Synthesis and Biological Activity of Bile Acid-Derived HMG-CoA Reductase Inhibitors. The Role of 21-Methyl in Recognition of HMG-CoA Reductase and the Ileal Bile Acid Transport System

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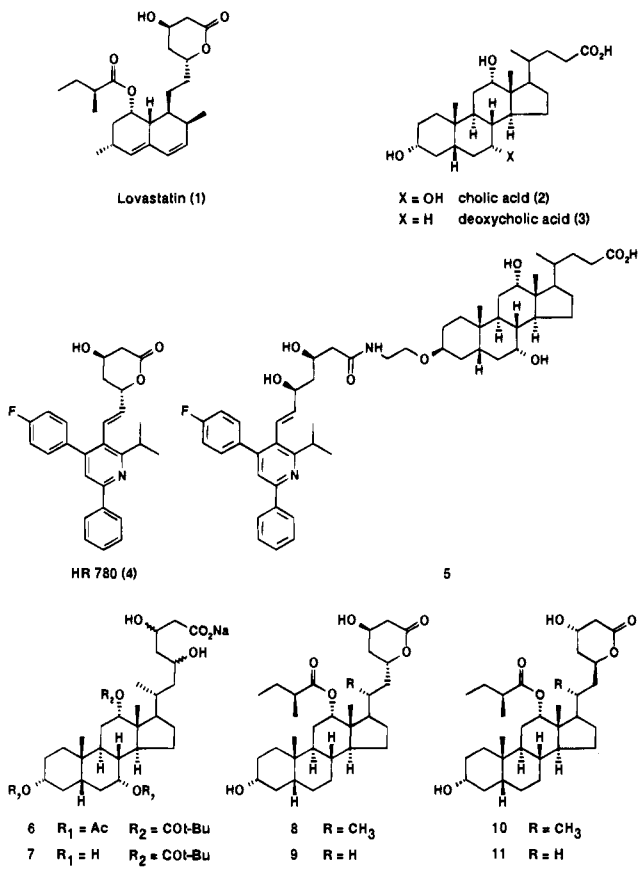
To increase hepatoselectivity of HMG-CoA reductase inhibitors by using the specific bile acid transport systems, deoxycholic acid-derived inhibitors **9** and **11** have been synthesized, on the basis of the concept of combining in one molecule structural requirements for specific inhibition of the HMG-CoA reductase and specific recognition by the ileal bile acid transport system. The 1-methyl-3-carboxylpropyl subunit of deoxycholic acid was replaced by the 3,5-dihydroxyheptanoic acid lactone of lovastatin, and position 12-OH was esterified with 2-methylbutyric acid. Compounds **9** and **11** were evaluated for their inhibitory activity on rat liver HMG-CoA reductase, cholesterol biosynthesis in HEP G2 cells, and [^{3}H]taurocholate uptake in rabbit brush border membrane vesicles and compared with methyl derivatives **8** and **10**. The steroidal 21-CH_3 group affects both activity on HMG-CoA reductase and recognition by the ileal bile acid transport system.

Inhibition of HMG-CoA reductase is a very effective way for lowering total and low-density lipoprotein (LDL) cholesterol levels in hypercholesterolemic patients.[1] Although lovastatin (**1**), pravastatin, and simvastatin are well tolerated in man and the incidence of adverse effects is low,[2] there has been a discussion on liver selective action of these drugs.[3] Since the liver is the major site of cholesterol regulation, lipoprotein production, and LDL removal,[4] an ideal HMG-CoA reductase inhibitor should exclusively act in the liver to minimize extrahepatic side effects arising from inhibition of nonhepatic HMG-CoA reductase. Considerable efforts have been made to modify the hexahydronaphthalene portion of lovastatin (**1**) and the other statins or to replace it by simplified aromatic or nonaromatic subunits.[1,3c,5] Data indicating whether these modifications have influenced liver selectivity are currently available only for a few examples.[3e–g]

To increase liver selectivity, we followed a rational approach based on the hepatotropism of bile acids and their specific physiological transport systems in the terminal ileum and the liver. Bile acids are synthesized from cholesterol in the liver, secreted into the small intestine, and recirculated back to the liver with portal blood. During this enterohepatic circulation, bile acids are transported by a specific sodium-dependent transport system in the sinusoidal membrane of hepatocytes[6,7] and the brush border membranes of ileocytes.[8–10] To make use of these transport systems, we followed two concepts. In a prodrug approach, bile acids were used as molecular shuttles. Therefore, in **5** we coupled the HMG-CoA reductase inhibitor **4** via linker with steroidal position 3-C of cholic acid (**2**).[11] In the second approach represented by compound **9** and reported herein, the hexahydronaphthalene part of lovastatin (**1**) was replaced by a modified deoxycholic acid ring system with the 2-methylbutyric acid side chain of lovastatin (**1**) attached in position 12-C. This is the concept of combining in one molecule structural requirements for specific recognition and inhibition of the HMG-CoA reductase and specific recognition and transport by the bile acid transport systems. From a series of compounds derived from various bile acids, **8** emerged as the most potent inhibitor of HMG-CoA reductase with an IC_{50} value of 0.65 μM and an affinity for the ideal bile acid transport system of the rabbit comparable to that of cholic acid.[12] Recent review of the literature has revealed that two other research groups are actively investigating bile acid-derived HMG-CoA reductase inhibitors. The patent from the Rhone–Poulenc–Rorer



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group claimed various analogues,[13a] but information on hepatoselectivity or effects on bile acid transport system were not disclosed. The Ciba–Geigy group reported[13b] diastereomerically unresolved mixtures of **6** and **7** bearing the steroidal 21-CH_3 group as weak inhibitors of HMG-CoA reductase. As the lactone moiety of HMG-CoA reductase inhibitors or its corresponding open chain dihydroxy acid is very sensitive to structural changes,[14] we expected that a 7-desmethyl (steroidal position 21-CH_3) derivative like **9** would exhibit much stronger inhibitory activity on HMG-CoA reductase compared to methyl derivatives like **6**, **7**, or **8**. But the key question was whether this structural change in addition to the elongation of the bile acid side chain by three carbon atoms and the additional two hydroxy groups would be tolerated by the ileal bile acid transporter. To answer this question, compound **9** was synthesized in diastereomerically pure form.

Chemistry

Deoxycholic acid (**3**) as its 3,12-diacetate was transformed in four steps to ketone **12** (Scheme 1) according to Barton et al.[15] following the sequence: (1) $SOCl_2$/pyridine; (2) MeOH; (3) $KMnO_4$; (4) O_2 (air), $Cu(OAc)_2$, 2,2′-dipyridyl, DABCO. Sodium borohydride reduction of **12** and subsequent elimination with bis[α,α-bis-(trifluoromethyl)benzenemethanolato]diphenylsulfur[16] provided olefin **13** as a single isomer; the 17-ene isomer could not be detected. In contrast, elimination of the mesylate with DBU/DMF at 110–120 °C for 7 h gave a mixture of 20-ene/17-ene = 60:40, whereas the corresponding triflate afforded predominantly the 17-ene (20-ene/17-ene = 20:80). Olefin **13** was transformed to aldehyde **14** using $OsO_4/NaIO_4$. Elongation of **14** to unsaturated aldehyde **15** was accomplished in two steps: (1) diisopropyl cyanomethylphosphonate/NaH; (2) DIBAH. During reduction, the acetyl groups were cleaved. Aldehyde **15** was reacted with 3.7 equiv of the dianion of *tert*-butyl acetoacetate, providing **16** as a 1:1 mixture of diastereomers which could not be separated at this stage. Diastereoselective reduction of **16** using Et_3B/MeOH, $NaBH_4$[17] provided a chromatographically separable 1:1 mixture of **17** and **18**. Stereochemistry of **17** and **18** was assigned unambiguously by transforming each isomer separately into the corresponding acetonide (Scheme 2) followed by ozonolysis, reductive workup ($NaBH_4$), and comparison of **20** and ent-**20** with the optical rotation of an authentic sample of **20** which had been prepared from malic acid.[18] For attachment of the 2-methylbutyric acid group to position 12-OH, the 3-OH was selectively protected as the acetate (Scheme 3) and the methylbutyryl was introduced using 2-*S*-methylbutyric anhydride/pyridine, DMAP. After cleavage of the acetonide (MeOH, *p*-TsOH), the double bond was hydrogenated (Rh/alumina). Treatment of **22** with sodium hydoxide followed by HCl/THF provided lactone **9**. Diastereomer **11** was synthesized from **18** following the same sequence of reactions via the intermediates **18** → **23** → **24** → **25** → **11**.

Biological Results and Discussion

Inhibitors were characterized biologically by (1) inhibition of rat liver microsomal HMG-CoA reductase (Table 1), (2) inhibition of cholesterol biosynthesis in HEP G2 cells (Table 1), and (3) inhibition of Na^+-dependent [^{3}H]taurocholate uptake in rabbit ileal brush border membrane vesicles (Table 2). Compounds **8**–**11** were tested as their sodium salts of the corresponding 3,5-dihydroxyheptanoates. In evaluation of HMG-CoA reductase inhibition, desmethyl derivative **9** (IC_{50} = 0.2 μM) was 3 times as active as the methyl derivative **8**[12] (IC_{50} = 0.65 μM) and 62 times as active as **7** (IC_{50} = 12.3 μM),[13b] whereas lovastatin (IC_{50} = 0.007 μM) was 29 times more active than **9**. As expected, diastereomers **10** and **11** with the unnatural configuration showed IC_{50} values of 7.0 (**10**) and 1 μM (**11**). In HEP G2 cells, on inhibition of cholesterol biosynthesis, similar observations were made. Desmethyl derivative **9** (IC_{50} = 1.8 μM) was 9 times as active as the methyl derivative **8** (IC_{50} = 16.1 μM), and lovastatin (IC_{50} = 0.029 μM) was 60 times as active as **9**. Again unnatural diastereomers **10** and **11** were much less active than **8** and **9**. Although compared to **8** desmethyl derivative **9** has increased inhibitory activity by a factor of 3 for HMG-CoA reductase and a factor of 9 for cholesterol biosynthesis in HEP G2 cells, the elimination of the 7-CH_3 group did not yield a compound with potency comparable to that of lovastatin. Other structural factors, for example, the 18-CH_3 group, the ring size of ring D, or the lack of a methyl group in ring D at position 16-C, might also influence the activity on HMG-CoA reductase significantly.

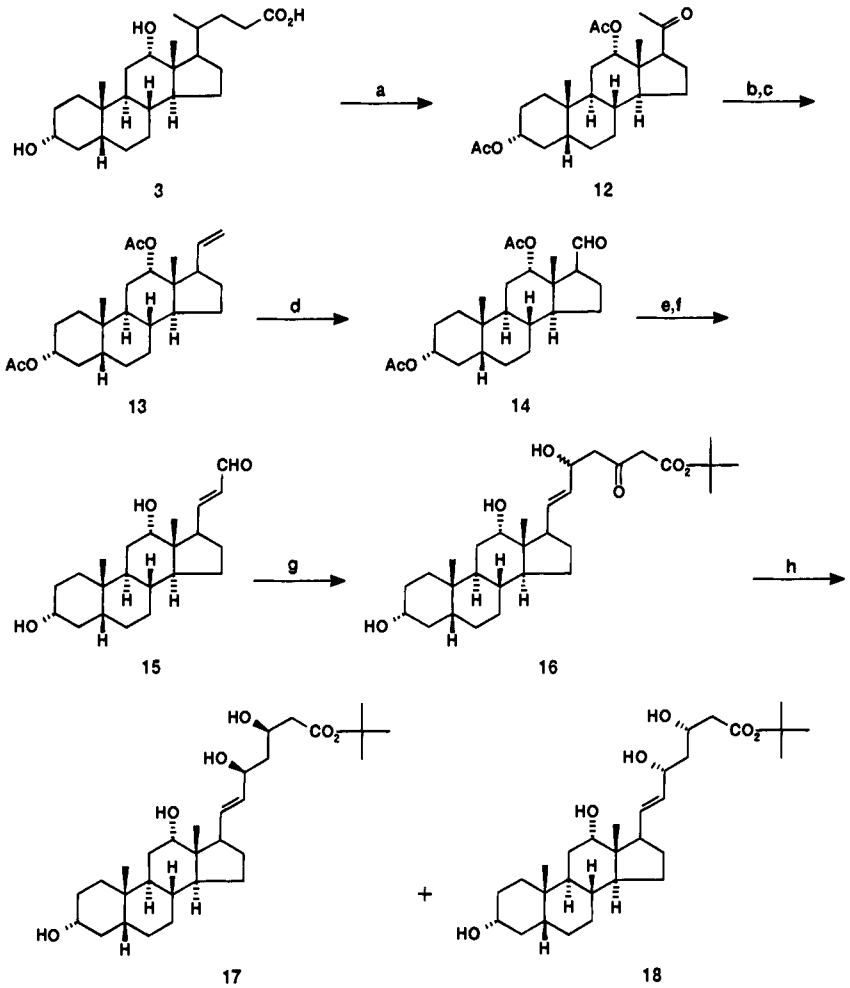
The effect on the bile acid transport system was studied by determining the inhibition of Na^+-dependent [^{3}H]taurocholate uptake in rabbit ileal brush border membrane vesicles. In these experiments, cholic acid served as the standard and taurocholate, the most potent physiological compound, as an internal control (Table 2). Lovastatin did not show any affinity for the ileal bile acid transporter. Methyl derivative **8** showed activity comparable to cholic acid, whereas desmethyl derivative **9** exhibited a slight decrease in activity by a factor of approximately 2. This result demonstrates that steroidal 21-CH_3 contributes to the interaction with the ileal bile acid transporter. This influence of the 21-CH_3 group is also reflected by comparison of the unnatural diastereomeres **10** and **11**. Comparison of **8** with **10** and **9** with **11** demonstrates that the configuration of the hydroxy groups at 3-C and 5-C of the heptanoic acid part does not play a significant role in molecular recognition by the bile acid transport system.

In summary, deoxycholic acid-derived HMG-CoA reductase inhibitor **9** lacking the steroidal 21-CH_3 group is still recognized by the Na^+-dependent ileal bile acid transport system, although with a slightly decreased affinity compared to cholic acid (**2**). The steroidal 21-CH_3 group contributes to the inhibitory activity at the HMG-CoA reductase by a factor of 3 relative to **8** but does not play a dramatic role overall. Therefore, to increase inhibitory activity at the HMG-CoA reductase, more substantial structural modifications of the bile acid moiety are required including modifications of the 18-CH_3 group and the D-ring, particularly ring size and substitution of position 16-C. Currently it is unknown how these modifications will influence the affinity for the ileal bile acid transporter.

Experimental Section

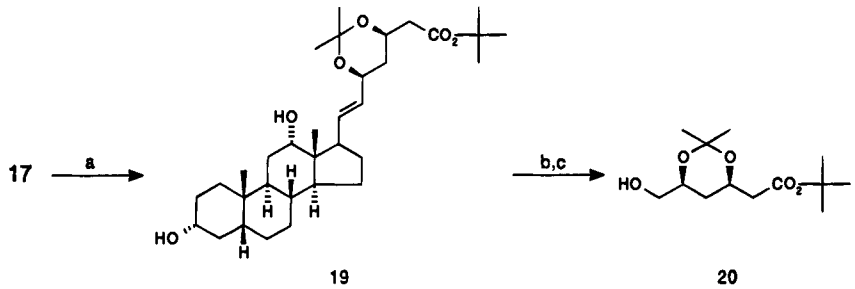
General Methods. Reactions with materials sensitive to air or moisture were run in dry glass apparatus under an argon atmosphere with dry solvents. Melting points were

Scheme 1[a]



[a] Reagents: (a) ref 15; (b) $NaBH_4$, MeOH; (c) $[C_6H_5C(CF_3)_2O]_2S(C_6H_5)_2$, CH_2Cl_2; (d) $OsO_4/NaIO_4$, dioxane/H_2O; (e) $(i\text{-PrO})_2P(O)CH_2CN$, NaH, THF; (f) DIBAH, toluene/ether; (g) $CH_3COCH_2CO_2t\text{-Bu}$, NaH, BuLi, THF; (h) Et_3B/MeOH, $NaBH_4$, THF.

Scheme 2[a]

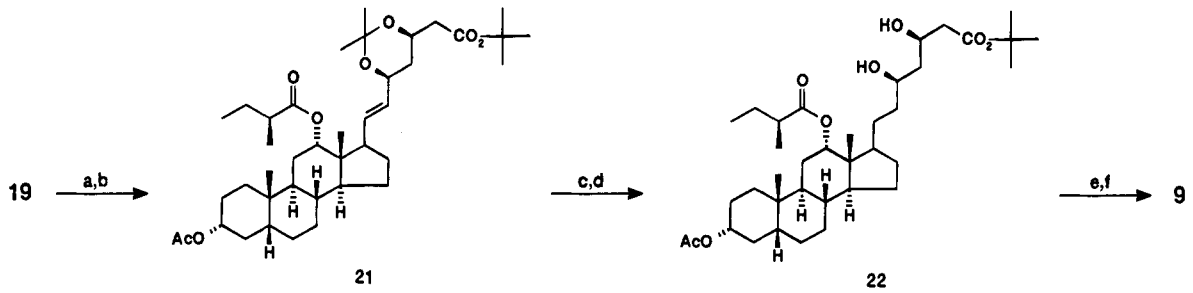


[a] Reagents: (a) $(MeO)_2CMe_2$, acetone, *p*-TsOH; (b) O_3, CH_2Cl_2/pyridine, −78 °C, Me_2S; (c) $NaBH_4$, MeOH.

determined on a Büchi capillary melting point apparatus (according to Dr. Tottoli) and are uncorrected. ^1H NMR spectra were recorded on a Bruker AM 270 or Varian Gemini 200 spectrometer. Significant ^1H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. FAB mass spectra were obtained on a Kratos MS 902 spectrometer in a 3-nitrobenzylic alcohol matrix (optionally in the presence of LiCl) using xenon as the target gas. DCI mass spectra were determined on a Kratos MS 80 RFA spectrometer using isobutane as reagent gas. Flash chromatography was carried out on E. Merck silica gel 60 (0.04−0.063 mm). Thin-layer chromatography was performed on silica gel F_{254} plates from E. Merck. Visualization was done using phosphomolybdic acid/cerium(IV) sulfate/H_2SO_4 and heating on a 100 °C plate. Elemental analyses were performed by Analytical Laboratories, Hoechst AG.

Synthesis of Compounds 17 and 18. 3α,12α-Diacetoxy-17β-ethenyl-5β-androstane (13). Sodium borohydride (50 mg, 1.30 mmol) was added to a stirred solution of **12** (600 mg, 1.44 mmol), prepared according to ref 15, in MeOH (10 mL). After 30 min at room temperature, removal of the solvent and flash chromatography (*n*-heptane/ethyl acetate = 6:4) gave 540 mg (90%) of 3α,12α-diacetoxy-17β-(1-(*RS*)-(hydroxyethyl)-5β-androstane: ^1H NMR ($CDCl_3$) δ 0.84 (s, 3H), 0.93 (s, 3H), 1.06 (d, 3H, J = 6), 2.02 (s, 3H), 2.14 (s, 3H), 3.44−3.56 (m, 1H), 4.64−4.78 (m, 1H), 5.28 (m, 1H); MS (FAB) 427.6 (M + Li). Anal. ($C_{25}H_{40}O_5$) C, H. A solution of 3α,12α-diacetoxy-17β-(1-(*RS*)-hydroxyethyl)-5β-androstane (2.1 g, 5.0 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of bis[α,α-bis(trifluoromethyl)benzenemethanolato]diphenylsulfur (5.0 g, 7.43 mmol) in CH_2Cl_2 (15 mL) at room temperature. After 3 h, the mixture was washed with 10% aqueous NaOH (2×) and water and dried. Evaporation and flash chromatography (*n*-

Scheme 3[a]



[a] Reagents: (a) CH_3COCl, pyridine; (b) 2-(*S*)-methylbutyric anhydride, pyridine, DMAP; (c) *p*-TsOH, MeOH, H_2O; (d) H_2, Rh/alumina (5%), ethyl acetate; (e) NaOH, EtOH; (f) THF, HCl.

Table 1. Inhibition of Rat Liver HMG-CoA Reductase[a] and Inhibition of Cholesterol Biosynthesis in HEP G2 Cells[a]

compd	IC_{50} (μM)[c] HMG-CoA reductase	cholesterol biosynthesis
1[b]	0.007 ± 0.002	0.029 ± 0.007
8[b]	0.65 ± 0.15	16.1 ± 3.8
9[b]	0.2 ± 0.05	1.8 ± 0.3
10[b]	7.0 ± 0.5	54.8 ± 7.2
11[b]	1.0 ± 0.3	8.2 ± 0.5

[a] Assay described in the Experimental Section. [b] Compound was tested as its sodium salt of the corresponding open chain 3,5-dihydroxyheptanoate. [c] The IC_{50} values are the mean ± SD of three independent experiments.

Table 2. Inhibition of Na^+-Dependent [^{3}H]Taurocholate Uptake into Ileal Brush Border Membrane Vesicles (Rabbit)[a]

compd	IC_{25} (μM)[c]	IC_{50} (μM)[c]	IC_{75} (μM)[c]
TCDC[b]	13 ± 1.6	28 ± 3.8	61 ± 5.8
1[d]	230	>250	≫250
2	40 ± 5	83 ± 32	152 ± 31
8[d]	37 ± 5.3	85 ± 10	148 ± 26
9[d]	87 ± 47	170 ± 54	>250
10[d]	47 ± 27	92 ± 25	166 ± 34
11[d]	87 ± 14	186 ± 31	>250

[a] Assay described in the Experimental Section. [b] TCDC = taurochenodeoxycholic acid. [c] The uptake of [^{3}H]taurocholate (50 μM) into ileal brush border membrane vesicles was measured for 60 s in the presence of 0, 25, 50, 100, 150, 200, and 250 μmol of the indicated compounds both in the presence and the absence of an inwardly directed Na^+ gradient. Inhibition values are expressed as the difference of uptake in the presence of Na^+ minus that in the absence of Na^+. The IC_x values are the mean ± SD of 3–10 independent experiments using different membrane preparations. [d] Compound was tested as its sodium salt of the corresponding open chain 3,5-dihydroxyheptanoate.

heptane/ethyl acetate = 9:1) afforded **13** (1.9 g, 95%): ^{1}H NMR ($CDCl_3$) δ 0.66 (s, 3H), 0.92 (s, 3H), 2.03 (s, 3H), 2.12 (s, 3H), 2.34–2.44 (m, 1H), 3.64–3.74 (m, 1H), 4.86 (dd, 1H, J_1 = 20, J_2 = 1.8), 4.90 (m, 1H), 4.97 (dd, 1H, J_1 = 11, J_2 = 1.8), 5.70 (m, 1H); MS (FAB) 409.3 (M + Li). Anal. ($C_{25}H_{38}O_4$) C, H.

3α,12α-Diacetoxy-17β-formyl-5β-androstane (14). To a vigorously stirred solution of **13** (3.5 g, 8.71 mmol) in dioxane (120 mL) and water (20 mL) was added OsO_4 (20 mg, 0.08 mmol) followed after 10 min by $NaIO_4$ (6.0 g, 28.04 mmol). After 2 h, saturated aqueous NaCl solution was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4) and evaporated and the residue purified by flash chromatography (cyclohexane/ethyl acetate = 4:1) providing **14** (2.80 g, 80%): ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3H), 0.94 (s, 3H), 2.03 (s, 3H), 2.14 (s, 3H), 2.58–2.64 (t, 1H, J = 7.4), 4.64–4.80 (m, 1H), 5.23 (m, 1H), 9.87 (d, 1H, J = 3); MS (FAB) 411.3 (M + Li). Anal. ($C_{24}H_{36}O_5$) C, H.

(*E*)-3-(3α,12α-Dihydroxy-5β-androstan-17β-yl)propenal (15). Diisopropyl cyanomethylphosphonate (2.5 g, 12.20 mmol) in THF (10 mL) was added to a stirred suspension of sodium hydride (480 mg, 55% oil dispersion, 11.0 mmol) in THF (20 mL) at 0 °C. After the solution was stirred at room temperature for 1 h and recooled to 0 °C, a solution of aldehyde **14** (2.29 g, 5.66 mmol) in THF (5 mL) was added dropwise. After 15 min, the mixture was poured into saturated aqueous NaCl solution and extracted with ethyl acetate (3×). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography (cyclohexane/ethyl acetate = 4:1) gave the desired nitriles (2.25 g, 97%) as a mixture of isomers, *E*/*Z* = 75:25, which was used subsequently for the next step.

DIBAH (23 mL of a toluene solution, 1.2 M, 27.6 mmol) was added dropwise to a stirred solution of nitriles (*E*/*Z*) in 40 mL of ether at −78 °C. After 30 min at −78 °C, 1 h at −30 °C, and 15 min at 0 °C, the reaction was quenched with 1 N aqueous HCL and the mixture warmed to room temperature. Water and ethyl acetate were added, and the aqueous layer was extracted with ethyl acetate (4×). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution and brine and dried (Na_2SO_4). Flash chromatography (*n*-heptane/ethyl acetate = 7:3) afforded the *E*-isomer **15** (1.19 g) and the corresponding *Z*-isomer (0.29 g), total yield 1.48 g (75% over two steps). *E*-Isomer: ^{1}H NMR ($CDCl_3$) δ 0.66 (s, 3H), 0.92 (s, 3H), 2.92–3.06 (m, 1H), 3.57–3.62 (m, 1H), 3.70–3.79 (m, 1H), 6.10–6.22 (dd, 1H, J_1 = 20, J_2 = 11), 6.75–6.85 (dd, 1H, J = 20, J = 10), 9.50 (d, 1H, J = 11); MS (FAB) 353.3 (M + Li). Anal. ($C_{22}H_{34}O_3$) C, H.

***tert*-Butyl (*E*)-7-(3α,12α-Dihydroxy-5β-androstan-17β-yl)-5-(*RS*)-hydroxy-3-oxo-6-heptenoate (16).** *tert*-Butyl acetoacetate (1.8 g, 12.67 mmol) was added dropwise to a stirred suspension of sodium hydride (480 mg, 50% dispersion, 10 mmol) in 25 mL of THF at 0 °C, warmed to room temperature until the solution became clear, and recooled to 0 °C. *n*-Butyllithium (1.6 M in hexane, 6.8 mL, 10.88 mmol) was added dropwise and the mixture stirred for another 30 min. At −70 °C, a solution of **15** (1.0 g, 2.89 mmol) in 10 mL of THF was added dropwise and the mixture stirred for 30 min. The reaction was quenched with saturated aqueous NH_4Cl solution and the mixture extracted with ethyl acetate (4×). The combined organic phases were dried (Na_2SO_4). Flash chromatography (ethyl acetate) gave **16** (1.12 g, 78%) as a mixture of diastereomers which could not be separated at this stage: ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H), 0.90 (s, 3H), 1.44 (s, 9H), 2.54–2.64 (m, 1H), 3.54–3.70 (m, 2H), 4.50–4.60 (m, 1H), 5.45–5.70 (m, 2H); MS (FAB) 511.4 (M + Li). Anal. ($C_{30}H_{48}O_6$) C, H.

***tert*-Butyl (*E*)-7-(3α,12α-Dihydroxy-5β-androstan-17β-yl)-(3*R*,5*S*)-dihydroxy-6-heptenoate (17) and *tert*-Butyl (*E*)-7-(3α,12α-Dihydroxy-5β-androstan-17β-yl)-(3*S*,5*R*)-dihydroxy-6-heptenoate (18).** Triethylborane (6 mL, 1 mol/L in THF, 6.0 mmol) was added at room temperature to a stirred solution of MeOH (8 mL) in THF (30 mL). After 1 h, **16** (1.0 g, 1.98 mmol) was added at −78 °C in THF (10 mL). After 1 h, $NaBH_4$ (240 mg, 6.33 mmol) was added. The mixture was stirred for 2 h, the reaction was quenched with saturated aqueous NH_4Cl solution warmed to room temperature, and the mixture was extracted with ethyl acetate (4×). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was dissolved in methanol and evaporated (3×). Flash chromatography (ethyl acetate) gave **17** (240 mg), **18** (270 mg), and a mixture of **17** and **18** (240 mg ≈ 1:1), total yield of diols 75%. **17** (unpolar isomer, R_f = 0.40, ethyl acetate/methanol = 20:1): ^{1}H NMR ($CDCl_3$) δ 0.58 (s, 3H), 0.90 (s, 3H), 1.45 (s,

9H), 2.20 (d, 2H, J = 5), 2.52–2.64 (m, 1H), 3.57–3.62 (m, 1H), 3.63–3.70 (m, 1H), 4.17–4.25 (m, 1H), 4.31–4.40 (m, 1H), 5.40 (dd, 1H, J_1 = 17, J_2 = 6), 5.63 (dd, 1H, J_1 = 17, J_2 = 7); MS (FAB) 513.3 (M + Li). Anal. ($C_{30}H_{50}O_6 \cdot {}^1/_2H_2O$) C, H. **18** (polar isomer, R_f = 0.32, ethyl acetate/methanol = 20:1): ^{1}H NMR ($CDCl_3$) δ 0.58 (s, 3H), 0.90 (s, 3H), 1.45 (s, 9H), 2.20 (d, 2H, J = 5), 2.52–2.64 (m, 1H), 3.57–3.62 (m, 1H), 3.63–3.70 (m, 1H), 4.17–4.25 (m, 1H), 4.31–4.40 (m, 1H), 5.40 (dd, 1H, J_1 = 17, J_2 = 6), 5.63 (dd, 1H, J_1 = 17, J_2 = 7); MS (FAB) 513.3 (M + Li). Anal. ($C_{30}H_{50}O_6 \cdot {}^1/_2H_2O$) C, H.

Synthesis of Compound 9 from Compound 17. *tert*-Butyl (*E*)-7-(3α,12α-Dihydroxy-5β-androstan-17β-yl)-(3*R*,5*S*)-(isopropylidenedioxy)-6-heptenoate (19). **17** (200 mg, 0.40 mmol), 2,2-dimethoxypropane (0.1 mL), acetone (5 mL), and *p*-toluenesulfonic acid (5 mg) were stirred at room temperature for 1 h. Triethylamine (0.05 mL) was added and the mixture evaporated. Flash chromatography (*n*-heptane/ethyl acetate = 7:3) afforded **19** (210 mg, 97%): ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H), 0.90 (s, 3H), 1.38 (s, 3H), 1.43 (s, 9H), 1.48 (s, 3H), 2.28 (dd, 1H, J_1 = 16, J_2 = 6), 2.33 (dd, 1H, J_1 = 16, J_2 = 6), 3.53–3.64 (m, 2H), 4.20–4.40 (m, 2H), 5.43 (dd, 1H, J_1 = 17, J_2 = 6), 5.58 (dd, 1H, J_1 = 17, J_2 = 7); MS (FAB) 553.4 (M + Li). Anal. ($C_{33}H_{54}O_6$) C, H.

***tert*-Butyl (E)-7-[3α-Acetoxy-12α-(2-(*S*)-methyl-1-oxobutoxy)-5β-androstan-17β-yl]-(3*R*,5*S*)-(isopropylidenedioxy)-6-heptenoate (21).** Acetyl chloride (0.034 mL, 0.48 mmol) was added to a stirred solution of **19** (200 mg, 0.37 mmol) in 5 mL of pyridine at 0 °C. After 6 h at 0 °C and 1 h at room temperature, ethyl acetate/water = 1:1 was added. The aqueous layer was extracted with ethyl acetate (3×), and the combined organic phases were dried (Na_2SO_4) and evaporated. Flash chromatography (*n*-heptane/ethyl acetate = 7:3) gave the 3-acetyl derivative of **19** (170 mg, 79%). The previous compound (80 mg, 0.14 mmol) was dissolved in pyridine (5 mL). At room temperature, DMAP (10 mg, 0.08 mmol) was added followed by 2-(*S*)-methylbutyric anhydride (0.05 mL, 0.25 mmol). After 7 h, ethyl acetate/water = 1:1 was added and the aqueous phase was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), and the solvent was removed. The residue was flash chromatographed (*n*-heptane/ethyl acetate = 4:1) providing **21** (70 mg, 82%): ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H), 0.90 (s, 3H), 0.94 (t, 3H, J = 7), 1.18 (d, 3H, J = 7), 1.39 (s, 3H), 1.45 (s, 9H), 1.48 (s, 3H), 2.00 (s, 3H), 2.24–2.36 (m, 3H), 4.20–4.53 (m, 2H), 4.62–4.74 (m, 1H), 4.80–4.84 (m, 1H), 5.24 (dd, 1H, J_1 = 16, J_2 = 6), 5.52 (dd, 1H, J_1 = 16, J_2 = 7); MS (FAB) 679.5 (M + Li). Anal. ($C_{40}H_{64}O_8$) C, H.

***tert*-Butyl 7-[3α-Acetoxy-12α-(2-(*S*)-methyl-1-oxobutoxy)-5β-androstan-17β-yl]-(3*R*,5*R*)-dihydroxyheptanoate (22).** For removal of the acetonide moiety, *p*-toluenesulfonic acid monohydrate (5 mg, 0.004 mmol) was added to a stirred solution of **21** (80 mg, 0.12 mmol) in 2.5% aqueous MeOH (5 mL) at room temperature. After 3 h, Et_3N (0.05 mL) was added and the mixture was evaporated. The residue was purifed by flash chromatography (*n*-heptane/ethyl acetate = 7:3) to afford 70 mg (93%) of deprotected **21**. This material (70 mg, 0.11 mmol) was dissolved in ethyl acetate (5 mL) and hydrogenated (1 atm) with 5% Rh on alumina (40 mg) for 2 h. The catalyst was filtered off and the solvent removed. Flash chromatography (*n*-heptane/ethyl acetate = 7:3) provided **22** (60 mg, 86%): ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H), 0.90 (s, 3H), 0.92 (t, 3H, J = 7), 1.45 (s, 9H), 2.02 (s, 3H), 2.36–2.44 (m, 3H), 3.70–3.80 (m, 1H), 4.13–4.23 (m, 1H), 4.60–4.68 (m, 1H), 4.92–4.98 (m, 1H); MS (FAB) 641.5 (M + Li). Anal. ($C_{37}H_{62}O_8$) C, H.

2-(*R*)-[2-[3α-Hydroxy-12α-(2-(*S*)-methyl-1-oxobutoxy)-5β-androstan-17β-yl]ethyl]tetrahydro-4-(*R*)-hydroxy-2*H*-pyran-6-one (9). To **22** (50 mg, 0.08 mmol) dissolved in ethanol (5 mL) was added 1 mL of 1 N aqueous NaOH at room temperature. After stirring for 1 h, the solvent was removed and the residue was dried at high vacuum for 4 h. The crude reaction product was dissolved in THF (5 mL), and 0.5 mL of hydrochloric acid (18% in ether) was added. After 1 h at room temperature, the reaction was quenched with 25% aqueous Na_2HPO_4 solution and the mixture extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4) and evaporated. Flash chromatography (cyclohexane/ethyl acetate = 1:3) afforded **9** (38 mg, 89%): ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H), 0.90 (s, 3H), 0.91 (t, 3H, J = 7), 1.16 (d, 3H, J = 7), 2.40 (m, 1H), 2.59 (m, 1H), 2.72 (dd, 1H, J_1 = 16, J_2 = 6), 3.55–3.68 (m, 1H), 4.32–4.39 (m, 1H), 4.52–4.62 (m, 1H), 4.94–4.98 (m, 1H); MS (FAB) 525.4 (M + Li). Anal. ($C_{31}H_{50}O_6 \cdot {}^1/_2H_2O$) C, H.

Synthesis of Compound 11 from Compound 18. *tert*-Butyl (*E*)-7-(3α,12α-Dihydroxy-5β-androstan-17β-yl)-(3*S*,5*R*)-(isopropylidenedioxy)-6-heptenoate (23). **23** was prepared from **18** according to the preparation of **19**: ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H), 0.90 (s, 3H), 1.38 (s, 3H), 1.43 (s, 9H), 1.48 (s, 3H), 2.28 (dd, 1H, J_1 = 16, J_2 = 6), 2.33 (dd, 1H, J_1 = 16, J_2 = 6), 3.53–3.64 (m, 2H), 4.20–4.40 (m, 2H), 5.43 (dd, 1H, J_1 = 17, J_2 = 6), 5.58 (dd, 1H, J_1 = 17, J_2 = 7); MS (FAB) 553.4 (M + Li). Anal. ($C_{33}H_{54}O_6$) C, H.

***tert*-Butyl (*E*)-7-[3α-Acetoxy-12α-(2-(*S*)-methyl-1-oxobutoxy)-5β-androstan-17β-yl]-(3*S*,5*R*)-(isopropylidenedioxy)-6-heptenoate (24).** **24** was prepared from **23** according to the preparation of **21**: ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H), 0.90 (s, 3H), 0.94 (t, 3H, J = 7), 1.18 (d, 3H, J = 7), 1.39 (s, 3H), 1.45 (s, 9H), 1.48 (s, 3H), 2.00 (s, 3H), 2.24–2.36 (m, 3H), 4.20–4.53 (m, 2H), 4.62–4.74 (m, 1H), 4.80–4.84 (m, 1H), 5.24 (dd, 1H, J_1 = 16, J_2 = 6), 5.52 (dd, 1H, J_1 = 16, J_2 = 7); MS (FAB) 679.5 (M + Li). Anal. ($C_{40}H_{64}O_8$) C, H.

***tert*-Butyl 7-[3α-Acetoxy-12α-(2-(*S*)-methyl-1-oxobutoxy)-5β-androstan-17β-yl]-(3*S*,5*S*)-dihydroxyheptanoate (25).** **25** was prepared from **24** according to the preparation of **22**: ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H), 0.90 (s, 3H), 0.92 (t, 3H, J = 7), 1.45 (s, 9H), 2.02 (s, 3H), 2.36–2.44 (m, 3H), 3.74–3.84 (m, 2H), 4.13–4.23 (m, 1H), 4.60–4.68 (m, 1H), 4.92–4.98 (m, 1H); MS (FAB) 641.5 (M + Li). Anal. ($C_{37}H_{62}O_8$) C, H.

2-(*S*)-[2-[3α-Hydroxy-12α-(2-(*S*)-methyl-1-oxobutoxy)-5β-androstan-17β-yl]ethyl]tetrahydro-4-(*S*)-hydroxy-2*H*-pyran-6-one (11). **11** was prepared from **25** according to the preparation of **9**: ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H), 0.90 (s, 3H), 0.91 (t, 3H, J = 7), 1.16 (d, 3H, J = 7), 2.40 (m, 1H), 2.59 (m, 1H), 2.72 (dd, 1H, J_1 = 16, J_2 = 6), 3.55–3.68 (m, 1H), 4.32–4.39 (m, 1H), 4.52–4.62 (m, 1H), 4.94–4.98 (m, 1H); MS (FAB) 524.4 (M + Li). Anal. ($C_{31}H_{50}O_6$) C, H.

Biological Tests. Preparation of Sodium Salt Solutions of 9 and 11. For the preparation of aqueous solutions of the sodium salt of the corresponding open chain carboxylic acids of **9** and **11**, the respective lactone was dissolved in ethanol, 1 equiv of 0.1 N aqueous NaOH was added, and the mixture was stirred for 2 h at room temperature. The mixture was evaporated and the residue dried at high vacuum and redissolved in water in the appropriate test concentration.

Enzymatic Activity of HMG-CoA Reductase.[19] For investigations of the effects of HMG-CoA reductase inhibitors on the enzymatic activity of HMG-CoA reductase, microsomes from rat liver were prepared as follows. Male Wistar rats (weighing 200–220 g) were fed a standard diet (Altromin) containing 2% cholestyramine for 10 days under reversed light cycle. The animals were sacrificed at the midpeak of the dark cycle by decapitation on day 11. The livers were removed and chilled on ice. The minced livers were homogenized in buffer A (ca. 2 g of liver in 3 mL, 100 mM sucrose, 50 mM KCl, and 40 mM KH_2PO_4, and adjusted to pH 7.2 by addition of a solution of 0.23 mM EDTA in 1 M KOH). The supernatant after centrifugation at 10000*g* was collected. Centrifugation was repeated after homogenization of the pellet in the same volume of buffer A. The combined supernatants were then subjected to ultracentrifugation at 100000*g* for 1 h. The supernatant was discarded, and centrifugation was repeated with the pellet homogenized in buffer B (10 mM DTT in buffer A). The resulting pellet was resuspended in buffer B and kept frozen at −80 °C in 200 μL portions.

For the determination of enzyme activity, the complete assay medium contained the following in a total volume of 100 μL at pH 7.4: $K_xH_yPO_4$, 100 mM; KCl, 50 mM; EDTA, 2 mM; DDT, 5 mM; NADP, 3 mM; glucose-6-phosphate, 32 mM, glucose-6-phosphate dehydrogenase, 0.3 units; and rat liver microsomes corresponding to 40 μg of protein. Test compounds were added to the assay system in 5 μL volumes at multiconcentration levels. After a 10 min preincubation at 37 °C, a

solution of nonradioactive HMG-CoA and 36 nCi (1.3 kBq) of [^{14}C]HMG-CoA was added to give a final substrate concentration of 30 μM in the assay. The complete assay was incubated at 37 °C with shaking for a further 15 min, and the reaction was stopped by addition of 400 μL of Dowex WX4 cationic exchange resin suspended in 1 M HCl. After 30 min of shaking at room temperature, the whole sample volume was applied to a 0.6 × 8.0 cm column containing 100–200 mesh AG1-X8, formiate form (Bio-Rad). The column was prewashed with 500 μL of water, and the mevanololactone was eluted with an additional 1.75 mL of water. After addition of 15 mL of Quickszint 212 (Zinsser), samples were measured in a scintillation counter. Inhibition of HMG-CoA reductase activity at six concentration levels between 2 orders of magnitude around the IC_{50} values was determined in triplicate. IC_{50} values were obtained graphically after plotting the percentage inhibition against the logarithm of the concentration of the test compound. IC_{50} values are the means ± SD of three independent experiments.

Measurement of Cholesterol Biosynthesis in HEP G2 Cells. Cholesterol biosynthesis was determined by measuring the incorporation of [^{14}C]acetate incorporation into total cholesterol according to ref 20 with the following alterations. Subconfluent HEP G2 cells were cultured at 37 °C in a 5% CO_2 atmosphere and seeded at a density of 5 × 10^4 cells/cm^2 on 10 cm^2 multiwell dishes. The cells were maintained in RPMI 1640 medium containing 10% fetal calf serum for 24 h. This medium was replaced by RPMI 1640 medium supplemented with 10% LPSD (lipoprotein deficient serum). After 24 h in LPDS-containing medium, the cells were fed RPMI 1640 medium containing 10% LPDS and the various test compounds. Stock solutions of the drugs were prepared in DMSO, the final DMSO concentration being 1% in both controls and all the test samples. After a preincubation for 1 h with inhibitors, [^{14}C]acetate was added (0.5 μM final concentration, 4.8 × 10^6 dpm/mmol) and the cells were pulsed for 17 h. Then the medium was removed, the cell layer was rinsed four times with ice-cold 0.9% NaCl, and the cells were scraped from the wells into 0.9% NaCl. Lipids were saponified with ethanolic KOH for 1 h at 80 °C and extracted with chloroform/methanol (2:1). This organic extract was evaporated under a stream of nitrogen, and the lipids were redissolved in chloroform and spotted on TLC plates (silica, Merck, Darmstadt, OF 254). The chromatograms were developed in chloroform/acetone (1/10, v/v) for 1 h. The cholesterol band was stained with iodine vapor and scraped into scintillation vials for quantitation. For each dish, a correction was made for the recovery with 20 nCi [1,2-^{3}H]cholesterol, which was added to the cell suspension prior to saponification. The extraction recovery averaged 70%. Cholesterol biosynthesis was expressed as nmol of acetate incorporated into total cholesterol/mg of cell protein. Protein content of cell layers was determined from aliquots of the starting cell suspension according to the procedure of Lowry.[21]

Preparation of Ileal Brush Border Membrane Vesicles. Brush border membrane vesicles from the ileum of male white New Zealand rabbits (weighing 4–5 kg) were prepared by the Mg^{2+} precipitation method[22] as described previously.[10,23–25] The brush border membranes were enriched (17.1 ± 4)-fold with regard to aminopeptidase N (EC 3.4.11.2), (16.6 ± 7.3)-fold for γ-glutamyltransferase (EC 2.3.2.2.), and 14.4 ± 4)-fold for alkaline phosphatase (EC 3.1.3.1.). Immediately after preparation, the vesicles were stored in liquid nitrogen without loss of transport and enzymatic activity for at least 4 weeks. The intactness of the vesicles was determined by measuring Na^+-dependent D-glucose uptake after 15 s of incubation; usually the overshoot uptake at 15 s was greater than 20-fold. The enzymatic activities of aminopeptidase N, γ-glutamyltransferase, and alkaline phosphatase were determined with Merckotest kits (Merck, Darmstadt, Germany), and the protein concentration was determined according to Bradford[26] using the Bio-Rad assay (Bio-Rad, München, Germany).

Transport Measurements. Uptake of radiolabeled substrates by brush border membrane vesicles was determined by the membrane filtration method[22] as described previously.[23–25] Typically, the transport reaction was initiated by adding 10 μL of the vesicle suspension (50 μg of protein) equilibrated with 10 mM Tris-Hepes buffer (pH 7.4), 300 mM mannitol to 90 μL of incubation medium containing the radioactively labeled substrate kept at 30 °C. The composition of the incubation medium for measurements in the presence of a Na^+ gradient usually was 10 mM Tris-HCl (pH 7.4), 100 mM NaCl, 100 mM mannitol and in the absence of a Na^+ gradient 10 mM Tris-HCl (pH 7.4), 100 mM KCl, 100 mM mannitol. For measurement of taurocholate uptake, these media contained 50 μM (0.75 μCi) [^{3}H]taurocholate. At 60 s, the transport reaction was terminated by addition of 1 mL of ice-cold stop solution (10 mM Tris-Hepes (pH 7.4), 150 mM KCl). The entire content was pipetted onto the middle of a prewashed, prechilled filter kept under suction with the aid of a vacuum controller. The filter was rinsed immediately with 5 mL of ice-cold stop solution and then solubilized in scintillator Quickszint 361. The radioactivity remaining on the filter was counted with standard liquid scintillation techniques. After correction of medium radioactivity bound to the filter in the absence of membrane vesicles, inhibition values were expressed as the difference of uptake in the presence of Na^+ minus uptake in the absence of Na^+. The uptake of [^{3}H]-taurocholate (50 μM) was measured in the presence of 0, 25, 50, 100, 150, 200, and 250 μM of the indicated compound. The inhibition value, IC_x, is the concentration of inhibitor where Na^+-dependent [^{3}H]taurocholate uptake was inhibited by x%.

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