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Synthetic Studies on Stevastelins. 2. Synthesis of Lipidic- and Peptidic-Modified Analogues

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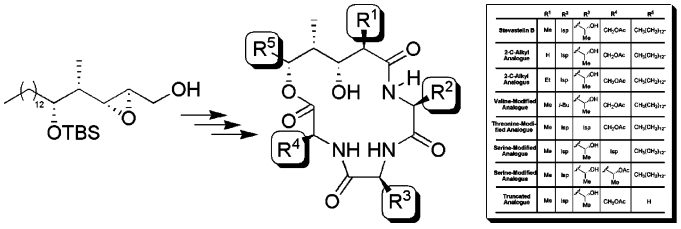
Received March 31, 2005



The synthesis of a series of stevastelin analogues with modification of the susbstituent at the C-2 position of the stearic acid chain (compounds **28** and **31**), variation of the amino acids (compounds **41**, **42**, **73**, and **78**), or lacking the lipidic chain (compound **91**) is described. The replacement of L-valine and L-threonine with other amino acids proceeded without difficulties for the synthesis of analogues **41** and **42**; however, the substitution of L-serine with simple amino acids, such as glycine or L-alanine, proved to be elusive, which was adscribed to factors of conformational flexibility. Finally, the substitution with L-valine or L-threonine proceeded without difficulties to provide the analogues **73** and **78** respectively.

Introduction

The preceding article in this issue,[1] detailed synthetic studies on the stevastelins, a new class of [13]- and [15]-macrocyclic depsipeptides with intriguing immunosuppressive properties,[2] that culminated with the total synthesis of stevastelin B3[3,4] following the synthetic strategy delineated by Chida's group,[4b] which proved to be the most effective, and stevastelin B3[5] via a translactonization reaction from the [15]-membered ring derivative. In recapping our research studies involving the search for new stevastelin-type immunosuppressive agents, we decided to undertake the preparation of a series of analogues. Taking into consideration previous studies on the biological properties of the truncated stevastelins,[6] which lack the lipidic chain, and the data revealing that this structural component was essential for the immunosuppressive activity, we deemed it of importance to ascertain the biological value of the substituents R^1−R^4 along the lipidic and peptidic fragments. To explore these variations, a primary set of analogues capable of elucidating the biological relevance of the 2-C-alkyl substituent and the amino acids was designed (Scheme 1). Accordingly, the synthetic strategy utilized by Chida's group[4b] proved to be the most appropriate route for the preparation of the stevastelin analogues through a convergent and flexible synthesis that permits the facile modification of key structural elements by the incorporation of the appropriate building blocks along a common synthetic sequence, utilizing the epoxyalcohol

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(1) See the preceding article on this issue: Sarabia, F.; Chammaa, S. *J. Org. Chem.* **2005**, *70*, 7846−7857.

(2) For isolation of stevastelins, see: (a) Morino, T.; Masuda, A.; Yamada, M.; Nishimoto, M.; Nishikiori, T.; Saito, S.; Shimada, N. *J. Antibiot.* **1994**, *47*, 1341−1343. For structural determination, see: (b) Morino, T.; Shimada, K.-I.; Masuda, A.; Nishimoto, M.; Saito, S. *J. Antibiot.* **1996**, *49*, 1049−1051. For biological properties, see: (c) Morino, T.; Shimada, K.-I.; Masuda, A.; Noriyuki, Y.; Nishimoto, M.; Nishikiori, T.; Saito, S. *J. Antibiot.* **1996**, *49*, 564−568. (d) Shimada, K.-I.; Morino, T.; Masuda, A.; Sato, M.; Kitagawa, M.; Saito, S. *J. Antibiot.* **1996**, *49*, 569−574.

(3) For synthetic approaches of [15]-membered stevastelins, see: (a) Chakraborty, T. K.; Ghosh, S.; Dutta, S. *Tetrahedron Lett.* **2001**, *42*, 5085−5088. (b) Sarabia, F.; Chammaa, S.; Sánchez-Ruiz, A.; López-Herrera, F. J. *Tetrahedron Lett.* **2003**, *44*, 7671−7675.

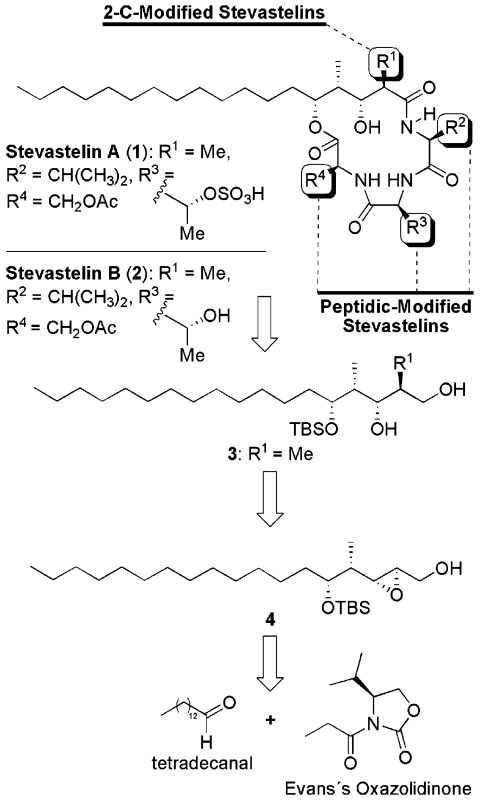
(4) For total synthesis of stevastelin B, see: (a) Kohyama, N.; Yamamoto, Y. *Synlett* **2001**, 694−696. (b) Kurosawa, K.; Nagase, T.; Chida, N. *Chem. Commun.* **2002**, 1280−1281.

(5) For synthetic approaches of [13]-membered stevastelins, see: (a) Sarabia, F.; Chammaa, S.; López-Herrera, F. J. *Tetrahedron Lett.* **2002**, *43*, 2961−2965. For total synthesis of stevastelin B3, see: (b) Kurosawa, K.; Matsuura, K.; Chida, N. *Tetrahedron Lett.* **2005**, *46*, 389−392. (c) Chakraborty, T. K.; Ghosh, S.; Laxman, P.; Dutta, S.; Samanta, R. *Tetrahedron Lett.* **2005**, *46*, 5447−5450.

(6) Hamaguchi, T.; Masuda, A.; Morino, T.; Osada, H. *Chem. Biol.* **1997**, *4*, 279−286.

10.1021/jo050628y CCC: $30.25
Published on Web 08/30/2005

SCHEME 1. Structures of Stevastelins A (1) and B (2) and Retrosynthetic Program for Stevastelin B Analogues

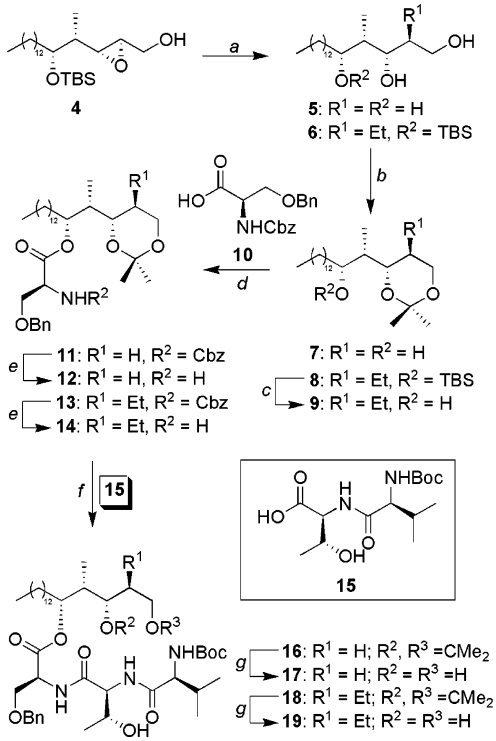


4 as the common intermediate for delivery of analogues, as mentioned in the previous article.

Results and Discussion

Synthesis of 2-C-Alkyl Stevastelin Analogues. The synthesis of the 2-C-alkyl-modified analogues commenced with the treatment of epoxyalcohol **4**[1] with various nucleophiles, including red-Al[7] and ethylmagnesium bromide/CuI,[8] to yield the oxirane ring-opening products **5** and **6**, respectively, noting that for **5** the oxirane ring opening was accompanied with TBS deprotection.[9] Protection of the 1,3-diol as the acetal, followed by TBS deprotection for **8**, provided the corresponding alcohols **7** and **9** in good yields. The couplings of these alcohols with the L-serine derivative **10** was accomplished by use of the Yamaguchi protocol,[10] to furnish the esters **11** and **13** in a 75% average yield but with 5−8% epimerization at C-2 of the serine residue, as was observed with the esterification of the corresponding esteric fragment contained in the natural compound. The introduction of the rest of the peptidic chain was undertaken under similar conditions as described in the previous article, by coupling of amines **12** and **14** with dipeptide **15**, to obtain acyclic depsipeptides **16** and **18**. The macrocyclization

SCHEME 2. Synthesis of the Acyclic Precursors 17 and 19 of Stevastelin Analogues 28 and 31[a]



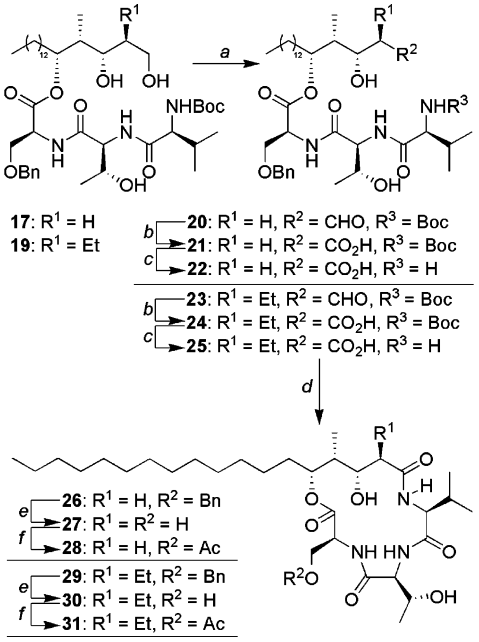
[a] Reagents and Conditions: (a) i. 2.2 equiv of red-Al, THF, 0 → 25 °C, 18 h, 80% for **5**; ii. 2.0 equiv of CuI, 6.0 equiv of 1.0 M EtMgBr, −20 °C, 3 h, 97% for **6**. (b) 3.0 equiv of $Me_2C(OMe)_2$, 0.05 equiv of CSA, DMF, 0 °C, 2 h, 57% for **7** plus 24% for the 3,5-acetal derivative, 93% for **8**. (c) 3.0 equiv of TBAF, THF, 25 °C, 4 days, 78% from **4**. (d) 6.4 equiv of **10**, 9.0 equiv of 2,4,6-$Cl_3C_6H_2COCl$, 9.0 equiv of Et_3N, THF/toluene, 0 °C, 1.5 h, then 0.5 equiv of 4-DMAP, 0 °C, 2.5 h, 86% (6:1 epimeric mixture) for **11**, 70% (6:1 epimeric mixture) for **13**. (e) 0.1 equiv of 10% Pd/C−ethylenediamine complex, H_2, MeOH, 25 °C, 0.5 h. (f) 1.5 equiv of **15**, 1.5 equiv of HOBt, 2.6 equiv of EDCI, DMF, 25 °C, 0.5 h, 80% for **16** from **11**, 80% for **18** from **13**. (g) AcOH/H_2O, THF, 25 °C, 18 h, 83% for **17**, 81% for **19**.

process was initiated with the acetal cleavage of **16** and **18** (Scheme 2) and selective oxidation of the primary hydroxyl group by the sequential action of TEMPO/NaClO[11] and $NaClO_2$ of the resulting diols **17** and **19** to the acids **21** and **24** through aldehydes **20** and **23**, respectively (Scheme 3).

With the acyclic stevastelin precursors **21** and **24** in hand, we proceeded with the macrocyclization reactions[12] by treatment of the resulting seco amino acids **22** and **25**, from the Boc deprotection of **21** and **24**, with diethyl cyanophosphonate (DECP)[13] as previously described for natural stevastelin B, to furnish the macrocyclic depsipeptides **26** and **29** in 23% and 33% yields from diols **17** and **19**, respectively. The preparation of the final 2-C-alkyl analogues was accomplished in two steps, including

(7) Ma, P.; Martin, V. S.; Masamune, S.; Sharpless, K. B.; Viti, S. M. *J. Org. Chem.* **1982**, *47*, 1378−1380.
(8) Nicolaou, K. C.; Oshima, T.; Murphy, F.; Barluenga, S.; Xu, J.; Winssinger, N. *Chem. Commun.* **1999**, 809−810.
(9) Rajashekhar, B.; Kaiser, E. T. *J. Org. Chem.* **1986**, *51*, 1625−1627.
(10) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989−1993.
(11) Hansen, T. M.; Florence, G. J.; Lugo-Mas, P.; Chen, J.; Abrams, J. N.; Forsyth, C. J. *Tetrahedron Lett.* **2003**, *44*, 57−59.
(12) (a) Hale, K. J.; Bhatia, G. S.; Frigerio, M. In *The Chemical Synthesis of Natural Products*; Hale, K. J., Ed.; Sheffield Academic Press: Sheffield, 2000; pp 349−415. (b) Sarabia, F.; Chammaa, S.; Sánchez-Ruiz, A.; Martín-Ortiz, L.; López-Herrera, F. J. *Curr. Med. Chem.* **2004**, *11*, 1309−1332.
(13) (a) Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595−1598. (b) Takuma, S.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147−3153.

SCHEME 3. Total Synthesis of 2-C-Alkyl Stevastelin Analogues 28 and 31[a]

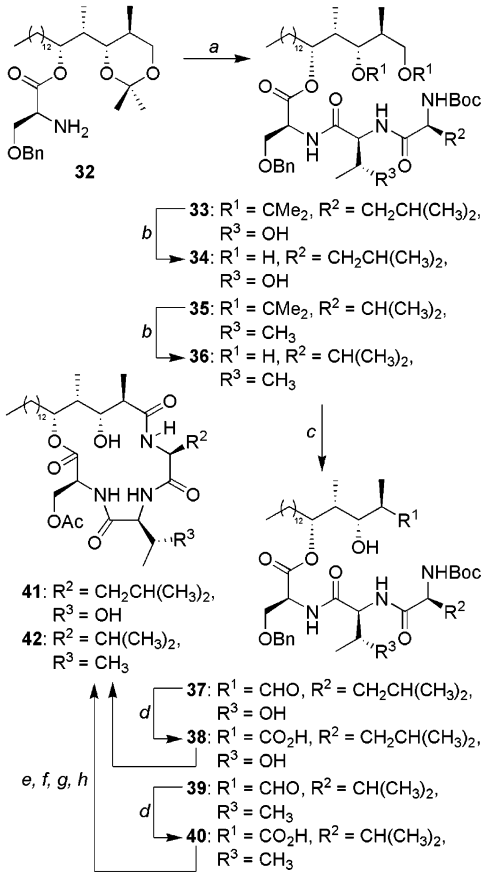


[a] Reagents and Conditions: (a) 0.01 equiv of TEMPO, 0.1 equiv of KBr, 15.0 equiv of $NaHCO_3$, 2.4 equiv of NaClO, CH_2Cl_2/H_2O, 0 °C, 1 h. (b) 5.0 equiv of $NaClO_2$, 4.0 equiv of NaH_2PO_4, 87.0 equiv of 2-methyl-2-butene, *t*-BuOH/H_2O, 25 °C, 10 min. (c) TFA, CH_2Cl_2, 0 °C, 1 h. (d) 4.9 equiv of DEPC, 5.4 equiv of Et_3N, DMF (1.0 mM based on triols **17** and **19**), 0 → 25 °C, 23% for **26** from **17**, 33% for **29** from **19**. (e) H_2, 10% Pd/C, MeOH, 25 °C, 2 h. (f) 4.3 equiv of Ac_2O, pyridine, 0 °C, 2 h, 17% for **28** from **17**, 33% for **31** from **19**.

debenzylation and selective monoacetylation to yield the 2-demethyl and 2-ethyl stevastelin analogues **28** and **31** (Scheme 3) in 1.0% and 3.0% overall yields in 21 and 22 steps, respectively, from Evans's oxazolidinone (see Scheme 1).[14]

Synthesis of Peptidic-Modified Stevastelins. The same route was projected for the preparation of the peptidic-modified stevastelins, replacing the alkyl side chains present in the natural stevastelins by groups that may elucidate the biological role of the different R^2, R^3, and R^4 groups. In particular, the exchange of the isopropyl, hydroxyl, and hydroxymethyl groups corresponding to the valine, threonine, and serine residues, respectively, by isobutyl, methyl, and hydrogen groups was considered as suitable changes to identify the effect of these amino acid substituents on the biological activities. In this context, the synthesis of the valine- and threonine-modified analogues commenced from the advanced intermediate **32**,[1] which was coupled with the corresponding dipeptides Boc-Leu-Thr(OH)-OH[15] and Boc-Val-Val-OH,[16] by the action of EDCI/HOBt,[17] to afford compounds **33** and **35** in 99% and 83% yields, respectively. Proceeding in a similar manner as before for compounds **22** or

SCHEME 4. Synthesis of the Peptidic-Modified Stevastelins 41 and 42[a]



[a] Reagents and Conditions: (a) 0.55 equiv of Boc-Leu-Thr-OH or 0.55 equiv of Boc-Val-Val-OH, 1.07 equiv of HOBt, 1.6 equiv of EDCI, DMF, 25 °C, 0.5 h, 99% for **33** and 83% for **35**. (b) AcOH/H_2O, THF, 25 °C, 18 h. (c) 0.02 equiv of TEMPO, 0.1 equiv of KBr, 15.0 equiv of $NaHCO_3$, 3.0 equiv of NaClO, CH_2Cl_2/H_2O, 0 °C, 1.5 h. (d) 2.0 equiv of $NaClO_2$, 2.8 equiv of NaH_2PO_4, 100.0 equiv of 2-methyl-2-butene, *t*-BuOH/H_2O, 25 °C, 15 min. (e) 5.0 equiv of TFA, CH_2Cl_2, 0 °C, 2 h. (f) 5.0 equiv of DEPC, 5.5 equiv of Et_3N, DMF (1.0 mM based on **33** and **35** respectively), 0 → 25 °C, 48 h. (g) i. H_2, 10% Pd/C, MeOH, 25 °C, 2 h for **41**; ii. H_2, 10% $Pd(OH)_2$, MeOH, 25 °C, 4 h for **42**. (h) 4.3 equiv of Ac_2O, pyridine, 0 °C, 2−3 h, 9% for **41** from **33**, 18% for **42** from **35**.

25, the acyclic acids **38** and **40** were efficiently synthesized through diols **34** and **36** and aldehydes **37** and **39**. From these acids, the synthesis of the peptidic analogues **41** and **42** was achieved by implementation of the same synthetic sequence as that described for stevasletin B (**5**), with no significant difficulties along the synthetic route, to give the stevastelin analogues **41** and **42** in 9% and 18% overall yields from **33** and **35**, respectively (Scheme 4).

The replacement of the L-serine residue with another amino acid was our next objective in this investigation

(14) The overall yield of the total synthesis for all new stevastelin analogues is referred to the starting oxazolidinone described in the preceding article, according to Evans methodology: (a) Evans, D. A. *Aldrichimica Acta* **1982**, *15*, 318−327. (b) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127−2129. (c) Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737−1739. (d) Evans, D. A.; Mathre, D. J.; Scott, W. L. *J. Org. Chem.* **1985**, *50*, 1830−1835. (e) Evans, D. A.; Scott, J. M.; Ennis, M. D. *J. Org. Chem.* **1993**, *58*, 471−485.

(15) Dipeptide Boc-Leu-Thr(OH)-OH was prepared from commercially available Boc-Thr(OH)-OH and Boc-Leu-OH through the allyl ester of L-threonine (H-Thr(OH)-Oallyl), which was coupled with Boc-Leu-OH by treatment with EDCI/HOBt in a 72% overall yield from Boc-Thr(OH)-OH. Finally, the dipeptide derivative Boc-Leu-Thr(OH)-OAllyl was transformed into the acid by the action of $Pd[PPh_3]_4$/NMO.

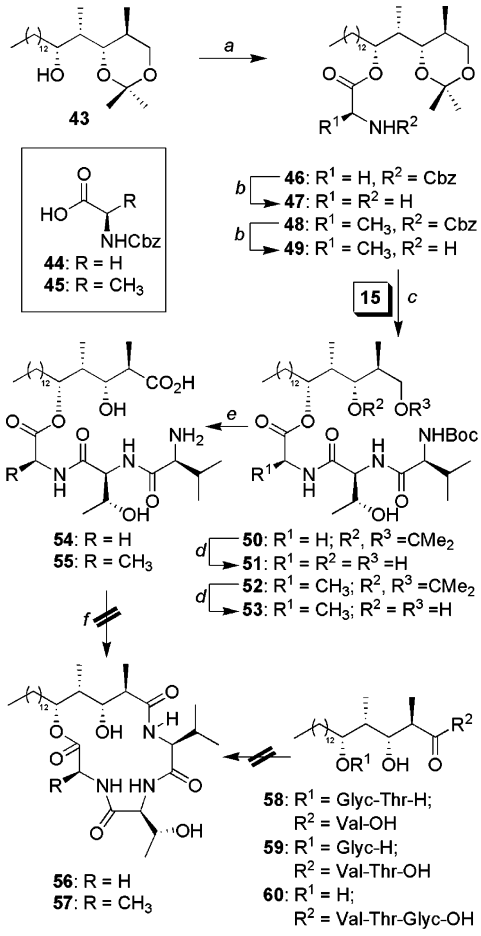
(16) Uccello-Barretta, G.; Luliano, A.; Menicagli, R.; Peluso, P.; Pieroni, E.; Salvadori, P. *Chirality* **1997**, *9*, 113−121.

(17) Gao, X.-M.; Ye, Y.-H.; Bernd, M.; Kutscher, B. *J. Pept. Sci.* **2002**, *8*, 418−430.

to complete the first set of representative stevastelin analogues for structure−activity relationship (SAR) studies. As indicated before, we deemed of interest the removal or the replacement of the acetyloxymethylene group of L-serine by other groups, leading us to consider the amino acids glycine or L-alanine as the most appropriate candidates. Thus, coupling of alcohol **43** with amino acid derivatives **44** and **45** was undertaken according to the Keck procedure[18] to afford the corresponding esters **46** and **48** in 85% and 70% yields, respectively. The cleavage of the *N*-Cbz group of these products by hydrogen in the presence of Pd/C−ethylendiamine complex catalyst[19] was followed by the linkage to the dipeptide **15**, delivering the tripeptide derivatives **50** and **52**, respectively. The preparation of these compounds for the macrocyclization process was conducted using the same synthetic sequence as that for the previously described analogues, with no difficulties encountered during the acetal hydrolysis, to provide triols **51** and **53**, followed by selective oxidation and Boc cleavage, yielding the acyclic depsipeptide precursors **54** and **55**. The macrocyclization reactions were performed under similar conditions as previously described, but unfortunately, these macrolactamization reactions did not proceed as desired, resulting in a complex mixture of products, without the formation of the cyclized products **56** and **57**. Similarly, unsuccessful results were encountered when we attempted all the possible macrocyclizations for the glycine-modified stevastelin analogue, from the other three possible acyclic precursors **58**, **59**, and **60** (Scheme 5). Presumably, the large degree of conformational flexibility that these precursors possess, as a consequence of the replacement of the bulky benzyloxymethyl group of the L-serine amino acid residue by the more flexible glycine or L-alanine, could explain these disappointing results in the macrocyclization reactions.[20]

In light of these discouraging although interesting results, we opted to introduce L-valine and L-threonine moeities instead of a L-serine residue, with the goal of demonstrating the importance of the conformational flexibility for the macrocyclization reaction, as well as preparing analogues to be considered for biological evaluations. Thus, esters **61** and **63**, prepared by esterification of alcohol **43** with the commercially available amino acids derivatives *Z*-Val-OH and *Z*-Thr(Bn)-OH via the Yamaguchi and Keck procedures, respectively, were subjected to chemical modifications, in a similar way as before, through derivatives **62**, **65**, **66**, and **69** for the L-valine-modified analogue and **64**, **67**, **68**, and **71** for the L-threonine derivative, to yield the corresponding acyclic precursors **70** and **72**. To our delight, Boc deprotections of **70** and **72** followed by macrocyclizations of their resulting ammonium trifluoroacetate salts under the action of DEPC, furnished the cyclic depsipeptides **73** and **74** in reasonably good yields (30% overall yield from **65** for **73** and 25% overall yield from **67** for **74**) as compared to previous macrolactamizations. While the stevastelin analogue **73** was already accomplished, the preparation of the stevastelin analogue **78** required additional steps for completion, which were carried out without difficulty through a sequence of selective protection−deprotection reactions, including silylation of **74**, debenzylation of the resultant silyl ether **75**, acetylation, and final desilylation of the acetate **77**, completing the synthesis in 24 steps for the longest linear sequence and 2.0% overall yield from the Evans's oxazolidinone (see Schemes 1 and 6).

SCHEME 5. Toward Serine-Modified Stevastelin Analogues[a]



[a] Reagents and Conditions: (a) 2.0 equiv of **44** or **45**, 2.0 equiv of DCC, 1.0 equiv of 4-DMAP, 0.5 equiv of CSA, CH_2Cl_2, 25 °C, 0.5 h, 85% for **46**, 70% for **48**. (b) 0.1 equiv of 10% Pd/C−ethylenediamine complex, H_2, MeOH, 25 °C, 15 min. (c) 1.5 equiv of **15**, 1.7 equiv of HOBt, 2.8 equiv of EDCI, DMF, 25 °C, 0.5 h, 72% for **50** from **46**, 62% for **52** from **48**. (d) AcOH/H_2O, 25 °C, 14 h, 91% for **51**, 99% for **53**. (e) Exact conditions as steps a, b, and c in Scheme 3, 75% for **54**, 83% for **55**. (f) 5.0 equiv of DEPC, 5.5 equiv of Et_3N, DMF, 25 °C, 20−72 h, decomposition in both cases.

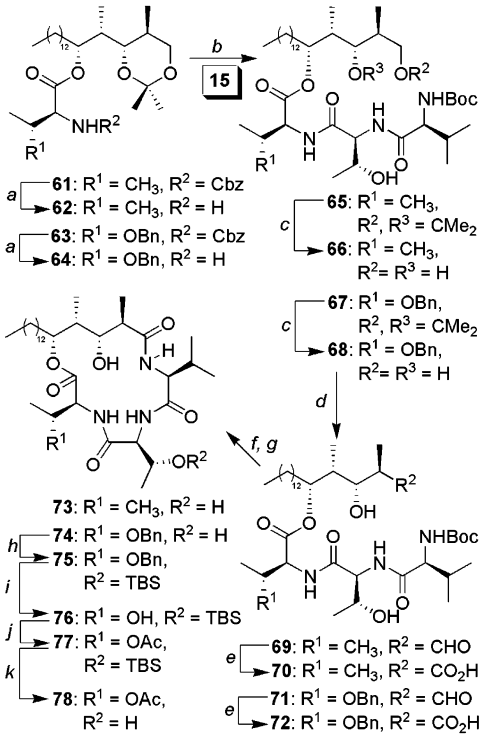
Synthesis of Truncated Stevastelins. Finally, we decided to examine the influence of the lipidic chain in the biological properties of stevastelins. Despite the fact that truncated stevastelins proved to be inactive, demonstrating that the lipidic chain seemed to be essential for their biological activities,[6] it was not demonstrated, however, if the reason for this lack of activity was caused exclusively by the absence of the lipidic chain or by the combination of the lack of either the lipidic chain as the

(18) (a) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394−2395. (b) Kiho, T.; Nakayama, M.; Kogen, H. *Tetrahedron* **2003**, *59*, 1685−1697.

(19) Sajiki, H.; Hattori, K.; Hirota, K. *J. Org. Chem.* **1998**, *63*, 7990−7992.

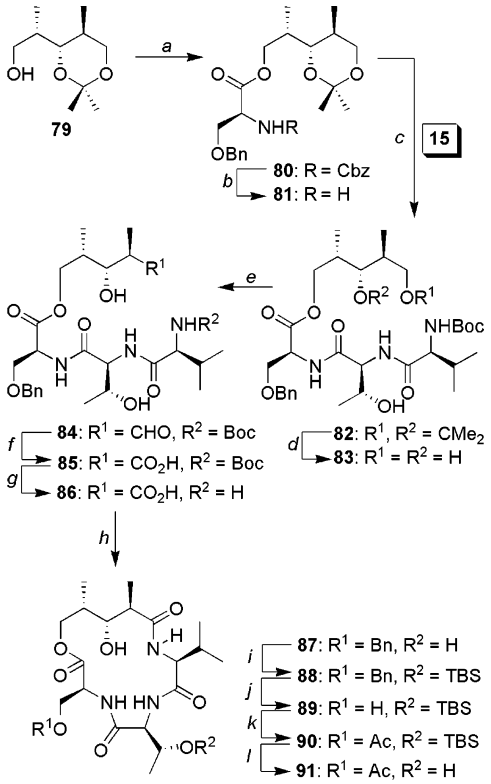
(20) Glycine residues contained in linear peptidic chains are known to enhance cyclization processes, see: (a) Kessler, H.; Hass, B. *Int. J. Pept. Protein Res.* **1993**, *39*, 36. Despite this, we found in the literature examples of glycine-containing peptides which did not cyclize via a macrolactamization reaction. See: (b) Jeremic, T.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2004**, *87*, 3056−3079.

SCHEME 6. Synthesis of the Peptidic-Modified Stevastelins 73 and 78[a]



[a] Reagents and Conditions: (a) 0.1 equiv of 10% Pd/C−ethylenediamine complex, H_2, MeOH, 25 °C, 15 min, 90% for **62**, 97% for **64**. (b) 1.2 equiv of **15**, 1.2 equiv of HOBt, 1.8 equiv of EDCI, DMF, 25 °C, 0.5 h, 51% from **61** for **65**, 83% from **63** for **67**. (c) AcOH/H_2O, THF, 25 °C, 14 h, 99% for **66** and **68**. (d) 0.02 equiv of TEMPO, 0.1 equiv of KBr, 20.0 equiv of $NaHCO_3$, 3.0 equiv of NaClO, CH_2Cl_2/H_2O, 0 °C, 0.5 h, 88% for **69** and **71**. (e) 2.5 equiv of $NaClO_2$, 2.0 equiv of NaH_2PO_4, 96.0 equiv of 2-methyl-2-butene, *t*-BuOH/H_2O, 25 °C, 15 min, 96% for **70**, 99% for **72**. (f) 5.0 equiv of TFA, CH_2Cl_2, 0 °C, 1 h, 99%. (g) 5.0 equiv of DEPC, 5.5 equiv of Et_3N, DMF (1.0 mM based on **65** and **67**), 0 → 25 °C, 20 h, 30% for **73** from **65**, 25% for **74** overall yield from **67**. (h) 1.3 equiv of TBSOTf, 2.0 equiv of 2,6-lutidine, CH_2Cl_2, 0 °C, 15 min. (i) H_2, 10% $Pd(OH)_2$, MeOH, 25 °C, 0.5 h. (j) 4.3 equiv of Ac_2O, 4-DMAP cat., pyridine, 0 °C, 1 h. (k) HF·pyridine (excess), THF, 0 °C, 0.5 h, 36% overall yield from **74**.

SCHEME 7. Synthesis of the Truncated Stevastelin 91[a]



[a] Reagents and Conditions: (a) 1.5 equiv of **10**, 1.5 equiv of EDCI, 0.1 equiv of 4-DMAP, CH_2Cl_2, 0 °C, 1 h, 80%. (b) 0.1 equiv of 10% Pd/C−ethylenediamine complex, H_2, MeOH, 25 °C, 15 min, 99%. (c) 1.1 equiv of **15**, 1.1 equiv of HOBt, 1.6 equiv of EDCI, DMF, 25 °C, 0.5 h, 72%. (d) AcOH/H_2O, THF, 25 °C, 18 h. (e) 0.02 equiv of TEMPO, 0.1 equiv of KBr, 15.0 equiv of $NaHCO_3$, 3.0 equiv of NaClO, CH_2Cl_2/H_2O, 0 °C, 1.5 h, 97%. (f) 2.0 equiv of $NaClO_2$, 2.8 equiv of NaH_2PO_4, 100.0 equiv of 2-methyl-2-butene, *t*-BuOH/H_2O, 25 °C, 15 min, 90%. (g) 5.0 equiv of TFA, CH_2Cl_2, 0 °C, 2 h. (h) 5.0 equiv of DEPC, 5.5 equiv of Et_3N, DMF (1.0 mM based on **82**), 0 → 25 °C, 48 h, 30% overall yield from **82**. (i) 1.3 equiv of TBSOTf, 2.0 equiv of 2,6-lutidine, CH_2Cl_2, 0 °C, 5 min. (j) H_2, 10% $Pd(OH)_2$, MeOH, 25 °C, 0.5 h. (k) 10.0 equiv of Ac_2O, 0.02 equiv of 4-DMAP, pyridine, 0 °C, 3 h. (k) HF·pyridine (excess), THF, 0 °C, 0.5 h, 56% overall yield from **88**.

tetrad system contained at C-2/C-4 positions. At this juncture, we chose analogue **91** in order to clarify such uncertainty. The synthesis, outlined in Scheme 7, commenced with the advanced precursor **79**[21] and proceeded in a synthetic course identical to that for previous analogues that afforded in an efficient way the coveted analogue **91**, through compounds **80**−**90** without major difficulties along its synthesis route (Scheme 7).

Conclusions

In conclusion, we have described the synthesis of a series of stevastelin analogues, in which either the 2-C-methyl group was replaced by another alkyl group, the amino acids contained in the structure of the natural substance were substituted by other residues, or the lipidic chain was removed. The resultant series of stevastelin analogues **28**, **31**, **41**, **42**, **73**, **78**, and **91** represent an interesting set of compounds capable of providing essential information about the structure−activity relationships for the stevastelin family. Chemically, we have demonstrated that the route designed by the Chida group proved to be the most adequate to reach the stevastelin group of cyclic depsipeptides. However, the conformational flexibility of the acyclic precursors seems to be essential for a successful macrocyclization reaction, thus concluding that this conformational restriction represents a significant hurdle for the implementation of the synthetic strategy for the synthesis of a library of amino acid-modified stevastelins. From the biological point of view, preliminary inhibition studies against different phosphatases (VHR, CD45, and PP2B) revealed a notable decrease of activity for all these analogues compared with stevastelin B (IC_{50} = 19.8 μM against VHR), providing interesting information about the highly specific binding to the VHR receptor. Further biological investigations, including inhibition studies of T-cell proliferation, of these and other related compounds

(21) (a) Ziegler, F. E.; Kneisley, A.; Thottathil, J. K.; Wester, R. T. *J. Am. Chem. Soc.* **1988**, *110*, 5434−5442. (b) Horita, K.; Oikawa, Y.; Yonemitsu, O. *Chem. Pharm. Bull.* **1989**, *37*, 1698−1704.

are in progress, which may represent high interest for the discovery and design of new potential therapeutic agents.[22]

Experimental Section

Acyclic Depsipeptides 22 and 25. General Procedure. To a solution of the crude dihydroxyacid (~0.069 mmol) in anhydrous CH_2Cl_2 (7.0 mL) was added TFA (1.3 mL) at 0 °C. The reaction mixture was stirred for 1 h at that temperature, and after that time, the solvents were evaporated under reduced pressure, and the crude product was diluted with toluene (5.0 mL) and concentrated again, repeating this operation twice. The resulting ammonium trifluoroacetate salt (**22** and **25**), obtained in quantitative yield, was used for the next step not requiring further purification.

Trihydroxy Cyclodepsipeptide 27. The resulting ammonium trifluoroacetate **22** (obtained from 152 mg of triol **17**, ~0.191 mmol) was dissolved in anhydrous DMF (190 mL, 1 mM based on the triol **17**), and the solution was cooled to 0 °C. DEPC (156 μL, 0.936 mmol, 4.9 equiv) and anhydrous TEA (143 μL, 1.03 mmol, 5.4 equiv) were sequentially added at 0 °C, and the reaction mixture was stirred for 18 h at ambient temperature. After this time, the crude mixture was diluted with diethyl ether and washed with a saturated aqueous NH_4Cl solution. After separation of both phases, the aqueous layer was extracted with more diethyl ether, and the final combined organic solution was sequentially washed with saturated aqueous NH_4Cl solution (2 × 10 mL), H_2O (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 35% EtOAc and 5% MeOH in hexanes) to afford impure cyclic depsipeptide **26** (30 mg, 23% overall yield from **17**). (FAB HRMS (NBA): *m/e* 712.4512, M + Na^+; calcd for $C_{38}H_{63}N_3O_8$ 712.4513.) This crude was dissolved in MeOH (3 mL), and 10% $Pd(OH)_2/C$ (40 mg) was added. The reaction was allowed to proceed under an atmosphere of H_2 at ambient temperature, and after 1 h, the suspension was filtered through a silica gel pad. The solid was washed with MeOH and CH_2Cl_2, and the combined clear organic solution was concentrated under reduced pressure to obtain crude product **27** which was purified by flash column chromatography (silica gel, 40% EtOAc and 10% MeOH in hexanes) to afford cyclodepsipeptide **27** (19 mg, 17% over 5 steps from triol **17**) as a colorless oil: R_f = 0.31 (silica gel, 50% EtOAc and 10% MeOH in hexanes). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.82−0.92 (m, 12 H, CH_3CH$_2$, CH(CH_3), CH(CH_3)$_2$), 1.02 (d, J = 5.9 Hz, 3 H, (CH_3)CH(OH)), 1.17−1.25 (m, 22 H, 11 × CH$_2$), 1.46−1.56 (m, 3 H, CH_2CHOC(=O), CH(CH$_3$)), 1.86−1.96 (m, 1 H, CH(CH$_3$)$_2$), 2.11 (dd, J = 13.4, 6.4 Hz, 1 H, CH_2CONH), 2.28 (dd, J = 13.4, 5.9 Hz, 1 H, CH_2-CONH), 3.58−3.70 (m, 2 H, CH_2OH), 3.84−3.92 (m, 3 H, CHNH(Val), CH(OH), (CH$_3$)CH(OH)), 4.03 (dt, J = 7.5, 6.4 Hz, 1 H, CHNH(Ser)), 4.12−4.20 (m, 1 H, CHNH(Thr)), 4.58 (d, J = 4.8 Hz, 1 H, OH), 4.68−4.75 (m, 1 H, CHOC(=O)), 4.83 (dd, J = 5.9, 5.3 Hz, 1 H, CH$_2$OH), 4.96 (d, J = 5.3 Hz, 1 H, OH), 7.22 (d, J = 7.0 Hz, 1 H, NH(Thr)), 8.00 (d, J = 9.1 Hz, 1 H, NH(Val)), 8.32 (d, J = 7.5 Hz, 1 H, NH(Ser)). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 10.1, 14.0, 19.2, 19.3, 20.2, 22.1, 25.5, 28.7, 28.8, 28.95, 29.0, 29.03, 29.5, 29.7, 31.3, 41.1, 42.4, 54.9, 58.5, 59.8, 61.2, 66.7, 67.4, 77.9, 169.7, 170.1, 171.1, 171.5. FAB HRMS (NBA): *m/e* 622.4044, M + Na^+; calcd for $C_{31}H_{57}N_3O_8$ 622.4043.

Cyclic Depsipeptide 28. To a solution of triol **27** (15 mg, 0.025 mmol, 1.0 equiv) in pyridine (3.0 mL) was added acetic anhydride (23 μL, 0.25 mmol, 10.0 equiv) at 0 °C. After stirring for 2 h at this temperature, MeOH was added, and after 5 min, the resulting solution was concentrated in vacuo. The obtained crude product was purified by flash column chromatography (silica gel, 4% MeOH in $CHCl_3$) to obtain cyclic depsipeptide **28** (7 mg, 44%) as a white solid: R_f = 0.28 (silica gel, 8% MeOH in $CHCl_3$). $[\alpha]^{25}_D$ = −60.6° (c = 0.35, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.85 (t, J = 7.0 Hz, 3 H, CH_3CH$_2$), 0.87−0.92 (m, 9 H, CH(CH_3), CH(CH_3)$_2$), 0.99 (d, J = 5.9 Hz, 3 H, (CH_3)CH(OH)), 1.17−1.27 (m, 22 H, 11 × CH$_2$), 1.37−1.49 (m, 1 H, CH_2CHOC(=O)), 1.53−1.64 (m, 2 H, CH_2-CHOC(=O), CH(CH$_3$)), 1.89−1.99 (m, 1 H, CH(CH$_3$)$_2$), 1.98 (s, 3 H, OCOCH_3), 2.13 (dd, J = 12.9, 5.9 Hz, 1 H, CH_2CONH), 2.28 (dd, J = 12.9, 7.5 Hz, 1 H, CH_2CONH), 3.77 (sext, J = 5.9 Hz, 1 H, (CH$_3$)CH(OH)), 3.83 (dd, J = 9.1, 8.6 Hz, 1 H, CHNH(Val)), 3.91−3.99 (m, 1 H, CH(OH)), 4.15−4.24 (m, 2 H, CHNH(Ser), CHNH(Thr)), 4.29 (dd, J = 11.1, 7.5 Hz, 1 H, CH_2OAc), 4.34 (dd, J = 11.1, 5.9 Hz, 1 H, CH_2OAc), 4.59 (d, J = 4.8 Hz, 1 H, CH(OH)), 4.69−4.75 (m, 1 H, CHOC(=O)), 4.85 (d, J = 5.4 Hz, 1 H, (CH$_3$)CH(OH)), 6.97 (d, J = 8.6 Hz, 1 H, NH(Ser)), 8.08 (d, J = 8.6 Hz, 1 H, NH(Val)), 8.75 (d, J = 7.0 Hz, 1 H, NH(Thr)).^{13}C NMR (100 MHz, DMSO-d_6): δ = 10.5, 14.2, 19.6, 19.7, 20.0, 20.8, 22.3, 25.6, 28.9, 29.0, 29.20, 29.26, 29.29, 29.31, 29.6, 30.2, 31.5, 41.4, 42.5, 51.6, 58.1, 61.5, 61.9, 67.2, 67.5, 79.0, 168.9, 170.3, 170.4, 171.3, 171.5. FAB HRMS (NBA): *m/e* 664.4144, M + Na^+; calcd for $C_{33}H_{59}N_3O_9$ 664.4149.

Cyclic Depsipeptide 29. The resulting ammonium trifluoroacetate **25** (obtained from 76 mg of triol **19**, ~0.092 mmol) was subjected to the macrolactamization reaction in a manner similar to that for the preparation of **26** to afford cyclic depsipeptide **29** (22 mg, 33% over four steps from triol **19**) as a colorless oil: R_f = 0.38 (silica gel, 55% EtOAc and 5% MeOH in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (t, J = 7.0 Hz, 3 H, CH_3CH$_2$), 0.90−0.98 (m, 9 H, CH(CH_3)$_2$, CH-(CH$_2$CH_3)), 1.01 (d, J = 7.0 Hz, 3 H, CH(CH_3)), 1.12 (d, J = 6.4 Hz, 3 H, (CH_3)CH(OH)), 1.17−1.29 (m, 22 H, 11 × CH$_2$), 1.39−1.50 (m, 1 H, CH_2CHOC(=O)), 1.66−1.76 (m, 4 H, CH_2-CHOC(=O), CH(CH_2CH$_3$), CH(CH$_3$)), 1.98−2.10 (m, 1 H, CH(CH$_3$)$_2$), 2.24−2.32 (m, 1 H, CH(CH$_2$CH$_3$)), 3.66 (bs, 1 H, CH(OH)), 3.90 (dd, J = 9.6, 5.3 Hz, 1 H, CH_2OBn), 3.92−4.00 (m, 1 H, CH_2OBn), 4.11−4.20 (m, 1 H, CHNH(Val)), 4.44 (dq, J = 6.4, 2.1 Hz, 1 H, (CH$_3$)CH(OH)), 4.43−4.50 (m, 2 H, CHNH(Ser), CHNH(Thr)), 4.48 (d, J = 11.3 Hz, 1 H, CH$_2$Ph), 4.53 (d, J = 11.3 Hz, 1 H, CH$_2$Ph), 4.87 (dt, J = 7.5, 6.4 Hz, 1 H, CHOC(=O)), 6.70 (bs, 1 H, NH), 7.21−7.35 (m, 6 H, Ph, NH), 7.61 (d, J = 8.1 Hz, 1 H, NH). FAB HRMS (NBA): *m/e* 740.4827, M + Na^+; calcd for $C_{40}H_{67}N_3O_8$ 740.4826.

Cyclic Depsipeptide 31. Hydrogenolysis of **29** (18 mg, 0.025 mmol) and subsequent selective monoacetylation of the resulting trihydroxy cyclodepsipeptide **30** (15 mg) were performed exactly as described above for the preparation of **28** to furnish, after purification by flash column chromatography (silica gel, 4% MeOH in $CHCl_3$), cyclic depsipeptide **31** (7 mg, 44% overall two steps), as a white solid, together with the diacetylated compound (5 mg, 28%). **[31]**: R_f = 0.34 (silica gel, 50% EtOAc and 10% MeOH in hexanes). $[\alpha]^{25}_D$ = −29.7° (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.72 (d, J = 7.0 Hz, 3 H, CH(CH_3)), 0.79−0.90 (m, 12 H, CH_3CH$_2$, CH-(CH_3)$_2$, CH(CH$_2$CH_3)), 0.99 (d, J = 6.4 Hz, 3 H, (CH_3)CH(OH)), 1.16−1.29 (m, 22 H, 11 × CH$_2$), 1.29−1.40 (m, 1 H, CH_2CHOC-(=O)), 1.46−1.57 (m, 3 H, CH_2CHOC(=O), CH(CH_2CH$_3$)), 1.60−1.70 (m, 1 H, CH(CH$_3$)), 1.93−2.00 (m, 1 H, CH(CH$_2$-CH$_3$)), 1.97 (s, 3 H, CH_3CO$_2$), 2.04−2.13 (m, 1 H, CH(CH$_3$)$_2$), 3.68−3.72 (m, 1 H, CH(OH)), 3.89 (dd, J = 10.7, 7.0 Hz, 1 H, CH_2OAc), 4.05 (dd, J = 10.7 Hz, 1 H, CHNH(Val)), 4.14−4.24 (m, 1 H, (CH$_3$)CH(OH)), 4.28 (dd, J = 9.7, 2.1 Hz, 1 H, CHNH-(Thr)), 4.40 (dd, J = 10.7, 6.4 Hz, 1 H, CH_2OAc), 4.74−4.81 (m, 1 H, CHNH(Ser)), 4.89 (d, J = 4.8 Hz, 1 H, (CH$_3$)CH(OH)), 4.94 (dt, J = 9.1, 4.8 Hz, 1 H, CHOC(=O)), 5.60 (d, J = 5.4 Hz, 1 H, CH(OH)), 7.75 (d, J = 8.6 Hz, 1 H, NH(Ser)), 7.95 (d, J = 10.2 Hz, 1 H, NH(Val)), 8.42 (d, J = 9.7 Hz, 1 H, NH(Thr)). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 6.6, 12.2, 14.2, 19.3, 19.7, 20.79, 20.83, 22.3, 24.8, 25.5, 28.9, 29.0, 29.1, 29.19, 29.23, 29.25, 29.29, 30.2, 30.6, 31.5, 32.3, 40.8, 50.0, 54.7, 57.9, 61.3, 62.7, 67.0, 75.3, 79.2, 169.8, 170.5, 170.6, 171.8, 174.1. FAB

(22) Bialy, L.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 3814−3839.

HRMS (NBA): *m/e* 692.4464, M + Na^+; calcd for $C_{35}H_{63}N_3O_9$ 692.4462.

Cyclic Depsipeptide 41. To a solution of the crude acid **38** (~0.064 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added TFA (0.5 mL) at 0 °C. The reaction mixture was stirred for 2 h at that temperature, and after that time, the solvents were evaporated under reduced pressure to obtain the corresponding ammonium trifluoroacetate salt, which was used for the next step without further purification. The resulting ammonium trifluoroacetate (~0.064 mmol) was dissolved in anhydrous DMF (64 mL, 1 mM based on the triol **34**), and the solution was cooled to 0 °C. DEPC (53 μL, 0.32 mmol, 5.0 equiv) and TEA (49 μL, 0.35 mmol, 5.5 equiv) were sequentially added at 0 °C, and the reaction mixture was stirred for 48 h at 25 °C. After this time, the reaction mixture was concentrated under high vacuum (0.5 mm of Hg) at 50 °C, and the resultant crude product was purified by flash column chromatography (silica gel, 40% toluene, 56% EtOAc and 4% MeOH) to obtain the corresponding cyclic depsipeptide (12 mg, partially impurified).

To a solution of this macrocycle (12 mg) in MeOH (2 mL) was added 10% Pd/C (20 mg), and the reaction was allowed to proceed under an atmosphere of H_2 at 25 °C. After 2 h, the suspension was filtered, and the solid was washed with MeOH and CH_2Cl_2. The resulting organic solution was concentrated in vacuo, and the obtained crude product was dissolved in pyridine (1.5 mL) and subjected to the action of acetic anhydride (14 μL) at 0 °C. After stirring for 2 h at this temperature, the reaction mixture was quenched by addition of MeOH and concentrated. The crude product was purified by flash column chromatography (silica gel, 3% MeOH in $CHCl_3$) to obtain stevastelin analogue **41** (4 mg, 9% overall yield from **34**) as a white solid: R_f = 0.30 (silica gel, 5% MeOH in $CHCl_3$). $[\alpha]^{25}_D$ = −26.0° (*c* = 0.20, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.75 (d, *J* = 7.0 Hz, 3 H, CH(C*H*$_3$)), 0.83−0.88 (m, 6 H, C*H*$_3$CH$_2$, CH(C*H*$_3$)$_2$), 0.90 (d, *J* = 6.4 Hz, 3 H, CH(C*H*$_3$)$_2$), 0.97 (d, *J* = 5.9 Hz, 3 H, (C*H*$_3$)CH(OH)), 1.10 (d, *J* = 7.5 Hz, 3 H, CH(C*H*$_3$)), 1.17−1.30 (m, 22 H, 11 × CH_2), 1.43−1.59 (m, 4 H, C*H*$_2$CHO(=O), C*H*$_2$C*H*(CH$_3$)$_2$), 1.67−1.80 (m, 2 H, C*H*(CH$_3$), C*H*$_2$CH(CH$_3$)$_2$), 1.98 (s, 3H, OC(=O)C*H*$_3$), 2.21 (dq, *J* = 7.5, 3.2 Hz, 1 H, C*H*(CH$_3$)), 3.61−3.64 (m, 1 H, C*H*(OH)), 3.97 (dd, *J* = 10.7, 6.4 Hz, 1 H, C*H*$_2$OAc), 4.14−4.19 (m, 1 H, (CH$_3$)C*H*(OH)), 4.22 (dd, *J* = 9.7, 2.7 Hz, 1 H, C*H*NH(Thr)), 4.37 (dd, *J* = 10.7, 7.0 Hz, 1 H, C*H*$_2$OAc), 4.38−4.45 (m,1 H, C*H*NH(Leu)), 4.67 (ddd, *J* = 7.5 Hz, 1 H, C*H*NH-(Ser)), 4.84−4.89 (m, 1 H, C*H*OC(=O)), 4.92 (d, *J* = 4.3 Hz, 1 H, (CH$_3$)CH(O*H*)), 5.35 (d, *J* = 5.4 Hz, 1 H, CH(O*H*)), 7.92 (d, *J* = 8.6 Hz, 1 H, N*H*(Ser)), 7.95 (d, *J* = 10.2 Hz, 1 H, N*H*(Leu)), 8.19 (d, *J* = 9.1 Hz, 1 H, N*H*(Thr)). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 7.1, 14.1, 16.0, 20.5, 20.7, 22.0, 22.3, 22.9, 24.7, 25.6, 28.87, 28.91, 29.07, 29.13, 29.17, 29.21, 31.5, 46.6, 50.2, 53.0, 57.9, 62.5, 66.8, 74.8, 79.3, 169.5, 170.3, 172.3, 175.0, 175.5.

Cyclic Depsipeptide 42. To a solution of the crude acid **40** (~0.052 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added TFA (0.5 mL) at 0 °C. The reaction mixture was stirred for 1.5 h at that temperature, and after that time, the solvents were evaporated under reduced pressure to obtain the corresponding ammonium trifluoroacetate salt, which was used for the next step without further purification. The resulting ammonium trifluoroacetate (~0.052 mmol) was dissolved in anhydrous DMF (52 mL, 1 mM based on the diol **36**), and the solution was cooled to 0 °C. DEPC (43 μL, 0.26 mmol, 5.0 equiv) and TEA (41 μL, 0.29 mmol, 5.5 equiv) were sequentially added at 0 °C, and the reaction mixture was stirred for 48 h at 25 °C. After this time, the reaction mixture was concentrated under high vacuum (0.5 mm of Hg) at 50 °C, and the resultant crude product was purified by flash column chromatography (silica gel, 70% EtOAc in hexanes) to obtain the corresponding cyclic depsipeptide (11 mg).

To a solution of this macrocycle (11 mg) in MeOH (2 mL) was added 20% Pd(OH)$_2$ (20 mg), and the reaction was allowed to proceed under an atmosphere of H_2 at 25 °C. After 4 h, the suspension was filtered, and the solid was washed with MeOH and CH_2Cl_2. The resulting organic solution was concentrated in vacuo, and the obtained crude product was dissolved in pyridine (2.0 mL) and subjected to the action of acetic anhydride (29 μL) at 0 °C. After stirring for 3 h at this temperature, the reaction mixture was quenched by addition of MeOH and concentrated. The crude product was purified by flash column chromatography (silica gel, 10% AcOEt in hexanes) to obtain stevastelin analogue **42** (4 mg, 18% overall yield from **36**) as a white solid: R_f = 0.52 (silica gel, 100% EtOAc). $[\alpha]^{25}_D$ = −43.3° (*c* = 0.30, $CHCl_3$).^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.73 (d, *J* = 7.0 Hz, 3 H, CH(C*H*$_3$)), 0.80 (d, *J* = 7.0 Hz, 3 H, CH(C*H*$_3$)$_2$), 0.82−0.87 (m, 9 H, C*H*$_3$CH$_2$, CH-(C*H*$_3$)$_2$), 0.89 (d, *J* = 6.4 Hz, 3 H, CH(C*H*$_3$)$_2$), 1.14 (d, *J* = 7.5 Hz, 3 H, CH(C*H*$_3$)), 1.16−1.30 (m, 22 H, 11 × CH_2), 1.37−1.47 (m,1 H, C*H*$_2$CHOC(=O)), 1.49−1.59 (m, 1 H, C*H*$_2$CHOC(=O)), 1.64−1.72 (m, 1 H, C*H*(CH$_3$)), 1.96 (s, 3 H, OC(=O)CH$_3$), 2.00−2.17 (m, 2 H, 2 × C*H*(CH$_3$)$_2$), 2.21 (dq, *J* = 7.5, 3.2 Hz, 1 H, C*H*(CH$_3$)), 3.60−3.64 (m, 1 H, C*H*(OH)), 3.96 (dd, *J* = 10.7 Hz, 1 H, C*H*NH(Val)), 4.03 (dd, *J* = 10.7 Hz, 1 H, C*H*$_2$OAc), 4.29 (dd, *J* = 9.7, 4.8 Hz, 1 H, C*H*NH(Val)), 4.48 (dd, *J* = 11.3, 4.8 Hz, 1 H, C*H*$_2$OAc), 4.66−4.73 (m, 1 H, C*H*NH(Ser)), 4.84−4.90 (m, 1 H, C*H*OC(=O)), 5.43 (d, *J* = 3.2 Hz, 1 H, CH(O*H*)), 7.89 (d, *J* = 10.2 Hz, 1 H, N*H*(Val)), 7.98 (d, *J* = 9.7 Hz, 1 H, N*H*(Ser)), 8.02 (d, *J* = 8.1 Hz, 1H, N*H*(Val)). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 7.2, 14.1, 16.1, 17.4, 19.2, 19.4, 19.5, 20.7, 22.2, 25.4, 28.8, 28.9, 29.0, 29.1, 29.2, 29.8, 31.2, 31.4, 31.7, 46.2, 49.9, 57.1, 61.4, 62.7, 74.8, 78.9, 169.3, 170.3, 170.6, 171.1, 175.2. FAB HRMS (NBA): *m/e* 676.4518, M + Na^+; calcd for $C_{35}H_{63}N_3O_8$ 676.4513.

Cyclic Depsipeptides 73 and 74. The transformation of compounds **65** (93 mg, 0.121 mmol) and **67** (77 mg, 0.085 mmol) to cyclic depsipeptide **73** and **74**, respectively, followed the same synthetic sequence as that described above for cyclic depsipeptide **26**, through triols **66** (88 mg, 99%) and **68** (75 mg, 99%), aldehydes **69** (77 mg, 88%) and **71** (66 mg, 88%), acids **70** (76 mg, 96%) and **72** (66 mg, 99%), and their corresponding ammonium trifluoroacetates, to obtain, after purifications by flash column chromatography (silica gel, 35% EtOAc, 5% MeOH in hexanes and 33% acetone in toluene, respectively) cyclic depsipeptides **73** (23 mg, 30% yield over 5 steps) and **74** (15 mg, 25% yield over 5 steps) as colorless oils. **[73]:** R_f = 0.33 (silica gel, 50% EtOAc, 5% MeOH in hexanes). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.72 (d, *J* = 7 Hz, 3 H, CH(C*H*$_3$)), 0.84 (d, *J* = 7 Hz, 9 H), 0.91 (d, *J* = 7 Hz, 12 H, 4 × C*H*$_3$CH), 1.02 (d, *J* = 6.4 Hz, 3 H, (C*H*$_3$)CH(OH)), 1.12 (d, *J* = 7.5 Hz, 3 H,), 1.17−1.37 (m, 37 H), 1.36−1.46 (m, 1 H, C*H*(CH$_3$)), 1.47−1.59 (m, 1 H, C*H*(CH$_3$)), 1.74−1.84 (m, 1 H, C*H*(CH$_3$)), 2.04−2.24 (m, 1 H, C*H*(CH$_3$)), 2.31−2.34 (s, 1 H, C*H*(CH$_3$)$_2$), 3.57−3.63 (m, 3 H,), 3.96 (t, *J* = 8.6 Hz, 1 H, C*H*O−), 4.06−4.18 (m, 6 H), 4.22 (dd, *J* = 9.1, 2.1 Hz, 2 H,), 4.30 (t, *J* = 7 Hz, 2 H, (C*H*NH), 4.81−4.87 (m, 1 H), 4.98−5.06 (m, 1 H), 7.49 (d, *J* = 8.1 Hz, 1 H, CHN*H*(Thr(OH)), 7.92 (d, *J* = 10.2 Hz, 1 H, N*H*), 8.25 (d, *J* = 8.6 Hz, 1 H, N*H*). FAB HRMS (NBA): *m/e* 648.8705, M + Na^+; calcd for $C_{34}H_{63}N_3O_7$ 648.8697. **[74]**: R_f = 0.25 (silica gel, 33% acetone in toluene). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.77 (d, *J* = 7.3 Hz, 3 H, CH(C*H*$_3$)), 0.81−0.88 (m, 6 H, C*H*$_3$CH$_2$, CH(C*H*$_3$)), 0.91 (d, *J* = 7.3 Hz, 3 H, CH(C*H*$_3$)), 0.98 (d, *J* = 6.1 Hz, 3 H, CH(C*H*$_3$)), 1.01 (d, *J* = 6.1 Hz, 3 H, CH(C*H*$_3$)), 1.16 (d, *J* = 6.7 Hz, 3 H, CH(C*H*$_3$)), 1.09−1.27 (m, 22 H, 11 × CH_2), 1.40−1.64 (m, 2 H, C*H*$_2$CHOC(=O)), 1.72−1.87 (m, 1 H, C*H*(CH$_3$)), 1.94−2.09 (m, 1 H, C*H*(CH$_3$)$_2$), 2.13−2.26 (m, 1 H, C*H*(CH$_3$)), 3.64−3.74 (m, 1 H, C*H*(OH)), 3.96 (dd, *J* = 11.0, 10.4 Hz, 1 H, C*H*NH(Val)), 4.03−4.16 (m, 2 H, (CH$_3$)C*H*(OBn), (CH$_3$)C*H*(OH)), 4.26 (dd, *J* = 9.8, 1.2 Hz, 1 H, C*H*NH(Thr)), 4.36 (d, *J* = 6.1 Hz, 1 H, CH(O*H*)), 4.45 (d, *J* = 11.6 Hz, 1 H, C*H*$_2$Ph), 4.59 (d, *J* = 11.6 Hz, 1 H, C*H*$_2$Ph), 4.70 (dd, *J* = 9.2, 3.0 Hz, 1 H, C*H*NH(Thr)), 4.81−4.93 (m, 1 H, C*H*OC(=O)), 5.08 (d, *J* = 4.9 Hz, 1 H, CH(O*H*)), 7.31 (s, 5 H, Ph), 7.64 (d, *J* = 9.2 Hz, 1 H, N*H*), 7.69 (d, *J* = 9.8 Hz, 1 H, N*H*), 8.31 (d, *J* = 9.8 Hz, 1 H, N*H*).

Silyl Ether 75. A solution of **74** (15 mg, 0.0208 mmol, 1.0 equiv) in CH_2Cl_2 (1.0 mL) was treated at 0 °C with 2,6-lutidine

(5 μL, 0.0416 mmol, 2.0 equiv) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (6.2 μL, 0.027 mmol, 1.3 equiv). After stirring for 15 min at 0 °C, MeOH was added, and the crude mixture was diluted with diethyl ether. The organic solution was washed with a saturated aqueous NH_4Cl solution, and the resulting biphasic mixture was separated, the aqueous layer extracted with diethyl ether, and the combined organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure to give silyl ether **75** (18 mg), which was used in the next step without purification.

Diol 76. A solution of compound **75** (18 mg, 0.0208 mmol) in MeOH (2.5 mL) was treated with 10% $Pd(OH)_2$−C/25 mg), and the reaction was allowed to proceed under an H_2 atmosphere at ambient temperature. After 30 min, the reaction was complete, as judged by TLC, and the suspension was filtered, the solids washed with MeOH and CH_2Cl_2, and the resulting organic solution concentrated under vacuum, to obtain diol **76** (14 mg), which was used in the next step without purification.

Cyclic Depsipeptide 78. To a solution of cyclic depsipeptide **76** (14 mg, 0.019 mmol) in pyridine (2.0 mL) was added Ac_2O (28 μL) and 4-DMAP (0.4 mg) at room temperature. After stirring for 1 h at this temperature, the reaction was quenched by addition of MeOH, and the resulting crude mixture was concentrated under reduced pressure to obtain crude product **77**. After solving the resulting product **77** in THF (2.0 mL), HF·pyridine (70% HF, 2.0 mL) was added at 0 °C. After stirring at this temperature for 40 min, a saturated aqueous $NaHCO_3$ solution was added, followed by addition of CH_2Cl_2. After separation of the phases, the organic layer was washed with water and brine, filtered, dried ($MgSO_4$), and concentrated under reduced pressure. The resulting crude product was subjected to purification by flash column chromatography (silica gel, 4% MeOH in $CHCl_3$) to obtain cyclic depsipeptide **78** (5 mg, 36% overall yield from **67**) as a white solid: R_f = 0.34 (silica gel, 10% MeOH in $CHCl_3$). $[\alpha]^{25}_D = -34.4°$ (c = 0.25, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.72 (d, J = 7.0 Hz, 3 H, CH(CH_3)), 0.82−0.87 (m, 6 H, CH_3CH$_2$, CH(CH_3)), 0.90 (d, J = 6.4 Hz, 3 H, CH(CH_3)), 1.03 (d, J = 6.4 Hz, 3 H, CH(CH_3)), 1.11 (d, J = 7.5 Hz, 3 H, CH(CH_3)), 1.19 (d, J = 6.4 Hz, 3 H, CH(CH_3)), 1.17−1.27 (m, 22 H, 11 × CH$_2$), 1.35−1.46 (m, 1 H, CH_2CHOC(=O)), 1.48−1.59 (m, 1 H, CH_2CHOC(=O)), 1.73−1.82 (m, 1 H, CH(CH$_3$)), 2.00 (s, 3 H, OC(=O)CH_3), 2.04−2.13 (m, 1 H, CH(CH$_3$)$_2$), 2.20 (dq, J = 7.5, 4.3 Hz, 1 H, CH(CH$_3$)), 3.64 (bs, 1 H, CH(OH)), 3.97 (dd, J = 10.7, 10.2 Hz, 1 H, CHNH(Val)), 4.04−4.11 (m, 1 H, (CH$_3$)CH(OH)), 4.25 (dd, J = 9.7, 2.7 Hz, 1 H, CHNH(Thr(OH))), 4.68 (dd, J = 9.1, 5.9 Hz, 1 H, CHNH(Thr(OAc))), 4.82−4.87 (m, 1 H, CHO(C=O)), 4.90 (d, J = 5.9 Hz, 1 H, CH(OH)), 5.00 (d, J = 4.8 Hz, 1 H, (CH$_3$)CH(OH)), 5.20 (dq, J = 6.4, 5.9 Hz, 1 H, (CH$_3$)-CH(OAc)), 7.70 (d, J = 9.1 Hz, 1 H, NH(Thr(OAc))), 7.88 (d, J = 10.2 Hz, 1 H, NH(Val)), 8.25 (d, J = 9.7 Hz, 1 H, NH(Thr-(OH))). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 7.3, 14.0, 15.8, 16.6, 19.0, 19.5, 20.8, 21.1, 22.1, 25.5, 28.7, 28.97, 29.04, 29.9, 31.3, 46.2, 54.1, 58.6, 61.1, 66.8, 68.6, 74.2, 78.0, 168.5, 169.7, 170.5, 171.2, 175.1. FAB HRMS (NBA): *m/e* 692.4461, M + Na^+; calcd for $C_{35}H_{63}N_3O_9$ 692.4462.

Cyclic Depsipeptide 91. Desilylation of compound **90** was carried out exactly in the same manner as that described before for cyclic depsipeptide **78**, by treatment with HF·pyridine (70% HF, 2.0 mL) to obtain, after purification by flash column chromatography (silica gel, 75% EtOAc and 5% MeOH in hexanes), cyclic depsipeptide **91** (10 mg, 56% from **88** over 3 steps) as a colorless oil: R_f = 0.24 (silica gel, 85% EtOAc, 10% hexanes, 5% MeOH). $[\alpha]^{25}_D = -110°$ (c = 0.11, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6): δ =0.73 (d, J = 6.4 Hz, 3H, CH_3CH), 0.90 (d, J = 6.4 Hz, 3H, CH_3CH), 0.92 (d, J = 6.4 Hz, 3H, C$H3$CH), 1.01 (d, J = 6.4 Hz, 3H, CH_3CHOH), 1.10 (d, J = 7.5 Hz, 3H, CH_3CHCONH), 1.92−2.07 (m, 2H, CH3CH, CH(CH$_3$)$_2$), 2.00 (s, 3H, CH_3CO$_2$), 2.20−2.30 (m, 1H, CH$_3$CH-CONH), 3.64−3.70 (m, 1H, CHOH), 3.86 (dd, J = 10.7 Hz, 1H, CH_2OCO), 3.94−4.01 (m, 2H, CH_2OCO, CHCH(CH$_3$)$_2$), 4.03−4.10 (m, 1H, CH$_3$CHOH), 4.18−4.25 (m, 2H, CH_2OAc), 4.26 (dd, J = 9.1, 2.1 Hz, 1H, CHCHOH), 4.58 (d, J = 6.4 Hz, 1H, CHOH), 4.60−4.66 (m, 1H, CHCH$_2$OAc), 5.13 (d, J = 4.8 Hz, CH$_3$CHOH), 7.55 (d, J = 9.7 Hz, 1H, NH(Val)), 7.76 (d, J = 8.1 Hz, 1H, NH(Ser)), 7.83 (d, J = 9.7 Hz, 1H, NH(Thr)).^{13}C NMR (100 MHz, DMSO-d_6): δ = 9.1, 13.7, 19.4, 19.8, 20.8, 30.8, 33.3, 46.3, 51.9, 58.6, 61.4, 62.9, 66.5, 67.0, 71.3, 168.2, 170.4, 171.1, 171.5, 175.5.

Acknowledgment. This work was financially supported by Fundación Ramón Areces and the Dirección General de Investigación (BQU2001-1576) and fellowships from Fundación Ramón Areces (S.C.). We thank Dr. J. I. Trujillo from Pfizer (St. Louis, MO) for assistance in the preparation of this manuscript. We thank Unidad de Espectroscopía de Masas of Seville and Granada Universities for exact mass spectroscopic assistance.

Supporting Information Available: Experimental procedures and spectroscopic data for all other compounds, and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO050628Y