Synthesis and Biological Evaluation of a New Series of Sterols as Potential Hypocholesterolemic Agents

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A new series of sterols was synthesized and tested in a CHO cell-based LDL receptor/luciferase (LDLR/Luc) assay to investigate the capability of derepressing the transcription of LDL receptor promoter in the presence of 25-hydroxycholesterol. The effect of various substitutions on antagonizing the repressing effect mediated by 25-hydroxycholesterol was also studied in terms of regio- and stereochemistry, lipophilicity, steric bulk, and π -electron density. Except 12, compounds active in the primary LDLR/Luc assay were not active in the secondary simian virus 40/luciferase (SV40/Luc) assay, demonstrating the specificity of their *in vitro* activity. Eight active compounds of various structural types were selected and screened in a [1-¹⁴C-*acetate*]cholesterol biosynthesis inhibition assay; none has shown any interference with the cholesterol biosynthesis in CHO cells. In hypercholesterolemic hamsters, generally, compounds that were active *in vitro* were active *in vivo* and *vice versa*, with the exception of three *in vitro* inactive compounds: 3β -ols **3a'** and **3c'** as well as 3-ketone **2a**. Experimental results from the livers of hamsters revealed that the *in vivo* conversion of **3a'** or **2a** to **3a** has in part contributed to the observed *in vivo* activity, and it is also anticipated that **3c'** may similarly be converted to **3c** in hamsters.

Cholesterol is a vital component of mammalian cell membrane of all tissues and also serves as a precursor of steroid hormones. It is of critical importance that the tissues are constantly supplied with cholesterol. However, regulatory mechanisms must be operative to balance the dietary cholesterol absorption, endogenous cholesterol synthesis from mevalonate, and cholesterol excretion to avoid cholesterol accumulation during a life span.¹ When regulatory mechanisms are not operative due to genetic or environmental causes, the level of cholesterol in the blood elevates, particularly lowdensity lipoprotein cholesterol (LDL-C), and the resultant hypercholesterolemia can further cause the development of atherosclerosis and incidence of coronary heart disease (CHD).²⁻⁵ To date, atherosclerosis and CHD are the leading causes of mortality in the western world; accordingly, there is considerable interest in drugs that can lower plasma LDL-C levels.^{5,6}

Brown and Goldstein began their work in the early 1970s in an attempt to understand a human genetic disease, termed familial hypercholesterolemia (FH), characterized by elevated plasma LDL-C.⁷ Their studies eventually led to the discovery of important cell surface receptors in the liver for plasma low-density lipoprotein (LDL) and increased the understanding of the molecular regulation of LDL receptor expression.⁸⁻¹⁰ A regulatory segment in the promoter region of human LDL receptor gene that exhibited positive and negative transcriptional activities in response to the absence and presence of 25-hydroxycholesterol was identified and designated as the sterol regulatory element (SRE).⁸

Stimulation of the expression of LDL receptors in the liver is a major mechanism for clearing plasma LDL- $C.^{11,12}$ Under conditions of high plasma LDL-C levels, expression of hepatic LDL receptors is low. Thus, by

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pharmacological interaction with bile acid enterohepatic circulation or by inhibition of cholesterol biosynthesis in the liver, the expression of hepatic LDL receptors can be increased in animals and man.¹³⁻¹⁵ Since sterols, including 25-hydroxycholesterol, are potent repressors of LDL receptor transcription, we are interested in searching for therapeutic agents that are capable of preventing the down-regulation of LDL receptor expression mediated by 25-hydroxycholesterol. A screening LDL receptor/luciferase (LDLR/Luc) assay¹⁶ was set up by stably transfecting a gene construct in Chinese hamster ovary (CHO) cells, in which a DNA segment containing the promoter and regulatory control elements of the LDL receptor gene was fused to the firefly luciferase reporter gene.^{17,18}

It is $known^{8,9,19-21}$ that in cultured cells (1) the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and the number of cell surface LDL receptors are coordinately regulated in response to changes in sterol availability, such as 25-hydroxycholesterol, and (2) the mRNA levels of HMG-CoA reductase and LDL receptors are also regulated in parallel, suggesting that a common mechanism may be responsible for controlling the expression of these two genes in vitro. Therefore, agents capable of derepressing the expression of LDL receptors in the presence of 25-hydroxycholesterol can also up-regulate the expression of HMG-CoA reductase; however, the increase in HMG-CoA reductase would anticipatedly be subject to negative multiregulations and result in decreasing the rate of cholesterol synthesis to normal.8

For the purpose of discriminating against those leads lacking promoter specificity, a secondary simian virus 40/luciferase (SV40/Luc) assay¹⁶ was set up using CHO cell lines transfected with a gene construct, in which a DNA segment containing the simian virus promoter gene was fused to the firefly luciferase reporter gene.

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^a Reagents: (a) Li, NH₃, *t*-BuOH, THF, -78 °C; substituted allyl bromide or iodide, -78 °C; (b) K-selectride, THF, -78 °C to room temperature or Dibal, CH₂Cl₂, 0 °C.

Since our compounds structurally belong to the sterol class, active compounds of various structural types were selected and screened in a CHO cell-based [1-¹⁴C-*acetate*]cholesterol biosynthesis inhibition (¹⁴CACBI) assay to investigate the potential inhibition of cholesterol biosynthesis.

In this publication we explore a new series of 3α sterols capable of derepressing the transcription of LDL receptors in the presence of 25-hydroxycholesterol in the LDLR/Luc assay. The new compounds will probe the effects of regio- and stereochemistry, lipophilicity, steric bulk, and π -electron density on antagonizing the repressing effect mediated by 25-hydroxycholesterol. More importantly, we also want to address the correlation of *in vitro* and *in vivo* activities in hamsters.

Results and Discussion

Synthesis. As shown in Scheme 1, reductive alkylation²² of enone 1 with various analogs of allyl bromide or iodide²³ yielded ketones 2, which in turn were reduced with K-selectride²⁴ to provide 3α -ols 3 as the only product. A mixture of 3α -ols 3 and 3β -ols 3' was obtained when Dibal was used as a reducing agent. Interestingly, in addition to 3j, acetylenic 4 was also isolated from the K-selectride reduction of 2j, presumably through dehydrobromination.

In order to introduce substituents of different geometric and electronic natures onto the double bond of the 4α -allyl, **3a** was first converted in two steps to silylprotected aldehyde **5**, which was then converted to *cis*olefin **6** via Wittig olefination²⁵ and deprotection. Alternatively, **5** was converted to diol **8** after three consecutive steps: Wadsworth-Emmons coupling,²⁶ Dibal reduction, and deprotection (Scheme 2).

At this juncture, we decided to modify the D-ring side chain by introducing a lipophilic ethyl at C-24 or a hydrophilic hydroxyl at C-25. Therefore, following the Scheme 2^a



^a Reagents: (a) TBDMSCl, imidazole, DMF; (b) O_3 , CH_2Cl_2 , -78 °C; (c) (Ph)₃PC₂H₅⁺Br⁻, KN(TMS)₂, THF, -15 to 0 °C; (d) Bu₄NF, THF, reflux; (e) (MeO)₂P(O)CH₂CO₂Me, KN(TMS)₂, THF, -15 °C; (f) Dibal, CH₂Cl₂, 0 °C.



Figure 1

same procedures as depicted in Scheme 1, **9–11** were synthesized in two steps from β -sitostenone, which was readily derived from Oppenauer oxidation²⁷ of β -sitosterol. Similarly, **12** was prepared in three steps from 25-hydroxycholesterol (Figure 1).

One of our target compounds was 15, a 5-dehydro derivative of **3a**; because of its structural similarity to cholesterol, it was predicted to be very active. Cholestenone 1 was quantitatively converted to labile dienamine **13**, following a literature procedure,²⁸ which was selectively allylated at C-4, and the resultant iminium salt was carefully hydrolyzed with water at 50 °C to give the labile β , γ -enone **14**. Without further purification, the crude product was reduced immediately with K-selectride²⁴ to provide the desired dienol **15** (Scheme 3).

To demonstrate the importance of the 4α -allyl substitution to the activity, **3a** and **3c** were hydrogenated respectively to yield the saturated analogs **16** and **17**, and **5** was converted to the 4α -vinyl analog **20** following Grieco's protocol²⁹ (Scheme 4).

We were also interested in investigating the biological effect associated with the location of the allyl group. Thus, according to literature precedent,³⁰ cholestenone 1 was alkylated with allyl iodide to give 2α -allyl enone 21, subsequent dissolving-lithium and Dibal reductions yielded a mixture of 2α -allyl alcohols 23 and 23'. Alternatively, 21 was subject to consecutive reductive alkylation²² and K-selectride reduction to afford the 2α , 4α -diallyl alcohol 25 (Scheme 5).

In addition, compounds **30** and **31**, with an allyl at C-6, were also prepared. Thus, 5α , 6α -epoxycholestan- 3β -ol **26** underwent epoxide ring opening with allyl-



^a Reagents: (a) pyrrolidine, benzene, reflux; (b) allyl bromide, dioxane, 55-60 °C; H₂O, 50 °C; (c) K-selectride, THF, -78 °C to room temperature.

Scheme 4^a



 a Reagents: (a) H₂, PtO₂, EtOAc; (b) Dibal, CH₂Cl₂, 0 °C; (c) (2-nitrophenyl)selenocyanate, PBu₃, THF; MCPBA, CH₂Cl₂; (d) Bu₄NF, THF, reflux.

magnesium bromide and subsequent Oppenauer oxidation²⁷ to give 6β -allyl enone **27** containing less than 5% inseparable 6α -allyl isomer; epimerization presumably occurred during the oxidation reaction. The mixture was then equilibrated in a basic medium to form an inseparable mixture of **27** and its 6α -allyl isomer in a ratio of 1/10. Further dissolving-lithium and K-selectride reductions provided 6α -allyl alcohol **30** and 6β allyl alcohol **31** (Scheme 6).

Another interesting investigation was the biological effect caused by changing the stereochemistry of the A/B-ring junction from *trans* to *cis*. Therefore **34** and **34**' were synthesized in two steps from 5β -cholestanone **32** following documented regio- and stereoselective alkylation³¹ and subsequent NaBH₄ reduction (Scheme 7).

At this point, it seemed advisable to replace the 4α allyl with the electron-rich 4α -benzyl with the anticipation that the latter would enhance the binding to the receptor through a stronger $\pi - \pi$ interaction. Most of 3α -ols **36** and 3β -ols **36'** were synthesized following the same procedures as depicted in Scheme 1;³² while **361** and **361'** were derived from NaBH₄ reduction of **351**, **360** and **360'** were derived from Dibal reduction of **351**. In addition, **36t** was obtained from hydrogenolysis of **36s**, while **36n** and **36p** were obtained respectively from **36m** through base hydrolysis and Dibal reduction (Scheme 8 and Table 4). Scheme 5^a



^a Reagents: (a) NaN(TMS)₂, THF, allyl iodide, -78 °C; (b) Li, NH₃, *t*-BuOH, THF, -78 °C; (c) Dibal, CH₂Cl₂, 0 °C; (d) Li, NH₃, *t*-BuOH, THF, -78 °C; allyl iodide, -78 °C; (e) K-selectride, THF, -78 °C to room temperature.

Scheme 6^a



^a Reagents: (a) allyl bromide, Mg, Et₂O; (b) cyclohexanone, Al(*i*-OPr)₃, toluene, reflux; (c) 2% KOH/MeOH, THF; (d) Li, NH₃, *t*-BuOH, THF, -78 °C; (e) K-selectride, THF, -78 °C to room temperature.

In Vitro Biology. Compounds were evaluated for their up-regulating or derepressing capability against 25-hydroxycholesterol in the LDLR/Luc assay,¹⁶ in which stably transfected, stationary CHO cells were exposed to 25-hydroxycholesterol (0.5 μ g/mL) and compound (0-20 μ g/mL) in serum free medium for 24 h. Cells were then washed and lysed and homogenates were evaluated for luciferase-catalyzed light production. EC₃₀ (μ M) refers to the effective concentration of compound required to increase the specific light induction

Scheme 7^a



^a Reagents: (a) NaN(TMS)₂, allyl iodide, THF, -78 °C; (b) NaBH₄, Et₂O/MeOH, 0 °C to room temperature.

Scheme 8^a



^a Reagents: (a) NaBH₄, MeOH/Et₂O, 0 °C to room temperature; (b) Dibal, CH₂Cl₂, 0 °C; H₃O⁺; (c) LiOH, MeOH/THF; H₃O⁺; (d) 10% Pd/C, H₂, EtOAc.

by 30% over the control. Due to solubility limitations of the sterol class in the test medium, MA (%) only refers to the maximum activity, or the percentage specific light induction, of agent over the control at the highest test concentration of 20 μ g/mL.

From the broad screening, 3α -sterol **3b** arose as a lead in the LDLR/Luc assay; presumably, **3b** was able to prevent the down-regulation of LDL receptor expression mediated by 25-hydroxycholesterol (a 3β -sterol) via antagonizing the binding to a putative oxysterol receptor. In order to discover more active compounds, an extensive, well-designed study of the structure-activity relationship (SAR) was thus carried out.

First we investigated the effect of substitution on the double bond of 4α -allyl on the *in vitro* activity in terms

of the geometric position, steric bulk, and lipophilicity. As indicated in Table 1, 3a is one of the most active compounds in the assay, with an EC₃₀ of 2.9 μ M. Compounds with substitution at the 2-position of 4α allyl, such as **3b** with a methyl, **3g** with a chloro, and 3j with a bromo, have as good a potency as 3a. However, comparing the EC_{30} values of **3b**, **3c**, and **6** clearly reveals that the geometric position of the methyl substitution on the double bond of 4α -allyl affects the biological activity with a decreasing order of potency: 3b > 3c > 6, namely 2 > 3-trans > 3-cis. A similar order of decreasing potency is also observed for the chloro compounds: 3g and 3h have comparable, good potency, but 3i is inactive. Data shown in Table 1 also reveal that steric bulk and lipophilicity affect the activity. For instance, **3c** with a methyl at the 3-trans position has an EC₃₀ of 11 μ M, whereas 3d with a slightly larger ethyl and **3k** with a phenyl at the same position turn out to be inactive in the assay. A comparison of the EC_{30} values of **3e** and **3f** vs **3b**, **3c**, and 6 once again demonstrates that compounds with bulkier dimethyl substitutions tend to lose the potency relative to the ones with monomethyl substitution. In addition, when the polarity of substitution at the 3-trans position increases, for instance 8 with a hydroxymethyl, activity completely vanishes.

It is remarkable to point out that, as shown in Table 1, all the active compounds have the 3-hydroxyl positioned in the α -space, whereas **3a'**, **3e'**, and **3g'**, with the 3-hydroxyl positioned in the β -space, are devoid of activity. The same stereospecificity is also observed later in the 4 α -benzyl series.

We also investigated the biological significance of adding a lipophilic ethyl at the C-24 of **3a**, **3b**, and **3g**; consequently, compounds **9–11** were synthesized and are shown to be 2-fold less potent. Because of 25hydroxycholesterol's active role in the regulation of cellular cholesterol metabolism, compound **12**, a 25hydroxy derivative of **3a**, was also prepared with the anticipation that it would enhance the binding to receptor site against 25-hydroxycholesterol. However, test results show that **12** is 3-fold less potent than **3a** in terms of EC₃₀ values shown in Table 1.

One of our target compounds was compound 15, a 5-dehydro derivative of 3a. Owing to its structural similarity to cholesterol, 15 could represent a better substrate to either enzyme or receptor and was expected to be more potent than others. However, test results reveal that 15 is only as potent as 3a.

The importance of the 4α -allyl substitution to the activity has clearly been demonstrated in Table 2. Saturated derivatives 16 and 17 are less active than **3a** and **3c**. Compounds 4, a 4α -propargyl derivative, and **20**, a 4α -vinyl derivative, show no activity (Table 2).

At this juncture, we learned a great deal from our SAR studies in the C-4 region and concluded that **3a** was the compound with locally optimized *in vitro* activity. Attention was then directed toward exploring other positions around the cholesterol skeleton that hopefully would lead to the discovery of more potent compounds. As indicated in Table 3, compound **23**, a 2α -allyl analog, shows some activity with an EC₃₀ of 41 μ M, but its 3β -ol epimer **23**' shows no activity. The results coincide with what has previously been observed

Table 1. Formulas, Properties, and in Vitro Biological Activities of 4α-Allyl Analogs

				LDLR/Luc ^d			
compd	formula ^a	$analyses^b$	mp (°C) (recrystn solv) ^c	EC ₃₀ (µM) ^e	MA (%) ^f	$SV40/Luc^d$	$^{14}\mathrm{CACBI}^d$
3a	$C_{30}H_{52}O$	C,H	117–119 (A)	2.9 (5)	127	NA ^g (5)	neg ^h
3a′	$C_{30}H_{52}O$	C,H	135–137 (A)	$NA^{g}(2)$			U U
3b	$C_{31}H_{54}O$	C,H	129–131 (B)	2.8(3)	124	$NA^{g}(2)$	
3c	$C_{31}H_{54}O$	C,H	100-102(A)	11 (5)	86	$NA^{g}(5)$	neg^h
3c′	$C_{31}H_{54}O$	C,H	115–117 (A)	NA ^g (2)			U
3d	$C_{32}H_{56}O$	C,H	92-94 (A)	NA ^g (2)			
6	$C_{31}H_{54}O$	C,H	99-101 (A)	26 (3)	72	$NA^{g}(2)$	
3e	$C_{32}H_{56}O$	C,H	122–123 (A)	18 (3)	72	$NA^{g}(2)$	
3f	$C_{32}H_{56}O$	C,H	185–187 (A)	$NA^{g}(2)$			
3g	C ₃₀ H ₅₁ ClO	C,H,Cl	130-132 (B)	1.8 (4)	141	$NA^{g}(2)$	
3g	$C_{30}H_{51}ClO$	C,H,Cl	122-124 (A)	$NA^{g}(2)$			
3ĥ	$C_{30}H_{51}ClO$	C,H,Cl	139–141 (A)	2.0(4)	169	$NA^{g}(2)$	neg^h
3i	$C_{30}H_{51}ClO$	C, H, Cl	140-142 (A)	$NA^{g}(2)$			0
3j	$C_{30}H_{51}BrO$	C,H,Br	155–157 (A)	3.2(5)	131	$NA^{g}(2)$	
3k	$C_{36}H_{56}O$	C,H	139-140 (A)	$NA^{g}(2)$			
8	$C_{31}H_{54}O_2$	C,H	178–180 (A)	$NA^{g}(2)$			
9	$C_{32}H_{56}O$	C,H	118-120 (B)	8.2 (3)	95	$NA^{g}(2)$	neg^h
10	$C_{33}H_{58}O$	C,H	136–137 (B)	3.1 (3)	165	$NA^{g}(2)$	U
11	$C_{32}H_{55}ClO$	C, H^i	148-150 (B)	4.2 (3)	120	$NA^{g}(2)$	
12	$C_{30}H_{52}O_2$	C,H	145-147 (B)	9.4 (3)	111	$active^{j}(2)$	
15	$C_{30}H_{50}O$	C,H	101–103 (B)	3.5 (5)	96	$NA^{g}(2)$	\mathbf{neg}^h

^a Each compound had proton NMR consistent with the proposed structure and expected M and/or M + H ions seen in MS. ^b Analyses for indicated elements were within 0.4% of the expected values for the formula. ^c Key: A = MeOH/Et₂O; B = CH₃CN/Et₂O. ^d See the Experimental Section. ^e Effective concentration at 30% specific light induction over control. The number of experiments (*n*) is given in parentheses. ^f Maximum activity of specific light induction at 20 μ g/mL. ^g Not active within the test range 0–20 μ g/mL. ^h Negative: no effect on the biosynthesis of cholesterol. ⁱ Analysis of percentage halogen was not performed. ^j At 10 μ g/mL, 45% specific light induction over control was recorded.

				$LDLR/Luc^d$			
compd	$formula^a$	$analyses^b$	mp (°C) (recrystn solv) ^c	EC ₃₀ (µM) ^e	MA (%)f	$SV40/Luc^d$	$^{14}\mathrm{CACBI}^d$
16 17 4 20	$\begin{array}{c} C_{30}H_{54}O\\ C_{31}H_{56}O\\ C_{30}H_{50}O\\ C_{29}H_{50}O\end{array}$	С,Н С,Н С,Н С,Н С,Н	120–123 (A) 104–105 (A) 146–148 (A) 137–138 (B)	38 (4) 20 (4) NA ^g (2) NA ^g (2)	48 88	NA ^g (2) NA ^g (2)	neg^h

 a^{-h} See footnotes a^{-h} of Table 1.

Table 3. Formulas, Properties, and in Vitro Biological Activities of 23, 23', 25, 30, 31, 34, and 34'

			$\mathrm{LDLR}/\mathrm{Luc}^d$				
compd	formula ^a	$analyses^b$	mp (°C) (recrystn solv) ^c	EC ₃₀ (µM) ^e	MA (%)f	$SV40/Luc^d$	$^{14}\mathrm{CACBI}^d$
23 23′ 25	$C_{30}H_{52}O \\ C_{30}H_{52}O \\ C_{50}H_{52}O \\ C_{50}H_{52}O \\ C_{50}H_{50}O $	С,Н С,Н С.Н	72-74 (B) 102-103 (B) 101-103i	41 (4) NA ^g (2)	60	NA ^g (2)	neg^h
23 30 31 34 34'	$\begin{array}{c} C_{30}H_{52}O\\ C_{30}H_{52}O\\ C_{30}H_{52}O\\ C_{30}H_{52}O\\ C_{30}H_{52}O\end{array}$	C,H C,H C,H C,H C,H	$\begin{array}{c} \text{gum} \\ 101-103 \text{ (A)} \\ \text{gum} \\ 63-65^i \end{array}$	NA ^g (2) NA ^g (2) NA ^g (2) NA ^g (2)			

 a^{-h} See footnotes a^{-h} of Table 1. ⁱ Isolated from chromatographic purification without further recrystallization.

in the 4 α -allyl series. It was disappointing to find out that compound **25**, a 2 α ,4 α -diallyl analog, showed no activity despite the anticipation that it might have the synergistic activity of both compounds **3a** and **23**. When an allyl substitution was introduced at C-6, neither **30** nor **31** showed any activity. Attention was then directed toward changing the stereochemistry of the A/B-ring junction from *trans* to *cis*; however, test results proved that the effort was fruitless: both **34** and **34**' showed no activity (Table 3).

Since previous studies had indicated that 4α -allyls **3a** and **3c** were more active than their saturated analogs **16** and **17**, it seemed advisable to replace the 4α -allyl with the electron-rich 4α -benzyl in hope of enhancing the binding to the receptor through a stronger $\pi - \pi$ interaction. To our delight, as shown in Table 4, unsubstituted benzyl **36e**, 4-halogenated benzyls **36fi**, and other 4-substituted benzyls **36j**-**m** and **36q** were

shown to be active; among them, 4-iodobenzyl 36i was the most active compound, with an EC₃₀ of 2.8 μ M, comparable to that of **3a**. However compounds with polar and/or bulkier substitution at the 4-position of benzyl, such as **36n-p** and **36s**,t, showed no activity in the LDLR/Luc assay. In light of 4-(trifluoromethoxy)benzyl **36q** with an EC₃₀ of 15 μ M, we cannot explain the lack of activity of 4-methoxybenzyl 36r. Test results also revealed that compounds with substitutions at the 2- and/or 3-positions of benzyl, such as 36a-d and 36uw, were not active. Apparently, in addition to lipophilicity and steric bulk, there is a regiochemistry requirement for substitution to be located at the 4-position of the benzyl to have activity. Finally, in line with the previous results, none of the 3β -ols **36**' showed any activity.

In summary, our studies have shown that the following structural features are important to the *in vitro*

Table 4. Formulas, Properties, and in Vitro Biological Activities of 4α -Benzyls 36

					LDLR/Luc ^a			
compd	R	formula ^a	analyses ^b	mp (°C) (recrystn solv) ^c	EC ₃₀ (µM) ^e	MA (%) ^f	$SV40/Luc^d$	$^{14}\mathrm{CACBI}^d$
36a	2-F	C ₃₄ H ₅₃ FO	C,H,F	129-131 (B)	NA ^g (2)			
36b	3-F	C ₃₄ H ₅₃ FO	C,H,F	174-176 (B)	$NA^{g}(2)$			
36c	3-I	C ₃₄ H ₅₃ IO	C, H^{i}	63-65 (A)	$NA^{g}(2)$			
36d	3-Me	$C_{35}H_{56}O$	C,H	122-124 (B)	$NA^{g}(2)$			
36e	н	$C_{34}H_{54}O$	C,H	145-146 (A)	32 (2)	44	$NA^{g}(2)$	neg^h
36f	4-F	$C_{34}H_{53}FO$	C,H,F	174–176 (B)	17 (3)	80	$NA^{g}(2)$	_
36g	4-C1	$C_{34}H_{53}ClO$	C,H,Cl	171–173 (B)	42 (3)	63	$NA^{g}(2)$	
36h	4-Br	C ₃₄ H ₅₃ BrO	C,H,Br	168–170 (A)	6.0 (4)	57	$NA^{g}(2)$	
36i	4-I	$C_{34}H_{53}IO$	C,H,I	187–189 (B)	2.8 (3)	80	$NA^{g}(2)$	neg^h
36j	4-Me	$C_{35}H_{56}O$	C,H	153–154 (B)	35 (3)	45	$NA^{g}(2)$	
36k	$4-CF_3$	$C_{35}H_{53}F_{3}O$	C,H,F	187–188 (B)	12 (3)	58	$NA^{g}(2)$	
361	4-CN	$C_{35}H_{53}NO$	C,H,N	216-218 (B)	9.9 (3)	55	$NA^{g}(2)$	
36m	$4-CO_2Me$	$C_{36}H_{56}O_3$	C,H	214-216 (B)	14 (2)	48	$NA^{g}(2)$	
36n	$4-CO_2H$	$C_{35}H_{54}O_3$ ·THF	C,H	$270 \; (dec)^k$	$NA^{g}(2)$			
360	4-CHO	$C_{35}H_{54}O_2$	C,H	235 (dec) (B)	$NA^{g}(2)$			
36p	$4-CH_2OH$	$C_{35}H_{56}O_2$	C,H	237–238 (B)	$NA^{g}(2)$			
36q	$4-OCF_3$	$C_{35}H_{53}F_3O_2$	C,H,F	164–166 (B)	15 (3)	71	$NA^{g}(2)$	
36r	4-OMe	$C_{35}H_{56}O_2$	C,H	204-206 (B)	NA ^g (2)			
36s	$4-OCH_2Ph$	$C_{41}H_{60}O_2$	C,H	208–210 (B)	$NA^{g}(2)$			
36t	4-OH	$C_{34}H_{54}O_2$	C,H	215 (dec) (B)	NA ^g (2)			
36u	$2,3-F_2$	$C_{34}H_{52}F_2O$	C,H,F	145–147 (B)	$NA^{g}(2)$			
36v	$2,4-F_2$	$C_{34}H_{52}F_2O$	C,H,F	89-91 (A)	$NA^{g}(2)$			
36w	3,4-Cl ₂	$C_{34}H_{52}Cl_2O$	C,H,Cl	168–170 (B)	NA ^g (2)			

 a^{-i} See footnotes a-i of Table 1. ^j 36a', 36c', 36e', 36f', 36h', 36h', 36h', 36h', 36h', 36h', 36h', and 36w' were not active in the assay. ^k 36n was recrystallized from CH₃CN/THF.

activity: (1) The 3-hydroxyl needs to be positioned in the α -space to have activity; none of the β -epimers are active. (2) The 4α -position is critical to the activity; moving the allyl substitution to the 2α -, 6α -, or 6β position resulted in either a decrease or loss of activity. (3) The substitution pattern on the double bond of 4α allyl and the phenyl of 4α -benzyl also affected the activity. In the 4α -allyl series, compounds with small, lipophilic substitution at the 2- or 3-trans-position of allyl are the most active. In the 4α -benzyl series, compounds with small, lipophilic substitution at the 4-position of the phenyl are the most active. (4) Introduction of an ethyl at C-24, a hydroxyl at C-25, or an unsaturation at C-5 did not lead to an improvement in activity. (5) Replacing the 4α -allyl with a vinyl, a propargyl, or a propyl only resulted in loss of activity. (6) None of the 3-keto precursors, from which the 3-hydroxy compounds were synthesized, showed any activity (data not shown).

As also indicated in Tables 1–4, except for 12, compounds active in the LDLR/Luc assay did not show any nonspecific induction in the SV40/Luc assay, nor did any of the eight compounds selected for the ¹⁴CACBI assay interfere with the cholesterol biosynthesis in CHO cells.

In Vivo Biology. Golden Syrian hamsters were fed a cholesterol diet for 2 weeks to induce hypercholesterolemia. The hamsters were separated into groups of six animals, each group having approximately the same mean serum cholesterol levels. Selected compounds were incorporated into the cholesterol test diet at 0.2% (w/w) and fed to the hamsters for 1 week. A control group of hamsters continued on the cholesterol test diet during the same 1 week period. **3a** was used as a positive control in each experiment.

Data in Table 5 have shown that, in general, compounds that are active *in vitro* are active *in vivo* and *vice versa*. It is surprising to observe that two 3β -ols, **3a'** and **3c'**, are active *in vivo* and the parent 3-ketone **2a** is also active. These three compounds did not show any activity in the *in vitro* assays. Experiments to assess the possible *in vivo* conversion of isomers have shown that in the livers of hamsters fed **3a'** or **2a**, **3a** was found through qualitative GC/mass analysis (no further effort was made to quantitate the percentage conversion); however, we have not observed any **3a'** in the livers of hamsters fed **3a** or **2a**. Apparently the *in vivo* conversion of **3a'** or **2a** to **3a** contributed in part to the observed *in vivo* activity. In light of the observed *in vivo* activity with **3c'**, it is anticipated that **3c'** may similarly be converted to **3c** in hamsters.

Compound **3a** was selected for a cholesterol absorption inhibition study in hamsters, and results have been reported³³ that reduction in cholesterol absorption from the gut was not able to account for the observed drug effect; thereby, we anticipate that cholesterol absorption inhibition could occur with other active 3α -sterols and potentially contribute in part to the observed activity in hamsters.

In conclusion, we have identified a new series of 3α sterols capable of preventing the down-regulation of LDL receptor expression mediated by 25-hydroxycholesterol, and the derepressing effect is presumably derived from antagonizing the binding to a putative oxysterol receptor. More importantly, in general, compounds that are active in vitro are active in vivo and vice versa. None of the eight selected compounds gave positive results in the ¹⁴CACBI assay, indicating that the new series of 3α -sterols apparently does not interfere with the cholesterol biosynthesis in CHO cells; however, owing to the observed absorption inhibition of dietary cholesterol by 3a,33 cholesterol absorption inhibition could potentially contribute in part to the observed activity in hamsters. Compound 3a has been selected for further development, and results of the extensive studies will be reported in due course.

Experimental Section

Materials. Reagents were used as supplied from commercial sources unless otherwise noted. 4-Cholesten-3-one (98%) was purchased from Aldrich, and 25-hydroxycholesterol,

	total	cholesterol	10104
compd	(mg/dL) ^a	to control (%) ^b	EDLR/Luc ECas (μ M) ^e
control	919 (94)		
39	167(24)	-47	20
3a'	198 (11)	-97	NA
30	210(14)	-33	11
3c'	245 (14)	-22	NA
23	209 (14)	-33	41
36e	202 (13)	-35	32
control	366 (24)		
3a	174 (7)	-53	2.9
2a	267 (21)	-27	NA
35e	312 (23)	[-15]	NA
36f	181 (15)	-50	17
control	306 (27)		
3a	151 (8)	-51	2.9
15	163 (9)	-47	3.5
16	222(13)	-27	38
36t	261 (6)	[-15]	NA
control	320 (14)		
3a	168 (12)	-47	2.9
36q	209 (15)	-34	15
control	383 (13)	40	
3a	197 (16)	-49	2.9
361	240 (30)	-37	2.8
control	274 (23)	01	0.0
38. 055	188 (10)	-31	2.9 NA
001 00 <i>8</i>	333 (30) 997 (95)	[+22] [± 5]	INA NA
301 96h	207 (20) 160 (6)	[+0] -49	60
control	333 (93)	-42	0.0
39	199 (15)	-40	29
30	200 (12)	-40	1.8
Sh	185 (15)	-44	2.0
9	217(15)	-35	8.2
10	196 (4)	-41	3.1
control	425 (58)		
3a	213 (17)	-50	2.9
3i	316 (10)	[-26]	NA
11	241 (27)	-43	4.2
36j	283 (33)	-33	35

^a Test compounds were administered in the diet at a dose of 0.2% of diet (approximately 100 mg/kg/day) for 1 week. The data are mean (SEM) of n = 6 observations per group. ^b Data listed in the brackets are not statistically different from that of controls at P < 0.05 using Dunnett's two-tailed test. ^c See footnotes d, e, and g of Tables 1-4.

 $5\alpha, 6\alpha$ -epoxycholestan- 3β -ol, and 5β -cholestan-3-one were purchased from Sigma. β -Sitosterol was supplied internally at Lilly. Reactions were run under a dry nitrogen or argon atmosphere unless otherwise noted. Silica gel (E. Merck, 230-400 mesh ASTM) was used for flash column chromatography. Reactions were monitored, and the homogeneity of the products was checked by TLC on silica gel 60 F₂₅₄ plates (E. Merck). ¹H NMR spectra were recorded on a General Electric QE-300 instrument. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Infrared (IR) spectra were determined on a Nicolet MX-1 FT-IR. Field desorption mass spectral data (FDMS) were obtained on a VG Analytical Zab-3F spectrometer. Elemental analyses were determined on a (Perkin-Elmer Model 240C) elemental analyzer and are within 0.4% of theory unless otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected.

In Vitro Assay: (A) Media. Both the growth medium and assay medium were prepared from the same basal medium, which consisted of three parts of Dulbecco's modified Eagle's medium and one part (by volume) of Ham's F-12 medium with the addition of $10^{-8} \mu$ M selenium, 50 μ M ethanolamine, and 20 mM 4-(2-hydroxyethyl)piperazineethanesulfonic acid. Growth medium was basal medium supplemented with 5% v/v fetal bovine serum. Assay medium was basal medium supplemented with 0.5% Bovuminar Cohn fraction V powder (bovine albumin) and 0.5 μ g/mL 25-hydroxycholesterol. A stock solution of 25-hydroxycholesterol in absolute ethanol (0.5 mg/mL) was prepared and diluted in the assay medium to achieve the indicated concentration of 0.5 μ g/mL.

(B) Cell Culture. The recombinant CHO cell lines (clone S27/B30 or SV40/LN9)¹⁶ were seeded at 5×10^4 cells/well into 24-well plates in 0.5 mL of growth medium and incubated at 37 °C in a humidified air atmosphere containing 5% CO₂. At confluence (2–3 days after seeding), the growth medium was removed, the monolayers were rinsed once with assay medium (0.5 mL/well), and then assay medium or assay media containing test compound at various concentrations (0.30, 0.60, 1.2, 2.5, 5.0, 10, and 20 µg/mL) were added to triplicate wells. A stock solution of test compound in absolute ethanol (4.0 mg/mL) was prepared and diluted in the assay medium to achieve the previously indicated concentrations. The plates were incubated for an additional 24 h and then assayed for luciferase activity.

(C) Luciferase Activity (LDLR/Luc and SV40/Luc Assays).¹⁶ Each well was washed once in phosphate-buffered saline without Ca²⁺ or Mg²⁺ (500 μ L/well), and the cell monolayer was lysed by addition of 100 $\mu L/well$ assay buffer containing 1% Triton X-100, 25 mM glycylglycine (pH 7.8), 15 mM MgSO₄, 1 mM dithiothreitol, and 1 mM ATP. A 50 μ L aliquot of each lysate was diluted up $425 \,\mu L$ in the above assay buffer and placed in a LMB luminometer and the reaction was initiated by the injection of 25 μ L of 1 mM luciferin. Light output from the luciferase reaction was expressed as peak luminescence and was proportional to luciferase concentration. Total protein was determined in each lysate (5 μ L) by Coomassie Brilliant Blue G250 binding with a protein assay kit (Bio-Rad Laboratories). Luciferase activity is expressed as light units/ μ g of protein (specific activity). EC₃₀ (μ M) is defined as the effective concentration of agent required to increase the luciferase specific activity by 30% relative to the control. Owing to a solubility limitation of the sterol class in test medium, MA (%) only refers to the maximum activity, or the percentage specific light induction, of agent over the control at the highest test concentration of 20 μ g/mL.

(D) [1-14C-acetate]Cholesterol Biosynthesis Inhibition Assay (¹⁴CACBI). Replicate 100 mm dishes of confluent CHO cells were incubated with 8 mL of assay medium (without the 25-hydroxycholesterol supplement) containing either the ethanol vehicle (0.5% v/v) or compound at 20 μ g/mL. After a 20 h incubation, [1-14C]-acetic acid, sodium salt (NEN), was added $(8 \ \mu L)$ directly to each dish (final concentration of 1 $\mu Ci/mL$), and the dishes were incubated for an additional 4 h. Cell monolayers were then harvested by scraping and the cell pellets were suspended in water and sonicated for 10 s. After an aliquot was removed for protein determination,³⁴ lipids were extracted with chloroform/methanol (2:1 v/v).35 After normalizing for protein content, the lipid fraction was resuspended using chloroform/methanol (4:1 v/v) in a small volume (100 μ L or less), and lipids were separated by high performance thin-layer chromatography on Kieselgel 60 silica plates with a mobile phase consisting of hexane/heptane/diethyl ether/ acetic acid (63:18.5:18.5:1, v/v). Lipids were visualized by dipping the plate for 3 s in a solution of hexane/ethanol/sulfuric acid (64:35:1, v/v) and then placed for 15 min in a 110 $^\circ\mathrm{C}$ oven.³⁶ The cholesterol band was scraped and counted for radioactivity on a Beckman LS6500 scintillation counter.

In Vivo Testing. Golden Syrian hamsters were fed a cholesterol diet made of 10% coconut oil and 0.12% cholesterol (by weight) in Purina 5001 rodent chow (cholesterol test diet) to induce hypercholesterolemia. After 2 weeks on the cholesterol diet, the hamsters were bled from the orbital sinium under light CO_2 anesthesia. Serum was prepared and analyzed for cholesterol using a commercial test kit (Cholesterol High Performance, Single Vial, product of Boehringer Mannheim Corp., Indianapolis, IN). The hamsters were separated into groups of six animals, such that each group had approximately the same mean serum cholesterol level. Selected compounds were incorporated into the cholesterol group of hamsters continued on the cholesterol test diet during the

same 1 week period. The dose of test compound was equivalent to 100 mg/kg/day on the basis of the weight of the hamsters and their diet consumption. Upon completion of the test period, the animals were bled, and serum cholesterol was determined as above.

Syntheses. The following synthesis of 3a and 3a' represents a general procedures for the synthesis of related analogs.

(4α,5α)-4-(2-Propenyl)cholestan-3-one (2a). A lithium chip (27.1 mg, 3.90 mmol) and a glass-coated stir bar were placed in a flame-dried, three-necked, round-bottomed flask fitted with a dry ice condenser under argon. Liquid ammonia (30 mL) was collected in the flask at -78 °C to form a deep blue solution, followed by the addition of dry THF (20 mL). A solution of (+)-4-cholesten-3-one (500 mg, 1.30 mmol) and t-BuOH (0.122 mL, 1.30 mmol) in dry THF (20 mL) was added dropwise to the deep blue solution. Upon completion of the addition, the resultant blue solution was stirred for 15 min before it was treated with 1,3-pentadiene (0.5 mL) to quench the excess of lithium. After 15 min, allyl iodide (0.357 mL, 3.90 mmol) was added to the white suspension and the resultant mixture was stirred at -78 °C for 2.5 h. Saturated aqueous NH₄Cl (10 mL) was carefully added to the white suspension. The cold bath was removed and the mixture, with the evaporation of ammonia, was allowed to warm to ambient temperature. After extraction with EtOAc (30 mL \times 2), the combined organic layers were washed with saturated aqueous NaCl (10 mL), dried over MgSO₄, filtered, and concentrated. After flash chromatography on silica (gradient 4-8% ethyl acetate/hexane), 2a (323 mg, 58%) was obtained as a white solid, which was recrystallized from Et₂O/MeOH: mp 108.0-110.0 °C; IR (KBr) 2933, 1706, 913 cm⁻¹; ¹H NMR (CDCl₃) δ 0.60-1.65 (m, 22H), 0.68 (s, 3H, CH₃), 0.87 (d, 6H, J = 6.5Hz, CH₃), 0.91 (d, 3H, J = 6.6 Hz, CH₃), 1.06 (s, 3H, CH₃), 1.65-1.91 (m, 3H), 1.91-2.08 (m, 2H), 2.18-2.55 (m, 5H), 4.95-5.08 (m, 2H, =CH₂), 5.70-5.88 (m, 1H, -CH=); FDMS m/e 426 (M⁺). Anal. (C₃₀H₅₀O) C, H.

(3a,4a,5a)-4-(2-Propenyl)cholestan-3-ol (3a). K-Selectride (0.419 mL, 1 M in THF) was added to a stirred solution of 2a (119 mg, 0.279 mmol) in dry THF (4 mL) at -78 °C under argon. Upon completion of addition, the cold bath was removed and the resultant yellowish solution was stirred for 2 h. The reaction mixture was then cooled to 0 °C before it was sequentially treated with methanol (0.5 mL), 5 N NaOH (0.446 mL, 2.23 mmol), and 30% H₂O₂ (0.171 mL, 1.68 mmol). The cold bath was removed and the mixture was stirred vigorously for 2 h. Half-saturated aqueous NaCl (10 mL) and EtOAc (30 mL) were added to the mixture, and the organic layer was separated, washed with half-saturated aqueous NaCl (10 mL \times 2), dried over MgSO₄, filtered, and concentrated. After flash chromatography on silica (gradient 0-8%ethyl acetate/toluene), 3a (117 mg, 98%) was obtained as a white solid, which was recrystallized from Et₂O/MeOH: mp 117.0-119.0 °C; IR (KBr) 3493 (br), 2934 cm⁻¹; ¹H NMR (CDCl₃) & 0.65-2.05 (m, 32H), 0.66 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.87 (d, 6H, J = 6.5 Hz, CH₃), 0.91 (d, 3H, J = 6.5 Hz, CH₃), 2.20–2.35 (m, 1H), 3.91 (br s, 1H, -CH(OH)-), 4.98– 5.15 (m, 2H, $=CH_2$), 5.78–5.98 (m, 1H, -CH=); FDMS m/e $428 \ (M^+). \ Anal. \ (C_{30}H_{52}O) \ C, \ H.$

3a and Its 3\beta-ol Isomer 3a'. A solution of diisobutylaluminum hydride (2.20 mL, 1.0 M in toluene) was added dropwise to a stirred solution of 2a (626 mg, 1.47 mmol) in dry CH₂Cl₂ (5.0 mL) at 0 °C under argon, and the resultant solution was stirred at 0 °C for 1 h. After addition of saturated aqueous Rochelle's salt (10 mL), the mixture was stirred vigorously at ambient temperature for 1.5 h. Subsequent extractive workup with EtOAc (40 mL) and flash chromatography on silica (gradient 2-10% ethyl acetate/toluene) yielded 3a (295 mg, 47%) and 3a' (302 mg, 48%) as white solids. 3a' was recrystallized from Et₂O/MeOH: mp 135.0–137.0 °C; IR (KBr) 3455 (br), 2931 cm⁻¹; ¹H NMR (CDCl₃) δ 0.55–1.90 (m, 30H), 0.66 (s, 3H, CH₃), 0.84 (s, 3H, CH₃), 0.87 (d, 6H, J = 6.6Hz, CH₃), 0.90 (d, 3H, J = 6.5 Hz, CH₃), 1.90–2.00 (m, 1H), 2.15-2.27 (m, 1H), 2.40-2.55 (m, 1H), 3.32-3.45 (m, 1H), 5.00-5.18 (m, 2H, =CH₂), 5.78-5.94 (m, 1H, -CH=); FDMS m/e 429 (M⁺ + 1), 428 (M⁺). Anal. (C₃₀H₅₂O) C, H.

(3a,4a,5a)-4-(2-Bromo-2-propenyl)cholestan-3-ol (3j) and (3a,4a,5a)-4-(2-Propynyl)cholestan-3-ol (4). K-Selectride (4.87 mL, 1 M in THF) was added to a stirred solution of 2j (2.05 g, 4.06 mmol) in dry THF (30 mL) at $-78 \text{ }^{\circ}\text{C}$ under argon. Upon completion of addition, the cold bath was removed and the resultant yellowish solution was stirred for 2 h. The reaction mixture was then cooled to 0 °C before it was sequentially treated with methanol (1.5 mL), 5 N NaOH (3.25 mL, 16.2 mmol), and 30% H_2O_2 (1.49 mL, 14.6 mmol). The cold bath was removed and the mixture was stirred vigorously for 2 h. After usual extractive workup and flash chromatography on silica (gradient 0-2% ethyl acetate/toluene), 3j (793 mg, 39%) and 4 (910 mg, 53%) were obtained as white solids, which were recrystallized from Et₂O/MeOH. 3j: mp 155.0-157.0 °C; IR (KBr) 3601, 3478 (br), 2931 cm⁻¹; ¹H NMR (CDCl₃) δ 0.67 (s, 3H, CH₃), 0.70-1.90 (m, 30H), 0.87 (s, 3H, CH₃), 0.88 (d, 6H, J = 6.8 Hz, CH₃), 0.92 (d, 3H, J = 6.5 Hz, CH₃), 1.99 (br d, 1H, J = 12.3 Hz), 2.40 (dd, 1H, J = 14.0 and 10.5 Hz), 2.55 (dd, 1H, J = 14.0 and 4.5 Hz), 3.93 (br s, 1H), 5.48 (s, 1H, =CH-), 5.68 (s, 1H, =CH-); FDMS m/e 506 (M+, ⁷⁹Br), 508 (M⁺, ⁸¹Br). Anal. (C₃₀H₅₁BrO) C, H, Br. 4: mp 146.0–148.0 °C; IR (KBr) 3539, 3276 (br), 2925, 2105 cm $^{-1}$ ¹H NMR (CDCl₃) δ 0.65–1.90 (m, 30H), 0.66 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.87 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J =6.6 Hz, CH₃), 1.98 (br d, 1H, J = 12.2 Hz), 2.04 (t, 1H, J = 2.5Hz, acetylenic proton), 2.21 (br dd, 1H, J = 16.8 and 9.3 Hz), 2.37 (br d, 1H, J = 16.8 Hz), 4.20 (br s, 1H); FDMS m/e 426 (M^+) . Anal. $(C_{30}H_{50}O) C, H$.

(3a,4a,5a)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]cholestane-4-acetaldehyde (5). A mixture of 3a (3.00 g, 7.00 mmol), tert-butyldimethylsilyl chloride (1.30 g, 8.50 mmol), and imidazole (0.578 g, 8.50 mmol) in dry DMF (20 mL) was stirred at ambient temperature under argon for 10 h. The reaction mixture was then poured into water (75 mL). Subsequent extractive workup with diethyl ether $(70 \text{ mL} \times 2)$ and flash chromatography on silica gel (gradient 0-4% ethyl acetate/hexane) provided silvlated product (3.34 g, 89%) as a white solid. Ozone was bubbled through a stirred solution of the silvlated product (20.0 g, 36.6 mmol) in CH₂Cl₂ (400 mL) at -78 °C until the solution became blue, then it was stirred for an additional 30 min. Argon was bubbled through the solution until the color dissipated before it was treated with triphenylphosphine (24.0 g, 92.2 mmol). The mixture was stirred overnight at ambient temperature. After concentration, the crude product was purified by preparative HPLC (5% ethyl acetate/hexane) to yield 5 (15.5 g, 78%) as a white solid, which was recrystallized from Et₂O/MeOH: mp 86.0-88.0 °C; IR (KBr) 2948, 1728 cm⁻¹; ¹H NMR (CDCl₃) δ 0.00 (s, 3H, CH₃), 0.03 (s, 3H, CH₃), 0.64 (s, 3H, CH₃), 0.65-1.88 (m, 28H), 0.83 $(s, 3H, CH_3), 0.86 (d, 6H, J = 6.6 Hz, CH_3), 0.90 (s, 9H, CH_3),$ $0.91 (d, 3H, J = 6.6 Hz, CH_3), 1.92-2.10 (m, 2H), 2.27-2.47$ (m, 2H), 3.82 (br s, 1H), 9.80 (br s, 1H, CHO); FDMS m/e 516 $(M^+ - CO)$. Anal. $(C_{35}H_{64}O_2Si) C, H$.

 $[3\alpha,4\alpha(Z),5\alpha]$ -4-(2-Butenyl)cholestan-3-ol (6). A solution of KN(TMS)₂ (1.98 mL, 0.5 M in toluene) was added dropwise to a stirred suspension of ethyltriphenylphosphonium bromide (409 mg, 1.10 mmol) in dry THF (4 mL) at -15 °C under argon, and the resultant orange solution was stirred for 15 min before it was treated dropwise with a solution of 5 (300 mg, 0.550 mmol) in dry THF (4 mL). The reaction mixture was then stirred at 0 °C for 3 h. After sequential treatment with acetone (1 mL) and hexane (10 mL), the mixture was stirred at ambient temperature for 15 min before it was filtered through a short pad of silica (10% ethyl acetate/hexane). The filtrate was concentrated and the oily residue was subject to flash chromatography on silica (gradient 0-2% ethyl acetate/hexane) to provide a cis-olefinic product (305 mg, 99%). Subsequently the cis-olefinic product was dissolved in dry THF (3 mL) and treated with tetrabutylammonium fluoride (4.0 mL, 1 M in THF), and the stirred mixture was heated to reflux under argon for 36 h. At ambient temperature, the mixture was diluted with Et₂O (30 mL), washed with half-saturated aqueous NaCl (10 mL \times 2), dried over MgSO₄, filtered, and concentrated. The oily residue was subject to flash chromatography on silica (gradient 0-4% ethyl acetate/hexane) to yield 6 (227 mg, 93%) as a white solid, which was recrystallized from Et₂O/MeOH: mp 99.0–101.0 °C; IR (KBr) 3486 (br), 2937 cm⁻¹; ¹H NMR (CDCl₃) δ 0.65–1.90 (m, 30H), 0.66 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.87 (d, 3H, J = 6.6 Hz, CH₃), 0.88 (d, 3H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.6 Hz, CH₃), 1.66 (d, 3H, J = 5.7 Hz, CH₃), 1.90–2.10 (m, 2H), 2.13–2.25 (m, 1H), 3.90 (br s, 1H), 5.40–5.60 (m, 2H, –CH=CH–); FDMS *m/e* 442 (M⁺). Anal. (C₃₁H₅₄O) C, H.

(E)-4-[$(3\alpha, 4\alpha, 5\alpha)$ -3-[[(1, 1-Dimethylethyl)dimethylsilyl]oxy]cholestan-4-yl]-2-butenoic Acid Methyl Ester (7). A solution of KN(TMS)₂ (2.22 mL, 0.5 M in toluene) was added dropwise to a stirred solution of methyl (dimethylphosphono)acetate (0.180 mL, 1.11 mmol) in dry THF (3 mL) at -15°C under argon, and the resultant solution was stirred for 15 min before it was treated dropwise with a solution of 5 (405 mg, 0.743 mmol) in dry THF (3 mL). The reaction mixture was then stirred at -15 °C for 1.5 h. After treatment with acetone (1 mL) and subsequent extractive workup with Et₂O (30 mL), the crude product was subject to flash chromatography on silica (gradient 0-3% ethyl acetate/hexane) to afford 7(368 mg, 82%) as a white solid, which was recrystallized from Et₂O/MeOH: mp 158.0-158.5 °C; IR (KBr) 2933, 1728 cm⁻¹; ¹H NMR (CDCl₃) δ 0.03 (s, 3H, CH₃), 0.05 (s, 3H, CH₃), 0.64- $1.90 (m, 29H), 0.65 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.87 (d,$ 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.5 Hz, CH₃), 0.92 (s, 9H, CH₃), 1.97 (br d, 1H, J = 11.9 Hz), 2.03–2.18 (m, 1H), 2.21-2.33 (m, 1H), 3.73 (s, 3H, OCH₃), 3.82 (br s, 1H), 5.83 (d, 1H, J = 15.6 Hz, -CH=), 6.94-7.06 (m, 1H, -CH=); FDMSm/e 602 (M⁺ + 1). Anal. (C₃₈H₆₈O₃Si) C, H.

 $[3\alpha,4\alpha(E),5\alpha]$ -4-(4-Hydroxy-2-butenyl)cholestan-3-ol (8). A solution of diisobutvlaluminum hydride (0.798 mL, 1.0 M in toluene) was added dropwise to a stirred solution of 7 (160 mg, 0.266 mmol) in dry CH₂Cl₂ (4 mL) at 0 °C under argon, and the resultant solution was stirred at 0 °C for 1 h. After addition of saturated aqueous Rochelle's salt (5 mL), the mixture was stirred vigorously at ambient temperature for 1.5 h. After usual extractive workup with EtOAc (30 mL), the crude alcohol (162 mg) was dissolved in dry THF (1 mL) and treated with tetrabutylammonium fluoride (3.0 mL, 1 M in THF), and the stirred mixture was heated to reflux under argon for 48 h. At ambient temperature, the mixture was diluted with Et_2O (30 mL), washed with half-saturated aqueous NaCl (10 mL \times 2), dried over MgSO₄, filtered, and concentrated. The residue was subject to flash chromatography on silica (gradient 40-60% ethyl acetate/hexane) to yield $8\,(117\text{ mg},96\%)$ as a white solid, which was recrystallized from $Et_2O/MeOH:\ mp\ 178.0-180.0\ ^\circ\text{C};\ IR\ (KBr)\ 3421\ (br),\ 2932$ cm⁻¹; ¹H NMR (CDCl₃) δ 0.65-1.90 (m, 31H), 0.66 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.87 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.5 Hz, CH₃), 1.90–2.04 (m, 2H), 2.20–2.30 (m, 1H), 3.90 $(br s, 1H), 4.12 (d, 2H, J = 3.9 Hz, CH_2O), 5.65-5.83 (m, 2H)$ -CH=CH-); FDMS m/e 458 (M⁺). Anal. (C₃₁H₅₄O₂) C, H.

3-(1-Pyrrolidinyl)cholesta-3,5-diene (13). A stirred solution of pyrrolidine (33.0 mL, 395 mmol) and 4-cholesten-3-one (30.4 g, 79.0 mmol) in dry benzene (120 mL) was heated to reflux under argon with continuous removal of water for 24 h. After concentration, **13** (34.9 g, 100%) was obtained as a yellowish solid, which was recrystallized from anhydrous benzene/hexane: mp 146.0-148.5 °C; IR (KBr) 2930, 1610 cm⁻¹; ¹H NMR (C₆D₆) δ 0.71 (s, 3H, CH₃), 0.89 (d, 6H, J = 6.6 Hz, CH₃), 0.98-1.90 (m, 26H), 1.00 (d, 3H, J = 6.5 Hz, CH₃), 1.15 (s, 3H, CH₃), 2.00-2.35 (m, 4H), 2.85-2.93 (m, 4H, -NCH₂-), 5.04 (s, 1H, -CH=), 5.34 (br s, 1H, -CH=); FDMS m/e 437 (M⁺). Anal. (C₃₁H₅₁N) C, H, N.

 $(3\alpha,4\alpha)$ -4-(2-Propenyl)cholest-5-en-3-ol (15). A stirred solution of allyl bromide (9.55 mL, 110 mmol) and 13 (19.3 g, 44.1 mmol) in dry THF (200 mL) was heated in an oil bath at 55–60 °C under argon for 4.5 h. The oil bath temperature was then cooled to 50 °C, at which point water was added to the reaction solution and the resultant mixture was stirred at 50 °C for 1 h. At ambient temperature the mixture was diluted with Et₂O (100 mL) and washed with saturated aqueous NaCl (100 mL), and the aqueous layer was extracted with THF (100 mL)/Et₂O (50 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over MgSO₄, filtered, and concentrated *in vacuo* at 30 °C to give a crude reddish oil of 14 (19.5 g). Then a solution

of K-selectride (66.2 mL, 1 M in THF) was added to a stirred solution of crude 14 in dry THF (150 mL) at -78 °C under argon. Upon completion of the addition, the cold bath was removed and the reaction solution was stirred for 2 h. The reaction solution was then cooled to 0 °C before it was treated sequentially and cautiously with 5 N NaOH (59.6 mL) and 30% H₂O₂ (30.4 mL). The cold bath was removed and the mixture was stirred vigorously for 2 h. Solid NH₄Cl (19.1 g) was added to the mixture before it was extracted with THF/ Et_2O (ratio 1:1, 150 mL \times 2). The combined organic layers were washed with saturated aqueous NaCl (100 mL \times 2), dried over MgSO₄, filtered, and concentrated. After two sequential flash chromatographic separations on silica (first, 0-2% ethyl acetate/toluene; second, 50-100% CH₂Cl₂/hexane), 15 (3.33 g, 18%) was obtained as a white solid, which was recrystallized from Et₂O/CH₃CN: mp 101.0-103.0 °C; IR (KBr) 3440 (br), 2937 cm⁻¹; ¹H NMR (CDCl₃) δ 0.70 (s, 3H, CH₃), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.93 (d, 3H, J = 6.5 Hz, CH₃), 0.95-1.92 (m, 25H), 1.06 (s, 3H, CH₃), 2.00-2.46 (m, 5H), 3.90-3.94 (m, 1H), 5.03-5.16 (m, 2H, =CH₂), 5.44 (br d, 1H, J = 5.5 Hz, -CH=), 5.77-5.93 (m, 1H, -CH=); FDMS m/e 426 (M⁺). Anal. (C₃₀H₅₀O) C, H.

The following synthesis of **16** represents a general procedure for the synthesis of related analogs.

(3a,4a,5a)-4-Propylcholestan-3-ol (16). A mixture of 3a (2.00 g, 4.67 mmol) and PtO₂ (200 mg) in EtOAc (50 mL) was stirred under hydrogen for 8 h. After filtration and concentration, the residue was passed through a short pad of silica (10% ethyl acetate/hexane) to provide 16 (1.54 g, 77%) as a white solid, which was recrystallized from Et₂O/MeOH: mp 120.0–123.0 °C; IR (KBr) 3458 (br), 2929 cm⁻¹; ¹H NMR (CDCl₃) δ 0.60–1.90 (m, 34H), 0.64 (s, 3H, CH₃), 0.80 (s, 3H, CH₃), 0.86 (d, 6H, J = 6.6 Hz, CH₃), 0.89 (d, 3H, J = 6.2 Hz, CH₃), 0.90 (t, 3H, J = 6.5 Hz, CH₃), 1.90–2.00 (m, 1H), 3.91 (br s, 1H); FDMS *m/e* 430 (M⁺). Anal. (C₃₀H₅₄O) C, H.

(3α,4α,5α)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]cholestane-4-ethanol (18). The procedure used for the preparation of 3a and 3a' was repeated with 5 (5.00 g, 9.18 mmol) and Dibal (13.8 mL, 1.0 M in toluene). After extractive workup and flash chromatography on silica (gradient 10-20% ethyl acetate/hexane), 18 (4.92 g, 98%) was obtained as a white solid, which was recrystallized from CH₂Cl₂/CH₃CN: mp 109.0-111.0 °C; IR (KBr) 3357 (br), 2931 cm⁻¹; ¹H NMR (CDCl₃) δ 0.03 (s, 3H, CH₃), 0.05 (s, 3H, CH₃), 0.64 (s, 3H, CH₃), 0.65-1.90 (m, 32H), 0.79 (s, 3H, CH₃), 0.86 (d, 6H, J = 6.6 Hz, CH₃), 0.89 (s, 9H, CH₃), 0.90 (d, 3H, J = 6.2 Hz, CH₃), 1.93-1.98 (m, 1H), 3.57-3.70 (m, 2H, -CH₂O-), 3.85 (s, 1H); FDMS m/e 547 (M⁺ + 1). Anal. (C₃₅H₆₆O₂Si) C, H.

(3a,4a,5a)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4ethenylcholestane (19). 2-Nitrophenyl selenocyanate (627 mg, 2.76 mmol) and tributylphosphine (0.688 mL, 2.76 mmol) were added to a stirred solution of 18 (500 mg, 0.920 mmol) in dry THF (12 mL) at 0 °C under argon. Upon completion of addition, the cold bath was removed and the reaction mixture was stirred for 2 h. The mixture was then diluted with EtOAc (36 mL) before it was filtered through a short pad of Celite. The filtrate was washed with saturated aqueous NaCl (20 mL), dried over MgSO₄, filtered, and concentrated. The residue was subject to flash chromatography on silica (gradient 5-10%ethyl acetate/hexane) to yield a yellow foam (668 mg, quantitative), the desired selenide. To a stirred suspension of the yellow selenide (638 mg, 0.870 mmol) in dry \dot{CH}_2Cl_2 (10 mL) at 0 °C was added CaCO₃ (522 mg, 5.22 mmol) and MCPBA (390 mg, 0.870 mmol). The resultant mixture was then stirred at ambient temperature for 1 h. After dilution with CH₂Cl₂ (40 mL) and subsequent filtration through a short pad of Celite, the filtrate was washed with saturated aqueous NaCl (20 mL), dried over MgSO₄, filtered, and concentrated. The residue was subject to flash chromatography on silica (hexane) to provide the desired 4 α -vinyl compound 19 (460 mg, 99%) as a white solid, which was recrystallized from CH_2Cl_2/CH_3 -CN: mp 87.0-90.0 °C; IR (KBr) 2935, 2867 cm⁻¹; ¹H NMR $(CDCl_3) \delta -0.01$ (s, 3H, CH₃), 0.00 (s, 3H, CH₃), 0.64 (s, 3H, CH_3), 0.65–1.70 (m, 27H), 0.80 (s, 3H, CH_3), 0.86 (d, 6H, J =6.6 Hz, CH₃), 0.89 (d, 3H, J = 6.2 Hz, CH₃), 0.90 (s, 9H, CH₃), $1.72-2.00 \ (m, 3H), 3.77 \ (br s, 1H), 4.86-4.96 \ (m, 2H, =CH_2), 5.62-5.78 \ (m, 1H, -CH=); FDMS \ m/e \ 529 \ (M^+ + 1), 471 \ (M^+ - C_4H_9).$

(3α,4α,5α)-4-Ethenylcholestan-3-ol (20). Following the previous procedure of forming 8, compound 19 (250 mg, 0.470 mmol) was desilylated with tetrabutylammonium fluoride (1.88 mL, 1 M in THF) to provide 20 (175 mg, 89%) as a white solid, which was recrystallized from Et₂O/CH₃CN: mp 137.0–138.0 °C; IR (KBr) 3448 (br), 2937 cm⁻¹; ¹H NMR (CDCl₃) δ 0.64 (s, 3H, CH₃), 0.70–1.90 (m, 29H), 0.82 (s, 3H, CH₃), 0.86 (d, 6H, J = 6.6 Hz, CH₃), 0.90 (d, 3H, J = 6.6 Hz, CH₃), 1.92–2.11 (m, 2H), 3.82 (br s, 1H), 5.00–5.13 (m, 2H, =CH₂), 5.73–5.87 (m, 1H, -CH=); FDMS *m/e* 415 (M⁺ + 1). Anal. (C₂₈H₅₀O) C, H.

(2a)-2-(2-Propenyl)cholest-4-en-3-one (21). A solution of 4-cholesten-3-one (500 mg, 1.30 mmol) in dry THF (10 mL) was added dropwise to a stirred solution of NaN(TMS)2 (1.69 mL, 1 M in THF) in dry THF (10 mL) at -78 °C under argon, and the resultant golden solution was stirred for 45 min before it was treated with allyl iodide (0.238 mL, 2.60 mmol). Then the reaction solution was stirred at -78 °C for 6 h. After treatment with acetic acid (0.5 mL), the solution was allowed to warm to ambient temperature, where it was diluted with EtOAc (30 mL) and washed with saturated aqueous NaCl (10 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated. The oily residue was subjected to flash chromatography on silica (gradient 2–10% ethyl acetate/ hexane) to provide 21 (422 mg, 76%) as a colorless oil: IR $(CHCl_3)$ 2933, 1673 cm⁻¹; ¹H NMR $(CDCl_3) \delta 0.72$ (s, 3H, CH₃), 0.75-1.70 (m, 20H), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.92 (d, 3H, J = 6.5 Hz, CH₃), 1.20 (s, 3H, CH₃), 1.78–1.91 (m, 2H), $1.98-2.14 \ (m,\ 3H),\ 2.20-2.48 \ (m,\ 3H),\ 2.68-2.77 \ (m,\ 1H),$ 5.02-5.10 (m, 2H, =CH₂), 5.72 (d, 1H, J = 1.0 Hz, -CH=), 5.75-5.88 (m, 1H, -CH=); FDMS m/e 424 (M+). Anal. (C30H48O) C, H.

(2a,5a)-2-(2-Propenyl)cholestan-3-one (22). A lithium chip (24.6 mg, 3.54 mmol) and a glass-coated stir bar were placed in a flame-dried, three-necked, round-bottomed flask fitted with a dry ice condenser under argon. Liquid ammonia (30 mL) was collected in the flask at -78 °C to form a deep blue solution, followed by the addition of dry THF (15 mL). A solution of 21 (430 mg, 1.01 mmol) and t-BuOH (95.2 mL, 1.01 mmol) in dry THF (15 mL) was added dropwise to the deep blue solution. Upon completion of addition, the resultant blue solution was stirred for 5 min before it was treated with 1,3pentadiene (0.5 mL) to quench the excess of lithium. Saturated aqueous NH₄Cl (10 mL) was carefully added to the white suspension, then cold bath was removed and the solution was allowed to warm to ambient temperature. After extraction with EtOAc (25 mL \times 2), the combined organic layers were washed with saturated aqueous NaCl (10 mL), dried over MgSO₄, filtered, and concentrated. After flash chromatography on silica (gradient 50-100% toluene/hexane), 22 (407 mg, 94%) was obtained as a colorless oil: IR (neat) 2929, 1711 cm⁻ ¹H NMR (CDCl₃) δ 0.69 (s, 3H, CH₃), 0.70-2.15 (m, 29H), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.5 Hz, CH₃), 1.06 (s, 3H, CH₃), 2.29–2.50 (m, 2H), 2.52–2.62 (m, 1H), 4.99–5.06 (m, 2H, =CH₂), 5.71-5.86 (m, 1H, -CH=); FDMS m/e 427 (M⁺ + 1). Anal. (C₃₀H₅₀O) C, H.

 $(2\alpha, 3\alpha, 5\alpha)$ -2-(2-Propenyl)cholestan-3-ol (23) and Its 3 β ol Isomer (23'). The procedure used for the preparation of 3a and 3a' was repeated with 22 (150 mg, 0.352 mmol) and Dibal (0.527 mL, 1 M in toluene) in CH₂Cl₂ (3 mL). After usual extractive workup with EtOAc (40 mL) and subsequent flash chromatographic separation on silica (gradient 8-20% ethyl acetate/hexane), 23 (66.5 mg, 44%) and 23' (83.9 mg, 56%) were obtained as white solids, which were recrystallized respectively from Et₂O/CH₃CN. 23: mp 72.0-74.0 °C; IR (KBr) 3376 (br), 2931 cm⁻¹; ¹H NMR (CDCl₃) δ 0.65–1.70 (m, 29H), 0.66 (s, 3H, CH₃), 0.80 (s, 3H, CH₃), 0.88 (d, 6H, J = 6.6 Hz, CH₃), $0.91 (d, 3H, J = 6.5 Hz, CH_3), 1.75-1.90 (m, 1H), 1.95-2.06$ (m, 2H), 2.09-2.21 (m, 1H), 3.89 (br s, 1H), 5.00-5.12 (m, 2H, =CH₂), 5.76–5.91 (m, 1H, –CH=); FDMS m/e 429 (M⁺ + 1). Anal. (C₃₀H₅₂O) C, H. 23': mp 102.0-103.0 °C; IR (KBr) 3365 (br), 2931 cm⁻¹; ¹H NMR (CDCl₃) δ 0.56-1.90 (m, 30H), 0.66 $(s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.6 Hz, CH_3),$

0.91 (d, 3H, J = 6.6 Hz, CH₃), 1.91–2.04 (m, 2H), 2.37–2.45 (m, 1H), 3.25–3.36 (m, 1H), 5.00–5.12 (m, 2H, =CH₂), 5.80–5.96 (m, 1H, -CH=); FDMS *m/e* 429 (M⁺ + 1). Anal. (C₃₀H₅₂O) C, H.

(2α,4α,5α)-2,4-Bis(2-propenyl)cholestan-3-one (24). The procedure used for the preparation of 2a was repeated with 21 (810 mg, 1.91 mmol). After the usual extractive workup with EtOAc and subsequent flash chromatographic separation on silica (gradient 2–10% ethyl acetate/hexane), 24 (392 mg, 44%) was obtained as a colorless oil: IR (film) 2930, 1709 cm⁻¹; ¹H NMR (CDCl₃) δ 0.65–1.65 (m, 22H), 0.68 (s, 3H, CH₃), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.6 Hz, CH₃), 1.12 (s, 3H, CH₃), 1.66–2.05 (m, 5H), 2.12 (dd, 1H, J = 13.0 and 5.6 Hz), 2.22–2.62 (m, 5H), 4.95–5.06 (m, 4H, =CH₂), 5.73–5.91 (m, 2H, -CH=); FDMS *m/e* 466 (M⁺). Anal. (C₃₃H₅₄O) C, H.

(2α,3α,4α,5α)-2,4-Bis(2-propenyl)cholestan-3-ol (25). The procedure used for the preparation of 3a was repeated with 24 (200 mg, 0.429 mmol) and K-selectride (0.857 mL, 1 M in THF). After the usual extractive workup with EtOAc and subsequent flash chromatographic separation on silica (gradient 20-100% toluene/hexane), 25 (92.0 mg, 46%) was obtained as a white solid: mp 101.0-103.0 °C; IR (KBr) 3631, 3440 (br), 2929 cm⁻¹; ¹H NMR (CDCl₃) δ 0.65-1.90 (m, 29H), 0.66 (s, 3H, CH₃), 0.83 (s, 3H, CH₃), 0.88 (d, 6H, J = 6.7 Hz, CH₃), 0.91 (d, 3H, J = 6.6 Hz, CH₃), 1.93-2.10 (m, 3H), 2.10-2.35 (m, 2H), 3.76 (t, 1H, J = 1.9 Hz), 4.98-5.14 (m, 4H, =CH₂), 5.75-5.95 (m, 2H, -CH=); FDMS *m/e* 468 (M⁺). Anal. (C₃₃H₅₆O) C, H.

(66)-6-(2-Propenvl)cholest-4-en-3-one (27), Allylmagnesium bromide (30.0 mL, 1.0 M in Et₂O) was added dropwise to a stirred solution of 5α , 6α -epoxycholestan- 3β -ol **26** (1.21 g, 3.00 mmol) in dry Et₂O (30 mL) at ambient temperature under argon, and the resultant mixture was stirred for 3 h. Methanol (6 mL) and 1 N HCl (14 mL) were sequentially and cautiously added to the reaction mixture. After extraction with EtOAc (30 mL), the organic layer was washed with saturated aqueous NaHCO₃ (15 mL) and NaCl (30 mL), dried over MgSO₄, filtered, and concentrated. Subsequent flash chromatography on silica (gradient 40-60% ethyl acetate/hexane) provided the desired allylated diol (1.34 g, 100%) as a white solid. A stirred mixture of allylated diol (1.34 g, 3.00 mmol), cyclohexanone (6.2 mL, 60 mmol), and aluminum isopropoxide (575 mg, 2.81 mmol) in dry toluene (20 mL) was heated to reflux under argon with continuous removal of water for 5 h. After being cooled to ambient temperature, the mixture was diluted with Et₂O (10 mL) and cautiously treated with 2.5 N HCl (10 mL). The resultant mixture was then stirred vigorously for 3 h. After the usual extractive workup and flash chromatography on silica (gradient 3-9% ethyl acetate/hexane), 27 (1.00 g, 78%) was obtained as a white solid, which contained less than 5% inseparable 6α-allyl isomer: mp 58.0–63.0 °C; IR (KBr) 2950, 1675 cm⁻¹; ¹H NMR (CDCl₃) δ 0.65–1.95 (m, 22H), 0.74 (s, 3H, CH₃), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.93 (d, 3H, J = 6.5Hz, CH₃), 1.25 (s, 3H, CH₃), 1.98-2.08 (m, 2H), 2.20-2.56 (m, 5H), $4.95-5.05 (m, 2H, =CH_2)$, 5.60-5.82 (m, 1H, -CH=), 5.72(s, 1H, -CH=); FDMS m/e 424 (M⁺). Anal. (C₃₀H₄₈O) C, H.

Mixture of 28 and 29. A solution of 27 (593 mg, 1.40 mmol) and 2% KOH/MeOH (20 mL) in THF (10 mL) was stirred vigorously at ambient temperature for 48 h. At 0 °C, 5 N HCl (1.5 mL) and saturated aqueous NaCl (10 mL) were added to the mixture before it was extracted with EtOAc (50 mL). The organic layer was washed with saturated aqueous NaCl (10 mL), dried over MgSO₄, filtered, and concentrated to give an inseparable mixture of 27 and its 6α -isomer which was subject to subsequent reduction following the procedure as described above for the preparation of 22. A mixture of 28 and 29 (372 mg, 62%, ratio 10:1) was obtained as a white solid after flash chromatography on silica (gradient 3-8% ethyl acetate/hexane): mp 80.0-82.0 °C; IR (KBr) 2949, 1711 cm⁻¹; ¹H NMR (CDCl₃) $\delta \ \bar{0.60}$ -1.90 (m, 25H), 0.69 (s, 3H, CH₃), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.5 Hz, CH₃), 1.03 (s, 3H, CH₃), 1.92-2.20 (m, 4H), 2.22-2.57 (m, 3H), 4.95-5.06 (m, 2H, =CH₂), 5.65-5.80 (m, 1H, -CH=); FDMS m/e 426 (M^+) . Anal. $(C_{30}H_{50}O) C, H$.

(3a,5a,6a)-6-(2-Propenyl)cholestan-3-ol (30) and Its 66-Allyl Isomer (31). The procedure used for the preparation of 3a was repeated with the mixture of 28 and 29 (267 mg, 0.626 mmol, ratio 10:1) and K-selectride (0.939 mL, 1 M in THF). After flash chromatography on silica (gradient 3-14% ethyl acetate/hexane), $\mathbf{30}~(227~\text{mg},\,85\%)$ and $\mathbf{31}~(21.4~\text{mg},\,8\%)$ were obtained as an oil and white solid, respectively; 31 was recrystallized from Et₂O/MeOH. 30: IR (neat) 3200 (br), 2910 cm⁻¹; ¹H NMR (CDCl₃) δ 0.60–1.90 (m, 31H), 0.66 (s, 3H, CH₃), $0.80 (s, 3H, CH_3), 0.87 (d, 6H, J = 6.7 Hz, CH_3), 0.91 (d, 3H, CH_3)$ J = 6.6 Hz, CH₃), 1.98 (br d, 1H, J = 11.9 Hz), 2.10–2.22 (m, 1H), 4.08 (br s, 1H), 4.95-5.02 (m, 2H, =CH₂), 5.70-5.85 (m, 1H, -CH=); FDMS m/e 428 (M⁺). Anal. (C₃₀H₅₂O) C, H. 31: mp 101.0-103.0 °C; IR (KBr) 3347 (br), 2935 cm⁻¹; ¹H NMR $(CDCl_3) \delta 0.65-2.04 \text{ (m, 32H)}, 0.69 \text{ (s, 3H, CH}_3), 0.86 \text{ (s, 3H, }$ CH₃), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.92 (d, 3H, J = 6.5 Hz, CH₃), 2.07-2.16 (m, 1H), 4.14 (br s, 1H), 4.92-5.00 (m, 2H, =CH₂), 5.60-5.75 (m, 1H, -CH=); FDMS *m/e* 428 (M⁺). Anal. $(C_{30}H_{52}O) C, H.$

 $(4\beta,5\beta)$ -4-(2-Propenyl)cholestan-3-one (33). The procedure used for the preparation of 21 was repeated with 5β -cholestan-3-one 32 (431 mg, 1.11 mmol). After flash chromatography on silica gel (gradient 50-100% toluene/ hexane), 33 (345 mg, 77%) was obtained as a white solid, which was recrystallized from Et₂O/CH₃CN: mp 59.0-60.0 °C; IR (KBr) 2952, 1712 cm⁻¹; ¹H NMR (CDCl₃) δ 0.69 (s, 3H, CH₃), 0.80-1.92 (m, 25H), 0.87 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, $3H, J = 6.5 Hz, CH_3$, 1.01 (s, $3H, CH_3$), 1.98–2.08 (m, 2H), 2.15-2.50 (m, 4H), 2.65-2.75 (m, 1H), 4.95-5.06 (m, 2H, =CH₂), 5.72-5.87 (m, 1H, -CH=); FDMS m/e 426 (M⁺). Anal. $(C_{30}H_{50}O) C, H.$

 $(3\alpha, 4\beta, 5\beta)$ -4-(2-Propenyl)cholestan-3-ol (34) and Its 3 β ol Isomer (34'). NaBH₄ (13.2 mg, 0.349 mmol) was added to a stirred solution of $\boldsymbol{33}$ (149 mg, 0.349 mmol) in dry Et_2O (5 mL) at 0 °C, followed by the addition of methanol (1 ml). The resultant mixture was stirred at ambient temperature for 1 h before it was treated with 5 N NaOH (0.558 mL) and stirred vigorously for 1 h. After the usual extractive workup and subsequent flash chromatography on silica (gradient 2-8%ethyl acetate/hexane), 34 (62.0 mg, 41%) and 34' (16.8 mg, 11%), in addition to some unhydrolyzed borates, were obtained as an oil and white solid, respectively. 34: IR (neat) 3280 (br), 2920 cm^-1; ¹H NMR (CDCl₃) δ 0.66 (s, 3H, CH₃), 0.80–2.03 (m, 31H), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.5Hz, CH₃), 0.94 (s, 3H, CH₃), 2.15-2.25 (m, 1H), 2.45-2.55 (m, 1H), 3.43 (td, 1H, J = 10.5 and 4.6 Hz), 5.02-5.16 (m, 2H, =CH₂), 5.80-5.95 (m, 1H, -CH=); FDMS m/e 428 (M⁺). Anal. (C₃₀H₅₂O) C, H. 34': mp 63.0-65.0 °C; IR (KBr) 3394 (br), 2929 cm⁻¹; ¹H NMR (CDCl₃) δ 0.67 (s, 3H, CH₃), 0.80–2.06 (m, 32H), 0.88 (d, 6H, J = 6.6 Hz, CH₃), 0.91 (d, 3H, J = 6.5Hz, CH₃), 0.98 (s, 3H, CH₃), 2.25-2.35 (m, 1H), 3.98 (br s, 1H), 5.02-5.18 (m, 2H, =CH₂), 5.84-6.00 (m, 1H, -CH=); FDMS m/e 428 (M⁺). Anal. (C₃₀H₅₂O) C, H.

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References

- (1) (a) Grundy, S. M.; Vega, G. L. Causes of High Blood Cholesterol. Circulation 1990, 81, 412-427. (b) Goldstein, J. L.; Brown, M. S. Progress in Understanding the LDL Receptor and HMG-CoA Reductase, Two Membrane Proteins that Regulate the Plasma Cholesterol. J. Lipid Res. 1984, 25, 1450-1461.
- (2) (a) Steinberg, D. Lipoproteins and Atherosclerosis: A Look Back and a Look Ahead. Arteriosclerosis 1983, 3, 283-301. (b) Steinberg, D. The Cholesterol Controversy Is Over. Why Did It Take So Long? Circulation 1989, 80, 1070-1078. (c) Steinberg,

D.; Wiztum, J. L. Lipoproteins and Atherosclerosis: Current Concepts. JAMA 1990, 264, 3047-3052.

- (3)Tyroler, H. A. Lowering Plasma Cholesterol Levels Decrease Risk of Coronary Heart Disease: An Overview of Clinical Trials. In Hypercholesterolemia and Atherosclerosis; Steinberg, D., Olefsky, J. M., Eds.; Churchill Livingstone: New York, 1987; pp 99-116. (4) Goldstein, J. L.; Brown, M. S. The Low-Density Lipoprotein
- Pathway and its Relation to Atherosclerosis. Annu. Rev. Biochem. **1977**, 46, 897–903.
- Grundy, S. M. Cholesterol and Coronary Heart Disease: Future (5)Directions. JAMA 1990, 264, 3053-3059.
- (a) Larsen, S.; Spilman, C. H. New Potential Therapies for the Treatment of Atherosclerosis. Annu. Rep. Med. Chem. 1993, 28, 217-226. (b) McCarty, P. A. New Approaches to Atherosclerosis: An Overview. Med. Res. Rev. 1993, 13, 139-159. (c) Brown, M. S.; Goldstein, J. L. Drugs Used in the Treatment of Hyperlipoproteinemias. In *The Pharmacological Basis of Therapeutics*; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 874-896.
 (7) Goldstein, J. L.; Kita, T.; Brown, M. S. Defective Lipoprotein
- Receptors and Atherosclerosis. N. Engl. J. Med. 1983, 309, 288-296.
- Sudhof, T. C.; Russell, D. W.; Brown, M. S.; Goldstein, J. L. 42 (8)bp Element from LDL Receptor Gene Confers End-Product Repression by Sterols when Inserted into Viral TK Promoter. Cell 1987, 48, 1061-1069.
- (9)(a) Dawson, P. A.; Hofmann, S. L.; van der Westhuyzen, D. R.; Sudhof, T. C.; Brown, M. S.; Goldstein, J. L. Sterol-Dependent Repression of Low Density Lipoprotein Receptor Promoter Mediated by 16-Base Pair Sequence Adjacent to Binding Site for Transcription Factor Sp1. J. Biol. Chem. 1988, 263, 3372-3379. (b) Osborne, T. F.; Gil, G.; Goldstein, J. L.; Brown, M. S. Operator Constitutive Mutation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Abolishes Protein Binding to Sterol Regulatory Element. J. Biol. Chem. 1988, 263, 3380-3387.
- (10) Smith, J. R.; Osborne, T. F.; Goldstein, J. L.; Brown, M. S. Identification of Nucleotides Responsible for Enhancer Activity of Sterol Regulatory Element in Low Density Lipoprotein Receptor Gene. J. Biol. Chem. 1990, 265, 2306-2310.
- Brown, M. S.; Goldstein, J. L. A Receptor-Mediated Pathway for Cholesterol Homeostasis. Science 1986, 232, 34-47.
 Myant, N. B. In Cholesterol Metabolism, LDL, and the LDL
- Receptor: Academic Press: San Diego, 1990.
- (13) Kovanen, P. T.; Bilheimer, J. L.; Goldstein, J. L.; Jaramillo, J. J.; Brown, M. S. Regulatory Role for Hepatic Low Density Lipoprotein Receptors in Vivo in the Dog. Proc. Natl. Acad. Sci. U.S.A. 1981, 78, 1194-1198.
- (14) Rudling, M.; Reihner, E.; Einarsson, K.; Ewerth, S.; Angelin, B. Low Density Lipoprotein Receptor-Binding Activity in Human Tissues: Quantitative Importance of Hepatic Receptors and Evidence for Regulation of their Expression in Vivo. Proc. Natl. Acad. Sci. U.S.A. 1990, 87, 3469-3473.
- (15) Reihner, E.; Rudling, M.; Stahlberg, D.; Berglund, L.; Ewerth, S.; Bjorkhem, I.; Einarsson, K.; Angelin, B. Influence of Pravastatin, a Specific Inhibitor of HMG CoA Reductase, on Hepatic Metabolism of Cholesterol. N. Engl. J. Med. 1990, 323, 224-228
- (16) Archer, R. A.; Beavers, L. S.; Gadski, R. A.; Lin, H.-S.; McClure, D. B.; McCowan, J. R.; Pawlak, J. M.; Rampersaud, A. A.; Schmidt, R. J.; Schreyer, T. A.; Yu, M. J. Steroid Derivatives. EP 0,562,849 A2, 1993.
- (17) (a) Brown, M. S.; Goldstein, J. L.; Russell, D. W.; Sudhof, T. C. Sterol Regulatory Elements. U.S. Patent 4,935,363, 1990. (b) Dewet, J. R.; Wood, K. V.; DeLuca, M.; Helinski, D. R.; Subramani, S. Mol. Cell Biol. 1987, 7, 725-737. (c) Pons, M.; Gagne, D.; Nicolas, J. C.; Mehtali, M. BioTechniques 1990, 9, 450–459.
- (18) Chen, L.-S. K. Transcriptional Modulation: A New Approach to Drug Discovery. Med. Chem. Res. 1992, 2, 397-409.
- (19) Goldstein, J. L.; Brown, M. S.; Anderson, R. G. W.; Russell, D. W.; Schneider, W. J. Receptor-Mediated Endocytosis: Concepts Emerging from the LDL Receptor System. Annu. Rev. Cell Biol. 1985, 1, 1-39.
- (20) Goldstein, J. L.; Brown, M. S. Regulation of the Mevalonate Pathway. *Nature* 1990, 343, 425-430.
 (21) Molowa, D. T.; Cimis, G. M. Co-ordinate Regulation of Low-
- Density-Lipoprotein Receptor and 3-Hydroxy-3-methylglutaryl-CoA Reductase and Synthase Gene Expression in HepG2 Cells. Biochem. J. 1989, 260, 731-736.
- (22) (a) Rychnovsky, S. D.; Mickus, D. E. Synthesis of ent-Cholesterol, the Unnatural Enantiomer. J. Org. Chem. 1992, 57, 2732-2736. (b) Stork, G.; McMurry, J. E. Stereospecific Total Synthesis of Steroids via Isoxazole Annelation. dl-D-Homotestosterone and *dl*-Progesterone. J. Am. Chem. Soc. **1967**, 89, 5464-5465. (c) Stork, G.; Rosen, P.; Goldman, N.; Coombs, R. V.; Tsuji, J. Alkylation and Carbonation of Ketones by Trapping the Enclates from the Reduction of α,β -Unsaturated Ketones. J. Am. Chem. Soc. 1965, 87, 275-286.

- (23) Allyl iodides were prepared from corresponding allyl chlorides (22) hily indices we prepared its of responding any distributions by analogy to: Letsinger, R. L.; Traynham, J. G. The Preparation of Allyl Iodide. J. Am. Chem. Soc. 1948, 70, 2818.
 (24) Tal, D. M.; Frisch, G. D.; Elliott, W. H. Bile Acid LXIX. Selective K-Selectride Reduction of 3,7-Diketo Steroids. Tetrahedron
- 1984, 40, 851-854.
- (25)For review, see: Maryanoff, B. E.; Reitz, A. B. The Wittig Olefination Reaction and Modifications Involving Phosphoryl-Stabilized Carbanions. Stereochemistry, Mechanism, and Selected Synthetic Aspects. Chem. Rev. 1989, 89, 863-927.
- (26) For reviews, see: (a) Wadsworth, W. S. Synthetic Applications of Phosphoryl-Stabilized Anions. Org. React. 1977, 25, 73-253.
 (b) Boutagy, J.; Thomas, R. Olefin Synthesis with Organic Phosphonate Carbanions. Chem. Rev. 1974, 74, 87-99.
 (27) Eastham, J. F.; Teranishi, R. Cholest-4-en-3-one. Org. Synth.
- Coll. Vol. 4 1963, 192–195.
- (28) (a) Carruthers, N. I.; Garshasb, S. Synthesis of Corticoids from 9a-Hydroxyandrost-4-ene-3,17-dione. J. Org. Chem. 1992, 57, 961–965. (b) Stork, G.; Brizzolara, A.; Landesman, H.; Szmusz-kovica, J.; Terrell, R. The Enamine Alkylation and Acylation of
- Carbonyl Compounds. J. Am. Chem. Soc. 1963, 85, 207-222.
 (29) Majetich, G.; Grieco, P. A.; Nishizawa, M. Total Synthesis of β-Elemenone. J. Org. Chem. 1977, 42, 2327-2329.
 (30) (a) Vidari, G.; Ferrino, S.; Grieco, P. A. Quassinoids: Total Synthesis of dl-Quassin. J. Am. Chem. Soc. 1984, 106, 3539-2548 (b) Wards V. Sardta in Soc. 1984, 106, 3539-2548 (b) Wards V. Sardta in Soc. 1984, 106, 3539-2548 (c) Wards V. Sardta in Soc. 1984 (c) Wards V. Sardta in Sardta i 3548. (b) Yasuda, K. Synthesis of 2α-Halo-4-en-3-oxo-steroids. Chem. Pharm. Bull. 1964, 12, 1217-1224.

- (32) Benzyl iodides were prepared from corresponding benzyl alcohols according to literature procedure: Olah, G. A.; Hussian, A.; Singh, B. P.; Mehrotra, A. K. Synthetic Methods and Reactions. 112. Synthetic Transformations with Trichloromethylsilane/ Sodium Iodide Reagent. J. Org. Chem. 1983, 48, 3667-3672.
- (33) Bensch, W. R.; Kauffman, R. F.; Beavers, L. S.; Schmidt, R. J.; Bean, J. S.; Perry, D. N.; Eacho, P. I.; McClure, D. B.; Archer, R. A.; Gadski, R. A. Effects of LY295427, a Novel Hypocholesterolemic Agent, on Low Density Lipoprotein (LDL) Receptor Gene Transcription in Vitro and on Serum Cholesterol in Hypercholesterolemic Animals. FASEB J. 1994, 8, A373.
- (34) Bradford, M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 1976, 72, 248-254.
- (35) Folch, J.; Lees, M.; Sloane Stanley, G. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. J. Biol. Chem. 1957, 226, 497-509.
- Kurantz, M.; Maxwell, R.; Kwoczak, R.; Taylor, F. Rapid and (36)Sensitive Method for the Quantitation of Non-Polar Lipids by High-Performance Thin-Layer Chromatography and Fluorodensitometry. J. Chromatogr. 1991, 549, 387-399.

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