Total Synthesis of HUN-7293

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Abstract: The first total synthesis of the cyclic heptadepsipeptide HUN-7293 (**1**), a potent inhibitor of cell adhesion molecule expression exhibiting anti-inflammatory properties, is detailed. The most effective approach relied on an unusually efficient macrocyclization with the formation of the MLEU^3−LEU^4 secondary amide that potentially benefits from intramolecular H-bonding preorganization of the acyclic substrate. The requisite linear depsipeptide was convergently assembled with the late stage introduction of the linking ester enlisting a Mitsunobu esterification that occurs with inversion of the DGCN α-center permitting the utilization of a readily available L-amino acid precursor to the D α-hydroxy carboxylic acid residue. An alternative and similarly attractive approach of direct macrolactonization of a substrate necessarily incorporating a D-DGCN subunit proved viable albeit less effective. Biological evaluation in cellular assays for vascular adhesion molecule expression confirmed that synthetic HUN-7923 (**1**) is essentially indistinguishable from the naturally occurring cyclodepsipeptide.

The cyclic heptadepsipeptide HUN-7293 (**1**, Figure 1) was first isolated in 1992 from a fungal broth during a screen for potent inhibitors of inducible cell adhesion molecule expression. On the basis of its activity in a cell-based enzyme-linked immunosorbant assay (ELISA), HUN-7293 (**1**) was purified by assay-guided fractionation and the two- and three-dimensional structure was subsequently determined.[1] Independently, the same cyclodepsipeptide was isolated by a Japanese group from a different fungal species based on a screen for anti-HIV compounds.[2]

Macrocyclic natural products often exhibit unique biological properties and thus are attractive candidates for drug development in many disease indications.[3] HUN-7293 (**1**) represents such a compound class with novel anti-inflammatory actions, due to its potent inhibition of the vascular cell adhesion molecule 1 (VCAM-1).[4] Endothelial cell-associated molecules such as intercellular adhesion molecule 1 (ICAM-1), VCAM-1, and E-selectin play a critical role in the immune response by regulating leukocyte migration and cell-to-cell interactions.[5] Modulation of these interactions in vivo by appropriate treatment with a compound like HUN-7293 (**1**) could have therapeutic potential in a variety of inflammatory disorders and autoimmune diseases.[6]

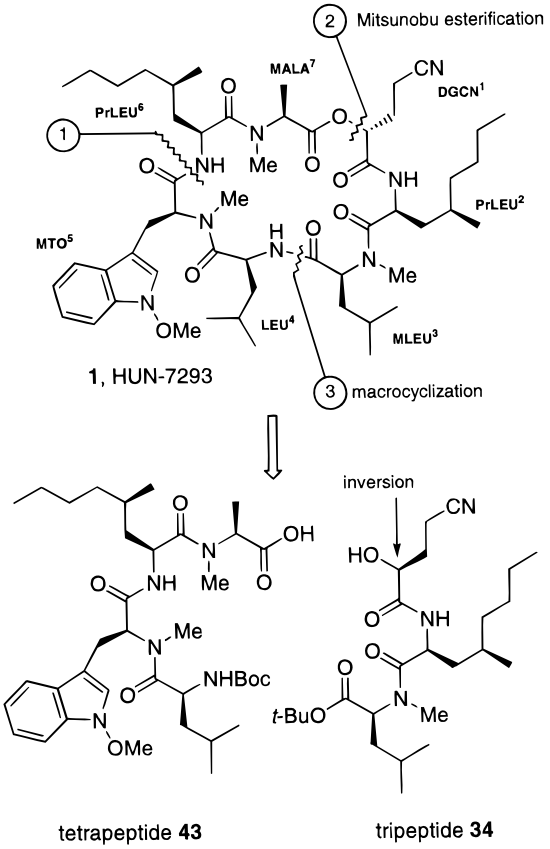

Figure 1.

HUN-7293 (**1**) is a cyclodepsipeptide containing six L-amino acid residues and a D α-hydroxy carboxylic acid residue (DGCN) coupled to form a 21-membered ring. The structure and stereochemistry of HUN-7293 (**1**) has been determined by

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(1) Hommel, U.; Weber, H.-P.; Oberer, L.; Naegeli, H. U.; Oberhauser, B.; Foster, C. A. *FEBS Lett.* **1996**, *379*, 69.
(2) Itazaki, H.; Fujiwara, T.; Sato, A.; Kawamura, Y.; Matsumoto, K. Jpn Patent, Kokai Tokkyo Koho 07109299, 1995, Appl. 93/255592, 13 Oct. 1993; *Chem. Abstr.* **1995**, *123*, 110270.
(3) Schreiber, S. L.; Crabtree, G. R. *Immunol. Today* **1992**, *13*, 136.
(4) Foster, C. A.; Dreyfuss, M.; Mandak, B.; Meingassner, J. G.; Naegeli, H. U.; Nussbaumer, A.; Oberer, L.; Scheel, G.; Swoboda, E.-M. *J. Dermatol.* **1994**, *21*, 847.
(5) (a) Springer, T. A. *Cell* **1994**, *76*, 301. (b) Ley, K. *Cardiovasc. Res.* **1996**, *32*, 733. (c) Campbell, J. J.; Hedrick, J.; Zlotnik, A.; Siani, M. A.; Thompson, D. A.; Butcher, E. C. *Science* **1998**, *279*, 381.

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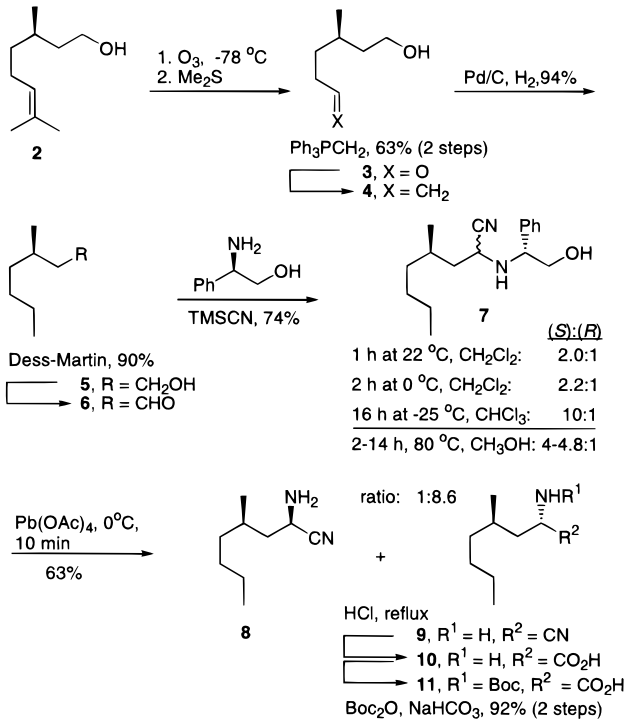
^{1}H NMR spectroscopy and X-ray crystallography.[3] Only one of the six L-amino acid residues is incorporated in its unmodified naturally occurring form, three are *N*-methylated, and four of the component residues are novel: (*R*)-2-hydroxy-4-cyanobutyric acid (DGCN), two (4*R*)-5-propyl-L-leucine (PrLEU) residues, and $N^{1'}$-methoxy-*N*-methyl-L-tryptophan (MTO). Although *N*-methoxyindoles have been isolated as early as the 1970s,[4] an $N^{1'}$-methoxytryptophan derivative has only recently been observed in one other natural product.[5]

The conformation of HUN-7293 (**1**) incorporates two cis peptide bonds in both the solution and crystalline states and two transannular H-bonds adding stability and rigidity to the backbone structure.[3] The cis peptide bonds occur at two of the three *N*-methyl amide sites and are found between PrLEU^2 and MLEU^3 and between LEU^4 and MTO^5. The transannular H-bonds are observed between PrLEU^6-CO/PrLEU^2-NH and MLEU^3-CO/PrLEU^6-NH stabilizing the compact conformation. The overall shape of the cyclopeptolide is bent or cup-shaped, allowing the DGCN, LEU, and MTO residues to reside in close proximity to one another on the concave side of the peptide. The two PrLEU side chains are located on the convex face of the peptide and extend in the same direction. Thus, the *N*-methyls may be regarded as conformationally significant structural features as well as contributing to improved protease resistance analogous to cyclosporin A.[6]

Herein we report the first total synthesis of **1** and the initial results from degradation/resynthesis studies of the natural product conducted as part of a systematic investigation of the structure−activity relationships of HUN-7293. The approach relied on a key macrocyclization reaction with formation of the MLEU^3−LEU^4 secondary amide, a reaction that proved to be unusually effective potentially benefitting from intramolecular H-bonding preorganization of the acyclic substrate. In turn, the requisite linear depsipeptide was assembled convergently with the late stage introduction of the linking ester enlisting a Mitsunobu esterification. This occurs with the inversion of the stereochemistry of the DGCN α-center which in turn permitted the utilization of a readily available L-amino acid precursor to the D α-hydroxy carboxylic acid residue. The requisite tetrapeptide **43** and tripeptide **34** were assembled convergently from the appropriate amino acid constituents (Figure 1).

Preparation of the Amino Acid Constituents. PrLEU (**10**, (4*R*)-5-propyl-L-leucine) was synthesized as shown in Scheme 1 starting with commercially available (*R*)-(+)-citronellol (**2**). Ozonolysis and Wittig reaction of the resulting aldehyde **3** with methylenetriphenylphosphorane gave alcohol **4** in 63% yield for the two steps. Hydrogenation of **4** provided the saturated alcohol **5**[7,8] (94%) which was oxidized to the corresponding aldehyde **6**[9] by treatment with the Dess−Martin reagent[10] and directly used in the next step without further purification. Asymmetric Strecker reaction utilizing (*R*)-phenylglycinol and

Scheme 1



trimethylsilyl cyanide[11] afforded **7** as an 8.6:1 mixture of diastereomers in favor of the expected and desired (2*S*)-diastereomer. This reaction proved to be sensitive to the reaction conditions, and both kinetic and thermodynamic control could be used to selectively generate the (2*S*)-diastereomer. Thus, low-temperature condensation (−25 °C, $CHCl_3$, 16 h) conducted under kinetically controlled reaction conditions provided **7** (74%, 10:1), and thermal equilibration (CH_3OH, 80 °C, 2−14 h) of a mixture of C2 diastereomers provided an equilibrium ratio of 4−4.8:1 with the (2*S*)-diastereomer predominating. Although separation of the diastereomers by crystallization or chromatography was not successful at this stage, the free amine **9**, derived by oxidative cleavage of **7** with lead tetraacetate,[12] could easily be separated from its C2 diastereomer **8** by column chromatography (SiO_2, 40% EtOAc−hexane, $\alpha = 1.17$). The absolute stereochemistry of the diastereomers was determined by synthesis and comparison of the ^{1}H NMR spectra of their Mosher[13] derivatives and ultimately confirmed upon incorporation into **1**. Hydrolysis of the nitrile to the free acid **10** and Boc protection of the amine completed of the synthesis of the PrLEU precursor **11**.

$N^{1'}$-Methoxytryptophan derivative **23** was initially prepared from *N*-methoxyindole (**12**)[14] as shown in Scheme 2. Phenylacetic acid was condensed with methoxyamine hydrochloride[15] (98%) and subsequent ring closure was achieved following a

(6) (a) Lobb, R. R.; Hemler, M. E. *J. Clin. Invest.* **1994**, *94*, 1722. (b) Foster, C. A. *J. Allergy Clin. Immunol.* **1996**, *98*, S270. (c) Cornejo, C. J.; Winn, R. K.; Harlan, J. M. *Adv. Pharmacol.* **1997**, *39*, 999.

(7) (a) Bergdahl, M.; Nilsson, M.; Olsson, T.; Stern, K. *Tetrahedron* **1991**, *47*, 9691. (b) Fang, C.; Ogawa, T.; Seumune, H.; Sakai, K. *Tetrahedron: Asymmetry* **1991**, *2*, 389. (c) Bezuidenhoudt, B. C. B.; Castle, G. H.; Innes, J. E.; Ley, S. V. *Recl. Trav. Chim. Pays-Bas* **1995**, *114*, 184. (d) Mori, K.; Kato, M. *Liebigs Ann. Chem.* **1985**, *10*, 2083. (e) Norsikian, S.; Marek, I.; Normant, J.-F. *Tetrahedron Lett.* **1997**, *38*, 7523. (f) Orsini, F.; Rinaldi, S. *Tetrahedron: Asymmetry* **1997**, *8*, 1039. (g) Marek, I.; Alexakis, A.; Mangeney, P.; Normant, J. F. *Bull. Soc. Chim. Fr.* **1992**, *129*, 171. (h) Somfai, P.; Tanner, D.; Olsson, T. *Tetrahedron* **1985**, *41*, 5973.

(8) Breitenbach, R.; Chiu, C. K.-F.; Masset, S. S.; Meltz, M.; Murtiashaw, C. W.; Pezzullo, S. L.; Staigers, T. *Tetrahedron: Asymmetry* **1996**, *7*, 435.

(9) Naemura, K.; Kittaka, K.; Murata, M.; Ida, H.; Hirose, H. *Enantiomer* **1996**, *1*, 219.

(10) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.

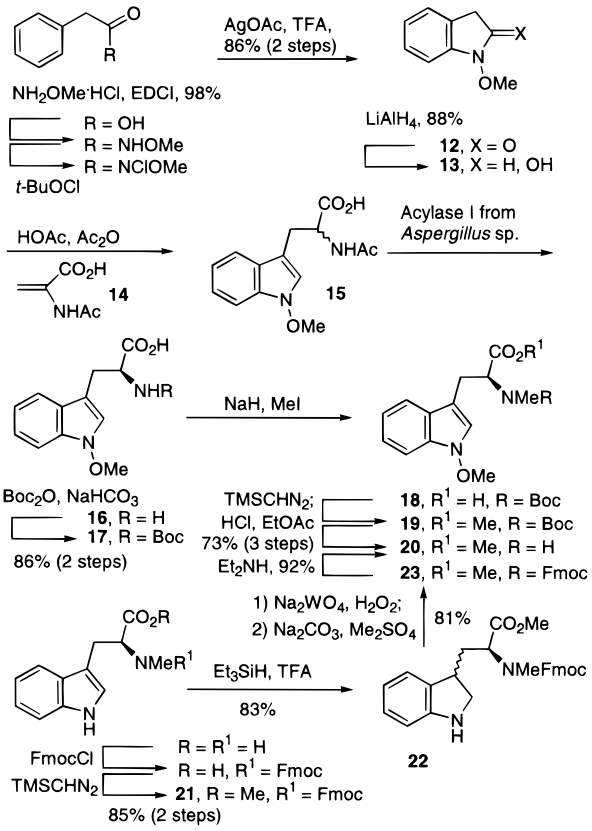
(11) (a) Inaba, T.; Kozono, I.; Fujita, M.; Ogura, K. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2359. (b) Chakraborty, T. K.; Reddy, G. V.; Hussain, K. A. *Tetrahedron Lett.* **1991**, *32*, 7597.

(12) Gawley, R. E.; Rein, K.; Chemburkar, S. *J. Org. Chem.* **1989**, *54*, 3002.

(13) (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512. (b) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543. (c) The stereochemistry of the undesired (*R*,*R*)-diastereomer was established by the synthesis of both Mosher derivatives and the comparison of the ^{1}H NMR spectra. The difference in the chemical shift of C3-proton δ (C3−H (*R*-derivative)−C3−H (*S*-derivative)) was found to be −0.05. According to the correlations established by Mosher,[13a] the configuration at C2 was established to be *R*. Details are provided in the Supporting Information.

(14) (a) Acheson, R. M.; Hunt, P. G.; Littlewood, D. M.; Murrer, B. A.; Rosenberg, H. E. *J. Chem. Soc., Perkin Trans. I* **1978**, 1117. (b) Hanke, F. J.; Kubo, I. *J. Nat. Prod.* **1989**, *52*, 1237.

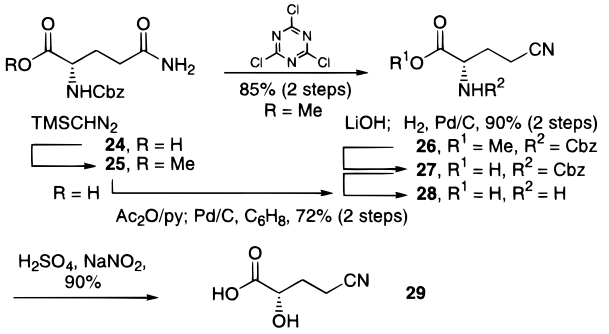
Scheme 2



sequence developed by Kikugawa,[16] which involved *N*-chlorination[17] followed immediately by Ag(I)-catalyzed ring closure to give **12** (86%). The amide was reduced cleanly to the hemiaminal **13** using $LiAlH_4$ (88%), which could be converted directly to the racemic tryptophan derivative **15** by heating with α-acetamidoacrylic acid (**14**) in HOAc−Ac_2O presumably proceeding through the *N*-methoxyindole.[18] Resolution of **15** by enantioselective enzymatic hydrolysis of the *N*-acetate using Acylase I[19] from *Aspergillus* sp. afforded $N^{1\prime}$-methoxy-L-tryptophan (**16**). The crude reaction mixture containing $N^{1\prime}$-methoxy-L-tryptophan and *N*-acetyl-$N^{1\prime}$-methoxy-D-tryptophan was submitted to standard *N*-Boc protection conditions which, upon chromatographic separation, gave pure **17** in 86% (43%) yield for the 2 steps. *N*-Methylation of **17** utilizing MeI−NaH followed by conversion of **18** to the methyl ester **19** upon reaction with $TMSCHN_2$[20] and removal of the Boc group (HCl−EtOAc) provided the MTO derivative **20** in 75% yield over the last 3 steps. The optical purity of intermediate **19** was established to be 98.6% ee by chiral phase HPLC (Chiralcel OD, 0.45 × 25 cm, 3% *i*-PrOH/hexane 1 mL/min flow rate, $\alpha = 1.19$, $t_R =$ 9.86 (D) and 11.73 (L) min), indicating that the enzymatic resolution was highly effective and, importantly, that the subsequent *N*-methylation step proceeded without detectable racemization.

Upon completion of the synthesis a shorter and more effective preparation of **20** was developed employing an indirect approach

Scheme 3



to the N^1-oxidation of a *N*-methyltryptophan derivative which itself was not successful. Thus, reduction of the indole **21** to the corresponding indoline **22** (83%) followed by *N*-oxidation ($Na_2WO_4-H_2O_2$) with in situ reoxidation to the indole and subsequent *O*-methylation provided the protected MTO derivative **23** (Scheme 2). Fmoc deprotection afforded **20** and provided a much more concise route to this key amino acid subunit.

The precursor **29** to the (*R*)-2-hydroxy-4-cyanobutyric acid (DGCN) constituent was synthesized according to Scheme 3 starting with the *N*-BCbz-L-GLN (**24**). Formation of the methyl ester **25** by treatment with $TMSCHN_2$ and subsequent dehydration utilizing cyanurchloride[21] gave the desired nitrile **26** (85%, 2 steps).[22] Hydrolysis of the methyl ester with LiOH and Cbz hydrogenolysis (H_2, Pd−C) gave the free amino acid **28** (>90%, 2 steps), which provided (*S*)-2-hydroxy-4-cyanobutyric acid (**29**) upon treatment with $NaNO_2$ and H_2SO_4 (>90%). Alternatively, direct amide dehydration of *N*-Cbz-GLN followed by transfer hydrogenolysis removal of the *N*-Cbz protecting group also provided **28** (72% overall).[22] Alcohol esterification of (*S*)-**29** under Mitsunobu conditions would proceed with inversion of the α-stereochemistry and, as such, permitted the use of a readily available L-amino acid precursor.[23]

Synthesis of Tripeptide 34. *N*-Boc-MLEU (**30**) was treated with isobutene and H_2SO_4 in CH_2Cl_2 to generate the *tert*-butyl ester **31** (89%) with simultaneous removal of the Boc group (Scheme 4). Following conversion of the amine to the HCl salt (HCl−EtOAc), **31** was coupled with *N*-Boc-PrLEU (**11**) utilizing EDCI−HOAt[24] and $NaHCO_3$ as base to provide **32** (54%). Removal of the Boc group was accomplished by treatment with HCO_2H (30 min, 22 °C),[25] and the *tert*-butyl ester was stable under these reaction conditions. Without purification, coupling of **33** with the DGCN residue precursor **29** utilizing EDCI−HOAt and $NaHCO_3$ as base provided the desired tripeptide **34** (65%, 2 steps) as a single diastereomer as determined by ^1H NMR.

Synthesis of Tetrapeptide 41. MALA-OCH_3 (**35**) was coupled with *N*-Boc-PrLEU (**11**, EDCI−HOAt, $NaHCO_3$, 74%)

(15) (a) De Rosa, M.; Alonso, J. L. T. *J. Org. Chem.* **1978**, *43*, 2639. (b) Gassman, P. G.; Campbell, G. A.; Metha, G. *Tetrahedron* **1972**, *28*, 2749.

(16) Kikugawa, Y.; Kawase, M. *J. Am. Chem. Soc.* **1984**, *106*, 5728.

(17) Mintz, M. J.; Walling, C. *Org. Synth.* **1969**, *49*, 9.

(18) Snyder, H. R.; Smith, C. W. *J. Am. Chem. Soc.* **1944**, *66*, 350.

(19) (a) Cho, H.-Y.; Tanizawa, K.; Tanaka, H.; Soda, K. *Agric. Biol. Chem.* **1987**, *51*, 2793. (b) Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354.

(20) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475.

(21) Maetz, P.; Rodriguez, M. *Tetrahedron Lett.* **1997**, *38*, 4221.

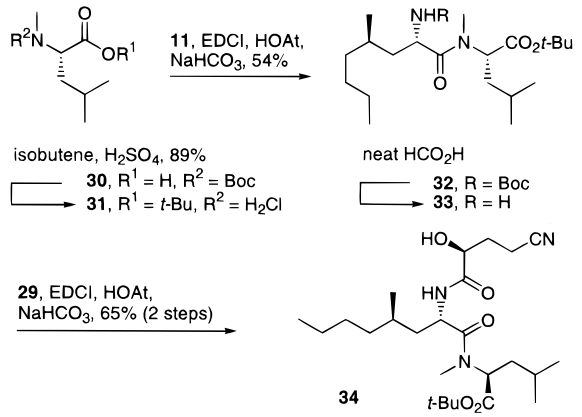
(22) Yoneta, T.; Shibahara, S.; Fukatsu, S.; Seki, S. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 3296. Boehlein, S. K.; Rosa-Rodriguez, J. G.; Schuster, S. M.; Richards, N. G. J. *J. Am. Chem. Soc.* **1997**, *119*, 5785. **26** has been prepared previously by esterification of Cbz-Glu (SO_2Cl_2, CH_3OH) and dehydration with $BnSO_2Cl$ or Burgess reagent; see the following: Van, T. T.; Kojro, E.; Grzonka, Z. *Tetrahedron* **1977**, *33*, 2299. Claremon, D. A.; Phillips, B. T. *Tetrahedron Lett.* **1988**, *29*, 2155. For **28**, see the following: Boehlein, S. K.; Rosa-Rodriguez, J. G.; Schuster, S. M.; Richards, N. G. J. *J. Am. Chem. Soc.* **1997**, *119*, 5785.

(23) The corresponding (*R*)-**29** was prepared from D-**24** in an analogous fashion and utilized for the preparation of **55** as detailed in Scheme 9.

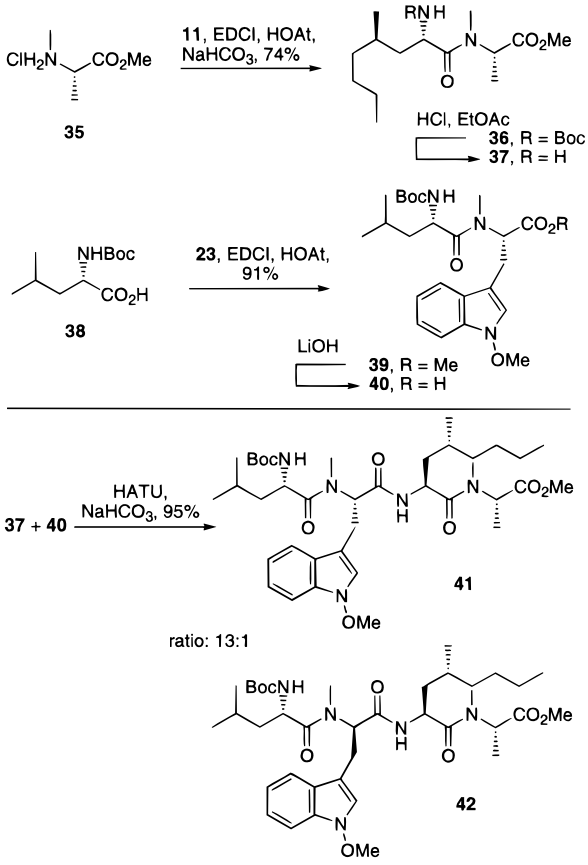
(24) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(25) Bodanszky, M.; Bodanszky, A. *Int. J. Pept. Res.* **1984**, *23*, 565.

Scheme 4

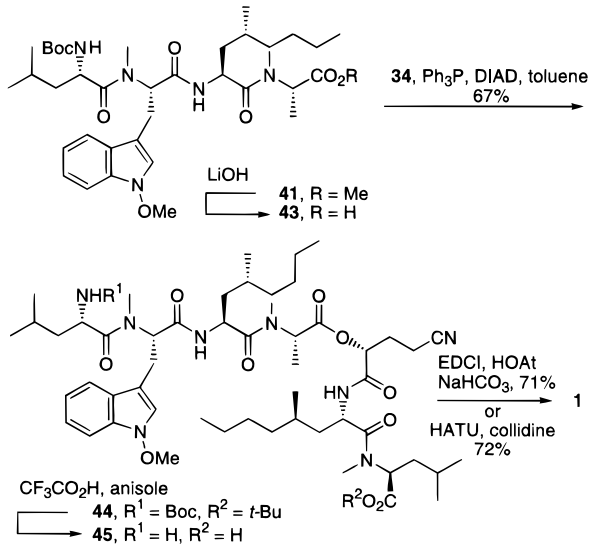


Scheme 5



to give the desired dipeptide **36** in which the extent of racemization during the coupling was less than 10% as determined by ^{1}H NMR (Scheme 5). Removal of the Boc group was accomplished by treatment with HCl−EtOAc to give **37** as the HCl salt which was used directly in the next coupling reacting without further purification. The dipeptide **40** was prepared from the tryptophan derivative **20** and *N*-Boc-LEU (**38**) in a coupling reaction that proceeded smoothly to give **39** as a single isomer in 91% yield. Hydrolysis of the methyl ester by treatment with LiOH in *t*-BuOH−H_2O gave the free acid **40** without evidence that racemization occurred under the reaction conditions[26] and in a reaction that was sufficiently clean that crude product could be used directly in the next reaction.

Scheme 6



Coupling of the dipeptides **37** and **40** (HATU, $NaHCO_3$) gave the desired tetrapeptide **41** together with small amounts of an undesired diastereomer **42** in 95% yield. Alternative coupling reagents including EDCI−HOAt provided larger amounts of **42**. Although attempts to separate the isomers by SiO_2 chromatography were unsuccessful, separation by semipreparative reversed-phase HPLC was easily accomplished to give clean samples of both isomers (13:1).

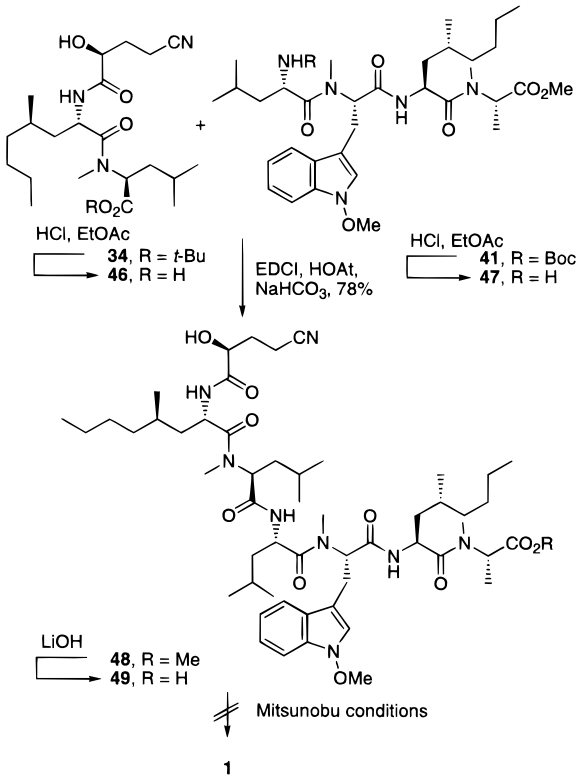
Final Steps: Esterification and Macrocyclic Ring Closure. Tetrapeptide **41** was hydrolyzed to the free acid **43** by treatment with LiOH, and the crude product was subjected to the following esterification step without further purification (Scheme 6). Reaction of **43** with alcohol **34** under modified Mitsunobu conditions[27] (Ph_3P−DIAD) proceeded smoothly to give the desired linear heptadepsipeptide **44** in good conversion. In optimizing this esterification, we found that the selection of the reaction conditions was critical. The combination of Ph_3P−DIAD in toluene gave the desired product in 67% yield. The use of Bu_3P instead of Ph_3P resulted in poor yields and various byproducts, and toluene was found to be a superior solvent over benzene and THF. The less hindered DEAD gave results comparable with DIAD although the yields were generally lower. Alternative attempts to generate **44** enlisting the corresponding (2*R*) versus (2*S*) DGCN precursor and direct esterification with carboxylate activation were not nearly as successful. Thus, coupling promoted by DCC required the presence of DMAP (0.1−2 equiv) to effect reasonable conversions at useful rates (−20 to 22 °C, 2−6 h, 18−69%) but provided a 1−2:1 ratio of diastereomers derived from racemization of the intermediate activated carboxylate together with significant amounts (∼20%) of the *N*-acyl urea. Epimerization is problematic because of the requirement for activation of a *N*-methyl *C*-terminus carboxylate, and this is strategically avoided through use of the Mitsunobu esterification.

Removal of the Boc group and the *tert*-butyl ester was accomplished in one step by treatment with CF_3CO_2H at 22 °C. The use of HCl−EtOAc or HCl−dioxane resulted in the formation of a byproduct derived from hydrolysis of the nitrile to the corresponding amide. Cyclization of the crude **45** utilizing EDCI−HOAt, $NaHCO_3$ proceeded smoothly within 6 h at 0 °C to give **1** in 71% overall yield identical in all respects with

(26) Treatment of **40** with $TMSCHN_2$ gave the methyl ester **39** which was indistinguishable from the ^{1}H NMR of the authentic starting material sample, indicating that no racemization occurred during the hydrolysis.

(27) (a) Mitsunobo, O.; Yamada, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380. (b) Mitsunobo, O. *Synthesis* **1981**, 1. (c) Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1988**, *110*, 6487.

Scheme 7

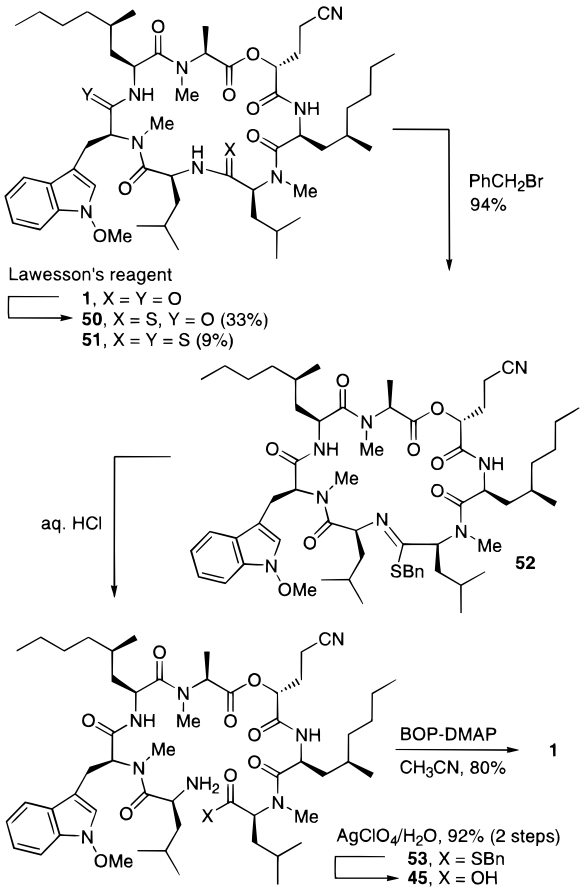


authentic HUN-7293[28] (^{1}H NMR, ^{13}C NMR, HPLC, TLC, $[\alpha]_D$, IR). Although not examined in detail, the use of DMF as solvent in initial trials was superior to CH_2Cl_2 or a mixture of CH_2Cl_2−DMF (5:1), which gave only traces of the desired product. Similarly, closure of crude **45** effected by treatment with HATU (3 equiv, 9 equiv of collidine, CH_2Cl_2, 25 °C, 10 h) provided **1** in superb conversion (72%), and in this case substantial racemization was observed in CH_3CN but not CH_2Cl_2. PyBroP (3 equiv, 6 equiv of iPr$_2$NEt, CH_2Cl_2 0−25 °C, 3−4 h, ∼70−80%) also promoted ring closure, but difficulty was encountered in removing reaction byproducts in the final purification of **1**. Closures conducted with DPPA (5 equiv, 4 equiv of $NaHCO_3$, DMF, 0 °C, 24 h, trace of **1**), BOP−Cl (3 equiv, DMAP, CH_3CN, 25 °C), or HATU (2 equiv, 5 equiv of $NaHCO_3$, DMF, 0 °C, 4 h, 20% **1**) were less effective in their initial examinations.

Additional strategically attractive attempts that reverse the order of the last amide coupling and esterification reaction such that the final macrocyclization step entailed formation of the sensitive ester were not nearly as successful (Scheme 7). Enlisting the substrate with the (2*R*)-DGCN subunit and the corresponding direct esterification (DCC, DMAP) was only modestly successful as detailed in the next section, and Mitsunobu displacement enlisting **49** with inversion of the DGCN α-center accompanying esterification was unsuccessful.[29]

Degradation and Resynthesis of HUN-7293. The most successful of the ring closures was independently shown to be

Scheme 8



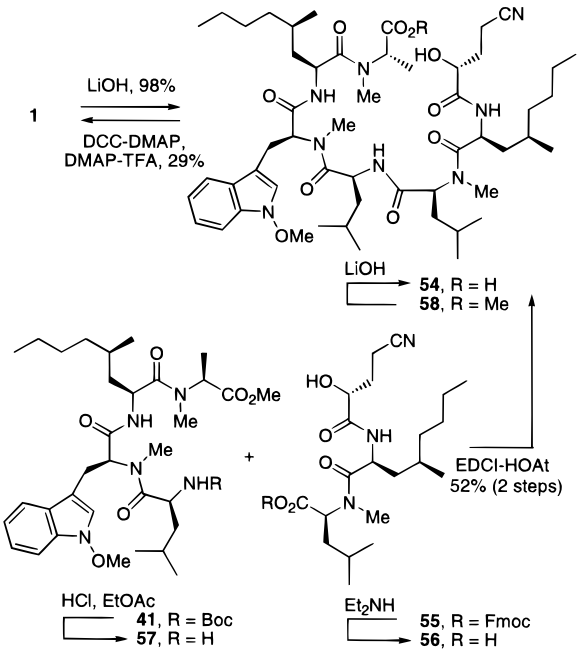
effective on the corresponding seco-derivative derived from the natural product via a selective ring-opening procedure at the MLEU^3–LEU^4 amide bond. Upon treatment of the natural product with 0.5 equiv of Lawesson's reagent, the amide bond between MLEU and LEU was primarily attacked and converted into the corresponding thioamide **50** (Scheme 8). This preference most likely reflects the accessibility of this amide, which in the crystal structure is the only amide to form a H-bond to a solvent molecule. As the only side product, a double thioamide with the additional introduction of sulfur at the MTO^5−PrLEU^6 amide bond was formed. Benzylation of the mono-thioamide was achieved in a two-phase system with benzyl bromide in CH_2Cl_2 and aqueous NaOH. The resulting benzylthioamidate **52** could quantitatively be cleaved by short treatment with aqueous acid (aqueous HCl/*t*-BuOH) at 55 °C without affecting the lactone. In a second step, the benzylthioester **53** at the newly formed C-terminus was selectively cleaved to the unprotected depsipeptide **45** by silver-promoted hydrolysis in aqueous *t*-BuOH. The crude material after gel filtration was subjected directly to ring closure in acetonitrile using BOP as condensing agent and DMAP as base. Using this complement to the ring closure detailed in Scheme 6, HUN-7293 was formed in a clean reaction (80%), most probably facilitated by a conformational preorganization of the precursor, due to a preformed H-bond of the activated ester C-terminus and the PrLEU^2 NH and partial *cis*-geometry of the methylated amide bonds.

The less successful ring closure via macrolactonization was also examined both with synthetic material as well as with that derived from the natural product (Scheme 9). The lactone bond in HUN-7293 was selectively cleaved under alkaline conditions (LiOH, THF/H_2O), producing the lithium salt of the corre-

(28) Dreyfuss, M. M.; Foster, C. A.; Naegeli, H.-U.; Oberhauser, B. PCT Int. Appl. 9603430, 1996; *Chem. Abstr.* **1996**, *125*, 34177.

(29) For **55**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.74 (d, J = 7.5 Hz, 2H), 7.52−7.56 (m, 2H), 7.37−7.41 (m, 2H), 7.28−7.33 (m, 2H), 7.04 (d, J = 8.0 Hz, 1H), 5.23 (dd, J = 10.7, 5.2 Hz, 1H), 4.88 (m, 1H), 4.54 (m, 2H), 4.22 (dd, J = 7.5, 4.0 Hz, 1H), 4.18 (t, J = 5.9 Hz, 1H), 2.77 (s, 3H), 2.50 (dd, J = 16.5, 7.7 Hz, 1H), 2.40 (ddd, J = 16.9, 8.3, 5.5 Hz, 1H), 2.09−2.15 (m, 1H), 1.90−1.97 (m, 1H), 1.17−1.59 (m, 12H), 0.94 (d, J = 6.0 Hz, 3H), 0.86 (d, J = 6.5 Hz, 3H), 0.84 (t, J = 7.0 Hz, 3H), 0.81 (d, J = 6.5 Hz, 1H); FABHRMS (NBA−CsI) *m*/*z* 722.2541 (M + Cs$^+$, $C_{35}H_{47}N_3O_5$ requires 722.2570). For **58**: FABHRMS (NBA−CsI) *m*/*z* 1141.5740 (M + Cs$^+$, $C_{54}H_{88}N_8O_{10}$ requires 1141.5678).

Scheme 9



sponding hydroxy acid **54**. Due to 15−20% racemization in this saponification at the α-carbon of MALA,[7] we obtained **54** as an inseparable mixture together with its D-MALA[7] isomer. Recyclization to HUN-7293 was achieved applying a modified protocol (acetonitrile, DCC/DMAP/DMAP•TFA, 55 °C) of the Keck method[30] which gave the natural product in 29% yield. For the cyclization, acetonitrile was found to be as efficient a solvent as chloroform which is usually used for this reaction. Efforts to form the (1*S*) analogue of HUN-7293 by a Mitsunobu reaction with inversion of the configuration were not successful.

This same hydroxy acid **54** incorporating the (*R*)-DGCN subunit was also prepared from tetrapeptide **41** and the corresponding tripeptide **55**[29] constituting a second, albeit less effective, total synthesis of HUN-7293 (Scheme 9). Notably, hydrolysis of **58** to provide **54** also proceeded with extensive racemization (20−30%), further detracting from this approach.

Biological Activity of HUN-7293. To compare the biological activity of the naturally occurring HUN-7293 [natural **1**] with the cyclodepsipeptide achieved by total synthesis [synthetic **1**], we evaluated both compounds in parallel using a cell-based ELISA for inducible adhesion molecules in human endothelial cells. The vascular proteins VCAM-1, ICAM-1, and E-selectin, due to their prominent roles in regulating leukocyte extravasation,[5,6] were investigated in primary cells derived from umbilical vein (HUVEC) and a microvascular cell line (HMEC-1). Figure 2 shows that natural and synthetic **1** potently and dose-dependently inhibited VCAM-1 expression in HUVEC to a similar extent, with IC_{50} (concentration resulting in 50% inhibition) values of 3 and 6 nM, respectively (Table 1). Complete down-regulation of VCAM-1 occurred by 37 nM, essentially equal to background levels in the unstimulated group, whereas the cell density was not altered under these experimental conditions (Figure 2). Natural and synthetic **1** also showed similar effects on VCAM-1 in the cell line HMEC-1 (Table 1), confirming earlier results with the naturally occurring HUN-7293.[4]

Compared to VCAM-1 inhibition, natural and synthetic **1** were both 8- to 20-fold less effective in suppressing ICAM-1 up-regulation in HUVEC and HMEC-1 cells, respectively (Table 1). In addition, approximately 13-fold higher concentrations of both compounds were needed to inhibit E-selectin expression in HUVEC, relative to their effect on VCAM-1 (Table 1). The present results with natural **1** are in agreement with previous studies, such as in HMEC-1 cells where the mean IC_{50} for VCAM-1 was 2 nM (range 0.3−4 nM in 12 experiments) and that for ICAM-1 was 39 nM (range 11−89 nM in 7 experiments). Therefore, considering the usual variablility seen in such biological assays, our data indicate that the synthesis of HUN-7293 (**1**) has provided a compound that is indistinguishable from the naturally occurring cyclodepsipeptide.

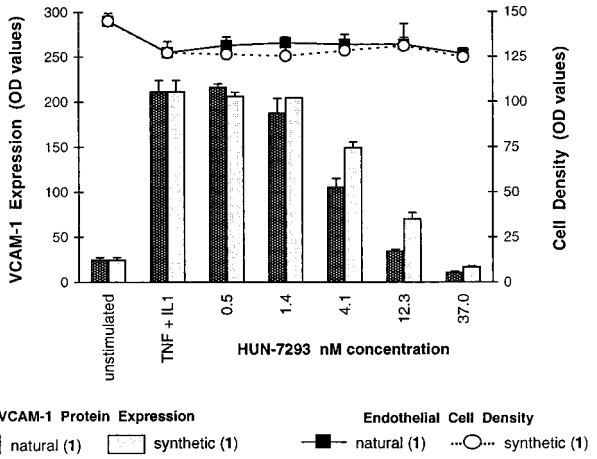


Figure 2. A representative cell-ELISA comparing the inhibitory effect of HUN-7293 natural (**1**) versus synthetic (**1**) on VCAM-1 expression in primary human endothelial cells (HUVEC). Optical density (OD 550 nm) values ± standard deviation indicate VCAM-1 protein levels on the left *y*-axis and relative cell density on the right axis, using triplicate wells/experimental group. Briefly, cells in 96-well microtiter plates were incubated with serial dilutions of HUN-7293 for 4 h and then cytokine-stimulated with TNF_α (tumor necrosis factor alpha) + IL-1 (interleukin 1) (100 units/mL each) for 16 h, as previously described.[4] Possible mitogenic, cytostatic, or cytotoxic effects were analyzed in the same plate by subsequently quantifying the cell number (absorbance of Giemsa nuclear dye), relative to control wells.

Table 1. Comparison of HUN-7293 Natural (**1**) Versus Synthetic (**1**) Biological Activity (nM IC_{50}) in Cell ELISA for VCAM-1, ICAM-1, and E-selectin Expression Using Primary Endothelial Cells (HUVEC) and a Human Micro-Vascular Cell Line (HMEC-1)

	VCAM-1 IC_{50}		ICAM-1 IC_{50}		E-sel
HUN-7293	HUVEC	HMEC	HUVEC	HMEC	HUVEC
natural (**1**)	3	1	26	24	44
synthetic (**1**)	6	2	49	42	69

Experimental Section

***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-Butyloxycarbonyl)amino]-4-methylheptanoyl]-*N*-methyl-L-leucine *tert*-Butyl Ester (32).** A solution of **11**[31] (43.7 mg, 160 μmol) and **31**[31] (56.8 mg, 1.5 equiv) in 0.4 mL of CH_2Cl_2−DMF (5:1) was treated with $NaHCO_3$ (20.1 mg, 1.5 equiv), HOAt (26.0 mg, 1.2 equiv), and EDCI (60.9 mg, 2 equiv) at 0 °C. The mixture was stirred for 32 h at 22 °C and diluted with 2 mL of aqueous 1 M HCl. The mixture was extracted with EtOAc (2 × 2 mL), and the combined organic phase was washed with 1 mL of H_2O and dried over $MgSO_4$. The mixture was filtered, and the solvent was removed under reduced pressure. Chromatography (SiO_2, 25% EtOAc−hexane) afforded **32** (39.5 mg, 54%) as a white solid: $[\alpha]_D^{22}$ −31 (*c* 0.50, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.19 (dd, *J* = 5.2, 10.7 Hz), 5.15 (d, *J* = 9.2 Hz), 4.61 (m, 1H, C2−H), 2.92 (s, 3H), 1.40 (s, 9H),

(30) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.

(31) Details of the preparation may be found in the Supporting Information.

1.39 (s, 9H), 1.20−1.70 (m, 12H), 0.96 (d, J = 6.3 Hz, 3H), 0.87−0.92 (m, 6H), 0.86 (t, J = 5.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.8, 170.8, 81.5, 79.4, 55.1, 48.8, 40.4, 37.3, 37.0, 30.8, 29.2, 29.1, 28.3, 28.0, 24.8, 23.3, 22.8, 21.4, 19.2, 14.0; IR (neat) υ_{max} 2958, 2928, 1732, 1716, 1652, 1368, 1272, 1254, 1170 cm^{-1}; FABHRMS (NBA) m/z 457.3629 (M + H$^+$, $C_{25}H_{48}N_2O_5$ requires 457.3641).

***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-2-Hydroxy-4-cyanobutanoyl]amino]-4-methylheptanoyl]-*N*-methyl-L-leucine *tert*-Butyl Ester (34).** A solution of **32** (29.0 mg, 64.2 μmol) in 3 mL of HCO_2H was stirred for 1 h at 22 °C. The solvent was removed with a steam of N_2, and the residue was dissolved in 0.5 mL of HCl−EtOAc. The solvent was removed with a stream of N_2, and the residue was dissolved in 0.5 mL of CH_2Cl_2−DMF (5:1). A sample of **29**[31] (16.8 mg, 1.5 equiv), $NaHCO_3$ (7.3 mg, 1 equiv), HOAt (17.7 mg, 1.5 equiv), and EDCI (33.1 mg, 2 equiv) was added at 0 °C. The mixture was stirred for 2 h at 0 °C and diluted with 2 mL of aqueous 1 M HCl. The mixture was extracted with EtOAc (2 × 2 mL), and the combined organic phase was washed with 1 mL of H_2O and dried over $MgSO_4$. The mixture was filtered, and the solvent was removed under reduced pressure. Chromatography (SiO_2, 25% EtOAc−hexane) afforded **34** (19.5 mg, 65%) as a white solid: $[\alpha]_D^{22}$ −58 (c 0.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.26 (d, J = 8.9 Hz, 1H), 5.08 (dd, J = 10.7, 5.2 Hz, 1H), 4.92 (m, 1H), 4.17 (m, 1H), 2.97 (s, 3H), 2.39−2.51 (m, 2H), 2.13−2.25 (m, 2H), 1.94 (m, 1H), 1.20−1.71 (m, 11H), 1.41 (s, 9H), 0.83−0.96 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.8, 172.4, 170.3, 119.3, 81.8, 69.8, 55.5, 47.3, 39.4, 37.2, 36.9, 31.0, 30.5, 29.5, 29.1, 24.8, 23.2, 22.8, 21.4, 18.9, 14.0, 13.0; IR (neat) υ_{max} 2958, 2931, 2872, 1732, 1634, 1520, 1368, 1274, 1160, 1127, 1090 cm^{-1}; FABHRMS (NBA) m/z 468.3424 (M + H$^+$, $C_{25}H_{45}N_3O_5$ requires 468.3437).

***N*-[(2*S*,4*R*)-2-[*N*-(*tert*-Butyloxycarbonyl)amino]-4-methylheptanoyl]-*N*-methyl-L-alanine Methyl Ester (36).** A solution of **11**[31] (6.5 mg, 24 μmol) and **35**[32] (5.5 mg, 36 μmol) in 0.25 mL of CH_2Cl_2−DMF (5:1) was treated with $NaHCO_3$ (2.9 mg, 35 μmol), HOAt (6.5 mg, 48 μmol), and EDCI (9.1 mg, 48 μmol) at 0 °C. The mixture was stirred for 35 h at 22 °C and diluted with 2 mL of aqueous 1 M HCl. The mixture was extracted with EtOAc (3 × 2 mL), and the combined organic phase was washed with 1 mL of H_2O and dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure. Chromatography (SiO_2, 25% EtOAc−hexane) afforded **36** (6.5 mg, 74%) as a white solid: $[\alpha]_D^{22}$ −6.8 (c 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.25 (q, J = 7.0 Hz, 1H), 5.18 (d, J = 9.0 Hz, 1H), 4.65 (m, 1H), 3.69 (s, 3H), 2.97 (s, 3H), 1.45−1.61 (m, 3H), 1.41 (s, 9H), 1.15−1.29 (m, 6H), 0.98 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H), 0.87 (t, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 172.1, 155.7, 79.4, 52.2, 52.0, 48.8, 40.9, 37.3, 30.9, 29.24, 29.17, 28.3, 22.8, 19.2, 14.2, 14.0; IR (neat) υ_{max} 2960, 2928, 1746, 1709, 1647, 1497, 1461, 1366, 1248, 1174, 1093 cm^{-1}; FABHRMS (NBA) m/z 373.2694 (M + H$^+$, $C_{19}H_{36}N_2O_5$ requires 373.2702).

***N*-[*N*-(*tert*-Butyloxycarbonyl)-L-leucinyl]-*N*$^{1\prime}$-methoxy-*N*-methyl-L-tryptophan Methyl Ester (39).** A solution of **20**[31] (36.3 mg, 139 μmol) and *N*-Boc-LEU (**38**, 64 mg, 277 μmol) in 1.4 mL of CH_2Cl_2 was treated with HOAt (38 mg, 277 μmol) and EDCI (53 mg, 277 μmol) at 0 °C. The mixture was stirred for 20 h at 22 °C and concentrated to dryness under reduced pressure. Chromatography (SiO_2, 33% EtOAc−hexane) afforded **39** (59.8 mg, 91%) as a pale yellow solid: $[\alpha]_D^{22}$ −53 (c 0.56, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55 (d, J = 7.9 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.21 (t, J = 8.1 Hz, 1H), 7.09 (t, J = 7.9 Hz, 1H), 7.08 (s, 1H), 5.33 (dd, J = 5.7, 10.1 Hz, 1H), 5.14 (d, J = 8.6 Hz, 1H), 4.56 (m, 1H), 3.99 (s, 3H), 3.71 (s, 3H), 3.42 (ddd, J = 1.0, 5.6, 15.6 Hz, 1H), 3.18 (dd, J = 10.1, 15.8 Hz, 1H), 2.90 (s, 3H), 1.51−1.77 (m, 3H), 1.39 (s, 9H), 0.91 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.7, 171.3, 155.6, 132.3, 123.6, 122.5, 121.4, 119.7, 118.6, 108.3, 106.9, 79.5, 65.6, 57.4, 52.3, 49.1, 42.2, 32.3, 28.3, 24.6, 24.1, 23.4, 21.7; IR (neat) υ_{max} 3346, 2957, 1742, 1709, 1650, 1504, 1454, 1440, 1392, 1367, 1251, 1169, 1047, 1024, 739 cm^{-1}; FABHRMS (NBA) m/z 476.2772 (M + H$^+$, $C_{25}H_{37}N_3O_6$ requires 476.2761).

***N*-[*N*-(*tert*-Butyloxycarbonyl)-L-leucinyl]-*N*$^{1\prime}$-methoxy-*N*-methyl-L-tryptophan (40).** A solution of **39** (100 mg, 211 μmol) dissolved in 1 mL of *t*-BuOH−H_2O (2:1) was treated with LiOH (10.1 mg, 422 μmol) at 0 °C. After being stirred for 2 h at 0 °C, the mixture was diluted with aqueous 1 N HCl (5 mL) and extracted with EtOAc (3 × 8 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure to give **40** (101 mg, quantitative) as a pale yellow solid: $[\alpha]_D^{22}$ −60 (c 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz, both rotamers) δ 7.55 (d, J = 7.9 Hz, 1H), 7.38 (t, J = 8.2 Hz, 1H), 7.22 (m, 1H), 7.02−7.13 (m, 2H), 5.23−5.28 (m, 1.66H), 4.87 (dd, J = 3.7, 10.7 Hz, 0.33H), 4.54 (m, 0.66H), 4.09 (m, 0.33H), 4.01 and 4.00 (s, 3H), 3.42−3.50 (m, 1H), 3.25 (dd, J = 10.7, 15.8 Hz, 0.66H), 3.13 (dd, J = 10.7, 15.1 Hz, 0.33H), 2.98 and 2.91 (s, 3H), 1.70 (m, 0.66H), 1.16−1.52 (m, 2.33H), 1.39 (s, 9H), 0.92 (d, J = 6.6 Hz, 2H), 0.89 (d, J = 6.6 Hz, 2H), 0.44 (d, J = 6.3 Hz, 1H), 0.14 (d, J = 6.3 Hz, 1H), −0.17 (m, 0.33H), ^{13}C NMR ($CDCl_3$, 125 MHz, both rotamers) δ 174.5, 174.2, 173.5, 170.6, 156.8, 155.8, 132.3, 123.6, 123.2, 123.0, 122.6, 122.0, 121.5, 120.4, 119.8, 118.6, 118.4, 108.6, 108.4, 106.7, 106.0, 81.4, 79.7, 65.9, 65.7, 61.2, 58.3, 49.2, 47.6, 41.7, 39.3, 33.0, 29.5, 28.25, 28.17, 24.6, 24.1, 23.9, 23.7, 23.4, 22.8, 21.6, 19.7; IR (neat) υ_{max} 3317, 2959, 1716, 1652, 1616, 1506, 1456, 1367, 1252, 1168, 1100, 1024, 955, 739 cm^{-1}; FABHRMS (NBA−NaI) m/z 484.2407 (M + Na$^+$, $C_{24}H_{35}N_3O_6$ requires 484.2424).

***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butyloxycarbonyl)-L-leucinyl]-*N*$^{1\prime}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-*N*-methyl-L-alanine Methyl Ester (41).** A solution of **36** (29.3 mg, 79.0 μmol) in 1.0 mL of EtOAc was saturated with HCl(g), at 0 °C and the mixture was stirred for 70 min at 0 °C. The solvent was removed with a stream of N_2, and the residue was dried under vacuum to give **37** (27 mg) as a white solid. A sample of **40** (33.0 mg, 71.9 μmol) in 1.0 mL of CH_2Cl_2−DMF (5:1) was added at 0 °C, followed by $NaHCO_3$ (10.0 mg, 119 μmol) and HATU (54.6 mg, 144 μmol). The mixture was stirred for 3 h at −30 °C and 1 h at 0 °C before being diluted with 2 mL of aqueous 1 M HCl. The mixture was extracted with EtOAc (3 × 2 mL), and the combined organic phase was washed with 1 mL of H_2O and dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure. Chromatography (SiO_2, 60% EtOAc−hexane) afforded the mixture of **41** and **42** (48.8 mg, 95%) as a white solid. Further purification by semipreparative HPLC (reverse-phase C18, 80% CH_3OH−H_2O) gave 45.0 mg of the desired diastereomer **41** (88%) and 3.5 mg (7%) of the undesired diastereomer **42** as white solids. For **41**: $[\alpha]_D^{22}$ −81.6 (c 0.58, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz, both rotamers) δ 8.23 (d, J = 8.1 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.36 (t, J = 9.9 Hz, 2H), 7.22 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 7.7 Hz, 1H), 7.06−7.11 (m, 2H), 6.96 (s, 1H), 6.59 (d, J = 8.5 Hz, 1H), 5.42 (t, J = 7.7 Hz, 1H), 5.38 (q, J = 7.8 Hz, 1H), 5.19 (q, J = 7.4 Hz, 1H), 5.14 (d, J = 8.9 Hz, 1H), 5.04 (m, 1H), 4.92 (t, J = 8.5 Hz, 1H), 4.76 (m, 1H), 4.57 (m, 1H), 3.99 (s, 3H), 4.00 (s, 3H), 3.67 (s, 3H), 3.69 (s, 3H), 3.38 (dd, J = 3.3, 15.5 Hz, 1H), 3.30 (dd, J = 7.4, 15.5 Hz, 1H), 3.13 (dd, J = 8.1, 15.5 Hz, 1H), 3.08 (dd, J = 10.7, 15.5 Hz, 1H), 3.07 (s, 3H), 2.99 (s, 3H), 2.94 (s, 3H), 2.92 (s, 3H), 1.10−1.78 (m, 23H), 1.41 (s, 9H), 1.38 (s, 9H), 0.96 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.84−0.92 (m, 18H), 0.42 (d, J = 6.7 Hz, 3H), 0.00 (d, J = 6.7 Hz, 3H), −0.43 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz, two rotamers) δ 174.3, 173.9, 172.8, 172.4, 172.1, 169.7 and 168.4, 156.3 and 155.7, 132.2 and 132.1, 123.7 and 123.5, 122.8, 122.4, 122.1, 121.3, 120.2, 119.6, 118.8 and 118.7, 108.5 and 108.2, 106.7 and 106.6, 80.3 and 79.6, 65.9 and 65.6, 60.9, 56.2, 52.2, 52.1, 52.0, 51.7, 49.1, 47.7 and 47.6, 47.3, 42.2, 40.0, 39.2, 38.7, 37.4, 37.1, 31.0, 30.9, 30.8, 30.6, 29.4, 29.3, 29.2, 29.1, 28.30 and 28.25, 24.6, 23.6, 23.5, 23.4, 23.0, 22.8, 21.5, 19.3, 19.0, 18.9, 14.4, 14.2, 14.1; IR (neat) υ_{max} 3303, 2956, 2930, 1744, 1707, 1637, 1453, 1408, 1366, 1251, 1169, 1097, 1045, 1023, 956, 739 cm^{-1}; FABHRMS (NBA−CsI) m/z 848.3548 (M + Cs$^+$, $C_{38}H_{61}N_5O_8$ requires 848.3574).

***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-2-[*N*-[(2*S*,4*R*)-2-[[*N*-[*N*-(*tert*-Butyloxycarbonyl)-L-leucinyl]-*N*$^{1\prime}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]amino]-4-methylheptanoyl]-*N*-methyl-L-leucine *tert*-Butyl Ester (44).** A solution of **41** (7.6 mg, 10.6 μmol) was dissolved in 0.3 mL of *t*-BuOH−H_2O (2:1) and treated with LiOH (0.5 mg, 21.2 μmol) at 0 °C. After being stirred for 100 min at 0 °C, the mixture was diluted with aqueous 1 N HCl (1 mL) and extracted with EtOAc (3 × 2 mL).

(32) Olsen, R. K. *J. Org. Chem.* **1970**, *35*, 1912.

The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure to give a pale yellow solid. A sample of **34** (4.4 mg, 9.4 μmol) and Ph_3P (13.9 mg, 53.1 μmol) in 200 μL of toluene was added, and the mixture was cooled to 0 °C. Diisopropylazodicarboxylate (10.4 μL, 52.9 μmol) was added and stirring was continued for 16 h at 22 °C. Evaporation of the solvent and chromatography (SiO_2, 60% EtOAc−hexane) afforded 19.5 mg of crude product which was contaminated with large amounts of reagent. Further purification by semipreparative HPLC (reverse-phase C18, 90% CH_3OH-H_2O) gave **44** (7.2 mg, 67%) as a white solid: $[\alpha]_D^{22}$ −94 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz, major rotamer) δ 8.08 (d, J = 9.2 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.19 (t, J = 7.3 Hz, 1H), 7.06−7.12 (m, 1H), 7.08 (s, 1H), 5.46 (dd, J = 7.0, 8.7 Hz, 1H), 5.30 (dd, J = 4.8, 10.4 Hz, 1H), 5.25 (d, J = 9.1 Hz, 1H), 5.12 (dd, J = 4.3, 7.3 Hz, 1H), 4.99 (t, J = 7.6 Hz, 1H), 4.86 (m, 1H), 4.56 (t, J = 8.9 Hz, 1H), 3.99 (s, 3H), 3.30 (dd, J = 6.5, 15.6 Hz, 1H), 3.12−3.22 (m, 1H), 3.08 (s, 3H), 3.04 (s, 3H), 2.93 (s, 3H), 1.92−2.31 (m, 5H), 1.10−1.70 (m, 42H), 0.81−0.99 (m, 21H), 0.78 (d, J = 6.6 Hz, 3H), 0.65 (d, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 173.5, 172.0, 170.8, 169.9, 168.2, 155.7, 132.1, 123.7, 122.4, 121.4, 119.7, 118.8, 108.2, 106.8, 81.4, 79.4, 73.2, 65.6, 58.0, 56.1, 55.1, 49.3, 48.1, 46.7, 42.2, 39.5, 38.1, 37.5, 36.9, 36.8, 35.8, 30.8, 30.6, 29.34, 29.30, 29.06, 29.02, 28.3, 28.1, 27.1, 24.7, 24.6, 24.1, 23.5, 23.1, 23.0, 22.9, 21.5, 21.3, 19.5, 18.9, 14.1, 13.6, 13.5; IR (neat) υ_{max} 3306, 2928, 2871, 2248, 1646, 1634, 1539, 1546, 1368, 1165, 1096, 1046, 955, 803, 739 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1283.6604 (M + Cs^+, $C_{62}H_{102}N_8O_{12}$ requires 1283.6672).

HUN-7293 (1). A sample of **44** (2.0 mg, 1.74 μmol) was dissolved in 50 μL of anisole, 250 μL of trifluoroacetic acid was added, and the mixture was stirred for 60 min at 22 °C. The solvent was removed with a stream of N_2, and the residue was dissolved in 100 μL of 3.5 M HCl−EtOAc. The solvent was removed with a stream of N_2, and the residue was dissolved in 1 mL of DMF (freshly distilled). $NaHCO_3$ (0.3 mg, 3.5 μmol), HOAt (0.5 mg, 3.5 μmol), and EDCI (0.7 mg, 3.5 μmol) were added at 0 °C. The mixture was stirred for 3 h at 0 °C, and additional EDCI (0.7 mg, 3.5 μmol) was added. Stirring was continued for 3 h at 0 °C, and the solvent was removed under reduced pressure. Chromatography (SiO_2, 10% *i*-PrOH−toluene) afforded **1** (1.2 mg, 71%) as a white solid: $[\alpha]_D^{22}$ −221 (*c* 0.06, CH_3OH), lit.[28] $[\alpha]_D^{22}$ −234 (*c* 1.11, CH_3OH); ^{1}H NMR ($CDCl_3$, 500 MHz, major rotamer) δ 8.48 (d, J = 10.1 Hz, 1H), 8.05 (d, J = 9.7 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.19 (t, J = 7.3 Hz, 1H), 7.06−7.12 (m, 1H), 7.08 (s, 1H), 5.46 (dd, J = 7.0, 8.7 Hz, 1H), 5.30 (dd, J = 4.8, 10.4 Hz, 1H), 5.25 (d, J = 9.1 Hz, 1H), 5.12 (dd, J = 4.3, 7.3 Hz, 1H), 4.99 (t, J = 7.6 Hz, 1H), 4.86 (mc, 1H), 4.56 (t, J = 8.9 Hz, 1H), 3.99 (s, 3H), 3.30 (dd, J = 6.5, 15.6 Hz, 1H), 3.12−3.22 (m, 1H), 3.08 (s, 3H), 3.04 (s, 3H), 2.93 (s, 3H), 1.76−2.31 (m, 6H), 1.10−1.67 (m, 21H), 0.81−0.99 (m, 21H), 0.78 (d, J = 6.6 Hz, 3H), 0.65 (d, J = 6.4 Hz, 3H), −0.41 (m, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz, major rotamer) δ 172.9, 172.6, 171.2, 170.8, 169.8, 168.1, 167.4, 132.3, 123.6, 122.9, 122.3, 120.2, 119.9, 118.8, 108.7, 106.8, 73.8, 65.9, 61.3, 59.5, 57.3, 47.3, 46.9, 39.7, 38.9, 37.7, 37.4, 37.0, 36.9, 36.7, 29.4, 29.2, 29.1, 28.8, 28.4, 26.6, 24.6, 23.9, 23.7, 23.6, 23.0, 22.9, 22.7, 21.8, 19.8, 19.0, 18.6, 14.2, 14.1, 13.7; IR (neat) υ_{max} 3280, 2957, 2928, 2822, 2249, 1750, 1652, 1634, 1558, 1539, 1456, 1287, 1194, 1097, 957, 740 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1109.5369 (M + Cs^+, $C_{53}H_{84}N_8O_9$ requires 1109.5416).

General Procedure for Cyclization Experiments. A sample of **44** (10.3 mg, 9 μmol) was dissolved in 0.1 mL of anisole, 0.5 mL of trifluoroacetic acid was added, and the mixture was stirred for 60 min at 22 °C. The solvent was removed with a stream of N_2, and the residue was dissolved in 0.2 mL of 3.5 M HCl−EtOAc. The solvent was removed with a stream of N_2, and the residue was dissolved in 10 mL of solvent. Base (2,6-collidine, 10 μL, 9 equiv) and reagent (HATU, 10.2 mg, 3 equiv) were added at 0 °C. The mixture was stirred at the temperature indicated (25 °C, 10 h), and the solvent was removed under reduced pressure. Chromatography (SiO_2,10% *i*-PrOH−toluene) afforded **1** (7.5 mg, 73%) as a pale yellow solid.

Thioamide 50. A solution of 1.17 g of natural HUN-7293 and 0.36 g of Lawesson's reagent in 50 mL of xylene was warmed at 130 °C for 30 min. The mixture was evaporated in vacuo, filtered over SiO_2 (toluene/CH_3OH 95:5), and purified by chromatography (SiO_2, 0.5−4% CH_3OH−toluene, gradient) to give 393 mg (33%) of the thioamide **50** (colorless solid foam) and 110 mg (9%) of the bis-thioamide **51** (colorless solid foam) and recovered starting material. For **50**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.38 (br d, 1H), 8.02 (br d, J = 9 Hz, 1H), 7.49 (d, J = 9.5 Hz, 1H), 7.48 (d, J = 8 Hz, 1H), 7.41 (d, J = 8 Hz, 1H), 7.22 (ddd, J = 7, 8, 1 Hz, 1H), 7.11 (s, 1H), 7.07 (ddd, J = 7, 8, 1 Hz, 1H), 5.32 (ddd, J = 9.5, 8.7, 5.5 Hz, 1H), 5.16 (ddd, J = 4.6, 7.5, 8 Hz, 1H), 5.13 (dd, J = 9.9, 2.7 Hz, 1H), 5.02 (ddd, J = 12, 9, 2.4 Hz, 1H), 4.19 (dd, J = 9, 7 Hz, 1H), 4.05 (s, 3H), 3.73 (dd, J = 5.3, 14.7 Hz, 1H), 3.72 (q, J = 7 Hz, 1H), 3.47 (s, 3H), 3.45 (m, 1H), 3.30 (dd, J = 8.6, 14.7 Hz, 1H), 3.29 (s, 3H), 2.37 (s, 3H), 1.50 (d, J = 7 Hz, 3H), 1.00 (d, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.4, 175.5, 172.8, 169.8, 168.9, 168.3, 168.0, 132.5, 123.5, 122.6, 122.0, 119.7, 118.5, 118.1, 108.8, 108.5, 75.5, 73.2, 66.7, 65.8, 59.7, 54.2, 48.9, 46.1, 40.9, 40.0, 39.0, 38.7, 38.5, 38.1, 37.4, 37.4, 37.4, 29.5, 29.2, 29.1, 28.8, 27.7, 25.5, 25.1, 23.6, 23.2, 23.0, 23.0, 22.8, 22.3, 21.8, 19.2, 19.16, 14.6, 14.2, 14.1, 13.6; ESIMS *m*/*z* 993.8 (M + H^+, $C_{53}H_{84}N_8O_8S$ requires 993.6); Anal. calcd for $C_{53}H_{84}N_8O_8S$: C, 64.08; H, 8.52; N, 11.28. Found: C, 63.79; H, 8.51; N, 11.16.

Benzylthioimidate 52. A mixture of aqueous NaOH (30%, 1 mL) and a CH_2Cl_2 solution containing 37.8 mg of thioamide **50** and 100 μL of benzyl bromide (5 mL) was stirred for 30 min at 25 °C with repeated sonication. The mixture was neutralized with aqueous 4 N HCl and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated and the residue subjected to chromatography (Sephadex LH-20, CH_3OH/EtOAc 1:1) to yield 38 mg (94%) of **52** as a colorless solid foam; ^{1}H NMR ($CDCl_3$, 500 MHz, 330 K) δ 7.93 (br d, 1H), 7.87 (br d, J = 8 Hz, 1H), 7.81 (br d, J = 8.5 Hz, 1H), 7.35 (d, J = 8 Hz, 1H), 7.27−7.23 (m, 5H), 7.23 (s, 1H), 7.17 (ddd, J = 7, 8, 1 Hz, 1H), 7.08 (ddd, J = 7, 8, 1 Hz, 1H), 5.27 (dd, J = 6.8, 3.4 Hz, 1H), 5.23 (dd, J = 11.1, 2.2 Hz, 1H), 5.16 (ddd, J = 9.5, 9.3, 5.3 Hz, 1H), 4.74 (ddd, J = 12, 8.5, 2 Hz, 1H), 4.48 (dd, J = 9.4, 4.5 Hz, 1H), 4.26 (d, J = 13.5 Hz, 1H), 4.02 (s, 3H), 3.83 (d, J = 13.5 Hz, 1H), 3.59 (q, J = 7 Hz, 1H), 3.42 (dd, J = 14, 9 Hz, 1H), 3.12 (s, 3H), 3.03 (s, 3H), 2.94 (s, 3H), 1.46 (d, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, 330 K) δ 174.6, 171.8, 171.2, 169.5, 168.7, 138.0, 132.6, 128.8, 128.4, 127.0, 124.7, 123.2, 122.0, 119.9, 119.6, 119.4, 108.1, 108.1, 72.6, 66.0, 65.4, 59.8, 59.2, 56.6, 49.2, 47.1, 42.5, 40.1, 38.8, 37.3, 37.3, 37.2, 36.0, 35.7, 33.5, 31.1, 29.8, 29.4, 29.0, 28.9, 27.4, 25.9, 25.7, 23.7, 23.5, 23.1, 22.9, 22.8, 22.1, 21.3, 20.1, 18.6, 18.6, 14.0, 13.9, 13.5, 13.3; FABMS (NBA) *m*/*z* 1083 (M + H^+), 1051 (M − OCH_3^+).

Ring-opening: Benzylthioester Hydrochloride 53. A solution of 26 mg of benzyl thioimidate **52** in 2 mL of *t*-BuOH and 200 μL of concentrated HCl was warmed at 55 °C for 20 min. The reaction mixture was diluted with EtOAc and evaporated in vacuo to give 30 mg of **53** as a colorless solid: ^{1}H NMR ($CDCl_3$, 500 MHz, main conformer, characteristic signals) δ 7.96 (br d, J = 9 Hz), 7.55 (d, J = 8 Hz, 1H), 7.36 (d, J = 8 Hz, 1H), 7.3−7.2 (m), 7.12 (s, 1H), 7.3−7.2 (m), 7.06 (ddd, J = 8, 7, 1 Hz, 1H), 5.55 (m, 1H), 5.24 (dd, J = 8, 4 Hz, 1H), 5.00 (m, 1H), 4.78 (ddd, J = 11, 8, 3 Hz, 1H), 4.33 (m, 1H), 4.16 (d, J = 13.8 Hz, 1H), 4.01 (s, 3H), 3.99 (d, J = 13.8 Hz, 1H), 3.79 (q, J = 7 Hz, 1H), 3.08 (s, 6H), 2.93 (s, 3H), 1.46 (d, J = 7 Hz, 1H), 0.63 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 199.8, 174.7, 171.6, 169.9, 169.7, 169.4, 168.6, 137.2, 132.3, 128.7, 128.5, 127.4, 123.5, 122.6, 122.0, 119.8, 119.4, 119.0, 108.3, 106.5, 72.9, 65.8, 61.9, 58.9, 57.4, 50.1, 48.3, 47.4, 47.0, 39.7, 39.6, 37.3, 37.0, 36.9, 36.6, 36.4, 33.1, 31.0, 29.7, 29.3, 29.2, 29.1, 29.0, 28.9, 27.4, 24.7, 24.4, 24.2, 23.2, 23.2, 23.0, 22.9, 21.3, 21.2, 19.7, 18.8, 14.1, 13.8, 13.6; ESIMS *m*/*z* 1101.4 (M + H^+, $C_{60}H_{92}N_8O_9S$ requires 1101.7), 1135.4 (M + Cl^-, requires 1135.6).

***N*-[(2*S*,4*R*)-2-[*N*-[(*S*)-2-[*N*-[(2*S*,4*R*)-2-[[*N*-L-Leucinyl-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl[amino-4-methylheptanoyl]-*N*-methyl-L-alanyloxy]-4-cyanobutanoyl]amino-4-methylheptanoyl]-*N*-methyl-L-leucine (45).** From **53**: The crude benzylthioester hydrochloride **53** (30 mg) was taken up in 2 mL of *t*-BuOH and 100 μL of water, and an excess of $AgClO_4$ was added (20 mg). After 5 min at 25 °C, the precipitated silver thiobenzylate was removed by filtration. Excess silver was removed by extraction of the solution with saturated aqueous NaCl/EtOAc. After removal of the AgCl by filtration over Celite, the organic

layer was dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography (Sephadex LH-20) to give 22.3 mg (92%, two steps) of **45** as a colorless solid identical to that described earlier.

Macrocyclization of 45 with BOP. A solution of 11 mg of the linear peptide **45** was dissolved in 20 mL of CH_3CN, and 12.3 mg of DMAP and 22 mg of BOP were added at 25 °C. After 20 min, the reaction mixture was diluted with EtOAc/aqueous HCl, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by chromatography (Sephadex LH-20, CH_3OH/EtOAc 1:1) to give 11.5 mg of the cyclization product HUN-7293. The identity with the natural product was confirmed by ^{1}H NMR (80% purity of the crude material after gel filtration), SiO_2, and C-8 reverse-phase TLC.

***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-[*N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-Hydroxy-4-cyanobutanoyl]amino]-4-methylheptanoyl]-*N*-methyl-L-leucinyl]-L-leucinyl]-*N*$^{1'}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-*N*-methyl-L-alanine Methyl Ester (54). (A) Saponification of HUN-7293.** A 200 mg (0.2 mmol) sample of HUN-7293 was dissolved in THF (5 mL). Then, 0.5 M aqueous LiOH (0.6 mL) was added at 25 °C and the mixture was stirred for 36 h. The reaction mixture was diluted with EtOAc/cyclohexane (1:2) and washed with 1 M aqueous HCl (10 mL) and water (3 × 10 mL). Drying over Na_2SO_4 and evaporation of the solvent gave the crude free acid. Final purification was performed by size exclusion chromatography on Sephadex LH-20 (CH_3OH) providing 199 mg (98%) of **54**. For characterization by NMR, the lithium salt was prepared. For this, after saponification the solvent was evaporated and the residue obtained redissolved in CH_3OH and purified by size exclusion chromatography on Sephadex LH-20 (CH_3OH). ^{1}H NMR (CD_3OD, 500 MHz, main conformer, characteristic signals) δ 7.60 (d, J = 8 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.22 (dd, J = 8.2, 7 Hz, 1H), 7.20 (s, 1H), 7.09 (dd, J = 8, 7 Hz, 1H), 5.14 (q, J = 7.3 Hz, 1H), 5.12 (m, 1H), 4.95 (dd, J = 11, 3.6 Hz, 1H), 4.89 (dd, J = 12.1, 3 Hz, 1H), 4.81 (dd, J = 7.7, 3 Hz, 1H), 4.37 (dd, J = 12.1, 3 Hz, 1H), 4.11 (dd, J = 7.3, 3.8 Hz, 1H), 4.04 (s, 3H), 3.41 (dd, J = 13, 3.6 Hz, 1H), 3.12 (s, 3H), 3.08 (dd, J = 13, 11 Hz, 1H), 3.01 (s, 3H), 2.92 (s, 3H), 2.55 (m, 1H), 2.47 (m, 1H), 2.07 (m, 1H), 1.89 (m, 1H), 1.36 (d, J = 7.3 Hz, 3H), 1.12 (m, 1H), 0.83 (d, J = 6.0 Hz, 3H), 0.39 (d, J = 6.6 Hz, 3H), 0.05 (d, J = 6.6 Hz, 3H), −0.52 (ddd, J = 13.9, 11, 3 Hz, 1H); ESIMS m/z 995.6 (M + H$^+$, $C_{53}H_{86}N_8O_{10}$ requires 995.7).

(B) From 56 and 57. A solution of **56** (1.1 mg, 2.7 μmol) and **57** (1.7 mg, 2.7 μmol) in 100 μL of CH_2Cl_2/DMF (5:1) was treated with $NaHCO_3$ (0.2 mg, 2.7 μmol), HOAt (0.7 mg, 5.4 μmol), and EDCI (1.0 mg, 5.4 μmol) at −30 °C. The mixture was stirred for 1 h at −30 °C and for 1 h at 0 °C. The crude reaction mixture was subjected to chromatography (SiO_2, 60% EtOAc−hexane) to give **58** (1.4 mg, 52%) as a white solid. The sample of **58** was dissolved in 100 μL of *t*-BuOH/H_2O (2:1) and treated with LiOH (0.1 mg, 4.2 μmol) at 0 °C. Stirring was continued for 1 h at 0 °C. The reaction mixture was diluted with EtOAc and washed with 1 M aqueous HCl and H_2O. Drying over $MgSO_4$ and evaporation of the solvent gave crude **54**, which was further purified by filtration over Sephadex G-15 (CH_3OH) providing the **54** (1.4 mg, 100%) as a white solid: FABHRMS (NBA−CsI) m/z 1127.5563 (M + Cs$^+$, $C_{53}H_{86}N_8O_{10}$ requires 1127.5521).

Macrolactonization of 54, Preparation of HUN-7293 (1). A solution of DCC (62 mg, 0.30 mmol), DMAP (55 mg, 0.45 mmol), and DMAP·TFA (70 mg, 0.30 mmol) in anhydrous CH_3CN (20 mL) was treated with **54** (100 mg, 0.1 mmol) in CH_3CN (5 mL) continuously added at 60 °C over a period of 5 h, and the resulting solution was stirred overnight. The solvent was evaporated, and the residue was dissolved in EtOAc (50 mL), extracted with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, and dried over Na_2SO_4. Oligomers were removed by size exclusion chromatography on Sephadex LH-20 (CH_3OH), and the sample was further purified by chromatography (SiO_2, 1−5% CH_3OH/toluene gradient). Traces of silica gel were removed again by filtration over Sephadex LH-20 (CH_3OH) yielding 29 mg (29%) of **1**. The identity with the natural product was confirmed by ^{1}H NMR, HPLC, and TLC.

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Supporting Information Available: Experimental details for the preparation of **3**−**11**, **13**−**23**, **25**−**29**, and **31** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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