Design, Synthesis, and Evaluation of a Depsipeptide Mimic of Tendamistat

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The cyclic hexadepsipeptide framework of enniatin B was identified as a template matching the β-turn tripeptide of tendamistat. The modified analog **1** was synthesized as a tendamistat mimic and compared to the acyclic derivative **2** and the tripeptide Ac-Try-Arg-Tyr-OMe. These compounds were assembled from the dimeric esters **3**−**5**. As an inhibitor of α-amylase, **1** is twice as potent as **2** and comparable to the tripeptide. NMR studies of **1** reveal four conformers in equilibrium in a 50:25:15:10 ratio; the ring conformation of the major component is similar to that of the enniatin B template, with the *cis* geometry of the α-hydroxyisovaleryl-*N*-methylvaline amide linkage; the other conformers differ in the position or presence of the *cis* amide linkage.

Introduction

β-Turns are common motifs in peptide−protein interactions. Their convex structure presents the amino acid side chains in a dense array, providing variability and, thus, specificity in an efficient format. This secondary structure consists of four residues in which the carbonyl oxygen of the *i* residue is hydrogen bonded to the amide hydrogen of the $i + 3$ residue.[1−3] β-Turns are central to many physiologically important interactions,[4−6] which has prompted the design of a number of rigid mimics and strategies for inducing the conformation in an oligopeptide.[7−11] One of the most straightforward methods of inducing a β-turn is to incorporate the desired sequence in a cyclic peptide,[12] an approach that has been used successfully in designing mimics of somatostatin,[13,14] tendamistat,[15,16] and other ligands.[17−20]

Tendamistat, a potent microbial inhibitor of α-amylase,[21] has been used as a design target for β-turn templates because of the prominent role that the tripeptide sequence ^{18}Trp-^{19}Arg-^{20}Tyr plays in the interaction with the enzyme active site.[22,23] The crystal and NMR structures of tendamistat show these residues to be in a slightly distorted type I β-turn conformation.[24,25] A number of approaches to the design of small molecule inhibitors of tendamistat have been described (Figure 1): incorporating the Trp-Arg-Tyr sequence in a cyclic hexapeptide framework,[15,16] complexing it with copper ion,[26] and embedding it in a rigid, polycyclic template.[27]

The previously described designs of tendamistat mimics[15,26,27] have used the program CAVEAT to identify templates that match the backbone of the β-turn and that can position the amino acid side chains of the ^{18}Trp-^{19}Arg-^{20}Tyr unit in the same orientation.[28] A CAVEAT search of the Cambridge Structural Database[29] also revealed that the backbone and side-chain atoms of enniatin B (CSD code VHVIMH10) provide a very close match to those of the tendamistat binding loop (see Figure 1d);[30] enniatin B is a cyclic hexa*depsi*peptide with DLLLLL stereochemistry.[31] The cyclic hexadepsipeptide is an intriguing template for structure-based design for several reasons. The macrocycle itself provides an entropic advantage in binding if the molecule is constrained in the correct conformation. The cyclic structure and N-methylation convey protease resistance and oral bioavailability, as exemplified by cyclosporin A[32,33] and a number of somatostatin and oxytocin mimics.[13,34] Additionally, the template is comprised of readily available α-hydroxy acid and *N*-methylamino acid subunits.

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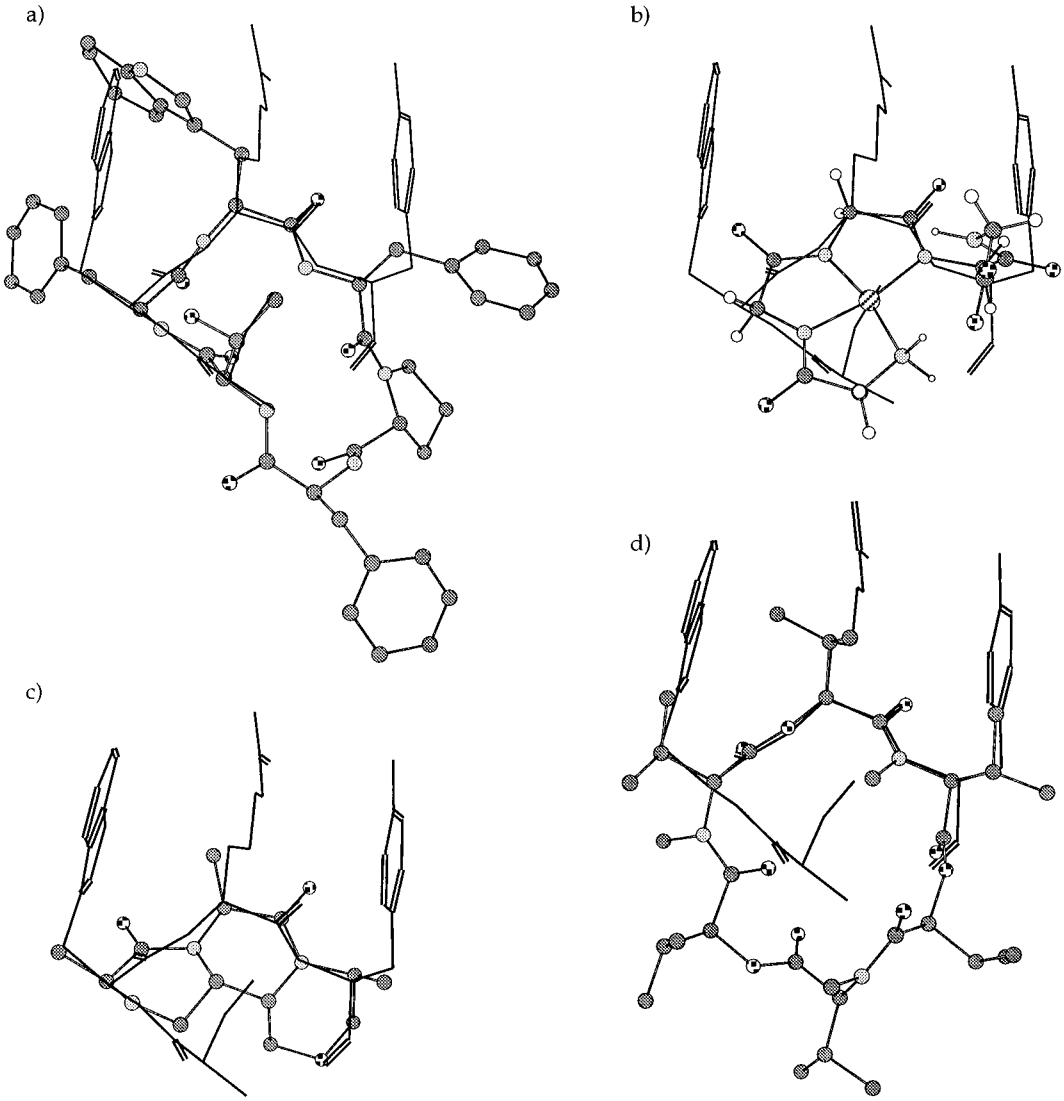

Figure 1. Tendamistat β-turn[24] (bare lines) superimposed on peptidomimetic templates (spherical atoms): (a) a cyclic hexapeptide;[15,16] (b) a tetrapeptide−copper complex;[26] (c) a rigid tricycle;[27] and (d) the cyclic hexadepsipeptide enniatin B.

Transformation of this structural lead into a potential tendamistat mimic involves replacement of the isopropyl side chains at the matching positions of enniatin B with the indolylmethyl, guanidinopropyl, and hydroxybenzyl groups of the tendamistat β-turn (Figure 2). In this paper, we describe the synthesis of this derivative, **1**, a comparison of its binding affinity with other tendamistat mimics and the acyclic analog **2**, and determination of its solution conformations by NMR.

Results

Synthesis. In principle, the cyclic hexadepsipeptide **1** could be synthesized from the hydroxy and amino acid subunits by a number of strategies; however, the ease with which tertiary amides with free hydroxyacyl groups would lactonize argued against assembly of the linear hexamer from the monomers in a linear stepwise sequence or by a convergent strategy in which the amide linkages would be formed first (Scheme 1). Consequently, we formed the dimeric esters **3**−**5** at the outset and assembled the linear hexamer without exposing the hydroxyl groups (Scheme 2). Since the branched side-chain subunits were expected to present the greatest problem in the coupling reactions, the amides adjacent to *N*-methylvaline and α-hydroxyisovalerate residues were formed first; cyclization involved formation of the amide between the hydroxy-Arg and *N*-Me-Tyr precursors. Orthogonal protecting groups were employed to allow selective deprotection of the side chain, amine, or carboxylic acid functional groups. Moreover, we used the ornithine side chain as an arginine precursor to avoid problems that the reactive guanidino group could present in the difficult coupling steps.

The forward synthesis is outlined in Schemes 3−5. L-α-Hydroxyisovaleric acid, **6a**, (Scheme 3) was prepared from L-valine by diazotization[35] and protected as the 2-(trimethylsilyl)ethyl (TMSE) ester, **6b**.[36] The yield was low in this esterification step (ca. 40%) since dimerization of the hydroxy acid was a significant side reaction. Nevertheless, the TMSE protecting group was advanta-

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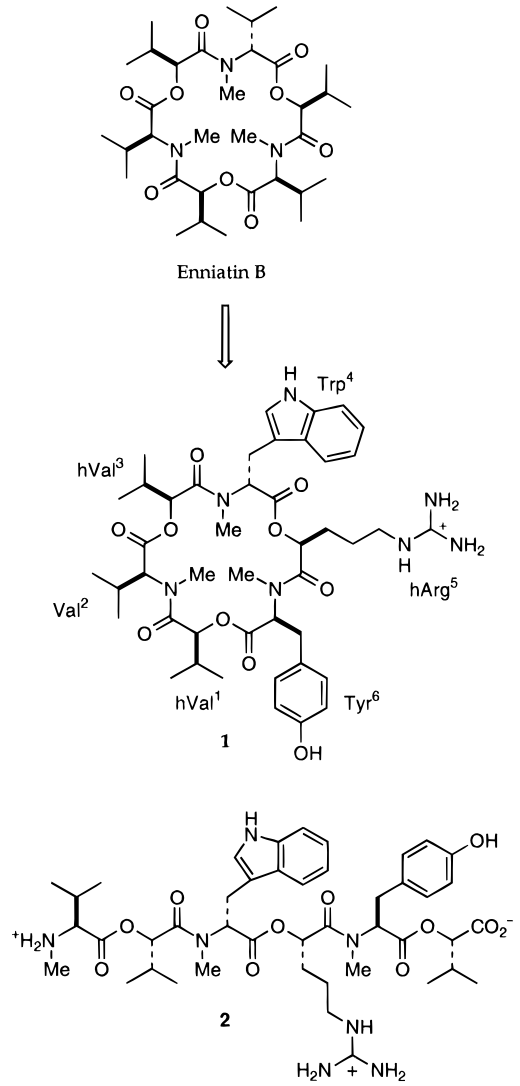
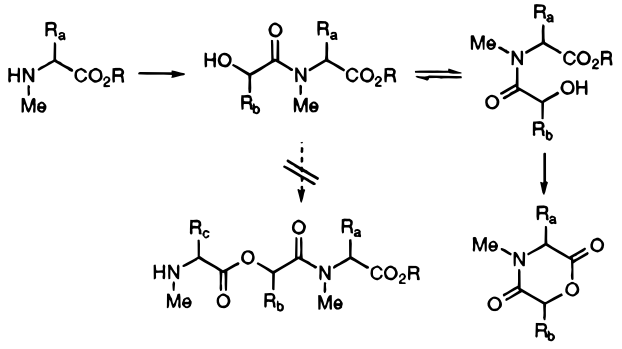


Figure 2. Enniatin B as scaffolding for peptidomimetic **1** and acyclic comparison compound **2**.

Scheme 1



geous since it can be cleaved in the presence of the depsipeptide esters. N-Methylation of *N*-Boc-valine and *N*-Boc-tyrosine benzyl ether was accomplished as described by McDermott and Benoiton.[19] A variety of methods were explored for the subsequent esterification reactions to give **3** and **4**. While the divalyl depsipeptide **4** was formed under standard coupling conditions with EDC and DMAP in pyridine, the formation of **3** required the more active isopropenyl chloroformate reagent. For deprotection of the TMSE esters (e.g., **4** → **4a**), we found that silica-supported tetrabutylammonium fluoride (TBAF),[37] a nonhygroscopic source of fluoride ion, minimized hydrolysis of the depsipeptide esters, which was a significant side reaction with the standard TBAF/THF solution. Also, because of the sensitive depsipeptide esters, Boc deprotection (e.g., **3** → **3a**) was accomplished with dilute TFA in methylene chloride, which gave a cleaner product than the standard conditions with neat TFA. After carboxyl deprotection of **3** and amine deprotection of **4**, the dimers were coupled to form tetramer **7a** with bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP),[38,39] a highly activated coupling reagent that works well with hindered *N*-methyl substrates. The TMSE ester was cleaved to prepare tetramer **7b** for the next coupling step.

The α-hydroxy analog of ornithine was formed by direct diazotization of the diamino acid and protected as the *N*-Cbz, TMSE ester **9c** (Scheme 4).[40,41] In preparing the *N*-methyl-D-tryptophan derivative **10c**, the indole nitrogen was first protected as the *tert*-butyldimethylsilyl derivative to prevent alkylation at this position.[42] Coupling of these two components and subsequent protecting group manipulations afforded the third dimeric intermediate, **5**.

Although the reasons are not readily apparent, the TBDMS group had to be removed prior to coupling the dimeric and tetrameric fragments **5b** and **7b**; otherwise, this reaction proceeded very poorly. HCl in ethyl acetate was used for Boc-deprotection of **5** and a subsequent intermediate (**11b**), since this reagent appeared to minimize *tert*-butylation of the indole ring. PyBroP was used both to couple tetramer **7b** with dimer **5b** and, after *N*- and C-deprotection, to cyclize the linear hexamer **11c** (Scheme 5). Hydrogenolysis of the Cbz and benzyl protecting groups and introduction of the guanidino side chain then afforded the target compound **1**.

The linear analog **2** was synthesized in a similar fashion using the three dimeric fragments from preparation of the cyclic compound (Scheme S1 in the Supporting Information). The coupling and deprotection steps were altered to produce the linear hexadepsipeptide with the residues mimicking the Trp-Arg-Tyr sequence at positions 3−5. After the final coupling step, the side-chain protection was removed by hydrogenolysis and the guanidino group was introduced prior to removal of the terminal protecting groups.

Inhibition. The inhibition constants for the cyclic and linear mimic **1** and **2** were determined as described previously (Table 1).[27] However, due to the poor solubility of **1** in water, the assays were carried out in 5% DMSO/H_2O; K_i values of the control tripeptides WRY and Ac-WRY-OMe were redetermined in this solvent to allow comparison with previous results. Compared to water alone, there is a 2-fold increase in the K_i values of these tripeptides in 5% DMSO/water, which suggests that hydrophobic effects make a significant contribution to the binding energy.[27]

The cyclic hexadepsipeptide **1** has only a 2-fold greater affinity for α-amylase than the linear analog **2**, and it is weaker than the cyclic hexapeptides (e.g., cyclo[D-

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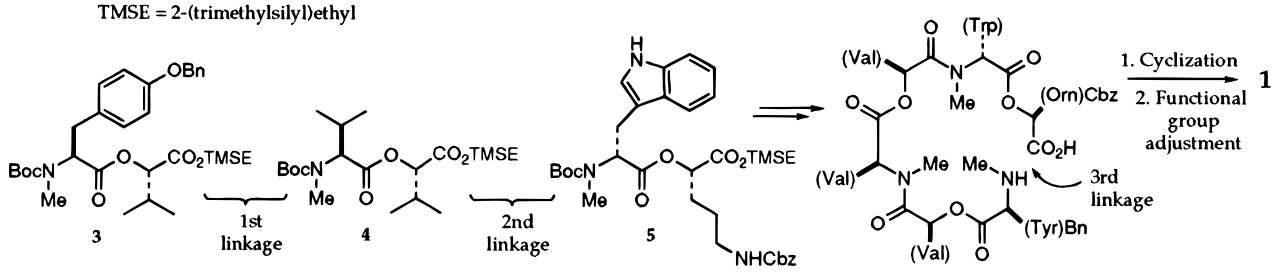
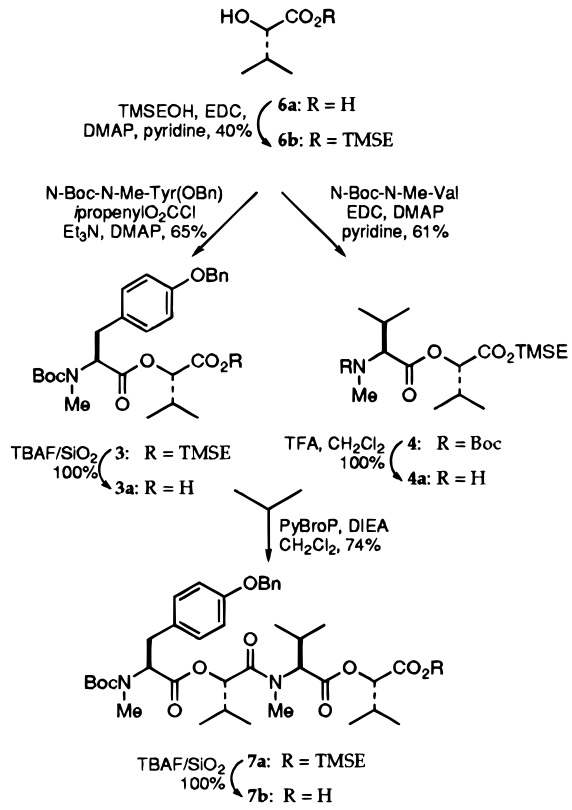
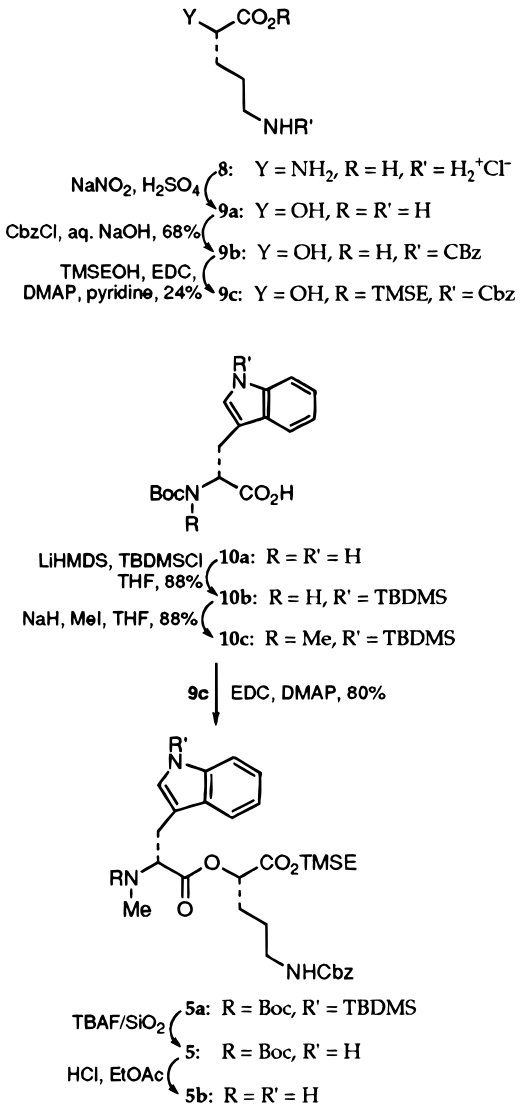
Scheme 2



Scheme 3



Scheme 4



PFAWRY][15,16] or peptide–copper complexes[26] reported previously. To discern whether the cyclic compound is constrained in a conformation that does not complement the active site of α-amylase, the solution structure of compound **1** was determined by NMR.

NMR Structure of 1. Although compound **1** was purified to homogeneity by reversed-phase HPLC, four sets of peaks were observed in the ^{1}H-NMR spectrum reflecting a population composition of 50:25:15:10 at room temperature. A NOESY spectrum was recorded at 50 °C to determine whether the extra peaks arose from coeluting impurities or conformational heterogeneity of **1**. The molecule showed positive nuclear Overhauser enhancements under these conditions, yet a number of the cross peaks were of the opposite sign. These cross peaks arise from chemical exchange, which indicates that the multiplicity of signals at room temperature reflect different conformations of the macrocycle. The structural studies were therefore carried out at 0 °C to ensure that the molecule did not undergo conformational exchange during the mixing time of the NOESY experiments. At this temperature, exchange cross peaks were no longer present, but only two sets of signals were observed because signals from the two least populated conformers were below the detection limits. Thus, only the two most abundant conformers (referred to as "major" and "minor") were studied under these conditions.

For further studies, compound **1** was dissolved in 40% CD_3OD/D_2O with a KH_2PO_4 buffer containing NaCl and $CaCl_2$, and the pH was adjusted to 7.0 with DCl to simulate assay conditions. CD_3OD was used instead of DMSO-d_6 as a cosolvent because satisfactory shimming could not be obtained at 0 °C with the more viscous DMSO solution. Because of the known ability of depsipeptides to chelate metals,[43] spectra were recorded in the absence as well as presence of the sodium and

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Scheme 5

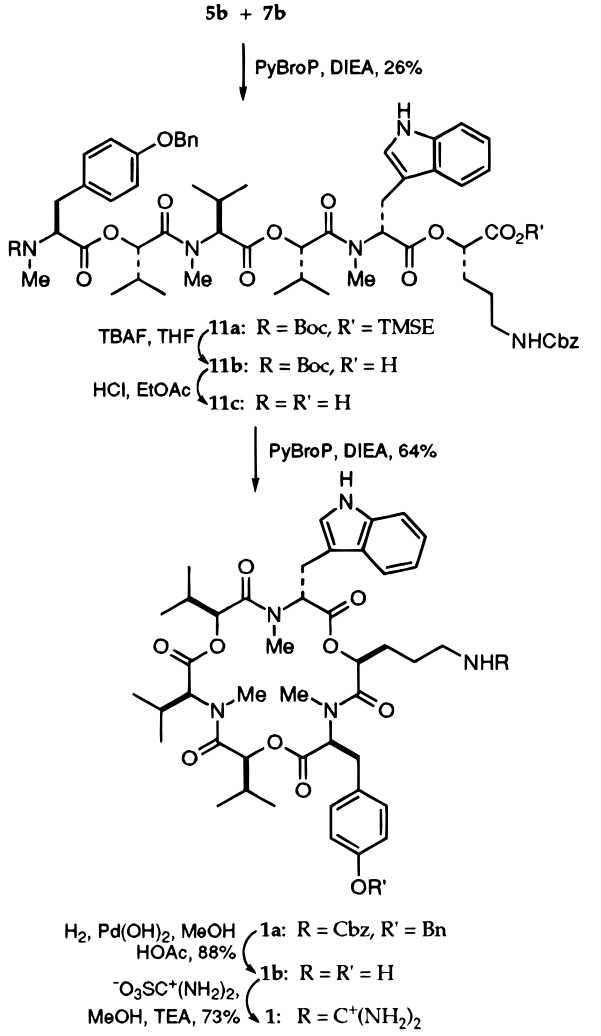


Table 1. Inhibitors of α-Amylase[a]

inhibitor	K_i (μM) in water	K_i (μM) in 5% aqueous DMSO
tendamistat[b]	0.0002	
GWR-D-Y·Cu^{+2} complex	2.4 ± 0.2[c]	
Trp-Arg-Tyr	520 ± 20[d]	870 ± 30
Ac-Trp-Arg-Tyr-OMe	100 ± 3[d]	190 ± 10
c[D-PFAWRY]	14 ± 2[b]	30[e]
2		290 ± 10
1		140 ± 10

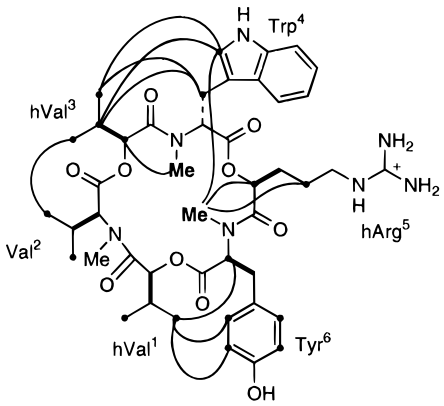
[a] pH 7, 30 °C, using *p*-nitrophenyl maltotrioside as substrate.[27] [b] From ref 21. [c] From ref 26. [d] From ref 15. [e] Estimated from a two-point determination.

calcium salts. No difference was observed, suggesting that cation complexation is not a factor in determining the conformations of the cyclic mimic **1** in 40% CD_3OD/D_2O.

TOCSY and NOESY spectra of **1** were recorded at 0 °C. Interpretation of the TOCSY spectrum was complicated by the three similar Val and hVal patterns and the two benzylic AMX spin systems (see side chain labels in Figure 2);[44] only the resonances from the hArg side chain could be identified uniquely from the TOCSY experiment. The Trp and Tyr residues could be distinguished by NOE cross peaks from the β-proton resonances to their unique

(44) We refer to the side chains by their amino acid equivalents, using the prefix "h" to designate the α-hydroxy analog of the amino acid.

a) *cis* amide: $hVal^1 \rightarrow$ N-Me-Val^2



b) *cis* amide: $hArg^5 \rightarrow$ N-Me-Tyr^6

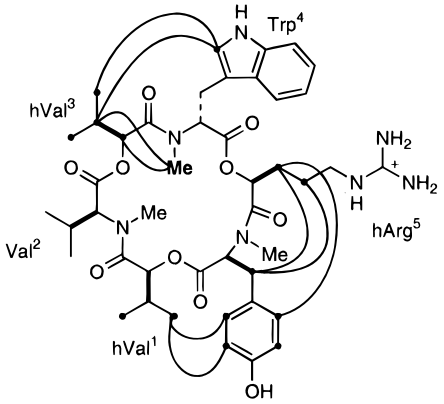


Figure 3. Inter-residue NOEs observed for major (a) and minor (b) conformers of **1**.

aromatic side-chain patterns. The Val and hVal resonances were then differentiated on the basis of sequential NOE's to either the Trp or the Tyr residues (Figure 3). All hydrogens for the major conformer and most of the hydrogens for the minor conformer were assigned in this manner.

The presence of NOE cross peaks from $hVal^3$ αH to Trp^4 N−Me and from Arg^5 αH to Tyr^6 N−Me for the major conformer indicates that these amide bonds are in the *trans* configuration; in turn, the lack of an $hVal^1$ αH to Val^2 N−Me NOE cross peak suggests that this amide is *cis* (see Figure 3). For the less populated component, the $hVal^3$-Trp^4 amide is *trans* (αH to N−Me NOE observed), while the absence of a Arg^5 αH to Tyr^6 N−Me cross peak suggests that the latter amide is *cis*. NOE's between the Arg^5 and Tyr^6 side chains support this assignment, since *cis* peptide bonds bring these side chains closer together than *trans* linkages do. For the minor conformer, the position of the $hVal^1$ αH to Val^2 N−Me NOE cross peak was obscured by the water signal so it could not be confirmed; however, molecular-modeling studies suggest that low-energy conformations for the hexadepsipeptide contain only one amide in the *cis* configuration, in analogy to enniatin B itself.[31] If the *cis* amide is found at the $hVal^1$-Val^2 and Arg^5-Tyr^6 positions in the most abundant conformers, it is a reasonable assumption that one of the least populated components observed at room temperature contains the *cis* linkage between $hVal^3$ and Trp^4 and that the other is all *trans*.

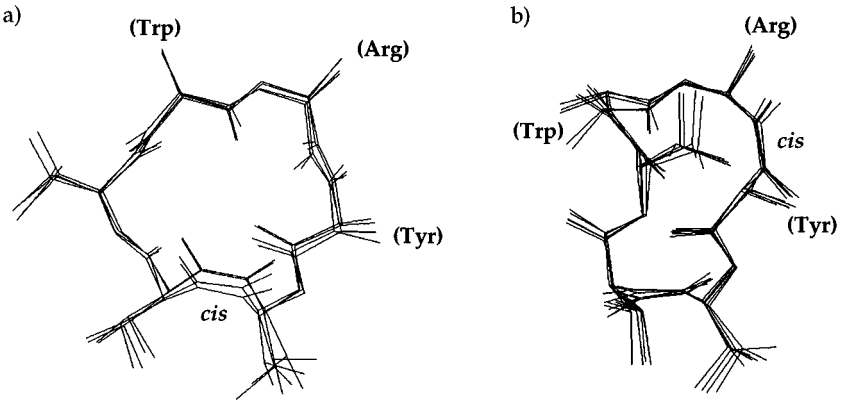


Figure 4. Superposition of low-energy structures of major (a) and minor (b) conformers of **1** that satisfy observed NOE constraints; Trp^4, $hArg^5$, and Tyr^6 side chains omitted for clarity.

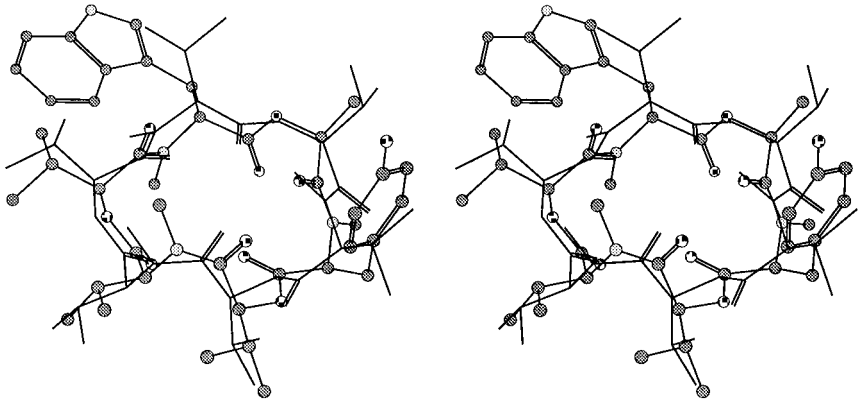


Figure 5. Enniatin B from crystal structure[31] (bare lines) superimposed with lowest energy structure of major conformer of **1** (spherical atoms); $hArg^5$ side chain omitted for clarity.

The absence of NH protons made it difficult to obtain torsional constraints to define backbone ϕ angles. However, Monte Carlo conformational searches of the four combinations of amide configurations, followed by clustering by the backbone atoms,[28] resulted in only a few, low energy conformations for each ring system; almost all of the conformational heterogeneity arises from side-chain torsions. The structures of the two major components ($hVal^1$-Val^2 or Arg^5-Tyr^6 amide *cis*) were then filtered against the relevant set of NOE constraints (Figure 3) to give a set of closely related structures that were both low energy as well as in accord with the spectral information (Figure 4). The backbone of the lowest energy structure of the primary component (*cis* $hVal^1$-Val^2 amide) overlays reasonably well with the crystal structure of enniatin B (rmsd = 1.1 Å for all backbone atoms, see Figure 5) because the *cis* amide bond is located in the same position. However, the structure for the *cis* Arg^5-Tyr^6 amide, as well as the modeled structures for the other amide isomers, have significantly different ring conformations, and it appears unlikely that they could bind to α-amylase.

The underlying design of mimic **1** is therefore partially validated, in that the structure of the template is largely preserved in the major conformer. The relative orientation of the side chain Cα−Cβ bonds is similar to that in tendamistat, although not as closely aligned as in the cyclic peptide cyclo[D-PFAWRY].[15] However, the multiple conformations of the cyclic hexadepsipeptide backbone detract from the binding affinity of **1**. If the major conformer of **1** is the only one able to bind to α-amylase, correcting for the inactive components projects an inhibition constant for this form of 70 μM. This inhibitor is somewhat weaker than the best cyclic and complexed peptides (Table 1), but 3-fold better than the linear control Ac-Trp-Arg-Tyr-OMe. Comparing the backbone conformation of the major form of **1** with the binding loop of tendamistat suggests why this compound is not a better inhibitor of α-amylase. As shown in Figure 6, the backbone turn of the depsipeptide is wider than the tripeptide. The recently reported high-resolution structure of the tendamistat/α-amylase complex[45] shows that the tendamistat packs tightly into the active site of the enzyme around the ^{18}Trp-^{19}Arg-^{20}Tyr tripeptide. This structure indicates that the backbone and bulky isopropyl side chains at the far side of the depsipeptide ring may clash sterically with the enzyme pocket.

Experimental Section[46]

2-(Trimethylsilyl)ethyl (*S*)-2-Hydroxy-3-methylbutanoate, 6b. EDC (17.0 g, 84.3 mmol) and a catalytic amount of DMAP (2.0 g, 16.41 mmol) were added to a solution of 8.8 g (74.6 mmol) of (*S*)-2-hydroxy-3-methylbutanoic acid, **6a** (synthesized according to Li et al.[35]), and 53.5 mL (373 mmol) of 2-(trimethylsilyl)ethanol in 100 mL of pyridine at 0 °C under Ar; the mixture was allowed to warm to 25 °C and stirred for 36 h. The pyridine was removed under reduced pressure, and the residue was dissolved in 250 mL of ethyl acetate, washed with 100 mL of 1 N HCl and 100 mL of H_2O, and dried over $MgSO_4$. The solvent was removed with a rotary evaporator at room temperature, and the excess 2-(trimethylsilyl)ethanol was recovered by vacuum distillation. Purification by frac-

(45) Wiegand, G.; Epp, O.; Huber, R. *J. Mol. Biol.* **1995**, *247*, 99−110.

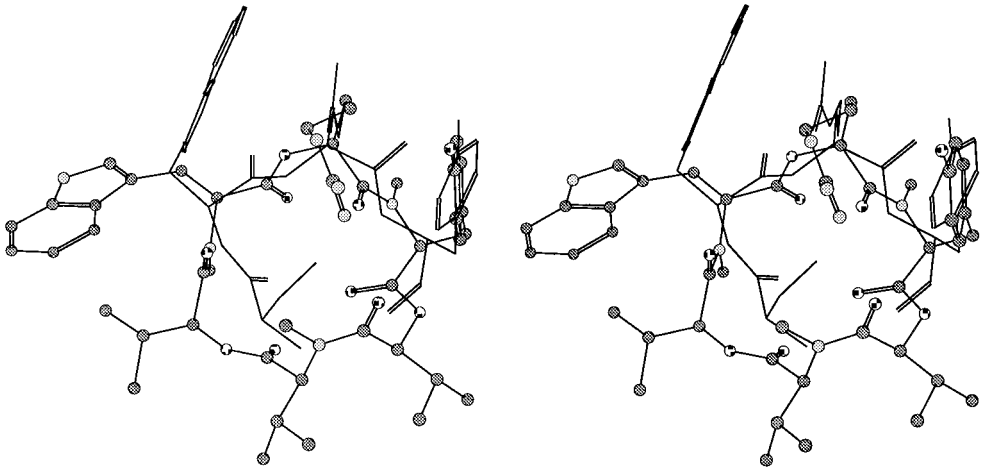


Figure 6. Tendamistat β-turn[24] (bare lines) superimposed with lowest energy structure of major conformer of **1** (spherical atoms), aligning by Cα and Cβ atoms of Trp-Arg-Tyr residues.

tional distillation under reduced pressure gave 6.52 g (40%) of ester **6b** as a colorless liquid: bp 65 °C/100 mm Hg; $[\alpha]^{20}_D$ −6.6° (c = 20 mg/mL, ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (m, 2), 4.08 (d, 1, J = 6.61), 2.27 (m, 1), 1.02 (m, 6), 0.06 (s, 11); ^{13}C NMR (100 MHz) δ 169.4, 64.3, 64.1, 32.6, 18.7, 17.4, −1.6; IR (CH_2Cl_2) 1750, 1470, 1170, 1050, 993, 942, 867, 845 cm^{-1}; MS (EI) m/z 218 (M^+), 191 (9.81), 174 (11.41), 157 (43.61), 145 (35.51), 117 (47.56), 101 (65.84), 93 (75.39); HRMS (EI) m/z calcd for $C_{10}H_{22}O_3Si$ 218.1347, found 218.1336.

(2*S*)-2-[[*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*O*-benzyl-L-tyrosyl]oxy]-3-methylbutanoic Acid 2-(Trimethylsilyl)-ethyl Ester, 3. DIEA (1.4 mL) and DMAP (178 mg, 1.45 mmol) were added to a solution of *N*-Boc-*N*-Me-*O*-Bn-L-tyrosine (2.8 g, 7.27 mmol) and hydroxy ester **6b** (1.75 g, 8.00 mmol) in 7 mL of CH_2Cl_2. The mixture was cooled to −78 °C, and freshly distilled isopropenyl chloroformate (875 μL, 8.00 mmol) was added dropwise with stirring over 10 min. The mixture was allowed to warm to 0 °C; after 1 h, EtOAc (100 mL) was added and the solution was washed with 5% aqueous $KHSO_4$ (2 × 50 mL), 5% aqueous $NaHCO_3$ (2 × 50 mL), and brine (50 mL). The organic layer was worked up, and the crude product was chromatographed (20% EtOAc/hexanes) to give 2.76 g (65%) of a light yellow oil: $[\alpha]^{20}_D$ 35.2° (c = 10 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.29 (m, 5), 7.15−7.08 (m, 2), 6.89 (d, 2, J = 8.4), 5.03 (s, 2), 4.89−4.70 (m, 2), 4.27−4.21 (m, 2), 3.33−2.86 (m, 2), 2.4 and 2.5 (2s, 3), 2.24 (bs, 1), 1.38 and 1.35 (2s, 9), 1.08−0.92 (m, 8), 0.05 (s, 9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 169.4, 157.5, 154.9, 137.0, 129.8, 128.5, 127.8, 127.4, 114.8, 80.3, 79.8, 77.5, 76.7, 70.0, 63.6, 63.5, 59.8, 56.6, 56.4, 34.1, 34.0, 32.6, 29.9, 28.2, 28.0, 27.6, 19.0, 18.9, 18.8, 17.4, 17.2, 17.1, −1.6; IR ($CHCl_3$) 2470, 1710, 1513, 1420, 1362, 1040, 930 cm^{-1}; MS (EI) m/z 609 (MH + Na^+), 586 (MH^+); HRMS (FAB) m/z calcd for $C_{32}H_{48}NO_7Si$ (MH^+) 586.3200, found 586.3183.

(2*S*)-2-[[*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-L-valyl]oxy]-3-methylbutanoic Acid 2-(Trimethylsilyl)ethyl Ester, 4. EDC (2.49 g, 13.0 mmol) and a catalytic amount of DMAP (309 mg, 2.53 mmol) were added to a solution of *N*-Boc-*N*-Me-L-valine (2.93 g, 12.65 mmol) and hydroxy ester **6b** (2.5 g, 11.5 mmol) in 20 mL of pyridine at 0 °C under Ar. The mixture was allowed to warm to 25 °C and stirred for 36 h. After the pyridine was removed under reduced pressure, the residue was dissolved in 200 mL of EtOAc and washed with 100 mL of 1 N HCl and 100 mL of H_2O, the solution was worked up, and the crude product was chromatographed (10% EtOAc/hexanes) to give 3.03 g (61%) of the ester **4** as a colorless oil: $[\alpha]^{20}_D$ 33.6° (c = 23.0 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.80 (d, 1, J = 4.6), 4.33−4.17 (m, 3), 2.85−2.76 (2 br s, 3), 2.45−2.18 (m, 2), 1.57 (bs, 9), 1.15−0.98 (m, 12), 0.06−0.03 (m, 11); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 169.9, 169.1, 77.9, 76.98, 76.4, 64.3, 63.9, 63.6, 32.5, 30.1, 28.4, 28.0, 20.6, 19.6, 18.7, 18.1, 17.8, 17.4, 17.2, −1.6; IR ($CHCl_3$) 2470, 1740, 1520, 1425, 1390, 1370, 1040, 930 cm^{-1}; MS (EI) 431 (M^+), 293 (1.89), 260 (1.59), 157 (47.13), 145 (43.95), 130 (55.27), 101 (65.35); HRMS (FAB) m/z calcd for $C_{21}H_{41}O_6NSi$ (MH^+) 432.2786, found 432.2786.

(2*S*)-2-[[*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*O*-benzyl-L-tyrosyl]oxy]-3-methylbutanoic Acid, 3a. Didepsipeptide **3** (910 mg, 1.6 mmol) was dissolved in 50 mL of THF in a round-bottomed flask under Ar. TBAF on SiO_2 (4 g of 1.1 mmol fluoride/g) was added, and the suspension was stirred at room temperature for 5 h. The yellow mixture was poured onto 25 mL of saturated NH_4Cl and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, and the solvent was evaporated. The crude product was chromatographed (95:5:0.1 CH_2Cl_2/MeOH/HOAc) to give 770 mg (100%) of acid **3a** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.30 (m, 5), 7.32−7.08 (m, 2), 6.90 (d, 2, J = 8.51), 5.03 (s, 2), 4.71 and 4.58 (2m, 1), 3.25 (m, 1), 3.10−2.92 (m, 2), 2.76 and 2.67 (2s, 3), 1.72 (m, 1), 1.40 and 1.32 (2s, 9), 1.02−0.96 (m, 6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 156.2, 155.1, 136.9, 129.9, 129.5, 128.5, 128.2, 127.8, 127.3, 127.3, 114.8, 114.7, 80.3, 80.2, 69.9, 61.4, 51.9, 34.4, 33.9, 32.4, 32.1, 28.2, 28.1, 25.0, 20.0, 14.1, 13.5; IR ($CHCl_3$) 2450, 1740, 1685, 1510, 1455, 1390, 1370, 1330, 1220, 1030, 930, 910 cm^{-1}; MS (FAB) m/z 486 (MH^+); HRMS (FAB) m/z calcd for $C_{27}H_{36}NO_7$ (MH^+) 486.2492, found 486.2481.

(2*S*)-2-[(*N*-Methyl-L-valyl)oxy]-3-methylbutanoic Acid 2-(Trimethylsilyl)ethyl Ester, 4a. Didepsipeptide **4** (700 mg, 1.62 mmol) was dissolved in 5 mL of CH_2Cl_2 in a flame-dried round-bottomed flask purged with Ar. Dry TFA (190 μL, 2.44 mmol) was added dropwise, and the solution was stirred for 24 h at room temperature. The solvent was evaporated, and the crude product was chromatographed (10% EtOAc/hexane) to give 536 mg (100%) of the deprotected amine **4a** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (d, 1, J = 4.54), 4.18 (m, 3), 2.19−2.15 (m, 2), 2.09 (s, 3), 0.99−0.91 (m, 12), 0.15−0.05 (m, 11); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.0, 77.4, 63.6, 31.6, 30.0, 28.3, 22.7, 20.6, 18.7, 17.4, 17.3,

(46) **General**. Reagents and solvent were obtained from commercial suppliers and were used as received, unless otherwise noted. Moisture- or air-sensitive reactions were conducted under argon in distilled solvents. The standard reaction workup involved drying the solution of crude product over $MgSO_4$, removing the solvent under reduced pressure, and purifying the residue by flash chromatography[47] with the eluting solvent indicated. *N*-Boc-*N*-methyl-*O*-benzyl-L-tyrosine and *N*-Boc-*N*-methyl-L-valine were prepared from the corresponding Boc-protected amino acids with sodium hydride and methyl iodide in analogy to the method of McDermott and Benoiton for the Cbz-protected amino acids.[19] Routine NMR spectra were obtained in $CDCl_3$ unless otherwise noted. J values are given in Hz. The following abbreviations are used: Boc = *N*-(*tert*-butoxycarbonyl); DMAP = N,N-dimethyl-4-aminopyridine; EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; TFA = trifluoroacetic acid; TBAF = tetrabutylammonium fluoride; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DIEA = diisopropylethylamine.

(47) Still, W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978**, *43*, 2923.

14.1, −1.5, −1.8; IR ($CHCl_3$) 3000, 2960, 2395, 1735, 1470, 1415, 1040, 925 cm^{-1}; MS (FAB) *m*/*z* 332 (MH^+); HRMS (FAB) *m*/*z* calcd for $C_{16}H_{34}NO_4Si$ (MH^+) 332.2252, found 332.2263.

(2*S*)-2-[[*N*-[(2*S*)-2-[[N-(*tert*-Butoxycarbonyl)-*N*-methyl-*O*-benzyl-L-tyrosyl]oxy]-3-methylbutanoyl]-*N*-methyl-L-valyl]oxy]-3-methylbutanoic Acid 2-(Trimethylsilyl)-ethyl Ester, 7a. Compounds **3a** (1.58 g, 3.3 mmol) and **4a** (809 mg, 2.2 mmol) were dissolved in 3 mL of freshly distilled CH_2Cl_2, and PyBroP (1.54 g, 3.3 mmol) was added. The mixture was placed under Ar and cooled to 0 °C, DIEA (1.54 mL, 8.8 mmol) was added dropwise with stirring, and the mixture was stirred for 6 h as it slowly warmed to room temperature. The solvent was evaporated, and the residue was dissolved in 100 mL of EtOAc; this solution was washed with 1 N HCl (2 × 100 mL), 1 N NaOH (2 × 100 mL), H_2O (2 × 100 mL), and 100 mL of brine and worked up. The crude product was chromatographed (30% EtOAc/hexane) to give 1.46 g (84%) of the tetramer **7a** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.5−7.3 (m 5), 7.1 (m, 2), 6.9 (br d, 2), 5.03 (s, 2), 4.8 (m, 2), 4.2−4.3 (m, 4), 3.0−3.5 (m, 2), 2.6−3.0 (m, 6), 2.2−2.3 (m, 3), 2.1 (s, 9), 1.1−0.9 (m, 18), 0.05 (m, 11); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.2, 157.4, 154.4, 137.9, 130.3, 129.9, 128.5, 128.2, 127.8, 127.4, 114.8, 114.7, 80.4, 75.4, 70.0, 61.5, 33.8, 31.3, 29.8, 28.2, 27.9, 27.5, 20.7, 19.8, 19.5, 18.8, 17.7, 17.6, 17.0, −1.5, −1.8; IR ($CHCl_3$) 2390, 1730, 1680, 1510, 1470, 1420, 1040, 925 cm^{-1}; MS (FAB) *m*/*z* 799 (MH^+); HRMS (FAB) *m*/*z* calcd for $C_{43}H_{67}N_2O_{10}Si$ (MH^+) 799.4565, found 799.4599.

(2*S*)-2-[[*N*-[(2*S*)-2-[[*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*O*-benzyl-L-tyrosyl]oxy]-3-methylbutanoyl]-*N*-methyl-L-valyl]oxy]-3-methylbutanoic Acid, 7b. Cleavage of the 2-(trimethylsilyl)ethyl ester was carried out as described for preparation of **3a**; the crude product was chromatographed (95:5:0.1 CH_2Cl_2/MeOH/HOAc) to give a quantitative yield of the tetrameric acid **7b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−6.87 (m, 9), 5.05 (s, 2), 5.09−4.84 (m, 3), 4.69 (m, 1), 3.37−2.07 (m, 11), 1.47−0.83 (m, 27); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 170.5, 157.5, 157.4, 137.0, 130.3, 129.9, 128.5, 128.2, 127.8, 127.3, 114.8, 114.7, 80.4, 75.7, 70.0, 61.5, 33.8, 31.3, 29.8, 28.2, 27.9, 27.5, 20.7, 19.8, 19.5, 18.8, 17.7, 17.6, 17.0; IR ($CHCl_3$) 2395, 1735, 1675, 1505, 1480, 1390, 1365, 1020, 925 cm^{-1}; MS (FAB) *m*/*z* 699 (MH^+); HRMS (FAB) *m*/*z* calcd for $C_{38}H_{55}N_2O_{10}$ (MH^+) 699.3826, found 699.3887.

(*S*)-2-Hydroxy-5-[(benzyloxycarbonyl)amino]pentanoic Acid, 9b. L-Ornithine·HCl (10 g, 60 mmol) was dissolved in 10 mL of water and loaded on an Amberlite IRA-120 (H^+) column (80 mL of the resin), which was eluted first with water (300 mL) and then with 3% NH_4OH (400 mL). The solution was lyophilized to give 9.2 g of an oil. The oil was dissolved in 30 mL of water and cooled to 0 °C, and 10.5 mL of concd H_2SO_4 (3 equiv) was added. A solution of 8.3 g of $NaNO_2$ (2 equiv) in 30 mL of water was added dropwise over a period of 60 min. The resulting solution was left to stand as the temperature rose from 0 °C to rt, and the reaction progress was followed by ^{13}C NMR. The reaction product, (*S*)-2-hydroxy-5-aminopentanoic acid, **9a**,[40] was characterized by NMR: ^{1}H NMR (400 MHz, D_2O) δ 3.87 (t, 1, *J* = 6.2), 2.80 (q, 2, *J* = 6.4), 1.4−1.6 (m, 4); ^{13}C NMR (100 MHz, D_2O) δ 183.7, 74.3, 42.2, 33.6, 25.8.

After 46 h, the reaction was complete, and excess $NaNO_2$ was quenched by adding urea until the solution gave a negative KI−starch test. The mixture was brought to pH 10 with 2 N NaOH and cooled in an ice bath; benzyloxycarbonyl chloride (CbzCl, 10 mL, 1.1 equiv) and 2 N NaOH were added alternately over a period of 2 h with vigorous stirring, keeping the pH at about 10. The mixture was stirred at rt for another 2 h. The resulting suspension was washed with ether (3 × 150 mL), and the clear aqueous layer was acidified with 6 N HCl and extracted with EtOAc (4 × 50 mL), washed with saturated NaCl (200 mL), and worked up to provide 9.2 g (68%) of *N*-protected α-hydroxy acid **9b** as a white solid after chromatography on silica gel 60G (80% EtOAc/hexanes): mp 102−6 °C (lit.[41] mp 107−108 °C); $[\alpha]_D$ +1.5° (*c* 0.0098 g/mL, MeOH); TLC: R_f 0.26 ($CHCl_3$/MeOH/HOAc 9:1:0.5); ^{1}H NMR (400 MHz, CD_3CN) δ 7.34 (m, 5), 5.70 (bs, 1), 5.03 (s, 2), 4.11 (t, 1, *J* = 6.7), 3.11 (q, 2, *J* = 6.4), 1.73 (m, 1), 1.56 (m, 3); ^{13}C NMR (100 MHz, CD_3CN) δ 176.0, 157.5, 138.5, 129.5, 128.8, 128.6, 70.6, 66.7, 41.2, 32.0, 26.4.

(*S*)-2-Hydroxy-5-[(benzyloxycarbonyl)amino]pentanoic Acid 2-(Trimethylsilyl)ethyl Ester, 9c. To a solution of the acid **9b** (5.1 g, 19 mmol) and 2-(trimethylsilyl)ethanol (13.6 mL, 5 equiv) in pyridine (25 mL, freshly distilled from CaH_2) at 0 °C were added EDC (4.4 g, 1.2 equiv) and DMAP (0.5 g, 0.2 equiv), and the resulting mixture was stirred at 0 °C to rt for 50 h. Pyridine was evaporated under reduced pressure, and the oily residue was dissolved in EtOAc (150 mL), washed successively with saturated NH_4Cl (125 mL), H_2O (125 mL), and saturated NaCl (100 mL), and worked up. The crude product was chromatographed (0−40% EtOAc/hexanes gradient) to give 1.7 g (24%) of the ester **9c** as a colorless oil: TLC R_f 0.62 (1:1 EtOAc/hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (m, 5), 5.06 (s, 2), 5.00 (s, 1), 4.24 (m, 2), 4.13 (s, 1), 3.21 (m, 2), 3.07 (s, 1), 1.82 (m, 1), 1.62 (m, 3), 1.00 (m, 2), 0.03 (s, 9); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 156.4, 136.5, 128.4, 128.0, 70.0, 66.5, 64.1, 40.5, 31.2, 25.4, 17.3, −1.63; MS(FAB) *m*/*z* 368.2 (MH^+); HRMS(FAB) *m*/*z* calcd for $C_{18}H_{30}NO_5Si$ (MH^+) 368.1893, found 368.1887.

N$^\alpha$-(*tert*-Butoxycarbonyl)-*N*$^\epsilon$-(*tert*-butyldimethylsilyl)-D-tryptophan, 10b.[42] A 1 M solution of LiHMDS (28.22 mL, 28.22 mmol) was added slowly to a solution of 1.53 g of TBSCl (10.2 mmol) and 2.53 g of *N*$^\alpha$-Boc-D-tryptophan (8.3 mmol) in 42 mL of THF at −78 °C with stirring under Ar. After 24 h at −78 °C, 25 mL of saturated aqueous NH_4Cl was added, and the mixture was warmed to 25 °C over 1 h and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with saturated NH_4Cl (2 × 50 mL) and 50 mL of brine and worked up. The crude product was chromatographed (90:10:0.1 CH_2Cl_2/MeOH/HOAc) to give 3.04 g (88%) of the silyl derivative **10b** as a white solid: $[\alpha]^{20}_D$ −20.2° (*c* = 21.3 mg/mL, $CHCl_3$) (lit.[42] $[\alpha]^{20}_D$−21.2°); mp 90−93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (br s, 1), 7.44 (d, 1, *J* = 7.7), 7.07 (m, 2), 7.01 (s, 1), 5.08 (br s, 1), 4.59 (br s, 1), 3.35 (m, 1), 3.23 (m, 1), 1.37 (br s, 9), 0.88 (s, 9), 0.55 (s, 6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 155.5, 141.3, 131.0, 130.0, 121.5, 119.6, 118.7, 113.9, 112.3, 79.9, 76.7, 54.2, 28.3, 27.8, 27.7, 26.2, 25.6, 19.4; IR (CH_2Cl_2) 3450, 1720, 1460, 1170, 980, 850 cm^{-1}; UV−vis (*c* = 0.1 mM, MeOH) λ_{max} 279.5 nm; MS (EI) *m*/*z* 418 (M^+), 344 (28.60), 244 (79.85), 188 (41.65), 130 (60.27); HRMS (EI) *m*/*z* calcd for $C_{22}H_{34}N_2O_4Si$ 418.2288, found 418.2270.

***N*$^\alpha$-(*tert*-Butoxycarbonyl)-*N*$^\epsilon$-(*tert*-butyldimethylsilyl)-*N*$^\alpha$-methyl-D-tryptophan, 10c.** Sodium hydride (0.27 g, 6.0 mmol) was added to a solution of 0.878 g of the silyl derivative **10b** (2.1 mmol) and 1.05 mL of CH_3I (17 mmol) in 8.0 mL of THF at 0 °C under Ar, and the suspension was stirred and allowed to warm to 25 °C. After 72 h, the mixture was partitioned between 10 mL of EtOAc and 10 mL of water; the aqueous layer was acidified to pH 2 with 3 N HCl and extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with 25 mL of water, 5% aqueous $Na_2S_2O_3$ (2 × 25 mL), 25 mL of water, and 25 mL of brine and worked up. The crude product was chromatographed (95:5:0.1 CH_2Cl_2/MeOH/HOAc) to give 0.79 g (88% yield) of the *N*-methyl derivative **10c** as an off-white foam: $[\alpha]^{20}$ −39.55° (*c* = 22.2 mg/mL, EtOAc); ^{1}H NMR (400 MHz, acetone-d_6) δ 7.64 (d, 1, *J* = 7.8), 7.52 (d, 1, *J* = 7.8), 7.13−7.07 (m, 3), 4.92 (m, 1), 3.1−3.5 (m, 2), 2.65 and 2.8 (2s, 3), 1.36 and 1.10 (2s, 9), 0.92 (s, 9), 0.607 and 0.592 (2s, 6); ^{13}C NMR (100 MHz, acetone-d_6) δ 173.2, 156.5, 155.6, 142.5, 142.3, 132.0, 131.8, 130.4, 129.8, 122.1, 120.2, 119.4, 114.7, 114.6, 79.8, 79.5, 60.1, 59.4, 31.4, 28.5, 28.1, 26.7, 26.6, 25.8, 25.2, 20.1, 20.0, −3.8, −3.9; IR (CH_2Cl_2) 3490, 1720, 1400, 1375, 1170, 792 cm^{-1}; UV−vis (*c* = 0.1 mM, MeOH) λ_{max} 279.5 nm; MS (EI) m/z 432 (M^+), 359 (14.44), 332 (14.97), 301 (22.41), 188 (36.38), 144 (71.23), 57 (99.39); HRMS (EI) *m*/*z* calcd for $C_{23}H_{36}N_2O_4Si$ 432.2433, found 432.2453.

(*S*)-5-[(Benzyloxycarbonyl)amino]-2-[[*N*$^\alpha$-(*tert*-butoxycarbonyl)-*N*$^\epsilon$-(*tert*-butyldimethylsilyl)-*N*$^\alpha$-methyl-D-tryptophanyl]oxy]pentanoic Acid 2-(Trimethylsilyl)ethyl Ester, 5a. To a solution of the hydroxy ester **9c** (2.6 g, 7.1 mmol) and the tryptophan derivative **10c** (3.5 g, 8.1 mmol) in CH_2Cl_2 (25 mL) at 0 °C under Ar were added EDC (1.61 g, 1.2 equiv) and DMAP (180 mg, 0.2 equiv), and the mixture was

stirred at 0 °C to rt for 24 h to give a clear yellow solution. The solution was diluted with 20 mL of CH_2Cl_2, washed with 1 N HCl (2 × 20 mL), H_2O (2 × 30 mL), and saturated NaCl (30 mL), and worked up. The residue was chromatographed (20% EtOAc/hexanes) to give 4.42 g (80%) of the dimeric ester as a yellow foam: TLC R_f 0.78 (1:2 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, 1, J = 7.8), 7.45 (d, 1, J = 7.8), 7.30 (m, 5), 7.12 (m, 2), 6.95 (s, 1), 5.21 (m, 1), 5.07 (s, 2), 4.99 (t, 1, J = 5.4), 4.87 and 4.73 (s, 1), 4.22 (m, 2), 3.42 (m, 1), 3.18 (m, 3), 2.84 and 2.75 (s, 3), 1.85 (m, 2), 1.60 (m, 2), 1.38 and 1.15 (2s, 9), 1.01 (m, 2), 0.89 (s, 9), 0.56 (s, 3), 0.55 (s, 3), 0.04 (s, 9); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.1, 169.5, 156.3, 141.4, 136.5, 130.5, 129.3, 128.8, 128.5, 128.0, 121.4, 119.5, 118.4, 113.8, 113.8, 113.2, 113.0, 79.9, 72.5, 66.6, 64.0, 60.3, 58.7, 57.9, 40.3, 31.2, 30.3, 28.3, 28.1, 27.9, 26.2, 25.6, 25.2, 24.3, 24.3, 21.0, 19.4, 17.4, 14.2, −1.6, −4.0; MS(FAB) m/z 781.3 (M^+); HRMS (FAB) m/z calcd for $C_{41}H_{64}N_3O_8Si_2$ (MH^+) 782.4232, found 782.4221.

5-[(Benzyloxycarbonyl)amino]-(2*S*)-2-[[*N*-[(2*S*)-2-[[*N*-[(2*S*)-2-[[*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*O*-benzyl-L-tyrosyl]oxy]-3-methylbutanoyl]-*N*-methyl-L-valyl]oxy]-3-methylbutanoyl]-*N*-methyl-D-tryptophanyl]oxy]-pentanoic Acid 2-(Trimethylsilyl)ethyl Ester, 11a. To a solution of the ester **5a** (1.3 g, 1.7 mmol) in THF (40 mL) under Ar at rt was added TBAF on SiO_2 (1.85 g, 1.1 mmol fluoride/g, 5 equiv), and the mixture was stirred for 20 min. TLC (1:1 EtOAc/hexanes) showed that **5a** (R_f 0.87) was cleanly converted to a new compound (**5**, R_f 0.65). The reaction mixture was poured onto saturated NH_4Cl (100 mL) and extracted with EtOAc (3 × 50 mL), and the organic layer was washed with saturated NaCl (40 mL) and worked up. ^{1}H NMR (400 MHz, $CDCl_3$) showed that only the TBDMS group had been removed. This material was then dissolved in EtOAc saturated with anhydrous HCl gas (15 mL) and left to stand at rt for 1 h. Evaporation of the solvent afforded the *N*-deblocked ester **5b** as a yellow oil. To a solution of this material (ca. 1.7 mmol), the tetradepsipeptide acid **7b** (1.18 mg, 1 equiv), and PyBroP (873 mg, 1 equiv) in CH_2Cl_2 (7.5 mL) at 0 °C under Ar was added DIEA (326 μL, 1.1 equiv) dropwise, and the mixture was allowed to stir as it warmed from 0 °C to rt for 20 h. The reaction mixture was diluted with EtOAc (50 mL), washed with 1 N HCl (3 × 50 mL), 1 N NaOH (3 × 50 mL), H_2O (50 mL), and saturated NaCl (50 mL), and worked up. The residue was chromatographed (40% EtOAc/hexanes) to give the coupled product **11a** as a white foam. The product was further purified by normal phase MPLC on a Merck 40−63 μM silica column, eluting with 40% EtOAc/hexanes at 20 mL/min and monitoring the effluent by UV absorbance. The total yield of **11a** was 554 mg (26%) of a yellow foam: TLC R_f 0.64 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, 1), 7.62−7.02 (m, 16), 6.86 (d, 2), 5.73−5.52 (m, 2), 5.15−5.06 (m, 8), 5.05 (s, 2), 5.01 (s, 2), 4.71−4.64 (m, 1), 4.21 (m, 2), 3.75−3.71 (m, 1), 3.55−3.45 (m, 1), 3.33−3.02 (m, 5), 3.01 (s, 3), 2.99 (s, 3), 2.69 and 2.58 (2s, 3), 2.21−2.14 (m, 1), 1.94−1.83 (m, 1), 1.65−1.55 (m, 2), 1.36 and 1.32 (2s, 9), 1.10−0.88 (m, 9), 0.80 (d, 3, J = 6.24), 0.66 (bd, 3), 0.51 (d, 3, J = 6.64), 0.03 (s, 9); MS(FAB) m/z 1248.1 (MH^+); HRMS (FAB) m/z calcd for $C_{68}H_{94}N_5O_{15}Si$ (MH^+) 1248.6516, found 1248.6524.

5-[(Benzyloxycarbonyl)amino]-(2*S*)-2-[[*N*-[(2*S*)-2-[[*N*-[(2*S*)-2-[(*N*-methyl-*O*-benzyl-L-tyrosyl)oxy]-3-methylbutanoyl]-*N*-methyl-L-valyl]oxy]-3-methylbutanoyl]-*N*-methyl-D-tryptophanyl]oxy]pentanoic Acid, 11c. Compound **11a** (124 mg, 0.099 mmol) was dissolved in DMF (1 mL), and TBAF (1.0 M in THF, 0.25 mL, 0.25 mmol) was added. After 15 min, the mixture was diluted with EtOAc (80 mL), and the organic layer was washed with 0.5 N HCl (3 × 40 mL), H_2O (40 mL), and saturated NaCl (40 mL) and worked up to give 114 mg (100%) of the free acid **11b** as a white foam. ^{1}H-NMR indicated the complete removal of the 2-(trimethylsilyl)-ethyl ester, and the material was used without further purification.

A solution of the free acid **11b** in EtOAc (15 mL) was purged with Ar via a glass pipet and cooled to −40 °C (dry ice/acetonitrile bath), and dry HCl was bubbled vigorously through it via a glass pipet for 10 min. The solution was then purged with Ar for 15 min at −40 °C to remove excess HCl and for an additional 5 min while the solution warmed to room temperature. The solution was then concentrated with a rotary evaporator without heating, and the residual solvent was removed *in vacuo* for 30 min. The resultant clear glass was lyophilized from benzene to yield compound **11c** (103 mg, 100%) as a white powder: ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (d, 1, J = 7.8), 7.26−7.40 (m, 12), 7.23 (d, 2, J = 8.6), 7.10 (s, 1), 7.07 (t, 1, J = 7.2), 6.99 (t, 1, J = 7.1), 6.94 (d, 2, J = 8.6), 5.55 (dd, 1, J = 4.2, 11.4), 5.20 (d, 1, J = 5.5), 5.06 (s, 2), 5.03 (s, 2), 5.02 (m, 2), 4.94 (m, 1), 3.37 (t, 1, J = 5.9), 3.48 (dd, 1, J = 4.7, 15.3), 3.25 (m, 3), 3.17 (t, 2, J = 6.8), 3.06 (s, 3), 3.03 (s, 3), 2.70 (s, 3), 2.17 (m, 2), 1.90 (m, 2), 1.64 (m, 3), 0.97 (d, 3, J = 6.9), 0.95 (d, 3, J = 6.7), 0.92 (d, 3, J = 6.7), 0.77 (d, 3, J = 6.6), 0.63 (d, 3, J = 6.9), 0.40 (d, 3, J = 6.6); ^{13}C NMR (100 MHz, CD_3OD) δ 172.9, 171.8, 171.6, 171.3, 171.1, 169.0, 160.0 (TFA), 158.8, 138.6, 138.4, 138.2, 131.9, 129.5, 129.5, 129.0, 128.9, 128.8, 128.5, 128.4, 128.4, 126.6, 124.5, 122.4, 119.8, 119.1, 116.6 (TFA), 112.4, 110.8, 78.5, 77.1, 74.2, 74.0, 70.9, 67.4, 63.4, 62.7, 59.0, 41.5, 35.4, 32.9, 32.7, 31.7, 30.9, 30.5, 29.5, 28.2, 26.4, 25.5, 20.1, 19.2, 19.2, 19.1, 17.7, 16.8; MS (FAB) m/z calcd for $C_{58}H_{74}N_5O_{13}$ (MH^+) 1048.5283, found 1048.5290; 1070 (MNa^+), 1048 (MH^+), 958, 948, 914.

Cyclo[5-[(benzyloxycarbonyl)amino]-(2*S*)-2-[[*N*-[(2*S*)-2-[[*N*-[(2*S*)-2-[(*N*-methyl-*O*-benzyl-L-tyrosyl)oxy]-3-methylbutanoyl]-*N*-methyl-L-valyl]oxy]-3-methylbutanoyl]-*N*-methyl-D-tryptophanyl]oxy]pentanoic acid], 1a. Precursor **11c** (351 mg, 335 μmol) and PyBroP (315 mg, 670 μmol) were dissolved in CH_2Cl_2 (300 mL), and the solution was cooled to 0 °C. DIEA (250 μL, 1.34 mmol) was added, and the ice bath was allowed to warm to room temperature. After 4 days, the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (100 mL) and washed with 1 N HCl (5 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and brine (50 mL). The organic layer was worked up, and the residue was chromatographed (90% ether:10% MeOH followed by 90% $CHCl_3$:10% MeOH (v/v)) to give 220 mg (64%) of the cyclic product **1a** as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1), 7.55 (d, 1, J = 8.7), 7.26 (m, 11), 7.10 (m, 5), 6.84 (d, 2, J = 8.5), 5.74 (m, 2), 5.34 (d, 1, J = 6.3), 5.07 (m, 3), 4.97 (s, 2), 4.82 (m, 2), 3.87 (d, 1, J = 10.8), 3.42 (m, 2), 3.31 (m, 1), 3.25 (s, 3), 3.10 (s, 3), 2.87 (m, 2), 2.78 (s, 3), 2.30 (m, 2), 2.04 (m, 2), 1.85 (m, 1), 1.68 (m, 2), 1.01 (m, 9), 0.88 (m, 3), 0.79 (d, 3, J = 6.6), 0.62 (d, 3, J = 6.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.3, 169.4, 168.7, 167.8, 157.3, 156.4, 136.9, 136.6, 136.2, 130.0, 129.9, 129.2, 128.5, 128.1, 127.8, 127.4, 124.7, 127.0, 122.7, 122.0, 119.5, 118.2, 115.3, 114.6, 111.3, 109.8, 76.7, 75.5, 74.2, 70.0, 67.1, 66.5, 55.0, 40.3, 32.8, 31.9, 31.7, 31.1, 30.4, 30.0, 29.6, 29.3, 28.2, 27.1, 26.2, 26.1, 25.7, 25.2, 20.6, 19.7, 19.5, 19.3, 18.0, 17.9, 17.5, 17.3, 14.1; MS (FAB) m/z calcd for MNa^+ $C_{58}H_{71}N_5O_{12}Na$ 1052.4997, found 1052.4997; 1068 (MK^+), 1052 (MNa^+), 962, 918, 616, 537, 515, 444.

Cyclo[5-amino-(2*S*)-2-[[*N*-[(2*S*)-2-[[*N*-[(2*S*)-2-[(*N*-methyl-L-tyrosyl)oxy]-3-methylbutanoyl]-*N*-methyl-L-valyl]-oxy]-3-methylbutanoyl]-*N*-methyl-D-tryptophanyl]oxy]-pentanoic acid], 1b. Compound **1a** (9.0 mg, 9.0 μmol) and $Pd(OH)_2$ (Degussa 20 wt % Pd, 11.8 mg, 22.0 μmol) were suspended in MeOH (6 mL) and HOAc (63 μL, 111 μmol). After being purged thoroughly with nitrogen, the mixture was stirred vigorously under an H_2 atmosphere for 40 min. The reaction mixture was filtered through a 0.45-μm filter, the filtrate was concentrated on a rotary evaporator, and the remaining solvent was removed *in vacuo* to afford 6.1 mg (88%) of compound **1b**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (m, 1), 7.31 (m, 1), 7.07 (m, 5), 6.71 (m, 2), 5.70 (m, 1), 5.30 (d, 1, J = 8.6), 5.24 (m, 1), 5.06 (m, 1), 4.85 (m, 1), 3.96 (d, 0.7, J = 10.8), 3.89 (d, 0.3, J = 10.8), 3.32−3.38 (m, 2), 3.22 (s, 3), 3.06−3.17 (m, 1), 3.05 (s, 3), 2.90 (s, 3), 2.80 (m, 2), 2.06−2.33 (m, 2), 1.45−1.79 (m, 2), 1.28 (m, 3), 0.57−1.18 (m, 12), 0.46 (d, 2, J = 6.8), 0.37 (d, 1, J = 6.8); MS (FAB) m/z calcd for $C_{43}H_{60}N_5O_{10}$ (MH^+) 806.4340, found 806.4349; 844 (MK^+), 828 (MNa^+), 806 (MH^+), 328, 283, 237.

Cyclo[5-guanidino-(2*S*)-2-[[*N*-[(2*S*)-2-[[*N*-[(2*S*)-2-[(*N*-methyl-L-tyrosyl)oxy]-3-methylbutanoyl]-*N*-methyl-L-valyl]oxy]-3-methylbutanoyl]-*N*-methyl-D-tryptophanyl]-oxy]pentanoic acid], 1. The deprotected intermediate **1a**

(7.4 mg, 9.2 μmol) and aminoiminomethanesulfonic acid[48,49] (2.3 mg, 18.4 μmol) were dissolved in MeOH (6 mL), and Et_3N (6.4 μL, 46 μmol) was added. After being stirred for 7 h, the solution was concentrated with a rotary evaporator and the residue was dissolved in MeOH. The material was purified over a preparative Vydac Proteins and Peptides (25 cm × 25 mm) 10 μm C_{18} reversed phase column with a solvent gradient from 30% to 70% of 10% H_2O/90% acetonitrile/0.1% TFA (v/v) (solvent B) and 90% H_2O/10% acetonitrile/0.1% TFA (v/v) (solvent A); detection at 214 nm revealed that the product eluted at ~45% B:A. The appropriate fractions were pooled and lyophilized to afford 5.7 mg (73%) of the target compound **1** as a white flocculent powder, which was insoluble in H_2O, partially soluble in 5−10% DMSO/H_2O, and completely soluble in 50% DMSO/H_2O or DMSO: ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (m, 1), 7.30 (m, 1), 7.08 (m, 5), 6.74 (d, 0.5, J = 8.5), 6.80 (d, 1.5, J = 8.5), 5.70 (dd, 1, J = 6.0, 10.8), 5.28 (d, 0.5, J = 8.7), 5.23 (d, 1.5, J = 6.5), 4.94 (m, 2), 3.97 (d, 0.7, J = 10.8), 3.89 (d, 0.3, J = 10.8), 3.33 (m, 2), 3.26 (s, 3), 3.19 (m, 1), 3.05 (s, 3), 2.91 (s, 3), 2.83 (m, 2), 2.34 (m, 1), 2.08 (m, 1), 1.68 (m, 1), 1.60 (m, 1), 1.50 (m, 1), 1.28 (m, 2), 1.00 (m, 13), 0.67 (d, 2, J = 6.7), 0.46 (d, 2, J = 6.9), 0.34 (d, 1, J = 6.8); MS (FAB) m/z calcd for $C_{44}H_{62}N_7O_{10}$ (MH^+) 848.4558, found 848.4556; 886 (MK^+), 870 (MNa^+), 848 (MH^+).

NMR General. Approximately 2 mg of cyclic hexamer **1** was dissolved in 300 μL of KD_2PO_4/NaOD (0.05 M/0.029 M) in D_2O, with 5 mM $CaCl_2$, 50 mM NaCl, and 200 μL of CD_3OD. The pH was adjusted to 6.98 with DCl. 1D ^{1}H-NMR spectra were recorded at 27 and 0 °C using presaturation to suppress the water signal; 2D spectra were recorded at 0 °C. Spectra were processed on a Bruker X-32 data station running UxNMR. Time proportional phase incrementation (TPPI)[50] was used to obtain phase-sensitive spectra. T1's were measured using an inversion-recovery experiment.

TOCSY (500 MHz). Pulse sequence according to Bax et al. with MLEV-17 spin-lock[51] and DANTE presaturation[52] during the relaxation delay, 512 experiments of 32 scans each, relaxation delay of 1.5 s, mixing time of 50 ms, acquisition time for one scan 0.184 s, size 2 K, spectral width in F2 and F1 11.1 ppm, zero filling in F2 and F1 to 2 K, apodization in both dimensions with squared sinebell shifted by π/2.

NOESY (300 MHz). Pulse sequence according to Bodenhausen et al.,[53] with presaturation during the relaxation delay and the mixing time, 1.5 s presaturation during the relaxation delay, 700 ms of presaturation during the mixing time, acquisition time of 0.67 s, size 2 K, spectral width in F2 and F1 11.1 ppm, apodization in both dimensions with squared sinebell shifted by π/2. Interresidue NOEs observed are depicted in Figure 3.

Molecular Modeling. All modeling was done using MacroModel/Batchmin software version 4.0.[54] Monte Carlo searches (10,000 iterations) and minimizations were run without constraints. Four searches were performed with each of the three amide bonds *cis* and all of them *trans*. Energy minimizations were carried out with MacroModel's TNCG method, using the Amber* force field[55] with GB/SA water solvation,[56] and the calculations were carried out until gradient convergence was achieved. The structures were sorted into families with a clustering algorithm[57] by comparing the peptide backbone atoms (Cα, amide N or ester O, amide carbonyl carbon). The families of the two major amide configurations were then filtered against the NOE data (see Figure 3) by requiring that two hydrogens showing an NOE cross peak be closer than 5.0 Å.

Enzymology. Due to the low solubility of compounds **1** and **2** in water, assays were performed in 5% DMSO/water. The kinetic constants for hydrolysis of *p*-nitrophenyl α-D-maltotrioside by porcine pancreatic α-amylase (PPA) were determined in water and in 5% DMSO/water as described previously.[27] K_m and V_{max} are slightly higher in 5% DMSO/water (K_m = 1.14 mM, V_{max} = 0.072 min^{-1}) than in water (K_m = 1.05 mM, V_{max} = 0.067 min^{-1}); however, K_i values were found to differ significantly. As a result, K_i values for the control compounds WRY (870 ± 30 μM) and AcN-WRY-OMe (190 ± 10 μM) were redetermined in 5% DMSO/water. Dixon plots for determination of the K_i values are provided in the Supporting Information.

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Supporting Information Available: Experimental procedures for synthesis of the linear control **2**; Dixon plots for K_i determinations; ^{1}H NMR spectra of compounds described above (20 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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