Structural and Stereoelectronic Requirements for the Inhibition of Mammalian 2,3-Oxidosqualene Cyclase by Substituted Isoquinoline Derivatives

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2,3-Oxidosqualene lanosterol-cyclase (OSC; EC 5.4.99.7) is an attractive target for the design of compounds that block hepatic cholesterol biosynthesis. (4aα,5α,6β,8aβ)-Decahydro-5,8a-dimethyl-2-(1,5,9-trimethyldecyl)-6-isoquinolinol (**1**) and simplified analogs have been devised to inhibit this enzyme by mimicking the postulated *pro*-C-8 high-energy intermediary carbocation occurring during the cyclization−rearrangement pathway. In order to gain an understanding into the mechanism by which these types of molecules inhibit OSC, we have synthesized a series of substituted isoquinoline derivatives **3** and investigated the structural and stereoelectronic requirements, and their stringency, that make **3** potential high-energy intermediate analogs of OSC. Determination of the IC_{50} values of the different compounds, with rat liver microsomal cyclase, allowed the study of the relative importance of (i) the nature and the stereochemistry of the nitrogen side chain, (ii) the presence of methyl groups at C-5 and C-8a (ring junction), (iii) the presence and stereochemistry of the C-6 hydroxyl group, (iv) the nature of the ring junction, and (v) the absolute configuration of the bicyclic system. The resulting structure−activity relationships seem to validate the mechanism of action of these inhibitors as analogs of a *pro*-C-8 high-energy intermediate and delineate the minimal requirements for the design of efficient isoquinoline-based, or simplified, OSC inhibitors.

Introduction

A prime target for the design of hypocholesterolemic drugs is HMG-CoA reductase, the major regulatory and rate-limiting enzyme in the biosynthetic pathway of cholesterol.[1] Inhibition of cholesterol synthesis at a later stage, however, which avoids possible limitations in mevalonate (a metabolite involved in many other functions[2]) is also of interest. Among the different possible target enzymes, 2,3-oxidosqualene lanosterol-cyclase (OSC; EC 5.4.99.7) is particularly attractive. In mammalian cells, inhibitors of this enzyme affect very efficiently the biosynthesis of cholesterol by a dual mechanism.[3−6] In addition to the direct inhibition of lanosterol formation, inhibition of the cyclase also results in the accumulation of (3*S*)-2,3-oxidosqualene and (3*S*,22*S*)-2,3:22,23-dioxidosqualene. This latter metabolite, which is preferentially cyclized into (24*S*)-24,25-epoxylanosterol,[7] leads ultimately to the formation of (24*S*)-24,25-epoxycholesterol, a known repressor of HMG-CoA reductase activity.[8,9] Thus, cyclase inhibitors can also affect cholesterol biosynthesis via a regulatory pathway; this mode of action might lead to an amplification of a normal enzyme inhibition.

Rational design of inhibitors, such as transition state analog, relies on the knowledge of the molecular mechanism of the target enzyme. The transformation of *all-trans*-2,3-oxidosqualene into lanosterol is a fascinating reaction for both the chemist and enzymologist. Its elusive mechanism has attracted much attention in the past[10] and a renewed interest during recent years. Work by the group of Corey[11] has emphasized substrate folding for the correct cyclization−rearrangement step. The cyclization is believed to be triggered by a general acid-catalyzed epoxide ring opening assisted by a neighboring π-bond.[12] The concertedness of the ensuing overall annulation and backbone rearrangement is a matter of debate.[13] However, for entropic reasons and from experimental evidence, the reaction is more likely to proceed through a series of discrete conformationally rigid carbocationic intermediates.[14] Thus, in some plants, a bicyclic intermediate occurring at position *pro*-C-8, besides its normal transformation into triterpenoids, can be trapped by a water molecule.[15] Moreover Johnson *et al.* have demonstrated the dramatic efficiency of intermediary carbocation-stabilizing auxiliary groups in biomimetic polyene cyclization reactions.[16] This indicates that in the cyclization of a molecule such as 2,3-oxidosqualene both conformational and electronic environments, provided by the active site of the cyclase, are of importance. Interestingly, a methylidene auxiliary group, when introduced at position C-29 of 2,3-oxidosqualene, was shown to lead to a mechanism-based type inhibitor of the cyclase,[17] presumably by trapping the intermediary proto-sterol carbocation.

Carbocationic high-energy intermediates occurring during the reaction pathway of enzymes catalyzing, for example, sterol biosynthesis, can be mimicked by structurally related molecules which bear strategically positioned positively charged groups, such as ammonium derivatives.[18] In many cases, these analogs proved to be very powerful inhibitors of these enzymes.[19] This approach was quite fruitful in inhibiting 2,3-oxidosqualene lanosterol-cyclase.[19,20] The first example along these lines was the design of 2-aza-2,3-dihydrosqualene and related compounds which were thought to mimic the charge deficiency at *pro*-C-2 of the substrate during the oxirane ring opening.[21] Importantly when an aza group was introduced at position 2 in 4,4,-

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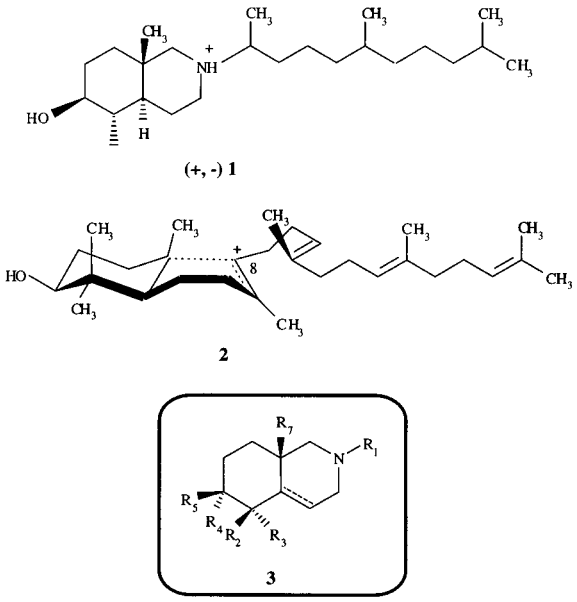


Figure 1. 2,3-Oxidosqualene lanosterol-cyclase inhibitor **1**, designed to mimic the high-energy intermediate **2** (B-ring closure), and the series of isoquinolines **3** studied in this paper.

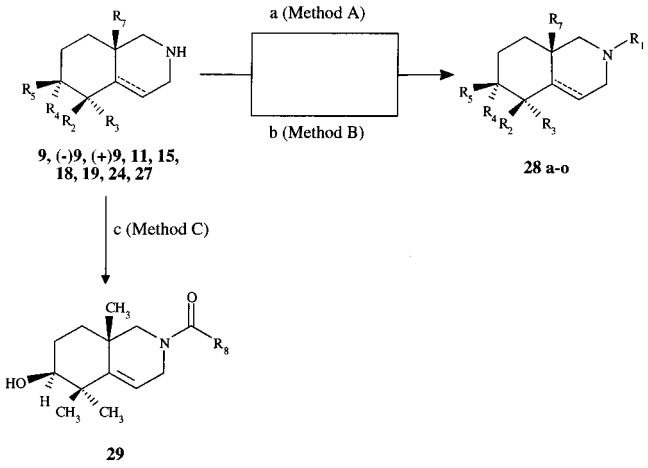
10β-trimethyl-*trans*-decal-3β-ol (TMD), *i.e.*, a decalin structure which was previously found to be a cyclase inhibitor,[22] much less inhibition was observed.[23] The same was true for the first generation of isoquinolines.[21] Mimicking the postulated *pro*-C-8 intermediary carbocation **2** (Figure 1) by (4aα,5α,6β,8aβ)-decahydro-5,-8a-dimethyl-2-(1,5,9-trimethyldecyl)-6-isoquinolinol (compound **1**, Figure 1) led, however, to an efficient cyclase inhibitor.[24] This indicates that these charged inhibitors seemingly need a hydrophobic tail, which mimics the long squalene molecule, in order to adopt a correct positioning in the enzyme active site. Many analogs of the other intermediary carbocationic intermediates along the cyclization−rearrangement pathway have since been synthesized and tested.[25]

Since the original publications on compound **1**, other workers have evaluated novel isoquinoline and simplified analogs.[26] In order to gain an understanding into the mechanism by which these types of molecules inhibit OSC, in the present study, we have synthesized and tested analogs structurally related to the proposed intermediate **2**, in an attempt to mimic the relevant structural attributes of the latter. Thus, a methyl group in position 5β of ring A and the carbon−carbon double bonds of the lateral side chain were reintroduced. This was done for the deca- and octahydroisoquinoline series. Subsequently, we evaluated the importance of different structural elements for the inhibition of OSC by investigating stereochemical and electronic aspects. The generalized sum of these structures is represented by the general structure **3** (Figure 1).

Chemistry

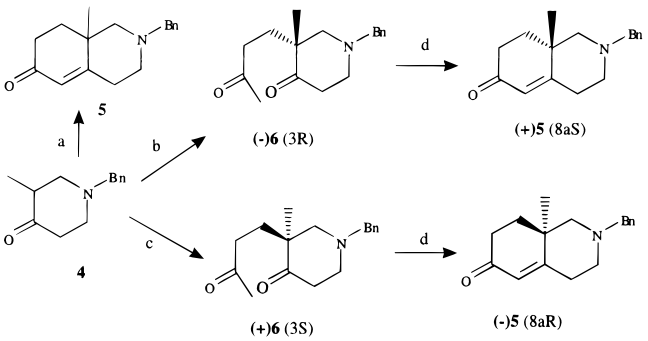
The compounds listed in Tables 1−3 were synthesized by the routes shown in Scheme 1. The preparation involved the alkylation of isoquinoline derivatives with either halides or sulfonates in acetonitrile in the presence of K_2CO_3 (method A) or with carbonyl derivatives using a reductive amination procedure with $NaBH_3CN$ (method B). Amide derivative was obtained by acylation

Scheme 1. General Methods A−C[a]



[a] Reagents: (a) R_1X, K_2CO_3, CH_3CN, reflux; (b) R_9COR_{10}, $NaBH_3CN$, CH_3OH; (c) R_8CO_2H, CDI, THF. Groups R_1−R_7 are defined in Tables 1 and 3.

Scheme 2[a]

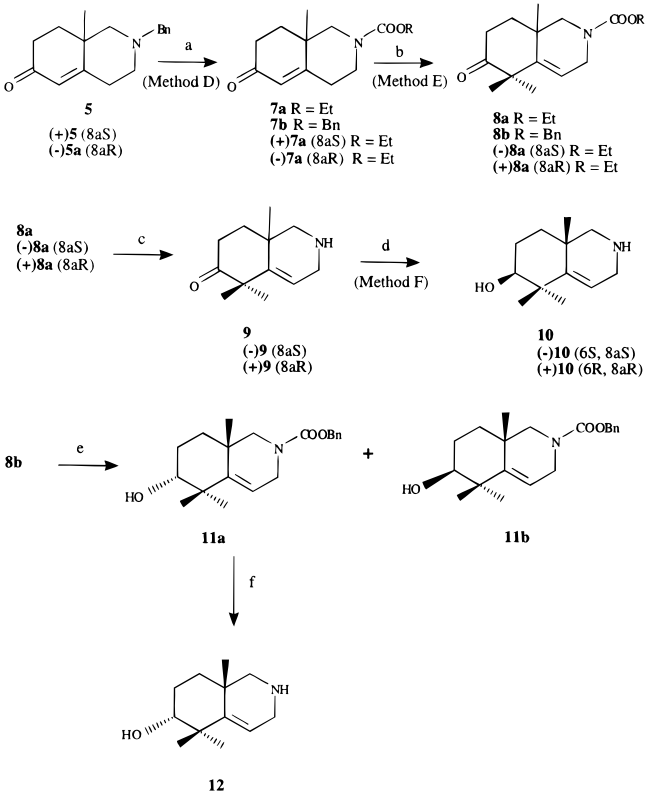


[a] Reagents: (a) MVK, CH_3ONa, CH_3OH, 50 °C; (b) (i) (*R*)-(+)-$PhCH(CH_3)NH_2$, toluene, (ii) MVK, THF, 50 °C; (c) (i) (*S*)-(−)-$PhCH(CH_3)NH_2$, toluene, (ii) MVK, THF, 50 °C; (d) CH_3ONa, CH_3OH, 50 °C.

of the isoquinoline with CDI-activated acid (method C). The bicyclic derivatives required for these syntheses were prepared by methods outlined in Schemes 2−7.

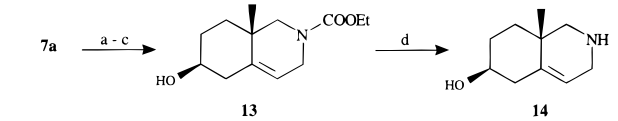
Compound **5** (Scheme 2) was prepared in a one-step procedure using Robinson annelation[27] starting from 1-(phenylmethyl)-3-methyl-4-piperidone (**4**) and methyl vinyl ketone (MVK). An alternative method, via a two-step process, according to Stork[28] was used for the synthesis of **21** (Scheme 6) starting from 1-(phenylmethyl)-4-piperidone (**20**) and ethyl vinyl ketone (EVK). Since these methods were not suitable for the enantioselective preparations of the optical isomers of **5**, we developed an original synthesis to prepare **(−)-5** and **(+)-5** via a three-step procedure by reference to D'Angelo's work[29] (Scheme 2). The first step of this reaction between 4-piperidone derivative **4** and (+)-α-methylbenzylamine on one hand and (−)-α-methylbenzylamine on the other hand provided a chiral imine−enamine equilibrium ensuring a good diastereofacial differentiation for MVK in the subsequent Michael reaction leading to **(−)-6** and **(+)-6**. Bicycles **(+)-5** and **(−)-5** were respectively obtained by cyclization of **(−)-6** and **(+)-6** with sodium methoxide in methanol. Optical purities of these compounds were determined by NMR analysis in the presence of the chiral $Eu(hfc)_3$ shift reagent and were better than 95%. The attribution of absolute stereochemistry was done using empirical D'Angelo's rules.[29]

Scheme 3. General Methods D−F[a]



[a] Reagents: (a) ROCOCl, K_2CO_3, reflux; (b) *tert*-BuOH, *tert*-BuOK, CH_3I; (c) Me_3SiCl, NaI, CH_3CN; (d) $NaBH_4$, CH_3OH, −30 °C; (e) $(i\text{-PrO})_3Al$, *i*-PrOH, reflux; (f) H_2, 10% Pd/C, CH_3CO_2H.

Scheme 4[a]

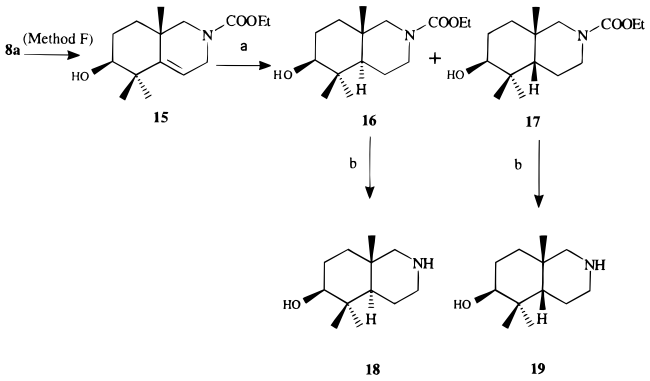


[a] Reagents: (a) H_2SO_4, isopropenyl acetate; (b) EtOH, $NaBH_4$; (c) EtOH, concentrated HCl; (d) CH_3Li−LiBr, THF.

Reaction of racemic **5** or its enantiomers **(+)-5** and **(−)-5** (Scheme 3) with ethyl or benzyl chloroformate provided respectively **7a**,**b**, **(+)-7a**, and **(−)-7a** (method D). Alkylation of these compounds according to Atwater[30] with iodomethane and potassium *tert*-butoxide in *tert*-butyl alcohol gave respectively **8a**,**b**, **(−)-8a**, and **(+)-8a** (method E). Cleavage of the carbamate group was performed using Me_3SiCl/NaI methodology[31] from **8**, **(+)-8a**, and **(−)-8a** leading to **9**, **(−)-9**, and **(+)-9**. Reduction of **9**, **(+)-9**, and **(−)-9** with sodium borohydride (method F) at −30 °C gave almost exclusively 6β-hydroxy derivatives **10**, **(−)-10**, and **(+)-10**. Reduction of **8b** to afford 6α-hydroxy derivatives was attempted according to various literature procedures.[32−35] The best result was obtained using aluminum isopropoxide and gave a 50:50 mixture of 6α-derivative **11a** and 6β-derivative **11b** which were separated by flash chromatography. Assignment of the stereochemistry of **11a**,**b** was made by ^{1}H NMR analysis using chemical shift, coupling constant, and half-height width[36] of the proton signal borne by the C-6. Selective hydrogenolysis of **11a** in the presence of palladium on carbon afforded **12**.

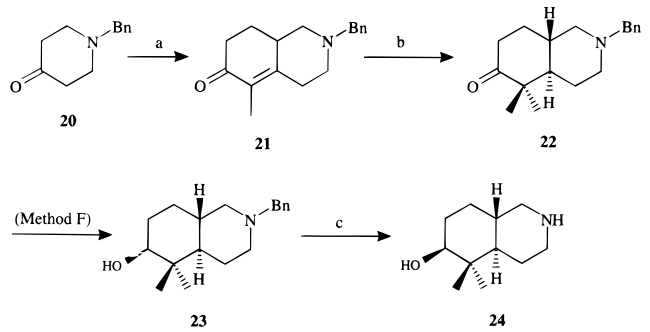
We prepared demethylated compound **28k** via intermediate **14** (Scheme 4). Deconjugation of the α,β-unsaturated ketone **7a** with isopropenyl acetate in acidic medium afforded an unstable enol acetate[37] which was

Scheme 5[a]



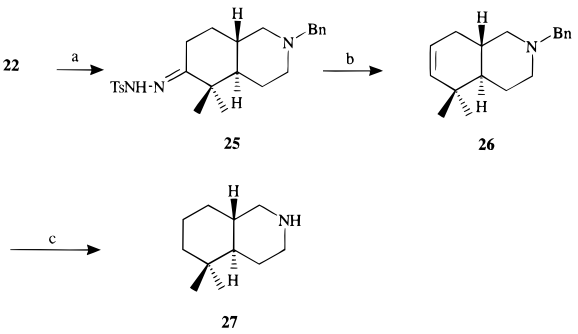
[a] Reagents: (a) H_2, PtO_2, CH_3CO_2H; (b) CH_3Li, THF, 0 °C.

Scheme 6[a]



[a] Reagents: (a) (i) pyrrolidine, toluene, (ii) EVK, toluene, reflux, (iii) CH_3ONa, toluene; (b) Li, NH_3, CH_3I, −60 °C; (c) H_2, Pd/C, ethyl acetate.

Scheme 7[a]



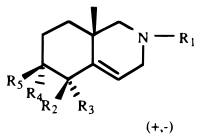
[a] Reagents: (a) $TsNHNH_2$, CH_3CO_2H; (b) NaH, toluene, reflux; (c) H_2, Pd/C, CH_3CO_2H.

reduced with $NaBH_4$ and then treated with acid to give **13** after purification by chromatography.[38] Deprotection of **13** using MeLi and LiBr gave **14**.

Decahydroisoquinolin-6-ols **18** and **19** were prepared as indicated in Scheme 5. Reduction of **8a** using method F gave **15**. Hydrogenation of **15** with PtO_2 as catalyst led to a 50:50 mixture of trans and cis isomers **16** and **17** which were separated by chromatography. The nature of the ring junction was supported by ^{1}H NMR analysis by analogy with studies in steroid series.[39] Compounds **18** and **19** were obtained after deprotection of **16** and **17** using MeLi in THF.[40]

The preparation of **24** starting from **20** is illustrated in Scheme 6. Robinson annelation of **20** led to the known compound **21**, which afforded **22**, the pure trans-fused ring, using the reductive alkylation procedure of Heathcock[41] or Stork.[42] After stereoselective reduction of the carbonyl function and hydrogenolysis of the benzyl protective group, **22** gave **24**.

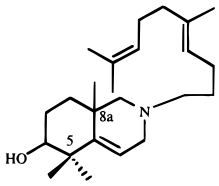
Table 1. 1,2,3,5,6,7,8,8a-Octahydroisoquinoline Derivatives



compd	R_1	R_2	R_3	R_4	R_5	mp, °C	yield, % (method)	formula[a]	OSC inh IC_{50}, μM
10	H	CH_3	CH_3	H	OH	157−162	50	$C_{12}H_{21}NO{\cdot}C_4H_4O_4$[f]	>100
28a	(*E*)-$CH(CH_3)(CH_2)_2CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	CH_3	CH_3	H	OH	oil[b]	66 (B)	$C_{25}H_{43}NO{\cdot}CH_4O_3S$[g]	0.6
28b	(*Z*)-$CH(CH_3)(CH_2)_2CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	CH_3	CH_3	H	OH	oil[c]	60 (B)	$C_{25}H_{43}NO$	6.8
28c	(*E*)-$(CH_2)_3CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	CH_3	CH_3	H	OH	118	70 (A)	$C_{24}H_{41}NO{\cdot}C_2H_2O_4$	1.0
28d	$CH(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	CH_3	CH_3	H	OH	oil[d]	30 (A)	$C_{20}H_{35}NO$	17.0
28e	*n*-$C_{12}H_{25}$	CH_3	CH_3	H	OH	106−108	(A)	$C_{24}H_{45}NO{\cdot}C_2H_2O_4$[h]	1.1
28f	*n*-$C_{14}H_{29}$	CH_3	CH_3	H	OH	53	40 (A)	$C_{26}H_{49}NO$	2.9
28g	*n*-$C_{18}H_{37}$	CH_3	CH_3	H	OH	59−62	50 (A)	$C_{30}H_{57}NO$	>100
28h	CH_2Ph	CH_3	CH_3	H	OH	88−105	(A)	$C_{19}H_{27}NO{\cdot}C_2H_2O_4$[h]	25
28i	$(CH_2)_3Ph$	CH_3	CH_3	H	OH	90	(A)	$C_{21}H_{31}NO{\cdot}C_2H_2O_4$[h]	>25
28j	(*E*)-$(CH_2)_3CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	CH_3	CH_3	OH	H	108	60 (A)	$C_{24}H_{41}NO{\cdot}C_2H_2O_4$[h]	20
28k	*n*-$C_{12}H_{25}$	H	H	H	OH	100	48 (A)	$C_{22}H_{41}NO{\cdot}C_2H_2O_4$[h]	16.4
29	(*E*)-$CO(CH_2)_2CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	CH_3	CH_3	H	OH	oil[e]	80 (C)	$C_{25}H_{39}NO_2$	0.68
TMD[i]									>25

[a] Analytical results are within ±0.4% of theoretical values unless otherwise noted. [b] $n^{20}D = 1.5180$. [c] $n^{20}_D = 1.5120$. [d] $n^{20}_D = 1.6560$. [e] $n^{20}_D = 1.5240$. [f] Fumarate. [g] Mesylate. [i] TMD = 4,4,10β-trimethyl-*trans*-decal-3β-ol.

Table 2. Optical Isomers of Compound **28c**



compd	config	mp, °C	α_D (deg)	yield, % (method)	formula[a]	OSC inh IC_{50}, μM
28l	6*S*,8a*S*	65−73	−38.4 (*c* 0.63, MeOH)	84 (A)	$C_{24}H_{41}NO{\cdot}C_2H_2O_4{\cdot}0.5H_2O$[b]	0.6
28m	6*R*,8a*R*	67−75	+38.4 (*c* 0.64, MeOH)	86 (A)	$C_{24}H_{41}NO{\cdot}C_2H_2O_4{\cdot}0.5H_2O$[b]	1.60

[a] Analytical results are within ±0.4% of theoretical values unless otherwise noted. [b] Oxalate.

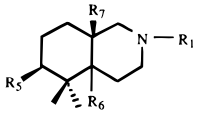
The preparation of **27** is illustrated in Scheme 7. Using the Bamford−Stevens[43,44] reaction, **26** was obtained from **22**, and a subsequent catalytic hydrogenation of **26** led to **27**.

Results and Discussion

2,3-Oxidosqualene lanosterol-cyclase inhibition was measured using rat liver microsomes and synthetic (*R*,*S*)-2,3-oxidosqualene. The inhibition potencies of our compounds, expressed as IC_{50} values, are presented in Tables 1−3. We have first examined the influence of the nature of the substituent linked to the nitrogen. The secondary amine **10** is totally devoid of inhibitory activity at a concentration as high as 100 μM. By sharp contrast, **28a** which bears a side chain which has the closest resemblance to that of the intermediate **2** is a good inhibitor with an IC_{50} of 0.6 μM. Interestingly **28b**, the *Z*-isomer of **28a**, is about 10-fold less potent. Moreover, removal of the terminal isoprenyl unit of **28a** afforded a 28-fold less active compound **28d**. Due to the presence of the methyl group carried by the side chain α to the nitrogen, **28a**,**b**,**d** are mixtures of diastereoisomers. In order to simplify the interpretation of results obtained with such compounds, we synthesized the corresponding normethylated analog of **28a**, **28c**, which is a single racemate. Its IC_{50} is 1.0 μM. A simple C_{12} alkyl straight chain with the same carbon condensation as the substituent of **28c** led to compound **28e** which has the same potency. Lengthening of the chain is detrimental for the inhibition as shown by compounds **28f**,**g**. Aryl-substituted alkyl chains are also not suitable for a good inhibition. Similar findings have already been described with other cyclase inhibitors.[25b,26a] That suggests that the active site of OSC contains a pocket which can only tolerate moieties closely related to the linear part of intermediate **2**. Interestingly, the amide **29** is as potent as the corresponding amine **28c** despite the absence of the fully charged nitrogen atom. A similar observation was made by Wannamaker *et al*. with a related series of compounds.[26a] It seems therefore possible that to mimic **2**, a full charge is not an absolute requisite and a dipole, provided by an amide or a sulfoxide,[25f] when suitably positioned could also interact with the putative group from the OSC active site that stabilizes the *pro*-C-8 carbocationic intermediate. Interestingly, the amide analog of 2-aza-2,3-dihydrosqualene, which was designed to mimic the high-energy intermediate generated in the A-ring formation, was a far less potent inhibitor than the parent compound.[21]

Then we focused our attention on the methyl groups of the isoquinoline ring. Comparison of **28e**,**k** clearly shows that substitution of the carbon C-5 is important for the inhibition, and results published for compound **1**[45] seem to indicate that the equatorial methyl plays probably the major role. On the other hand, removal of the C-8 axial methyl group in the *trans*-decahydroisoquinoline series led to a 9-fold increased inhibition (**28n** vs **28o**, Table 3).

Table 3. Decahydroisoquinoline Derivatives



compd	R_1	R_5	R_6	R_7	mp, °C	yield, % (method)	formula[a]	OSC inh IC_{50}, μM
28n	(*E*)-$(CH_2)_3CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	OH	Hα	H	97−98	72 (A)	$C_{23}H_{41}NO{\cdot}C_2H_2O_4{\cdot}0.5H_2O$[b]	0.10
28o	(*E*)-$(CH_2)_3CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	OH	Hα	CH_3	136	60 (A)	$C_{24}H_{43}NO{\cdot}C_2H_2O_4{\cdot}1.5H_2O$[b]	0.9
28p	(*E*)-$(CH_2)_3CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$	OH	Hβ	CH_3	124	61 (A)	$C_{24}H_{43}NO{\cdot}C_2H_2O_4{\cdot}H_2O$[b]	0.7
28q	*N*-$C_{12}H_{25}$	H	Hα	H	121−124	66 (A)	$C_{23}H_{45}N{\cdot}C_7H_8O_3S$[c]	>25

[a] Analytical results are within ±0.4% of theoretical values unless otherwise noted. [b] Oxalate. [c] Tosylate.

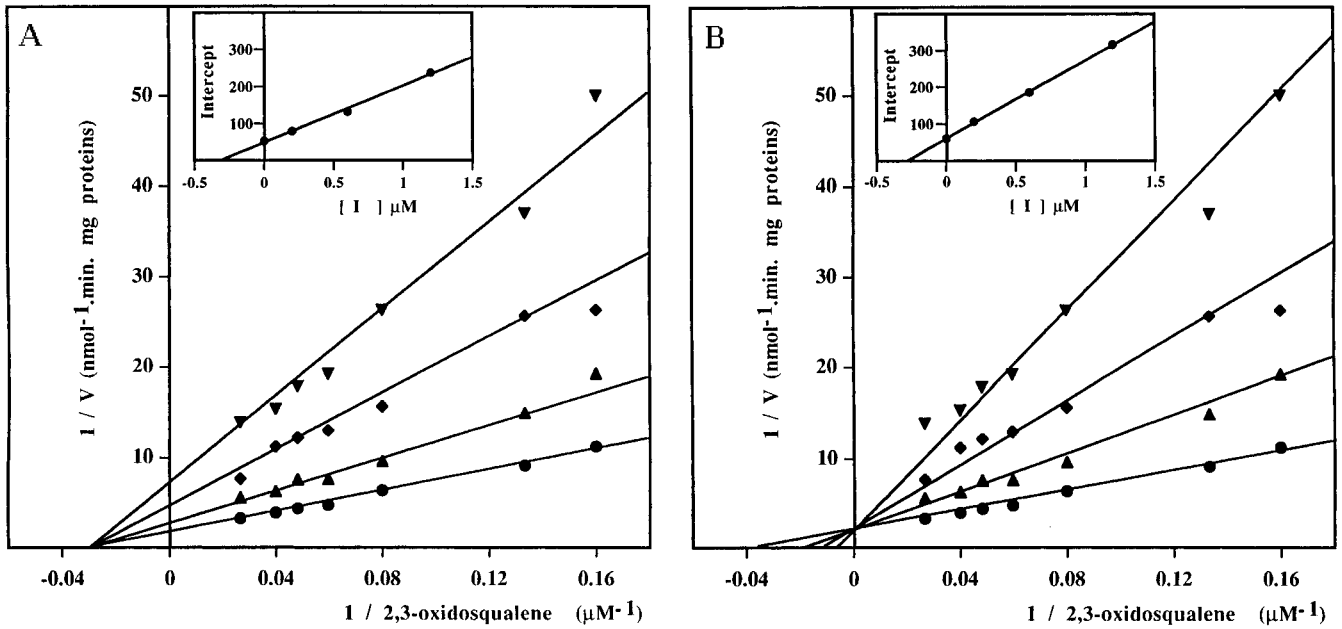


Figure 2. Lineweaver−Burk plots of the inhibition of rat microsomal oxidosqualene cyclase by different concentrations of compound **28a**. The insets show the plot of intercepts of the lines obtained in the Lineweaver−Burk plots versus the concentrations of compound **28a**. The concentrations of **28a** were (▼) 1.2, (◆) 0.6, (▲) 0.2, and (●) 0 μM. A: curves are plotted according to a noncompetitive inhibition; $K_m = 33 \pm 3.5$ μM, $V_{max} = 0.57 \pm 0.04$ nmol min^{-1} mg^{-1} of proteins, and $K_i = 0.40 \pm 0.02$ μM. B: curves are plotted according to a competitive inhibition; $K_m = 29 \pm 6$ μM, $V_{max} = 0.49 \pm 0.06$ nmol min^{-1} mg^{-1} of proteins, and $K_i = 0.30 \pm 0.04$ μM.

The nature of the ring junction has been also investigated. Comparison of compounds **28c**,**o**,**p** shows that they are equally potent.

We then considered the orientation of the hydroxyl group at position 6 with compounds **28j**,**c**. As expected, the α-isomer **28j** is 20-fold less active than the β-isomer **28c**. Finally, removal of the hydroxyl group led to **28q** (Table 3) which is totally inactive at 25 μM.

It is worth noting that all the above-mentioned data were obtained with racemic compounds. Since the mimicked intermediate **2** has the depicted absolute configuration, it was necessary to verify the importance of the absolute configuration on the inhibitory potency of our compounds. We chose to synthesize the enantiomers of **28c** as one of the best representative of intermediate **2**. The IC_{50} of **28l** (Table 2) which has the "natural" stereochemistry is 0.6 μM, whereas that of **28m** which has the "unnatural" stereochemistry is 1.6 μM. This small difference in activity between the two enantiomers was quite unexpected. Thus, the different results obtained here raise the question: Do these molecules really mimic a high-energy intermediate binding to the active site of the cyclase? The answer has to take into account several kinds of arguments that are discussed below.

Kinetic studies were undertaken to find out whether our compounds were competitive inhibitors. Using rat liver microsomal OSC, we found using Lineweaver−Burk plots (Figure 2) that the leading compound **28a**, contrary to our expectation, seems to be a noncompetitive inhibitor (K_i: 0.4 μM) (Figure 2, plot A) rather than a competitive one (Figure 2, plot B) when using the Akaike criterion test.[46] It was nevertheless difficult to distinguish between competitive and noncompetitive inhibition. However, when using the method of joint saturation (Figure 3) with increasing concentrations of substrate and inhibitor, at a fixed ratio *S*/*I*, it appears that $V_{(S+I)}$ is finite at high concentrations of substrate, albeit smaller than V_S. This result is consistent with a competitive inhibition, on account of the counterproductive competition between substrate and inhibitor, whereas with a noncompetitive inhibition, joint saturation by substrate and inhibitor will entrap all the enzyme into the unproductive ESI complex and $V_{(S+I)}$ will fall to zero.[47]

These contradictory results underline the difficulty in obtaining meaningful kinetic data, which rely on steady state kinetic equations, when dealing with membrane-associated enzymes (or integrated in micelles) and hydrophobic substrate and inhibitors. The

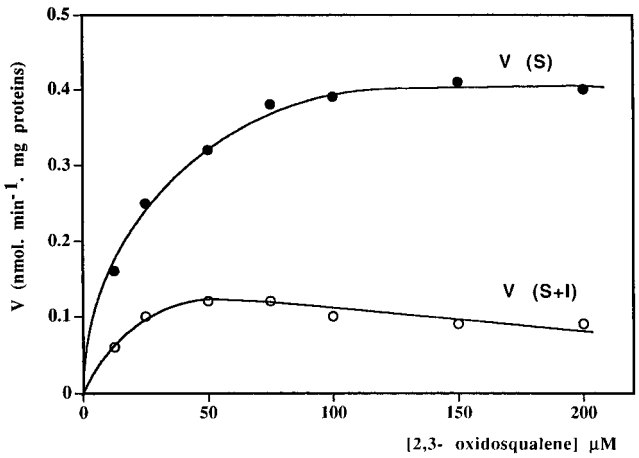


Figure 3. *V* versus [2,3-oxidosqualene] plots (using rat liver microsomal enzyme) in the absence of compound **28a** (●) and in the presence of increasing concentrations of a mixture of 2,3-oxidosqualene and compound **28a** at a fixed ratio of 60 (○).

presence of the lipid environment could have a profound effect on the apparent ability of the cyclase to bind hydrophobic compounds, whose respective amounts in the aqueous and membrane phases are determined by their partition coefficient.[48] The partitioning or micellization may affect the apparent association constants by making an unknown portion of substrate or inhibitor unavailable for binding. It is worth mentioning that it is the case with either an aqueous- or a lipid-faced enzyme active site. In such anisotropic systems, noncompetitive inhibition has been found with OSC inhibitors,[21] whereas with purified enzyme, (4α,5α,6β,8aβ)-decahydro-5,8a-dimethyl-2-(1-oxododecyl)-6-isoquinolinol, which is closely related to our series, has been found competitive with an apparent K_i of 28 nM.[26a] Furthermore, inhibitors such as **28a** designed to mimic high-energy intermediates (HEI), could also be slow-binding inhibitors with slow k_{on} and k_{off} rate constants, compared to diffusion rates, during the formation of the enzyme−inhibitor complex.[49] The observed noncompetitive behavior may therefore reflect a reduced amount of free enzyme. When the concentration of inhibitor increases, a fraction of OSC accumulates as an initial collision E−I complex, or as a more tight E−I* complex, which does not noticeably dissociate. To give support to this hypothesis, measurement of rate constants for inhibitor−enzyme formation and dissociation with purified enzyme would be necessary.

Most of the elements of the structure−activity relationships observed with the present series of compounds are in agreement with the hypothesis of mimicking **2**. Indeed, good inhibitors are obtained when the side chain on the nitrogen has a length and a stereochemistry similar to that of **2**. C-5 methyl groups appear important for activity, as is the case for the presence of a C-6 hydroxyl group with β-configuration. The negative influence of the C-8a methyl group is more difficult to explain, although it can be noticed that the corresponding methyl group of **2** is not fully axial so it does not occupy the same region of space as does the C-8a methyl of **28a**.

Results dealing with the absolute configuration of the ring system are more puzzling and could be explained as follows. If we assume that the key features for the recognition of the inhibitors by the active site of the cyclase are the oxygen of the hydroxyl group, the nitrogen of the ring and its side chain, and the equatorial C-5 methyl group, it appears from simple molecular model examination of the enantiomers **28l**,**m** that these four elements can be easily superimposed. On the other hand, the C-8a methyl groups are opposite in their orientation as are the axial C-5 methyl groups. It is interesting to note that the C-8a methyl group appears to be detrimental for activity and that the C-5 axial methyl group is not essential. Although at that point, it is difficult to conclude definitely, we do think that our results are compatible with the initially postulated hypothesis. It should also be kept in mind that, although formation of rings A and B can be rate-determining in biomimetic cyclization of 2,3-oxidosqualene analogs,[14,25f,50] we still ignore the relative heights of the energy barriers occurring along the OSC-catalyzed reaction pathway, and consequently it is hard to predict the interaction energy that could be expected by mimicking a transient intermediate such as **2**.

Conclusion

In the present study we have synthesized a series of compounds in order to investigate the structural requirements for the isoquinolinol-based inhibitors of the 2,3-oxidosqualene lanosterol-cyclase. The resulting structure−activity relationships seem to validate the mechanism of action of these inhibitors as analogs of a high-energy intermediate. In the course of this work, we discovered that besides crucial elements that were present in the initially designed compounds others are not essential, and are even detrimental, for a good inhibition. These findings could be helpful for the correct understanding of the mechanism of action of the cyclase and for the design of structurally simplified inhibitors; some examples will be described elsewhere.

Experimental Section

General Methods. Melting points were determined on a Büchi melting point apparatus and are uncorrected. IR spectra were measured on a Perkin Elmer 782 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Bruker AC 300 spectrometer using tetramethylsilane as internal reference. Structural assignments for all new compounds are consistent with their spectra. Specific rotation determinations were obtained using a Perkin Elmer 241 polarimeter. Elemental analyses were performed on a Perkin Elmer 240c apparatus. Molecular formulas followed by the symbols C,H,N indicate that elemental analyses were found to be within ±0.4% of the theoretical values for C, H, and N.

1,3,4,7,8,8a-Hexahydro-8a-methyl-2-(phenylmethyl)-6(2*H*)-isoquinolinone (5). To a solution of 1 L of 0.55 M sodium methoxide in methanol was added 101.5 g (0.5 mol) of **4**. After the mixture was stirred at room temperature for 45 min, it was cooled at 5 °C and 62.3 mL (0.75 mol) of 3-buten-2-one was added over a period of 2 h; then the mixture was stirred overnight at room temperature. Concentrated hydrochloric acid (55 mL) was added, and the mixture was evaporated. The residue was partitioned between water and ether, and the organic layer was washed with water, dried ($MgSO_4$), and concentrated. The crude material was purified by chromatography on silica gel and eluted with CH_2Cl_2−ether (9:1) to afford a solid (37.8 g, 37%): mp 106 °C; IR (KBr, 1%) ν (cm^{-1}) 1670, 1600, 1500; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 5.73 (d, J = 1.5 Hz, H_5), 3.60 (d, J = 13.4 Hz, H_{10}), 3.43 (d, J = 13.4 Hz, H_{10}), 3.02 (m, H_3), 2.72 (m, H_4), 2.63 (dd, J = 2, 11.2 Hz, H_1), 2.45 (m, H_7), 2.37 (m, H_7), 2.15 (m, H_4), 2.05 (m, H_3), 1.82 (d, J = 11.2 Hz, H_1), 1.78 (m, H_8), 1.63 (m, H_8), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 200 (C_6), 168 (C_{4a}), 124 (C_5), 67 (C_1), 63 (C_{10}), 55 (C_3), 38 (C_{8a}), 35 (C_8), 34 (C_7), 33 (C_4), 23 (C_9). Anal. ($C_{17}H_{21}NO$) C,H,N.

(3*R*)-3-Methyl-3-(3-oxobutyl)-1-(phenylmethyl)-4-piperidone [(−)-6)]. To a solution of 30 g (0.15 mol) of **4** in 300 mL of anhydrous toluene was added 23 mL (0.18 mol) of (*R*)-(+)-α-methylbenzylamine. The mixture was then stirred under reflux for 20 h with elimination of water. Toluene and excess of amine were evaporated to give 46 g of imine as a yellow oil. The crude oil was dissolved in 300 mL of anhydrous THF; then 15 mL (0.18 mol) of methyl vinyl ketone and 300 mg of hydroquinone were added. The mixture was stirred at 50 °C, sheltered from light for 2 days, and then diluted with 1 N HCl, made basic by adding 1 N NaOH, and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The crude material was chromatographed with toluene−AcOEt (9:1) to give 27.6 g (68%) of **(−)-6** as a yellow oil: $[\alpha]^{20}_D = -52.7°$ (c = 1.4, $CHCl_3$); IR (neat) ν (cm^{-1}) 1720, 1600, 1500; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5H), 3.59 (d, J = 13.2 Hz, 1H), 3.50 (d, J = 13.2 Hz, 1H), 2.89 (m, 1H), 2.58 (m, 3H), 2.37 (m, 1H), 2.24 (m, 4H), 2.11 (s, 3H), 1.72 (m, 1H), 0.99 (s, 3H).

(3*S*)-3-Methyl-3-(3-oxobutyl)-1-(phenylmethyl)-4-piperidone [(+)-6]. Following the procedure described above, **4** was reacted with (*S*)-(−)-α-methylbenzylamine and then with methyl vinyl ketone to give **(+)-6** as an oil (75%): $[\alpha]^{20}_D = +47.7°$ (c = 0.77, $CHCl_3$).

(8a*S*)-1,3,4,7,8,8a-Hexahydro-8a-methyl-2-(phenylmethyl)-6(2*H*)-isoquinolinone [(+)-5]. To a solution of 26 g (0.09 mol) of **(−)-6** in 100 mL of anhydrous methanol was added 200 mL of MeONa (8% in methanol). The mixture was stirred at 50 °C overnight and then diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The crude material was purified by chromatography with toluene−AcOEt (8:2) to afford 19.7 g (81%) of **(+)-5** as a light yellow powder: mp 87 °C; $[\alpha]^{20}_D = +209.2°$ (c = 0.81, $CHCl_3$).

(8a*R*)-1,3,4,7,8,8a-Hexahydro-8a-methyl-2-(phenylmethyl)-6(2*H*)-isoquinolinone [(−)-5]. Following the procedure described above, **(−)-5** was obtained (83%): mp 87 °C; $[\alpha]^{20}_D = -206.7°$ (c = 0.8, $CHCl_3$).

3,4,6,7,8,8a-Hexahydro-8a-methyl-6-oxo-2(1*H*)-isoquinolinecarboxylic Acid Phenylmethyl Ester (7b). Method D. A stirred mixture of 35 g (0.14 mol) of **5**, 99 mL (0.7 mol) of phenylmethyl chloroformate, and 28.6 g (0.2 mol) of potassium carbonate in 300 mL of chloroform was refluxed overnight; it was cooled and filtered. The filtrate was washed with water and dried ($MgSO_4$). Evaporation of solvent under reduced pressure afforded **7b** as a solid (36 g, 87%): mp 128 °C; IR (KBr, 1%) ν (cm^{-1}) 1710, 1675; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.37 (m, 5H), 5.77 (s, H_5), 5.19 (d, J = 12.3 Hz, 1H), 5.14 (d, J = 12.3 Hz, 1H), 4.38 (m, 1H), 4.05 (m, 1H), 2.83 (m, 1H), 5.65−2.39 (m, 4H), 2.23 (m, 1H), 1.78 (m, 2H), 1.24 (m, 3H).

3,4,6,7,8,8a-Hexahydro-8a-methyl-6-oxo-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester (7a). Following the procedure described above, **5** was reacted with ethyl chloroformate to give **7a** as a solid (90%): mp 98 °C; IR (KBr, 1%) ν (cm^{-1}) 1690, 1660, 1620, 1480; ^1H NMR (300 MHz, $CDCl_3$) δ 5.80 (s, H_5), 4.35 (m, H_3), 4.18 (m, H_{11}, H_{11}), 4.05 (m, H_1), 2.84 (m, H_3), 2.62 (m, H_4, H_7), 2.53 (m, H_1), 2.49 (m, H_4), 2.24 (m, H_7), 1.83 (m, H_8, H_8), 1.29 (m, 3H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 198.6 (C_6), 165.3 (C_{10}), 155.5 (C_{4a}), 125.2 (C_5), 61.6 (C_{11}), 55.5 (C_1), 44.1 (C_3), 37.2 (C_{8a}), 33.6 (C_4, C_8), 31.6 (C_7), 21.2 (C_{12}), 14.7 (C_9). Anal. ($C_{13}H_{19}NO_3$) C,H,N.

(8a*S*)-3,4,6,7,8,8a-Hexahydro-8a-methyl-6-oxo-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester [(+)-7a]. Following method D, **(+)-5** was reacted with ethyl chloroformate to give **(+)-7a** as a white solid (73%): mp 106 °C; $[\alpha]^{19}_D = +200.0°$ (c = 0.79, $CHCl_3$).

(8a*R*)-3,4,6,7,8,8a-Hexahydro-8a-methyl-6-oxo-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester [(−)-7a]. Following method D, **(−)-5** was reacted with ethyl chloroformate to give **(−)-7a** as a white solid (90%): mp 106 °C; $[\alpha]^{19}_D = -202.0°$ (c = 0.87, $CHCl_3$).

3,5,6,7,8,8a-Hexahydro-6-oxo-5,5,8a-trimethyl-2(1*H*)-isoquinolinecarboxylic Acid Phenylmethyl Ester (8b). Method E. To a solution of 35 g (0.12 mol) of **7b** in 300 mL of *tert*-butyl alcohol was added 39.4 g (0.35 mol) of potassium *tert*-butoxide. The stirred mixture was heated at 50 °C for 1 h; then a solution of 44 mL (0.7 mol) of methyl iodide in 50 mL of *tert*-butyl alcohol was added. After addition, the mixture was heated at 50 °C for a further 1 h and then allowed to cool at room temperature overnight. The mixture was poured onto water and extracted with ethyl acetate. The extracts were washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography eluting with toluene−AcOEt (9:1) to give **8b** as an oil (23.6 g, 62%): n_D^{31} 1.5375; IR (neat) ν (cm^{-1}) 1710; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 5H), 5.56 (s) and 5.48 (total 1H), 5.18 (s, 2H), 4.34 (m, 1H), 3.90 (dd, J = 12.5, 34.9 Hz, 1H), 3.71 (t, J = 15.5 Hz, 1H), 2.73 (dd, J = 11.9, 18.8 Hz, 1H), 2.56−2.43 (m, 2H), 1.77 (m, 2H), 1.25 (s, 6H), 1.10 (s) and 1.07 (s) (total 3H).

3,5,6,7,8,8a-Hexahydro-6-oxo-5,5,8a-trimethyl-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester (8a). This compound was obtained from **7a** as described above (65%): mp 65−67 °C; IR (KBr, 1%) ν (cm^{-1}) 1710; ^1H NMR (300 MHz, $CDCl_3$) δ 5.53 (d, J = 17.5 Hz, H_4), 4.31 (m, 1H), 4.19 (m, 2H), 3.87 (d, J = 12.4, 4.0 Hz, 1H), 3.68 (dd, J = 7.8, 18.3 Hz, 1H), 2.62 (m, 1H), 2.57 (m, 1H), 2.45 (m, 1H), 1.77 (m, 2H), 1.30 (t, J = 7.2 Hz, 3H), 1.26 (s, 6H), 1.09 (s, 3H). Anal. ($C_{15}H_{23}NO_3$) C,H,N.

(8a*S*)-3,4,6,7,8,8a-Hexahydro-6-oxo-5,5,8a-trimethyl-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester [(−)-8a]. Following method E **(−)-8a** was obtained from **(+)-7a** as a white solid (68%): mp 61 °C; $[\alpha]^{19}_D = -42.6°$ (c = 0.93, $CHCl_3$).

(8a*R*)-3,4,6,7,8,8a-Hexahydro-6-oxo-5,5,8a-trimethyl-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester [(+)-8a]. **(+)-8a** was prepared from **(−)-7a** according to method E: mp 61 °C; $[\alpha]^{19}_D = +42.5°$(c = 0.85, $CHCl_3$).

1,3,5,7,8,8a-Hexahydro-5,5,8a-trimethyl-6(2*H*)-isoquinolinone (9). To a solution of 2.7 g (10.3 mmol) of **8a** in 25 mL of CH_3CN were added 4.6 g (30.7 mmol) of sodium iodide and 2.6 mL (20.5 mmol) of trimethylsilyl chloride. The mixture was stirred under reflux for 3 days and concentrated. The residue was diluted with 1 N HCl and extracted with ethyl acetate. The aqueous layer was made basic by adding 10 N NaOH and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The crude material was purified by chromatography with CH_2Cl_2−MeOH−NH_4OH (8:2:0.5) to give 1.62 g (83%) of **9** as a white crystals: IR (neat) ν (cm^{-1}) 3300, 1715; ^1H NMR (300 MHz, $CDCl_3$) δ 5,57 (t, J = 3.1 Hz, H_4), 3.44 (d, J = 1 Hz, H_3, H_3), 3.17 (m large, NH), 2.80 (d, J = 12.4 Hz, H_1), 2.61 (d, J = 12.4 Hz, H_1), 2.59 (ddd, J = 17.9, 7.1, 10.3 Hz, H_7), 2.52 (ddd, J = 17.9, 7.7, 3.7 Hz, H_7), 1.76 (ddd, J = 13.4, 10.3, 7.7 Hz, H_8), 1.65 (ddd, J = 13.4, 7.1, 3.7 Hz, H_8), 1.25 (s, 6H), 1.14 (s, 3H).

(8a*R*)-1,3,5,7,8,8a-Hexahydro-5,5,8a-trimethyl-6(2*H*)-isoquinolinone [(+)-9]. Following the same procedure as above, **(+)-9** was obtained from **(+)-8a** as a colorless oil (82%): $[\alpha]^{19}_D = +0.38°$ (c = 1.03, $CHCl_3$).

(8a*S*)-1,3,5,7,8,8a-Hexahydro-5,5,8a-trimethyl-6(2*H*)-isoquinolinone [(−)-9]. Following the same procedure as above **(−)-9** was obtained from **(−)-8a** as a colorless oil (80%): $[\alpha]^{19}_D = -0.36°$ (c = 1.15, $CHCl_3$).

(6*β*,8a*β*)-1,2,3,5,6,7,8,8a-Octahydro-5,5,8a-trimethyl-6-isoquinolinol (10). Method F. To a solution of 1.43 g (7.4 mmol) of **9** in 100 mL of anhydrous methanol at −20 °C was added 370 mg (9.8 mmol) of $NaBH_4$. The mixture was stirred at −20 °C for 1 h and concentrated. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The product was purified by recrystallization of the fumarate salt in methanol−diisopropyl ether to afford 700 mg (50%) of the amine **10** as a white powder: mp 146 °C; IR (KBr, 1%) ν (cm^{-1}) 3350, 1470; ^1H NMR (300 MHz, $CDCl_3$) δ 5.53 (dd, J = 2.6, 3.4 Hz, H_4), 3.47 (dd, J = 2.6, 24.5 Hz, H_3), 3.39 (dd, J = 3.4, 24.5 Hz, H_3), 3.26 (dd, J = 4.8, 11.2 Hz, H_6), 2.64 (d, J = 12.2 Hz, H_1), 2.44 (d, J = 12.2 Hz, H_1), 1.84−1.70 (m, H_7, H_7, OH, NH), 1.44 (dt, J = 13.2, 3.4 Hz, H_8), 1.24 (s, 3H), 1.21 (m, H_8), 1.14 (s, 3H), 1.05 (s, 3H). Anal. ($C_{12}H_{21}NO$) C,H,N.

(6α*R*,8aα*R*)-1,2,3,5,6,7,8,8a-Octahydro-5,5,8a-trimethyl-6-isoquinolinol [(+)-10]. Following method F **(+)-10** was obtained from **(+)-9** as a white powder (46%): mp 144 °C; $[\alpha]^{19}_D = +102.6°$ (c = 0.71, $CHCl_3$).

(6*βS*,8a*βS*)-1,2,3,5,6,7,8,8a-Octahydro-5,5,8a-trimethyl-6-isoquinolinol [(−)-10]. Following method F **(−)-10** was prepared from **(−)-9** (47%, white powder): mp 144 °C; $[\alpha]^{19}_D = -102.8°$ ($c = 0.73$, $CHCl_3$).

(6α,8a*β*)-3,5,6,7,8,8a-Hexahydro-6-hydroxy-5,5,8a-trimethyl-2(1*H*)-isoquinolinecarboxylic Acid Phenylmethyl Ester (11a) and (6*β*,8a*β*)-3,5,6,7,8,8a-Hexahydro-6-hydroxy-5,5,8a-trimethyl-2(1*H*)-isoquinolinecarboxylic Acid Phenylmethyl Ester (11b). A solution of 7.76 g (23.7 mmol) of **8b** in 100 mL of 2-propanol with 9.8 g (48 mmol) of aluminum isopropoxide was stirred and heated for 7 h. The mixture was brought to dryness, and the resulting residue was stirred with 150 mL of 1 N HCl for 0.5 h and then extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography with cyclohexane−AcOEt (7.5:2.5) of the residue gave the 6*β*-hydroxy derivative **11b** as an oil (3 g, 38%). Further elution provided the 6α-hydroxy derivative **11a** as an oil (2.4 g, 30%).

11a: IR (neat) ν (cm^{-1}) 3485, 1700; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 5.47 (m) and 5.40 (m) (total 1H), 5.16 (s, 2H), 4.30 (m, 1H), 3.88−3.70 (m, 2H), 3.57 (m, 1H), 2.67 (dd, J = 12.9, 16.9 Hz, 1H), 2.14 (m, 1H), 1.67 (m, 2H), 1.25−0.83 (m, 11H).

11b: IR (neat) ν (cm^{-1}) 3480, 1700; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 5.53 (m) and 5.46 (m) (total 1H), 5.16 (s, 2H), 4.30 (m, 1H), 3.88−3.65 (m, 2H), 3.28 (m, 1H), 2.54 (dd, J = 13.0, 18.1 Hz, 1H), 1.80 (m, 2H), 1.53 (m, 1H), 1.27 (d, J = 10.4 Hz, 1H), 1.19−1.15 (m, 7H), 1.03 (m, 3H).

(6α,8a*β*)-1,2,3,5,6,7,8,8a-Octahydro-5,5,8a-trimethyl-6-isoquinolinol (12). A mixture of 2.1 g (6.37 mmol) of **11a** in 3 mL of acetic acid with a catalytic amount of 10% palladium on carbon was stirred under 1 atm of H_2 pressure for 8 h. The catalyst was filtered off and the filtrate evaporated. The resulting residue was partitioned between 15% aqueous sodium hydroxide solution and ethyl acetate. The extracts were washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to afford **12** as a solid (0.89 g, 72%): mp 136 °C; IR (KBr) ν (cm^{-1}) 3185; ^1H NMR (300 MHz, $CDCl_3$) δ 5.48 (s, 1H), 3.55−3.23 (m, 3H), 2.60 (m, 2H), 2.19−2.02 (m, 3H), 1.68−1.53 (m, 2H), 1.35−0.84 (m, 10H).

(6*β*,8a*β*)-3,5,6,7,8,8a-Hexahydro-6-hydroxy-8a-methyl-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester (13). A solution of 12 g (0.05 mol) of **7a** in 125 mL of isopropenyl acetate and 1 mL of concentrated H_2SO_4 was heated at 110 °C while acetone, which was produced, was slowly distillated for 2 h. After cooling, the solution was evaporated to give a black oil which was used without purification. This oil was dissolved in 170 mL of ethanol, and the stirred solution was cooled to −10 °C; then 2.83 g (75 mmol) of sodium borohydride was added portionwise. After completion of the addition, the ice bath was removed and the reaction mixture was stirred at room temperature for 24 h. Solvent was evaporated, and the residue was partitioned between water and ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$), and evaporated to give an oil. Purification by silica gel column chromatography with toluene−AcOEt (8:2) gave 4.95 g (47%) of a mixture (6:4) of 3,5,6,7,8,8a-hexahydro-6*β*-hydroxy-8a-methyl-2(1*H*)-isoquinolinecarboxylic acid ethyl ester and 3,4,6,7,8,8a-hexahydro-6*β*-hydroxy-8a-methyl-2(1*H*) isoquinolinecarboxylic acid ethyl ester. This mixture was dissolved in 100 mL of ethanol with 1 mL of concentrated HCl, and the solution was refluxed for 2 h. After cooling, solvent was evaporated and the resulting residue was purified by silica gel column chromatography with toluene−AcOEt (7:3) to give **13** as a white powder (2 g, 40%): mp 78−80 °C; IR (KBr) ν (cm^{-1}) 3400, 1700, 1440; ^1H NMR (300 MHz, $CDCl_3$) δ 5.32 (d, J = 10.3 Hz, 1H), 4.17 (m, 3H), 3.96 (d, J = 12.8 Hz) and 3.82 (d, J = 12.8 Hz) (total 1H), 3.56 (m, 2H), 2.60 (m, 1H), 2.39 (m, 1H), 2.16 (m, 1H), 1.90 (m, 1H), 1.58 (m, 3H), 1.26 (m, 4H), 1.10 (s, 3H).

(6*β*,8a*β*)-1,2,3,5,6,7,8,8a-Octahydro-8a-methyl-6-isoquinolinol (14). A solution of 1.8 g (7.7 mmol) of **13** in 20 mL of THF was cooled to 0 °C, and 17.5 mL (38 mmol) of 2.2 M methyllithium−lithium bromide complex in ether were carefully added. The reaction mixture was stirred at 0 °C for 2 h and then poured onto 50 mL of saturated ammonium chloride aqueous solution. The aqueous solution was extracted with ethyl acetate, and the organic layer was evaporated to give **14** as a brown oil (700 mg, 56%): IR (NaCl) ν (cm^{-1}) 3425, 1625, 1460; ^1H NMR (300 MHz, $CDCl_3$) δ 5.41 (m, H_4), 3.54 (m, H_6), 3.23 (m, H_3, H_3), 2.76 (d, J = 12.6 Hz, H_1), 2.47 (d, J = 12.6 Hz, H_1), 2.32 (m, H_5), 2.16 (m, H_5), 1.88 (m, H_7, OH, NH), 1.60 (m, H_7), 1.47 (m, H_8), 1.22 (m, H_8), 1.13 (s, 3H).

(6*β*,8a*β*)-3,5,6,7,8,8a-Hexahydro-6-hydroxy-5,5,8a-trimethyl-2(1*H*)isoquinolinecarboxylic Acid Ethyl Ester (15). Following method F **15** was obtained from **8a** as a colorless oil (80%): IR (neat) ν (cm^{-1}) 3480, 1700; ^1H NMR (300 MHz, $CDCl_3$) δ 5.40 (d, J = 13.3 Hz, 1H), 4.17 (m, 1H), 4.09 (m, 2H), 3.70 (m, 1H), 3.60 (m, 1H), 3.18 (dd, J = 4.5, 11.2 Hz, 1H), 2.38 (m, 1H), 2.30 (s, OH), 1.79−1.64 (m, 2H), 1.44 (m, 1H), 1.20 (m, 3H), 1.16 (m, 1H), 1.09 (s, 6H), 0.95 (s, 3H).

(4aα,6*β*,8a*β*)-Octahydro-6-hydroxy-5,5,8a-trimethyl-2-(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester (16) and (4a*β*,6*β*,8a*β*)-Octahydro-6-hydroxy-5,5,8a-trimethyl-2(1*H*)-isoquinolinecarboxylic Acid Ethyl Ester (17). To a solution of 3.07 g (11.5 mmol) of **15** in 15 mL of ethyl acetate were added 40 mL of acetic acid and 620 mg of platinum oxide. The mixture was stirred under 1 atm of H_2 at room temperature for 8 h. The catalyst was filtered and the filtrate evaporated to give a mixture of **16** and **17**. They were separated by chromatography with toluene−AcOEt (8:2) to afford 1.3 g of **16** (42%) and 1.6 g of **17** (52%) as colorless oils.

16: IR (neat) ν (cm^{-1}) 3500, 1750, 1700, 1450; ^1H NMR (300 MHz, $CDCl_3$) δ 4.35 (m, 1H), 4.13 (m, 2H), 3.75 (m, 1H), 3.27 (dt, J = 10.7, 5.5 Hz, 1H), 2.65 (m, 1H), 2.33 (m, 1H), 1.72 (m, 2H), 1.55 (m, 2H), 1.45 (m, 1H), 1.36 (d, J = 5.5 Hz, OH), 1.27 (m, 3H), 1.17 (m, 1H), 0.99 (s, 3H), 0.95 (s, 3H), 0.78 (s, 3H). Anal. ($C_{15}H_{27}NO_3$) C,H,N.

17: IR (neat) ν (cm^{-1}) 3500, 1750, 1700, 1440; ^1H NMR (300 MHz, $CDCl_3$) δ 4.12 (m, 3H), 3.56 (m, 2H), 2.63 (m, 2H), 1.75 (m, 3H), 1.65 (m, 1H), 1.62 (s, 1H, OH), 1.53 (m, 1H), 1.31 (m, 2H), 1.27 (m, 3H), 1.11 (s, 3H), 1.07 (s, 3H), 1.01 (s, 3H). Anal. ($C_{15}H_{27}NO_3$) C,H,N.

(4aα,6*β*,8a*β*)-Decahydro-5,5,8a-trimethyl-6-isoquinolinol (18). To a solution of 740 mg (2.75 mmol) of **16** in 10 mL of anhydrous THF was added, at 0 °C, 9.6 mL (13.4 mmol) of 1.4 M methyllithium in ether. The mixture was stirred at 0 °C for 1 h and concentrated. The residue was diluted with 5 mL of water and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated to afford **18** (540 mg, 99%) as a light yellow oil: ^1H NMR (300 MHz, $CDCl_3$) δ 3.26 (m, 1H), 3.20 (m, 1H), 2.58 (m, 1H), 2.52 (d, J = 11.9 Hz, 1H), 2.25 (d, J = 11.9 Hz, 1H), 1.80−1.55 (m, 2H), 1.47 (m, 2H), 1.36 (m, 1H), 1.26 (m, 1H), 1.23 (d, J = 6.2 Hz, 1H, OH), 1.14 (m, 1H), 1.07 (s, 3H), 0.96 (s, 3H), 0.93 (s, 1H), 0.79 (s, 3H).

(4a*β*,6*β*,8a*β*)-Decahydro-5,5,8a-trimethyl-6-isoquinolinol (19). Following the procedure described above, **19** was obtained from **17** (100%) as a light yellow oil: ^1H NMR (300 MHz, $CDCl_3$) δ 3.08 (m, 1H), 2.46 (s, 2H), 2.44 (m, 1H), 2.03 (m, 1H), 1.78−1.54 (m, 5H), 1.40 (m, 1H), 1.27 (m, 2H), 1.10 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H).

1,3,4,7,8,8a-Hexahydro-5-methyl-2-(phenylmethyl)-6-(2*H*)-isoquinolinone (21). To a solution of 15 g (79 mmol) of **20** in 70 mL of anhydrous toluene was added 10 mL (120 mmol) of pyrrolidine. The mixture was stirred under reflux for 20 h with elimination of water. Toluene and excess of amine were removed under reduced pressure. The residue was diluted in 70 mL of anhydrous toluene, and then 7.9 mL (79 mmol) of ethyl vinyl ketone and 100 mg (0.9 mmol) of hydroquinone were added. The mixture was stirred under reflux, sheltered from light, for 20 h. Then 200 mg (3.7 mmol) of sodium methoxide was added, and the mixture was stirred under reflux for 2 h. The solution was diluted with a saturated solution of ammonium chloride, made basic by adding NaOH, and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The crude material was chromatographed with toluene−AcOEt (7:3) to afford 15.3 g (76%) of **21** as a yellow oil which crystallized in pentane: mp 70 °C (lit.[19] mp 67 °C); IR (neat) ν (cm^{-1}) 1720, 1680, 1660, 1600, 1570, 1560, 750, 700; ^1H NMR (300 MHz,

$CDCl_3$) δ 7.31 (m, 5H, Ph), 5.57 (d, J = 13.1 Hz, H_{10}), 3.51 (d, J = 13.1 Hz, H_{10}), 3.03 (m, H_1, H_3), 2.77 (dt, J = 10, 1, 2.4 Hz, H_4), 2.62 (m, H_{8a}), 2.47 (dt, J = 16.1, 4.0 Hz, H_7), 2.30 (m, H_4, H_7), 2.05 (ddd, J = 2.9, 11.0, 13.8 Hz, H_3), 1.91 (m, H_8), 1.79 (s, H_9, $H_{9'}$, $H_{9''}$), 1.74 (d, J = 11.0 Hz, H_1), 1.52 (m, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.2 (C_6), 156.1 (C_5), 138.2 (C_4), 129.4 (C_{11}), 129.0 (C_{12}, C_{16}), 128.2 (C_{13}, C_{15}), 127.2 (C_{14}), 62.6 (C_{10}), 60.4 (C_1), 53.2 (C_3), 37.8 (C_{8a}), 36.7 (C_7), 30.5 (C_4), 26.0 (C_8), 10.5 (C_9). Anal. ($C_{17}H_{21}NO$) C,H,N.

(4aα,8aβ)-Octahydro-5,5-dimethyl-2-(phenylmethyl)-6(2*H*)-isoquinolinone (22). To 250 mL of freshly distilled ammonia was added 0.5 g (71.4 mmol) of lithium at −60 °C. A solution of 5 g (19.6 mmol) of **21** in 80 mL of THF was added dropwise. The mixture was stirred for 0.5 h, and 20 mL (321 mmol) of methyl iodide was added. Ammonia was evaporated, and the mixture was stirred at room temperature for 2 days. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The crude material was purified by chromatography with toluene−AcOEt (7:3) to give 2.1 g (50%) of **22** as a yellow solid: mp 84 °C; IR (KBr, 1%) ν (cm^{-1}) 1710, 1500, 1460, 750, 700; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H, Ph), 3.54 (d, J = 13.1 Hz, H_{11}), 3.47 (d, J = 13.1 Hz, H_{11}), 2.99 (dm, J = 10.5 Hz, H_3), 2.92 (dm, J = 10.9 Hz, H_1), 2.63 (dt, J = 6.3, 14.0 Hz, H_7), 2.27 (ddd, J = 2.3, 4.5, 14.0 Hz, H_7), 2.00−1.79 (m, H_3, H_{8a}, H_8), 1.63−1.55 (m, H_1, H_4, H_4), 1.30 (m, H_8), 1.09 (m, H_{4a}), 1.07 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.7 (C_6), 138.3 (C_{12}), 129.1 (C_{13}, C_{17}), 128.2 (C_{14}, C_{16}), 127.0 (C_{15}), 63.2 (C_{11}), 60.0 (C_1), 53.8 (C_3), 50.4 (C_{4a}), 47.5 (C_5), 37.4 (C_7), 35.3 (C_{8a}), 30.9 (C_8), 25.7 (C_4), 21.6−20.36 (C_9, C_{10}). Anal. ($C_{18}H_{25}NO$) C,H,N.

(4aα,6β,8aβ)-Decahydro-5,5-dimethyl-2-(phenylmethyl)-6-isoquinolinol (23). Following method F, **23** was obtained starting from **22** as a solid (95%): mp <40 °C; IR (neat) ν (cm^{-1}) 3400, 1600, 1470, 1460; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 5H, Ph), 3.50 (d, J = 13.2 Hz, H_{11}), 3.45 (d, J = 13.2 Hz, H_{11}), 3.23 (dd, J = 4.2, 11.5 Hz, H_6), 2.97 (dm, J = 11.0 Hz, H_3), 2.81 (dm, J = 8.8 Hz, H_1), 1.87 (dt, J = 2.3, 11.0 Hz, H_3), 1.75−1.32 (m, H_7, H_4, H_{8a}, H_1, $H_{7'}$, $H_{4'}$, H_8, OH), 1.01 (m, H8), 0.96 (s, 3H), 0.77 (s, 3H), 0.68 (m, H_{4a}). Anal. ($C_{18}H_{27}NO{\cdot}0.25H_2O$) C,H,N.

(4aα,6β,8aβ)-Decahydro-5,5-dimethyl-6-isoquinolinol (24). To a solution of 1.5 g (5.5 mmol) of **23** in 40 mL of methanol were added 400 mg of 5% Pd/C and 35 μL (6 mmol) of acetic acid. The mixture was stirred under 50 psi of H_2 pressure at 35 °C for 6 h. The catalyst was filtered and the filtrate evaporated. The residue was diluted with NaOH and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to give 0.81 g (81%) of **24** as a light yellow solid: mp 108 °C; IR (KBr, 1%) ν (cm^{-1}) 3400, 3250, 3100, 1450; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.23 (dd, J = 4.5, 11.6 Hz, H_6), 3.14 (dm, J = 12.0 Hz, H_3), 2.98 (ddd, J = 1.1, 3.9, 11.9 Hz, H_1), 2.53 (dt, J = 3.3, 12.0 Hz, H_3), 2.16 (dd, J = 10.6, 11.9 Hz, H_1), 1.75−1.64 (m, H_7, H_4, OH), 1.64−1.53 (m, $H_{7'}$), 1.46 (m, H_8), 1.32 (m, H_{8a}), 1.22 (m, H_4), 1.02 (m, $H_{8'}$), 0.96 (s, 3H), 0.82 (m, H_{4a}), 0.77 (s, 3H).

(4aα,8aβ)-5,5-Dimethyl-1,3,4,4a,5,7,8,8a-octahydro-2-(phenylmethyl)-6(2*H*)-isoquinolinone *N*-[(4-Methylphenyl)sulfonyl]hydrazone (25). To a solution of 15.4 g (56.7 mmol) of **22** in 100 mL of acetic acid was added 11.6 g (62.3 mmol) of *N*-[(4-methylphenyl)sulfonyl]hydrazine portionwise. The mixture was heated at 50 °C for 1 h and then allowed to be stirred for 24 h at room temperature. Acetic acid was evaporated, and the resulting residue was partitioned between ethyl acetate and 1 N NaOH. The extracts were concentrated, and the residue was purified by chromatography with toluene−acetone (7.5:2.5) to give **25** as a solid (7.85 g, 31%): mp 173 °C; IR (KBr) ν (cm^{-1}) 3210, 1600, 1500, 1325, 1160; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 8.3 Hz, 2H), 7.29 (m, 7H), 7.14 (s, NH), 3.46 (dd, J = 13.1, 19.8 Hz, 2H), 2.97 (d, J = 10.6 Hz, 1H), 2.82 (d, J = 9.9 Hz, 1H), 2.55 (m, 1H), 2.44 (s, 3H), 1.99−1.41 (m, 7H), 1.07 (s, 3H), 1.03−0.81 (m, 5H).

(4aα,8aβ)-5,5-Dimethyl-1,2,3,4,4a,5,8,8a-octahydro-2-(phenylmethyl)isoquinoline (26). A solution of 7.63 g (17.3 mmol) of **25** in 150 mL of toluene was cooled at 0 °C, and 2.4 g (80 mmol) of NaH (80% dispersion in mineral oil) was added portionwise. After completion of the addition, the mixture was refluxed for 4 h. The mixture was cooled, poured onto water, and then extracted with ethyl acetate. The organic phases were washed with water, dried ($MgSO_4$), and evaporated. The oil was purified by chromatography with toluene−acetone (9.5:0.5) to give the compound **26** as an oil (3.2 g, 72.7%): IR (NaCl) ν (cm^{-1}) 1500, 730, 700; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 5.48 (m, 1H), 5.36 (m, 1H), 3.48 (s, 2H), 3.11 (m, 2H), 1.92 (m, 2H), 1.78−1.39 (m, 5H), 1.02 (m, 1H), 0.96 (s, 3H), 0.88 (s, 3H).

(4aα,8aβ)-Decahydro-5,5-dimethylisoquinoline (27). To 35 mL of ethanol in a Parr hydrogenation bottle were added 2.85 g (11.1 mmol) of **26**, 250 mg of 10% palladium on carbon, and 0.7 mL of acetic acid. The mixture was hydrogenated under 45 psi of hydrogen pressure at 50 °C for 6 h. The mixture was filtered and the filtrate evaporated. The residue was partitioned between 1 N NaOH and ethyl acetate; the organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated to give **27** as an oil (1.4 g, 75%): IR (NaCl) ν (cm^{-1}) 3270, 1460; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.15 (dm, J = 12 Hz, 1H), 3.04 (s, NH), 2.96 (dd, J = 2.9, 11.9 Hz, 1H), 2.59 (dt, J = 2.7, 12.2 Hz, 1H), 2.21 (t, J = 10.9 Hz, 1H), 1.64 (dm, J = 10.9 Hz, 1H), 1.55−1.30 (m, 5H), 1.26−1.12 (m, 3H), 0.86 (m, 4H), 0.82 (s, 3H).

(6β,8aβ)-2-Dodecyl-1,2,3,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-6-isoquinolinol (28e). Method A. A solution of 0.5 g (2.5 mmol) of **9** (as a base), 0.7 g (2.7 mmol) of dodecyl bromide, 0.7 g of potassium carbonate, and a catalytic amount of sodium iodide in 20 mL of 4-methyl-2-pentanone was refluxed for 12 h. After cooling, the mixture was filtrated, the filtrate concentrated under reduced pressure, and the residue purified by chromatography with isopropyl ether to give an oil which was converted into oxalate and recrystallized from ethanol−ether to give **28e** (0.5 g, 50%): mp 106−108 °C. Anal. ($C_{24}H_{45}NO{\cdot}C_2H_2O_4$) C,H,N.

(6β,8aβ)-2-(1,5,9-Trimethyl-(*E*)-4,8-decadienyl)-1,2,3,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-6-isoquinolinol (28a). Method B. Acetic acid was added to a solution of 9.8 g (50 mmol) of **9** in 100 mL of methanol in order to adjust the pH of the mixture to 7.6; then 3.5 g (55 mmol) of sodium cyanoborohydride was added. After the mixture stirred at room temperature for 3 days, the reaction was quenched with HCl. Then the mixture was evaporated under reduced pressure. The residue was partitioned between 10 N NaOH and ether. The organic layer was washed with water, dried ($MgSO_4$), and evaporated to give an oil which was purified by flash chromatography with CH_2Cl_2−ether (8:2). The resulting oil was treated with a stoichiometric amount of methanesulfonic acid in ether, and the mixture was evaporated and then lyophilized to give **28a** (15.5 g, 66%) as an oil. Anal. ($C_{25}H_{43}NO{\cdot}CH_4O_3S$) C,H,N.

(6β,8aβ)-2-(5,9-Dimethyl-(*E*)-4,8-decadienoyl)-1,2,3,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-6-isoquinolinol (29). Method C. To a solution of 1 g (5.1 mmol) of 5,9-dimethyl-(*E*)-4,8-decadienoic acid in 20 mL of THF was added 1 g (6.7 mmol) of carbonyldiimidazole. The resulting mixture was cooled to 0 °C, and a solution of 1 g (5 mmol) of **9** in 5 mL of THF was added. The mixture was stirred at room temperature overnight. Solvent was evaporated, and the residue was taken up with water and ether. The organic layer was washed with water, dried ($MgSO_4$), and evaporated. The crude residue was purified by flash chromatography with CH_2Cl_2 as eluent to afford **29** as an oil (1.4 g, 80%): n_D^{20} 1.5240. Anal. ($C_{25}H_{35}NO_2$) C,H,N.

Preparation of Rat Liver Microsomal 2,3-Oxidosqualene Cyclase. Male Wistar rats weighing 250 g were used. Food was withdrawn 4 h before animals were killed. The livers were quickly removed, minced, and homogenized in ice cold 0.25 M sucrose in a Potter-Elvehjem type Teflon homogenizer at 2000 rpm. Homogenates were centrifuged for 15 min at 16000 g_{max}, and the supernatants collected were centrifuged again at 16000 g_{max}. Then the two-thirds upper part of the supernatant was collected and centrifuged at 100000 g_{max} for 1 h. Microsomal pellets were resuspended in one-half the initial volume and then centrifuged for 1 h at 100000 g_{max}. Pellets of washed microsomes were collected and fractionated

in small aliquots stored at −80 °C without loss of activity for at least 2 months.

2,3-Oxidosqualene Lanosterol-Cyclase (OSC) Assay. We used the original following procedure: A standard assay mixture consisted of a total volume of 500 μL containing 150 μg of microsomal protein and 100 μM (*R,S*)-2,3-oxidosqualene.[51] Incubation was for 1 h at 37 °C; then the reaction was stopped by adding 1 mL of 7% methanolic KOH and 20 ng of stigmasterol as internal standard. After 0.5 h at 80 °C for saponification, nonsaponifiable lipids were extracted twice with *n*-hexane and then evaporated and derivatized with 75 μL of BSTFA−1% TMCS and 25 μL of pyridine. TMCS ethers were chromatographed on a 30 m OV1 column at 260 °C. Retention times of TMCS-lanosterol and -stigmasterol ethers were respectively 17 and 15.5 min. The rat liver microsomal OSC has an apparent K_m for 2,3(*S*)-oxidosqualene of 30 μM. The inhibitory potencies of compounds were expressed as the concentration at which 50% inhibition of OSC activity was observed. Enzyme was also assayed in the presence of several different substrate and inhibitor concentrations or at different substrate/inhibitor ratios. The resulting *V* vs [S] curves were evaluated with EZ-Fit Enzyme Kinetic Model Fitting Software kindly provided by F. Perrella.[46]

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