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# Synthesis and Biological Property of Carba and 20-Deoxo Analogues of Arenastatin A

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**Abstract**—The carba analogue, in which a methylene group is substituted for the oxygen atom linked to C-15, and 20-deoxo analogue of arenastatin A, a potent cytotoxic spongean depsipeptide, were synthesized. Both analogues lacking the 15,20-ester function, which was easily metabolized in serum, showed good stability in serum as well as moderate cytotoxic activity against KB cells and better solubility. 

In the course of our search for new, biologically active principles from marine organisms, we isolated and characterized the extremely cytotoxic ($IC_{50}$ 5 pg/mL against KB cells) depsipeptide named arenastatin A (**1**), from the Okinawan marine sponge of *Dysidea arenaria* as a minute constituent.[1,2] Later, we achieved the total synthesis of **1**[3,4] and elucidated that **1** inhibited microtubule assembly[5,6] by binding to the rhizoxin/maytansine site on tubulin. A closely similar depsipeptide named cryptophycin 1 (**3**) has been found from a terrestrial cyanobacterium of *Nostoc* sp.[7] Cryptophycin 1 (**3**) showed potent cytotoxicity and excellent in vivo anti-tumor activity. On the other hand, a significant reduction of the in vivo anti-tumor activity of arenastatin A (**1**) was found. Furthermore, the metabolism of the 15,20-ester function in **1** was disclosed through the synthesis of three analogues containing an amide function in place of the ester linkage of **1** and the evaluation of their stability in serum. Among them, only triamide analogue-II (**2**) with a 15,20-amide function showed sufficient stability as well as moderate cytotoxicity ($IC_{50}$ 6 ng/mL). However, **2** showed very poor solubility in polar solvents (such as MeOH, DMSO, and water) applicable to in vivo anti-tumor testing.

This background prompted us to search for new analogues of **1** possessing good stability in serum as well as potent cytotoxicity and sufficient solubility applicable to in vivo anti-tumor testing. So far, more than 15 synthetic studies of cryptophycin 1 (**3**) and arenastatin A (**1**) have been reported.[8–11] Then, we have undertaken to synthesize carba analogue (**4**) having a 15,20-methylene bridge and 20-deoxo analogue (**5**) lacking the 20-carbonyl group in **1**. This paper describes the synthesis and biological assessment of the two analogues (**4**, **5**) (Chart 1).

Carba analogue (**4**), in which a methylene group is substituted for the oxygen atom linked to C-15 of **1**, was synthesized through condensation between segment A-B (**13**) and segment C-D (**12**) as illustrated in Scheme 1. The key segment **12** was prepared by the following procedure. Carbonyldiimidazole induced the coupling of *N*-Boc-β-alanine (**7**) and lithium enolate generated from *tert*-butyl acetate (**6**) to provide a ketoester (**8**) in 83% yield. $S_N2$ Nucleophilic substitution of the carbanion of **8** with a triflate **10**, which was converted from (*R*)-leucinic acid (**9**) through treatment with trimethylsilyldiazomethane followed by trifluoromethanesulfonylation, afforded a diastereomeric mixture **11** in 71% yield. Removal of the *tert*-butyl group in **11** and subsequent re-introduction of the Boc group gave a half-ester, which was subjected to decarboxylation under reflux in benzene to yield a ketoester. Saponification of the resulting ketoester by LiOH furnished the desired segment C-D (**12**) with high enantioselectivity in a ratio of 30:1 in 84% overall yield through four steps.

The absolute configuration of **12** was confirmed by Kusumi's method.[12] The proton signals due to the *iso*-butyl residue of (*S*)-PGME amide (**12a**) appeared at lower field than those of (*R*)-PGME amide (**12b**), while the proton signals assignable to the β-alanine portion of **12a** were observed at higher field than those of **12b** as

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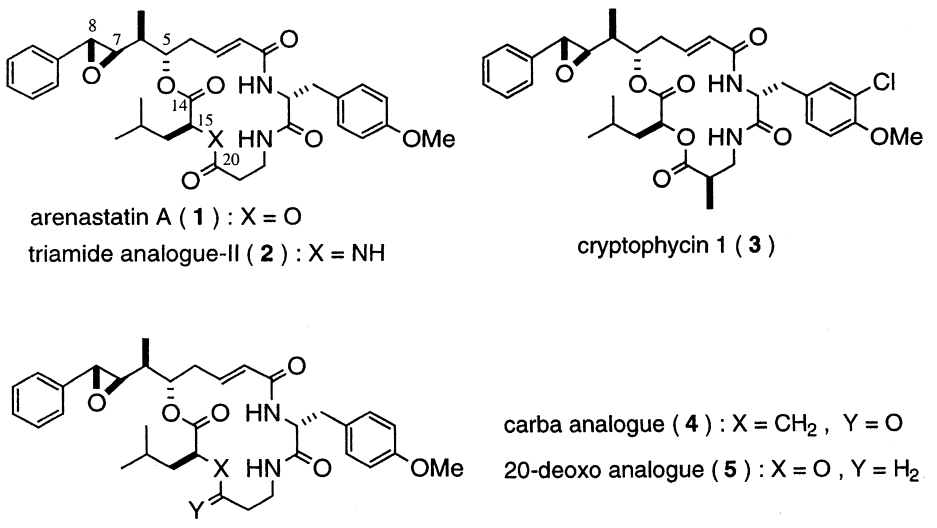
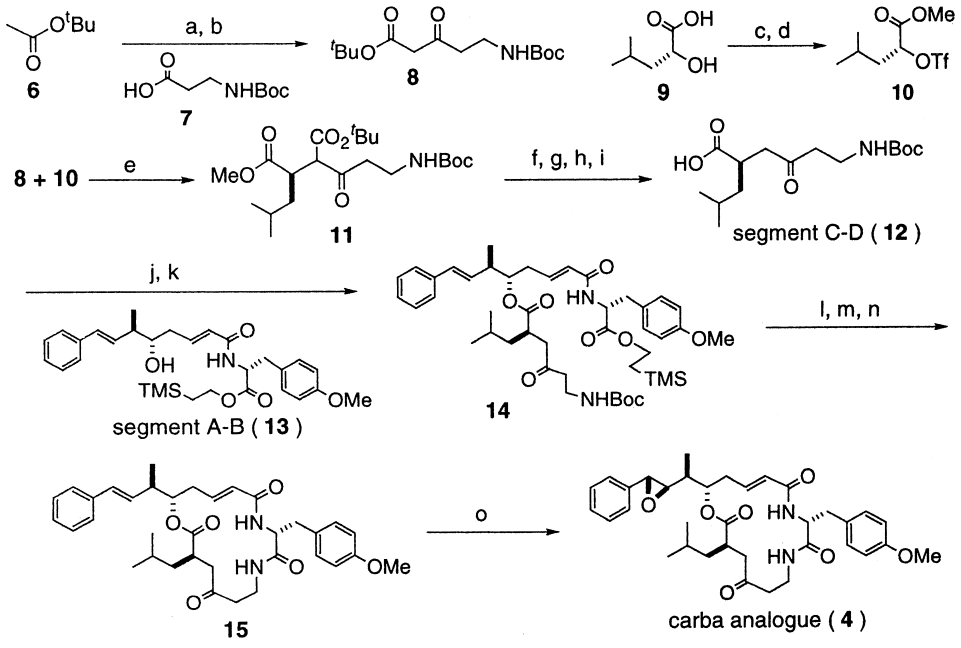



**Chart 1.**

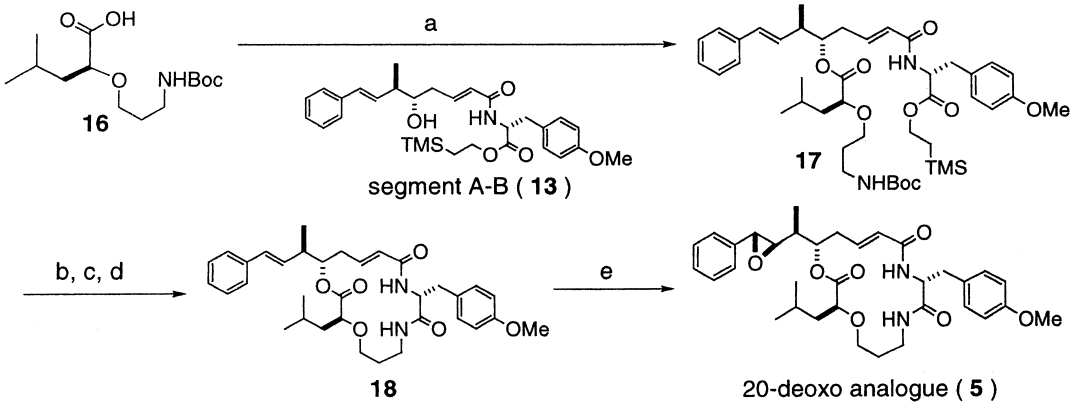



**Scheme 1.** Synthesis of carba analogue (**4**). Reagents and conditions: (a) LDA, THF, −78 °C; (b) carbonyldiimidazole, THF, 2 steps 83%; (c) $CHN_2TMS$, $CH_2Cl_2$; (d) $Tf_2O$, 2,6-lutidine, −20 °C, 2 steps 95%; (e) NaH, THF, 71%; (f) TFA; (g) $(Boc)_2O$, $Et_3N$, $CH_2Cl_2$; (h) benzene, reflux; (i) LiOH, THF:$H_2O$ (3:1), 4 steps 84%; (j) 2,4,6-trichlorobenzoyl chloride, $Et_3N$, THF; (k) $Et_3N$, DMAP, toluene, 2 steps 97%; (l) TFA, $CH_2Cl_2$; (m) HCl–$Et_2O$; (n) DPPA, $NaHCO_3$, DMF, 5 °C, 3 steps 66%; (o) dimethyldioxirane, $CH_2Cl_2$, 66%.

shown in Figure 1. Consequently, the absolute configuration at the C-15 position in **12** was confirmed to be the desired *R* configuration.

As a result of examining various activating reagents for the condensation of **12** and **13**, esterification was achieved by using 2,4,6-trichlorobenzoyl chloride in the presence of $Et_3N$ to furnish the desired diester **14** in 97% yield.[13] Simultaneous cleavage of two protecting groups in **14** followed by HCl treatment afforded a *seco* amino acid as a hydrochloride salt, which was subjected to intramolecular macrolactamization by use of diphenylphosphoryl azide (DPPA) and $NaHCO_3$ to give a cyclic depsipeptide **15** in 66% overall yield through three steps. Finally, epoxidation of **15** was successfully executed with dimethyldioxirane to furnish the desired carba analogue (**4**)[14] predominantly in a ratio of ca. 2:1.

Next, 20-deoxo analogue (**5**) lacking the 20-carbonyl group of **1** was synthesized as illustrated in Scheme 2. An alkoxy carboxylic acid **16** was prepared from D-leucine according to TenBrink's procedure.[15] The resulting carboxylic acid **16** was coupled with **13** using EDCI·HCl in the presence of DMAP in $CH_2Cl_2$ to give a conjugated product **17** quantitatively. After removal of two protecting groups followed by conversion to a hydrochloride salt, the resulting *seco* acid was also cyclized intramolecularly to give a cyclic depsipeptide **18** in moderate overall yield (74%). Treatment of **18** with dimethyldioxirane furnished the 20-deoxo analogue




**Scheme 2.** Synthesis of 20-deoxo analogue (**5**). Reagents and conditions: (a) EDCI·HCl, DMAP, $CH_2Cl_2$, quant; (b) TFA, $CH_2Cl_2$; (c) HCl–$Et_2O$; (d) DPPA, $NaHCO_3$, DMF, 5 °C, 3 steps 74%; (e) dimethyldioxirane, $CH_2Cl_2$, 65%.

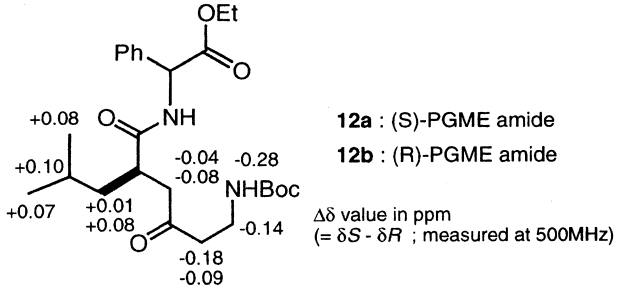



**Figure 1.** Confirmation of absolute configuration in **12**.

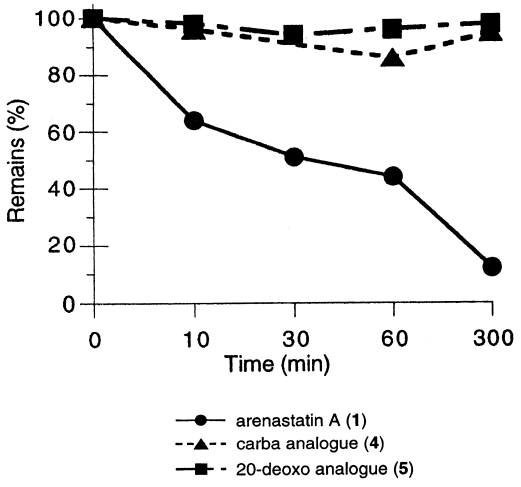



**Figure 2.** Stability of arenastatin A (**1**) and its analogues (**4**, **5**) in mouse serum. Each sample (10 μL of 0.1 mg/ml solution) was treated with fresh mouse serum (100 μL) and incubated at 37 °C for 0, 10, 30, 60, 300 min, respectively. After extraction of the reaction mixture with EtOAc, each extract was analyzed by reversed phase HPLC to determine the remain of **1**, **4** and **5**.

(**5**)[16,17] as a major oxygenated product (α-epoxide:β-epoxide = 1:2).

Carba (**4**) and 20-deoxo (**5**) analogues exhibited moderate cytotoxicity against KB cells with $IC_{50}$ values of 0.07 and 0.04 μg/mL, respectively. In addition, both analogues (**4**, **5**) also showed nearly complete stability in mouse serum as we expected (Fig. 2). Notably, the two analogues possessed better solubility for application to in vivo anti-tumor testing unlike the triamide analogue-II (**2**).[18] The significant decrease (10,000-fold less) of the cytotoxicity for **4** and **5** compared with that of arenastatin A (**1**) might be caused by the conformational difference in the 16-membered ring system between **1** and **4**, **5**, inasmuch as we have already clarified that the synthesized stereoisomers relative to epoxy, 6-methyl, and OMe-tyrosine did not show any potent cytotoxicity at concentrations below 0.1 μg/mL.[4] Conformational comparison of the ring structure between arenastatin A (**1**) and its analogues is currently under way.

## Acknowledgements

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## References and Notes

1. Kobayashi, M.; Aoki, S.; Ohyabu, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 7969.
2. Kobayashi, M.; Kurosu, M.; Ohyabu, N.; Wang, W.; Fujii, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2196.
3. Kobayashi, M.; Kurosu, M.; Wang, W.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2394.
4. Kobayashi, M.; Wang, W.; Ohyabu, N.; Kurosu, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1995**, *43*, 1598.
5. Koiso, Y.; Morita, K.; Kobayashi, M.; Wang, W.; Ohyabu, N.; Iwasaki, S. *Chem.-Biol. Interact.* **1996**, *102*, 183.
6. Morita, K.; Koiso, Y.; Hashimoto, Y.; Kobayashi, M.; Wang, W.; Ohyabu, N.; Iwasaki, S. *Biol. Pharm. Bull.* **1997**, *20*, 171.
7. Barrow, R. A.; Hemscheidt, T.; Liang, J.; Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 2479.
8. Varie, D. L.; Shih, C.; Hay, D. A.; Andis, S. L.; Corbett, T. H.; Gossett, L. S.; Janisse, S. K.; Martinelli, M. J.; Moher, E. D.; Schultz, R. M.; Toth, J. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 369.
9. Liang, J.; Hoard, D. W.; Khau, V. V.; Martinelli, M. J.; Moher, E. D.; Moore, R. E.; Tius, M. A. *J. Org. Chem.* **1999**, *64*, 1459.
10. Patel, V. F.; Andis, S. L.; Kennedy, J. H.; Ray, J. E.; Schultz, R. M. *J. Med. Chem.* **1999**, *42*, 2588.
11. White, J. D.; Hong, J.; Robarge, L. A. *J. Org. Chem.* **1999**, *64*, 6206.
12. Nagai, Y.; Kusumi, T. *Tetrahedron Lett.* **1995**, *36*, 1853.

13. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
14. **4**: $[\alpha]_D^{18}$ + 43.9° (*c* = 0.33, MeOH). IR (KBr) $cm^{-1}$: 2930, 2860, 1728, 1668, 1514. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.40–7.25 (5H, m, Ph), 7.18 (1H, m, 22-NH), 7.09 (2H, d, *J* = 8.5 Hz, 27-H), 6.82 (2H, d, *J* = 8.5, 28-H), 6.71 (1H, ddd, *J* = 8.0, 14.7, 15.3, 3-H), 5.73 (1H, d, *J* = 15.3, 2-H), 5.50 (1H, d, *J* = 7.3, 24-NH), 5.25 (1H, m, 5-H), 4.65 (1H, ddd, *J* = 5.5, 7.3, 7.4, 24-H), 3.79 (3H, s, 30-H), 3.69 (1H, d, *J* = 1.9, 8-H), 3.63 (1H, m, 22-Ha), 3.14 (1H, m, 22-Hb), 3.10 (1H, dd, *J* = 5.5, 14.0, 25-Ha), 3.05 (1H, dd, *J* = 7.4, 14.0, 25-Hb), 2.94 (1H, dd, *J* = 1.9, 7.3, 7-H), 2.90 (1H, dd, *J* = 8.5, 16.2, 15′-Ha), 2.87 (1H, m, 15-H), 2.61 (1H, m, 21-Ha), 2.53 (1H, m, 21-Hb), 2.33 (1H, brd, *J* = 16.2, 15′-Hb), 1.65–1.40 (3H, m, 16, 17-H), 1.40 (1H, m, 6-H), 1.13 (3H, d, *J* = 7.4, 6-$CH_3$), 0.86, 0.83 (both, 3H, d, *J* = 6.8, 18, 19-H). FABHRMS: Obsd; *m*/*z* 605.3224. Calcd for $C_{35}H_{45}N_2O_7$; *m*/*z* 605.3227 $(M+H)^+$. The stereochemistry of the epoxy moiety was established by comparison of the chemical shifts due to the 8-H and 6-Me groups with those of arenastatin A (**1**). The practical data of 8-H and 6-Me were as follows: **1**: δ 3.68, 1.14; α-epoxy isomer of **1**: δ 3.59, 1.05; α-epoxy isomer of **4**: δ 3.59, 1.04; α-epoxy isomer of **5**: δ 3.59, 1.03.
15. TenBrink, R. E. *J. Org. Chem.* **1987**, *52*, 418.
16. **5**: $[\alpha]_D^{18}$ + 44.1° (*c* = 0.11, $CHCl_3$). IR (KBr) $cm^{-1}$: 2928, 1745, 1660, 1535, 1514. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.36–7.23 (5H, m, Ph), 7.12 (2H, d, *J* = 8.5 Hz, 27-H), 6.84 (1H, ddd, *J* = 3.7, 11.0, 14.6, 3-H), 6.81 (2H, d, *J* = 8.5, 28-H), 6.52 (1H, brd, *J* = 6.7, 22-NH), 5.67 (1H, d, *J* = 14.6, 2-H), 5.66 (1H, d, *J* = 8.5, 24-NH), 5.33 (1H, ddd, *J* = 2.4, 5.5, 9.2, 5-H), 4.66 (1H, q-like, *J* = ca. 8.0, 24-H), 3.77 (3H, s, 30-H), 3.76 (1H, m, 22-Ha), 3.67 (1H, d, *J* = 1.8, 8-H), 3.60 (1H, dd, *J* = 3.1, 10.4, 15-H), 3.26 (1H, m, 22-Hb), 3.18 (1H, dd, *J* = 7.3, 14.6, 25-Ha), 3.08 (1H, m, 20-Ha), 2.92 (1H, dd, *J* = 1.8, 7.9, 7-H), 2.89 (1H, m, 25-Hb), 2.84 (1H, m, 20-Hb), 2.59 (1H, m, 4-Ha), 2.37 (1H, m, 4-Hb), 1.78 (3H, m, 6, 21-H), 1.64 (1H, m, 17-H), 1.49 (1H, m, 16-Ha), 1.23 (1H, m, 16-Hb), 1.14 (3H, d, *J* = 7.3, 6-$CH_3$), 0.84, 0.83 (both, 3H, d, *J* = 6.7, 18, 19-H). FABHRMS: Obsd; *m*/*z* 593.3217. Calcd for $C_{34}H_{44}N_2O_7$; *m*/*z* 593.3227 $(M+H)^+$.
17. Murakami, N.; Ito, T.; Tamura, S.; Aoki, S.; Kobayashi, M. *Abstract Papers, Part 2, P-160*, 118th Annual Meeting of the Japanese Society of Pharmaceutical Sciences, Kyoto, 31 March–2 April, 1998.
18. For example, the solubility of arenastatin A (**1**) and its analogues (**2**, **4**, **5**) in MeOH was 2.8, 0.20, 2.9 and 1.6 mg/mL, respectively.