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# A macrolactonization approach to the stevastelins

Francisco Sarabia,* Samy Chammaa and F. Jorge López-Herrera

*Departamento de Bioquímica, Biología Molecular y Química Orgánica, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain*

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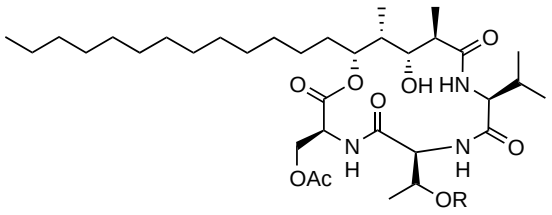
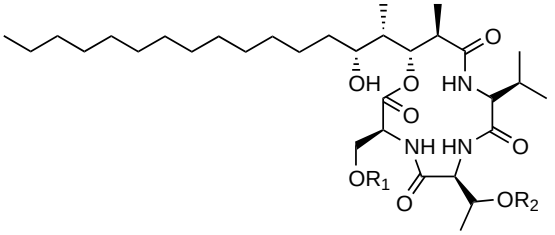
**Abstract**—A synthesis of the stevastelins, a novel class of immunosuppressant agents, is reported based on a macrolactonization approach. This synthesis commenced with the stereoselective preparation of the stearic acid segment from tetradecanal using Evans asymmetric synthesis methodology and an 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**). 

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 A[3] 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] tamandarins[8] or pateamine A[9] 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 $IC_{50}$ particularly striking for stevastelin B (**2**) of 1.8 μg/mL and for B3 (**4**) of 0.42



**Stevastelins** A (**1**): R = $SO_3H$

B (**2**): R = H



**Stevastelins** A3 (**3**): $R_1$ = Ac; $R_2$ = $SO_3H$

B3 (**4**): $R_1$ = Ac; $R_2$ = H

C3 (**5**): $R_1$ = H; $R_2$ = H

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

*Keywords*: stevastelin; immunosuppressant; natural products; cyclic depsipeptide; stereoselective synthesis; macrolactonization.

* Corresponding author. Tel.: +34-952134258; fax: +34-952131941; e-mail: frsarabia@uma.es

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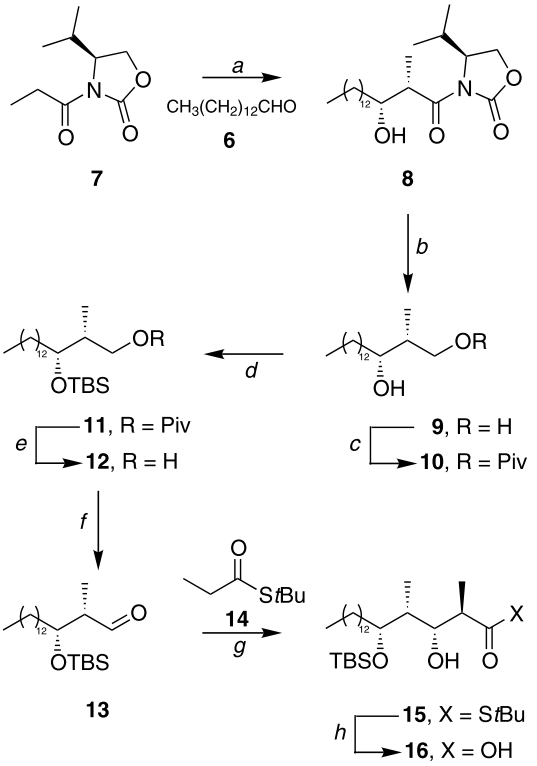
μg/mL. However, further biological investigations[11] revealed that these natural products, unlike the above mentioned immunosuppressants, did not inhibit the phosphatase activity of calcineurin. So it appears that the novel mechanism of immunosuppressive action that the stevastelins seem to be involved in remains unclear, and its elucidation could lead to the disclosure of new signal transduction pathways. These interesting and unprecedented biological findings, coupled with the fact that a total synthesis of the stevastelins has not been reported to date, make the stevastelins very attractive targets for chemists, biochemists and clinicians owing to their potential utility in medicine. Structurally,[12] the stevastelins are comprised of a family of 13- and 15-membered cyclic depsipeptides, characterized by two main frameworks: a stearic acid unit and a peptidic fragment that contains L-serine, L-threonine and L-valine which are connected by an ester and an amide linkage. In addition, the stearic acid moiety contains a tetrade fragment, whose absolute configuration has been established only for stevastelin B (**2**)[13] but no for other members of the family.

Thus, we decided to attempt the synthesis of these immunosuppressive agents, starting with the construction of the main structural units contained in the stevastelins: the stearic acid and the tripeptide derivative fragments. Scheme 1 outlines the synthesis of the first key important fragment. Accordingly, tetradecanal **6**, obtained by oxidation from the commercially available tetradecanol, was reacted with the chiral boron enolate of the oxazolidinone **7**, following Evans' asymmetric methodology,[14] to obtain in a 80% yield the *syn* aldol **8**. The conversion of this compound to the aldehyde **13** was achieved in five steps with excellent yields involving selective protections and deprotections through compounds **9**, **10**, **11** and **12** according to the synthetic sequence depicted in Scheme 1. In order to complete the synthesis of the stearic acid fragment, the *anti* aldol was required from aldehyde **13**. So, we proceeded with an aldol reaction of **13** with the boron enolate of *tert*-butyl thiopropionate **14**,[15] to obtain the desired compound **15** in good yield (85%) as the only diastereoisomer and containing the required relative configuration of the tetrade. Finally, thioester **15** was transformed into the acid **16** by reaction with hydrogen peroxide in the presence of lithium hydroxide in 85% yield. The correct absolute configuration of compound **16** was unequivocally confirmed by chemical methods.[16]

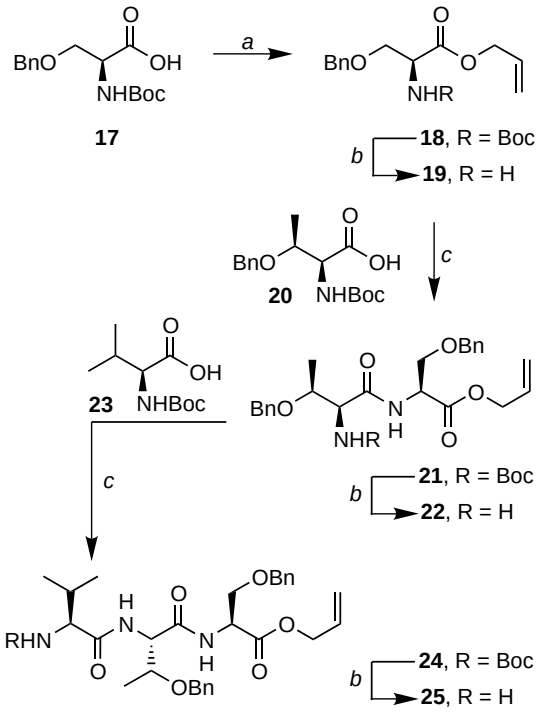
The synthesis of the peptide residue was achieved by conventional methods starting from the corresponding commercially available protected amino acids, which were coupled by standard solution phase synthesis of peptides,[17] as outlined in Scheme 2. Thus, after preparation of the allyl ester of the protected serine derivative **17**, the coupling with the threonine derivative **20** was carried out with *N*-ethyl-*N*′-[3-(dimethylamino)-propyl]carbodiimide (EDCI) in the presence of 1-hydroxybenzotriazole (HOBt) to obtain 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**[20] in a very good yield (90%) (Scheme 3).

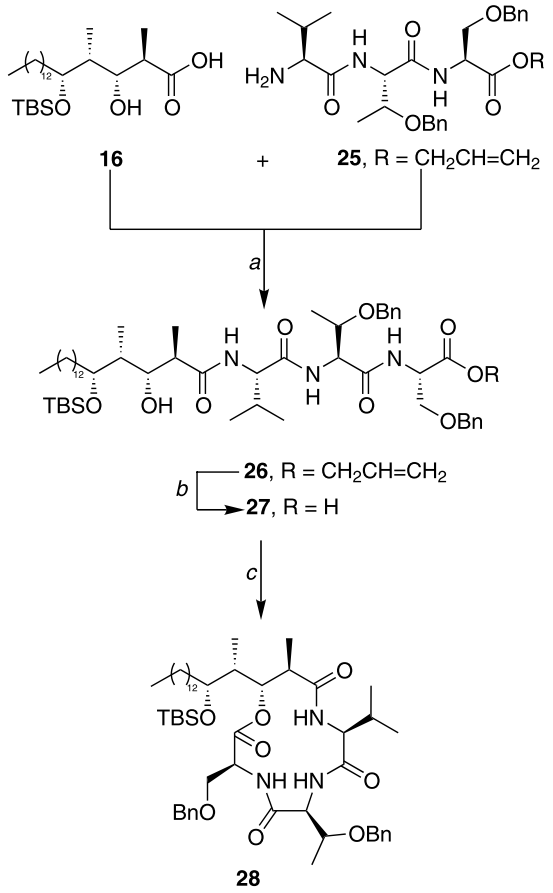
This methodology should provide a highly convergent route to other members of the stevastelins if we are to be able to accomplish the synthesis of the 15-membered macrolactone derivatives of the stevastelins starting from the same synthetic intermediates. Thus, we prepared the diol **29** by reaction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




**Scheme 1.** Synthesis of the fatty acid residue of stevastelins, the stearic acid derivative (**16**). *Reagents and conditions*: (a) 1.1 equiv. *n*-$Bu_2BOTf$, 1.1 equiv. $Et_3N$, $CH_2Cl_2$, 0°C, 0.5 h, then 1.0 equiv. of **6**, −78°C, 12 h, 80%. (b) 4.0 equiv. $LiBH_4$, THF, 0°C, 4 h, 95%. (c) 1.1 equiv. PivCl, $CH_2Cl_2$/pyr., 0°C, 2 h, 94%. (d) 1.2 equiv. TBSCl, 1.5 equiv. imidazole, DMF, 0°C, 12 h, 98%. (e) 2.1 equiv. DIBAL-H, $CH_2Cl_2$, −78°C, 0.5 h, 98%. (f) 2.0 equiv. $(COCl)_2$, 2.5 equiv. DMSO, 4.0 equiv. $Et_3N$, $CH_2Cl_2$, −78°C, 0.5 h, 98%. (g) 1.5 equiv. of **14**, 1.2 equiv. $(Chx)_2BCl$, 1.2 equiv. $Et_3N$, $CH_2Cl_2$, 0°C, 2 h, then 1.0 equiv. of **13**, −78°C, 12 h, 85%. (h) 5.0 equiv. $H_2O_2$, 5.0 equiv. LiOH, THF–$H_2O$, 0°C, 1 h, 85%.




**Scheme 2.** Synthesis of the peptide residue of stevastelins, the tripeptide (**25**). *Reagents and conditions*: (a) 1.5 equiv. EDCI, 0.1 equiv. 4-DMAP, 1.5 equiv. allylic alcohol, $CH_2Cl_2$, 25°C, 8 h, 88%. (b) 2.5 equiv. TFA, $CH_2Cl_2$, 25°C, 0.5 h, 98% for **19**, 98% for **22**, 98% for **25**. (c) 1.5 equiv. EDCI, 1.0 equiv. HOBt, $CH_2Cl_2$, 0→25°C, 0.5 h, 85% for **21**, 89% for **24**.




**Scheme 3.** Coupling of stevastelin fragments (**16**) and (**25**) and macrolactonization reaction. *Reagents and conditions*: (a) 1.5 equiv. EDCI, 1.0 equiv. HOBt, $CH_2Cl_2$, 0→25°C, 0.5 h, 90%. (b) 0.1 equiv. $Pd[PPh_3]_4$, 1.0 equiv. morpholine, THF, 0→25°C, 0.5 h, 98%. (c) 1.3 equiv. of 2,4,6-trichlorobenzoylchloride, 2.2 equiv. $Et_3N$, THF, 0°C, 1 h, then add to a solution of 2.2 equiv. 4-DMAP in toluene (0.005 M based on **27**), 75°C, 10 min, 90%.

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**[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 alternated substrate for the macrolactonization reaction. However, this strategy has already been attempted by Chakraborty et al.,[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**)[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.

## Acknowledgements

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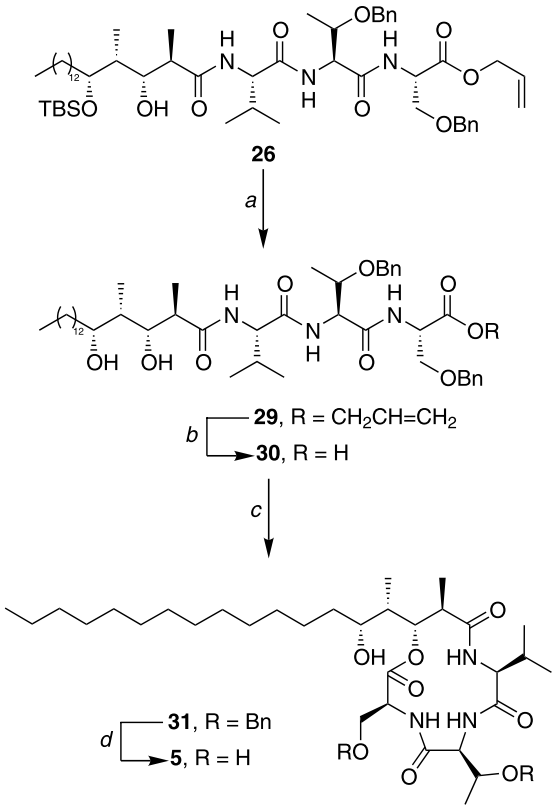


**Scheme 4.** Macrolactonization of dihydroxy acid (**30**). Synthesis of stevastelin C3 (**5**). *Reagents and conditions*: (a) 0.5 equiv. CSA, MeOH, 0→25°C, 3.0 h, 60%. (b) 0.1 equiv. $Pd[PPh_3]_4$, 10.0 equiv. morpholine, THF, 0→25°C, 0.5 h, 98%. (c) 1.3 equiv. of 2,4,6-trichlorobenzoylchloride, 2.2 equiv. $Et_3N$, 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. $BCl_3$, $CH_2Cl_2$, −78°C, 0.5 h, 90%.

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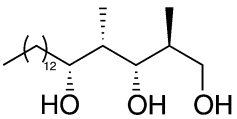
16. Reduction and silyl ether deprotection of the thioester **15** furnished triol **32**, which exhibited identical physical and spectroscopic properties as reported by Morino et al. (see Ref. 13), who obtained triol **32** by degradation of natural stevastelin B (**2**) during structural elucidation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



**32**

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20. **Compound 28**: $R_f = 0.60$ (silica gel, hexanes:AcOEt:MeOH, 15:4:1); $^1H$ NMR (400 MHz,

$CDCl_3$) $\delta$ 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, C*H*($CH_3$)), 2.12 (ds, 1H, $J=7.0$, 6.5 Hz, C*H*($CH_3$)), 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*H*$_3$)), 1.04 (d, 3H, $J=7.0$ Hz, CH(C*H*$_3$)), 0.92 (d, 3H, $J=7.0$ Hz, CH(C*H*$_3$)), 0.89 (d, 3H, $J=7.0$ Hz, CH(C*H*$_3$)), 0.83–0.75 (m, 18H, $SiC(CH_3)_3$, $CH_3(CH_2)_{12-}$, CH(C*H*$_3$)$_2$), 0.01 (s, 3H, $Si(CH_3)_2$), 0.00 (s, 3H, $Si(CH_3)_2$); $^{13}$C NMR (50.3 MHz, $CDCl_3$) $\delta$ 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, 77.2, 76.5, 74.7, 72.2, 71.3, 61.8, 58.3, 52.1, 44.2, 37.2, 34.8, 31.9, 31.2, 30.3, 29.8, 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 $C_{52}H_{85}N_3O_8Si$ 908.6184.

21. **Compound 31**: $R_f=0.60$ (silica gel, hexanes: AcOEt:MeOH, 12:7:1); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 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, C*H*($CH_3$)), 2.20–2.11 (m, 1H), 1.65–1.55 (m, 1H), 1.50–1.31 (m, 1H), 1.28–1.11 (m, 24H, $CH_3(CH_2)_{12-}$), 1.06 (d, 3H, $J=7.0$ Hz, CH(C*H*$_3$)), 0.93 (d, 3H, $J=7.0$ Hz, CH(C*H*$_3$)), 0.90 (d, 3H, $J=6.5$ Hz, CH(C*H*$_3$)), 0.86–0.75 (m, 9H, CH(C*H*$_3$)$_2$, C*H*$_3$CH$_2$); $^{13}$C NMR (50.3 MHz, $CDCl_3$) $\delta$ 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, 26.1, 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 $C_{46}H_{71}N_3O_8$ 794.5319.

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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**.