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Synthesis and Bioactivities of Steroid Derivatives as Antifungal Agents

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Abstract: A series of lanosterol and cholesterol derivatives with modified side chain structures, which might interfere with sterol C24-methyltransferase in the ergosterol biosynthesis as substrate analogs, have been synthesized. The *in vitro* bioassay studies have shown that some of these compounds, in particular with C24-amino- and thio-functionalities, possess potent antifungal activities. *in vivo*. Bioassays have also been carried out for the leading compounds.

Introduction

Systemic fungal infections continue to be a major clinical problem in infectious diseases chemotherapy, especially in immunocompromised individuals such as those afflicted with AIDS, undergoing cytotoxic chemotherapy, and receiving organ transplants. The polyene antifungals such as amphotericin B still remain the standard of therapy because of its broad spectrum and fungicidal activity. However, due to the various side effects associated with amphotericin B including toxicity, the azole antifungals such as fluconazole and itraconazole are now most widely used in the treatment of candidiasis. The azole antifungals disturb the integrity of fungal membranes by inhibiting the cytochrome P450-linked monooxygenase component of lanosterol C14-demethylase thus blocking the biosynthesis of ergosterol, a key membrane component. There has been an increasing number of reports on the development of resistance to some of azole antifungals, and this causes some concern over the continued research and development of antifungal agents based on this specific enzyme in the ergosterol biosynthetic pathway. Thus, there is a need for novel therapies for serious fungal diseases as well as for the management of the legions of topical fungal infections.[1]

In principle, any difference between parasitic fungi and the host human cells can be exploited as the target for the development of antifungal chemotherapy. It has frequently been suggested that biosyntheses of cell-wall components (e.g. chitin and glucan), membrane components (e.g. ergosterol) and cytoskeletal proteins (e.g. tubulin) may represent the most logical targets for the desired differentiation. In fact the currently available drugs or lead structures cover some of these targets.[1a]

Ergosterol is the dominant sterol in most fungi with the notable exception of the *Oomycete* genera

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Phthium and *Phytophthora* which apparently do not synthesize any sterol. Both ergosterol and cholesterol, the major mammalian sterol, are synthesized from acetyl-Co A via a series of enzymic reactions. Lanosterol represents the key branching point in the biosynthesis of ergosterol and cholesterol, since the biosynthetic steps leading to lanosterol are common to both fungi and animals. Conversion of lanosterol to ergosterol involves multistep processes that are catalyzed by membrane-bound enzymes. The precise sequence in which these reactions occur appears to be dependent upon fungal species. But in most of fungi with a notable exception of *Saccharomyces*, the first step is the C24 methylation and it is followed by sequential demethylations at C14 and C4. Once the methylation and demethylations have taken place, various double bond transformations occur and the exact order of the double bond transformation may also vary depending on organism.[2]

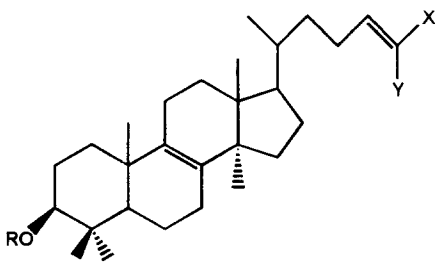
The C24-methylation is unique to the ergosterol biosynthesis, whereas the C4 and C14 demethylations are common to the biosynthesis of ergosterol and cholesterol. S-Adenosylmethionine (SAM) is known to be the source of the C24-methyl group. The responsible enzyme, methyltransferase, has been purified from yeast,[3] and the mechanism studied.[4] In general, several approaches are possible in the design of enzyme inhibitors: substrate analogs, transition state mimics, and mechanism based inhibitors.[5] In view of the reports that the antifungal activities of 25-azasterol, plakinamines and related compounds might be due to interfering with the C24 methylation,[6] we have examined as a potential means of achieving antifungal activity some substrate analogs that are structurally based on lanosteol and cholesterol nucleus and the side-chains are modified, and herein report the results of these studies.[7]

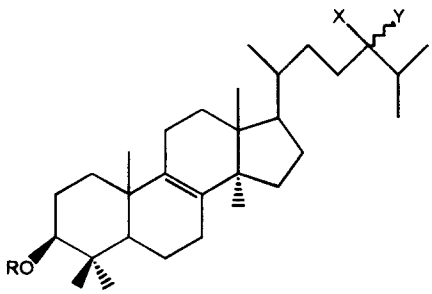
Results and Discussion

On the basis of the geometric and synthetic considerations, we initially investigated lanosterol derivatives **1-21** (Figure 1). Lanosterol acetate (**1b**) was ozonized in CH_2Cl_2 at -78 °C and worked up with Me_2S or Zn in HOAc[8] to give the aldehyde (**22b**). The Knoevenagel condensation of **22b** with various active methylene compounds (malononitrile, cyanoacetate, malonate) under the conventional[9a] or the neutral alumina-catalyzed conditions[9b] provided compounds **2b**, **3b**, and **5b** in moderate yields.[10] The alumina catalyzed Henry reaction[11] between **22b** and nitroethane also proceeded smoothly to give **4b**.[12] The *gem*-dihaloolefin compounds **6-8** were prepared from the aldehyde **22b** by employing the Wittig-like procedures based on $CF_2CBr_2/P(NMe_2)_3/Zn$ dust,[13] $Cl_3CP(O)(OEt)_2/nBuLi$,[14] and $CBr_4/Ph_3P/Zn$ dust,[14] respectively (Scheme 1). The acetylenic compound **9a** was prepared from the THP ether (**8c**) of the *gem*-dibromoolefin by successive treatments with n-BuLi in THF at -78 °C and methyl iodide,[14] followed by removal of the THP ether.

Syntheses of compounds **10-21** commenced with the hydroboration of lanosterol acetate (**1b**) with BH_3/SMe_2 in THF and then alkaline hydrogen peroxide to yield the C24 alcohol (**10b**)[15] in ca. 40 % yield. Alcohol **10b** was converted stepwise to the mesylate (**11b**) and then the azide (**12b**) in good yields by successive treatments with MsCl and Et_3N in CH_2Cl_2 at 0 °C and NaN_3 in aq. DMF at 95 °C. Reduction of the azide (**12b**)

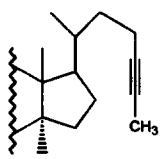
with $LiAlH_4$ in refluxing ether gave the amino compound (**15a**) in 76 %. Jones oxidation of **10b** readily provided ketone (**13b**) in good yield. The same ketone (**13b**) could perhaps be more conveniently obtained directly from lanosterol acetate (**1b**) by epoxidation with mCPBA followed by a rearrangement catalyzed by BF_3 etherate in CH_2Cl_2 in 43 % overall yield.[16] Reductive amination of **13b** in methanol with a variety of amines ($MeNH_2$, $EtNH_2$, $C_6H_{11}NH_2$, $BnNH_2$) in the presence of $ZnCl_2$ and $NaBH_3CN$ provided the amine derivatives **16-19** in moderate yields.[17] Reaction of **13b** with NH_2OH/HCl in pyridine-ethanol gave the corresponding oxime (**14b**), which could be conveniently reduced to **15a** with $LiAlH_4$ in refluxing THF (68 % overall yield). The mesylate (**11b**) in HMPA was reacted with mercaptans such as PhSH and BnSH in the presence of NaH to afford the substituted products (**20b** and **21b**) in modest yields.





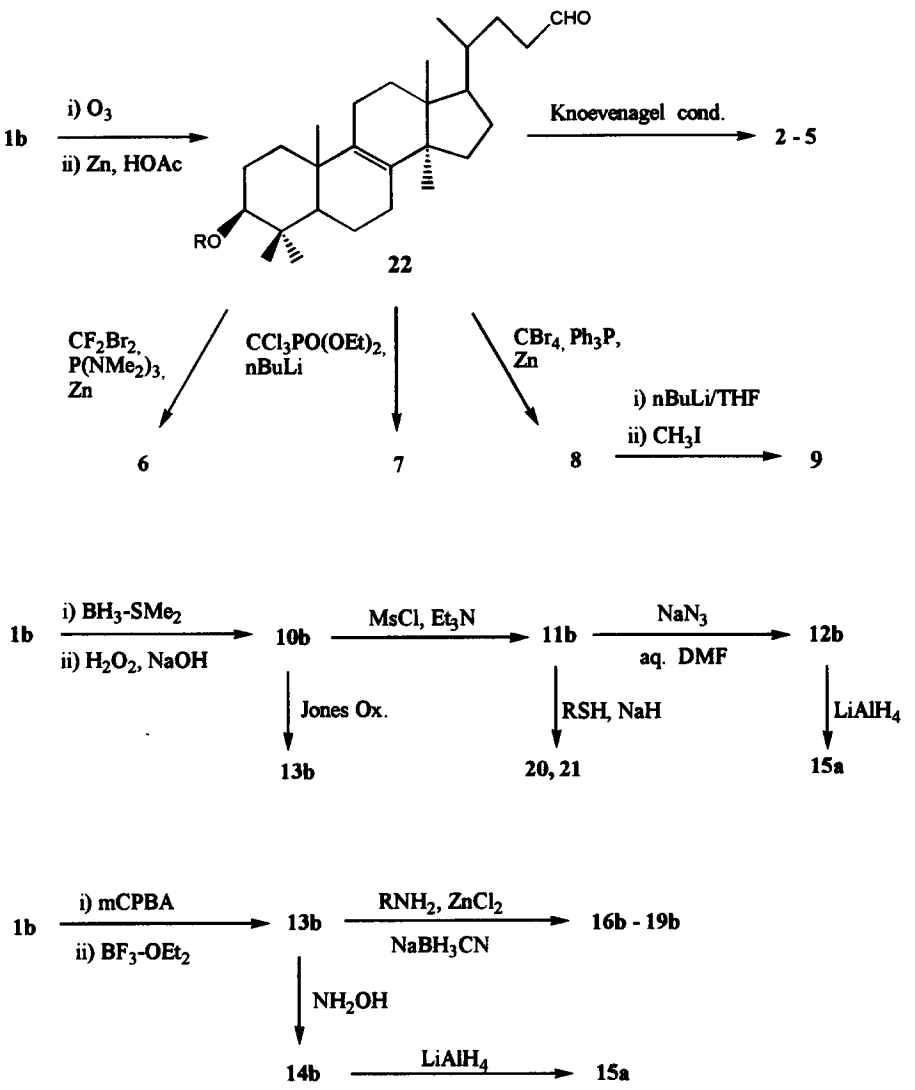
	X	Y
1	CH_3	CH_3
2	CN	CN
3	CO_2Et	CN
4	CH_3	NO_2
5	CO_2Et	CO_2Et
6	F	F
7	Cl	Cl
8	Br	Br

	X	Y
10	H	OH
11	H	OMs
12	H	N_3
13	X, Y = O	
14	X, Y = NOH	
15	H	NH_2
16	H	NHMe
17	H	NHEt
18	H	NHC_6H_{11}
19	H	NHBn
20	H	SPh
21	H	SBn



9

Figure 1. Synthetic Lanosterol Derivatives (**a**.R=H; **b**.R=Ac; **c**.R=THP)



Scheme 1. Preparation of *gem*-Dihaloolefin and Acetylene Derivatives

The antifungal activities of the compounds both as 3-OH and 3-OAc were initially assessed *in vitro* against a number of fungal strains. No appreciable activity was found for compounds **1-14**. Despite the apparent geometric similarity (in terms of size and shape) of the side chain in some of these compounds to that of the lanosterol, none of these showed a promise as an antifungal lead. As expected, the electronic environment around the C-24/25 double bond appears to be critically important. However, interesting activities were observed for the amino- and thio-compounds, and they are listed in Table 1. Several points are noteworthy. Generally, the 3-OH forms showed higher activities that the 3-OAc (see **16a** vs. **16b**; **17a** vs. **17b**). The

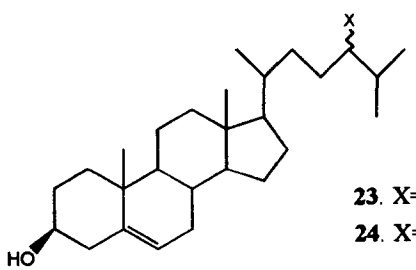
antifungal activities, especially against *Cryptococcus neoformans*, which is considered to be important in connection with AIDS, begin to appear in the C24-azido-lanosterol derivative (**12a**), and improve along the order of ethyl- (**17a**), cyclohexyl- (**18a**), benzyl- (**19a**), methyl- (**16a**) and primary- (**15a**) amino derivatives. The *in vitro* activities of **16a** measured after 24 hr and 48 hr cultivations of the fungal inoculums (data not shown) have suggested that the activities are largely fungistatic rather than fungicidal.

Table 1. *in vitro* Antifungal Activities (MIC, μg/mL)

Fungal strain	**12a**	**15a**	**16a**	**16b**	**17a**	**17b**	**18a**	**19a**	**20a**	**21a**	KCZ[a]
C.albicans A10231[b]	100	100	100	100	25	100	50	100	100	50	12.5
C.krusei A28838	100	100	100	100	50	100	100	100	100	50	0.39
C.parapsilosis A7330	100	100	100	100	100	100	100	100	100	50	12.5
C.pseudotropicalis K11648[c]	100	100	100	100	100	100	100	100	100	50	0.2
Cr.neoformans A34144	25	0.10	0.78	100	25	50	12.5	3.13	25	25	0.05
S.carlsbergensis A9080	50	25	6.25	100	25	50	50	50	50	25	0.10
S.cervisiae A36375	100	25	6.25	100	12.5	100	50	50	50	25	0.10
T.candida KCTC 1491	100	100	100	100	100	100	100	100	100	50	1.56
C.glabrata A2001	100	50	25	100	25	100	100	100	50	50	0.20

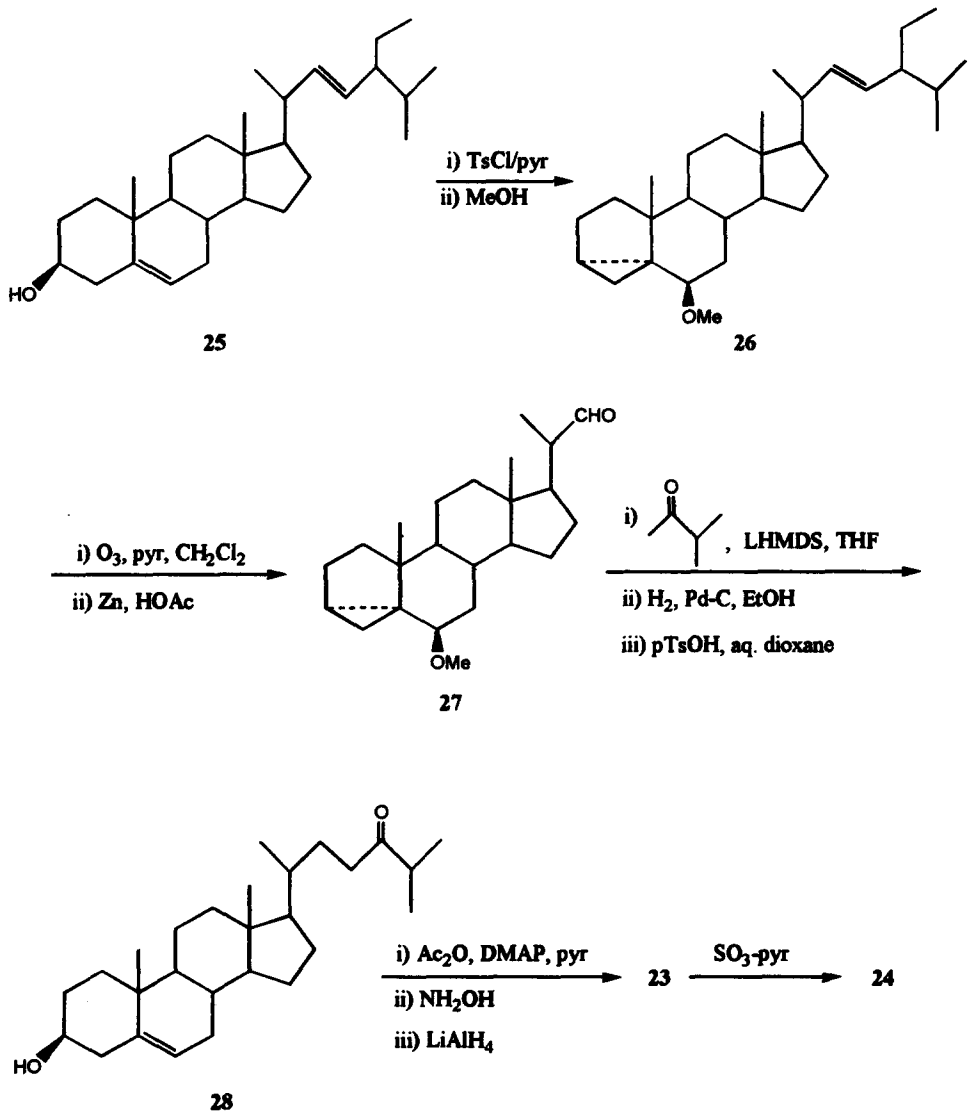
a. ketoconazole; b. ATCC; c. KFCC

These results prompted us to examine another type of steroid nucleus with modified side chain that are readily accessable, i.e. cholesterol derivatives **23** and **24** (Figure 2). The homoallylic alcohol moiety of commercially available stigmasterol (**25**) could be most conveniently protected in the form of *i*-steroid by conversion to the corresponding tosylate and subsequent solvolysis in methanol.[18a] This protected form of *i*-steroid could be readily reverted back to the homoallylic alcohol form by treatment with pTsOH in aq. acetone.[18b] Thus, compound **26** was ozonized in CH_2Cl_2 containing ca. 1 % pyridine at -78 °C and reductively worked up with Zn in HOAc to provide the aldehyde (**27**) in 65 % yield (Scheme 2).



23. X=NH_2
24. X=$NHSO_3H$

Figure 2. Synthetic Cholesterol Derivatives



Scheme 2. Synthesis of Cholesterol Derivatives

Compounds **23** was readily derived from aldehyde **27**. Thus, the aldol condensation of **27** with methyl isopropyl ketone in THF with lithium hexamethyldisilazane (LHMDS) as base,[19] followed by hydrogenation (H_2/Pd-C/EtOH) gave ketone **28** in 65 % overall yield. Reductive amination of the ketone intermediate (**28**) was most efficiently performed in the following manner. The hydroxyketone (**28**) was first acetylated with Ac_2O, DMAP and pyridine in CH_2Cl_2, and then converted to the corresponding oxime by treatment with NH_2OH-HCl in pyridine and ethanol. The $LiAlH_4$ reduction of the oxime in refluxing THF gave C24-aminocholesterol (**23**). The *N*-sulfonation of compound **23** made it more water-soluble. Thus, treatments of (**23**) with SO_3-pyridine

complex in chloroform[20] yielded the aminosulfated compound (**24**) in 89 % yield. The selective *O*-sulfonation of the protonated form of compound **23** with the same reagent was unsuccessfully tried.

The initial *in vitro* bioassays against a number of fungal strains[16] indicated much improved activities for compounds **23** and **24** over those of C24-aminolanosterol derivatives. The MIC values of these compounds are compared with those of fluconazole and itraconazole in Table 2. Both compounds **23** and **24** showed much higher potencies than fluconazole or itraconazole. Furthermore, the *in vitro* activities of **23** measured after 24 hr and 48 hr cultivations of the fungal inoculums (data not shown) indicated that the antifungal activities are of fungicidal nature rather than fungistatic. In general, the *in vitro* tests provide information on the impact of the structure on activity against a variety of organisms, but their correlation with activities is known to be unreliable in the case of azole antifungals.[21] Therefore, *in vivo* tests were performed with murine systemic candidiasis models.

Table 2. MIC (μg/mL) values

Fungal strains	**15**	**23**	**24**	FCZ[a]	ICZ[b]
C.albicans B02630	100	1.56	6.25	100	100
C.albicans A10231	>100	0.78	3.13	100	100
C.albicans A11651	100	3.13	6.25	100	100
C.albicans IFO1385	6.25	0.39	0.78	6.25	6.25
C.tropicalis A13803	100	0.78	3.13	100	100
C.pseudotropicalis K11658	100	3.13	3.13	6.25	0.78
C.krusei K11655	100	3.13	6.25	12.5	0.78
C.parapsilosis A7330	100	0.39	0.78	12.5	100
C.glabrata B16205	12.5	0.20	0.78	100	100
Cr.neoformans B42419	0.78	0.39	0.10	12.5	0.39
Cr.neoformans IFM40092	0.10	≤0.05	0.20	3.13	0.10
Cr.neoformans A34144	0.20	≤0.05	0.39	6.25	0.39
A.niger A16404	3.13	0.39	3.13	100	0.78
T.mentagrophytes A9129	≤0.05	≤0.05	≤0.05	25	≤0.05
T.mentagrophytes B32663	≤0.05	≤0.05	≤0.05	3.13	≤0.05

a. fluconazole; b. itraconazole

Table 3. summarizes the therapeutic effects on lethal models causing total death of untreated groups within 4-6 days of infection. All the mice treated with single *iv* dose of fluconazole (20 mg/kg) survived until day 8 after infection and showed 50 % death rate on day 10. However, all the infected mice died when treated with

compound **24** at the dose ranges of 0.31-5 mg/kg and only 10 % of mice survived at the highest dose of 20 mg/kg. Oral dose of fluconazole for 3 days showed excellent therapeutic efficacy with ED_{50} of 1.2 mg/kg on day 10, whereas compound **15** and **23** were not active at doses upto 8 mg/kg against the infection, and the death rate was higher than 90 % within 3 days after the challenge. Although compounds **15**, **23** and **24** have potent *in vitro* antifungal activities, unfortunately these activities are not translated into *in vivo* activities. We are currently attempting to understand the underlying cause of the low correlation in terms of possible metabolic degradation and excessive serum binding.

Table 3. ED_{50} (mg/kg) against *C.albicans* B02630 infection to mice

Compound	ED_{50} (*po*)	ED_{50} (*iv*)
fluconazole	1.20	20
23	>16.0	nt[a]
24	nt	>20
15	>32.0	nt

a. not tested

Conclusion

In an attempt to generate lead structures that might have antifungal activities by inhibiting the C24-methylation step of the ergosterol biosynthesis, we have synthesized a series of lanosterol and cholesterol derivatives with modified side chain structures. The *in vitro* bioassay studies have shown that some of these compounds, in particular compound **15**, **23** and **24** possess potent antifungal activities. However, the *in vitro* bioactivities have not been linearly translated into *in vivo* protection data for some unknown reasons. The potential underlying causes of the low correlation, such as metabolic degradation and excessive serum binding, are currently under examination.

Experimental Section

General.

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained on a BOMEM FT-IR M100-C15 spectrometer with the compound in thin film on a NaCl plate, KBr pellet, or solution. NMR spectra were taken on a Bruker AM 300 (300 MHz) spectrometer. Chemical shifts are reported in δ ppm relative to tetramethylsilane for ^{1}H and ^{13}C NMR, and to CF_3COOH for ^{19}F NMR spectra. Coupling constants, J are reported in Hz. Mass spectra were determined on a Kratos MS 25 RFA (EI and FAB) system. High resolution MS were performed by Korea Basic Science Center.

Analytical TLC was performed on Merck 60 F254 silica gel plate (0.25 mm layer thickness) and visualization was done with UV light and/or a spray with 5% phosphomolybdic acid in ethanol followed by

charring with a heat gun. Column chromatography was carried out on silica gel 60 (e. Merck, 70-230 mesh). All reactions were carried out under N_2 or Ar atmosphere in oven-dried glassware, all commercial chemicals were used as obtained, and all solvents were carefully dried and distilled by standard methods prior to use. The standard extractive work-up procedure consisted of pouring the reaction mixture into a large amount of water, extracting with the organic solvent indicated, washing the combined extracts successively with water and brine, drying the extract over anhydrous magnesium sulfate, and evaporating the solvent to afford the crude product.

Lanosta-8,24-dien-3β-yl acetate (1b)

To a solution of lanosterol (25 g, 58.7 mmol, 50-60 % pure). 4-DMAP (cat. amount) and pyridine (38 ml, 468 mmol) in CH_2Cl_2 (200 ml) at RT, was added acetic anhydride (7 ml, 56.3 mmol), and the resulting solution was stirred for 5 h. A standard extractive work-up with CH_2Cl_2 including extensive washing with 5 % HCl and 10 % aqueous $NaHCO_3$ gave **1b** as a solid (26.9 g): mp 118-120 ^{0}C (lit. mp 129-130 ^{0}C)[22]; δ_H ($CDCl_3$) 2.03 (s, 3H), 4.49 (dd, 1H, *J*, 11.24, 4.77), 5.09 (t, 1H, *J* 7.11); IR (KBr) 1735, 1244 cm^{-1}.

3β-Acetoxy-25,26,27-trisnor-lanost-8-en-24-al (22b)

A solution of **1b** (5.38 g, 11.9 mmol) in $CHCl_3$ (100 ml) was ozonolyzed at -78 ^{0}C and then treated with zinc (5.7 g) and acetic acid (20 ml) for 3 h at RT.[8] The precipitate was filtered and washed with $CHCl_3$. The organic phase was extensively washed with sat. sodium bicarbonate and water, dried and evaporated to give a crude product, which was chromatographed on silica gel to yield the aldehyde (**22b**, 1.90 g) as a solid: mp 155-156 ^{0}C; δ_H ($CDCl_3$) 2.2-2.5 (m, 2H), 4.5 (dd, 1H, *J* 11.2, 4.6), 9.8 (t, 1H, *J* 2 Hz); IR (KBr) 2743, 1730, 1448 cm^{-1}.

Knoevenagel Condensation of 22b

Method 1. A solution of aldehyde (**22b**, 433 mg, 1 mmol) and malononitrile (66 mg, 1 mmol) in benzene (20 ml) was vigorously stirred over activated basic alumina (0.3 g) for 10 min.[9b] The precipitate was filtered and washed extensively with $CHCl_3$. The organic phase was washed with water, dried and evaporated to give a crude product, which was chromatographed on silica gel to yield **2b** (400 mg, 82 %) as a solid: mp 182 ^{0}C; δ_H ($CDCl_3$) 2.35-2.65 (m, 2H), 4.52 (dd, 1H, *J* 11.3, 4.7), 7.28 (t, 1H, *J* 8.0 Hz); IR (KBr) 2236, 1720, 1600, 1256 cm^{-1}; MS (EI) *m/z* 490 (M^+). The corresponding alcohol (**2a**) was readily obtained by hydrolyzing the acetate in ethanolic KOH at elevated temperature. The alumina catalyzed condensations of **22b** were similarly carried out to give the following compounds.

3b: mp 114-115 ^{0}C; δ_H ($CDCl_3$) 2.4-2.7 (m, 2H), 4.25 (q, 2H, *J* 7.1), 4.55 (dd, 1H, *J* 11.3, 4.7), 7.64 (t, 1H, *J* 7.9); IR (KBr) 2235, 1733, 1619, 1251 cm^{-1}; MS (EI) *m/z* 537 (M^+).

4b: mp172-174 ^{0}C; δ_H ($CDCl_3$) 2.16 (s, 3H), 2.2-2.35 (m, 2H), 4.48 (dd, 1H, *J* 11.2, 4.6), 7.12 (t, 1H, *J* 7.9); IR (KBr) 2235, 1733, 1619, 1251 cm^{-1}; MS (EI) *m/z* 499 (M^+).

Method 2. A solution of **22b** (150 mg, 0.35 mmol), diethyl malonate (57 μl, 0.37 mmol), acetic acid (4 μl, 0.068 mmol) and piperidine (6.8μl, 0.068 mmol) in CH_2Cl_2 was stirred at RT for 2 h,[9a] diluted with CH_2Cl_2, washed with dil. HCl, sat. $NaHNO_3$, water and brine, dried and evaporated to give a crude product, which was chromatographed to yield **5b** (123 mg, ca. 60 %): mp 85-86 ^{0}C; δ_H ($CDCl_3$) 2.1-2.4 (m, 2H), 4.15-4.3 (each q,

2H, *J* 7.1), 4.52 (dd, 1H, *J* 11.4, 4.8), 6.98(t, 1H, *J* 7.9); MS (EI) *m/z* 584 (M^+).

***gem*-Dihaloolefination of 22b**

Compound **6b**. To a solution of **22b** (500 mg, 1.16 mmol) in THF at 0 ^{0}C under Ar, were added hexamethylaminophosphorus triamide (1.02 ml, 5.65 mmol) and dibromodifluoromethane (0.52 ml, 5.65 mmol) via syringe.[13] After addition of zinc dust (370 mg, 5.65 mmol) in one portion, the mixture was stirred at RT for 12 h. The mixture was filtered, and the filtrate was evaporated and chromatographed on silica gel to give the difluoroolefin (**6b**, 191 mg, 35 %): mp 119-120 ^{0}C; δ_H ($CDCl_3$) 4.17 (m, 1H), 4.50 (dd, 1H, *J* 11.5, 4.7); δ_F ($CDCl_3$) -17.69 (dd, *J* 53.2, 20.9), -15.28 (d, *J* 53.2); δ_C ($CDCl_3$) 171.0, 134.4, 134.2, 80.9; IR (KBr) 1735, 1425, 1372, 1055 cm^{-1}; MS (EI) *m/z* 476 (M^+).

Compound **7a**. To a solution of diethyl trichloromethanesulfonate (638 mg, 2.50 mmol) in THF (25 ml) and ether (30 ml) at -78 ^{0}C under Ar, were slowly added n-butyllithium (1.56 ml of 1.6 M solution in hexane, 2,50 mmol) and then a solution of **22b** (1.0 g, 2.31 mmol) in ether (10 ml) via syringe.[14] The mixture was slowly warmed up to RT and then refluxed for 1 h. The sloution was cooled to -78 ^{0}C again and treated with 2N H_2SO_4. A standard extractive work-up with ether followed by chromatography on silica gel provided the dichloroolefin (**7a**, 360 mg, 33 %): mp 120-121 ^{0}C; δ_H ($CDCl_3$) 3.25 (dd, 1H, *J* 11.3 ,4.6), 6.30 (t, 1H, *J* 7.5); δ_C ($CDCl_3$) 134.46, 134.34, 130.44, 79.00; IR (KBr) 3330, 2940, 1480. 1410, 1040 cm^{-1}; MS (EI) *m/z* 466 (M^+), 468 $(M+2)^+$ and 470 $(M+4)^+$.

Compound **8a**. To a mixture of triphenylphosphine (7.86 g, 29.95 mmol), carbon tetrabromide (9.93 g, 29.95 mmol) and zinc dust (1.96 g, 29.95 mmol) in CH_2Cl_2 (70 ml), which had been stirred at RT for 1 day under N_2, was added the solution of **22b** (4.0 g, 9.26 mmol) in CH_2Cl_2 (20 ml).[14] The mixture was stirred for 4 h at RT, diluted with cold ether and filtered. The filtrate was evaporated and chromatographed on silica gel to give the dibromoolefin (**8a**, 2.33 g, ca. 45 %): mp 125-126 ^{0}C; δ_H ($CDCl_3$) 3.23 (dd, 1H, *J* 11.4 ,4.7), 6.38 (t, 1H, *J* 7.2); δ_C ($CDCl_3$) 139.24, 134.3; IR (KBr) 3330, 2940, 1480. 1410, 1040 cm^{-1}; MS (EI) *m/z* 554 (M^+), 556 $(M+2)^+$ and 558 $(M+4)^+$.

Compound **9a**. A solution of **8a** (2.33 g, 4.18 mmol), dihydropyran (0.71 g, 8.38 mmol) and pyridinium p-toluenesulfonate (PPTS, 0.25 g, 1.0 mmol) in CH_2Cl_2 (50 ml) was stirred for 6 h at RT. The solution was diluted with ether and extractively worked up to give 3β-THP protected compound (**8c**) as a syrup (2.50 g, 93 %): δ_H ($CDCl_3$) 3.01-3.13 (dd, ca. 0.5H, *J* 11.2 ,4.2), 3.21-3.32 (dd, ca. 0.5H, *J* 11.2 ,4.2), 3.42-3.53 (m, 1H), 3.90-4.01 (m, 1H), 4.58 (m, ca. 0.5H), 4.75 (m, ca. 0.5H), 6.37 (t, 1H, *J* 7.3); IR (film) 2932, 1456, 1371, 1025 cm^{-1}. To **8c** (1.15 g, 1.80 mmol) in THF (70 ml) at -78 ^{0}C under N_2, was added via syringe n-butyllithium (1.5 ml of 2.5 M solution in hexane, 3.6 mmol). The resulting solution was stirred for 2 h at RT and cooled to -78 ^{0}C before addition of methyl iodide (0.40 g, 2.70 mmol). After stirring for 1 h at this temperature, the reaction mixture was extractively worked up with pentane to provide **9c** as a syrup. A solution of crude **9c** (0.1 g, 207 mmol) and PPTS (12.6 mg) in ethanol (10 ml) was heated at 55 ^{0}C for 10 h, and extractively worked up to give **9a** (62 mg, ca. 73 %) as a solid: mp 130-131 ^{0}C; δ_H ($CDCl_3$) 2.17 (s, 3H), 3.23 (dd, 1H, *J* 11.4, 4.7); δ_C ($CDCl_3$)

134.49, 134.36, 85.20, 78.99; IR (KBr) 3350, 2980, 2180, 1475, 1390, 1040 cm^{-1}; MS (EI) *m/z* 410 (M^+).

24-Hydroxy-lanost-8-en-3β-yl acetate (10b)

To a solution of **1b** (5 g, ca. 6.4 mmol) in THF at 0 ^0C, was added borane dimethylsulfide (3.55 ml of 2 M solution in THF), and the resulting solution was stirred for 5 h at RT. Water (0.39 ml) was added and the mixture was stirred for 1 h, and cooled to 0 ^0C. The mixture was treated with 3N NaOH and 30 % H_2O_2 at 55 ^0C for 3 h, and extractively worked up with ether to give after chromatography on silica gel **10b** (1.25 g, ca. 40 %) as a solid: mp 142-143 ^0C; δ_H ($CDCl_3$) 2.04(s, 3H, OAc), 3.32 (m, 1H, 24-H), 4.50 (dd, 1H, *J* 11.3, 4.7, 3α-H); IR (KBr) 3453, 1726, 1254 cm^{-1}; MS (EI) *m/z* 486 (M^+).

24-Mesyl-lanost-8-en-3β-yl acetate (11b)

A solution of **10b** (443 mg, 0.91 mmol), methanesulfonyl chloride (0.10 ml, 1.27 mmol) and triethylamine (0.22 ml, 1.56 mmol) in CH_2Cl_2 was stirred for 30 min at -10-0 ^0C, and extractively worked up to give after chromatography on silica gel **11b** (342 mg, 67 %): mp 135-136 ^0C; δ_H ($CDCl_3$) 2.04(s, 3H, OAc), 3.00 (s, 3H, OMs), 4.51 (m, 2H, 3α-H and 24-H); IR (KBr) 1732, 1356, 1251, 1174, 1032, 907 cm^{-1}; MS (EI) *m/z* 564 (M^+).

24-Azido-lanost-8-en-3β-yl acetate (12b)

A solution of **11b** (52 mg, 0.092 mmol), sodium azide (78 mg, 1.20 mmol) in water (0.1 ml) and DMF (3 ml) was heated at 95 ^0C for 3 h, and extractively worked up with ether to give **12b** (40 mg, ca. 85 %) as a light yellow solid: mp 107-109 ^0C; δ_H ($CDCl_3$) 2.04 (s, 3H), 3.02 (m, 1H, 24-H), 4.50 (dd, 1H, *J* 11.2, 4.7, 3α-H); IR (KBr) 2094, 1720, 1374, 1265 cm^{-1}; MS (EI) *m/z* 511 (M^+).

24-Oxo-lanost-8-en-3β-yl acetate (13b)

Method 1. A solution of **10b** (520 mg, 1.07 mmol) in chloroform (2ml) and acetone (13 ml) was oxidized with Jones reagent (1 ml) at RT over 30 min. The solution was treated with isopropanol and water, and extractively worked up to give after chromatography on silica gel **13b** (380 mg, 73 %): mp 128-130 ^0C (lit. mp 128-129.5 ^0C)[15]; δ_H ($CDCl_3$) 2.04 (s, 3H, OAc), 2.35-2.45 (m, 2H, 23-H_2), 2.61 (m, 1H, 25-H), 4.50 (dd, 1H, *J* 11.3, 4.8, 3α-H); IR (KBr) 1736, 1708, 1241 cm^{-1}; MS (EI) *m/z* 484 (M^+).

Method 2. A solution of **1b** (7.7 g, 7.95 mmol) and m-CPBA (2.28 g, ca. 60 % pure, 7.95 mmol) in CH_2Cl_2 (30 ml) was stirred at RT for 3 h. After dilution with CH_2Cl_2, the solution was washed with 10 % Na_2SO_3, sat. $NaHCO_3$ and water, dried and evaporated to give the corresponding epoxide: mp 171-172 ^0C; δ_H ($CDCl_3$) 2.04 (s, 3H, OAc), 2.68 (t, 1H, *J* 6.1), 4.50 (dd, 1H, *J* 11.2, 4.7, 3α-H); IR (KBr) 2934, 1732, 1462, 1374, 1250, 1030 cm^{-1}. The epoxide was dissolved in CH_2Cl_2 (100 ml) and treated with BF_3 etherate (0.14 ml, 1.1 mmol) at RT for 10 min.[16] An extractive work-up followed by a chromatography on silica gel gave **13b** (3.4 g, 88 %).

24-Oximino-lanost-8-en-3β-yl acetate (14b)

A solution of **13b** (3 g, 6.19 mmol) , NH_2OH-HCl (0.52 g, 7.43 mmol) and pyridine (0.75 ml, 9.28 mmol) in anhydrous EtOH (50 ml) was stirred at RT for 3 h. A standard extractive work-up with CH_2Cl_2 followed by chromatography on silica gel provided **14b** (2.99 g, 97 %): mp 163-164 ^0C; δ_H ($CDCl_3$) 2.09 (s, 3H, OAc), 2.22

(m, 1H), 2.39 (m, 1H), 2.51 (m, 1H), 4.53 (dd, 1H, *J* 11.6, 4.8, 3α-H); IR (KBr) 2946, 1735 cm^{-1}; MS (FAB) *m/z* 500 $(M+H)^+$.

24-Amino-lanosterol (15a)

Method 1. To the mixture of $LiAlH_4$ (0.6 g, 14 mmol) in THF (50 ml) at 0 ^0C under N_2, was carefully added a solution of **14b** (1 g, 2 mmol) in THF (100 ml). The mixture was refluxed for 24 h and quenched carefully with sat. NH_4Cl solution. After filtration, the mixture was extractively worked up to give after chromatography on silica gel **15a** (600 mg, 68 %): mp 154-156 ^0C; δ_H ($CDCl_3$) 2.46 (m, 1H, 24-H), 3.25 (dd, 1H, *J* 10.0, 4.8, 3α-H); δ_C ($CDCl_3$)134.87, 134.80,79.29; IR (KBr) 3331, 1933, 1460, 1370, 1033 cm^{-1}; MS (EI) *m/z* 443 (M^+); HRMS (FAB) calcd for $C_{30}H_{54}NO$ $(M+1)^+$: 444.4208, found: 444.4205.

Method 2. Compound **12** (569 mg, 1.1 mmol) was similarly reduced with $LiAlH_4$ (260 mg, 6.6 mmol) in ether (20 ml) to provide **15a** (372 mg, 76 %).

Reductive Amination of Ketone (13b).

To a solution of **13b** (50 mg, 0.1 mmol), $ZnCl_2$ (25 mg, 0.18 mmol) and methylamine (0.036 ml, 0.42 mmol) in methanol (10 ml) was added $NaBH_3CN$ (24 mg, 0.36 mmol). The mixture was stirred for 16 h at RT, treated with 0.1N NaOH (2 ml). A standard extractive work-up with ether followed by chromatography on silica gel gave **16b** (22 mg, 42 %): mp 132-133 ^0C; δ_H ($CDCl_3$) 2.03 (s, 3H, OAc), 2.08 (m, 1H, 24-H), 2.38 (d, 3H, *J* 2.4, NMe), 4.49 (dd, 1H, *J* 11.3, 4.7, 3α-H); IR (KBr) 3440, 1736, 1249 cm^{-1}; MS (EI) *m/z* 499 (M^+), 484 $(M-15)^+$, 456 (100 %, $M-43)^+$. The following compounds were similarly prepared.

Compound **17b**: mp 122-124 ^0C; δ_H ($CDCl_3$) 2.04 (s, 3H, OAc), 2.19 (m, 1H, 24-H), 2.60 (m, 2H, NCH_2), 4.50 (dd, 1H, *J* 11.3, 4.8, 3α-H); IR (KBr) 3465, 1736, 1247 cm^{-1}; MS (EI) *m/z* 470 (100 %, $M-43)^+$.

Compound **18b**: mp 125-127 ^0C; δ_H ($CDCl_3$) 2.04 (s, 3H, OAc), 2.29 (m, 1H, 24-H), 2.38 (m, 1H, *J* 2.4, NCH), 4.49 (dd, 1H, *J* 11.3, 4.7, 3α-H); IR (KBr) 3429, 1738, 1247 cm^{-1}; MS (EI) *m/z* 567 (M^+), 552 $(M-15)^+$, 524 (100 %, $M-43)^+$.

Compound **19b**: mp 115-116 ^0C; δ_H ($CDCl_3$) 2.04 (s, 3H, OAc), 2.26 (m, 1H, 24-H), 3.75 (m, 2H, NCH_2), 4.49 (dd, 1H, *J* 11.3, 4.7, 3α-H), 7.30 (m, 5H, aromatic); IR (KBr) 3452, 1730, 1247, 1029 cm^{-1}; MS (EI) *m/z* 575 (M^+), 560 $(M-15)^+$, 532 (100 %, $M-43)^+$.

24-Phenylthio-lanost-8-en-3β-yl acetate (20b)

To a mixture of NaH (20 mg, 0.51 mmol) and thiophenol (0.046 ml, 0.44 mmol) in HMPA (4 ml) was added dropwise a solution of **11b** (206 mg, 0.37 mmol) in HMPA (6 ml). The mixture was stirred at RT for 6 h and quenched carefully with water (30 ml). A standard extractive work-up incorporating washing with sat. $NaHCO_3$, followed by chromatography on silica gel gave **20b** (62 mg, 24 %): mp 113-115 ^0C; δ_H ($CDCl_3$) 1.97 (s, 3H, OAc), 2.88 (m, 1H, 24-H), 7.09-7.30 (m, 5H, aromatic); IR (KBr) 1733, 1247 cm^{-1}; MS (EI) *m/z* 578 (M^+), 563 $(M-15)^+$.

24-Benzylthio-lanost-8-en-3β-yl acetate (21b) was prepared in a similar manner to that described for **20b**: mp

107-108 ^{0}C; δ_H ($CDCl_3$) 2.04 (s, 3H, OAc), 2.27 (m, 1H, 24-H), 3.68 (s, 2H, SCH_2), 4.49 (dd, 1H, *J* 11.3, 4.7, 3α-H), 7.25 (m, 5H, aromatic); IR (KBr) 1731, 1247 cm^{-1}; MS (EI) *m/z* 592 (M^+), 577 $(M-15)^+$.

Compound 27

A solution of stigmasterol (25 g, ca. 57 mmol, 95 % pure), 4-DMAP (catalytic amount) and tosyl chloride (23 g, 120 mmol) in pyridine (250 ml) was stirred at RT for 6 h. The solution was poured into 10 % $NaHCO_3$ (1000 ml), and the precipitate was filtered, washed extensively with water, dried and recrystallized from acetone to give the corresponding tosylate (30 g, 99 %): mp 147-148 ^{0}C (lit. mp 148-149 ^{0}C)[18a]; δ_H ($CDCl_3$) 2.49 (s, 3H, OTs), 4.34 (m, 1H, 3α-H), 5.02 (dd, 1H, *J* 15.2, 8.4), 5.16 (dd, 1H, *J* 15.2, 8.5), 5.32 (d, 1H, *J* 5.2), 7.35 (d, 2H, *J* 8.3), 7.82 (d, 2H, *J* 8.3); IR (KBr) 2951, 2867, 1456, 1359, 1175, 1089 cm^{-1}.

The tosylate (20 g, 35.3 mmol) and pyridine (8.5 ml, 3 eq.) was dissolved in anhydrous methanol (200 ml) and refluxed for 6 h. The solution was evaporated, and a standard extractive work-up and chromatography gave *i*-stigmasteryl methyl ether (**26**, 14 g, 60 %): mp 52-53 ^{0}C (lit. mp 54-55 ^{0}C)[18a]; δ_H ($CDCl_3$) 0.42 (m, 1H), 0.64 (t, 1H), 2.76 (t, 1H, *J* 3.0), 3.33 (s, 3H), 5.01 (dd, 1H, *J* 15.2, 8.5), 5.15 (dd, 1H, *J* 15.2, 8.5); δ_C ($CDCl_3$) 138.80,129.61, 82.82; IR ($CHCl_3$) 2947, 1458, 1376, 1097 cm^{-1}.

i-Stigmasteryl methyl ether (11 g, 25.9 mmol) and pyridine (5 g, 62.3 mmol) were dissolved in CH_2Cl_2 (250 ml), ozonized at -78 ^{0}C, and reductively worked up with zinc dust (20 g) and acetic acid (20 ml) over 3 h. The mixture was filtered and the filtrate was washed with sat. $NaHCO_3$ and water, dried, evaporated, and chromatographed to give **27** (8 g, 90 %): mp 80-82 ^{0}C; δ_H ($CDCl_3$) 0.46 (m, 1H), 0.76 (t, 1H, *J* 3.9), 2.39 (br, 1H), 2.79 (t, 1H, *J* 2.6), 3.35 (s, 3H), 9.59 (d, 1H, *J* 3.2); IR ($CHCl_3$) 2938, 1724, 1456, 1379, 1095 cm^{-1}.

3β-Hydroxy-5-cholesten-24-one (28)

To a solution of lithium bis(trimethylsilyl)amide (LHMDS, 8.7 ml, 10.44 mmol, 1 M in THF) in THF (50 ml) at -78 ^{0}C, was added dropwise 3-methyl-2-butanone (1.2 ml, 10.44 mmol) in THF (3 ml). The solution was stirred for 2 h at that temperature before addition of **27** (3 g, 8.7 mmol) in THF (15 ml). The resulting mixture was stirred for 20 min at -78 ^{0}C and 3 h at RT. The reaction was quenched by addition of water. An extractive work-up followed by chromatography yielded the enone product (2.5 g): mp 112-113 ^{0}C (lit. mp 115-116 ^{0}C)[19]; δ_H ($CDCl_3$) 0.46 (dd, 1H, *J* 8.0, 5.1), 0.64 (t, 1H, *J* 4.7), 2.29 (m, 1H), 2.77 (br, 1H), 2.83 (m, 1H), 3.33 (s, 3H), 6.07 (d, 1H, *J* 15.6), 6.72 (dd, 1H, *J* 15.6, 9.0); δ_C ($CDCl_3$) 204.95, 153.03, 126.46, 82.72; IR ($CHCl_3$) 2941, 2360, 1671 cm^{-1}.

The enone compound (2.5 g) in EtOH (100 ml) was hydrogenated under H_2 (40 psi) over Pd/C for 3 h and worked up in the usual fashion to give the saturated ketone, which was recrystallized from ethyl acetate (2.3 g, 64.4 % over two steps): mp 92-93 ^{0}C; δ_H ($CDCl_3$) 0.43 (dd, 1H, *J* 8.0, 5.1), 0.64 (t, 1H, *J* 4.8), 2.36-2.47 (m, 2H), 2.61 (m, 1H), 2.77 (br, 1H), 3.32 (s, 3H); δ_C ($CDCl_3$) 215.23, 82.80; IR ($CHCl_3$) 2947, 1712, 1461, 1378, 1098 cm^{-1}.

A solution of the saturated ketone (2.33 g, 5.62 mmol), p-TsOH (0.1 g) in aqueous dioxane (1:9, 50 ml) was heated at 80 ^{0}C for 3 h before the evaporation of dioxane. The residue was extractively worked up with $CHCl_3$

and chromatographed on silica gel to give compund **28** (2.18 g, 97 %): mp 137-138 ^{0}C; δ_H ($CDCl_3$) 0.68 (s, 3H), 1.01 (s, 3H), 2.36-2.59 (m, 2H), 2.61 (m, 1H), 3.53 (m, 1H), 5.35 (d, 1H, *J* 3.9); δ_C ($CDCl_3$) 215.79, 141.16, 122.07; IR (KBr) 3428, 2935, 1709, 1055 cm^{-1}; MS (FAB) *m/z* 401 $(M+H)^+$.

24-Amino-cholesterol (23)

A solution of **28** (3 g, 7.5 mmol), 4-DMAP (a catalytic amount) and pyridine (6 ml, 75 mmol) in CH_2Cl_2 (50 ml) was reacted with acetic anhydride (1.42 ml, 15 mmol) for 3 h at RT. An extractive work-up with CH_2Cl_2 provided the acetylated product (3.25 g, 98 %): mp 117-118 ^{0}C; δ_H ($CDCl_3$) 0.67 (s, 3H), 1.01 (s, 3H), 2.03 (s, 3H), 2.30-2.33 (br, 2H), 2.36-2.46 (m, 2H), 2.61 (m, 1H), 4.60 (m, 1H), 5.37 (d, 1H, *J* 4.8); δ_C ($CDCl_3$) 215.78, 170.89, 140.02, 122.97; IR ($CHCl_3$) 2945, 1730, 1709 cm^{-1}.

A solution of the acetylated ketone (3 g, 6.8 mmol), NH_2OH-HCl (0.7 g, 10.2 mmol), pyridine (1.1 ml, 13.6 ml) in EtOH was stirred for 3 h at RT, and extractively worked up with $CHCl_3$ to give the oxime (2.98 g, 96 %): mp 186-187 ^{0}C; δ_H ($CDCl_3$) 0.68 (s, 3H), 1.02 (s, 3H), 2.03 (s, 3H), 2.12-2.22 (m, 2H), 2.31-2.38 (m, 3H), 2.48 (m, 1H), 4.60 (m, 1H), 5.38 (d, 1H, *J* 4.5); δ_C ($CDCl_3$) 170.94, 166.86, 140.05, 123.07; IR ($CHCl_3$) 3265, 2935, 1733, 1653, 1461, 1243, 1033 cm^{-1}; MS (FAB) *m/z* 458 $(M+H)^+$.

A mixture of $LiAlH_4$ (1.28 g, 30 mmol), the oxime (2 g, 4.38 mmol) in THF was refluxed for 3 h, and extractively worked up with $CHCl_3$, and chromatographed on silica gel to give compound **23** (1.1 g, 63 %): mp 132-133 ^{0}C; δ_H ($CDCl_3$) 0.68 (s, 3H), 0.98 (s, 3H), 1.99 (m, 2H), 2.27 (m, 2H), 2.44 (m, 1H), 3.47 (m, 1H), 5.34 (d, 1H, *J* 4.8); δ_C ($CDCl_3$) 141.32, 121.91; IR ($CHCl_3$) 3366, 2921, 1644, 1217 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{48}NO$ $(M+H)^+$: 402.3736, found: 402.3741.

24-Amino-cholesterol N-sulfate (24)

A solution of **23** (50 mg, 0.12 mmol) and SO_3-pyridine (19 mg, 0.12 mmol) in $CHCl_3$ (10 ml) was stirred for 3 h at RT. After filtration through a bed of celite, the filtrate was evaporated, and chromatographed on silica gel to provide **24** (51.2 mg, 89 %): mp 307-308 ^{0}C; δ_H (CD_3OD) 0.73 (s, 3H), 1.07 (s, 3H), 2.21-2.22 (m, 2H), 2.97 (m, 1H), 3.36 (m, 1H), 5.33 (d, 1H, *J* 4.2); IR (KBr) 3369, 2920, 1613, 1521, 1460, 1378, 1233, 1060, 1013 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{48}NO_4S$ $(M+H)^+$: 482.3304, found: 482.3313.

Antifungal Bioassays

The *in vitro* antifungal activities were assayed with Kimmig's broth and microdilution method.[23] Cultures grown on yeast malt extract agar were used to prepare the inoculum which were adjusted to 1×10^4 CFU/ml. Test compounds were serially diluted to provide range of 0.05-100 ug/ml. Microtiter plates were incubated for 48 hrs at 30 ^{0}C and minimal inhibitory concentration (MIC) were determined as the concentration showing 90 % inhibition of growth by visual inspection relative to drug-free control.

The *in vivo* efficacy against systemic murine candidiasis was measured as follows. Male ICR mice weighing 23-25 g were infected with *C. albicans* B02630 by injecting 0.7-1.0 $\times 10^7$ CFU into tail vein. Test compounds suspended in 50 % PEG200 were orally administered at the dose range of 0.25-32 mg/kg by gavage once a day for 3 days starting at 1 hr postinfection. For single *i.v.* administration, the infected mice were treated with the

test compounds at the dose range of 0.31-20 mg/kg via tail vein after infection. The survival rates were recorded for a period of 10 days. The 50 % effective dose (ED_{50}) values were calculated from the survival rates of each group by Lichfield and Wilcoxon method.[24]

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