A macrolactonization approach to the stevastelins

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Abstract—A synthesis of the stevastelins, a novel class of immunosuppressant agents, is reported based on a macrolactonization approach. This synthesis commenced with the stereoselective preparation of the stearic acid segment from tetradecanal using Evans asymmetric synthesis methodology and an aldol reaction with a thioester. After a high yielding coupling reaction between the fatty acid residue and the corresponding tripeptide, we proceeded with the macrolactonization key step. Thus, macrolactonizations of hydroxy acid 27 and dihydroxy acid 30, according to Yamaguchi conditions, afforded the corresponding 13-membered ring stevastelin derivatives 28 and 31 in 90 and 82% yields, respectively. In this latter case, the corresponding 15-membered lactone was not formed. Finally, depsipeptide derivative 31 was converted into stevastelin C3 (5). © 2002 Elsevier Science Ltd. All rights reserved.

Stevastelins A (1), B (2), A3 (3), B3 (4) and C3 (5) (Fig. 1), recently isolated from culture broths of Penicillium sp. NK374186,1,2 represent a new class of natural cyclic depsipeptides with intriguing biological properties as immunosuppressive agents. The great level of interest that immunosuppressants have elicited in the scientific community is clearly indicated by the immunosuppressive agents cyclosporin A3 or FK506,4 whose discoveries, biological evaluations and subsequent uses in medicine have led to a substantial increase in the success of organ and bone marrow transplantations. In addition to these relevant and outstanding applications in surgery, these compounds represent valuable biochemical tools for the investigation of signal transduction pathways at the molecular level.5 Therefore, immunosuppressants have occupied a prominent position in the forefront of total synthesis, chemical biology and medicine research and new natural products such as rapamycin,6 sanglifehrin A,7 tamandarins8 or pateamine A9 have also rapidly captured the attention of the chemical and biomedical communities on account of their broad range of immunosuppressant actions. Initially, the stevastelins were discovered as inhibitors of the IL-2 and IL-6 gene expressions. Thus, like cyclosporin A or FK506,10 they exhibited growth-inhibition activities against OKT3-stimulated human T cell proliferation with an IC₅₀ particularly striking for stevastelin B (2) of 1.8 μg/mL and for B3 (4) of 0.42

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Structures of Stevastelins A (1), B (2), A3 (3), B3 (4) and C3 (5).

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The synthesis of the peptide residue was achieved by conventional methods starting from the corresponding commercially available protected amino acids, which were coupled by standard solution phase synthesis of depsipeptides, as outlined in Scheme 2. Thus, after preparation of the dipeptide 21 in very good yield. Following the same synthetic sequence, the tripeptide 25 was prepared in high yields (Scheme 2). In this way, both key fragments, the acid 16 and the peptide 25, were ready for the coupling reaction.

This coupling was performed again by using EDCI and HOBt as coupling reagents, to obtain the amide 26 in 90% yield. The final step towards the corresponding hydroxy acid, prior to the final macrolactonization, was conducted with the cleavage of the allyl ester of 26 mediated by palladium(0) in the presence of morpholine\(^{18}\) to obtain the hydroxy acid 27. This hydroxy acid would give access to the 13-membered stevastelins, and, in fact, macrolactonization of this compound, under Yamaguchi conditions,\(^{19}\) afforded the corresponding depsipeptide 28\(^{10}\) in a very good yield (90%) (Scheme 3).

This methodology should provide a highly convergent route to other members of the stevastelins if we are to be able to accomplish the synthesis of the 15-membered macrolactone derivatives of the stevastelins starting from the same synthetic intermediates. Thus, we prepared the diol 29 by reaction with camphorsulfonic acid (CSA) in methanol in a moderate yield (60%) and, after

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\text{Scheme 1. Synthesis of the fatty acid residue of stevastelins, the stearic acid derivative (16). Reagents and conditions: (a) 1.1 equiv. } n\text{-BuBO}_{2}F, 1.1 \text{ equiv. Et}_{3}N, \text{ CH}_{2}Cl_{2}, 0^\circ C, 0.5 \text{ h, then 1.0 equiv. of 6, } -78^\circ C, 12 \text{ h, 80%}. (b) 4.0 \text{ equiv. LiBH}_{4}, \text{ THF, } 0^\circ C, 4 \text{ h, 95%}. (c) 1.1 \text{ equiv. PivCl, CH}_{2}Cl_{2}/\text{pyr.}, 0^\circ C, 2 \text{ h, 94%}. (d) 1.2 \text{ equiv. TBSCI, 1.5 equiv. imidazole, DMF, } 0^\circ C, 12 \text{ h, 98%}. (e) 2.1 \text{ equiv. Dibal-H, CH}_{2}Cl_{2}, -78^\circ C, 0.5 \text{ h, 98%}. (f) 2.0 \text{ equiv. (COCl)}_{2}, 2.5 \text{ equiv. DMSO, 4.0 equiv. Et}_{3}N, \text{ CH}_{2}Cl_{2}, -78^\circ C, 0.5 \text{ h, 98%}. (g) 1.5 \text{ equiv. of 14, 1.2 equiv. (Chx)BCl, 1.2 equiv. Et}_{3}N, \text{ CH}_{2}Cl_{2}, 0^\circ C, 2 \text{ h, then 1.0 equiv. of 13, } -78^\circ C, 12 \text{ h, 85%}. (h) 5.0 \text{ equiv. H}_{2}O, 5.0 \text{ equiv. LiOH, THF–H}_{2}O, 0^\circ C, 1 \text{ h, 85%}.}
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cleavage of the allyl ester, the resulting dihydroxy acid 30 was subjected to a macrolactonization reaction under Yamaguchi conditions. However, we obtained the 13-membered macrolactone 31\textsuperscript{21} as a single product (82%), not detecting any formation of the desired 15-membered cyclic depsipeptide. To address this result, we could include a protection step of the hydroxyl group at C-3 of 26, followed by the cleavage of the silyl ether of the hydroxyl group at C-5, to give an alternative substrate for the macrolactonization reaction. However, this strategy has already been attempted by Chakraborty et al.,\textsuperscript{22} who has recently reported an approach to the stevastelins wherein they describe no success with the modified strategy. With the 13-membered macrolactone of the stevastelins in hand, we undertook deprotection of the benzyl ethers of the depsipeptide 31 by the action of boron trichloride to obtain stevatelin C3 (5)\textsuperscript{23} (Scheme 4).

In conclusion, we have designed an approach to the stevastelins with the aim of delivering not only the natural compounds but also analogues thereof. Initial results have proven the convergence and efficiency of our synthetic approach towards 13-membered ring stevastelins; however, an efficient synthetic approach for the corresponding 15-membered rings has proved elusive. Thus, the next challenge to address in this research is to synthesize the corresponding 15-membered lactones, and, in fact, we are currently exploring different strategies to accomplish this goal and thereby give access to a broad family of stevastelins and analogues for further biological evaluations.

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equiv. Et₃N, THF, 0°C, 98%. (c) 1.3 equiv. of 2,4,6-trichlorobenzoyl chloride, 2.2 min, 82%. (d) 2.5 equiv. BCl₃, CH₂Cl₂, 0.1 equiv. 4-DMAP in toluene (0.005 M based on Pd[PPh₃]₄, 10.0 equiv. morpholine, THF, 0°C, CSA, MeOH, 0°C), furnished triol 32, which exhibited identical physical and spectroscopic properties as reported by Morino et al. (see Ref. 13), who obtained triol 32 by degradation of natural stevastatin B (2) during structural elucidation studies.

References

16. Reduction and silyl ether deprotection of the thioester 15 furnished triol 32, which exhibited identical physical and spectroscopic properties as reported by Morino et al. (see Ref. 13), who obtained triol 32 by degradation of natural stevastatin B (2) during structural elucidation studies.

![Scheme 4. Macrolactonization of dihydroxy acid (30). Synthesis of stevastatin C3 (5). Reagents and conditions: (a) 0.5 equiv. CSA, MeOH, 0→25°C, 3.0 h, 60%. (b) 0.1 equiv. Pd[PPh₃]₄, 10.0 equiv. morpholine, THF, 0→25°C, 0.5 h, 98%. (c) 1.3 equiv. of 2,4,6-trichlorobenzoyl chloride, 2.2 equiv. Et₃N, THF, 0°C, 1 h, then add to a solution of 2.2 equiv. 4-DMAP in toluene (0.005 M based on 30), 75°C, 10 min, 82%. (d) 2.5 equiv. BCl₃, CH₂Cl₂, ~78°C, 0.5 h, 90%.

![Compound 28: R₂=0.60 (silica gel, hexanes:AcOEt:MeOH, 15:4:1); ¹H NMR (400 MHz, DMSO-d₆)](image)
CDCl$_3$ δ 7.30–7.09 (m, 11H, CH aromatics, NH), 6.66 (d, 1H, $J = 8.8$ Hz, –NH), 6.60 (d, 1H, $J = 8.8$ Hz, –NH), 5.15 (dd, 1H, $J = 8.8, 2.3$ Hz, CHOC–O), 4.50–4.43 (m, 3H), 4.37–4.30 (m, 4H), 4.12 (dc, 1H, $J = 5.9, 2.3$ Hz), 3.79–3.73 (m, 1H), 3.68 (dd, 1H, $J = 7.6, 2.9$ Hz), 3.62–3.51 (m, 1H), 2.34 (dc, 1H, $J = 7.6$ Hz, 7.0 Hz, CH(CH$_3$)), 2.12 (ds, 1H, $J = 7.0$ Hz, CH(CH$_3$)), 1.70–1.57 (m, 1H), 1.50–1.32 (m, 1H), 1.25–1.07 (m, 27H, CH$_3$(CH$_2$)$_{12}$−, CH(C$_3$H$_3$)), 1.04 (d, 3H, $J = 7.0$ Hz, CH(CH$_3$)), 0.92 (d, 3H, $J = 7.0$ Hz, CH(CH$_3$)), 0.89 (d, 3H, $J = 7.0$ Hz, CH(CH$_3$)), 0.83–0.75 (m, 18H, SiC(CH$_3$)$_3$, CH(CH$_3$)$_2$), 0.01 (s, 3H, Si(CH$_3$)$_2$), 0.00 (s, 3H, Si(CH$_3$)$_2$); $^{13}$C NMR (50.3 MHz, CDCl$_3$) δ 175.7, 171.6, 160.0, 156.6, 146.1, 137.8, 137.7, 133.2, 129.5, 128.4, 128.3, 127.8, 127.7, 127.6, 121.3, 78.5, 74.7, 72.2, 71.4, 61.8, 58.6, 52.2, 44.3, 38.2, 35.2, 31.9, 31.1, 29.7, 29.6, 29.5, 29.3, 25.8, 25.4, 22.7, 19.3, 18.0, 17.8, 16.4, 14.3, 14.1, 5.8, −3.6, −4.6; FAB HRMS (NBA) m/e 908.6175, M+1 calcd for C$_{52}$H$_{85}$N$_3$O$_8$Si 908.6184.

21. Compound 31: $R_f = 0.60$ (silica gel, hexanes: AcOEt:MeOH, 12:7:1); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.31–7.10 (m, 11H, aromatic CH, NH), 6.76 (d, 1H, $J = 8.8$ Hz, NH), 6.44 (d, 1H, $J = 8.2$ Hz, NH), 5.15 (dd, 1H, $J = 6.5, 2.3$ Hz, CHOC–O), 4.51–4.43 (m, 3H), 4.38–4.28 (m, 4H), 4.12 (dc, 1H, $J = 5.9, 2.3$ Hz), 3.84 (dd, 1H, $J = 5.9, 2.3$ Hz), 3.83–3.71 (m, 2H), 2.41 (dc, 1H, $J = 7.6, 7.0$ Hz, CH(CH$_3$)), 2.20–2.11 (m, 1H), 1.65–1.55 (m, 1H), 1.50–1.31 (m, 1H), 1.28–1.11 (m, 24H, CH$_3$(CH$_2$)$_{12}$−, CH(CH$_3$)), 1.06 (d, 3H, $J = 7.0$ Hz, CH(CH$_3$)), 0.93 (d, 3H, $J = 7.0$ Hz, CH(CH$_3$)), 0.90 (d, 3H, $J = 6.5$ Hz, CH(CH$_3$)), 0.86–0.75 (m, 9H, CH(CH$_3$)$_2$, CH$_2$CH$_3$); $^{13}$C NMR (50.3 MHz, CDCl$_3$) δ 175.9, 171.5, 160.0, 156.5, 137.8, 137.7, 137.6, 133.1, 129.4, 128.4, 128.3, 127.8, 127.7, 127.6, 121.3, 78.5, 74.7, 72.2, 71.4, 61.8, 58.6, 52.2, 44.3, 38.2, 35.2, 31.9, 31.1, 29.7, 29.6, 29.3, 25.8, 22.7, 19.3, 18.0, 16.4, 14.7, 14.1, 4.9; FAB HRMS (NBA) m/e 794.5287, M+1 calcd for C$_{46}$H$_{71}$N$_3$O$_8$ 794.5319.


23. All new compounds exhibited satisfactory spectroscopic and analytical and/or accurate mass data. For the specific case of stevastelin C3 (5), spectroscopic data of natural stevastelin C3 were not reported by the Japanese group (see Ref. 1), and this compound is pending to be compared with an authentic sample of 5.