A TOTAL SYNTHESIS OF ARENASTATIN A, AN EXTREMELY POTENT CYTOTOXIC DEPSIPEPTIDE, FROM THE OKINAWAN MARINE SPONGE *DYSIDEA ARENARIA*

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An asymmetric synthesis of a cyclic depsipeptide arenastatin A (**1**), which was isolated from the marine sponge *Dysidea arenaria* and exhibited extremely potent cytotoxicity with IC_{50} 5 pg/ml for KB cells, has been accomplished.

KEYWORDS marine sponge; *Dysidea arenaria*; depsipeptide; arenastatin A; cytotoxic

During our continuing investigations searching for new bioactive substances in marine organisms,[1] we have been investigating the chemical constituents of the Okinawan marine sponge *Dysidea arenaria* and have recently isolated an extremely potent cytotoxic (IC_{50} 5 pg/ml for KB cells) depsipeptide designated arenastatin A (**1**) *via* bioassay-guided separation. The plane structure of arenastatin A (**1**) was first elucidated on the basis of 2D-NMR analysis.[2] Then the absolute stereostructure of **1** was determined partially by HPLC identification of the fragmental D-amino acid and 2(*S*)-hydroxy acid derivatives[2] and finally by a synthesis of a tetrahydrofuranoid **2**, which was obtained by methanolysis of **1** with K_2CO_3-MeOH and subsequent imidazole treatment.[3] Due to scarcity of the natural supply of **1** for further biological evaluation, our effort has been directed toward the total synthesis. In this paper, we report an asymmetric total synthesis of arenastatin A (**1**).

Chart 1 depicts strategic bond disconnections and retrosynthetic analysis of arenastatin A (**1**). Arenastatin A (**1**), having an epoxy moiety adjacent to a phenyl group and a cyclic diester structure, is fairly unstable under both acidic and alkaline conditions. Thus, the construction of the cyclic skeleton has been designed by an intramolecular Wittig-Horner reaction of **4**, and the epoxy function has been introduced in the final stage.

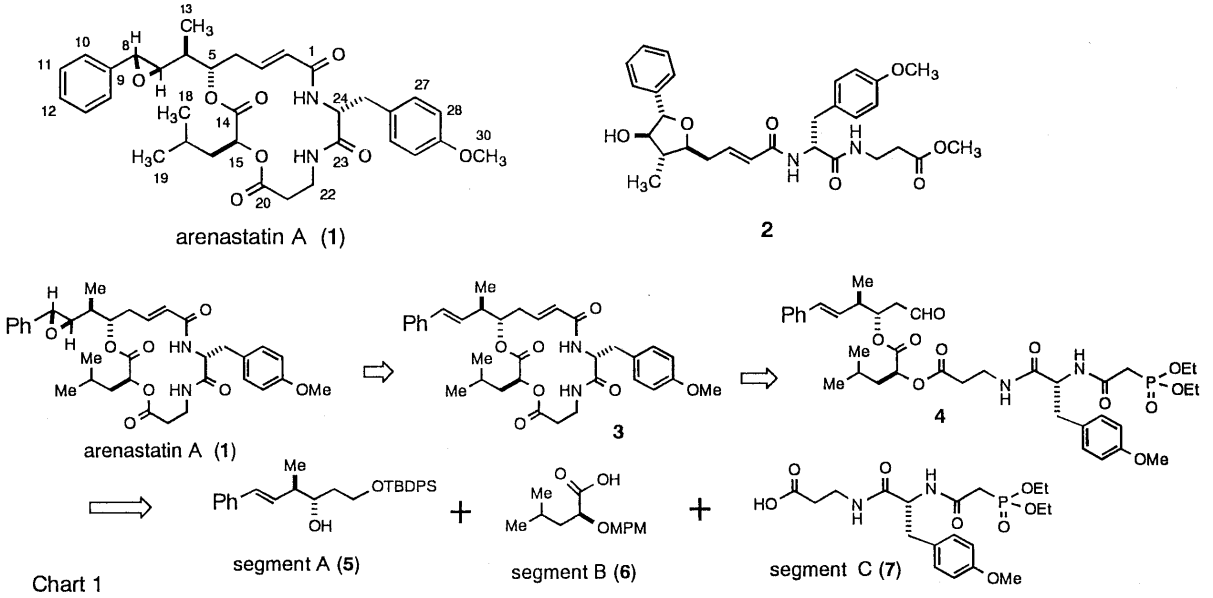
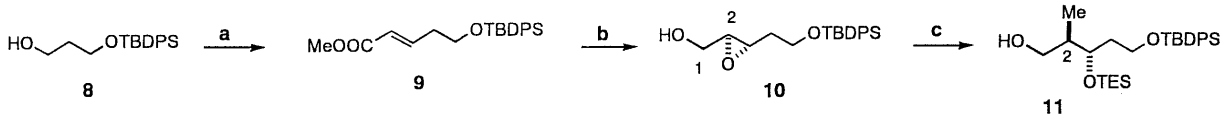
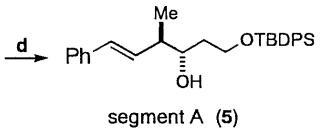


Chart 1

Segment A (**5**) has been synthesized starting from 1-*O*-TBDPS-1,3-pentanediol **8** as shown in Chart 2. Dess-Martin oxidation of **8** followed by Wittig-Horner reaction gave an α,β-unsaturated ester **9**, which, upon DIBAL reduction and subsequent Sharpless asymmetric epoxidation[4] using L-diethyl tartrate, was converted to a 2*S*,3*S*-epoxide **10** in 90% yield with 96%ee. Epoxide **10** was treated with Me_2CuLi and then subjected to $NaIO_4$ oxidation to furnish a 1,3*S*-

diol, and selective protection of the 3-hydroxyl moiety was effected *via* three steps to give **11** in 65% yield from **10**. Dess-Martin oxidation of **11** provided an aldehyde while preserving the C-2 methyl configuration, and the aldehyde was further subjected to Wittig reaction using a semistabilized ylide in benzene[5] and subsequently to selective deprotection of the TES group to give segment A (**5**)[6] in 63% yield from **11**.





segment A (**5**)

Chart 2

Reagents and Conditions: **a**) i) Dess-Martin periodinane, CH_2Cl_2, ii) $(MeO)_2P(O)CH_2COOMe$, NaH, THF, 2 steps 75%; **b**) i) DIBAL, ii) L-DET, $Ti(O^iPr)_4$, TBHP, 2 steps 90%; **c**) i) Me_2CuLi, ether, ii) $NaIO_4$, ether-water, iii) PivCl, pyridine, iv) TESCl, pyridine, v) DIBAL, CH_2Cl_2, 5 steps 65%; **d**) i) Dess-Martin periodinane, CH_2Cl_2, ii) Ph_3PCH_2PhCl, ^{n}BuLi, benzene, ii) HF•pyridine, THF-CH_2Cl_2, 3 steps 63%.
(TBDPS: ^{t}butyldiphenylsilyl TES: triethylsilyl)

Segment B (**6**) has been prepared from L-leucine as shown in Chart 3. Thus, 2(*S*)-hydroxy-4-methylpentanoic acid (**12**), obtained by sodium nitrite and aqueous sulfuric acid treatment of L-leucine, was first esterified with diazomethane, and then the hydroxyl moiety was protected with a *p*-methoxybenzyl (MPM) group to afford **13** in 65% yield with 100%ee. Treatment of **13** with a diluted aqueous KOH-dioxane solution furnished segment B (**6**) without racemization at C-2.

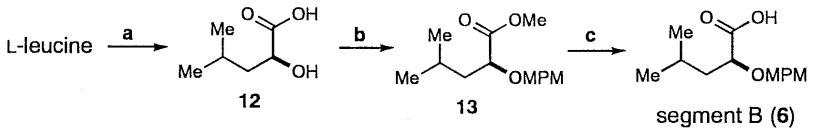
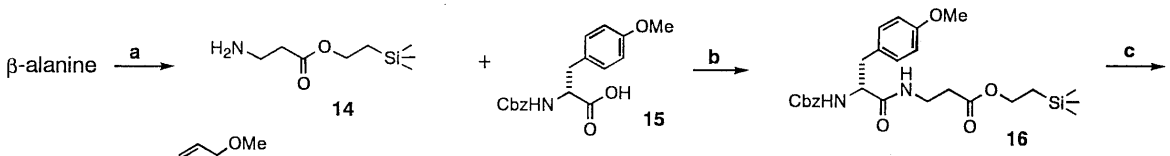
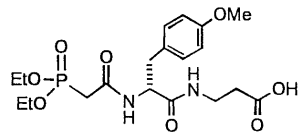


Chart 3

Reagents and Conditions: **a**) 1*N* aq.H_2SO_4, $NaNO_2$, 70%; **b**) i) CH_2N_2, ether, ii) $MPMOC(NH)CCl_3$, TfOH, ether, 2 steps 65%; **c**) 1*N* aq.KOH, dioxane, 88%.

Next, segment C (**7**) has been synthesized starting from β-alanine through a reaction sequence as shown in Chart 4. A 2-(trimethylsilyl)ethyl ester **14**, prepared from β-alanine in three steps, was coupled with a D-*O*-methyltyrosine derivative **15** using DEPC[7] to afford **16** in 82% yield. Removal of the Cbz group of **16**, followed by phosphonoacetylation in the presence of WSCI[8] and subsequent deprotection of the 2-(trimethylsilyl)ethyl group, provided segment C (**7**)[9] in 90% yield.





segment C (**7**)

Chart 4

Reagents and Conditions: **a**) i) ZCl, NaOH, ii) 2-(trimethylsilyl)ethanol, DCC, DMAP, CH_2Cl_2, iii) H_2/ Pd-C, HCOOH, MeOH then aq.$NaHCO_3$, 3 steps 95%; **b**) DEPC, Et_3N, DMF, 82%; **C**) i) H_2/Pd-C, HCOOH, MeOH, then aq.$NaHCO_3$, ii) $(EtO)_2P(O)CH_2COOH$, WSCI•HCl, DMAP, THF, iii) BF_3•OEt_2, CH_3CN, 3 steps 90%.
(DEPC: diethyl phosphorocyanidate WSCI: water-soluble carbodiimide)

Then the connection of these segments A, B, and C was carried out as summarized in Chart 5. Segment A (**5**) was first coupled with an acid chloride **17**, which was prepared by $(COCl)_2$ treatment of segment B (**6**), in the presence of Et_3N and DMAP to furnish **18** in 90% yield. Removal of the MPM protecting group in **18** using PhSH and BF_3·OEt_2 and subsequent coupling with segment C (**7**) using IPCF[10] afforded diester **19** in 81% yield. Removal of the TBDPS group in **19** with *n*-Bu4NF-AcOH[11] and successive Dess-Martin oxidation yielded aldehyde **4**, which was then subjected to an intramolecular Wittig-Horner reaction[12] to give cyclic depsipeptide **3**[13] in 40% yield.

Finally, compound **3** was treated with dimethyldioxirane[14] to furnish arenastatin A (**1**) and its 7,8-epoxy-stereoisomer **20**[15] in 2.2:1 ratio totally in 80% yield.[16] The compound **1** thus synthesized has been found identical with the natural authentic sample by HPLC, IR, ^{1}H-NMR, $[\alpha]_D$, and CD comparisons. The detailed biological activities of arenastatin A (**1**) and allied compounds are under investigation.

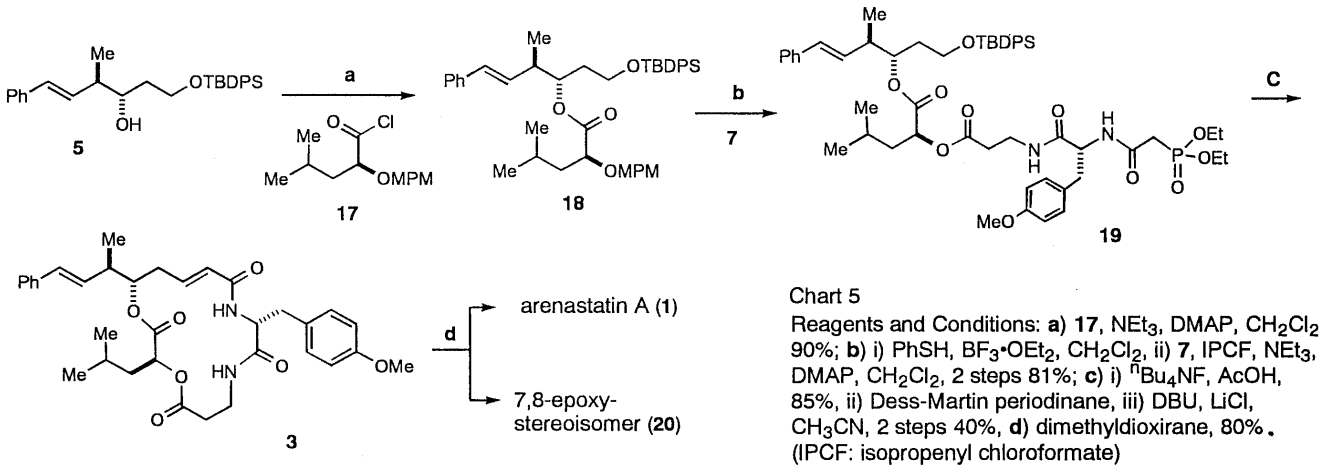


Chart 5

Reagents and Conditions: **a)** **17**, NEt_3, DMAP, CH_2Cl_2, 90%; **b)** i) PhSH, BF_3•OEt_2, CH_2Cl_2, ii) **7**, IPCF, NEt_3, DMAP, CH_2Cl_2, 2 steps 81%; **c)** i) $^nBu_4NF$, AcOH, 85%, ii) Dess-Martin periodinane, iii) DBU, LiCl, CH_3CN, 2 steps 40%, **d)** dimethyldioxirane, 80%. (IPCF: isopropenyl chloroformate)

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

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4) Y. Gao, R. M. Hanson, J. M. Klunder, S. Y. Ko, H. Masamune, K. B. Sharpless, *J. Am. Chem. Soc.*, **109**, 5765 (1987).

5) The *E*-selectivity of the double bond in **8** was 91% in benzene, while 81% in THF and 71% in DMF.

6) **5**: ^{1}H-NMR (270 MHz, $CDCl_3$)δ: 7.2-7.7 (15H), 6.42 (1H, d, *J*=15.8 Hz), 6.24 (1H, dd, *J*=15.8, 7.9), 3.9 (3H, m), 2.4 (1H, m), 1.7 (2H, m), 1.15 (3H, d, *J*=6.9), 1.06 (9H, s). MS: *m/z* 426 (M^+-H_2O) ($C_{29}H_{34}OSi$ by HR-MS).

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9) **7**: ^{1}H-NMR (270 MHz, $CDCl_3$)δ: 7.12 (2H, d, *J*=8.6 Hz), 6.80 (2H, d, *J*=8.6), 4.71 (1H, m), 4.05 (4H, m), 3.76 (3H, s), 3.42 (2H,m), 3.0 (2H, m), 2.88, 2.81 (both 1H, d, *J*=3.3), 2.45 (2H, m), 1.25 (6H, m). FAB-MS: *m/z* 467 $(M+Na)^+$ ($C_{19}H_{29}N_2O_8PNa$ by HR FAB-MS).

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11) The 1,3-acylmigration was restrained in the presence of AcOH.

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13) **3**: ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 7.12 (2H, d, *J*=8.6 Hz), 6.82 (2H, d, *J*=8.6), 6.71 (1H, m, H-3), 6.40 (1H, d, *J*=15.8, H-8), 6.01 (1H, dd, *J*=15.8, 8.6, H-7), 5.72 (1H, d, *J*=16.2, H-2), 5.05 (1H, m, H-5), 4.90 (1H, m, H-15), 4.70 (1H, m, H-24), 3.78 (3H, s, H-30), 3.50 (2H, m, H-22), 1.14 (3H, d, *J*=6.8, H-13), 0.75, 0.72 (both 3H, d, *J*=6.4). FAB-MS: *m/z* 591 $(M+H)^+$ ($C_{34}H_{43}N_2O_7$ by HR FAB-MS). CD maxima (MeOH): $[\Theta]_{253}$ +15000, $[\Theta]_{228}$ -10000, $[\Theta]_{219}$ -40000.

14) R. W. Murray, R. Jeyaraman, *J. Org. Chem.*, **50**, 2847 (1985).

15) **20**: ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 7.13 (2H, d, *J*=8.6 Hz), 6.82 (2H, d, *J*=8.6), 6.72 (1H, m, H-3), 5.78 (1H, d, *J*=15.4, H-2), 5.20 (1H, m, H-5), 5.00 (1H, m, H-15), 4.76 (1H, m, H-24), 3.79 (3H, s), 3.61 (1H, d, *J*=1.7, H-8), 2.90 (1H, dd, *J*=8.1, 1.7, H-7), 1.05 (3H, d, *J*=7.3, H-13), 0.90, 0.88 (both 3H, d, *J*=6.4). FAB-MS: *m/z* 607 $(M+H)^+$ ($C_{34}H_{43}N_2O_8$ by HR FAB-MS). CD maxima (MeOH): $[\Theta]_{267}$ +12000, $[\Theta]_{224}$ +4500.

16) The MCPBA oxidation of **3** in the presence of Na_2HPO_4 yielded **1** and **20** in poor yields with less stereoselectivity.

(Received August 29, 1994; accepted October 14, 1994)