Total Synthesis of Stevastelin B, a Novel Immunosuppressant

Naoki Kohyama, Yukio Yamamoto*

Graduate School of Human and Environmental Studies, Kyoto University, Sakyo-ku, Yoshida, Kyoto 606-8501, Japan
Fax +81-75-753-2999; E-mail: yuyamamo@ip.media.kyoto-u.ac.jp

Received 5 March 2001

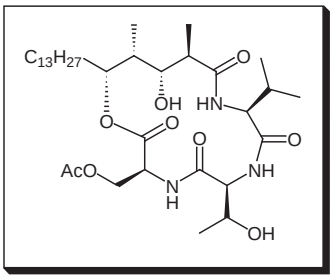
Abstract: Total synthesis of stevastelin B is described. Evans asymmetric aldol methodology and Roush asymmetric allylation were used to construct four consecutive stereo-centers on the octadecanoic acid moiety of stevastelin B. Subsequent coupling with a dipeptide and macrolactamization gave stevastelin B. The flexibility of this route could allow the synthesis of many analogues for biological tests, which cannot be obtained from natural sources.

Key words: total synthesis, stevastelin B, immunosuppressant, asymmetric aldol reaction, asymmetric allylation

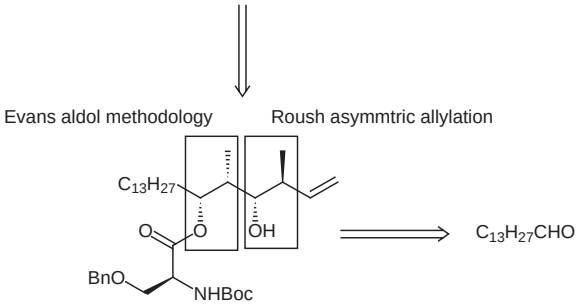
Stevastelins are novel depsipeptides, isolated from a culture broth of *penicillium* sp. NK374186 as immunosuppressants in 1994.[1] Among stevastelins, stevastelin B is the most abundant. Stevastelin B (**1**) contains four components, namely valine, threonine, *O*-acetyl-serine and 3,5-dihydroxy-2,4-dimethyloctadecanoic acid. The absolute structure of stevastelin B was determined in 1996.[2] Stevastelin B shows the inhibitory activity not only against T cell activation but also B cell.[1] Together with its low toxicity, stevastelins may be useful tools for investigation of T cell and B cell activation mechanisms and have applications as immunosuppressants.[1,3]

Total synthesis of stevastelin B has not been reported yet, although it has a very interesting structure and biological activity. So we decided to make stevastelin B our target of total synthesis. The synthetic plans are shown in Scheme 1. For the appropriate construction of four consecutive stereo-centers in synthetic intermediate **3**, which was a crucial point in this total synthesis, we decided to use Evans asymmetric aldol methodology[4] and Roush asymmetric allylation.[5]

Evans asymmetric aldol reaction followed by hydrolysis and esterification gave methyl ester **4** (Scheme 2). MTPA ester derived from methyl ester **4** was used to determine the enantiomeric purity. By ^{19}F NMR, methyl ester **4** proved to be >99% ee. Reduction of **4** with $LiBH_4$ gave a diol,[6] whose primary alcohol was selectively protected with a TBS group to give alcohol **5**. Coupling of **5** with Boc-Ser(Bn)-OH followed by deprotection of the TBS group under acidic conditions gave alcohol **6**. Alcohol **6** was oxidized to the corresponding aldehyde by TPAP,[7] which was converted to homoallyl alcohol **3** by Roush asymmetric allylation using (*R,R*)-boronate **7**. The diastereomers, which might be yielded by the asymmetric allylation, were not found at all. This boronate **7** can construct the 3,4-*anti* configuration, regardless of the configuration of aldehydes.[5] In fact, the desired stereochemistry was correctly constructed as described below. Olefin **3** was



1; Stevastelin B

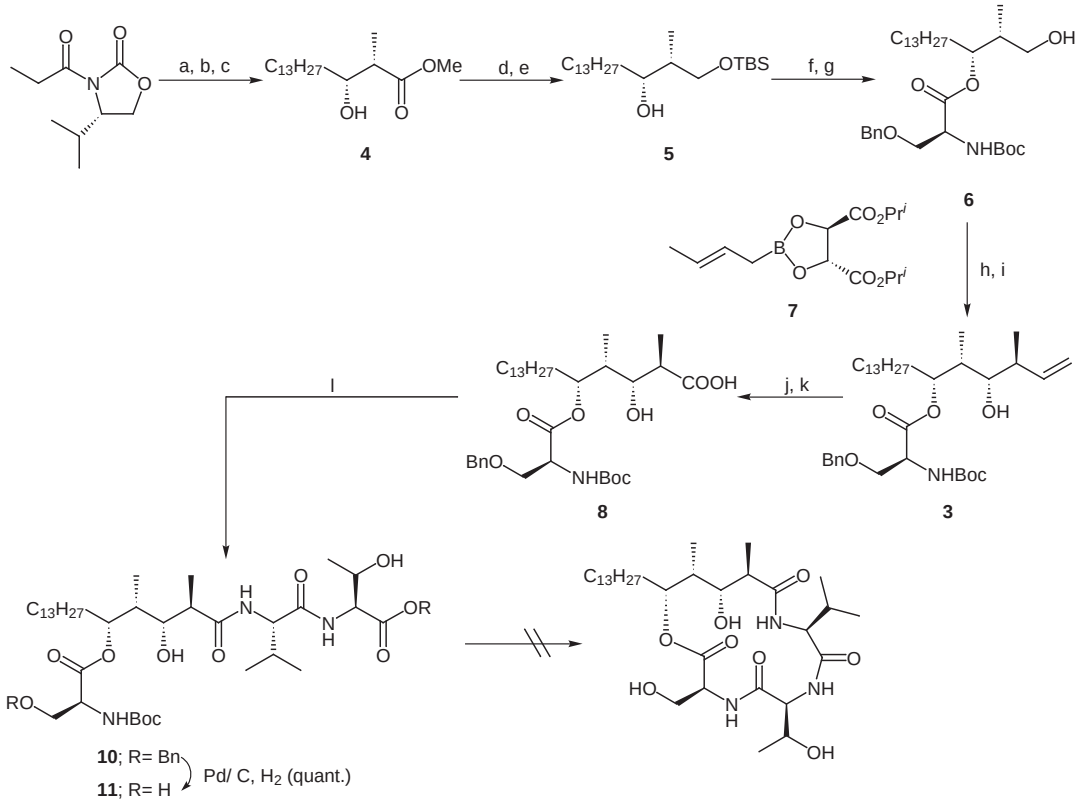


Scheme 1

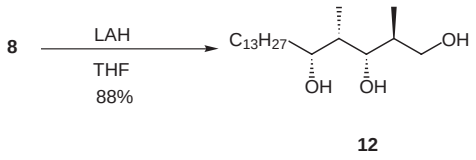
oxidized to acid **8** by ozonolysis and subsequent $NaClO_2$-NaH_2PO_4 oxidation.

Acid **8** was reduced to triol **12**[8] which had been derived from stevastelin B itself (Scheme 3). The ^1H NMR spectra and the specific rotation of **12** thus obtained were identical with the reported data.[2b] Moreover, benzyl ether **13**, prepared by the same method for **3**, was converted to acetonide **14** to confirm the *syn*-stereochemistry of two hydroxyl groups, independently. The *gem*-methyl groups of **14** were observed at 19.55 and 30.11 ppm, respectively (in ^{13}C NMR). The ^{13}C NMR spectra of *syn*-1,3-diol acetonides are reported to show an axial methyl group at 19 ppm and an equatorial methyl group at 30 ppm, while the two methyl groups of *anti*-1,3-diol acetonides appear at 24 ppm.[9] Therefore, the relative stereochemistry of this 1,3-diol was unambiguously determined as *syn* (Scheme 4).

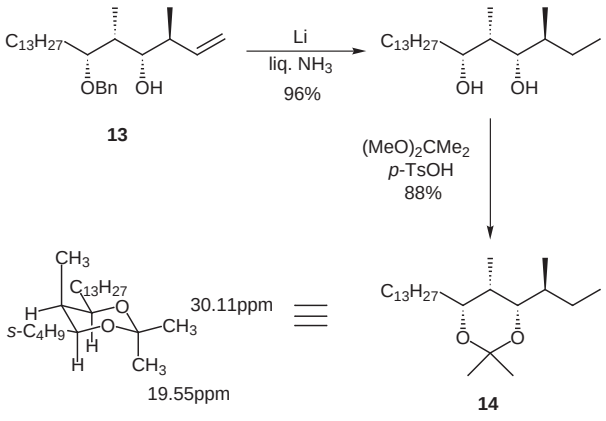
Coupling of acid **8** with dipeptide **9** gave **10**. Removal of the benzyl groups in **10** by hydrogenation with Pd/C gave **11**. After removal of the Boc group in **11**, macrolactamization under several conditions was examined, but the desired macrocyclic product was not obtained. The free serine hydroxyl group seemed to affect the cyclization reaction. When we changed the position in which the Val-Thr dipeptide was introduced, hydroxyl group of serine



Scheme 2 Reagents and conditions: a) *n*-Bu_2BOTf, Et_3N, $C_{13}H_{27}CHO$; b) H_2O_2, LiOH; c) CH_2N_2, 89% for 3 steps; d) $LiBH_4$, MeOH, 99%; e) TBSCl, Et_3N, DMAP, 86%; f) Boc-Ser(Bn)-OH, DCC, HOBt, quant.; g) 3 N $HCl_{aq.}$/ THF, 92%; h) TPAP, NMO, 70%; i) **7**, -78 °C, 82%; j) O_3, Ph_3P, -78 °C, 85%; k) $NaClO_2$, NaH_2PO_4, 2-Methyl-2-butene, THF/ *t*-BuOH/ H_2O, quant.; l) H-Val-Thr-OBn (**9**), DCC, HOBt, 55%



Scheme 3



Scheme 4

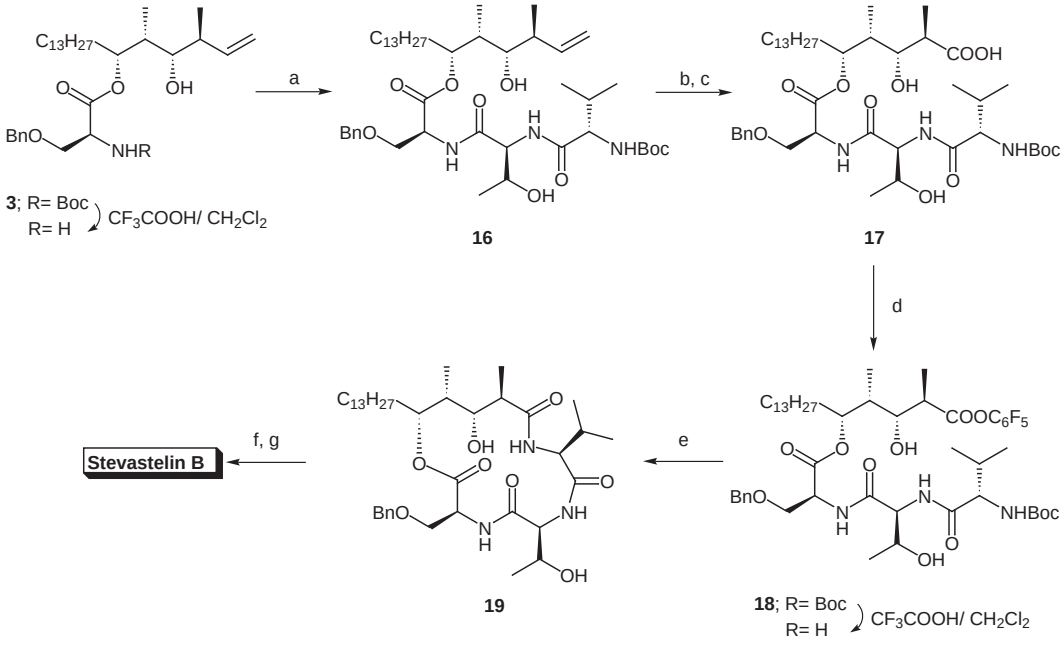
was successfully protected until the completion of macrolactamization.

Removal of the Boc group in homoallyl alcohol **3** followed by coupling with dipeptide **15** gave **16** (Scheme 5). Olefin **16** was oxidized to acid **17** by ozonolysis and $NaClO_2$-NaH_2PO_4 oxidation. Esterification of **17** with pentafluorophenol and DCC gave active ester **18**. Removal of the Boc group followed by macrolactamization in the presence of large excess of triethylamine gave the desired macrocyclic benzyl ether **19**.[10] Hydrogenation of **19** with $Pd(OH)_2$/C followed by selective acetylation of the primary hydroxyl group in the serine residue gave stevastelin B (**1**).[11] The ^1H and ^{13}C NMR spectra were coincident with those of the natural compound.

Thus, the total synthesis of stevastelin B has been achieved (18 steps, overall yield 3% from tetradecanal) and the present synthetic strategy of stevastelin B allows systematic syntheses of not only other natural stevastelins but also other unnatural analogues which may show activities of biological interest. The total synthesis of stevastelin B3, our next target, is now under course.

References and Notes

(1) Morino, T.; Masuda, A.; Yamada, M.; Nishimoto, M.; Nishikiori, T.; Saito, S.; Shimada, N. *J. Antibiotics* **1994**, *47*, 1341- 1343.



Scheme 5 Reagents and conditions: a) Boc-Val-Thr-OH (**15**), DCC, HOBt, quant.; b) O_3, Ph_3P, -78 °C, 87%; c) $NaClO_2$, NaH_2PO_4, 2-Methyl-2-butene, THF/ *t*-BuOH/ H_2O, 62%; d) C_6F_5OH, DCC, 66%; e) high dilution, Et_3N (20 equiv), 47% for 2 steps; f) $Pd(OH)_2$/ C, H_2, 56%; g) Ac_2O, pyridine, 81%.

(2) a) Morino, T.; Shimada, K.; Masuda, A.; Yamashita, N.; Nishimoto, M.; Nishikiori, T.; Saito, S. *J. Antibiotics* **1996**, *49*, 565- 568. b) Shimada, K.; Morino, T.; Masuda, A.; Sato, M.; Kitagawa, M.; Saito, S. *J. Antibiotics* **1996**, *49*, 569- 574.
(3) Hamaguchi, T,; Masuda, A.; Morino, T.; Osada, H. *Chem. Biol.* **1997**, *4*, 279- 286.
(4) a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127- 2129. b) Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099- 3111.
(5) a) Roush, W. R.; Ando, K.; Powers, D.B.; Palkowitz, A. D.; Halterman, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 6339- 6348. b) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990**, *112*, 6348- 6359. c) Scheidt, K. A.; Tasaka, A.; Bannister, T. D.; Wendt, M. D.; Roush, W. R. *Angew. Chem. Int. Ed.* **1999**, *38*, 1652- 1655.
(6) Soai, K.; Ookawa, A. *J. Org. Chem.* **1986**, *51*, 4000- 4005.
(7) a) Griffith, W. P.; Ley, S. V. *Aldrichimica Acta* **1990**, *23*, 13- 19. b) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625- 1627.
(8) Triol **12**: $[\alpha]_D^{21}$ +16.6 (*c* 1.0, $CHCl_3$); ^1H NMR δ_H (270 MHz, $CDCl_3$): 4.38 (1H, br s), 3.85-3.61 (5H, m), 3.37 (1H, br s), 2.85 (1H, br s), 1.89 (1H, m), 1.29-1.23 (24H, m), 0.91 (3H, t, *J* = 7.1), 0.87 (3H, d, *J* = 6.6), 0.75 (3H, d, *J* = 6.9); ^{13}C NMR δ_C (68 MHz, $CDCl_3$): 83.6, 77.8, 69.3, 37.9, 37.4, 35.6, 32.0, 29.7, 29.7, 29.4, 26.1, 22.8, 14.2, 13.3, 4.2; HRMS (FAB) calcd for $C_{20}H_{43}O_3$ $(M+H)^+$ 331.3212, found 331.3205.
(9) Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945- 948.
(10) Benzyl ether **19**: $[\alpha]_D^{25}$ -11.3 (*c* 1.2, $CHCl_3$); ^1H NMR δ_H (500 MHz, $CDCl_3$): 8.10 (1H, d, *J* = 7.3, N*H*), 7.49 (1H, d, *J* = 7.9, N*H*), 7.35-7.27 (5H, m, Ph), 4.99 (1H, m, C*H*OCO), 4.62 (1H, m, MeC*H*Me), 4.50 (2H, s, PhCH_2), 4.44 (1H, dd, *J* = 7.6, 2.8, COC*H*NH), 4.33 (1H, m, MeC*H*OH), 4.00 [1H, dd, *J* = 8.9, 4.0, MeCHC*H*(OH)CHMe], 3.88 (1H, dd, *J* = 9.2, 3.7, CHC*H*HOBn), 3.68 (1H, dd, *J* = 9.2, 3.7, CHCH*H*OBn), 3.28 (1H, m, BnOCH$_2$C*H*), 3.23 (1H, m, Me_2CHC*H*), 2.27 (1H, m, MeC*H*CO), 1.94 [1H, m, MeC*H*CH(OH)], 1.60 (1H, m, C*H*HCHOCO), 1.50 (1H, m, CH*H*CHOCO), 1.32 [3H, d, *J* = 7.3, CH(OH)*Me*CHCONH], 1.28-1.21 [18H, m, $(CH_2)_9$], 1.17 [3H, d, *J* = 6.1, *Me*CH(OH)CH], 1.08 [3H, d, *J* = 6.7, CHOCH*Me*CH(OH)], 0.98 (3H, d, *J* = 6.7, *Me*CHMe), 0.88 (3H, t, *J* = 7.0, CH_2*Me*), 0.84 (3H, d, *J* = 6.7, MeCH*Me*); ^{13}C NMR δ_C (126 MHz, $CDCl_3$): 175.4, 171.8, 171.1, 169.6, 137.0, 128.5, 128.0, 127.7, 80.4, 74.9, 73.5, 69.2, 66.6, 60.1, 56.5, 53.6, 49.8, 41.1, 31.9, 31.4, 30.9, 29.7, 29.6, 29.5, 29.4, 29.3, 25.6, 22.7, 19.5, 17.9, 16.1, 14.1, 12.5, 9.6; IR ν ($CHCl_3$): 3300, 2926, 2855, 2361, 1827, 1734, 1647, 1558, 1541, 1458, 1420, 1364, 1215, 1117, 1055, 984, 935, 872, 756, 696, 669; HRMS (FAB) calcd for $C_{39}H_{66}N_3O_8$ $(M+H)^+$ 704.4849, found 704.4825.
(11) Stevastelin B (**1**): $[\alpha]_D^{25}$ -18.1 (*c* 1.0, $CHCl_3$) (The rotation of the natural compound is not reported.); ^1H NMR δ_H (500 MHz, $CDCl_3$): 7.73 (1H, d, *J* = 7.9, N*H*), 7.34 (1H, d, *J* = 7.9, N*H*), 6.74 (1H, d, *J* = 6.7, N*H*), 5.01 (1H, m, C*H*OCO), 4.55 (1H, m, MeC*H*Me), 4.54 (1H, dd, *J* = 7.9, 3.7, COC*H*NH), 4.35 (1H, m, MeC*H*OH), 4.25 (1H, t, *J* = 6.7, COC*H*NH), 4.11 [1H, dd, *J* = 8.6, 3.7, MeCHC*H*(OH)], 3.95 (1H, dd, *J* = 11.6, 4.3, CHC*H*HOAc), 3.88 (1H, dd, *J* = 11.6, 4.3, CHCH*H*OAc), 3.32 (1H, m, COC*H*NH), 2.13 (1H, m, MeC*H*CONH), 2.04 (3H, s, MeCO), 1.97 [1H, m, MeC*H*CH(OH)], 1.37 (3H, d, *J* = 7.3, *Me*CHMe), 1.35-1.21 [24H, m, $(CH_2)_{12}$], 1.18 [(1H, d, *Me*CH(OH)CH], 1.10 (3H, d, *J* = 7.3, MeCH*Me*), 1.00-0.98 [6H, m, *Me*CHCH(OH)CH*Me*], 0.88 (3H, t, *J* = 6.7, CH_2*Me*); ^{13}C NMR δ_C (126 MHz, $CDCl_3$): 172.3, 172.0, 171.9, 170.7, 169.7, 80.7, 75.0, 66.7, 62.4, 60.1, 57.7, 55.4, 49.7, 40.9, 31.9, 31.3, 30.4, 29.7, 29.6, 29.5, 29.4, 29.3, 29.3, 25.6, 22.7, 19.3, 18.6, 18.4, 14.1, 12.5, 9.6; IR ν ($CHCl_3$): 3308, 3088, 2926, 2855, 2361, 1828, 1734, 1641, 1541, 1458, 1379, 1200, 1128, 1080, 986, 939, 870, 756, 667; HRMS (FAB) calcd for $C_{34}H_{62}N_3O_9$ $(M+H)^+$ 656.4486, found 656.4497.

Article Identifier:
1437-2096,E;2001,0,05,0694,0696,ftx,en;Y04501ST.pdf