Restricted Conformation Analogues of an Anthelmintic Cyclodepsipeptide

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Six analogues of the anthelmintic cyclodepsipeptide PF1022A were prepared, each containing a small ring fused to the macrocycle to restrict the number of conformations the larger ring can adopt. It was anticipated that such conformational changes could lead to enhanced biological activity and selectivity. The analogues form two series of three members each. In one series, a carbon-based molecular bridge joins the methyl of a leucine residue with the methyl of its closest lactic acid residue to form five-, six-, and seven-membered lactam rings. In the second series, a leucine residue is replaced with five-, six-, and seven-membered nitrogen heterocycles. Decreasing the size of the small ring in the lactam series increasingly distorts the macrocycle and consistently decreases activity relative to PF1022A. In the leucine series, a similar trend is observed. Molecular modeling of PF1022A along with the analogues described herein suggests that the ability to exist in a highly symmetrical conformational state is a necessary condition for biological activity.

Introduction

Helminths, especially parasitic nematodes, cause substantial health problems in humans and domestic animals. Three broad classes of drugs are currently used to treat gastrointestinal nematodes in veterinary medicine: benzimidazoles, imidazothiazoles, and macrolactones.[1a] None of these drugs is ideally suited for all therapeutic situations, and certain nematode strains have developed resistance to them. The potent antiparasitic activity of cyclodepsipeptide (CDP) PF1022A **1** (Figure 1) was discovered by Japanese scientists.[1b] Because **1** is unique both structurally and in its mode of action, it represents a promising new class of anthelmintics. Following our total synthesis of **1**,[2] we began investigating the effects on biological activity resulting from restricting the number of conformations that **1** can adopt. It is well-established that geometry-constraining effects, such as those produced by macrocyclic rings, are nature's way of rendering peptides biologically active.[3] Additional constraints offer the possibility of increasing both potency and selectivity. It was anticipated that molecular modeling techniques applied to constrained analogues of **1** might reveal information about the conformational requirements of the receptor binding site, which are presently unknown. With these objectives in mind, we set about constructing a series of conformationally restricted analogues of **1** at two distinct molecular sites: (1) between the *N*-methyl group of a leucine residue and the methyl group of its adjacent lactic acid residue where the construction of carbon-based, molecular bridges led to CDPs **2**−**4**; (2) at a leucine residue where replacing it with nitrogen heterocycles produced CDPs **5**−**7**.

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Chemistry

Compound **1** consists of eight residues in a floppy, 24-membered ring: four *N*-methyl-L-leucines (MLeu), two D-lactic acids (Lac), and two 3-phenyl-D-lactic acids (PLac). Constructing a carbon-based bridge between the *N*-methyl group of a leucine residue and the methyl group of the adjacent lactic acid residue had the effect of fusing a lactam ring to the macrocycle, thereby reducing the number of conformations the macrocycle can adopt. Five-, six-, and seven-membered lactams (hereafter designated γ, δ, and ϵ) were prepared and elaborated as analogues of **1** (CDPs **2**−**4**).

γ-Lactam CDP 2. A five-membered lactam (γ-lactam) containing two chiral centers was prepared in four steps from D-malic acid **8** and L-leucine benzyl ester **13** (Scheme 1). Stirring D-malic acid with 2,2-dimethoxypropane, which served as both reactant and solvent, along with a catalytic amount of pyridinium *p*-toluenesufonate (PPTS) at room temperature for 2 days produced dioxolanone acid **10** in 97% yield. The initial two-phase reaction mixture slowly gave way to a homogeneous solution and occasionally produced a small amount of the related methyl ester **9**, which could be removed by recrystallization or chromatography. Often the crude material was used and any ester present removed by chromatography following subsequent reactions. Aldehyde **12**, required for conversion to γ-lactam **15**, was most easily obtained by first reducing acid **10** to alcohol **11** with borane·THF (∼100% yield) and then oxidizing the alcohol to aldehyde **12** with PCC (73% yield). Secondary amine **14** resulting from the reductive amination of aldehyde **12** with L-leucine benzyl ester **13** and sodium cyanoborohydride in MeOH was not isolable but immediately underwent an intramolecular displacement of acetone from the dioxolanone ring to give γ-lactam benzyl ester **15** in 53% yield.

This was elaborated to γ-lactam CDP **2** using conventional methods of peptide synthesis (Scheme 2). *N*-Boc-*N*-methyl-L-leucine **16** was coupled with γ-lactam

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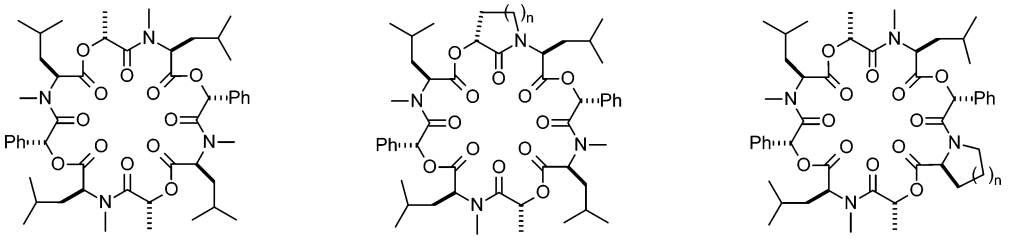


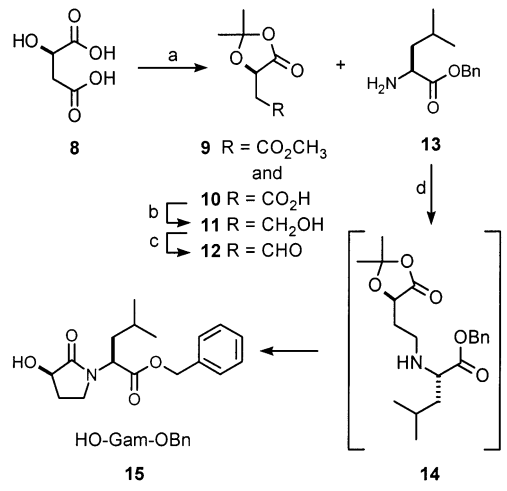
1 (PF1022A)

2 n = 1
3 n = 2
4 n = 3

5 n = 1
6 n = 2
7 n = 3

Figure 1.

Scheme 1[a]



8 **9** R = CO_2CH_3 and **10** R = CO_2H **11** R = CH_2OH **12** R = CHO **13** **14** **15** HO-Gam-OBn

[a] (a) $(CH_3)_2C(OCH_3)_2$, PPTS, 25 °C, 53 h; (b) BH_3·THF, THF, 25 °C; (c) PCC, CH_2Cl_2, 25 °C; (d) HOAc, $NaBH_3CN$, MeOH, 25 °C.

benzyl ester **15** (86%) and the benzyl group was removed catalytically (98%) to give γ-lactam free acid **17**. This was coupled with benzyl 3-phenyl-D-lactate[4] **18** (87%) followed by removal of the benzyl group (95%) to give tetradepsipeptide free acid **19**. Tetradepsipeptide free amine **25** was prepared by coupling didepsipeptide free acid **21** with didepsipeptide free amine **23** (80%) to give tetradepsipeptide **24** followed by removal of the Boc group with TFA (92%). Didepsipeptide free acid **21** was prepared by removing the benzyl group (98%) from didepsipeptide **20**.[2] Didepsipeptide free amine **23** was prepared by removing the Boc group (90%) from didepsipeptide **22**.[2] Coupling acid **19** with amine **25** (55%) followed first by removal of the Boc group (~100%) and then the benzyl group (97%) gave octadepsipeptide amino acid **26**, which underwent macrolactamization with BOP reagent at high dilution (1 mM) to give γ-lactam CDP **2** in 27% yield.

δ-Lactam CDP 3. The first attempt to prepare a six-membered lactam (δ-lactam) by extending the synthetic sequence used to prepare the γ-lactam gave the expected product, **31**, but proved impractical for elaboration to a CDP because of poor yields in critical steps (Scheme 3). However, because both chiral centers were present in the starting materials, this synthetic sequence did allow us to establish unambiguously the stereochemistry of the δ-lactam resulting from a higher yielding, alternative synthesis described later. Aldehyde **12** was homologated by treating it with methoxymethyltriphenylphosphonium chloride and phenyllithium in ether to give vinyl ether **27** as a 5:1 mixture of *E* and *Z* stereoisomers in low yield (27−36%). Hydrolysis of the vinyl ether to aldehyde **29** was problematic, but treatment with undried acetone containing a trace of sulfuric acid at room temperature produced sufficient quantities of the homologous aldehyde. The modest yield (44%) can be partly accounted for by formation in 13% yield of the corresponding dimethylacetal, **28**. Aldehyde **29** was subjected to reductive amination with L-leucine benzyl ester **13** to give a 4:1 mixture of secondary amine **30** and δ-lactam benzyl ester **31** in approximately 33% yield. The secondary amine was unstable with respect to intramolecular cyclization and slowly underwent lactamization over a period of 5 days at room temperature to give a 6:1 mixture of δ-lactam benzyl ester **31** and amine **30**. Heating amine **30** in toluene at 70 °C for 90 min resulted in essentially complete conversion to δ-lactam **31** having the *R*,*S* stereochemistry.

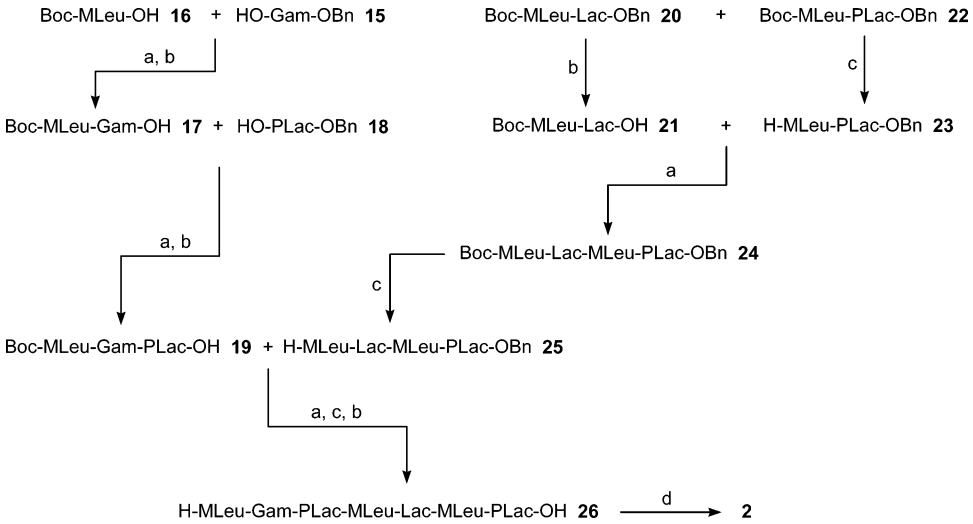
δ-Lactam **39**, the *S*,*S* diastereomer of δ-lactam **31**, was prepared in better overall yield but required adjusting a chiral center late in the synthesis (Scheme 4). Methyl 5-bromovalerate **32** and L-leucine benzyl ester **13** were heated at 130 °C in DMF in the presence of powdered sodium bicarbonate for 70−90 min to give secondary amine **33** in 91% yield.

Unlike the γ-lactam synthesis where spontaneous lactamization occurred between the secondary amine moiety and its attached dioxolanone ring, here the much less reactive ester moiety of secondary amine **33** required strong heating. During 18 h in refluxing xylene, amine **33** underwent lactamization to produce δ-lactam **34** in 66% yield.

Hydroxylation of the α position of δ-lactam **34** was accomplished in two steps. Bromination of **34** gave **35** in yields of 85−94%, which upon being heated at 150 °C for 65 min in formamide containing 1 equiv of water produced the hydroxylated δ-lactam **36** in 87−92% yields as a 1:1 mixture of *R*,*S* and *S*,*S* diastereomers. Prolonged heating and higher temperatures led to a reduction in yield due to O-formylation of the product to give formate **37**. In one such case, the hydroxylated lactam was obtained in only 53% yield, while the formylated lactam was formed in 21% yield.

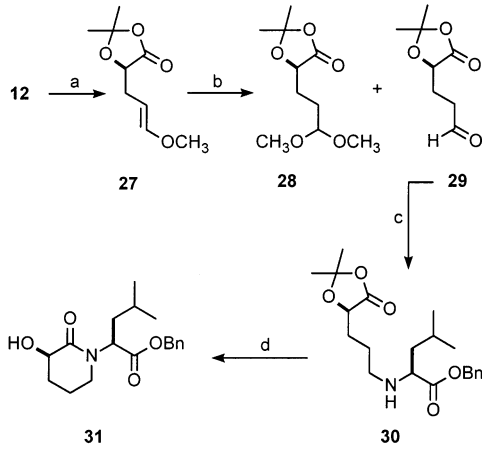
Although either diastereomer was suitable for elaboration, the mixture of both diastereomers was clearly unacceptable. Therefore, δ-lactam **36** was subjected to a Swern oxidation, which gave ketone **38** in 70−75% yields. Reduction with D-glucose and Baker's yeast produced predominantly δ-lactam benzyl ester **39** having the *S*,*S* stereochemistry but was not very consistent: while ≥95% de (50% yield) was obtained on a 3 g scale.

Scheme 2[a]



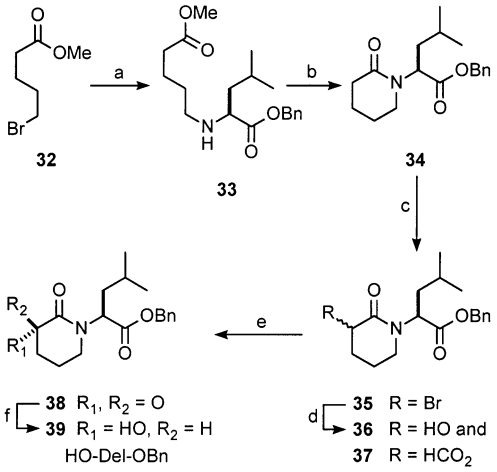
[a] (a) DCC, DMAP, CH_2Cl_2; (b) H_2 (3 atm), 10% Pd/C, EtOH; (c) TFA, CH_2Cl_2, 25 °C; (d) BOP reagent, NMM, CH_2Cl_2, 1 mM, 0−25 °C, 3 days.

Scheme 3[a]



[a] (a) $Ph_3P^+CH_2OCH_3\ Cl^-$, PhLi, 70:30 cyclohexane/Et_2O, −78 °C; (b) H_2SO_4 (trace), acetone, 25 °C; (c) **13**, HOAc, $NaBH_3CN$, MeOH, 25 °C; (d) neat, 25 °C, 5 days or dry toluene, 70 °C, 90 min.

Scheme 4[a]



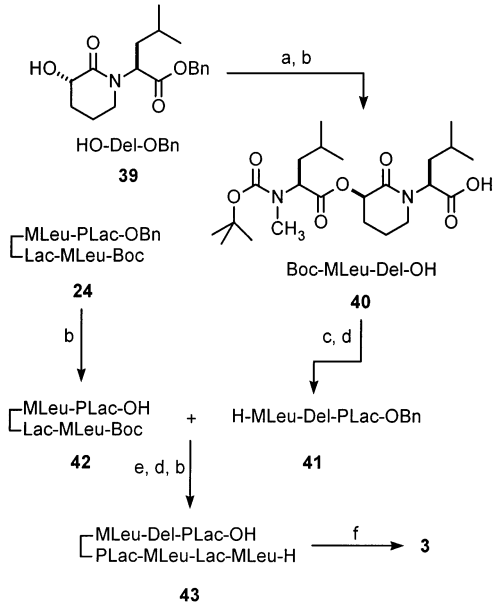
[a] (a) **13**, $NaHCO_3$ (powder), DMF, 130 °C, 90 min; (b) mixed xylenes, reflux, 18 h; (c) $(CH_3)_3SiCl$, I_2, Br_2, Et_3N, CH_2Cl_2, −20 °C; (d) H_2O (1 equiv), $HCONH_2$, 150 °C, 65 min; (e) **36**, oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C; (f) Baker's yeast, D-glucose, H_2O, 25 °C.

An earlier experiment on a 1 g scale gave only 70% de although in 68% yield. (Reduction with K-selectride gave inferior stereoselectivity: 58% de and 63% yield.) Ratios of the two diastereomers were determined by averaging relative peak heights for several absorbances in the ^{13}C NMR spectrum. Comparing the ^1H and ^{13}C NMR spectra of this δ-lactam with those of δ-lactam **31** whose stereochemistry (*R*,*S*) was unambiguously established enabled us to assign the *S*,*S* stereochemistry to δ-lactam **39**.

δ-Lactam benzyl ester **39** (≥95% de) was coupled with *N*-Boc-*N*-methyl-L-leucine **16** using Mitsunobu chemistry (84%) followed by removal of the benzyl group (99%) to give δ-lactam free acid **40** with the required (inverted) stereochemistry at the α position of the lactam ring (Scheme 5). This was coupled with benzyl 3-phenyl-D-lactate **18** (48%), and the Boc group was removed (94%) to give tetradepsipeptide free amine **41**. Removal of the benzyl group from tetradepsipeptide **24** gave tetradepsipeptide free acid **42**. Coupling amine **41** with acid **42** (58%) followed by first removing the Boc group (99%) and then the benzyl group (93%) produced octadepsipeptide amino acid **43**. Macrolactamization of **43** with BOP-Cl at high dilution (1 mM) produced δ-lactam CDP **3** in 65% yield.

ϵ-Lactam CDP 4. The synthetic sequence used successfully in the preparation of the δ-lactam failed when applied to the synthesis of the ϵ-lactam because the corresponding homologous ester was too unreactive to form a seven-membered lactam ring. The dioxolanone sequence proved to be more successful (Scheme 6). The required two-carbon homologation was achieved by treating aldehyde **12** with (triphenylphosphoranylidene)acetaldehyde in a Wittig reaction, which gave unsaturated aldehyde **44** in 38−54% yields. The reaction produced a ratio of *E*/*Z* isomers that varied considerably between batches, ranging from 4:1 to 15:1. In preliminary work, aldehyde **44** was subjected to reductive amination with L-leucine benzyl ester **13**. Instead of the expected secondary amine **47**, this aldehyde gave methoxylamine **45** in 39% yield as a 1:1 mixture of both epimers, the methoxyl group having arisen from the unexpected 1,4-addition of methanol to **44** prior to

Scheme 5[a]



[a] (a) **16**, DEAD, Ph_3P, THF, 0−25 °C; (b) H_2 (3 atm), 10% Pd/C, EtOH; (c) **18**, DCC, DMAP, CH_2Cl_2, 25 °C; (d) TFA, CH_2Cl_2, 25 °C; (e) DCC, DMAP, CH_2Cl_2; (f) BOP-Cl, DPEA, CH_2Cl_2, 1 mM, 0−25 °C, 48 h.

reductive amination. Compound **45** provided an opportunity to test our hypothesis that the dioxolanone ring is more reactive than an ordinary ester. Indeed, when methoxylamine **45** was heated in refluxing xylene, a slow conversion to ϵ-lactam **46** resulted on the basis of HPLC and mass spectral analyses. Heating for 90 h was required to achieve a 90% conversion.

The problem caused by addition of methanol was averted by reducing the double bond of aldehyde **44**. Hydrogenation at balloon pressure (72−90%) gave saturated aldehyde **48** contaminated with acid **49**. The abundance of acid **49** varied from 6% to 26% and may have been due to air oxidation of **44** during handling.[5] Washing with aqueous $NaHCO_3$ was not successful in removing acid **49** probably because of its tendency to chelate the sodium ion, which rendered the carboxylate salt ether-soluble. Chromatography was used to remove the acid from the more seriously contaminated batches. Reductive aminations carried out on saturated aldehyde **48** using L-leucine benzyl ester **13** (65−74%) produced secondary amine **50** having two chiral centers. When heated in refluxing xylene, amine **50** underwent lactamization to give ϵ-lactam benzyl ester **51** in 54−79% yield. The length of time (115−225 h) required to achieve reasonable conversion varied considerably from batch to batch, suggesting catalysis or inhibition by an unknown agent whose abundance varied. The product appears to have excellent thermal stability. NMR and HPLC analyses showed ϵ-lactam **51** to be optically pure; all of the several batches of ϵ-lactam gave a consistent optical rotation of −36° to −37°.

ϵ-Lactam **51** was further elaborated to ϵ-lactam CDP **4** (Scheme 7). *N*-Boc-*N*-methyl-L-leucine **16** was coupled with ϵ-lactam benzyl ester **51** (71%), and the benzyl group was removed (∼100%) to give ϵ-lactam free acid **52**. Coupling this product with benzyl 3-phenyl-D-lactate[4] **18** gave the tetradepsipeptide (42%) along with a lesser amount of diastereomer, which was easily removed by chromatography. Removal of the Boc group (91%) gave tetradepsipeptide free amine **53**. Amine **53** was coupled with tetradepsipeptide **42** (78%) and the Boc group was removed (97%) followed by removal of the benzyl group (94%) to give octadepsipeptide amino acid **54**. Macrolactamization of **54** with BOP-Cl (43%) at high dilution (1 mM) gave ϵ-lactam CDP **4**.

To further investigate the effect on anthelmintic activity of restricting the shape of the macrocycle, we replaced one of the leucine residues of **1** with small, nitrogen-containing heterocycles of five, six, and seven members to give prolyl, pipecolyl, and azepanyl CDP analogues (CDPs **5**−**7**).

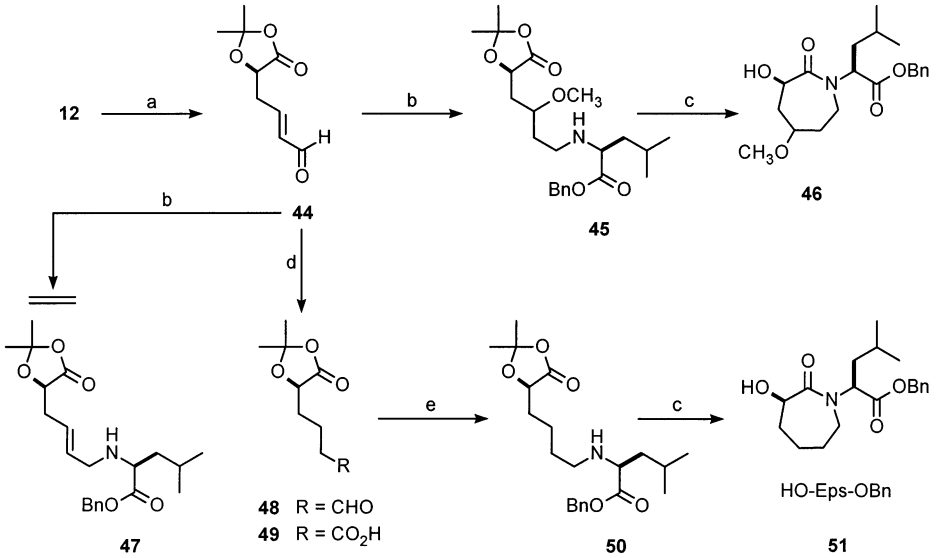
Prolyl CDP 5 (Scheme 8). *N*-Boc-Proline **55** and methyl D-lactate **56** were coupled (74%) and the methyl ester was hydrolyzed (70%) to give didepsipeptide free acid **57**. Tetradepsipeptide free acid **58** was prepared by coupling acid **57** and didepsipeptide free amine **23** (82%) followed by removal of the benzyl group (98%). Coupling acid **58** with tetradepsipeptide free amine **25** (61%) followed by first removing the Boc group (90%) and then the benzyl group (95%) gave octadepsipeptide amino acid **59**. Macrolactamization of **59** with BOP reagent at high dilution (1 mM) gave prolyl CDP **5** in 12% yield.

Pipecolyl CDP 6 (Scheme 9). Hexadepsipeptide free acid **61** was prepared by coupling tetradepsipeptide free acid **60**[2] with didepsipeptide free amine **23** (80%) followed by removal of the benzyl group (81%). *N*-Boc-L-pipecolic acid **62** was coupled with benzyl L-lactate[6] **63** using Mitsunobu chemistry (24%) to invert the stereochemistry of the lactic acid residue. Removal of the Boc group (74%) gave didepsipeptide free amine **64**. Coupling acid **61** and amine **64** (24%) followed first by removing the Boc group (84%) and then the benzyl group (88%) gave octadepsipeptide amino acid **65**. Subsequent macrolactamization of **65** at moderate dilution (4 mM) using 1-methyl-2-chloropyridinium iodide in CH_2Cl_2 gave pipecolyl CDP **6** in 50% yield.

Azepanyl CDP 7. Preparation of azepanyl CDP **7** required the prior synthesis of 1-(*tert*-butoxycarbonyl)-2-azepanecarboxylic acid **71** (Scheme 10). We prepared oxazinone **66** following a literature procedure[7] and converted it in five steps to azepane **71**. Generation of the enolate of oxazinone **66** with sodium hexamethyldisilylamide and treatment with 1-iodo-5-chloropentane furnished the homologated chloride, **67**, in 73% yield. Removal of the Boc group (87%) gave free amine **68**, which was cyclized to give bicyclic oxazine **69** in 45% yield. Catalytic hydrogenation over $PdCl_2$ (96%) gave azepane **70**, which upon treatment with di-*tert*-butyl dicarbonate gave the Boc-protected azepane free acid **71** in 60% yield.

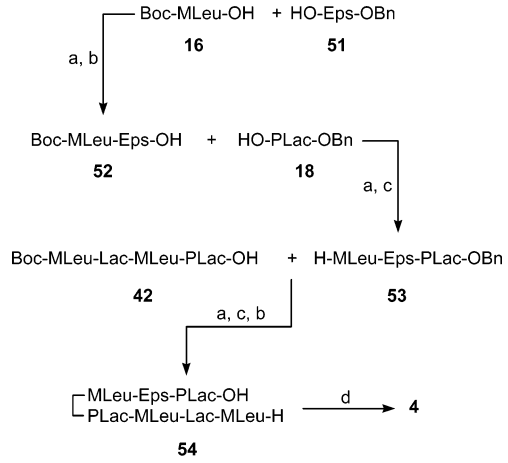
Acid **71** was coupled with benzyl D-lactate **72** (50%), and the Boc group was removed (89%) to give didepsipeptide free amine **73** (Scheme 11). This was coupled with hexadepsipeptide **61** (48%) followed first by removal of the BOC group (90%) and then the benzyl group (90%) to give octadepsipeptide amino acid **74**. This underwent macrolactamization when treated with 1-methyl-2-chloropyridinium iodide at moderate dilution (7 mM) to give azepanyl CDP **7** in 34% yield.

Scheme 6[a]



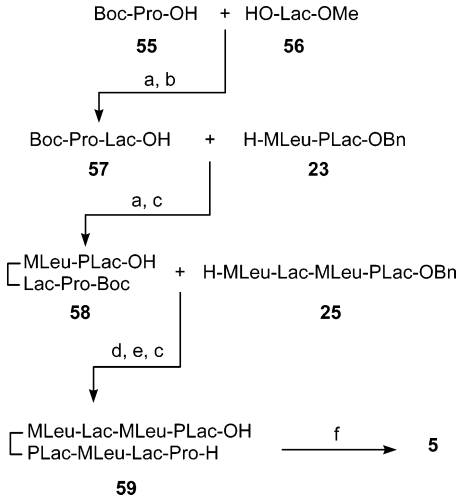
[a] (a) $Ph_3PCHCHO$, toluene, 80 °C, 90 min; (b) **13**, HOAc, $NaBH_3CN$, MeOH, 0−25 °C; (c) mixed xylenes, reflux, 115 h; (d) H_2 (balloon pressure), 5% Pd/C, EtOAc, 3 h; (e) **48**, **13**, HOAc, $NaBH_3CN$, MeOH, 0−25 °C.

Scheme 7[a]



[a] (a) DCC, DMAP, CH_2Cl_2; (b) H_2 (3 atm), 10% Pd/C, EtOH; (c) TFA, CH_2Cl_2, 25 °C; (d) BOP-Cl, DPEA, CH_2Cl_2, 1 mM, 0−25 °C, 22 h.

Scheme 8[a]



[a] (a) DIC, DMAP, CH_2Cl_2; (b) 1 N NaOH, MeOH, 25 °C, 20 min; (c) H_2 (3 atm), 10% Pd/C, EtOH; (d) DCC, DMAP, CH_2Cl_2; (e) TFA, CH_2Cl_2, 25 °C; (f) BOP reagent, NMM, CH_2Cl_2, 1 mM, 25 °C, 16 h.

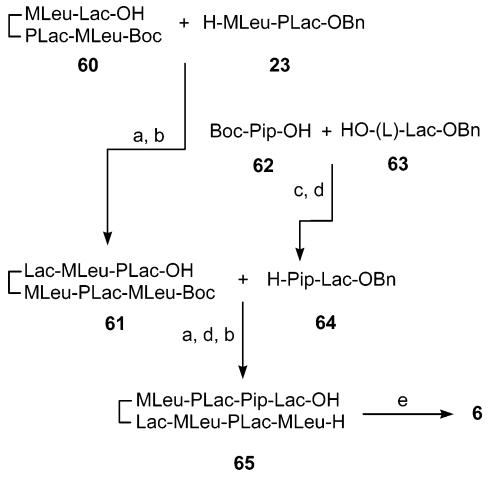
Biology

The relative potency of **1** and the CDPs of this report was determined in an anthelmintic assay utilizing immunosuppressed jirds, *Meriones unguiculatus*, infected with the ruminant parasite, *Haemonchus contortus*, which has demonstrated utility as a model to study anthelmintic efficacy[8] and resistance.[9] The conformational constraints introduced by the small rings in both series of CDPs had a profound effect upon biological activity and the shape of the macrocycle. The five-membered rings in both series exhibited significantly reduced biological activity relative to **1** (Table 1). In the lactam series (CDPs **2**−**4**), increasing the size of the small ring tended to restore the macrocycle toward its original shape and biological activity, suggesting that the effects were indeed of a conformational nature and not merely the result of a change in molecular composition. The situation is more complex in the case of the three compounds (CDPs **5**−**7**) in which a leucine residue was replaced with a nitrogen heterocycle. Of these three CDPs, CDP **6** containing the six-membered pipecolyl ring had by far the best activity, suggesting that **6** is capable of attaining a biologically active conformation unavailable to **5**. The activity of CDP **6** was comparable to that of CDP **3**. The seven-membered azepanyl ring of CDP **7** seemingly constrained the macrocycle the least. However, CDP **7** had essentially no biological activity possibly because of the increased bulk of the azepanyl ring relative to leucine. This is consistent with earlier observations that replacing a leucine residue often has a deleterious effect on anthelmintic activity.[10] Molecular modeling of our CDPs has provided a further explanation.

Molecular Modeling

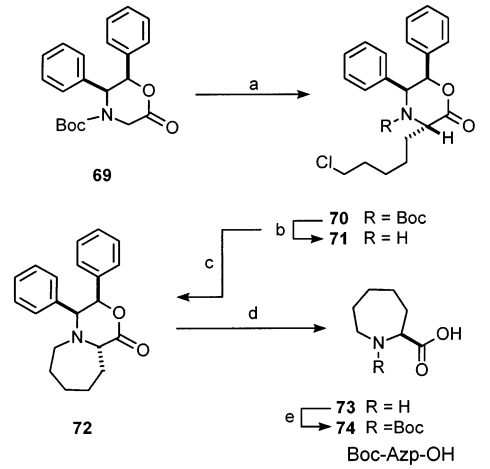
To study the relationship between biological activity and the structure of a compound, conformational analysis and molecular matching were performed. Since no structural information is available for these compounds, except for NMR data on **1**, and because of their extreme

Scheme 9[a]



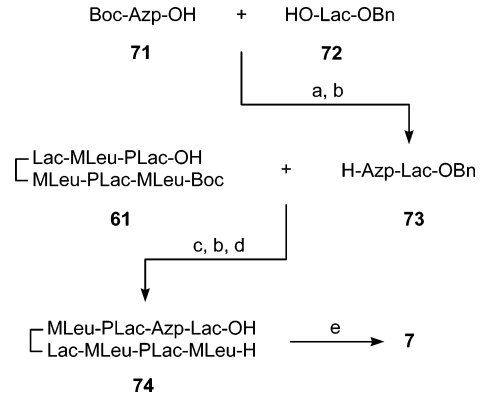
[a] (a) DIC, DMAP, CH_2Cl_2; (b) H_2 (3 atm), 10% Pd/C, EtOH; (c) DEAD, Ph_3P, Et_2O, 25 °C; (d) TFA, CH_2Cl_2, 25 °C; (e) 1-methyl-2-chloropyridinium iodide, Et_3N, CH_2Cl_2, 4 mM, 25 °C, 16 h.

Scheme 10[a]



[a] (a) 1-Iodo-5-chloropentane, $[(CH_3)_3Si]_2NNa$, HMPA, THF, −78 °C; (b) TFA, CH_2Cl, 25 °C, 4 h ; (c) K_2CO_3, CH_3CN, reflux, 24 h; (d) H_2 (50 psi), $PdCl_2$, 1:2 THF/EtOH, 20 h; (e) $[(CH_3)_3CO]_2O$, Et_3N, 7:3 THF/H_2O, 25 °C, 16 h.

Scheme 11[a]



[a] (a) DIC, DMAP, CH_2Cl_2; (b) TFA, CH_2Cl_2, 25 °C; (c) BOP-Cl, Et_3N, CH_2Cl_2, 0−25 °C, 16 h; (d) H_2 (3 atm), 10% Pd/C, EtOH; (e) 1-methyl-2-chloropyridinium iodide, Et_3N, CH_2Cl_2, 7 mM, 25 °C, 16 h.

flexibility, it is very difficult to identify the biologically active conformation. Given these limitations, we attempted to make some qualitative comparisons using

Table 1. Percent Reduction of *Haemonchus contortus* in the Jird by CDPs When Dosed Orally[a]

	reduction of *Haemonchus contortus* (%) at the following CDP dosages				
CDP	6.00 mg/jird	3.00 mg/jird	1.00 mg/jird	0.33 mg/jird	0.11 mg/jird
1					100
2	89[a]	69[b]			
3		99	89	75	36
4		100	100	99	91
5	69				
6		100	96	76	
7			3	19	

[a] Result from dosing at 5.75 mg/jird. [b] Result from dosing at 2.75 mg/jird.

the NMR-based structures of **1** as references. NMR studies[11,12] show that **1** exists as a mixture of conformers in solution: one is *asymmetric*, showing eight magnetically nonequivalent residues; the other is *symmetric*, showing only four magnetically nonequivalent residues. From the rotating-frame Overhauser enhancement spectroscopy (ROSEY) and nuclear Overhauser effect spectroscopy (NOESY) data, it is clear that the *asymmetric* conformer possesses a cis amide bond between the Lac and MLeu residues. Two solution conformers of **1** were obtained by using two sets of NMR NOESY data obtained from methanol, one for the *asymmetric* conformer (38 NOEs) and the other for the *symmetric* conformer (34 NOEs). An ensemble of NMR structures was generated using the method of dynamical simulated annealing[13] from a random array of atoms with the upper bound distance restraints from the NOEs as follows: very strong, <2.2 Å; strong, <2.7 Å; medium, <3.3 Å; weak, <5.0 Å; very weak, <5.5 Å. The calculation produced a set of 25 structures with the lowest restraint violations. For the analogues of **1**, the Monte Carlo multiple minimum (MCMM) technique[14] was applied to search conformational space by random changes in torsion angles within the window of 10 kcal/mol from the global minimum energy conformation. Each structure was minimized with the Amber* potential energy force field,[15] and only the unique structures were kept. The number of conformations generated for each compound with 5000 Monte Carlo steps ranged from 419 for CDP **5** to 688 for CDP **4**, reflecting the flexibility of the compound. In a recent publication,[11] Scherkenbeck, et al. obtained the *symmetric* conformation of **1** by MD simulations in $CDCl_3$ using NMR-derived NOEs as constraints. In this conformation, there are two type II′ β-turns with Lac and MLeu as R_i and R_{i+3}, respectively, causing hydrophobic collapse of the two PLac-MLeu residues and the side chains of two MLeu residues. At least one of their active analogues also resembles the *symmetric* conformation of **1**.

We identified this conformation among the 25 NMR structures for the *symmetric* conformation of **1**. This conformation is shown in Figure 2. Assuming that this might be the active conformation, we searched for a similar conformation among the multiple conformations of the analogues of **1**. Two of the active analogues, CDP **3** and CDP **4**, contain this conformation. The *symmetric* conformation of each of these compounds is overlaid on the *symmetric* conformation of **1** in Figures 3 and 4.

As are shown in the side views of these compounds, there are hydrophobic collapses between the side chains

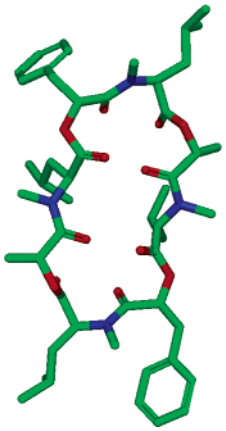


Figure 2. Symmetric conformation of **1** from NMR data.

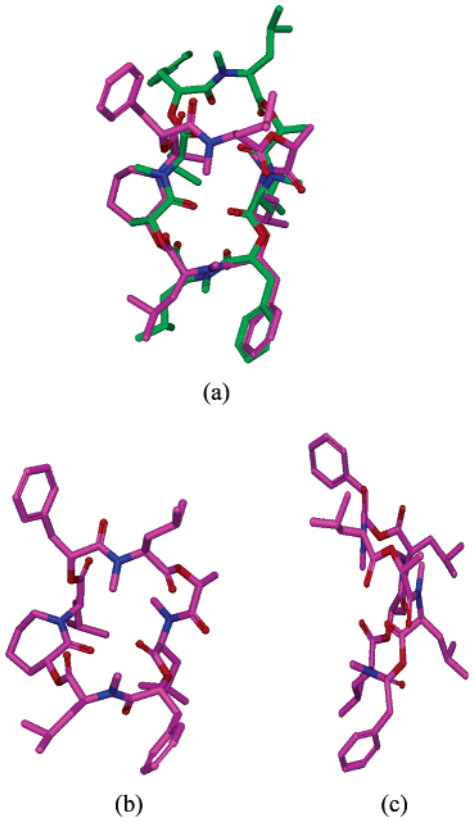


Figure 3. (a) Overlay of CDP4 (pink) on **1** (green). (b) Front view of CDP4. (c) Side view of CDP4.

of MLeu and PLac as well as between the side chains of the MLeu residues. Also seen from the front view, these compounds with their fused rings cause the overall conformation to fold more compared with **1**. In CDP6, the other active analogue, hydrophobic interactions between different pairs of groups were observed, for example, strong interactions between the phenyl ring in the PLac and the six-membered heterocycle and also between the side chains of the PLac and MLeu residues. A similar *symmetric* conformation was not found in the inactive analogues (CDPs **2**, **5**, and **7**), although different types of hydrophobic collapses were observed.

Given the limited experimental data, we cannot conclusively identify the active conformations of **1** and its analogues, but assuming that the *symmetric* conformation of **1** is the biologically active one, then the most salient difference between our active and inactive

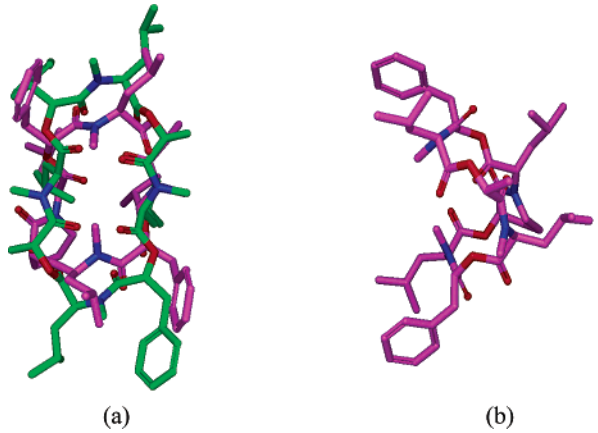


Figure 4. (a) Overlay of CDP3 (pink) on **1** (green). (b) Side view of CDP3.

analogues is their relative ability to achieve a symmetrical conformational state, as our modeling has demonstrated.

Conclusion

We prepared six conformationally constrained analogues of the anthelmintic cyclodepsipeptide **1** by fusing five-, six-, and seven-membered rings to its macrocycle. The lactam CDPs **2**−**4** exhibit a steady progression in relative biological activity as the size of the fused ring increases, thereby reducing macrocyclic strain. Such a progression in the leucine series is suggested by the relative activities of CDPs **5** and **6**. In the absence of molecular modeling, the remaining analogue, **7**, seems to belie this progression. However, modeling indicates that, despite the larger size of its fused ring, CDP **7** was unable to achieve the symmetrical conformational state necessary for biological activity.

Experimental Section

Chemistry. Analytical Methods. Mass spectra from electron impact (EI) ionization were determined with a Finnigan MAT 8230B double-focusing spectrometer. Samples were introduced through the AUDEVAP probe inlet. A MicroMass Platform II mass spectrometer was used to obtain electrospray ionization (ES) data. FAB spectra were obtained via the liquid secondary ion mass spectrometry (LSIMS) ionization technique on a VG70SE double-focusing spectrometer utilizing a cesium ion gun. These samples were analyzed in a matrix of 2-hydroxyethyl disulfide. ^{1}H and ^{13}C NMR spectra were obtained using Bruker 300 and 400 MHz instruments. Proton spectra of many of the intermediates showed the presence of more than one conformer on the NMR time scale. The isopropylmethyl groups of leucine often appeared as two, three, or more sets of doublets because of conformers and are designated (2d, 6H), (3d, 6H), etc. in the Experimental Section of this report. Similar results were sometimes found with the *tert*-butyl groups and *N*-methyl groups when a Boc group was present and are designated (2s, 9H), (3s, 3H), etc. Other protons were less likely to exhibit this effect. IR spectra were recorded using a Digilab model FTS40 spectrometer. Samples were prepared in a mineral oil mull, and the spectra were collected from 4000 to 500 cm^{-1} at a resolution of 2 cm^{-1} through the addition of 16 scans. A Perkin-Elmer Lambda 7 UV/vis spectrophotometer was used to record UV spectra. Optical rotations were obtained in MeOH using a Perkin-Elmer model 241 polarimeter.

Materials. D-Malic acid was purchased from Lancaster Synthesis Inc., and *N*-Boc-*N*-methyl-L-leucine was purchased from Bachem California. *N*-Boc-L-proline, methyl d-lactate, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP Reagent), bis(2-oxo-3-oxazolidinyl)phos-

phinic chloride (BOP-Cl), 1,3-dicyclohexylcarbodiimide (DCC), 1,3-diisopropylcarbodiimide (DIC), diethyl azodicarboxylate (DEAD), *N*,*N*-diisopropylethylamine (DPEA), *N*-methylmorpholine (NMM), 2-chloro-1-methylpyridinium iodide, L-pipecolic acid, methyl 5-bromovalerate, and pyridinium *p*-toluenesulfonate (PPTS) were purchased from Aldrich Chemical Co., Inc. Methylene chloride, mixed xylenes, and DMF were dried over 4 Å molecular sieves. Methanol was dried over 3 Å molecular sieves. THF was dried and purified by distillation from the sodium ketyl of benzophenone.

General Chemical Procedures. 1. General Procedure for Removing a Boc Protecting Group. The substrate was dissolved in CH_2Cl_2 and sufficient TFA was added with stirring to give a 10−20% TFA solution. The resulting reaction mixture was stirred at 25 °C under an atmosphere of dry N_2. Reaction progress was monitored by TLC. The reaction was usually done within 60 min. The reaction mixture was concentrated in vacuo, and the residue was taken up in CH_2Cl_2 and washed twice with saturated $NaHCO_3$ and then with H_2O followed by saturated NaCl. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. Final drying under high vacuum gave the product.

2. General Procedure for Removing a Benzyl Protecting Group. The substrate was dissolved in absolute EtOH. Palladium (10%) on activated carbon was added, and the reaction mixture was hydrogenolyzed in a Parr shaker at 45−50 psi of H_2. The reaction was usually complete within 3 h. The reaction mixture was flushed with N_2 and filtered through Celite. The Celite cake was washed thoroughly with absolute EtOH, and the filtrates were combined and concentrated. Residual EtOH, which could interfere with any subsequent coupling reaction, was removed by twice dissolving the product in EtOAc and concentrating. Drying under high vacuum at 25 °C gave the product. When both Boc and benzyl protecting groups were to be removed, we found it generally advantageous to remove the Boc group first followed by removal of the benzyl group. This avoided the problem associated with retrieving the resulting zwitterionic peptide from an acidic medium.

3. General Procedure for Coupling Peptides Using DCC. The amine or alcohol was dissolved in CH_2Cl_2 along with the carboxylic acid under an atmosphere of dry N_2. The solution was cooled to 0 °C, and DCC was added, usually in the form of a 1 M solution of DCC in CH_2Cl_2. This was followed immediately by the addition of solid DMAP. A precipitate of dicyclohexylurea usually appeared within 2 min. The cooling bath was removed, and the reaction mixture was stirred at 25 °C until the starting alcohol or amine had been mostly consumed as indicated by TLC (usually within 3 h); reactions seldom went to completion. The reaction mixture was filtered to remove the urea and then concentrated. Any urea still present was removed by dissolving the crude product in Et_2O and filtering a second time. The filtrate was concentrated and dried under high vacuum to give the crude product.

4. General Procedure for Coupling Peptides Using DIC. The carboxylic acid and amine were dissolved in CH_2Cl_2, and the solution was cooled to 0 °C under an atmosphere of dry N_2. DIC (neat) was added followed immediately by addition of DMAP, and the mixture was stirred at room temperature. The reaction mixture was filtered to remove the precipitated urea and the filtrate was concentrated to give crude product.

5. Chromatography. Preparative low-pressure chromatography was performed using EM Science silica gel (230−400 mesh) contained in a glass column or in a sintered glass funnel. Products were eluted from the absorbent with varying concentrations of EtOAc in hexane except where otherwise noted. Small amounts of material, e.g., <1 g, were purified by centrifugal elution from silica gel bonded to a glass rotor contained in a Chromatotron.

Synthesis of γ-Lactam Cyclodepsipeptide (2). 2-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]acetic Acid (10). Powdered D-malic acid **8** (13.94 g, 104 mmol) was combined with 2,2-dimethoxypropane (50 mL), PPTS (2.23 g, 8.91 mmol) was added, and the two-phase mixture was stirred at 25 °C under N_2. The malic acid and PPTS slowly formed a gum, which was broken up with a spatula after 5 h. The gum slowly gave way to a clear solution with stirring overnight. The reaction mixture was stirred for 53 h and then concentrated. The residue was taken up in EtOAc and filtered through silica gel to remove the PPTS. The effluent was concentrated to give dioxolanone acid **10** (97%) as an off-white solid, which sometimes contained a small amount of ester **9**. The crude material was often used without further purification. Recrystallization from CH_2Cl_2/hexane gave pure dioxolanone acid **10** (13.80 g, 76%) as a white, crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56 (s, 3H), 1.61 (s, 3H), 2.85 (dd, J = 6.5, 17.3 Hz, 1H), 2.99 (dd, J = 3.9, 17.3 Hz, 1H), 4.71 (dd, J = 3.9, 6.5 Hz, 1H), 10.64 (bs, 1H). MS (EI) *m*/*z*: 159 [M − CH_3]. Anal. ($C_7H_{10}O_5$) C, H. Chromatography (20% EtOAc in hexane) of the crude reaction product occasionally produced a small amount (≤3 wt %) of ester **9**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56 (s, 3 H), 1.61 (s, 3 H), 2.80 (dd, J = 6.6, 17.0 Hz, 1 H), 2.94 (dd, J = 3.9, 17.0 Hz, 1 H), 3.74 (s, 3 H), 4.72 (dd, J = 3.9, 6.6 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.20, 27.13, 36.46, 52.49, 71.02, 111.51, 169.95, 172.35. MS (ES) *m*/*z*: 211 [M + Na].

2-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]ethanol (11). Dioxolanone acid **10** (13.6 g, 78.1 mmol) was dissolved in THF (40 mL), and the mixture was cooled to 0 °C under an atmosphere of N_2. A solution of BH_3 in THF (1 M, 100 mL, 100 mmol) was added dropwise over a period of 47 min. The reaction mixture was stirred for 60 min at 0 °C and then for 2 h at 24 °C. The mixture was cooled to 0 °C, MeOH (50 mL) was added dropwise, and the mixture was stirred for 5 min to destroy the remaining borane. The mixture was concentrated at or below 35 °C, MeOH (100 mL) was added, and the resulting solution was concentrated. A final concentration from EtOAc (100 mL) gave dioxolanone alcohol **11** (12.5 g, ∼100%) as a clear, colorless oil. It was stored under N_2 at 0 °C for several days without deterioration. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 1.61 (s, 3H), 1.97 (m, 1H), 2.14 (m, 1H), 3.82 (m, 2H), 4.56 (dd, J = 4.9, 7.1 Hz, 1H).

2-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]acetaldehyde (12). Dioxolanone alcohol **11** (17.8 g, 111 mmol) was dissolved in CH_2Cl_2 (700 mL) and cooled to 0 °C. Solid pyridinium chlorochromate (120 g, 555 mmol) was added all at once, and the cooling bath was removed. The reaction mixture was stirred at 25 °C for 4 h and then poured into Et_2O (1 L). The residual solid in the reaction flask was triturated several times with Et_2O, bringing the total volume of Et_2O to 2 L. The combined Et_2O solutions were filtered through Celite, giving a clear, dark-orange filtrate. This was treated with activated carbon (Darco G-60), and the mixture was stirred intermittently for 20 min, after which it was filtered through Celite giving a clear, pale-yellow filtrate. This was concentrated at less than 42 °C. The residue was dissolved in EtOAc and similarly concentrated followed by drying under high vacuum to give dioxolanone aldehyde **12** (12.9 g, 73%) as a clear, brown-green oil. ^{1}H NMR spectroscopy showed the product to contain 90 mol % aldehyde and 10 mol % acid, giving an adjusted yield of 65%. This material was stored under N_2 at 0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.57 (s, 3H), 1.62 (s, 3H), 2.91 (dd, J = 7.2, 18.4 Hz, 1H), 3.09 (dd, J = 3.5, 18.3 Hz, 1H), 4.78 (dd, J = 3.5, 7.0 Hz, 1H), 9.74 (s, 1H).

L-Leucine Benzyl Ester (13). L-Leucine (13.1 g, 100 mmol) was combined with dioxane (60 mL) and H_2O (40 mL), and the resulting slurry was cooled to 0 °C. A solution of NaOH (1 N, 200 mL) was added followed by molten di-*tert*-butyl dicarbonate (49.3 g, 226 mmol). Additional dioxane (50 mL) was used to rinse the di-*tert*-butyl dicarbonate into the reaction flask. The cooling bath was removed, and the reaction mixture was stirred at 25 °C for 23 h. The reaction mixture was diluted with H_2O (200 mL) and washed with pentane (4 × 300 mL). The aqueous layer was cautiously acidified (foaming) with citric acid to pH 1−3, and the mixture was then extracted with EtOAc (2 × 350 mL). The extracts were combined, washed in turn with H_2O and saturated NaCl, and then dried over anhydrous Na_2SO_4. Filtration and concentration of the filtrate

followed by drying under high vacuum gave *N*-Boc-L-leucine (25.5 g, ~100%) as a clear, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (d, J = 6.5 Hz, 6H), 1.45 (s, 9H), 1.5−1.8 (m, 3H), 4.32 (bs, 1H), 4.93 (d, J = 7.9 Hz, 1H), 6.13 (bs, 1H). The bulk of this material (23.1 g, 100 mmol) was dissolved in DMF (200 mL), and the solution was cooled to 0 °C. Cesium carbonate (32.6 g, 100 mmol) was added, and the mixture was stirred at 0 °C for 45−60 min. Benzyl bromide (11.9 mL, 17.1 g, 100 mmol) was added, and the reaction mixture was stirred at 0 °C for 30 min and then at 25 °C overnight. The mixture was poured into H_2O (800 mL) and extracted with hexane (4 × 250 mL). The extracts were combined, washed in turn with H_2O (200 mL) and saturated NaCl, and then dried over Na_2SO_4. Filtration, concentration, and drying under high vacuum gave benzyl *N*-Boc-L-leucinate (30.5 g, 95%) as clear, colorless oil. $[\alpha]_D$ −38° (*c* 0.99, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (2d, 6H), 1.44 (s, 9H), 1.46−1.54 (m, 1H), 1.60−1.73 (m, 2H), 4.36 (m, 1H), 4.90 (m, 1H), 5.14 (d, J = 12.3 Hz, 1H), 5.20 (d, J = 12.5 Hz, 1H), 7.37 (s, 5H). HRMS (FAB) *m*/*z* calcd for $C_{18}H_{27}NO_4 + H_1$: 322.2018. Found: 322.2020. A portion of this material (10.8 g, 33.6 mmol), CH_2Cl_2 (280 mL), and TFA (70 mL) were combined and processed according to the general procedure for removing a Boc protecting group, which gave L-leucine benzyl ester **13** (7.58 g, ~100%) as a clear, pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (2d, 6H), 1.47 (m, 1H), 1.60 (m, 1H), 1.79 (m, 1H), 2.15 (bs, 2H), 3.55 (m, 1H), 5.16 (s, 2H), 7.35 (bs, 5H).

Benzyl (2*S*)-2-[(3*R*)-3-Hydroxy-2-oxopyrrolidinyl]-4-methylpentanoate (15) [γ-Lactam Benzyl Ester 15]. A solution of dioxolanone aldehyde **12** (6.40 g, 40.5 mmol) in MeOH (25 mL) was added to a solution of L-leucine benzyl ester **13** (8.96 g, 40.5 mmol) in MeOH (100 mL) at 0 °C under an atmosphere of dry N_2. Glacial HOAc (6.0 mL, 6.3 g, 105 mmol) was added, and the mixture was stirred for 30 min. Powdered $NaBH_3CN$ (1.28 g, 20.3 mmol) was added, and the mixture was stirred for 3 h at 25 °C. The reaction mixture was poured into saturated $NaHCO_3$ (200 mL), and the mixture was swirled until CO_2 evolution had abated. The mixture was then extracted with Et_2O (3×). The extracts were combined, washed with saturated NaCl, dried (Na_2SO_4), and filtered. The filtrate was concentrated to a turbid yellow oil. Chromatography (30−70% EtOAc in hexane) gave γ-lactam benzyl ester **15** (6.56 g, 53%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 6.9 Hz, 3H), 1.47 (septet, J = 6.8 Hz, 1H), 1.73 (dd, J = 7.4, 7.8 Hz, 2H), 1.92 (m, 1H), 2.42 (m, 1H), 3.33 (m, 2H), 4.15 (bs, 1H), 4.37 (dd, J = 8.4, 8.7 Hz, 1H), 4.91 (dd, J = 7.7, 8.4 Hz, 1H), 5.11 (d, J = 12.3 Hz, 1H), 5.15 (d, J = 12.3 Hz, 1H), 7.34 (m, 5H). HRMS (EI) *m*/*z* calcd for $C_{17}H_{23}NO_4$ 305.1627. Found: 305.1615.

***N*-Boc-*N*-Methyl-L-leucyl-γ-lactam Free Acid (17).** *N*-Boc-*N*-Methyl-L-leucine **16** (2.42 g, 9.86 mmol), γ-lactam benzyl ester **15** (3.01 g, 9.86 mmol), a solution of DCC in CH_2Cl_2 (1 M, 9.9 mL, 9.9 mmol), DMAP (60 mg, 0.49 mmol), and CH_2Cl_2 (50 mL) were combined and processed according to the general procedure for coupling peptides using DCC to give crude product, which upon chromatography (20% EtOAc in hexane) produced the elaborated γ-lactam benzyl ester (4.50 g, 86%) as a clear, colorless, viscous oil. MS (FAB) *m*/*z*: 533 [M + H]. This material (4.46 g, 8.37 mmol), absolute EtOH (100 mL), and 10% Pd on activated carbon (700 mg) were combined and processed according to the general procedure for removing a benzyl protecting group to give γ-lactam free acid **17** (3.63 g, 98%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89−1.00 (~3d, 12H), 1.43 (s, 9H), 1.52 (m, 2H), 1.75 (m, 4H), 1.96 (m, 1H), 2.53 (m, 1H), 2.76 and 2.78 (2s, 3H), 3.37 (m 1H), 3.50 (m, 1H), 4.70 (m, 0.5H), 4.86 (m, 1.5H), 5.38 (dd, J = 8.1, 17.5 Hz, 1H), 8.0 (bs, 1H). MS (FAB) *m*/*z*: 443 [M + H], 465 [M + Na]. Anal. ($C_{22}H_{38}N_2O_7$) C, H, N.

***N*-Boc-*N*-methyl-L-leucyl-γ-lactam-3-phenyl-D-lactic Acid (19).** γ-Lactam free acid **17** (3.50 g, 7.91 mmol), benzyl 3-phenyl-D-lactate[4] **18** (2.03 g, 7.91 mmol), a solution of DCC in CH_2Cl_2 (1 M, 7.9 mL, 7.9 mmol), DMAP (48 mg, 0.40 mmol), and CH_2Cl_2 (75 mL) were combined and processed according to the general procedure for coupling peptides using DCC to give crude product, which upon chromatography (20% EtOAc in hexane) produced the tetradepsipeptide intermediate (4.93 g, 87%) as a clear, pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.87−1.99 (~3d, 12H), 1.40 (m, 1H), 1.46 (s, 9H), 1.59 (m, 3H), 1.73 (m, 3H), 2.18 (m, 1H), 2.77 and 2.80 (2s, 3H), 3.10 (m, 2H), 3.15 (m, 2H), 4.73 (m 0.5H), 4.92 (m, 1.5H), 5.09 (d, J = 12.1 Hz, 1H), 5.15 (d, J = 12.0 Hz, 1H), 5.22 (m, 1H), 5.31 (m, 1H), 7.05−7.40 (m, 10H). MS (FAB) *m*/*z*: 681 [M + H]. This intermediate (2.48 g, 3.64 mmol), absolute EtOH (100 mL), and 10% Pd on activated carbon (300 mg) were combined and processed according to the general procedure for removing a benzyl protecting group to give tetradepsipeptide free acid **19** (2.05 g, 95%) as a glass. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85−1.00 (~4d, 12H), 1.35−1.49 (m, 1H), 1.46 (s, 9H), 1.60 (m, 3H), 1.72 (m, 2H), 1.87 (m, 1H), 2.32 (m, 1H), 2.78 and 2.80 (2s, 3H), 3.14 (m, 1H), 3.19−3.38 (m, 3H), 4.78−4.98 (m, 2H), 5.30 (m, 2H), 7.16−7.37 (m, 6H). MS (EI) *m*/*z*: 590 [M].

***N*-Boc-*N*-Methyl-L-leucyl-D-lactic Acid (21).** Benzyl *N*-Boc-*N*-methyl-L-leucyl-D-lactate[2] **20** (7.62 g, 18.7 mmol), absolute EtOH (150 mL), and 10% Pd on activated carbon (1.50 g) were combined and processed according to the general procedure for removing a benzyl protecting group to give didepsipeptide free acid **21** (5.82 g, 98%) as a slightly turbid, pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89−1.00 (2d, 6H), 1.45 and 1.46 (2s, 9H), 1.50−1.80 (m, 6H), 2.81 (s, 3H), 4.74 and 4.84 (2m, 1H), 5.12 (m, 1H), 8.91 (bs, 1H).

Benzyl *N*-Methyl-L-leucyl-3-phenyl-D-lactate (23). Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactate[2] **22** (8.57 g, 17.7 mmol), CH_2Cl_2 (50 mL), and TFA (6 mL) were combined and processed according to the general procedure for removing a Boc protecting group to give didepsipeptide free amine **23** (6.09 g, 90%) as a clear, pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75−0.82 (2d, 6H), 1.29 (t, J = 7.2 Hz, 2H), 1.42 (bs, 1H), 1.49 (m, 1H), 2.20 (s, 3H), 3.08 (dd, J = 9.8, 14.2 Hz, 1H), 3.18 (t, J = 7.2 Hz, 1H), 3.28 (dd, J = 4.0, 14.3 Hz, 1H), 5.15 (d, J = 12.2 Hz, 1H), 5.20 (d, J = 12.2 Hz, 1H), 5.32 (dd, J = 4.0, 9.9 Hz, 1H), 7.17−7.40 (m, 10H). HRMS (FAB) *m*/*z* calcd for $C_{23}H_{29}NO_4 + H_1$: 384.2175. Found: 384.2183.

Benzyl *N*-Boc-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactate (24). Didepsipeptide free acid **21** (5.02 g, 15.8 mmol), didepsipeptide free amine **23** (6.06 g, 15.8 mmol), a solution of DCC in CH_2Cl_2 (1 M, 16 mL, 16 mmol), DMAP (97 mg, 0.8 mmol), and CH_2Cl_2 (150 mL) were combined and processed according to the general procedure for coupling peptides using DCC. Chromatography (10−20% EtOAc in hexane) of the crude reaction product gave tetradepsipeptide **24** (8.68 g, 80%) as a clear, nearly colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80−1.00 (~4d, 12H), 1.30−1.90 (m, 9H), 1.43 and1.46 (2s, 9H), 2.75 and 2.92 (2s, 1H), 2.84 (3s, 5H), 2.92 (s, 0.5H), 3.18 (bs, 2H), 4.68−5.42 (m, 4H), 5.09 (d, J = 12.1 Hz, 1H), 5.16 (d, J = 11.9 Hz, 1H), 7.10−7.40 (m, 10H). HRMS (FAB) *m*/*z* calcd for $C_{38}H_{54}N_2O_9 + H_1$: 683.3907. Found: 683.3911.

Benzyl *N*-Methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactate (25). Tetradepsipeptide **24** (1.58 g, 2.31 mmol), CH_2Cl_2 (108 mL), and TFA (27 mL) were combined and processed according to the general procedure for removing a Boc protecting group to give tetradepsipeptide free amine **25** (1.24 g, 92%) as a clear, pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80−0.98 (~5d, 12H), 1.28−1.90 (m, 7H), 1.46 (d, J = 6.9 Hz, 3H), 2.38 (s, 3H), 2.77 (s, 0.5H), 2.80 (s, 2H), 2.84 (s, 0.5H), 3.16 (m, 2H), 3.30 (t, J = 7.3 Hz, 1H), 5.08 (d, J = 12.1 Hz, 1H), 5.14 (d, J = 13.0 Hz, 1H), 5.25 (dd, J = 5.2, 7.2 Hz, 1H), 5.34 (dd, J = 5.0, 10.9 Hz, 1H), 5.48 (dd, J = 6.8, 13.5 Hz, 1H), 7.10−7.41 (m, 10H). HRMS (FAB) *m*/*z* calcd for $C_{33}H_{46}N_2O_7 + H_1$: 583.3383. Found: 583.3375.

***N*-Methyl-L-leucyl-γ-lactam-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactic Acid (26).** Tetradepsipeptide free acid **19** (1.27 g, 2.15 mmol), tetradepsipeptide free amine **25** (1.29 g, 2.21 mmol), a solution of DCC (2.6 mL, 1 M, 2.6 mmol), DMAP (16 mg, 0.13 mmol), and CH_2Cl_2 (75 mL) were combined and processed according to the general procedure for coupling peptides using DCC.

Chromatography of the crude reaction product gave the octadepsipeptide (1.48 g) as a clear, colorless oil. Proton NMR showed 92 wt % product and 8 wt % EtOAc for a yield of 55%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79−1.00 (∼6d, 24H), 1.43 (s, 9H), 1.38−1.50 (m, 4H), 1.50−1.99 (m, 13H), 2.70−3.30 (m, 15H), 4.60−5.50 (m, 10H), 7.08−7.40 (m, 15H). HRMS (FAB) *m/z* calcd for $C_{64}H_{90}N_4O_{15}$ + Na_1: 1177.6300. Found: 1177.6275. This material (1.45 g, 1.25 mmol), CH_2Cl_2 (100 mL), and TFA (25 mL) were combined and processed according to the general procedure for removing a Boc protecting group to give the octadepsipeptide free amine (1.32 g, ∼100%) as a clear, colorless, viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78−1.02 (∼6d, 24H), 1.15−1.96 (m, 18H), 2.40 (s, 3H), 2.43−3.33 (m, 12H), 3.48 (m, 1H), 4.91 (dd, J = 5.2, 10.6 Hz, 1H), 5.00−5.39 (m, 7H), 5.49 (m, 1H), 7.10−7.40 (m, 15H). HRMS (FAB) *m/z* calcd for $C_{59}H_{82}N_4O_{13}$ + H_1: 1055.5956. Found: 1055.5920. The octadepsipeptide free amine (1.29 g, 1.22 mmol), absolute EtOH (150 mL), and 10% Pd on activated carbon (225 mg) were combined and processed according to the general procedure for removing a benzyl protecting group to give octadepsipeptide amino acid **26** (1.14 g, 97%) as a tan powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75−1.20 (m, 24H), 1.20−2.02 (m, 14H), 1.28 (d, J = 7.1 Hz, ∼2.5H), 1.41 (d, J = 6.8, ∼0.5H), 2.30−3.52 (m, ∼9H), 2.51 (s, ∼1H), 2.56 (s, ∼1H), 2.72 (s, ∼1H), 2.80 (s, ∼1H), 2.88 (s, ∼1H), 3.00 (s, ∼1H), 3.60 (m, 1H), 4.76−4.90 (m, 1H), 5.06−5.51 (m, 5H), 5.57 (dd, J = 7.1, 13.8 Hz, 1H), 6.88 (bs, 2H), 7.11−7.32 (m, 10H). HRMS (FAB) *m/z* calcd for $C_{52}H_{76}N_4O_{13}$ + H_1: 965.5487. Found: 965.5464.

γ-Lactam Cyclodepsipeptide (2). Octadepsipeptide amino acid **26** (1.11 g, 1.15 mmol) was dissolved in CH_2Cl_2 (1150 mL) to give a concentration of 1 mM, and the solution was cooled to 0 °C. BOP reagent (534 mg, 1.21 mmol) was added, and the mixture was stirred until it was completely dissolved. *N*-Methylmorpholine (0.13 mL, 1.21 mmol) was added, and the reaction mixture was stirred at 0 °C for 30 min and then for 3 days at 25 °C. The reaction mixture was concentrated to about 100 mL and washed with saturated NH_4Cl (250 mL). The layers were separated, and the organic layer was dried ($MgSO_4$), filtered through Celite, and concentrated. The residue was dissolved in EtOAc and again concentrated. Drying under high vacuum gave a light, tan foam. This was further purified by chromatography (30−60% EtOAc in hexane) to give γ-lactam cyclodepsipeptide **2** (289 mg, 27%) as a white solid. $[\alpha]^{25}_D$ −93° (*c* 0.62, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75−1.00 (m, 24H), 1.00−1.80 (m, 14H), 1.05 (d, J = 6.3 Hz, ∼0.7H), 1.12 (d, J = 6.9 Hz, ∼0.8H), 1.47 (d, J = 6.8 Hz, 1.5H), 2.80 (s, 2.2H), 2.81 (s, 1.4H), 2.82 (s, 1.3H), 2.86 (s, 1.4H), 2.88 (s, 1.7H), 3.01 (s, 1H), 3.01−3.50 (m, 6H), 4.44 (m, 1H), 4.73−5.71 (m, 7H), 7.17−7.34 (m, 10H). MS (FAB) *m/z*: 947 [M + H], 1079 [M + Cs]. HRMS (FAB) *m/z* calcd for $C_{52}H_{74}N_4O_{12}$ + Na_1: 969.5201. Found: 969.5228. HPLC: t_R = 13.48 min (RP 8 column, 99.2% pure, gradient from 65% to 95% acetonitrile + 0.1% TFA/H_2O + 0.1% TFA over 20 min); t_R = 13.41 min (RP 4 column, 99.0% pure, gradient from 40% to 90% acetonitrile + 0.1% TFA/H_2O + 0.1% TFA over 20 min).

Synthesis of δ-Lactam Cyclodepsipeptide (3). Synthesis of δ-Lactam Benzyl Ester (31). (5*R*)-5-[(*E*)-3-Methoxy-2-propenyl]-2,2-dimethyl-1,3-dioxolan-4-one (27). A solution of phenyllithium in 70:30 cyclohexane/ether (1.8 M, 13.6 mL, 24.4 mmol) was added dropwise over a period of 10 min to a slurry of (methoxymethyl)triphenylphosphonium chloride (8.37 g, 24.4 mmol) in anhydrous ether (50 mL) at −20 to −5 °C under N_2, which produced a red solution along with some solid. The reaction mixture was stirred at 0 °C for 30 min and then cooled to −78 °C. A solution of aldehyde **12** (1.93 g, 12.2 mmol) was added dropwise over a period of 15 min. The reaction mixture was stirred at −78 °C for 45 min and then at 25 °C for 2 h. Saturated NH_4Cl (25 mL) was added dropwise. Further dilution with H_2O caused all solids to dissolve. The mixture was shaken briskly, and the layers were separated. The aqueous layer was extracted with ether, and the organic layers were combined, washed in turn with H_2O, saturated $NaHCO_3$, and saturated NaCl and then dried (Na_2SO_4). The solution was filtered, concentrated, dissolved in EtOAc, and again concentrated to a yellow solid (4.64 g). Chromatography (10% EtOAc in hexane) gave a 5:1 *E*/*Z* mixture of vinyl ether **27** (620 mg, 27%) as a clear, pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.52 (s, 3H), 1.57 (s, 3H), 2.32−2.70 (m, 2H), 3.50 (s, 2.5 H), 3.60 (s, 0.5 H), 4.40 (m, 1H), 4.70 (m, 1H), 6.02 (d, J = 6.2, 0.5 H), 6.40 (d, J = 12.6, 2.5 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.3 (*Z*), 26.4 (*E*), 26.7 (*Z*), 27.4 (*E*), 30.2 (*E*), 56.2 (*E*), 60.0 (*Z*), 74.3 (*Z*), 75.0 (*E*), 95.7 (*E*), 99.4 (*Z*), 110.8 (*E*), 115.6 (*Z*), 149.3 (*Z*), 150.7 (*E*), 172.9 (*E*, *Z*). MS (EI) *m/z*: 186 [M].

3-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]propanal (29) and (5*R*)-5-(3,3-Dimethoxypropyl)-2,2-dimethyl-1,3-dioxolan-4-one (28). Vinyl ether **27** (490 mg, 2.63 mmol) was dissolved in acetone (25 mL) and treated with sulfuric acid (≪1 drop) at 25 °C. After 70 min, TLC showed that all of the aldehyde had been consumed. The reaction mixture was treated with several drops of saturated $NaHCO_3$ and concentrated to dryness. The residue was taken up in Et_2O and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a yellow oil (275 mg) consisting of a 78:22 molar ratio of aldehyde **29** (44%) to acetal **28** (13%). This material was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.54 (s, 3H), 1.58 (s, 3H), 1.77 (m, 0.6 H), 1.95 (m, 0.2 H), 2.09 (m, 0.8 H), 2.25 (m, 0.8 H), 2.62 (m, 1.6 H), 3.31 (s, 0.6 H), 3.33 (s, 0.6 H), 4.40 (m, 0.4 H), 4.47 (m, 0.8 H), 4.73 (s, 0.8).

Benzyl (2*S*)-2-[(3*R*)-3-Hydroxy-2-oxopiperidinyl]-4-methylpentanoate (31) [δ-Lactam Benzyl Ester 31] and Benzyl (2*S*)-2-({3-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]propyl}amino)-4-methylpentanoate (30). Aldehyde **29** (76 mg, 0.44 mmol) was dissolved in MeOH (1.5 mL), and the mixture was cooled to 0 °C. L-Leucine benzyl ester **13** (98 mg, 0.44 mmol) was added followed by HOAc (0.06 mL), and the reaction mixture was stirred for 30 min. Sodium cyanoborohydride (14 mg, 0.22 mmol) was added, the mixture was stirred for 5 min, and the cooling bath was removed. The progress of the reaction was monitored by TLC, which showed many spots. The reaction mixture was stirred for 4 h, more $NaCNBH_3$ (20 mg) was added, and the mixture was stirred overnight, after which TLC showed fewer spots. The mixture was poured into saturated $NaHCO_3$, mixed thoroughly, and twice extracted with Et_2O . The extracts were combined, washed with 10% citric acid, dried ($MgSO_4$), filtered, and concentrated. A second concentration from EtOAc and drying under high vacuum gave a colorless, slightly turbid oil. This was further purified by chromatography (20% EtOAc in hexane) to give δ-lactam benzyl ester **31** (46 mg, 33%), free of secondary amine **30**, as a clear, colorless oil. In the case where the reaction time was only 2 h, a 4:1 mixture (18% combined yield) of secondary amine **30** and δ-lactam **31** was obtained. After standing 5 days at 25 °C, most of the amine had lactamized to give a 6:1 mixture of δ-lactam to amine. Complete conversion to δ-lactam **31** was achieved by heating the mixture at 70 °C in dry toluene for 90 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (s, 3H), 0.96 (s, 3H), 1.52 (m, 1H), 1.57−1.96 (m, 6H), 2.28 (m, 1H), 3.22 (m, 2H), 4.07 (dd, J = 4.73, 6.46 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 5.17 (m, 1H), 5.18 (d, J = 12.0 Hz, 1H), 7.34 (m, 5H). HRMS (FAB) *m/z* calcd for $C_{18}H_{25}NO_4$ + H_1: 320.1862. Found: 320.1862 (sic).

Synthesis of δ-Lactam Benzyl Ester (39), a Diastereomer of δ-Lactam Benzyl Ester (31). Methyl 5-[*N*-((1*S*)-1-Benzyloxycarbonyl-3-methylbutyl)amino]pentanoate (33). Methyl 5-bromovalerate **32** (5.47 g, 28.1 mmol), L-leucine benzyl ester **13** (6.21 g, 28.1 mmols), and powdered $NaHCO_3$ (4.71 g, 56.1 mmol) were combined, and DMF (25 mL) was added. The reaction mixture was heated at 130 °C for 90 min and then cooled to room temperature. The mixture was diluted with H_2O and extracted with Et_2O (3×). The extracts were combined, washed in turn with H_2O and saturated NaCl, and then dried (Na_2SO_4). Filtration followed by concentration of the filtrate gave secondary amine **33** (8.53 g, 91%) as a clear, pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 1.49 (m, 3H), 1.67 (m, 4H), 1.83 (bs, 1H), 2.30 (m, 2H), 2.45 (m, 1H), 2.59 (m, 1H), 3.32

(m, 1H), 3.68 (s, 3H), 5.17 (s, 2H), 7.35 (s, 5H). MS (FAB) *m*/*z*: 336 [M + H].

Benzyl (2*S*)-4-Methyl-2-(2-oxo-1-piperidinyl)pentanoate (34). Secondary amine **33** (8.53 g, 25.4 mmol) was combined with mixed xylenes and heated under reflux for 42 h, during which time the reaction mixture became orange but remained clear. The solvent was removed under vacuum, and the residue was purified by chromatography (20−30% EtOAc in hexane) to give *δ*-lactam **34** (5.10 g, 66%) as a clear, light-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.94 (2d, 6H), 1.50 (m, 1H), 1.75 (m, 6H), 2.43 (m, 2H), 3.19 (m, 2H), 5.10 (d, *J* = 12.4 Hz, 1H), 5.18 (d, *J* = 12.3 Hz, 1H), 5.39 (dd, *J* = 6.4, 9.5 Hz, 1H), 7.35 (m, 5H). MS (EI) *m*/*z*: 303 [M].

Benzyl (2*S*)-2-(3-Bromo-2-oxo-1-piperidinyl)-4-methylpentanoate (35). *δ*-Lactam **34** (3.93 g, 13.0 mmol) was dissolved in CH_2Cl_2 (100 mL), Et_3N (9.0 mL, 65 mmol) was added, and the reaction mixture was cooled to −20 °C. Chlorotrimethylsilane (3.3 mL, 26 mmol) was added, and the mixture was stirred for 7 min. Small crystals of iodine (4.9 g, 19 mmol) were added, and stirring was continued at −20 °C for another 15 min, after which bromine (5.0 mL, 97 mmol) was added and the reaction mixture was stirred at 0 °C for 2.3 h. The reaction was quenched by adding 10% Na_2SO_3 (50 mL). The mixture was transferred to a separatory funnel where more Na_2SO_3 solution (100 mL) was added. The mixture was shaken vigorously, causing it to become extremely dark. After addition of another 100 mL of Na_2SO_3 solution and more vigorous shaking, the mixture became light-yellow. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2×). The extracts were combined and washed with 10% Na_2SO_3 (150 mL), causing both layers to become pale-amber in color. The layers were separated, and the organic material was washed in turn with H_2O and saturated NaCl and then dried over Na_2SO_4. Filtration, concentration, and drying under high vacuum gave a red-orange oil. This was purified by chromatography (20% EtOAc in hexane) to give bromo-*δ*-lactam **35** (4.20 g, 85%) as a mixture of diastereomers in the form of a clear, very pale-yellow oil, which solidified on standing for 30 min at room temperature. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.94 (3d, 6H), 1.51 (m, 1H), 1.79 (m, 3H), 2.22 (m, 3H), 3.30 (m, 2H), 4.60 (m, 1H), 5.08−5.25 (m, 3H), 7.37 (m, 5H). HRMS (FAB) *m*/*z* calcd for $C_{18}H_{24}BrNO_3 + H_1$: 382.1018. Found: 382.1021. Anal. ($C_{18}H_{24}BrNO_3$) C, H, N, Br.

Benzyl (2*S*)-2-(3-Hydroxy-2-oxo-1-piperidinyl)-4-methylpentanoate (36) and Benzyl (2*S*)-2-[3-(Formyloxy)-2-oxo-1-piperidinyl]-4-methylpentanoate (37). Bromo-*δ*-lactam **35** (3.90 g, 10.0 mmol) was dissolved in dry formamide (75 mL) to which had been added H_2O (0.18 mL, 10.0 mmol). The reaction mixture was heated at 150 °C for 65 min, cooled to room temperature, diluted with H_2O, and extracted with Et_2O (3×). The extracts were combined, washed with H_2O (2×) and saturated NaCl, and then dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (40% EtOAc in hexane) to give hydroxy-*δ*-lactam **36** (2.54 g, 80%) as a mixture of diastereomers in the form of a clear, light-yellow oil, which solidified on standing to a waxy solid. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.91−0.96 (3d, *J* ≈ 6.6 Hz, 6H), 1.50 (m, 1H), 1.60−1.98 (m, 5H), 2.28 (m, 1H), 3.14−3.20 (m, 2H), 3.48 (bs, 1H), 4.05 (m, 1H), 5.06−5.21 (m, 2H), 5.26 (dd, *J* = 5.4, 10.7 Hz, 1H), 7.35 (m, 5H). When this reaction was performed on a larger scale (9.74 g of **35**) and over a longer reaction time (∼2 h), only a 53% yield (4.20 g) of hydroxy-*δ*-lactam **36** was obtained along with a 21% yield (1.80 g) of formate **37**. These were separated by chromatography (15−20% EtOAc in hexane) to give **37** as a clear, light-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.90−0.96 (3d, *J* ≈ 6.7 Hz, 6H), 1.50 (m, 1H), 1.68−2.03 (m, 5H), 2.20 (m, 1H), 3.27 (m, 2H), 5.08−5.42 (m, 4H), 7.36 (m, 5H), 8.15 (2s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 20.3, 20.5, 21. 8, 21.9, 23.5, 23.6, 25.3, 25.4, 27.1, 27.2, 37.2, 37.3, 43.5, 44.3, 54.9, 55.1, 67.3, 67.3, 68.9, 69.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 135.9, 160.4, 160.4, 167.3, 167.7, 171.5, 171.5. MS (FAB) *m*/*z*: 348 [M + H], 695 [2M + H]. Anal. ($C_{19}H_{25}NO_5$) C, H, N.

Benzyl (2*S*)-2-(2,3-Dioxo-1-piperidinyl)-4-methylpentanoate (38). A solution of DMSO (2.0 mL, 28.8 mmol) in CH_2Cl_2 (6 mL) was added in a slow stream to a solution of oxalyl chloride (1.2 mL, 14.4 mmol) in CH_2Cl_2 (25 mL) at −78 °C, and the mixture was stirred for 10 min. A solution of hydroxy-*δ*-lactam **36** (4.18 g, 13.1 mmol) in CH_2Cl_2 (8 mL) was added in a slow stream, and the reaction mixture was stirred at −78 °C for 60 min. Triethylamine (8.0 mL, 57.6 mmol) was added in a slow stream at −78 °C. The cooling bath was removed, and the mixture was stirred for 45 min at ambient temperature. The reaction mixture was poured into H_2O and extracted with CH_2Cl_2 (2×). The organic layers were combined and washed in turn with 1 N H_2SO_4 and saturated $NaHCO_3$ and then dried (Na_2SO_4), filtered and concentrated under high vacuum to a red oil, which solidified on standing overnight. This was triturated with boiling hexane containing a small amount of EtOAc and then chilled at 0 °C for 3 h. The solid was removed by filtration and air-dried. Further drying to constant weight under vacuum at 60 °C gave keto-*δ*-lactam **38** (3.12 g, 75%) as an off-white powder. $[\alpha]^{25}_D$ −47° (*c*, 0.97, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.93−0.97 (2d, *J* ≈ 6.6 Hz, 6H), 1.50 (m, 1H), 1.72−1.89 (m, 2H), 2.07−2.28 (m, 2H), 3.38−3.56 (m, 2H), 5.12 (d, *J* = 12.2 Hz, 1H), 5.20 (d, *J* = 12.2 Hz, 1H), 5.40 (dd, *J* = 5.33, 12.2 Hz, 1H), 7.34 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 21.6, 22.1, 23.6, 25.4, 37.3, 38.9, 44.1, 55.5, 67.6, 128.6, 128.8, 129.0, 135.6, 158.5, 171.0, 191.5. HRMS (EI) *m*/*z* calcd for $C_{18}H_{23}NO_4$: 317.1627. Found: 317.1621. Anal. ($C_{18}H_{23}NO_4$) C, H, N.

Benzyl (2*S*)-2-[(3*S*)-3-Hydroxy-2-oxopiperidinyl]-4-methylpentanoate (39) [*δ*-Lactam Benzyl Ester 39]. Baker's yeast (60 g) and D-glucose (6.0 g) were combined, H_2O (160 mL) was added, and the mixture was swirled until the yeast was completely wetted. Keto-*δ*-lactam **38** (3.03 g, 9.55 mmol) was added as a powder. The clumps of solid, which formed during the first hour, were broken up with a stirring rod. The reaction mixture was stirred overnight at room temperature, during which it increased severalfold in volume. The mixture was filtered through Celite, an arduous process that required repeated scraping of the Celite pad to facilitate filtration. When the H_2O had been mostly removed, the residue was mixed with a large amount of sand, transforming the solids into a more granular mixture, which facilitated stirring. The mixture was stirred with EtOAc, and the solvent was removed by filtration. This process was repeated four times. The filtrates were combined and partially dried (Na_2SO_4), filtered, and concentrated to a brown oil. The oil was taken up in Et_2O and filtered to remove the insoluble material. Concentration of the filtrate produced a clear, brown oil, which partially solidified on standing at room temperature. Purification by chromatography (20−40% EtOAc in hexane) gave *δ*-lactam benzyl ester **39** (1.81 g, 59%) as a clear, amber-colored oil. This material was triturated with Et_2O/hexane while warming, which caused it to solidify. Filtration and air-drying gave *δ*-lactam benzyl ester **39** (1.52 g, 50%) as a nearly white powder. ^{1}H and ^{13}C NMR showed the presence of only one diastereomer, suggesting an optical purity of ≥95% de as determined by comparison with *δ*-lactam benzyl ester **31** whose stereochemistry was unambiguously established by an alternative synthesis.[16] ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.90−0.96 (2d, *J* ≈ 6.6 Hz, 6H), 1.48 (m, 1H), 1.60−1.84 (m, 4H), 1.84−1.98 (m, 1H), 2.27 (m, 1H), 3.15−3.30 (m, 2H), 3.63 (bs, 1H), 4.06 (dd, *J* = 6.5, 10.9 Hz, 1H), 5.12 (d, *J* = 12.3 Hz, 1H), 5.19 (d, *J* = 12.3 Hz, 1H), 5.25 (dd, *J* = 10.7, 5.4 Hz, 1H), (7.34 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 20.2, 21.7, 23.5, 25.3, 28.3, 37.3, 43.1, 54.8, 67.3, 68.2, 128.5, 128.8, 129.0, 135.8, 171.7, 174.2. Anal. ($C_{18}H_{25}NO_4$) C, H, N.

***N*-Boc-*N*-Methyl-L-leucyl-*δ*-lactam Free Acid (40).** *δ*-Lactam benzyl ester **39** (1.49 g, 4.66 mmol), *N*-Boc-*N*-methyl-L-leucine **16** (1.14 g, 4.66 mmol), and triphenylphosphine (1.22 g, 4.66 mmol) were combined. THF (100 mL) was added, and the mixture was stirred until all solids had dissolved. The solution was cooled to 0 °C, and DEAD (0.88 mL, 5.60 mmol) was added. The reaction mixture was stirred at room temperature for 110 min and then concentrated. Chromatography

(20% EtOAc in hexane) of the residue produced 2.30 g of clear, colorless oil, which ^{1}H NMR showed to contain 93 wt % product and 7 wt % EtOAc. This amounts to 2.14 g (84%) of the elaborated δ-lactam benzyl ester. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3d, 12 H), 1.45 (s, 9H), 1.48−2.19 (m, 10 H), 2.79 (m, 3H), 3.22 (m, 2H), 4.69−5.27 (m, 2H), 5.08 (d, J = 12.3 Hz, 1H), 5.17 (d, J = 12.3 Hz, 1H), 5.30 (m, 1H), 7.32 (m, 5H). This material (2.11 g, 3.86 mmol), absolute EtOH (100 mL), and 10% Pd on activated carbon (300 mg) were combined and processed according to the general procedure for removing a benzyl protecting group. This produced 1.83 g of white foam and glass, which ^{1}H NMR showed to contain 95 wt % product and 5 wt % EtOAc. This amounts to 1.74 g (99%) of δ-lactam free acid **40**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3d, 12 H), 1.38−2.24 (m, 10 H), 1.45 (s, 9H), 2.76 (2s, 3H), 3.30 (bs, 2H), 4.73 and 4.93 (2m, 1H), 5.22 (m, 2H), 9.40 (bs, 1H).

Benzyl *N*-Methyl-L-leucyl-δ-lactam-3-phenyl-D-lactate (41). δ-Lactam free acid **40** (1.71 g, 3.75 mmol), benzyl 3-phenyl-D-lactate[4] **18** (0.96 g, 3.75 mmol), a solution of DCC in CH_2Cl_2 (1 M, 4.1 mL, 4.1 mmol), DMAP (23 mg, 0.19 mmol), and CH_2Cl_2 (50 mL) were combined and processed according to the general procedure for coupling peptides using DCC. The crude reaction product was chromatographed (15−20% EtOAc in hexane), which produced 1.28 g of clear, colorless oil. ^{1}H NMR showed this to contain 98 wt % product and 2 wt % EtOAc. This amounts to 1.25 g (48%) of tetradepsipeptide. HPLC analysis showed this material to consist of a 11:1 mixture of two diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3d, 12 H), 1.46 (s, 9H), 1.35−2.03 (m, 10 H), 2.80 (2s, 3H), 3.01 (m, 2H), 3.16 (m, 2H), 4.70−5.55 (m, 6H), 7.12 (m, 2H), 7.27 (m, 5H), 7.36 (m, 3H). A second chromatography fraction (0.82 g) contained a different mix of diastereomers. The tetradepsipeptide (600 mg, 0.863 mmol), CH_2Cl_2 (16 mL), and TFA (4 mL) were combined and processed according to the general procedure for removing a Boc protecting group. This produced tetradepsipeptide free amine **41** (483 mg, 94%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (4d, 12 H), 1.37−2.37 (m, 11 H), 2.47 (s, 3H), 2.96−3.02 (m, 2H), 3.15 (m, 2H), 3.33 (t, J = 7.25 Hz, 1H), 5.10 (d, J = 12.1 Hz, 1H), 5.15 (d, J = 12.0 Hz, 1H), 5.21−5.33 (m, 2H), 5.43 (dd, J = 5.20, 10.4 Hz, 1H), 7.11 (m, 2H), 7.27 (m, 5H), 7.37 (m, 3H).

***N*-Boc-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactic Acid (42).** Tetradepsipeptide **24** (11.8 g, 17.3 mmol), absolute EtOH (200 mL), and 10% Pd on activated carbon (2.5 g) were combined and processed according to the general procedure for removing a benzyl protecting group, which gave tetradepsipeptide free acid **42** (10.3 g, ∼100%) as a white, solid foam. $[\alpha]^{25}_D$ −26° (*c* 1.13, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (m, 12 H), 1.23−1.86 (m, 18 H), 2.74−3.05 (5s, 6H), 3.07−3.47 (m, 2H), 4.35−5.62 (8m, 4H), 7.02−7.37 (m, 5H), 7.42 (bs, 1H). HRMS (FAB) *m*/*z* calcd for $C_{31}H_{48}N_2O_9 + H_1$: 593.3438. Found: 593.3445. Anal. ($C_{31}H_{48}N_2O_9$) C, H, N.

***N*-Methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-δ-lactam-3-phenyl-D-lactic Acid (43).** Tetradepsipeptide free acid **42** (461 mg, 0.778 mmol), tetradepsipeptide free amine **41** (463 mg, 0.778 mmol), a solution of DCC in CH_2Cl_2 (1 M, 0.8 mL, 0.8 mmol), DMAP (4.8 mg, 0.039 mmol), and CH_2Cl_2 (20 mL) were combined and processed according to the general procedure for coupling peptides using DCC. This produced a solid foam and glass (940 mg), which was purified by chromatography (25−30% EtOAc in hexane). From the appropriate fraction, 576 mg of clear, yellow oil was obtained, which ^{1}H NMR showed to contain 92 wt % product and 8 wt % EtOAc. This amounts to 530 mg (58%) of octadepsipeptide. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (4d, 24 H), 1.30−2.01 (m, 19 H), 1.45 (m, 9H), 2.75−3.28 (2s + m, 15 H), 4.41−5.50 (m, 8H), 5.09 (d, J = 12.0 Hz, 1H), 5.14 (d, J = 12.0 Hz, 1H), 7.05−7.44 (m, 15 H). The octadepsipeptide (512 mg, 0.437 mmol), CH_2Cl_2 (16 mL), and TFA (4 mL) were combined and processed according to the general procedure for removing a Boc protecting group. This gave the octadepsipeptide free amine (462 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78−1.05 (5d, 24 H), 1.17−2.12 (m, 20 H), 2.40−3.30 (3s + m, 15 H), 3.47 (m, 1H), 5.09 (d, J = 12.1 Hz, 1H), 5.14 (d, J = 12.0 Hz, 1H), 5.19−5.57 (m, 7H), 7.03−7.41 (m, 15 H). The octadepsipeptide free amine (452 mg, 0.423 mmol), absolute EtOH (100 mL), and 10% Pd on activated carbon (80 mg) were combined and processed according to the general procedure for removing a benzyl protecting group. This produced 403 mg of cream-colored solid, which ^{1}H NMR showed to consist of 96 wt % octadepsipeptide amino acid and 4 wt % EtOAc. This amounts to 387 mg (93%) of octadepsipeptide amino acid **43**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78−1.08 (4d, 24 H), 1.28 (m, 4H), 1.40−2.02 (m, 16 H), 2.43−2.70 (s + m, 3H), 2.70−3.38 (2s + m, 12 H), 3.74 (m, 1H), 5.00−5.60 (m, 7H), 6.69 (bs, 1H), 7.25 (m, 10 H).

δ-Lactam Cyclodepsipeptide (3). Octadepsipeptide amino acid **43** (388 mg, 0.396 mmol) was dissolved in CH_2Cl_2 (400 mL) to give a 1 mM solution, which was then cooled to 0 °C. DPEA (0.17 mL, 1.0 mmol) was added followed by BOP-Cl (121 mg, 0.475 mmol), and the reaction mixture was stirred at 25 °C for 24 h. When TLC showed the reaction to be incomplete, more DPEA (0.20 mL) and BOP-Cl (143 mg) were added. After the mixture was stirred an additional 24 h, the reaction was essentially complete. The reaction mixture was washed with aqueous $NaHCO_3$, filtered through Na_2SO_4, and dried ($MgSO_4$). Filtration, concentration, and drying under high vacuum produced a solid, which was dissolved in EtOAc and filtered through Celite to remove a small amount of insoluble material. The filtrate was concentrated and then chromatographed (40−50% EtOAc in hexane) to give an off-white solid. To remove residual EtOAc, the solid was twice dissolved in 4:1 hexane/CH_2Cl_2 and concentrated. Drying under high vacuum gave δ-lactam cyclodepsipeptide **3** (249 mg, 65%) as a nearly colorless, solid foam and glass. HPLC analysis showed this material to be 99.8% pure and free of diastereomers (t_R was 16.7 min on a 250 mm Vydac pH stable RP C_8 column, with product eluting with 40−90% CH_3CN/H_2O at 1 mL/min). ^{1}H NMR showed the product to consist of a mixture of conformers. $[\alpha]^{25}_D$ −87° (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70−1.20 (m, 27 H), 1.20−2.30 (m, 19 H), 2.67−3.00 (5s, 9H), 3.00−3.37 (m, 6H), 4.44 (m, 1H), 4.78 (m, 0.5 H), 5.05 (m, 0.5 H), 5.18−5.45 (m, 4H), 5.55−5.68 (m, 1H), 5.80−5.90 (m, 1H), 7.25 (m, 10 H). MS (FAB) *m*/*z*: 961 [M + H], 983 [M + Na], 1093 [M + Cs]. HRMS (FAB) *m*/*z* calcd for $C_{53}H_{76}N_4O_{12} + H_1$: 961.5538. Found: 961.5528. IR (KBr, cm^{-1}): 1743 (ester carbonyl), 1663 (amide carbonyl). UV (1%, 1 cm, MeOH) λ_{max} (ε): 250 (5.0), 257 (4.6), 263 (3.4). Anal. ($C_{53}H_{76}N_4O_{12}$) C, H, N.

Synthesis of ε-Lactam Cyclodepsipeptide (4). (*E*)-4-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]-2-butenal (44). Dioxolanone aldehyde **12** (9.65 g, 61.0 mmol) and (triphenylphosphoranylidene)acetaldehyde (18.6 g, 61.1 mmol) were combined with toluene (200 mL), and the mixture was heated at 80 °C for 90 min. The reaction mixture was cooled to room temperature and filtered through silica gel with Et_2O. The filtrate was concentrated to a mushy solid, which was triturated with 1:1 Et_2O/hexane. Filtration followed by concentration of the filtrate and drying under high vacuum gave crude product (9.09 g) as a clear, orange oil. Chromatography (20% EtOAc in hexane) produced α,β-unsaturated aldehyde **44** (3.94 g, 46%) as an orange oil. [This material is sensitive to air oxidation.] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56 (s, 3H), 1.61 (s, 3H), 2.75 (m, 1H), 2.91 (m, 1H), 4.57 (m, 1H), 6.25 (m, 1H), 6.80 (m, 1H), 9.54 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.0, 27.4, 34.7, 72.8, 111.5, 136.3, 150.4, 172.0, 193.5. HRMS (EI) *m*/z calcd for $C_9H_{12}O_4$: 184.0735. Found: 184.0743. [In one case, high vacuum-drying of a faster eluting chromatography fraction gave a small amount of methyl ester **9** as a clear, yellow oil. This material sometimes formed during the preparation of dioxolanone acid **10** and was harmlessly carried forward in a synthesis until it was removed during a later chromatography.]

Benzyl (2*S*)-2-({3-Methoxy-4-[(4*R*)-2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl]butyl}amino)-4-methylpentanoate (45). L-Leucine benzyl ester **13** (401 mg, 1.81 mmol) was dissolved

in MeOH (5 mL), and the solution was cooled in an ice/water bath under a nitrogen atmosphere. α,β-Unsaturated aldehyde **44** (334 mg, 1.81 mmol) was added followed by glacial HOAc (0.2 mL, 210 mg, 3.49 mmol). The reaction mixture was stirred at 0 °C for 30 min. Powdered $NaBH_3CN$ (56 mg, 0.89 mmol) was added, and the mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with Et_2O and poured into a 1:1 solution of saturated $NaHCO_3$ and H_2O. The mixture was shaken, the layers were separated, and the aqueous layer was extracted with Et_2O . The organic layers were combined, washed in turn with Et_2O and saturated NaCl, and then dried (Na_2SO_4). Filtration and concentration of the filtrate led to a slightly turbid, yellow oil (696 mg). This was purified by chromatography (20% EtOAc in hexane) to give methoxylamine **45** (296 mg, 39%) as a clear, light-yellow oil. NMR spectroscopy showed this to be a 1:1 mixture of both epimers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (m, 6 H), 1.50 (m, 2 H), 1.55 (2s, 3 H), 1.60 (s, 3 H), 1.71 (m, 4 H), 1.90 (m, 1 H), 2.09 (m, 1 H), 2.56 (m, 1 H), 2.70 (m, 1 H), 3.30 (s, 1.5 H), 3.31 (m, 1 H), 3.34 (s, 1.5 H), 3.50 (m 0.5 H), 3.58 (m, 0.5 H), 4.47 (m, 0.5 H), 4.53 (m, 0.5 H), 5.18 (s, 2 H), 7.35 (s, 5 H). HRMS (FAB) *m/z* calcd for $C_{23}H_{35}NO_6 + H_1$: 422.2542. Found: 422.2552. The slow conversion of this material to ϵ-lactam **46** by heating it in refluxing xylene was monitored by mass spectrometry on selected HPLC fractions during the 90 h required for 90% completion.

4-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]butanal (48) and 4-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]butanoic Acid (49). α,β-Unsaturated aldehyde **44** (3.19 g, 17.3 mmol) was dissolved in EtOAc (200 mL). Palladium on activated carbon (5 wt %, 180 mg) was added, and the mixture was hydrogenated at balloon pressure with stirring at room temperature for 3 h. When ^{1}H NMR indicated incomplete reaction, the reaction mixture was flushed with N_2, more catalyst was (205 mg) added, and hydrogenation continued for another 3 h. After being flushed with N_2, the reaction mixture was vacuum-filtered through Celite and the filtrate was concentrated to an oily residue (3.13 g). Since ^{1}H NMR revealed an unacceptably high level of acid **49** (26 mol % acid and 74 mol % saturated aldehyde) apparently arising from air oxidation of **44**, the crude product was chromatographed (20−30% EtOAc in hexane), which gave saturated aldehyde **48** (1.23 g) as a clear, colorless oil. NMR indicated that this material was still contaminated with acid **49** (9 wt %). Nonetheless, the material was judged to be suitable for further synthesis.[17] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53 (s, 3H), 1.61 (s, 3H), 1.79 (m, 3H), 1.95 (m, 1H), 2.50 (m, 2H), 4.40 (m, 1H), 9.78 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.9, 26.0, 27.5, 31.1, 43.5, 74.1, 110.9, 173.2, 201.8. MS (ES) *m/z*: 187 [M + H], 209 [M + Na]. Acid **49** (485 mg) was obtained free of aldehyde as a clear, pale-yellow oil from a slower eluting chromatography fraction. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53 (s, 3H), 1.60 (s, 3H), 1.80 (m, 3H), 1.98 (m, 1H), 2.43 (m, 2H), 4.40 (m, 1H), 7.2−9.0 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 26.0, 27.5, 31.0, 33.6, 74.1, 111.0, 173.3, 179.2. MS (ES) *m/z*: 201 [M − H], 203 [M + H], 225 [M + Na].

Benzyl (2*S*)-2-({4-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]butyl}amino)-4-methylpentanoate (50). A solution of saturated aldehyde **48** (4.95 g, 26.6 mmol) in MeOH (50 mL) was added to a solution of L-leucine benzyl ester **13** (5.88 g, 26.6 mmol) in MeOH (50 mL) at 0 °C under an atmosphere of dry N_2. Glacial HOAc (2.3 mL, 2.39 g, 39.9 mmol) was added, and the mixture was stirred for 7 min (prolonged stirring is deleterious) at 0 °C. A solution of $NaBH_3CN$ in THF (1 M, 17.8 mL, 17.8 mmol) was then added, and the reaction mixture was stirred at room temperature for 30−45 min. The reaction mixture was diluted with Et_2O (200 mL) and poured into aqueous $NaHCO_3$ (200 mL). The mixture was shaken, and the layers were separated. The aqueous layer was extracted with Et_2O (200 mL). The organic layers were combined and washed with H_2O (200 mL) and then with saturated NaCl (200 mL). Filtration, concentration, and drying under high vacuum gave crude product (10.8 g). Chromatography (20% EtOAc in hexane) produced secondary amine **50** (6.89 g, 74%) as a clear, light-yellow oil. $[\alpha]^{25}_D$ −7° (*c* 1.22, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 1.50 (m, 6H), 1.52 (s, 3H), 1.60 (s, 3H), 1.69 (m, 2H), 1.88 (m, 1H), ∼2.0 (bs, 1H), 2.48 (m, 1H), 2.60 (m, 1H), 3.32 (t, *J* = 7.3 Hz, 1H), 4.35 (dd, *J* = 4.3, 7.3 Hz, 1H), 5.18 (s, 2H), 7.36 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.8, 22.9, 22.9, 25.2, 26.1, 27.5, 29.9, 31.7, 42.9, 48.0, 60.4, 66.7, 74.3, 110.7, 128.6, 128.7, 128.9, 136.1, 173.6, 176.0. HRMS (FAB) *m/z* calcd for $C_{22}H_{33}NO_5 + H_1$: 392.2437. Found: 392.2437 (sic). Anal. ($C_{22}H_{33}NO_5$) C, H, N.

Benzyl (2*S*)-2-[(3*R*)-3-Hydroxy-2-oxoazepanyl]-4-methylpentanoate (51) [ϵ-Lactam Benzyl Ester 51]. Secondary amine **50** (2.55 g, 6.51 mmol) was dissolved in mixed xylenes and heated at reflux under an atmosphere of dry N_2 for 115 h. The reaction mixture was concentrated to a dark-brown oil (2.56 g), which was dissolved in Et_2O and treated with activated charcoal for 20 min at room temperature. Filtration through Celite and concentration gave crude product as a clear, light-yellow oil. Chromatography (20% EtOAc in hexane) followed by drying under high vacuum produced ϵ-lactam benzyl ester **51** (1.71 g, 79%) as a clear, pale-yellow oil. $[\alpha]^{25}_D$ −37° (*c* 1.12, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 1.30 (m, 1H), 1.42−1.74 (m, 5H), 2.82 (m, 2H), 2.00 (m, 2H), 3.27 (m, 2H), 4.28 (dd, *J* = 2.3, 11.4 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 5.17 (d, *J* = 12.2 Hz, 1H), 5.31 (dd, *J* = 4.4, 10.3 Hz, 1H), 7.36 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.1, 23.5, 25.4, 27.5, 28.4, 33.9, 38.2, 46.2, 57.4, 67.4, 71.0, 128.5, 128.8, 129.0, 135.8, 171.5, 177.3. HRMS (EI) *m/z* calcd for $C_{19}H_{27}NO_4$: 333.1940. Found: 333.1948. HRMS (FAB) *m/z* calcd for $C_{19}H_{27}NO_4 + H_1$: 334.2018. Found: 334.2030. IR (mull, cm^{-1}): 1739 (ester carbonyl), 1642 (amide carbonyl). UV (1%, 1 cm, MeOH) λ_{max} (ϵ): 257 (6.8), 262 (5.5), 266 (3.5). Anal. ($C_{19}H_{27}NO_4$) C, H, N.

***N*-Boc-*N*-Methyl-L-leucyl-ϵ-lactam Free Acid (52).** *N*-Boc-*N*-methyl-L-leucine **16** (1.19 g, 4.86 mmol), ϵ-lactam benzyl ester **51** (1.62 g, 4.86 mmol), a solution of DCC in CH_2Cl_2 (1 M, 4.9 mL, 4.9 mmol), DMAP (30 mg, 0.24 mmol), and CH_2Cl_2 (25 mL) were combined and processed according to the general procedure for coupling peptides using DCC. Chromatography (20% EtOAc in hexane) of the crude reaction product and high vacuum-drying gave the elaborated ϵ-lactam benzyl ester (1.92 g, 71%) as a clear, colorless oil. $[\alpha]^{25}_D$ −33° (*c* 1.14, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 0.97 (m, 12 H), 1.21−2.02 (m, 12 H), 1.46 (2s, 9H), 2.88 (2s, 3H), 3.30 (m, 2H), 4.81, (m, 0.5 H), 4.98 (m, 0.5 H), 5.06−5.26 (m, 3H), 5.31 (m, 1H), 7.34 (bs, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.4, 21.3, 21.6, 22.3, 23.0, 23.4, 23.6, 23.7, 24.9, 25.2, 25.4, 26.6, 26.7, 28.4, 28.7, 29.7, 30.4, 31.1, 31.9, 37.7, 38.0, 38.4, 45.7, 56.5, 56.8, 56.9, 57.3, 67.1, 74.0, 80.0, 80.4, 128.4, 128.5, 128.9, 136.0, 171.1, 171.6. HRMS (FAB) *m/z* calcd for $C_{31}H_{48}N_2O_7 + H_1$: 561.3539. Found: 561.3552. This material (1.89 g, 3.37 mmol), absolute EtOH (150 mL), and 10% Pd on activated carbon (400 mg) were combined and processed according to the general procedure for removing a benzyl protecting group, which gave ϵ-lactam free acid **52** (1.62 g, ∼100%) as a white, solid foam. $[\alpha]^{25}_D$ −27° (*c* 1.13, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (m, 12 H), 1.45 (s, 9H), 1.50−2.05 (m, 12 H), 2.85 (s, 3H), 3.32 (m, 1H), 3.49 (m, 1H), 4.78 (m, 0.5 H), 4.95 (m, 0.5 H), 5.07 (m, 1H), 5.31 (m, 1H), 6.43−8.02 (bs, 1H). HRMS (FAB) *m/z* calcd for $C_{24}H_{42}N_2O_7 + H_1$: 471.3070. Found: 471.3075.

Benzyl *N*-Methyl-L-leucyl-ϵ-lactam-3-phenyl-D-lactate (53). ϵ-Lactam free acid **52** (2.96 g, 6.29 mmol), benzyl 3-phenyl-D-lactate[4] **18** (1.69 g, 6.46 mmol), a solution of DCC in CH_2Cl_2 (1 M, 7.9 mL, 7.9 mmol), DMAP (40 mg, 0.33 mmol), and CH_2Cl_2 (75 mL) were combined and processed according to the general procedure for coupling peptides using DCC. Chromatography (15% EtOAc in hexane) of the crude reaction product followed by high vacuum-drying gave the tetradepsipeptide (1.88 g, 42%) as a clear, colorless oil. $[\alpha]^{25}_D$ −19° (*c* 1.14, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (m, 12 H), 1.10−2.00 (m, 12 H), 1.48 (s, 9H), 2.85−3.06 (m, 5H), 3.18 (bs, 2H), 4.78−5.44 (4 H), 5.06 (d, *J* = 12.1 Hz, 1H), 5.13 (d, *J* = 12.0 Hz, 1H), 7.06−7.45 (m, 10 H). ^{13}C NMR (100 MHz,

$CDCl_3$): δ 14.5, 21.3, 21.6, 22.2, 23.0, 23.5, 23.8, 24.9, 25.1, 26.9, 28.4, 28.7, 30.0, 31.9, 35.0, 37.4, 37.7, 38.1. 5.5, 55.7, 55.8, 56.6, 57.3, 67.6, 73.9, 80.4, 127.4, 128.8, 128.9, 129.0, 130.3, 135.2, 135.8, 169.3, 171.4, 171.5. MS (ES) *m/z*: 731 [M + Na], 707 [M − H]. HRMS (FAB) *m/z* calcd for $C_{40}H_{56}N_2O_9 + H_1$: 709.4064. Found: 709.4059. A slower eluting chromatography fraction upon drying under high vacuum produced a diastereomer of the tetradepsipeptide (1.04 g, 23%), which had ^{1}H NMR and ^{13}C NMR spectra that were very similar to the above. $[\alpha]^{25}_D$ +8° (*c* 0.94, MeOH). MS (ES) *m/z*: 731 [M + Na], 707 [M − H]. HRMS (FAB) *m/z* calcd for $C_{40}H_{56}N_2O_9 + H_1$: 709.4064. Found: 709.4052. An intermediate fraction contained a mixture of the two diastereomers (1.25 g, 28%) for a total yield of coupled product amounting to 4.17 g (93%). The major tetradepsipeptide diastereomer (1.72 g, 2.43 mmol), CH_2Cl_2 (20 mL), and TFA (5 mL) were combined and processed according to the general procedure for removing a Boc protecting group, which gave tetradepsipeptide free amine **53** (1.34 g, 91%) as a clear, colorless, viscous oil. $[\alpha]^{25}_D$ +6° (*c* 1.23, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (m. 12 H), 1.19 (m, 1H), 1.40−2.03 (m, 11 H), 2.52 (s, 3H), 2.90−3.13 (m, 3H), 3.19 (m, 2H), 3.49 (t, J = 7.3 Hz, 1H), 5.06 (d, J = 12.0 Hz, 1H), 5.15 (d, J = 12.0 Hz, 1H), 5.26 (t, J = 5.7 Hz, 1H), 5.39 (m, 2H), 7.12−7.45 (m, 10 H). HRMS (FAB) *m/z* calcd for $C_{35}H_{48}N_2O_7 + H_1$: 609.3539. Found: 609.3553.

***N*-Methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-ϵ-lactam-3-phenyl-D-lactic Acid (54).** Tetradepsipeptide free acid **42** (1.26 g, 2.13 mmol), tetradepsipeptide free amine **53** (1.30 g, 2.13 mmol), a solution of DCC in CH_2Cl_2 (1 M, 2.3 mL, 2.3 mmol), DMAP (13 mg, 0.11 mmol), and CH_2Cl_2 (60 mL) were combined and processed according to the general procedure for coupling peptides using DCC. Chromatography of the crude reaction product followed by drying under high vacuum gave the octadepsipeptide (1.99 g, 78%) as a white solid foam and clear glass. $[\alpha]^{25}_D$ −51° (*c* 1.02, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (m, 24 H), 1.32−2.08 (m, 21 H), 1.44 (bs, 9H), 2.74−3.29 (m, 12 H), 3.01 (s, 3H), 4.39−5.57 (6m, 8H), 5.05 (d, J = 12.0 Hz, 1H), 5.13 (d, J = 12.0 Hz, 1H), 7.02−7.40 (m, 15 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5, 17.0, 21.6, 22.2, 23.5, 23.7, 25.0, 25.1, 25.2, 27.0, 28.7, 37.4, 37.9, 38.1, 55.7, 67.6, 68.2, 73.9, 74.2, 127.4, 128.7, 128.8, 128.9, 129.0, 129.9, 130.3, 135.8, 169.3, 171.4. MS (ES) *m/z*: 1206 [M + Na], 1184 [M + H]. This material (1.98 g, 1.67 mmol), CH_2Cl_2 (30 mL), and TFA (7.5 mL) were combined and processed according to the general procedure for removing a Boc protecting group, which gave the octadepsipeptide free amine (1.75 g, 97%) as a white solid foam. $[\alpha]^{25}_D$ −39° (*c* 1.35, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (m, 24 H), 1.06−2.05 (m, 22 H), 2.52 (m, 2H), 2.79−3.25 (m, 13 H), 3.58 (m, 1H), 5.04 (d, J = 12.0 Hz, 1H), 5.12 (d, J = 12.0 Hz, 1H), 5.17−7.59 (m, 7H), 7.00−7.40 (m, 15 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6, 22.2, 22.6, 22.8, 23.5, 23.6, 25.0, 25.1, 25.2, 32.1, 37.4, 38.1, 67.6, 73.9, 127.4, 128.7, 128.9, 128.9, 129.0, 129.8, 130.3, 135.8, 169.3, 171.4. HRMS (FAB) *m/z* calcd for $C_{61}H_{86}N_4O_{13} + H_1$: 1083.6270. Found: 1083.6304. The octadepsipeptide free amine (1.72 g, 1.59 mmol), absolute EtOH (150 mL), and 10% Pd on activated carbon (360 mg) were combined and processed according to the general procedure for removing a benzyl protecting group, which gave octadepsipeptide amino acid **54** (1.48 g, 94%) as a tan, solid foam and glass. $[\alpha]^{25}_D$ −23° (*c* 0.75, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (m, 24 H), 1.12−2.08 (m, 21 H), 2.60 (m, 2H), 2.79 (m, 13 H), 3.89 (m, 1H), 5.00−5.61 (m, 7H), 7.28 (m, 10 H), 7.69 (bs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5, 16.6, 21.6, 21.9, 22.6, 23.2, 23.5, 23.6, 25.3, 25.3, 37.8, 60.7, 72.5, 126.8, 127.5, 128.5, 128.9, 129.8, 129.9, 170.7, 171.3. HRMS (FAB) *m/z* calcd for $C_{54}H_{80}N_4O_{13} + H_1$: 993.5800. Found: 993.5825.

ϵ-Lactam Cyclodepsipeptide (4). DPEA (0.65 mL, 480 mg, 3.73 mmol) was added to a 1 mM solution of octadepsipeptide amino acid **54** (1.48 g, 1.49 mmol) in CH_2Cl_2 (1.5 L) at 0 °C. After the mixture was stirred for 5 min, powdered BOP-Cl (455 mg, 1.79 mmol) was added and the reaction mixture was stirred at room temperature. After 22 h, the reaction came to a halt although TLC indicated the presence of unreacted starting materials; addition of more DPEA and BOP-Cl was without effect. The reaction mixture was concentrated to 800 mL and washed with aqueous $NaHCO_3$. The resulting emulsion was broken up by adding saturated NaCl. The organic layer was filtered through anhydrous Na_2SO_4 and dried (Na_2SO_4). Filtration, concentration, and drying under high vacuum gave crude product. This was further purified by chromatography (50% EtOAc in hexane) followed by high vacuum-drying to give ϵ-lactam cyclodepsipeptide **4** (628 mg, 43%) as a white, solid foam. $[\alpha]^{25}_D$ −97° (*c* 0.92, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70−1.08 (m, 27H), 1.20−2.20 (m, 18 H), 2.65 (s, ∼0.2 H), 2.75 (s, ∼0.2 H), 2.78 (s, ∼2.8 H), 2.80 (s, ∼0.1 H), 2.82 (s, ∼2.8 H), 2.90 (s, ∼0.2 H), 2.97 (s, ∼2.7 H), 3.00−3.49 (m, 6H), 4.45 (t, J = 7.5 Hz, 1H), 5.06 (dd, J = 6.8, 13.8 Hz, 1H), 5.30 (dd, J = 4.3, 11.8 Hz, 1H), 5.36 (dd, J = 2.7, 12.9 Hz, 1H), 5.44 (dd, J = 4.2, 12.3 Hz, 1H), 5.53 (dd, J ≈ 3, 8.1 Hz, 1H), 5.65 (t, J = 7.6 Hz, 1H), 5.71 (t, J = 7.7 Hz, 1H), 7.23 (m, 10 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.4, 16.1, 21.3, 21.4, 21.5, 22.1, 23.6, 23.7, 24.4, 25.0, 25.5, 27.2, 28.4, 29.7, 30.8, 30.8, 31.5, 31.9, 36.7, 36.9, 38.0, 38.1, 38.4, 45.3, 54.5, 54.5, 55.9, 57.5, 69.0, 70.7, 71.6, 73.1, 127.4, 127.5, 128.8, 128.9, 128.9, 129.8, 135.4, 135.5, 169.9, 170.3, 170.5, 170.6, 171.1, 171.3, 171.4, 171.7. MS (ES) *m/z*: 997 [M + Na]. HRMS (FAB) *m/z* calcd for $C_{54}H_{78}N_4O_{12} + H_1$: 975.5694. Found: 975.5711. Infrared (mull, cm^{-1}): 1744 (ester carbonyl), 1666 (amide carbonyl). UV (1%, 1 cm, MeOH) λ_{max} (ϵ): 250 (4.7), 263 (3.7). Anal. ($C_{54}H_{78}N_4O_{12}$) C, H, N.

Synthesis of Proline Cyclodepsipeptide (5). *N*-Boc-L-Prolyl-D-lactic Acid (57). *N*-Boc-L-proline **55** (2.15 g, 10 mmol), methyl D-lactate **56** (1.2 g, 12 mmol), DIC (1.6 mL, 10 mmol), DMAP (0.6 g, 5 mmol), and CH_2Cl_2 (25 mL) were combined and processed according to the general procedure for coupling peptides using DIC (reaction time of 1 h). In an exception to the general procedure, the crude product was partitioned between CH_2Cl_2 (150 mL) and 0.3 N HCl (100 mL). The organic layer was washed with 5% K_2CO_3 (100 mL), dried ($MgSO_4$), and concentrated to give the didepsipeptide as an oil (2.22 g, 74%), which slowly solidified. $[\alpha]^{25}_D$ −26.3° (*c* 0.89, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 and 1.45 (s, 9H), 1.4−1.6 (m, 3H), 1.8−2.4 (m, 4H), 3.4−3.7 (m, 2H), 3.74 and 3.75 (2s, 3H), 4.3−4.4 (m, 1H), 5.13 and 5.18 (2q, 1H). HRMS (FAB) *m/z* calcd for $C_{14}H_{23}NO_6 + H_1$: 302.1603. Found: 302.1602. This material (2.1 g, 7.0 mmol) was dissolved in MeOH (25 mL) and treated with 1 N NaOH (8 mL, 8 mmol) at room temperature for 20 min. The mixture was poured into H_2O (20 mL) and extracted with Et_2O (2 × 30 mL). The aqueous layer was acidified with 1 N HCl (40 mL). The resulting mixture was extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated to give didepsipeptide free acid **57** as an oil (1.4 g, 70%), which slowly solidified. $[\alpha]^{25}_D$ −39.7° (*c* 0.90, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 and 1.46 (s, 9H), 1.54 (d, 3H), 1.8−2.5 (m, 4H), 3.4−3.7 (m, 2H), 4.3−4.4 (m, 1H), 5.12 and 5.28 (q, 1H). HRMS (FAB) *m/z* calcd for $C_{13}H_{21}NO_6 + H_1$: 288.1447. Found: 288.1449.

***N*-Boc-L-prolyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactic Acid (58).** Didepsipeptide free acid **57** (5.47 g, 19 mmol), didepsipeptide free amine **23** (7.2 g, 19 mmol), DIC (3.27 mL, 20.9 mmol), DMAP (464 mg, 3.8 mmol), and CH_2Cl_2 (50 mL) were combined and processed according to the general procedure for coupling peptides using DIC (reaction time of 16 h). The residue was chromatographed (10% EtOAc in hexane) to give the tetradepsipeptide as an oil (10 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.9−2.3 (m, 23H), 2.7−2.9 (m, 3H), 3.0−3.6 (m, 4H), 4.3−5.4 (m, 6H), 7.1−7.4 (m, 10H). This material (5.0 g, 7.7 mmol), absolute EtOH (100 mL), and 10% Pd on activated carbon (1.2 g) were combined and processed according to the general procedure for removing a benzyl protecting group to give tetradepsipeptide free acid **58** (4.18 g, 98%) as a solid. $[\alpha]^{25}_D$ −29.7° (*c* 0.80, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8−2.3 (m, 23H), 2.9−3.6 (m, 7H), 4.1−5.7 (m, 4H), 7.2−7.4 (m, 5H). HRMS (FAB) *m/z* calcd for $C_{29}H_{42}N_2O_9 + H_1$: 563.2968. Found: 563.2980.

L-Prolyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactic Acid (59). Tetradepsipeptide free acid **58** (0.82 g, 1.46 mmol), tetradepsipeptide free amine **25** (0.895 g, 1.54 mmol), a solution of DCC in CH_2Cl_2 (1 M, 1.65 mL, 1.65 mmol), DMAP (40 mg, 0.33 mmol), and CH_2Cl_2 (20 mL) were combined and processed according to the general procedure for coupling peptides using DCC. The residue was chromatographed (10−20% acetone in hexane) to give the octadepsipeptide as a solid (1 g, 61%). $[\alpha]^{25}_D$ −51.8° (*c* 0.35, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8−2.3 (m, 42H), 2.7−3.6 (m, 15H), 4.3−5.6 (m, 10H), 7.1−7.5 (m, 15H). HRMS (FAB) *m*/*z* calcd for $C_{62}H_{86}N_4O_{15}$ + Na_1: 1149.5986. Found: 1149.5994. The octadepsipeptide (1.0 g, 0.89 mmol), CH_2Cl_2 (27 mL), and TFA (3 mL) were combined and processed according to the general procedure for removing a Boc protecting group with the exception that the reaction mixture was slowly poured into saturated K_2CO_3 (30 mL) with rapid stirring. The mixture was transferred to a separatory funnel and shaken, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 and the organic layers were combined, washed with H_2O, dried (Na_2SO_4), filtered, and concentrated to give octadepsipeptide free amine as an oil (0.84 g, 90%). This material (0.80 g, 0.78 mmol), absolute EtOH (100 mL), and 10% Pd on activated carbon (0.2 g) were combined and processed according to the general procedure for removing a benzyl protecting group to give octadepsipeptide amino acid **59** (0.7 g, 95%) as a solid. It was used without further purification.

Proline Cyclodepsipeptide (5). Octadepsipeptide amino acid **59** (550 mg, 0.59 mmol) was dissolved in CH_2Cl_2 (590 mL) to give a 1 mM solution, which was treated with BOP reagent (262 mg, 0.59 mmol) and *N*-methylmorpholine (0.065 mL, 0.60 mmol) at room temperature (reaction time of 16 h). The residue was purified by chromatography (50% EtOAc in hexane) to give proline cyclodepsipeptide **5** (60 mg, 12%) as a solid. $[\alpha]^{25}_D$ −65° (*c* 0.80, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8−2.4 (m, 37H), 2.7−3.3 (m, 15H), 3.5−6.0 (m, 8H), 7.2−7.4 (m, 10H). HRMS (FAB) *m*/*z* calcd for $C_{50}H_{70}N_4O_{12}$ + H_1: 941.4888. Found: 941.4905. Anal. ($C_{50}H_{70}N_4O_{12}\cdot H_2O$) C, H, N.

Synthesis of Pipecolyl Cyclodepsipeptide (6). *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactic Acid (61). *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactic acid[2] **60** (4.0 g, 6.8 mmol), didepsipeptide free amine **23** (2.6 g, 6.8 mmol), CH_2Cl_2 (50 mL), DIC (1.17 mL, 7.5 mmol), and DMAP (0.1 g, 0.8 mmol) were combined and processed according to the general procedure for coupling peptides using DIC. Chromatography of the crude reaction product (20% acetone in hexane) gave the hexadepsipeptide as an oil (3.4 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8−1.9 (m, 45H), 2.6−3.3 (m, 13H), 4.1−5.5 (m, 8H), 7.1−7.4 (m,15H). MS (FAB) *m*/*z*: 958 [M + H]. This material (3.4 g, 3.55 mmol), absolute EtOH (100 mL), and 10% Pd on activated carbon (1.1 g) were combined and processed according to the general procedure for removing a benzyl protecting group. The residue was dried under high vacuum to give hexadepsipeptide free acid **61** (2.5 g, 81%) as a semisolid. It was used without further purification. $[\alpha]^{25}_D$ −43.5° (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.7−1.8 (m, 45H), 2.6−3.3 (m, 13H), 4.6−5.5 (m, 6H), 7.2−7.4 (m, 10H). HRMS (FAB) *m*/*z* calcd for $C_{21}H_{29}N_1O_6$ + H_1: 392.2073. Found: 392.2086.

***N*-Boc-L-pipecolic Acid (62).** L-Pipecolic acid (517 mg, 4 mmol) was suspended in THF (20 mL) and treated with di-*tert*-butyl dicarbonate (960 mg, 4.4 mmol) and Et_3N (1.1 mL, 8 mmol). The mixture was refluxed for 2 h, cooled to room temperature, and then stirred for 16 h. The precipitate was removed by filtration, and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with 0.5 N HCl (20 mL). The organic layer was separated, dried ($MgSO_4$), and concentrated to give *N*-Boc-L-pipecolic acid **62** (640 mg, 2.8 mmol, 70%).

Benzyl L-Pipecolyl-D-lactate (64). *N*-Boc-L-pipecolic acid **62** (640 mg, 2.8 mmol) was dissolved in Et_2O (20 mL), and triphenylphosphine (880 mg, 3.36 mmol) and benzyl L-lactate[6] **63** (0.5 g, 2.8 mmol) were added. The resulting mixture was treated with a solution of DEAD (0.5 mL, 3.17 mmol) in Et_2O (5 mL) at room temperature over 20 min. The mixture was stirred for an additional 1 h, and the precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by chromatography (10% EtOAc in hexane) to give the didepsipeptide (0.37 g, 24%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.1−2.3 (m, 18H), 2.8−3.0 (m, 1H), 3.8−4.1 (m, 1H), 4.7−5.2 (m, 4H), 7.2−7.4 (m, 5H). HRMS (FAB) *m*/*z* calcd for $C_{21}H_{29}NO_6$ + H_1: 392.2073. Found: 392.2086. The didepsipeptide (370 mg, 0.95 mmol), CH_2Cl_2 (13 mL), and TFA (2 mL) were combined and processed according to the general procedure for removing a Boc protecting group to give didepsipeptide free amine **64** (0.204 g, 74%) as a clear, pale-yellow oil. It was used without further purification.

***N*-Methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-L-pipecolyl-D-lactic Acid (65).** Hexadepsipeptide free acid **61** (417 mg, 0.48 mmol), didepsipeptide free amine **64** (0.141 g, 0.48 mmol), DIC (0.09 mL, 0.51 mmol), DMAP (10 mg, 0.08 mmol), and CH_2Cl_2 (20 mL) were combined and processed according to the general procedure for coupling peptides using DIC (reaction time of 16 h). Chromatography of the residue (20% acetone in hexane) gave the octadepsipeptide as a semisolid (130 mg, 24%). HRMS (FAB) *m*/*z* calcd for $C_{63}H_{88}N_4O_{15}$ + H_1: 1141.6324. Found: 1141.6293. This material (130 mg, 0.11 mmol), CH_2Cl_2 (18 mL), and TFA (2 mL) were combined and processed according to the general procedure for removing a Boc protecting group to give the octadepsipeptide free amine as an oil (0.1 g, 84%). This material (100 mg, 0.096 mmol), absolute EtOH (50 mL), and 10% Pd on activated carbon (0.1 g) were combined and processed according to the general procedure for removing a benzyl protecting group to give octadepsipeptide amino acid **65** (0.08 g, 88%) as a solid. It was used without further purification.

Pipecolyl Cyclodepsipeptide (6). Octadepsipeptide amino acid **65** (80 mg, 0.084 mmol) was dissolved in CH_2Cl_2 (20 mL) to give a 4 mM solution. Triethylamine (0.05 mL, 0.36 mmol) was added followed by addition of 1-methyl-2-chloropyridinium iodide (30 mg, 0.12 mmol, 1.4 equiv) at room temperature. After being stirred for 16 h, the mixture was washed with 1 N HCl (30 mL) and the organic layer was separated, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (30% acetone in hexane) to give pipecolyl cyclodepsipeptide **6** (40 mg, 50%) as a solid. $[\alpha]^{25}_D$ −74.1° (*c* 0.48, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8−2.4 (m, 39H), 2.6−3.4 (m, 15H), 3.5−5.8 (m, 8H), 7.1−7.4 (m, 10H). HRMS (FAB) *m*/*z* calcd for $C_{51}H_{72}N_4O_{12}$ + Na_1: 955.5044. Found: 955.5039. Anal. ($C_{51}H_{72}N_4O_{12}\cdot 0.5H_2O$) C, H, N.

Synthesis of Azepanyl Cyclodepsipeptide (7). *tert*-Butyl (3*S*,5*S*,6*R*)-3-(5-Chloropentyl)-2-oxo-5,6-diphenyl-4-morpholinecarboxylate (66). To a solution of *tert*-butyl (2*R*,3*S*)-6-oxo-2,3-diphenyl-4-morpholinecarboxylate[7] **66** (4 g, 11.3 mmol), 1-iodo-5-chloropentane (7.7 g, 33 mmol), THF (160 mL), and HMPA (18 mL) was added a solution of sodium bis(trimethylsilyl)amide in THF (1 M, 17 mL, 17 mmol) dropwise at −78 °C. After 1.5 h, the dry ice bath was removed. After an additional 1 h, the reaction mixture was poured into EtOAc, washed with H_2O and brine, dried ($MgSO_4$), filtered, concentrated, and crystallized from hexanes to afford oxazinone **67** (3.8 g, 73%) as a yellow solid. $[\alpha]^{25}_D$ −62.8° (*c* 0.97, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 and 1.46 (2s, 9H), 1.3−2.4 (m, 8H), 3.57 (quintet, 2H), 4.8−5.3 (m, 2H), 5.94 (br s, 1H), 6.4−6.6 (m, 2H), 6.9−7.3 (m, 13H). HRMS (FAB) *m*/*z* calcd for $C_{26}H_{32}Cl_1NO_4$ + H_1: 458.2094. Found: 458.2098.

(3*R*,4*S*,10a*S*)-3,4-Diphenyloctahydro-1*H*-[1,4]oxazino-[4,3-*a*]azepin-1-one (69). Oxazinone **67** (2.55 g, 5.57 mmol) was dissolved in a solution of TFA in CH_2Cl_2 (20%, 25 mL). The reaction mixture was stirred for 3−4 h and then slowly poured into saturated K_2CO_3 (30 mL) with rapid stirring. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The organic phases were combined, washed with H_2O, dried ($MgSO_4$), filtered, and concentrated to give the deprotected oxazinone **68** (1.82 g, 87%) as an oil. This material

was dissolved in acetonitrile (25 mL) and treated with K_2CO_3 (786 mg, 5.70 mmol) and KI (946 mg, 5.70 mmol). The mixture was heated at reflux for 24 h, taken up in EtOAc (100 mL), and washed with H_2O. The organic layer was separated, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (20% Et_2O in hexane) to give bicyclic oxazine **69** (800 mg, 45%) as an oil, which slowly solidified. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.5−2.0 (m, 7H), 2.3−2.4 (m, 1H), 2.5−2.6 (m, 1H), 2.7−2.9 (m, 1H), 3.97 (dd, J = 3.5, 10.5 Hz, 1H), 4.24 (d, J = 3.0 Hz, 1H), 5.94 (d, J = 3.0 Hz, 1H), 6.7−7.3 (m, 10H). HRMS (FAB) m/z calcd for $C_{21}H_{23}NO_2 + H_1$: 322.1807. Found: 322.1804.

(2*S*)-2-Azepanecarboxylic Acid (70). To a solution of bicyclic oxazine **69** (100 mg, 0.31 mmol) in 1:2 THF/EtOH (21 mL) was added $PdCl_2$ (30 mg). The reaction mixture was hydrogenated at 50 psi for 20 h. The mixture was then purged with N_2, filtered through Celite to remove the catalyst, and concentrated. The residue was crystallized from MeOH/acetone/Et_2O to give azepanecarboxylic acid **70** as a white solid (43 mg, 96%). $[\alpha]^{25}_D$ −23° (*c* 0.76, MeOH). Lit. value:[18] $[\alpha]^{25}_D$ −21° (*c* 1.02, H_2O). ^{1}H NMR (400 MHz, CD_3OD): δ 1.6−2.1 (m, 7H), 2.3−2.5 (m, 1H), 3.2−3.4 (m, 2H), 4.1−4.2 (m, 1H).

(2*S*)-1-(*tert*-Butoxycarbonyl)-2-azepanecarboxylic Acid (71). Azepanecarboxylic acid **70** (330 mg, 2.3 mmol) was dissolved in 7:3 THF/H_2O (20 mL) and treated with di-*tert*-butyl dicarbonate (646 mg, 2.99 mmol) and Et_3N (0.83 mL, 6 mmol). The mixture was stirred for 16 h at room temperature and then partitioned between Et_2O (30 mL) and H_2O (20 mL). The layers were separated, and the aqueous layer was acidified to pH 2 with 1 N HCl. This was extracted with Et_2O (2 × 25 mL) and all organic layers were combined, dried ($MgSO_4$), and concentrated to give *N*-Boc-azepanecarboxylic acid **71** (335 mg, 60%), which slowly solidified. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.1−2.0 (m, 7H), 1.43 and 1.48 (2s, 9H), 2.2−2.4 (m, 1H), 2.9−3.1(m, 1H), 3.8−4.0 (m, 1H), 4.4−4.7 (2 m, 1H). HRMS (FAB) m/z calcd for $C_{12}H_{21}NO_4 + H_1$: 244.1549. Found: 244.1549.

Benzyl L-Azepanyl-D-lactate (73). *N*-Boc-azepanecarboxylic acid **71** (200 mg, 0.82 mmol), benzyl D-lactate[6] **72** (148 mg, 0.86 mmol), a solution of DIC in CH_2Cl_2 (1 M, 0.86 mL, 0.86 mmol), DMAP (10 mg, 0.08 mmol), and CH_2Cl_2 (20 mL) were combined and processed according to the general procedure for coupling peptides using DIC (reaction time of 16 h). The residue was chromatographed (10% EtOAc in hexane) to give the didepsipeptide as an oil (166 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.1−2.0 (m, 11H), 1.42 and 1.49 (2s, 9H), 2.2−2.4 (m, 1H), 2.8−3.0 (m, 1H), 3.8−4.0 (m, 1H), 4.4−4.8 (m, 1H), 5.1−5.2 (m, 3H), 7.2−7.4 (m, 5H). MS (EI) m/z: 405 [M]. HRMS (FAB) m/z calcd for $C_{22}H_{31}NO_6 + H_1$: 406.2229. Found: 406.2236. This material (150 mg, 0.37 mmol), CH_2Cl_2 (9 mL), and TFA (1 mL) were combined and processed according to the general procedure for removing a Boc protecting group to give didepsipeptide free amine **73** (100 mg, 89%) as a semisolid.

***N*-Methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-L-azepanyl-D-lactic Acid (74).** Hexadepsipeptide free acid **61** (347 mg, 0.40 mmol) and didepsipeptide free amine **73** (0.100 g, 0.333 mmol) were dissolved in CH_2Cl_2 (20 mL), and the solution was cooled to 0 °C. BOP-Cl (204 mg, 0.80 mmol) and Et_3N (0.14 mL, 1.0 mmol) were added, and the reaction mixture was slowly warmed to room temperature where it was stirred for 16 h. The reaction mixture was concentrated, and the residue was chromatographed (25% acetone in hexane) to give the octadepsipeptide as a semisolid (184 mg, 48%). HRMS (FAB) m/z calcd for $C_{64}H_{90}N_4O_{15} + Na_1$: 1177.6300. Found: 1177.6326. This material (180 mg, 0.16 mmol), CH_2Cl_2 (18 mL), and TFA (2 mL) were combined and processed according to the general procedure for removing a Boc protecting group (reaction time of 1.5 h) to give the octadepsipeptide free amine as a semisolid (148 mg, 90%). The amine (148 mg, 0.155 mmol), absolute EtOH (50 mL), and 10% Pd on activated carbon (50 mg) were combined and processed according to the general procedure for removing a benzyl protecting group. The residue was dried under high vacuum to give octadepsipeptide amino acid **74** (135 mg, 90%) as a solid.

Azepanyl Cyclodepsipeptide (7). Octadepsipeptide amino acid **74** (135 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (20 mL) to give a 7 mM solution, which was treated with Et_3N (0.08 mL, 0.56 mmol) and 1-methyl-2-chloropyridinium iodide (50 mg, 0.20 mmol, 1.4 equiv). The reaction mixture was stirred at room temperature for 16 h and washed with 1 N HCl (30 mL), and the organic layer was separated, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (35% acetone in hexane) to give azepanyl cyclodepsipeptide **7** (45 mg, 34%) as a solid. $[\alpha]^{25}_D$ −76.0° (*c* 0.48, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8−2.0 (m, 41H), 2.1−3.1 (m, 13H), 3.2−5.7 (m, 10H), 7.1−7.4 (m, 10H). HRMS (FAB) m/z calcd for $C_{52}H_{74}N_4O_{12} + H_1$: 947.5381. Found: 947.5401. Anal. ($C_{52}H_{74}N_4O_{12}\cdot H_2O$) C, H, N.

Biology. The relative potency of PF1022 A and the CDPs of this report was determined in an anthelmintic assay utilizing immunosuppressed jirds, *Meriones unguiculatus*, infected with the ruminant parasite *Haemonchus contortus*. Outbred female jirds weighing 30−35 g (Charles River Laboratories, Stone Ridge, NY) were used. Upon arrival, three jirds each were randomly assigned to translucent polypropylene cages (26.7 cm × 21.6 cm × 14.0 cm) containing wood shavings and given commercial rodent chow (Purina 5002) and water ad libitum. After a 2-day acclimation period, the jirds were switched to a modification of the commercial chow containing 0.02% hydrocortisone (medicated) and remained on this chow for the duration of the study. Five days after being placed on medicated food, each jird was inoculated with ∼1000 exsheathed infective stage larvae, which had been incubated for 1 h in Earle's balanced salt solution as described by Conder and Johnson.[8] Inoculations were administered per os using an 18 gauge dosing needle fitted to a 1 cm^3 syringe. The lavae were a strain of *H. contortus* originally obtained from Dr. K. S. Todd, Jr. (University of Illinois, Urbana, Illinois), which has been maintained continuously in sheep at Pharmacia and Upjohn. The jirds were allocated randomly by cage to treatment groups (three animals/group) on day 10 postinoculation (PI). The test compound was suspended in DMSO (17%)/carboxymethylcellulose (83%) vehicle using a vortex mixer, and the desired treatment dose was administered orally to each jird in a 0.2 mL volume using an 18 gauge dosing needle fitted to a 1 cm^3 syringe. Control animals for each experiment were dosed with vehicle alone. Treatment, necropsy, examination of stomach contents, and determination of compound activity were done as described by Conder.[8] On day 3 posttreatment (day 13 PI), all jirds were killed by CO_2 inhalation, their stomachs removed, longitudinally opened, and put in separate scintillation vials containing 14 mL of distilled water. The vials were vortexed and placed in a 37 °C water bath and incubated for 5 h. Following incubation, 1 mL of formaldehyde solution was added to each vial and the vials were stored for subsequent examination. Upon examination, the contents of each vial were vortexed, the tissue was removed, and the entire vial contents were examined for worms using a dissecting microscope (15−45×). Percentage clearance for each compound was determined using the following formula:

$$\text{percentage clearance} = \{[(\text{mean number of worms recovered from vehicle control group}) - (\text{mean number of worms recovered from treated group})]/(\text{mean number of worms recovered from vehicle control group})\} \times 100$$

Uniformity studies were run to refine the jird model and to provide estimates of variance components to be used in defining a mathematical model for the distribution of worm burdens in the animals. Untreated vehicle control and standard anthelmintic treatments were evaluated in test groups of three, six, and nine jirds per treatment group. Information from these trials showed little effect in increasing the number of jirds from three to nine in each treatment group/trial. A

cutoff was chosen that would result in no more than a 5% error for a true 90% clearance compound when three jirds per trial were used.

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(16) In one case where this reaction was performed on a 1 g scale, ^{1}H NMR showed the product to consist of an 85:15 mixture (70% de) of δ-lactam benzyl ester **39** and δ-lactam benzyl ester **31**, but the yield was 68%.

(17) This reaction gave variable results. Other batches required much less reaction time, typically 1−3 h, and smaller amounts of catalyst, and they contained fewer acid byproducts.

(18) Seebach, D.; Dziadulewicz, Ed.; Behrendt, L.; Cantoreggi, S.; Fitzi, R. Synthesis of nonproteinogenic (*R*)- or (*S*)-amino acids. Analogs of phenylalanine, isotopically labelled and cyclic amino acids from *tert*-butyl 2-(*tert*-butyl)-3-methyl-4-oxo-1-imidazolidinecarboxylate (Boc-BMI). *Liebigs Ann. Chem.* **1989**, *12*, 1215−1232.

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