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Total Synthesis and Antibacterial Testing of the A54556 Cyclic Acyldepsipeptides Isolated from *Streptomyces hawaiiensis*

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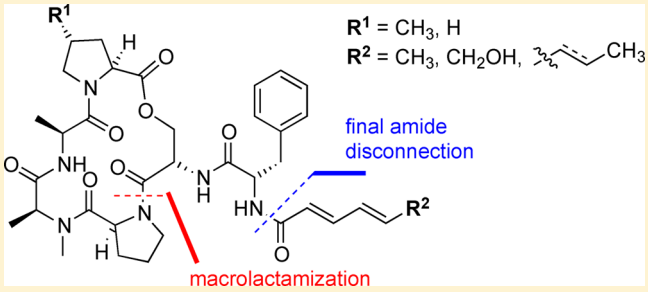
Supporting Information

ABSTRACT: The first total synthesis of all six known A54556 acyldepsipeptide (ADEP) antibiotics from *Streptomyces hawaiiensis* is reported. This family of compounds has a unique mechanism of antibacterial action, acting as activators of caseinolytic protease (ClpP). Assembly of the 16-membered depsipeptide core was accomplished via a pentafluorophenyl ester-based macrolactamization strategy. Late stage amine deprotection was carried out under neutral conditions by employing a mild hydrogenolysis strategy, which avoids the formation of undesired ring-opened depsipeptide side products encountered during deprotection of acid-labile protecting groups. The free amines were found to be significantly more reactive toward late stage amide bond formation as compared to the corresponding ammonium salts, giving final products in excellent yields. A thorough NMR spectroscopic analysis of these compounds was carried out to formally assign the structures and to aid with the spectroscopic assignment of ADEP analogues. The identity of two of the structures was confirmed by comparison with biologically produced samples from *S. hawaiiensis*. An X-ray crystallographic analysis of an ADEP analogue reveals a conformation similar to that found in cocrystal structures of ADEPs with ClpP protease. The degree of antibacterial activity of the different compounds was evaluated *in vitro* using MIC assays employing both Gram-positive and Gram-negative strains and a fluorescence-based biochemical assay.



Despite the achievements of modern antibiotic therapies, a disturbing trend of increased bacterial resistance has prompted a need for the development of novel, effective, and safe antibiotics with new mechanisms of action as a means to combat cross-resistance.[1] Natural products from actinomycetes (soil bacteria), particularly those of the *Streptomyces* genus, have served as a promising source of new structural leads in this area, a trend that will likely continue well into the future.[2] The cyclic depsipeptide class of antibiotics has provided several examples of such compounds, including the antibiotic daptomycin.[3] In addition to their antibiotic activity, naturally occurring cyclic depsipeptides display a broad range of biological activities, and their complex chemical structures serve as challenging total synthesis targets and as medicinal chemistry leads.[4,5] In the early 1980s, scientists from Eli Lilly and Company described the isolation of eight acyldepsipeptides (ADEPs, previously described as A54556 factors A−H) from *Streptomyces hawaiiensis* NRRL 15010, some of which showed notable antibiotic activity against Gram-positive bacteria.[6] Of these eight compounds, only six were structurally assigned (Figure 1, **1**−**6**). In 1991, the group of Isono isolated the structurally related enopeptins A (**7**) and B (**8**) from *Streptomyces* sp. RK-1051.[7] His group was the first to assign the correct configuration and position of the methyl group at C-4 in the *trans*-4-methyl-L-proline in **7**, which for some of the A54556 factors was incorrectly assigned to C-17 with ambiguous stereochemistry. Schmidt and co-workers reported the first total synthesis of enopeptin B (**8**),[8] featuring a pentafluorophenyl ester based macrolactamization strategy.[9]

More recently, ADEPs have gained considerable attention in the medicinal chemistry and biological communities owing to their biologically distinct mechanism of action. Using reversed genomics techniques,[10] scientists at Bayer were the first to identify the biological target of ADEPs as caseinolytic protease (ClpP).[11] ClpP comprises the core proteolytic unit in a large family of bacterial serine protease complexes.[12] There is a wealth of crystal structure data available for ClpP from a large variety of bacterial species.[13] Moreover, the structural changes underlying the ADEP-mediated ClpP activation mechanism have been elucidated using X-ray crystallography, with two cocrystal structures reported for *Bacillus subtilis* ClpP−ADEP[14] and *Escherichia coli* (*E. coli*) ClpP−ADEP complexes.[15] In

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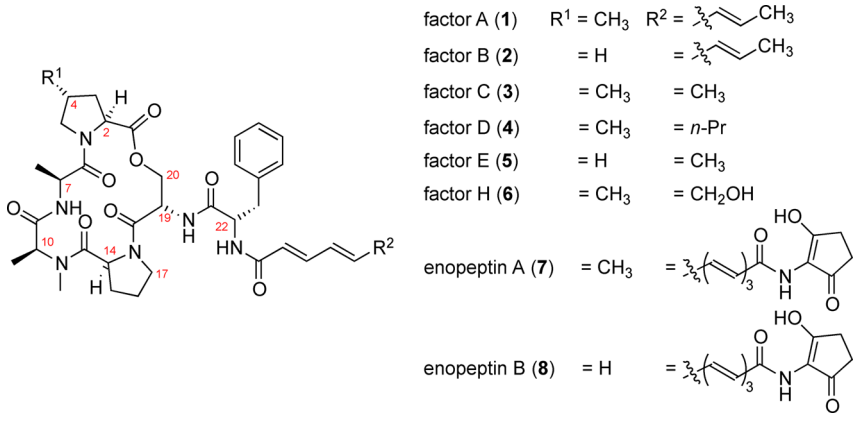
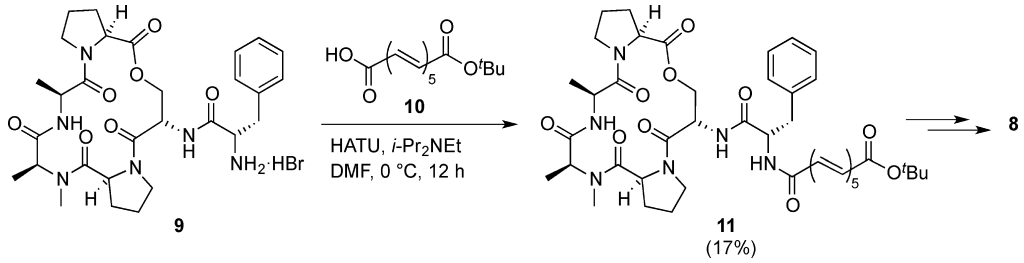
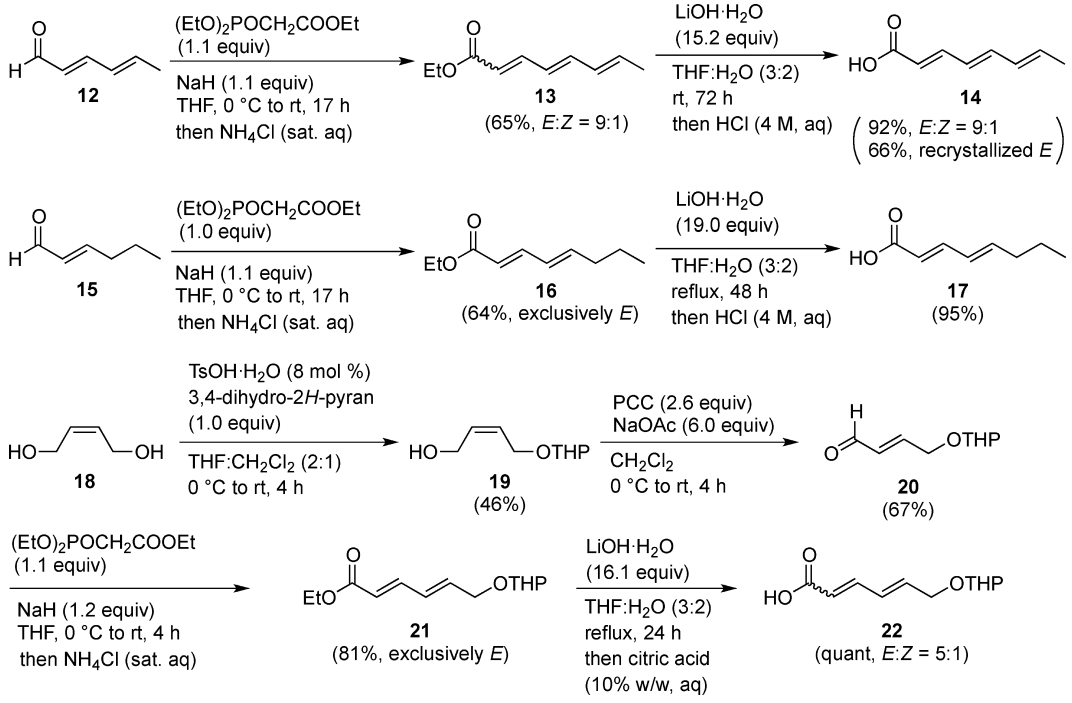


Figure 1. Structures of A54556 factors (**1**–**6**) and enopeptins A (**7**) and B (**8**).

Scheme 1. Late-Stage Amide Coupling in Schmidt's Total Synthesis of Enopeptin B (8)



Scheme 2. Synthesis of Conjugated Enoic Acids 14, 17, and 22



addition to mechanistic studies of ADEPs, QSAR studies have also been reported.[16] More recently, it has been shown that coadministration of ADEPs with rifampicin leads to complete eradication of highly resistant *Staphylococcus aureus* (*Staph. aureus*) biofilms *in vitro* and in mouse models.[17] Furthermore, we and other research groups have discovered small-molecule scaffolds, which have also been explored with respect to their ability to both activate[18] and inhibit[19] ClpP.

Despite all of the literature surrounding the ADEP natural products as structurally important lead compounds, there are to the best of our knowledge no known reports of the total synthesis of the originally assigned A54556 factors (**1**–**6**, Figure 1). In addition, the spectroscopic data[6] that is available for these natural products (and also many of the synthetic intermediates reported in the literature) are limited. A full spectroscopic analysis for the natural products will also facilitate the

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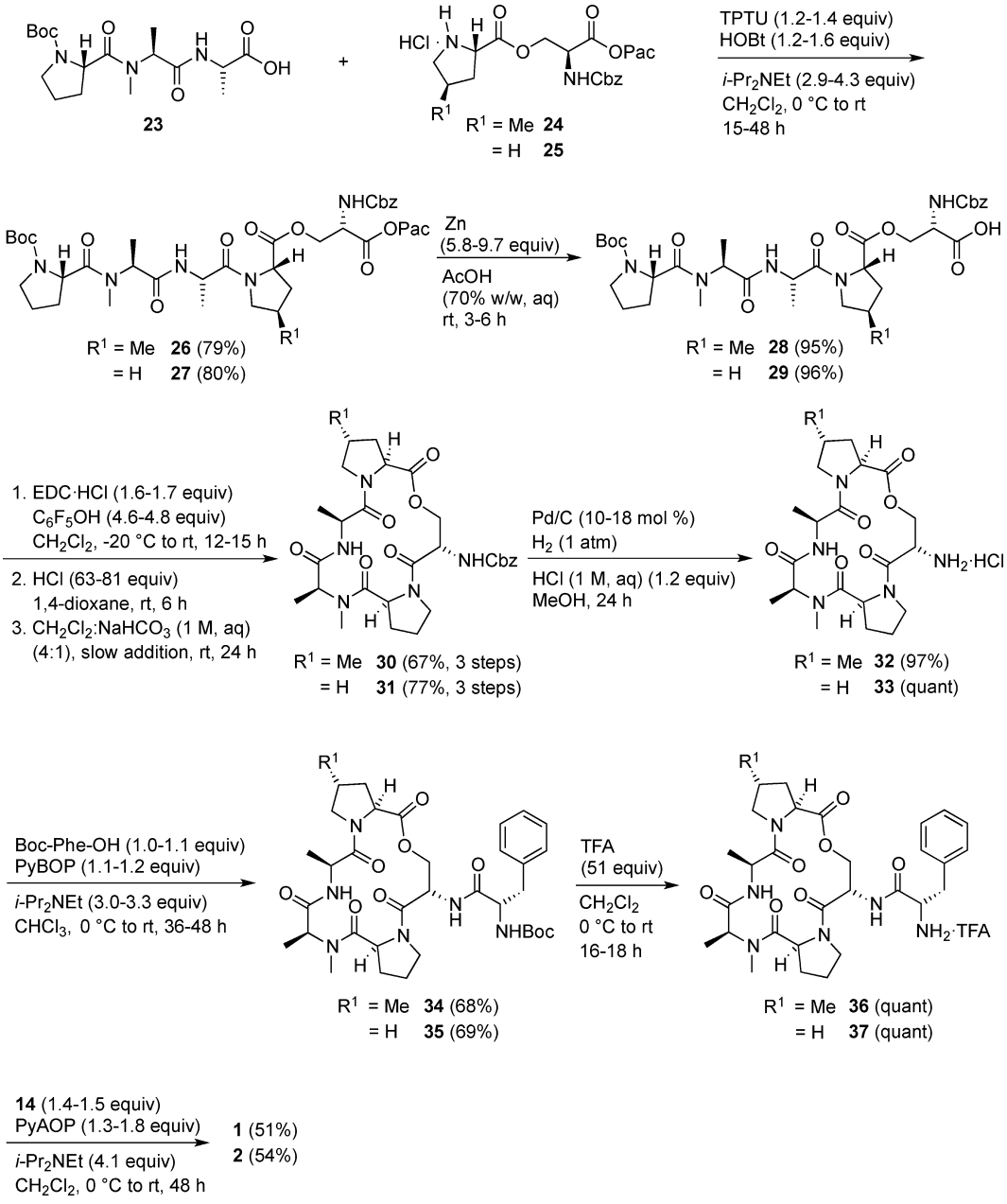
Scheme 3. Initial Strategy to Cyclic Acyldepsipeptide Natural Products 1 and 2



spectroscopic assignment of ADEP analogues and provide information on their solution-phase conformations. The synthetic challenges posed by these compounds include the development of an efficient macrocyclization protocol (a general problem for cyclic peptide/depsipeptide synthesis),[20] as well as identifying an effective strategy for the attachment of the exocyclic acylated amino acid group. The latter problem is exemplified by the serious difficulties encountered in the late-stage amide coupling in Schmidt's total synthesis of enopeptin B (**8**), which proceeded through the coupling of hydrobromide salt **9** with acid **10** to generate the corresponding amide **11** in only 17% yield (Scheme 1).[8] Herein, we now report the total synthesis of all six previously reported A54556 factors (**1**–**6**), including a detailed spectroscopic assignment of these compounds and comparison of **1** and **2** with biologically produced samples from *S. hawaiiensis*. Also included is measurement of the ClpP activation ability of compounds **1**–**6** and their antibacterial effect on Gram-positive and Gram-negative bacteria.

RESULTS AND DISCUSSION

The requisite *trans*-enoic acids (**14**, **17**, and **22**) for the final amide couplings of ADEPs **1**, **2**, **4**, and **6** were furnished through a two-step sequence using Horner–Wadsworth–Emmons (HWE) olefination[21] followed by hydrolysis (Scheme 2). Ester **13** was obtained in 65% yield as an inseparable mixture of geometric isomers (*E*:*Z* = 9:1) from the HWE olefination of sorbic aldehyde **12**. Basic hydrolysis of ester **13** followed by acidic workup afforded acid **14** in 92% yield (geometric purity unchanged). Geometrically pure **14** was obtained in 66% yield by recrystallization from hot chloroform and minimal ethyl acetate. Diene **17** was synthesized in an analogous fashion. The synthetic route to acid **22** utilized *cis*-2-butene-1,4-diol **18** as a precursor for the synthesis of aldehyde

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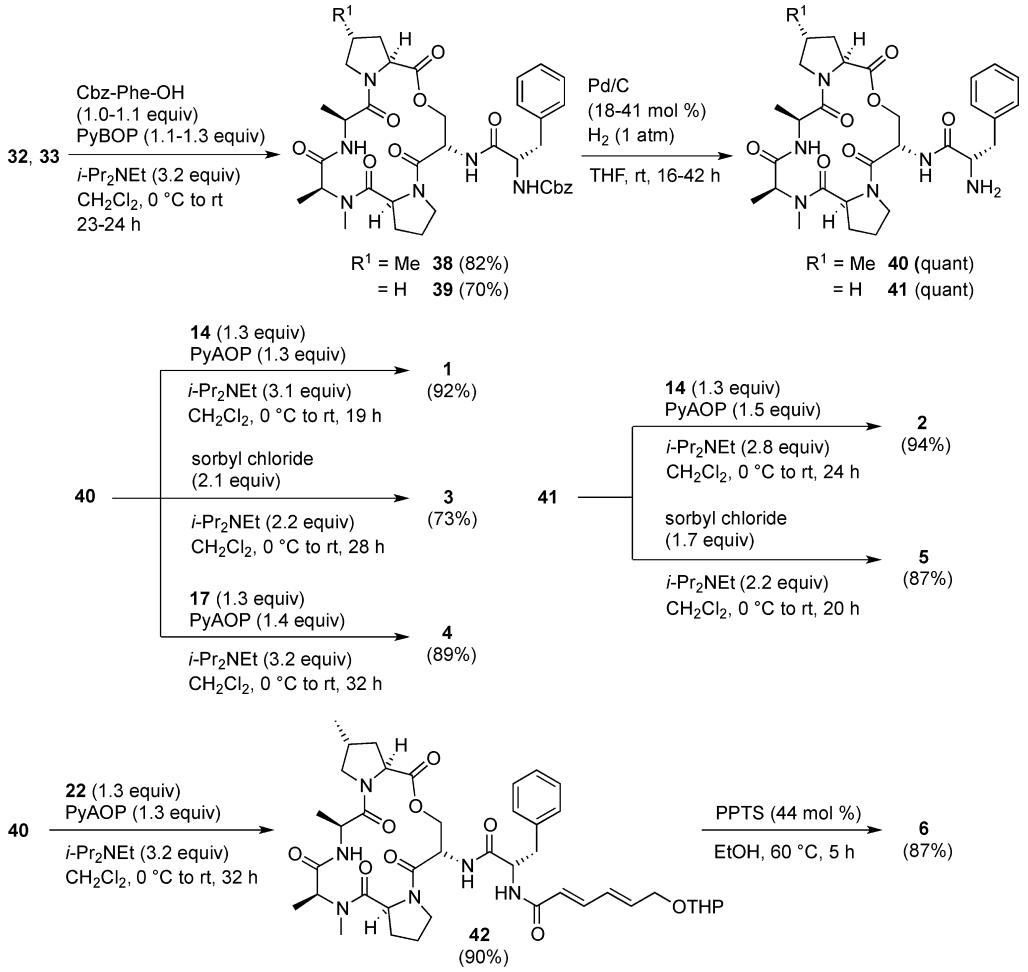
Scheme 4. Revised Strategy to Compounds 1−6



intermediate **20**.[22,23] Subsequent HWE olefination of aldehyde **20** gave ester **21** in 81% yield as a single geometric isomer; however basic hydrolysis of ester **21** required heating to obtain full conversion, and after mild acidic workup with citric acid (with no loss of the THP protecting group), acid **22** was obtained in quantitative yield as a mixture of geometric isomers (*E*:*Z* = 5:1).

The pentapeptide precursors required for the key macrolactamization were assembled from didepsipeptide and tripeptide fragments using standard solution-phase peptide chemistry (Scheme 3).[24] Pentadepsipeptides **26** and **27** were obtained in good yields by the amide coupling of didepsipeptide hydrochloride salts **24** and **25** with tripeptide **23** using the uronium-based coupling reagent TPTU.[25,26] Although this particular reagent class, similar to aminium-based reagents (e.g., HATU[27]), has found widespread use in peptide synthesis[26] and has been employed for late-stage amide formations in previous ADEP syntheses,[8,16,24] we found that separation of the *N*,*N*,*N*′,*N*′-tetramethyl urea byproduct from the pentadepsipeptide products is a challenge due to their comparable polarity on silica gel (the same is true for subsequent hexadepsipeptide intermediates). Similar purification issues occurred using polar aprotic solvents for these reactions (e.g., DMF and NMP). To circumvent these issues, further peptide coupling reactions were carried out in halogenated solvents (e.g., CH_2Cl_2 or $CHCl_3$) using phosphonium-based coupling reagents (e.g., PyBOP and PyAOP), as the corresponding phosphoramide byproduct is easily separable.[26] Phenacyl (Pac) deprotection of pentapeptides **26** and **27** using Zn/AcOH yielded the cyclization precursors **28** and **29**. The use of a pentafluorophenyl ester based cyclization procedure[9] for **28** and **29** afforded the *N*-Cbz-protected macrolactams **30** and **31** as single diastereomers in good overall yields. Palladium on carbon catalyzed hydrogenolysis of the Cbz group in **30** and **31** required the addition of a protic additive (i.e., HCl) to give hydrochloride salts **32** and **33**. Attempts to remove the Cbz group in the absence of a protic additive were unsuccessful due to catalyst poisoning by the free amine, resulting in minimal conversion as indicated by TLC analysis after 24 h.

Peptide coupling of the hydrochloride salts **32** and **33** with *N*-Boc-L-phenylalanine gave *N*-Boc-protected hexadepsipeptides **34** and **35** in good yields. In Schmidt's total synthesis of enopeptin B (**8**), unusually harsh acidic conditions (HBr in AcOH) were required for Boc deprotection.[8] Other conditions that have been reported in the literature for this deprotection validate these findings (TFA:H_2O, 9:1).[16c] In our hands, the latter conditions led to mixtures of the desired cyclic Boc-deprotected TFA salt accompanied by minor amounts of the corresponding depsipeptide ring-opened TFA salt (seco acid). Similarly, attempts to obtain the analogous ammonium hydrochloride salts from Boc-protected hexadepsipeptides through the use of HCl in dioxane were not successful. It was found however that the undesired ring-opened byproducts from Boc-protected hexadepsipeptides **34** and **35** could be

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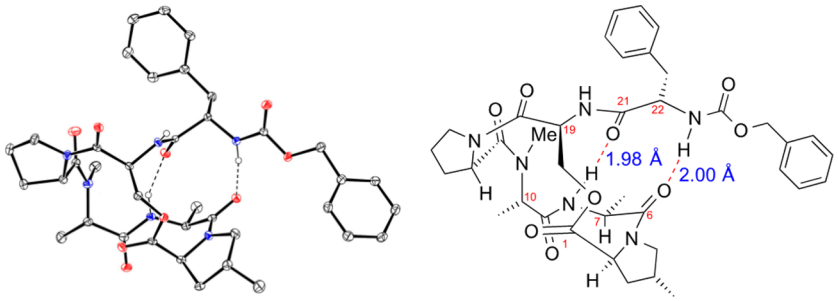

Figure 2. X-ray crystal structure of *N*-Cbz-protected hexadepsipeptide **38** showing intramolecular H-bonding (methanol solvate was omitted for clarity). Left: ORTEP diagram (showing 30% probability ellipsoids for the non-H atoms). Right: Structural representation.

avoided by using strictly anhydrous TFA in CH_2Cl_2, giving TFA salts **36** and **37** in quantitative yields. It is plausible that depsipeptide ring-opening may have occurred as a result of adventitious water during this step in Schmidt's synthesis (resulting in a poor amide yield in the next step, Scheme 1), since we have found that even small quantities of water present in the TFA can still promote the formation of this side product. Amide coupling of the trifluoroacetic acid salts **36** and **37** with trienoic acid **14** using PyAOP furnished the natural products **1** and **2** in only modest yields despite prolonged reaction time (48 h). Our initial attempts to optimize this coupling step revealed that adding more equivalents of the acid component or Hünig's base did not increase yields appreciably. Interestingly, the corresponding free amines of compounds **36** and **37**, initially obtained in low yields by extraction from $NaHCO_3$ (sat. aq) and CH_2Cl_2 (likely due to appreciable water solubility), underwent the same amide formations in excellent yields under identical reaction conditions.

Problems with acidic deprotection of the Boc groups and the increased amide coupling yields observed with free amines prompted the use of an alternative amine protecting group strategy that could allow for neutral cleavage under mild reaction conditions, providing direct access to the free amines from the cyclic hexadepsipeptides. Accordingly, it was found that the analogous *N*-Cbz-protected hexadepsipeptides **38** and **39** could be deprotected in quantitative yields under neutral conditions without the need for protic additives, yielding the free amines **40** and **41** (Scheme 4). PyAOP-based amide coupling of amines **40** and **41** with the acid **14** or **17** or with sorbyl chloride resulted in excellent yields of the depsipeptide natural products **1**−**5**. Similarly, peptide coupling of **40** with THP-protected acid **22** gave ADEP precursor **42** in 90% yield. Subsequent deprotection using catalytic PPTS in EtOH at 60 °C gave natural product **6** in 87% yield, without any observed ring-opening or *O* → *N*-acyl transfer side products. The triene-containing ADEPs **1** and **2** were observed to be both thermally and photochemically unstable compounds upon isolation, and special precautions were necessary in their handling and storage to avoid decomposition.[28]

An X-ray crystal structure of *N*-Cbz-protected hexadepsipeptide **38** was obtained confirming the stereochemical integrity of the synthetic sequence (Figure 2). The conformation of **38** shows two stabilizing intramolecular hydrogen bonds: (i) the phenylalanine C═O with the alanine NH (1.98 Å) and (ii) the alanine C═O with the phenylalanine NH (2.00 Å), which is Cbz-protected. These intramolecular interactions impose a significant amount of conformational bias and give ADEP molecules their unique conformation. The conformation of **38** is the same as that observed with the known ClpP−ADEP complexes.[14,15] Each of the dihedral angles (ψ and ϕ) of the amide backbone of **38** fall within the normal range associated with the amino acid residues found in peptides.[29]

It is plausible to propose that, based on the crystal structure data, the decreased reactivity of the TFA salts **36** and **37** (Scheme 3) toward amide coupling reactions may be attributed to the strong conformational preference of the depsipeptide ring. The resultant intramolecular H-bonding interaction between the alanine C═O with the phenylalanine-NH_3^+ group in **36** and **37** could lead to the formation of a tight ion pair, resistant to deprotonation by Hünig's base.

Spectroscopic Analysis. The assignment of the ^{1}H NMR spectrum of **1** was accomplished using a combination of 2D NMR techniques including COSY, TOCSY, HSQC, and ROESY[30] (Table 1). The assignment of the ^{13}C NMR spectrum of **1** utilized a combination of HSQC (for carbons with attached hydrogens) and HMBC[30] spectra (for carbonyl carbons) (Table 1 and Figure 3). Selected NOE correlations observed in the ROESY of **1** (Figure 3) (i) provided unambiguous assignment of relative stereochemistry for all hydrogens in both proline residues and (ii) further support a molecular conformation in solution that is consistent with the conformation for the X-ray crystal structure of Cbz-protected hexapeptide **38**, based upon the transannular interactions observed across the macrocyclic ring. Prominent interactions include (i) NH(1)−H-19 (distance in X-ray crystal structure of **38** = 2.66 Å), (ii) H-2−H-7 (2.19 Å), (iii) H-8−H-12 (C-8−C-12 = 4.25 Å), (iv) H-10−H-14 (1.95 Å), (v) H-11−H-15b (C-11−H-15b = 3.41 Å), and (vi) H-14−H-19 (2.15 Å). Weak transannular NOE correlations (not shown) were also observed for NH(2)−H-5a (2.51 Å) and H-14−H-20a (2.74 Å).

Many of the same relative stereochemical relationships for the proline residues in **2** are similar to **1**; hence the majority of assignments for **2** (Table 2) were made by comparison of the ^{1}H, ^{13}C, HSQC, and ROESY[31] spectra with those of **1**. The only structural difference between **1** and **2** is at C-4, where the methyl group (4-Me) present in **1** is absent in **2**. The methylene hydrogens in **2** were not resolved at all in the ^{1}H NMR spectrum and, hence, could not be individually assigned. Similarly, the diastereotopic methylene H-23 hydrogens were not sufficiently resolved in the ^{1}H NMR spectra to be individually assigned for any of the final compounds. Spectroscopic assignment for the remainder of compounds **3**−**6** was made by analogy depending on whether the C-4 position was methylated (related to **1**) or not (related to **2**), each compound having only minor structural differences in their acyl side chains.

The concentration effect on spectral appearance can be quite pronounced for ADEP compounds due to their high degree of

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Table 1. NMR Data (600 MHz, $CDCl_3$) of Synthetic 1[a]

no.	δ_C, type	δ_H (J in Hz)	COSY	HMBC[b]
1	172.7, C			
2	60.2, CH	4.45, d (8.5)	H-3b	1, 3, 4, 5
3a	38.90, CH_2	2.06, dd (13.0, 7.0)	H-3b	1, 2, 4, 5
3b		1.77,[c] m	H-2, H-3a	1, 2, 4, 4-Me
4	29.6, CH	2.36,[c] m	4-Me	
4-Me	18.4, CH_3	0.98, d (6.5)	H-4	3, 4, 5
5a	54.5, CH_2	3.49, dd (12.0, 9.0)	H-5b	3, 4
5b		3.08, dd (12.0, 8.5)	H-5a	3, 4, 4-Me
6	170.00, C			
7	47.9, CH	4.89, dq (9.5, 6.5)	NH(1), H-8	6, 8
8	17.7, CH_3	1.37, d (6.5)	H-7	6, 7
9	170.07, C			
10	56.4, CH	4.76, q (6.5)	H-11	9, 11, 12, 13
11	15.8, CH_3	1.51, d (6.5)	H-10	9, 10
12	31.05, N-CH_3	2.83, s		10, 13
13	172.3, C			
14	56.5, CH	5.13, dd (8.0, 3.0)	H-15a	15, 16, 17
15a	31.06, CH_2	2.34,[c] m	H-14, H-15b	13, 16
15b		1.96,[c] m	H-15a, H-16a	
16a	23.3, CH_2	2.16, m	H-16b	15, 17
16b		1.95,[c] m	H-16a	15
17a	46.7, CH_2	3.74, ddd (12.0, 8.0, 5.0)	H-17b	15, 16
17b		3.54,[c] m	H-17a	14, 15, 16
18	164.7, C			
19	51.3, CH	4.51, ddd (9.0, 9.0, 1.5)	NH(3), H-20a, H-20b	18, 20, 21
20a	65.0, CH_2	4.84, dd (12.0, 1.5)	H-19, H-20b	1, 19
20b		3.52,[c] m	H-19, H-20a	1, 19
21	171.6, C			
22	55.3, CH	4.69, m	NH(2), H-23	21, 23, 24, 28
23	38.93, CH_2	2.97,[d] m	H-22	21, 22, 24, 25
24	136.3, C			
25	129.5, CH	7.15, d (7.0)	H-26, H-27	23, 24, 27
26	128.7, CH	7.27,[c] m	H-25, H-27	24, 25
27	126.9, CH	7.19, t (7.0)	H-25, H-26	25, 26
28	166.3, C			
29	123.1, CH	6.27, d (15.0)	H-30	28, 31
30	141.5, CH	7.26,[c] m	H-29, H-31	28, 31, 32
31	128.1, CH	6.25, dd (14.5, 11.5)	H-30, H-32	29, 30, 33
32	139.9, CH	6.50, dd (14.5, 11.0)	H-31, H-33	30, 33, 34
33	131.5, CH	6.13, m	H-32, H-34	31, 35
34	134.0, CH	5.89, dq (14.0, 7.0)	H-33, H-35	32, 35
35	18.6, CH_3	1.81, m	H-34, H-33	32, 33, 34
NH(1)		8.54, d (9.5)	H-7	9
NH(2)		6.96, d (8.0)	H-22	21, 22, 28
NH(3)		6.83, br d (9.0)	H-19	21

[a] c = 16 mg/mL. [b] HMBC correlations, optimized for 8.0 Hz, are from proton(s) stated to the indicated carbon. [c] Denotes values were taken from correlations observed in a $^1H-^{13}C$ HSQC NMR experiment. [d] Protons are not resolved.

intramolecular H-bonding in solution. As the concentration is increased, an increased propensity for aggregation[32] in solution occurs due to competing intermolecular H-bonds. This effect is shown at various compound concentrations (c = 7−35 mg/mL) in the ^1H NMR spectra of **6** (Figure 4). The ^1H NMR signal of the solvent-exposed amide NH(3) shifts dramatically downfield ($\Delta\delta$ +0.46 for c = 7 and 35 mg/mL) with increasing concentration in comparison with NH(1) (not shown, $\Delta\delta$ +0.01) and NH(2) ($\Delta\delta$ +0.02), which are involved in intramolecular H-bonding. Similarly, the rate of deuterium exchange for NH(3) is much faster than for NH(1) and NH(2), which is apparent by the significant broadening of this signal at low concentration. The change in spectroscopic appearance with concentration is not limited to the amide NH signals, but also is apparent for the α-hydrogen H-22 signal (close proximity to intramolecular hydrogen bond), which undergoes a noticeable downfield shift with increasing concentration ($\Delta\delta$ +0.11), resulting in eventual overlap with the H-10 signal at c = 29 mg/mL. More subtle upfield shifts at higher concentrations were also observed for H-8 (not shown, $\Delta\delta$ −0.04), H-12 (not shown, $\Delta\delta$ −0.03), H-20a ($\Delta\delta$ −0.02), and the aromatic phenylalanine H-25, H-26, and H-27 signals ($\Delta\delta$ −0.02, −0.03, and −0.04, respectively), whereas H-17b was slightly downfield shifted (not shown, $\Delta\delta$ +0.05). All other hydrogen signals from the molecule did not change appreciably in terms of chemical shift with changing concentration. These effects made initial spectroscopic comparison of the synthetic natural products with biologically produced samples[33] from *S. hawaiiensis* slightly more challenging. However, comparable NMR data sets for **1** and **2** with the isolated natural products were collected at similar concentrations and using the same frequency spectrometer.[34]

Biological Data. To assess and compare the antibacterial activity of **1**−**6**, two types of biological assays were conducted. A fluorometric assay revealed that *E. coli* ClpP in the presence of compounds **1** and **4**, and to a lesser extent compound **2** (at 5 μM concentration), was able to effectively degrade a fluorescein isothiocyanate-labeled casein substrate (casein-FITC), demonstrating the ability of the compounds to act as ClpP activators.[35] In contrast, compounds **3**, **5**, and **6**, incorporating shorter side chains, were not effective. Thus, the length and degree of hydrophobicity of the acyl side chains appear to play a pivotal role in *E. coli* ClpP activation, with longer hydrophobic side chains tending to be more far more active than shorter chains. This is perhaps not surprising given that this moiety is surrounded largely by hydrophobic residues in the reported *E. coli* ClpP−**1** cocrystal structure.[15] Interestingly, compounds **1** and **4** incorporating the *trans*-4-methyl-proline residue are more effective *E. coli* ClpP activators than the corresponding proline-based compound **2**. This could be rationalized based on subtle differences in their binding affinities for ClpP. For compounds **1** and **2**, we have previously measured their dissociation constants (K_d) by isothermal titration calorimetry, which were found to be 0.3 ± 0.1 and 0.7 ± 0.2 μM, respectively.[18]

The antibacterial activities of ADEPs **1**−**6** are summarized in Table 5. The activity of the compounds was initially confirmed by testing them against previously susceptible Gram-positive organisms, namely, *Staphylococcus aureus* and *Streptococcus pneumoniae*.[6,11a] For these bacterial species, the glycopeptide antibiotic vancomycin was used as a positive control. Compounds **1**−**5** all showed good to moderate activity against *Staph. aureus* (MICs in the range 0.5−16 μg/mL), while compound **6**, which incorporates a more hydrophilic acyl side

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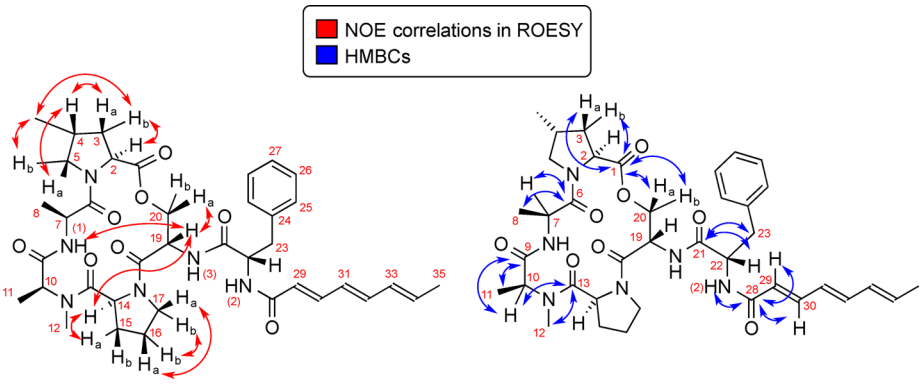

Figure 3. Left: Selected NOE correlations observed in the ROESY. Right: Selected HMBCs for **1**.

chain, was found to be largely inactive (MIC 128 μg/mL). Interestingly, compound **4** (MIC 0.5 μg/mL) was found to be 8 times more potent against *Staph. aureus* than the next most active ADEPs **1** and **3** (MIC 4 μg/mL). This increased activity is somewhat suprising given that **1** and **4** have identical chain lengths and differ only in the degree of unsaturation of the acyl group. Unlike the case with *Staph. aureus*, all of the ADEPs **1**–**6** were found to be effective against *S. pneumoniae* (MICs in the range <0.0625 to 0.5 μg/mL). In particular, both compounds **1** and **4** showed the highest levels of activity (MICs <0.0625 μg/mL) and were found to be at least 32 times more potent than vancomycin. Similar to the trends observed for *Staph. aureus*, compounds **1**–**5** showed good activity against the Gram-negative species *Neisseria meningitidis* (MICs in the range <0.0625 to 4 μg/mL), with compounds **4** and **1** having the highest activities, while compound **6** was found to have more modest activity (MIC 64 μg/mL). For this species, the aminoglycoside kanamycin A was used as a positive control. In contrast to related studies,[6,36] compounds **1**–**6** were found to be sufficiently active against this Gram-negative strain and did not necessitate the use of an external membrane permeabilizing agent in the culture broth.[37] In general, the observed antibacterial trends suggest that compounds lacking a C-4 methyl group (i.e., **2** and **5**) show lower activity than their methylated counterparts **1** and **3**, while compounds with longer acyl side chains (i.e., **1**, **2**, and **4**) show higher activity than comparable compounds with shorter side chains (i.e., **3** and **5**).

Conclusions. In conclusion, we have completed the first total syntheses of all of the originally isolated ADEPs **1**–**6** from *S. hawaiiensis*, compounds that are increasingly attracting significant attention due to their unique mode of antibacterial activity. Several practical challenges were encountered during the course of our synthetic studies. These problems were addressed, aiding future studies toward the synthesis of related natural products and ADEP analogues. Notably, the use of a late stage Cbz-deprotection strategy was found to offer significant advantages in terms of the general synthesis of these compounds. Thus, quantitative yields of free amine species can be obtained under mild conditions using hydrogenolysis, avoiding formation of depsipeptide ring-opened side products (sometimes encountered under strongly acidic deprotection conditions). The free amines, in contrast with the corresponding ammonium salts, reliably undergo the final amide formations in excellent yields.

An X-ray crystal structure analysis of one of the synthetic intermediates revealed that the conformation of both the cyclic depsipeptide ring and the side chain is the same as that observed in the two known ClpP–ADEP cocrystal structures. This conformation is locked by the presence of hydrogen bonds that occur between two of the cyclic depsipeptide ring amide residues and the two amide linkages of the phenylalanine side chain. The first formal assignment of the ^{1}H and ^{13}C NMR spectra for the ADEPs using a combination of 2D NMR techniques (COSY, TOCSY, HSQC, HMBC, and ROESY) and a direct comparison of biologically produced and synthetic **1** and **2** from *S. hawaiiensis* provided sufficient spectroscopic evidence for structural confirmation. A pronounced concentration effect for the ^{1}H NMR spectra was also observed, presumably reflecting the breakdown of the intramolecular H-bond network and/or increased aggregation at higher concentrations. Similar effects and conformational preferences are likely for related ADEP analogues currently being investigated as part of medicinal chemistry studies. Finally, the antibacterial activities obtained *in vitro* confirm activity trends of the compounds from two well-studied Gram-positive bacterial strains (*Staph. aureus* and *S. pneumoniae*), as well as demonstrating good levels of activity against a previously untested and clinically relevant Gram-negative bacterial species, *N. meningitidis*.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were performed under nitrogen in flame-dried glassware. Tetrahydrofuran was freshly distilled from sodium/benzophenone ketyl under nitrogen. Dichloromethane was freshly distilled from calcium hydride under nitrogen. Anhydrous dimethylformamide and methanol were obtained as ≥99.9% pure and stored under argon. All other solvents were ACS grade or better from commercial suppliers and used as received. Flash chromatography on silica gel (60 Å, 230–400 mesh) was performed with reagent grade solvents. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates and visualized with a UV_{254} lamp. Solvent ratios for chromatography and R_f values are reported as v/v ratios. Melting points are uncorrected and obtained on >95% pure compounds without any further recrystallization. All 1D (^{1}H, ^{13}C) and 2D (COSY, TOCSY, HSQC, HMBC, and ROESY) NMR spectra were obtained on 300, 400, 400, 500, and 600 MHz spectrometers as solutions in deuterated solvents. Chemical shifts are reported in δ ppm values. ^{1}H chemical shifts were internally referenced to tetramethylsilane (δ 0.00) for $CDCl_3$ or to the residual proton resonance in CD_3OD (δ 3.31) and DMSO-d_6 (δ 2.49). Carbon chemical shifts were internally referenced to the solvent resonances in $CDCl_3$ (δ 77.16 ppm), CD_3OD (δ 49.15 ppm), or DMSO-d_6 (δ 39.51 ppm). Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; *J*, coupling constant in Hz and rounded to the nearest 0.5 Hz. Exact mass measurements were performed on a time-of-flight mass

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Table 2. NMR Data (500 MHz, $CDCl_3$) of Synthetic 2[a]

no.	δ_C, type	δ_H (J in Hz)	COSY	HMBC[b]
1	172.8, C			
2	59.3, CH	4.44, d (8.0)	H-3b	1, 3, 4, 5
3a	30.8, CH_2	2.00,[c] m	H-3b	1, 4, 5
3b		2.16,[c] m	H-2, H-3a	1, 2, 4
4	21.3, CH_2	1.88,[c,d] m		3
5a	47.1, CH_2	3.60,[c] m	H-5b	4
5b		3.30, m	H-5a	3, 4
6	170.06, C			
7	48.0, CH	4.89, dq (9.5, 6.5)	NH(1), H-8	6, 8
8	17.6, CH_3	1.36, d (6.5)	H-7	6, 7
9	170.08, C			
10	56.4, CH	4.78, q (7.0)	H-11	9, 11, 12
11	15.8, CH_3	1.52, d (7.0)	H-10	9, 10
12	31.0,[e] N-CH_3	2.82, s		10, 13
13	172.2, C			
14	56.6, CH	5.15, dd (8.0, 3.0)	H-15a, H-15b	15, 16, 17
15a	31.0,[e] CH_2	2.35, m	H-14, H-15b	13, 14, 16
15b		1.97,[c] m	H-15a	
16a	23.3, CH_2	2.16,[c] m	H-16b	15, 17
16b		1.97,[c] m	H-16a	15
17a	46.7, CH_2	3.74, ddd (12.0, 8.0, 5.0)	H-17b	15, 16
17b		3.58,[c] m	H-17a	14, 15, 16
18	164.8, C			
19	51.2, CH	4.51, ddd (9.5, 9.5, 1.5)	NH(3), H-20a, H-20b	18, 20, 21
20a	65.1, CH_2	4.82, dd (11.0, 1.5)	H-19, H-20b	1, 19
20b		3.53, dd (11.0, 9.5)	H-19, H-20a	1, 19
21	171.6, C			
22	55.3, CH	4.72, m	NH(2), H-23	21, 23, 24, 28
23	39.0, CH_2	2.97,[d] m	H-22	21, 22, 24, 25
24	136.5, C			
25	129.6, CH	7.15, m	H-26, H-27	23, 24, 27
26	128.7, CH	7.27,[c] m	H-25, H-27	24, 25
27	126.8, CH	7.18, m	H-25, H-26	25, 26
28	166.3, C			
29	123.1, CH	6.29, d (14.5)	H-30	28, 31, 32
30	141.5, CH	7.30, dd (14.5, 11.0)	H-29, H-31	28, 29, 32
31	128.3, CH	6.25, dd (14.5, 11.0)	H-30, H-32	29, 30, 33
32	139.8, CH	6.50, dd (14.5, 11.0)	H-31, H-33	29, 30, 33, 34
33	131.5, CH	6.12, m	H-32, H-34, H-35	31, 32, 35
34	134.0, CH	5.89, dq (14.0, 7.0)	H-33, H-35	32, 35
35	18.6, CH_3	1.81, m	H-34, H-33	31, 32, 33, 34
NH(1)		8.56, d (9.5)	H-7	9
NH(2)		7.01,[f] m	H-22	22, 28
NH(3)		7.00,[f] m	H-19	19, 21

[a] c = 26 mg/mL. [b] HMBC correlations, optimized for 8.0 Hz, are from proton(s) stated to the indicated carbon. [c] Denotes values were taken from correlations observed in a $^1H-^{13}C$ HSQC NMR experiment. [d] Protons are not resolved. [e] Carbon signals are overlapping. [f] Denotes values were taken from correlations observed in a $^1H-^{13}C$ HMBC NMR experiment.

spectrometer utilizing electrospray ionization (ESI), direct analysis in real time ionization (DART), and electron impact ionization (EI).

General Procedure A: Synthesis of Amides Using Coupling Reagents. To an ice bath-cooled solution of amine, acid, and coupling reagent in CH_2Cl_2 was added Hünig's base dropwise. The reaction was stirred overnight with slow warming to room temperature under nitrogen. The reaction mixture was diluted with water and extracted with three portions of CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$ and concentrated *in vacuo*, and the crude product was then purified using flash chromatography to give the corresponding amide.

General Procedure B: Synthesis of Amides Using Acid Chlorides. To an ice bath-cooled solution of amine in CH_2Cl_2 was added via cannula transfer the acid chloride in CH_2Cl_2, followed by the dropwise addition of Hünig's base. The reaction was stirred overnight with slow warming to room temperature under nitrogen. The reaction mixture was then diluted with water and extracted with three portions of CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$ and concentrated *in vacuo*, and the crude product was then purified using flash chromatography to give the corresponding amide.

For ADEP compounds **1**–**6**, analytically pure material (used for characterization and biological testing) was obtained by semi-preparative reversed-phase HPLC of the product previously isolated by flash chromatography. HPLC fractions that contained the product by LRMS (ESI^+) were combined into a lyophilization flask, frozen solid in a dry ice–acetone bath, and then lyophilized overnight, yielding solid compounds.

A55456 Factor A (1).[6] This was synthesized according to general procedure A using amine **40** (99.3 mg, 0.166 mmol, 1.0 equiv), acid **14** (30.4 mg, 0.220 mmol, 1.3 equiv), PyAOP (122.0 mg, 0.234 mmol, 1.3 equiv), CH_2Cl_2 (5 mL), and Hünig's base (90.0 μL, 0.517 mmol, 3.1 equiv), 19 h. Note: The reaction flask was covered in tin foil to protect the coupling reagent and product from light. Flash chromatography (19:1 EtOAc–MeOH) of the crude organic extracts gave 110.3 mg of **1** in 92% yield. Semipreparative reversed-phase HPLC of the aforementioned silica gel purified product gave **1** as a white solid: mp 175–176 °C (CH_2Cl_2); R_f 0.55 (19:1 EtOAc–MeOH); $[\alpha]^{24}_D$ −48.0 (c 0.24, $CHCl_3$); IR (thin film in CH_2Cl_2) ν_{max} 3500 (br), 3293 (br), 2960, 2933, 2876, 1650, 1437, 1271, 1111, 1011 cm^{-1}; ^1H and ^{13}C NMR see Table 1; LRMS (ESI^+) m/z (rel intensity) 741.4 (97) $[M + Na]^+$, 719.4 (100) $[M + H]^+$, 452.3 (18); HRMS (ESI^+) m/z calcd for $C_{38}H_{51}N_6O_8$ $[M + H]^+$ 719.3762, found 719.3774; analytical HPLC (C_{18} column, 5 μm, 4.6 × 150 mm, gradient elution with water–acetonitrile, flow rate 2.5 mL/min, column temp 23 °C, detector λ 280 nm, t_R 6.48 min).

A55456 Factor B (2).[6] This was synthesized according to general procedure A using amine **41** (96.9 mg, 0.166 mmol, 1.0 equiv), acid **14** (31.1 mg, 0.225 mmol, 1.3 equiv), PyAOP (127.0 mg, 0.243 mmol, 1.5 equiv), CH_2Cl_2 (4 mL), and Hünig's base (80.0 μL, 0.459 mmol, 2.8 equiv), 24 h. Note: The reaction flask was covered in tin foil to protect the coupling reagent and product from light. Flash chromatography (19:1 EtOAc–MeOH) of the crude organic extracts gave 109.8 mg of **2** in 94% yield. Semipreparative reversed-phase HPLC of the aforementioned silica gel purified product gave **2** as a white solid: mp 187–188 °C (CH_2Cl_2); R_f 0.25 (19:1 EtOAc–MeOH); $[\alpha]^{24}_D$ −46.2 (c 0.37, $CHCl_3$); IR (thin film in CH_2Cl_2) ν_{max} 3440 (br), 3304 (br), 3061, 3026, 2982, 2936, 2887, 1650 (br), 1437, 1267, 1007 cm^{-1}; ^1H and ^{13}C NMR see Table 2; LRMS (ESI^+) m/z (rel intensity) 727.4 (8) $[M + Na]^+$, 705.4 (100) $[M + H]^+$; HRMS (ESI^+) m/z calcd for $C_{37}H_{49}N_6O_8$ $[M + H]^+$ 705.3603, found 705.3606; analytical HPLC (C_{18} column, 5 μm, 4.6 × 150 mm, gradient elution with water–acetonitrile, flow rate 2.5 mL/min, column temp 23 °C, detector λ 280 nm, t_R 6.04 min).

A55456 Factor C (3).[6] This was synthesized according to general procedure B using amine **40** (77.4 mg, 0.129 mmol, 1.0 equiv) in CH_2Cl_2 (2 mL), sorbyl chloride (36.0 mg, 0.276 mmol, 2.1 equiv) in CH_2Cl_2 (2 mL), and Hünig's base (50.0 μL, 0.287 mmol, 2.2 equiv), 28 h. Flash chromatography (19:1 EtOAc–MeOH) of the crude organic extracts gave 65.1 mg of **3** in 73% yield. Semipreparative reversed-phase HPLC of the aforementioned silica gel purified product

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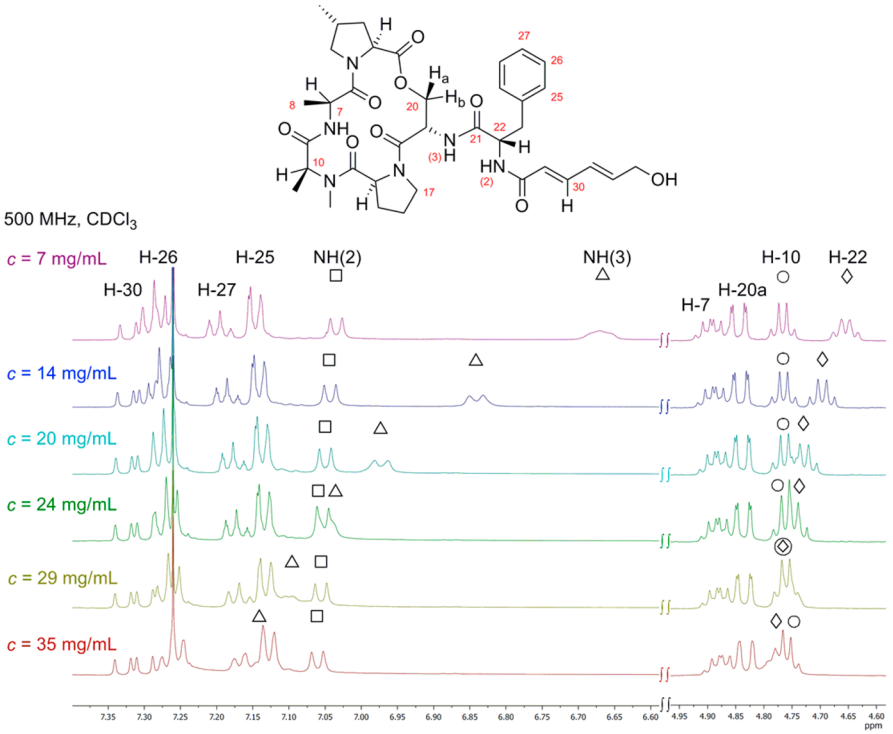

Figure 4. Effect of concentration on spectroscopic appearance for compound **6**.

Table 5. *In Vitro* Antibacterial Activity of A54556 Factors (1−6) against Gram-Positive and Gram-Negative Pathogens[a]

	MIC (μg/mL)		
Compound	*Staph. aureus* (ATCC 29213)	*S. pneumoniae* (ATCC 46919)	*N. meningitidis* (H44/76)
1	4	<0.0625	0.125
2	16	0.5	0.5
3	4	0.125	2
4	0.5	<0.0625	<0.0625
5	16	0.25	4
6	128	0.25	64
vancomycin	2	2	–[b]
kanamycin A	–	–	64

[a]MIC values shown have been determined by standard broth-microdilution techniques.[38] [b]Symbol "–" indicates not determined.

gave **3** as a white solid: decomposition temp 113 °C (CH_2Cl_2); R_f 0.32 (19:1 EtOAc−MeOH); $[\alpha]^{24}_D$ −39.1 (*c* 0.18, $CHCl_3$); IR (thin film in CH_2Cl_2) ν_{max} 3500 (br), 3284 (br), 2961, 2934, 2876, 1736, 1650 (br), 1441, 1111, 1001 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, *c* 20 mg/mL) δ 8.55 (1H, d, *J* = 9.5 Hz, NH(1)), 7.32−7.21 (3H, m, H-26, H-30), 7.20−7.12 (3H, H-25, H-27), 6.99 (1H, d, *J* = 9.5 Hz, NH(3)), 6.95 (1H, d, *J* = 8.5 Hz, NH(2)), 6.28−6.15 (2H, m, H-29, H-31), 6.14−6.04 (1H, m, H-32), 5.13 (1H, dd, *J* = 9.0, 3.0 Hz, H-14), 4.88 (1H, dq, *J* = 9.5, 6.5 Hz, H-7), 4.83 (1H, dd, *J* = 12.0, 1.5 Hz, H-20a), 4.80−4.66 (2H, m, H-10, H-22), 4.51 (1H, ddd, *J* = 9.5, 9.5, 1.5 Hz, H-19), 4.44 (1H, d, *J* = 8.0 Hz, H-2), 3.73 (1H, ddd, *J* = 11.5, 8.0, 5.0 Hz, H-17a), 3.62−3.45 (3H, m, H-5a, H-17b, H-20b), 3.08 (1H, dd, *J* = 12.0, 8.5 Hz, H-5b), 3.03−2.92 (2H, m, H-23), 2.82 (3H, s, N−CH_3 (H-12)), 2.44−2.28 (2H, m, H-4, H-15a), 2.22−2.10 (1H, m, H-16a), 2.06 (1H, dd, *J* = 13.0, 7.0 Hz, H-3a), 2.02−1.90 (2H, m, H-15b, H-16b), 1.88−1.74 (4H, m, H-33, H-3b), 1.51 (3H, d, *J* = 7.0 Hz, H-11), 1.35 (3H, d, *J* = 6.5 Hz, H-8), 0.99 (3H, d, *J* = 7.0 Hz, 4-Me); ^{13}C NMR ($CDCl_3$, 100 MHz, *c* 20 mg/mL) δ 172.7 (C, C-1), 172.2 (C, C-13), 171.6 (C, C-21), 170.07 (C, C-9), 169.96 (C, C-6), 166.5 (C, C-28), 164.8 (C, C-18), 141.6 (CH, C-30), 137.7 (CH, C-32), 136.4 (C, C-24), 130.1 (CH, C-31), 129.6 (CH, C-25), 128.7 (CH, C-26), 126.9 (CH, C-27), 122.0 (CH, C-29), 65.1 (CH_2, C-20), 60.2 (CH, C-2), 56.5 (CH, C-14), 56.4 (CH, C-10), 55.2 (CH, C-22), 54.5 (CH_2, C-5), 51.3 (CH, C-19), 47.9 (CH, C-7), 46.7 (CH_2, C-17), 38.89 (CH_2, C-23), 38.84 (CH_2, C-3), 31.06 (CH_2, C-15), 31.03 (N-CH_3, C-12), 29.6 (CH, C-4), 23.3 (CH_2, C-16), 18.7 (CH_3, C-33), 18.4 (CH_3, 4-Me), 17.6 (CH_3, C-8), 15.8 (CH_3, C-11); LRMS (ESI^+) *m*/*z* (rel intensity) 715.4 (72) $[M + Na]^+$, 693.4 (100) $[M + H]^+$, 452.3 (13); HRMS (ESI^+) *m*/*z* calcd for $C_{36}H_{49}N_6O_8$ $[M + H]^+$ 693.3606, found 693.3631; analytical HPLC (C_{18} column, 5 μm, 4.6 × 150 mm, gradient elution with water−acetonitrile, flow rate 2.5 mL/min, column temp 23 °C, detector λ 280 nm, t_R 5.62 min).

A55456 Factor D (4).[6] This was synthesized according to general procedure A using amine **40** (85.9 mg, 0.143 mmol, 1.0 equiv), acid **17** (26.2 mg, 0.187 mmol, 1.3 equiv), PyAOP (102.7 mg, 0.197 mmol, 1.4 equiv), CH_2Cl_2 (4 mL), and Hünig's base (80.0 μL, 0.459 mmol, 3.2 equiv), 32 h. Note: The reaction flask was covered in tin foil to protect the coupling reagent from light. Flash chromatography (19:1 EtOAc−MeOH) of the crude organic extracts gave 91.5 mg of **4** in 89% yield. Semipreparative reversed-phase HPLC of the aforementioned silica gel purified product gave **4** as a white solid: mp 140−142 °C (CH_2Cl_2); R_f 0.51 (19:1 EtOAc−MeOH); $[\alpha]^{24}_D$ −51.9 (*c* 0.18, $CHCl_3$); IR (thin film in CH_2Cl_2) ν_{max} 3500 (br), 3287 (br), 2959, 2932, 2874, 1732, 1650 (br), 1439, 1271, 1111, 1001 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, *c* 17 mg/mL) δ 8.54 (1H, d, *J* = 9.5 Hz, NH(1)), 7.32−7.22 (3H, m, H-26, H-30), 7.21−7.10 (3H, m, H-25, H-27), 6.93 (1H, d, *J* = 8.0 Hz, NH(2)), 6.87 (1H, d, *J* = 9.5 Hz, NH(3)), 6.26−6.16 (2H, m, H-29, H-31), 6.12−6.03 (1H, m, H-32), 5.13 (1H, dd, *J* = 9.0, 3.0 Hz, H-14),

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4.89 (1H, dq, J = 9.5, 6.5 Hz, H-7), 4.84 (1H, dd, J = 12.0, 1.5 Hz, H-20a), 4.76 (1H, q, J = 7.0 Hz, H-10), 4.73−4.66 (1H, m, H-22), 4.51 (1H, ddd, J = 9.5, 9.5, 1.5 Hz, H-19), 4.44 (1H, d, J = 8.0 Hz, H-2), 3.78−3.69 (1H, m, H-17a), 3.62−3.46 (3H, m, H-5a, H-17b, H-20b), 3.09 (1H, dd, J = 12.0, 8.5 Hz, H-5b), 3.03−2.92 (2H, m, H-23), 2.82 (3H, s, N-CH_3 (H-12)), 2.44−2.29 (2H, m, H-4, H-15a), 2.22−2.03 (4H, m, H-3a, H-16a, H-33), 2.02−1.91 (2H, m, H-15b, H-16b), 1.86−1.74 (1H, m, H-3b), 1.51 (3H, d, J = 7.0 Hz, H-11), 1.44 (2H, sextet, J = 7.5 Hz, H-34), 1.36 (3H, d, J = 6.5 Hz, H-8), 0.98 (3H, d, J = 6.5 Hz, 4-Me), 0.90 (3H, t, J = 7.5 Hz, H-35); ^{13}C NMR ($CDCl_3$, 100 MHz, c 17 mg/mL) δ 172.8 (C, C-1), 172.3 (C, C-13), 171.6 (C, C-21), 170.1 (C, C-9), 170.0 (C, C-6), 166.5 (C, C-28), 164.8 (C, C-18), 143.0 (CH, C-30), 141.8 (CH, C-32), 136.4 (C, C-24), 129.6 (CH, C-25), 128.8 (CH, C-31), 128.7 (CH, C-26), 126.9 (CH, C-27), 122.1 (CH, C-29), 65.0 (CH_2, C-20), 60.2 (CH, C-2), 56.5 (CH, C-14), 56.4 (CH, C-10), 55.2 (CH, C-22), 54.5 (CH_2, C-5), 51.3 (CH, C-19), 47.9 (CH, C-7), 46.7 (CH_2, C-17), 38.89 (CH_2, C-23), 38.83 (CH_2, C-3), 35.1 (CH_2, C-33), 31.06 (CH_2, C-15), 31.03 (N-CH_3, C-12), 29.6 (CH, C-4), 23.3 (CH_2, C-16), 22.1 (CH_2, C-34), 18.4 (CH_3, 4-Me), 17.6 (CH_3, C-8), 16.0 (CH_3, C-11), 13.8 (CH_3, C-35); LRMS (ESI$^+$) m/z (rel intensity) 743.4 (100) $[M + Na]^+$, 721.4 (81) $[M + H]^+$, 452.3 (14); HRMS (ESI$^+$) m/z calcd for $C_{38}H_{53}N_6O_8$ $[M + H]^+$ 721.3919, found 721.3888; analytical HPLC (C_{18} column, 5 μm, 4.6 × 150 mm, gradient elution with water−acetonitrile, flow rate 2.5 mL/min, column temp 23 °C, detector λ 280 nm, t_R 6.94 min).

*A55456 Factor E (**5**).*[6] This was synthesized according to general procedure B using amine **41** (74.6 mg, 0.128 mmol, 1.0 equiv) in CH_2Cl_2 (4 mL), sorbyl chloride (29.0 mg, 0.222 mmol, 1.7 equiv) in CH_2Cl_2 (2 mL), and Hünig's base (50.0 μL, 0.287 mmol, 2.2 equiv), 20 h. Flash chromatography (19:1 EtOAc−MeOH) of the crude organic extracts gave 75.4 mg of **5** in 87% yield. Semipreparative reversed-phase HPLC of the aforementioned silica gel purified product gave **5** as a white solid: decomposition temp 145 °C (CH_2Cl_2); R_f 0.28 (19:1 EtOAc−MeOH); $[\alpha]^{24}_D$ −49.1 (c 0.35, $CHCl_3$); IR (thin film in CH_2Cl_2) ν_{max} 3500 (br), 3281 (br), 2982, 2936, 1732, 1650 (br), 1437, 1265, 1159, 1111, 1003 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, c 16 mg/mL) δ 8.56 (1H, d, J = 9.5 Hz, NH(1)), 7.32−7.22 (3H, m, H-26, H-30), 7.21−7.13 (3H, m, H-25, H-27), 7.13−6.96 (2H, m, NH(2), NH(3)), 6.27−6.17 (2H, m, H-29, H-31), 6.14−6.04 (1H, m, H-32), 5.15 (1H, dd, J = 8.5, 3.0 Hz, H-14), 4.89 (1H, dq, J = 9.5, 6.5 Hz, H-7), 4.82 (1H, dd, J = 11.5, 1.5 Hz, H-20a), 4.78 (1H, q, J = 7.0 Hz, H-10), 4.73−4.65 (1H, m, H-22), 4.51 (1H, ddd, J = 9.5, 9.5, 1.5 Hz, H-19), 4.44 (1H, d, J = 8.0 Hz, H-2), 3.73 (1H, ddd, J = 11.5, 8.0, 5.0 Hz, H-17a), 3.66−3.50 (3H, m, H-5a, H-17b, H-20b), 3.34−3.26 (1H, m, H-5b), 3.07−2.90 (2H, m, H-23), 2.82 (3H, s, N-CH_3 (H-12)), 2.41−2.29 (1H, m, H-15a), 2.22−2.10 (2H, m, H-3b, H-16a), 2.03−1.92 (3H, m, H-3a, H-15b, H-16b), 1.91−1.78 (5H, m, H-4, H-33), 1.52 (3H, d, J = 7.0 Hz, H-11), 1.36 (3H, d, J = 6.5 Hz, H-8); ^{13}C NMR ($CDCl_3$, 100 MHz, c 16 mg/mL) δ 172.9 (C, C-1), 172.3 (C, C-13), 171.6 (C, C-21), 170.1 (C, C-9), 170.0 (C, C-6), 166.5 (C, C-28), 164.8 (C, C-18), 141.6 (CH, C-30), 137.7 (CH, C-32), 136.4 (C, C-24), 130.2 (CH, C-31), 129.6 (CH, C-25), 128.7 (CH, C-26), 126.9 (CH, C-27), 121.8 (CH, C-29), 65.1 (CH_2, C-20), 59.3 (CH, C-2), 56.6 (CH, C-14), 56.4 (CH, C-10), 55.3 (CH, C-22), 51.2 (CH, C-19), 48.1 (CH, C-7), 47.1 (CH_2, C-5), 46.7 (CH_2, C-17), 39.0 (CH_2, C-23), 31.0 (2C; N-CH_3, C-12 and CH_2, C-15), 30.8 (CH_2, C-3), 23.3 (CH_2, C-16), 21.3 (CH_2, C-4), 18.7 (CH_3, C-33), 17.6 (CH_3, C-8), 15.8 (CH_3, C-11); LRMS (ESI$^+$) m/z (rel intensity) 701.4 (100) $[M + Na]^+$, 679.4 (27) $[M + H]^+$; HRMS (ESI$^+$) m/z calcd for $C_{35}H_{47}N_6O_8$ $[M + H]^+$ 679.3449, found 679.3462; analytical HPLC (C_{18} column, 5 μm, 4.6 × 150 mm, gradient elution with water−acetonitrile, flow rate 2.5 mL/min, column temp 23 °C, detector λ 280 nm, t_R 5.14 min).

*A55456 Factor H (**6**).*[6] To a solution of THP-protected depsipeptide **42** (62.2 mg, 0.784 mmol, 1.0 equiv) in EtOH (4 mL) was added PPTS (8.7 mg, 0.035 mmol, 44 mol %), and the reaction mixture was heated in a 60 °C oil bath under nitrogen (flask fitted with a water-cooled reflux condenser). Complete consumption of the starting material was observed by TLC after 5 h, and the reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were then dried over $MgSO_4$ and concentrated *in vacuo*. Flash chromatography (19:1 EtOAc−MeOH) of the crude organic extracts gave **6** in 87% yield. Semipreparative reversed-phase HPLC of the aforementioned silica gel purified product gave **6** as a white solid: decomposition temp 168 °C (CH_2Cl_2); R_f 0.35 (9:1 EtOAc−MeOH); $[\alpha]^{24}_D$ −47.3 (c 0.32, $CHCl_3$); IR (thin film in CH_2Cl_2) ν_{max} 3439 (br), 3289 (br), 2961, 2934, 2876, 1732, 1650 (br), 1441, 1271, 1111, 1007 cm^{-1}; ^{1}H NMR ($CDCl_3$, 700 MHz, c 20 mg/mL) δ 8.53 (1H, d, J = 9.5 Hz, NH(1)), 7.31 (1H, dd, J = 15.5, 11.0 Hz, H-30), 7.29−7.23 (2H, m, H-26), 7.18 (1H, t, J = 7.5 Hz, H-27), 7.14 (2H, d, J = 7.5 Hz, H-25), 7.06 (1H, d, J = 8.0 Hz, NH(2)), 6.98 (1H, d, J = 9.5 Hz, NH(3)), 6.48−6.43 (1H, m, H-31), 6.34 (1H, d, J = 15.5 Hz, H-29), 6.20 (1H, dt, J = 15.5, 5.0 Hz, H-32), 5.13 (1H, dd, J = 8.5, 3.0 Hz, H-14), 4.89 (1H, dq, J = 9.5, 6.5 Hz, H-7), 4.82 (1H, dd, J = 12.0, 1.5 Hz, H-20a), 4.76 (1H, q, J = 7.0 Hz, H-10), 4.74−4.69 (1H, m, H-22), 4.51 (1H, ddd, J = 9.5, 9.5, 1.5 Hz, H-19), 4.44 (1H, d, J = 8.5 Hz, H-2), 4.29−4.25 (2H, br m, H-33), 3.76−3.71 (1H, m, H-17a), 3.57 (1H, ddd, J = 11.5, 7.0, 7.0 Hz, H-17b), 3.53 (1H, dd, J = 12.0, 9.5 Hz, H-20b), 3.48 (1H, dd, J = 12.0, 9.0 Hz, H-5a), 3.09 (1H, dd, J = 12.0, 9.0 Hz, H-5b), 3.01−2.94 (2H, m, H-23), 2.82 (3H, s, N-CH_3 (H-12)), 2.44−2.32 (2H, m, H-4, H-15a), 2.20−2.13 (1H, m, H-16a), 2.07 (1H, dd, J = 13.0, 7.0 Hz, H-3a), 2.00−1.93 (2H, m, H-15b, H-16b), 1.88 (1H, br s, OH(1)), 1.83−1.76 (1H, m, H-3b), 1.41 (3H, d, J = 7.0 Hz, H-11), 1.34 (3H, d, J = 6.5 Hz, H-8), 0.99 (3H, d, J = 6.5 Hz, 4-Me); ^{13}C NMR ($CDCl_3$, 175 MHz, c 20 mg/mL) δ 172.7 (C, C-1), 172.2 (C, C-13), 171.5 (C, C-21), 170.0 (2C; C, C-6 and C, C-9), 166.1 (C, C-28), 164.7 (C, C-18), 140.4 (CH, C-30), 139.7 (CH, C-32), 136.2 (C, C-24), 129.6 (CH, C-25), 128.7 (CH, C-26), 128.5 (CH, C-31), 126.9 (CH, C-27), 124.4 (CH, C-29), 65.1 (CH_2, C-20), 63.0 (CH_2, C-33), 60.2 (CH, C-2), 56.5 (CH, C-14), 56.4 (CH, C-10), 55.4 (CH, C-22), 54.4 (CH_2, C-5), 51.3 (CH, C-19), 47.9 (CH, C-7), 46.7 (CH_2, C-17), 38.92 (CH_2, C-23), 38.87 (CH_2, C-3), 31.0 (2C; N-CH_3, C-12 and CH_2, C-15), 29.6 (CH, C-4), 23.3 (CH_2, C-16), 18.5 (CH_3, 4-Me), 17.6 (CH_3, C-8), 15.8 (CH_3, C-11); LRMS (ESI$^+$) m/z (rel intensity) 731.4 (99) $[M + Na]^+$, 709.4 (100) $[M + H]^+$; HRMS (ESI$^+$) m/z calcd for $C_{36}H_{49}N_6O_9$ $[M + H]^+$ 709.3555, found 709.3553; analytical HPLC (C_{18} column, 5 μm, 4.6 × 150 mm, gradient elution with water−acetonitrile, flow rate 2.5 mL/min, column temp 23 °C, detector λ 280 nm, t_R 3.71 min).

Antibacterial Activity Evaluation. MIC values for compounds **1**−**6** were determined by standard protocols for broth microdilution.[38] The Gram-positive strains *Staph. aureus* (ATCC 29213) and *S. pneumoniae* (ATCC 49619) were provided by Dr. Don Low, Mount Sinai Hospital, Toronto. The Gram-negative strain *N. meningitidis* (H44/76) was provided by Dr. Andy Gorringe, University of Bath, UK. *Staph. aureus* cells were grown on LB agar (Invitrogen), and all the other strains were grown on GC agar (BD Biosciences) supplemented with 1% IsoVitaleX (BD Biosciences) for 16−18 h at 37 °C in a moist atmosphere containing 5% CO_2. Cells were harvested and diluted in brain heart infusion broth (BHI, BD Biosciences) supplemented with 1% IsoVitaleX. The optical density of each inoculum (λ = 600 nm for *Staph. aureus* and *S. pneumoniae*, λ = 550 nm for *N. meningitidis*) was measured, and the inoculum was adjusted to ∼10^6 cfu/mL. DMSO stock solutions were made for compounds **1**−**6** at 5 mg/mL. Each compound was then diluted in BHI broth supplemented with 1% IsoVitaleX to 256 μg/mL (dilute 51.2 μL of DMSO stock solution into 948.8 μL of BHI + 1% IsoVitaleX, DMSO at 5.12% after dilution). A serial dilution at 0.5-fold per step, with 256 μg/mL being the highest concentration and 0.125 μg/mL being the lowest, was prepared for each compound. The analogous serial dilutions were also done for the antibiotics vancomycin (BioShop) and kanamycin A (BioShop) and were used as positive controls. The serial dilutions were then transferred onto sterile round-bottom 96-well microtiter plates (Corning) at 50 μL per well. Bacterial cells were then applied at 50 μL per well using the ∼10^6 cfu/mL inoculum prepared. This yielded an inoculum at ∼5 × 10^5 cfu/mL per well and the following concentrations of compounds: 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 μg/mL. Negative control wells (3), which contained only DMSO instead of the test compound/antibiotic, and blank control wells (3), which contained only the growth media but

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without bacteria, were set in the plate as well. A total of three independently constructed plates were prepared for each bacterial strain. All plates were sealed with a Breath-Easy sealing membrane (Sigma-Aldrich), and they were incubated for 16−18 h at 37 °C in a moist atmosphere containing 5% CO_2. Cell growth was determined by visually examining for the presence of cell pellets or turbidity of the wells. The lowest concentration of compound used that did not exhibit any visible cell growth was taken as the MIC value.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental procedures and compound data pertaining to other synthesized intermediates, copies of 1D NMR spectra (^{1}H and ^{13}C) for all synthesized numbered compounds, 2D NMR spectra (COSY, TOCSY, HSQC, HMBC, and ROESY) for selected compounds **1**−**6**, comparison ^{1}H and ^{13}C NMR spectra of synthetic and isolated for compounds **1** and **2**, analytical HPLC chromatograms for compounds **1**−**6**, semipreparative HPLC chromatograms for compounds **1**−**6** and **26**−**29**, ORTEP of X-ray crystal structure and CIF for compound **38**, Ramachandran plot for compound **38**, and fluorometric assay data for compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(27) HATU = 1-[(dimethylamino)(dimethyliminium)methyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-3-oxide hexafluorophosphate.

(28) In the final amide coupling step for compounds **1** and **2**, the reaction was protected from light by wrapping the vessel in aluminum foil. Following aqueous workup, the compounds were immediately purified by flash chromatography and concentrated into amber vials. To minimize decomposition, the compounds should be stored as dry solids in a cold freezer (−10 °C or colder) when not in use, as they are not stable at room temperature for prolonged periods of time.

(29) For a Ramachandran plot for compound **38**, see the Supporting Information.

(30) For a detailed labeled ROESY spectrum of **1** showing all ^{1}H−^{1}H NOE correlations pertinent to stereochemical assignment and for a labeled HMBC spectrum showing all ^{1}H−^{13}C interactions pertinent to the assignment of carbonyl carbons, see the Supporting Information.

(31) NOE correlations observed for H-2 in the ROESY of **2** showed that H-3b is significantly shifted downfield in the ^{1}H NMR (δ 2.16) when compared with the analogous signal in **1** (δ 1.77). For a detailed labeled ROESY spectrum of **2** showing all ^{1}H−^{1}H NOE correlations pertinent to stereochemical assignment see the Supporting Information.

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(33) For experimental details on the isolation of **1** and **2** from *S. hawaiiensis*, see the Supporting Information.

(34) For a comparison of the ^{1}H and ^{13}C NMR data of **1** and **2** with the isolated natural products from *S. hawaiiensis*, see the Supporting Information.

(35) For a more detailed explanation of the fluorometric assay, see the Supporting Information.

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(37) Addition of the membrane permeabilizing agent polymyxin B nonapeptide to the culture broth had no measureable beneficial effect on the observed antibacterial activity.

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