Total Synthesis of Cyclotheonamide A

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Summary: The potent serine protease inhibitor cyclotheonamide A was prepared in a convergent strategy from D-phenylalanine (D-Phe), vinylogous L-tyrosine (L-Vty), L-diaminopropanoic acid (L-Dpr), L-proline (L-Pro), and a hydroxy acid derivative of L-arginine. Macrocyclic ring closure between the D-Phe and the L-Vty residues was performed via the pentafluorophenyl ester, and the Dess–Martin periodinane was used for the oxidation of the hydroxyamide to the α-ketoarginine (L-Kar) residue.

The novel 19-membered cyclic peptides, cyclotheonamide A and B, have been isolated by Fusetani et al. from a marine sponge of the genus *Theonella*.[1] Cyclotheonamide A (1) displays a slow- and tight-binding mechanism of action on human thrombin and plasmin and bovine trypsin. Its biological profile shows a K_i of 0.2 nM for trypsin, while ca. 5- and 60-fold higher values are observed for thrombin and plasmin.[2] Cyclotheonamide A also inhibits dose-dependently the aggregation of human platelets with an IC_{50} of 1.5 μM.

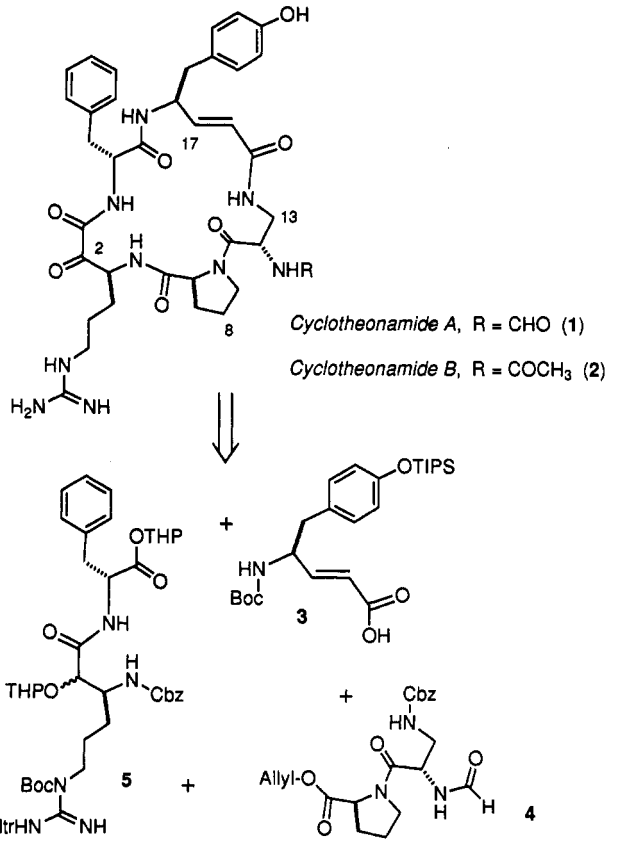
The unique structural features and the potential of these cyclopeptides to serve as lead structures for the development of new antithrombotic agents have prompted us to initiate an effort toward the total synthesis, structural and mechanistic investigations, and the preparation of analogues for SAR studies.[3]

Since our recent report on the preparation of a C(1) to N(14) segment of cyclotheonamide A,[4] Schreiber and Hagihara published the total synthesis of cyclotheonamide B and assigned the (*S*)-stereochemistry for C(3) and C(18).[5,6] In this paper, we present our strategy for the total synthesis of the major *Theonella* sp. metabolite, cyclotheonamide A. In a convergent approach, two dipeptide units and the L-Vty residue **3** are combined to give the highly functionalized macrocycle (Scheme I).

The unusual vinylogous amino acid **3** was prepared via activation of L-Boc-tyrosine (**6**) with isobutyl chloroformate and *N*-methyl morpholine[7] and conversion to the Weinreb amide **7** (Scheme II).[8] Phenol protection with triisopropylsilyl chloride in the presence of DMAP and imidazole, followed by reduction to the aldehyde and Wittig–Horner condensation with $(EtO)_2P(O)CH_2CO_2SiMe_3$,[9] gave the α,β-unsaturated amino acid **3** directly after mild aqueous workup. After mixed anhydride formation with diphenylphosphinic chloride,[10] condensation of the L-Vty residue **3** with hydrobromide **8**[4] resulted in a 44% overall yield of the *right side* segment **9** of cyclotheonamide A. The use of the mixed anhydride of **3** and isobutyl chloroformate provided tripeptide of lesser purity.

For the preparation of the *left side* of cyclotheonamide A, the chain-extended arginine derivative **10**[4] was saponified, activated to the mixed anhydride, and condensed with D-phenylalanine methyl ester (Scheme III). A change in the protective group scheme of **11** was crucial for the successful completion of the total synthesis. In order to reduce the still considerable nucleophilicity and basicity of the Mtr-protected[11] guanidino function, the N_ϵ-Cbz in **10** was changed to the more base-resistant Boc group. The conversion of the methyl to the THP ester[12] allowed a mild demasking of the C-terminus of the dipeptide (*vide*

Scheme I



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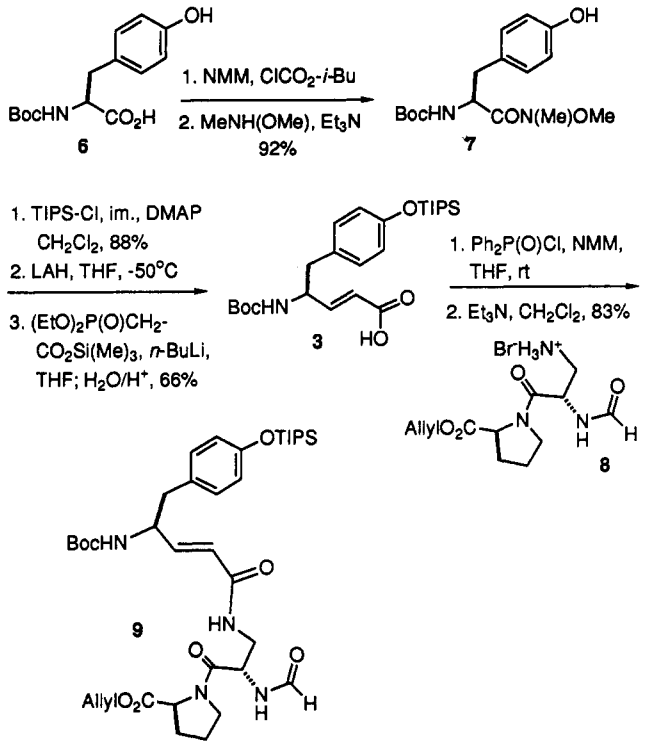
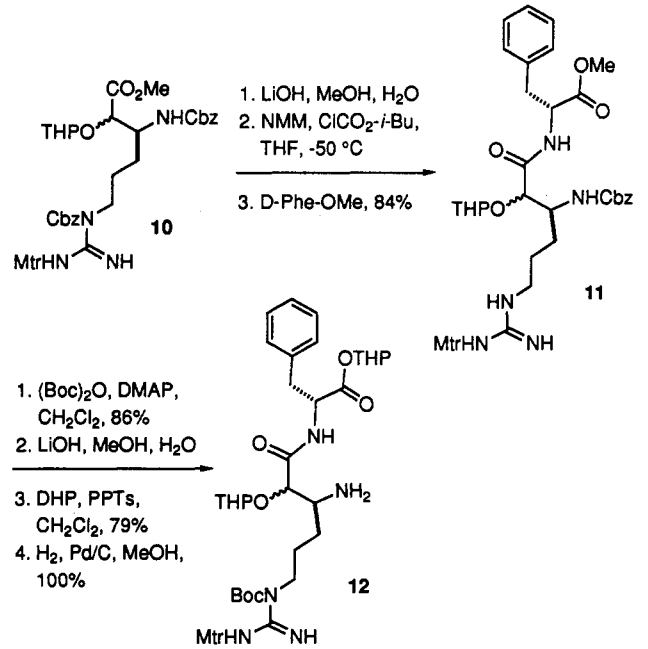
Scheme II



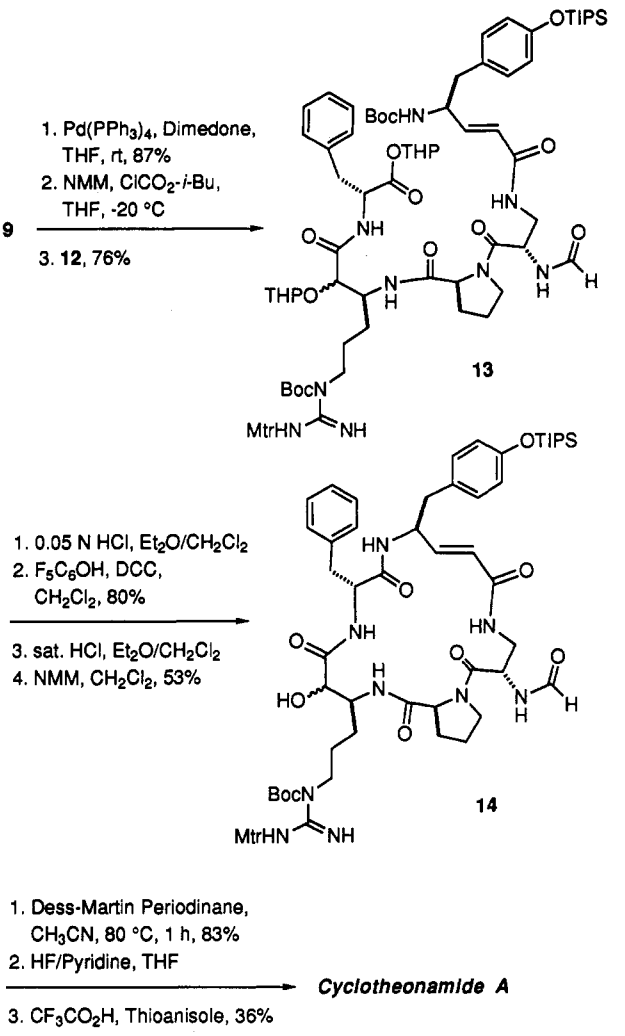
Scheme III



infra). Catalytic hydrogenolysis of the N_α-Cbz group, finally, provided amine **12** in 57% yield from arginine derivative **10**.

C-Terminal deprotection of tripeptide **9** with Pd(0) in the presence of dimedone,[13] followed by segment condensation with dipeptide **12**, gave the acid-sensitive pentapeptide **13** in 66% yield (Scheme IV). Hydrolysis of the THP ester with dilute HCl, conversion to the pentafluorophenyl ester,[14] and selective removal of the

Scheme IV



N(19)-Boc group with anhydrous HCl in Et_2O/CH_2Cl_2 (which simultaneously removed the THP ether) set the stage for a macrolactamization in CH_2Cl_2 in the presence of NMM at room temperature for 12 h and provided cyclopeptide **14** in 42% yield from seco-ester **13**.

Oxidation of the α-hydroxy amide in **14** to the α-keto function proved to be considerably more delicate than anticipated. Even in the presence of a large excess of Dess–Martin periodinane,[15] only very low conversions (10–15%) were observed at room temperature in CH_2Cl_2. High-temperature oxidation in acetonitrile, however, in the presence of 2.5 equiv of periodinane, led to a complete conversion to the desired keto amide cyclopeptide. Quite possibly, conformational preferences of the cyclopeptide backbone shield the hydroxy function against attack by the bulky periodinane at low temperatures.

Subsequently, desilylation with HF/pyridine in THF and removal of both Boc and Mtr protective groups with trifluoroacetic acid in the presence of 50 equiv of thioanisole led to cyclotheonamide A in 30% yield ($[\alpha]^{22}_D$ −12.7°, *c* 0.1, MeOH).[16] Synthetic cyclotheonamide A (10 mg) was fully characterized (FAB-MS, ^{1}H NMR, ^{13}C NMR)

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(16) Deprotection in trifluoroacetic acid at rt for 1 h led to an approximately 4:1 mixture of cyclotheonamide A and the corresponding guanidino-Mtr derivative. Both compounds were isolated by RP-HPLC (C-18, $CH_3CN:H_2O$ = 60:40), and the Mtr derivative was recycled.
(17) A sample of natural cyclotheonamide A was kindly provided by Professor Fusetani.

and was identical (spectroscopic data, RP-HPLC and TLC retention times, $[\alpha]_D$) with the natural product.[17]

The total synthesis of cyclotheonamide A confirms the stereochemical reassignments for the Vty and Kar residues of cyclotheonamides that were made by Schreiber and Hagihara in the context of their synthesis of cyclotheonamide B. Noteworthy features of our approach are the convergent strategy toward the highly modified cyclopentapeptide, which lends itself easily to the preparation of analog structures for mechanism of action and SAR investigations, as well as the protective group tactics in segment condensation and macrolactamization steps. The presence of strongly nucleophilic (guanidino group) and electrophilic (α-keto amide) functions in close spatial proximity requires a double protection of the guanidino group and is probably responsible for the instability of this compound in solution.

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Supplementary Material Available: Experimental procedures and compound characterization data (6 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.