

IMPROVED TOTAL SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF ARENASTATIN A, A POTENT CYTOTOXIC SPONGEAN DEPSIPEPTIDE

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An efficient asymmetric synthesis of a cyclic depsipeptide arenastatin A (**1**) is described. **1**, isolated from the marine sponge *Dysidea arenaria*, exhibited extremely potent cytotoxicity with IC₅₀ of 5 pg/ml for KB cells, and in this context the structure-activity relationship among several stereoisomers of **1** and allied compounds has also been examined.

KEY WORDS marine sponge; *Dysidea arenaria*; depsipeptide; arenastatin A; cytotoxicity

Recently, we have reported an asymmetric total synthesis²⁾ of an extremely cytotoxic depsipeptide designated arenastatin A (**1**), which we isolated from the Okinawan marine sponge *Dysidea arenaria* through bioassay-guided separation.³⁾ In order to define the structural requirement for exhibiting such potent cytotoxicity of arenastatin A (**1**), a larger amount of **1** was required and thus an efficient synthetic route has been explored. In this paper, we describe improved asymmetric total synthesis of **1** which has provided enough material for studies of the structure-activity relationship as summarized below.

Intramolecular cyclization by the Wittig-Horner reaction (method **a**, Chart 1) that we employed at the final depsipeptide ring construction in the previous total synthesis,²⁾ proceeded in 40% yield. Further investigations on the final cyclization reaction for building up **2** (a desepoxy derivative of **1**) led us to find that the macrolactamization (method **b**, Chart 1) proceeds in favorable yield. Strategic disconnections and retrosynthetic analysis of arenastatin A (**1**) are depicted in Chart 1 (segments A (**4**) to D (**7**)). Since arenastatin A (**1**), with an epoxy moiety adjacent to a phenyl group as well as a cyclic diester structure, is fairly unstable under both acidic and alkaline conditions,^{2,3)} the epoxy function is introduced at the final stage.

Segment A (**4**) was synthesized starting from *trans*-styrylacetic acid by taking advantage of Evans asymmetric aldol reactions as shown in Chart 2.⁴⁾ Oxazolidyl carboximide **8**, prepared from *trans*-

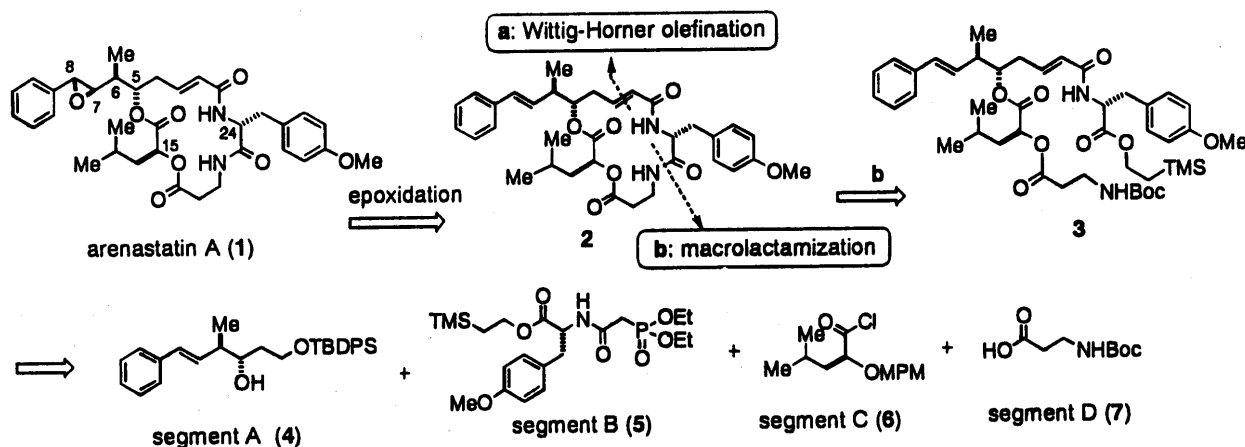
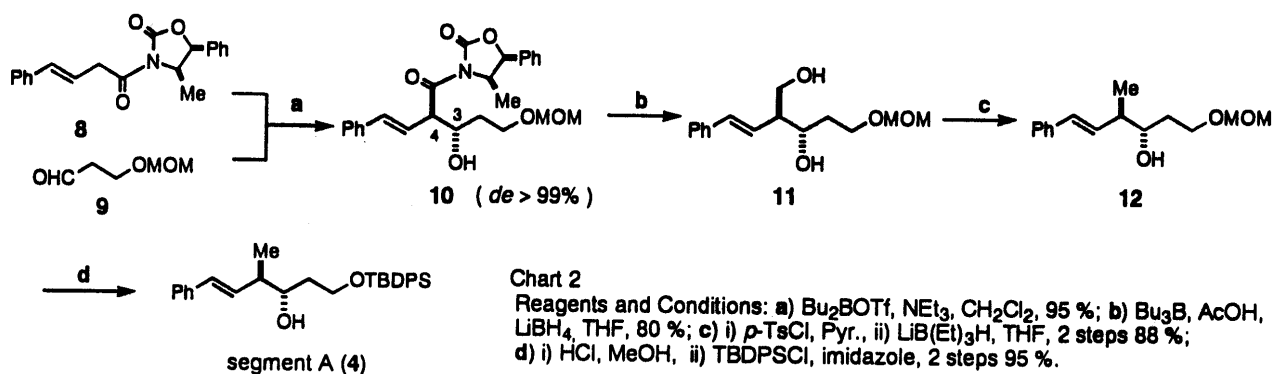


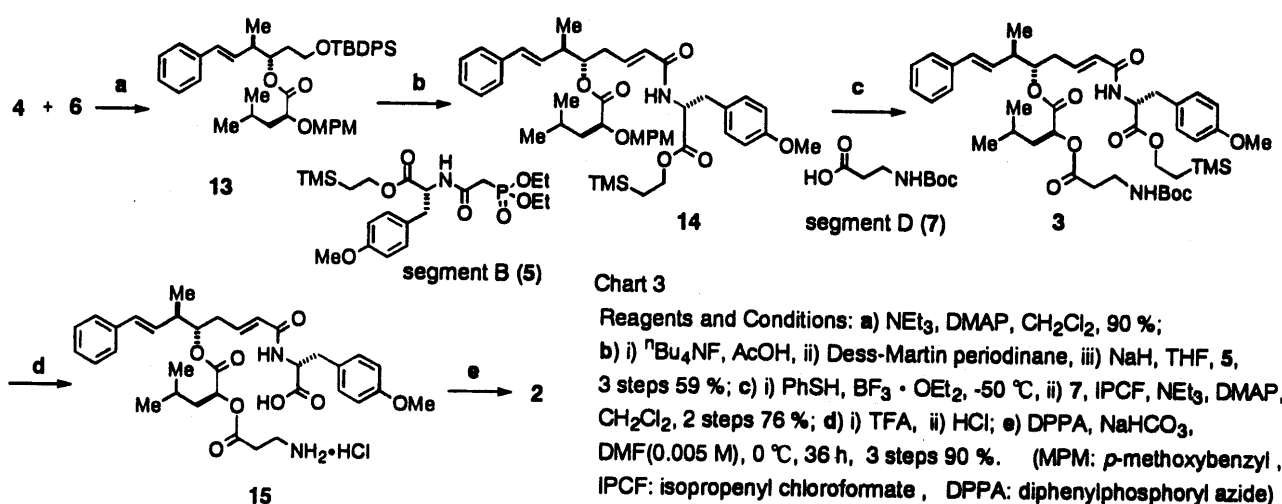
Chart 1

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styrylacetic acid and (4*R*,5*S*)-oxazolidinone, was treated with an aldehyde **9** to afford a 3*S*-hydroxy-4*R* adduct **10**⁵⁾ stereoselectively in 95% yield (*de* >99%). Reductive removal of the asymmetric auxiliary group in **10** using LiBH_4 gave a diol **11**, which was then converted to a 3*S*-hydroxy-4*R*-methyl derivative **12**⁶⁾ in 71% yield from **10**. Exchange of the protective group of **12** furnished segment A (**4**) in 64% overall yield from **8**. Segment A (**4**) thus prepared was proved identical with the authentic sample synthesized previously²⁾ by direct comparison.

Next, connection of three segments A (**4**), B (**5**), and C (**6**) was carried out as summarized in Chart 3. Segment A (**4**) was first coupled with segment C (**6**), synthesized from L-leucine,²⁾ in the presence of NEt_3 and DMAP to furnish **13** in 90% yield. Removal of the TBDPS group in **13** and subsequent Dess-Martin oxidation furnished an aldehyde, which was then coupled with segment B (**5**) by the Wittig-Horner reaction to give **14**⁷⁾ in 59% yield. Removal of the MPM group in **14** using PhSH and $\text{BF}_3 \cdot \text{OEt}_2$ and subsequent coupling reaction with segment D (**7**) using IPCF⁸⁾ afforded a triester **3**⁹⁾ in 76% yield. Removal of the 2-(trimethylsilyl)ethyl group as well as *tert*-butoxy carbonyl (Boc) group in **3** provided **15**, which was then subjected to intramolecular macrolactamization using DPPA¹⁰⁾ to give a cyclic depsipeptide **2** in 90% yield. By dimethyldioxirane oxidation, compound **2** was already converted to arenastatin A (**1**) and its 7,8-epoxy isomer **16** in 2.2:1 ratio, totally in 80 % yield.²⁾



Then, in order to study the structure-cytotoxicity relationship concerning arenastatin A (**1**), we further synthesized the following four stereoisomers **17-20** in an analogous manner.¹¹⁻¹⁵⁾ Among these

synthesized allied compounds, only **1** showed extremely potent cytotoxicity (IC_{50} 5 $\mu\text{g/ml}$ for KB cells) while the others such as **2** and **16-20** did not show any potent cytotoxicity at concentrations below 0.1 $\mu\text{g/ml}$ (Chart 4). The detailed action mechanism of arenastatin A (**1**) is currently under investigation.

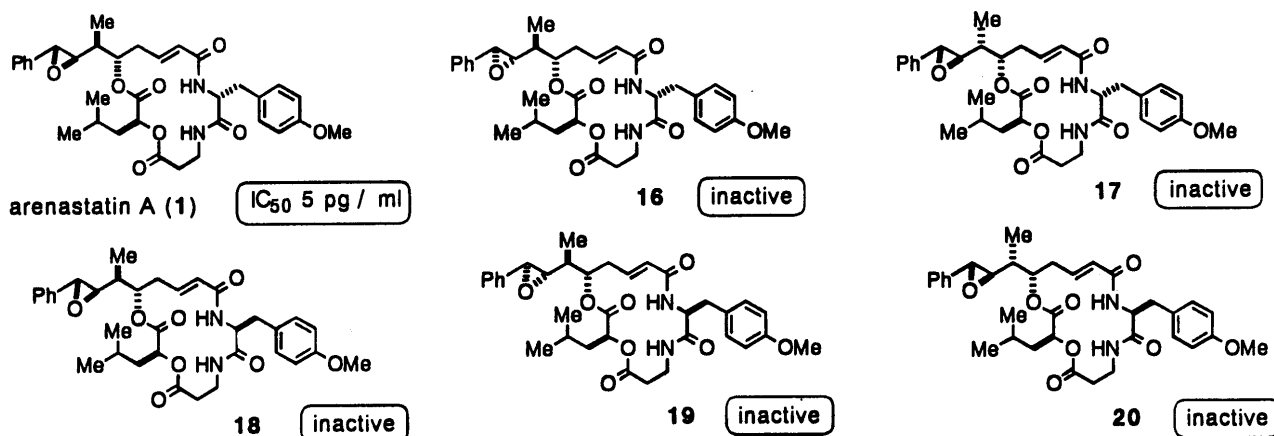


Chart 4. The Structures and Cytotoxicities of Arenastatin A (**1**) and its Diastereoisomers **16-20**

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- 5) **10**: $[\alpha]_D +51^\circ$ ($c=0.19$, CHCl_3), IR (KBr) cm^{-1} : 3518, 1728, 1693, 1195, 1120. $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ : 7.4-7.2 (10H), 6.74 (1H, d, $J=16$ Hz, H-6), 6.39 (1H, dd, $J=9, 16$, H-5), 5.69 (1H, d, $J=7.5$, H-5'), 4.82 (1H, dq, $J=7.5, 7$, H-4'), 4.72 (1H, dd, $J=9, 4$ Hz, H-4), 4.64, 3.37 (MOM), 4.33 (1H, m, H-3), 3.74 (2H, m, H-1), 0.87 (3H, d, $J=7$ Hz, 4'-CH₃). FAB-MS: m/z 440 ($\text{M}+\text{H}^+$) ($\text{C}_{25}\text{H}_{30}\text{O}_6\text{N}$ by HR FAB-MS).
- 6) **12**: $[\alpha]_D +39^\circ$ ($c=0.11$, CHCl_3), IR (KBr) cm^{-1} : 3477, 1149, 1109. $^1\text{H-NMR}$ (CDCl_3) δ : 7.4-7.2 (5H), 6.45 (1H, d, $J=16$ Hz, H-6), 6.21 (1H, dd, $J=8, 16$, H-5), 4.64, 3.37 (MOM), 3.75 (3H, m, H-1,3), 1.16 (3H, d, $J=7$, 4-CH₃). FAB-MS: m/z 273 ($\text{M}+\text{Na}^+$) ($\text{C}_{15}\text{H}_{22}\text{O}_3\text{Na}$ by HR FAB-MS).
- 7) **14**: $[\alpha]_D -20^\circ$ ($c=0.3$, CHCl_3), IR (KBr) cm^{-1} : 3290, 1739, 1180. $^1\text{H-NMR}$ (CDCl_3) δ : 7.4-6.70 (14H), 6.40 (1H, d, $J=16$ Hz, H-8), 6.06 (1H, dd, $J=16, 9$, H-7), 5.86 (1H, d, $J=16$, H-2), 5.09 (1H, m, H-5), 4.83 (1H, m, α -H of Me-Tyr), 4.57-4.23 (both 1H, $J=11$, MPM), 4.16, 0.95 (both 2H, m, TMS-ET), 3.90 (1H, dd, $J=3.5, 9.5$, α -H of Leu), 3.78, 3.76 (both 3H, s), 1.12 (3H, d, $J=7$, H-13), 0.78, 0.74 (both 3H, d, $J=6.5$). FAB-MS: m/z 758 ($\text{M}+\text{H}^+$) ($\text{C}_{44}\text{H}_{60}\text{O}_8\text{NSi}$ by HR FAB-MS).
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- 9) **3**: $[\alpha]_D -13.4^\circ$ ($c=0.63$, CHCl_3), IR (KBr) cm^{-1} : 3325, 1736, 1678, 1641, 1176. $^1\text{H-NMR}$ (CDCl_3) δ : 7.4-6.7 (10H), 6.40 (1H, d, $J=16$ Hz, H-8), 6.01 (1H, dd, $J=16, 7.5$, H-7), 5.87 (1H, d, $J=15.5$, H-2), 5.0-4.8 (3H, m, H-5, 15, 24), 4.18, 0.95 (both 2H, m, TMS-ET), 3.37 (3H, s), 3.38 (2H, m, H-22), 1.42 (9H, s, Boc), 1.11 (3H, d, $J=7$, H-13), 0.85, 0.81 (both 3H, d, $J=6.5$). FAB-MS: m/z 809 ($\text{M}+\text{H}^+$) ($\text{C}_{44}\text{H}_{65}\text{O}_{10}\text{N}_2\text{Si}$ by HR FAB-MS).
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- 11) The IR and $^1\text{H-NMR}$ data for these compounds were consistent with their structures and the details will be presented in our forthcoming paper.
- 12) **17**: $[\alpha]_D +31^\circ$ ($c=0.23$, MeOH). FAB-MS: m/z 607 ($\text{M}+\text{H}^+$) ($\text{C}_{34}\text{H}_{43}\text{O}_8\text{N}_2$ by HR FAB-MS).
- 13) **18**: $[\alpha]_D -124^\circ$ ($c=0.52$, CHCl_3). FAB-MS: m/z 607 ($\text{M}+\text{H}^+$) ($\text{C}_{34}\text{H}_{43}\text{O}_8\text{N}_2$ by HR FAB-MS).
- 14) **19**: $[\alpha]_D -116^\circ$ ($c=0.52$, CHCl_3). FAB-MS: m/z 607 ($\text{M}+\text{H}^+$) ($\text{C}_{34}\text{H}_{43}\text{O}_8\text{N}_2$ by HR FAB-MS).
- 15) **20**: $[\alpha]_D -111^\circ$ ($c=0.56$, CHCl_3). FAB-MS: m/z 607 ($\text{M}+\text{H}^+$) ($\text{C}_{34}\text{H}_{43}\text{O}_8\text{N}_2$ by HR FAB-MS).

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