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-^{14}C-*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 low-density 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 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) assay[16] 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) assay[16] 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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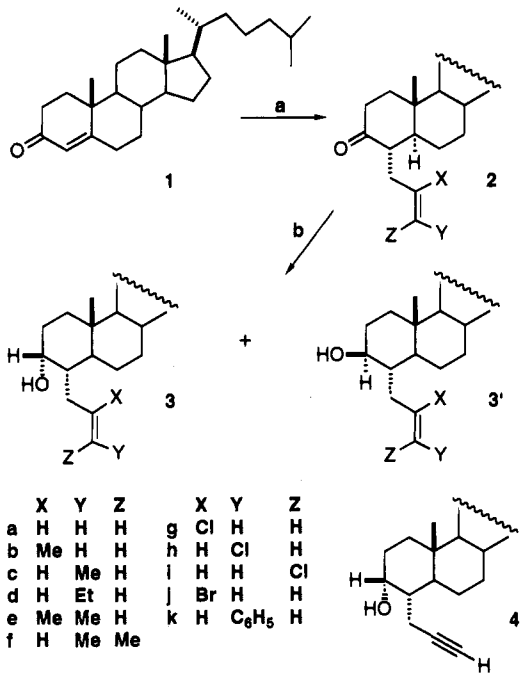
Scheme 1[a]



	X	Y	Z		X	Y	Z
a	H	H	H	g	Cl	H	H
b	Me	H	H	h	H	Cl	H
c	H	Me	H	i	H	H	Cl
d	H	Et	H	j	Br	H	H
e	Me	Me	H	k	H	C_6H_5	H
f	H	Me	Me				

[a] Reagents: (a) Li, NH_3, *t*-BuOH, THF, −78 °C; substituted allyl bromide or iodide, −78 °C; (b) K-selectride, THF, −78 °C to room temperature or Dibal, CH_2Cl_2, 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-^{14}C-*acetate*]cholesterol biosynthesis inhibition (^{14}CACBI) 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.

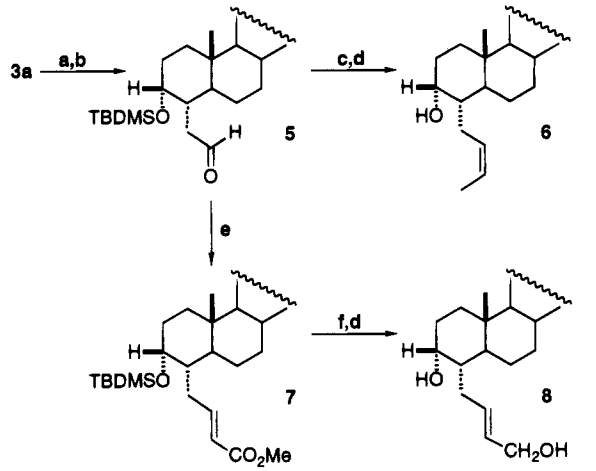
Results and Discussion

Synthesis. As shown in Scheme 1, reductive alkylation[22] of enone **1** with various analogs of allyl bromide or iodide[23] yielded ketones **2**, which in turn were reduced with K-selectride[24] 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.

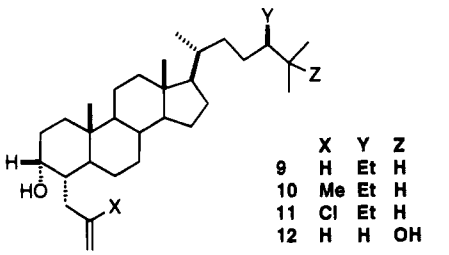
In order to introduce substituents of different geometric and electronic natures onto the double bond of the 4α-allyl, **3a** was first converted in two steps to silyl-protected aldehyde **5**, which was then converted to *cis*-olefin **6** via Wittig olefination[25] and deprotection. Alternatively, **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 same procedures as depicted in Scheme 1, **9**−**11** were synthesized in two steps from β-sitostenone, which was readily derived from Oppenauer oxidation[27] of β-sitosterol. Similarly, **12** was prepared in three steps from 25-hydroxycholesterol (Figure 1).

Scheme 2[a]



[a] Reagents: (a) TBDMSCl, imidazole, DMF; (b) O_3, CH_2Cl_2, −78 °C; (c) $(Ph)_3PC_2H_5^+Br^-$, $KN(TMS)_2$, THF, −15 to 0 °C; (d) Bu_4NF, THF, reflux; (e) $(MeO)_2P(O)CH_2CO_2Me$, $KN(TMS)_2$, THF, −15 °C; (f) Dibal, CH_2Cl_2, 0 °C.



	X	Y	Z
9	H	Et	H
10	Me	Et	H
11	Cl	Et	H
12	H	H	OH

Figure 1

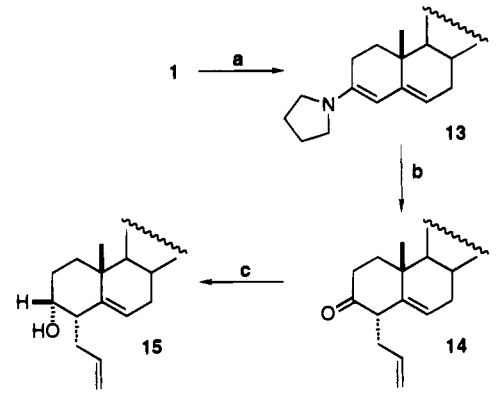
One of our target compounds was **15**, a 5-dehydro derivative of **3a**; because of its structural similarity to cholesterol, it was predicted to be very active. Cholestenone **1** was quantitatively converted to labile dienamine **13**, following a literature procedure,[28] which was selectively allylated 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-selectride[24] 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 protocol[29] (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 alkylation[22] and K-selectride reduction to afford the 2α,4α-diallyl alcohol **25** (Scheme 5).

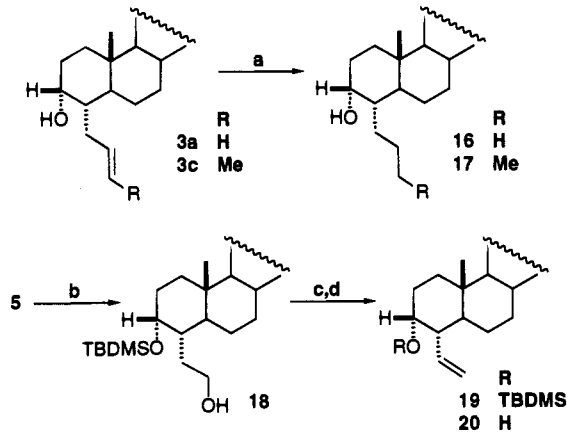
In addition, compounds **30** and **31**, with an allyl at C-6, were also prepared. Thus, 5α,6α-epoxycholestan-3β-ol **26** underwent epoxide ring opening with allyl-

Scheme 3[a]



[a] Reagents: (a) pyrrolidine, benzene, reflux; (b) allyl bromide, dioxane, 55–60 °C; H_2O, 50 °C; (c) K-selectride, THF, −78 °C to room temperature.

Scheme 4[a]



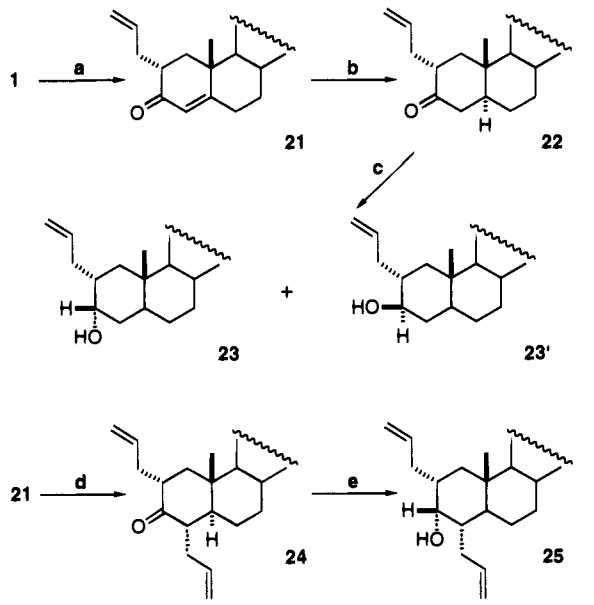
[a] Reagents: (a) H_2, PtO_2, EtOAc; (b) Dibal, CH_2Cl_2, 0 °C; (c) (2-nitrophenyl)selenocyanate, PBu_3, THF; MCPBA, CH_2Cl_2; (d) Bu_4NF, THF, reflux.

magnesium bromide and subsequent Oppenauer oxidation[27] 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 alkylation[31] and subsequent $NaBH_4$ reduction (Scheme 7).

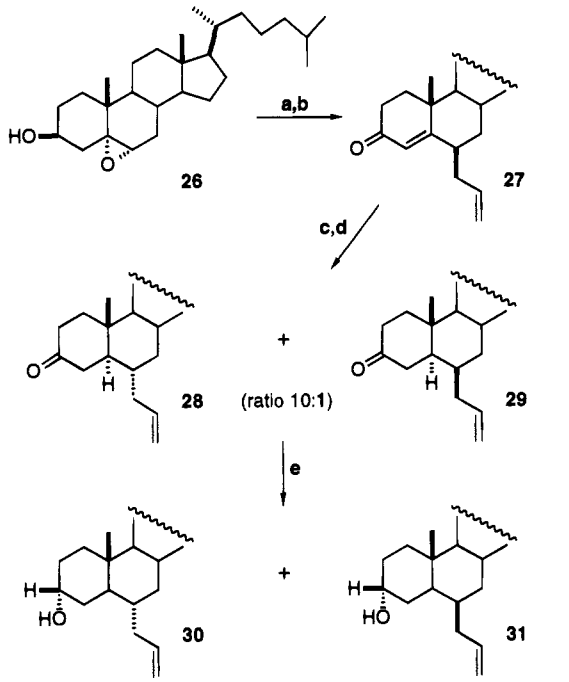
At this point, it seemed advisable to replace the 4α-allyl with the electron-rich 4α-benzyl with the anticipation that the latter would enhance the binding to the receptor through a stronger π−π interaction. Most of 3α-ols **36** and 3β-ols **36′** were synthesized following the same procedures as depicted in Scheme 1;[32] while **36l** and **36l′** were derived from $NaBH_4$ reduction of **35l**, **36o** and **36o′** were derived from Dibal reduction of **35l**. 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, NH_3, *t*-BuOH, THF, −78 °C; (c) Dibal, CH_2Cl_2, 0 °C; (d) Li, NH_3, *t*-BuOH, THF, −78 °C; allyl iodide, −78 °C; (e) K-selectride, THF, −78 °C to room temperature.

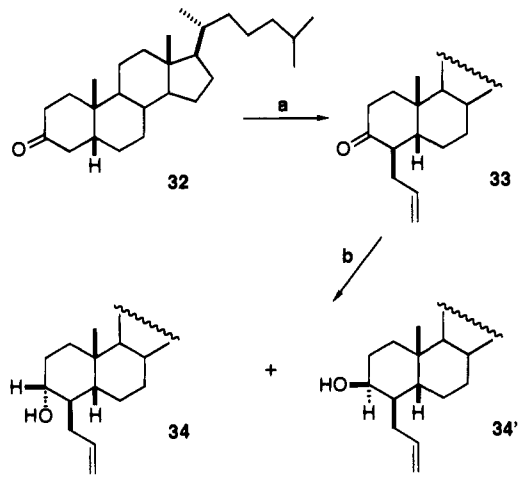
Scheme 6[a]



[a] Reagents: (a) allyl bromide, Mg, Et_2O; (b) cyclohexanone, Al(*i*-OPr)$_3$, toluene, reflux; (c) 2% KOH/MeOH, THF; (d) Li, NH_3, *t*-BuOH, THF, −78 °C; (e) K-selectride, THF, −78 °C to room temperature.

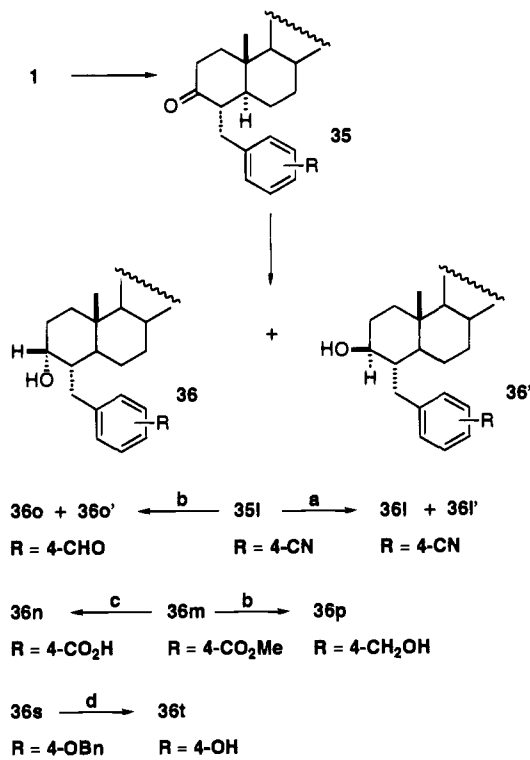
***In Vitro* Biology.** Compounds were evaluated for their up-regulating or derepressing capability against 25-hydroxycholesterol in the LDLR/Luc assay,[16] in which stably transfected, stationary CHO cells were exposed to 25-hydroxycholesterol (0.5 μg/mL) and compound (0–20 μg/mL) in serum free medium for 24 h. Cells were then washed and lysed and homogenates were evaluated for luciferase-catalyzed light production. EC_{30} (μM) 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) $NaBH_4$, Et_2O/MeOH, 0 °C to room temperature.

Scheme 8[a]



[a] Reagents: (a) $NaBH_4$, MeOH/Et_2O, 0 °C to room temperature; (b) Dibal, CH_2Cl_2, 0 °C; H_3O^+; (c) LiOH, MeOH/THF; H_3O^+; (d) 10% Pd/C, H_2, 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 μg/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 EC_{30} of 2.9 μM. 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 EC_{30} of 11 μM, 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 4α-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 25-hydroxycholesterol's active role in the regulation of cellular cholesterol metabolism, compound **12**, a 25-hydroxy 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 EC_{30} 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 EC_{30} of 41 μM, 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

compd	formula[a]	analyses[b]	mp (°C) (recrystn solv)[c]	LDLR/Luc[d] EC$_{30}$ (μM)[e]	LDLR/Luc[d] MA (%)[f]	SV40/Luc[d]	^{14}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)			
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)			
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)			
3h	$C_{30}H_{51}ClO$	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)			
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)	
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)	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/Et$_2$O; B = CH$_3$CN/Et$_2$O. [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 μg/mL. [g] Not active within the test range 0–20 μg/mL. [h] Negative: no effect on the biosynthesis of cholesterol. [i] Analysis of percentage halogen was not performed. [j] At 10 μg/mL, 45% specific light induction over control was recorded.

Table 2. Formulas, Properties, and *in Vitro* Biological Activities of **16**, **17**, **4**, and **20**

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

[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′**

compd	formula[a]	analyses[b]	mp (°C) (recrystn solv)[c]	LDLR/Luc[d] EC$_{30}$ (μM)[e]	LDLR/Luc[d] MA (%)[f]	SV40/Luc[d]	^{14}CACBI[d]
23	$C_{30}H_{52}O$	C,H	72–74 (B)	41 (4)	60	NA[g] (2)	neg[h]
23′	$C_{30}H_{52}O$	C,H	102–103 (B)	NA[g] (2)			
25	$C_{33}H_{56}O$	C,H	101–103[i]	NA[g] (2)			
30	$C_{30}H_{52}O$	C,H	gum	NA[g] (2)			
31	$C_{30}H_{52}O$	C,H	101–103 (A)	NA[g] (2)			
34	$C_{30}H_{52}O$	C,H	gum	NA[g] (2)			
34′	$C_{30}H_{52}O$	C,H	63–65[i]	NA[g] (2)			

[a–h] See footnotes *a–h* of Table 1. [i] Isolated from chromatographic purification without further recrystallization.

in the 4α-allyl series. It was disappointing to find out that compound **25**, a 2α,4α-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 **36f–i**, 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 EC$_{30}$ of 2.8 μM, 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 EC$_{30}$ of 15 μM, 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 **36u–w**, 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**

compd	R	formula[a]	analyses[b]	mp (°C) (recrystn solv)[c]	LDLR/Luc[d,j] EC_{30} (μM)[e]	LDLR/Luc[d,j] MA (%)[f]	SV40/Luc[d]	^{14}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	H	$C_{34}H_{54}O$	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-Cl	$C_{34}H_{53}ClO$	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	$C_{35}H_{53}F_3O$	C,H,F	187–188 (B)	12 (3)	58	NA[g] (2)	
36l	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)			
36o	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′**, **36i′**, **36k′**, **36l′**, **36o′**, and **36w′** were not active in the assay. [k] **36n** was recrystallized from CH_3CN/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 ^{14}CACBI 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 reported[33] 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 ^{14}CACBI 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,

Table 5. Serum Cholesterol in Hamsters after 1 Week of Drug Treatment

compd	total cholesterol (mg/dL)[a]	cholesterol change relative to control (%)[b]	LDLR/Luc EC_{30} (μM)[c]
control	**313** (24)		
3a	167 (7)	−47	2.9
3a'	198 (11)	−37	NA
3c	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)		
3a	197 (16)	−49	2.9
36i	240 (30)	−37	2.8
control	274 (23)		
3a	188 (10)	−31	2.9
35f	335 (30)	[+22]	NA
36f'	287 (25)	[+5]	NA
36h	160 (6)	−42	6.0
control	333 (23)		
3a	199 (15)	−40	2.9
3g	200 (12)	−40	1.8
3h	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.

5α,6α-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 F_{254} plates (E. Merck). ^{1}H 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} μM selenium, 50 μM 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 μg/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 μg/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% CO_2. 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 Ca^{2+} or Mg^{2+} (500 μL/well), and the cell monolayer was lysed by addition of 100 μL/well assay buffer containing 1% Triton X-100, 25 mM glycylglycine (pH 7.8), 15 mM $MgSO_4$, 1 mM dithiothreitol, and 1 mM ATP. A 50 μL aliquot of each lysate was diluted up 425 μL in the above assay buffer and placed in a LMB luminometer and the reaction was initiated by the injection of 25 μL 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 μL) by Coomassie Brilliant Blue G250 binding with a protein assay kit (Bio-Rad Laboratories). Luciferase activity is expressed as light units/μg of protein (specific activity). EC_{30} (μM) 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 μg/mL.

(D) [1-^{14}C-*acetate*]Cholesterol Biosynthesis Inhibition Assay (^{14}CACBI). 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 μg/mL. After a 20 h incubation, [1-^{14}C]-acetic acid, sodium salt (NEN), was added (8 μL) directly to each dish (final concentration of 1 μCi/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 μL 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 °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 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. 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 NH_4Cl (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 × 2), the combined organic layers were washed with saturated aqueous NaCl (10 mL), dried over $MgSO_4$, 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 Et_2O/MeOH: mp 108.0−110.0 °C; IR (KBr) 2933, 1706, 913 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.60−1.65 (m, 22H), 0.68 (s, 3H, CH_3), 0.87 (d, 6H, J = 6.5 Hz, CH_3), 0.91 (d, 3H, J = 6.6 Hz, CH_3), 1.06 (s, 3H, CH_3), 1.65−1.91 (m, 3H), 1.91−2.08 (m, 2H), 2.18−2.55 (m, 5H), 4.95−5.08 (m, 2H, $=CH_2$), 5.70−5.88 (m, 1H, −CH=); FDMS *m/e* 426 (M^+). Anal. ($C_{30}H_{50}O$) C, H.

(3α,4α,5α)-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% H_2O_2 (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 × 2), dried over $MgSO_4$, 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 Et_2O/MeOH: mp 117.0−119.0 °C; IR (KBr) 3493 (br), 2934 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.65−2.05 (m, 32H), 0.66 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.87 (d, 6H, J = 6.5 Hz, CH_3), 0.91 (d, 3H, J = 6.5 Hz, CH_3), 2.20−2.35 (m, 1H), 3.91 (br s, 1H, −C*H*(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 3β-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 CH_2Cl_2 (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 Et_2O/MeOH: mp 135.0−137.0 °C; IR (KBr) 3455 (br), 2931 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.55−1.90 (m, 30H), 0.66 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.87 (d, 6H, J = 6.6 Hz, CH_3), 0.90 (d, 3H, J = 6.5 Hz, CH_3), 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, $=CH_2$), 5.78−5.94 (m, 1H, −CH=); FDMS *m/e* 429 (M^+ + 1), 428 (M^+). Anal. ($C_{30}H_{52}O$) C, H.

(3α,4α,5α)-4-(2-Bromo-2-propenyl)cholestan-3-ol (3j) and (3α,4α,5α)-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 °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 Et_2O/MeOH. **3j**: mp 155.0−157.0 °C; IR (KBr) 3601, 3478 (br), 2931 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (s, 3H, CH_3), 0.70−1.90 (m, 30H), 0.87 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.8 Hz, CH_3), 0.92 (d, 3H, J = 6.5 Hz, CH_3), 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^+, ^{79}Br), 508 (M^+, ^{81}Br). Anal. ($C_{30}H_{51}BrO$) C, H, Br. **4**: mp 146.0−148.0 °C; IR (KBr) 3539, 3276 (br), 2925, 2105 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.65−1.90 (m, 30H), 0.66 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.87 (d, 6H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.6 Hz, CH_3), 1.98 (br d, 1H, J = 12.2 Hz), 2.04 (t, 1H, J = 2.5 Hz, 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.

(3α,4α,5α)-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 mL × 2) and flash chromatography on silica gel (gradient 0−4% ethyl acetate/hexane) provided silylated product (3.34 g, 89%) as a white solid. Ozone was bubbled through a stirred solution of the silylated product (20.0 g, 36.6 mmol) in CH_2Cl_2 (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 Et_2O/MeOH: mp 86.0−88.0 °C; IR (KBr) 2948, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.00 (s, 3H, CH_3), 0.03 (s, 3H, CH_3), 0.64 (s, 3H, CH_3), 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.

[3α,4α(Z),5α]-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 Et_2O (30 mL), washed with half-saturated aqueous NaCl (10 mL × 2), dried over $MgSO_4$, 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 Et_2O/MeOH: mp 99.0–101.0 °C; IR (KBr) 3486 (br), 2937 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.65–1.90 (m, 30H), 0.66 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.87 (d, 3H, J = 6.6 Hz, CH_3), 0.88 (d, 3H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.6 Hz, CH_3), 1.66 (d, 3H, J = 5.7 Hz, CH_3), 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. ($C_{31}H_{54}O$) C, H.

(*E*)-4-[(3α,4α,5α)-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 Et_2O (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 Et_2O/MeOH: mp 158.0–158.5 °C; IR (KBr) 2933, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.03 (s, 3H, CH_3), 0.05 (s, 3H, CH_3), 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, CH_3), 0.91 (d, 3H, J = 6.5 Hz, CH_3), 0.92 (s, 9H, CH_3), 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, OCH_3), 3.82 (br s, 1H), 5.83 (d, 1H, J = 15.6 Hz, –CH=), 6.94–7.06 (m, 1H, –CH=); FDMS *m/e* 602 (M^+ + 1). Anal. ($C_{38}H_{68}O_3Si$) C, H.

[3α,4α(*E*),5α]-4-(4-Hydroxy-2-butenyl)cholestan-3-ol (8). A solution of diisobutylaluminum 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 CH_2Cl_2 (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 × 2), dried over $MgSO_4$, filtered, and concentrated. The residue was subject to flash chromatography on silica (gradient 40–60% ethyl acetate/hexane) to yield **8** (117 mg, 96%) as a white solid, which was recrystallized from Et_2O/MeOH: mp 178.0–180.0 °C; IR (KBr) 3421 (br), 2932 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.65–1.90 (m, 31H), 0.66 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.87 (d, 6H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.5 Hz, CH_3), 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. ($C_{31}H_{54}O_2$) 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 (C_6D_6) δ 0.71 (s, 3H, CH_3), 0.89 (d, 6H, J = 6.6 Hz, CH_3), 0.98–1.90 (m, 26H), 1.00 (d, 3H, J = 6.5 Hz, CH_3), 1.15 (s, 3H, CH_3), 2.00–2.35 (m, 4H), 2.85–2.93 (m, 4H, $-NCH_2-$), 5.04 (s, 1H, –CH=), 5.34 (br s, 1H, –CH=); FDMS *m/e* 437 (M^+). Anal. ($C_{31}H_{51}N$) C, H, N.

(3α,4α)-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 Et_2O (100 mL) and washed with saturated aqueous NaCl (100 mL), and the aqueous layer was extracted with THF (100 mL)/Et_2O (50 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$, 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% H_2O_2 (30.4 mL). The cold bath was removed and the mixture was stirred vigorously for 2 h. Solid NH_4Cl (19.1 g) was added to the mixture before it was extracted with THF/Et_2O (ratio 1:1, 150 mL × 2). The combined organic layers were washed with saturated aqueous NaCl (100 mL × 2), dried over $MgSO_4$, filtered, and concentrated. After two sequential flash chromatographic separations on silica (first, 0–2% ethyl acetate/toluene; second, 50–100% CH_2Cl_2/hexane), **15** (3.33 g, 18%) was obtained as a white solid, which was recrystallized from Et_2O/CH_3CN: mp 101.0–103.0 °C; IR (KBr) 3440 (br), 2937 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.93 (d, 3H, J = 6.5 Hz, CH_3), 0.95–1.92 (m, 25H), 1.06 (s, 3H, CH_3), 2.00–2.46 (m, 5H), 3.90–3.94 (m, 1H), 5.03–5.16 (m, 2H, $=CH_2$), 5.44 (br d, 1H, J = 5.5 Hz, –CH=), 5.77–5.93 (m, 1H, –CH=); FDMS *m/e* 426 (M^+). Anal. ($C_{30}H_{50}O$) C, H.

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

(3α,4α,5α)-4-Propylcholestan-3-ol (16). A mixture of **3a** (2.00 g, 4.67 mmol) and PtO_2 (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 Et_2O/MeOH: mp 120.0–123.0 °C; IR (KBr) 3458 (br), 2929 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.60–1.90 (m, 34H), 0.64 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.86 (d, 6H, J = 6.6 Hz, CH_3), 0.89 (d, 3H, J = 6.2 Hz, CH_3), 0.90 (t, 3H, J = 6.5 Hz, CH_3), 1.90–2.00 (m, 1H), 3.91 (br s, 1H); FDMS *m/e* 430 (M^+). Anal. ($C_{30}H_{54}O$) 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 CH_2Cl_2/CH_3CN: mp 109.0–111.0 °C; IR (KBr) 3357 (br), 2931 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.03 (s, 3H, CH_3), 0.05 (s, 3H, CH_3), 0.64 (s, 3H, CH_3), 0.65–1.90 (m, 32H), 0.79 (s, 3H, CH_3), 0.86 (d, 6H, J = 6.6 Hz, CH_3), 0.89 (s, 9H, CH_3), 0.90 (d, 3H, J = 6.2 Hz, CH_3), 1.93–1.98 (m, 1H), 3.57–3.70 (m, 2H, $-CH_2O-$), 3.85 (s, 1H); FDMS *m/e* 547 (M^+ + 1). Anal. ($C_{35}H_{66}O_2Si$) C, H.

(3α,4α,5α)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4-ethenylcholestane (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 $MgSO_4$, 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 CH_2Cl_2 (10 mL) at 0 °C was added $CaCO_3$ (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 CH_2Cl_2 (40 mL) and subsequent filtration through a short pad of Celite, the filtrate was washed with saturated aqueous NaCl (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was subject to flash chromatography on silica (hexane) to provide the desired 4α-vinyl compound **19** (460 mg, 99%) as a white solid, which was recrystallized from CH_2Cl_2/CH_3CN: mp 87.0–90.0 °C; IR (KBr) 2935, 2867 cm^{-1}; ^1H NMR ($CDCl_3$) δ −0.01 (s, 3H, CH_3), 0.00 (s, 3H, CH_3), 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, CH_3), 0.89 (d, 3H, J = 6.2 Hz, CH_3), 0.90 (s, 9H, CH_3),

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 Et_2O/CH_3CN: mp 137.0–138.0 °C; IR (KBr) 3448 (br), 2937 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.64 (s, 3H, CH_3), 0.70–1.90 (m, 29H), 0.82 (s, 3H, CH_3), 0.86 (d, 6H, J = 6.6 Hz, CH_3), 0.90 (d, 3H, J = 6.6 Hz, CH_3), 1.92–2.11 (m, 2H), 3.82 (br s, 1H), 5.00–5.13 (m, 2H, =CH_2), 5.73–5.87 (m, 1H, –CH=); FDMS *m/e* 415 (M^+ + 1). Anal. ($C_{29}H_{50}O$) C, H.

(2α)-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 ($CDCl_3$) δ 0.72 (s, 3H, CH_3), 0.75–1.70 (m, 20H), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.92 (d, 3H, J = 6.5 Hz, CH_3), 1.20 (s, 3H, CH_3), 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, =CH_2), 5.72 (d, 1H, J = 1.0 Hz, –CH=), 5.75–5.88 (m, 1H, –CH=); FDMS *m/e* 424 (M^+). Anal. ($C_{30}H_{48}O$) C, H.

(2α,5α)-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 1,3-pentadiene (0.5 mL) to quench the excess of lithium. Saturated aqueous NH_4Cl (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 × 2), the combined organic layers were washed with saturated aqueous NaCl (10 mL), dried over $MgSO_4$, 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^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (s, 3H, CH_3), 0.70–2.15 (m, 29H), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.5 Hz, CH_3), 1.06 (s, 3H, CH_3), 2.29–2.50 (m, 2H), 2.52–2.62 (m, 1H), 4.99–5.06 (m, 2H, =CH_2), 5.71–5.86 (m, 1H, –CH=); FDMS *m/e* 427 (M^+ + 1). Anal. ($C_{30}H_{50}O$) C, H.

(2α,3α,5α)-2-(2-Propenyl)cholestan-3-ol (23) and Its 3β-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 CH_2Cl_2 (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 Et_2O/CH_3CN. **23**: mp 72.0–74.0 °C; IR (KBr) 3376 (br), 2931 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.65–1.70 (m, 29H), 0.66 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 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, =CH_2), 5.76–5.91 (m, 1H, –CH=); FDMS *m/e* 429 (M^+ + 1). Anal. ($C_{30}H_{52}O$) C, H. **23′**: mp 102.0–103.0 °C; IR (KBr) 3365 (br), 2931 cm^{-1}; ^1H NMR ($CDCl_3$) 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, CH_3), 1.91–2.04 (m, 2H), 2.37–2.45 (m, 1H), 3.25–3.36 (m, 1H), 5.00–5.12 (m, 2H, =CH_2), 5.80–5.96 (m, 1H, –CH=); FDMS *m/e* 429 (M^+ + 1). Anal. ($C_{30}H_{52}O$) 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 ($CDCl_3$) δ 0.65–1.65 (m, 22H), 0.68 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.6 Hz, CH_3), 1.12 (s, 3H, CH_3), 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, =CH_2), 5.73–5.91 (m, 2H, –CH=); FDMS *m/e* 466 (M^+). Anal. ($C_{33}H_{54}O$) 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 ($CDCl_3$) δ 0.65–1.90 (m, 29H), 0.66 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.7 Hz, CH_3), 0.91 (d, 3H, J = 6.6 Hz, CH_3), 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, =CH_2), 5.75–5.95 (m, 2H, –CH=); FDMS *m/e* 468 (M^+). Anal. ($C_{33}H_{56}O$) C, H.

(6β)-6-(2-Propenyl)cholest-4-en-3-one (27). Allylmagnesium bromide (30.0 mL, 1.0 M in Et_2O) was added dropwise to a stirred solution of 5α,6α-epoxycholestan-3β-ol **26** (1.21 g, 3.00 mmol) in dry Et_2O (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 $NaHCO_3$ (15 mL) and NaCl (30 mL), dried over $MgSO_4$, 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 Et_2O (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 ($CDCl_3$) δ 0.65–1.95 (m, 22H), 0.74 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.93 (d, 3H, J = 6.5 Hz, CH_3), 1.25 (s, 3H, CH_3), 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. ($C_{30}H_{48}O$) 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 $MgSO_4$, 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 ($CDCl_3$) δ 0.60–1.90 (m, 25H), 0.69 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.5 Hz, CH_3), 1.03 (s, 3H, CH_3), 1.92–2.20 (m, 4H), 2.22–2.57 (m, 3H), 4.95–5.06 (m, 2H, =CH_2), 5.65–5.80 (m, 1H, –CH=); FDMS *m/e* 426 (M^+). Anal. ($C_{30}H_{50}O$) C, H.

(3α,5α,6α)-6-(2-Propenyl)cholestan-3-ol (30) and Its 6β-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), **30** (227 mg, 85%) and **31** (21.4 mg, 8%) were obtained as an oil and white solid, respectively; **31** was recrystallized from Et_2O/MeOH. **30**: IR (neat) 3200 (br), 2910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.60–1.90 (m, 31H), 0.66 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.87 (d, 6H, J = 6.7 Hz, CH_3), 0.91 (d, 3H, J = 6.6 Hz, CH_3), 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, $=CH_2$), 5.70–5.85 (m, 1H, −CH=); FDMS *m/e* 428 (M^+). Anal. ($C_{30}H_{52}O$) C, H. **31**: mp 101.0–103.0 °C; IR (KBr) 3347 (br), 2935 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.65–2.04 (m, 32H), 0.69 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.92 (d, 3H, J = 6.5 Hz, CH_3), 2.07–2.16 (m, 1H), 4.14 (br s, 1H), 4.92–5.00 (m, 2H, $=CH_2$), 5.60–5.75 (m, 1H, −CH=); FDMS *m/e* 428 (M^+). Anal. ($C_{30}H_{52}O$) C, H.

(4β,5β)-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 Et_2O/CH_3CN: mp 59.0–60.0 °C; IR (KBr) 2952, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (s, 3H, CH_3), 0.80–1.92 (m, 25H), 0.87 (d, 6H, J = 6.6 Hz, CH_3), 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, $=CH_2$), 5.72–5.87 (m, 1H, −CH=); FDMS *m/e* 426 (M^+). Anal. ($C_{30}H_{50}O$) C, H.

(3α,4β,5β)-4-(2-Propenyl)cholestan-3-ol (34) and Its 3β-ol Isomer (34′). $NaBH_4$ (13.2 mg, 0.349 mmol) was added to a stirred solution of **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 ($CDCl_3$) δ 0.66 (s, 3H, CH_3), 0.80–2.03 (m, 31H), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.5 Hz, CH_3), 0.94 (s, 3H, CH_3), 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, $=CH_2$), 5.80–5.95 (m, 1H, −CH=); FDMS *m/e* 428 (M^+). Anal. ($C_{30}H_{52}O$) C, H. **34′**: mp 63.0–65.0 °C; IR (KBr) 3394 (br), 2929 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (s, 3H, CH_3), 0.80–2.06 (m, 32H), 0.88 (d, 6H, J = 6.6 Hz, CH_3), 0.91 (d, 3H, J = 6.5 Hz, CH_3), 0.98 (s, 3H, CH_3), 2.25–2.35 (m, 1H), 3.98 (br s, 1H), 5.02–5.18 (m, 2H, $=CH_2$), 5.84–6.00 (m, 1H, −CH=); FDMS *m/e* 428 (M^+). Anal. ($C_{30}H_{52}O$) C, H.

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