Flexible and Convergent Total Synthesis of Cyclotheonamide B

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A convergent approach using two key intermediates, segment A [a L-proline-L-α-hydroxy-β-homoarginine-D-phenylalanine (Pro-hArg-D-Phe) tripeptide] and segment B [a vinylogous L-tyrosine-L-2,3-diaminopropanoic acid (vTyr-Dpr) dipeptide], was developed for the synthesis of cyclotheonamide B (Scheme 1). The starting compound for the preparation of the hArg moiety **7**, the predominant part of segment A, was N^{α}-(benzyloxycarbonyl)-N^{ω},$N^{\omega\prime}$-bis(*tert*-butyloxycarbonyl)-l-arginine methyl ester (**15**, Scheme 2), which was converted into the aldehyde **16** and subsequently homologated using [tris(methylthio)methyl]lithium as a carboxylic acid anion equivalent. Coupling with properly protected Pro and D-Phe derivatives gave smoothly the desired Pro-hArg-D-Phe tripeptide derivative **24**. The key feature of segment B, *i.e.*, the L-tyrosine-derived α,β-unsaturated γ-amino acid **4**, was prepared by a Wadsworth−Emmons olefination of the aldehyde **29** (Scheme 3) derived from *N*-(*tert*-butyloxycarbonyl), *O*-*tert*-butyl-L-tyrosine methyl ester (**28**). Selective *N*-(*tert*-butyloxycarbonyl) removal in the presence of the aryl *tert*-butyl ether present in the fully protected segment B, *i.e.*, **32**, was achieved by treatment with trimethylsilyl triflate/2,6-lutidine to give vTyr-Dpr dipeptide derivative **34** in quantitative yield. Coupling of the key intermediates **24** and **34** using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) afforded the protected linear pentapeptide **35** in high yield (Scheme 4). Treatment of **35** with $Pd(PPh_3)_4$/morpholine resulted in simultaneous removal of the *C*-terminal allyl group and the *N*-terminal allyloxycarbonyl group to yield **36**. Ring closure was effected under dilution conditions by treatment with TBTU/1-hydroxybenzotriazole/4-(dimethylamino)pyridine and gave the protected cyclopentapeptide **37** in 61% yield. Oxidation of the hydroxyl group with Dess−Martin periodinane (24 h, 40 °C) in the presence of *tert*-butyl alcohol gave **38**, which was then subjected to *O*,*N*-deprotection with trifluoroacetic acid/thioanisole. Subsequent HPLC purification afforded cyclotheonamide B in an overall yield of 1.8% in 17 steps.

Introduction

The cyclotheonamides A and B (**1a**,**b**, Figure 1) are cyclic pentapeptides from the marine sponge *Theonella swinhoei*, isolated and characterized as late as 1990. One might wonder why these metabolites have attracted so much attention: as much as 16 papers on isolation and characterization, synthetic aspects, enzyme inhibition, X-ray data, and conformational analysis have appeared in the literature since 1990.[1−16] The answer to this question lies in the unique structure as well as in the biological activity of these two closely related natural products. They are true peptide-mimics consisting of five amino acids, *viz.* L-proline (Pro), D-phenylalanine (D-Phe), L-2,3-diaminopropanoic acid (Dpr) (with a N^2 formyl or

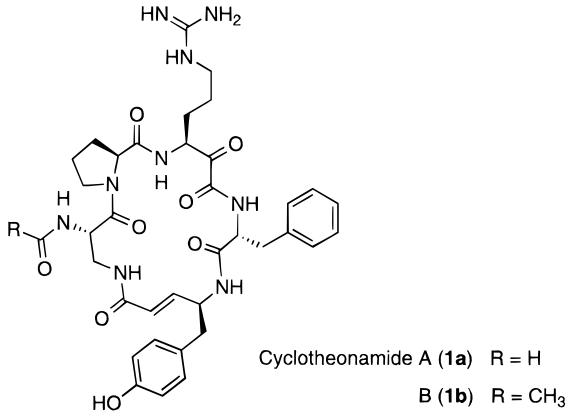

Figure 1.

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⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1997.

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acetyl group for cyclotheonamide A and B, respectively), α-oxo-L-β-homoarginine (**2**, kArg), and an L-tyrosine-derived α,β-unsaturated γ-amino acid (**4**, vTyr) (Scheme 1). The latter two amino acids are hitherto unknown compounds. These cyclotheonamides are potent inhibitors of several serine proteases. Of potential therapeutic value is their inhibitory activity toward thrombin, a serine protease that plays a crucial role in the bioregulation of thrombosis and hemostasis. The mode of action of cyclotheonamide A/B is partly based on the reversible, tetrahedral adduct formation of a hydroxyl group of the enzyme's active site with the α-keto group of the homoarginine moiety.[3,11] This intriguing mode of action, together with the unusual structural features, forms the basis for the design of novel, peptide-derived thrombin inhibitors, which was readily recognized by a variety of researchers at universities and research institutes as well as at pharmaceutical companies.

In this paper, our total synthesis of cyclotheonamide B including full experimental procedures is described.[17] Our synthesis strategy eventually turned out to have some features in common with approaches[3,11] published after the start of our investigations. Nevertheless, we feel that it deserves a place on its own; it provides a flexible and convergent route for the preparation of the title compound and of a series of analogues,[18] starting from the constituent amino acids and using a minimal set of conventional benzyl-, *tert*-butyl-, and allyl-based protecting groups in a straightforward fashion.

Results and Discussion

Retrosynthesis. In cyclotheonamide B, disconnection of the five lactam bonds obviously leads to the five constituent amino acids as starting materials (Scheme 1). As we planned to develop a concise and flexible synthesis for both cyclotheonamide B and analogues, a convergent approach using two key intermediates, a tripeptide and a dipeptide, was selected. A choice for one of the five possible sets of these peptides was made on the basis of a molecular modeling study of cyclotheonamide and thrombin.[19]

In this study, we found that the L-proline-L-α-hydroxy-β-homoarginine-D-phenylalanine (Pro-kArg-D-Phe) sequence of cyclotheonamide B interacts well with the active site of thrombin. The Pro ring occupies the S_2 pocket, and the kArg side chain resides in the S_1 pocket of thrombin. Consequently, the keto group of the kArg moiety is optimally located to form a hemiacetal with Serine-195 of thrombin. Furthermore, the aromatic side-chain of D-Phe is located in a lipophilic region of the enzyme. The L-tyrosine-L-2,3-diaminopropanoic acid (vTyr-Dpr) moiety, however, does not seem to contribute much to the binding of the natural product to thrombin as the hydroxyphenyl group of vTyr appears to be exposed to the solvent, and the acetyl substituent (or formyl in case of cyclotheonamide A) of the Dpr part is too small to effectively occupy the large hydrophobic S_3 pocket.[20] So, by employing a synthetic route with Pro-kArg-D-Phe as a common intermediate and modifying the two other amino acid residues, we would be able to conveniently prepare cyclotheonamide B as well as a set of analogues to explore the issues of selectivity and potency.

It was anticipated that the electrophilic keto group of α-oxo-β-homoarginine (kArg, **2**) would be very difficult to preserve during the entire synthesis. Therefore, it was decided that this α-keto amide was to be generated from α-hydroxy-β-homoarginine (hArg, **7**) in a late step of the total synthesis. Thus, conversion of the keto group into a hydroxyl group is the first step in our retrosynthetic analysis (Scheme 1, step 1). Subsequent disconnection of the Dpr-Pro amide bond (step 2) gives a linear sequence of amino acids, and subsequent breaking of the D-Phe-vTyr bond (step 3) results in the two desired key intermediates: the tripeptide Pro-hArg-D-Phe, *i.e.*, segment A, and the dipeptide vTyr-Dpr, *i.e.*, segment B. Further disconnection of the amide bonds of these segments gives five amino acids (steps 4−7). The three α-amino acids D-Phe (**3**), Pro (**6**), and Dpr (**9**) are commercially available; however, as Dpr is quite expensive, it was decided to prepare this amino acid from the relatively cheap L-aspartic acid (**10**) (step 8). The homologous amino acids vTyr (**4**) and hArg (**7**) can also be related to corresponding, commercially available α-amino acids, *viz.* L-tyrosine (Tyr, **11**) (step 9) and L-arginine (Arg, **8**) (step 10), respectively.

The presence of two highly functionalized, nonproteinogenic amino acids in the selected key intermediates, *viz.* hArg (**7**) and a vTyr (**4**), makes the proper choice of a orthogonal set of protecting groups especially important. Furthermore, the protecting group strategy should be straightforward in order to secure a fast and efficient route to both cyclotheonamide B and analogues.

Synthesis of Protected Segment A. When considering segment A, the synthetically most challenging part undoubtedly is the arginine-derived α-hydroxy-β-homoamino acid **7** (Scheme 1). A general method for the synthesis of α-hydroxy-β-homoamino acids, which starts from the corresponding α-amino acids, is based upon the addition of a carboxylate anion equivalent to a protected α-amino aldehyde, followed by hydrolytic and/or oxidative transformations. Several reagents have been used to yield α-hydroxyhomologate amino acids in this fashion, *e.g.*, [tris(methylthio)methyl]lithium,[21] α-(bromomagneso)ethyl vinyl ether,[22] and cyanide.[23] However, at the start of our investigations, homologation of Arg (**8**) to hArg (**7**) had not yet been reported.

Arginine is probably the most notorious of all natural amino acids; the synthesis of arginine-containing pep-

(17) A portion of the results reported here has been subject of a preliminary publication; see ref 5.

(18) The route described in this paper was also used to prepare a series of analogues. Their synthesis and biological activity will be reported elsewhere.

(19) These studies were carried out by P. D. J. Grootenhuis at N.V. Organon, Oss, The Netherlands. At the time these studies were performed, the solid-state structure of the cyclotheonamide−thrombin complex (refs. 3 and 11) was not available yet.

(20) The cyclotheonamide−thrombin interactions found in this study are in good agreement with the solid-state structure published by Maryanoff et al. (refs 3 and 11), except that the vTyr side-chain interaction assumed by Maryanoff was not observed by us. The contribution of this vTyr side chain to the overall cyclotheonamide-thrombin interaction was one of the issues to be clarified by studying cyclotheonamide analogues (see also ref 12).

(21) This method was originally developed by Seebach; see: Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 15. Seebach, D. *Chem. Ber.* **1972**, *105*, 487. For the preparation of an α-hydroxy-β-homolysine derivative, see, *e.g.:* Burkhart, J. P.; Peet, N. P.; Bey P. *Tetrahedron Lett.* **1990**, *31*, 1385.

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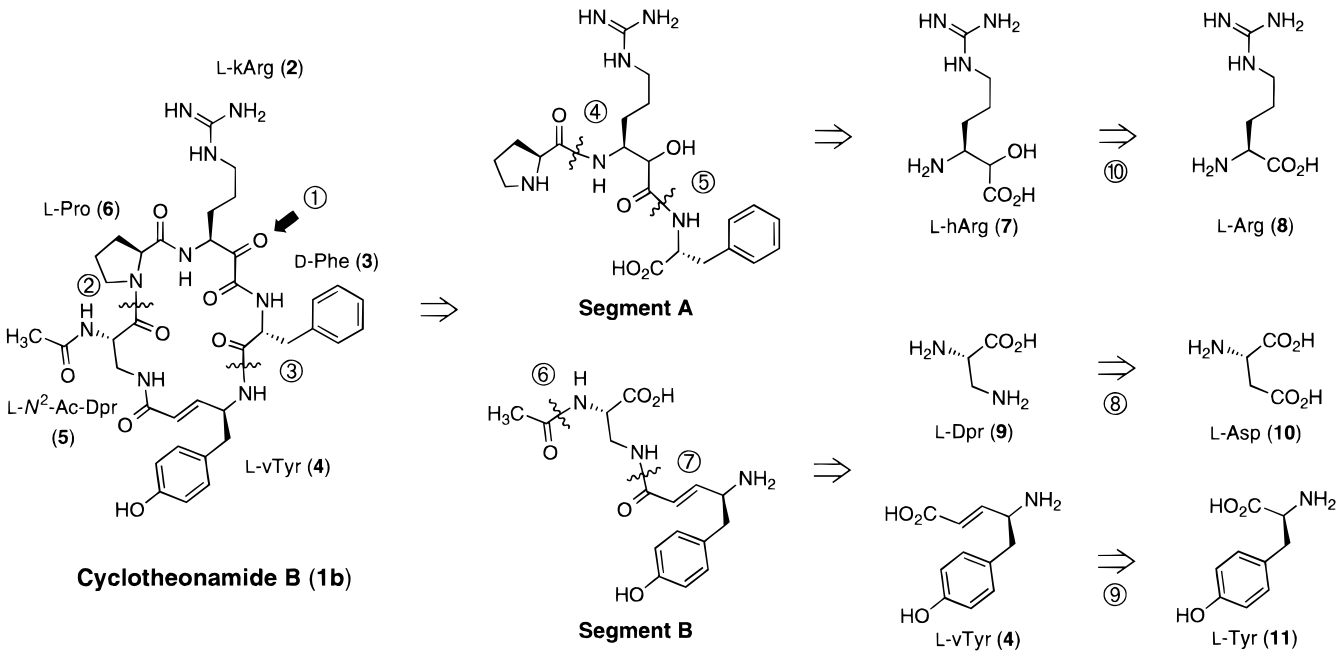
Scheme 1



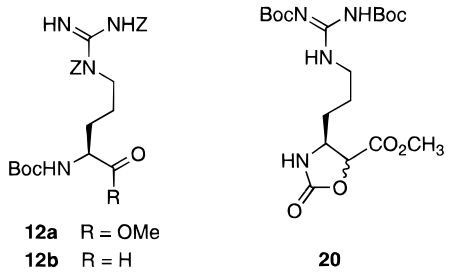


Figure 2.

tides is often very cumbersome due to problems with the (protected) guanidino group of the arginine side chain. In our study, additional problems were expected to arise as it was foreseen that successful homologation of Arg to hArg would be very dependent on a proper protection of the guanidino group. The orthogonally protected N^{α}-(*tert*-butyloxycarbonyl)-N^{δ},N^{ω}-bis(benzyloxycarbonyl)arginine methyl ester (**12a**) (Figure 2) was initially selected as starting material as we expected that the presence of two benzyloxycarbonyl (Z) groups at the guanidino moiety would give effective protection during homologation procedures. Furthermore, large-scale preparation of **12a** was found to be fast and inexpensive.[24]

Unfortunately, the N^{δ},N^{ω}-(bis-Z)-protected guanidino group of **12** proved to be very sensitive toward nucleophiles. Attempts to homologate the racemization-prone α-amino aldehyde **12b** derived from methyl ester **12a** by treatment with [tris(methylthio)methyl]lithium or α-(bromomagneso)ethyl vinyl ether were unsuccessful due to nucleophilic attack at the imino carbon atom, which resulted in deamidation yielding ornithine derivatives. Difficulties were also encountered during homologation attempts using less nucleophilic carbanions such as cyanide. However, by careful control of reaction conditions, the initially observed cleavage of the δ-Z group upon cyanohydrin formation from **12b** could be suppressed nearly completely. Nevertheless, under the basic conditions required for elaboration of the resulting arginine homologue into more advanced cyclotheonamide intermediates, the δ-Z group of the adduct from **12b** was rapidly cleaved. This time, the loss of the δ-Z group could not be prevented, not even under mild basic conditions. Furthermore, the sluggish cleavage of the remaining ω-Z group (CF_3CO_2H/thioanisole, rt, several days) in advanced α-keto amide intermediates was accompanied by severe degradation of the products. Therefore, we abandoned this protecting group strategy.

A second strategy, using N^{ω},$N^{\omega\prime}$-bis(*tert*-butyloxycarbonyl)-protected arginine methyl ester **15**, easily prepared from L-ornithine (**13**) in four steps,[25] was more successful (Scheme 2). We anticipated that deamidation in **15** was less likely to occur than previously observed with **12**, due to the decreased electrophilic nature of the imino carbon atom, the decreased leaving group character of N^{δ}, and the increased bulkiness of the protecting groups. Furthermore, final removal of the acid-labile *tert*-butyloxycarbonyl (Boc) groups in advanced α-keto amide derivatives was expected to be straightforward. Thus, **15** was reduced to aldehyde **16**, homologation of which, using [tris(methylthio)methyl]lithium, indeed yielded smoothly orthothioester **17**; products resulting from deamidation were not detected. Treatment of **17** with $HgCl_2$/HgO in MeOH/H_2O at room temperature for 64 h afforded α-hydroxy-β-homoarginine derivative **18** in an overall yield of 41% from **15**.

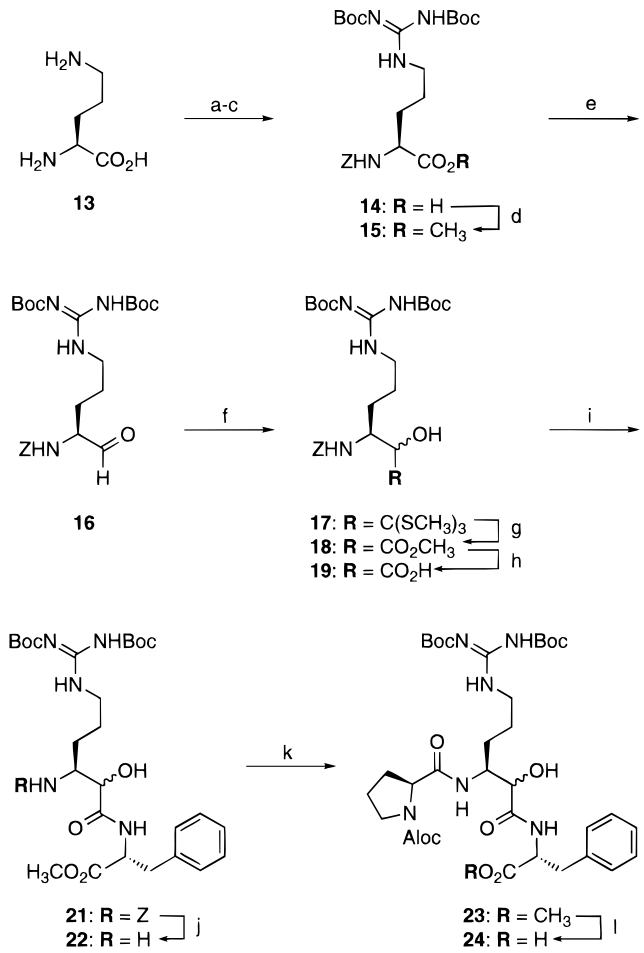
However, upon deprotection of methyl ester **18** with LiOH (3 equiv, THF/MeOH/H_2O (4:1:1), rt, 72 min), a considerable amount of cyclic carbamate **20**[26] (Figure 2) was isolated in addition to the desired acid **19**. Formation of **20** is probably due to an intramolecular nucleophilic attack by the hydroxyl group at the carbonyl group of the Z group followed by displacement of benzyl alcohol. Prevention of this side reaction by introduction of a protecting group at the hydroxyl group of several hArg derivatives proved to be quite cumbersome. Protection

(24) Jetten, M.; Peters, C. A. M.; van Nispen, J. W. F. M.; Ottenheijm, H. C. J. *Tetrahedron Lett.* **1991**, *33*, 6025.

(25) See also: Wu, Y.; Matsueda, G. R.; Bernatowicz, M. *Synth Commun.* **1993**, *23*, 3055.

(26) Formation of a cyclic carbamate was also observed by Maryanoff *et al.* (ref 11).

Scheme 2[a]



[a] Key: (a) $CuCO_3$, H_2O, 100 °C, 1 h, 87%; (b) N^1-(*N*,*N*′-bis(boc)amidino)pyrazole/DIPEA, formamide, rt, 24 h; 90%; (c) ZCl/EDTA/$NaHCO_3$, H_2O/acetone, rt, 12 h, 60%; (d) CH_2N_2, CH_2Cl_2/EtOH, 0 °C, 91%; (e) DIBALH, CH_2Cl_2, −65 °C, 75 min, 97%; (f) $LiC(SCH_3)_3$, THF, −65 °C, 5 h, 54%; (g) $HgCl_2$/HgO, MeOH/H_2O (92:8), rt, 64 h, 79%; (h) LiOH, THF/MeOH/H_2O (4:1:1), rt, 12 min, 96%; (i) DCC/HOBt, THF, 45 min, followed by D-phenylalanine methyl ester·HCl/DIPEA, 61%; (j) H_2/Pd−C, MeOH, 100%; (k) *N*-Aloc proline/TBTU/TEA, CH_2Cl_2, 2.5 h, 79%; (l) LiOH, THF/MeOH/H_2O (3.6:1:1), rt, 12 min, 100%.

with a *tert*-butyldimethylsilyl (TBDMS) group could eventually be achieved, but subsequent attempts to selectively hydrolyze the methyl ester failed. Therefore, we decided to investigate the rate of ester hydrolysis of **18** with LiOH. It was surprising and gratifying to find that after only 12 min the ^{1}H NMR signal of the methyl ester had completely disappeared and that no signals due to the formation of cyclic carbamate **20** had appeared yet. Hence, decreasing the reaction time from 72 to 12 min prevented the formation of detectable amounts of **20** and gave acid **19** as the only product in nearly quantitative yield.

Further elaboration into protected segment A was without appreciable problems. Thus, β-homoarginine derivative **19** was coupled to D-phenylalanine methyl ester using DCC and HOBt to furnish dipeptide **21** in 61% yield. Selective removal of the Z group by hydrogenolysis gave quantitatively amine **22**. Subsequent coupling of **22** with *N*-(allyloxycarbonyl)proline using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) afforded tripeptide **23** in 79% yield. Hydrolysis of the methyl ester of **23** with LiOH (12 min) gave tripeptide **24**, *i.e.*, protected segment A, in quantitative yield. Upon prolonged treatment of **23** with LiOH, cleavage of the Pro-hArg bond was observed, presumably again through nucleophilic attack of the hydroxyl group (*vide supra*).

Synthesis of Protected Segment B. Once we had established the nature of the protecting groups in segment A (**24**), we could focus on selecting the protecting groups for segment B, especially the one protecting the carboxylic acid group of the 2,3-diaminopropanoic acid unit. With an allyloxycarbonyl group on the nitrogen atom of the Pro unit of **24**, it would be advantageous to protect the carboxylic acid group of the Dpr unit of segment B as an allyl ester to enable simultaneous Pd(0)-catalyzed deprotection in the linear pentapeptide. Introduction of an acetyl group at the α-NH_2 of Dpr (as present in cyclotheonamide B) would allow regioselective coupling of the β-NH_2 group with a vTyr derivative.

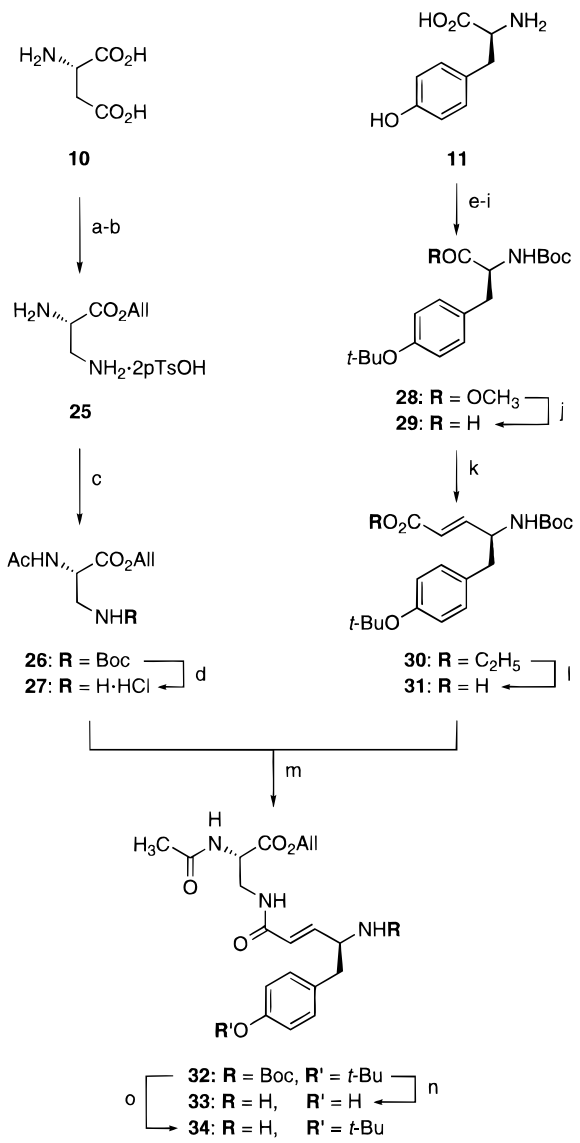
L-Dpr·HCl, prepared from L-aspartic acid (**10**) according to the method of Rao *et al.*,[27] was esterified using allyl alcohol/*p*-toluenesulfonic acid (*p*-TsOH) in refluxing benzene to give allyl ester **25** (Scheme 3). In a recent study on selective protection of 2,3-diaminopropanoic acid methyl ester, the introduction of a Boc group with a regioselectivity of N^3: N^2 = 8:1 was reported, yielding the pure N^3-Boc derivative in 70% yield after chromatography.[28] Also in our case, the regioselective introduction of the Boc group at N^3 of **25** yielded mainly the desired N^3-Boc isomer (N^3: N^2 = 8:1 in the crude product). However, aqueous work-up and chromatographic purification gave the desired product in only 11% yield. In an effort to optimize the yield, the allyl *N*-Boc-diaminopropanoate mixture of isomers was not workedup but treated directly with acetyl chloride. Subsequent aqueous workup and chromatography now furnished **26** in 64% yield. To allow coupling with a vTyr derivative, the N^3-Boc group in **26** was removed by treatment with an ethereal solution of HCl. Recrystallization from ethyl acetate afforded **27** as a colorless, crystalline solid in 86% yield.

The protected tyrosine derivative **28**, selected as starting material for the synthesis of the vTyr moiety, was prepared by a five-step sequence starting from L-tyrosine (**11**).[29] Reduction of **28** with diisobutylaluminum hydride (−72 °C, 20−25 min) gave the aldehyde **29**. Crude **29** was converted immediately by a Wadsworth−Emmons olefination using triethyl phosphonoacetate/NaH at −50 → 10 °C to give exclusively the (*E*)-alkene **30** in 94% yield (from **28**). Hydrolysis of the ethyl ester of **30** with NaOH in dioxane/H_2O gave acid **31** as a colorless crystalline solid in essentially quantitative yield, ready to be coupled to **27**.

Coupling of **27** and **31** was achieved by mixing these amino acid derivatives with stoichiometric amounts of TBTU and diisopropylethylamine (DIPEA) in CH_2Cl_2 to give, after 90 min reaction time and subsequent aqueous workup, the pure dipeptide **32** in 99% yield. Treatment of **32** with acid (*e.g.* CF_3CO_2H/H_2O or HCl/Et_2O) caused cleavage of both the Boc group and the *tert*-butyl ether to give **33**. Unfortunately, it was found that employment

(27) Rao, S. L. N. *Biochemistry* **1975**, *14*, 5218.
(28) Egbertson, M. S.; Homnick, C. F.; Hartman, G. D. *Synth. Commun.* **1993**, *23*, 703.
(29) (a) Boissonas, R. A.; Guttman, P. A.; Jaquenoud, P.-A.; Waller, J.-P. *Helv. Chim. Acta* **1955**, *38*, 1491. (b) Konishita, M.; Klostermeyer, H. *Liebigs Ann. Chem.* **1966**, *696*, 226. (c) Schröder, E. *Liebigs Ann. Chem.* **1963**, *670*, 127. (d) McNulty, J.; Still, I. W. J. *Synth. Commun.* **1992**, *22*, 979. (e) Methyl ester **28** is a known compound; see, *e.g.*: Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 307.

Scheme 3[a]



[a] Key: (a) NaN_3, 30% oleum/$CHCl_3$, 58 °C, 4 h, followed by H_2O, 58%; (b) allyl alcohol/*p*-TsOH, benzene, reflux, 5h, 77%; (c) Boc_2O/TEA, CH_2Cl_2, −68 → 2 °C, 4 h, followed by acetyl chloride, 2 → 20 °C, 16 h, 64%; (d) 3 M HCl in Et2O, 0 °C → rt, 1 h, 86%; (e) ref 29a: $SOCl_2$, MeOH, rt, 2 h, reflux 0.5 h, 100%; (f) ref 29b: ZCl/Na_2CO_3, CH_2Cl_2/H_2O, 0 °C → rt, 2.5 h, 98%; (g) ref 29c: isobutene/H_2SO_4, CH_2Cl_2, rt, 36 h, 89%; (h) H_2/Pd−C, MeOH, THF, rt, 6 h, 92%; (i) ref 29d: Boc_2O, DMF, 60 °C, 0.5 h, 91%; (j) DIBALH, CH2Cl2, −72 °C, 20 min; (k) triethyl phosphonoacetate/NaH, THF, −50 → 10 °C, 2.5 h, 94% (two steps); (l) NaOH, dioxane/H_2O, 14 h, 99%; (m) TBTU/DIPEA, CH_2Cl_2, rt, 90 min; 99%; (n) TFA/H_2O (9:1), 45 min, 100%; (o) TMS-triflate/2,6-lutidine, CH_2Cl_2, 0 °C → rt, 2 h, 100%.

of the reagent of choice (Dess-Martin periodinane, *vide infra*) to be used for the oxidation of α-hydroxy-*β*-homoarginine derivatives was incompatible with an unprotected hydroxyphenyl group as present in **33**. We decided to study the possibility of selective *N*-Boc cleavage in the presence of an aryl *tert*-butyl ether instead of devising a new protecting group strategy with established orthogonal *O*,*N*-protection for the synthesis of segment B. For this study, tyrosine derivative **28** was used as a model compound. However, no selective deprotection[30] was observed when **28** was treated with acids at different concentrations in various solvents, *e.g.*, HCl/Et_2O, CF_3CO_2H/H_2O (CH_2Cl_2), *p*-TsOH/CH_2Cl_2(Et_2O), HCO_2H/H_2O, aqueous H_2SO_4 (10%)/1,4-dioxane. Under some conditions the *tert*-butyl ether was found to be even more labile than the Boc group.

There is only a limited number of methods available for removal of a Boc-group avoiding the use of acidic conditions.[31] Ohfune *et al.*[31b,c] showed that transformation of a *N*-Boc group to a TBDMS carbamate employing TBDMS triflate/2,6-lutidine is compatible with the presence of other acid sensitive groups, *e.g.*, silyl ethers, acetonides, tetrahydropyranyl ethers, and *tert*-butyl esters. On the basis of this information, **28** was treated with trimethylsilyl triflate/2,6-lutidine in dry CH_2Cl_2. This indeed resulted in a clean and selective cleavage of the *N*-Boc group without affecting the aryl *tert*-butyl ether. Application of this method to dipeptide **32** also effected selective cleavage of the *N*-Boc group to give, after aqueous workup, amine **34** in quantitative yield and only slightly contaminated with 2,6-lutidine. Thus, the desired orthogonality of the *N*-Boc group and the aryl *tert*-butyl ether was achieved, and protected segment B (**34**) was now ready to be coupled with protected segment A (**24**).

Synthesis of Cyclotheonamide B from the Key Intermediates. TBTU was used to effect the coupling of **24** with **34** (Scheme 4). By adding the coupling reagent to a *mixture* of both reaction partners, *δ*-lactone formation in **24**, involving the unprotected hydroxyl group of the hArg moiety, could efficiently be suppressed. The protected linear pentapeptide **35** was isolated in 85% yield after purification by chromatography.

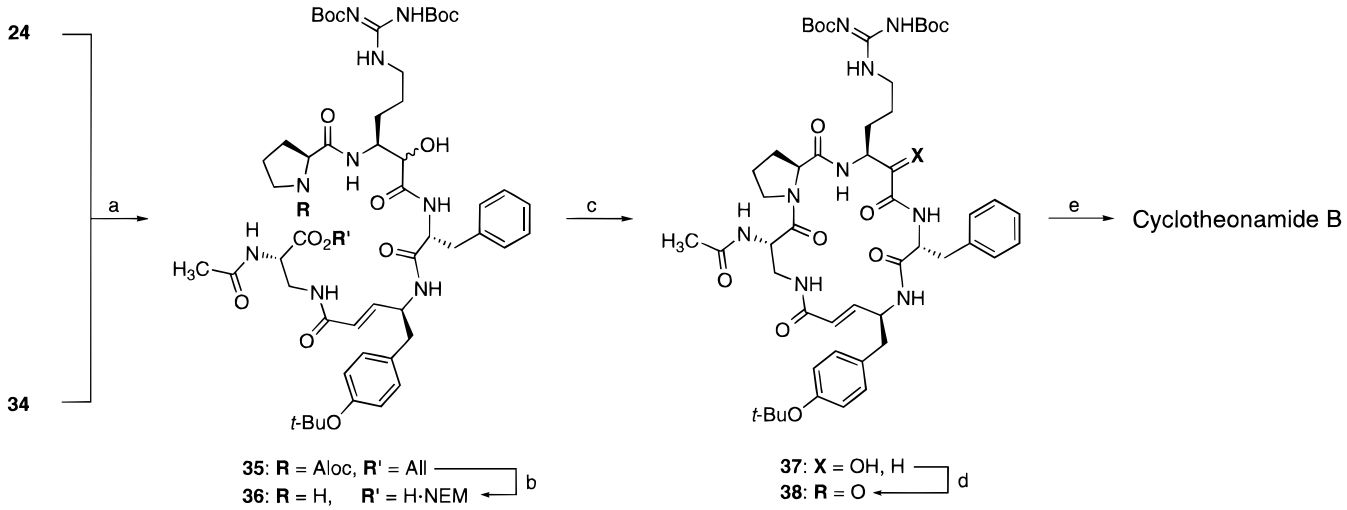
Before cyclization could take place, the *C*-terminal allyl group and the *N*-terminal allyloxycarbonyl group of **35** had to be removed. In the Pd(0)-catalyzed deprotection of allyl esters and allyl carbamates, the allyl group is transferred, *via* a *π*-allyl-Pd complex, to an acceptor. A series of nucleophilic allyl acceptors was tested, of which morpholine appeared to work nicely (50 equiv, THF, 7 mol % $Pd(PPh_3)_4$, rt, 45 min). After repeated evaporation to remove *N*-allylmorpholine and the excess of morpholine, the ^1H NMR spectrum still indicated the presence of morpholine, probably as an ion pair with the liberated *C*-terminal carboxyl group. A procedure to remove this residual morpholine was devised, as we were concerned that it would compete with the proline nitrogen during the cyclization step. We were able to exchange the residual morpholine for a tertiary amine, *i.e.*, *N*-ethylmorpholine (NEM), by chromatographic purification of the crude product on silica (preparative TLC, CH_2Cl_2/MeOH/NEM, 85:15:10).

As we had used TBTU successfully for several coupling reactions and as this reagent was also shown to be very effective for couplings involving a proline nitrogen, it was the reagent of choice for the macrolactamization. Although TBTU couplings normally proceed with little or no racemization, addition of HOBt is reported to suppress

(30) The extreme sensitivity of an *O*-(*tert*-butyl)tyrosine derivative toward careful treatment with HCl/ethyl acetate (1 M, 500 mol %) was also demonstrated recently in a study to achieve selective cleavage of *N*-Boc groups in the presence of other acid-labile protective groups such as *tert*-butyl esters, aliphatic *tert*-butyl ethers, *S*-Boc groups, and *S*-trityl ethers: Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216.

(31) TMS-triflate and TBDMS-triflate are known to effect transesterification of Boc groups to give TMS- and TBDMS-carbamates. When only deprotection is required, the use of TMS-triflate is superior to TBDMS-triflate because TMS-carbamates are more readily cleaved than TBDMS-carbamates; however, TBDMS-triflate appears to be more selective; *e.g.*: (a) Borgulya, J.; Bernauer, K. *Synlett* **1980**, 545. (b) Sakaitani, M.; Ohfune, Y. *Tetrahedron Lett.* **1985**, *26*, 5543. (c) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870.

Scheme 4[a]



[a] Key: (a) TBTU/DIPEA, CH_2Cl_2, rt, 3 h, 85%; (b) $Pd(PPh_3)_4$/morpholine, THF, rt, 45 min, followed by preparative TLC on SiO_2, CH_2Cl_2/MeOH/NEM, 73%; (c) TBTU/HOBt/DMAP, CH_2Cl_2 (0.5 mM), rt, 23 h, 61%; (d) Dess−Martin periodinane/*t*-BuOH, CH_2Cl_2/MeCN, 40 °C, 24 h, ca. 81%; (e) TFA/thioanisole, rt, 105 min, followed by HPLC, 51% from 35.

it completely.[32] Thus, a 0.5 mM solution of **36** in CH_2Cl_2 was treated with TBTU (3 equiv), HOBt (3 equiv), and (dimethylamino)pyridine (5 equiv), which furnished, after aqueous workup and preparative TLC, cyclopentapeptide **37** in 61% yield.

Exposure of **37** to the optimized oxidation conditions devised using some linear hArg-peptides as model compounds (1.5 equiv of Dess-Martin reagent/2-methyl-2-propanol, rt, 45 min) did not furnish the oxidized product. Even after treatment for 24 h at 40 °C with a large excess of Dess-Martin reagent/2-methyl-2-propanol (2.5 equiv) in CH_2Cl_2/MeCN, some starting material (≤10%, as shown by ^{1}H NMR) was still present. Similar observations have been described by Maryanoff[11] and Wipf[2] for the oxidation of the α-hydroxy-*β*-homoarginine residue in their cyclopentapeptide. Presumably, the ring geometry of the 19-membered macrocycle forces the hydroxyl group into a sterically and/or electronically unfavorable position, as can be concluded from the observation that linear α-hydroxy-*β*-homoarginine derivatives are oxidized much faster. Noteworthy in this context is our observation that an 18-membered ring analogue of cyclotheonamide was rapidly oxidized, whereas analogues with a 19-membered ring were oxidized much slower.[18]

The crude oxidation product **38** was treated with TFA/thioanisole at room temperature during 105 min to deprotect the arginine and tyrosine side chains. The resulting material was purified by reversed-phase HPLC to give cyclotheonamide B in 51% yield from **37**. Synthetic cyclotheonamide B was fully characterized (^{1}H NMR, ^{13}C NMR, HH-COSY, CH-COSY, FAB-MS, optical rotation, and bioassay) and was identical in all respects to the natural product.

Comparative 400 MHz ^{1}H NMR spectra of natural cyclotheonamide A (**1a**) (kindly provided by professor Fusetani) and synthetic cyclotheonamide B (**1b**) are depicted in Figure 3. The differences observed are solely due to the presence of a N^{α}-formyl group at the diaminopropanoic acid residue in cyclotheonamide A and a N^{α}-acetyl group in cyclotheonamide B. Noteworthy is the ^{13}C-chemical shift value of the α-carbon atom of the

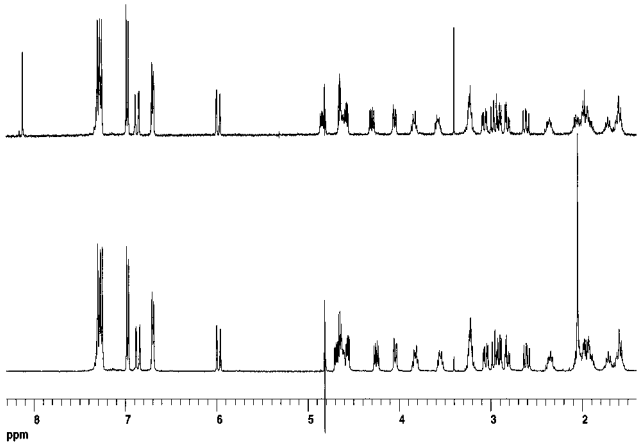


Figure 3. Comparative 400 MHz ^{1}H-NMR spectra of natural cyclotheonamide A (top panel) and synthetic cyclotheonamide B (bottom panel) in D_2O.

β-homoarginine unit ($\delta_{C\alpha}$ = 97.2 ppm), indicating that the electrophilic carbonyl group is present in its hydrated form (*gem*-diol) rather than as an α-keto amide as drawn. Adduct formation between cyclotheonamide and protic solvents such as water, methanol, and ethanol is also observed by FAB-MS. The formation of these adducts might contribute to the stability toward epimerization of the racemization-prone α-oxo-*β*-homoarginine residue and is the mechanistic rationale for the high affinity of the natural product for serine proteases.

Experimental Section

General Methods. THF was distilled from NaH and then from sodium benzophenone ketyl. Et_2O was distilled from NaH. CH_2Cl_2 and MeOH were dried over molecular sieves. Glassware was usually oven-dried overnight at 140 °C, assembled hot, and flushed with nitrogen before use. All reactions were performed under a nitrogen atmosphere, unless stated otherwise.

For preparative chromatography at atmospheric pressure (LC), silica gel 60 (Merck 7733) was used. Preparative medium-pressure (12 atm) liquid chromatography (MPLC) was performed on silica gel 60 H (Merck 7736) using UV (254 nm) and RI detection. Preparative thin-layer chromatography (TLC) was done with silica gel 60 F_{254} coated glass plates (2

(32) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.

mm, Merck 5717). Analytical thin-layer chromatography was carried out on silica gel 60 F_{254}-coated aluminum plates (0.2 mm, Merck 5554). Compounds were visualized using UV detection, iodine vapor, pyrolysis, or ninhydrin.

^{1}H-NMR spectra were recorded at either 200.1 or 400.1 MHz; chemical shifts (δ) are reported in ppm relative to TMS using the solvent signal as internal reference ($CHCl_3$, 7.27 ppm; DMSO, 2.50 ppm). ^{13}C-NMR spectra were recorded at either 50.29 or 100.63 MHz; chemical shifts (δ) are reported in ppm relative to TMS using the solvent signal as internal reference ($CHCl_3$, 77.0 ppm; DMSO, 39.5 ppm). Coupling constants (J) are given in Hz. 2D-NMR (H−H and C−H) COSY and DEPT-techniques were frequently used to support interpretation of 1D spectra.

FAB-MS and FAB-HRMS were determined using a spectrometer equipped with a WATV Cs ion gun, operated at a beam current of approx 2 μA at 25 KV.

Melting points were recorded on a hot stage apparatus under a microscope and are uncorrected.

Methyl 2(*S*)-[(Phenylmethoxycarbonyl)amino]-5-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]-methyl]amino]pentanoate (15). To a stirred suspension of Cu(N^{ω},$N^{\omega'}$-bis(Boc)arginine)$_2$[25] (73.4 g, 90.6 mmol) in H_2O (350 mL) were added EDTA·4Na·2H_2O (55.3 g, 0.109 mol) and $NaHCO_3$ (36.0 g, 0.426 mol). Subsequently, benzyloxycarbonyl chloride (ZCl, 35.0 mL, 0.238 mol) in acetone (250 mL) was added dropwise, upon which a dark blue solution was obtained. After 5 h at room temperature, the acetone was evaporated under reduced pressure and the residue was dissolved in H_2O (1 L). The aqueous solution was acidified to pH ≈ 3.0 with aqueous 6% $KHSO_4$ and extracted with EtOAc (5 × 200 mL). The organic phase was washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification of the crude product by LC (gradient from PE/EtOAc, 2:1 to PE/EtOAc/HOAc, 2:2:0.1) gave acid **14** as a colorless oil containing a small amount of EtOAc (55.6 g; 60.3%): ^{1}H NMR ($CDCl_3$) 1.50 (s, 9 H), 1.53 (s, 9 H), 1.58−2.04 (m, 4 H), 3.39 (m, 2 H), 4.42 (m, 1 H), 5.10 (bs, 1 H), 5.78 (d, J = 8.3, 1 H), 7.29−7.39 (m, 5 H), 8.43 (m, 1 H).

A stirred solution of **14** (7.51 g, 14.8 mmol) in CH_2Cl_2 (100 mL) containing EtOH (3 mL) was cooled with an ice bath while diazomethane, generated from Diazald in an apparatus described by Lombardi,[14] was bubbled through until a persistent yellow color was observed. The ice bath was removed, and the reaction mixture was flushed with nitrogen to remove the excess of diazomethane. After drying (Na_2SO_4) and concentration *in vacuo*, **15** was obtained as a colorless glass (6.99 g; 90.6%): ^{1}H NMR ($CDCl_3$) 1.45 (s, 18 H), 1.54−1.96 (m, 4 H), 3.40 (m, 2 H), 3.75 (s, 3 H), 4.37 (m, 1 H), 5.10 (bs, 1 H), 5.51 (d, J = 8.4, 1 H), 7.29−7.39 (m, 5 H), 8.28 (bt, J = 5.0, 1 H), 11.45 (bs, 1 H); ^{13}C NMR ($CDCl_3$) 25.6, 27.1, 27.4, 28.3, 39.3 , 51.4 , 52.9 , 65.8, 78.5, 82.3, 127.1, 127.4, 127.6, 135.6, 152.3, 154.7, 154.7, 162.6, 171.9; FAB-HRMS calcd for [$C_{25}H_{38}N_4O_8$ + H]$^+$ 523.2768, found 523.2786.

2(*S*)-[(Phenylmethoxycarbonyl)amino]-5-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)-amino]methyl]amino]pentanal (16). To a stirred solution of methyl ester **15** (6.22 g, 11.9 mmol) in CH_2Cl_2 (120 mL) at −65 °C was added diisobutylaluminum hydride (DIBALH, 1 N in hexanes, 42.7 mL, 42.7 mmol) at such a rate as to keep the temperature below −63 °C (*ca*. 45 min). After the reaction was complete (usually after an additional 30−45 min, monitored by TLC), a solution of EtOH/36% HCl_{aq} (2.4 mL, 9:1) was added slowly (the temperature was kept below −63 °C). The cold reaction mixture was then added to a vigorously stirred solution of HCl_{aq} (1 N, 350 mL) at 0 °C, stirred for 3 min, and the layers were separated. The aqueous phase was extracted with ice-cold CH_2Cl_2 (2 × 150 mL), and the combined organic extracts were washed, sequentially, with ice-cold HCl_{aq} (1 N, 100 mL), ice-cold H_2O (3 × 100 mL), and ice-cold brine (100 mL), dried (Na_2SO_4), filtrered, and concentrated under reduced pressure. The residue was in dissolved benzene, and again concentrated *in vacuo*, to give **16** as a white foam (5.68 g; 96.9%): ^{1}H NMR ($CDCl_3$) 1.39 (s, 9 H), 1.43 (s, 9 H), 1.47−1.96 (m, 4 H), 3.35 (m, 2 H), 4.25 (m, 1 H), 5.05 (bs, 1 H), 5.90 (d, J = 8.6, 1 H), 7.26−7.36 (m, 5 H), 8.35 (bt, J = 5.1, 1 H), 9.54 (s, 1 H), 11.40 (bs, 1 H); ^{13}C NMR ($CDCl_3$) 25.2, 25.6, 27.8, 28.0, 39.7, 59.7, 66.9, 79.2, 83.1, 127.9, 128.0, 128.3, 136.1, 153.0, 156.1, 156.1, 163.1, 199.2; FAB-HRMS calcd for [$C_{24}H_{36}N_4O_7$ + H]$^+$ 493.2662, found 493.2671.

1,1,1-Tris(methylthio)-3(*S*)-[(phenylmethoxycarbonyl)amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]amino]hexan-2(*R,S*)-ol (17). *n*-Butyllithium (BuLi, 1.6 M in hexanes, 30.6 mL, 49.0 mmol) was added over a period of 10 min to a stirred solution of tris(methylthio)methane (6.9 mL, 52.0 mmol) in THF (132 mL) at −65 °C. After 20 min, a precipitate had formed, and a precooled (−65 °C) solution of aldehyde **16** (5.68 g, 11.5 mmol) in THF (50 mL) was added in 30 min, upon which the precipitate dissolved. Stirring was continued for 5 h. Subsequently, the reaction mixture was poured onto a stirred mixture of saturated aqueous NH_4Cl/CH_2Cl_2 (400 mL, 1:12). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were washed with H_2O and brine, dried ($MgSO_4$), filtered, and evaporated to dryness *in vacuo*. Purification of the crude product by LC (gradient from CH_2Cl_2 to CH_2Cl_2/EtOAc, 9:1) afforded **17** as a thick oil (diastereomers (9:1), 4.00 g; 53.6%): ^{1}H NMR ($CDCl_3$) 1.46 (s, 18 H), 1.52−1.78 (m, 4 H), 2.17 and 2.22 (2 × s, 9 H, diast), 3.41 (m, 2 H), 4.69 and 3.92 (2 × bs, 1 H, diast), 4.12 (m, 1 H), 4.98−5.12 (m, 2 H), 5.56 (d, J = 8.6, 1 H), 7.22−7.40 (m, 5 H), 8.35 (bt, J = 4.9, 1 H), 11.49 (bs, 1 H); ^{13}C NMR ($CDCl_3$) 13.9, 25.4, 28.1, 28.2, 33.1, 40.6, 50.4, 65.9, 74.0, 76.7, 79.2, 83.0, 127.9, 128.1, 128.3, 136.8, 153.2, 156.0, 156.1, 163.5; FAB-HRMS calcd for [$C_{28}H_{46}N_4O_7S_3$ + H]$^+$ 647.2607, found 647.2660.

Methyl 2(*R,S*)-Hydroxy-3(*S*)-[(phenylmethoxycarbonyl)amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]amino]hexanoate (18). A solution of orthothioester **17** (4.00 g, 6.18 mmol) in MeOH (118 mL) and H_2O (8.5 mL) was vigorously stirred with $HgCl_2$ (5.66 g, 20.8 mmol) and HgO (1.69 g, 7.82 mmol) for 72 h at rt. The reaction mixture was filtered over Celite, and the residue was washed with CH_2Cl_2 (300 mL), MeOH (50 mL), and H_2O (50 mL). The biphasic filtrate was separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic phases were sequentially washed with saturated aqueous NH_4OAc (3 × 150 mL) and saturated aqueous NH_4Cl (2 × 150 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give, after purification by MPLC (EtOAc/PE, 2:3), **18** as white foam (diastereomers (9:1), 2.70 g; 79.0%): ^{1}H NMR ($CDCl_3$) 1.47 (s, 18 H), 1.51−1.71 (m, 4 H), 3.22−3.57 (m, 2 H), 3.70 and 3.76 (2 × s, 3 H), 4.10 (m, 1 H), 4.18 and 4.30 (2 × bs, 1 H, diast), 4.98−5.11 (m, 2 H), 5.49 and 5.57 (2 × bd, J = 9.6, 1 H), 7.20−7.38 (m, 5 H), 8.31 (bt, J = 5.3, 1 H), 11.44 (bs, 1 H); ^{13}C NMR ($CDCl_3$) 25.8 and 26.0 (diast), 27.8, 27.9, 28.2, 40.2, 52.6, 53.4, 66.5 and 66.7 (diast), 72.0 and 72.6 (diast), 79.1, 83.0, 127.7, 121.8, 128.3, 136.5, 153.1, 155.9, 156.1, 163.2, 173.6; FAB-HRMS calcd for [$C_{26}H_{40}N_4O_9$ + H]$^+$ 553.2874, found 553.2867.

2(*R,S*)-Hydroxy-3(*S*)-[(phenylmethoxycarbonyl)amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]amino]hexanoic Acid (19). To a vigorously stirred solution of ester **18** (5.33 g, 9.64 mmol) in THF/MeOH/H_2O (313 mL, 4:1:1) was added finely powdered LiOH·H_2O (1.12 g, 26.7 mmol). After exactly 12 min, aqueous $KHSO_4$ (6%, 73 mL) was added, and THF was evaporated under reduced pressure. The residual, turbid liquid was diluted with H_2O (75 mL), acidified to pH ≈ 2 with aqueous 6% $KHSO_4$, and extracted with EtOAc (3 × 100 mL). The combined organic phases were washed with H_2O and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford pure **19** as a white foam (diastereomers (14:1), 5.01 g; 96.4%): ^{1}H NMR ($CDCl_3$) 1.43 (s, 9 H), 1.48 (s, 9 H), 1.45−1.70 (m, 4 H), 3.35 (m, 2 H), 4.09 (m, 1 H), 4.12 and 4.29 (2 × m, 1 H, diast), 4.98−5.12 (m, 2 H), 6.68 (d, J = 8.6, 1 H), 5.70 (bs, 1 H), 7.20−7.41 (m, 5 H), 8.52 (m, 1 H); FAB-HRMS calcd for [$C_{25}H_{38}N_4O_9$ + H]$^+$ 539.2727, found 539.2733.

Methyl 2(*R*)-[[2(*R,S*)-Hydroxy-3(*S*)-[(phenylmethoxycarbonyl)amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]amino]hexanoyl]amino]-3-phenylpropanoate (21). To a stirred solution of acid **19**

(4.78 g, 8.87 mmol) and 1-hydroxybenzotriazole (HOBt, 2.40 g, 17.8 mmol) in CH_2Cl_2 (65 mL) at 0 °C was added dicyclohexylcarbodiimide (DCC, 2.01 g, 9.72 mmol). After 45 min, a solution of D-phenylalanine methyl ester hydrochloride (2.10 g, 9.74 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 1.81 mL, 10.4 mmol) in CH_2Cl_2 (25 mL) was added. After another 30 min the ice bath was removed, and the reaction mixture was stirred for 18 h. Subsequently, the reaction mixture was filtered, diluted with EtOAc (500 mL), and washed sequentially with H_2O (2 ×), aqueous $NaHCO_3$ (5%, 3 ×), H_2O, aqueous $KHSO_4$ (6%, 3 ×), H_2O, and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified by MPLC (EtOAc/PE, 1:1), to give dipeptide **21** as a white foam (3.81 g; 61.3%): ^{1}H NMR (major isomer, $CDCl_3$) 1.43 (s, 9 H), 1.47 (s, 9 H), 1.45−1.65 (m, 4 H), 3.01 (d, J = 6.7, 2 H), 3.35 (m, 2 H), 3.68 (s, 3 H), 3.98 (m, 1 H), 4.15 (m, 1 H), 4.73−4.90 (m, 2 H), 5.04 (bs, 2 H), 5.73 (bd, J = 8.3, 1 H), 7.00−7.09 (m, 2 H), 7.14−7.49 (m, 8 H), 8.33 (bt, J = 5.4, 1 H), 11.42 (bs, 1 H); ^{13}C NMR (major isomer, $CDCl_3$) 25.7, 26.7, 27.9, 28.0, 38.0, 40.0, 52.1, 52.6, 53.3, 67.8, 72.9, 79.3, 83.0, 127.0, 127.9, 127.9, 128.3, 128.4, 129.0, 135.6, 136.1, 153.0, 156.2, 156.7, 163.1, 171.5, 171.8; FAB-HRMS calcd for $[C_{35}H_{49}N_5O_{10} + H]^+$ 700.3558, found 700.3585.

Methyl 2(*R*)-[[2(*R*,*S*)-Hydroxy-3(*S*)-[[[1-(allyloxycarbonyl)pyrrolidin-2(*S*)-yl]carbonyl]amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]-methyl]amino]hexanoyl]amino]-3-phenylpropanoate (23). A solution of **21** (6.30 g, 9.00 mmol) in MeOH (25 mL) was stirred vigorously with Pd−C (5%, 0.92 g) in a H_2 atmosphere during 6 h. Filtration and evaporation of the solvent *in vacuo* yielded **22** as a white foam (5.28 g; 100%): ^{1}H NMR (major isomer, $CDCl_3$) 1.48 (s, 18 H), 1.48−1.80 (m, 4 H), 2.96 (dd, J = 13.7, 7.4, 1 H), 3.08−3.20 (m, 2 H), 3.37 (m, 2 H), 3.70 (s, 3 H), 3.79 (d, J = 2.4, 1 H), 4.91 (m, 1 H), 7.02−7.18 (m, 2 H), 7.18−7.31 (m, 3 H), 7.49 (bd, J = 8.4, 1 H), 8.32 (bt, J = 5.4, 1 H), 11.44 (bs, 1 H); ^{13}C NMR (major isomer, $CDCl_3$) 25.9, 27.9, 28.1, 29.8, 38.1, 40.3, 52.2, 52.3, 52.5, 72.7, 79.1, 83.0, 126.9, 128.4, 129.0, 135.9, 153.1, 156.0 , 163.0, 171.6, 172.7.

To a stirred solution of crude **22** (4.31 g, 7.62 mmol) and *N*-(allyloxycarbonyl)proline (1.67 g, 8.38 mmol) in CH_2Cl_2 (50 mL) were added DIPEA (1.40 mL, 8.04 mmol) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 2.57 g, 8.00 mmol). After 2.5 h, the reaction mixture was diluted with EtOAc (250 mL) and washed sequentially with H_2O (4×), aqueous $NaHCO_3$ (5%, 3×), H_2O, aqueous $KHSO_4$ (6%, 3×), H_2O, and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give, after purification by MPLC (EtOAc/PE/EtOH, 60:40:2), tripeptide **23** as a white foam (4.50 g; 79.1%): ^{1}H NMR (major isomer, $CDCl_3$, 400.1 MHz) 1.49 (s, 18 H), 1.45−1.70 (m, 5 H), 1.75−1.93 (m, 2 H), 2.07 (m, 1 H), 3.10 (d, J = 6.6, 2 H), 3.33 (m, 1 H), 3.39−3.52 (m, 2 H), 3.57 (m, 1 H), 3.70 (s, 3 H), 4.04−4.16 (m, 1 H), 4.25 (m, 1 H), 4.55 (m, 2 H), 4.78−4.95 (m, 2 H), 5.24 (m, 2 H), 5.79 (m, 1 H), 7.10−7.20 (m, 2 H), 7.20−7.34 (m, 5 H), 8.34 (bt, J = 5.0, 1 H), 11.49 (bs, 1 H); ^{13}C NMR (major isomer, $CDCl_3$, 100.6 MHz) 23.7, 24.5, 28.1, 28.3, 29.7, 31.4, 38.1, 40.2, 47.3, 52.3, 52.8, 53.0, 61.0, 66.3, 73.1, 79.3, 83.1, 117.7, 125.5, 127.1, 128.6, 132.7, 135.9, 153.2, 155.6, 156.3, 163.4, 171.7, 171.8, 173.3; FAB-HRMS calcd for $[C_{36}H_{54}N_6O_{11} + H]^+$ 747.3929, found 747.3892.

2(*R*)-[[2(*R*,*S*)-Hydroxy-3(*S*)-[[[1-(allyloxycarbonyl)-pyrrolidin-2(*S*)-yl]carbonyl]amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]-amino]hexanoyl]amino]-3-phenylpropanoic Acid (24). To a vigorously stirred solution of **23** (4.30 g 5.76 mmol) in THF/MeOH/H_2O (187 mL, 3.6:1:1) was added thoroughly powdered LiOH·H_2O (727 mg, 17.3 mmol). After 12 min, aqueous $KHSO_4$ (6%, 40 mL) was added, and the organic solvents were evaporated under reduced pressure. The turbid residue was diluted with H_2O (100 mL), acidified to pH ≈ 2.5 with aqueous $KHSO_4$ (6%), and extracted with EtOAc (3 × 80 mL). The combined organic extracts were washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield almost pure **24** as a white foam (4.21 g; 99.7%): ^{1}H NMR ($CDCl_3$) 1.47 (s, 9 H), 1.49 (s, 9 H), 1.47−2.08 (m, 8 H), 2.93−3.60 (m, 6 H), 3.77−4.26 (m, 3 H), 4.37−4.83 (m, 3 H), 5.23 (m, 2 H), 5.86 (m, 1 H), 7.12−7.37 (m, 6 H), 7.40 (bd, J = 8.3, 1 H), 8.42 (bs, 1 H); FAB-HRMS calcd for $[C_{35}H_{52}N_6O_{11} + H]^+$ 733.3772, found 733.3759.

2(*S*),3-Diaminopropanoic Acid Monohydrochloride (9). L-Aspartic acid (**10**) was converted to L-2,3-diaminopropanoic acid hydrochloride according to the method of Rao *et al.*[12] to give **9** as off-white needles in 58.3% yield: ^{1}H NMR (6% DCl in D_2O) 3.50 (d, J = 7.0, 2 H) and 3.99 (t, J = 7.0, 1 H); $[\alpha]^{30}_D$ +24.8° (c = 2, 0.5 N HCl), [lit. $[\alpha]^{27}_D$ +25.2° (c = 2, 0.5 N HCl)].

Allyl 2(*S*),3-Diaminopropanoate Bis(*p*-toluenesulfonate) (25). A stirred suspension of **9** (14.1 g, 100 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O, 47.1 g, 247 mmol) in allyl alcohol/benzene (270 mL, 17:10) was refluxed in a reaction flask equipped with a Dean−Stark trap. After 2 h, a suspension of *p*-TsOH·H_2O (7.50 g, 40.0 mmol) in allyl alcohol (8.0 mL) was added; this addition was repeated three times (at 4, 6, and 8 h). After the last addition, the reaction mixture (which had become clear by now) was refluxed for an additional 4 h, cooled to 45 °C, and added while stirring to ice-cold Et_2O (1 L). The precipitate was collected by filtration and dried *in vacuo* at 40 °C and, subsequently, in a desiccator over P_2O_5. The crude product was crystallized from MeOH/EtOAc to give allyl ester **25** as colorless needles (37.7 g; 77.2%): mp 159−162 °C dec; $[\alpha]^{30}_D$ +21.3° (c = 1, MeOH); ^{1}H NMR (DMSO-d_6) 2.38 (s, 6 H), 3.25 (m, 2 H), 4.35 (bt, J = 6.7, 1 H), 4.70 (m, 2 H), 5.36 (m, 2 H), 5.91 (m, 1 H), 7.11 and 7.49 (AB-system, J = 8.0, 8 H), 8.10 (bs, 3 H), 8.52 (s, 3 H); FAB-HRMS calcd for $[C_6H_{12}N_2O_6 + H]^+$ 145.0977, found 145.0936.

Allyl 2(*S*)-(Acetylamino)-3-[(*tert*-butyloxycarbonyl)-amino]propanoate (26). Triethylamine (TEA, 14.1 mL, 100 mmol) was slowly added to a stirred suspension of **25** (12.2 g, 25.0 mmol) in CH_2Cl_2 (750 mL) at −62 °C. To the resulting solution was added di-*tert*-butyl dicarbonate (Boc_2O, 5.42 g, 24.8 mmol) in CH_2Cl_2 (40 mL) over a period of 2 h. Subsequently, the reaction mixture was cooled in an ice bath, and stirring was continued for 2 h, after which time acetyl chloride (7.85 mL, 100 mmol, freshly distilled) was added dropwise. The reaction mixture was stirred overnight (temperature <20 °C), concentrated under reduced pressure at 20 °C to *ca*. 250 mL, and poured onto saturated aqueous $NaHCO_3$ (250 mL). The organic layer was sequentially washed with H_2O, aqueous $KHSO_4$ (6%, 3 ×), H_2O, and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give, after purification by MPLC (CH_2Cl_2/EtOAc, 84:16), **26** as a colorless oil that solidified upon standing (4.60 g; 64.3%): mp 71−72 °C; $[\alpha]^{30}_D$ + 11.8° (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 1.41 (s, 9 H), 2.01 (s, 3 H), 3.53 (t, J = 5.8, 2 H), 4.53−4.72 (m, 3 H), 4.90 (bt, J = 5.7, 1 H), 5.30 (m, 2 H), 5.90 (m, 1 H), 6.70 (bd, J = 7.0, 1 H); ^{13}C NMR ($CDCl_3$) 23.1, 28.2, 42.1, 53.9, 66.3, 80.1, 119.0, 131.4, 156.7, 170.1, 170.3; FAB-HRMS calcd for $[C_{13}H_{22}N_2O_5 + H]^+$ 287.1607, found 287.1584.

Allyl 2(*S*)-(Acetylamino)-3-aminopropanoate Hydrochloride (27). To a stirred solution of **26** (4.08 g, 14.2 mmol) in Et_2O (50 mL) was added an etheral solution of HCl (4.0 M, 100 mL) at 5 °C. After 40 min, the turbid reaction mixture was concentrated under reduced pressure. The residue was triturated with Et_2O, dried under reduced pressure, and further dried in a desiccator over KOH and P_2O_5 to give pure **27** as an amorphous powder (3.18 g; 100%). Recrystallization from EtOAc/MeOH furnished **27** as colorless needles (2.73 g; 86.4%): mp 142.5−144 °C; $[\alpha]^{30}_D$ −30.4° (c = 1, MeOH); ^{1}H NMR (DMSO-d_6) 1.89 (s, 3 H), 2.98−3.31 (m, 2 H), 4.48−4.67 (m, 3 H), 5.30 (m, 2 H), 5.89 (m, 1 H), 8.28 (bs, 3 H), 8.66 (bd, J = 8.2, 1 H); FAB-HRMS calcd for $[C_8H_{14}N_2O_3 + H]^+$ 187.1083, found 187.1098.

Methyl 2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-[(4-(*tert*-butyloxy)phenyl]propanoate (28). **28** was prepared in a five step sequence from L-tyrosine using literature procedures[29] to give **28** as a colorless solid, after purification by MPLC (EtOAc/PE, 3:1), in an overall yield of 69.6%: mp 99−100 °C (lit.[29e] mp 99−100 °C); $[\alpha]^{30}_D$ +46.5° (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 1.30 (s, 9 H), 1.39 (s, 9 H), 3.00 (m, 2 H), 3.68 (s, 3 H), 4.53 (m, 1 H), 4.98 (bd, J = 8.3, 1 H), 6.90, 7.00 (AB-system, J = 9.2, 4 H); ^{13}C NMR ($CDCl_3$) 28.0, 28.6, 37.4, 51.8, 54.3, 78.0, 79.4, 123.9, 129.4, 130.7, 154.1, 154.8, 172.2; FAB-HRMS calcd for $[C_{19}H_{29}NO_5 + H]^+$ 352.2123, found 352.2135.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-[4-(*tert*-butyloxy)phenyl]propanal (29). To a stirred solution of **28** (8.79 g, 25.0 mmol) in CH_2Cl_2 (250 mL) at −70 °C was added DIBALH (1 N in hexanes, 62.5 mL, 62.5 mmol) over 15 min, after which time the reaction was usually complete (monitored by TLC). Subsequently, a solution of EtOH/36% HCl_{aq} (9:1, 6.0 mL) was added slowly (the temperature was kept below −65 °C). The reaction mixture was added to a vigorously stirred solution of HCl_{aq} (1 N, 400 mL) at 0 °C. The layers were separated, and the aqueous layer was extracted with ice-cold CH_2Cl_2 (2 × 150 mL). The combined organic layers were washed with ice-cold HCl_{aq} (1 N, 2 × 150 mL), ice-cold H_2O (2 × 150 mL), and ice-cold brine, dried (Na_2SO_4), and concentrated *in vacuo* at ambient temperature, to give aldehyde **29** as a colorless oil that was used as such without delay (8.04 g; 100% crude yield): ^{1}H NMR ($CDCl_3$) 1.31 (s, 9 H), 1.42 (s, 9 H), 3.07 (bd, J = 6.7, 2 H), 4.43 (m, 1 H), 5.06 (bd, J = 8.1, 1 H), 6.91, 7.01 (AB-system, J = 9.2, 4 H), 9.61 (s, 1 H).

Ethyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-5-[4-(*tert*-butyloxy)phenyl]pent-2(*E*)-enoate (30). A dispersion of NaH (60% in mineral oil, 2.00 g, 50.0 mmol) was washed with Et_2O (2×). THF (130 mL) was added. The stirred suspension was cooled with an ice bath, and triethyl phosphonoacetate (10.5 mL, 52.5 mmol) was carefully added (H_2 evolution!). Subsequently, the reaction mixture was stirred at room temperature for 10 min. The resulting solution was placed again in an ice bath and added dropwise to a solution of **29** (8.04 g, max 25.0 mmol) in THF (200 mL) at −50 °C. After an additional 30 min at −50 °C, the reaction mixture was warmed to 10 °C in 2.5 h. The resulting yellow solution was added to a vigorously stirred mixture of aqueous $NaHCO_3$ (5%)/EtOAc (500 mL, 2:3), the layers were separated, and the aqueous layer was extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with H_2O (2×) and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give, after purification by LC (EtOAc/PE, 1:4), **30** as a slightly yellow oil (9.09 g, 93.7%): ^{1}H NMR ($CDCl_3$) 1.25 (t, J = 6.7, 3 H), 1.30 (s, 9 H), 1.39 (s, 9 H), 2.80 (m, 2 H), 4.16 (q, J = 6.7, 2 H), 4.39−4.62 (m, 2 H), 5.80 (dd, J = 16.7, 1.5, 1 H), 6.67 (dd, J = 16.7, 5.0, 1 H), 6.90, 7.02 (AB-system, J = 9.2, 4 H); FAB-HRMS calcd for $[C_{22}H_{33}NO_5 + H]^+$ 392.2437, found 392.2460.

4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-5-[4-(*tert*-butyloxy)phenyl]pent-2(*E*)-enoic Acid (31). To stirred solution of **30** (9.00 g, 23.0 mmol) in 1,4-dioxane/H_2O (390 mL, 35:4) was added aqueous NaOH (1 N, 23.0 mL) over a period of 2 h so as to maintain the pH at approximately 12. The reaction mixture was stirred overnight (the reaction was complete as checked by TLC), acidified to pH ≈ 2.5 with aqueous 6% $KHSO_4$, diluted with H_2O (100 mL), and extracted with EtOAc (3 × 250 mL). The combined organic extracts were washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give, after purification by LC ($CHCl_3$/MeOH, 98:2), acid **31** as a colorless crystalline solid (8.15 g, 97.5%). The crude product could also be crystallized from $CHCl_3$/hexane: mp 133−135 °C dec; $[\alpha]^{30}_D$ +9.2° (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400.1 MHz) 1.31 (s, 9 H), 1.39 (s, 9 H), 2.84 (m, 2 H), 4.46−4.70 (m, 2 H), 5.83 (dd, J = 15.7, 1.6, 1 H), 6.94 and 7.02 (AB-system, J = 8.3, 4 H), 6.99 (dd, J = 15.7, 5.1, 1 H); ^{13}C NMR ($CDCl_3$) 28.1, 28.6, 39.9, 52.3, 77.3, 78.3, 120.3, 124.1, 129.6, 131.0, 150.0, 154.1, 154.9, 170.7; FAB-HRMS calcd for $[C_{20}H_{29}NO_5 + H]^+$ 364.2124, found 364.2139.

Allyl 2(*S*)-Acetylamino-3-[[4(*S*)-[(*tert*-butyloxycarbonyl)amino]-5-[4-(*tert*-butyloxy)phenyl]pent-2(*E*)-enoyl]-amino]propanoate (32). To a stirred solution of acid **31** (2.00 g, 5.50 mmol) and amine **27** (1.33 g, 5.97 mmol) in CH_2Cl_2 (50 mL) was added DIPEA (2.09 mL, 12.0 mmol) followed by TBTU (1.92 g, 6.00 mmol). The reaction was complete after 1.5 h (checked by TLC). The reaction mixture was diluted with EtOAc (200 mL) and sequentially washed with H_2O (3×), aqueous $NaHCO_3$ (5%, 3×), H_2O, aqueous $KHSO_4$ (6%, 3×), H_2O (2×) and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give **32** as a colorless glass (2.89 g, 98.8%): mp 134−135 °C; $[\alpha]^{30}_D$ −49.2° (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 1.30 (s, 3 H), 1.39 (s, 9 H), 2.01 (s, 3 H), 2.79 (m, 2 H), 3.70 (t, J = 5.8, 2 H), 4.44−4.71 (m, 5 H), 5.30 (m, 2 H), 5.79 (d, J = 15.6, 1 H), 5.90 (m, 1 H), 6.30 (bs, 1 H), 6.79 (dd, J = 15.6, 4.9, 1 H), 6.90 and 7.06 (AB-system, J = 8.4, 4 H) , 7.05 (m overlapping, 1 H); ^{13}C NMR ($CDCl_3$) 22.6, 28.0, 28.5, 40.0, 40.9, 52.4, 53.1, 66.1, 78.0, 79.3, 119.7, 122.6, 123.8, 129.5, 131.1, 131.4, 144.0, 153.7, 154.9, 166.5, 170.0, 170.6; FAB-HRMS calcd for $[C_{28}H_{41}N_3O_7 + H]^+$ 532.3023, found 532.3042.

Allyl 2(*S*)-(Acetylamino)-3-[[4(*S*)-[[2(*R*)-[[2(*R*,*S*)-hydroxy-3(*S*)-[[[1-(allyloxycarbonyl)pyrrolidin-2(*S*)-yl]carbonyl]-amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]amino]hexanoyl]amino]-3-phenylpropanoyl]amino]-5-[4-(*tert*-butyloxy)phenyl]pent-2-(*E*)-enoyl]amino]propanoate (35). To a vigorously stirred solution of dipeptide **32** (1.06 g, 2.00 mmol) and 2,6-lutidine (1.17 mL, 10.0 mmol) in CH_2Cl_2 (4.0 mL) at 0 °C was added trimethylsilyl riflate (1.60 mL, 8.00 mmol). After 15 min, the ice bath was removed, and stirring was continued for 95 min. The reaction mixture was placed again in an ice bath, and ice-cold saturated NH_4Cl_{aq} (16 mL) was added. Subsequently, the quenched reaction mixture was extracted with EtOAc (2× 30 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give amine **34** (contaminated with some 2,6-lutidine) as a viscous oil (1.00 g, essentially quantitative): ^{1}H NMR ($CDCl_3$) 1.30 (s, 3 H), 2.01 (s, 3 H), 2.31 (bs, 2 H), 2.60 (dd, J = 4.9, 17.9, 1 H), 2.88 (dd, J = 8.4, 17.9, 1 H), 3.61−3.82 (m, 3 H), 4.58−4.71 (m, 3 H), 5.30 (m, 2 H), 5.89 (m, 1 H), 5.92 (dd, J = 15.4, 1.5, 1 H), 6.51 (bt, 1 H), 6.72 (dd, J = 15.5, 5.1, 1 H), 6.88 (bs, 1 H), 6.92 and 7.08 (AB-system, J = 8.2, 4 H); FAB-HRMS calcd for $[C_{23}H_{33}N_3O_5 + H]^+$ 431.2420, found 431.2441.

To a stirred solution of dipeptide **34** (206 mg, 0.400 mmol + lutidine) and tripeptide **24** (267 mg, 0.365 mmol) in CH_2Cl_2 (10 mL) was added DIPEA (140 mL, 0.804 mmol) followed by TBTU (129 mg, 0.402 mmol). After 3 h, the reaction mixture was diluted with EtOAc (100 mL), sequentially washed with H_2O (3×), aqueous $NaHCO_3$ (5%, 3×), H_2O, aqueous $KHSO_4$ (6%, 3×), H_2O (2×), and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give, after preparative TLC (EtOAc/CH_2Cl_2, 6:4), **35** as a white foam (356 mg, 85.3%), pure according to analytical TLC: ^{1}H NMR (DMSO-d_6, 400.1 MHz) 1.07−1.43 (m, 4 H), 1.19 (s, 9 H), 1.39 (s, 9 H), 1.47 (s, 9 H), 1.67−1.80 (m, 3 H), 1.81 (s, 3 H), 1.97 (m, 1 H), 2.53−2.72 (m, 3 H), 2.82 (m, 1 H), 3.19 (m, 2 H), 3.27−3.40 (m, 3 H), 3.51 (m, 1 H), 3.82 (m, 1 H), 3.90 (m, 1 H), 4.18 (m, 1 H), 4.31−4.60 (m, 7 H), 5.03−5.32 (m, 4 H), 5.73−5.92 (m, 4 H), 6.60 (dd, J = 15.2, 5.3, 1 H), 6.87 (part of AB-system, J = 8.4), 6.89−6.97 (m, 2 H), 7.08−7.24 (m, 5 H), 7.47−7.59 (m, 2 H), 8.14−8.29 (m, 4 H), 11.50 (bs, 1 H); ^{13}C NMR ($CDCl_3$) 22.7, 24.5, 25.6, 28.0, 28.2, 28.7, 29.5, 31.1, 38.4, 39.4, 40.6, 40.8, 46.9, 51.1, 51.6, 53.2, 54.8, 60.8, 66.2, 66.4, 71.8, 78.2, 79.2, 83.0, 117.7, 118.7, 122.4, 123.9, 127.0, 128.5, 129.3, 129.8, 131.2, 131.5, 132.5, 136.5, 143.2, 153.1, 154.0, 154.8, 155.4, 156.1, 163.3, 167.7, 170.4, 170.4, 171.2, 172.4, 172.6.

***N*-Ethylmorpholinium 2(*S*)-(Acetylamino)-3-[[4(*S*)-[[2(*R*)-[[2-(*R*,*S*)-hydroxy-3(*S*)-[[[1-(allyloxycarbonyl)-pyrrolidin-2(*S*)-yl]carbonyl]amino]-6-[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]-amino]hexanoyl]amino]-3-phenylpropanoyl]amino]-5-[4-(*tert*-butyloxy)phenyl]pent-2(*E*)-enoyl]amino]propanoate (36).** To a stirred solution of **35** (316 mg, 0.270 mmol) and morpholine (1.18 mL, 13.5 mmol) in THF (38 mL) was added $Pd(PPh_3)_4$ (20.1 mg, 0.017 mmol). After 45 min, the volatiles were evaporated *in vacuo*. Purification by preparative TLC (CH_2Cl_2/MeOH/NEM, 85:15:10) gave **36** as an off-white powder (224 mg; 72.9%): ^{1}H NMR (DMSO-d_6) 0.99 (t, J = 6.7, 3 H), 1.05−2.00 (m, 8 H), 1.20 (s, 9 H), 1.38 (s, 9 H), 1.48 (s, 9 H), 2.23−2.89 (m, 6 H), 1.81 (s, 3 H), 2.55−3.05 (m, 4 H), 3.07−3.61 (m, 10 H), 3.70 (m, 1 H), 3.81−4.00 (m, 2 H), 4.08 (m, 1 H), 4.41−4.63 (m, 2 H), 5.92 (bd, J = 15.3, 1 H), 6.02 (bs, 1 H), 6.59 (dd, J = 15.3, 4.7, 1 H), 6.87 (part of AB-system, J = 8.2, 2 H), 6.90−7.00 (m, 2 H), 7.03−7.28 (m, 5 H), 7.60 (d, J = 8.2, 1 H), 7.79 (d, J = 7.0, 1 H), 7.96 (bt, J = 6.0, 1 H), 8.08 (d, J = 8, 1 H), 8.18−8.45 (bt, 2 H), 11.49 (bs, 1 H); FAB-HRMS calcd for $[C_{51}H_{75}N_9O_{13} + H]^+$ 1022.5563, found 1022.5546.

(2*S*,5*S*,11*S*,14*R*,17(*R*,*S*),18*S*)-*N*-[11-[4-(*tert*-Butyloxy)-benzyl]-14-benzyl-17-hydroxy-18-[[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]-amino]propyl]-4,8,13,16,20-pentaoxo-2,3,4,5,6,7,8,11,12,13,14,15,16,17,18,19,20,20a-octadecahydro-1*H*-3a,7,12,15,19-pentaazacyclopentacyclononadecen-5-yl]acetamide (37). To a stirred solution of **36** (223 mg, 0.196 mmol) in CH_2Cl_2 (393 mL) were added 4-(dimethylamino)pyridine (DMAP, 96.5 mg, 0.790 mmol), HOBt (53.0 mg, 0.393 mmol), and TBTU (126.6 mg, 0.393 mmol). After 1.5 h again DMAP (24.1 mg, 0.197 mmol), HOBt (26.5 mg, 0.197 mmol) and TBTU (63.1 mg, 0.197 mmol) were added. After 23 h, the solvent was slowly evaporated *in vacuo* at ambient temperature, and the residue was partitioned between EtOAc and H_2O. The organic layer was sequentially washed with H_2O (2×), aqueous $NaHCO_3$ (5%, 2×), H_2O (2×), aqueous $KHSO_4$ (6%, 2×), H_2O (2×), and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give, after preparative TLC (CH_2Cl_2/THF/EtOH, 70:30:1.5), **37** as a white foam (119 mg, 60.6%): ^{1}H NMR (DMSO-d_6, 400.1 MHz) 1.22 (s, 9 H), 1.24 (m, 1 H), 1.39 (s, 9 H), 1.46 (s, 9 H), 1.39−1.58 (m, 3 H), 1.81 (s, 3 H), 1.71−1.88 (m, 2 H), 1.92 (m, 1 H), 2.09 (m, 1 H), 2.39−2.61 (m, 3 H), 2.69 (m, 1 H), 2.97 (m, 1 H), 3.28 (m, 2 H), 3.39 (m, 1 H), 3.59 (m, 1 H), 3.92−4.09 (m, 4 H), 4.41−4.55 (m, 3 H), 4.60 (m, 1 H), 5.95 (dd J = 15.4, 2.2, 1 H), 6.68 (dd, J = 15.3, 2.0, 1 H), 6.75−6.83 (m, 2 H), 6.92 and 7.29 (AB-system, J = 8.5, 4 H), 7.05−7.12 (m, 3 H), 7.90 (d, J = 9.7, 1 H), 8.08 (d, J = 8.3, 1 H), 8.19 (d, J = 6.7, 1 H), 8.27 (d, J = 8.6, 1 H), 8.32 (bt, J = 6.0, 1 H), 8.45 (d, J = 10.6, 1 H), 11.53 (bs, 1 H); ^{13}C NMR ($CDCl_3$) 22.7, 24.5, 26.6, 27.9, 28.3, 28.6 , 27.0, 30.6, 38.3, 39.8, 40.2, 40.4, 47.9, 49.2, 50.1, 53.0, 55.7, 60.3, 72.3, 78.3, 79.4, 82.9, 123.8, 124.2, 127.1, 128.5, 129.3, 129.7, 129.9, 136.1, 141.2, 153.0, 154.3, 156.7, 163.2, 165.0, 169.1, 169.9, 170.1, 171.1, 172.6; FAB-HRMS calcd for $[C_{51}H_{73}N_9O_{12} + H]^+$ 1004.5457, found 1004.5452.

(2*S*,5*S*,11*S*,14*R*,18*S*)-*N*-[11-[4-(*tert*-Butyloxy)benzyl]-14-benzyl-18-[[[[(*tert*-butyloxycarbonyl)imino][(*tert*-butyloxycarbonyl)amino]methyl]amino]propyl]-4,8,13,16,17,20-hexaoxo-2,3,4,5,6,7,8,11,12,13,14,15,16,17,18,19,20,20a-octadecahydro-1*H*-3a,7,12,15,19-pentaazacyclopentacyclononadecen-5-yl]acetamide (38). A solution of **37** (155 mg, 0.154 mmol), Dess−Martin periodinane (177 mg, 0.417 mmol), and *t*-BuOH (37 mL, 0.386 mmol) in CH_2Cl_2/MeCN (7.75 mL, 2:1) was stirred at 40 °C for 23 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (20 mL), and vigorously shaken with a solution of $NaS_2O_3 \cdot 5H_2O$ (4.0 g) in saturated aqueous $NaHCO_3$ (10 mL) until a clear biphasic system appeared. The organic layer was sequentially washed with saturated aqueous $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* at room temperature to give **38** as a white foam (157 mg, 89%). The ^{1}H NMR spectrum showed the presence of small amounts of starting material (≤10%) and EtOAc: ^{1}H NMR ($CDCl_3$) 1.32 (s, 9 H), 1.35−2.27 (m, 8 H), 1.47 (s, 9 H), 1.49 (s, 9 H), 1.97 (s, 3 H), 2.50−2.81 (m, 2 H), 2.88−3.67 (m, 7 H), 4.13−4.40 (m, 3 H), 4.46 (m, 3 H), 4.67 (m, 1 H), 4.87 (m, 1 H), 5.47 (bd, J = 8.2, 1 H), 5.62 (dd, J = 15.3, 1.8, 1 H), 6.77−6.92 (m, 5 H), 6.92 (dd overlapping, 1 H), 7.1−7.23 (m, 2 H), 7.23−7.39 (m, 3 H), 7.45 (bd, J = 8.7, 1 H), 7.55−7.82 (m, 2 H), 8.42 (bt, J = 6.0, 1 H), 11.53 (bs, 1 H); FAB-HRMS calcd for $[C_{51}H_{71}N_9O_{12} + H]^+$ 1002.5300, found 1002.5265.

(2*S*,5*S*,11*S*,14*R*,18*S*)-*N*-[11-(4-Hydroxybenzyl)-14-benzyl-18-[3-[(iminoaminomethyl)amino]propyl]-4,8,13,16,17,20-hexaoxo-2,3,4,5,6,7,8,11,12,13,14,15,16,17,18,19,20,20a-octadecahydro-1*H*-3a,7,12,15,19-pentaazacyclopentacyclononadecen-5-yl]acetamide: Cyclotheonamide B (1b). To a stirred suspension of **38** (69.1 mg, *ca.* 67.8 μmol, *vide supra*) in thioanisole (0.3 mL) was added trifluoroacetic acid (2.7 mL). The resulting solution was stirred for 105 min. Subsequently, the volatiles were evaporated *in vacuo*. The crude product was purified by reversed-phase HPLC; column: Supelco LC-18 DB 250 × 50 mm; mobile phase: (MeCN/H_2O, 3:2)/H_2O/phosphate buffer (0.5 M, pH = 2.1), gradient from 20:60:20 to 60:20:20 in 42 min; flow: 20 mL/min. The product was desalted on the same column (MeCN/H_2O, 3:2)/H_2O/HCl_{aq} (0.1 N), 0:80:20, 15 min, then 80:18:2) and lyophilized to give cyclotheonamide B·1.1HCl·9.6H_2O (based on peptide content) as a fluffy white powder (32.8 mg, 50.8% from **37**): analytical HPLC, ≥96.5%; $[\alpha]^{23}_D$ −13.7° (c = 0.2, MeOH) [lit. $[\alpha]^{23}_D$ −13.6° (c = 0.2, MeOH)]; ^{1}H NMR (D_2O, 400.1 MHz) 1.47−1.60 (m, 2 H), 1.67 (m, 1 H), 1.80−2.09 (m, 4 H), 1.99 (s, 3 H), 2.30 (m, 1 H), 2.54 (dd, J = 14.0, 10.3, 1 H), 2.77 (dd, J = 13.7, 4.6, 1 H), 2.86 (dd, J = 13.7, 6.0, 1 H), 2.89 (t, J = 11.4, 1 H), 2.99 (dd, J = 14.0, 4.9, 1 H), 3.16 (m, 2 H), 3.49 (m, 1 H), 3.76 (m, 1 H), 3.98 (dd, J = 10.9, 2.4, 1 H), 4.20 (dd, J = 12.8, 5.9, 1 H), 4.50 (m, 1 H), 4.54 (m, 1 H), 4.58 (m, 1 H), 4.62 (m, 1 H), 5.91 (dd, J = 15.6, 2.1, 1 H), 6.63 (m, 2 H), 6.80 (dd, J = 15.6, 2.6, 1 H), 6.91 (part of AB-system, J = 8.4, 2 H), 7.17−7.29 (m, 5 H); ^{13}C NMR ($CDCl_3$) 24.0, 25.9, 27.2, 27.5, 33.0, 40.4, 41.7, 41.8, 43.4, 51.4, 53.1, 55.4, 56.8, 57.2, 63.3, 97.2, 118.4, 125.3, 130.0, 131.2, 132.1, 132.4, 133.3, 138.0, 146.1, 157.2, 159.4, 170.3, 173.6, 173.8, 174.4, 176.1, 176.7; FAB-MS m/z 746 (MH^+) and 764 ($MH^+ + H_2O$).

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Supporting Information Available: Copies of ^{1}H NMR spectra for **14**, **15**, **18**, **19**, **21**, **23**−**25**, **27**−**32**, and **34**−**38** and of ^{13}C NMR spectra for **16**, **17**, **22**, and **26** (25 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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