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Total Synthesis of Cyclotheonamide B, a Facile Route towards Analogues

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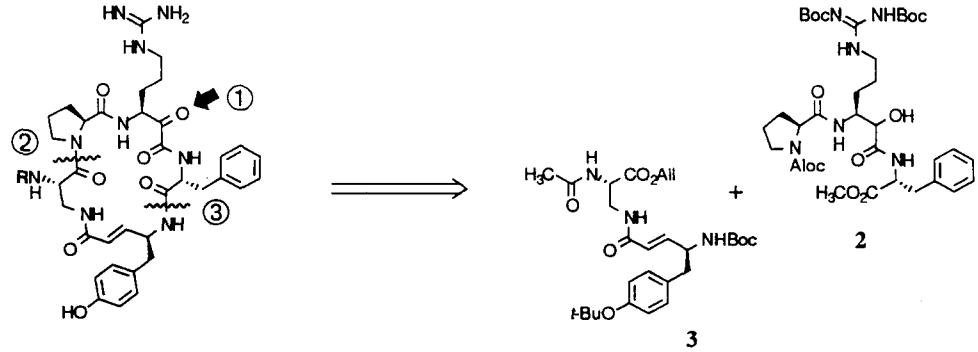
Abstract: *A flexible, convergent synthesis of Cyclotheonamide B (**1b**) was developed, starting from the constituent amino acids, using conventional benzyl-, t-butyl- and allyl-based protecting groups. By modification of the key intermediates, this approach allows the preparation of cyclotheonamide analogues.*

Cyclotheonamides A/B (**1a,b**), metabolites isolated from a marine sponge,[1] are cyclic pentapeptides, whose 19-membered ring features five stereocenters and one *E*-double bond. Besides the residues of three α-amino acids (L-proline (Pro), L-2,3-diaminopropanoic acid (Dpr) and D-phenylalanine (D-Phe)), those of an α,β-unsaturated γ-amino acid derived from L-tyrosine (Tyr) and of α-keto L-β-homoarginine (kArg) are present (scheme 1). The latter two amino acids were unknown as constituents of natural products up to the isolation of the Cyclotheonamides. These unique structural features, in combination with their serine-protease inhibiting properties have prompted many researchers to study these compounds.[2-9] Among the proteases inhibited is thrombin, which plays a crucial role in the process of thrombosis and hemostasis. Thus, the Cyclotheonamides can serve as valuable lead compounds in the development of a new class of antithrombotic agents.

Scheme 1

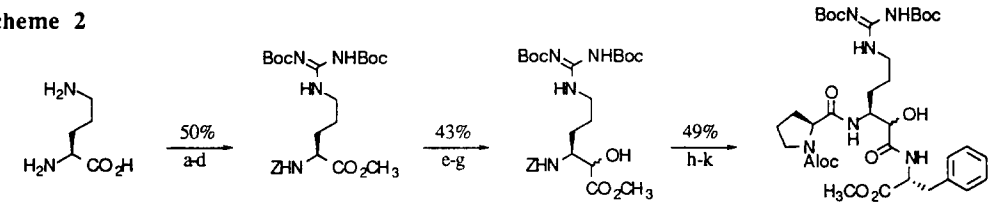


1a Cyclotheonamide A (**R** = formyl)
1b Cyclotheonamide B (**R** = acetyl)

As shown by crystallographic studies of the Cyclotheonamide-human α-thrombin-hirugen complex, the α-keto group of the β-homoarginine unit (see ① in scheme 1) forms a highly stabilized hemiketal with Ser^{195} of the enzyme, similar to the transition state of peptide hydrolysis.[3] Moreover, these studies showed that, besides the conventional Pro-Arg binding mode, the remainder of the macrocycle has no strong interactions with the enzyme, so that it is to be expected that proper modifications of the molecular structure of the natural product can improve its binding properties.

In this paper a convergent, [2 + 3] fragment-condensation route to Cyclotheonamide B is described.[10] This route was designed to allow the synthesis of a series of Cyclotheonamide analogues, to probe and to improve the selectivity and potency of the natural product with respect to its thrombin inhibiting properties. The key fragment in Cyclotheonamide A/B undoubtedly is the α-keto L-β-homoarginine unit. Because of the high reactivity of α-keto carboxylic acid derivatives it was decided that this unit was to be formed from an α-hydroxy carboxylic acid in a late step in the total synthesis (step ① in the retrosynthetic analysis, scheme 1). Disconnection of the Dpr-Pro amide bond (step ②) and subsequent breaking of the Phe-Tyr bond (step ③) gives tripeptide **2** with an α-hydroxy L-β-homoarginine moiety as the central part, and dipeptide **3** containing the vinylogous tyrosine unit. The synthesis of these properly protected key intermediates was achieved as follows.

Scheme 2



4 **5** **6** **2**

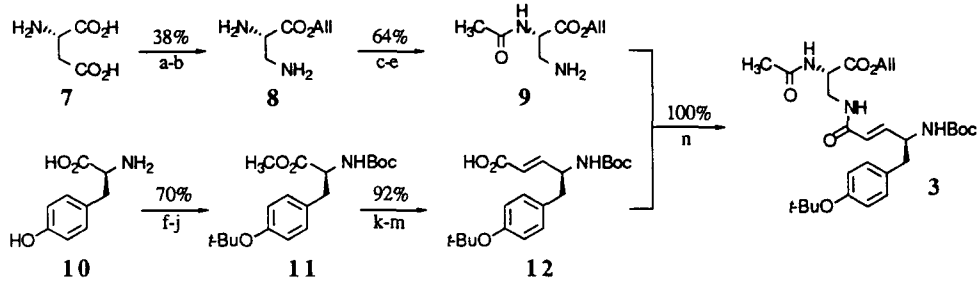
a) $CuCO_3$, H_2O, 100 °C, 1h; b) N^1-(*N,N'*-bis(Boc)amidino)pyrazole/DiPEA, formamide, 24h; c) EDTA/benzylchloroformate, H_2O/aceton, 12h; d) CH_2N_2; e) DiBAH, CH_2Cl_2, - 72 °C, 2h; f) $LiC(SCH_3)_3$, THF, - 72 °C, 5h; g) $HgO/HgCl_2$, CH_3OH/H_2O, 68 h; h) LiOH, $THF/CH_3OH/H_2O$, 12 min; i) DCC/HOBt/H-D-Phe-OCH_3, CH_2Cl_2, 14h; j) H_2/Pd/C, CH_3OH; k) Aloc-Pro-OH /TBTU, CH_2Cl_2, 1.5h.

Starting compound for the preparation of the α-hydroxy β-homoarginine moiety, the predominant part of tripeptide **2**, was N^{ω}, $N^{\omega'}$-bis(Boc) N^{α}-Z L-arginine methyl ester (**5**), prepared in 4 steps (50% yield) from L-ornithine (**4**) (scheme 2). Reduction of **5** to the corresponding aldehyde and subsequent addition of tris(methylthio)methyllithium, followed by treatment with $HgCl_2/HgO$ in aqueous methanol, yielded the orthogonally protected methyl ester of α-hydroxy β-homoarginine **6** (mixture of diastereomers *SR*:*SS* = 8:1).[11] Upon hydrolysis of **6** under standard conditions (LiOH 3 eq, $THF/MeOH/H_2O$, 4/1/1, rt, 72 min) a considerable amount of cyclic carbamate was found, due to intramolecular nucleophilic attack by the hydroxyl group at the benzyloxycarbonyl group.[12] Decreasing the reaction time to 12 min prevented this side-reaction and gave the α-hydroxy acid as the only product. Subsequent coupling (DCC/HOBt) with D-phenylalanine methyl ester followed by hydrogenolysis to remove the Z group and coupling (TBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate) with *N*-allyloxycarbonyl proline, gave the desired tripeptide **2** in 10.5% yield from **4**.

For the synthesis of the other intermediate, *i.e.* dipeptide **3**, the allyl ester of L-2,3-diaminopropanoic acid (**8**) (prepared from L-aspartic acid (**7**), scheme 3) was reacted with Boc_2O/TEA under high-dilution conditions to give mainly the mono N^{β}-Boc regioisomer (N^{α} : N^{β} = 1 : 8) of 2,3-diaminopropanoic acid allyl ester.[13] The crude reaction mixture was treated with acetyl chloride yielding, after chromatographic purification and removal of the Boc group with an etheral solution of HCl, N^{α}-acetyl 2,3-diaminopropanoic acid allyl ester (**9**). The tyrosine-derived α,β-unsaturated γ-amino acid **12** was prepared by a Wadsworth-Emmons olefination of the aldehyde prepared from *N*-Boc *O*-*t*-butyl tyrosine methyl ester (**11**), which in its turn was prepared (5 steps, 70% yield) from L-tyrosine (**10**). This Wittig-type reaction gave in quantitative yield the ethyl ester

exclusively having the *E*-alkene geometry,[14] which, after hydrolysis to **12**, was coupled (TBTU) with **9**, to yield dipeptide **3** in 64.4% yield from **10**.

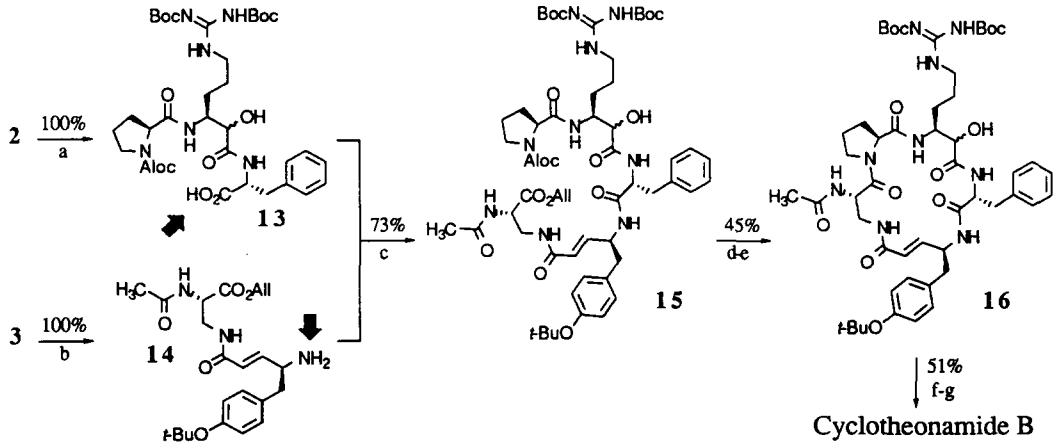
Scheme 3



a) NaN_3, 30 % oleum/$CHCl_3$, 58 °C, 4h, H_2O; b) allyl alcohol/pTSOH, benzene, reflux, 5.5h; c) Boc_2O/TEA, CH_2Cl_2, - 68 °C, 3h; d) acetyl chloride/TEA, CH_2Cl_2, 14h; e) 3M HCl, Et_2O, 0 °C→rt, 1h; f) $SOCl_2$, CH_3OH, reflux, 0.5h; g) benzyl-chloroformate /$NaCO_3$, H_2O/$CHCl_3$, 5 °C, 2h; h) isobutene/H_2SO_4, CH_2Cl_2 36h; i) H_2/Pd/C, CH_3OH; j) Boc_2O, DMF, 50 °C, 0.5h; k) DiBAH, CH_2Cl_2, - 72 °C, 0.5h; l) triethylphosphonacetate/NaH, THF, -50 →10 °C, 2.5h; m) NaOH, dioxane/H_2O, 14h; n) 7/TBTU, CH_2Cl_2, 2.5h.

Selective deprotection of the *C*-terminus of **2** was achieved by saponification (LiOH, rt, 12 min) to yield **13** (scheme 4), which was used without further purification. A longer reaction time caused hydrolysis of the Pro-Arg peptide bond, probably *via* intramolecular nucleophilic attack by the free hydroxyl group (*vide supra*). Treatment of **3** with acid gave the corresponding *O,N*-deprotected dipeptide. Unfortunately, it was found that the conditions for oxidation of an α-hydroxy β-homoarginine moiety to the corresponding α-keto carboxylic acid derivative are incompatible with the presence of a free phenolic hydroxyl group.[15] Consequently, we faced the challenge to achieve selective *N*-Boc removal in the presence of an aryl *t*-butylether. Treatment of **3** with conventional acids in different concentrations and different solvents was unsuccessful.[16] It was gratifying to observe that treatment of **3** with trimethylsilyl trifluoromethane-sulphonate/2,6-lutidine effected selective removal of the *N*-Boc group to yield **14** in quantitative yield.[17]

Scheme 4



a) LiOH, THF/CH_3OH/H_2O, 12 min; b) TMS-triflate/2.6-lutidine, CH_2Cl_2, 0 °C→rt, 3h; c) TBTU, CH_2Cl_2, 3h; d) $Pd(PPh_3)_4$ /morpholine, THF, 45 min; e)TBTU/HOBt/DMAP, 0.5 mM CH_2Cl_2, 18h; f) Dess-Martin periodane/*t*-BuOH, CH_2Cl_2, rt, 24h, g) TFA/thioanisole, 80 min.

Coupling (TBTU) of tripeptide **13** with dipeptide **14** yielded smoothly (73%) the fully protected pentapeptide **15**. Treatment of **15** with 5 mol% of $Pd(PPh_3)_4$, in the presence of a 50-fold excess of morpholine, caused simultaneous removal of the *C*-terminal allyl group and the *N*-terminal allyloxycarbonyl group, to give (73%), after chromatographic purification [silica; CH_2Cl_2/CH_3OH/*N*-ethylmorpholine (NEM), 85/15/10], the NEM-salt of the *C,N*-terminal deprotected pentapeptide. This compound (0.5 mM solution in CH_2Cl_2) was cyclized by treatment with TBTU/HOBt/DMAP to give (61%) the protected cyclopentapeptide **16**. Oxidation of the α-hydroxy group with Dess-Martin periodane (room temperature, 24 h) in the presence of *t*-butylalcohol, followed by *O,N*-deprotection with TFA/thioanisole, and subsequent HPLC-purification led to Cyclotheonamide B (**1b**) in 51% yield.[18]

The convergent synthesis outlined here and the use of conventional benzyl-, *t*-butyl- and allyl-based protecting groups in combination with the straightforward synthesis of the key fragment, *i.e.* N^{ω}, $N^{\omega'}$-bis(Boc) N^{β}-Z α-hydroxy β-homoarginine (**6**), provide an efficient route for the preparation of Cyclotheonamide analogues. In combination with molecular modeling studies, this synthetic strategy will enable us to explore a new class of low molecular-weight thrombin inhibitors based on Cyclotheonamide B, a natural transition-state analogue, as lead compound.[19]

REFERENCES AND NOTES

1. Fusetani, N. and Matsunaga, S. *J. Am. Chem. Soc.* **1990**, *112*, 7053-7054.
2. Hagihara, M. and Schreiber, S.L. *J. Am. Chem. Soc.* **1992**, *114*, 6570-6571.
3. a) Maryanoff, B.E.; Qiu, X.; Padmanabhan, K.P.; Tulinsky, A.; Almond Jr., H.R.; Andrade-Gordon, P.; Greco, M.N.; Kaufmann, J.A.; Nicolaou, K.C.; Liu, A.; Brungs, P.H. and Fusetani, N. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8048-8052. b) Maryanoff, B.E.; Greco, M.N.; Zhang H.-C.; Andrade-Gordon, P.; Kauffman J.A.; Nicolaou, K.C.; Liu, A.; Brungs, P.H. *J. Am. Chem. Soc.* **1995**, *117*, 1225-1239.
4. a) Wipf, P. and Kim, H-Y. *J. Org. Chem.* **1993**, *58*, 5592-5594. b) Wipf, P. and Kim, H-Y. *Tetrahedron. Lett.* **1992**, *33*, 4275-4278.
5. Deng, J; Hamada, Y.; Shioiri; Matsunaga S. and Fusetani N. *Angew. Chem.* **1994**, *106*, 1811-1813.
6. Roth, P. and Metternich, R. *Tetrahedron. Lett.* **1992**, *33*, 3993-3996.
7. Lewis, S.D.; Ng, A.S.; Baldwin, J.J.; Fusetani, N.; Naylor, A.M. and Shafer J.A. *Thrombosis Res.* **1993**, *70*, 173-190.
8. Lin, Z. and Johnson, E. *Protein and Peptide Lett.* **1994**, *1*, 9-14.
9. Lee, A.Y.; Hagihara, M.; Karmacharya, R.; Albers, M.W.; Schreiber, S.L. and Clardy, J. *J. Am. Chem. Soc.* **1993**, *115*, 12619-12620.
10. This [2+3] fragment-condensation and macrocyclization strategy has also been employed by Maryanoff *et al.* (ref. 3). Other synthetic studies towards the total synthesis of Cyclotheonamides A/B feature different fragment strategies (ref. 4a and 5) or a linear approach (ref. 2). Two syntheses of fragments have also been reported (ref. 4b and 6).
11. This principle of carboxylic acid homologation to α-hydroxy carboxylic acids has been described by Seebach, and has been applied to the synthesis of an α-hydroxy β-homoarginine derivative by Schreiber *et al.* (ref. 2, and references cited therein).
12. ^{1}H-NMR-analysis of the resulting cyclic carbamates (*anti* and *syn* oxazolidinones) allowed assigment of the *S,R* configuration to the major α-hydroxy β-homoarginine isomer.
13. Egbertson, M.S.; Homnick, C.F. and Hartman, G.D. *Synth. Commun.* **1993**, *23*, 703-709.
14. An elaborate discussion on the formation of *E/Z*-isomers in this reaction yielding analogues of **12** is found in ref. 6. Under the conditions eployed by us (triethylphosphonacetate /NaH, THF, -50 →10 °C, 2.5h) the Z-alkene was not found.
15. See also: Wipf, P. and Kim, Y. *Tetrahedron. Lett.* **1992**, *33*, 5477-5480.
16. This lack of selectivity upon treatment with acids was recently also demonstrated by Gibson, F.S.; Bergmeier, S.C. and Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216-3218.
17. The scope and limitations of this *N*-Boc vs aryl *t*-butyl ether/ester discrimination upon treatment with TMS-triflate/2,6-lutidine are subject of a forthcoming report. See also: Ohfune Y. and Sakaitani M. *J. Org. Chem.* **1990**, *55*, 870-876.
18. Cyclotheonamide B was purified by RP-HPLC (column: Supelco LC-18 DB 250 x 25 mm; mobile phase CH_3CN/H_2O /phosphate buffer pH = 2.1; flowrate 20 mL/min; detection by UV at 210 nm; the product was desalted on the same column with CH_3CN/H_2O/0.1 N HCl as mobile phase). After freeze-drying, Cyclotheonamide B.HCl was obtained as a colorless amorphous solid, $[\alpha]_D^{23}$ = -13.7 (c = 0.2, CH_3OH); its ^{1}H and ^{13}C NMR spectra and FAB-MS were in complete accordance with data of the natural product (a sample of natural Cyclotheonamide A was kindly provided by professor Fusetani).
19. The preparation of analogues, and the evaluation of their biological activity is now in progress. Experimental details will be reported in a forthcoming full account.

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