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 IC_{50} 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 synthesis[2)] 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*-

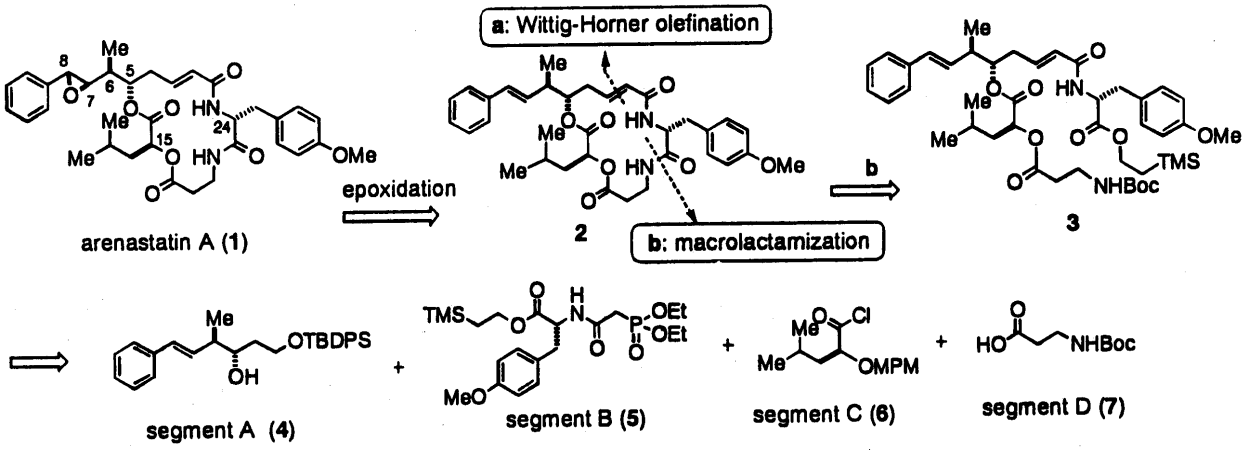

Chart 1

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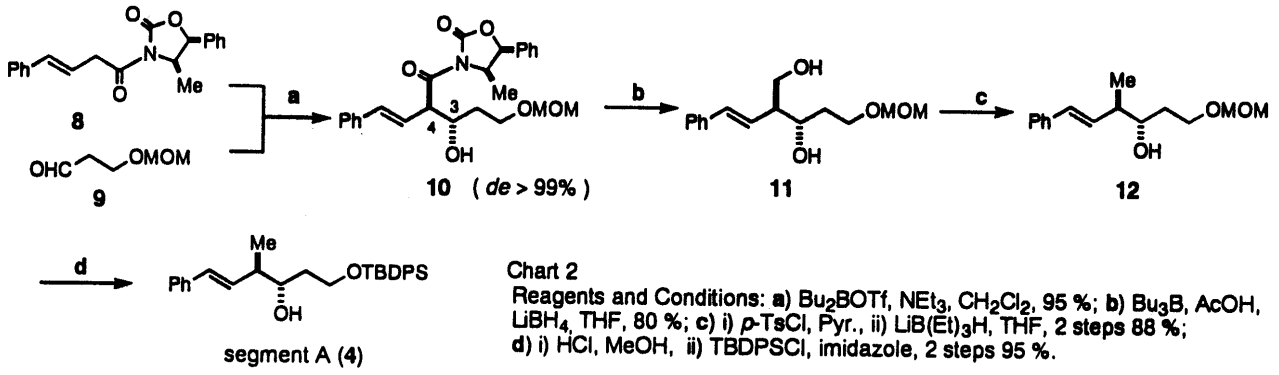
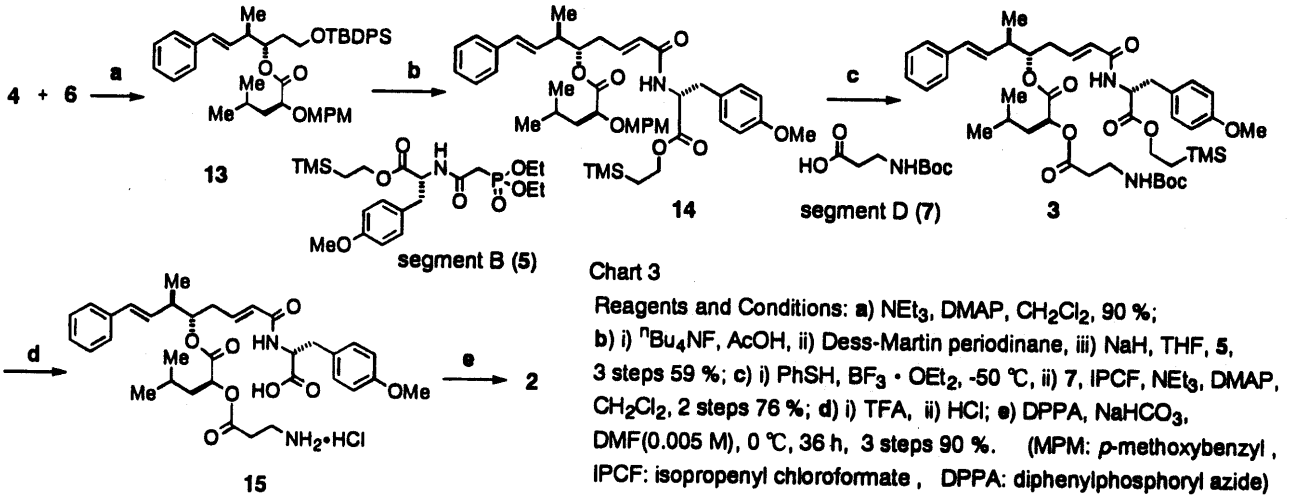


styrylacetic acid and (4*R*,5*S*)-oxazolidinone, was treated with an aldehyde **9** to afford a 3*S*-hydroxy-4*R* adduct **10**[5] stereoselectively in 95% yield (*de* >99%). Reductive removal of the asymmetric auxiliary group in **10** using $LiBH_4$ gave a diol **11**, which was then converted to a 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 previously[2] 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 $BF_3 \cdot OEt_2$ and subsequent coupling reaction with segment D (**7**) using IPCF[8] 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 DPPA[10] 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 pg/ml for KB cells) while the others such as **2** and **16-20** did not show any potent cytotoxicity at concentrations below 0.1 μg/ml (Chart 4). The detailed action mechanism of arenastatin A (**1**) is currently under investigation.

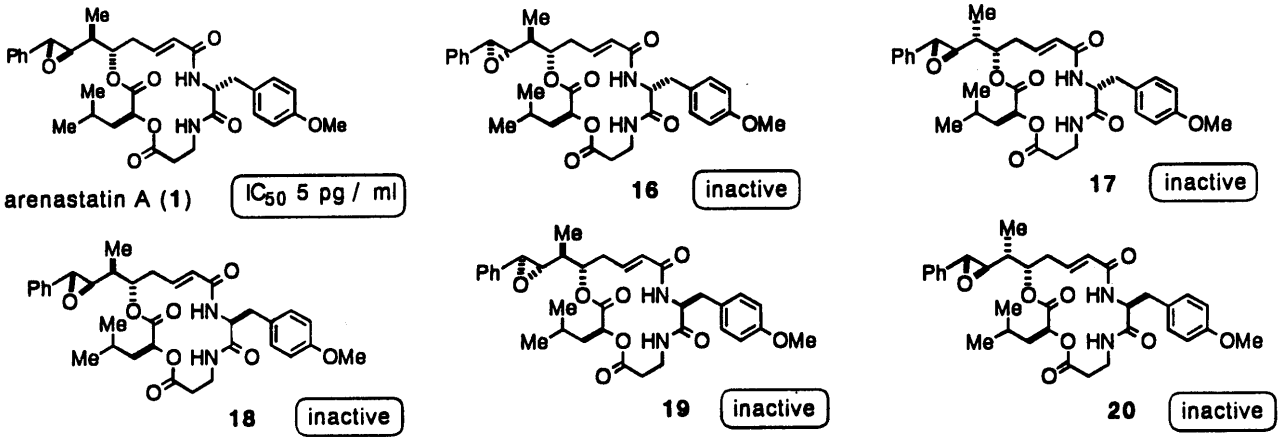


Chart 4. The Structures and Cytotoxicities of Arenastatin A (**1**) and its Diastereoisomers **16-20**

ACKNOWLEDGEMENT The authors are grateful to the Ministry of Education, Science, and Culture of Japan for financial support.

REFERENCES AND NOTES

1) Present address: *Faculty of Pharmaceutical Sciences, Kinki University, Kowakae 3-4-1, Higashiosaka, Osaka 577, Japan.*
2) Kobayashi M., Kurosu M., Wang W., Kitagawa I., *Chem. Pharm. Bull.*, **42**, 2394-2396 (1994).
3) a) Kobayashi M., Aoki S., Ohyabu N., Kurosu M., Wang W., Kitagawa I., *Tetrahedron Lett.*, **35**, 7969-7972 (1994); b) Kobayashi M., Kurosu M., Ohyabu N., Wang W., Fujii S., Kitagawa I., *Chem. Pharm. Bull.*, **42**, 2196-2198 (1994).
4) Evans D. A., Dow R. L., Shih T. L., Takacs J. M., Zahler R., *J. Am. Chem. Soc.*, **112**, 5290-5313 (1990).
5) **10**: $[\alpha]_D$ +51° (*c*=0.19, $CHCl_3$), IR (KBr) cm^{-1}: 3518,1728, 1693, 1195, 1120. ^{1}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'-CH_3). FAB-MS: *m/z* 440 $(M+H)^+$ ($C_{25}H_{30}O_6N$ by HR FAB-MS).
6) **12**: $[\alpha]_D$ +39°(*c*=0.11, $CHCl_3$), IR (KBr) cm^{-1}: 3477, 1149, 1109. ^{1}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-CH_3). FAB-MS: *m/z* 273 $(M+Na)^+$ ($C_{15}H_{22}O_3Na$ by HR FAB-MS).
7) **14**: $[\alpha]_D$ -20° (*c*=0.3, $CHCl_3$), IR (KBr) cm^{-1}: 3290, 1739, 1180. ^{1}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 $(M+H)^+$ ($C_{44}H_{60}O_8NSi$ by HR FAB-MS).
8) Jouin P., Castro B., Zeggaf C., Pantaloni A., Senet J. P., Lecolier S., Sennyey G., *Tetrahedron Lett.*, **28**, 1661-1664 (1987).
9) **3**: $[\alpha]_D$ -13.4° (*c*=0.63, $CHCl_3$), IR (KBr) cm^{-1}: 3325, 1736, 1678 , 1641, 1176. ^{1}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 $(M+H)^+$ ($C_{44}H_{65}O_{10}N_2Si$ by HR FAB-MS).
10) Brady S. F., Freidinger R. M., Paleveda W. J., Colton C. D., Homnick C. F., Whitter W. L., Curley P., Nutt R. F., Veber D. F., *J. Org. Chem.*, **52**, 764-769 (1987).
11) The IR and ^{1}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° (*c*=0.23, MeOH). FAB-MS: *m/z* 607 $(M+H)^+$ ($C_{34}H_{43}O_8N_2$ by HR FAB-MS).
13) **18**: $[\alpha]_D$ -124° (*c*=0.52, $CHCl_3$). FAB-MS: *m/z* 607 $(M+H)^+$ ($C_{34}H_{43}O_8N_2$ by HR FAB-MS).
14) **19**: $[\alpha]_D$ -116° (c=0.52, $CHCl_3$). FAB-MS: *m/z* 607 $(M+H)^+$ ($C_{34}H_{43}O_8N_2$ by HR FAB-MS).
15) **20**: $[\alpha]_D$ -111°(*c*=0.56, $CHCl_3$). FAB-MS: *m/z* 607 $(M+H)^+$ ($C_{34}H_{43}O_8N_2$ by HR FAB-MS).

(Received June 13, 1995; accepted August 7, 1995)