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Total synthesis of stevastelins B3 and C3: structure confirmation of stevastelin B3 and revision of stevastelin C3

Kazuo Kurosawa, Keigo Matsuura and Noritaka Chida*

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

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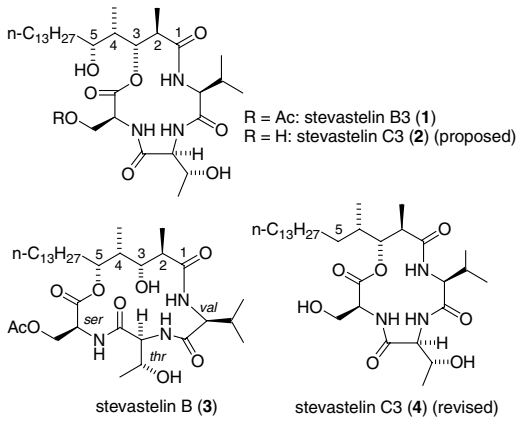
Abstract—The total synthesis of stevastelin B3, stevastelin C3 and 5-deoxy derivative of stevastelin C3, novel 13-membered cyclic depsipeptides, is described. This study unambiguously confirmed the proposed absolute structure of stevastelin B3, and revealed that the structure of stevastelin C3 is incorrect. The correct structure of stevastelin C3 was established by the total synthesis to be 5-deoxy derivative of the proposed structure.

Stevastelins B3 (**1**) and C3 (**2**), members of stevastelin family, are novel cyclic depsipeptides isolated from a culture broth of *Penicillium* by Nippon Kayaku group and reported to show a potent immunosuppressive activity.[1,2] The structural study by spectral, degradation and synthetic methods of stevastelin B (**3**), the most abundant congener of stevastelin family, established that stevastelin B consists of (2*S*,3*S*,4*S*,5*R*)-3,5-dihydroxy-2,4-dimethylstearic acid, L-serine, L-threonine and L-valine, and possesses 15-membered ring structure.[3] Based on spectroscopic analyses of other stevastelins, it has been proposed that stevastelin B3 (**1**) is an isomer of stevastelin B with a 13-membered ring structure and stevastelin C3 (**2**) is a de-*O*-acetyl derivative of stevastelin B3.[1] Their interesting mode of action, repression of both T cells and B cells,[1] as well as their unique structures have attracted the synthetic attention, and total syntheses of stevastelins B[4] and the proposed structure of C3,[5] synthetic approach[6] and preparation and biological assessment of simple analogues[7] have been reported to date. Although the proposed absolute structure of stevastelin B has been determined by the total synthesis,[4b] those of stevastelins B3 and C3 have not been synthetically confirmed. Here we report a total synthesis of stevastelins B3 (**1**), C3 (**2**) and a 5-deoxy derivative of stevastelin C3 (**4**), which resulted in the structure confirmation of the proposed absolute structure of stevastelin B3 (**1**) and the structure revision of stevastelin C3 to **4**.



Synthesis of stevastelin B3 commenced from acetonide **6**, which was previously prepared from L-quebrachitol (**5**) in our total synthesis of stevastelin B[4b] (Scheme 1). Benzylation of the hydroxy group in **6**, followed by acid treatment gave diol **7**.[8] The primary hydroxy group in **7** was selectively protected as a TES ether to give **8** in 96% yield from **6**. Since direct acylation of **8** with Boc-Val-Thr-Ser(Bzl)[9] under various reaction conditions was found to be fruitless due to the steric congestion of the hydroxy group, stepwise introduction of the peptide

Keywords: Stevastelin B3; Stevastelin C3; Total synthesis; Structure revision.
* Corresponding author. Tel./fax: +81 45 566 1573; e-mail: chida@applc.keio.ac.jp

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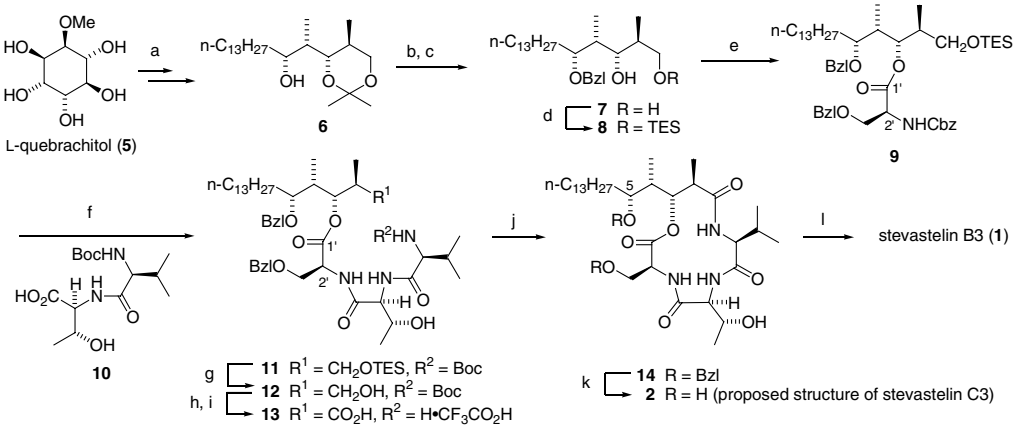
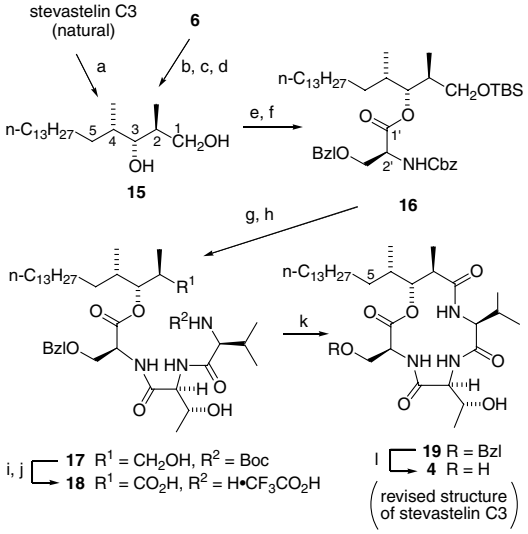


Scheme 1. TES = $-SiEt_3$, Bzl = $-CH_2Ph$, Cbz = $-C(O)OCH_2Ph$. Reagents and conditions: (a) see Ref. 4b; (b) $(Me_3Si)_2NK$, BzlBr, THF; (c) AcOH–H_2O (4/1); (d) TESCl, Et_3N, CH_2Cl_2; (e) Cbz–Ser(Bzl), 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, THF; (f) H_2, 10% Pd–C ethylenediamine complex, MeOH, rt, then **10**, WSC·HCl, HOBt, DMF, rt; (g) AcOH–THF–H_2O (3/3/1); (h) TEMPO, KBr, NaOCl, $NaHCO_3$, aq CH_2Cl_2, 0 °C, then $NaClO_2$, $HOSO_2NH_2$, NaH_2PO_4, *t*-BuOH–H_2O, 0 °C; (i) TFA, CH_2Cl_2, −18 °C; (j) DEPC, Et_3N, DMF; (k) H_2, 10% Pd–C, MeOH; (l) Ac_2O, pyridine, rt.

moiety was carried out. Condensation of **8** with Cbz–Ser(Bzl) under the modified Yamaguchi's conditions[10] afforded **9**. Unfortunately racemization of the serine moiety during the coupling process was observed and **9** and its diastereoisomer were obtained as an inseparable mixture in a ratio of ca. 1:1 in 80% yield. Hydrogenolysis of a mixture of **9** and its diastereomer in the presence of 10% Pd–C ethylenediamine complex[11] deprotected *N*-Cbz group selectively to give an amine, which, without isolation, was coupled with Boc-Val-Thr **10**[9] by the action of ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC·HCl) and 1-hydroxybenzotriazole (HOBt) to provide **11** and its diastereomer in 94% yield. Acid hydrolysis of **11** gave diol **12**. At this stage, diastereomers were cleanly separated by silica gel chromatography and **12** was obtained in pure form in 45% yield from **11**. The primary hydroxy group in **12** was selectively oxidized to give a carboxylic acid, whose *N*-Boc group was deprotected to generate **13**. Macrolactamization of **13** was successfully accomplished under Shioiri's protocol[12] [(diethyl phosphorocyanidate (DEPC) in DMF (0.01 mol dm^{-3})] to give macrocycle **14** in 29% yield from **12**. Removal of the *O*-benzyl group in **14** by hydrogenolysis afforded the compound possessing the proposed structure of stevastelin C3 **2**[13,14] in 74% yield. Treatment of **2** with acetic anhydride in pyridine at room temperature furnished stevastelin B3 (**1**)[14] (38% yield).

The direct comparison of synthetic **1** and **2** with natural stevastelins B3 and C3, kindly provided by Nippon Kayaku group revealed that the synthetic **1** is unambiguously identical with natural stevastelin B3. Thus, the proposed structure of stevastelin B3 was fully confirmed by this first total synthesis. However, spectral data of synthetic **2** were not identical with those of natural stevastelin C3. Further analysis of natural stevastelin C3 by NMR suggested the possibility that the natural product might be a deoxy derivative of the proposed structure. The molecular ion peak observed at *m/z* 598 ($C_{32}H_{59}N_3O_7$ + H) by FABMS also supported the deoxygenated structure.

To elucidate the correct structure of stevastelin C3, the natural product was subjected to degradation (Scheme 2). Treatment of natural stevastelin C3 with $LiBH_4$ afforded diol **15**[14] in 24% yield. The structure of **15** was determined based on spectral analysis, and finally confirmed by comparison with the synthetic specimen (vide infra). On the other hand, acid hydrolysis of the natural product, followed by HPLC analysis (MIC GEL CRS 10W) of the hydrolysates revealed that the amino acids constituting stevastelin C3 are the same as those of stevastelins B and B3 (L-serine, L-threonine and L-valine). From these results, it was presumed that natural stevastelin C3 should be a 5-deoxy derivative of the proposed structure.



Scheme 2. TBS = $-SiMe_2$ (*t*-Bu). Reagents and conditions: (a) $LiBH_4$, THF, reflux; (b) NaH, CS_2, THF, then MeI; (c) Bu_3SnH, AIBN, toluene, reflux; (d) CSA, MeOH, rt; (e) TBSCl, Et_3N, CH_2Cl_2; (f) Cbz–Ser(Bzl), 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, THF, 0 °C; (g) H_2, 10% Pd–C ethylenediamine complex, MeOH, rt, then **10**, WSC·HCl, HOBt, DMF; (h) AcOH–THF–H_2O (14/3/6); (i) TEMPO, KBr, NaOCl, $NaHCO_3$, aq CH_2Cl_2, 0 °C, then $NaClO_2$, $HOSO_2NH_2$, NaH_2PO_4, *t*-BuOH–H_2O, 0 °C; (j) TFA, CH_2Cl_2, −18 °C; (k) DEPC, Et_3N, DMF; (l) H_2, 10% Pd–C, MeOH.

With these structural information, we turned to the synthesis of the expected structure of stevastelin C3. The hydroxy group in **6** was removed via xanthate ester by Barton's method,[15] and the product was treated with acid to give diol **15** in 73% yield from **6**. The spectral data of **15** were fully identical with those of the diol obtained by reductive degradation of natural stevastelin C3. The primary hydroxy group in **15** was selectively protected as a TBS ether to give secondary alcohol, which was then condensed with Cbz–Ser(Bzl) under the modified Yamaguchi's conditions to give **16** and its C-2′ epimer as an inseparable mixture in a ratio of 1.5:1 in 90% yield. Deprotection of *N*-Cbz group in **16** and subsequent condensation with **10**, followed by deprotection of *O*-TBS group gave **17** and its diastereomer, which were cleanly separated by silica gel chromatography to afford pure **17**[13] in 37% from **15**. Compound **17** was transformed into macrocycle **19** in 28% overall yield by the same procedure as described for preparation of **14** from **12**. Removal of the *O*-benzyl group in **19** furnished **4**,[14] whose spectral data as well as $[\alpha]_D$ value showed good accordance with those of natural stevastelin C3. Based on this synthesis, it was concluded that the structure of natural stevastelin C3 should be revised to **4**.

In summary, total synthesis of stevastelins B3 and C3, which fully established the absolute structures of these natural products has been achieved. The methodology developed in this work would be applicable to the synthesis of other stevastelins as well as other cyclic depsipeptides. It is also interesting that the fatty acid moiety of stevastelin C3 is different from that of stevastelins B and B3 although the stevastelins are produced by the same microorganism.

Acknowledgements

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8. All new compounds described in this paper were characterized by 300 MHz ^{1}H NMR, 75 MHz ^{13}C NMR, IR and mass spectrometric and/or elemental analyses.
9. These peptides were prepared by condensation (WSC·HCl, HOBt, DMF) of appropriate protected amino acids which were purchased from Peptide Institute, Inc. (Osaka, Japan).
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13. The absolute structures of amino acids in this compound were confirmed by acid hydrolysis, followed by HPLC analyses (MIC GEL CRS 10W, Mitsubishi Chemical Industries, Ltd) of the hydrolysates.
14. *Spectral data of compound* **2**: $[\alpha]_D^{25.5}$ − 39 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.52 (3H, d, J = 6.9 Hz), 0.86 (3H, t, J = 6.9 Hz), 0.88 (3H, d, J = 6.0 Hz), 0.93 (3H, d, J = 6.6 Hz), 1.07 (3H, d, J = 6.3 Hz), 1.09 (3H, d, J = 7.2 Hz), 1.14–1.41 (24H, m), 1.66 (1H, m), 2.04 (1H, m), 2.86 (1H, m), 3.54 (1H, m), 3.85 (1H, m), 3.92 (1H, m), 4.03 (2H, m), 4.13 (1H, dd, J = 9.9 and 2.4 Hz), 4.31 (1H, d, J = 5.7 Hz), 4.60 (1H, m), 4.91 (1H, m), 5.03 (1H, dd, J = 4.8 and 5.1 Hz), 5.14 (1H, d, J = 4.8 Hz), 7.54 (1H, d, J = 9.9 Hz), 7.84 (1H, d, J = 9.9 Hz) and 7.88 (1H, d, J = 9.9 Hz); HRMS (FAB) *m*/*z* 614.4407, calcd for $C_{32}H_{60}N_3O_8$ (M+H) 614.4380. *Spectral data of compound* **1**: $[\alpha]_D^{26}$ − 53 (*c* 0.1, $CHCl_3$), {natural stevastelin B3, $[\alpha]_D^{25.5}$ − 51 (*c* 0.255, $CHCl_3$) (measured in our laboratory)}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.54 (3H, d, J = 6.8 Hz), 0.85 (3H, t, J = 6.6 Hz), 0.90 (3H, d, J = 6.8 Hz), 0.93 (3H, d, J = 6.6 Hz), 1.08 (3H, d, J = 6.1 Hz), 1.12 (3H, d, J = 6.8 Hz), 1.17–1.35 (24H, m), 1.72 (1H, m), 2.01 (3H, s), 2.05 (1H, m), 2.90 (1H, m), 3.84 (1H, m), 4.01 (2H, m), 4.13 (1H, m), 4.18 (1H, m), 4.35 (1H, m), 4.40 (1H, d, J = 5.4 Hz), 4.92 (2H, m), 5.24 (1H, d, J = 4.4 Hz), 7.50 (1H, m), 7.67 (1H, br) and 7.72 (1H, d, J = 9.0 Hz) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 9.2, 13.8, 13.9, 19.0, 19.2, 20.7, 20.9, 22.1, 25.7, 28.7, 29.0, 29.1 and 29.1, 31.3, 34.9, 39.1, 41.1, 50.2, 59.4, 61.6, 63.2, 65.2, 69.0, 80.2, 168.7, 170.1, 170.5, 170.7 and 170.8; HRMS (FAB) *m*/*z* 656.4494, calcd for $C_{34}H_{62}N_3O_9$ (M+H) 656.4486. The ^{1}H and ^{13}C NMR data were fully identical with those of natural stevastelin B3.[2] *Spectral data of compound* **15**: $[\alpha]_D^{21}$ + 10 (*c* 1.74, $CHCl_3$); ν_{max} (neat) 3320 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.79 (3H, d, J = 7.1 Hz), 0.86 (3H, d, J = 7.1 Hz), 0.87 (3H, t, J = 7.0 Hz), 1.15–1.42 (24 H, m), 1.60 (3 H, m), 1.85 (1H, dddq, J = 9.0, 7.8, 3.4 and 7.1 Hz), 2.12–3.18 (2H, m), 3.46 (1H, dd, J = 9.0 and 2.4 Hz), 3.63 (1H, dd,J = 10.7 and 7.8 Hz) and 3.71 (1H, dd, J = 10.7 and 3.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.2, 13.5, 14.1, 22.7, 27.4, 29.4, 29.7, 29.9, 31.9, 34.0, 35.1, 37.3, 68.8 and 80.3; HRMS (FAB) *m*/*z* 315.3283, calcd for $C_{20}H_{43}O_2$ (M+H) 315.3263. *Spectral data of compound* **4**: $[\alpha]_D^{25}$ − 67 (*c* 0.13, MeOH), {natural stevastelin C3, $[\alpha]_D^{27.5}$ − 66 (*c* 0.305, MeOH) (measured in

our laboratory)}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.59 (3H, d, J = 6.8 Hz), 0.85 (3H, t, J = 6.8 Hz), 0.87 (3H, d, J = 6.3 Hz), 0.92 (3H, d, J = 6.6 Hz), 1.07 (3H, d, J = 6.3 Hz), 1.10 (3H, d, J = 7.6 Hz), 1.13–1.30 (25H, m), 1.49 (1H, m), 1.74 (1H, m), 2.05 (1H, m), 2.74 (1H, m), 3.54 (1H, m), 3.92 (1H, m), 4.03 (2H, m), 4.13 (1H, dd, J = 10.3 and 2.4 Hz), 4.61 (2H, m), 5.04 (1H, dd, J = 5.1 and 4.9 Hz), 5.16 (1H, d, J = 4.6 Hz), 7.52 (1H, m) and 7.85 (2 H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.9, 15.4, 18.9, 19.3, 21.0, 22.1, 25.9, 28.7, 28.9, 29.0, 29.2, 31.3, 32.3, 34.4, 42.1, 53.7, 59.5, 61.0, 65.2, 80.5, 170.0, 170.4 and 170.8; HRMS (FAB) m/z 598.4422, calcd for $C_{32}H_{60}N_3O_7$ (M+H) 598.4431. The ^{1}H and ^{13}C NMR data were fully identical with those of natural stevastelin C3.

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