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Development and Characterization of Potent Cyclic Acyldepsipeptide Analogues with Increased Antimicrobial Activity

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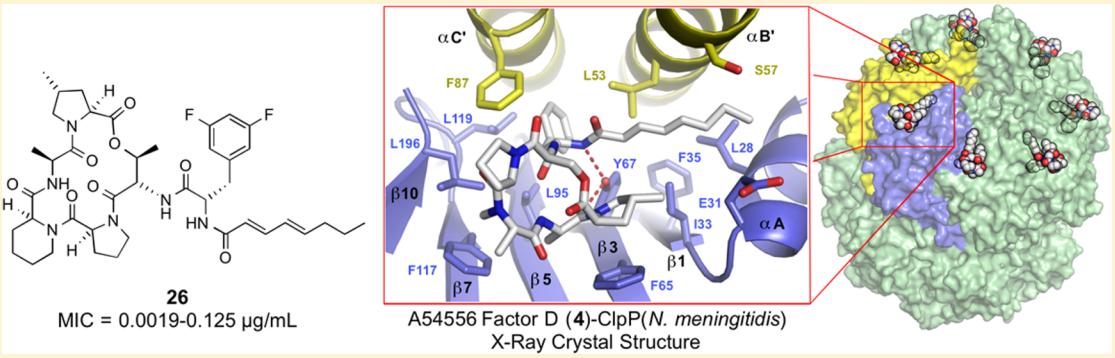
Supporting Information



ABSTRACT: The problem of antibiotic resistance has prompted the search for new antibiotics with novel mechanisms of action. Analogues of the A54556 cyclic acyldepsipeptides (ADEPs) represent an attractive class of antimicrobial agents that act through dysregulation of caseinolytic protease (ClpP). Previous studies have shown that ADEPs are active against Gram-positive bacteria (e.g., MRSA, VRE, PRSP (penicillin-resistant *Streptococcus pneumoniae*)); however, there are currently few studies examining Gram-negative bacteria. In this study, the synthesis and biological evaluation of 14 novel ADEPs against a variety of pathogenic Gram-negative and Gram-positive organisms is outlined. Optimization of the macrocyclic core residues and *N*-acyl side chain culminated in the development of **26**, which shows potent activity against the Gram-negative species *Neisseria meningitidis* and *Neisseria gonorrheae* and improved activity against the Gram-positive organisms *Staphylococcus aureus* and *Enterococcus faecalis* in comparison with known analogues. In addition, the co-crystal structure of an ADEP−ClpP complex derived from *N. meningitidis* was solved.

INTRODUCTION

In recent years, there has been an alarming increase in the number of cases reporting antibacterial resistance in hospitals.[1] Every class of antibiotic to date has encountered the problem of resistance, in some instances only a few years after their introduction.[2,3] Judicious use of current antibiotics can certainly slow the spread of resistance, but it cannot prevent it. As a result, many health agencies have expressed the urgent need for the discovery and development of novel antibiotics,[4] especially compounds that can effectively treat multidrug-resistant strains.[5] Despite the need to address the issue of resistance, the process of antibacterial drug discovery poses unique challenges distinct from other therapeutic areas and is an inherently difficult process.[6] Currently, there exists a significant innovation gap in the antibacterial pipeline as the majority of compounds presently undergoing evaluation are derivatives of known drug classes for which clinical resistance has already developed.[7] To avoid cross-resistance with currently used drugs, new classes of antibiotics having novel mechanisms of action are actively being sought.

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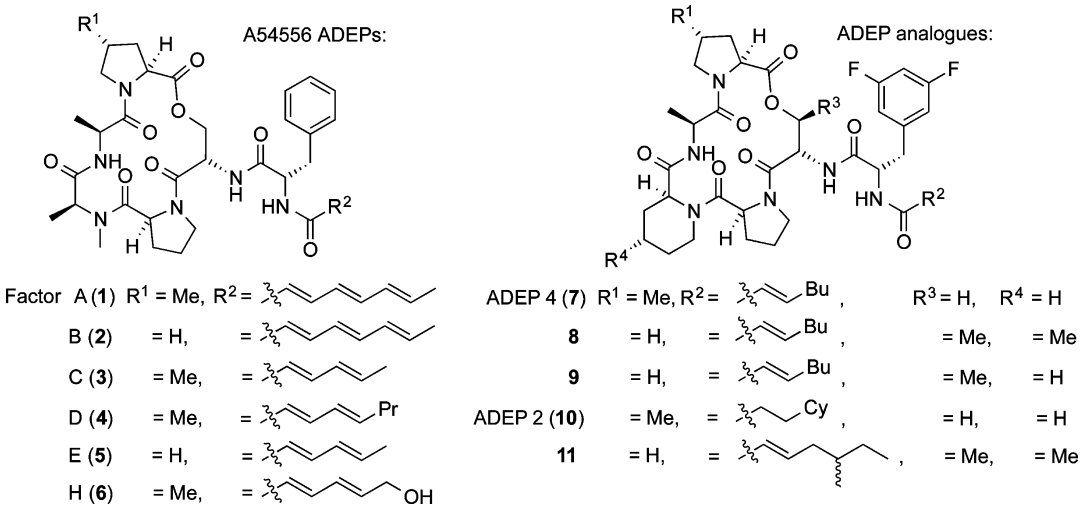

Figure 1. Structures of the A54556 natural products (**1**−**6**) and synthetic ADEP analogues (**7**−**11**).

Over the past decade, studies involving bacterial proteases have revealed a number of potential antibacterial targets.[8−10] ClpP, for example, has been the subject of numerous investigations. ClpP is a cytoplasmic serine protease and is a member of a major family of cylindrical protease complexes common to bacteria and eukaryotes.[11] ClpP has been extensively studied, and X-ray structures from several different organisms have been reported.[12,13] The catalytically active form of the protease exists as two stacked heptameric rings, forming a cylindrical structure which encloses the 14 active sites (Ser-His-Asp) in a central chamber. Normally, the proteolytic activity of ClpP is strictly regulated by hexameric ATPase chaperones of the AAA+ superfamily such as ClpX, ClpA, and ClpC.[14] These chaperones form ATPase−ClpP complexes which mediate the unfolding and degradation of protein substrates.

Studies have shown that ClpP and its cognate ATPases[15,16] are compelling antibacterial targets. To date, there have been several reports on different classes of small molecules that exhibit antibacterial activity via the targeting of ClpP; these are further classified as either inhibitors[17−20] or activators[21−25] of ClpP. The A54556 cyclic acyldepsipeptides (ADEPs, also denoted "factors A−H" **1**−**6**, Figure 1) comprise a small family of antibacterial natural products isolated from *Streptomyces hawaiiensis*[26] and were the first class of Clp-activators to be discovered.[25] Many of the ADEP natural products are active against a broad range of Gram-positive bacteria.[26−28] ClpP was first identified as the target of ADEPs by scientists at Bayer through sequencing ADEP-resistant strains of *Escherichia coli*.[25] Preliminary SAR studies led to the development of ADEP 4 (**7**, Figure 1), an improved synthetic derivative that shows potent activity against PRSP, MRSA, and VRE and demonstrated therapeutic efficacy in mouse models of bacterial infection.[25,28,29] Recent studies by Sello's group have shown that macrocyclic analogues that are further rigidified, through the use of amino acid residues such as 4-methylpipecolate (4-Me-Pip)[30] and *allo*-threonine (**8** and **9**, Figure 1),[31] exhibit improved antibacterial activity. Generally, most Gram-negative bacteria are resistant to ADEPs due to efficient cellular efflux mechanisms and/or limited penetration of the outer membrane.[25] Similarly, ADEPs tend to show diminished activity against Mycobacteria; however, their activity can be enhanced by the addition of efflux pump inhibitors[32] or by coadministration with truncated ADEP-derived fragments.[22]

The structural changes underlying ClpP activation by ADEPs have been examined by X-ray crystallography, and a number of ADEP−ClpP cocrystal structures from different organisms have been reported, including: *Bacillus subtilis* (**1**− and **10**−*Bs*ClpP, Figure 1),[33] *E. coli* (**1**−*Ec*ClpP),[34] and most recently, *Mycobacterium tuberculosis* (**11**−*Mt*ClpP1P2).[35] Analysis of these structures revealed that ADEPs bind in hydrophobic pockets located between subunits on the apical surface of ClpP. Although structural information about the ATPase−ClpP complex remains elusive, it has been proposed that these hydrophobic pockets constitute the binding sites for the Ile-Gly-Phe/Leu (IGF/L) loops of *Ec*ClpP ATPases, ClpX and ClpA (in addition to related loop motifs found in other bacterial ATPases).[34] Much like ATPase binding,[36] ADEPs stabilize the active conformation of ClpP.[37] This in turn allows substrates to access the proteolytic chamber for degradation in an uncontrolled fashion and leads to cell death.[38,39]

Here, we describe the synthesis and biological evaluation of a small library of novel ADEPs, some of which show potent activity against two susceptible pathogenic Gram-negative species, *Neisseria meningitidis* and *Neisseria gonorrheae*, by selectively targeting ClpP. In addition, these ADEPs were also tested against a diverse group of Gram-positive organisms and their activities were compared with previously reported analogues. Finally, we report the first crystal structure of A54556 factor D (**4**, Figure 1) bound to *N. meningitidis* ClpP.

■ RESULTS AND DISCUSSION

Synthesis of ADEP Analogues. The ADEP analogues used in this study were accessed by one of two synthetic routes. The first involves a solution-phase sequence developed by Schmidt (Scheme 1),[40,41] which employs standard peptide coupling reagents[42] and protecting groups,[43] while the second route, recently developed by our group, involves a solid-phase peptide synthesis (SPPS)/dysprosium(III) triflate-mediated macrolactonization sequence (Scheme 2).[44] For the first approach, tripeptide (**12a**[31] and **12b**[45]) and dipeptide/depsipeptide (**13a**−**d**)[27,45] fragments were initially coupled to give linear pentapeptide/depsipeptide intermediates (**14a**−**f**), which following deprotection of the phenacyl (Pac) group (**15a**−**f**) were cyclized using a pentafluorophenyl ester-based

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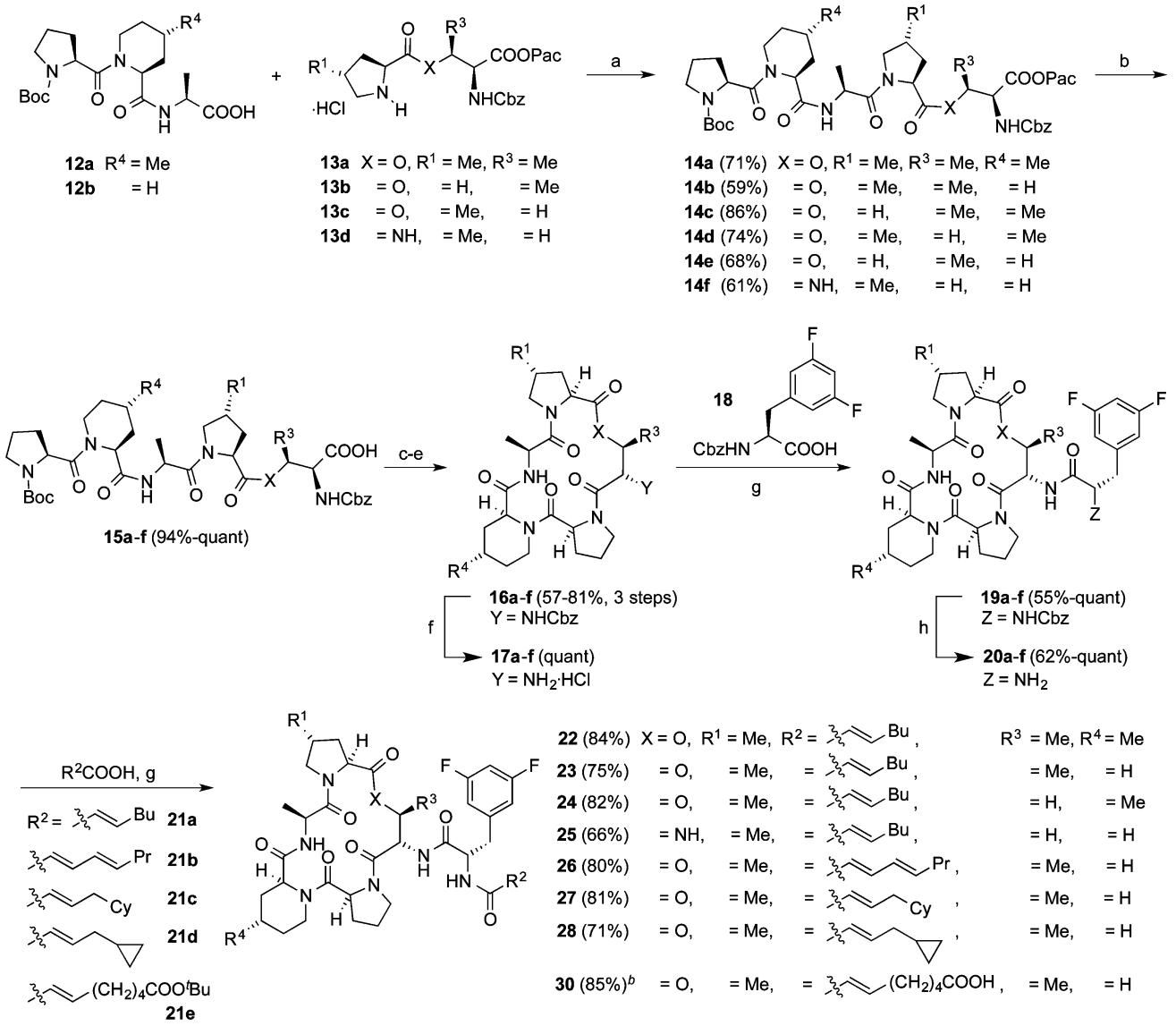
Scheme 1. Solution-Phase Synthesis of ADEP Analogues 22–28 and 30[a]



[a]Reagents and conditions: (a) PyBOP, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 48–72 h; (b) Zn, 70% v/v AcOH (aq), rt, 6–12 h; (c) EDC·HCl, pentafluorophenol, CH_2Cl_2, −20 °C to rt, 12 h; (d) HCl, dioxane, rt, 6 h; (e) CH_2Cl_2:$NaHCO_3$ (1 M, aq) (4:1), slow addition, rt, 24 h; (f) cat. Pd/C, H_2, HCl (1 M, aq), MeOH, rt, 24 h; (g) PyAOP, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 24–48 h; (h) cat. Pd/C, H_2, THF, rt, 24 h; (i) TFA, CH_2Cl_2, 0 °C to rt, 12 h. [b]Coupling yield of intermediate *tert*-Bu ester **29** is given in parentheses. A subsequent deprotection step (condition (i)) was carried out in quantitative yield to obtain free acid **30**.

macrolactamization to give the corresponding 16-membered macrocycles (**16a–f**) in good overall yields. Palladium-catalyzed hydrogenolysis of the Cbz group in the presence of stoichiometric HCl (aq) yielded the corresponding hydrochloride salts (**17a–f**), which were subsequently coupled with Cbz-3,5-difluorophenylalanine (**18**) to give cyclic hexapeptide/depsipeptide intermediates (**19a–f**). Cleavage of the final Cbz group in the absence of acid gave free amines (**20a–f**), which were then coupled to various acrylic acids (**21a–e**) in high yields, giving the corresponding ADEP analogues. It should be noted that previous studies by our group on the total synthesis of the ADEP natural products (**1–6**, Figure 1) have shown that higher yields were generally obtained for related late-stage amide couplings via free amine species as opposed to ammonium salts derived from the corresponding Boc-protected cyclic hexadepsipeptides.[27] In addition to rigidified analogues **8** and **9**[31] (Figure 1, synthesized in 83% and 70% yield from amines **20c** and **20e**, respectively), a series of related analogues (**22–24**) were also synthesized to determine if the *trans*-4-methyl-proline (4-Me-Pro) residue present in some of the natural products (**1**, **3**, **4**, and **6**, Figure 1)[27] and synthetic Bayer analogues (e.g., **7** and **10**, Figure 1)[25] could be combined with 4-Me-Pip and/or *allo*-threonine to further increase the antibacterial activity. In addition, an all-amide ADEP (**25**, Scheme 1) was synthesized as a potentially more biologically stable and soluble derivative. Lastly, in addition to heptenoic acid derivatives, other unsaturated side chain analogues (**26–28** and **30**, Scheme 1) were also synthesized to further expand on previous SAR studies.[28]

The second approach was used to access ADEP 4 (**7**, Figure 1) and its corresponding *N*-methylalanine (MeAla) analogue (**31**, Scheme 2).[28,44,45] In addition, macrocyclic backbone

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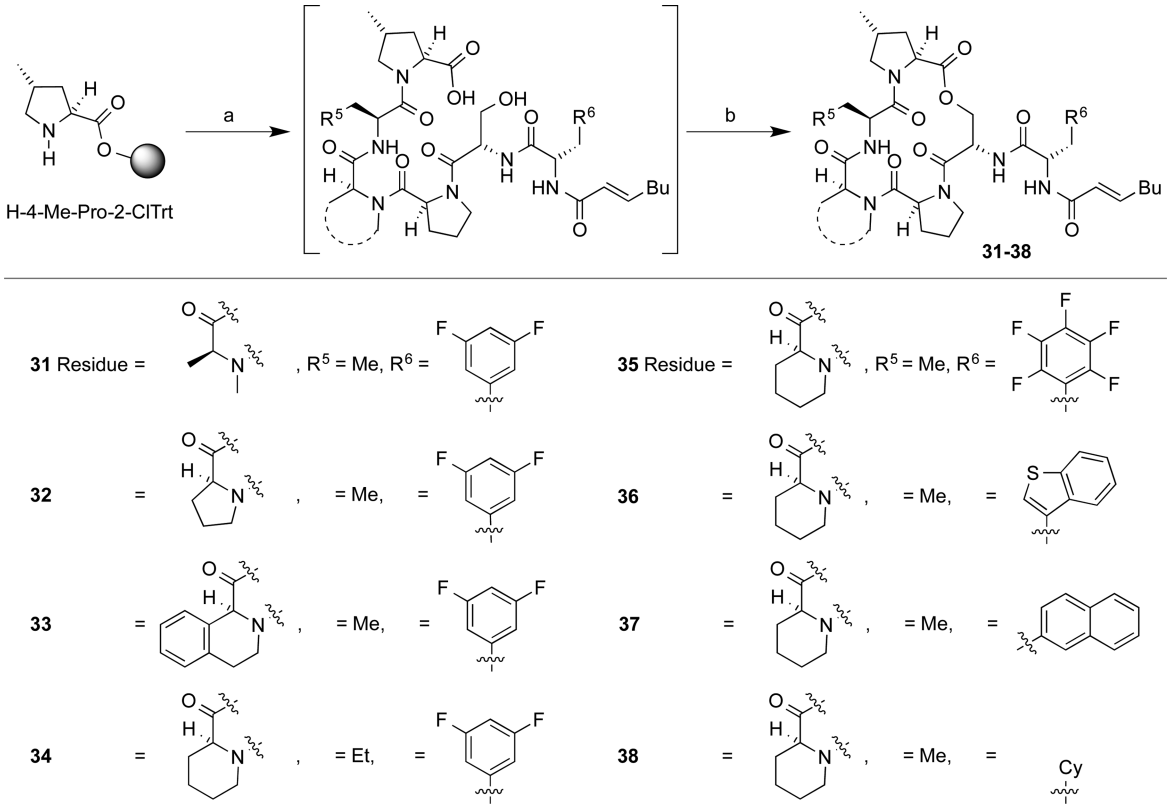
Scheme 2. Synthesis of ADEP Analogues (31−38) via a SPPS/Dysprosium(III) Triflate-Mediated Macrolactonization Sequence[a,44]



[a]Reagents and conditions: (a) SPPS; (b) $Dy(OTf)_3$, 2-methyl-6-nitrobenzoic anhydride, i-Pr_2NEt, CH_2Cl_2, concn = 2.0 mM, slow addition, 24 h.

analogues which replaced the pipecolic acid (Pip) moiety with alternative cyclic residues such as proline (**32**, Scheme 2)[44,45] and tetrahydroisoquinoline-3-carboxylic acid (Tic) (**33**), or the alanine with 2-aminobutyric acid (**34**), were synthesized. A series of analogues replacing the 3,5-difluorophenylalanine (Phe(3,5-F_2)) moiety with alternative aryl residues (**35**−**37**, Scheme 2) or cyclohexylalanine (**38**) were also synthesized by this method.

Antibacterial Testing of ADEP Analogues. Previous studies by our group on ADEP natural products found that A54556 factor D (**4**, Figure 1) showed the highest overall antibacterial activity against two susceptible Gram-positive strains (i.e., *S. aureus*, *S. pneumoniae*) in addition to *N. meningitidis*, a previously untested Gram-negative bacterium.[27] To further expand on this work, we evaluated the antibacterial activities of our ADEP analogues and compared their activity against a set of Gram-negative and Gram-positive organisms.[46]

The Gram-negative species examined include *N. meningitidis*, *N. gonorrheae*, *P. aeruginosa*, and two strains of *E. coli* (Table 1). Interestingly, natural product **4** having the MeAla residue remained one of the most potent compounds against *N. meningitidis* and *N. gonorrheae* (MICs = 0.0313 and 0.0156 μg/mL, respectively), being 2-fold more active than its corresponding Phe(3,5-F_2)/heptenoic acid side chain analogue (**31**). For ADEPs having different combinations of rigidifying amino acids together with the Phe(3,5-F_2)/heptenoic acid moieties, (**7**−**9** and **22**−**24**), compound **23** having the 4-Me-Pro, Pip, and *allo*-threonine combination was found to be highly active against both *Neisseria* spp. (MIC = 0.0625 μg/mL). With this same combination of macrocyclic residues, we examined alternative lipophilic side chains (**26**−**28** and **30**); the octadienoic acid derivative (**26**) was equally potent as **4** against *N. meningitidis* and 2-fold more potent against *N. gonorrheae* (MICs = 0.0313 and 0.0078 μg/mL, respectively). Stability studies on ADEP derivative **26** show that the diene functionality is much more stable at ambient conditions than the triene-containing natural products **1** and **2** (Figure 1).[47] The all-amide ADEP derivative **25** was found to have increased aqueous solubility relative to its endo ester analogue (**7**), but it was found to be significantly less active. This observation suggests that substitution of the depsipeptide ester bond may be leading to antibacterial analogues with improved pharmacokinetic profiles.[48] Similarly, other unnatural Phe mimics (**35**−**38**) were found to be largely inactive. To test whether the observed antibacterial effects against *Neisseria* species were the result of ADEP-mediated ClpP activation, a *N. meningitidis* Δ*clpP* strain (in which the *clpP* gene was deleted[24]) was treated with ADEPs. ADEPs that were potently active against wild-type *N. meningitidis* (MICs ≤ 0.0625 μg/mL: **4**, **7**, **22**, **23**, **26**, **27**, **31**) were inefficacious against this mutant strain (MIC > 128 μg/mL). This suggests that ClpP is the target of the ADEPs in *N. meningitidis*. Consistent with previous studies, none of the ADEPs showed activity against wild type *E. coli*[25] or *P. aeruginosa*; this is perhaps not surprising given that both of these strains employ efficient efflux mechanisms.[53] In addition to wild-type *E. coli*, we also examined a mutant strain with a compromised outer membrane, *lptD*-4213.[54−56] This strain is susceptible to moenomycin A and chlorobiphenyl vancomycin, two antibiotics which are unable to penetrate the outer membrane of Gram-negative bacteria.[55] While nearly all of our

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Table 1. Antibacterial Activities of ADEPs against Gram-Negative Bacteria[a]

compd	MIC (μg/mL)					
	Nm[b]	*Nm*[c]	*Ng*[R d]	*Ec*[e]	*Ec*[f]	*Pa*[g]
4	0.0313	>128	0.0156	>128	>128	>128
7	0.0625	>128	0.125	>128	>128	>128
8	0.125	–[h]	0.125	>128	>128	>128
9	0.25	–	0.125	>128	>128	>128
22	0.0625	>128	0.125	>128	>128	>128
23	0.0625	>128	0.0625	>128	>128	>128
24	0.125	–	0.0625	>128	>128	>128
25	1	–	1	>128	>128	>128
26	0.0313	>128	0.0078	>128	4	>128
27	0.0625	>128	0.125	>128	>128	>128
28	0.25	–	0.125	>128	>128	>128
30	4	–	1	>128	>128	>128
31	0.0625	>128	0.0313	>128	>128	>128
32	4	–	4	>128	>128	>128
33	8	–	8	>128	>128	>128
34	0.25	–	0.125	>128	>128	>128
35	32	–	32	>128	>128	>128
36	32	–	>128	>128	>128	>128
37	32	–	>128	>128	>128	>128
38	32	–	32	>128	>128	>128
CHL[i]	0.5	1	64	3.1[49]	2[50]	8[51]

[a]MIC values shown have been determined by standard broth-microdilution techniques.[52] [b]*Neisseria meningitidis* H44/76. [c]*Neisseria meningitidis* H44/76 Δ*clpP*. [d]*Neisseria gonorrheae* N.279 (chloramphenicol-resistant strain). [e]*Escherichia coli* MC4100. [f]*Escherichia coli* MC4100 *lptD*-4213. [g]*Pseudomonas aeruginosa* PA01. [h]Symbol "–" indicates not determined. [i]CHL = chloramphenicol.

ADEP analogues demonstrated no difference in efficacy relative to wild type, one compound, **26**, showed activity (MIC = 4 μg/mL). The acyl side chain of **26** resembles that of polymixin B, a class of lipopeptide antibiotics. Studies of polymixin and the corresponding delipidated nonapeptide demonstrated the importance of the lipid chain for antimicrobial activity,[57,58] although whether this has any bearing in the context of ADEP function is not known.

Several antibiotics that are generally inactive against Gram-negative bacteria, such as lantibiotics, ramoplanin, and moenomycin A, are effective against *Neisseria* spp.[59,60] This increased susceptibility is attributed to the increased inherent permeability of the *Neisseria* outer membrane.[61] In Neisseriaceae, the outer leaflet of the outer membrane contains phospholipids in addition to lipopolysaccharide[62] and the presence of phospholipids in the outer leaflet, which leads to bilayer regions, may provide a point of entry for hydrophobic molecules.[63,64] However, it remains to be explicitly shown whether the pronounced ADEP activity against *Neisseria* spp. is a consequence of these physiological differences in outer membrane structure/composition leading to increased permeability.[62,65]

ADEPs were also evaluated for activity in several Gram-positive organisms, including clinical isolates of *S. aureus* and *Enterococcus faecalis* (Table 2). Previous reports have inferred that there is a measurable increase in antibacterial activity of ADEPs against Gram-positive organisms, namely *S. aureus* and *E. faecalis*, through the use of rigidifying residues such as 4-Me-Pip and *allo*-threonine compared with their corresponding less rigid analogues (which contained Pip and serine residues, respectively).[30,31] We found that inclusion of the *allo*-threonine residue was generally beneficial in terms of activity against Gram-positive strains, however, the inclusion of the 4-Me-Pip

Table 2. Antibacterial Activities of ADEPs against Gram-Positive Bacteria[a]

compd	MIC (μg/mL)						
	Sa[R b]	*Sa*[c]	*Sa*[d]	*Ef* [R e]	*Sp*[f]	*Bs*[g]	*Li*[h]
4	0.5	0.25	0.5	0.125	≤0.0625	0.125	≤0.0625
7	0.125	0.5	0.0156	0.125	≤0.0625	≤0.0625	≤0.0625
8	1	0.5	0.0078	0.0156	≤0.0625	≤0.0625	≤0.0625
9	0.125	0.0625–0.125	0.0078	0.0078	≤0.0625	≤0.0625	≤0.0625
22	8	0.25	0.0078	0.0156	≤0.0625	≤0.0625	≤0.0625
23	0.125	0.0625	0.0039	0.0078	≤0.0625	≤0.0625	≤0.0625
24	4	0.5	0.0156	0.0156	≤0.0625	≤0.0625	≤0.0625
25	16	16	4	0.5	2	4	≤0.0625
26	0.125	0.0625	0.0019	0.0039	≤0.0625	≤0.0625	≤0.0625
27	0.125	0.0625	0.0078	0.0078	0.0625–0.125	≤0.0625	≤0.0625
28	0.125	0.0625	0.0078	0.0078	≤0.0625	≤0.0625	≤0.0625
30	64	64	32	8	0.125	16	0.5
31	0.5	0.125	0.125	0.0156	≤0.0625	≤0.0625	≤0.0625
32	2	2	2	0.25	0.5	0.125	≤0.0625
33	4	8	4	0.25	0.5	2	0.125
34	1	1	0.125	0.0156	0.0625–0.125	≤0.0625	≤0.0625
35	>128	>128	>128	16	>128	128	4
36	>128	8	8	8	8	8	0.125
37	>128	>128	>128	32	2	128	0.5
38	4	1	1	1	0.5	2	≤0.0625
VAN[i]	1.3[66]	1[67]	2	64[68]	0.25[69]	0.16[70]	1[71]

[a]MIC values shown have been determined by standard broth-microdilution techniques.[52] [b]*Staphylococcus aureus* 1784A (MRSA clinical isolate). [c]*Staphylococcus aureus* subsp. *aureus* Newman (clinical isolate). [d]*Staphylococcus aureus* ATCC 29213. [e]*Enterococcus faecalis* V583 (VRE clinical isolate). [f]*Streptococcus pneumoniae* D39. [g]*Bacillus subtilis* PY79. [h]*Listeria innocua*. [i]VAN = vancomycin.

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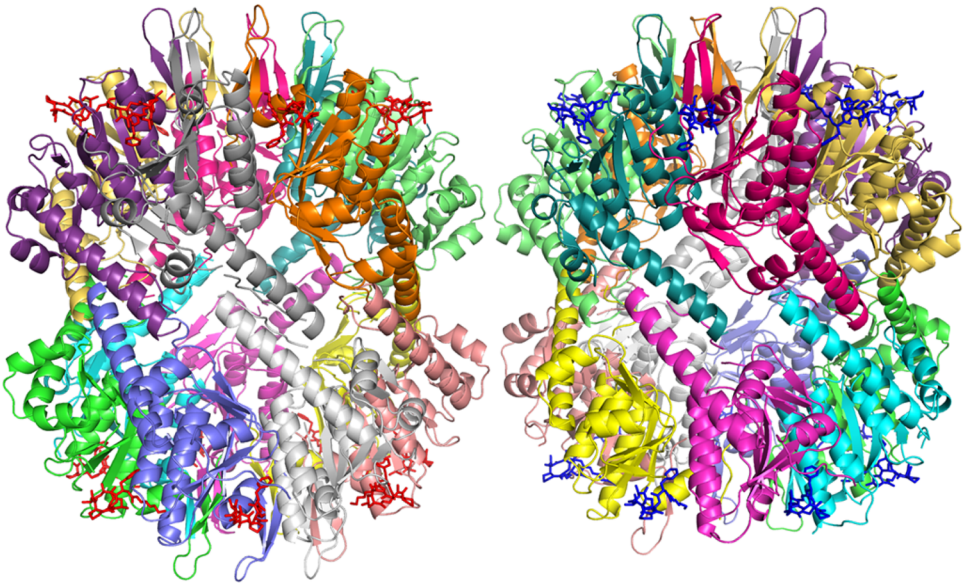

Figure 2. Two tetradecamers (depicted as rainbow ribbons) as arranged in the asymmetric unit of A54556 factor D (**4**)−*Nm*ClpP crystals.[73] The 28 molecules of **4** are shown as red sticks for the first tetradecamer (left, chains A−N) and blue sticks for the second tetradecamer (right, chains a−n).

residue did not increase the activity against any of the strains we tested (activity: **9** ≥ **8**). Similarly, when combining one or both of these rigidifying residues with the 4-Me-Pro residue that has been shown to increase activity (as opposed to proline),[27,28] it was found that the 4-Me-Pro/*allo*-threonine combination was optimal in this case (activity: **23** > **22** > **24**). Against MRSA (1784A), a number of compounds showed activity below 1 μg/mL, with several having MICs of 0.125 μg/mL; these compounds, **7**, **9**, and **23**, all contain a heptenoic acid side chain, however, compounds bearing octadienoic acid (**26**), 4-cyclohexylbut-2-enoic acid (**27**), and 4-cyclopropylbut-2-enoic acid (**28**) side chain analogues were equally effective. Against *S. aureus* strains, Newman and ATCC, compounds **23** and **26**, both of which contain the 4-Me-Pro/*allo*-threonine residue combination with similar side chains (heptenoic acid and octadienoic acid, respectively), showed 2−4-fold improved activity over previously reported ADEPs. Similarly, compound **26** was found to be more active against VRE (V583) than previously reported ADEP analogues (MIC = 0.0039 μg/mL). Additionally, many of the ADEP analogues were found be highly effective against other Gram-positive strains, including *S. pneumoniae*, *B. subtilis*, and *Listeria innocua* (MICs ≤ 0.0625 μg/mL).[72] Compounds **25**, **30**, **32**, **33**, and **35**−**38** which were inactive against *Neisseria* spp. had poor activity or were completely inactive against all tested Gram-positive strains.

A54556 Factor D (4)−*Nm*ClpP Crystal Structure. In an effort to better understand the interaction between ADEPs and *Neisseria* ClpPs, we obtained a co-crystal structure of A54556 factor D (**4**, Figure 1) bound to the *N. meningitidis* enzyme (*Nm*ClpP) (Figures 3−4 and Supporting Information, Figure S1). The asymmetric unit of these crystals contained 28 copies of the protein, labeled A−N for tetradecamer 1 and a−n for tetradecamer 2, arranged into two double rings, with each ring consisting of seven subunits (Figure 2). Although noncrystallographic symmetry (NCS) constraints applied during the refinement process restricted structural differences between the various subunits, the two resulting tetradecamer models fit the electron density very well (Figure 2). While for subunit c alone no interpretable electron density could be seen for the first 21 amino acids, the other subunits lacked clear density only for the first five or six residues. The following N-terminal segment of *Nm*ClpP (residues 7−22), with the exception of subunit c, shows varying degrees of enhanced mobility but allowed the tracing of most of the backbone arranged in the β-hairpin feature (β-1/β0 loop) observed in other ADEP−ClpP structures in addition to a variety of apo ClpP structures from various organisms. It is interesting to note that there is no obvious correlation between the degree of mobility and possible crystal−crystal contacts. Generally, ADEP−ClpP structures which have well-resolved N-terminal β-hairpins show additional stabilization through the formation of intraside chain salt bridges between charged residues of the β-1/β0 loop and the neighboring αA-helix.[34,35] In our case, no extensive salt bridge formation was observed, the only exception being a potential, weak interaction between the side chains of Glu13 on the β-1/β0 loop and Arg27 and Lys30 on the αA-helix.

A second stretch of weaker electron density was found for residues ^{130}LISGGLGGQA^{139}. These residues mark the beginning of the handle region of ClpP and typically form antiparallel β-sheets between intercalating monomers from opposing heptameric rings (Supporting Information, Figure S2).[74] Although, in *Nm*ClpP, this stretch of amino acids still adopted an extended, β-strand-like conformation the insertion of extra glycine residues led to significant higher mobility and the formation of a highly mobile, bulge-like structure at Gly133 and Gly134 (Supporting Information, Figure S2). This lowered structural stability when compared to other ClpP molecules of known three-dimensional structure can easily be explained by the presence of an amino acid sequence (SGGLGG) that is very similar to those used as flexible linkers in protein design.

Similar to the previously reported structures of ADEP−ClpP homotetradecamers,[33,34] each tetradecamer contained 14 ADEPs located between *Nm*ClpP subunits (Figure 2). The bound orientation of **4**, its corresponding interactions with surface residues (Figure 3), and the resulting ∼20 Å diameter axial pore of *Nm*ClpP, which is created by ADEP-binding, are all consistent with other reported ADEP−ClpP complexes.[33−35] The phenyl ring of the exocyclic Phe residue of

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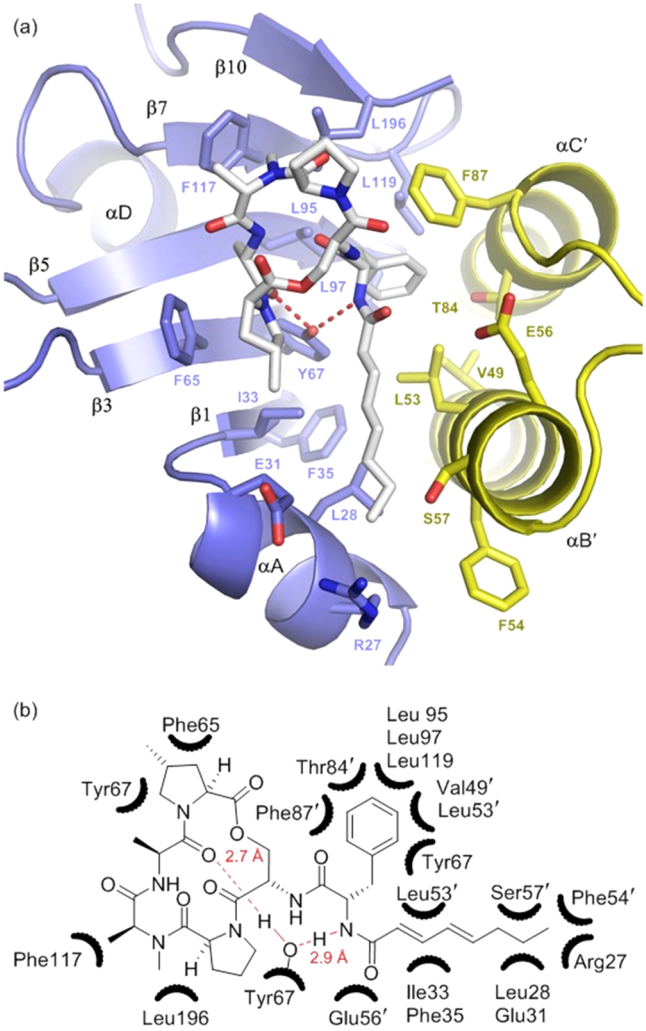

Figure 3. Binding site of factor D (**4**) to *Nm*ClpP. (a) The ligand (in gray stick representation) is located between two adjacent *Nm*ClpP monomers, indicated by yellow and lavender ribbons, respectively. Residues lining the binding site are also shown in stick representation. Hydrogen bonding interactions are denoted by red dashed lines. (b) Schematic diagram showing the interactions between **4** and *Nm*ClpP. Interacting residues on different *Nm*ClpP monomers are distinguished by the prime (′) denotation. The measured distance between heteroatoms is shown in Å.

4 resides deep in a hydrophobic pocket defined by Tyr67, Leu95, Leu97, and Leu119 from one monomer and Val49′, Leu53′, Thr84′, and Phe87′ from an adjacent monomer. ADEP analogues **35**−**38** (Scheme 2), bearing a variety of Phe alternatives, showed lower activity against *Neisseria* spp., likely due to unfavorable steric interactions within the Phe-binding pocket.

One wall of the ADEP-binding pocket is stabilized through binding of a K^+ ion to a highly negatively charged surface depression. The cation interacts strongly with the backbone carbonyl oxygens of Met85, Ile88, and Pro90, as well as up to three water molecules (Supporting Information, Figure S1a). The octadienoic acid side chain of **4** lies along a narrow hydrophobic channel defined by Arg27, Leu28, Glu31, Ile33, Phe35, and Tyr67 from one monomer and Leu53′, Phe54′, and Ser57′ from an adjacent monomer (Figure 3). In previously reported ClpP structures, the carboxyl oxygen of the *N*-acyl side chain is stabilized through a water-mediated hydrogen bond to Glu56′.[34] However, our model lacks distinct density for this water. Rather, in subunits c, h, and k, we observed density for a Na^+ ion at an equivalent position, which we assigned due to its close contact to the glutamic acid side chain and consistent *B*-factors (Supporting Information, Figure S1b). All subunits in the factor D−*Nm*ClpP complex, however, do bind a water molecule in a negatively charged part of the pocket (Figure 4 and Supporting Information, Figure S1b), where it interacts with residues of the neighboring subunit, the backbone oxygen of Leu53, the side chain carboxylate of Glu56, and more weakly with the main chain nitrogen of Ser57.

Bulky aliphatic side chains, such as those in **27** and **28** (Scheme 1), did not abolish antibacterial activity; however, they were generally found to be slightly more tolerated in terms of activity against Gram-positive organisms as opposed to Gram-negative bacteria when compared with **23** and **26** (Scheme 1) which instead have linear side chains. This can likely be explained in terms of differences in the *N*-acyl side chain binding pockets between Gram-positive and Gram-negative species. For example, in the X-ray structure of **4**−*Nm*ClpP, the hydrophobic groove where the acyl side chain of an ADEP resides is comprised (in part) by Glu31 residue from one *Nm*ClpP monomer and Ser57 from an opposing monomer (Figure 3); these polar residues are conserved in other Gram-negative species (e.g., *N. gonnorhoeae* and *E. coli*, Supporting Information, Figure S2) and would disfavor binding large hydrophobic groups. Conversely, the corresponding residues in a variety of Gram-positive species (e.g., *S. aureus*, *E. faecalis*, *B. subtilis*, and *S. pneumoniae*, Supporting Information, Figure S2) are replaced by Asp and Ala, respectively, making the pocket better able to accommodate large substituents. Taken together, C_7 or C_8 length acyl side chains, that may also include a small cyclic component, could lead to analogues of increased potency. Similarly, ADEP side chains bearing a polar functional group (e.g., alcohol, amine, etc.) at the β position may displace the ordered water molecule bound to ClpP (Figure 4 and Supporting Information, Figure S1b). ADEP analogue **30**, which contains an acid-terminated C_8 acyl side chain, was synthesized in an effort to form a salt bridge with Arg27, a highly conserved residue (Supporting Information, Figure S2), however, this modification was deleterious to activity. Macrocyclic residues of **4** are stabilized by van der Waals contacts with one of the ClpP monomers. For example, the 4-Me-Pro residue of **4** interacts with the alkyl part of the side chain of Glu31, Ile33, and Phe65 (this interaction accounts for the increased activity relative to ADEPs which contain only proline), and similarly the MeAla residue interacts with Phe117 and Leu196. The phenolic hydroxyl group of Tyr67 makes key hydrogen bonds to both the alanine C═O and the phenylalanine NH residue of **4**. Mutation studies have shown that this particular tyrosine residue is important for ADEP-mediated activation of *Bs*ClpP (e.g., the Y62A mutant has decreased proteolytic activity compared to wild type),[25] and furthermore this dual hydrogen bonding interaction has been observed in every reported ADEP−ClpP crystal structure to date.[33−35] A sequence alignment of representative bacterial ClpPs shows that the majority of residues involved in ADEP binding are conserved across both Gram-negative and Gram-positive species (many of which are relevant to the current study) as well as *M. tuberculosis* (Supporting Information, Figure S2).

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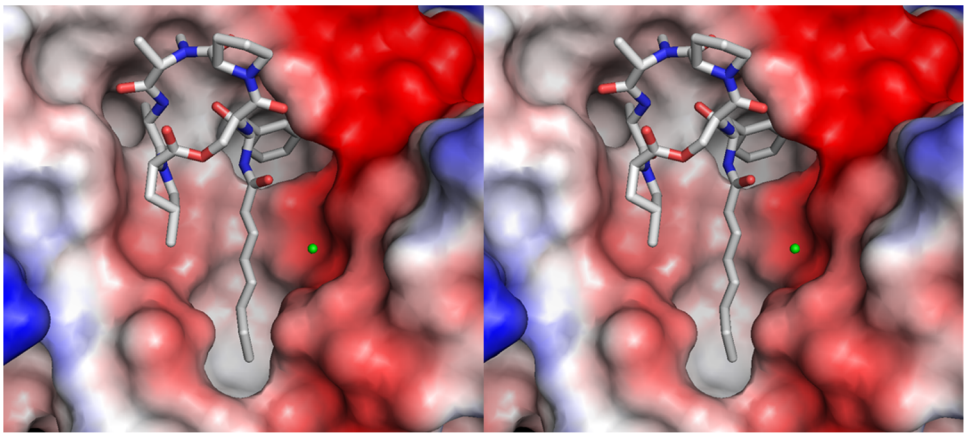

Figure 4. Stereo (wall-eyed) representation of the factor D (**4**) binding site on *Nm*ClpP. The protein surface is colored according to its electrostatic potential. Positive potential is shown in blue and negative potential in red. The ligand (**4**) is shown in gray stick representation. The green sphere indicates a water molecule found in all 28 *Nm*ClpP subunits.

■ CONCLUSION

We have synthesized a small library of novel ADEP analogues using a combination of solution- and solid-phase approaches and have evaluated their antibacterial activity against a variety of different Gram-negative and Gram-positive organisms. Taken together, these approaches permit the rapid synthesis of ADEP analogues bearing modifications at various positions. The ability of ADEPs to eradicate persistent *S. aureus* biofilm infections when combined with rifampicin exemplifies the potential of these compounds as antibacterial agents.[29] Previous work established ADEPs as highly effective in the treatment of Gram-positive organisms, and while we have previously shown that select natural ADEPs are efficacious against *Neisseria* sp., this work represents the first comprehensive evaluation of synthetic ADEP analogues in these sensitive Gram-negative strains. Through the course of our studies, we identified a lead analogue, **26**, which displays potent antibacterial activity in all tested Gram-positive strains, *Neisseria* spp. and an *E. coli* strain bearing an outer membrane defect. That **26** is able to target a compromised *E. coli* strain is encouraging and suggests that **26** or further optimized analogues may act as effective antibacterial agents in combination with compounds known to compromise the outer membrane of Gram-negatives such as LpxC inhibitors.[75] We also show that ADEPs exhibit potent antibacterial activity against wild-type *N. meningitidis* and *N. gonorrheae*, which are the primary causes of invasive meningococcal disease, and the sexually transmitted genital mucosa-associated gonorrhea, respectively. This susceptibility of *N. gonorrheae* to ADEPs in particular represents a significant finding given the oppressive public health burden of this bacterium (>100 million new infections per year globally) and the recent emergence of multidrug-resistant strains, which have led to untreatable gonococcal infections.[76] In this regard, our crystal structure of **4** bound to *N. meningitidis* ClpP will assist in the design of new ADEP analogues, or related peptidomimetics, with further improved abilities to target meningococcal- and gonococcal-specific cell death through the activation of ClpP.

■ EXPERIMENTAL SECTION

General Chemistry. 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 methanol was obtained as ≥99.9% pure and stored under argon. Peptide coupling reagents were purchased from Aapptec. All other reagents were purchased from Sigma-Aldrich and were used as received. Flash chromatography on silica gel (60 Å, 230−400 mesh) was performed with reagent grade solvents. Analytical TLC was performed on precoated Merck silica gel 60 F_{254} plates and visualized with a UV lamp and/or $KMnO_4$ stain. Solvent ratios for chromatography and R_f values are reported as v/v ratios. Melting points were obtained on a Fisher Johns apparatus and are uncorrected. Optical rotation measurements were obtained using a Rudolf AUTOPOL IV polarimeter. All ^{1}H and ^{13}C NMR spectra were obtained on 300, 400, 500, and 600 MHz spectrometers as solutions in deuterated solvents. Chemical shifts are reported in δ ppm values. ^{1}H NMR 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) or DMSO-d_6 (δ 2.49). ^{13}C NMR 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 spectrometer utilizing ESI or direct analysis in real time ionization (DART). All ADEP compounds used in antibacterial assays were repurified (following silica gel chromatography) via semipreparative HPLC using a method previously described by our group[27] to ≥95% purity established on a Agilent Eclipse XDB−C18 (5 μM, 4.6 mm × 150 mm, column temperature 23 °C) eluting with a gradient of 20−95% acetonitrile in water containing 0.1% formic acid (flow rate 2.0 mL/min) at 220 nm. Retention times (t_R) are expressed in minutes.

General Procedure A: Synthesis of Amides Using Coupling Reagents. To an ice bath-cooled solution of amine/ammonium hydrochloride salt (1.0 equiv), acid (1.0 equiv), and coupling reagent (1.1 equiv) in CH_2Cl_2 (0.1 M, with respect to acid) was added Hünig's base (4.0 equiv) dropwise. The reaction was stirred overnight with slow warming to room temperature. After 24−72 h, the reaction mixture was diluted with water and extracted with three portions of CH_2Cl_2. The organic extracts were then dried over $MgSO_4$, concentrated in vacuo, and the crude product was purified using silica gel chromatography (EtOAc−MeOH or hexanes−EtOAc or EtOAc) to give the corresponding amide.

General Procedure B: Synthesis of Acids via Deprotection of Phenacyl Esters. Zinc dust (6.0 equiv) was added to a solution of pentapeptide/pentadepsipeptide in 70% v/v acetic acid (aq) (0.05 M) and allowed to stir for 6−12 h. After complete consumption of the starting material by TLC, the reaction mixture was filtered with suction over a 2 in. plug of Celite and then rinsed with EtOAc before being concentrated in vacuo to yield a crude residue. The residue was taken up again in EtOAc and washed once with HCl (1 M, aq). The organic layer was then dried over $MgSO_4$ and concentrated in vacuo to give a

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crude acid product. Residual acetic acid was then removed by coevaporation with toluene (performed 2−3 times), and the crude acid was dried further under vacuum overnight. The resulting crude acid contained small amounts of residual acetophenone (<10% w/w by crude ^{1}H NMR) and was used without further purification. It should be noted that a small amount of the purified acid was obtained via semipreparative HPLC and was used for characterization.[27]

General Procedure C: Macrolactamization of Cyclic Peptides/Depsipeptides from Linear Pentapeptide/Pentadepsipeptide Intermediates. Pentafluorophenol (4.0 equiv) and EDC·HCl (1.4 equiv) were successively added to a cooled solution (−20 °C) of pentapeptide/pentadepsipeptide in CH_2Cl_2 (0.05 M) under nitrogen. The mixture was stirred overnight (12 h) with slow warming to room temperature. The following day the reaction mixture was concentrated in vacuo to give a crude residue which was subsequently dissolved in dioxane (0.1 M), treated with HCl (4 M in dioxane, 30 equiv), and stirred under nitrogen for 6 h. After this time, the reaction mixture was concentrated in vacuo to give a crude residue which was subsequently dissolved in CH_2Cl_2 (1 vol) and added overnight via syringe pump (over ∼20 h period) into a vigorously stirring biphasic solution of 4:1 CH_2Cl_2:$NaHCO_3$ (1 M, aq, 10 vol, final concn after addition ∼2 mM). After 24 h (includes time required for slow addition), the reaction mixture was transferred to a separatory funnel, diluted with water, shaken, and the resulting layers were separated. Following phase separation, the aqueous layer was extracted twice more with CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$, concentrated in vacuo, and the crude product was purified using flash chromatography (EtOAc−MeOH) to give the corresponding macrocycle.

General Procedure D: Synthesis of Amines/Ammonium Hydrochloride Salts via Palladium-Catalyzed Hydrogenolysis of Cbz-Protected Amines. Palladium on activated carbon (10% w/w, 15 mol %) was added to a solution of Cbz-protected amine in THF or MeOH (0.03 M). The reaction mixture was initially purged with nitrogen for 5 min, hydrogen for 5 min using a balloon, and then stirred under static hydrogen atmosphere overnight. After complete consumption of the starting material by TLC (24 h), the reaction mixture was filtered with suction over a 2 in. plug of Celite and then rinsed with 5% MeOH in CH_2Cl_2 before being concentrated in vacuo to yield the corresponding crude amine. Residual THF/MeOH was removed by evaporation from $CHCl_3$ in vacuo (performed twice) and drying under vacuum; the resultant crude amine was used without further purification. Note: To obtain the corresponding ammonium hydrochloride salt from the reaction, HCl (1 M, aq, 1.2 equiv) was added to the reaction mixture prior to the addition of hydrogen gas. Residual water was removed by coevaporation with toluene (performed 2−3 times) after filtration of the reaction mixture.

General Procedure E: Synthesis of Unsaturated Esters Using Horner−Wadsworth−Emmons Olefination. Triethylphosphonoacetate (1.1 equiv) was added dropwise to an ice-cold suspension of sodium hydride (60% dispersion in mineral oil, 1.1 equiv) in THF (0.1 M). After the addition, the mixture was stirred for 30 min, during which time the reaction mixture became clear. The aldehyde (1.0 equiv) was added dropwise, forming a thick mass during the addition (requires vigorous stirring to break up the mass). The reaction was stirred overnight with slow warming to room temperature under nitrogen (12 h). The reaction mixture was then quenched by dropwise addition of NH_4Cl (saturated aq) until effervescence ceased and the resulting solution was slightly acidic as judged by pH paper (pH 4−5). The reaction mixture was diluted with water and extracted with three portions of Et_2O. The combined organic extracts were washed once with brine, dried over $MgSO_4$, concentrated in vacuo, and the crude product was then purified using flash chromatography (hexanes−EtOAc) to give the corresponding unsaturated ester.

General Procedure F: Synthesis of Acids via Ester Hydrolysis. Lithium hydroxide monohydrate was added to a vigorously stirring solution of ester (1.0 equiv) in 3:2 THF:H_2O (0.02 M). Following complete consumption of the starting material by TLC, the reaction mixture was acidified with HCl (4 M, aq) until the resulting solution was acidic to pH paper (pH 2−3). The reaction mixture was diluted with water and extracted with three portions of $CHCl_3$. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo to yield corresponding crude acid product, which was then purified using flash chromatography (hexanes−EtOAc) to give the corresponding acid.

General Procedure G: Synthesis of Ammonium Salts via Cleavage of Boc-Protected Amines. To a solution of Boc-protected amine in dioxane (0.4 M) was added dropwise HCl (4.0 M in dioxane, 10.0 equiv). After complete consumption of the starting material by TLC (12−24 h), the reaction mixture was then concentrated in vacuo to yield the crude ammonium salt. Residual dioxane was removed by evaporation from $CHCl_3$ in vacuo (performed 2−3 times) and drying under vacuum; the resultant ammonium salt was used without further purification.

(*E*)-*N*-((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((6*S*,8a*S*,10*S*,14a*S*,20*S*,21*S*,23a*S*)-6,10,21-trimethyl-5,8,14,19,23-pentaoxodocosahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1,4,7,10,13]oxatetraazacyclohexadecin-20-yl)amino)propan-2-yl)hept-2-enamide (8).[31] ADEP **8** (48.9 mg, 83%) was prepared from amine **20c** and acid **21a**[44] according to general procedure A; white solid, mp 195−196 °C (CH_2Cl_2). HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 8.74 min); $[\alpha]_D^{24}$ − 68.1 (*c* 0.58, $CHCl_3$); R_f 0.47 (EtOAc). IR (thin film in CH_2Cl_2) ν_{max} 3291, 3053, 2957, 2932, 2874, 1728, 1643, 1597, 1520, 1435, 1117, 845 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (1H, d, *J* = 9.5 Hz), 7.02−6.89 (3H, m), 6.76−6.70 (2H, m), 6.65 (1H, tt, *J* = 9.0, 2.5 Hz), 6.15 (1H, dt, *J* = 15.5, 1.5 Hz), 5.23 (1H, dd, *J* = 9.0, 3.0 Hz), 5.11 (1H, qd, *J* = 6.5, 2.0 Hz), 4.95 (1H, dq, *J* = 9.5, 6.5 Hz), 4.72−4.66 (3H, m), 4.64 (1H, dd, *J* = 9.5, 2.0 Hz), 4.46 (1H, d, *J* = 8.0 Hz), 3.78 (1H, ddd, *J* = 11.5, 8.5, 5.0 Hz), 3.60 (1H, ddd, *J* = 12.0, 8.5, 8.5 Hz), 3.52 (1H, ddd, *J* = 11.5, 7.0, 7.0 Hz), 3.29 (1H, ddd, *J* = 12.0, 9.0, 3.5 Hz), 3.00 (1H, dd, *J* = 13.5, 8.0 Hz), 2.92 (1H, dd, *J* = 13.5, 5.5 Hz), 2.73−2.66 (1H, br m), 2.62 (1H, ddd, *J* = 13.5, 13.5, 2.5 Hz), 2.40−2.28 (1H, m), 2.27−2.06 (4H, m), 2.05−1.80 (5H, m), 1.68−1.54 (2H, m), 1.48−1.40 (2H, m), 1.38−1.27 (5H, m), 1.18 (3H, d, *J* = 6.5 Hz), 1.11−0.98 (2H, m), 0.95 (3H, d, *J* = 6.5 Hz), 0.89 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 171.4, 171.1, 169.8, 169.6, 166.3, 165.6, 163.0 (dd, *J* = 248.0, 13.0 Hz), 146.1, 140.4 (t, *J* = 9.0 Hz), 123.4, 112.6 (dd, *J* = 19.0, 5.5 Hz), 102.5 (t, *J* = 25.0 Hz), 70.0, 59.2, 57.2, 57.0, 54.4, 53.4, 47.9, 47.1, 46.6, 40.9, 38.0 (2C), 36.2, 33.5, 31.9, 30.8, 30.6, 28.1, 23.1, 22.4, 22.0, 21.3, 18.0, 14.0, 13.1. HRMS (ESI$^+$) *m*/*z* calcd for $C_{40}H_{55}F_2N_6O_8$ [M + H]$^+$, 785.4043; found, 785.4046.

(*E*)-*N*-((*S*)-3-(3,5-Difluorophenyl)-1-(((6*S*,8a*S*,14a*S*,20*S*,21-*S*,23a*S*)-6,21-dimethyl-5,8,14,19,23-pentaoxodocosahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1,4,7,10,13]oxatetraazacyclohexadecin-20-yl)amino)-1-oxopropan-2-yl)hept-2-enamide (9).[31] ADEP **9** (79.1 mg, 70%) was prepared from amine **20e** and acid **21a**[44] according to general procedure A; white solid, mp 144−145 °C (CH_2Cl_2). HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 8.11 min); $[\alpha]_D^{23}$ − 76.8 (*c* 0.81, $CHCl_3$); R_f 47 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3291, 3055, 2955, 2934, 1728, 1645, 1597, 1435, 1258, 1117 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amide protons were not observed due to deuterium exchange) δ 6.89−6.79 (3H, m), 6.75 (1H, tt, *J* = 9.5, 2.5 Hz), 6.24 (1H, dt, *J* = 15.5, 1.5 Hz), 5.32 (1H, dd, *J* = 8.5, 2.5 Hz), 5.20 (1H, qd, *J* = 6.5, 2.0 Hz), 5.02 (1H, q, *J* = 6.5 Hz), 4.80−4.77 (1H, m), 4.75 (1H, dd, *J* = 7.5, 6.0 Hz), 4.68 (1H, d, *J* = 2.0 Hz), 4.61−4.54 (1H, br m), 4.43 (1H, d, *J* = 8.0 Hz), 3.73−3.66 (1H, m), 3.62 (1H, ddd, *J* = 11.5, 8.5, 8.5 Hz), 3.48 (1H, ddd, *J* = 11.5, 7.0, 7.0 Hz), 3.31−3.25 (1H, m), 3.06 (1H, dd, *J* = 13.5, 7.5 Hz), 2.89 (1H, dd, *J* = 13.5, 6.0 Hz), 2.69−2.58 (2H, m), 2.53−2.42 (1H, m), 2.30−2.18 (3H, m), 2.11−1.88 (6H, m), 1.76 (1H, br d, *J* = 13.0 Hz), 1.69−1.55 (2H, m), 1.54−1.31 (9H, m), 1.23 (3H, d, *J* = 6.5 Hz), 0.94 (3H, t, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 173.5, 173.3, 172.4, 171.45, 171.38, 168.4, 167.8, 164.4 (dd, *J* = 246.5, 13.0 Hz), 146.0, 142.6 (t, *J* = 9.5 Hz), 124.6, 113.8 (dd, *J* = 19.5, 5.5 Hz), 103.0 (t, *J* = 25.5 Hz), 71.6, 61.0, 58.8, 59.7, 55.2, 54.9, 49.3, 48.4, 47.8, 42.6, 38.9, 32.9, 31.9, 31.7, 31.5, 28.9, 26.0, 24.1, 23.4, 22.47, 22.44, 18.3, 14.3, 13.5. HRMS (ESI$^+$) *m*/*z* calcd for $C_{39}H_{53}F_2N_6O_8$ [M + H]$^+$, 771.3887; found, 771.3897.

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(*S*)-2-((2*S*,4*S*)-1-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carbonyl)-4-methylpiperidine-2-carboxamido)propanoic Acid (12a).[31] Acid **12a** (1.53 g, 99%) was prepared from the corresponding methyl ester according to general procedure F using LiOH·H_2O (0.78 g, 18.57 mmol, 5.0 equiv), 24 h; white solid, mp 90−91 °C ($CHCl_3$); $[\alpha]_D^{24}$ − 82.1 (*c* 0.99, $CHCl_3$). IR (thin film in CH_2Cl_2) ν_{max} 3309, 3055, 2974, 2955, 2932, 2878, 1743, 1670, 1454, 1412, 1366, 1207, 1165 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, notes: carboxylic acid proton was not observed due to deuterium exchange and mixture of rotamers) δ 8.43 (0.5H, d, *J* = 7.0 Hz), 6.81 (0.25H, d, *J* = 7.0 Hz), 6.76 (0.25H, d, *J* = 7.0 Hz), 5.46−4.40 (4H, m), 3.94−3.38 (4H, m), 2.60−0.98 (20H, m), 0.97−0.90 (3H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 175.43, 175.36, 173.1, 172.6, 171.7, 171.4, 170.90, 170.84, 155.0, 154.7, 154.0, 80.6, 79.95, 79.89, 57.8, 57.4, 56.7, 55.8, 52.6, 52.5, 49.5, 48.4, 48.2, 47.0, 46.9, 46.7, 43.4, 43.2, 39.9, 34.5, 34.0, 33.9, 33.7, 33.5, 33.2, 30.5, 30.0, 29.8, 29.1, 28.63, 28.57, 28.54, 27.4, 26.9, 26.8, 25.0, 24.0, 23.5, 22.2, 22.04, 21.95 18.2, 18.0, 16.4. HRMS (ESI^+) *m*/*z* calcd for $C_{20}H_{33}N_3O_6Na$ $[M + Na]^+$, 434.2262; found, 434.2274.

(*S*)-2-((*S*)-1-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carbonyl)piperidine-2-carboxamido)propanoic Acid (12b).[45] Acid **12b** (4.30 g, quantitative yield) was prepared from the corresponding benzyl ester using the same procedure as outlined in general procedure D using Pd/C (764.0 mg, 0.718 mmol, 7 mol %) in THF; white solid, mp 71−72 °C ($CHCl_3$); $[\alpha]_D^{25}$ − 90.0 (*c* 1.02, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3302, 3055, 2978, 2940, 2878, 1736, 1670, 1454, 1420, 1366, 1165 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, note: mixture of rotamers) δ 9.07 (1H, br s), 8.39 (0.5 H, d, *J* = 7.0 Hz), 6.92−6.68 (0.5H, m), 5.26−4.16 (4H, m), 3.86−3.00 (4H, m), 2.82−1.00 (21H, m). ^{13}C NMR (100 MHz, $CDCl_3$, note: mixture of rotamers) δ 175.8, 175.46, 175.38, 173.2, 172.8, 172.1, 171.2, 170.70, 170.66, 154.8, 154.7, 154.1, 80.3, 80.04, 79.99, 57.7, 57.4, 56.4, 55.8, 52.6, 52.4, 49.2, 48.26, 48.19, 46.98, 46.93, 46.7, 43.5, 43.3, 40.0, 30.0, 29.7, 29.1, 28.55, 28.49, 26.3, 25.8, 25.7, 25.4, 25.0, 24.9, 24.8, 24.0, 23.4, 20.7, 20.3, 20.2, 18.1, 18.0, 16.4. HRMS (ESI^+) *m*/*z* calcd for $C_{19}H_{31}N_3O_6Na$ $[M + Na]^+$, 420.2105; found, 420.2117.

(2*S*,3*S*)-3-(((Benzyloxy)carbonyl)amino)-4-oxo-4-(2-oxo-2-phenylethoxy)butan-2-yl (2*S*,4*R*)-4-methylpyrrolidine-2-carboxylate Hydrochloride (13a). Ammonium salt **13a** (2.53 g, quantitative yield) was prepared as an inseparable mixture of diastereomers (*trans*-4-Me-L-Pro:*cis*-4-Me-L-Pro 4:1 by ^1H NMR) from the corresponding Boc-protected amine (dr 4:1) according to general procedure G; light-beige crystalline solid, mp 72−73 °C ($CHCl_3$); $[\alpha]_D^{24}$ − 20.1 (*c* 1.02, $CHCl_3$). IR (thin film in CH_2Cl_2) ν_{max} 3372 (br), 3223 (br), 2963, 2938, 1748, 1701, 1533, 1451, 1223 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (major diastereomer) δ 11.19 (1H, br s), 8.56 (1H, br s), 7.95−7.79 (2H, m), 7.62 (1H, t, *J* = 7.5 Hz), 7.48 (2H, t, *J* = 7.5 Hz), 7.40−7.23 (5H, m), 6.26 (1H, d, *J* = 8.5 Hz), 5.58 (1H, d, *J* = 16.5 Hz), 5.51−5.38 (1H, br m), 5.31 (1H, d, *J* = 16.5 Hz), 5.20−5.02 (2H, m), 4.90 (1H, dd, *J* = 8.5, 2.0 Hz), 4.61−4.39 (1H, br m), 3.68−3.47 (1H, br m), 3.01−2.77 (1H, br m), 2.62−2.24 (2H, m), 1.97−1.72 (1H, m), 1.47 (3H, d, *J* = 6.5 Hz), 1.05 (3H, d, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) (major diastereomer) δ 191.3, 168.5, 168.3, 156.4, 136.3, 134.4, 133.7, 129.1, 128.7, 128.3, 127.95, 127.88, 74.0, 67.3 (2C), 59.7, 56.7, 51.9, 36.5, 32.0, 16.8, 15.2. HRMS (ESI^+) *m*/*z* calcd for $C_{26}H_{31}N_2O_7$ $[M - Cl]^+$, 483.2126; found, 483.2120.

(2*S*,3*S*)-3-(((Benzyloxy)carbonyl)amino)-4-oxo-4-(2-oxo-2-phenylethoxy)butan-2-yl L-prolinate Hydrochloride (13b). Ammonium salt **13b** (1.97 g, quantitative yield) was prepared from the corresponding Boc-protected amine[31] according to general procedure G; white crystalline solid, mp 72−73 °C (CH_2Cl_2); $[\alpha]_D^{25}$ − 29.0 (*c* 1.02, $CHCl_3$). IR (thin film in CH_2Cl_2) ν_{max} 3370, 3215, 2940, 1748, 1701, 1533, 1450, 1225, 1180 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.12 (1H, br s), 8.60 (1H, br s), 7.93−7.82 (2H, m), 7.66−7.57 (1H, m), 7.49 (2H, t, *J* = 7.5 Hz), 7.42−7.24 (5H, m), 6.27 (1H, d, *J* = 8.5 Hz), 5.45 (1H, d, *J* = 16.5 Hz), 5.50−5.40 (1H, br m), 5.32 (1H, d, *J* = 16.5 Hz), 5.13 (1H, d, *J* = 12.5 Hz), 5.08 (1H, d, *J* = 12.5 Hz), 4.91 (1H, dd, *J* = 8.5, 3.0 Hz), 4.51−4.39 (1H, br m), 3.52−3.30 (2H, br m), 2.34−2.08 (2H, m), 2.06−1.83 (2H, m), 1.47 (3H, d, *J* = 6.5 Hz). C NMR (100 MHz, $CDCl_3$) δ 191.4, 168.5, 168.0, 156.4, 136.4, 134.4, 133.8, 129.1, 128.7, 128.3, 128.0, 127.9, 74.0, 67.35, 67.26, 59.5, 56.8, 46.0, 28.8, 23.7, 15.2. HRMS (ESI^+) *m*/*z* calcd for $C_{25}H_{29}N_2O_7$ $[M - Cl]^+$, 469.1969; found, 469.1977.

(*S*)-2-Oxo-2-phenylethyl 2-(((Benzyloxy)carbonyl)amino)-3-((2*S*,4*R*)-4-methylpyrrolidine-2-carboxamido)propanoate Hydrochloride (13d). Ammonium salt **13d** (328.1 mg, quantitative yield) was prepared as an inseparable mixture of diastereomers (*trans*-4-Me-L-Pro:*cis*-4-Me-L-Pro 4:1 by ^1H NMR) from the corresponding Boc-protected amine (dr 4:1) according to general procedure G; light-yellow solid, mp 135−136 °C (CH_2Cl_2); $[\alpha]_D^{24}$ − 15.4 (*c* 0.52, $CHCl_3$). IR (thin film in CH_2Cl_2) ν_{max} 3298, 3213, 3063, 3034, 2961, 2938, 1755, 1701, 1537, 1450, 1234, 752 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, note: mixture of rotamers) (major diastereomer) δ 10.89 (1H, br s), 8.64 (1H, br s), 8.02−7.67 (3H, m), 7.61 (1H, t, *J* = 7.0 Hz), 7.48 (2H, t, *J* = 7.0 Hz), 7.40−7.25 (5H, m), 6.77−6.37 (1H, m), 5.67−5.45 (1H, m), 5.44−5.24 (1H, m), 5.21−5.00 (2H, m), 4.88−4.48 (2H, m), 4.03−3.60 (2H, m), 3.56−3.35 (1H, m), 3.03−2.76 (1H, m), 2.32 (1H, br s), 2.24−2.12 (1H, m), 2.11−1.82 (1H, m), 1.10−0.90 (3H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) (major diastereomer) δ 192.7, 170.2, 170.0, 169.8, 156.4, 156.2, 136.4, 134.6, 134.5, 133.8, 133.7, 129.19, 129.13, 128.68, 128.66, 128.4, 128.30, 128.28, 128.18, 128.13, 67.4, 67.26, 67.21, 60.4, 60.0, 54.6, 54.5, 52.5, 52.4, 41.7, 41.6, 37.8, 37.7, 34.1, 32.4, 16.5, 16.0. HRMS (ESI^+) *m*/*z* calcd for $C_{25}H_{30}N_3O_6$ $[M - Cl]^+$, 468.2129; found, 468.2147.

(*S*)-*tert*-Butyl 2-((2*S*,4*S*)-2-(((*S*)-1-((2*S*,4*R*)-2-((((2*S*,3*S*)-3-(((Benzyloxy)carbonyl)amino)-4-oxo-4-(2-oxo-2-phenylethoxy)butan-2-yl)oxy)carbonyl)-4-methylpyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)-4-methylpiperidine-1-carbonyl)pyrrolidine-1-carboxylate (14a). Pentadepsipeptide **14a** (1.24 g, 71%) was prepared from acid **12a**[31] and ammonium salt **13a** according to general procedure A; white crystalline solid, mp 100−101 °C (CH_2Cl_2); $[\alpha]_D^{24}$ − 54.5 (*c* 0.89, $CHCl_3$); R_f 0.19 (3:1 EtOAc:hexanes). IR (thin film in CH_2Cl_2) ν_{max} 3304, 2972, 2959, 2932, 2874, 1748, 1699, 1643, 1520, 1452, 1402, 1368, 1236, 1211, 1173, 754 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, note: mixture of rotamers) δ 8.54−6.65 (11H, m), 6.63−5.02 (7H, m), 5.01−4.23 (4H, m), 4.15−2.84 (6H, m), 2.65−0.72 (33H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 191.2, 190.96, 190.92, 172.43, 172.39, 172.18, 172.15, 171.8, 171.6, 171.20, 171.17, 171.15, 170.4, 170.3, 170.0, 169.9, 169.01, 169.00, 168.91, 168.84, 168.80, 156.4, 156.2, 154.6, 154.0, 136.3, 134.29, 134.23, 134.18, 134.12, 134.11, 133.97, 133.95, 133.94, 80.29, 80.23, 80.19, 80.0, 79.9, 79.60, 79.58, 79.53, 71.58, 71.52, 71.3, 71.1, 67.25, 67.14, 67.10, 66.98, 66.87, 66.77, 61.1, 60.5, 60.28, 60.25, 59.9, 59.8, 59.24, 59.19, 59.13, 57.8, 57.7, 57.32, 57.26, 57.16, 56.8, 56.4, 56.3, 56.2, 56.0, 55.8, 55.7, 55.6, 53.87, 53.82, 53.7, 53.6, 52.67, 52.61, 52.52, 52.48, 48.3, 47.0, 46.9, 46.8, 46.7, 46.6, 43.5, 43.15, 43.10, 39.8, 36.75, 36.72, 36.5, 36.31, 36.29, 36.23, 34.7, 34.3, 34.16, 34.09, 33.95, 33.88, 33.6, 33.5, 33.43, 33.38, 32.8, 32.6, 32.5, 31.4, 31.0, 30.3, 30.2, 29.9, 29.81, 29.77, 29.71, 29.65, 29.3, 29.1, 28.62, 28.60, 28.4, 28.3, 27.3, 27.25, 27.19, 27.13, 27.09, 25.0, 24.02, 23.99, 23.4, 22.1, 22.03, 22.02, 21.9, 18.3, 18.1, 17.38, 17.33, 17.2, 16.6, 15.9, 15.5, 15.4, 15.3. HRMS (ESI^+) *m*/*z* calcd for $C_{46}H_{62}N_5O_{12}$ $[M + H]^+$, 876.4389; found, 876.4386.

(*S*)-*tert*-Butyl 2-((*S*)-2-(((*S*)-1-((2*S*,4*R*)-2-((((2*S*,3*S*)-3-(((Benzyloxy)carbonyl)amino)-4-oxo-4-(2-oxo-2-phenylethoxy)butan-2-yl)oxy)carbonyl)-4-methylpyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)piperidine-1-carbonyl)-pyrrolidine-1-carboxylate (14b). Pentadepsipeptide **14b** (1.06 g, 59%) was prepared from acid **12b**[45] and ammonium salt **13a** according to general procedure A; white crystalline solid, mp 113−114 °C (CH_2Cl_2); $[\alpha]_D^{25}$ − 90.4 (*c* 0.64, MeOH); R_f 0.35 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3400, 3304, 2972, 2943, 2876, 1748, 1705, 1643, 1450, 1421, 1169, 845 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, note: mixture of rotamers) δ 8.44−6.61 (11H, m), 5.80−5.01 (7H, m), 4.96−4.25 (4H, m), 3.92−2.91 (6H, m), 2.70−1.18 (28H, m), 1.14−0.80 (3H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 190.97, 190.95, 190.91, 172.5, 172.3, 171.78, 171.76, 171.65, 171.61, 171.26, 171.25, 171.17, 171.15, 170.2, 169.9, 169.0, 168.9, 156.2, 154.65, 154.60, 154.1, 136.4, 134.29,

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134.23, 133.99, 133.96, 133.95, 129.14, 129.10, 128.7, 128.34, 128.30, 128.22, 128.19, 128.11, 127.92, 127.85, 127.83, 80.2, 79.57, 79.55, 71.5, 71.1, 67.3, 67.0, 59.25, 59.21, 59.1, 57.2, 56.87, 56.85, 56.2, 55.7, 53.7, 53.60, 53.57, 52.5, 52.4, 48.3, 46.97, 46.90, 46.7, 43.7, 43.3, 39.9, 36.30, 36.24, 32.8, 32.6, 30.3, 29.7, 29.3, 28.66, 28.62, 28.60, 26.5, 26.0, 25.8, 25.5, 25.3, 25.06, 25.02, 24.0, 23.4, 20.9, 20.8, 20.7, 18.1, 17.4, 17.2, 16.0, 15.53, 15.46, 15.3. HRMS (ESI$^+$) m/z calcd for $C_{45}H_{60}N_5O_{12}$ [M + H]$^+$, 862.4233; found, 862.4247.

(*S*)-*tert*-Butyl 2-((2*S*,4*S*)-2-(((*S*)-1-((*S*)-2-((((2*S*,3*S*)-3-(((Benzyloxy)carbonyl)amino)-4-oxo-4-(2-oxo-2-phenylethoxy)butan-2-yl)oxy)carbonyl)pyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)-4-methylpiperidine-1-carbonyl)pyrrolidine-1-carboxylate (14c).[31] Pentadepsipeptide **14c** (750.0 mg, 86%) was prepared from acid **12a**[31] and ammonium salt **13b** according to general procedure A. White solid, mp 101−102 °C (CH_2Cl_2); $[\alpha]_D^{25}$ − 92.5 (*c* 0.69, MeOH); R_f 0.54 (19:1 EtOAc:MeOH); IR (thin film in CH_2Cl_2) ν_{max} 3304, 2976, 2955, 2878, 1748, 1699, 1643, 1516, 1452, 1418, 1173, 843 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, note: mixture of rotamers) δ 8.50−6.67 (11H, m), 6.25−5.63 (1H, m), 5.62−5.02 (6H, m), 5.00−4.32 (4H, m), 3.95−3.03 (6H, m), 2.52−0.70 (31H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 190.97, 190.96, 190.92, 172.4, 172.2, 171.8, 171.7, 171.6, 171.17, 171.14, 170.3, 170.0, 169.9, 169.0, 168.9, 156.25, 156.17, 156.12, 154.65, 154.62, 154.09, 154.05, 136.4, 134.28, 134.22, 133.0, 129.3, 129.13, 129.10, 128.65, 128.59, 128.32, 128.29, 128.12, 128.05, 127.99, 127.90, 127.83, 80.2, 79.9, 79.7, 79.6, 79.5, 71.7, 71.4, 71.1, 67.5, 67.3, 67.2, 67.1, 67.0, 59.99, 59.96, 59.95, 59.69, 59.67, 59.25, 59.20, 58.9, 57.31, 57.26, 57.11, 57.08, 57.05, 56.9, 56.3, 55.8, 53.6, 52.7, 52.6, 52.4, 48.4, 47.2, 47.1, 47.02, 46.97, 46.89, 46.80, 46.7, 43.5, 43.1, 39.8, 34.7, 34.2, 33.95, 33.92, 33.6, 33.4, 30.3, 29.7, 29.2, 29.1, 28.9, 28.65, 28.61, 28.4, 27.35, 27.29, 27.26, 27.19, 26.8, 25.04, 24.96, 24.0, 23.4, 22.1, 22.0, 21.9, 18.1, 18.0, 16.0, 15.50, 15.44, 15.2. HRMS (ESI$^+$) m/z calcd for $C_{45}H_{60}N_5O_{12}$ [M + H]$^+$, 862.4233; found, 862.4230.

(*S*)-*tert*-Butyl 2-((2*S*,4*S*)-2-(((*S*)-1-((2*S*,4*R*)-2-(((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-oxo-3-(2-oxo-2-phenylethoxy)propoxy)carbonyl)-4-methylpyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)-4-methylpiperidine-1-carbonyl)pyrrolidine-1-carboxylate (14d). Pentadepsipeptide **14d** (1.22 g, 74%) was prepared from acid **12a**[31] and ammonium salt **13c**[27,45] according to general procedure A; white crystalline solid, mp 95−96 °C (CH_2Cl_2); R_f 0.21 (3:1 EtOAc:hexanes); $[\alpha]_D^{25}$ − 62.7 (*c* 0.57, $CHCl_3$). IR (thin film in CH_2Cl_2) ν_{max} 3304, 2957, 2932, 2874, 1751, 1701, 1647, 1452, 1402, 1167 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, note: mixture of rotamers) δ 8.51−6.63 (11H, m), 5.84−5.75 (1H, m), 5.55−5.07 (5H, m), 4.85−4.76 (1H, m), 4.73−4.40 (5H, m), 3.84−2.95 (6H, m), 2.59−0.80 (30H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 191.04, 191.01, 191.00, 172.4, 172.2, 171.89, 171.83, 171.63, 171.61, 171.42, 171.37, 171.32, 171.30, 171.2, 170.3, 170.0, 169.9, 169.1, 156.13, 156.11, 156.05, 154.66, 154.64, 154.0, 136.3, 134.32, 134.28, 134.21, 134.16, 134.09, 134.00, 133.97, 133.94, 129.16, 129.14, 129.09, 129.05, 129.02, 128.65, 128.63, 128.61, 128.5, 128.31, 128.25, 128.1, 128.0, 127.87, 127.84, 127.81, 80.2, 79.60, 79.57, 67.26, 67.20, 67.10, 67.07, 64.6, 64.4, 59.23, 59.19, 59.17, 57.3, 57.2, 56.31, 56.29, 56.1, 55.8, 53.7, 53.57, 53.56, 53.4, 52.7, 52.6, 48.4, 46.97, 46.90, 46.88, 46.7, 43.4, 43.1, 39.8, 36.6, 34.7, 34.2, 33.93, 33.86, 33.6, 33.4, 32.9, 32.7, 30.3, 29.7, 29.25, 29.22, 28.68, 28.65, 28.60, 27.3, 27.2, 25.0, 24.0, 23.4, 22.1, 22.03, 22.01, 18.0, 17.3, 17.2, 15.9. HRMS (ESI$^+$) m/z calcd for $C_{45}H_{60}N_5O_{12}$ [M + H]$^+$, 862.4233; found, 862.4230.

(*S*)-*tert*-Butyl 2-((*S*)-2-(((*S*)-1-((*S*)-2-((((2*S*,3*S*)-3-(((Benzyloxy)carbonyl)amino)-4-oxo-4-(2-oxo-2-phenylethoxy)butan-2-yl)oxy)carbonyl)pyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)piperidine-1-carbonyl)pyrrolidine-1-carboxylate (14e).[31] Pentadepsipeptide **14e** (1.14 g, 68%) was prepared from acid **12b**[45] and ammonium salt **13b** according to general procedure A; white solid, mp 101−102 °C (CH_2Cl_2); $[\alpha]_D^{25}$ − 125.4 (*c* 0.48, MeOH); R_f 0.35 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3387, 3304, 2978, 2945, 2880, 1748, 1705, 1643, 1520, 1450, 1423, 1368, 1240, 1169, 845 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, note: mixture of rotamers) δ 8.50−6.63 (11H, m), 5.76−5.64 (1H, m), 5.60−5.51 (1H, m), 5.46−5.36 (1H, m), 5.29−5.24 (1H, m), 5.20−5.05 (3H, m), 4.92−4.37 (4H, m), 3.86−3.05 (6H, m), 2.52−1.24 (29H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 190.98, 190.96, 190.92, 172.5, 172.3, 171.8, 171.69, 171.67, 171.24, 171.16, 171.14, 170.3, 169.92, 169.89, 169.0, 168.9, 156.2, 154.66, 154.60, 154.1, 136.4, 134.29, 134.27, 134.23, 133.99, 133.98, 133.96, 129.14, 129.10, 128.66, 128.65, 128.34, 128.30, 128.1, 127.86, 127.84, 80.2, 79.57, 79.54, 71.5, 71.1, 67.3, 67.0, 59.27, 59.24, 58.9, 57.2, 56.9, 56.2, 55.7, 53.6, 52.5, 52.4, 48.4, 47.1, 47.0, 46.9, 46.8, 46.7, 43.6, 43.3, 39.9, 30.3, 29.7, 29.2, 28.8, 28.67, 28.62, 28.60, 26.5, 26.0, 25.8, 25.5, 25.2, 25.08, 25.06, 25.02, 24.98, 24.0, 23.4, 20.9, 20.8, 20.6, 18.1, 16.1, 15.52, 15.46, 15.2. HRMS (ESI$^+$) m/z calcd for $C_{44}H_{58}N_5O_{12}$ [M + H]$^+$, 848.4076; found, 848.4072.

(*S*)-*tert*-Butyl 2-((*S*)-2-(((*S*)-1-((2*S*,4*R*)-2-(((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-oxo-3-(2-oxo-2-phenylethoxy)propyl)carbamoyl)-4-methylpyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)piperidine-1-carbonyl)pyrrolidine-1-carboxylate (14f). Pentapeptide **14f** (299.6 mg, 61%) was prepared from acid **12b**[45] and ammonium salt **13d** according to general procedure A; white solid, mp 87−88 °C (CH_2Cl_2); $[\alpha]_D^{25}$ − 73.9 (*c* 0.44, MeOH); R_f 0.18 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3308, 2959, 2936, 2874, 1755, 1651, 1530, 1450, 1366, 1258, 1232, 1165 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, note: mixture of rotamers) δ 8.74−5.44 (13H, m), 5.40−5.00 (3H, m), 4.82−2.89 (14H, m), 2.68−0.70 (28H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 198.53, 198.50, 192.9, 192.41, 192.39, 171.77, 191.74, 191.39, 191.37, 173.5, 173.13, 172.99, 172.90, 172.8, 172.7, 172.6, 172.40, 172.33, 172.29, 172.23, 172.10, 172.06, 172.03, 171.98, 171.92, 171.84, 171.78, 171.64, 171.60, 171.3, 170.9, 170.32, 170.25, 170.17, 170.07, 169.96, 169.93, 169.86, 156.63, 156.57, 156.52, 156.42, 156.37, 156.31, 154.8, 154.69, 154.66, 164.61, 154.06, 154.02, 136.8, 136.5, 136.4, 136.3, 134.7, 134.6, 134.49, 134.43, 134.39, 134.16, 134.11, 134.06, 133.98, 133.8, 133.7, 133.6, 133.5, 129.3, 129.2, 129.08, 129.03, 129.01, 128.63, 128.57, 128.43, 128.36, 128.32, 128.28, 128.18, 128.10, 128.08, 128.03, 127.92, 127.86, 127.80, 127.77, 80.2, 80.10, 80.06, 79.98, 79.91, 79.61, 79.58, 79.55, 67.1, 67.0, 66.83, 66.77, 66.70, 65.5, 61.9, 61.67, 61.61, 61.4, 61.1, 60.8, 60.7, 60.6, 60.36, 60.32, 60.29, 57.4, 57.3, 57.2, 56.17, 56.15, 56.10, 56.08, 55.83, 55.76, 55.71, 55.68, 55.41, 55.36, 55.22, 55.16, 55.11, 54.29, 54.27, 54.1, 54.0, 53.8, 53.7, 53.6, 52.46, 52.43, 52.36, 52.33, 49.7, 49.59, 49.53, 49.48, 49.3, 49.2, 48.9, 48.8, 46.96, 46.94, 46.90, 46.87, 46.85, 46.81, 46.74, 46.65, 46.57, 43.6, 43.3, 41.14, 41.11, 41.0, 40.7, 39.95, 29.87, 39.82, 39.5, 39.4, 37.7, 37.6, 37.2, 36.4, 36.1, 35.0, 34.9, 33.7, 33.6, 33.5, 33.3, 33.2, 33.1, 32.9, 32.03, 32.00, 30.3, 30.2, 30.09, 30.05, 29.99, 29.87, 29.80, 29.75, 29.70, 29.6, 29.5, 29.42, 29.36, 29.33, 29.2, 26.5, 26.3, 26.1, 25.8, 25.67, 25.64, 25.5, 25.2, 25.02, 25.01, 24.97, 24.87, 24.80, 24.7, 24.0, 23.4, 22.8, 20.96, 20.91, 20.85, 20.7, 20.6, 18.1, 17.8, 17.6, 17.4, 17.33, 17.30, 17.20, 17.17, 17.13, 16.8, 16.02, 15.94, 15.89, 15.69, 15.64, 15.53, 15.48, 15.10, 15.05, 14.98, 14.2. HRMS (ESI$^+$) m/z calcd for $C_{44}H_{59}N_6O_{11}$ [M + H]$^+$, 847.4236; found, 847.4262.

(2*S*,3*S*)-2-(((Benzyloxy)carbonyl)amino)-3-(((2*S*,4*R*)-1-((*S*)-2-((2*S*,4*S*)-1-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carbonyl)-4-methylpiperidine-2-carboxamido)propanoyl)-4-methylpyrrolidine-2-carbonyl)oxy)butanoic Acid (15a). Pentadepsipeptide **15a** (910.0 mg, quantitative yield) was prepared from pentadepsipeptide **14a** according to general procedure B; white solid, mp 128−129 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −99.5 (*c* 0.69, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3308, 2961, 2932, 2874, 1724, 1647, 1526, 1456, 1368, 1258, 1211, 1180 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, notes: carboxylic acid proton was not observed due to deuterium exchange and mixture of rotamers) δ 8.42−6.84 (6H, m), 6.59−5.72 (1H, m), 5.39−5.00 (4H, m), 4.82−4.34 (4H, m), 3.95−2.94 (6H, m), 2.55−0.74 (33H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 173.43, 173.49, 172.7, 172.5, 172.4, 172.1, 171.8, 171.5, 171.4, 171.28, 171.24, 170.99, 170.96, 170.82, 170.75, 170.66, 170.60, 170.5, 170.34, 170.28, 170.23, 169.9, 156.2, 156.0, 154.8, 154.7, 154.1, 154.0, 136.7, 136.51, 136.45, 128.64, 128.60, 128.55, 128.50, 128.3, 128.11, 128.06, 128.00, 127.86, 80.4, 80.0, 79.85, 79.80, 72.8, 71.89, 71.80, 71.77, 67.2, 67.1, 67.0, 60.4, 60.0, 59.8, 59.34, 59.28, 59.23, 58.0, 57.5, 57.3, 57.1, 56.95, 56.88, 56.3, 55.8, 54.0, 53.78, 53.76, 53.71, 53.63, 53.56, 53.3, 52.8, 52.6, 48.4, 47.2,

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47.00, 46.96, 46.92, 46.7, 43.5, 43.2, 40.4, 40.0, 39.7, 39.3, 37.0, 36.2, 35.7, 35.4, 35.2, 34.6, 34.3, 34.1, 33.9, 33.8, 33.5, 33.4, 32.8, 32.7, 32.6, 30.4, 30.19, 30.14, 29.9, 29.7, 29.2, 28.62, 28.59, 28.52, 28.3, 27.3, 27.2, 27.0, 26.4, 26.2, 24.8, 24.1, 24.0, 23.5, 23.4, 22.1, 22.0, 21.8, 19.13, 19.10, 18.1, 17.94, 17.91, 17.8, 17.6, 17.5, 17.33, 17.31, 17.1, 16.9, 16.0, 15.85, 15.77, 15.70. HRMS (ESI$^+$) m/z calcd for $C_{38}H_{56}N_5O_{11}$ [M + H]$^+$, 758.3971; found, 758.3975.

(2*S*,3*S*)-2-(((Benzyloxy)carbonyl)amino)-3-(((2*S*,4*R*)-1-((*S*)-2-((*S*)-1-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carbonyl)-piperidine-2-carboxamido)propanoyl)-4-methylpyrrolidine-2-carbonyl)oxy)butanoic Acid (15b). Pentadepsipeptide **15b** (800.0 mg, 98%) was prepared from pentadepsipeptide **14b** according to general procedure B; white solid, mp 118−119 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −107.0 (c 0.34, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3304, 2972, 2938, 2874, 1724, 1647, 1533, 1456, 1368, 1258, 1246, 1180, 1165 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, notes: carboxylic acid proton was not observed due to deuterium exchange and mixture of rotamers) δ 8.41−6.84 (6H, m), 5.99−5.74 (1H, m), 5.38−4.99 (4H, m), 4.77−4.25 (4H, m), 3.93−2.90 (6H, m), 2.60−0.90 (31H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 172.7, 172.6, 172.4, 171.8, 171.6, 171.5, 171.3, 171.01, 170.99, 170.7, 170.3, 170.2, 156.2, 156.0, 154.8, 154.7, 154.1, 136.52, 136.47, 128.63, 128.57, 128.25, 128.18, 128.14, 126.2, 80.8, 80.4, 80.1, 80.0, 79.77, 79.74, 72.5, 72.0, 71.99, 71.83, 67.2, 67.1, 60.07, 60.04, 59.33, 59.29, 59.17, 57.7, 57.4, 57.3, 57.2, 56.7, 56.2, 55.8, 53.70, 53.65, 53.57, 53.5, 52.6, 52.4, 48.4, 46.9, 46.7, 43.7, 43.6, 43.3, 36.3, 32.9, 32.7, 30.2, 29.8, 29.2, 28.64, 28.61, 28.59, 26.5, 26.1, 26.0, 25.5, 25.4, 25.2, 25.0, 24.8, 24.0, 23.4, 20.9, 20.6, 20.4, 17.92, 17.88, 17.3, 17.1, 16.2, 16.0, 15.9, 15.8. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{54}N_5O_{11}$ [M + H]$^+$, 744.3814; found, 744.3815.

(2*S*,3*S*)-2-(((Benzyloxy)carbonyl)amino)-3-(((*S*)-1-((*S*)-2-((2*S*,4*S*)-1-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carbonyl)-4-methylpiperidine-2-carboxamido)propanoyl)pyrrolidine-2-carbonyl)oxy)butanoic Acid (15c).[31] Pentadepsipeptide **15c** (591.6 mg, 99%) was prepared from pentadepsipeptide **14c**[31] according to general procedure B; white solid, mp 127−128 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −87.3 (c 0.51, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3312, 2976, 2955, 2932, 2878, 1740, 1724, 1647, 1522, 1456, 1167 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, notes: carboxylic acid proton was not observed due to deuterium exchange and mixture of rotamers) δ 8.41−6.84 (6H, m), 6.51−5.74 (1H, m), 5.43−5.02 (4H, m), 4.93−4.28 (4H, m), 3.90−3.73 (1H, m), 3.67−3.00 (5H, m), 2.54−0.76 (31H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 173.8, 173.5, 173.3, 173.0, 172.7, 172.5, 172.4, 172.1, 171.8, 171.5, 171.37, 171.36, 171.2, 171.1, 171.02, 171.00, 170.9, 170.7, 170.64, 170.57, 170.52, 170.34, 170.36, 170.29, 156.3, 156.24, 156.19, 156.1, 155.3, 155.0, 154.9, 154.7, 154.1, 154.04, 154.01, 153.98, 136.7, 136.52, 136.47, 128.6, 128.5, 128.4, 128.3, 128.18, 128.14, 128.05, 128.00, 127.8, 127.7, 80.79, 80.73, 80.4, 80.08, 80.03, 79.85, 79.80, 72.85, 72.83, 72.7, 71.8, 71.75, 71.72, 67.1, 67.0, 66.7, 66.6, 60.9, 60.1, 59.9, 59.4, 59.31, 59.25, 57.6, 57.53, 57.52, 57.45, 57.3, 57.14, 57.07, 56.8, 56.3, 55.8, 55.2, 53.65, 53.56, 53.0, 52.81, 52.75, 52.66, 48.5, 47.6, 47.3, 47.12, 47.08, 47.04, 47.00, 46.93, 46.7, 46.6, 46.51, 46.44, 44.1, 43.7, 43.5, 43.3, 43.2, 42.0, 40.8, 40.0, 35.3, 34.6, 34.3, 34.1, 33.9, 33.8, 33.5, 33.4, 33.3, 30.2, 29.7, 29.4, 29.2, 28.76, 28.73, 28.59, 28.52, 28.46, 28.3, 27.3, 27.1, 27.0, 25.13, 25.08, 24.98, 24.8, 24.6, 24.0, 23.4, 22.1, 21.99, 21.94, 21.88, 19.1, 19.0, 18.0, 17.86, 17.82, 17.68, 17.66, 17.61, 15.99, 15.92, 15.85, 15.76. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{54}N_5O_{11}$ [M + H]$^+$, 744.3814; found, 744.3816.

(*S*)-2-(((Benzyloxy)carbonyl)amino)-3-(((2*S*,4*R*)-1-((*S*)-2-((2*S*,4*S*)-1-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carbonyl)-4-methylpiperidine-2-carboxamido)propanoyl)-4-methylpyrrolidine-2-carbonyl)oxy)propanoic Acid (15d). Acid **15d** (1.02 g, quantitative yield) was prepared from amide **14d** according to general procedure B; white solid, mp 109−110 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −84.7 (c 0.85, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3308, 2972, 2961, 2932, 2876, 1744, 1724, 1647, 1522, 1456, 1368, 1211, 1167 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, notes: carboxylic acid proton was not observed due to deuterium exchange and mixture of rotamers) δ 8.22−6.66 (6H, m), 6.21−5.40 (1H, m), 5.35−5.02 (3H, m), 4.88−4.13 (6H, m), 3.94−2.95 (6H, m), 2.58−0.77 (30H, m). ^{1}H NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 172.92, 172.85, 172.69, 172.61, 172.58, 171.8, 171.8, 171.7, 171.6, 171.5, 171.3, 171.06, 170.96, 170.94, 170.88, 170.81, 170.76, 170.46, 170.41, 170.33, 170.31, 170.0, 168.8, 156.34, 156.29, 156.02, 156.00, 155.84, 155.76, 155.1, 154.71, 154.70, 154.09, 154.03, 136.8, 136.38, 136.34, 136.2, 128.70, 128.65, 128.62, 128.5, 128.42, 128.40, 128.29, 128.21, 128.19, 128.07, 128.04, 127.98, 80.6, 80.00, 79.95, 79.88, 79.84, 79.78, 79.74, 67.3, 67.16, 67.13, 67.0, 65.6, 65.3, 64.7, 60.1, 59.61, 59.58, 59.07, 59.03, 58.9, 57.7, 57.53, 57.46, 57.36, 57.30, 57.2, 56.4, 56.0, 54.1, 53.9, 53.83, 53.77, 53.73, 53.60, 53.56, 53.1, 53.0, 52.9, 52.8, 52.7, 48.3, 47.6, 47.07, 47.03, 46.97, 46.93, 46.7, 43.63, 43.57, 43.4, 43.3, 43.2, 40.1, 38.9, 36.9, 36.8, 36.38, 36.34, 34.6, 34.3, 34.22, 34.16, 34.02, 33.96, 33.88, 33.81, 33.74, 33.71, 33.67, 33.5, 33.4, 33.2, 33.0, 32.9, 32.74, 32.70, 32.68, 30.35, 30.32, 30.16, 30.14, 29.82, 29.77, 29.3, 29.2, 28.60, 28.57, 28.47, 27.41, 27.35, 27.2, 27.1, 26.9, 26.3, 26.2, 24.7, 24.1, 24.0, 23.9, 23.6, 23.5, 23.44, 23.36, 22.3, 22.1, 22.0, 21.9, 21.5, 18.9, 18.4, 17.7, 17.6, 17.3, 17.23, 17.16, 17.10, 15.7, 15.6. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{54}N_5O_{11}$ [M + H]$^+$, 744.3814; found, 744.3812.

(2*S*,3*S*)-2-(((Benzyloxy)carbonyl)amino)-3-(((*S*)-1-((*S*)-2-((*S*)-1-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carbonyl)piperidine-2-carboxamido)propanoyl)pyrrolidine-2-carbonyl)oxy)-butanoic Acid (15e).[31] Pentadepsipeptide **15e** (850.0 mg, 94%) was prepared from pentadepsipeptide **14e**[31] according to general procedure B; white solid, mp 127−128 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −122.2 (c 0.25, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3304, 2976, 2939, 2880, 1744, 1717, 1647, 1522, 1456, 1406, 1165 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, notes: carboxylic acid proton was not observed due to deuterium exchange and mixture of rotamers) δ 8.43−6.67 (6H, m), 5.98−5.75 (1H, m), 5.30−4.99 (4H, m), 4.79−4.33 (4H, m), 3.88−3.31 (5H, m), 3.28−3.00 (1H, m), 2.54−1.12 (29H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 172.7, 172.6, 172.4, 171.9, 171.5, 171.4, 171.3, 171.09, 171.05, 170.99, 170.96, 170.8, 170.7, 170.5, 170.3, 170.2, 156.2, 156.1, 155.1, 154.8, 154.7, 154.13, 154.08, 136.51, 136.46, 136.44, 128.60, 128.54, 128.3, 128.24, 128.20, 128.16, 128.12, 80.7, 80.3, 80.0, 79.84, 79.78, 71.72, 71.68, 67.2, 67.09, 67.06, 59.9, 59.5, 59.28, 59.26, 59.19, 58.0, 57.5, 57.39, 57.35, 57.30, 57.07, 57.01, 56.7, 56.1, 55.7, 53.5, 52.6, 52.5, 48.5, 47.05, 47.01, 46.95, 46.91, 46.90, 46.7, 46.5, 46.4, 43.6, 43.3, 40.1, 30.3, 30.1, 29.80, 29.74, 29.2, 28.72, 28.69, 28.61, 28.56, 28.54, 26.4, 26.1, 26.0, 25.4, 25.10, 25.04, 24.98, 24.94, 24.8, 24.4, 24.1, 24.0, 23.4, 22.80, 22.79, 21.9, 20.8, 20.5, 20.4, 19.1, 19.0, 18.1, 17.82, 17.79, 16.9, 15.85, 15.78, 15.74. HRMS (ESI$^+$) m/z calcd for $C_{36}H_{52}N_5O_{11}$ [M + H]$^+$, 730.3658; found, 730.3665.

(*S*)-2-(((Benzyloxy)carbonyl)amino)-3-((2*S*,4*R*)-1-((*S*)-2-((*S*)-1-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carbonyl)piperidine-2-carboxamido)propanoyl)-4-methylpyrrolidine-2-carboxamido)propanoic Acid (15f). Pentapeptide **15f** (235.0 mg, quantitative yield) was prepared from pentapeptide **14f** according to general procedure B; white solid, mp 130−131 °C (CH_2Cl_2); $[\alpha]_D^{27}$ −76.1 (c 0.27, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3308, 2972, 2936, 2874, 1717, 1651, 1533, 1456, 1418, 1368, 1258, 1163 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, notes: carboxylic acid proton was not observed due to deuterium exchange and mixture of rotamers) δ 8.67−6.05 (8H, m), 5.32−5.03 (3H, m), 4.90−4.15 (4H, m), 3.98−3.00 (8H, m), 2.63−0.97 (28H, m). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 173.3, 173.1, 172.90, 172.84, 172.79, 172.7, 172.42, 172.37, 172.32, 172.27, 172.21, 172.20, 171.99, 171.92, 171.8, 171.7, 170.8, 170.42, 170.35, 156.5, 156.44, 156.36, 156.33, 156.25, 155.1, 154.9, 154.8, 154.65, 154.62, 154.1, 154.0, 136.7, 136.59, 136.52, 136.47, 136.42, 136.3, 128.56, 128.55, 128.47, 128.27, 128.23, 128.17, 128.15, 128.08, 127.98, 127.93, 80.5, 80.15, 80.14, 80.03, 79.98, 79.87, 79.85, 79.82, 67.1, 66.96, 66.91, 66.86, 61.6, 61.4, 61.2, 60.6, 60.4, 57.6, 57.4, 56.21, 56.17, 55.88, 55.82, 54.96, 54.90, 54.8, 54.7, 54.4, 53.1, 53.9, 53.7, 53.5, 52.8, 52.6, 52.4, 49.4, 49.2, 49.1, 48.0, 47.02, 47.00, 46.98, 46.89, 46.87, 46.7, 43.5, 43.3, 41.5, 41.4, 41.3, 40.12, 40.05, 39.95, 39.39, 39.33, 37.0, 36.3, 36.2, 35.8, 33.4, 33.1, 32.80, 32.79, 30.2, 30.04, 30.00, 29.8, 29.72, 29.69, 29.1, 28.59, 28.57, 28.54, 26.5, 26.4, 26.2, 26.0, 25.8, 25.4, 25.1, 25.0, 24.90, 24.87, 24.85, 24.77, 24.1, 24.0, 23.48, 23.45, 20.88, 20.82, 20.45, 20.37, 20.27, 17.8, 17.71, 17.68, 17.5, 17.4, 17.3, 17.2, 17.0, 15.88, 15.87, 15.3. HRMS

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(ESI+) m/z calcd for $C_{36}H_{53}N_6O_{10}$ [M + H]+, 729.3818; found, 729.3832.

Benzyl ((2*R*,6*S*,8a*S*,10*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,10,21-Tetramethyl-5,8,14,19,23-pentaoxodocosahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13]oxatetraazacyclohexadecin-20-yl)carbamate (16a). Cyclic pentadepsipeptide **16a** (570.4 mg, 81% over 3 steps) was prepared from pentadepsipeptide **15a** according to general procedure C; white crystalline solid, mp 125−126 °C (CH_2Cl_2); $[\alpha]_D^{25}$ +17.0 (*c* 0.72, MeOH); R_f 0.58 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3296, 2957, 2932, 2876, 1724, 1705, 1663, 1533, 1508, 1429, 1267, 1248 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (1H, d, *J* = 9.5 Hz), 7.45−7.24 (5H, m), 5.56 (1H, d, *J* = 9.5 Hz), 5.29 (1H, dd, *J* = 8.5, 3.0 Hz), 5.13−5.02 (3H, m), 4.99 (1H, dq, *J* = 9.5, 6.5 Hz), 4.71−4.65 (1H, m), 4.53−4.41 (2H, m), 4.33 (1H, dd, *J* = 9.5, 1.0 Hz), 3.79 (1H, ddd, *J* = 12.0, 8.0, 5.0 Hz), 3.68 (1H, dd, *J* = 12.0, 9.0 Hz), 3.50 (1H, ddd, *J* = 12.0, 7.0, 7.0 Hz), 3.21 (1H, dd, *J* = 12.0, 8.5 Hz), 2.70 (1H, br d, *J* = 13.0 Hz), 2.53 (1H, ddd, *J* = 13.5, 13.5, 2.0 Hz), 2.48−2.27 (2H, m), 2.18−2.04 (2H, m), 2.00−1.80 (3H, m), 1.66−1.51 (2H, m), 1.39 (3H, d, *J* = 6.5 Hz), 1.26 (3H, d, *J* = 6.5 Hz), 1.11−0.90 (8H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 171.8, 169.9, 168.6, 166.9, 156.4, 136.1, 128.58, 128.57, 128.3, 70.4, 68.2, 60.0, 57.2, 56.8, 56.2, 54.5, 48.1, 46.6, 40.9, 39.4, 36.5, 33.4, 30.7, 29.8, 28.1, 23.1, 22.0, 18.6, 18.2, 13.1. HRMS (ESI+) m/z calcd for $C_{33}H_{46}N_5O_8$ [M + H]+, 640.3341; found, 640.3342.

Benzyl ((2*R*,6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-Trimethyl-5,8,14,19,23-pentaoxodocosahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13]oxatetraazacyclohexadecin-20-yl)carbamate (16b). Cyclic pentadepsipeptide **16b** (351.7 mg, 57% over 3 steps) was prepared from pentadepsipeptide **15b** according to general procedure C; white solid, mp 193−194 °C (CH_2Cl_2); $[\alpha]_D^{25}$ +16.7 (*c* 0.55, MeOH); R_f 0.51 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3293, 2957, 2942, 2876, 1724, 1705, 1661, 1514, 1431, 1252, 1015, 735, 700 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (1H, d, *J* = 9.5 Hz), 7.43−7.28 (5H, m), 5.57 (1H, d, *J* = 9.5 Hz), 5.29 (1H, dd, *J* = 9.0, 3.0 Hz), 5.13−5.03 (3H, m), 5.01 (1H, dq, *J* = 9.5, 6.5 Hz), 4.71−4.65 (1H, m), 4.52−4.43 (2H, m), 4.31 (1H, dd, *J* = 9.5, 1.0 Hz), 3.79 (1H, ddd, *J* = 12.0, 8.5, 5.0 Hz), 3.68 (1H, dd, *J* = 12.0, 9.0 Hz), 3.51 (1H, ddd, *J* = 12.0, 7.0, 7.0 Hz), 3.21 (1H, dd, *J* = 12.0, 8.5 Hz), 2.76−2.67 (1H, br m), 2.52 (1H, ddd, *J* = 13.5, 13.5, 2.5 Hz), 2.48−2.28 (2H, m), 2.17−2.04 (2H, m), 2.01−1.90 (2H, m), 1.85 (1H, ddd, *J* = 13.0, 12.0, 8.5 Hz), 1.77−1.70 (1H, br m), 1.59 (1H, br d, *J* = 13.0 Hz), 1.52−1.22 (9H, m), 1.07 (3H, d, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 171.7, 169.8, 168.6, 166.8, 156.4, 136.1, 128.60, 128.58, 128.3, 70.4, 68.2, 60.1, 57.3, 56.7, 56.2, 54.5, 48.1, 46.6, 41.2, 39.5, 30.7, 29.8, 28.3, 25.0, 23.2, 21.5, 18.6, 18.2, 13.1. HRMS (ESI+) m/z calcd for $C_{32}H_{44}N_5O_8$ [M + H]+, 626.3184; found, 626.3202.

Benzyl ((6*S*,8a*S*,10*S*,14a*S*,20*S*,21*S*,23a*S*)-6,10,21-Trimethyl-5,8,14,19,23-pentaoxodocosahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13]oxatetraazacyclohexadecin-20-yl)carbamate (16c).[31] Cyclic pentadepsipeptide **16c** (350.5 mg, 76% over 3 steps) was prepared from pentadepsipeptide **15c**[31] according to general procedure C; white solid, mp 128−129 °C (CH_2Cl_2); $[\alpha]_D^{26}$ +17.6 (*c* 0.37, MeOH); R_f 0.48 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3293, 2953, 2934, 1724, 1701, 1659, 1512, 1429, 1265, 1252, 1086 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, note: mixture of rotamers) δ 8.48−8.30 (1H, m), 7.41−7.23 (5H, m), 6.28−5.52 (1H, m), 5.48−4.83 (5H, m), 4.78−4.27 (4H, m), 3.84−3.22 (4H, m), 3.21−2.48 (2H, m), 2.40−1.80 (8H, m), 1.64−1.54 (2H, m), 1.43−1.21 (6H, m), 1.18−0.90 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$, note: mixture of rotamers) δ 173.2, 171.7, 171.5, 171.1, 169.9, 169.7, 168.6, 168.1, 167.3, 166.9, 156.4, 156.3, 137.1, 136.1, 128.57, 127.56, 128.4, 128.3, 128.0, 127.8, 71.2, 70.4, 68.2, 66.8, 59.7, 59.2, 57.3, 57.2, 56.8, 56.2, 55.0, 53.0, 48.4, 48.2, 47.1, 46.5, 44.2, 40.9, 36.5, 34.2, 33.4, 33.2, 31.3, 30.7, 29.3, 28.7, 28.1, 27.5, 26.5, 25.5, 23.1, 22.0, 21.9, 21.5, 18.2, 17.7, 17.0, 16.9. HRMS (ESI+) m/z calcd for $C_{32}H_{44}N_5O_8$ [M + H]+, 626.3184; found, 626.3192.

Benzyl ((2*R*,6*S*,8a*S*,10*S*,14a*S*,20*S*,23a*S*)-2,6,10-Trimethyl-5,8,14,19,23-pentaoxodocosahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13]oxatetraazacyclohexadecin-20-yl)carbamate (16d). Cyclic pentadepsipeptide **16d** (459.1 mg, 59% over 3 steps) was prepared from pentadepsipeptide **15d** according to general procedure C; white solid, mp 144−145 °C (CH_2Cl_2); $[\alpha]_D^{27}$ +11.0 (*c* 0.72, MeOH); R_f 0.44 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3293, 2959, 2932, 2876, 1732, 1705, 1659, 1515, 1429, 1267, 1246, 735 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (1H, d, *J* = 9.5 Hz), 7.43−7.28 (5H, m), 5.62 (1H, d, *J* = 9.5 Hz), 5.19 (1H, dd, *J* = 8.5, 3.0 Hz), 5.09 (1H, d, *J* = 12.0 Hz), 5.05 (1H, d, *J* = 12.0 Hz), 5.00 (1H, dq, *J* = 9.5, 6.5 Hz), 4.80 (1H, dd, *J* = 11.5, 1.5 Hz), 4.73−4.66 (1H, m), 4.57−4.46 (2H, m), 4.22 (1H, ddd, *J* = 9.5, 9.5, 1.5 Hz), 3.78 (1H, ddd, *J* = 11.5, 8.0, 5.0 Hz), 3.71 (1H, dd, *J* = 12.0, 9.0 Hz), 3.62 (1H, dd, *J* = 11.5, 9.5 Hz), 3.54 (1H, ddd, *J* = 11.5, 7.0, 7.0 Hz), 3.22 (1H, dd, *J* = 12.0, 9.0 Hz), 2.76−2.65 (1H, br m), 2.62−2.44 (2H, m), 2.42−2.27 (1H, m), 2.20−2.04 (2H, m), 2.01−1.80 (3H, m), 1.66−1.52 (2H, m), 1.40 (3H, d, *J* = 6.5 Hz), 1.15−0.90 (8H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 171.7, 169.9, 168.7, 166.3, 156.4, 136.0, 128.7, 128.6, 128.3, 68.2, 66.0, 59.9, 57.3, 56.8, 54.4, 54.1, 48.1, 46.5, 41.0, 39.2, 36.6, 33.4, 30.8, 29.8, 28.1, 23.3, 22.0, 18.4, 18.2. HRMS (ESI+) m/z calcd for $C_{32}H_{44}N_5O_8$ [M + H]+, 626.3184; found, 626.3187.

Benzyl ((6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-6,21-Dimethyl-5,8,14,19,23-pentaoxodocosahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1,4,7,10,13]oxatetraazacyclohexadecin-20-yl)carbamate (16e).[31] Cyclic pentadepsipeptide **16e** (392.7 mg, 58% over 3 steps) was prepared from pentadepsipeptide **15e**[31] according to general procedure C; white solid, decomposition temp 260 °C; $[\alpha]_D^{25}$ +12.5 (*c* 0.46, MeOH); R_f 0.39 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3293, 2980, 2941, 2887, 1724, 1705, 1659, 1533, 1516, 1433, 1350, 1321, 1252, 1167, 1086, 1015, 735, 700 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.38 (1H, d, *J* = 9.5 Hz), 7.40−7.33 (4H, m), 7.32−7.28 (1H, m), 5.56 (1H, d, *J* = 9.5 Hz), 5.30 (1H, dd, *J* = 9.0, 3.0 Hz), 5.11−5.04 (3H, m), 5.01 (1H, dq, *J* = 9.5, 6.5 Hz), 4.71−4.66 (1H, m), 4.51−4.43 (2H, m), 4.32 (1H, dd, *J* = 9.5, 1.5 Hz), 3.79 (1H, ddd, *J* = 11.5, 8.5, 5.0 Hz), 3.71 (1H, ddd, *J* = 12.0, 8.5, 8.5 Hz), 3.55−3.46 (2H, m), 2.76−2.68 (1H, br m), 2.53 (1H, ddd, *J* = 13.5, 13.5, 3.0 Hz), 2.39−2.30 (1H, m), 2.25−2.16 (1H, m), 2.15−2.07 (1H, m), 2.06−1.99 (1H, m), 1.98−1.88 (4H, m), 1.77−1.70 (1H, br m), 1.59 (1H, br d, *J* = 12.5 Hz), 1.53−1.38 (5H, m), 1.37−1.28 (1H, m), 1.25 (3H, d, *J* = 6.5 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.2, 171.7, 169.8, 168.6, 166.9, 156.4, 136.1, 128.59, 128.58, 128.3, 70.4, 68.2, 59.2, 57.3, 56.8, 56.2, 48.3, 47.1, 46.6, 41.2, 31.3, 30.7, 28.4, 25.0, 23.2, 21.5 (2C), 18.2, 13.1. HRMS (ESI+) m/z calcd for $C_{31}H_{42}N_5O_8$ [M + H]+, 612.3028; found, 612.3040.

Benzyl ((2*R*,6*S*,8a*S*,14a*S*,20*S*,23a*S*)-2,6-Dimethyl-5,8,14,19,23-pentaoxodocosahydro-1*H*-pyrido[1,2-*d*]dipyrrolo[1,2-*a*:1′,2′-*j*][1,4,7,10,13]pentaazacyclohexadecin-20-yl)carbamate (16f). Cyclic pentapeptide **16f** (98.2 mg, 58% over 3 steps) was prepared from pentapeptide **15f** according to general procedure C; white solid, decomposition temp 143 °C; $[\alpha]_D^{25}$ −35.5 (*c* 0.51, MeOH); R_f 0.10 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3293, 2940, 2874, 1705, 1659, 1514, 1449, 1431, 1256, 735, 700 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (1H, d, *J* = 10.0 Hz), 7.41−7.28 (5H, m), 6.44 (1H, dd, *J* = 10.5, 2.5 Hz), 5.87 (1H, d, *J* = 8.5 Hz), 5.24 (1H, dd, *J* = 9.0, 3.5 Hz), 5.15−5.01 (3H, m), 4.78−4.74 (1H, m), 4.52−4.46 (1H, br m), 4.44 (1H, d, *J* = 8.5 Hz), 4.06 (1H, ddd, *J* = 14.5, 10.5, 1.0 Hz), 4.01 (1H, ddd, *J* = 8.5, 8.5, 1.0 Hz), 3.83−3.74 (2H, m), 3.56 (1H, ddd, *J* = 11.5, 7.0, 7.0 Hz), 3.18 (1H, dd, *J* = 12.5, 10.0 Hz), 2.78 (1H, ddd, *J* = 14.5, 8.5, 2.5 Hz), 2.70 (1H, br d, *J* = 13.0 Hz), 2.49 (1H, ddd, *J* = 13.5, 13.5, 2.5 Hz), 2.43−2.25 (2H, m), 2.16−2.07 (2H, m), 2.00−1.89 (2H, m), 1.85 (1H, ddd, *J* = 13.0, 12.0, 8.5 Hz), 1.77−1.67 (1H, br m), 1.58 (1H, br d, *J* = 13.5 Hz), 1.54−1.29 (6H, m), 1.07 (3H, d, *J* = 6.5 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.4, 172.4, 171.2, 169.7, 167.2, 157.3, 135.7, 128.70, 128.64, 128.5, 68.1, 61.3, 57.3, 56.7, 55.8, 55.1, 48.8, 46.6, 43.9, 41.5, 39.6, 30.8, 29.7, 28.2, 25.0, 23.3, 21.4, 18.3, 17.3. HRMS (ESI+) m/z calcd for $C_{31}H_{43}N_6O_7$ [M + H]+, 611.3188; found, 611.3171.

(2*R*,6*S*,8a*S*,10*S*,14a*S*,20*S*,21*S*,23a*S*)-20-Amino-2,6,10,21-tetramethylhexadecahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1,4,7,10,13]oxatetraazacyclohexadecine-5,8,14,19,23(8a*H*)-pentaone Hydrochloride (17a). Macrocyclic ammonium salt **17a** (351.2 mg, quantitative yield) was prepared from cyclic pentadep-

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sipeptide **16a** according to general procedure D; light-yellow solid, decomposition temp 205 °C; $[\alpha]_D^{25}$ −19.5 (*c* 0.54, MeOH). IR (solid) ν_{max} 2955, 1734, 1641, 1437, 1161, 1108, 1087 cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD, note: amide and ammonium protons were not observed due to deuterium exchange) δ 5.16 (1H, br q, *J* = 6.0 Hz), 4.88−4.80 (1H, m), 4.76 (1H, s), 4.64−4.59 (1H, m), 4.57 (1H, br s), 4.33−4.23 (2H, m), 3.84 (1H, dd, *J* = 11.5, 7.5 Hz), 3.78−3.70 (1H, m), 3.51 (1H, br ddd, *J* = 12.5, 8.0, 8.0 Hz), 3.16 (1H, dd, *J* = 11.5, 7.0 Hz), 2.74−2.63 (1H, m), 2.53−2.45 (2H, m), 2.41 (1H, ddd, *J* = 13.5, 13.5, 2.5 Hz), 2.33−2.25 (1H, m), 2.09−1.98 (2H, m), 1.94 (1H, br s), 1.87−1.77 (1H, br m), 1.77−1.66 (2H, m), 1.51−1.42 (1H, m), 1.38 (3H, d, *J* = 6.5 Hz), 1.29 (3H, d, *J* = 6.0 Hz), 1.14−0.94 (7H, m). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 175.8, 174.07, 174.04, 172.9, 172.20, 172.12, 166.7, 70.2, 62.2, 60.3, 57.75, 57.74, 56.8, 55.0, 51.0, 50.9, 49.6, 42.4, 39.2, 34.0, 33.9, 33.1, 31.8, 28.6, 23.2, 22.0, 18.4, 16.15, 16.11, 13.7. HRMS (ESI$^+$) *m*/*z* calcd for $C_{25}H_{40}N_5O_6$ [M − Cl]$^+$, 506.2973; found, 506.2972.

(2*R*,6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-20-Amino-2,6,21-trimethylhexadecahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1,4,7,10,13]oxatetraazacyclohexadecine-5,8,14,19,23(8a*H*)-pentaone Hydrochloride (17b). Macrocyclic ammonium salt **17b** (274.5 mg, quantitative yield) was prepared from cyclic pentadepsipeptide **16b** according to general procedure D; white solid, mp 190−191 °C (MeOH); $[\alpha]_D^{24}$ −18.2 (*c* 0.77, MeOH). IR (solid) ν_{max} 2954, 2875, 1732, 1640, 1440 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD, note: amide and ammonium protons were not observed due to deuterium exchange) δ 5.16 (1H, qd, *J* = 6.5, 2.5 Hz), 4.88−4.82 (1H, m), 4.77 (1H, d, *J* = 2.5 Hz), 4.62 (1H, dd, *J* = 8.5, 3.5 Hz), 4.55 (1H, br d, *J* = 3.5 Hz), 4.33−4.20 (2H, m), 3.84 (1H, dd, *J* = 11.5, 7.5 Hz), 3.74 (1H, ddd, *J* = 12.0, 8.0, 4.0 Hz), 3.51 (1H, ddd, *J* = 12.0, 8.0, 8.0 Hz), 3.16 (1H, dd, *J* = 11.5, 7.0 Hz), 2.76−2.62 (1H, m), 2.55−2.42 (2H, m), 2.38 (1H, ddd, *J* = 13.5, 13.5, 3.0 Hz), 2.29 (1H, ddd, *J* = 13.0, 6.5, 3.5 Hz), 2.10−1.69 (7H, m), 1.68−1.51 (1H, m), 1.50−1.34 (4H, m), 1.29 (3H, d, *J* = 6.5 Hz), 1.08 (3H, d, *J* = 6.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ 175.9, 174.1, 172.3, 172.1, 166.8, 70.2, 62.2, 60.2, 57.6, 56.8, 54.9, 50.9, 49.5, 42.4, 39.2, 33.1, 31.8, 25.7, 25.5, 23.2, 21.9, 18.4, 16.1, 13.6. HRMS (ESI$^+$) *m*/*z* calcd for $C_{24}H_{38}N_5O_6$ [M − Cl]$^+$, 492.2816; found, 492.2810.

(6*S*,8a*S*,10*S*,14a*S*,20*S*,21*S*,23a*S*)-20-Amino-6,10,21-trimethylhexadecahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1,4,7,10,13]oxatetraazacyclohexadecine-5,8,14,19,23(8a*H*)-pentaone Hydrochloride (17c).[31] Macrocyclic ammonium salt **17c** (216.4 mg, quantitative yield) was prepared from cyclic pentadepsipeptide **16c**[31] according to general procedure D; light-yellow solid, decomposition temp 205 °C; $[\alpha]_D^{25}$ −19.0 (*c* 0.39, DMSO). IR (solid) ν_{max} 2933, 1732, 1635, 1438, 1264, 1084 cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD, note: amide and ammonium protons were not observed due to deuterium exchange) δ 5.57−5.12 (1H, m), 5.01−4.38 (4H, m), 4.34−4.17 (2H, m), 3.79−3.57 (3H, m), 3.56−3.42 (1H, m), 2.60−2.28 (4H, m), 2.27−2.11 (2H, m), 2.09−1.65 (6H, m), 1.57−1.24 (7H, m), 1.22−0.93 (4H, m). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 175.8, 173.9, 173.5, 172.47, 172.42, 172.17, 171.09, 171.9, 170.0, 167.3, 166.7, 165.6, 71.7, 70.6, 70.1, 62.9, 62.0, 61.4, 61.1, 60.2, 59.7, 58.3, 57.7, 56.8, 55.9, 55.5, 54.5, 51.1, 51.0, 50.2, 49.5, 47.9, 45.4, 42.4, 35.1, 34.7, 34.2, 34.0, 33.9, 33.7, 33.1, 32.7, 31.8, 30.9, 30.6, 30.2, 29.8, 29.3, 28.81, 28.75, 28.6, 27.5, 26.6, 26.4, 24.0, 23.2, 23.0, 22.1, 22.0, 17.8, 17.2, 16.0, 13.7. HRMS (ESI$^+$) *m*/*z* calcd for $C_{24}H_{38}N_5O_6$ [M − Cl]$^+$, 492.2817; found, 492.2824.

(2*R*,6*S*,8a*S*,10*S*,14a*S*,20*S*,23a*S*)-20-Amino-2,6,10-trimethylhexadecahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1,4,7,10,13]oxatetraazacyclohexadecine-5,8,14,19,23(8a*H*)-pentaone Hydrochloride (17d). Macrocyclic ammonium salt **17d** (302.5 mg, quantitative yield) was prepared from cyclic pentadepsipeptide **16d** according to general procedure D; yellow solid, decomposition temp 205 °C; $[\alpha]_D^{24}$ −37.9 (*c* 0.76, MeOH). IR (solid) ν_{max} 2938, 1730, 1645, 1435, 1258, 1167, 1104, 1014 cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD, note: amide and ammonium protons were not observed due to deuterium exchange) δ 4.76 (1H, dd, *J* = 10.0, 2.0 Hz), 4.71 (1H, dd, *J* = 9.0, 3.0 Hz), 4.68−4.62 (2H, m), 4.56 (1H, br d, *J* = 5.0 Hz), 4.28−4.22 (1H, br m), 4.13 (1H, q, *J* = 7.0 Hz), 3.95 (1H, d, *J* = 12.5, 10.0 Hz), 3.90 (1H, dd, *J* = 11.5, 7.5 Hz), 3.71 (1H, ddd, *J* = 12.0, 8.0, 4.0 Hz), 3.52 (1H, ddd, *J* = 12.0, 7.5, 7.5 Hz), 3.09 (1H, dd, *J* = 11.5, 8.0 Hz), 2.68−2.59 (1H, m), 2.53−2.40 (3H, m), 2.28 (1H, ddd, *J* = 13.0, 6.5, 2.5 Hz), 2.08−1.99 (2H, m), 1.97−1.90 (1H, m), 1.87−1.78 (1H, m), 1.75−1.65 (2H, m), 1.50 (1H, ddd, *J* = 14.0, 12.5, 5.0 Hz), 1.38 (3H, d, *J* = 7.0 Hz), 1.15−1.04 (4H, m), 1.02 (3H, d, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 176.1, 173.9, 172.9, 172.3, 166.7, 64.8, 62.0, 60.4, 57.9, 55.0, 53.7, 51.2, 49.3, 42.5, 39.6, 34.1, 33.9, 33.1, 31.8, 28.5, 23.4, 22.0, 17.9, 15.9. HRMS (ESI$^+$) *m*/*z* calcd for $C_{24}H_{38}N_5O_6$ [M − Cl]$^+$, 492.2817; found, 492.2829.

(6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-20-Amino-6,21-dimethylhexadecahydropyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1,4,7,10,13]oxatetraazacyclohexadecine-5,8,14,19,23(8a*H*)-pentaone Hydrochloride (17e).[31] Macrocyclic ammonium salt **17e** (307.9 mg, quantitative yield) was prepared from cyclic pentadepsipeptide **16e**[31] according to general procedure D; yellow solid, decomposition temp 200 °C; $[\alpha]_D^{25}$ −25.7 (*c* 0.30, MeOH). IR (solid) ν_{max} 2937, 1730, 1646, 1440, 1257, 1166, 1083, 1013. ^{1}H NMR (400 MHz, CD_3OD, note: amide and ammonium protons were not observed due to deuterium exchange) δ 5.17 (1H, qd, *J* = 6.5, 2.5 Hz), 4.92−4.80 (1H, m), 4.75 (1H, d, *J* = 2.5 Hz), 4.62−4.51 (2H, m), 4.34−4.20 (2H, m), 3.74 (1H, ddd, *J* = 12.0, 8.0, 4.0 Hz), 3.70−3.62 (2H, m), 3.51 (1H, ddd, *J* = 12.0, 7.0, 7.0 Hz), 2.57−2.32 (4H, m), 2.28−2.11 (2H, m), 2.10−1.68 (7H, m), 1.67−1.52 (1H, m), 1.51−1.34 (4H, m), 1.29 (3H, d, *J* = 6.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ 175.9, 173.9, 172.4, 172.0, 166.7, 70.0, 62.0, 60.2, 57.5, 56.8, 51.0, 49.4, 48.3, 42.4, 33.1, 31.8, 25.7, 25.5, 24.0, 23.2, 21.9, 16.0, 13.6. HRMS (ESI$^+$) *m*/*z* calcd for $C_{23}H_{36}N_5O_6$ [M − Cl]$^+$, 478.2660; found, 478.2657.

(2*R*,6*S*,8a*S*,14a*S*,20*S*,23a*S*)-20-Amino-2,6-dimethylhexadecahydro-5*H*,14*H*,19*H*-pyrido[1,2-*d*]dipyrrolo[1,2-*a*:1′,2′-*j*]-[1,4,7,10,13]pentaazacyclohexadecine-5,8,14,19,23(8a*H*,20*H*)-pentaone Hydrochloride (17f). Macrocyclic ammonium salt **17f** (68.7 mg, quantitative yield) was prepared from cyclic pentapeptide **16f** according to general procedure D; light-yellow solid, decomposition temp 210 °C; $[\alpha]_D^{23}$ −69.3 (*c* 0.31, MeOH). IR (solid) ν_{max} 3370, 3290, 2936, 2877, 1643, 1548, 1516, 1454 cm^{-1}. ^{1}H NMR (500 MHz, $(CD_3)_2SO$, notes: amide and ammonium protons were not observed due to deuterium exchange and mixture of rotamers) δ 4.83−4.59 (1H, m), 4.40−4.10 (4H, m), 3.95 (1H, d, *J* = 9.0 Hz), 3.92−3.77 (1H, m), 3.74−3.61 (1H, m), 3.58−3.41 (2H, m), 3.22−3.01 (1H, m), 2.87 (1H, dd, *J* = 10.5 Hz), 2.57−2.12 (4H, m), 2.02−1.48 (8H, m), 1.40−1.10 (5H, m), 1.04−0.88 (3H, m). ^{13}C NMR (125 MHz, $(CD_3)_2SO$, note: mixture of rotamers) δ 172.53, 172.47, 171.9, 171.64, 171.60, 170.14, 170.11, 169.87, 169.85, 169.82, 169.79, 165.7, 61.4, 61.38, 61.1, 57.9, 57.1, 56.10, 56.08, 55.9, 54.9, 54.8, 53.9, 52.14, 52.08, 48.3, 48.2, 47.4, 47.3, 40.7, 40.2, 38.9, 31.16, 31.10, 29.1, 26.1, 25.8, 24.0, 23.7, 22.0, 21.8, 20.7, 20.5, 20.3, 20.2, 20.0, 16.83, 16.77, 16.72, 16.69, 16.68, 16.66, 16.4. HRMS (ESI$^+$) *m*/*z* calcd for $C_{23}H_{37}N_6O_5$ [M − Cl]$^+$, 477.2820; found, 477.2820.

Benzyl ((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,10-*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,10,21-tetramethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo-[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)-amino)propan-2-yl)carbamate (19a). Cyclic hexadepsipeptide **19a** (332.5 mg, 86%) was prepared from macrocyclic ammonium salt **17a** and Cbz-3,5-difluoro-L-phenylalanine (**18**) according to general procedure A; white solid, mp 169−170 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −17.2 (*c* 0.43, MeOH); R_f 0.86 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3289, 2957, 2934, 1728, 1663, 1640, 1597, 1514, 1452, 849 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: two of the amide protons were not observed due to deuterium exchange) δ 8.67 (1H, d, *J* = 9.5 Hz), 7.40−7.20 (5H, m), 6.93−6.71 (3H, m), 5.32 (1H, dd, *J* = 8.5, 3.0 Hz), 5.19 (1H, qd, *J* = 6.5, 1.5 Hz), 5.15 (1H, d, *J* = 12.5 Hz), 5.05 (1H, d, *J* = 12.5 Hz), 5.02−4.96 (1H, m), 4.81−4.74 (1H, m), 4.65 (1H, d, *J* = 1.5 Hz), 4.59−4.50 (1H, br m), 4.46 (1H, t, *J* = 7.5 Hz), 4.42 (1H, d, *J* = 8.5 Hz), 3.68 (1H, ddd, *J* = 11.5, 7.5, 5.0 Hz), 3.57 (1H, dd, *J* = 12.0, 9.5 Hz), 3.48 (1H, ddd, *J* = 11.5, 7.0, 7.0 Hz), 3.16−3.00 (2H, m), 2.82 (1H, dd, *J* = 13.5, 7.5 Hz), 2.71−2.56 (2H, m), 2.52−2.34 (2H, m), 2.10 (1H, dd, *J* = 13.0, 7.0 Hz), 2.07−1.91 (3H, m), 1.86 (1H, ddd, *J* = 13.0, 11.5, 8.5 Hz), 1.68−1.51 (2H, m),

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1.35 (3H, d, J = 6.5 Hz), 1.27−1.12 (4H, m), 1.09−0.87 (7H, m). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 173.6, 173.4, 172.8, 171.62, 171.56, 171.10, 171.08, 168.03, 168.02, 164.4 (dd, J = 246.5, 13.0 Hz), 158.2, 143.1 (t, J = 9.5 Hz), 138.4, 129.6, 129.2, 128.9, 113.7 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 26.0 Hz),71.6, 67.9, 61.8, 58.8, 58.68, 58.67, 56.8, 55.8, 55.0, 49.3, 47.8, 42.3, 39.8, 38.8, 37.2, 34.3, 31.8, 31.1, 29.4, 24.1, 22.2, 18.8, 18.36, 18.35, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{42}H_{53}N_6O_9$ [M + H]$^+$, 823.3837; found, 823.3825.

Benzyl ((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)-propan-2-yl)carbamate (19b). Cyclic hexadepsipeptide **19b** (323.5 mg, quantitative) was prepared from macrocyclic ammonium salt **17b** and Cbz-3,5-difluoro-L-phenylalanine (**18**) according to general procedure A; white solid, mp 205−206 °C (CH_2Cl_2); $[\alpha]_D^{23}$ −27.9 (c 0.54, MeOH); R_f 0.58 (19:1 EtOAc:MeOH). IR (solid) ν_{max} 3281, 2946, 1721, 1635, 1444, 840 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: two of the amide protons were not observed due to deuterium exchange) δ 8.69 (1H, d, J = 9.5 Hz), 7.39−7.18 (5H, m), 6.90−6.67 (3H, m), 5.31 (1H, dd, J = 9.0, 2.5 Hz), 5.19 (1H, qd, J = 6.5, 1.5 Hz), 5.16 (1H, d, J = 12.5 Hz), 5.08−4.97 (2H, m), 4.79−4.74 (1H, m), 4.65 (1H, d, J = 1.5 Hz), 4.56−4.49 (1H, br m), 4.46 (1H, dd, J = 7.5, 7.5 Hz), 4.42 (1H, d, J = 8.5 Hz), 3.73−3.64 (1H, m), 3.57 (1H, dd, J = 12.0, 9.0 Hz), 3.48 (1H, ddd, J = 11.5, 7.0, 7.0 Hz), 3.14−3.03 (2H, m), 2.82 (1H, dd, J = 13.5, 7.5 Hz), 2.70−2.56 (2H, m), 2.53−2.34 (2H, m), 2.10 (1H, dd, J = 13.0, 7.0 Hz), 2.08−1.93 (3H, m), 1.87 (1H, ddd, J = 13.0, 11.5, 8.5 Hz), 1.75 (1H, br d, J = 13.0 Hz), 1.67−1.53 (2H, m), 1.52−1.31 (5H, m), 1.21 (3H, d, J = 6.5 Hz), 0.98 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 173.6, 173.4, 172.8, 171.53, 171.47, 171.12, 171.10, 168.0, 164.4 (dd, J = 246.5, 13.0 Hz), 158.2, 143.1 (t, J = 9.5 Hz), 138.4, 129.6, 129.2, 128.9, 113.7 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 25.5 Hz), 71.6, 67.9, 61.8, 58.73, 58.69, 56.8, 55.8, 55.0, 49.2, 47.8, 42.6, 39.8, 38.8, 31.9, 31.1, 29.0, 26.0, 24.1, 22.4, 18.8, 18.4, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{51}F_2N_6O_9$ [M + H]$^+$, 809.3680; found, 809.3653.

Benzyl ((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((6*S*,8a*S*,10-*S*,14a*S*,20*S*,21*S*,23a*S*)-6,10,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)-propan-2-yl)carbamate (19c). Cyclic hexadepsipeptide **19c** (135.7 mg, 55%) was prepared from macrocyclic ammonium salt **17c**[31] and Cbz-3,5-difluoro-L-phenylalanine (**18**) according to general procedure A; white solid, mp 191−192 °C (CH_2Cl_2); $[\alpha]_D^{24}$ −23.4 (c 0.32, MeOH); R_f 0.62 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3291, 2955, 2934, 1728, 1659, 1597, 1506, 1445, 1350, 1319, 1267, 1117, 847 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: two of the amide protons were not observed due to deuterium exchange) δ 8.67 (1H, d, J = 9.5 Hz), 7.39−7.21 (5H, m), 6.94−6.71 (3H, m), 5.33 (1H, dd, J = 8.5, 3.0 Hz), 5.23−5.12 (2H, m), 5.07−4.95 (2H, m), 4.82−4.76 (1H, m), 4.65 (1H, d, J = 1.0 Hz), 4.58−4.50 (1H, br m), 4.49−4.43 (1H, m), 4.40 (1H, d, J = 8.5 Hz), 3.67 (1H, ddd, J = 12.0, 7.5, 5.0 Hz), 3.58−3.44 (2H, m), 3.33 (1H, ddd, J = 12.5, 8.5, 5.0 Hz), 3.08 (1H, dd, J = 13.5, 6.5 Hz), 2.82 (1H, dd, J = 13.5, 8.0 Hz), 2.71−2.57 (2H, m), 2.52−2.41 (1H, m), 2.29−2.14 (1H, m), 2.10−1.78 (6H, m), 1.68−1.51 (2H, m), 1.35 (3H, d, J = 6.5 Hz), 1.26−1.12 (4H, m), 1.04−0.89 (4H, m). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 173.62, 173.56, 172.8, 171.62, 171.56, 171.07, 171.05, 168.0, 164.4 (dd, J = 246.5, 13.0 Hz), 158.2, 143.1 (t, 9.0 Hz), 138.3, 129.6, 129.21, 129.17, 113.7 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 25.5 Hz), 71.7, 67.9, 61.0, 58.8, 58.69, 58.68, 56.8, 55.0, 49.4, 48.4, 47.8, 42.3, 38.8, 37.2, 34.3, 31.8, 31.6, 29.4, 24.1, 22.5, 22.2, 18.33, 18.32, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{51}F_2N_6O_9$ [M + H]$^+$, 809.3680; found, 809.3684.

Benzyl ((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,10-*S*,14a*S*,20*S*,23a*S*)-2,6,10-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)propan-2-yl)carbamate (19d). Cyclic hexadepsipeptide **19d** (252.9 mg, 68%) was prepared from macrocyclic ammonium salt **17d** and Cbz-3,5-difluoro-L-phenylalanine (**18**) according to general procedure A; white solid, mp 178−179 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −32.1 (c 0.25, MeOH); R_f 0.70 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3287, 2959, 2932, 1732, 1643, 1597, 1516, 1441, 845 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, note: two of the amide protons were not observed due to deuterium exchange) δ 8.47 (1H, d, J = 9.5 Hz), 7.39−7.27 (5H, m), 6.78−6.70 (2H, m), 6.65 (1H, tt, J = 9.0, 2.5 Hz), 5.23 (1H, d, J = 12.5 Hz), 5.12 (1H, dd, J = 9.0, 3.0 Hz), 5.04 (1H, d, J = 12.5 Hz), 4.94 (1H, dq, J = 9.5, 6.5 Hz), 4.79 (1H, d, J = 11.5 Hz), 4.71−4.63 (2H, m), 4.55−4.44 (2H, m), 4.35−4.28 (1H, m), 3.76 (1H, ddd, J = 11.5, 8.5, 5.0 Hz), 3.61−3.48 (3H, m), 3.07 (1H, dd, J = 12.0, 8.0 Hz), 3.02 (1H, dd, J = 13.5, 7.5 Hz), 2.90 (1H, dd, J = 13.5, 6.5 Hz), 2.74−2.59 (2H, m), 2.40−2.19 (2H, m), 2.18−2.08 (1H, m), 2.05 (1H, dd, J = 13.0, 7.0 Hz), 1.99−1.86 (2H, m), 1.81 (1H, ddd, J = 13.0, 11.5, 8.5 Hz), 1.68−1.54 (2H, m), 1.37 (3H, d, J = 6.5 Hz), 1.13−0.92 (5H, m), 0.90 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, note: mixture of rotamers) δ 172.8, 171.3, 170.9, 170.8, 169.6, 169.5, 165.21, 165.19, 163.1 (dd, J = 248.5, 13.0 Hz), 156.4, 140.5 (t, J = 9.5 Hz), 136.7, 128.6, 128.2, 128.0, 112.6 (dd, J = 19.0, 6.0 Hz), 102.6 (t, J = 25.0 Hz), 67.0, 65.01, 64.99, 59.8, 57.3, 56.9, 55.93, 55.89, 54.3, 51.6, 51.5, 47.8, 46.5, 41.0, 38.8, 37.9, 36.3, 33.5, 30.9, 29.7, 28.1, 23.2, 22.0, 18.6, 18.2. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{51}F_2N_6O_9$ [M + H]$^+$, 809.3680; found, 809.3669.

Benzyl ((*S*)-3-(3,5-Difluorophenyl)-1-(((6*S*,8a*S*,14a*S*,20*S*,21-*S*,23a*S*)-6,21-dimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa-[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)-1-oxopropan-2-yl)carbamate (19e). Cyclic hexadepsipeptide **19e** (351.6 mg, 97%) was prepared from macrocyclic ammonium salt **17e**[31] and Cbz-3,5-difluoro-L-phenylalanine (**18**) according to general procedure A; white solid, mp 181−182 °C (CH_2Cl_2); $[\alpha]_D^{24}$ −30.2 (c 0.60, MeOH); R_f 0.57 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3293, 2980, 2940, 1726, 1659, 1595, 1514, 1441, 1254, 1117, 845 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: two of the amide protons were not observed due to deuterium exchange) δ 8.70 (1H, d, J = 9.5 Hz), 7.41−7.21 (5H, m), 6.93−6.71 (3H, m), 5.32 (1H, dd, J = 9.0, 2.5 Hz), 5.23−5.12 (2H, m), 5.07−4.97 (2H, m), 4.80−4.74 (1H, m), 4.64 (1H, d, J = 1.0 Hz), 4.56−4.49 (1H, br m), 4.48−4.43 (1H, m), 4.40 (1H, d, J = 8.0 Hz), 3.74−3.63 (1H, m), 3.58−3.44 (2H, m), 3.38−3.32 (1H, m), 3.09 (1H, dd, J = 13.5, 6.5 Hz), 2.82 (1H, dd, J = 13.5, 8.0 Hz), 2.69−2.57 (2H, m), 2.52−2.40 (1H, m), 2.27−2.14 (1H, m), 2.10−1.80 (6H, m), 1.75 (1H, br d, J = 14.0 Hz), 1.67−1.53 (2H, m), 1.52−1.31 (5H, m), 1.20 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 173.61, 173.55, 172.8, 171.53, 171.47, 171.09, 171.07, 168.05, 168.04, 164.4 (dd, J = 246.5, 13.0 Hz), 158.2, 141.1 (t, J = 9.5 Hz), 138.3, 129.6, 129.21, 129.17, 113.7 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 26.0 Hz), 71.7, 67.9, 61.0, 58.76, 58.70, 58.69, 56.8, 55.0, 49.4, 48.4, 47.8, 42.6, 38.8, 31.9, 31.6, 29.0, 26.0, 24.1, 22.52, 22.46, 18.35, 18.33, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{49}F_2N_6O_9$ [M + H]$^+$, 795.3524; found, 795.3518.

Benzyl ((*S*)-3-(3,5-Difluorophenyl)-1-(((2*R*,6*S*,8a*S*,14a*S*,20-*S*,23a*S*)-2,6-dimethyl-5,8,14,19,23-pentaoxoicosahydro-1*H*,5*H*,14*H*-pyrido[1,2-*d*]dipyrrolo[1,2-*a*:1′,2′-*j*][1,4,7,10,13]-pentaazacyclohexadecin-20-yl)amino)-1-oxopropan-2-yl)-carbamate (19f). Cyclic hexapeptide **19f** (54.8 mg, 63%) was prepared from macrocyclic ammonium salt **17f** and Cbz-3,5-difluoro-L-phenylalanine (**18**) according to general procedure A; white solid, mp 167−168 °C (CH_2Cl_2); $[\alpha]_D^{23}$ −93.7 (c 0.68, MeOH); R_f 0.24 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3445, 2937, 2874, 1709, 1643, 1433 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amide and carbamate protons were not observed due to deuterium exchange) δ 7.43−7.22 (5H, m), 6.82 (2H, d, J = 6.5 Hz), 6.79−6.71 (1H, m), 5.29 (1H, d, J = 7.5 Hz), 5.18 (1H, d, J = 13.0 Hz), 5.11 (1H, d, J = 13.0 Hz), 4.97 (1H, q, J = 6.5 Hz), 4.81 (1H, br s), 4.49 (1H, d, J = 9.5 Hz), 4.39 (1H, br d, J = 13.5 Hz), 4.32 (1H, d, J = 8.5 Hz), 4.11 (1H, dd, J = 10.5, 8.5 Hz), 3.94−3.81 (2H, m), 3.75 (1H, ddd, J = 12.0, 6.0, 6.0 Hz), 3.49 (1H, ddd, J = 12.0, 6.5, 6.5 Hz), 3.20 (1H, dd, J = 14.0, 5.0 Hz), 3.04−2.89 (2H, m), 2.74−2.57 (3H, m), 2.51−2.39 (1H, m), 2.27−2.13 (1H, m), 2.06−1.91 (4H, m), 1.85 (1H, ddd, J = 12.5, 9.5, 9.5 Hz), 1.74 (1H, br d, J = 13.0 Hz), 1.67−1.27 (7H, m), 1.06 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 176.0, 174.4, 173.4,

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172.7, 171.5, 169.5, 164.5 (dd, J = 246.5, 13.0 Hz), 158.2, 144.3 (t, J = 8.5 Hz), 138.6, 129.7, 129.1, 128.3, 113.5 (dd, J = 19.5, 5.5 Hz), 102.8 (t, J = 26.0 Hz), 68.0, 62.9, 58.9, 58.8, 57.0, 56.3, 54.8, 50.2, 47.9, 44.1, 42.8, 40.4, 37.6, 31.9, 30.8, 28.9, 25.9, 24.3, 22.4, 18.5, 17.4. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{50}F_2N_7O_8$ [M + H]$^+$, 794.3683; found, 794.3680.

(*S*)-2-Amino-3-(3,5-difluorophenyl)-*N*-((2*R*,6*S*,8a*S*,10-*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,10,21-tetramethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo-[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)-propanamide (20a). Macrocyclic amine **20a** (229.5 mg, 98%) was prepared from cyclic hexadepsipeptide **19a** according to general procedure D; white solid, mp 169−170 °C ($CHCl_3$); $[\alpha]_D^{24}$ −18.9 (c 0.26, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3281, 2959, 2934, 2878, 1728, 1647, 1595, 1510, 1445, 1117, 847 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amine and one of the amide protons were not observed due to deuterium exchange) δ 8.84 (1H, d, J = 9.0 Hz), 6.92−6.84 (2H, m), 6.78 (1H, tt, J = 9.5, 2.5 Hz), 5.35 (1H, dd, J = 8.5, 3.0 Hz), 5.22 (1H, qd, J = 6.5, 1.5 Hz), 5.07−4.98 (1H, m), 4.82−4.78 (1H, m), 4.67 (1H, d, J = 1.5 Hz), 4.58−4.51 (1H, br m), 4.45 (1H, d, J = 8.5 Hz), 3.76−3.63 (2H, m), 3.60 (1H, dd, J = 7.5, 6.0 Hz), 3.49 (1H, ddd, J = 11.5, 7.0, 7.0 Hz), 3.14−3.01 (2H, m), 2.83−2.58 (3H, m), 2.57−2.41 (2H, m), 2.14 (1H, dd, J = 13.0, 9.5 Hz), 2.10−1.84 (4H, m), 1.68−1.53 (2H, m), 1.34 (3H, d, J = 6.5 Hz), 1.29 (3H, d, J = 6.5 Hz), 1.24−1.14 (1H, m), 1.07 (3H, d, J = 6.5 Hz), 1.05−0.94 (4H, m). ^{13}C NMR (125 MHz, CD_3OD) δ 176.3, 173.71, 173.65, 171.7, 170.9, 168.3, 164.6 (dd, J = 246.5, 13.0 Hz), 143.5 (t, J = 9.5 Hz), 113.7 (dd, J = 19.0, 5.5 Hz), 103.0 (t, J = 26.0 Hz), 71.6, 61.8, 58.71, 58.65, 56.8, 55.8, 55.1, 49.2, 47.8, 42.4, 41.5, 39.9, 37.2, 34.3, 31.8, 31.1, 29.4, 24.1, 22.2, 18.6, 18.3, 13.6. HRMS (ESI$^+$) m/z calcd for $C_{34}H_{47}F_2N_6O_7$ [M + H]$^+$, 689.3469; found, 689.3500.

(*S*)-2-Amino-3-(3,5-difluorophenyl)-*N*-((2*R*,6*S*,8a-*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)-propanamide (20b). Macrocyclic amine **20b** (153.6 mg, 89%) was prepared from cyclic hexadepsipeptide **19b** according to general procedure D; white solid, decomposition temp 190 °C; $[\alpha]_D^{25}$ −14.3 (c 0.38, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3273, 2943, 2876, 1732, 1647, 1597, 1516, 1441, 1117, 845 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amine and one of the amide protons were not observed due to deuterium exchange) δ 8.54 (1H, d, J = 9.5 Hz), 6.98−6.91 (2H, m), 6.86 (1H, tt, J = 9.0, 2.5 Hz), 5.35−5.23 (2H, m), 5.06 (1H, dq, J = 9.5, 6.5 Hz), 4.81−4.77 (1H, m), 4.63 (1H, d, J = 2.0 Hz), 4.57−4.50 (1H, br m), 4.48 (1H, d, J = 8.5 Hz), 3.92 (1H, dd, J = 8.0, 5.5 Hz), 3.76−3.63 (2H, m), 3.50 (1H, ddd, J = 11.5, 7.0, 7.0 Hz), 3.17 (1H, dd, J = 14.0, 5.5 Hz), 3.10 (1H, dd, J = 12.0, 8.5 Hz), 2.84 (1H, dd, J = 14.0, 8.0 Hz), 2.68−2.58 (2H, m), 2.57−2.44 (2H, m), 2.16 (1H, dd, J = 13.0, 7.0 Hz), 2.11−1.89 (4H, m), 1.76 (1H, br d, J = 12.5 Hz), 1.68−1.55 (2H, m), 1.52−1.24 (8H, m), 1.08 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 173.6, 173.4, 173.2, 171.52, 171.46, 171.40, 171.38, 167.8, 164.8 (dd, J = 247.5, 13.0 Hz), 141.5 (t, J = 9.5 Hz), 113.8 (dd, J = 19.5, 6.0 Hz), 103.8 (t, J = 25.5 Hz), 71.2, 61.8, 58.72, 58.70, 58.6, 56.0, 55.8, 55.6, 49.0, 47.9, 42.8, 39.9, 39.7, 32.0, 31.1, 28.9, 26.0, 24.1, 22.4, 18.7, 18.28, 18.26, 13.7. HRMS (ESI$^+$) m/z calcd for $C_{33}H_{45}F_2N_6O_7$ [M + H]$^+$, 675.3312; found, 675.3328.

(*S*)-2-Amino-3-(3,5-difluorophenyl)-*N*-((6*S*,8a*S*,10-*S*,14a*S*,20*S*,21*S*,23a*S*)-6,10,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)-propanamide (20c). Macrocyclic amine **20c** (88.3 mg, quantitative yield) was prepared from cyclic hexadepsipeptide **19c** according to general procedure D; white solid, mp 194−195 °C ($CHCl_3$); $[\alpha]_D^{24}$ −19.0 (c 0.49, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3281, 2955, 2934, 2884, 1728, 1651, 1597, 1514, 1445, 1269, 1117, 847 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amine and one of the amide protons were not observed due to deuterium exchange) δ 8.82 (1H, d, J = 9.5 Hz), 7.03−6.86 (2H, m), 6.78 (1H, tt, J = 9.5, 2.5 Hz), 5.36 (1H, dd, J = 8.5, 3.0 Hz), 5.21 (1H, qd, J = 6.5, 1.5 Hz), 5.08−4.98 (1H, m), 4.83−4.79 (1H, m), 4.67 (1H, d, J = 1.5 Hz), 4.59−4.51 (1H, br m), 4.44 (1H, d, J = 8.0 Hz), 3.69 (1H, ddd, J = 11.5, 7.5, 5.0 Hz), 3.65−3.56 (2H, m), 3.54−3.43 (2H, m), 3.09 (1H, dd, J = 13.5, 6.0 Hz), 2.76−2.58 (3H, m), 2.54−2.41 (1H, m), 2.32−2.17 (1H, m), 2.12−1.90 (6H, m), 1.69−1.53 (2H, m), 1.34 (3H, d, J = 6.5 Hz), 1.29 (3H, d, J = 6.5 Hz), 1.19 (1H, ddd, J = 13.0, 13.0, 5.0 Hz), 1.06−0.93 (4H, m). ^{13}C NMR (125 MHz, CD_3OD) δ 176.0, 173.8, 173.6, 171.7, 170.9, 168.3, 164.6 (dd, J = 246.5, 13.0 Hz), 143.4 (t, J = 9.5 Hz), 113.7 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 26.0 Hz), 71.5, 60.9, 58.71, 58.67, 56.7, 55.1, 49.3, 48.5, 47.8, 42.4, 41.4, 37.2, 34.3, 31.9, 31.8, 29.4, 24.1, 22.5, 22.2, 18.3, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{33}H_{45}F_2N_6O_7$ [M + H]$^+$, 675.3312; found, 675.3308.

(*S*)-2-Amino-3-(3,5-difluorophenyl)-*N*-((2*R*,6*S*,8a*S*,10-*S*,14a*S*,20*S*,23a*S*)-2,6,10-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)propanamide (20d). Macrocyclic amine **20d** (119.9 mg, 67%) was prepared from cyclic hexadepsipeptide **19d** according to general procedure D; white solid, mp 146−147 °C ($CHCl_3$); $[\alpha]_D^{25}$ −31.7 (c 0.41, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3269, 2959, 2932, 1732, 1659, 1595, 1504, 1435, 1269, 1117 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amine and amide protons were not observed due to deuterium exchange) δ 7.05−6.82 (2H, m), 6.78 (1H, tt, J = 9.5, 2.5 Hz), 5.25 (1H, dd, J = 8.5, 3.0 Hz), 5.02 (1H, q, J = 6.5 Hz), 4.91−4.80 (2H, m), 4.61−4.55 (1H, m), 4.54−4.48 (2H, m), 3.77−3.61 (3H, m), 3.58−3.53 (1H, m), 3.50 (1H, ddd, J = 11.5, 7.0, 7.0 Hz), 3.10 (1H, dd, J = 12.0, 8.5 Hz), 3.04 (1H, dd, J = 13.5, 6.5 Hz), 2.78−2.41 (5H, m), 2.18−1.84 (5H, m), 1.68−1.53 (2H, m), 1.33 (3H, d, J = 6.5 Hz), 1.24−1.15 (1H, m), 1.11−0.93 (7H, m). ^{13}C NMR (125 MHz, CD_3OD) δ 175.8, 174.0, 173.6, 171.8, 171.0, 167.8, 164.6 (dd, J = 247.0, 13.0 Hz), 143.2 (t, J = 9.5 Hz), 113.6 (dd, J = 19.0, 5.5 Hz), 103.1 (t, J = 25.5 Hz), 65.7, 61.6, 58.8, 58.6, 56.9, 55.7, 53.1, 49.1, 47.8, 42.4, 41.3, 39.6, 37.3, 34.3, 32.0, 31.0, 29.4, 24.2, 22.2, 18.6, 18.3. HRMS (ESI$^+$) m/z calcd for $C_{33}H_{45}F_2N_6O_7$ [M + H]$^+$, 675.3312; found, 675.3326.

(*S*)-2-Amino-3-(3,5-difluorophenyl)-*N*-((6*S*,8a*S*,14a*S*,20*S*,21-*S*,23a*S*)-6,21-dimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa-[4,7,10,13]tetraazacyclohexadecin-20-yl)propanamide (20e). Macrocyclic amine **20e** (34.8 mg, quantitative yield) was prepared from cyclic hexadepsipeptide **19e** according to general procedure D; white solid, decomposition temp 190 °C; $[\alpha]_D^{25}$ − 12.9 (c 0.43, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3277, 2947, 2891, 1732, 1659, 1597, 1512, 1449, 1258, 1121, 847 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amine and one of the amide protons were not observed due to deuterium exchange) δ 8.84 (1H, d, J = 9.5 Hz), 7.04−6.86 (2H, m), 6.78 (1H, tt, J = 9.5, 2.5 Hz), 5.36 (1H, dd, J = 9.0, 3.0 Hz), 5.21 (1H, qd, J = 6.5, 2.0 Hz), 5.10−5.00 (1H, m), 4.83−4.77 (1H, m), 4.66 (1H, d, J = 2.0 Hz), 4.58−4.50 (1H, br m), 4.44 (1H, d, J = 8.0 Hz), 3.77−3.56 (3H, m), 3.55−3.43 (2H, m), 3.09 (1H, dd, J = 14.0, 6.0 Hz), 2.72 (1H, dd, J = 14.0, 7.5 Hz), 2.69−2.59 (2H, m), 2.53−2.42 (1H, m), 2.32−2.16 (1H, m), 2.12−1.94 (6H, m), 1.76 (1H, br d, J = 13.0 Hz), 1.68−1.54 (2H, m), 1.53−1.23 (8H, m). ^{13}C NMR (125 MHz, CD_3OD, note: mixture of rotamers) δ 176.1, 173.8, 173.6, 171.64, 171.58, 170.97, 170.95, 168.3, 164.6 (dd, J = 247.0, 13.0 Hz), 143.4 (t, J = 9.5 Hz), 113.7 (dd, J = 19.5, 6.0 Hz), 103.1 (t, J = 25.5 Hz), 71.5, 60.9, 58.7, 58.6, 56.7, 55.1, 49.3, 48.5, 47.8, 42.7, 41.4, 31.9, 31.8, 29.0, 26.0, 24.1, 22.48, 22.47, 18.3, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{32}H_{43}F_2N_6O_7$ [M + H]$^+$, 661.3156; found, 661.3151.

(*S*)-2-Amino-3-(3,5-difluorophenyl)-*N*-((2*R*,6*S*,8a*S*,14a*S*,20-*S*,23a*S*)-2,6-dimethyl-5,8,14,19,23-pentaoxoicosahydro-1*H*,5*H*,14*H*-pyrido[1,2-*d*]dipyrrolo[1,2-*a*:1′,2′-*j*][1,4,7,10,13]-pentaazacyclohexadecin-20-yl)propanamide (20f). Macrocyclic amine **20f** (24.7 mg, 62%) was prepared from cyclic hexapeptide **19f** according to general procedure D; white solid, decomposition temp 250 °C; $[\alpha]_D^{24}$ −47.1 (c 1.0, MeOH). IR (thin film in CH_2Cl_2) ν_{max} 2876, 1636, 1435, 1254, 1169, 1150, 1117, 1015 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, notes: amine and amide protons were not observed due to deuterium exchange) δ 6.93−6.81 (2H, m), 6.77 (1H, tt, J = 9.0, 2.5 Hz), 5.29 (1H, dd, J = 8.5, 3.0 Hz), 4.93 (1H, q, J = 7.0 Hz), 4.88−4.81 (1H, m, obscured by H_2O signal), 4.54−4.48 (1H, br m), 4.46 (1H, dd, J = 9.5, 1.5 Hz), 4.35 (1H, d, J = 8.5 Hz), 4.00 (1H, dd, J = 11.5, 8.0 Hz), 3.84 (1H, dd, J = 14.0, 1.5 Hz), 3.70−3.63 (1H, m),

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3.57−3.48 (2H, m), 3.05−2.95 (2H, m), 2.86 (1H, dd, J = 14.0, 9.5 Hz), 2.72 (1H, dd, J = 13.5, 7.0 Hz), 2.68−2.58 (2H, m), 2.50−2.41 (1H, m), 2.39−2.29 (1H, m), 2.11−1.95 (4H, m), 1.89 (1H, ddd, J = 12.5, 8.5, 8.5 Hz), 1.74 (1H, br d, J = 13.0 Hz), 1.67−1.44 (3H, m), 1.43−1.32 (4H, m), 1.07 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 176.0, 175.8, 174.3, 173.0, 171.6, 169.2, 164.6 (dd, J = 249.5, 13.0 Hz), 143.4 (t, J = 9.0 Hz), 113.6 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 25.5 Hz), 63.0, 58.8 (2C), 57.0, 56.2, 54.2, 50.0, 47.8, 44.3, 42.7, 42.0, 40.2, 31.9, 30.9, 28.9, 25.9, 24.3, 22.4, 18.3, 17.3. HRMS (ESI$^+$) m/z calcd for $C_{32}H_{44}F_2N_7O_6$ [M + H]$^+$, 660.3316; found, 660.3317.

(*E*)-4-Cyclohexylbut-2-enoic Acid (21c).[77] Acid **21c** (142.5 mg, 62%) was prepared from ethyl (*E*)-4-cyclohexylbut-2-enoate[78] according to general procedure F using LiOH·H_2O (1.15 g, 27.38 mmol, 20.0 equiv), 96 h; light-yellow solid, mp 35−36 °C ($CHCl_3$); R_f 0.63 (2:1 hexanes:EtOAc). IR (thin film in CH_2Cl_2) ν_{max} 2928, 2853, 1686, 1647, 1429, 1308, 1234, 1182, 986, 953, 905 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.04 (1H, br s), 7.07 (1H, dt, J = 15.5, 7.5 Hz), 5.81 (1H, dt, J = 15.5, 1.5 Hz), 2.17−2.05 (2H, m), 1.81−1.59 (5H, m), 1.51−1.38 (1H, m), 1.31−1.07 (3H, m), 1.02−0.86 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 151.5, 121.6, 40.4, 37.4, 33.3, 26.4, 26.3. HRMS (ESI$^-$) m/z calcd for $C_{10}H_{15}O_2$ [M − H]$^-$, 167.1072; found, 167.1070.

(*E*)-4-Cyclopropylbut-2-enoic Acid (21d). Ethyl (*E*)-4-cyclopropylbut-2-enoate (233.2 mg, 39%) was prepared from 2-cyclopropylacetaldehyde according to general procedure E. Colorless liquid; R_f 0.44 (19:1 hexanes:EtOAc). IR (thin film in CH_2Cl_2) ν_{max} 3080, 3003, 2984, 1721, 1655, 1651, 1368, 1325, 1271, 1180, 1043 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (1H, dt, J = 15.5, 6.5 Hz), 5.91 (1H, dt, J = 15.5, 1.5 Hz), 4.19 (2H, q, J = 7.0 Hz), 2.09 (2H, ddd, J = 6.5, 6.5, 1.5 Hz), 1.29 (3H, t, J = 7.0 Hz), 0.89−0.71 (1H, m), 0.60−0.42 (2H, m), 0.19−0.02 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 148.6, 121.4, 60.3, 37.0, 14.4, 9.2, 4.5. HRMS (DART$^+$) m/z calcd for $C_9H_{15}O_2$ [M + H]$^+$, 155.1072; found, 155.1081. The title compound (77.3 mg, 78%) was prepared from the intermediate ethyl ester according to general procedure F using LiOH·H_2O (615 mg, 14.64 mmol, 15.0 equiv), 36 h; light-yellow oil; R_f 0.53 (2:1 hexanes:EtOAc). IR (thin film in CH_2Cl_2) ν_{max} 3080, 3007, 2683, 1694, 1647, 1418, 1323, 1287, 1246, 1209, 1018, 984, 924, 824 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.63 (1H, br s), 7.15 (1H, dt, J = 15.5, 6.5 Hz), 5.94 (1H, dt, J = 15.5, 1.5 Hz), 2.13 (2H, ddd, J = 6.5, 6.5, 1.5 Hz), 0.89−0.73 (1H, m), 0.62−0.43 (2H, m), 0.23−0.03 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 151.6, 120.7, 37.1, 9.1, 4.6. HRMS (DART$^-$) m/z calcd for $C_7H_9O_2$ [M − H] $^-$, 125.0602; found, 125.0604.

(*E*)-8-(*tert*-Butoxy)-8-oxooct-2-enoic Acid (21e). 8-(*tert*-Butyl) 1-ethyl (*E*)-oct-2-enedioate (305.0 mg, 75%) was prepared from *tert*-butyl 6-oxohexanoate according to general procedure E; colorless liquid; R_f 0.46 (9:1 hexanes:EtOAc). IR (neat) ν_{max} 2980, 2933, 2870, 1724, 1655, 1458, 1393, 1368, 1267, 1155, 1144, 1096, 1043, 982, 849 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (1H, dt, J = 15.5, 7.0 Hz), 5.81 (1H, dt, J = 15.5, 1.5 Hz), 4.17 (2H, q, J = 7.0 Hz), 2.27−2.14 (4H, m), 1.67−1.55 (2H, m), 1.54−1.38 (11H, m), 1.28 (3H, d, J = 7.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.0, 166.8, 148.8, 121.7, 80.3, 60.3, 35.4, 32.0, 28.3, 27.6, 24.7, 14.4. HRMS (DART$^+$) m/z calcd for $C_{14}H_{28}NO_4$ [M + NH_4]$^+$, 274.2018; found, 274.2052. The title compound (77.7 mg, 51%) was prepared from the intermediate diester according to general procedure F using LiOH·H_2O (423.0 mg, 10.071 mmol, 15.0 equiv), 36 h; light-yellow oil; R_f 0.47 (2:1 hexanes:EtOAc). IR (thin film in CH_2Cl_2) ν_{max} 2978, 2934, 1728, 1697, 1651, 1422, 1393, 1368, 1310, 1285, 1256, 1225, 1152, 984 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, note: acid proton was not observed due to deuterium exchange) δ 7.06 (1H, dt, J = 15.5, 7.0 Hz), 5.83 (1H, dt, J = 15.5, 1.5 Hz), 2.29−2.18 (4H, m), 1.69−1.56 (2H, m), 1.55−1.46 (2H, m), 1.44 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.8, 151.7, 121.0, 80.4, 35.4, 32.1, 28.3, 27.4, 24.7. HRMS (DART$^-$) m/z calcd for $C_{12}H_{19}O_4$ [M − H]$^-$, 227.1283; found, 227.1281.

(*E*)-*N*-((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,10-*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,10,21-tetramethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo-[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)-amino)propan-2-yl)hept-2-enamide (22). ADEP **22** (40.3 mg, 84%) was prepared from macrocyclic amine **20a** and acid **21a**[44] according to general procedure A; white solid, mp 136−137 °C (CH_2Cl_2); HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 9.26 min); $[\alpha]_D^{23}$ −69.2 (c 0.70, $CHCl_3$); R_f 0.45 (3:1 EtOAc:hexanes). IR (thin film in CH_2Cl_2) ν_{max} 3316, 2959, 2932, 2874, 1728, 1643, 1597, 1520, 1435, 1319, 1269, 1250, 1198, 1169, 1117, 982 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (1H, d, J = 9.5 Hz), 6.99−6.85 (3H, m), 6.76−6.70 (2H, m), 6.65 (1H, tt, J = 9.0, 2.5 Hz), 6.15 (1H, dt, J = 15.5, 1.5 Hz), 5.20 (1H, dd, J = 9.0, 3.0 Hz), 5.12 (1H, qd, J = 6.5, 2.0 Hz), 4.95 (1H, dq, J = 9.5, 6.5 Hz), 4.73−4.65 (3H, m), 4.63 (1H, dd, J = 10.0, 2.0 Hz), 4.47 (1H, d, J = 8.0 Hz), 3.78 (1H, ddd, J = 11.5, 8.5, 5.0 Hz), 3.56−3.44 (2H, m), 3.09 (1H, dd, J = 12.0, 8.5 Hz), 3.00 (1H, dd, J = 13.5, 8.5 Hz), 2.92 (1H, dd, J = 13.5, 5.5 Hz), 2.73−2.65 (1H, br m), 2.61 (1H, ddd, J = 13.5, 13.5, 2.0 Hz), 2.40−2.28 (2H, m), 2.26−2.18 (2H, m), 2.17−2.05 (2H, m), 1.99−1.89 (2H, m), 1.82 (1H, dd, J = 13.0, 11.5, 8.0 Hz), 1.67−1.55 (2H, m), 1.49−1.40 (2H, m), 1.39−1.29 (5H, m), 1.19 (3H, d, J = 6.5 Hz), 1.11−0.98 (5H, m), 0.95 (3H, d, J = 6.5 Hz), 0.89 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 171.4, 171.1, 169.7, 169.6, 166.3, 165.6, 163.0 (dd, J = 248.5, 13.0 Hz), 146.0, 140.4 (t, J = 9.0 Hz), 123.4, 112.6 (dd, J = 19.0, 5.5 Hz), 102.5 (t, J = 25.0 Hz), 69.9, 60.1, 57.2, 56.9, 54.5, 54.4, 53.4, 47.8, 46.6, 40.9, 39.0, 38.0, 36.1, 33.5, 31.9, 30.9, 30.6, 29.7, 28.1, 23.1, 22.4, 22.0, 18.8, 18.1, 14.0, 13.2. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{57}F_2N_6O_8$ [M + H]$^+$, 799.4200; found, 799.4196.

(*E*)-*N*-((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a-*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)-propan-2-yl)hept-2-enamide (23). ADEP **23** (79.2 mg, 75%) was prepared from macrocyclic amine **20b** and acid **21a**[44] according to general procedure A; white solid, mp 144−145 °C (CH_2Cl_2); HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 8.65 min); $[\alpha]_D^{23}$ −71.1 (c 0.64, $CHCl_3$); R_f 0.54 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3296, 3057, 2959, 2934, 2874, 1728, 1645, 1435, 1269, 1252, 1169, 1117, 1015, 989 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amide protons were not observed due to deuterium exchange) δ 6.90−6.79 (3H, m), 6.75 (1H, tt, J = 9.5, 2.5 Hz), 6.23 (1H, dt, J = 15.5, 1.5 Hz), 5.29 (1H, dd, J = 9.0, 3.0 Hz), 5.21 (1H, qd, J = 6.5, 2.0 Hz), 5.02 (1H, q, J = 6.5 Hz), 4.79−4.72 (2H, m), 4.66 (1H, d, J = 2.0 Hz), 4.60−4.53 (1H, br m), 4.44 (1H, d, J = 8.0 Hz), 3.69 (1H, ddd, J = 11.5, 8.0, 5.0 Hz), 3.52−3.42 (2H, m), 3.10 (1H, dd, J = 12.0, 8.5 Hz), 3.05 (1H, dd, J = 13.5, 7.5 Hz), 2.88 (1H, dd, J = 13.5, 6.0 Hz), 2.70−2.56 (2H, m), 2.53−2.40 (2H, m), 2.30−2.17 (2H, m), 2.12 (1H, dd, J = 13.0, 7.0 Hz), 2.09−1.93 (3H, m), 1.88 (1H, ddd, J = 13.0, 11.5, 8.0 Hz), 1.76 (1H, br d, J = 13.0 Hz), 1.68−1.54 (2H, m), 1.53−1.30 (9H, m), 1.23 (3H, d, J = 6.5 Hz), 1.03 (3H, d, J = 6.5 Hz), 0.93 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 173.6, 173.2, 172.4, 171.44, 171.36, 168.3, 167.8, 164.4 (dd, J = 246.5, 13.0 Hz), 147.0, 142.6 (t, J = 9.5 Hz), 124.6, 113.8 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 25.5 Hz), 71.5, 61.9, 58.8, 58.6, 55.9, 55.2, 55.0, 49.0, 47.8, 42.6, 39.7, 38.9, 32.9, 31.9, 31.8, 31.1, 28.9, 26.0, 24.1, 23.4, 22.5, 18.9, 18.3, 14.3, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{55}F_2N_6O_8$ [M + H]$^+$, 785.4044; found, 785.4054.

(*E*)-*N*-((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,10-*S*,14a*S*,20*S*,23a*S*)-2,6,10-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*]-[1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)propan-2-yl)hept-2-enamide (24). ADEP **24** (43.9 mg, 82%) was prepared from macrocyclic amine **20d** and acid **21a**[44] according to general procedure A; white solid, mp 194−195 °C (CH_2Cl_2); HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 8.98 min); $[\alpha]_D^{26}$ −59.4 (c 0.67, $CHCl_3$); R_f 0.42 (EtOAc). IR (thin film in CH_2Cl_2) ν_{max} 3296, 3059, 2959, 2932, 2874, 1738, 1645, 1593, 1506, 1441, 1116, 1016, 984 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (1H, d, J = 9.5 Hz), 7.16 (1H, d, J = 9.5 Hz), 7.00−6.90 (2H, m), 6.75−6.68 (2H, m), 6.64 (1H, tt, J = 9.0, 2.5 Hz), 6.20 (1H, dt, J = 15.5, 1.5 Hz), 5.12 (1H, dd, J = 9.0, 3.0 Hz), 4.95 (1H, dq, J = 9.5, 6.5 Hz), 4.79 (1H, dd, J = 11.5,

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1.5 Hz), 4.76−4.64 (3H, m), 4.54−4.47 (2H, m), 3.76 (1H, ddd, J = 11.5, 8.0, 5.0 Hz), 3.62−3.48 (3H, m), 3.10 (1H, dd, J = 12.0, 8.5 Hz), 2.98 (1H, dd, J = 13.5, 8.0 Hz), 2.91 (1H, dd, J = 13.5, 5.5 Hz), 2.73−2.66 (1H, br m), 2.62 (1H, ddd, J = 13.5, 13.5, 2.5 Hz), 2.51−2.30 (2H, m), 2.27−2.17 (2H, m), 2.16−2.05 (2H, m), 2.03−1.91 (2H, m), 1.82 (1H, ddd, J = 13.0, 11.5, 8.0 Hz), 1.68−1.55 (2H, m), 1.49−1.39 (2H, m), 1.38−1.29 (5H, m), 1.11−0.98 (5H, m), 0.95 (3H, d, J = 6.5 Hz), 0.89 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 171.3, 170.9, 169.9, 169.6, 166.2, 165.1, 163.0 (dd, J = 248.0, 13.0 Hz), 146.0, 140.4 (t, J = 9.5 Hz), 123.4, 112.6 (dd, J = 19.0, 5.5 Hz), 102.4 (t, J = 25.5 Hz), 65.2, 60.0, 57.3, 57.0, 54.5, 54.3, 51.4, 47.8, 46.6, 41.0, 38.7, 38.4, 36.3, 33.5, 31.9, 30.9, 30.6, 29.7, 28.1, 23.2, 22.4, 22.0, 18.6, 18.1, 14.0;. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{55}F_2N_6O_8$ [M + H]$^+$, 785.4043; found, 785.4035.

(*E*)-*N*-((*S*)-3-(3,5-Difluorophenyl)-1-(((2*R*,6*S*,8a*S*,14a*S*,20-*S*,23a*S*)-2,6-dimethyl-5,8,14,19,23-pentaoxoicosahydro-1*H*,5*H*,14*H*-pyrido[1,2-*d*]dipyrrolo[1,2-*a*:1′,2′-*j*][1,4,7,10,13]-pentaazacyclohexadecin-20-yl)amino)-1-oxopropan-2-yl)-hept-2-enamide (25). ADEP **25** (18.1 mg, 66%) was prepared from macrocyclic amine **20f** and acid **21a**[44] according to general procedure A; white solid, mp 144−145 °C (CH_2Cl_2). HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 6.83 min); $[\alpha]_D^{23}$ −165.6 (c 0.46, $CHCl_3$); R_f 0.22 (19:1 EtOAc:MeOH). IR (thin film in CH_2Cl_2) ν_{max} 3291, 2955, 2932, 2874, 1643, 1597, 1435, 1117 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amide protons were not observed due to deuterium exchange) δ 6.88−6.71 (4H, m), 6.02 (1H, dt, J = 15.5, 1.5 Hz), 5.26 (1H, dd, J = 8.5, 3.0 Hz), 4.90 (1H, q, J = 7.0 Hz), 4.84−4.79 (1H, m), 4.55−4.41 (3H, m), 4.34 (1H, d, J = 8.5 Hz), 4.02 (1H, dd, J = 11.5, 8.0 Hz), 3.84 (1H, dd, J = 14.0, 1.0 Hz), 3.73−3.63 (1H, m), 3.53 (1H, ddd, J = 11.5, 7.0, 7.0 Hz), 3.13 (1H, dd, J = 14.0, 5.5 Hz), 3.04−2.91 (2H, m), 2.82 (1H, dd, J = 14.0, 9.5 Hz), 2.69−2.57 (2H, m), 2.53−2.41 (1H, m), 2.40−2.29 (1H, m), 2.22 (2H, dtd, J = 7.0, 7.0, 1.5 Hz), 2.10−1.95 (4H, m), 1.88 (1H, ddd, J = 12.5, 12.5, 8.5 Hz), 1.74 (1H, br d, J = 13.0 Hz), 1.67−1.31 (11H, m), 1.07 (3H, d, J = 6.5 Hz), 0.93 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 175.9, 174.3, 173.1, 172.8, 171.6, 169.2, 168.7, 164.5 (dd, J = 246.5, 13.0 Hz), 146.8, 143.5 (t, J = 9.5 Hz), 124.5, 113.6 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 26.0 Hz), 63.1, 58.87, 58.81, 56.2, 56.0, 54.3, 50.1, 47.8, 44.2, 42.8, 40.3, 38.2, 32.8, 32.0, 31.8, 30.9, 28.8, 25.9, 24.3, 23.4, 22.4, 18.3, 17.5, 14.3. HRMS (ESI$^+$) m/z calcd for $C_{39}H_{54}F_2N_7O_7$ [M + H]$^+$, 770.4047; found, 770.4052.

(2*E*,4*E*)-*N*-((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a-*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)-propan-2-yl)octa-2,4-dienamide (26). ADEP **26** (28.4 mg, 80%) was prepared from macrocyclic amine **20b** and acid **21b**[27] according to general procedure A; white solid, mp 149−150 °C (CH_2Cl_2). HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 8.92 min); $[\alpha]_D^{23}$ −56.8 (c 0.41, $CHCl_3$); R_f 0.58 (95:5 EtOAc:MeOH). IR (solid) ν_{max} 3292, 2935, 1731, 1628, 1596, 1434, 838 cm^{-1}. ^{1}H NMR (500 M Hz, CD_3OD, note: two of the amide protons were not observed due to deuterium exchange) δ 8.65 (1H, d, J = 9.5 Hz), 7.20 (1H, dd, J = 15.0, 10.5 Hz), 6.85−6.79 (2H, m), 6.75 (1H, tt, J = 9.5, 2.5 Hz), 6.30−6.19 (2H, m), 6.15 (1H, dt, J = 15.0, 7.0 Hz), 5.29 (1H, dd, J = 8.5, 2.5 Hz), 5.21 (1H, qd, J = 6.5, 1.5 Hz), 5.06−4.98 (1H, m), 4.80−4.73 (2H, m), 4.66 (1H, d, J = 1.5 Hz), 4.61−4.53 (1H, br m), 4.43 (1H, d, J = 8.5 Hz), 3.74−3.64 (1H, m), 3.54−3.41 (2H, m), 3.14−3.00 (2H, m), 2.89 (1H, dd, J = 13.5, 6.0 Hz), 2.70−2.56 (2H, m), 2.53−2.39 (2H, m), 2.17 (2H, dt, J = 7.0, 7.0 Hz), 2.11 (1H, dd, J = 13.0, 7.0 Hz), 2.08−1.93 (3H, m), 1.87 (1H, ddd, J = 13.0, 11.5, 8.5 Hz), 1.76 (1H, br d, J = 13.0 Hz), 1.69−1.53 (2H, m), 1.52−1.32 (6H, m), 1.23 (3H, d, J = 6.5 Hz), 1.01 (3H, d, J = 6.5 Hz), 0.94 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 173.6, 173.2, 172.4, 171.45, 171.37, 168.8, 167.8, 164.4 (dd, J = 246.5, 13.0 Hz), 144.7, 143.3, 142.6 (t, J = 9.5 Hz), 130.1, 122.8, 113.8 (dd, J = 19.5, 6.0 Hz), 103.0 (t, J = 25.5 Hz), 71.6, 62.0, 58.8, 58.7, 55.8, 55.3, 55.0, 49.1, 47.8, 42.6, 39.7, 38.9, 36.2, 31.9, 31.1, 28.9, 26.0, 24.1, 23.3, 22.5, 18.8, 18.3, 14.1, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{55}F_2N_6O_8$ [M + H]$^+$, 797.4044; found, 797.4047.

(*E*)-4-Cyclohexyl-*N*-((*S*)-3-(3,5-difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]-dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]-tetraazacyclohexadecin-20-yl)amino)propan-2-yl)but-2-enamide (27). ADEP **27** (32.8 mg, 81%) was prepared from macrocyclic amine **20b** and acid **21c** according to general procedure A; white solid, mp 155−157 °C (CH_2Cl_2). HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 9.98 min); $[\alpha]_D^{23}$ −54.8 (c 0.42, $CHCl_3$); R_f 0.61 (19:1 EtOAc:MeOH). IR (solid) ν_{max} 3291, 2926, 1730, 1627, 1432, 836 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amide protons were not observed due to deuterium exchange) δ 6.88−6.78 (3H, m), 6.75 (1H, tt, J = 9.0, 2.5 Hz), 6.21 (1H, dt, J = 15.5, 1.5 Hz), 5.29 (1H, dd, J = 8.5, 3.0 Hz), 5.21 (1H, qd, J = 6.5, 2.0 Hz), 5.01 (1H, q, J = 6.5 Hz), 4.80−4.72 (2H, m), 4.66 (1H, d, J = 2.0 Hz), 4.61−4.54 (1H, m), 4.43 (1H, d, J = 8.5 Hz), 3.69 (1H, ddd, J = 12.0, 8.0, 5.0 Hz), 3.53−3.41 (2H, m), 3.10 (1H, dd, J = 12.0, 8.5 Hz), 3.05 (1H, dd, J = 13.5, 7.5 Hz), 2.88 (1H, dd, J = 13.5, 6.0 Hz), 2.69−2.56 (2H, m), 2.54−2.40 (2H, m), 2.23−1.94 (6H, m), 1.88 (1H, ddd, J = 13.0, 11.4, 8.5 Hz), 1.81−1.12 (20H, m), 1.08−0.88 (5H, m). ^{13}C NMR (125 MHz, CD_3OD) δ 173.6, 173.2, 172.5, 171.45, 171.37, 168.3, 167.8, 164.4 (dd, J = 246.5, 13.0 Hz), 145.7, 142.6 (t, J = 9.5 Hz), 125.5, 113.8 (dd, J = 19.5, 6.0 Hz), 103.0 (t, J = 25.5 Hz), 71.5, 61.9, 58.8, 58.7, 55.8, 55.2, 54.9, 49.2, 47.8, 42.6, 41.2, 39.7, 38.9 (2C), 34.5, 34.3, 31.9, 31.2, 28.9, 27.6, 27.53, 27.49, 26.0, 24.1, 22.5, 18.9, 18.3, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{43}H_{59}F_2N_6O_8$ [M + H]$^+$, 825.4357; found, 825.4364.

(*E*)-4-Cyclopropyl-*N*-((*S*)-3-(3,5-difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]-dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]-tetraazacyclohexadecin-20-yl)amino)propan-2-yl)but-2-enamide (28). ADEP **28** (26.5 mg, 71%) was prepared from macrocyclic amine **20b** and acid **21d** according to general procedure A; white solid, mp 240−241 °C (CH_2Cl_2). HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 8.03 min); $[\alpha]_D^{23}$ −73.6 (c 0.25, $CHCl_3$); R_f 0.46 (19:1 EtOAc:MeOH). IR (solid) ν_{max} 3291, 2936, 1729, 1627, 1596, 1435, 835 cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD, note: amide protons were not observed due to deuterium exchange) δ 6.92 (1H, dt, J = 15.5, 6.5 Hz), 6.85−6.79 (2H, m), 6.75 (1H, tt, J = 9.5, 2.5 Hz), 6.33 (1H, dt, J = 15.5, 1.5 Hz), 5.29 (1H, dd, J = 9.0, 3.0 Hz), 5.21 (1H, qd, J = 6.5, 2.0 Hz), 5.01 (1H, q, J = 6.5 Hz), 4.80−4.73 (2H, m), 4.67 (1H, d, J = 2.0), 4.61−4.53 (1H, br m), 4.44 (1H, d, J = 8.5 Hz), 3.69 (1H, ddd, J = 12.0, 8.0, 5.0 Hz), 3.53−3.44 (2H, m), 3.12 (1H, dd, J = 12.0, 8.5 Hz), 3.06 (1H, dd, J = 13.5, 7.5 Hz), 2.89 (1H, dd, J = 13.5, 6.0 Hz), 2.68−2.57 (2H, m), 2.52−2.41 (2H, m), 2.20−2.09 (3H, m), 2.08−1.94 (3H, m), 1.88 (1H, ddd, J = 13.0, 11.5, 8.5 Hz), 1.80−1.72 (1H, br m), 1.68−1.54 (2H, m), 1.53−1.43 (1H, m), 1.42−1.30 (4H, m), 1.23 (3H, d, J = 6.5 Hz), 1.03 (3H, d, J = 6.5 Hz), 0.88−0.78 (1H, m), 0.55−0.47 (2H, m), 0.18−0.10 (2H, m). ^{13}C NMR (125 MHz, CD_3OD) δ 173.6, 173.2, 172.5, 171.4, 171.3, 168.4, 167.8, 164.4 (dd, J = 246.5, 13.0 Hz), 146.1, 142.6 (t, J = 9.5 Hz), 124.5, 113.8 (dd, J = 19.5, 5.5 Hz), 103.0 (t, J = 25.5 Hz), 71.6, 61.9, 58.8, 58.7, 55.9, 55.2, 55.0, 49.1, 47.8, 42.6, 39.7, 38.9, 37.8, 31.9, 31.1, 28.9, 26.0, 24.1, 22.5, 18.9, 18.3, 13.5, 10.4, 5.1, 5.0. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{53}F_2N_6O_8$ [M + H]$^+$, 783.3887; found, 783.3893.

***tert*-Butyl (*E*)-8-(((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]-dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]-tetraazacyclohexadecin-20-yl)amino)propan-2-yl)amino)-8-oxooct-6-enoate (29).** ADEP **29** (32.8 mg, 85%) was prepared from macrocyclic amine **20b** and acid **21e** according to general procedure A; white solid, decomposition temp 176−177 °C; $[\alpha]_D^{23}$ −19.3 (c 0.83, MeOH); R_f 0.60 (19:1 EtOAc:MeOH). IR (solid) ν_{max} 3292, 2935, 2874, 1727, 1629, 1435 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD, note: two of the amide protons are not observed due to deuterium exchange) δ 8.64 (1H, d, J = 9.5 Hz), 6.91−6.79 (3H, m), 6.74 (1H, tt, J = 9.0, 2.5 Hz), 6.24 (1H, dt, J = 15.5, 1.5 Hz), 5.29 (1H, dd, J = 9.0, 3.0 Hz), 5.21 (1H, qd, J = 6.5, 1.5 Hz), 5.01 (1H, dq, J = 9.5, 6.5 Hz), 4.80−4.71 (2H, m), 4.66 (1H, d, J = 1.5 Hz), 4.56 (1H, br d, 1H, J =

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13.0 Hz), 4.43 (1H, d, J = 8.0 Hz), 3.75−3.63 (1H, m), 3.55−3.41 (2H, m), 3.10 (1H, dd, J = 12.0, 8.5 Hz), 3.06 (1H, dd, J = 13.5, 7.5 Hz), 2.88 (1H, dd, J = 13.5, 6.0 Hz), 2.70−2.56 (2H, m), 2.55−2.39 (2H, m), 2.32−2.18 (4H, m), 2.12 (1H, dd, J = 13.0, 7.0 Hz), 2.08−1.94 (3H, m), 1.87 (1H, ddd, J = 13.0, 11.5, 8.0 Hz), 1.76 (1H, br d, J = 13.0 Hz), 1.69−1.30 (20H, m), 1.23 (3H, d, J = 6.5 Hz), 1.03 (3H, d, J = 6.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ 174.8, 173.6, 173.2, 172.4, 171.5, 171.3, 168.3, 167.8, 164.4 (dd, J = 246.5, 13.0 Hz), 146.5, 142.6 (t, J = 9.5 Hz), 124.8, 113.8 (dd, J = 18.5, 6.5 Hz), 103.0 (t, J = 26.0 Hz), 81.5, 71.5, 61.9, 58.8, 58.7, 55.8, 55.2, 55.0, 49.2, 47.8, 42.6, 39.7, 38.9, 36.3, 32.8, 31.9, 31.1, 28.93, 28.88, 28.5, 26.0, 25.8, 24.1, 22.4, 18.9, 18.3, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{45}H_{63}F_2N_6O_{10}$ [M + H]$^+$, 885.4568; found, 885.4557.

(*E*)-8-(((*S*)-3-(3,5-Difluorophenyl)-1-oxo-1-(((2*R*,6*S*,8a-*S*,14a*S*,20*S*,21*S*,23a*S*)-2,6,21-trimethyl-5,8,14,19,23-pentaoxooctadecahydro-1*H*,5*H*,14*H*,19*H*-pyrido[2,1-*i*]dipyrrolo[2,1-*c*:2′,1′-*l*][1]oxa[4,7,10,13]tetraazacyclohexadecin-20-yl)amino)-propan-2-yl)amino)-8-oxooct-6-enoic Acid (30). To an ice bath-cooled solution of ADEP **29** (26.2 mg, 0.030 mmol, 1.0 equiv) in CH_2Cl_2 (3.0 mL) was added dropwise TFA (100.0 μL, 1.306 mmol, 44.1 equiv). The reaction was stirred overnight with slow warming to room temperature. After complete consumption of the starting material by TLC (12 h), the reaction mixture was then concentrated in vacuo to yield the crude product. Residual TFA was removed by evaporation from $CHCl_3$ in vacuo (performed 3 times) and drying on a high vacuum pump overnight giving 24.5 mg of the title compound in quantitative yield as a white solid, mp 157−158 °C ($CHCl_3$). HPLC (eluent, acetonitrile/water 20:80 to 95:5 for 15 min, t_R 6.04 min); $[\alpha]_D^{23}$ −51.9 (c 0.29, $CHCl_3$). IR (solid) ν_{max} 3277, 2937, 2876, 1729, 1627, 1596, 1444, 1168, 1115, 1014, 984, 845 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, note: amide protons were not observed due to deuterium exchange) δ 6.91−6.78 (3H, m), 6.75 (1H, tt, J = 9.0, 2.5 Hz), 6.25 (1H, dt, J = 15.5, 1.5 Hz), 5.29 (1H, dd, J = 8.5, 2.5 Hz), 5.21 (1H, qd, J = 6.5, 1.5 Hz), 5.01 (1H, q, J = 6.5 Hz), 4.79−4.70 (2H, m), 4.66 (1H, d, J = 1.5 Hz), 4.61−4.57 (1H, br m), 4.43 (1H, d, J = 8.5 Hz), 3.75−3.63 (1H, m), 3.55−3.41 (2H, m), 3.10 (1H, dd, J = 12.0, 8.5 Hz), 3.05 (1H, dd, J = 13.5, 7.5 Hz), 2.88 (1H, dd, J = 13.5, 6.0 Hz), 2.70−2.56 (2H, m), 2.54−2.40 (2H, m), 2.36−2.22 (4H, m), 2.11 (1H, dd, J = 13.0, 7.0 Hz), 2.09−1.93 (3H, m), 1.88 (1H, ddd, J = 13.0, 11.5, 8.5 Hz), 1.76 (1H, br d, J = 13.0 Hz), 1.71−1.31 (11H, m), 1.23 (3H, d, J = 6.5 Hz), 1.03 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 177.8, 173.6, 173.2, 172.4, 171.4, 171.3, 168.3, 167.8, 164.4 (dd, J = 246.5, 13.0 Hz), 146.5, 142.6 (t, J = 9.5 Hz), 124.8, 113.8 (dd, J = 19.0, 5.5 Hz), 103.0 (t, J = 25.5 Hz), 71.5, 61.9, 58.8, 58.7, 55.8, 55.2, 55.0, 49.2, 47.8, 42.6, 39.7, 38.9, 35.2, 32.9, 31.9, 31.2, 29.1, 28.9, 26.0, 25.9, 24.1, 22.5, 18.9, 18.3, 13.5. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{55}F_2N_6O_{10}$ [M + H]$^+$, 829.3942; found, 829.3940.

Antibacterial Activity Evaluation. Strains used in this study are outlined in Supporting Information, Table S1. In general, MIC values for numbered ADEP compounds were determined by standard protocols for broth-microdilution,[52] and the optical density of each inoculum was measured at λ = 600 nm for all strains, except for *N. meningitidis* and *N. gonorrheae*, which were measured at λ = 550 nm. *S. aureus* ATCC 29213 cells were grown on LB agar (Invitrogen), and *Neisseria* spp. cells were grown on GC-agar (BD Biosciences) supplemented with 1% IsoVitaleX (BD Biosciences) for 16−18 h at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were harvested and diluted in brain heart infusion broth (BHI, BD Biosciences) supplemented with 1% IsoVitaleX. For testing *S. aureus* ATCC 29213 and *Neisseria* spp., the following protocol was used: After growing overnight, optical density was measured, and the inoculum was adjusted to ∼10^6 cfu/mL. DMSO stock solutions were made for ADEPs 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 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 0.0039 μg/mL being the lowest, was prepared for each compound. The analogous serial dilutions were also done for the antibiotics vancomycin (BioShop) and chloramphenicol (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 with the following concentrations of compounds: 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156, 0.0078, 0.0039, and 0.0019 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 without bacteria, were set in the plate as well. A total of three (or more) 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.

S. pneumoniae were grown in THY (Todd Hewitt broth mixed with 0.5% yeast extract) supplemented with catalase (10^3 U/mL) with at 37 °C in a moist atmosphere containing 5% CO_2. *S. aureus* strains 1784 and Newman, as well as *E. faecalis*, *L. innocua*, and *P. aeruginosa*, were grown in TSB (tryptic soy broth) at 37 °C. *E. coli* and *B. subtilis* were grown in LB (Luria-Burtani broth) at 37 °C. Cultures were inoculated the afternoon or evening prior to MIC determination. On the day of determination, innocula were diluted to OD_{600} of 0.001 (∼10^6 cells) in the same media, as described above.

ADEPs were dissolved in DMSO to 50 mM. From these stocks, working dilutions were prepared by (2-fold) serial dilution into DMSO ranging from 12.8 mg/mL down to 0.19 mg/mL. To flat-bottom 96-well microtiter plates (Corning), 1.5 μL of compound was added, along with 150 μL of inoculum. Rows A and H and columns 1 and 12 were not used for testing due to variability and evaporation; to these wells media alone was added. Plates were covered with plastic lids, placed in an orbital shaker and incubated at 37 °C (∼200 rpm) and protected from light. The exception to this was D39, which was placed in an incubator with a moist atmosphere and containing 5% CO_2 at 37 °C without shaking. DMSO alone (1.5 μL) and chloramphenicol, at 100 μg/mL (Cm100), were used as positive and negative controls, respectively. MICs were determined after 16 h; plates were shaken (particularly D39) prior to collecting OD_{600} values. Plates were also examined by eye to corroborate MIC, as defined above. Values shown correspond to a minimum of two agreements for MIC out of at least three independent experiments.

A54556 Factor D (4)−*Nm*ClpP Crystal Structure Determination and Refinement. For crystallization experiments, a solution of 10 mg/mL *Nm*ClpP in 50 mM Tris·HCl, pH 7.5, 200 mM KCl, 1 mM DTT, 10% glycerol, and 1 mM A54556 factor D (**4**) was incubated at rt for 1 h. Then, 1 μL of this solution was mixed with 1 μL of precipitant solution (200 mM ammonium acetate, 40% MPD) and equilibrated against the precipitant in a hanging drop setup (Hampton Research Corp., Aliso Viejo, CA). Screening and optimization of crystal growth were done in an incubator at 20.0 ± 0.5 °C. Optimization resulted in cube-shaped crystals ca. 100 × 100 × 100 μm^3 in size that grew over the course of 2 weeks. For data collection, a crystal was flash frozen directly in boiling nitrogen and diffraction data were collected at the Advanced Photon Source, IMCA-CAT beamline 17-ID-B with a Pilatus 6 M pixel array detector (Dectris Ltd., Baden, CH) by X-CHIP Technologies, Toronto, ON. Reflections were indexed and integrated using IMCA-CAT's autoPROC program running the CCP4 software package.[79] Molecular replacement employing the EMPR program[80] and using apo-*Nm*ClpP (in turn, solved using heptamers of *Ec*ClpP, PDB code 1YG6) as template structure yielded a solution with 28 copies of the complex in the asymmetric crystallographic unit, consistent with the Matthews coefficient.[81] The initial electron density map guided the location of 28 molecules of factor D (**4**). Iterative manual rebuilding, density modification, water molecule selection, and refinement were performed in CNS,[82] PHENIX,[83] REFMAC5.5,[84] and Coot.[85] The last rounds of refinement used the torsion angle NCS constraints option in PHENIX for residues 22 to 196 to avoid overfitting. All

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computations were performed using SBGrid-compiled software.[86] Data processing and refinement statistics are provided in Table 3. The coordinates and structure factors have been deposited in the Protein Data Bank[87] and assigned the identifier 5DKP.

Table 3. Crystallographic Data Collection and Refinement Statistics[a]

Data Collection and Processing	
wavelength (Å)	1.000
resolution range (Å)	20−2.4 (2.5−2.4)
space group	$P2_1$
unit cell parameters	
a, b, c (Å)	117.6, 198.9, 144.0
α, β, γ (deg)	90.0, 97.8, 90.0
total reflections	1,840,313 (372,431)
unique reflections	259,622 (53,296)
multiplicity	7.1 (7.0)
completeness (%)	99.4 (99.3)
mean $I/\sigma(I)$	10.8 (3,6)
R_{merge}	17.3 (57.5)
Refinement Statistics	
R_{work} (%)	19.6
R_{free} (%)	23.9
no. of atoms	43,749
protein	41,408
ligands	1,487 (1,456 ADEP; 28 K^+, 3 Na^+)
water	854
RMS bond lengths (Å)	0.009
RMS bond angles (deg)	1.30
Ramachandran	
favored (%)	96.8
outliers (%)	0.46
average B-factor (Å^2)	26.6

[a]Statistics for the highest-resolution shell are shown in parentheses.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01451.

Copies of ^{1}H and ^{13}C NMR spectra for all synthesized compounds, analytical HPLC traces of all biologically tested ADEP analogues, supplementary structural figures, sequence alignments of ClpPs from various organisms showing key interactions with ADEP, and a list of bacterial strains used in this study (PDF)

Molecular formula strings(CSV)

Accession Codes

The coordinates and structure factors for the complex of A54556 factor D (**4**) bound to *N. meningitidis* ClpP have been deposited in the Protein Data Bank with PDB code 5DKP.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AAA+, ATPases associated with various cellular activities; ADEP, cyclic acyldepsipeptide; ATCC, American Type Culture Collection; ATPase, adenosine triphosphatase; Clp, caseinolytic protease; Dap, (*S*)-2,3-diaminopropanoic acid; DTT, DL-dithiothreitol; Phe(3,5-F_2), 3,5-difluorophenylalanine; EDC·HCl, *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride; MeAla, *N*-methyl-L-alanine; 4-Me-Pip, (2*S*,4*S*)-4-methylpiperidine-2-carboxylic acid; MPD, (±)-2-methyl-2,4-pentanediol; NCS, noncrystallographic symmetry; Pac, phenacyl; Pip, L-pipecolic acid; PRSP, penicillin-resistant *Streptococcus pneumoniae*; PyAOP, (7-azabenzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; sp., species (singular); spp., species (multiple); SPPS, solid-phase peptide synthesis; Tic, (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride; U, unit

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