Design and Synthesis of Seco-oxysterol Analogs as Potential Inhibitors of 3-Hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) Reductase Gene Transcription

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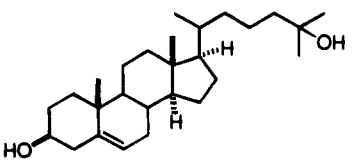
The synthesis and biological activity of a series of seco-oxysterol analogs designed to be inhibitors of transcription of the gene for 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGR) are described. The compound possessing the most significant activity, [1α(*E*),4β]-3-[2-(4-hydroxy-1-methylcyclohexyl)ethenyl]-α,α-dimethylbenzenepentanol (**4**, U-88156), inhibited (IC_{50} = 10 μM) the expression of β-galactosidase (β-gal) in a transfected human HepG2 cell line wherein the *β-gal* gene was driven by a 5 kB segment of the promoter for hamster HMGR. Furthermore, using wild-type HepG2 cells, it was shown that 10 μM **4** reduced HMGR mRNA levels by 73% while *stimulating* LDL-receptor activity by 47%. In the same system, the related oxysterol, 25-hydroxycholesterol (**1**), at 10 μM lowered *both* HMGR mRNA levels and LDL-receptor activity by 58% and 64%, respectively. Overall HMGR activity in wild-type HepG2 cells was inhibited 30% by **4** at 10 μM. These findings collectively demonstrate that a seco-oxysterol analog is capable of regulating HMGR gene expression and that this regulation can occur without a concomitant attenuation of the level of LDL-receptor activity.

Regulation of cholesterol biosynthesis is believed to be dependent on the level of activity of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGR).[1] Although direct inhibitors of this enzyme have proven to be highly effective antihypercholesterolemic agents,[2] the natural regulatory mechanism apparently is dependent primarily on the amount of HMGR protein present rather than its inherent level of activity. Brown and Goldstein have postulated that mevalonate-derived intermediates in the steroid and isoprenoid biosynthetic pathways orchestrate a multivalent feedback mechanism entailing control of the expression of the gene for HMGR.[3] Although the precise identity of the endogenous regulatory agent(s) has not been firmly established, a sizeable body of evidence supports the hypothesis that oxygenated derivatives of cholesterol and/or lanosterol are largely responsible. In fact, a number of these "oxysterols" have been shown to effectively regulate HMGR activity *in vitro*.[4] One of the most potent oxysterols, 25-hydroxycholesterol (**1**), effects this regulation at both transcriptional and post-transcriptional levels.[3c] Collectively, SAR studies of oxysterols have identified three structural features important for this activity: an oxygen functionality at the 3-carbon (optimally 3-β-hydroxy), a complete cholesterol side chain, and at least one other oxygen functionality in the molecule.[5]

The precise mechanism by which oxysterols exert their effect on HMGR gene expression is not clear. One attractive hypothesis is that they bind to a cytosolic protein (the so-called "oxysterol receptor"), increasing that protein's affinity for DNA.[6] This would be analogous to the mechanism by which other steroid hormones regulate gene expression.[7] This hypothesis is supported by an impressive correlation between the binding affinity of oxysterols to this binding protein and their ability to attenuate overall HMGR activity.[5a] Some oxysterols may act indirectly in this regard by inhibiting lanosterol 14-α-methyl demethylase, the enzyme which catalyzes the first step in the conversion of lanosterol to cholesterol. This induces the accumulation of 32-oxygenated lanosterols, which have been postulated to be endogenous regulators of HMGR gene expression.[4g,h,k] There is substantial evidence, however, that this regulation may be post-transcriptional.

Despite recognition for over 20 years that oxysterols are regulators of HMGR, very few antihypercholesterolemic agents have been discovered within this class.[8] One possible reason is the generally rapid metabolism of sterol derivatives observed *in vivo*. Efforts by medicinal chemists to endow oxysterols with metabolic stability have met with only limited success.[9] An alternative approach is to consider non-steroidal analogs of oxysterols which might be less susceptible to typical steroidal metabolic pathways. A classical example of a non-steroidal agonist for a steroid receptor is diethylstilbestrol (**2**). Several factors led us to consider the stilbestrol template as a model for an oxysterol mimic. First, it lacks the steroidal B and C rings, both of which are primary sites for oxidation of the cholesterol nucleus.[10] Second, a recent report that 25-hydroxyvitamin D_3 (**3**) inhibits HMGR suggested that an intact



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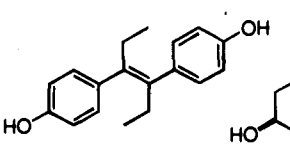

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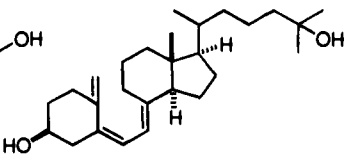

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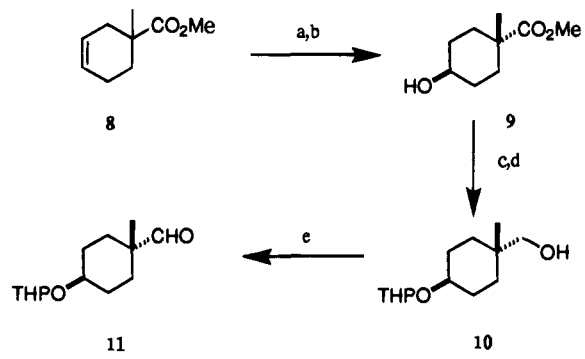
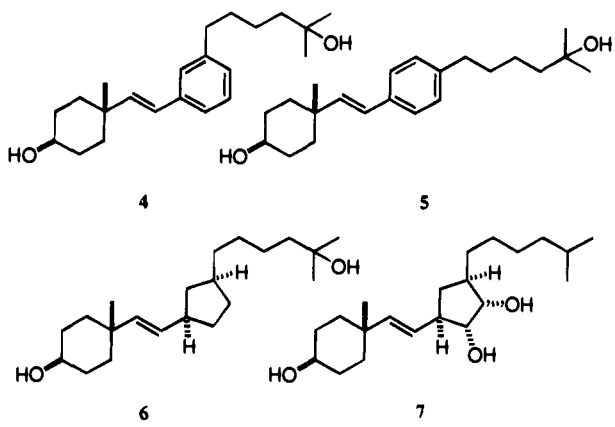
Scheme 1[a]



[a] Reagents: (a) $Hg(OAc)_4$; (b) $NaBH_4$; (c) dihydropyran, TsOH; (d) $LiAlH_4$; (e) SO_3–pyridine.

steroid nucleus might not be required for oxysterol-like activity.[11] Finally, the oxysterol binding protein itself is apparently not highly substrate-specific, considering the diverse array of oxysterols capable of regulating HMGR.

A set of prototypical seco-oxysterol analogs **4**–**7** was targeted to determine if the steroidal B and C rings could be dissected without losing HMGR gene expression-inhibiting activity. Compounds **4**–**6** were selected as 25-hydroxycholesterol mimics possessing either a cyclopentyl "D" ring or an isosteric phenyl "D" ring. Compound **7** was included to evaluate a seco-oxysterol analog oxygenated on the "D" ring. 15-Oxygenated sterols comprise a sizeable class of very potent inhibitors of HMGR, particularly those possessing a 15-α-hydroxyl.[12]

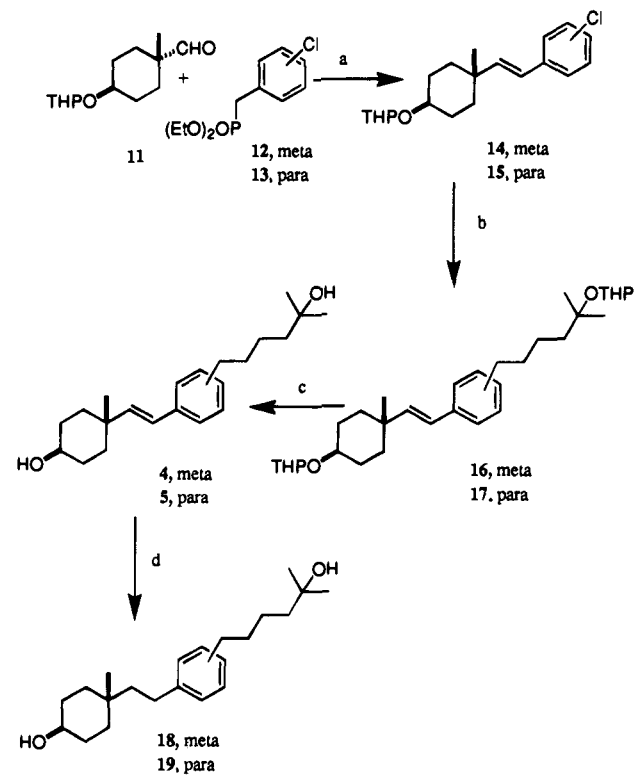


Chemistry

Synthesis of the "A" ring of the seco-oxysterol analogs is outlined in Scheme 1. Stereoselective oxymercuration of cyclohexenecarboxylate **8**[13] was achieved via a modification of the literature procedure.[14] The 15:1 diastereomer ratio of the resulting hydroxy ester **9** could be improved to >40:1 by saponification of the ester, recrystallization of the resulting carboxylic acid, and reesterification. Protection of the alcohol as the corresponding THP ether followed by hydride reduction then afforded alcohol **10**.[14] Oxidation to the requisite aldehyde **11** was smoothly effected with sulfur trioxide–pyridine complex.[15]

Completion of the construction of the two aromatic seco-oxysterol analogs is presented in Scheme 2. Phosphonates **12** and **13**, each obtained by standard Arbuzov reaction of the corresponding benzyl chlorides with

Scheme 2[a]



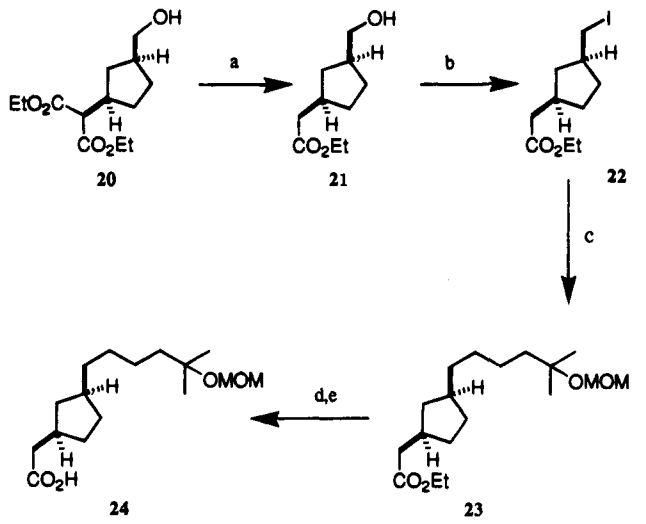
[a] Reagents: (a) NaOMe; (b) $BrMg(CH_2)_4C(Me)_2OTHP$, $Ni(dppp)Cl_2$; (c) $HOAc/THF/H_2O$; (d) H_2, Pd/C.

triethyl phosphite, were subjected to Horner–Emmons coupling with aldehyde **11**. This afforded in excellent yields olefins **14** and **15**, each exclusively trans. The crucial attachment of the side chain was efficiently accomplished via nickel-catalyzed coupling[16] with the Grignard reagent derived from the THP ether of 6-bromo-2-methylhexan-2-ol. Hydrolysis of the THP ethers with aqueous acetic acid then gave the crystalline seco-oxysterol analogs **4** and **5**. Hydrogenation of the olefins was undertaken to allow biological evaluation of the more flexible saturated analogs **18** and **19**.

The known hydroxy malonate **20**[17] provided a convenient starting point for elaboration of the "D" ring of seco-oxysterol analog **6** (Scheme 3). Krapcho decarboxylation[18] afforded monoester **21**. The primary alcohol was quantitatively converted to the corresponding iodide with triphenylphosphine/iodine[19] in preparation for the pivotal attachment of the side chain, which was effected via copper-catalyzed coupling[20] with the Grignard reagent derived from the MOM ether of 5-bromo-2-methylhexan-2-ol. An excess of the Grignard reagent was required to obtain a good yield of **23** due to extensive homocoupling. Saponification of the ester to acid **24** then completed the synthesis of the "D" ring fragment.

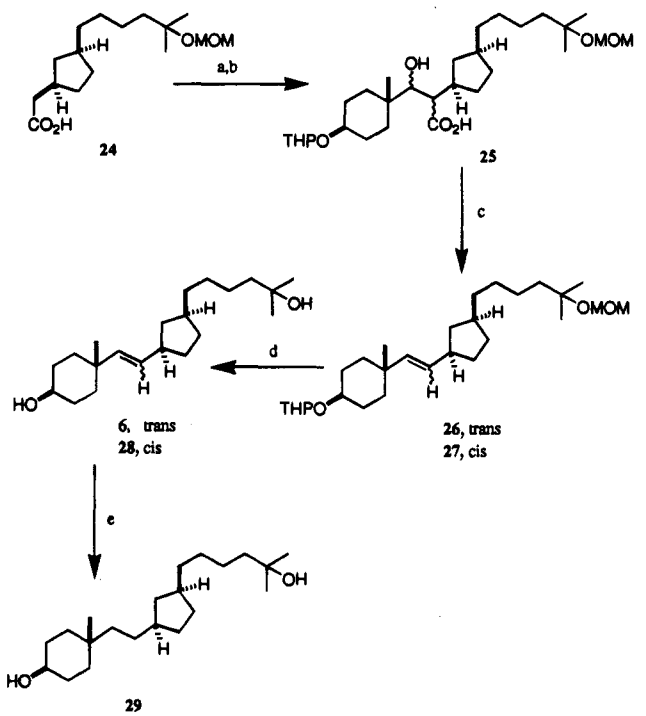
Scheme 4 outlines the final steps in the synthesis of seco-oxysterol analog **6**. Aldol condensation of the dianion of **24** with aldehyde **11** provided β-hydroxy acid **25** as a mixture of diastereomers. No attempt was made to purify the unstable mixture, which was directly subjected to decarboxylative dehydration,[21] affording a mixture of olefins **26** and **27** (1.4:1) in good overall yield. Following separation on silver nitrate-impregnated silica gel, the corresponding diols **6** and **28** were liberated with methanolic HCl. Hydrogenation of a mixture

Scheme 3[a]



[a] Reagents: (a) LiCl, aqueous DMSO, 180 °C; (b) Ph_3P, I_2, imidazole; (c) $BrMg(CH_2)_3C(Me)_2OMOM$, Li_2CuCl_4; (d) NaOH; (e) HCl.

Scheme 4[a]



[a] Reagents: (a) 2 equiv of LDA; (b) **11**; (c) $Me_2NCH(OCH_2C(Me)_3)_2$; (d) concentrated HCl, MeOH; (e) H_2, 5% Rh/C, 40 psi.

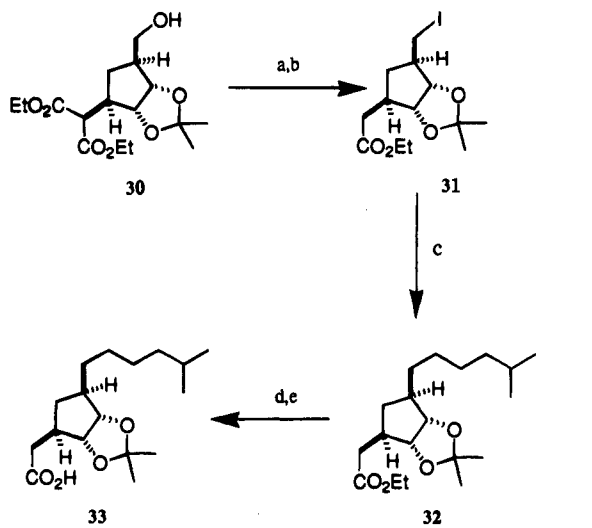
Table 1. Inhibition of HMG-CoA Reductase Transcription by Seco-oxysterol Analogs

compound	% inhibition[a] (IC_{50})	compound	% inhibition[a] (IC_{50})
25-hydroxy-cholesterol	70.0 ± 2.3 (0.3 μM)	**18**	29.3 ± 13.4
		19	0
4	46.3 ± 5.8 (10 μM)	**28**	0
5	0	**29**	0
6	0	**37**	0
7	0		

[a] Values are presented as mean ± SEM. Transfected HepG2 cells were treated for 24 h with test compound at 10^{-5} M.

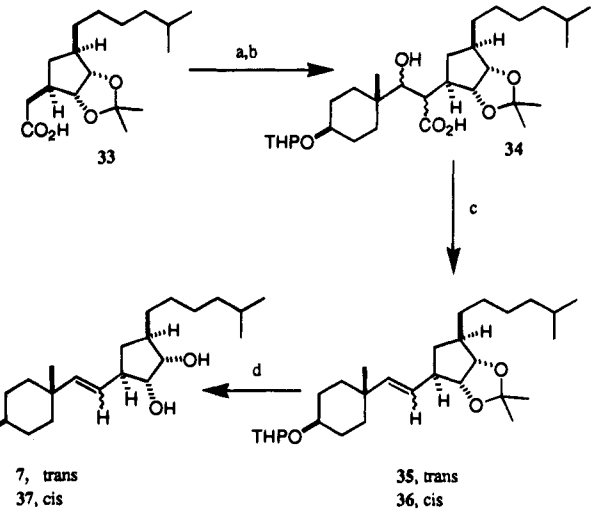
of the olefins gave a single crystalline diol **29**. It was found necessary to employ Rh/C as the catalyst in this reduction since apparent isomerization at the allylic methine position was observed with Pd/C.

Scheme 5[a]



[a] Reagents: (a) LiCl, aqueous DMSO, 180 °C; (b) Ph_3P, I_2, imidazole; (c) $BrMg(CH_2)_3CH(Me)_2$, Li_2CuCl_4; (d) NaOH; (e) HCl.

Scheme 6[a]



[a] Reagents: (a) 2 equiv of LDA; (b) **11**; (c) $Me_2NCH(OCH_2C(Me)_3)_2$; (d) 1 M HCl, MeOH.

Preparation of the "D"-ring oxygenated seco-oxysterol analog **7** was undertaken in a manner exactly analogous to that described for **6** (Schemes 5 and 6). Malonate **30**[17] was transformed to carboxylic acid **33** in four steps in good overall yield. Aldol condensation with aldehyde **11** and subsequent decarboxylative dehydration produced a mixture of trans and cis olefins **35** and **36**. Chromatographic separation and deprotection of the alcohols gave the desired triols **7** and **37**.

Biology

Each of the diols and triols produced in the foregoing schemes was evaluated for its ability to suppress the promoter for the HMGR gene. The assay was performed using transfected human HepG2 cells wherein transcription of the gene for β-galactosidase (*β-gal*) is controlled by a 5 kB segment of the hamster HMGR 5′-promoter sequence. The positive control used was 25-hydroxycholesterol, and inhibition was detected by measuring *β-gal* activity spectrophotometrically. Results are summarized in Table 1.

Table 2. Inhibitory Effects[a] of Compound 4 (U-88156) on Hepatic HMG-CoA Reductase and LDL Receptor Expression

assay	compound 4	25-hydroxy-cholesterol
HMG-CoA reductase mRNA	72.7 ± 3.9	58.2 ± 10.8
HMG-CoA reductase activity	29.7 ± 1.4	48.4 ± 3.7
LDL-receptor binding activity	−47.0 ± 2.1[b]	64.0 ± 4.8

[a] Values are presented as percent inhibition (mean ± SD). HepG2 cells were treated for 24 h with compound 4 or 25-hydroxycholesterol at 10^{-5} M. [b] The negative sign indicates that LDL receptor binding was *increased* 47% by 4.

Only two of the seco-oxysterol analogs exhibited significant inhibition of the HMGR promoter, both of which possess the meta-disubstituted phenyl "D" ring (**4** and **18**). The unsaturated analog **4** (U-88156) was considerably more active, with an IC_{50} of 10 μM. The weaker and more variable activity of **18** precluded accurate calculation of an IC_{50}. The preliminary SAR gleaned from this limited study indicates, rather surprisingly, that the phenyl ring of **4** apparently is functioning more effectively as a bioisostere for the D ring of the steroid nucleus than is the isolated cyclopentane ring of **6**. Molecular modeling studies were not very useful in providing a rationale for this, as both **4** and **6** were capable of achieving equally reasonable overlap with the cholesterol nucleus.

Additional *in vitro* evaluation of analog **4** to confirm the primary assay results was undertaken by measuring both HMGR mRNA levels and overall HMGR enzyme activity. Furthermore, because the LDL receptor gene is coordinately regulated with the HMGR gene and is down-regulated by oxysterols such as 25-hydroxycholesterol,[22] LDL binding by HepG2 cells was also measured to determine if the compound had any effect on the level of LDL receptor activity. This last result was considered of paramount importance in assessing the potential of **4** to function as an antihypercholesterolemic agent since the LDL receptor plays a key role in cholesterol clearance from the blood. The results of these experiments are summarized in Table 2.

Wild type, nontransfected HepG2 cells were treated for 24 h with 10 μM **4** or 25-hydroxycholesterol. HMGR mRNA levels, determined by slot-blot analysis, were decreased 73% by treatment with **4** and were decreased 58% by treatment with 25-hydroxycholesterol. Overall HMGR enzyme activity in similarly treated HepG2 cells was reduced 30% in cells treated with **4** and was reduced 48% in cells treated with 25-hydroxycholesterol.

In contrast to the effects of **4** on HMGR transcription, mRNA levels and enzyme activity, each of which mirrored the effects observed with 25-hydroxycholesterol, this seco-oxysterol *increased* HepG2 cell LDL receptor binding activity by 47%. As expected, 25-hydroxycholesterol decreased LDL receptor binding activity 64% in the same assay. It is not possible at this point to ascertain whether the observed stimulation with **4** reflects a direct positive effect on LDL-receptor expression or is merely an indirect effect consisting of natural up-regulation of LDL-receptor activity induced by the lower intracellular cholesterol levels that would be expected to result from HMGR inhibition.

Although there reside in the literature reports of compounds capable of reducing levels of HMGR protein without a concomitant decrease in LDL-receptor activity,[23] it has not been established that the regulation of HMGR in these cases is occurring at the level of *transcription*, the only point at which coordinate regulation of the LDL receptor would be expected. Post-transcriptional regulation, for example inhibition of HMGR mRNA translation or stimulation of HMGR degradation, would on the other hand be anticipated to ultimately result in an overall increase in LDL-receptor activity. The results of the present study are thus particularly significant in that the seco-oxysterol analog **4** is, to the best of our knowledge, the first compound to unambiguously attenuate transcription of the HMGR gene without an accompanying decrease in overall LDL-receptor activity.

Conclusion

In summary, we have demonstrated that a seco-oxysterol analog (**4**, U-88156) is capable of regulating transcription of the gene for HMGR *in vitro* in a transfected HepG2 cell model. Further testing confirmed that **4** reduced the level of HMGR mRNA and inhibited overall HMGR activity in wild-type HepG2 cells. Of particular interest was the observation that LDL-receptor binding activity in these cells was not attenuated as it is by 25-hydroxycholesterol, but in fact was *increased*. This apparent disruption of the normal coordinate regulation of HMGR and LDL-receptor activities increases the likelihood that **4** or a related compound will be useful in the reduction of serum cholesterol levels.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on a Digilab Model FTS-40 spectrophotometer. Combustion analyses and mass spectra were obtained by the Physical and Analytical Chemistry Department of The Upjohn Co. Mass spectra were obtained by electron ionization unless otherwise noted. ^1H and ^{13}C NMR spectra were recorded at 300 MHz with a Bruker Aspect 3000 spectrometer. All dry solvents used were Aldrich anhydrous grade packaged in Sure/Seal bottles. Moisture- and air-sensitive reactions were run under an atmosphere of nitrogen. Unless otherwise specified, all reagents used were commercially available.

(1α,4β)-1-Methyl-4-hydroxycyclohexanecarboxylic Acid, Ethyl Ester (9). A slight modification of the reported procedure[14] was employed. To a solution of mercuric acetate (62.06 g, 195 mmol) in water (200 mL) was added THF (200 mL). To the resulting bright orange mixture was added a solution of ester **8**[13] (23.10 g, 149.8 mmol) in THF (25 mL) at room temperature with vigorous mechanical stirring. The mixture was stirred for 30 min at room temperature, becoming a clear, colorless solution. A modified workup was employed to minimize saponification of the product ester.[24] Ether (250 mL) was added, and the mixture was cooled to 0 °C in an ice bath. NaOH (3 M, 130 mL, ice cold) was added over 10 min, followed by 0.5 M sodium borohydride in water (195 mL, ice cold) over another 10 min. Finally, solid sodium chloride (155 g) was added, and the mixture was stirred at 0 °C for 5 min. The ether layer was separated, and the aqueous phase was extracted rapidly with more ether (2 × 300 mL). Drying over magnesium sulfate and concentration *in vacuo* left a colorless oil. NMR analysis revealed a ca. 15:1 ratio of diastereomeric alcohols had been formed. Kugelrohr distillation (95–115 °C, 0.3 mm) afforded 19.89 g (77%). The diastereomer ratio could be further improved to > 40:1 by saponification of the ester, recrystallization of the resulting carboxylic acid from chloroform/hexane (mp 136–137 °C), and reesterification: ^1H NMR ($CDCl_3$) δ 3.75 (m, 1H), 3.62 (s, 3H), 2.15 (bs, 1H), 1.45–1.85 (m, 8H), 1.15 (s, 3H); ^{13}C NMR ($CDCl_3$) 178.2, 67.6, 51.6, 42.0,

30.6, 30.2, 23.9; IR (film, cm^{-1}) 3400 brd, 1730, 1713; MS *m/z* (rel intensity) 172 (M^+, 6), 95 (100).

(*R,S*)-(1α,4β)-1-Methyl-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]cyclohexanecarboxaldehyde (11). Hydroxy ester **9** was converted to monoprotected diol **10** as previously reported.[14] To a mixture of alcohol **10** (22.24 g, 97.4 mmol) in dry DMSO (485 mL) and dry triethylamine (200 mL) cooled in a 15 °C water bath was added sulfur trioxide–pyridine complex (62.0 g, 390 mmol) over about 1 min with vigorous stirring. The brown mixture was then stirred vigorously for 45 min at room temperature. The reaction was diluted with ether/hexane (700 mL/300 mL) and washed with water (1 × 750 mL, 2 × 200 mL). Drying over magnesium sulfate and concentration left 21.78 g of pale yellow oil. Kugelrohr distillation (90–100 °C, 0.2 mm) afforded 20.35 g (92%) of aldehyde **11** as a colorless oil: ^1H NMR ($CDCl_3$) δ 9.44 (s, 1H), 4.68 (m, 1H), 3.91, 3.49 (2 m, 2H), 3.71 (m, 1H), 1.5–1.9 (m, 14H), 1.06 (s, 3H); IR (film, cm^{-1}) 1726; MS *m/z* (rel intensity) 226 (M^+, 1), 85 (100). Anal. ($C_{13}H_{22}O_3$) C,H.

[(3-Chlorophenyl)methyl]phosphonic Acid, Diethyl Ester (12). 3-Chlorobenzyl chloride (25.10 g, 155.9 mmol) and triethyl phosphite (28.1 mL, 163.7 mmol) were mixed without solvent and heated slowly under a reflux condenser to 190 °C. Gas evolution ensued at about 155 °C and was fairly vigorous at 175 °C. Upon reaching 190 °C, the reaction mixture was stirred for 30 min. The solution was cooled and fractionally distilled under vacuum. After some forerun at 35–45 °C (0.2 mm), the major fraction was collected at 120–130 °C (0.05 mm), affording a colorless oil (35.62 g, 87%): ^1H NMR ($CDCl_3$) δ 7.15–7.3 (m, 4H), 4.03 (quintet, 4H, J = 7 Hz), 3.12 (d, 2H, J = 22 Hz), 1.26 (t, 6H, J = 7 Hz); MS *m/z* (rel intensity) 264 (M + 2, 12), 262 (M^+, 36), 125 (100). Anal. ($C_{11}H_{16}ClO_3P$) C, H.

(*R,S*)-[1α,4β(*E*)]-2-[[4-[2-(3-Chlorophenyl)ethenyl]-4-methylcyclohexyl]oxy]tetrahydro-2*H*-pyran (14). To a 0 °C solution of aldehyde **11** (679 mg, 3.00 mmol) and phosphonate **12** (1.58 g, 6.00 mmol) in dry DMF (7 mL) was added solid sodium methoxide (370 mg, 6.8 mmol) in a single portion. The mixture turned pale yellow and then gradually became a solution over about 10 min at 0 °C. After a total of 15 min at that temperature, the reaction mixture was diluted with hexane (25 mL) and washed with water (1 × 15 mL, 2 × 8 mL). Drying of the hexane layer over magnesium sulfate and concentration *in vacuo* afforded a colorless oil (1.24 g). Flash chromatography (36 g of silica, 10% ethyl acetate/hexane) provided 1.03 g (100%) of a colorless oil: ^1H NMR ($CDCl_3$) δ 7.35 (bs, 1H), 7.1–7.2 (m, 3H), 6.27, 6.19 (AB q, 2H, J = 16 Hz), 4.71 (m, 1H), 3.93, 3.50 (2 m, 2H), 3.67 (m, 1H), 1.2–1.9 (m, 14H), 1.11 (s, 3H); IR (film, cm^{-1}) 1647 weak, 1594, 1565; MS *m/z* (rel intensity) 334 (M+, 2), 85 (100). Anal. ($C_{20}H_{27}ClO_2$) C, H, Cl.

***d,l*-[1α(*E*),4β]-2-[[1,1-Dimethyl-5-[3-[2-[1-methyl-4-[(tetrahydro-2*H*-pyran-2-yl)oxy]cyclohexyl]ethenyl]phenyl]pentyl]oxy]tetrahydro-2*H*-pyran (16).** The THP ether of 6-bromo-2-methyl-2-hexanol was prepared as follows: A solution of ethyl 5-bromovalerate (7.9 mL, 50 mmol) in THF (100 mL) was cooled to −78 °C with mechanical stirring under nitrogen. Methylmagnesium bromide (65 mL, 3.1 M in ether, 200 mmol) was added over about 5 min via syringe. The thick white mixture was then stirred at −23 °C for 3.5 h, becoming thinner, but not homogeneous. While maintaining the temperature, saturated ammonium chloride (100 mL) was cautiously added. The resulting mixture was allowed to warm above 0 °C, water was added until the aqueous phase was clear, and the mixture was extracted with ether (2 × 100 mL). Drying over magnesium sulfate and concentration (rotovap only) left 9.76 g of 6-bromo-2-methyl-2-hexanol as a colorless oil. NMR analysis indicated the crude product contained about 4 wt % THF but was otherwise pure. The yield was thus 9.3 g (95%). A solution of the crude alcohol, dihydropyran (6.3 mL, 68 mmol) and *p*-toluenesulfonic acid (43 mg, 0.23 mmol) in ether (55 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with ether (100 mL) and washed with saturated sodium bicarbonate (2 × 50 mL). Drying over magnesium sulfate and concentration left 12.8 g of an amber oil. Flash chromatography (400 g of silica, 10% ethyl acetate/hexane) provided 10.21 g (80%) of 6-bromo-2-methyl-2-hexanol, THP ether as a colorless oil: ^1H NMR ($CDCl_3$) δ 4.71 (m, 1H), 3.94 (m, 1H), 3.45 (m, 1H), 3.42 (t, 2H, J = 7 Hz), 1.85 (m, 3H), 1.65 (m, 1H), 1.5 (m, 8H), 1.22 (s, 3H), 1.20 (s, 3H).

Magnesium turnings (250 mg, 10.2 mmol) were crushed in a mortar and pestle and placed in a 25-mL conical flask. Under nitrogen flow, the flask was flame-dried and then cooled. The flask was then charged with dry THF (9 mL) and a trace of iodine. While stirring at 30 °C (water bath), 6-bromo-2-methyl-2-hexanol, THP ether (2.23 mL, 9.25 mmol) was added neat via syringe over about 5 min. Initiation of the Grignard formation occurred almost immediately after the first few drops of bromide were added, as indicated by the disappearance of the iodine color. The mixture was then stirred at 35 °C for 1 h. The Grignard solution was added via syringe dropwise to a mixture of aryl chloride **14** (1.24 g, 3.70 mmol) and [1,3-bis(diphenylphosphino)propane]nickel(II) chloride (Ni(dppp)Cl_2) (75 mg, 0.14 mmol) in dry ether (4.6 mL) at room temperature. The brown suspension slowly became a yellow solution during the addition. The reaction was then stirred at 31 °C for 22 h. The brown solution was poured into saturated ammonium chloride (35 mL) and water (15 mL) and extracted with ether (1 × 50 mL, 1 × 35 mL). Drying over magnesium sulfate and concentration *in vacuo* left 2.93 g. Flash chromatography (175 g of silica, 13% ethyl acetate/hexane, 35-mL fractions) provided 1.69 g (92%) of **16** as a colorless viscous oil in fractions 16–26: ^1H NMR ($CDCl_3$) δ 7.18 (m, 3H), 7.02 (m, 1H), 6.31, 6.17 (AB q, 2H, J = 16 Hz), 4.70 (m, 2H), 3.93 (m, 2H), 3.66 (m, 1H), 3.4–3.55 (m, 2H), 2.60 (t, 2H, J = 7 Hz), 1.3–1.9 (m, 26H), 1.20 (s, 3H), 1.18 (s, 3H), 1.11 (s, 3H); MS *m/z* (rel intensity) no M^+, 312 (5), 295 (4), 143 (3), 103 (13), 85 (100). Anal. ($C_{32}H_{50}O_4$) C, H.

[1α(*E*),4β]-3-[2-(4-Hydroxy-1-methylcyclohexyl)ethenyl]-α,α-dimethylbenzenepentanol (4). A solution of **16** (1.69 g, 3.39 mmol) in acetic acid (50 mL), THF (25 mL), and water (12.5 mL) was stirred at room temperature for 29 h. The solvent was evaporated *in vacuo*, leaving a viscous oil. The crude product was dissolved in ether (50 mL) at reflux, and hexane (150 mL) was added. The solution was boiled until most of the ether was gone (temperature of distilling vapor: 62 °C). The solution was cooled slowly to room temperature with seeding and addition of ether to prevent oiling out (required about 8 mL). Once crystallization had commenced, the mixture was allowed to stand at room temperature for 2 h and then at 0 °C overnight, affording 886 mg (79%) of white crystals (mp 74–76 °C): ^1H NMR ($CDCl_3$) δ 7.2 (m, 3H), 7.02 (dm, 1H, J = 7 Hz), 6.32, 6.17 (AB q, 2H, J = 16 Hz), 3.72 (m, 1H), 2.61 (t, 2H, J = 7 Hz), 1.35–1.9 (m, 14H), 1.21 (s, 6H), 1.12 (s, 3H); ^{13}C NMR ($CDCl_3$) δ (mult) 142.9 (s), 140.6 (d), 137.9 (s), 128.5, 127.1, 126.1, 125.9, 123.4 (all d), 71.0 (s), 69.6 (d), 43.8 (t), 36.0 (t), 35.4 (t), 34.3 (t), 32.1 (t), 30.7 (t), 29.3 (q), 24.1 (t); IR (mull, cm^{-1}) 3300 brd, 1603, 1464, 1457, 1377, 1362, 1192, 1154, 1063, 971, 963, 907, 697; MS *m/z* (rel intensity) 330 (M^+, 2), 312 (52), 279 (21), 269 (36), 169 (37), 158 (94), 145 (60), 117 (98), 95 (51), 59 (100). Anal. ($C_{22}H_{34}O_2$) C, H.

[1α(*E*),4β]-4-[2-(4-Hydroxy-1-methylcyclohexyl)ethenyl]-α,α-dimethylbenzenepentanol (5). The compound was prepared via a multistep protocol precisely analogous to that described for **4**, starting with benzyl chloride **13**: ^1H NMR ($CDCl_3$) δ 7.27, 7.11 (AB q, 4H, J = 8 Hz), 6.31, 6.14 (AB q, 2H, J = 16 Hz), 3.70 (m, 1H), 2.60 (t, 2H, J = 7 Hz), 1.3–1.9 (m, 14H), 1.20 (s, 6H), 1.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ (mult) 141.3 (s), 139.9 (d), 135.3 (s), 128.3 (d), 125.8 (d), 125.4 (d), 70.9 (s), 69.4 (d), 43.6 (t), 35.5 (t), 35.2 (s), 34.2 (t), 31.9 (t), 30.6 (t), 29.1 (q), 24.9 (q), 23.9 (t); IR (mull, cm^{-1}) 3330 brd, 1513, 1365, 1063; MS *m/z* (rel intensity) 330 (M^+, 17), 313 (98), 312 (92), 295 (37), 211 (30), 129 (37), 117 (55), 95 (100). Anal. ($C_{22}H_{34}O_2$) C, H.

***trans*-3-[2-(4-Hydroxy-1-methylcyclohexyl)ethyl]-α,α-dimethylbenzenepentanol (18).** A mixture of **4** (300 mg, 0.908 mmol) and 10% palladium on carbon (100 mg) in absolute ethanol (20 mL) was hydrogenated in a Parr bottle at 40 psi at room temperature for 3 h. The mixture was diluted with chloroform (25 mL) and filtered through a pad of Celite. The filtrate was concentrated, leaving a viscous oil (304 mg) which crystallized on standing. The crude solid was

dissolved in boiling hexane (100 mL), and the solution was decanted off the insoluble residue. The flask was rinsed with another 20 mL of boiling hexane, and the combined hexane solutions were concentrated to a volume of 100 mL by boiling. Cooling to room temperature and then to 0 °C afforded 263 mg (87%) of fine white needles (mp 84–85 °C): ^{1}H NMR ($CDCl_3$) δ 7.18 (t, 1H, J = 8 Hz), 6.99 (m, 3H), 3.62 (m, 1H), 2.60 (t, 2H, J = 7 Hz), 2.54 (m, 2H), 1.2–1.85 (m, 16H), 1.21 (s, 6H), 1.00 (s, 3H); ^{13}C NMR ($CDCl_3$) δ (mult) 143.2 (s), 142.6 (s), 128.3 (d), 128.1 (d), 125.5 (d), 70.9 (s), 70.6 (d), 46.2 (t), 43.6 (t), 35.8 (t), 35.0 (t), 32.1 (s), 32.0 (t), 30.9 (t), 30.1 (t), 29.1 (q), 24.0 (t), 22.5 (q); IR (film, cm^{-1}) 3350 brd, 1607, 1588, 1367, 1151, 1065, 700; MS *m/z* (rel intensity) no M$^+$ in EI spectrum, 315 (12), 314 (51), 244 (14), 145 (20), 117 (33), 105 (24), 95 (100); exact mass calcd for $C_{22}H_{37}O_2$ 333.2793 (M + 1, FAB), found 333.2805. Anal. ($C_{22}H_{36}O_2$·0.1 H_2O) H, C.

***trans*-4-[2-(4-Hydroxy-1-methylcyclohexyl)ethyl]-α,α-dimethylbenzenepentanol (19).** The compound was prepared as described for **18**: ^{1}H NMR ($CDCl_3$) δ 7.09 (s, 4H), 3.61 (m, 1H), 2.59 (t, 2H, J = 7 Hz), 2.53 (m, 2H), 1.2–1.8 (m, 16H), 1.21 (s, 6H), 0.99 (s, 3H); IR (mull, cm^{-1}) 3400 brd, 1514, 1361, 1190, 1156, 1068, 1061, 951, 906, 832, 757; MS *m/z* (rel intensity) 332 (M+, 1), 315 (24), 314 (100), 244 (32), 187 (18), 95 (37). Anal. ($C_{22}H_{36}O_2$) C, H.

***d,l-cis*-3-(Hydroxymethyl)cyclopentaneacetic Acid, Ethyl Ester (21).** A solution of malonate **20**[17] (7.42 g, 28.7 mmol) and lithium chloride (6.87 g, 162 mmol) in DMSO (135 mL) and water (1.35 mL) was degassed by repeated cycles of evacuation and nitrogen purging. While under nitrogen, the solution was immersed in an oil bath at 178 °C and stirred for 4 h, 15 min. After cooling, the turbid yellow mixture was diluted with ether (600 mL) and washed with water (1 × 150 mL, 1 × 100 mL, 1 × 75 mL). The aqueous washes were saturated with solid NaCl and extracted with ether (1 × 125 mL). This extract was washed with water (25 mL) and then combined with the original ether solution. Drying of the ether solution over magnesium sulfate followed by concentration *in vacuo* left 5.07 g. Flash chromatography (160 g of silica, 1/1 ethyl acetate/hexane) gave 4.47 g of colorless oil. Pure **21** was obtained by Kugelrohr distillation (bp 110–140 °C, 0.3 mm) (3.70 g, 69%): ^{1}H NMR ($CDCl_3$) δ 4.12 (q, 2H, J = 7 Hz), 3.53 (bd, 2H, J = 7 Hz), 1.65–2.4 (m, 7H), 1.4–1.5 (bm and m, 2H), 1.26 (overlapping t and m, 4H, J = 7 Hz), 0.87 (m, 1H); IR (film, cm^{-1}) 3412 brd, 1735; MS *m/z* (rel intensity) 187 (M + 1, 5), 186 (M$^+$, 1), 81 (100); exact mass calcd for $C_{10}H_{18}O_3$ 186.1256, found 186.1250. Anal ($C_{10}H_{18}O_3$) H; C: calcd, 64.49; found, 60.54; compound was homogeneous by TLC analysis.

***d,l-cis*-3-(Iodomethyl)cyclopentaneacetic Acid, Ethyl Ester (22).** To a 0 °C solution of triphenylphosphine (5.49 g, 20.9 mmol) and imidazole (1.43 g, 20.9 mmol) in ether (50 mL) and acetonitrile (20 mL) was added iodine (5.31 g, 20.9 mmol). After being stirred vigorously at 0 °C for 10 min, the initially brown mixture was yellow. To this mixture was added a solution of alcohol **21** (2.60 g, 14.0 mmol) in ether (10 mL). The ice bath was removed, and the reaction mixture was stirred for 2 h. The resulting dark brown mixture was poured into hexane (400 mL) and washed with saturated sodium bicarbonate (150 mL) and water (150 mL). The hexane solution was dried over magnesium sulfate and concentrated *in vacuo*, leaving an oil and solid. The solid triphenylphosphine oxide was removed by filtration, using a minimum volume of hexane. Concentration of the filtrate afforded an unstable pale yellow oil (4.19 g, ca. 100%), sufficiently pure to use in the next step: ^{1}H NMR ($CDCl_3$) δ 4.12 (q, 2H, J = 7 Hz), 3.21 (d, 2H, J = 7 Hz), 2.05–2.4 (m, 5H), 1.88 (m, 2H), 1.35 (m, 2H), 1.26 (t, 3H, J = 7 Hz), 0.89 (m, 1H).

***cis*-3-[5-(Methoxymethoxy)-5-methylhexyl]cyclopentaneacetic Acid, Ethyl Ester (23).** 5-Bromo-2-methyl-2-pentanol was prepared from ethyl 4-bromobutyrate and methylmagnesium bromide as outlined in the procedure for **16**. The crude alcohol (8.9 g, ca. 50 mmol) was dissolved in dimethoxymethane (150 mL, distilled off Na) and chloroform (150 mL, ethanol-free). After the solution was cooled to −23 °C, phosphorus pentoxide (30 g) was added. After the mixture was stirred at −23 °C for 1 h, TLC indicated no significant reaction, so the mixture was warmed to 0 °C. Reaction was then quite rapid. After 1 h, 50 min at 0 °C, the phosphorus pentoxide had become gummy and turned nearly black. The reaction was quenched with 8 M KOH (60 mL) and added slowly at 0 °C, followed by water (250 mL). The mixture was extracted with chloroform (1 × 150 mL, 1 × 100 mL), and the extracts were washed with water (1 × 100 mL), dried over magnesium sulfate, and concentrated *in vacuo* (rotovap only), leaving a brown oil. Flash chromatography (255 g of silica, 10% ethyl acetate/hexane) provided 7.63 g (69%) of the MOM ether of 5-bromo-2-methyl-2-pentanol as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.70 (s, 2H), 3.42 (t, 2H, J = 7 Hz), 3.36 (s, 3H), 1.95 (m, 2H), 1.62 (m, 2H), 1.23 (s, 6H).

Magnesium turnings (160 mg, 2.99 mmol) which had been crushed in a mortar and pestle were placed in a conical flask and flame-dried under nitrogen flow. After cooling, a trace of iodine was added before charging the flask with THF (6.0 mL). While the mixture was stirred at 25 °C (water bath), 5-bromo-2-methyl-2-pentanol, MOM ether (1.09 mL, 5.98 mmol) was added without solvent over 10 min. Initiation of the Grignard formation was rapid, as judged by the disappearance of the iodine color. Following addition, the mixture was stirred at 30–35 °C for 1 h. The Grignard solution was then added dropwise to a 0 °C solution of iodide **22** (885 mg, 2.99 mmol) in a solution of dilithium tetrachlorocuprate (0.1 M in THF, 3.0 mL). Following the addition, the dark blue solution was stirred at 0 °C for 30 min. The reaction was quenched by the addition of saturated ammonium chloride (15 mL) and then extracted with ether (3 × 15 mL), shaking during the first extraction until the organic phase was colorless and the aqueous phase was a clear blue. Drying over magnesium sulfate and concentration *in vacuo* left a colorless oil. Flash chromatography (45 g of silica, methylene chloride (one column volume) and then 5% ether/methylene chloride) provided **23** (688 mg, 73%) as a colorless oil followed by homocoupled Grignard reagent (470 mg, 54% based on starting bromide): ^{1}H NMR ($CDCl_3$) δ 4.70 (s, 2H), 4.12 (q, 2H, J = 7 Hz), 3.36 (s, 3H), 2.25 (m, 3H), 1.99 (m, 1H), 1.8 (m, 3H), 1.47 (m, 2H), 1.25 (t, 3H, J = 7 Hz), 1.20 (s, 6H), 1.1–1.4 (m, 8H), 0.72 (m, 1H); IR (film, cm^{-1}) 1737; MS *m/z* (rel intensity) no M$^+$, 299 (M$^+$ − CH_3, 4), 103 (73), 45 (100). Anal. ($C_{18}H_{34}O_4$) C, H.

***cis*-3-[5-(Methoxymethoxy)-5-methylhexyl]cyclopentaneacetic Acid (24).** A solution of ester **23** (1.14 g, 3.62 mmol) in 95% ethanol (28 mL) and 1.0 M aqueous NaOH (9.3 mL) was stirred at room temperature for 8.5 h. Most of the solvent was evaporated *in vacuo*. The residue was dissolved in water (20 mL), acidified with 1.0 M aqueous HCl (ca. 10 mL), and extracted with ether (1 × 50 mL). The aqueous phase was saturated with solid NaCl and further extracted with ether (1 × 30 mL). Drying over magnesium sulfate and concentration *in vacuo* left 1.01 g (98%) of acid **24** as a viscous oil, sufficiently pure to carry directly into the next step: ^{1}H NMR ($CDCl_3$) δ 4.71 (s, 2H), 3.37 (s, 3H), 2.2–2.4 (m, 3H), 2.03 (m, 1H), 1.7–1.9 (m, 3H), 1.48 (m, 2H), 1.15–1.4 (m, 8H), 1.20 (s, 6H), 0.75 (m, 1H); IR (film, cm^{-1}) 2400–3600, 1734, 1709; MS *m/z* (rel intensity) no M$^+$, 241 (3), 225 (3), 209 (9), 103 (67), 45 (100).

Compounds 26 and 27. To a 0 °C solution of diisopropylamine (0.58 mL, 4.1 mmol) in THF (6 mL) was added a solution of *n*-butyllithium (1.53 M in hexane, 2.46 mL, 3.76 mmol). The solution was stirred at 0 °C for 5 min before it was slowly added via syringe to a −78 °C solution of acid **24** (489 mg, 1.71 mmol) in THF (9 mL). The cooling bath was then removed, and the yellow solution was allowed to stir for 2.5 h., turning light amber. After cooling again to −78 °C, aldehyde **11** (388 uL, 1.80 mmol) was added dropwise without solvent. The mixture was then allowed to come to room temperature and stir for 2 h. Most of the color disappeared. The solution was poured into ice-cold aqueous HCl (10 mmol in 40 mL) and ether (50 mL) and partitioned. The aqueous phase was then further extracted with ether (2 × 50 mL). The combined extracts were dried over magnesium sulfate and concentrated *in vacuo*, leaving a sticky white solid (0.92 g). The crude hydroxy acid **25** was a mixture of at least three diastereomers, judging by proton NMR and TLC, and was used without further purification.

Acetonitrile (22 mL) was added to crude hydroxy acid **25** (860 mg, ca. 1.6 mmol). To the resulting suspension was added

N,N-dimethylformamide dineopentyl acetal (0.95 mL, 3.4 mmol). Upon addition, the hydroxy acid **25** slowly dissolved. The resulting solution was then stirred at 72 °C for 6.5 h. The reaction was cooled and concentrated *in vacuo*, leaving a light amber oil. This was partitioned between water (25 mL) and hexane (35 mL), forming an emulsion. Addition of a small amount of solid sodium chloride dispersed the emulsion. After the hexane layer was separated, the aqueous phase was further extracted with ether (1 × 25 mL). The combined organic extracts were then washed with water (2 × 20 mL). Drying over magnesium sulfate and concentration *in vacuo* left 0.63 g of pale amber oil. NMR analysis indicated that a 1.4:1 mixture of trans:cis olefin isomers was present. Silica gel was prepared for flash chromatography as follows:[25] 35 g of 230–400-mesh silica was slurried with a solution of silver nitrate (4.38 g) in acetonitrile (100 mL). The solvent was removed on a rotovap, and then the coated silica was dried *in vacuo* overnight at 40 °C and 0.1 mm of pressure in the dark. The column was packed, and the crude product was loaded onto the column with methylene chloride. Elution followed with 10% ether/methylene chloride (150 mL) and then 20% ether/methylene chloride (250 mL). The trans olefin **26** eluted first (224 mg), followed by a mixture of isomers (1.7:1 trans:cis, 130 mg) and then the cis olefin **27** (159 mg). The total yield was thus 513 mg (71% overall from acid **24**). Trans isomer **26**: ^{1}H NMR ($CDCl_3$) δ 5.29 (m, 2H), 4.69 (overlapping s and m, 3H), 3.92 (m, 1H), 3.61 (m, 1H), 3.47 (m, 1H), 3.36 (s, 3H), 2.36 (m, 1H), 1.2–2.1 (m, 28H), 1.20 (s, 6H), 0.98 (s, 3H), 0.84 (m, 1H); ^{13}C NMR ($CDCl_3$) δ (mult) 138.7 (d), 130.6 (d), 96.6 (d), 90.8 (t), 76.2 (s), 73.8 (d), 62.7 (t), 54.9 (q), 43.4 (d), 41.8 (t), 41.1 (t), 39.8 (d), 36.5 (t), 34.9 (t), 34.6 (t), 34.5 (s), 32.2 (t), 31.6 (t), 31.2 (t), 29.1 (t), 28.9 (t), 26.9 (t), 26.2 (q), 25.4 (t), 24.7 (q), 24.1 (t), 19.9 (t); IR (film, cm^{-1}) (absorbances diagnostic for olefin geometry are underlined) 1145, 1130, 1110, 1090, 1075, 1030, 995, 980, 965; MS *m/z* (rel intensity) (FAB) 451 (M + H, 1), 389 (3), 85 (100). Cis isomer **27**: ^{1}H NMR ($CDCl_3$) δ 5.20, 5.12 (AB q with one-half further split to dd, 2H, J = 12 Hz (vicinal), 10 Hz (allylic)), 4.71 (s, 2H), 4.68 (m, 1H), 3.90 (m, 1H), 3.68 (m, 1H), 3.48 (m, 1H), 3.39 (s, 3H), 2.85 (m, 1H), 1.2–2.0 (m, 28H), 1.21 (s, 6H), 1.12 (s, 3H), 0.79 (m, 1H); ^{13}C NMR ($CDCl_3$) δ (mult) significant peaks only 137.1 (d), 135.7 (d), 96.6 (d), 90.8 (t), 76.2 (s), 72.8 (d), 62.7 (t), 54.9 (q); IR (film, cm^{-1}) 1145, 1130, 1110, 1090, 1075, 1030, 995, 740; MS *m/z* (FAB) virtually identical to the trans olefin.

***d,l*-[1α[*E*(*trans*)],3α]-4-[2-[3-(5-Hydroxy-5-methylhexyl)cyclopentyl]ethenyl]-4-methylcyclohexanol (6).** To a solution of **26** (264 mg, 0.586 mmol) in methanol (12 mL) was added concentrated aqueous HCl (2 drops). The reaction mixture was stirred at room temperature for 6.5 h. Concentration *in vacuo* left 217 mg of light amber oil. Flash chromatography (12.8 g of silica, 1/1 ethyl acetate/hexane) provided 156 mg (83%) of **6** as a white crystalline solid: ^{1}H NMR ($CDCl_3$) δ 5.31 (m, 2H), 3.67 (m, 1H), 2.39 (m, 1H), 1.2–1.95 (m, 22H), 1.21 (s, 6H), 0.98 (s, 3H), 0.84 (m, 1H); ^{13}C NMR ($CDCl_3$) δ (mult) 138.3 (d), 130.8 (d), 70.9 (s), 69.7 (d), 43.9 (t), 43.4 (d), 41.1 (t), 39.8 (d), 36.5 (t), 34.4 (t), 32.2 (t), 31.6 (t), 30.7 (t), 29.10 (q), 29.07 (t), 25.0 (q), 24.4 (t); IR (mull, cm^{-1}) 3295 brd, 1365, 1066, 964; MS *m/z* (rel intensity) no M$^+$, 304 (45), 286 (56), 202 (47), 191 (59), 165 (32), 121 (30), 108 (39), 95 (100). Anal. ($C_{21}H_{38}O_2$) C, H.

***d,l*-[1α[*Z*(*trans*)],3α]-4-[2-[3-(5-Hydroxy-5-methylhexyl)cyclopentyl]ethenyl]-4-methylcyclohexanol (28).** A solution of **27** (211 mg, 0.468 mmol) and concentrated aqueous HCl (2 drops) in methanol (12 mL) was stirred at room temperature for 6.5 h. The solvent was evaporated *in vacuo*, leaving a viscous pale amber oil (162 mg). Flash chromatography (12 g of silica, 1/1 ethyl acetate/hexane) gave 135 mg (89%) of a white crystalline solid: ^{1}H NMR ($CDCl_3$) δ 5.19 (d, half of AB q, 1H, J = 11.9 Hz), 5.12 (dd, half of AB q, J = 11.9, 9.2 Hz), 3.75 (m, 1H), 2.88 (m, 1H), 1.2–1.95 (m, 22H), 1.20 (s, 6H), 1.12 (s, 3H), 0.80 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 136.7, 136.0, 71.0, 68.6, 44.0, 41.8, 40.2, 39.6, 36.5, 35.5, 35.3, 32.8, 31.8, 30.7, 29.2, 26.6, 24.6; IR (mull, cm^{-1}) 3298 brd, 1361, 1065, 740; FAB MS *m/z* (rel intensity) 323 (M + 1, 9), 322 (M$^+$, 1), 305 (17), 287 (48), 191 (16), 121 (26), 107 (28), 95 (100), 81 (94), 67 (92), 55 (93); exact mass calcd for $C_{21}H_{39}O_2$ (FAB, M + 1) 323.2950, found 323.2988. Anal. ($C_{21}H_{38}O_2$·0.1H_2O) C, H.

***d,l*-[1α(*trans*),3α]-4-[2-[3-(5-Hydroxy-5-methylhexyl)cyclopentyl]ethyl]-4-methylcyclohexanol (29).** A mixture of olefins **6** and **28** (560 mg, 1.73 mmol) and 5% rhodium on carbon (485 mg) in absolute ethanol (60 mL) was hydrogenated in a Parr apparatus at 40 psi for 3.5 h. The mixture was filtered through Celite and concentrated *in vacuo*. The crystalline residue was taken up in boiling hexane (115 mL), and the hot mixture was filtered to remove insoluble residue. Cooling of the filtrate to room temperature afforded 407 mg (71%) of **29** as a fine white solid (mp 90–92 °C): ^{1}H NMR ($CDCl_3$) δ 3.58 (m, 1H), 1.92 (m, 1H), 1.1–1.8 (m, 26H), 1.21 (s, 6H), 0.87 (s, 3H), 0.62 (m, 1H); ^{13}C NMR ($CDCl_3$) δ (mult) 70.9 (d), 70.8 (s), 43.9 (t), 42.9 (t), 40.7 (d), 40.6 (t), 39.9 (d), 36.5 (t), 35.1 (t), 31.7 (s), 31.6 (t), 31.5 (t), 31.0 (t), 30.3 (t), 29.1 (q), 24.5 (t), 22.5 (q); IR (mull, cm^{-1}) 3257 brd, 3185 brd, 1365, 1077, 1068; FAB MS *m/z* (rel intensity) 325 (M + H, 5), 307 (12), 305 (14), 289 (16), 263 (5), 95 (100), 81 (38), 67 (46), 55 (46); exact mass calcd for $C_{21}H_{41}O_2$ (M + H) 325.3106, found 325.3137. Anal. ($C_{21}H_{40}O_2$·0.1H_2O) C, H.

***d,l*-[1α,2α,3β[*E*(*trans*)],5β]-3-[2-(4-Hydroxy-1-methylcyclohexyl)ethenyl]-5-(5-methylhexyl)-1,2-cyclopentanediol (7).** The cis and trans olefins **35** and **36** were prepared from the known malonate 30[17] by procedures exactly analogous to those described for the conversion of **20** to **26** and **27** (see Schemes 5 and 6). To a solution of trans olefin **35** (376 mg, 0.813 mmol) in methanol (60 mL) was added 1 M aqueous HCl (6 drops). The solution was stirred at room temperature for 5 h. The solvent was removed *in vacuo*, leaving a white solid. The crude material was suspended in boiling hexane (50 mL) and dissolved by the slow addition of ether (40 mL). The volume was reduced to 50 mL by boiling. Cooling to room temperature and then to 0 °C afforded 198 mg (72%) of **7** as white crystals (mp 107–108 °C): ^{1}H NMR ($CDCl_3$) δ 5.48 (d, half of AB q, 1H, J = 15.7 Hz), 5.29 (dd, half of AB q, 1H, J = 7.6, 15.7 Hz), 3.55–3.72 (m, 3H), 2.41 (m, 1H), 2.02 (m, 1H), 1.1–1.9 (m, 18H), 1.01 (s, 3H), 0.87 and 0.85 (2 s, 6H), 0.9 (m, 1H); ^{13}C NMR ($CDCl_3$) δ (mult) 141.7 (d), 127.1 (d), 77.5 (d), 77.3 (d), 69.6 (d), 47.9 (d), 44.6 (d), 38.8 (t), 34.7 (s), 34.6 (t), 34.4 (t), 34.2 (t), 30.6 (t), 28.1 (t), 27.8 (d), 27.4 (t), 24.7 (q), 22.5 (q); IR (mull, cm^{-1}) 3338 brd, 1128, 1060, 1039, 971; MS *m/z* (rel intensity) 338 (M$^+$, 17), 320 (43), 250 (19), 207 (100), 191 (21), 181 (44); exact mass calcd for $C_{21}H_{38}O_3$ 338.2281, found 338.2276. Anal. ($C_{21}H_{38}O_3$·0.1H_2O) C, H.

***d,l*-[1α,2α,3β[*Z*(*trans*)],5β]-3-[2-(4-Hydroxy-1-methylcyclohexyl)ethenyl]-5-(5-methylhexyl)-1,2-cyclopentanediol (37).** A solution of cis olefin **36** (274 mg, 0.592 mmol) in methanol (40 mL) and 1 M aqueous HCl (4 drops) was stirred at room temperature for 7 h. After the solvent was removed *in vacuo*, the crude oil was purified by flash chromatography (9 g silica, 3/1 ethyl acetate/hexane (30 mL), then ethyl acetate), providing 156 mg (78%) of a colorless viscous oil which crystallized very slowly upon standing (mp 83–85 °C): ^{1}H NMR ($CDCl_3$) δ 5.42 (d, half of AB q, 1H, J = 12.0 Hz), 5.10 (t, half of AB q, 1H, J = 12.0 Hz), 3.75 (m, 1H), 3.69 and 3.61 (2 m, 2H), 3.03 (m, 1H), 2.33 (d, 1H, J = 3.6 Hz), 2.19 (d, 1H, J = 5.1 Hz), 2.02 (m, 1H), 1.0–1.95 (m, 18H), 1.16 (s, 3H), 0.87 and 0.85 (2 s, 6H), 0.8 (m, 1H); ^{13}C NMR ($CDCl_3$) δ (mult) 140.7 (d), 132.0 (d), 78.9 (d), 77.4 (d), 68.7 (d), 44.8 (d), 43.9 (d), 38.9 (t), 35.8 (t), 35.5 (t and s?), 35.0 (t), 34.7 (t), 30.7 (t), 30.6 (t), 28.2 (t), 27.9 (d), 27.5 (t), 26.6 (q), 22.6 (q); IR (mull, cm^{-1}) 3295 brd, 1365, 1352, 1125, 1100, 1060, 1043, 726; MS *m/z* (rel intensity) 338 (M$^+$, 6), 320 (26), 250 (15), 207 (100), 181 (46), 121 (30), 109 (40), 95 (68). Anal. ($C_{21}H_{38}O_3$) C, H.

Biological Methods. Human hepatoma, HepG2, cells were purchased from American Type Culture Collection (Rockville, MD) and maintained in MEM with Earle's salts, L-glutamine, and 10% fetal bovine serum (all from GibcoBRL, Grand Island, NY) in a humidified atmosphere of 5% CO_2 in air at 37 °C. Human LDL and lipoprotein-deficient serum (LPDS) were isolated as described previously.[26] The LDL were iodinated using *N*-chlorobenzenesulfonamide attached to polystyrene beads (Iodo-Beads, Pierce, Rockford, IL) as described.[26]

HepG2 cells were transfected with a plasmid, pRed3lacZ, that contains 5 kB of hamster HMGR 5'-sequence originally described in pRedCAT-3 by Osborne *et al.*[27] The promoter sequence that is contained in pRed3lacZ controls transcription of β-galactosidase (*β-gal*). Transfection of the cells was accomplished using Lipofectin reagent (GibcoBRL, Grand Island, NY) according to the instructions provided by the manufacturer. A single cell clone was selected by limiting dilution. This clone, designated G52, expresses a high level of *β-gal*, and the expression of *β-gal* is regulated by 25-hydroxycholesterol. These transfected cells were treated for 24 h with several concentrations of 4 or 25-hydroxycholesterol. The compounds were added in ethanol to MEM containing 10% LPDS in place of the fetal bovine serum. After 24 h the culture medium was aspirated, and "Z" buffer[28] containing 0.1% Triton X-100 was placed on the cells. The cells were lysed with three freeze–thaw cycles. *β-Gal* activity in cell lysates was assayed using 4-methylumbelliferyl β-D-galactoside as substrate.

Nontransfected, wild type HepG2 cells were treated for 24 h with 10 μM **4** or 25-hydroxycholesterol. Total RNA was extracted from the cells using RNAzol (Tel-Test, Inc., Friendswood, TX), and HMGR mRNA levels were determined by slot-blot analysis. A 270 base fragment of the hamster reductase structural gene (bases 1469–1738) was used as a probe. Actin mRNA was used as the internal standard. The mRNA was quantitated using a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA).

HepG2 cell HMGR activity was determined using the method reported by others,[29] with slight modifications. After treatment for 24 h with 10 μM **4** or 25-hydroxycholesterol, the cells were scraped from the plates and microsomal fractions were prepared. Microsomal HMGR activity was assayed by measuring the conversion of DL-3-hydroxy-3-methyl[3-^{14}C]-glutaryl-CoA (DuPont NEN, Boston, MA) to ^{14}C-mevalonate. [5-^{3}H]Mevalonolactone (DuPont NEN, Boston, MA) was included to monitor recovery. Activities were determined as picomoles of HMG-CoA converted to mevalonate per minute per milligram of microsomal protein.

Total LDL receptor binding activity by HepG2 cells was determined after treatment for 20 h with 10 μM **4** or 25-hydroxycholesterol. After treatment the media containing the compounds was aspirated, the cells were washed with ice-cold PBS, and fresh media containing 5 mg/mL [^{125}I]LDL was added. After a 3-h incubation at 4 °C the cells were washed extensively with PBS. The cells were solubilized by adding a mixture (1:1, vol:vol) of 1 N NaOH and 1% sodium deoxycholate and incubating the plates at 50 °C for 20 min. The solubilized cells were transferred to tubes, and the amount of radioactivity that had been bound to the cells was determined using a γ counter. Total specific binding of LDL by the cells was calculated by subtracting the amount of [^{125}I]LDL bound in the presence of a 50-fold excess of unlabeled LDL from that bound in the absence of excess LDL.

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