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 IC₅₀ 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 (IC₅₀ 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 derivatives²⁾ and finally by a synthesis of a tetrahydrofuranoid **2**, which was obtained by methanolysis of **1** with K₂CO₃-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.

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 epoxidation⁴⁾ 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 Me₂CuLi and then subjected to NaIO₄ 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 benzene⁵⁾ and subsequently to selective deprotection of the TES group to give segment A (**5**)⁶⁾ in 63% yield from **11**.

Chart 2

Reagents and Conditions: a) i) Dess-Martin periodinane, CH₂Cl₂, ii) (MeO)₂P(O)CH₂COOMe, NaH, THF, 2 steps 75%; b) i) DIBAL, ii) L-DET, Ti(OⁱPr)₄, TBHP, 2 steps 90%; c) i) Me₂CuLi, ether, ii) NaIO₄, ether-water, iii) PivCl, pyridine, iv) TESCl, pyridine, v) DIBAL, CH₂Cl₂, 5 steps 65%; d) i) Dess-Martin periodinane, CH₂Cl₂, ii) Ph₃PCH₂PhCl, ⁿBuLi, benzene, ii) HF·pyridine, THF-CH₂Cl₂, 3 steps 63%.

(TBDPS: ^tbutyldiphenylsilyl 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) 1N aq. H₂SO₄, NaNO₂, 70%; b) i) CH₂N₂, ether, ii) MPMOC(NH)CCl₃, TiOH, ether, 2 steps 65%; c) 1N 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 DEPC⁷⁾ to afford **16** in 82% yield. Removal of the Cbz group of **16**, followed by phosphonoacetylation in the presence of WSCI⁸⁾ and subsequent deprotection of the 2-(trimethylsilyl)ethyl group, provided segment C (**7**)⁹⁾ in 90% yield.

Chart 4

Reagents and Conditions: a) i) ZCl, NaOH, ii) 2-(trimethylsilyl)ethanol, DCC, DMAP, CH₂Cl₂, iii) H₂/Pd-C, HCOOH, MeOH then aq. NaHCO₃, 3 steps 95%; b) DEPC, Et₃N, DMF, 82%; c) i) H₂/Pd-C, HCOOH, MeOH, then aq. NaHCO₃, ii) (EtO)₂P(O)CH₂COOH, WSCI·HCl, DMAP, THF, iii) BF₃·OEt₂, CH₃CN, 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 Et₃N and DMAP to furnish **18** in 90% yield. Removal of the MPM protecting group in **18** using PhSH and BF₃·OEt₂ and subsequent coupling with segment C (**7**) using IPCF¹⁰⁾ afforded diester **19** in 81% yield. Removal of the TBDPS group in **19** with *n*-Bu₄NF·AcOH¹¹⁾ and successive Dess-Martin oxidation yielded aldehyde **4**, which was then subjected to an intramolecular Wittig-Horner reaction¹²⁾ to give cyclic depsipeptide **3**¹³⁾ in 40% yield.

Finally, compound **3** was treated with dimethyldioxirane¹⁴) 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, ¹H-NMR, [α]_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**, NEt₃, DMAP, CH₂Cl₂, 90%; **b**) i) PhSH, BF₃·OEt₂, CH₂Cl₂, ii) **7**, IPCF, NEt₃, DMAP, CH₂Cl₂, 2 steps 81%; **c**) i) ⁿBu₄NF, AcOH, 85%, ii) Dess-Martin periodinane, iii) DBU, LiCl, CH₃CN, 2 steps 40%, **d**) dimethyldioxirane, 80%. (IPCF: isopropenyl chloroformate)

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- 5**: ¹H-NMR (270 MHz, CDCl₃) 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⁺-H₂O) (C₂₉H₃₄O₅Si by HR-MS).
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- The MCPBA oxidation of **3** in the presence of Na₂HPO₄ yielded **1** and **20** in poor yields with less stereoselectivity.

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