Synthesis and Biological Evaluation of Dihydroeptastatin, a Novel Inhibitor of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase[1]

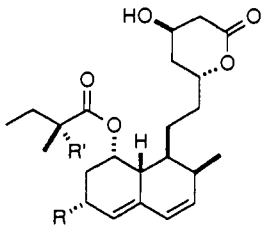
Elisabeth A. Bone, Alan H. Davidson, Christopher N. Lewis,* and Richard S. Todd

Departments of Medicinal Chemistry and Biology, British Bio-technology Limited, Watlington Road, Cowley, Oxford OX4 5LY, U.K. *Received November 14, 1991*

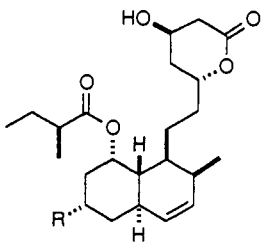
The total synthesis of the novel hydroxylated HMG-CoA reductase inhibitor dihydroeptastatin (7) is described. The key C-3 hydroxyl group is introduced via a Baeyer–Villiger reaction on the methyl ketone 17 which is obtained in three high-yielding steps from the known tricyclic lactone 12. In an isolated enzyme assay dihydroeptastatin had a similar IC_{50} to mevinolin but in cellular assays using Hep G2 and HES 9 cell lines, dihydroeptastatin was much less potent. No selectivity between the two cell lines was observed.

Introduction

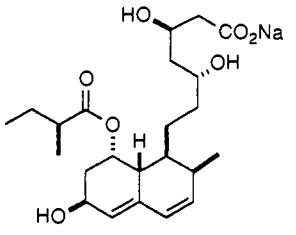
Coronary heart disease (CHD) and atherosclerosis are the major causes of mortality in the western world. Epidemiological studies have revealed that there is a strong correlation between the incidence of CHD and levels of low-density lipoprotein (LDL) cholesterol in the blood. Over the past 15 years a series of fungal metabolites, the mevinic acids 1–6, have been shown to be highly effective plasma cholesterol lowering agents, through their ability to inhibit the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[2]



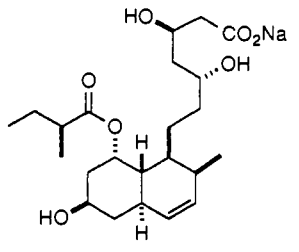
1 R = H ; R' = H Compactin
2 R = Me ; R' = H Mevinolin
3 R = Me ; R' = Me Simvastatin



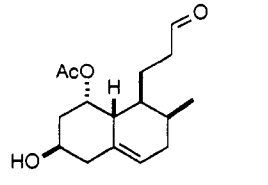
4 R = H Dihydrocompactin
5 R = Me Dihydromevinolin



6 Eptastatin



7 Dihydroeptastatin



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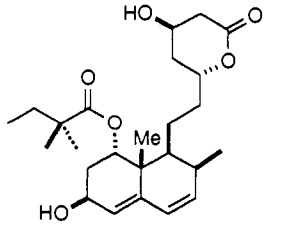

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The liver is the major site of cholesterol biosynthesis and as such, controls the levels of circulating LDL-cholesterol. Hence an important property of any clinical HMG-CoA reductase inhibitor should be that it preferentially inhibits cholesterol biosynthesis in the liver. Data have been presented for the currently marketed[3] agents mevinolin (also known as lovastatin) (2), simvastatin (3), and eptastatin (pravastatin) (6) which show that this selectivity can be achieved in two different ways. For mevinolin and simvastatin, the selectivity is ascribed to efficient first-pass uptake of the lactones which are then enzymatically hydrolyzed to the active dihydroxy acid form.[4] For eptastatin the selectivity is attributed to differential uptake between cells from different organs.[5] The reason why eptastatin should show this selectivity is not clear. It could be related to the fact that the compound is given as the sodium salt of the dihydroxy acid, or it may be a consequence of the presence of the equatorial hydroxyl group at C-3.[6]

As part of our work on developing novel HMG-CoA reductase inhibitors we developed an efficient route to the key intermediate 12 from which we produced a series of inhibitors with C-3 alkenyl substituents.[7] It occurred to us that 12 could also be used for the production of dihydroeptastatin (7), the missing member of the mevinic acid family, and that this would allow us to study the selectivity of this class of inhibitor. In this paper we describe the first synthesis of dihydroeptastatin and studies on its cell selectivity.

Chemistry

Although no total synthesis of eptastatin or dihydroeptastatin has been published to date, workers at Roche have outlined the synthesis of a possible intermediate (8) from

(1) Presented in part as a poster at the 22nd National Medicinal Chemistry Conference of the American Chemical Society, Austin, Texas, July 29–Aug 2, 1990.

(2) Tobert, J. A. New Developments in Lipid-Lowering Therapy: The Role of Inhibitors of Hydroxymethylglutaryl Coenzyme A Reductase. *Circulation*, **1987**, *76*, 534–538.

(3) Mevinolin as Mevacor, simvastatin as Zocor, eptastatin as Pravacol.

(4) Germershausen, J. I.; Hunt, V. M.; Bostedor, R. G.; Bailey, P. J.; Karkas, J. D.; Alberts, A. W. Tissue Selectivity of the Cholesterol-Lowering Agents Lovastatin, Simvastatin and Pravastatin in Rats in Vivo. *Biochem. Biophys. Res. Commun.*, **1989**, *158*, 667–675.

(5) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Kaneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fujii, S. CS-514, A Competitive Inhibitor of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase: Tissue Selective Inhibition of Sterol Synthesis and Hypolipidemic Effect on Various Animal Species. *Biochim. Biophys. Acta* **1986**, *877*, 50–60.

(6) The numbering of the mevinic acids is not standardized; we prefer to label the decalin ring so that the ester (highest priority) group is at C-1. Systematic names are used in the Experimental Section.

(7) Bone, E. A.; Cunningham, E. M.; Davidson, A. H.; Galloway, W. A.; Lewis, C. N.; Morrice, E. M.; Reeve, M. M.; Todd, R. S.; White, I. M. The Design and Biological Evaluation of a Series of 3-Hydroxy-3-Methylglutaryl Coenzyme A (HMG-CoA) Reductase Inhibitors Related to Dihydromevinolin, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 223–228.

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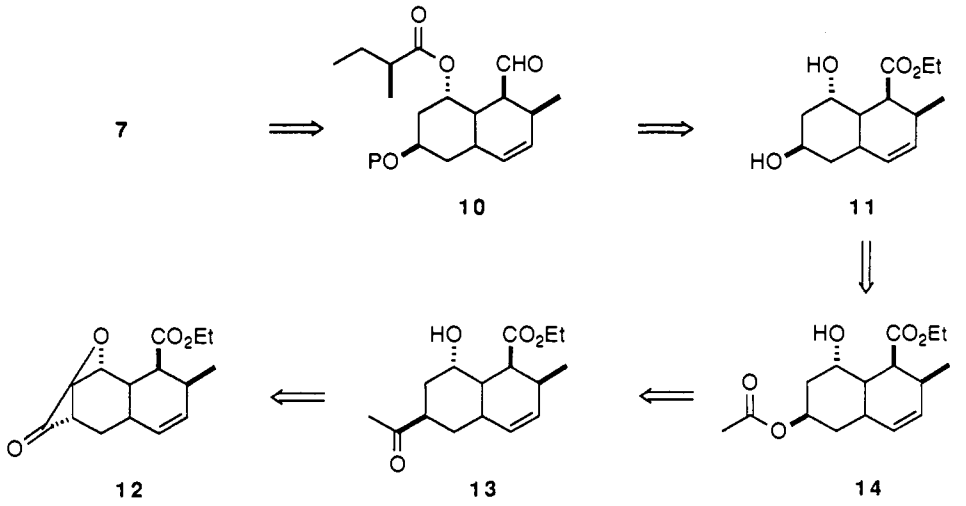
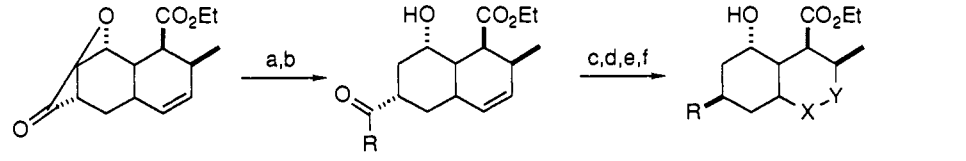
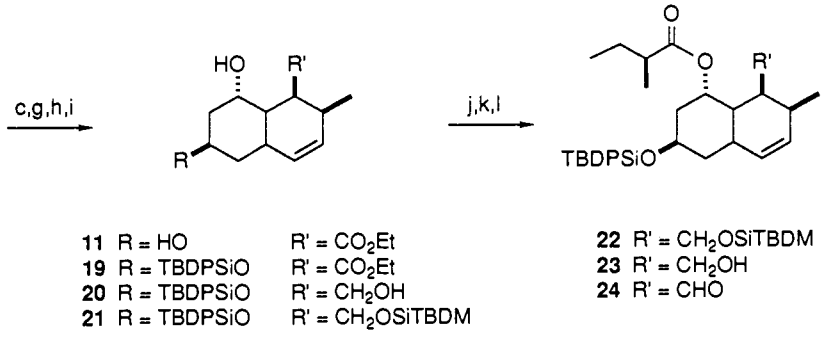
Scheme I



Scheme II[a]





[a] (a) $MeCO_2{}^tBu$, LiHMDS, THF, −70 °C; (b) TFA; (c) NaOEt, EtOH, room temperature; (d) Br_2, CCl_4, −20 °C; (e) CF_3CO_3H, DCM, 0 °C; (f) Zn, AcOH, Et_2O, (g) TBDPSiCl, imidazole, DMF, room temperature; (h) $LiAlH_4$, Et_2O; (i) TBDMSiCl, DCM, room temperature; (j) $(C_2H_5(CH_3)CHCO)_2O$, DMAP, pyr, room temperature; (k) TsOH, MeOH; (l) DMSO, $(COCl)_2$, Et_3N, DCM.

(*S*)-pulegone,[8] and of an analogue (**9**) from 1β-hydroxydehydroepiandrosterone.[9] Our retrosynthetic analysis of dihydroeptastatin is shown in Scheme I. The aldehyde **10**, which would be converted to **7** by applying the methodology developed by Heathcock,[10] would be obtained through a series of simple functional group conversions from the key intermediate alcohol **11**. This in turn would be derived from the known[11] homochiral tricyclic lactone **12**, via conversion to a methyl ketone, epimerization to **13**, and Baeyer–Villiger reaction.

The synthesis therefore commenced with the addition of the anion of *tert*-butyl acetate to the homochiral lactone **12** (Scheme II). Removal of the *tert*-butyl group and concomitant decarboxylation gave the axial methyl ketone **16** which was directly epimerized with sodium ethoxide to give the equatorially substituted ketone **13** in 87% yield. It was found that epoxidation of the 5,6 double bond of **13** was always more rapid than the required Baeyer–Villiger oxidation but that this unwanted epoxidation reaction could be avoided by protection of the double bond of **13** by bromination. Baeyer–Villiger oxidation of the resulting ketone **17** with trifluoroperacetic acid gave the acetate **18**

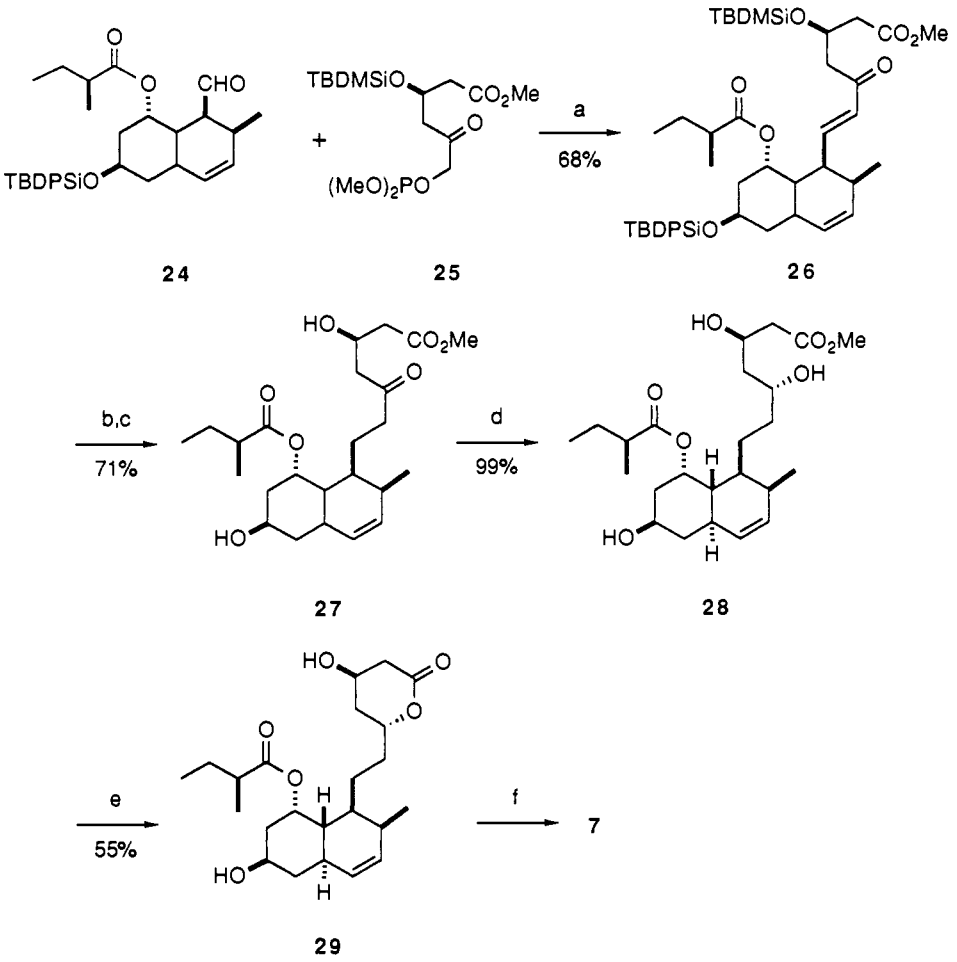
(8) Barrish, J. C.; Wovkulich, P. M.; Tang, P. C.; Batcho, A. D.; Uskokovic, M. R. An Approach to the Synthesis of the Hexahydronaphthalene Unit of Pravastatin, *Tetrahedron Lett.* **1990**, *31*, 2235–2238.

(9) Wovkulich, P. M.; Madison, V. S.; Batcho, A. D.; Tang, P. C.; Uskokovic, M. R. Inhibitors of HMG-CoA Reductase. Total Synthesis of Compactin Analogues. New Aspects Org. Chem. 1, Proc. Int. Kyoto Conf., 4th, Meeting Date 1988; Yoshida, Z., Shiba, T., Ohshiro, Y., Eds.; Kodansha: Tokyo, Japan; pp 499–507.

(10) Hecker, S. J.; Heathcock, C. H. Total Synthesis of (+)-Dihydromevinolin. *J. Am. Chem. Soc.* **1986**, *108*, 4586–4594. Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. Total Synthesis and Biological Evaluation of Structural Analogues of Compactin and Dihydromevinolin, *J. Med. Chem.* **1987**, *30*, 1858–1873.

(11) Davidson, A. H.; Jones, A. J.; Floyd, C. D.; Lewis, C. N.; Myers, P. L. The Enantiospecific Synthesis of Dihydromevinolin from L-Glutamic Acid, *J. Chem. Soc., Chem. Commun.* **1987**, 1786–1787.

Scheme III[a]



[a] (a) LiHMDS, THF, room temperature, 66 h; (b) NaHTe, EtOH, NH_4Cl, room temperature, 4 h; (c) HF, aqueous MeCN, room temperature, 6 h; (d) Et_3B, MeOH THF, $NaBH_4$, -70 °C, 18 h; (e) TsOH, C_6H_6, room temperature; (f) NaOH, H_2O MeOH.

and debromination with activated zinc restored the double bond to give the correctly functionalized decalin **14** in 65% yield from **13**, without requiring purification of the intermediates.[12]

At this point it was necessary to protect the C-3 hydroxyl group in a form that would allow the incorporation of the correct functionality at C-1 and C-8. The acetate of **14** was cleaved with sodium ethoxide to give the diol **11** and the C-3 hydroxyl group silylated with *tert*-butyldiphenylsilyl chloride. This silylation was completely selective for the equatorial (C-3) hydroxyl group in the presence of the axial (C-1) group. Reduction of the ethyl ester of **19**, protection of the resulting primary alcohol, and acylation of the secondary alcohol were all routine operations. Despite literature claims for such selectivity,[13] it was not possible to selectively remove the *tert*-butyldimethylsilyl group from the primary alcohol of **22** without partially removing the *tert*-butyldiphenylsilyl group from the secondary alcohol. However, with careful monitoring of the reaction and one recycle of the recovered starting material the completed decalin portion **23** of dihydroeptastatin was obtained.

The remainder of the synthesis used a modification of Heathcock's procedure.[10] The free alcohol **23** was oxidized to the aldehyde **24** which was reacted with the keto phosphonate **25** using lithium hexamethyldisilazide as a base (Scheme III). These conditions were found to give the enone **26** without any epimerization of the aldehyde or β-elimination of the siloxy group, both serious side reactions that occurred when Roush–Masamune[14] conditions were used in the syntheses of compactin and dihydromevinolin. Reduction of the α,β double bond was carried out using sodium hydrogen telluride in ethanol[15] buffered with ammonium chloride. The use of a buffer with this reagent was essential since otherwise the reaction mixture became sufficiently basic to cause elimination of the C-3′ silyloxy group. The C-3 and C-3′ hydroxy groups were next cleanly deprotected with hydrofluoric acid, to give the keto alcohol **27**, and a chelation-controlled reduction[16] used to

(12) Funk and Zeller used a similar bromination reaction followed by base-catalyzed double debromination to introduce the diene system in their partial synthesis of compactin. The dibrominated intermediate **18** therefore may be a precursor in a total synthesis of eptastatin. Funk, R. L.; Zeller, W. E. Hypocholesterolemic Agent Compactin (ML-236B). Total Synthesis of the Hexahydronaphthalene Portion. *J. Org. Chem.* **1982**, *47*, 180–182.

(13) Prakash, C.; Saleh, S.; Blair, I. A. Selective Deprotection of Silyl Ethers. *Tetrahedron Lett.* **1989**, *30*, 19–22.

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(15) Yamashita, M.; Kato, Y.; Suemitsu, R. Selective Reduction of α,β-Unsaturated Carbonyl Compounds By NaHTe. *Chem. Lett.* **1980**, 847–848.

(16) Chen, K-M.; Gunderson, K. G.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. A Novel Method for the In Situ Generation of Alkoxydialkylboranes and Their Use in the Selective Preparation of 1,3-Syn Diols. *Chem. Lett.* **1987**, 1923–1926.

Table I

	IC_{50} values (nM)		
	enzyme	Hep G2	HES 9
mevinolin (lactone) 2	ND[a]	37 ± 16	13 ± 2
mevinolin (salt)	13 ± 1	29 ± 5	17 ± 2
simvastatin (salt)	3 ± 0.5	12 ± 2	6.5 ± 3
dihydroeptastatin (lactone) 29	ND	1400 ± 500	7000 ± 1000
dihydroeptastatin (salt) 7	14 ± 0.4	2220 ± 270	7280 ± 580

[a] ND = not determined.

relay the stereochemistry at the 3′ hydroxyl group to the 5′ position yielding the required *syn*-diol **28**. None of the anti isomer could be detected in the ^{13}C NMR spectrum of the crude product. Acid-catalyzed ring closure then gave the lactone **29**, which was purified by recrystallization prior to hydrolysis to the final product **7**.

Biological Studies

In an assay using a rat liver microsomal preparation[17] of HMG-CoA reductase, dihydroeptastatin (sodium salt) gave an IC_{50} value of 14 nM, indistinguishable from mevinolin (as the sodium salt) used as a standard.

The potential selectivity of dihydroeptastatin and the clinically used compounds mevinolin and simvastatin was studied in a cell-based model. To aid interpretation the lactone and sodium salt of each compound was tested. The production of labeled cholesterol from [^{14}C]acetate in the presence of the HMG-CoA inhibitors was examined in two cultured human cell lines, Hep G2 (a hepatoma-derived cell line considered to be a reasonable system for the study of hepatic lipoprotein metabolism) and HES 9 (embryonic skin fibroblasts). The results are shown in Table I. All of the compounds assayed, both as the lactones or as the salts, showed similar IC_{50} values in the two cell lines. This indicates firstly that none of the compounds have any inherent selectivity between these two cell types, and secondly that the form administered (lactone or salt) does not affect the selectivity. Surprisingly, however, dihydroeptastatin was 100-fold less potent against the cell lines than against the isolated enzyme. This is in contrast to mevinolin and simvastatin whose IC_{50} values in the isolated enzyme assay were similar to those obtained in both the HES 9 and Hep G2 cellular assays. The finding that eptastatin (as the sodium salt) is less potent than expected in Hep G2 cells and human skin fibroblasts, whereas mevinolin (hydrolyzed and presented as the salt) is not, has recently been reported by other workers.[18] However it should be noted that in experiments using freshly isolated rat hepatocytes there is little difference between the enzymic and cellular IC_{50} values for eptastatin (salt).[5] These results suggest that the C-3 hydroxyl group of dihydroeptastatin and eptastatin is important in determining the activity of the compounds in some (but not all) cell types.

In summary, we have presented the first synthesis of the C-3 hydroxylated mevinic acid dihydroeptastatin (**7**) and shown it to be a potent HMG-CoA reductase inhibitor. In cultured human cell lines the compound is surprisingly less active. This suggests that the C-3 hydroxyl group of dihydroeptastatin and eptastatin prevents the compounds from reaching the site of enzyme activity in these cell lines.

Experimental Section

All nonaqueous reactions were carried out under an argon atmosphere using oven-dried glassware. Aqueous workup solutions were saturated unless otherwise noted. Organic solutions were dried over sodium sulfate or magnesium sulfate, and evaporated under reduced pressure. Chromatography was carried out using Woelm 32–60-μm silica. NMR spectra were recorded on a Bruker AC-250E at ambient temperature in deuteriochloroform at 250 MHz for proton and 62.9 MHz for carbon-13. All chemical shifts are given in parts per million referenced to tetramethylsilane. Coupling constants are in hertz. Infrared spectra were recorded using a Perkin-Elmer 197 in potassium bromide discs, or solution in $CHCl_3$ as indicated. Mass spectra were obtained on a VG autospec operating at 8 kV.

Mevinolin and simvastatin were extracted with $CHCl_3$ from powdered commercially available tablets, and recrystallized from aqueous MeOH.

***tert*-Butyl 3-[(1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*)-[6-[1-(Ethoxycarbonyl)-1,2,4a,5,6,7,8,8a-octahydro-8-hydroxy-2-methylnaphthalenyl]]-3-oxopropionate (15).** A solution of freshly distilled *tert*-butyl acetate (4.4 g, 38 mmol) in dry tetrahydrofuran (THF) (5 mL) was added dropwise to a well-stirred solution of lithium hexamethyldisilazide in THF (1.0 M; 38 mmol) at −78 °C. After 3.5 h, a solution of (+)-(1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*)-1-(ethoxycarbonyl)-1,2,4a,5,6,7,8,8a-octahydro-2-methyl-6,8-naphthalenecarbolactone[11] (**12**) (2.0 g, 7.58 mmol) in dry THF (5 mL) was added dropwise, the solution stirred for 90 min at −70 °C and then quenched with NH_4Cl solution (15 mL). The mixture was warmed to room temperature and then separated and the aqueous phase extracted with CH_2Cl_2 (2 × 40 mL). The combined organic phases were washed with brine (20 mL), then dried, and evaporated to leave a gum (3.34 g) which was purified by column chromatography eluting with 4:1 to 1:1 hexane–EtOAc to give **15** (1.6 g, 56%) as a white powder: mp 90–95 °C; IR (KBr disc) ν_{max} 3480 (br), 2960, 1725, 1710, 1640 cm^{-1}; ^1H NMR δ 5.50 (1 H, ddd, *J* = 9.8, 4.5, 2.5), 5.31 (1 H, d, *J* = 9.8), 4.16 (1 H, m), 4.07 (2 H, m), 3.69 (1 H, m), 3.44 (2 H, d, *J* = 1.8), 2.95 (1 H, t, *J* = 6.3), 2.76 (1 H, dd, *J* = 11.6, 6.0), 2.52 (1 H, m), 2.18 (1 H, br d, *J* = 16.3), 2.13–2.0 (2 H, m), 1.78 (1H, ddd, *J* = 15.2, 6.4, 3.5), 1.45–1.28 (11 H, m), 1.18 (3 H, t, *J* = 7) and 0.82 (3 H, d *J* = 7); ^{13}C NMR δ 210.0, 173.3, 166.3, 131.4, 129.6, 81.9, 64.7, 59.8, 48.1, 46.0, 44.4, 40.1, 32.5, 32.2, 32.0, 29.4, 27.8, 17.4, 14.1. Anal. ($C_{21}H_{32}O_6 \cdot {}^1/_2H_2O$) C, H.

Ethyl (1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*)-1,2,4a,5,6,7,8,8a-Octahydro-8-hydroxy-2-methyl-6-(1-oxoethyl)naphthalene-1-carboxylate (13). CF_3CO_2H (3.75 mL, 48.7 mmol) was added to a stirred solution of the keto ester **15** (1.5 g, 3.9 mmol) in CH_2Cl_2 (4 mL), followed by water (0.3 mL). The solution was stirred for 75 min, then brine (10 mL) and CH_2Cl_2 (10 mL) were added, and the aqueous phase was separated and extracted with CH_2Cl_2 (3 × 10 mL). The combined organics were washed with Na_2CO_3 solution (20 mL) and brine (10 mL), dried, and evaporated to give a solid. The solid (1.1 g) was dissolved in sodium ethoxide solution (prepared by dissolving sodium (70 mg, 3.04 mmol) in absolute EtOH (20 mL)), and after 1 h the solvent was evaporated to give a gum, which was partitioned between brine (20 mL) and CH_2Cl_2 (50 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (50 mL), and the combined organic phases were washed with brine (20 mL), dried, and evaporated to leave a gum, which was purified by column chromatography eluting with 1:2 EtOAc–hexane to give **13** (0.945 g, 87%) as a gum: IR (thin film) ν_{max} 3600–3300, 2930, 1730 and 1710 cm^{-1}; ^1H NMR δ 5.57 (1 H, ddd, *J* = 9.8, 4.4, 2.7), 5.42 (1 H, d, *J* = 9.8), 4.43 (1 H, br s), 4.23–4.07 (2 H, m), 2.9 (1 H, tt, *J* = 12.6, 3.7), 2.84 (1 H, dd, *J* = 11.6, 5.9), 2.64 (1 H, m), 2.40 (1 H, m), 2.16 (3 H, s), 2.15–1.9 (2 H, m), 1.68–1.32 (3 H, m), 1.27 (3 H, t, *J* = 7), 1.25 (1 H, m), 0.93 (3 H, d, *J* = 7); ^{13}C NMR δ 210.0, 173.5, 131.0, 130.0, 65.9, 60.0, 45.4, 44.9, 39.4, 35.4, 34.3, 33.2, 32.4, 28.0, 17.3, 14.2; MS *m/z* found 280.1681, calcd for $C_{16}H_{24}O_4$ 280.1675; 280 (10%), 219 (25), 173 (23) and 145 (100). Anal. ($C_{16}H_{24}O_4$) H; C: calcd, 68.55; found, 67.98.

Ethyl (1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*)-6-Acetoxy-1,2,4a,5,6,7,8,8a-octahydro-8-hydroxy-2-methylnaphthalene-1-carboxylate (14). A solution of Br_2 (0.54 g, 3.4 mmol) in CCl_4 (25 mL) was

(17) Ness, G. C.; Spindler, C. D.; Moffler, M. H. Purification of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase from Rat Liver. *Arch. Biochem. Biophys.* **1979**, *197*, 493–499.

(18) Shaw, M. K.; Newton, R. S.; Sliskovic, D. R.; Roth, B. D.; Ferguson, E.; Krause, B. R. HEP-G2 cells and Primary Rat Hepatocytes Differ in their Response to Inhibitors of HMG-CoA reductase. *Biochem. Biophys. Res. Commun.* **1990**, *170*, 726–734.

added dropwise to a cold (−20 °C), stirred solution of the alkene **13** (0.94 g, 3.37 mmol) in a mixture of the same solvent (25 mL) and absolute EtOH (1.5 mL). The pale orange solution was stirred for 10 min and then washed with a solution of $NaHSO_3$, followed by $NaHCO_3$ solution (30 mL). The organic solution was dried and evaporated to leave **17** (1.61 g) as a solid, which was used directly in the next step: ^1H NMR δ 4.87 (1 H, m), 4.53 (1 H, m), 4.35 (1 H, m), 4.22–4.09 (2 H, m), 3.40 (1 H, dd, J = 11.6, 5.1), 2.99 (1 H, tt, J = 12.3, 3.6), 2.77 (1 H, m), 2.49 (1 H, tt, J = 11.3, 3.6), 2.16 (3 H, s), 2.15–1.54 (5 H, m), 1.32 (3 H, d, J = 7), 1.26 (3 H, t, J = 7.2); ^{13}C NMR δ 211.5, 173.1, 65.5, 60.3, 57.5, 57.4, 43.9, 42.0, 39.2, 35.2, 35.0, 33.7, 32.1, 27.9, 19.5, 14.1.

$(CF_3CO)_2O$ (7.75 mL, 55.5 mmol) was added to a well-stirred mixture of H_2O_2 solution (60% w/v, 1.2 mL, 20.8 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 15 min, the clear solution was added rapidly to a stirred ice-cold solution of crude **17** (1.52 g) in CH_2Cl_2 (6 mL) and then the cooling bath was removed. After 30 min, more CH_2Cl_2 (25 mL) was added, the solution recooled to 0 °C, and Na_2CO_3 solution added slowly until the mixture reached pH 8. The aqueous phase was separated and extracted with CH_2Cl_2 (2 × 25 mL), and the combined organic extracts were dried and evaporated to leave crude **18** (1.37 g) as a white foam: ^1H NMR δ (key peaks) 5.17 (1 H, tt, J = 11.5, 4.4) and 2.02 (3 H, s).

Freshly prepared active Zn (1.91 g, 29.2 mmol) was added in several portions to a stirred solution of **18** (1.33 g) and AcOH (0.5 mL) in dry ether (15 mL). After the refluxing had subsided, the mixture was stirred for 15 min and then filtered, and the solid was washed with ether. The filtrate was washed with Na_2CO_3 solution, dried, and evaporated. The residue was purified by column chromatography, eluting with 1:4 EtOAc–hexane, to give **14** (645 mg, 65% from **13**) as needles, mp 90–92 °C; IR (soln) ν_{max} 3610, 3000, 1725 cm^{-1}; ^1H NMR δ 5.56 (1 H, ddd, J = 10.0, 4.4, 2.7), 5.39 (1 H, d, J = 10.0), 5.12 (1 H, tt, J = 11.5, 4.5), 4.45 (1 H, m), 4.21–4.08 (3 H, m), 2.83 (1 H, dd, J = 11.6, 6.0), 2.67–2.39 (2 H, m), 2.21–1.09 (5 H, m), 2.0 (3 H, s), 1.26 (3 H, t, J = 7.2), 0.91 (3 H, d, J = 7.0); ^{13}C NMR δ 173.5, 170.5, 131.0, 129.7, 69.3, 67.0, 60.0, 44.6, 39.2, 37.8, 32.4, 32.2, 21.2, 17.4, 14.2. Anal. ($C_{16}H_{24}O_5 \cdot {}^1/_2H_2O$) C, H.

Ethyl (1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*)-1,2,4a,5,6,7,8,8a-Octahydro-6,8-dihydroxy-2-methylnaphthalene-1-carboxylate (11). The acetate **14** (615 mg, 2.08 mmol) was dissolved in a solution of sodium ethoxide (8 mL), prepared using sodium metal (50.2 mg, 2.18 mmol). After 30 min, the solvent was evaporated and the residue partitioned between brine (25 mL) and CH_2Cl_2 (25 mL). The brine was extracted with more CH_2Cl_2 (2 × 25 mL), and the combined organic extracts were dried and evaporated to leave **11** as a solid (0.476 g, 90%): mp 142–145 °C; IR (solution) ν_{max} 3600, 2980, 1725 cm^{-1}; ^1H NMR δ (key peaks) 4.05 (1 H, tt, J = 11.3, 4.5); ^{13}C NMR δ 173.6, 130.9, 130.1, 67.4, 66.3, 60.0, 44.8, 42.9, 41.8, 39.2, 32.5, 17.4, 14.2. Anal. ($C_{14}H_{22}O_4 \cdot 0.33H_2O$) C, H.

(1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-1,2,4a,5,6,7,8,8a-octahydro-1-(hydroxymethyl)-2-methylnaphthalene (20). *tert*-Butyldiphenylsilyl chloride (0.44 mL, 1.71 mmol) was added dropwise to a stirred solution of **11** (416 mg, 1.64 mmol) and imidazole (223 mg, 3.27 mmol) in dry DMF (3 mL). After 16 h, brine (10 mL) was added and the mixture extracted with ether (2 × 20 mL), which was then washed sequentially with 2 M HCl (10 mL), water (2 × 10 mL), and Na_2CO_3 solution (10 mL). The organic solution was dried and evaporated to leave crude **19** (740 mg): ^1H NMR δ (key peaks) 7.74–7.32 (10 H, m), 1.06 (9 H, s).

A solution of **19** (0.74 g) in dry ether (6 mL) was added dropwise to a solution of $LiAlH_4$ (0.19 g, 4.92 mmol) in dry ether (5 mL) and cooled in a cold water bath. After 1 h, water (0.2 mL) was added slowly to the stirred, cooled mixture, followed by NaOH solution (15%; 0.2 mL) and then more water (0.6 mL). The mixture was filtered and the filtrate evaporated to leave a gum (0.74 g), which was purified by column chromatography eluting with 1:2 EtOAc–hexane to give **20** (0.67 g, 91%): IR ν_{max} 3660–3100, 3100–3000 cm^{-1}; ^1H NMR δ (key peaks) 3.67–3.52 (2 H, m); ^{13}C NMR δ 135.7, 134.7, 134.6, 131.9, 131.0, 129.4, 127.4, 68.0, 67.8, 64.9, 42.4, 42.2, 42.0, 40.9, 34.5, 33.7, 27.0, 19.1, 15.5.

(1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*)-1-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-6-[(*tert*-butyldiphenylsilyl)oxy]-1,2,4a,5,6,7,8,8a-octahydro-8-hydroxy-2-methylnaphthalene (21). *tert*-Butyldimethylsilyl chloride (0.228 g, 1.51 mmol) was added in portions to a stirred solution of **20** (0.622 g, 1.38 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 18 h and then more CH_2Cl_2 (20 mL) was added, and the organic phase was washed successively with 1 M H_3PO_4 (10 mL), $NaHCO_3$ solution (10 mL), and brine (10 mL). The CH_2Cl_2 was dried and evaporated to leave a gum, which was purified by column chromatography eluting with 19:1 hexane–EtOAc to give **21** as a gum (0.668 g, 86%): ^1H NMR δ (key peaks) 0.89 (9 H, s), 0.076 (3 H, s) and 0.073 (3 H, s).

8-[(1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-1,2,4a,5,6,7,8,8a-octahydro-1-(hydroxymethyl)-2-methylnaphthalenyl] 2(*S*)-Methylbutyrate (23). A solution of **21** (0.668 g, 1.18 mmol), 2(*S*)-methylbutyric anhydride (1.32 g, 7.1 mmol), DMAP (22 mg), and dry pyridine (2.00 mL, 24.9 mmol) in CH_2Cl_2 (2.0 mL) was stirred for 26 h. MeOH (10 mL) was added and after stirring for 2 h, ether (100 mL) was added and the solution washed with 1 M H_3PO_4 (30 mL), water (2 × 25 mL), and $NaHCO_3$ solution (25 mL). The organic solution was dried and evaporated to leave **22** as a gum (0.734 g) which was used in the next step without further purificiation. ^1H NMR δ (key peaks) 5.03 (1 H, m), 2.15 (1 H, sextet, J = 6.9), 1.6–1.2 (2 H, m), 0.97 (3 H, d, J = 6.8) and 0.80 (3 H, t, J = 7.4).

22 was dissolved in a solution of $TsOH \cdot H_2O$ (200 mg, 1.07 mmol) in MeOH (250 mL). After 2 h, $NaHCO_3$ solution (100 mL) was added, and the mixture stirred for 5 min and then concentrated to about 30-mL volume. The residue was extracted with CH_2Cl_2 (3 × 100 mL); the CH_2Cl_2 was dried and evaporated to leave a gum (0.728 g) which was purified by column chromatography eluting with 9:1 hexane–EtOAc to 4:1 hexane–EtOAc to give **23** (418 mg) plus unreacted **22** (137 mg). This was recycled using the same ratio of reagents and purified by column chromatography as above to give a further 68 mg of **23**, a combined total yield of 486 mg (85%): ^1H NMR δ (key peaks) absence of signals at 0.87, 0.053, and 0.023 for *tert*-butyldimethylsilyl group; ^{13}C NMR δ 175.7, 135.6, 134.4, 134.1, 132.6, 129.7, 129.5, 127.4, 69.8, 68.2, 61.8, 41.9, 41.3, 40.0, 39.3, 39.2, 34.8, 31.5, 26.9, 26.2, 19.0, 16.3, 15.4, 11.4.

Methyl 7-{1-[(1*R*,2*R*,4a*R*,6*R*,8*S*,8a*S*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-8-[(2(*S*)-methyl-1-oxobutyl)oxy]-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthylenyl]}-3(*R*)-[(*tert*-butyldimethylsilyl)oxy]-5-oxohepten-6-oate (26). A solution of dry DMSO (178 mg, 2.28 mmol) in dry CH_2Cl_2 (0.7 mL) was added to a stirred solution of $(COCl)_2$ (145 mg, 1.14 mmol) in dry CH_2Cl_2 (1.8 mL) at −70 °C. After 5 min, a solution of the alcohol **23** (486 mg, 0.91 mmol) in dry CH_2Cl_2 (1.8 mL) was added rapidly. After a further 10 min, a second batch of "activated DMSO" (prepared as above) was added to the stirred mixture. Then after an additional 10 min, dry Et_3N (1.14 mL, 8.2 mmol) was added rapidly. After 5 min more at −70 °C, the solution was allowed to warm to room temperature, ether (50 mL) was added, and the organic phase washed successively with 1 M H_3PO_4 (20 mL), water (2 × 20 mL), $NaHCO_3$ solution (20 mL), and dried. Evaporation gave a gum which was purified by rapid column chromatography eluting with 1:15 EtOAc–hexane to give **24** (398 mg, 82%) which was used immediately: ^1H NMR δ (key peaks) 9.69 (1 H, d, J = 2.5, CHO), 2.52 (1 H, ddd, J = 11.3, 5.7, 2.5, 1-H); ^{13}C NMR δ (key peak) 203.2.

Lithium bis(trimethylsilyl)amide in THF (1.0 M; 1.42 mmol) was added slowly to a stirred solution of methyl 3(*R*)-[(*tert*-butyldimethylsilyl)oxy]-6-(dimethoxyphosphonyl)-5-oxohexanoate[10] **25** (0.68 g, 1.78 mmol) in THF (5 mL) at −70 °C. After 1 hour, a solution of the aldehyde **24** (378 mg, 0.71 mmol) in THF (5 mL) was added dropwise, the solution warmed to room temperature, and stirred for 66 h. NH_4Cl solution (5 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 25 mL). The combined CH_2Cl_2 extracts were dried and evaporated, and the residue was purified by column chromatography eluting with 19:1 hexane–EtOAc to give **26** (380 mg, 68%) as an oil: IR (solution) ν_{max} 2980, 1740, 1675, 1630 cm^{-1}; ^1H NMR δ (key peaks) 6.74 (1 H, dd, J = 15.75, 10.2), 5.92 (1 H, d, J = 15.75), 4.89 (1 H, m), 4.60 (1 H, pentet, J = 6.3), 3.65 (3 H, s), 2.74 and 2.49 (m, 2 × 2 H), 1.02 (9 H, s); ^{13}C NMR δ 197.1, 174.6, 171.4, 147.9, 135.6, 134.3, 133.9, 131.9, 131.8, 129.9, 129.5, 127.4, 70.2, 68.1, 65.9, 51.3, 47.5, 42.7, 42.4, 41.7, 41.1, 40.4, 39.7, 35.7, 34.0, 26.8, 26.1, 25.6, 19.0, 17.8, 16.5, 16.3, 11.7, −4.8, −5.1; MS m/z found 731.3794, (M−57), calcd for $C_{42}H_{59}O_7Si_2$ (M − C_4H_9) 731.3799; 788 (1%), 747 (8), 731 (28),

629 (7), 599 (10), 497 (9), 431 (17), 283 (43) and 199 (100). Anal. ($C_{46}H_{68}O_7Si_2 \cdot {}^1/_2CH_2Cl_2$) C, H.

Methyl 7-{1-[(1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*)-6-(Hydroxy-8-[(2-(*S*)-methyl-1-oxobutyl)oxy]-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthalenyl]}-3(*R*)-hydroxy-5-oxoheptanoate (27). A mixture of Te (0.137 g, 1.075 mmol) and $NaBH_4$ (93 mg, 2.47 mmol) in deoxygenated EtOH (5 mL) was heated to reflux for 1 h. The purple solution[15] was cooled and finely ground NH_4Cl (0.58 g, 10.8 mmol) added, followed by a solution of the enone **26** (340 mg, 0.43 mmol) in deoxygenated EtOH (5 mL). After stirring for 2 h, a second batch of sodium hydrogen telluride was prepared (on one-fifth of the scale above) and added to the reaction, together with more NH_4Cl (116 mg, 2.16 mmol). The mixture was stirred for a further 2 h and then NH_4Cl solution (2 mL) added. The mixture was concentrated to a small volume and extracted with CH_2Cl_2 (3 × 20 mL), and the CH_2Cl_2 was dried and evaporated to leave a gum (355 mg). A solution of the gum (315 mg) in 1:19 40% aqueous HF–CH_3CN (20 mL) was stirred for 6 h at room temperature. $NaHCO_3$ solution (50 mL) was added carefully and the mixture concentrated under reduced pressure and then extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried and evaporated to leave a crude gum (220 mg), which was purified by column chromatography eluting with 1:1 EtOAc–hexane to give **27** (125 mg, 71%): mp 89–91 °C; IR (solution) ν_{max} 3680, 3600, 3000, 1725 cm^{-1}; ^1H NMR δ 5.61 (1 H, ddd, J = 9.8, 4.8, 2.7), 5.40 (1 H, br d, J = 9.8), 5.28 (1 H, m), 4.43 (1 H, m), 3.89 (1 H, tt, J = 11.2, 4.5), 3.70 (3 H, s), 2.62–2.59 (2 H, m), 2.51–2.48 (2 H, d, J = 6.6), 2.46–1.02 (17 H, m), 1.13 (3 H, d, J = 7.1), 0.89 (3 H, t, J = 7.4), 0.82 (3 H, d, J = 7.0); ^{13}C NMR δ 209.7, 175.7, 172.1, 132.4, 129.8, 69.4, 66.5, 64.3, 51.7, 48.0, 41.6, 41.5, 41.0, 40.5, 40.4, 39.5, 36.5, 35.1, 31.3, 26.5, 21.6, 16.6, 14.7, 11.5. Anal. ($C_{24}H_{38}O_7$) C, H.

(1*S*,2*S*,4a*R*,6*R*,8*S*,8a*S*,4′*R*,6′*R*,2″*S*)-6′-[2-[1,2,4a,5,6,7,8,8a-octahydro-6-hydroxy-2-methyl-8-[(2″-methyl-1″-oxobutyl)oxy]-1-naphthalenyl]ethyl]tetrahydro-4′-hydroxy-2*H*-pyran-2′-one (29). Et_3B in THF (1.0 M; 0.66 mmol) was added to a stirred mixture of MeOH/THF (1:4, 6.6 mL) at room temperature. After 1 h the solution was cooled to −70 °C, and a solution of the ketone **27** (125 mg, 0.285 mmol) in MeOH–THF (1:4, 6.6 mL) was added dropwise. The solution was stirred for 90 min, then $NaBH_4$ (13 mg, 0.342 mmol) added in one portion, and the solution left at −70 °C for 18 h. NH_4Cl solution (10 mL) was added, the mixture allowed to warm to room temperature, and then water added until the solids just dissolved. The aqueous solution was extracted with EtOAc (3 × 50 mL) and the combined organics washed with brine (50 mL) and dried. The solvents were evaporated and the residue dissolved in MeOH (25 mL), which was warmed and then evaporated again. This process was repeated four times to leave **28** as a gum (125 mg); ^1H NMR δ (key peaks) 4.23 (1 H, m), 3.89–3.67 (2 H, m).

A mixture of **28** (125 mg) and tosic acid monohydrate (20 mg) in dry benzene (15 mL) was stirred for 30 min, and then the solvent was evaporated under reduced pressure and replaced with fresh dry benzene (15 mL). After stirring for 3.5 h, the solvent was evaporated and the residue partitioned between EtOAc and $NaHCO_3$ solution. The organic layer was separated, dried, and evaporated. The crude product was recrystallized from EtOAc/hexane to give **29** (49.6 mg). The mother liquor was evaporated and the residue crystallized as above to give a second crop of the title compound (14.6 mg). The two crops were combined and dissolved in EtOAc, and then the solvent allowed to evaporate slowly, giving a total yield of 64.2 mg (55%) from **27**: mp 158–159 °C; ^1H NMR δ 5.64 (1 H, m), 5.41 (1 H, br d, J = 9.9), 5.30 (1 H, m), 4.60 (1 H, m), 4.36 (1 H, m), 3.91 (1 H, m), 2.74 (1 H, dd, J = 17.6 and 5.1), 2.61 (1 H, m), 2.44–1.04 (17 H, m), 1.14 (3 H, d, J = 6.9), 0.91 (3 H, t, J = 7.4), 0.86 (3 H, d, J = 7.0); ^{13}C NMR δ 176.0, 170.3, 132.7, 129.6, 76.0, 69.6, 66.7, 62.5, 41.7, 41.6, 41.0, 39.7, 38.5, 37.2, 35.9, 35.1, 32.8, 31.4, 26.6, 23.4, 16.7, 14.7, 11.6. Anal. ($C_{23}H_{36}O_6$) C, H.

Dihydroeptastatin. Sodium 7-{1-[(1*S*,2*S*,4a*R*,6*R*,8*S*,-8a*S*)-6-Hydroxy-8-[(2(*S*)-methyl-1-oxobutyl)oxy]-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthalenyl]}-3(*R*),5-(*R*)-dihydroxyheptanoate (7). A solution of **29** (4.0 mg; 9.8 μmol) in a mixture of water (50 μL) and NaOH in MeOH (0.1 M; 10.8 μmol) was stirred at room temperature for 18 h, and the solvent was evaporated to leave the sodium salt **7** as a gum. TLC (1:9:90 AcOH–MeOH–CH_2Cl_2) showed a single spot.

Cellular Assay. Cells were grown in medium containing 10% foetal calf serum (FCS) to approximately 90% confluence. Twenty-four hours prior to the experiment the medium was changed to one containing 1% FCS in order to upregulate HMG-CoA reductase. Sodium [^{14}C]acetate was added together with the test compound, and incubation continued for 3 h. The extraction, isolation, and quantitation of the ^{14}C-labeled sterols were carried out according to the method of Kandutsch.[19]

Registry No. **7**, 138505-91-6; **7** free base, 142507-51-5; **11**, 138505-81-4; **12**, 116097-23-5; **13**, 138505-77-8; **14**, 138505-80-3; **15**, 142437-80-7; **17**, 142437-81-8; **18**, 142437-82-9; **19**, 138523-70-3; **20**, 138505-82-5; **21**, 138505-83-6; **22**, 138505-84-7; **23**, 138505-85-8; **24**, 138505-86-9; **25**, 142437-83-0; **26**, 138505-87-0; **27**, 138505-88-1; **28**, 138505-89-2; **29**, 138505-90-5; (*S*,*S*)-[[$C_2H_5(CH_3)CHCO]_2O$], 84131-91-9; HMG-CoA, 37250-24-1.

(19) Kandutsch, A. A.; Saucier, S. E. Prevention of Cyclic and Triton-induced Increases in Hydroxymethylglutaryl Coenzyme A Reductase and Sterol by Puromycin. *J. Biol. Chem.* **1969**, *244*, 2299–2305.