N*-Boc-L-proline (1.95 g, 8.5 mmol) were added directly to the reaction mixture, and were stirred for 15 min. The isocyanide 2 (780 mg, 6.9 mmol) was added, and the reaction stirred for 2–5 days. Solvent was

removed under vacuum and crude mixture was purified on a Si gel column with 7:3 EtOAc/hexanes eluent.

Diastereomeric ratio determinations of the saponified Joullié-Ugi 3CR products were determined using a Haisil-100 C18 column (5 micron,  $150 \times 4.6$  mm). The flow rate was 1.5 mL/min. The method consisted of 20–65% MeCN in H<sub>2</sub>O + 0.1% TFA over 20 min. The detector was set to 214 nm. The retention times of the diastereomeric products were 7.63 and 8.58 min. The retention time of authentic *N*-Boc-L-Pro-L-Pip-L-Ala-COOH, prepared according to the literature,<sup>8</sup> was 7.65 min.

**5.2.2.1.** *N*-Boc-Pro-pipecolate-Ala-OMe (3). Yield: 2.16 g (5.2 mmol, 76%).  $R_f = 0.3$  (7:3 EtOAc/hexanes) <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of diasteromers) 8.45 (d, 0.5H), 7.38, (d, 0.5 H), 4.52 (m, 1H), 4.48 (m, 1H), 4.42 (m, 1H), 3.85 (d, 0.5H), 3.67 (s, 3H), 3.44 (1H, m), 3.40 (1H, m), 2.45 (1H, m), 2.08 (2H, m), 1.87 (3H, m), 1.64 (2H, m) 1.35 (12H, s), 0.99 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 173.3, 172.1, 170.0, 154.4, 79.6, 55.4, 52.0, 49.9, 48.1, 46.7, 43.4, 39.4, 34.4, 29.3, 28.0 (3C), 25.7, 24.5, 20.3, 16.6. HRMS (FAB): calcd for  $C_{20}H_{33}N_3O_6Na$  [M+Na]<sup>+</sup> 434.2267, obsd 434.2267 ( $\varDelta = 5.8$  ppm).

**5.2.2. N-Boc-Pro-4-methylpipecolate-Ala-OMe (4).** Yield: 2.47 g (5.8 mmol, 84%).  $R_f = 0.3$  (7:3 EtOAc/hexanes) <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of diasteromers): 8.39 (d, 0.5H), 7.28, (d, 0.5 H), 4.55 (m, 1H), 4.48 (m, 1H), 4.40 (m, 1H), 3.85 (d, 0.5H), 3.65 (s, 3H), 3.49 (1H, m), 3.41 (1H, m), 2.43 (1H, m), 2.05 (2H, m), 1.83 (3H, m), 1.64 (2H, m) 1.38 (12H, s), 0.99 (m, 2H), 0.85 (3H, d). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 173.4, 172.1, 170.1, 154.6, 79.8, 56.4, 52.2, 48.5, 47.0, 43.4, 34.4, 33.1, 29.5, 29.4, 28.4 (3C), 27.1, 24.9, 21.8, 16.9. HRMS (FAB): calcd for C<sub>21</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> 448.2424, obsd 448.2406 ( $\Delta$  = 4.1 ppm).

#### 5.2.3. General procedure for the Ugi 4CR

Each ketone (9.48 mmol) was added to a solution of 40% methylamine in MeOH (4.75 mL, 9.48 mmol) and allowed to precondense for 1.5 h. *N*-Boc-L-proline (2.04 g, 9.48 mmol) was added and the resulting mixture stirred for 15 min, then isocyanide derived from L-alanine methyl ester (1.08 g, 9.48 mmol) was added. The reaction was stirred 5 days at room temperature, then concentrated in vacuo. Crude product was loaded onto a  $80 \times 50$  mm Si gel resin bed and eluted with 7:3 EtOAc/hexanes. By TLC, all Ugi products had an *R*<sub>f</sub> of approximately 0.2 (7:3 EtOAc/hexanes) and stained white with a *p*-anisaldehyde reagent.

**5.2.3.1. 3-Pentanone Ugi tripeptide (5).** Yield 3.03g (7.11 mmol, 75%). Yellow oil <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 6.35 (1H, d), 4.59 (1H, m), 3.70 (3H, s), 3.50 (1H, m), 3.45 (1H, m), 3.08 (3H, s), 2.23 (2H, m), 2.10 (2H, m), 1.85 (4H, m), 1.48 (3H, d), 1.38 (9H, s), 0.87 (3H, m) 0.78 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 173.9, 173.6, 172.9, 157.1, 154.7, 79.6, 68.7, 68.2, 58.0, 57.7, 52.0, 50.5, 48.5, 46.9, 32.5, 29.2, 28.5 (3C), 26.1, 24.2, 24.0, 17.7. HRMS (FAB): calcd for  $C_{21}H_{37}N_3O_6Na [M+Na]^+$  450.2580, obsd 450.2566 ( $\Delta = 3.1$  ppm).

**5.2.3.2. Cyclobutanone Ugi tripeptide (6).** Yield: 3.62 g (8.81 mmol, 93%). Yellow oil <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 7.65 (1H, d), 4.42 (2H, m), 3.52 (3H, s), 3.45 (1H, m), 3.40 (1H, m), 2.87 (3H, s), 2.54 (4H, m), 2.03 (3H, m), 1.65 (2H, m), 1.54 (1H, m), 1.38 (3H, d), 1.28 (9H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 173.7, 173.3, 173.2, 154.7, 153.6, 79.7, 65.6, 60.5, 56.7, 53.6.0, 52.2, 48.1, 46.9, 33.2, 31.8, 28.5 (3C), 24.5, 23.4, 17.5, 14.5. HRMS (FAB): calcd for C<sub>20</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> 434.2267, obsd 434.2282 ( $\Delta = 3.4 \text{ ppm}$ ).

**5.2.3.3.** Cyclopentanone Ugi tripeptide (7). Yield: 3.12 g (7.33 mmol, 78%). Yellow oil <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 7.65 (1H, d), 4.54 (1H, m), 4.40 (1H, m), 3.65 (3H, s), 3.60 (1H, m),

3.45 (1H, m), 3.00 (3H, s), 2.52 (1H, m), 2.11–1.52 (12H, m) 1.43 (9H, s), 1.38 (3H, d). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 175.4, 173.6, 171.0, 154.4, 153.5, 79.4, 73.4, 73.0, 60.2, 58.0, 57.9, 52.0, 50.5, 48.2, 46.9, 36.2, 35.5, 34.8, 33.0, 29.2, 28.3 (3C), 24.2, 24.0, 23.1, 22.4, 17.6, 14.1. HRMS (FAB): calcd for  $C_{21}H_{35}N_3O_6Na$  [M+Na]<sup>+</sup> 448.2424, obsd 448.2432 ( $\Delta$  = 1.8 ppm).

**5.2.3.4. Cyclohexanone Ugi tripeptide (8).** Yield: 3.75 g (8.53 mmol, 90%). Yellow oil <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 6.72 (1H, m), 4.51 (1H, m), 4.37 (1H, m) 3.54 (3H, s), 3.45 (1H, m), 3.35 (1H, m), 2.98 (3H, s), 2.11–1.52 (14H, m) 1.43 (9H, s), 1.38 (3H, d). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 175.6, 173.7, 171.1, 154.5, 79.4, 66.6, 65.8, 60.2, 58.6, 52.2, 48.1, 46.7, 32.9, 32.4, 31.8, 30.4, 29.5, 28.5 (3C), 24.2, 22.8, 21.9, 20.9, 18.1, 17.7, 14.1. HRMS (FAB): calcd for C<sub>22</sub>H<sub>37</sub>N<sub>3</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> 462.2580, obsd 462.2562 ( $\Delta$  = 3.9 ppm).

### 5.2.4. General procedure for the saponification of tripeptides

The tripeptide methyl esters (1.55 g, 3.62 mmol) were dissolved in 10.4 mL of 1:1 THF/H<sub>2</sub>O solution and LiOH·H<sub>2</sub>O (356 mg, 8.47 mmol) was added. The reaction was stirred for 2.5 h then quenched with 10 mL 1 N HCl. THF was removed under vacuum and aqueous layer was extracted with 2  $\times$  20 mL EtOAc. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum.

# 5.2.5. Synthesis of *N*-2-heptenoyl-3-fluorophenylalanine-COOH (9)

N-Boc-L-3-fluorophenyl-L-alanine (500 mg, 1.76 mmol) and NaHCO<sub>3</sub> (300 mg, 3.6 mmol) were dissolved in 10 mL DMF. MeI (1 mL, 16 mmol) was added dropwise and the mixture stirred for 24 h. The reaction mixture was then partitioned in 25 mL EtOAc 25 mL 0.1 N HCl and 25 mL brine, then dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. Yield: 500 mg (1.68 mmol, 96%). N-Boc-L-3fluorophenyl-L-alanine methyl ester (500 mg, 1.7 mmol) was dissolved in 6.75 mL DCM and 4.3 mL 9:1 TFA/H<sub>2</sub>O was added at 0 °C. The reaction was allowed to warm up to room temperature and stirred for 2 h, then  $2 \times 100$  mL toluene successively added and removed in vacuo. Yield: 510 mg of TFA salt of L-3-fluorophenyl-L-alanine methyl ester (1.63 mmol, 93%). The product (650 mg, 2.09 mmol) was dissolved in 25 mL of DCM and then 3 mL of DIEA was added. The mixture was transferred to a separate flask containing HATU (1.14 g, 3 mmol) and trans-2-heptenoic acid (350 µL, 2.6 mmol) in 15 mL DCM. The reaction was stirred overnight. The reaction mixture was then washed with 30 mL 0.1 N HCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum, then purified by flash Si gel chromatography with 1:1 EtOAc/hexanes as eluent. *R*<sub>f</sub> = 0.95 (1:1 EtOAc/hexanes).

*N*-2-heptenoyl-3-fluorophenylalanine methyl ester (400 mg, 1.3 mmol) was dissolved in 4 mL of 1:1 THF/H<sub>2</sub>O, then LiOH·H<sub>2</sub>O (149 mg, 3.55 mmol) was added and the reaction stirred for 30 min. 1 N HCl was added to adjust the pH to 1, THF was removed under vacuum and the resulting aqueous layer was extracted with  $2 \times 20$  mL EtOAc. The organic layer was then dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. Yield: 400 mg (100%) of **9**. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 7.18 (1H, m), 6.88 (4H, m), 6.37 (1H, d, *J* = 8 Hz), 5.77 (1H, d, *J* = 16 Hz), 4.91 (1 H, d, *J* = 8 Hz), 3.22 (1H, m) 3.12, (1H, m), 2.13 (2H, m), 1.33 (4H, m), 0.86, (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 174.2, 167.0, 164.1, 161.7, 147.5, 138.5, 130.3, 125.4, 122.7, 116.6, 114.4, 53.7, 37.1, 32.0, 30.2, 22.3, 13.9. HRMS (FAB): calcd for C<sub>16</sub>H<sub>20</sub>NO<sub>3</sub>FNa [M+Na]<sup>+</sup> 316.1325, obsd 316.1306 ( $\Delta$  = 6.0 ppm).

# 5.2.6. General procedure for synthesis of acyldepsipeptides (10a-h)

**5.2.6.1. Linear pentapeptolide formation and deprotec-tion.** Each tripeptide (3.9 mmol), TPTU (1.45 g, 4.88 mmol), HOBt

(770 mg, 5.7 mmol) and DIEA (2 mL, 11.5 mmol) were dissolved in 20 mL dry DCM and allowed to stir under N<sub>2</sub>, at rt for 10 min. Depsipeptide (NH<sub>2</sub>-Pro-N-CBz-Ser-phenacylester)<sup>8</sup> (2.2 g, 3.9 mmol) was dissolved in 10 mL dry DCM and added dropwise over 5 min. After 15 h, the reaction mixture was concentrated in vacuo and then redissolved in 50 mL EtOAc. The EtOAc solution was extracted sequentially with 20 mL each of 0.2 N HCl, saturated NaHCO<sub>3</sub> and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The product was purified over a 110x 55 mm bed of silica gel using 20:0.5 EtOAc/MeOH. By TLC, all pentapeptolide products had an  $R_{\rm f}$  of approximately 0.3 (20:0.5 EtOAc/methanol) and stained blue with ceric ammonium molybdenate.

Phenacyl pentapeptolides (1.3 mmol) were deprotected by dissolving in 1.45 mL 90% AcOH in H<sub>2</sub>O, then Zn dust (580 mg, 9.07 mmol) added and reaction stirred for 30 min. Zn was filtered through cotton and residue washed with 10 mL EtOAc and 10 mL 1 N HCl. 30 mL EtOAc was added to the filtrate and it was washed with 3 × 20 mL 1 N HCl. Organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. Yield: 945 mg (100%).  $R_{\rm f}$  = 0.2 (20:1 EtOAc/MeOH).

5.2.6.2. Macrocyclization. Pentafluorophenol esters were prepared from phenacyl deprotected linear peptides (1.37 mmol), by dissolving in 5.25 mL dry DCM and adding pentafluorophenol (1 g, 5.43 mmol) and EDC (285 mg, 1.64 mmol) at -78 °C. The reaction was allowed to warm to room temperature overnight with stirring, then concentrated under vacuum. Crude pentafluorophenol esters (approximately 2 g) were Boc deprotected by treatment with 8.35 mL 3 N HCl in EtOAc at 0 °C and stirred at 0 °C for 3.5 h. For cyclization, the crude reaction mixture was diluted into 417 mL DCM and added dropwise at a rate of  $\sim$ 1 drop/s to a vigorously stirring solution of 324 mL satd NaHCO3 and 557 mL DCM. The reaction was left to stir overnight. The organic layer was collected and dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo, then purified over a  $100 \times 25$  mm Si gel column with 20:1 EtOAc/MeOH eluent. In the case of diastereomers derived from **3** and **4**, the compound with an  $R_f = 0.3$  is (2S)-pipecolate and the compound with an  $R_f = 0.2$  is (2R)-pipecolate.

Spectral data for S-Pip-CBz-macrocycle derived from **3**: <sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.30 (1H,d, *J* = 8 Hz), 7.28 (4H, m), 5.68 (1H, d, *J* = 8 Hz), 5.15 (1H, m), 5.03 (2H, m), 4.99 (1H, m), 4.74 (1H, d, *J* = 8 Hz), 4.66 (1H, s), 4.49 (2H, m), 4.17 (1H, t), 4.06 (1H, dd), 3.72 (2H, m), 3.65 (1H, m), 3.50 (2H, m), 2.68 (1H, d, *J* = 12 Hz), 2.52 (1H, m), 2.31 (1H, m), 2.11 (3H, m), 1.91 (7H, m), 1.68 (1H, m), 1.58 (1H, m), 1.36 (5H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 173.3, 171.5, 169.6, 168.5, 166.2, 156.2, 135.9, 128.5, 128.3 (3H), 128.0, 67.9, 65.8, 60.3, 58.9, 57.1, 56.6, 53.9, 53.4, 48.0, 46.8, 46.3, 41.0, 30.9, 30.6, 28.2, 24.8, 23.1, 21.3, 18.0, 14.1. HRMS (FAB): calcd for  $C_{30}H_{39}N_5O_8$ . Na [M+Na]<sup>+</sup> 620.2696, obsd 620.2681 ( $\Delta$  = 2.4 ppm).

Spectral data for S-4-Me-Pip-CBz-macrocycle derived from **4**: <sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.31 (1H,d, J = 8 Hz), 7.28 (4H, m), 5.63 (1H, d, J = 8 Hz), 5.17 (1H, m), 5.03 (2H, m), 4.99 (1H, m), 4.76 (1H, d, J = 8 Hz), 4.67 (1H, s), 4.50 (2H, m), 4.19 (1H, t), 4.08 (1H, dd), 3.72 (2H, m), 3.66 (1H, m), 3.49 (2H, m), 2.68 (1H, d, J = 12 Hz), 2.53 (1H, m), 2.31 (1H, m), 2.12 (3H, m), 1.94 (7H, m), 1.56 (2H, m), 1.36 (3H, m), 1.02 (2H, m), 0.96 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 173.2, 171.5, 169.7, 168.6, 166.2, 156.2, 135.8, 128.4, 128.3 (3H), 128.0, 67.9, 65.8, 60.3, 58.8, 57.1, 56.7, 53.9, 48.0, 46.9, 46.3, 40.7, 36.3, 33.1, 30.8, 30.5, 27.9, 23.0, 21.7, 21.2, 17.9. HRMS (FAB): calcd for C<sub>30</sub>H<sub>39</sub>N<sub>5</sub>O<sub>8</sub>Na [M+Na]<sup>+</sup> 634.2853, obsd 634.2868 ( $\Delta = 2.4$  ppm).

**5.2.6.3. Deprotection of macrocycles.** Cbz-protected macrocycles (0.16 mmol) were deprotected using 75 mg 10% Pd/C and 100 mg NH<sub>4</sub>COOH dissolved in 4 mL isopropanol heated in a microwave reactor for 8 min at 85 °C.<sup>35</sup> The reaction mixture was

then filtered through celite, the residue was washed with MeOH and the filtrate was concentrated under vacuum. The CBz deprotected macrocycles (0.1 mmol) were coupled to **9** (44 mg, 0.15 mmol) in 800  $\mu$ L DMF containing TPTU (50 mg, 0.17 mmol), HOBt (20 mg, 0.15 mmol), and 60  $\mu$ L diisopropylethyamine. The reaction was stirred overnight at room temperature.

Purification of the acyldepsipeptides (**10a**–**h**) was accomplished using a Phenomenex Gemini C18 (10 micron,  $150 \times 10$  mm, 110 Å). The method consisted of 20–80% MeCN in H<sub>2</sub>O + 0.1% TFA over 20 min. The detector was set to 214 nm. The flow rate was 10 mL/min.

**5.2.6.4. Acyldpesipeptide 10a.** Isolated yield from deprotected peptidolactone: 25 mg (0.034 mmol, 34%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.55 (1H, d, *J* = 8 Hz), 7.26 (0.5H, m), 7.22 (0.5H, m), 6.99 (1H, m), 6.89 (3H, m), 6.52 (1H, d, *J* = 12 Hz), 6.17 (1H, d, *J* = 16 Hz), 5.12 (1H, m), 4.98 (1H, m), 4.75 (1H, d, *J* = 12 Hz), 4.67 (2H, m), 4.55 (1H, m), 4.48 (2H, m), 3.73 (1H, m), 3.61 (1H, m), 3.51 (2H, m), 3.30 (1H, m), 2.94 (2H, m), 2.71 (1H, m), 2.62 (1H, m), 1.40 (1H, m), 1.38 (3H, m), 1.32 (4H, m), 0.88 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 172.5, 171.2, 170.9, 169.4, 166.4, 165.0, 164.0, 161.5, 146.2, 138.6, 130.0, 125.2, 123.1, 116.5, 116.4, 113.8, 113.6, 65.0, 59.0, 57.1, 56.8, 54.7, 51.2, 47.8, 47.0, 46.4, 41.3, 38.4, 31.8, 30.7, 30.4, 28.0, 24.9, 23.1, 22.2, 21.3, 18.0, 13.8. HRMS (FAB): calcd for C<sub>38</sub>H<sub>51</sub>N<sub>6</sub>O<sub>8</sub>FNa [M+Na]<sup>+</sup> 761.3650, obsd 761.3625 ( $\Delta$  = 3.3 ppm).

**5.2.6.5. Acyldpesipeptide 10b.** Isolated yield from deprotected peptidolactone: 26 mg (0.035 mmol, 35%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 8.03 (1H, m), 7.45 (1H, m), 7.18 (1H, m), 6.99 (4H, m), 6.85 (1H, m), 6.75 (1H, m), 6.56 (0.6H, m), 5.90 (1H, d), 5.80 (1H, d), 5.38 (1H, d), 5.30 (2H, m), 4.92 (3H, m), 4.76 (2H, m), 4.37 (1H, m), 4.33 (1 H, m), 3.97 (1H, m), 3.80 (1H, m), 2.65 (1H, m), 2.10 (4H, m), 2.01 (10H, m), 1.52 (2H, m), 1.45 (4H, m), 1.38 (3H, m), 0.85 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 172.2, 170.9, 170.3, 167.7, 167.3, 166.0, 163.8, 161.1, 129.7, 129.6, 125.0, 123.3, 116.3, 113.3, 61.2, 60.0, 56.1, 53.1, 48.5, 47.5, 47.2, 46.3, 44.7, 31.7, 30.1, 28.8, 28.7, 26.2, 25.6, 25.5, 25.0, 22.1, 20.6, 17.3, 13.4. HRMS (FAB): calcd for C<sub>38</sub>H<sub>51</sub>N<sub>6</sub>O<sub>8</sub>FNa [M+Na]<sup>+</sup> 761.3650, obsd 761.3638 ( $\Delta$  = 1.6 ppm).

**5.2.6.6. Acyldpesipeptide 10c.** Isolated yield from deprotected peptidolactone: 23.4 mg (0.034 mmol, 31%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.89 (1H, br s), 8.51 (1H, d, J = 12 Hz), 8.42 (0.5H, br s) 7.93 (0.5H, br s), 7.16 (1H, m), 6.88 (4H, m), 6.20 (1H, d, J = 16 Hz), 5.14 (1H, m), 4.98 (1H, m), 4.73 (3H, m), 4.67 (1H, m), 4.50 (2H, m), 3.75 (1H, m), 3.55 (3H, m), 3.30 (1H, m), 2.94 (2H, m), 2.70 (2H, m), 2.31 (1H, m), 2.14 (3H, m), 1.88 (5H, m), 1.62 (2H, m), 1.38 (3H, m), 1.30 (5H, m), 0.94 (3H, m), 0.86 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 172.5, 171.1, 170.8, 169.6, 167.1, 165.1, 164.0, 161.6, 147.0, 138.4, 130.2, 125.2, 122.9, 116.5, 114.0, 65.0, 59.1, 57.1, 57.2, 57.0, 55.2, 51.1, 47.9, 47.1, 46.6, 41.0, 38.4, 36.2, 33.3, 31.9, 30.8, 30.4 (2C), 28.0, 23.1, 22.2, 21.9, 18.0, 13.8. HRMS (FAB): calcd for C<sub>39</sub>H<sub>53</sub>N<sub>6</sub>O<sub>8</sub>FNa [M+Na]<sup>+</sup> 775.3807, obsd 775.3820 ( $\Delta = 1.7$  ppm).

**5.2.6.7. Acyldpesipeptide 10d.** Isolated yield from deprotected peptidolactone: 22.1 mg (0.03 mmol, 30%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 8.02 (1H, m), 7.74 (1H, br s), 7.51 (2H, m), 7.16 (1H, m), 6.96 (4H, m), 6.74 (1H, m), 5.96 (1H, m), 5.35 (0.5H, m), 5.30 (1H, m), 5.20 (0.5H, m), 4.82 (3H, m), 4.75 (1H, m), 4.40 (1H, m), 4.22 (1H, m), 4.01 (1H, m), 3.56 (3H, m), 3.25 (2H, m), 2.94 (1H, m), 2.62 (1H, m), 2.25 (3H, m), 2.02 (6H, m), 1.86 (5H, m), 1.62 (1H, m), 1.38 (3H, m), 1.30 (4H, m), 0.93 (3H, m), 0.85

(3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 172.1, 171.8, 171.3, 170.5, 170.2, 168.8, 161.5, 145.4, 140.2, 129.7, 124.8, 123.4, 116.4, 116.1, 113.4, 61.0, 60.3, 57.6, 56.6, 54.2, 53.3, 50.9, 47.5, 47.2, 46.4, 44.4, 40.9, 38.0, 33.9, 33.2, 32.8, 31.9, 30.4, 29.7, 28.9, 28.2, 27.3, 26.2, 25.7, 22.2, 21.7, 18.1, 15.1, 13.9. MS (ESI, *m/z*): 775.8 [M+Na]<sup>+</sup> for C<sub>39</sub>H<sub>53</sub>N<sub>6</sub>O<sub>8</sub>F.

**5.2.6.8.** Acyldpesipeptide 10e. Isolated yield from deprotected peptidolactone: 22.0 mg (0.037 mmol, 37%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 7.69 (1H, d), 6.99 (1H, d), 7.18 (1H, m), 6.94 (4H, m), 6.75 (1H, m), 5.94 (1H, d), 5.75 (1H, m), 5.58 (1H, m), 5.02 (1H, m), 4.90 (1H, m), 4.80 (2H, m), 4.62 (2H, m), 4.00 (1H, m), 3.70 (3H, m), 3.60 (2H, m), 3.28 (2H, m), 2.92 (1H, m), 2.60 (1H, m), 2.27 (3H, m), 2.02 (6H, m), 1.86 (5H, m), 1.72 (1H, m), 1.38 (3H, m), 1.30 (4H, m), 0.93 (3H, m), 0.85 (3H, m). MS (ESI, *m/z*): 777.4 [M+Na]<sup>+</sup> for C<sub>39</sub>H<sub>53</sub>N<sub>6</sub>O<sub>8</sub>F.

**5.2.6.9. Acyldpesipeptide 10f.** Isolated yield from deprotected peptidolactone: 25 mg (0.034 mmol, 34%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 7.52 (1H, d), 7.47 (1H, d), 7.15 (1H, m), 6.82 (4H, m), 5.90 (1H, d, *J* = 16 Hz), 5.30 (1H, d), 4.92 (2H, m), 4.54 (1H, m), 4.40 (1H, m), 4.23, 1H, m), 3.85 (1H, d, *J* = 12 Hz), 3.58 (3H, m), 3.34 (1H, m), 2.91 (3H, s), 2.85 (1H, m), 2.62 (2H, m), 2.06 (4H, m), 1.98 (4H, m), 1.81 (2H, d), 1.23 (4H, m), 0.85 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 172.4, 172.2, 171.0, 166.6, 161.6, 145.5, 140.2, 129.9, 125.3, 123.3, 116.5, 113.6, 66.8, 61.1, 60.2, 58.0, 56.9, 54.3, 51.2, 47.5, 38.0, 34.2, 32.9, 31.9, 30.3, 29.7, 29.4, 28.6, 28.1, 26.4, 26.2, 25.7, 23.3, 22.7, 22.2, 15.6, 15.3, 14.1, 13.9. MS (ESI, *m/z*): 761.3 [M+Na]<sup>+</sup> for  $C_{38}H_{51}N_6O_8F$ .

**5.2.6.10. Acyldpesipeptide 10g.** Isolated yield from deprotected peptidolactone: 23 mg (0.030 mmol, 30%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 7.70 (1H, m), 7.49 (1H, m), 7. 20 (1H, m), 6.97 (2H, m), 6.88 (1H, m), 6.72 (1H, m), 5.92 (1H, d, J = 16 Hz), 5.35 (1H, d, J = 12 Hz), 4.90 (2H, m), 4.83 (1H, m), 4.61 (1H, d), 4.37 (1H, m), 3.86 (1H, d, J = 12 Hz), 3.69 (2H, m), 3.58 (1H, m), 3.49 (1H, m), 3.40 (1H, m), 3.19 (3H, s), 2.91 (1H, m), 2.60 (1H, m), 2.21 (2H, m), 2.10 (2H, m), 2.00 (2H, m), 1.92 (2H, m), 1.33 (3H, m), 0.85 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 174.0, 173.2, 172.6, 171.8, 171.2, 170.2, 167.3, 166.7, 163.9, 161.5, 155.6, 140.5, 129.6, 125.1, 116.4, 113.2, 79.5, 74.6, 61.0, 60.8, 60.0, 59.4, 59.0, 56.2, 51.1, 47.5, 46.1, 38.5, 37.9, 36.9, 35.5, 35.1, 33.4, 31.9, 29.4, 28.2, 27.9, 26.4, 25.8, 25.0, 24.6, 23.5, 22.6, 18.2, 15.6, 14.1. MS (ESI, m/z): 775.6 [M+Na]<sup>+</sup> for C<sub>39</sub>H<sub>55</sub>N<sub>6</sub>O<sub>8</sub>FNa.

**5.2.6.11. Acyldpesipeptide 10h.** Isolated yield from deprotected peptidolactone: 24 mg (0.032 mmol, 32%) white amorphous powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) 7.76 (1H, d, J = 8 Hz), 7.48 (1H, d, J = 4 Hz), 7.20 (1H, m), 6.82 (4H, m), 5.90 (1H, d, J = 16 Hz), 5.36 (1H, d, J = 8 Hz), 4.92 (2H, m), 4.72 (1H, m), 4.37 (1H, m), 3.70 (1H, m), 3.58 (2H, m), 3.39 (2H, m), 3.18 (3H, s), 2.24 (2H, m), 2.05 (6H, m), 1.83 (1H, m), 1.63 (1H, m), 1.38 (3H, m), 0.85 (3H, m). <sup>13</sup>C NMR (CDCl<sub>3</sub>, mixture of rotamers) 174.7, 171.4, 170.7, 170.3, 167.2, 166.4, 145.9, 140.3, 129.7, 124.9, 123.3 (2C), 116.3, 113.4, 69.9, 61.1, 60.2, 60.0, 59.2, 54.4, 51.0, 47.4, 46.4, 40.3, 38.1, 34.5, 33.8, 33.4, 31.9, 29.4, 26.3, 25.7, 23.3, 22.7, 22.2, 18.5, 18.1, 14.2, 13.8. MS (ESI, m/z): 789.6 [M+Na]<sup>+</sup> for  $C_{40}H_{55}N_6O_8FNa$ .

# 5.3. Biological assays

Minimum inhibitory concentrations (MIC) were determined in duplicate using methods outlined by The Clinical and Laboratory Standards Institute. Compounds were dissolved in DMSO and fresh cation-adjusted Mueller–Hinton broth (SMHB; Difco Laboratories, Sparks, MD, USA; 25  $\mu$ g/mL calcium and 12.5  $\mu$ g/mL magnesium) was used to dilute antimicrobial agents in a serial twofold schedule. Colony counts for the inoculum verification were determined using Tryptic Soy Agar plates (TSA, Difco, Becton Dickinson Co., Sparks, MD, USA). Following incubation, 100  $\mu$ L of broth was subcultured onto TSA for MBC determination. MIC and MBC determination can be found in Table 2 and 3. Control isolates were obtained from the American Type Culture Collection (ATCC) and were as follows: Methicillin-resistant *S. epidermidis* (MRSE ATCC 35984); MSSA (ATCC 35556) and, VRE (ATCC 2030).

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#### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.032.

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