to the 3^{10}-helix of normal peptides, but contains a bulge at the methoxyethyl insert and is C-capped with a type I like β-turn element at the Pro-Gly linkage.[9]

In conclusion, simple, prototype vinylogous polypeptides are seen to be rich in secondary structure. These would appear to be a promising new class of polypeptide-like substances with altered backbones. It will be interesting to determine the receptor-binding properties of these potential ligands. Indeed, the following communication illustrates a naturally occurring vinylogous polypeptide that functions as a high affinity ligand.

Acknowledgment. Support of this research by the AIDS Initiative of the National Institute of General Medical Sciences (GM-44993) is gratefully acknowledged. M.H. thanks Ube Industries, Ltd., for support as a visiting scientist. We are especially grateful to Soroosh Shambayati, whose insights and suggestions were influential throughout this investigation.

Supplementary Material Available: ^{1}NMR spectra (500 MHz) of **10–13**, stereoviews of **4**, **6b**, **7**, and **8**, and tables of crystal data, atomic coordinates, isotropic and anisotropic displacement coefficients, and bond lengths and angles for **3**, **4**, **6b**, **7–9**, and **13** (62 pages). Ordering information is given on any current masthead page.

(9) We note that the β-oxo γ-amino acid (statine-like) moiety is a common structural element in many natural products of biological significance, including statine, dolastatine 10, and didemnin B.

Reassignment of Stereochemistry and Total Synthesis of the Thrombin Inhibitor Cyclotheonamide B

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Modern methods of molecular and structural biology provide excellent opportunities to characterize the interactions between natural products and their cellular receptors.[1] In the process, much can be learned about cellular pathways sensitive to the natural products.[2] Marine sponges have been a rich source of natural products whose influences on cellular function have proved especially illuminating. Examples include okadaic acid,[3] the microcystins,[4] and calyculin[5] that, together with the bacterial natural products FK506 and cyclosporin A,[6] have revealed the significant role of soluble protein phosphatases in signal transduction pathways.[7] More recently, a family of cyclic peptides obtained from the marine sponge genus *Theonella*[8,9] had been characterized that appear to be promising candidates for structural and mechanistic investigations. Of these, the cyclotheonamides[10] attracted our attention for two main reasons. First, it was exciting to discover, in a natural product, a residue we had thus far considered nonnatural but whose synthesis and conformational properties we had studied in some detail. Vinylogous tyrosine (V-Tyr) belongs to the larger class of vinylogous amino acids that can form polymers of regular secondary structure.[11] Second, the presence of an α-keto amide moiety was provocative and strongly suggestive of a mechanism of action for these low molecular weight[12] inhibitors of a protease of the blood coagulation cascade, thrombin (see below). As a first step toward elucidating the structural basis for thrombin–cyclotheonamide complexation, we now report the total synthesis of cyclotheonamide B (CyB) and the modification of the previously reported stereostructure.

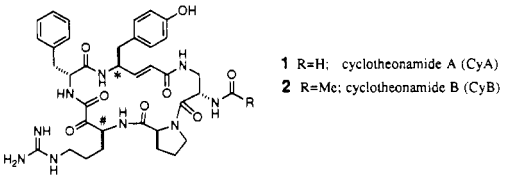
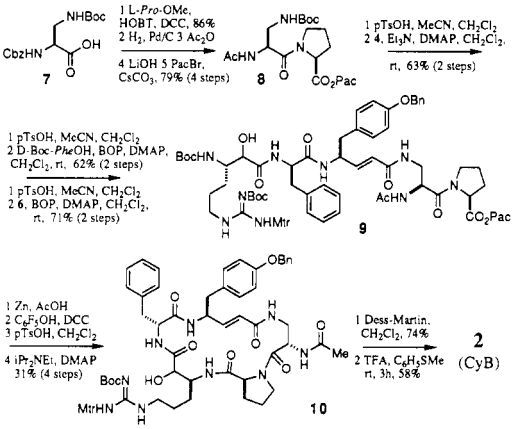


Figure 1. Revised stereostructures of the cyclotheonamides.

Scheme I



In the early phase of our studies, we developed syntheses of both the *R* and *S* stereoisomers at the Arg-like residue (see # in Figure 1; this site was not defined in the original studies) of the proposed structure of CyB (having the proposed *R* stereochemistry at the V-Tyr residue (*S* is shown); see *), using reaction sequences analogous to that shown in Scheme I. The spectral properties of our synthetic samples, however, differed significantly from those of natural CyB kindly provided by Professor Fusetani.[10] We then proposed an alternative stereostructure in Figure 1, containing the *S* stereochemistry at the V-Tyr residue, upon considering the potential pitfalls in the earlier degradative studies[9] and our analyses of NMR spectra from the synthetic and natural samples. The *S* stereochemistry of the Arg-like residue followed from the potent inhibition of thrombin by the *R*(*),*S*(#) synthetic isomer[13] and our speculation concerning thrombin inhibition. (The α-keto group of the cyclotheonamides may function as an electrophilic mimic of the ArgX scissile amide bond of thrombin substrates.) This stereochemical reassignment was confirmed by a synthesis of CyB described below.

L-Proline methyl ester and the protected aminoserine **7**, which was readily synthesized according to the Izumiya procedure,[14] were coupled with DCC/HOBT to yield a dipeptide that was acetylated at its N-terminus (formylation would be required for the synthesis of CyA **1**) and alkylated at its C-terminus with phenacyl (Pac) bromide (Scheme I). Three consecutive amide couplings in the C → N direction converted **8** into the seco-CyB precursor **9**. The

(1) Rosen, M. K.; Schreiber, S. L. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 384–400.
(2) Schreiber, S. L. *Science* **1991**, *251*, 283–287.
(3) Tachibana, K.; Scheuer, P. J.; Tsukitani, Y.; Kikuchi, Y.; Van Engen, D.; Clardy, J.; Gopichand, Y.; Schmitz, F. J. *J. Am. Chem. Soc.* **1981**, *103*, 2469–2471.
(4) Dilip de Silva, E.; Williams, D. E.; Anderson, R. J.; Klix, H.; Holmes, C. F. B.; Allen, T. M. *Tetrahedron Lett.* **1992**, *33*, 1561–1564.
(5) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780–2781.
(6) Schreiber, S. L.; Crabtree, G. R. *Immunol. Today* **1992**, *13*, 136–142.
(7) Cohen, P.; Holmes, C. F. B.; Tsukitani, Y. *TIBS* **1990**, *15*, 98–102.
(8) Orbiculamides: Fusetani, N.; Sugawara, T.; Matsunaga, S.; Hirota, H. *J. Am. Chem. Soc.* **1991**, *113*, 7811–7812.
(9) Keramimides: Kobayashi, J.; Itagaki, F.; Shigemori, H.; Ishibashi, M.; Takahashi, K.; Ogura, M.; Nagasawa, S.; Nakamura, T.; Hirota, H.; Ohta, T.; Nozoe, S. *J. Am. Chem. Soc.* **1991**, *113*, 7812–7813.
(10) Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Tukebayashi, Y. *J. Am. Chem. Soc.* **1990**, *112*, 7053–7054.
(11) Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, preceding communication in this issue.
(12) In contrast to protein inhibitors of thrombin such as hirudin, see: (a) Haruyama, H.; Wüthrich, K. *Biochemistry* **1989**, *28*, 4301–4312. (b) Rydel, T. J.; Ravichandran, K. G.; Tulinsky, A.; Bode, W.; Huber, R.; Roitsch, C.; Fenton, J. W., II *Science* **1990**, *249*, 277–280.
(13) Unpublished results, M.H. and S.L.S.
(14) Waki, M.; Kitajima, Y.; Izumiya, N. *Synthesis* **1981**, 266–268.

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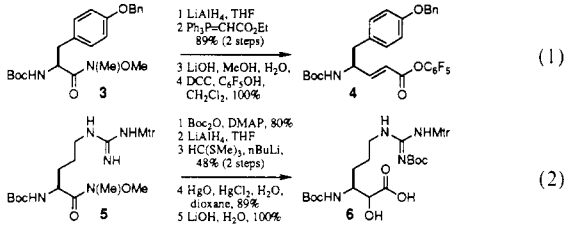
first of these involved homologation with the vinylogous tyrosine derivative **4**, which was prepared from the protected tyrosine derivative **3** by application of our standard V-amino acid synthesis[11] followed by a pentafluorophenyl ester activation protocol[15] (eq 1). Following a second homologation with D-*N*-Boc-phenylalanine,, the third coupling was achieved with the α-hydroxy acid **6**, which was prepared from the Weinreb amide[16] of a protected arginine **5** (eq 2). The guanidinium of **6** was doubly protected with Boc and 2,5,6-trimethyl-4-methoxybenzenesulfonyl (Mtr) groups to prevent nucleophilic addition to an intermediate arginine aldehyde, which was homologated to the α-hydroxy acid **6** by Seebach's procedure.[17]



Macrolactamization was achieved by a four-step process. Removal of the phenacyl group (Zn, AcOH) was followed by pentafluorophenyl ester formation at the C-terminus. Selective removal of the N-terminal Boc group with *p*-TsOH[18] illustrates the utility of the novel guanidinium protecting group strategy used in this study. After neutralization of the ammonium tosylate with Hünig's base, treatment with DMAP resulted in smooth conversion to the cyclic peptide **10**. After oxidation of the hydroxyl, which is left unprotected during the synthesis, the entire regiment of protecting groups could be removed in a single step with TFA and thioanisole. The ^{1}H NMR spectrum of the product, synthetic CyB **2** ($[\alpha]^{23}_D = -13.5°$, $c = 0.2$, MeOH),[19,20] was the same as that obtained from the natural sample. The identity of synthetic CyB, prepared in this manner, to natural CyB is in stark contrast to the products obtained by analogous procedures using the enantiomer of **3**, which results in the originally proposed structure, and the enantiomer of **5** (the stereochemistry at the arginine-like residue was not previously defined).[21] Thus, the cyclotheonamides are represented by structures **1** and **2**. With the full stereostructue and an efficient total synthesis in hand, we are presently investigating the structural features of thrombin–CyB interactions and the potential role of a vinylogous amino acid in this context.

Acknowledgment. Support of this research by the AIDS Initiative of the National Institute of General Medical Sciences (GM-44993) is gratefully acknowledged. M.H. thanks Ube Industries, Ltd., for support as a visiting scientist. We thank Michael K. Rosen and Soroosh Shambayati for their assistance with the CyB structural analysis and their suggestions for several synthetic transformations.

Supplementary Material Available: Spectral data, ^{1}H NMR spectra, and TOCSY spectra for the compounds mentioned in the text (14 pages). Ordering information is given on any current masthead page.

(15) (a) Schmidt, U.; Lieberknecht, A.; Griesser, H.; Utz, R.; Beuttler, T.; Bartkowiak, F. *Synthesis* **1986**, 361-366. (b) Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1063-1072.

(16) (a) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815-3818. (b) Fehrentz, J.; Castro, B. *Synthesis* **1983**, 676-678. (c) Lubell, W. D.; Rapoport, H. *J. Am. Chem. Soc.* **1987**, *109*, 236-239.

(17) (a) Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 442-444. (b) Seebach, D. *Chem. Ber.* **1972**, *105*, 487-510. (c) Dailey, O. D., Jr.; Fuchs, P. L. *J. Org. Chem.* **1980**, *45*, 216-236. (d) Beautement, K.; Clough, J. M. *Tetrahedron Lett.* **1987**, *28*, 475-478.

(18) This reaction required careful monitoring; attempts to achieve this transformation with TFA failed to bring about the selective unmasking of the N-terminal Boc group.

(19) Synthetic CyB was purified by HPLC (column: TOSO ODS80TM 12.5 cm; mobile phase: MeCN:H_2O = 75:25, 0.1% TFA; flow rate: 1 mL/min; detection: UV 254 nm).

(20) Natural CyA: $[\alpha]^{23}_D = -13°$, $c = 0.2$, MeOH.[10]

(21) The potent inhibition of thrombin by synthetic CyB and investigations of the influence of stereochemistry on thrombin inhibition will be reported separately.

Redox Chemistry of *meso*-Octaethylporphyrinogen: Formation and Opening of a Cyclopropane Ring§

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The oxidative aromatization of the *meso*-tetraalkylhexahydroporphyrinogen to porphyrin is one of the most interesting chemical and biochemical pathways.[1-3] *meso*-Octaalkylporphyrinogen,[4] for which the oxidative aromatization is prevented by the presence of two alkyl substituents at each meso carbon, shows an unexpected redox chemistry.

During our studies on the interaction of the *meso*-octaethylporphyrinogen tetraanion **2** with transition metals,[5-7] we discovered its transformation into an oxidized dianionic form **4**, which can be reduced back to the original tetraanion, as shown in Scheme I.

The attempt to complex Pd(II) with **2** led to the reduction of Pd(II) to Pd metal and the isolation of **4**,[8] whose hydrolysis led to the diprotic ligand **6**.[9] The cyclopropane form **4** can be reduced back to the tetraanion **2** by the use of lithium metal.[10] The C–C

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(1) *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1978-1979; Vols. 1-7. Smith, K. M. *Porphyrins and Metalloporphyrins*; Elsevier: Amsterdam, 1975.

(2) (a) Mashiko, T.; Dolphin, D. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R. D., McCleverty, J. A., Eds.; Pergamon: Oxford, 1987; Vol. 2, Chapter 21.1, p 855. (b) Kim, J. B.; Adler, A. D.; Longo, R. F. In *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1978; Vol. 1, Part A, p 85. Mauzerall, D. *Ibid.* Vol. 2, p 91. (c) von Maltzan, B. *Angew. Chem., Int Ed. Engl.* **1982**, *21*, 785. (d) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827. Lindsey, J. S.; Wagner, R. W. *J. Org. Chem.* **1989**, *54*, 828.

(3) (a) Franck, B. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 343. (b) Johansen, J. E.; Piermattie, V.; Angst, C.; Diener, E.; Kratky, C.; Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 261. (c) Kratky, C.; Waditschatka, R.; Angst, C.; Johansen, J. E.; Plaquevent, J. C.; Schreiber, J.; Eschenmoser, A. *Helv. Chim. Acta* **1985**, *68*, 1312. (d) Waditschatka, R.; Diener, E.; Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 631. (e) Angst, C.; Kajiwara, M.; Zass, E.; Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 140. (f) Waditschatka, R.; Kratky, C.; Jaun, B.; Heinzer, J.; Eschenmoser, A. *J. Chem. Soc., Chem. Commun.* **1985**, 1604.

(4) (a) Fischer, H.; Orth, H. *Die Chemie des Pyrrols*; Akademische Verlagsgesellschaft: Leipzig, 1934; p 20. (b) Baeyer, A. *Chem. Ber.* **1886**, *19*, 2184. (c) Dennstedt, M.; Zimmermann, J. *Chem. Ber.* **1887**, *20*, 850 and 2449; **1888**, *21*, 1478. (d) Dennstedt, D. *Chem. Ber.* **1890**, *23*, 1370. (e) Chelintzev, V. V.; Tronov, B. V. *J. Russ. Phys. Chem. Soc.* **1916**, *48*, 105, 127. (f) Sabalitschka, Th.; Haase, H. *Arch. Pharm.* **1928**, *226*, 484. (g) Rothemund, P.; Gage, C. L. *J. Am. Chem. Soc.* **1955**, *77*, 3340.

(5) Jacoby, D.; Floriani, C.; Chiesi-Villa, A.; Rizzoli, C. *J. Chem. Soc., Chem. Commun.* **1991**, 220.

(6) Jacoby, D.; Floriani, C.; Chiesi-Villa, A.; Rizzoli, C. *J. Chem. Soc., Chem. Commun.* **1991**, 790.

(7) Jubb, J.; Jacoby, D.; Floriani, C.; Chiesi-Villa, A.; Rizzoli, C. *Inorg. Chem.* **1992**, *31*, 1306.

(8) Preparation of **4**: Palladium dichloride (1.17 g, 6.71 mmol) was added to a solution of **2** (4.66 g, 5.47 mmol) in toluene (200 mL), and the mixture was allowed to stir overnight. The filtrate, kept at −15 °C for 2 weeks, gave orange-red crystals of **4** (25%). Anal. Calcd for $C_{44}H_{64}Li_2N_4O_2$: C, 75.98; H, 9.21; N, 8.06. Found: C, 75.58; H, 9.12; N, 7.88.

(9) Preparation of **6**: **4** (0.25 g, 0.42 mmol) was hydrolyzed with a few drops of aqueous HCl, followed by the addition of Et_2O. The yellow etheric phase gave, after evaporation to dryness, a pale yellow powder (88%): ^{1}H NMR (CD_2Cl_2) δ 11.6 (bs, 2 H, NH), 7.52 (d, 2 H, C_4H_2N), 6.71 (d, 2 H, C_4H_2N), 5.88 (m, 2 H, C_4H_2N), 5.78 (m, 2 H, C_4H_2N), 2.79 (q, 2 H, Et), 2.14 (m, 6 H, Et), 1.94 (m, 8 H, Et), 1.03 (m, 6 H, Et), 0.75 (m, 12 H, Et), 0.39 (t, 6 H, Et).

(10) **4** (0.50 g, 0.72 mmol) was added to THF (200 mL) under argon followed by metallic lithium sand (0.01 g, 1.44 mmol). The mixture was refluxed overnight. After concentration to dryness, **2** was isolated (89%).

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