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TOTAL SYNTHESIS OF (-)-HEMIASTERLIN, A STRUCTURALLY NOVEL TRIPEPTIDE THAT EXHIBITS POTENT CYTOTOXIC ACTIVITY

Raymond J. Andersen* and John E. Coleman

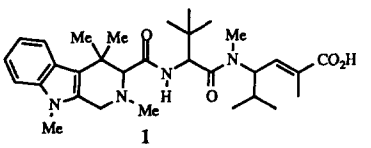
Departments of Chemistry and Oceanography–Earth & Ocean Sciences, University of British Columbia Vancouver, British Columbia, Canada V6T 1Z1

Edward Piers* and Debra J. Wallace

Department of Chemistry, University of British Columbia Vancouver, British Columbia, Canada V6T 1Z1

Abstract: *The total synthesis of (-)-hemiasterlin (2), a structurally novel, naturally occurring tripeptide that exhibits potent cytotoxic and antimitotic activity against human breast cancer MCF7 cells, is described.* Copyright © 1996 Elsevier Science Ltd

Milnamide A (**1**),[1] hemiasterlin (**2**),[2-4] hemiasterlin A (**3**),[3] and criamide A (**4**)[3] are members of a small family of tri- and tetrapeptides containing two highly modified amino acids. These naturally occurring substances, which have been isolated from marine sponges,[1-4] show potent *in vitro* cytotoxicity against murine leukemia P388 (**2**: IC_{50} 4.57 × 10-5 μg/mL) and human breast, ovarian, colon, and lung cancer cell lines.[3] The hemiasterlins **2** and **3** are antimitotic agents that inhibit tubulin polymerization by binding to the Vinca alkaloid site.[5,6] Against human breast cancer MCF7 cells, compounds **2** and **3** are more potent cytotoxins and mitotic blockers than vincristine, taxol, and nocodazole.[5] In order to provide the quantities of hemiasterlins, criamides, and structural analogs required for complete evaluation[7] of their potential as anticancer drugs, we have developed a general synthetic route to this family of peptides. We describe herein the application of this methodology to the total synthesis of the natural product (-)-hemiasterlin (**2**).



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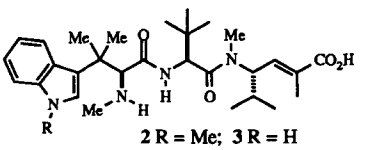
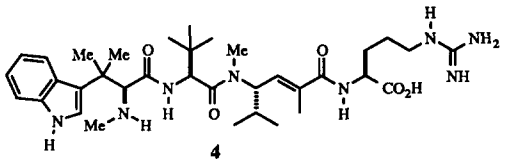


2 R = Me; **3** R = H



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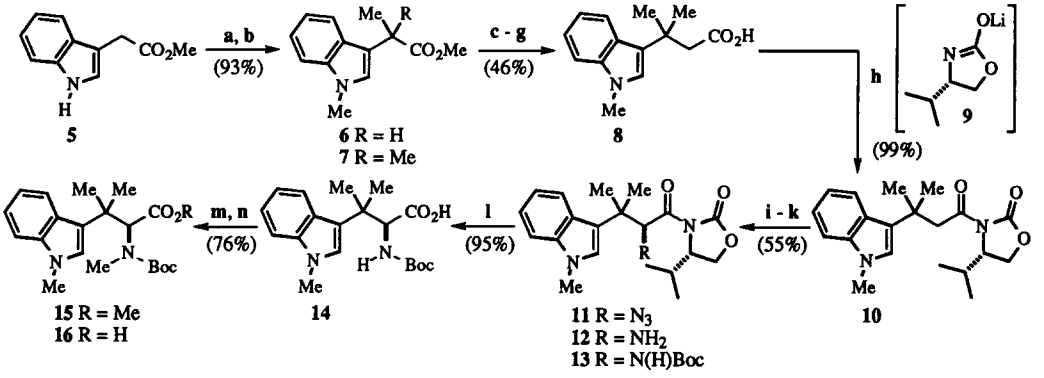
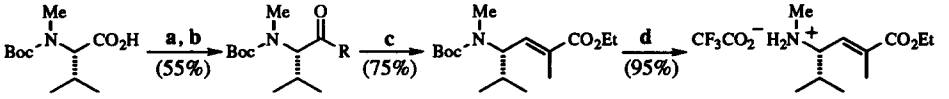
The preparation of the structurally modified tryptophan **16** is outlined in **Scheme 1**. Bis-methylation of the ester **5** (readily derived by treatment of indole-3-acetic acid with CH_2N_2 in Et_2O) provided **6**,[8] which, upon subjection to a second alkylation reaction, gave the required trimethyl derivative **7**. Homologation of **7** was accomplished via a straightforward sequence of reactions. Reduction of the ester function of **7**, followed by oxidation of the resultant primary alcohol with tetrapropylammonium perruthenate (TPAP) in the presence of *N*-methylmorpholine oxide (NMO),[9] gave the corresponding aldehyde. Wittig olefination of this carbonyl compound with methoxymethylenetriphenylphosphorane,[10] acid hydrolysis of the derived alkenyl ether, and oxidation of the resultant aldehyde with sodium chlorite[11] produced the carboxylic acid **8**. Reaction of this acid with 2,2-dimethylpropanoyl chloride in the presence of Et_3N gave the corresponding mixed anhydride, which, upon treatment with the lithiated oxazolidone **9**,[12] afforded **10**. Sequential treatment[13] of **10** with base and 2,4,6-triisopropylbenzenesulfonyl azide in THF produced the azide **11** (70% yield, diastereoselectivity >98%). Reduction[14] of **11**, followed by *in situ* treatment[14] of the resultant amine **12** with di-*tert*-butyl dicarbonate, afforded the *tert*-butoxycarbonyl (Boc) derivative **13** in 55% yield from **10**. Lithium hydroperoxide-promoted removal[15] of the chiral auxiliary from **13** gave the acid **14**, which was *bis*-methylated to give **15**. Base hydrolysis of the ester function in **15** provided the functionalized tryptophan **16**.



Scheme 1. **a**: $KN(SiMe_3)_2$ (3 equiv), THF, -78 to 0°C, 3 h; MeI, -78 to 0°C, 2 h. **b**: $KN(SiMe_3)_2$ (1.5 equiv), then as in **a**. **c**: *i*-Bu_2AlH, Et_2O. **d**: TPAP, NMO, CH_2Cl_2, 4Å molecular sieves. **e**: Ph_3P=CHOMe, THF, r.t. **f**: *p*-TsOH, H_2O, dioxane, 60°C, 16 h. **g**: $NaClO_2$, NaH_2PO_4, 2-methylbut-2-ene, *t*-BuOH, H_2O, 0°C. **h**: Me_3CCOCl, Et_3N, THF, -78°C; **9**, THF, -78°C. **i**: $KN(SiMe_3)_2$ (1.1 equiv), THF, -78°C; 2,4,6-triisopropylbenzenesulfonyl azide, THF, -78°C, 1 min; HOAc, 30-40°C, 1 h. **j**: $SnCl_2$, dioxane, H_2O, r.t., 36 h. **k**: $(Me_3CO_2C)_2O$, dioxane, H_2O, r.t., 16 h. **l**: LiOOH, THF, H_2O, r.t., 16 h; citric acid, H_2O. **m**: NaH (~6 equiv), DMF; MeI, r.t., 16 h. **n**: LiOH, MeOH, HO, 60°C, 24 h; citric acid, H_2O.

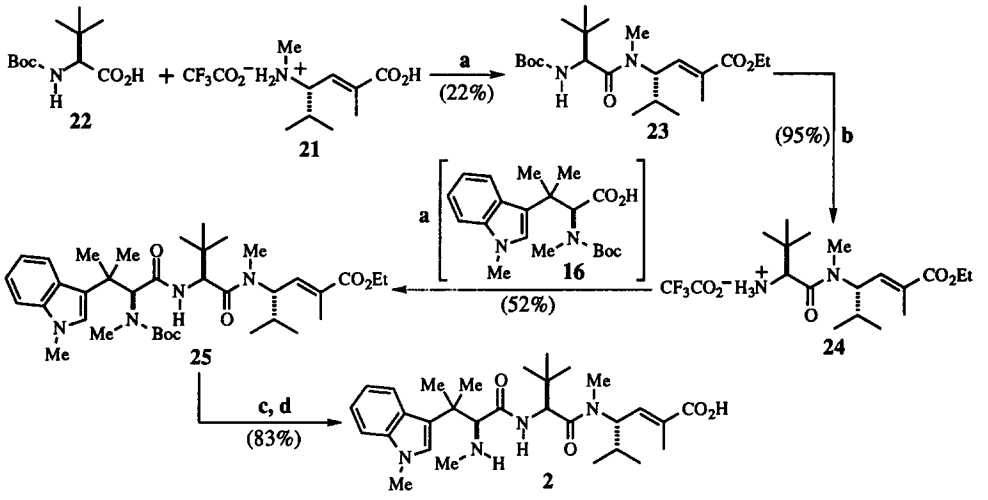
The structurally novel amino acid salt **21** was prepared as summarized in **Scheme 2**. Conversion of commercially available (*S*)-*N*-Boc-*N*-methylvaline (**17**) into the corresponding amide derivative **18**, followed by reduction[16,17] of the latter substance with $LiAlH_4$, gave the aldehyde **19**. Reaction of **19** with [(1-ethoxycarbonyl)ethylidene]triphenylphosporane in CH_2Cl_2 afforded, stereoselectively, the *E*-2-alkenoate **20**.

Trifluoroacetic acid mediated cleavage of the *N*-Boc group led to the isolation of the required ammonium trifluoroacetate derivative **21** in excellent yield.



Scheme 2. **a**: $[H_2N(OMe)Me]Cl$, DCC, *i*-Pr_2NEt, MeCN, 0°C to r.t., 1 h. **b**: $LiAlH_4$, THF, -78°C, 30 min; $Na_2SO_4 \cdot 10H_2O$. **c**: $Ph_3P{=}C(Me)CO_2Et$, CH_2Cl_2. **d**: 1:1 CF_3CO_2H-CH_2Cl_2, r.t., 30 min.

The synthesis of (-)-hemiasterlin (**2**) from the amino acid derivatives **22**, **21** and **16** is outlined in **Scheme 3**. PyBroP-mediated coupling[18] of (*S*)-*N*-Boc-*tert*-leucine (**22**) with the amino acid salt **21** in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) provided, albeit in mediocre yield,[19] the functionalized dipeptide **23**, which was smoothly converted into trifluoroacetate salt **24**. Coupling of the latter material with the carboxylic acid **16**, again mediated by PyBroP[18] in the presence of DMAP, afforded **25**. Base hydrolysis of the ethyl ester function of **25**, followed by acid-promoted removal of the *N*-Boc group and purification of the resultant crude product by reversed phase HPLC, provided (-)-hemiasterlin (**2**) as an amorphous white solid that produced spectroscopic data (^1H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6), HRFABMS) identical with those derived from natural (-)-**2**.[3]



Scheme 3. **a**: PyBroP (1.1 equiv), 4-(*N*,*N*-dimethylamino)pyridine (3 equiv.), CH_2Cl_2, r.t., 16 h. **b**: 1:1 CF_3CO_2H-CH_2Cl_2, r.t., 30 min. **c**: LiOH, MeOH, HO, r.t., 16 h; citric acid, H_2O. **d**: as in **b**, then reversed phase (C_{18}) HPLC, 45% H_2O (containing 0.05% CF_3CO_2H), 55% MeOH.

The *in vitro* cytotoxic and antimitotic IC_{50}s against human breast cancer MCF7 cells for synthetic (-)-hemiasterlin (**2**) were found to be identical with the IC_{50}s for the natural product isolated from the sponge

Cymbastela sp.[3] The total syntheses of other members of this family of peptides as well as the preparation of structural analogs of these novel substances are underway. A thorough assessment of the potential of these compounds as anticancer agents will be carried out. The results of these studies will be reported in due course.

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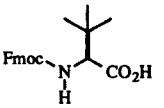

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