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Synthesis and evaluation of new 6-hydroximinosteroid analogs as cytotoxic agents

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Abstract—Taking into account the structural requirements for cytotoxicity, several new hydroximinosteroid derivatives have been prepared and evaluated for their cytotoxic activity against A-549, H116, PSN1, and T98G cultured tumor cell lines in order to obtain further information on the potential pharmacophoric core of this type of compound. The influence of the oxygenated position in the A ring, the presence of an additional oxygenated position at C-7 and C-16, and a fluorinated position at C-5 were considered in order to study the structure–activity relationships. The results reveal the importance of oxygenated positions in the A ring (e.g., 4,5-epoxide showed an IC_{50} value against HCT-116 under micromolar level) for an increase in cytotoxic activity in this type of compound. Furthermore, they showed an important selectivity toward colon tumor line (HCT-116).

1. Introduction

As part of our search for new antitumor compounds from marine organisms, in 1997 we isolated from two morphospecies of *Cinachyrella alloclada* and *C. apion* sponges the 6*E*-hydroximino-4-ene steroids **1** and **2**, which constitute the first examples of marine steroids bearing an oxime group.[1] The selective cytotoxic activity shown by **1** against several cancer cells prompted us to consider this type of steroid as potentially biologically active compounds. Indeed, synthetic oxime steroids with analogous structures were found to be efficient aromatase inhibitors[2] and, as a result, they were considered as potential antitumor agents.[3] On the other hand, several hydroximino derivatives of some 16*E*-arylidenosteroids were active in a 60-cell line antitumor pre-screen, showed interesting ip and sc scores in the in vivo hollow fiber bioassay and have been referred to the Biological Evaluation Committee for Cancer Drugs for further detailed in vivo testing.[4]

The previously developed synthetic methodology enabled us to prepare a new series of 6*E*-hydroximino-4-ene steroid analogs to study the pharmaceutical utility and the structural requirements for activity as cytotoxic agents for this type of compound.[5] Our previous report concerning the chemical synthesis and the pharmacological evaluation of these synthetic analogs highlighted a suitable structure for the development of new antitumor agents.

Two key features in the cytotoxic profile were taken into account, that is, strong activity and remarkable selectivity. Indeed, the synthetic analog **3**, bearing an additional oxygenated position at C-2 in relation to that of natural compound **1**, showed a very promising level of selective cytotoxicity. Furthermore, the synthesis of several 6*E*-hydroximino-4-ene steroids with different side chains and degrees of unsaturation on ring A identified important features related to the structure/cytotoxic activity relationship for this type of compound: the presence of a cholesterol-type side chain, which appears to play a major role in determining the biological activity, and an elevated degree of oxidation on ring A resulted in a bioactivity that proved to be higher than that encountered with other structural motifs.

In this paper, we wish to report the design and synthesis of a new series of 6-hydroximinosteroid derivatives taking into account the previously found structural

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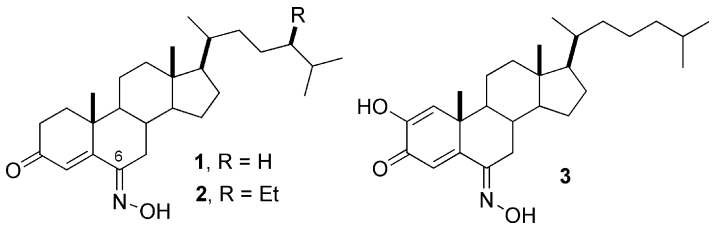


Figure 1. Natural 6*E*-hydroximino-4-ene steroids, compounds **1** and **2**, and the synthetic analog **3**.

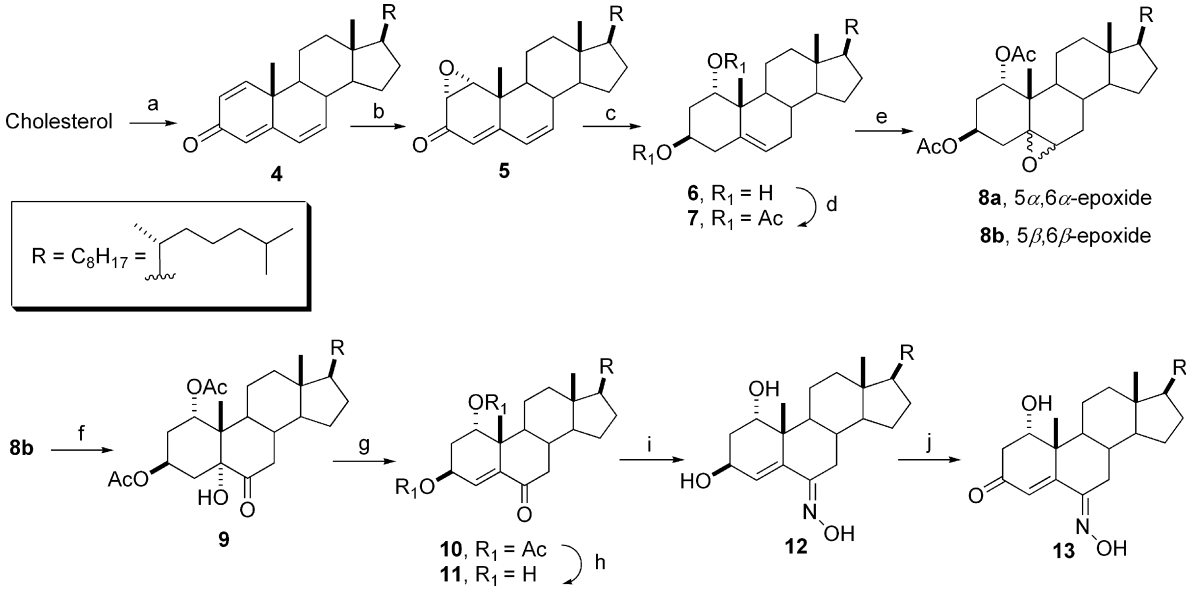
requirements for cytotoxicity. The aim of this study was to obtain additional information on the potential pharmacophoric core and to expand the study of the structure–activity relationships for this type of compound (Fig. 1).

2. Results and discussion

2.1. Chemistry

The new analogs were prepared from commercially available low cost starting materials, such as cholesterol and diosgenin, to maintain the cholesterol-type side chain and a good level of cytotoxicity.[5] Initially, in order to analyze the influence of the oxygenated position in the A ring, we carried out the synthesis of several analogs and varied the location of this position around this ring.

2.1.1. Synthesis of analogs with an additional hydroxyl group at C-1 (Scheme 1). Analogs **12** and **13**, with a hydroxyl group at C-1, were synthesized according to the sequence shown in Scheme 1. The introduction of the α-OH group at C-1 in the steroid skeleton was achieved using the methodology developed by Fürst et al.[6,7] The oxidation of cholesterol with DDQ gave trienone **4**, which was further oxidized with H_2O_2 in the presence of alcoholic NaOH to yield epoxide **5**. The lithium/ammonia reduction of **5** under carefully controlled anhydrous conditions gave the required 1α,3β-dihydroxycholest-5-ene (**6**), in which the hydroxyl groups at C-1 and C-3 of **6** were protected by acetylation. Subsequent epoxidation of the resulting diacetate **7** gave a mixture of α- and β-epoxides, **8a** and **8b**, in a 1:2 ratio. The epoxide configurations were established by analysis of the proton and carbon NMR chemical shifts at C-6.[8] Resonances due to H-6 at 2.80 ppm (d, $J = 3.0$ Hz) and C-6 at 61.7 ppm were in perfect agreement with an α-disposition of the epoxide in **8a**, while the chemical shifts found for H-6 at 3.14 ppm (d, $J = 2.0$ Hz) and C-6 at 56.3 ppm were indicative of a β-configuration of the epoxide in **8b**. Several attempts at oxidation of **8a** and **8b** to the corresponding 5α-hydroxy-6-ketones with CrO_3/H_2O,[9] NBS/dioxane,[10] or $HClO_4$/dioxane[11] were unsuccessful. Finally, oxidation of **8b** with Jones' reagent[12] in Ac_2O at 0 °C gave the desired 1α,3β-diacetoxy-5α-hydroxy-6-one steroid **9** in 72% yield, whereas epoxide **8a** was found to be unreactive under the same conditions. Subsequent hydroxyl elimination at C-5 using thionyl chloride in pyridine afforded the diacetoxy-α,β-unsaturated ketone **10**, which, after removal

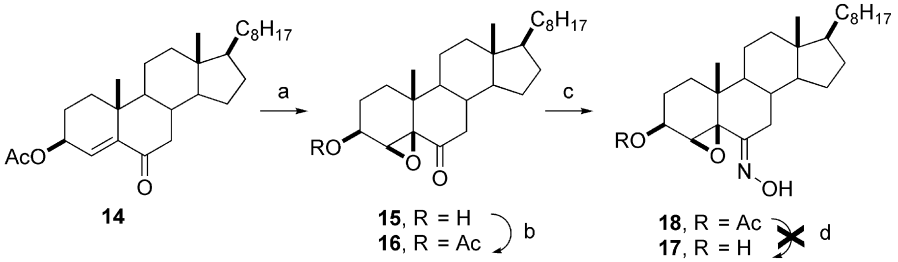


Scheme 1. Reagents: (a) DDQ, dioxane (45%); (b) 35% H_2O_2, NaOH/MeOH (5:95) (70%); (c) i—Li, NH_3, THF; ii—NH_4Cl (25%); (d) Ac_2O, Py (99%); (e) MCPBA, $CHCl_3$ (97%); (f) Jones' reagent, Ac_2O (92%); (g) $SOCl_2$, Py (85%); (h) KOH/MeOH (5:95) (72%); (i) $NH_2OH{\cdot}HCl$, Py (90%); (j) MnO_2, $CHCl_3$ (75%) or CrO_3, Py (60%).

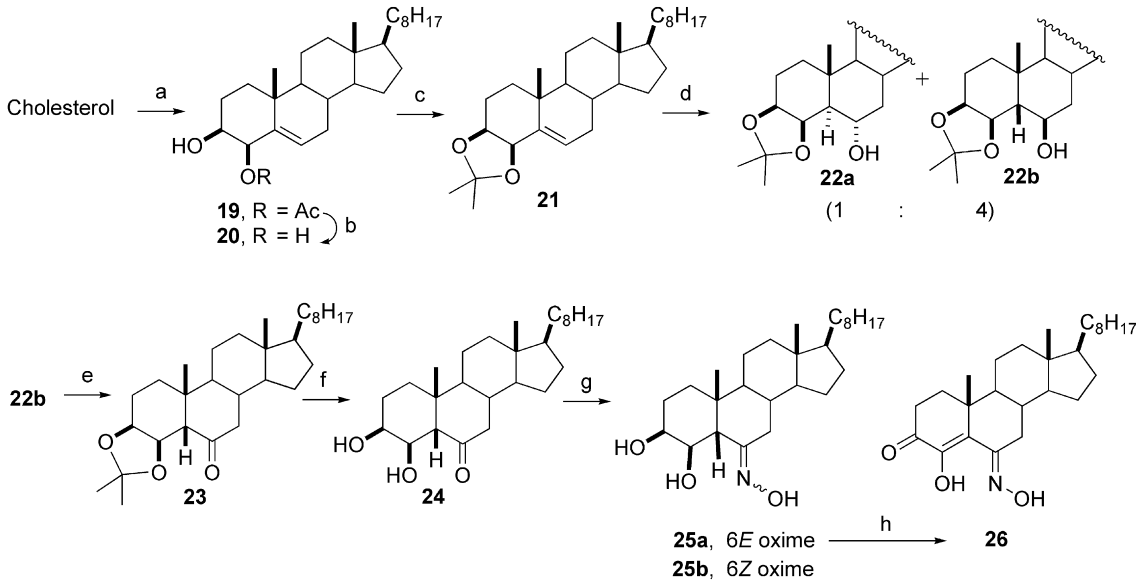
of the acetate groups with alcoholic KOH, was treated with hydroxylamine hydrochloride to provide the required 6*E*-hydroximinocholest-4-en-1α,3β-diol (**12**). The downfield chemical shift of H-7β at 3.33 ppm (d, $J = 12.5$ Hz) confirmed the *E* configuration of the oxime group.[1] Finally, allylic oxidation of the 3β-hydroxy group of **12** to give **13** was achieved under different conditions. Although the oxidation with CrO_3 in pyridine was faster, a better yield was obtained when MnO_2 in $CHCl_3$ was used.

2.1.2. Synthesis of analogs involving a 4β,5β-epoxide (Scheme 2). In an effort to evaluate the presence of an epoxide group on the cytotoxic activity of this type of compound, we designed the synthesis of 4β,5β-epoxide analogs. The 4β,5β-epoxide was prepared from 3β-acetoxycholest-4-en-6-one (**14**)[5] using alkaline hydrogen peroxide[13] to give 4β,5β-epoxy-3β-hydroxycholestan-6-one (**15**) in 88% yield.[14] Compound **15** was converted to the unstable 4β,5β-epoxide analog **17** using hydroxylamine hydrochloride in pyridine. In order to obtain a more stable analog, 4β,5β-epoxy-3β-hydroxycholestan-6-one (**15**) was first acetylated and then oximinated using our standard procedure to yield the epoxide analog **18**, which was found to be stable. Attempts to hydrolyze **18** with alcoholic K_2CO_3 to give analog **17** were unsuccessful.

2.1.3. Synthesis of analogs with an additional hydroxyl group at C-4 (Scheme 3). It is well known that 4-hydroxyandrost-4-en-3,17-dione, which bears a 4-hydroxy-4-en-3-one moiety, is a potent aromatase inhibitor as well as effective agent in the treatment of advanced estrogen-dependent breast cancer.[15] Based on this information we became interested in the combination of the 6-hydroximine-4-en-3-one grouping in conjunction with a 4-hydroxy-substituent. We therefore addressed the synthesis of the analogs **25a**–**b** and **26**, which are depicted in Scheme 3. The introduction of the β-OH group onto C-4 of cholesterol was attempted by the chemo- and stereoselective allylic acetoxylation of the Δ^5 double bond (Petrows' rearrangement). Reaction of cholesterol with bromine in chloroform and subsequent treatment with silver acetate in pyridine gave the 4β-acetoxy-3β-hydroxy steroid **19**.[16] Hydrolysis of the acetate with alcoholic KOH followed by protection of the resulting 3,4-diol **20** as the acetonide gave dioxolane **21**. Hydroboration of the double bond followed by oxidation of the resulting alkylborane with alkaline hydrogen peroxide gave a 1:4 mixture of the two



Scheme 2. Reagents: (a) 35% H_2O_2, NaOH/MeOH (1:9) (88%); (b) Ac_2O, Py (99%); (c) $NH_2OH{\cdot}HCl$, Py (99%); (d) K_2CO_3, MeOH.



Scheme 3. Reagents: (a) Br_2, AcOAg, $CHCl_3$, Py (65%); (b) KOH/MeOH (5:95) (90%); (c) TsOH, Acetone (85%); (d) i—$BH_3{\cdot}THF$ ii—35% H_2O_2, NaOH, THF (60%); (e) Dess–Martin, CH_2Cl_2 (90%); (f) 1 M HCl/THF (1:1) (99%); (g) $NH_2OH{\cdot}HCl$, AcONa, EtOH, H_2O (85%); (h) DMSO, TFAA, Et_3N, CH_2Cl_2 (20%).

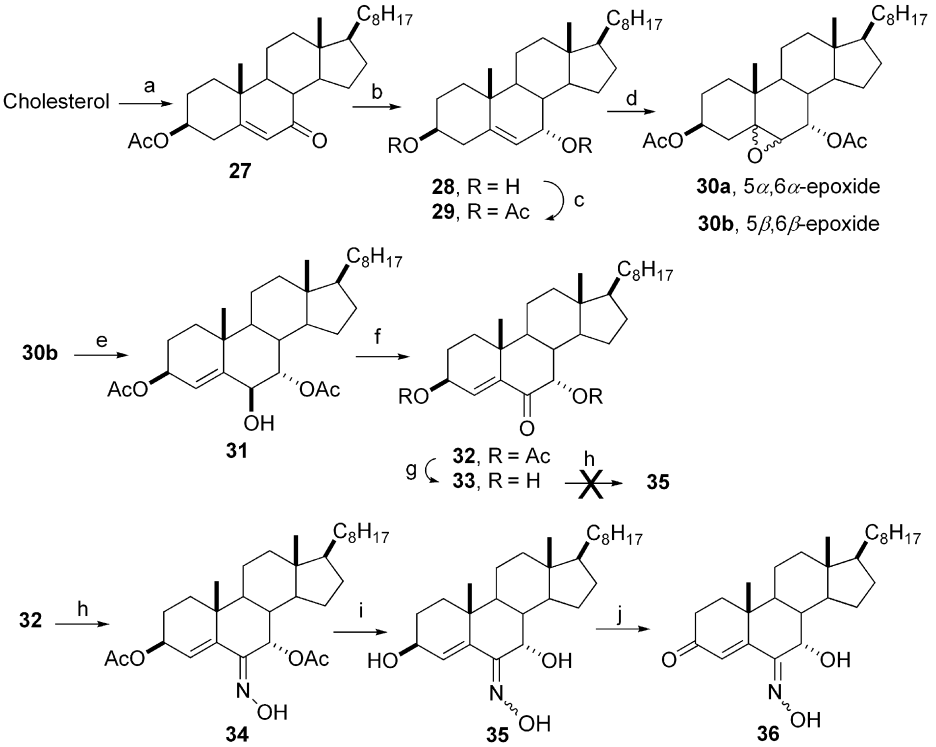
isomeric C-6 alcohol acetonides **22a** and **22b**. The downfield carbon and proton chemical shift of the C-19 methyl group in compound **22a** (1.03 ppm and 15.1 ppm) in relation to **22b** (1.15 ppm and 24.9 ppm) allowed us to establish the stereochemistry of these compounds.[17] After chromatographic separation of both isomers, Dess–Martin oxidation of the 5β-isomer **22b** followed by cleavage of the acetonide under acidic conditions yielded the diol ketone **24**. Oximination of **24** with hydroxylamine hydrochloride in aqueous ethanol and sodium acetate gave a separable *E*/*Z* mixture of oximes **25a** and **25b** in a 2.2:1 ratio. Final oxidation of the *E*-isomer with dimethylsulfoxide activated with trifluoroacetic anhydride gave the 6*E*-hydroximino-4-ene steroid analog **26**.

2.1.4. Synthesis of 6-hydroximino-4-ene steroids with a hydroxyl group at C-7 (Scheme 4). In order to evaluate the influence of the oxidation degree in the B ring on the cytotoxic activity, we designed new 6-hydroximine analogs with an additional oxygenated position at C-7 while keeping the enone functionality in the A ring.

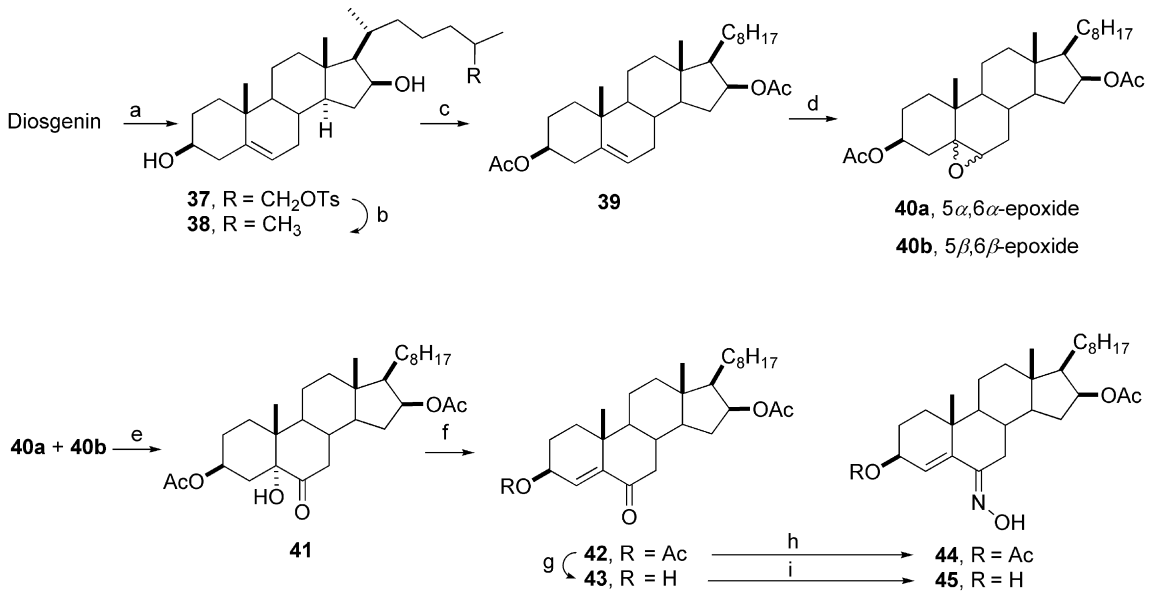
The reaction sequence began with the copper-catalyzed allylic oxidation[18] of cholesteryl acetate by *tert*-butylhydroperoxide in benzene to give enone **27**, which was chemoselectively reduced with L-selectride[19] to yield diol **28**. Protection of the hydroxyl groups at C-3 and C-7 of **28** as acetates, followed by epoxidation of **29**, gave a mixture of epoxides **30a** and **30b** in a 2:1 ratio. The stereochemistry of epoxides α- and β-**30a** and **30b** was assigned by comparison of their NMR data with the literature data.[20] Once again, attempts to open epoxides **30** using CrO_3 in H_2O, NBS in dioxane, and $HClO_4$ in dioxane were unsuccessful. Finally, regioselective ring opening of the β-**30b** epoxide was achieved using $CeCl_3{\cdot}7H_2O$ in dry CH_3CN via the chlorohydrin[21] to give 3β,7α-diacetoxy-6-hydroxycholest-4-ene (**31**),[22,23] which was subsequently converted into diacetylated enone **32** by treatment with the Dess–Martin reagent. Deprotection with alcoholic KOH gave diol enone **33**, which afforded an intractable mixture of compounds upon treatment with hydroxylamine hydrochloride. To circumvent this problem, diacetylated enone **32** was oximinated using our standard procedure followed by deprotection with alcoholic KOH to give a mixture of the *E*,*Z* oximes **35**. Final oxidation of **35** with CrO_3 in pyridine gave the required analog **36** also as mixture of the *E*,*Z* oximes.

2.1.5. Synthesis of 6*E*-hydroximino-4-ene steroids with an oxygenated position at C-16 (Scheme 5). To further evaluate the influence of the oxidation degree in the D ring on the cytotoxic activity, we designed new 6-hydroximine analogs with an additional oxygenated position at C-16.

Clemmensen reduction of diosgenin[24] followed by selective tosylation of the primary alcohol with *p*-toluenesulfonyl chloride in pyridine gave the monotosylated diol **37** in a variable yield. Reduction of **37** with $LiAlH_4$ in ether furnished diol **38** and acetylation of this compound gave diacetylated product **39**. Epoxidation of **39** using two different methods (MCPBA or $KMnO_4$/



Scheme 4. Reagents: (a) i—Ac_2O, Py (99%); ii—*t*-BuOOH, CuI, C_6H_6 (40%); (b) L-selectride, THF (95%); (c) Ac_2O, Py (99%); (d) MCPBA, $CHCl_3$ (85%); (e) $CeCl_3{\cdot}7H_2O$, CH_3CN/THF (2:1) (60%); (f) Dess–Martin, CH_2Cl_2 (82%); (g) KOH/MeOH (5:95) (90%); (h) $NH_2OH{\cdot}HCl$, Py (90%); (i) KOH/MeOH (5:95) (99%); (j) CrO_3, Py (13%).

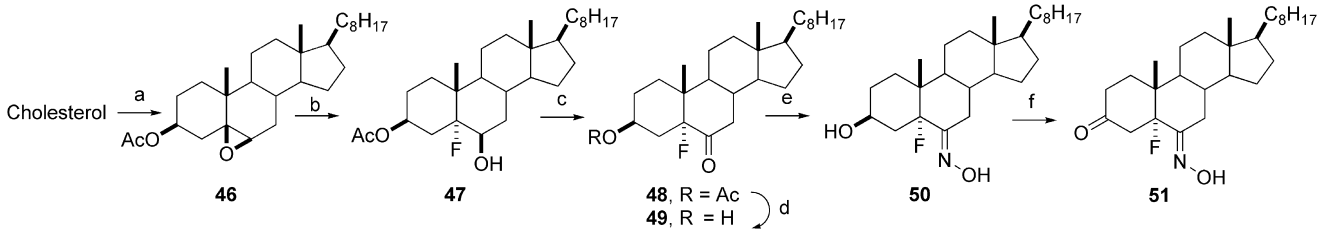


Scheme 5. Reagents: (a) i—Zn, HCl, EtOH (90%); ii—TsCl, Py (80–40%);(b) $LiAlH_4$, Et_2O (70%); (c) Ac_2O, Py (99%); (d) MCPBA, $CHCl_3$ (70%) or $KMnO_4/Fe_2(SO_4)_3{\cdot}nH_2O/CH_2Cl_2$ (40%); (e) Jones' reagent, Ac_2O (72%); (f) $SOCl_2$, Py (75%); (g) KOH/MeOH (5:95) (50%); (h) $NH_2OH{\cdot}HCl$, Py (60%); (i) $NH_2OH{\cdot}HCl$, Py (16%).

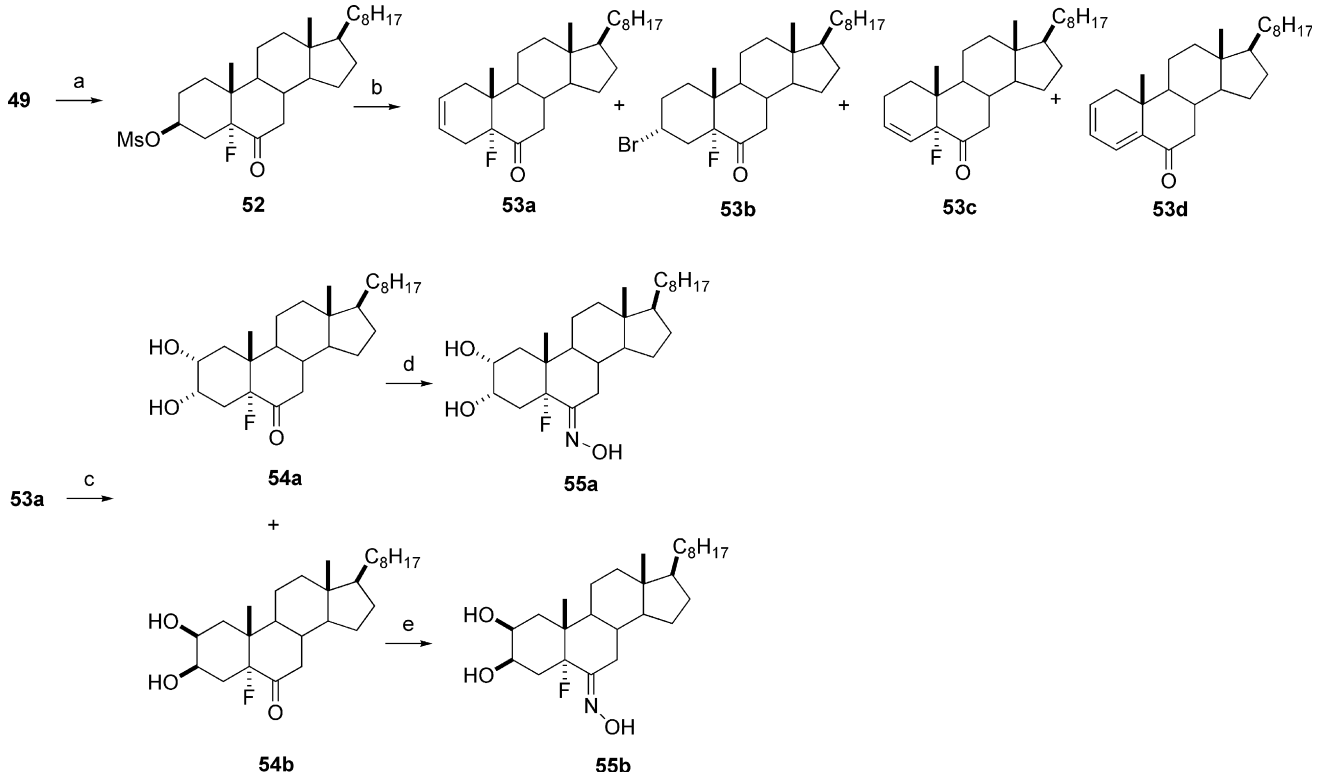
$Fe_2(SO_4)_3{\cdot}nH_2O$)[25] afforded an inseparable mixture of α- and β-epoxides, **40a** and **40b**, respectively. The configurations of the epoxides were established from the proton and carbon chemical shifts at C-6 in the same way as described for compounds **8**. Oxidation of the mixture of **40a** and **40b** with Jones' reagent in Ac_2O at 0 °C gave the desired 1,16-diacetoxy-5-hydroxy-6-one steroid **41**, whereas epoxide **40a** proved to be unreactive under these conditions. Elimination of the hydroxyl group at C-5 in **41** with thionyl chloride in pyridine provided **42**. Further oximination of **42** with hydroxylamine hydrochloride afforded the 16-acetoxy-6*E*-hydroximinocholest-4-en-3β-ol (**44**), while hydrolysis of the acetate group on the hydroxyl group at C-3 of **42** with alcoholic KOH, followed by oximination of the resultant product **43**, gave the analog **45** (Scheme 5).

2.1.6. Synthesis of 6*E*-hydroximinosteroids fluorinated at C-5 (Schemes 6 and 7). The use of fluorine to influence the biological activity of compounds has been studied mainly due to the steric similarity between fluorine and hydrogen, coupled with fluorine's much larger electronegativity. In an effort to estimate the influence of the presence of fluorine on the cytotoxic activity of this type of compound, we prepared compounds **50**, **51**, **55**, and **56**. The fluoro-substituent was introduced using a similar strategy to that described by Galagovsky and coworkers[12] to obtain new fluorinated brassinosteroid analogs.

The stereo- and regioselective epoxidation of cholesteryl acetate with $KMnO_4$ and $Fe_2(SO_4)_3{\cdot}nH_2O$[25] in dichloromethane gave the 5β,6β-epoxide **46**. The *trans*-diaxial acid-catalyzed opening with BF_3 etherate allowed the introduction of an α-fluoro-substituent at C-5 with the β-OH group at C-6 in 80% yield. The chemical shift of C-5 at 98.6 ppm as a doublet with a $^1J_{C,F}$ of 166.9 Hz in the ^{13}C NMR spectrum, along with the signal at −156.4 ppm in the ^{19}F NMR spectrum of **47**, confirmed the introduction of the fluoro-substituent. Subsequent oxidation with Dess–Martin reagent gave ketone **48** and the 3β-hydroxyl group was deprotected by mild alkaline hydrolysis with K_2CO_3 to yield **49**. Introduction of the oxime group at C-6 in **49** with hydroxylamine hydrochloride in pyridine gave compound **50**. Subsequent oxidation of the 3β-hydroxy group of **50** with Jones' reagent yielded the fluorinated analog **51** in 56% yield, which has since been optimized to 76% when CrO_3 in dry pyridine was used as the oxidant.



Scheme 6. Reagents: (a) i—Ac_2O, Py (95%); ii—$KMnO_4/Fe_2(SO_4)_3{\cdot}nH_2O/CH_2Cl_2$ (65%); (b) BF_3/Et_2O (80%); (c) Dess–Martin, CH_2Cl_2 (90%); (d) K_2CO_3/MeOH (84%); (e) $NH_2OH{\cdot}HCl$/Py (96%); (f) CrO_3, Py (76%) or Jones' reagent (56%).



Scheme 7. Reagents: (a) MsCl, Py (99%); (b) LiBr, DMF (60%); (c) OsO_4, NMO, THF (30%); (d) $NH_2OH{\cdot}HCl$/Py (88%); (e) $NH_2OH{\cdot}HCl$/Py (90%).

The preparation of the 5-fluoro-6*E*-hydroximinosteroids **55** and **56** from cholesterol is described in Scheme 7. Treatment of the intermediate **49** (Scheme 6) with MsCl in pyridine yielded the mesylate **52**. This compound was heated under reflux with LiBr in dry DMF to provide the desired Δ^2 steroid **53a** along with other steroid derivatives (**53b**–**d**). Osmium dihydroxylation with NMO on **53a** gave the dihydroxy steroids 2α,3α-**54a** and 2β,3β-**55b** in a 1:1 ratio after separation by flash chromatography. Oximination of both diol steroids using our standard procedure yielded the hydroximinosteroid analogs **55a** and **56b** in high yield.

2.2. Biological evaluations

All of these novel 6*E*-hydroximino-4-ene steroids were studied in vitro on A-549, HCT-116, PSN1, and T98G tumor cells. The results, expressed as IC_{50} values in μM, are reported in Table 1.

First, we evaluated the influence of the oxygenated position on ring A. Although compound **12**, with a hydroxyl group at C-1, and compounds **25a**, **25b**, and **26**, each with a hydroxyl group at C-4, showed a slight increase in cytotoxic activity against H116 tumor cell in comparison to that of the 'parent' natural compound **1**, they were less active than compound **3**. The configuration of the oxime group does not seem to have an influence on the level of cytotoxic activity since compounds **25a** and **25b** have similar activity patterns. Compound **18**, bearing an epoxide group between positions C-4 and C-5, was found to be the most active derivative and displayed similar activity as compound **3**.

Table 1. The in vitro cytotoxic activities (IC_{50} in μM) of the synthetic hydroximinosteroid analogs

Compound	A-549	HCT-116	PSN1	T98G
1	12.10	12.10	12.10	24.21
3	2.34	0.23	1.17	2.34
12	11.60	2.32	2.32	23.20
13	>25	>25	>25	25
18	1.06	0.21	1.06	1.06
25a	11.54	1.15	11.54	>23.09
25b	11.54	1.15	23.09	23.09
26	11.65	2.33	>23.31	23.31
35	>25	>25	>25	>25
36	>25	>25	>25	>25
44	19.41	1.94	19.41	19.41
45	21.14	21.14	21.14	>25
50	22.98	22.98	22.98	>25
51	11.54	11.54	11.54	11.54
55a	2.22	2.22	2.22	2.22
55b	>25	>25	>25	>25

The influence of an additional oxygenated position on rings B and D was evaluated next. The presence of a hydroxyl group at C-7 in the B ring (compounds **35** and **36**) resulted in the loss of activity. On the other hand, compounds **44** and **45**, with an oxygenated position at C-16 in the D ring, showed very moderate activity – although this was selective against H116 in the case of **44**.

The presence of a fluorinated position at C-5 in **51**, instead of the Δ^4 position as in **1**, led to moderate activity. However, the higher cytotoxicity shown by compound **55a** confirmed the importance of the 2,3-oxygenation pattern for increased activity.

3. Conclusions

We have prepared a new series of hydroximinosteroid derivatives with different oxygenated positions in the A, B, and D ring and we have introduced fluor at the C-5 position. These results reveal the importance of oxygenated positions in the A ring (e.g., 4,5-epoxide showed an IC_{50} value against HCT-116 under μM level) for an increase in cytotoxic activity in this type of compound. It is worth underlining the selective activity shown by the most active hydroximinosteroids against H116 tumor cells.

4. Experimental

Nuclear magnetic resonance spectra (proton and carbon) were recorded on Bruker AC 200 F and 300 or 500 Avance spectrometers at the University of A Coruña, using $CDCl_3$ and CD_3OD as the solvents and internal standards. Multiplicities of ^{13}C signals were obtained by DEPT. Medium-pressure chromatographic separations were carried out on silica gel 60 (230–400 mesh). Melting points were determined on a Büchi 510 apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-1000 polarimeter, with Na (589 nm) lamp and filter. LREIMS and LRFABMS were recorded on a VG-Quattro instrument, while (+)-HRESIMS were measured on QSTAR Elite of Applied Biosystem and FTMS Apex III Bruker spectrometers.

4.1. Cholesta-1,4,6-trien-3-one (4)

A solution of cholesterol (5 g, 13 mmol) and dichlorodicyanobenzoquinone (DDQ) (12.5 g, 55 mmol) in dry dioxane (40 mL) was heated under reflux for 5 h. The mixture was cooled, filtered, and applied to a short column of silica gel (CH_2Cl_2) to give a crude product, which was purified by column chromatography (silica gel, hexanes/ethyl acetate 9:1) to afford cholesta-1,4,6-trien-3-one (**4**, 2.25 g, 45%): white solid; $[\alpha]_D$ −16.9 (hexane/Et_2O (1:1), $c = 1$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.05 (H1, 1H, d, $J = 10$ Hz); 6.0–6.3 (H2, H4, H6, H7, 4H, m); 1.01 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.2$ Hz); 0.86 (H26, H27, 6H, d, $J = 7.0$ Hz); 0.68 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 186.3 (C3, s); 162.8 (C5, s); 153.0 (C1, d); 138.8 (C7, d); 128.0 (C2, d); 127.4 (C6, d); 123.6 (C4, d); 55.9; 52.5; 48.3; 42.9; 41.2; 38.2; 36.9; 36.5; 36.0; 35.7; 30.2; 28.0; 27.9; 23.8; 23.6; 22.7; 21.8; 20.6; 18.6 (C19); 11.9 (C18). LREIMS (70 eV, *m/z* %): 380 (M^+, 6); 84 (100).

4.2. 1α,2α-Epoxycholesta-4,6-dien-3-one (5)

H_2O_2 (35%, 12 mL) and a solution of 5% NaOH/MeOH (20 mL) were added to a solution of cholest-1,4,6-trien-3-one (**4**, 3 g, 7.88 mmol). The mixture was stirred at room temperature for 5 h. The reaction mixture was poured into 20 mL of water and extracted with ethyl acetate (2 × 30 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate 8:2) to give 1α,2α-epoxycholest-4,6-dien-3-one (**5**, 2.55 g, 70%): white solid; $[\alpha]_D$ +182.5 (hexane/Et_2O (1:1), $c = 1$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 6.08 (H6, H7, 2H, s); 5.65 (H4, 1H, dd, $J = 2.0$, 0.5 Hz); 3.59 (H1β, 1H, d, $J = 4$ Hz); 3.45 (H2β, 1H, dd, $J = 4.0$, 2.0 Hz); 1.19 (H19, 3H, s); 0.94 (H21, 3H, d, $J = 6.3$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.3$ Hz); 0.78 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 185.4 (C3); 158.9 (C5); 140.6 (C7); 127.6 (C6); 119.3 (C4); 59.4; 55.9; 54.6; 53.2; 45.9; 42.9; 39.4; 39.2; 38.8; 37.5; 36.0; 35.7; 28.0; 27.9; 23.8; 23.7; 22.7; 22.5; 21.1; 18.6; 18.4 (C19); 11.8 (C18,). LREIMS (70 eV, *m/z* %): 396 (M^+, 5); 95 (100).

4.3. 1α,3β-Dihydroxycholest-5-ene (6)

A three-necked flask was fitted with a dropping funnel, a cold-finger condenser filled with liquid N_2, and an inlet tube connected to an ammonia source, with the gas dried using KOH. Argon was swept through the system for 10 min and then ammonia (40 mL) was trapped in the flask. Lithium wire was cut into short pieces and added. After being stirred for 1 h, 1α,2α-epoxycholest-4,6-dien-3-one (**5**, 0.6 g, 1.5 mmol) in THF (20 mL) was added dropwise during 30 min. The cooling bath was removed and the mixture was allowed to warm to −40 °C for 20 min. The flask was dipped into a cooling bath and anhydrous NH_4Cl was added during 2 h (note: take care! vigorous reaction). The mixture turned white and pasty. Most of the ammonia was removed in a stream of argon. The residue was diluted with ether, washed with brine, and dried. Evaporation left a white solid that was subjected to column chromatography (silica gel, hexanes/ethyl acetate 7:3) to afford 1α,3β-dihydroxycholest-5-ene (**6**, 0.18 g, 25%): white solid; $[\alpha]_D$ −9.9 (hexane/Et_2O (9:1), $c = 0.5$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.59 (H6, 1H, d, $J = 4.5$ Hz); 3.96 (H3α, 1H, m); 3.84 (H1β, 1H, br s); 1.03 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.4$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.68 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 137.3 (C5, s); 125.5 (C6, d); 72.8 (C3, d); 66.3 (C1, d); 56.6; 56.1; 42.3; 41.6; 41.3; 40.2; 38.2; 37.2; 36.2; 35.7; 31.8; 31.7; 30.7; 28.2; 27.9; 24.3; 23.8; 22.7; 22.5; 20.2; 19.4; 18.7; 11.8. LREIMS (70 eV, *m/z* %): 402 (M^+, 52); 384 (M^+−OH, 96); 366 (M^+−2 OH, 22); 105 (100).

4.4. 1α,3β-Diacetoxycholest-5-ene (7)

1α,3β-Dihydroxycholest-5-ene (**6**, 1.2 g, 3 mmol) and acetic anhydride/pyridine (1:1, 20 mL) were stirred at room temperature for 22 h and heated at 60 °C for 2 h. After removal of the solvents, the residue was dissolved in 30 mL of ethyl acetate and the solution washed with $NaHCO_3$ (25 mL), 5% HCl (25 mL) and dried over anhydrous Na_2SO_4. The resulting organic phase was evaporated under vacuum to give 1α,3β-diacetoxycholest-5-ene (**7**, 1.2 g, 99%): white solid; $[\alpha]_D$ −150.7 (CH_2Cl_2, $c = 0.25$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.53 (H6, 1H, d, $J = 5.4$ Hz); 5.07 (H1β, 1H, t, $J = 5.8$, 2.9 Hz); 4.92 (H3α, 1H, m); 2.05 (OAc, 3H, s); 2.01 (OAc, 3H, s); 1.08 (H19, 3H, s); 0.90 (H21, 3H, d, $J = 6.2$ Hz); 0.86 (H26, H27, 6H, d, $J = 6.8$ Hz); 0.67 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.4

(OAc, s); 170.3 (OAc, s); 136.0 (C5, s); 125.2 (C6, d); 74.6 (C3, d); 69.4 (C1, d); 56.6; 56.0; 42.2; 42.0; 40.4; 39.5; 38.1; 37.3; 36.1; 35.7; 31.9; 31.6; 28.1; 27.9; 25.2; 24.2; 23.7; 22.7; 22.5; 21.3; 21.0; 20.4; 19.4; 18.6; 11.8. (+)-LRFABMS, *m/z* (%): 509 ($[M+Na]^+$, 5); 486 ($[M+H]^+$, 7); 365 (100).

4.5. 1α,3β-Diacetoxy-5*a*,6*a*-epoxycholestane (8a) and 1α,3β-diacetoxy-5β,6β-epoxycholestane (8b)

1α,3β-Diacetoxycholest-5-ene (**7**, 0.4 g, 0.7 mmol) was dissolved in 20 mL of $CHCl_3$ at 0 °C. A solution of *m*-chloroperbenzoic acid (0.4 g, 2 mmol) in 20 mL of $CHCl_3$ was added dropwise to the reaction mixture and the solution was stirred for 20 h. 5% Na_2SO_3 (50 mL) was added to the mixture with cooling (ice/water bath) and the mixture was kept at this temperature for 6 h. The final aqueous phase was extracted twice with $CHCl_3$ (20 mL) and, after removal of the solvent, the residue was subjected to chromatography (silica gel, hexane/ethyl acetate, 7:3) to give a mixture of 1α,3β-diacetoxy-5*a*,6*a*-epoxycholestane (**8a**, 0.15 g) and 1α,3β-diacetoxy-5β,6β-epoxycholestane (**8b**, 0.25 g) in a 1:2 ratio with a yield of 97%. Compound **8a**: white solid; $[\alpha]_D$ +16.8 (CH_2Cl_2, $c = 0.1$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.21 (H3α, 1H, m); 5.02 (H1β, 1H, dt, $J = 4.0$, 2.0 Hz); 2.80 (H6β, 1H, d, $J = 3.0$ Hz); 2.07 (OAc, 3H, s); 1.96 (OAc, 3H, s); 1.09 (H19, 3H, s); 0.84 (H21, 3H, d, $J = 3.4$ Hz); 0.80 (H26, H27, 6H, d, $J = 6.3$ Hz); 0.57 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.7 (OAc, s); 169.9 (OAc, s); 73.6 (C3, d); 67.1 (C1, d); 63.3 (C5, s); 61.7 (C6, d); 56.3; 56.0; 42.4; 42.2; 39.6; 39.4; 38.9; 37.2; 36.5; 36.0; 35.6; 32.2; 29.0; 28.0; 27.9; 24.1; 23.6; 22.8; 22.5; 21.3; 21.2; 21.0; 18.6; 16.5; 11.7. EIMS (70 eV, *m/z* %): 502 (M^+, 8); 442 (M^+-CH_3COOH, 4); 382 ($M^+-2\ CH_3COOH$, 40); 105 (100). Compound **8b**: white solid; $[\alpha]_D$ −15.1 (CH_2Cl_2, $c = 0.3$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.12 (H1β, 1H, dt, $J = 4.0$, 2.0 Hz); 5.05 (H3α, 1H, m); 3.14 (H6α, 1H, d, $J = 2.0$ Hz); 2.12 (OAc, 3H, s); 2.04 (OAc, 3H, s); 1.10 (H19, 3H, s); 0.85 (H21, 3H, d, $J = 6.0$ Hz); 0.81 (H26, H27, 6H, d, $J = 5.2$ Hz); 0.59 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.3 (OAc, s); 169.8 (OAc, s); 74.5 (C3, d); 67.1 (C1,d); 63.0 (C5, s); 56.3 (C6, d); 56.1; 55.5; 42.3; 42.0; 39.1; 39.0; 38.6; 37.1; 36.8; 36.8; 35.3; 32.8; 29.4; 28.2; 27.2; 24.6; 23.4; 22.8; 22.5; 21.8; 21.6; 21.3; 18.2; 16.0; 11.4. (+)-LRFABMS, *m/z* (%): 525 ($[M+Na]^+$, 100); 503 ($[M+H]^+$, 12).

4.6. 1α,3β-Diacetoxy-5α-hydroxycholestan-6-one (9)

A solution of 1α,3β-diacetoxy-5β,6β-epoxycholestane (**8b**, 0.25 g, 0.5 mmol) in Ac_2O (12 mL) was treated with Jones' reagent (2.5 mL) and the mixture was stirred at 0 °C for 6 h. The reaction mixture was poured into 10 mL of MeOH and extracted with ether (2 × 20 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate 8:2) to give 1α,3β-diacetoxy-5α-hydroxycholestan-6-one (**9**, 0.18 g, 72%): white solid; $[\alpha]_D$ +5.2 (CH_2Cl_2, $c = 0.1$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.24 (H3α, 1H, m); 5.12 (H1β, 1H, t, $J = 2.1$, 0.5 Hz); 2.83 (H7β, 1H, t, $J = 12.5$, 12.2 Hz); 2.16 (OAc, 3H, s); 2.03 (OAc, 3H, s); 0.93 (H19, 3H, s); 0.89 (H21, 3H, d, $J = 6.8$ Hz); 0.85 (H26, H27, 6H, d, $J = 5.8$ Hz); 0.64 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 209.2 (C6, s); 170.2 (OAc, s); 168.9 (OAc, s); 81.5 (C5, s); 76.2 (C3, d); 66.6 (C1,d); 63.0; 56.6; 56.1; 55.8; 42.4; 39.4; 39.2; 38.3; 36.9; 36.0; 35.8; 35.6; 32.0; 29.8; 28.0; 27.9; 23.9; 23.7; 22.7; 22.5; 21.1; 20.1; 18.6; 16.3; 11.9. (+)-LRFABMS, *m/z* (%): 541 ($[M+Na]^+$, 52); 399 (100).

4.7. 1α,3β-Diacetoxycholest-4-en-6-one (10)

Thionyl chloride (0.2 mL, 2.6 mmol) was added dropwise to a solution of 1α,3β-diacetoxy-5α-hydroxycholestan-6-one (**9**, 0.16 g, 0.3 mmol) in 5 mL of dry pyridine at 0 °C. The mixture was stirred for 1 h and then poured into water (20 mL). The resulting precipitate was extracted with ethyl acetate (2 × 20 mL) and the combined extracts washed (10% HCl followed by brine), dried, and evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate, 8:2) to give 1α,3β-diacetoxycholest-4-en-6-one (**10**, 0.14 g, 92%): white solid; $[\alpha]_D$ −28.6 (CH_2Cl_2, $c = 0.1$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 6.26 (H4, 1H, s); 5.45 (H3α, 1H, ddd, $J = 9.8$, 6.8, 2.4 Hz); 5.09 (H1β, 1H, d, $J = 2.4$ Hz); 2.58 (H7β, 1H, d, $J = 12.6$ Hz); 2.08 (OAc, 3H, s); 2.06 (OAc, 3H, s); 1.10 (H19, 3H, s); 0.91 (H21, 3H, d, $J = 6.3$ Hz); 0.86 (H26, H27, 6H, d, $J = 6.8$ Hz); 0.69 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 201.2 (C6, s); 170.4 (OAc, s); 170.2 (OAc, s); 144.4 (C5, s); 128.6 (C4, d); 73.8 (C3, d); 66.5 (C1, d); 56.7; 56.0; 55.9; 45.9; 44.7; 43.6; 42.5; 41.4; 39.4; 39.2; 36.0; 35.6; 33.4; 28.0; 27.9; 26.4; 23.9; 22.9; 21.0; 20.9; 20.8; 20.4; 18.6; 11.9. LREIMS (70 eV, *m/z* %): 500 (M^+, 2); 440 (M^+-CH_3COOH, 8); 398 ($M^+-2\ CH_3COOH+H_2O$, 22); 383 ($M^++H-2\ CH_3COO$, 13); 84 (100).

4.8. 1α,3β-Dihydroxycholest-4-en-6-one (11)

1α,3β-Diacetoxycholest-4-en-6-one (**10**, 0.2 g, 0.4 mmol) was dissolved in a 5% methanolic potassium hydroxide solution (15 mL) under argon. The mixture was stirred at room temperature for 1 h and then the solvent was evaporated under reduced pressure. This solution was poured into ice/water (25 g) and the product extracted with ethyl acetate (2 × 20 mL). The combined extracts were washed with saturated brine, dried, and evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 7:3) to give 1α,3β-dihydroxycholest-4-en-6-one (**11**, 0.15 g, 75%): white solid; $[\alpha]_D$ +5.5 (CH_2Cl_2, $c = 0.4$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 6.36 (H4, 1H, s); 4.55 (H3α, 1H, m); 3.95 (H1β, 1H, s); 3.65 (OH, 1H, br s); 2.58 (H7β, 1H, d, $J = 12.7$ Hz); 1.04 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.3$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.3$ Hz); 0.72 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 202.4 (C6, s); 142.9 (C5, s); 132.6 (C4, d); 72.1 (C3, d); 64.0 (C1, d); 60.4; 56.6; 56.0; 46.0; 43.2; 42.5; 43.4; 39.2; 36.0; 35.6; 34.9; 33.6; 27.9; 23.9; 23.7; 22.7; 21.1; 21.0; 20.5; 18.6; 14.1; 11.9. LREIMS (70 eV, *m/z* %): 416 (M^+, 9); 398 (M^+-OH, 5); 84 (100).

4.9. 6*E*-Hydroximinocholest-4-en-1α,3β-diol (12)

1α,3β-Dihydroxycholest-4-en-6-one (**11**, 0.1 g, 0.24 mmol) and hydroxylamine hydrochloride (0.12 g, 1.7 mmol) were dissolved in dry pyridine (10 mL). The resulting mixture was stirred at room temperature for 22 h and the solvent was removed under reduced pressure. The residue was diluted with water (20 mL) and extracted with ethyl acetate (20 mL). The extract was dried, evaporated, and the residue subjected to chromatography (silica gel, hexanes/ethyl acetate, 6:4) to give 6*E*-hydroximinocholest-4-en-1α,3β-diol (**12**, 92 mg, 92%): white solid; $[\alpha]_D$ +64.6 (MeOH, $c = 0.1$); ^{1}H NMR (200 MHz, CD_3OD) δ_H: 5.76 (H4, 1H, s); 4.38 (H3α, 1H, ddd, $J = 8.3, 6.3, 1.9$ Hz); 3.83 (H1β, 1H, d, $J = 2.9$ Hz); 3.33 (H7β, 1H, dd, $J = 3.9, 12.0$ Hz); 0.97 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 5.2$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.72 (H18, 3H, s). ^{13}C NMR (50 MHz, CD_3OD) δ_C: 159.4 (C6, s); 140.0 (C5, s); 127.4 (C4, d); 72.4 (C3, d); 64.7 (C1, d); 58.0; 57.7; 45.6; 43.3; 42.6; 40.8; 40.7; 37.3; 37.0; 35.9; 35.8; 30.6; 29.2; 29.1; 25.2; 24.9; 23.2; 22.9; 21.6; 20.5; 19.2; 12.4. LREIMS (70 eV, *m/z* %): 431 (M^+, 6); 415 (M^+-OH, 32); 414 (M^+-H_2O, 100); 382 ($M^+-NOH-H_2O$, 22). (+)-HRESIMS: *m/z* 432.3459 $[M+H]^+$ (calcd for $C_{27}H_{46}NO_3$, 432.3472).

4.10. 1α-Hydroxy-6*E*-hydroximinocholest-4-en-3-one (13)

Method A. A mixture of 6*E*-hydroximinocholest-4-en-1α,3β-diol (**12**, 0.1 g, 0.23 mmol) and activated MnO_2 (0.4 g, 4.55 mmol) in dry chloroform (25 mL) was shaken vigorously at room temperature for 17 h. The reaction was subjected to chromatography (silica gel, hexanes/ethyl acetate, 1:1) and the product was purified by HPLC (Sharlau C18, flow rate 1 mL/min, retention time 49 min, MeOH/H_2O, 9:1) to give 1α-hydroxy-6*E*-hydroximinocholest-4-en-3-one (**13**, 75 mg, 72%). *Method B.* A solution of 6*E*-hydroximinocholest-4-en-1α,3β-diol (**12**, 0.02 g, 0.046 mmol) in 2 mL of pyridine was added dropwise to the chromium trioxide/pyridine complex prepared by the addition of CrO_3 (0.06 g, 0.6 mmol) to 2 mL of pyridine at 0 °C. The reaction mixture was stirred at room temperature for 75 min and then diluted with 15 mL of ethyl acetate. The resulting precipitate was filtered off and the filtrate was washed (10% HCl, 10% $NaHCO_3$, and brine), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to chromatography (silca gel, hexane/ethyl acetate, 1:1) to give **13** (12 mg, 60%): white solid; $[\alpha]_D$ +199.8 (MeOH, $c = 0.1$); ^{1}H NMR (500 MHz, CD_3OD) δ_H: 6.12 (H4, 1H, s); 4.12 (H1β, 1H, br s); 3.43 (H7β, 1H, dd, $J = 12.2, 4.4$ Hz); 2.84 (H2, 1H, dd, $J = 17.6, 2.7$ Hz); 2.49 (H2′, 1H, dd, $J = 17.6, 2.0$ Hz); 1.16 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.3$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.72 (H18, 3H, s). ^{13}C NMR (125 MHz, CD_3OD) δ_C: 200.2 (C3, s); 156.8 (C6, s); 136.7 (C5, s); 128.6 (C4, d); 72.2 (C1, d); 57.9 (C14, s); 57.4 (C17, d); 51.0 (C9, d); 44.67 (C13, s); 44.6 (C24, t); 43.7 (C12, t); 43.5 (C10, s); 40.6 (C22, t); 37.2 (C20, d); 37.0 (C2, t); 33.9 (C8, d); 30.2 (C7, q); 29.2 (C16, t); 29.1 (C25, q); 25.1 (C15, t); 24.9 (C23, t); 23.2 (C26, q); 22.9 (C27, q); 21.3 (C11, t); 19.2 (C21, q); 18.7 (C19, q); 12.3 (C18, q). EIMS (70 eV, *m/z* %): 429 (M^+, 6); 412 (M^+-H_2O, 67); 385 ($M^+-NOH-H_2O$, 25); 172 (100). (+)-HRESIMS: *m/z* 430.3300 $[M+H]^+$ (calcd for $C_{27}H_{44}NO_3$, 430.3316).

4.11. 4β,5β-Epoxy-3β-hydroxycholestan-6-one (15)

H_2O_2 (35%, 5 mL) and a solution of 10% NaOH/MeOH (1.4 mL) were added to a solution of 3β-acetoxycholest-4-en-6-one[5] (**14**, 1 g, 2.25 mmol). The mixture was stirred at 0 °C for 20 h. The reaction mixture was poured into 20 mL of water and extracted with ethyl acetate (2 × 30 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 7:3) to give 4β,5β-epoxy-3β-hydroxycholestan-6-one (**15**, 0.88 g, 88%): white solid; $[\alpha]_D$ −15.6 (CH_2Cl_2, $c = 0.5$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 4.03 (H3α, 1H, d, $J = 3.7$ Hz); 3.28 (H4α, 1H, d, $J = 3.7$ Hz); 2.61 (H7β, 1H, dd, $J = 11.2, 0.2$ Hz); 1.04 (H19, 3H, s); 0.94 (H21, 3H, d, $J = 6.2$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.8$ Hz); 0.71 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 209.6 (C6, s); 67.9 (C3, s); 66.4 (C5, s); 61.2 (C4, d); 58.3 (C14, d); 55.1 (C17, d); 55.0; 48.6; 46.3; 42.3; 39.9; 38.5; 36.5; 36.1; 35.2, 35.1; 30.2; 28.2; 26.5; 25.5; 24.2; 23.3; 22.7; 21.6; 18.8; 17.4 (C19, q); 13.5 (C18, q). LREIMS (70 eV, *m/z* %): 416 (M^+, 14); 398 (M^+-H_2O, 6); 84 (100).

4.12. 3β-Acetoxy-4β,5β-epoxycholestan-6-one (16)

4β,5β-Epoxy-3β-hydroxycholestan-6-one (**15**, 0.3, 0.72 mmol) and acetic anhydride/pyridine (1:1, 10 mL) were stirred at room temperature for 24 h. A similar work up used for epoxidation of compound **7** gave a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate, 8:2) to give 3β-acetoxy-4β,5β-epoxycholestan-6-one (**16**, 0.3 g, 99%): white solid; $[\alpha]_D$ −9.7 (CH_2Cl_2, $c = 1$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 5.11 (H3α, 1H, m); 3.32 (H4α, 1H, d, $J = 3.0$ Hz); 2.61 (H7β, 2H, dd, $J = 6.2, 0.5$ Hz); 2.11 (OAc, 3H, s); 1.08 (H19, 3H, s); 0.98 (H21, 3H, d, $J = 6.9$ Hz); 0.89 (H26, H27, 6H, d, $J = 6.8$ Hz); 0.72 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 206.5 (C6, s); 170.1 (OAc, s); 68.2 (C3, d); 66.7 (C5, s); 61.2 (C4, d); 55.9; 55.5; 49.6; 46.2; 42.2; 39.0; 38.8; 37.2; 35.6; 35.2; 34.3; 33.2; 32.2; 27.9; 27.5; 23.3; 22.3; 22.0; 21.4; 21.0; 20.5; 18.2; 17.2; 11.3. LREIMS (70 eV, *m/z* %): 458 (M^+, 5); 83 (100).

4.13. 4β,5β-Epoxy-3β-hydroxy-6*E*-hydroximinocholestane (17)

4β,5β-epoxy-3β-hydroxycholestan-6-one (**15**, 0.5 g, 1.2 mmol) was reacted with hydroxylamine hydrochloride (0.6 g, 8.4 mmol) in a similar way as compound **11** to give a residue which was subjected to chromatography (silica gel, hexane/ethyl acetate, 6:4) to afford 4β,5β-epoxy-3β-hydroxy-6*E*-hydroximinocholestane (**17**, 0.5 g, 90%): white solid; $[\alpha]_D$ −4.9 (CH_2Cl_2, $c = 0.5$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 4.15 (H3α, 1H, m); 3.43 (H7β, 1H, dd, $J = 3.9, 4.4$ Hz); 3.23 (H4α, 1H, d, $J = 3.0$ Hz); 1.12 (H19, 3H, s); 0.94 (H21, 3H, d,

J = 6.8 Hz); 0.88 (H26, H27, 6H, d, J = 6.8 Hz); 0.69 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 155.6 (C6, s); 87.6 (C5, s); 84.9 (C3, d); 64.1 (C4, d); 55.4; 55.2; 48.6; 44.8; 43.6; 39.7; 36.6; 36.2; 35.4; 34.4; 34.0; 33.8; 32.6; 37.4; 27.1; 26.5; 25.2; 24.6; 23.3; 22.1; 21.3 (C19, q); 20.6, 12.2 (C18, q). LREIMS (70 eV, *m/z* %): 432 ($[M+H]^+$, 100); 414 ($[M+H]^+ - H_2O$, 65).

4.14. 3β-Acetoxy-4β,5β-epoxy-6*E*-hydroximinocholestane (18)

3β-Acetoxy-4β,5β-epoxycholestan-6-one (**16**, 0.2 g, 0.35 mmol) was reacted with hydroxylamine hydrochloride (0.7 g, 2.1 mmol) in a similar way as compound **11** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate, 8:2) to afford 3β-acetoxy-4β,5β-epoxy-6*E*-hydroximinocholestane (**18**, 0.16 g, 80%): white solid; $[\alpha]_D$ −0.2 (CH_2Cl_2, c = 0.1); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 7.91 (OH, 1H, br s); 5.14 (H3α, 1H, m); 3.39 (H7β, 1H, dd, J = 9.9, 2.6 Hz); 3.28 (H4α, 1H, d, J = 2.0 Hz); 2.03 (OAc, 3H, s); 1.02 (H19, 3H, s); 0.93 (H21, 3H, d, J = 6.7 Hz); 0.86 (H26, H27, 6H, d, J = 6.9 Hz); 0.66 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.6 (OAc, s); 157.3 (C6, s); 67.1 (C3, d); 64.4 (C5, s); 60.8 (C4, d); 55.9; 55.6; 48.1; 42.2; 39.0; 36.5; 35.6; 35.2; 33.3; 32.6; 29.7; 29.0; 27.6; 27.5; 23.7; 23.5; 22.5; 22.3; 22.1; 21.9; 21.0; 18.6; 17.8; 11.9. (+)-HRESIMS: *m/z* 474.3569 $[M+H]^+$ (calcd for $C_{29}H_{48}NO_4$, 474.3578); 496.3387 $[M+Na]^+$ (calcd for $C_{29}H_{47}NNaO_4$, 496.3397).

4.15. 4β-Acetoxycholest-5-en-3β-ol (19)

To a stirred solution of cholesterol (3.00 g, 7.37 mmol) in chloroform (40 mL) at −20 °C was added bromine (0.40 mL, 7.80 mmol) followed, after 30 min, by silver acetate (5.40 g, 32 mmol) in pyridine (12 mL). The reaction mixture was warmed to room temperature in darkness and filtered after 14 h. The filtrate was poured into a solution of 5% hydrochloric acid and the steroid was extracted into a petroleum ether/ethyl acetate (1:1) mixture. The organic phase was washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate, from 9:1 to 8:2) to give 4β-acetoxycholest-5-en-3β-ol (**19**, 2.13 g, 65%): white solid; mp 166–169 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 5.83 (H6, 1H, dd, J = 1.9, 4.9 Hz); 5.38 (H4α, 1H, d, J = 2.5 Hz); 3.64 (H3α, 1H, m); 2.08 (OAc, 3H, s); 1.10 (H19, 3H, s); 0.91 (H21, 3H, d, J = 6.6 Hz); 0.87 (H26, H27, 6H, d, J = 6.6 Hz); 0.67 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 171.3 (O*C*OCH$_3$, s); 138.9 (C5, s); 131.7 (C6, d); 79.4 (C4, d); 71.9 (C3, d); 56.9; 56.2; 50.3; 42.4; 39.7; 39.6; 36.9; 36.3; 36.1; 35.9; 32.2; 31.7; 28.3; 28.1; 25.9; 24.3; 23.9; 22.9; 22.6; 21.7; 20.7; 20.5; 18.8; 11.9. (+)-LRESIMS, *m/z*, (%): 467 ($[M + Na]^+$, 100), 499 ($[M+Na+MeOH]^+$, 57).

4.16. 5-Cholestene-3β,4β-diol (20)

4β-Acetoxycholest-5-en-3β-ol (**19**, 2.37 g, 5.33 mmol) was dissolved in 5% KOH in methanol (120 mL). The reaction mixture was stirred for 1 h at room temperature, the solvent was evaporated under reduced pressure to a volume of 50 mL, poured into ice-water (100 mL), and extracted with ethyl acetate (3 × 100 mL). The organic phase was washed with water (2 × 200 mL) and brine (2 × 200 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate, 7:3) to give 5-cholestene-3β,4β-diol (**20**, 1.94 g, 90%): white solid; mp 168–172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 5.68 (H6, 1H, dd, J = 1.4, 4.6 Hz); 4.14 (H4α, 1H, d, J = 3.3 Hz); 3.56 (H3α, 1H, m); 1.18 (H19, 3H, s); 0.91 (H21, 3H, d, J = 6.5 Hz); 0.87 (H26, H27, 6H, d, J = 6.6 Hz); 0.68 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 142.9 (C5, s); 128.9 (C6, d); 77.4 (C4, d); 72.6 (C3, d); 57.0; 56.2; 50.3; 42.4; 39.8; 39.6; 37.0; 36.3; 36.1; 35.9; 32.2; 31.9; 28.3; 28.1; 25.5; 24.4; 23.9; 22.9; 22.7; 21.1; 20.6; 18.8; 12.0. (+)-LRESIMS, *m/z* (%): 425 ($[M+Na]^+$, 100), 457 ($[M+Na+MeOH]^+$, 80).

4.17. 3β,4β-Methylethylidenebis(oxy)cholest-5-ene (21)

To a solution of 5-cholesten-3β,4β-diol (**20**, 2.00 g, 4.97 mmol) in acetone (120 mL) were added activated molecular sieves (4 Å, 70 g) and *p*-toluenesulfonic acid (0.72 g, 3.68 mmol). The reaction mixture was stirred at room temperature for 1 h and then quenched with $NaHCO_3$ (1.50 g), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β,4β-methylethylidenebis(oxy)cholest-5-ene (**21**, 1.87 g, 85%): white solid; mp 130–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 5.81 (H6, 1H, dd, J = 1.8, 4.4 Hz); 4.41 (H4α, 1H, d, J = 5.8 Hz); 4.10 (H3α, 1H, m); 1.53 ($O_2C(Me)_2$, 3H, s); 1.35 ($O_2C(Me)_2$, 3H, s); 1.17 (H19, 3H, s); 0.92 (H21, 3H, d, J = 6.5 Hz); 0.87 (H26, H27, 6H, d, J = 6.6 Hz); 0.69 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 138.4 (C5, s); 131.0 (C6, d); 108.1 ($O_2C(Me)_2$, s); 80.8 (C4, d); 75.7 (C3, d); 57.1; 56.2; 48.7; 42.5; 39.9; 39.6; 36.3; 36.2; 35.9; 32.9; 32.1; 31.8; 28.3; 28.2; 28.1; 26.0; 25.8; 24.3; 23.9; 22.9; 22.7; 21.5; 20.9; 18.8; 12.1. (+)-LRFABMS, *m/z* (%): 443 ($[M+H]^+$, 8); 385 ($[M-CH_3COCH_3+H]^+$, 100).

4.18. 3β,4β-Methylethylidenebis(oxy)-5α-cholestan-6α-ol (22a) and 3β,4β-methylethylidenebis(oxy)-5β-cholestan-6β-ol (22b)

A stirred solution of 3β,4β-methylethylidenebis(oxy)cholest-5-ene (**21**, 0.20 g, 0.46 mmol) in dry THF (3 mL) at 0 °C was treated with 1 M BH_3·THF solution (2 mL, 2 mmol). The mixture was kept at 0 °C for 10 min and then 25 °C for 1 h. A mixture of aqueous NaOH (3N, 2 mL) and H_2O_2 (35%, 2 mL) was added at 0 °C and the reaction mixture was stirred overnight. Water (15 mL) was added and the reaction mixture was extracted with ether (3 × 30 mL), the combined organic extracts were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by flash chromatography (silica gel, hexanes/ethyl acetate, from 9:1 to 8:2) to give 3β,4β-methylethylidenebis(oxy)-5α-cholestan-6α-ol (**22a**, 0.025 g, 10%) and 3β,4β-methylethylidenebis(oxy)-5β-cholestan-6β-ol (**22b**, 0.107 g, 50%) (60% global; α/β 1:4). Compound **22a**: white solid;

^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.49 (H4α, 1H, t, J = 4.5 Hz); 4.02 (H3α, 1H, m); 3.89 (H6β, 1H, td, J = 10.8, 4.4 Hz); 1.52 ($O_2C(Me)_2$, 3H, s); 1.34 ($O_2C(Me)_2$, 3H, s); 1.03 (H19, 3H, s); 0.90 (H21, 3H, d, J = 6.5 Hz); 0.87 (H26, H27, 6H, d, J = 6.6 Hz); 0.66 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 108.8 ($O_2C(Me)_2$, s); 74.7 (C3, d); 72.4 (C4, d); 66.9 (C6, d); 56.4; 56.3; 54.4; 52.7; 42.8; 41.4; 39.9; 39.6; 36.2; 35.9; 35.8; 35.6; 34.6; 28.8; 28.3; 28.1; 26.4; 26.0; 24.2; 23.9; 22.9; 22.6; 20.9; 18.7 (C21, q); 15.1 (C19, q); 12.2 (C18, q). (+)-LRFABMS, m/z (%): 443 ([M+H−H_2O]$^+$, 8); 385 (100). Compound **22b**: white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.23 (H3α, 1H, q, J = 5.2 Hz); 4.19 (H6α, 1H, br s); 3.84 (H4α, 1H, dd, J = 10.3, 5.2 Hz); 1.50 ($O_2C(Me)_2$, 3H, s); 1.34 ($O_2C(Me)_2$, 3H, s); 1.15 (H19, 3H, s); 0.90 (H21, 3H, d, J = 6.5 Hz); 0.86 (H26, H27, 6H, d, J = 6.6 Hz); 0.68 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 108.0 ($O_2C(Me)_2$); 75.3 (C3, d); 73.6 (C4, d); 68.9 (C6, d); 56.5; 56.4; 52.2; 44.8; 42.8; 40.1; 39.6; 36.3; 35.8; 35.4; 34.7; 32.2; 30.0; 28.4; 28.3; 28.1; 26.1; 24.9 (C19, q); 24.3; 23.9; 22.9; 22.6; 22.2; 20.9; 18.8 (C21, q); 12.1 (C18, q). (+)-LRFABMS, m/z (%): 461 ([M+H]$^+$, 14); 385 (100).

4.19. 3β,4β-Methylethylidenebis(oxy)-5β-cholestan-6-one (23)

To a stirred solution of 3β,4β-methylethylidenebis(oxy)-5β-cholestan-6β-ol (**22b**, 0.180 g, 0.39 mmol) in dichloromethane (3 mL) was added Dess–Martin periodinane (0.210 g, 0.48 mmol). After 20 min, the homogeneous solution was diluted with 10 mL of ether and poured into 20 mL of saturated aqueous $NaHCO_3$ containing 4 g of $Na_2S_2O_3$ (25 g per 100 mL). The mixture was stirred for 10 min. The layers were separated and extracted with ether 10 mL. The organic phase was washed with saturated $NaHCO_3$ (30 mL) and water (30 mL), dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β,4β-methylethylidenebis(oxy)-5β-cholestan-6-one (**23**, 0.16 g, 90%): white solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 4.34 (H3, H4, 2H, m); 2.28 (H5, H7, 2H, m); 1.53 ($O_2C(Me)_2$, 3H, s); 1.35 ($O_2C(Me)_2$, 3H, s); 0.88 (H19, H21, H26, H27, 12H, m); 0.65 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 211.7 (C6, s); 108.8 ($O_2C(Me)_2$, s); 74.7 (C3, d); 72.7 (C4, d); 63.0; 56.8; 56.1; 43.6; 43.2; 43.0; 40.3; 39.5; 39.4; 37.3; 36.0; 35.6; 30.2; 28.1; 28.0; 27.9; 25.9; 23.9; 23.7; 22.7; 22.6; 22.5; 22.2; 21.1; 18.6; 11.9. (+)-LRFABMS, m/z(%): 458 (M$^+$, 3); 401 ([M−CH_3COCH_3+H]$^+$, 100).

4.20. 3β,4β-Dihydroxy-5β-cholestan-6-one (24)

3β,4β-Methylethylidenebis(oxy)-5β-cholestan-6-one (**23**, 0.180 g, 0.395 mmol) was dissolved in 1 M HCl/THF 1:1 (20 mL). The reaction mixture was stirred at room temperature for 24 h and then quenched with $NaHCO_3$, extracted with ethyl acetate (3 × 30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, CH_2Cl_2/MeOH, 98:2) to give 3β,4β-dihydroxy-5β-cholestan-6-one (**24**, 0.164 g, 99%): white solid; mp 130-134°C; $[\alpha]_D$ −31.7 ($CHCl_3$, c = 0.2); ^{1}H NMR (200 MHz, CD_3OD) δ_H: 4.10 (H3α, 1H, dd, J = 11.3, 3.0 Hz); 3.92 (H4α, 1H, m); 0.94 (H21, 3H, d, J = 6.4 Hz); 0.87 (H26, H27, 6H, d, J = 6.5 Hz); 0.83 (H19, 3H, s); 0.68 (H18, 3H, s); ^{13}C NMR (50 MHz, CD_3OD) δ_C: 217.1 (C6, s); 72.6 (C3, d); 70.7 (C4, d); 64.3; 58.9; 58.5; 45.3; 45.2; 43.6; 43.2; 41.9; 41.6; 40.5; 38.3; 38.0; 30.2; 30.1; 28.2; 26.0; 25.9; 24.7; 24.1; 23.9; 23.5; 20.1; 13.4. (+)-LRESIMS, m/z (%): 441 ([M+Na]$^+$, 100).

4.21. 6*E*-Hydroximino-5β-cholestan-3β,4β-diol (25a) and 6*Z*-hydroximino-5β-cholestan-3β,4β-diol (25b)

A solution of 3β,4β-dihydroxy-5β-cholestan-6-one (**24**, 0.09 g, 0.215 mmol) in ethanol (5 mL) was treated with a solution of hydroxylamine hydrochloride (0.134 g, 1.91 mmol) in 50% aqueous ethanol (2 mL) and sodium acetate trihydrate (0.152 g, 1.10 mmol) in 50% aqueous ethanol (2.0 mL). The resulting mixture was stirred at room temperature for 24 h and the solvent was removed under reduced pressure. The residue was diluted with water (5.0 mL) and extracted with ethyl acetate (3 × 5 mL). The extract was dried and evaporated and the residue was subjected to flash chromatography (silica gel, CH_2Cl_2/MeOH, from 98:2 to 9:1) to give 6*E*-hydroximino-5β-cholestan-3β,4β-diol (**25a**, 0.054 g, 58%) as a white solid and 6*Z*-hydroximino-5β-cholestan-3β,4β-diol (**25b**, 0.025 g, 27%), (85% global, *E*/*Z* 2.2:1). Compound **25a**: white solid; $[\alpha]_D$ −2.8 ($CHCl_3$, c = 0.4); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 4.04 (H3α, 1H, br s); 3.85 (H4α, 1H, br d, J = 10.5 Hz); 3.24 (H7β, 1H, br d, J = 10.5 Hz); 2.27 (H5, 1H, br d, J = 10.3 Hz); 0.88 (H19, H21, H26, H27, 12H, m); 0.64 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 160.4 (C6, s); 69.3 (C4, d); 68.3 (C3, d); 56.8; 56.2; 51.3 (C5, d); 43.0; 41.6; 39.8; 39.4; 39.0; 36.1; 35.7; 35.2; 28.2; 28.0; 25.9 (C7, t); 25.4; 24.1; 23.8; 23.2; 22.7; 22.5; 21.2; 18.6; 12.0. (+)-LRFABMS, m/z(%): 434 ([M+H]$^+$, 100), 456 ([M+Na]$^+$, 10). (+)-HRESIMS: m/z 434.3625 [M+H]$^+$ (calcd for $C_{27}H_{48}NO_3$, 434.3629). Compound **25b**: white solid; $[\alpha]_D$ +18.2 ($CHCl_3$, c = 0.3); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 4.04 (H3α, 1H, br s); 3.86 (H4α, 1H, br d, J = 10.3 Hz); 3.48 (H5, 1H, br d, J = 10.7 Hz); 2.30 (H7, 1H, br d, J = 11.7 Hz); 0.88 (H19, H21, H26, H27, 12H, m); 0.64 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 160.9 (C6, s); 70.2 (C3, d); 68.1 (C4, d); 56.5; 56.2; 43.9 (C5, d); 42.8; 41.5; 39.7; 39.4; 38.8; 36.3; 36.1; 35.7; 33.4 (C7, t); 28.2; 27.9; 25.2; 24.0; 23.8; 23.2; 22.7; 22.5; 21.0; 18.6; 12.0; (+)-LRFABMS, m/z (%): 434 ([M+H]$^+$, 100), 456 ([M+Na]$^+$, 26). (+)-HRESIMS: m/z 434.3639 [M+H]$^+$ (calcd for $C_{27}H_{48}NO_3$, 434.3629); 456.3444 [M+Na]$^+$ (calcd for $C_{27}H_{47}NNaO_3$, 456.3448).

4.22. 6*E*-Hydroximino-4-hydroxycholest-4-en-3-one (26)

A magnetically stirred solution of dimethylsulfoxide (0.030 mL, 0.423 mmol) in dichloromethane (1.8 mL) maintained under an argon atmosphere at ca. −60 °C was treated in a dropwise fashion with TFAA (0.054 mL, 0.378 mmol). The resulting clear colorless solution was stirred at −60 °C for 10 min and then a solution of 6*E*-hydroximino-5β-cholestan-3β,4β-diol

(**25a**, 0.059 g, 0.136 mmol) in dichloromethane (1 mL) was added in a dropwise fashion. The clear colorless solution was stirred at −60 °C for 1.5 h. After the dropwise addition of triethylamine (0.130 mL, 0.928 mmol), the pale yellow reaction mixture was stirred for a further 1.5 h at −60 °C, warmed to rt, diluted with dichloromethane (4 mL), poured into aqueous 1 M HCl (5 mL), and extracted with dichloromethane (2 × 5 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, CH_2Cl_2/MeOH, from 100:0 to 9:1) to give 6*E*-hydroximino-4-hydroxycholest-4-en-3-one (**26**, 12 mg, 20%): white solid; $[\alpha]_D$ −46.9 ($CHCl_3$, $c = 0.1$); ^1H NMR (500 MHz, $CDCl_3$) δ_H: 3.08 (H7β, 1H, dd, $J = 18.1$, 6.3 Hz); 2.89 (H2, 1H, m); 2.66 (H2′, 1H, br d, $J = 18.1$ Hz); 2.29 (H7α, 1H, dd, $J = 18.1$, 10.6 Hz); 2.18 (H1, 1H, br d, $J = 13.5$ Hz); 1.84 (H1′, 1H, td, $J = 13.5$, 4.5 Hz); 1.32 (H19, 3H, s); 0.96 (H21, 3H, d, $J = 6.5$ Hz); 0.89 (H26, H27, 6H, d, $J = 6.6$ Hz); 0.77 (H18, 3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 185.6 (C3, s); 159.9 (C6, s); 156.5 (C4, s); 138.6 (C5, s); 56.8; 56.0; 48.7; 42.8; 39.6; 39.0; 38.3 (C1, t); 36.8 (C2, t); 36.2; 35.8; 33.4; 32.5; 28.1; 28.0; 26.1 (C7, t); 24.5; 23.9; 22.8 (C26, C27, q); 20.8; 19.2 (C19, q); 18.8 (C21, q); 12.1 (C18, q). (+)-LRFABMS, *m/z* (%): 452 ($[M+Na]^+$, 3); 434 ($[M-H_2O+Na]^+$, 8), 412 ($[M-H_2O+H]^+$, 100). (+)-HRESIMS: *m/z* 412.3218 $[M-H_2O+H]^+$ (calcd for $C_{27}H_{42}NO_2$, 412.3216), *m/z* 434.3048 $[M-H_2O+Na]^+$ (calcd for $C_{27}H_{41}NO_2Na$, 434.3035).

4.23. 3β-Acetoxycholest-5-en-7-one (27)

tert-Butylhydroperoxide (15.4 mL) was added dropwise to a solution of 3β-acetoxy-5-cholestene (5.5 g, 12.8 mmol) and CuI (0.1 g, 0.5 mmol) in dry benzene (50 mL). The mixture was heated under reflux for 24 h and then cooled. The reaction mixture was dropped into a solution of Na_2SO_3 (30 mL). The resulting precipitate was extracted with ether and the organic phase was washed (10% HCl, 10% $NaHCO_3$, and brine), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 9:1), to give 3β-acetoxycholest-5-en-7-one (**27**, 2.1 g, 40%): white solid; $[\alpha]_D$ −78.1 (CH_2Cl_2, $c = 1.0$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.70 (H6, 1H, s); 4.72 (H3α, 1H, m); 2.53 (H8, 1H, m); 2.06 (OAc, 3H, s); 1.02 (H19, 3H, s); 0.93 (H21, 3H, d, $J = 6.3$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.6$ Hz); 0.67 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 210.8 (C7, s); 170.2 (OAc, s); 163.8 (C5, s); 126.6 (C6, d); 72.1 (C3, d); 56.8; 56.0; 50.2; 42.2; 39.6; 39.4; 36.8; 36.2; 35.9; 35.7; 32.0; 31.6; 27.9; 27.7; 25.8; 24.2; 22.7; 22.5; 21.7; 21.3; 20.6; 20.3; 17.2 (C19, q); 11.9 (C18, q). (+)-LRFABMS, *m/z* (%): 465 ($[M+Na]^+$, 5); 399 (100).

4.24. 3β,7α-Dihydroxycholest-5-ene (28)

To a solution of 3β-acetoxycholest-5-en-7-one (**27**, 1.9 g, 4.4 mmol) in dry THF (50 mL) was added L-selectride (14 mL) dropwise over 15 min. The reaction mixture was stirred at −78 °C for 1 h. The residue was diluted with water (20 mL) and extracted with CH_2Cl_2 (50 mL). The organic phase was washed (10% HCl, 10% $NaHCO_3$, brine), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 8:2) to give 3β,7α-dihydroxycholest-5-ene (**28**, 1.82 g, 95%): white solid; $[\alpha]_D$ −130.2 (CH_2Cl_2, $c = 1.0$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.59 (H6, 1H, d, $J = 4.9$ Hz); 3.84 (H7β, 1H, m); 3.58 (H3α, 1H, m); 0.99 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.2$ Hz); 0.85 (H26, H27, 6H, d, $J = 6.6$ Hz); 0.68 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 146.2 (C5, s); 123.8 (C6, d); 71.3 (C3, d); 65.3 (C7, d); 56.2; 48.6; 42.3; 39.4; 39.2; 37.2; 36.0; 35.8; 35.6; 34.7; 33.3; 31.2; 30.7; 28.0; 27.9; 24.5; 23.7; 22.7; 22.5; 21.4; 20.6; 18.2 (C19, q); 11.6 (C18, q). LREIMS (70 eV, *m/z* %): 402 (M^+, 4); 385 (M^+-OH, 30); 384 (M^+-H_2O, 13); 368 (M^+-2 OH, 4); 237 (100).

4.25. 3β,7α-Diacetoxycholest-5-ene (29)

3β,7α-Dihydroxycholest-5-ene (**28**, 3 g, 7.4 mmol) was acetylated in a similar way as compound **15** to give 3β,7α-diacetoxycholest-5-ene (**29**, 3 g, 99%): white solid; $[\alpha]_D$ −407.9 (CH_2Cl_2, $c = 0.2$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.58 (H6, 1H, d, $J = 4.6$ Hz); 4.96 (H7β, 1H, dd, $J = 8.7$, 4.6 Hz); 4.64 (H3α, 1H, m); 2.35 (H4, 2H, d, $J = 7.8$ Hz); 2.04 (OAc, 3H, s); 2.02 (OAc, 3H, s); 1.01 (H19, 3H, s); 0.91 (H21, 3H, d, $J = 6.7$ Hz); 0.85 (H26, H27, 6H, d, $J = 6.2$ Hz); 0.66 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.7 (OAc, s); 170.4 (OAc, s); 146.4 (C5, s); 120.8 (C6, d); 73.1 (C3, d); 68.2 (C7, d); 56.2; 55.2; 48.9; 45.5; 42.3; 39.5; 37.4; 36.0; 35.5; 34.3; 33.3; 31.6; 29.7; 29.0; 28.0; 27.9; 24.4; 24.0; 23.7; 22.7; 22.5; 21.4; 20.4; 18.1 (C19, q); 11.4 (C18, q). (+)-LRFABMS, *m/z* (%): 509 ($[M+Na]^+$, 16); 367 (100).

4.26. 3β,7α-Diacetoxy-5α,6α-epoxycholestane (30a) and 3β,7α-diacetoxy-5β,6β-epoxycholestane (30b)

3β,7α-Diacetoxycholest-5-ene (**29**, 2.8 g, 5.7 mmol) was epoxidized in a similar way as compound **7** to give a residue which was subjected to chromatography (silica gel hexanes/ethyl acetate, from 9:1 to 8:2) to afford 3β,7α-diacetoxy-5α,6α-epoxycholestane (**30a**, 1.65 g) and 3β,7α-diacetoxy-5β,6β-epoxycholestane (**30b**, 0.82 g) in a 2:1 ratio with a yield of 85%. Compound **30a**: white solid; $[\alpha]_D$ −109.1 (CH_2Cl_2, $c = 1.0$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 4.98 (H7β, 1H, d, $J = 4.5$, 1.8 Hz); 4.89 (H3α, 1H, m); 3.29 (H6β, 1H, d, $J = 4.5$ Hz); 2.12 (OAc, 3H, s); 1.98 (OAc, 3H, s); 1.04 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.9$ Hz); 0.86 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.59 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.6 (OAc, s); 170.5 (OAc, s); 71.4 (C7, d); 70.1. (C3, d); 63.3 (C5, s); 62.0 (C6, d); 56.0; 43.4; 42.4; 39.8; 37.8; 37.0; 36.5; 35.4; 34.8; 33.2; 32.5; 32.1; 30.7; 29.4; 28.0; 27.6; 25.4; 23.2; 22.7; 22.3; 21.0; 20.7; 20.5; 18.1 (C19, q); 11.8 (C18, q). LREIMS (70 eV, *m/z* %): 502 (M^+, 3); 442 (M^+-2 CH_3COOH, 10); 382 (100). Compound **30b**: white solid; $[\alpha]_D$ −19.2 (CH_2Cl_2, $c = 1.0$); ^1H NMR (200 MHz, $CDCl_3$) δ_H:

5.17 (H7β, 1H, d, J = 4.9, 2.3 Hz); 4.75 (H3α, 1H, m); 3.08 (H6α, 1H, d, J = 2.3 Hz); 2.10 (OAc, 3H, s); 2.04 (OAc, 3H, s); 1.02 (H19, 3H, s); 0.91 (H21, 3H, d, J = 6.2 Hz); 0.85 (H26, H27, 6H, d, J = 6.6 Hz); 0.60 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.6 (OAc, s); 170.4 (OAc, s); 71.0 (C7, d); 69.9 (C3, d); 63.3 (C5, s); 59.6 (C6, d); 56.7; 48.7; 43.7; 37.9; 37.5; 36.8; 35.4; 35.3; 35.0; 33.9; 33.1; 26.5; 24.7; 23.7; 23.2; 23.0; 22.7; 22.5; 21.6; 21.3; 20.8; 18.4; 17.0 (C19, q); 15.4; 11.4 (C18, q). (+)-LRFABMS, m/z (%): 525 ($[M+Na]^+$, 70); 501 ($[M+H]^+$, 3); 441 (M^+-2CH_3COOH, 4); 383 (100).

4.27. 3β,7α-Diacetoxy-6β-hydroxycholest-4-ene (31)

A solution of $CeCl_3{\cdot}7H_2O$ (1.56 g, 4.2 mmol) in dry CH_3CN (30 mL) was added dropwise to a solution of 3β,7α-diacetoxy-5β,6β-epoxycholestane (**30b**, 4.2 g, 8.3 mmol) in a mixture of CH_3CN/THF (2:1, 30 mL). The reaction mixture was heated under reflux for 8 h and the solvent was removed under reduced pressure. The residue was diluted with water (20 mL) and extracted with ethyl acetate (20 mL). The extract was dried, evaporated, and the residue subjected to chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β,7α-diacetoxy-6-hydroxycholest-4-ene (**31**, 1.26 g, 60%): white solid; $[\alpha]_D$ −66.6 (CH_2Cl_2, c = 0.2); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 5.46 (H4, 1H, s); 5.28 (H3α, 1H, m); 4.83 (H7β, 1H, dd, J = 4.8, 2.4 Hz); 3.99 (H6α, 1H, d, J = 2.4 Hz); 2.04 (OAc, 3H, s); 2.03 (OAc, 3H, s); 0.98 (H19, 3H,s); 0.91 (H21, 3H, d, J = 6.4 Hz); 0.85 (H26, H27, 6H, d, J = 6.5 Hz); 0.69 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.8 (OAc, s); 170.5 (OAc, s); 146.0 (C5, s); 127.6 (C4, d); 74.3 (C3, d); 73.7 (C7, d); 70.4 (C6, d); 56.0; 49.8; 46.7; 43.2; 39.8; 39.7; 38.9; 35.7; 35.5; 34.7; 33.1; 26.7; 24.6; 24.2; 23.5; 22.7; 22.4; 22.1; 21.5; 21.0; 20.9; 19.4; 18.6 (C19, q); 11.6 (C18, q). (+)-LRFABMS, m/z (%): 525 ($[M+Na]^+$, 100).

4.28. 3β,7α-Diacetoxycholest-4-en-6-one (32)

A solution of 3β,7α-diacetoxy-6-hydroxycholest-4-ene (**31**, 3.5 g, 6.9 mmol) in dry CH_2Cl_2 (30 mL) was treated with the Dess–Martin reagent (3.5 g, 8.4 mmol) in dry CH_2Cl_2 (30 mL) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into 100 mL of $Na_2S_2O_3{\cdot}5H_2O$ and extracted with ether (2 × 20 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β,7α-diacetoxycholest-4-en-6-one (**32**, 3.3 g, 82%): white solid; $[\alpha]_D$ −363.3 (CH_2Cl_2, c = 0.1); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 5.89 (H4, 1H, s); 5.32 (H3α, 1H, m); 4.99 (H7β, 1H, d, J = 2.3 Hz); 2.06 (OAc, 6H, s); 1.11 (H19, 3H, s); 0.89 (H21, 3H, d, J = 6.4 Hz); 0.86 (H26, H27, 6H, d, J = 6.6 Hz); 0.67 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 198.2 (C6, s); 170.6 (OAc, s); 169.4 (OAc, s); 147.2 (C5, s); 129.0 (C4, d); 76.7 (C7, d); 68.9 (C3, d); 76.7 (C7); 56.8; 48.9; 44.3; 42.6; 38.9; 38.1; 36.6; 36.1; 35.7; 28.7; 25.4; 24.9; 24.4; 23.9; 23.2; 22.7; 22.1; 21.6; 21.5; 20.7; 20.6; 18.6 (C19, q); 11.5 (C18, q). (+)-LRFABMS, m/z (%): 523 ($[M+Na]^+$, 7); 500 ($[M+H]^+$, 4).

4.29. 3β,7α-Dihydroxycholest-4-en-6-one (33)

3β,7α-Diacetoxycholest-4-en-6-one (**32**, 0.1 g, 0.2 mmol) was hydrolyzed as compound **10** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate, 7:3) to give 3β,7α-dihydroxycholest-4-en-6-one (**33**, 0.1 g, 90%): white solid; $[\alpha]_D$ −25.3 (CH_2Cl_2, c = 0.1); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 6.39 (H4, 1H, s); 4.68 (H7β, 1H, d, J = 2.4 Hz); 3.51 (H3α, 1H, m); 1.13 (H19, 3H, s); 0.91 (H21, 3H, d, J = 6.3 Hz); 0.87 (H26, H27, 6H, d, J = 6.4 Hz); 0.66 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 196.3 (C6, s); 143.7 (C5, s); 131.0 (C4, d); 77.7 (C7, d); 63.3 (C3, d); 55.3; 48.7; 46.7; 44.5; 42.4; 39.5; 38.6; 37.9; 36.6; 34.4; 27.9; 26.8; 24.7; 23.3; 23.1; 22.8; 22.6; 22.1; 20.8; 20.2; 17.1 (C19, q); 11.2 (C18, q). (+)-LRFABMS, m/z (%): 439 ($[M+Na]^+$, 6); 399 (100).

4.30. 3β,7α-Diacetoxy-6*Z*-hydroximinocholest-4-ene (34)

3β,7α-Diacetoxycholest-4-en-6-one (**32**, 0.8 g, 2 mmol) was treated with hydroxylamine hydrochloride (1 g, 15 mmol) in a similar way as compound **11** to give a residue which was subjected to chromatography (hexanes/ethyl acetate, 9:1) to give 3β,7α-diacetoxy-6*Z*-hydroximinocholest-4-ene (**34**, 0.74 g, 90%): white solid; $[\alpha]_D$ +27.6 (CH_2Cl_2, c = 0.1); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 7.97 (OH, 1H, br s); 6.05 (H4, 1H, d, J = 2.4 Hz); 5.61 (H7β, 1H, s); 5.34 (H3α, 1H, m); 2.05 (OAc, 6H, s); 1.03 (H19, 3H, s); 0.89 (H21, 3H, d, J = 6.3 Hz); 0.85 (H26, H27, 6H, d, J = 6.5 Hz); 0.67 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.9 (OAc, s); 169.8 (OAc, s); 156.0 (C6, s); 141.9 (C5, s); 125.1 (C4, d); 69.4 (C7, d); 64.3 (C3, d); 56.4; 50.2; 46.7; 43.4; 38.6; 38.0; 37.8; 36.7; 36.1; 35.8; 35.6; 34.3; 27.9; 24.6; 24.6; 23.9; 23.7; 23.5; 22.5; 21.5; 20.5; 19.8; 18.6 (C19, q); 11.63 (C18, q). (+)-LRFABMS, m/z (%): 538 ($[M+Na]^+$, 100).

4.31. 3β,7α-Dihydroxy-6*E*/*Z*-hydroximinocholest-4-ene (35)

3β,7α-Diacetoxy-6*Z*-hydroximinocholest-4-ene (**34**, 0.1 g, 0.2 mmol) was hydrolyzed in a similar way as compound **10** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate, 7:3) to give a mixture of 3β,7α-dihydroxy-6*E*/*Z*-hydroximinocholest-4-ene (**35**, 0.1 g, 99%): white solid; $[\alpha]_D$ +19.2 (CH_2Cl_2, c = 0.1); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 5.85/5.74 (H4, 1H, br s); 5.01 (H7β, 1H, s); 4.14 (H3α, 1H, m); 0.98 (H19, 3H, s); 0.89 (H21, 3H, d, J = 6.4 Hz); 0.85 (H26, H27, 6H, d, J = 6.1 Hz); 0.64 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 160.3/157.6 (C6, s); 139.9/137.6 (C5, s); 136.0/131.1 (C4, d); 79.8 (C7, d); 61.2 (C3, d); 57.2; 45.5; 44.3; 42.5; 40.8; 39.9; 39.4; 39.0; 38.7; 38.2; 37.8; 36.5; 36.4; 35.5; 28.7; 28.4; 23.7; 23.3; 21.3; 20.2; 18.4 (C19, q); 11.7 (C18, q). (+)-LRFABMS, m/z (%): 454 ($[M+Na]^+$, 24); 414 (100). (+)-HRESIMS: m/z 432.3453 $[M+H]^+$ (calcd for $C_{27}H_{46}NO_3$, 432.3472).

4.32. 7α-Hydroxy-6*E*/*Z*-hydroximinocholest-4-en-3-one (36)

A solution of 3β,7α-dihydroxy-6*E*/*Z*-hydroximinocholest-4-ene (**35**, 15 mg, 0.03 mmol) in 5 mL of pyridine was added dropwise to the chromium trioxide/pyridine complex prepared by the addition of CrO_3 (0.5 g, 0.6 mmol) to 5 mL of pyridine at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then diluted with 10 mL of ethyl acetate. The resulting precipitate was filtered off and the filtrate was washed (10% HCl, 10% $NaHCO_3$, brine), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to chromatography (hexanes/ethyl acetate, 8:2, 7:3) and the product was purified by HPLC (Sharlau C18, flow rate 1 mL/min, retention time 59 min, MeOH/H_2O, 9:1) to give a mixture of 7α-hydroxy-6 *E*/*Z*-hydroximinocholest-4-en-3-one (**36**, 2 mg, 13%): white solid; $[\alpha]_D$ +6.2 (MeOH, $c = 0.1$); ^1H NMR (300 MHz, CD_3OD) δ_H: 6.15/6.01 (H4, 1H, s); 5.16 (H7β, 1H, s); 2.57 (2H, dt, $J = 5.2$, 16.4 Hz); 2.37 (2H, br d, $J = 15.3$ Hz); 1.15 (H19, 3H, s); 0.98 (H21, 3H, d, $J = 6.6$ Hz); 0.90 (H26, H27, 6H, d, $J = 6.6$ Hz); 0.77 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 201.8/201.6 (C3, s); 164.3 (C6, s); 158.9/157.6 (C5, s); 129.8/125.1 (C4, d); 61.7 (C7, d); 57.4 (C14, d); 50.8 (C17, d); 50.2 (C9, d); 48.6 (C13, d); 48.1 (C24, t); 44.8 (C12, t); 43.5 (C10, s); 40.6 (C22, t); 39.9 (C20, d); 37.1 (C1,t); 35.9 (C2, t); 34.9 (C8,d); 29.2 (C16, t); 29.1 (C25, q); 24.9 (C15, t); 24.4 (C23, t); 23.1 (C26, q); 22.9 (C27, q); 21.8 (C11, t); 19.2 (C21, q); 17.2 (C19, q); 12.1 (C18, q). LREIMS (70 eV, *m*/*z* %): 430 (M^+, 1); 414 (M^+-H_2O, 7); 149 (100).

4.33. (25*R*)-3β,16β-Dihydroxycholesterol 26-tosylate (37)

To a 250 mL flask were added sequentially diosgenin (2 g, 4.8 mmol), zinc dust (40 g, 675 mmol), and 100 mL absolute ethanol. The mixture was stirred and heated under reflux and 40 mL of concentrated HCl was added dropwise during 1 h. The reaction mixture was heated under reflux for an additional 30 min and filtered to remove the zinc dust. The solution was collected and distilled water was added until a precipitate appeared. The solution was heated until transparent and then slowly cooled. The resulting precipitate was collected by suction filtration and washed three times with cold water. The crystals were then dried to yield (25*R*)-cholest-5-en-3β,16β, 26-triol[24] as a white solid (1.8 g, 90%). To a solution of (25*R*)-cholest-5-en-3β,16β, 26-triol (0.5 g, 1.2 mmol) in 5 mL of dry pyridine, cooled in an ice bath, was added *p*-toluenesulfonyl chloride (1.4 g, 7 mmol) in 5 mL of dry pyridine and the mixture was stirred for 2 h at room temperature. The mixture was poured into water and extracted with ether. The organic layer was washed with 2 N HCl and water. Evaporation of the solvent gave a solid, which was applied to a silica gel column (hexanes/ethyl acetate 7:3) to afford (25*R*)-3β,16β-dihydroxycholesterol 26-tosylate (**37**, 0.4 g, 80%) and (25*R*)-16β-hydroxycholesterol 3,26-ditosylate. Compound **37**: white solid; $[\alpha]_D$ −6.6 (MeOH, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 7.78 (2H, d, aryl-H, $J = 8$ Hz); 7.31 (2H, d, aryl-H, $J = 8$ Hz); 5.35 (H6, 1H, m); 4.33 (H16α, 1H, m); 3.72 (2H, m, 26-CH_2); 3.3–3.6 (H3α, 1H, m); 2.43 (3H, s, aryl-CH_3); 0.91 (H19, 3H, s); 0.78 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 144.3; 140.8 (C5, s); 136.4; 128.5; 128.5; 127.2; 127.2; 121.8 (C6, d); 74.7 (C16, d); 71.6 (C3, d); 65.4; 62.5; 55.4; 49.8; 42.5; 39.5; 36.5; 35.5; 35.4; 35.2; 33.6; 32.4; 31.8; 30.7; 29.5; 29.4; 28.9; 23.2; 22.8; 22.1; 21.3; 20.7; 20.4; 19.7; 18.4; 17.3; 16.5; 13.3. EIMS (70 eV, *m*/*z* %): 572 (M^+, 3); 91 (100). (25*R*)-16β-*Hydroxycholesterol* 3,26-*ditosylate*: white solid; $[\alpha]_D$ −24.0 (CH_2Cl_2, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 7.79 (2H, d, aryl-H, $J = 8$ Hz); 7.34 (2H, d, aryl-H, $J = 8$ Hz); 5.30 (H6, 1H, m); 4.32 (H16α, 1H, m); 3.76–3.90 (2H, m, 26-CH_2); 2.45 (3H, s, aryl-CH_3); 0.94 (H19, 3H, s); 0.82 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 144.6; 144.4; 138.9 (C5); 134.7; 133.2; 129.7; 129.7; 127.8; 127.8; 127.6; 123.2 (C6, d); 82.2; 75.1 (C16, d); 72.2 (C3, d); 61.3; 60.4; 54.3; 49.8; 42.1; 39.6; 38.8; 36.7; 36.3; 35.8; 32.9; 32.7; 31.7; 31.3; 29.6; 28.5; 23.4; 21.6; 21.0; 19.1; 18.1; 16.4; 14.7; 12.4. LREIMS (70 eV, *m*/*z* %): 726 (M^+, 2); 367 (100).

4.34. 3β,16β-Dihydroxycholest-5-ene (38)

To a solution of (25*R*)-3β,16β-dihydroxycholesterol 26-tosylate (**37**, 0.5 g, 0.85 mmol) in 10 mL of dry ether, cooled in an ice bath, was added lithium aluminum hydride (0.3 g, 8 mmol) in 10 mL of dry ether and the mixture was stirred for 20 h at room temperature. The mixture was poured into water and extracted with ether. The organic layer was washed with 5% HCl and water. Evaporation of the solvent gave a yellow solid, which was applied to a silica gel column (hexanes/ethyl acetate 8:2) to give 3β,16β-dihydroxycholest-5-ene (**38**, 0.35 g, 70%): white solid; $[\alpha]_D$ +6.0 (MeOH, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 5.35 (H6, 1H, d, $J = 5.2$ Hz); 4.35 (H16α, 1H, m); 3.51 (H3α, 1H, m); 1.02 (H19, 3H, s); 0.89 (H21, 3H, d, $J = 6.3$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.3$ Hz); 0.86 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 140.8 (C5, s); 121.4 (C6, d); 72.5 (C16, d); 71.7 (C3, d); 61.4; 54.5; 50.1; 42.3; 42.2; 39.8; 39.5; 37.2; 36.6; 36.4; 36.2; 31.7; 31.6; 31.4; 29.8; 28.1; 24.1; 22.8; 22.5; 20.6; 19.3; 18.2; 13.1. LREIMS (70 eV, *m*/*z* %): 402 (M^+, 24); 384 (M^+-H_2O, 26); 105 (100).

4.35. 3β,16β-Diacetoxycholest-5-ene (39)

3β,16β-Dihydroxycholest-5-ene (**38**, 0.6 g 1.5 mmol) was acetylated in a similar way as compound **15** to give a residue which was subjected to chromatography (hexanes/ethyl acetate 8:2) to give 3β,16β-diacetoxycholest-5-ene (**39**, 0.6 g, 99%): white solid; $[\alpha]_D$ −10.2 ($CHCl_3$, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 5.37 (H6, 1H, d, $J = 4.5$ Hz); 5.20 (H16α, 1H, m); 4.60 (H3α, 1H, m); 2.03 (OAc, 3H, s); 2.01 (OAc, 3H, s); 1.03 (H19, 3H, s); 0.96 (H21, 3H, d, $J = 5.9$ Hz); 0.88 (H26, H27, 6H, d, $J = 5.9$ Hz); 0.84 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 170.2 (OAc); 170.1 (OAc); 139.3 (C5, s); 121.9 (C6, d); 74.8 (C16, d); 73.4 (C3, d); 59.5; 54.1; 49.5; 42.0; 39.8; 38.8; 37.6; 36.4; 36.1; 35.3; 34.4; 31.2; 30.9; 29.5; 27.5; 27.3; 24.1; 23.8; 22.3; 20.9; 20.5;

18.8; 18.6; 17.7; 12.7. LREIMS (70 eV, *m/z* %): 486 (M^+, 2); 426 ($M^+ - CH_3COOH$, 18); 84 (100).

4.36. 3β,16β-Diacetoxy-5*a*,6*a*-epoxycholestane (40a) and 3β,16β-diacetoxy-5β,6β-epoxycholestane (40b)

Method A. 3β,16β-Diacetoxycholest-5-ene (**39**, 0.5 g, 1 mmol) was epoxidized as compound **7** to give a residue which was subjected to chromatography (hexanes/ethyl acetate, 8:2) to give 0.35 g of a mixture of 3β,16β-diacetoxy-5*a*,6*a*-epoxycholestane (**40a**) and 3β,16β-diacetoxy-5β,6β-epoxycholestane (**40b**) in a 1:2 ratio in 70% yield. *Method B.* A mixture of $KMnO_4$ (0.2 g, 1.3 mmol) and $Fe_2(SO_4)_3{\cdot}nH_2O$ (0.1 g) was ground to a fine powder, water (10 μL) was added, and the mixture transferred to the reaction flask. To a stirred suspension of this mixture in CH_2Cl_2 (5 mL), 3β,16β-diacetoxycholest-5-ene (**39**, 60 mg, 1 mmol) was added followed by *tert*-butyl alcohol (0.5 mL). After 3 h at room temperature the reaction was complete (TLC control) and the product was separated from inorganic residue by adding ether (5 mL), stirring for 5 min, and filtering through a pad of Celite. The filtrate was washed with water and dried over $MgSO_4$. After removal of the solvent, the residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 8:2) to give a mixture of 3β,16β-diacetoxy-5*a*,6*a*-epoxycholestane (**40a**) and 3β,16β-diacetoxy-5β,6β-epoxycholestane (**40b**) in a 1:4 ratio in 40% yield. Compound **40a**: white solid; $[\alpha]_D$ +26.3 (CH_2Cl_2, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 5.21 (H16α, 1H, m); 4.96 (H3α, 1H, m); 2.88 (H6β, 1H, d, $J = 4.4$ Hz); 2.01 (OAc, 3H, s); 2.00 (OAc, 3H, s); 1.01 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.4$ Hz); 0.84 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.80 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 170.2 (OAc); 170.0 (OAc); 74.6 (C16, d); 70.8 (C3, d); 63.3; 62.8; 58.7; 58.4; 53.1; 49.2; 42.2; 40.0; 39.7; 37.4; 36.7; 35.2; 34.7; 34.3; 34.2; 31.6; 29.6; 28.7; 27.3; 25.4; 24.7; 23.3; 21.9; 21.6; 20.8; 15.7; 11.9. EIMS (70 eV, *m/z* %): 502 (M^+, 8); 442 ($M^+ - CH_3COOH$, 38); 84 (100). Compound **40b**: white solid; $[\alpha]_D$ +27.4 (CH_2Cl_2, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 5.20 (H16α, 1H, m); 4.74 (H3α, 1H, m); 3.05 (H6α, 1H, d, $J = 1.9$ Hz); 2.02 (OAc, 3H, s); 2.01 (OAc, 3H, s); 1.02 (H19, 3H, s); 0.93 (H21, 3H, d, $J = 6.6$ Hz); 0.85 (H26, H27, 6H, d, $J = 6.6$ Hz); 0.81 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 170.1 (OAc); 169.9 (OAc); 76.8 (C16, d); 70.8 (C3, d); 64.6; 62.9; 59.7; 59.4; 54.4; 50.2; 42.0; 41.9; 41.8; 39.4; 37.7; 35.5; 34.9; 34.7; 31.8; 29.1; 28.7; 26.3; 25.4; 23.7; 23.3; 22.4; 21.9; 19.9; 17.7; 16.6; 12.1. LREIMS (70 eV, *m/z* %): 502 (M^+, 4); 442 ($M^+ - CH_3COOH$, 10); 84 (100).

4.37. 3β,16β-Diacetoxy-5α-hydroxycholestan-6-one (41)

A solution of a mixture of 3β,16β-diacetoxy-5*a*,6*a*-epoxycholestane (**40a**) and 3β,16β-diacetoxy-5β,6β-epoxycholestane (**40b**) in a 1:2 ratio (0.1 g, 0.2 mmol) in Ac_2O (4 mL) was treated with Jones' reagent (1 mL) and the mixture was stirred at 0 °C for 6 h. The reaction mixture was poured into 5 mL of MeOH and extracted with ether (2 × 15 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate 8:2) to give 3β,16β-diacetoxy-5α-hydroxycholestan-6-one (**41**, 72 mg, 72%): white solid; $[\alpha]_D$ −18.6 (CH_2Cl_2, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 5.21 (H16α, 1H, m); 5.03 (H3α, 1H, m); 2.73 (H7β, 1H, t, $J = 12.0$, 6.0 Hz); 2.03 (OAc, 3H, s); 2.01 (OAc, 3H, s); 1.03 (H19, 3H, s); 0.95 (H21, 3H, d, $J = 6.4$ Hz); 0.90 (H18, 3H, s); 0.87 (H26, H27, 6H, d, $J = 6.7$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 210.9 (C6, s); 170.4 (OAc); 170.2 (OAc); 79.9 (C5, s); 76.7 (C16, d); 76.4 (C3, d); 74.3; 69.9; 59.6; 53.7; 43.4; 42.8; 41.9; 40.9; 38.9; 38.8; 36.2; 35.2; 33.9; 32.1; 29.4; 28.9; 27.5; 25.8; 23.8; 22.2; 21.9; 21.3; 17.6; 13.4; 12.3. LREIMS (70 eV, *m/z* %): 518 (M^+, 5); 458 ($M^+ - CH_3COOH$, 8); 83 (100).

4.38. 3β,16β-Diacetoxycholest-4-en-6-one (42)

3β,16β-diacetoxy-5α-hydroxycholestan-6-one (**41**, 0.1 g, 0.15 mmol) was treated with thionyl chloride (0.1 mL, 1.2 mmol) in a similar way as compound **9** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate, 8:2) to give 3β,16β-diacetoxycholest-4-en-6-one (**42**, 75 mg, 75%): white solid; $[\alpha]_D$ −6.6 (CH_2Cl_2, $c = 0.1$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 6.07 (H4, 1H, s); 5.32 (H16α, 1H, m); 5.23 (H3α, 1H, m); 2.52 (H7β, 1H, d, $J = 12$ Hz); 2.06 (OAc, 3H, s); 2.01 (OAc, 3H, s); 1.02 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.3$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.3$ Hz); 0.84 (H18, 3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 201.8 (C6); 170.6 (OAc); 170.4 (OAc); 147.8 (C5, s); 132.4 (C4, d); 69.2 (C16, d); 68.1 (C3, d); 66.8; 59.8; 54.4; 51.0; 45.9; 42.7; 39.2; 39.1; 38.7; 35.7; 34.6; 33.9; 33.6; 30.4; 29.6; 24.7; 24.2; 22.9; 22.6; 22.2; 21.7; 20.4; 12.6; 10.9. LREIMS (70 eV, *m/z* %): 500 (M^+, 5); 83 (100).

4.39. 16β-Acetoxy-3β-hydroxycholest-4-en-6-one (43)

3β,16β-Diacetoxycholest-4-en-6-one (**42**, 15 mg, 0.1 mmol) was hydrolyzed in a similar way as compound **10** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate, 7:3) to afford 16β-acetoxy-3β-hydroxycholest-4-en-6-one (**43**, 7 mg, 50%): white solid; $[\alpha]_D$ +5.7 ($CHCl_3$, $c = 0.05$); ^1H NMR (300 MHz, $CDCl_3$) δ_H: 6.16 (H4, 1H, s); 5.21 (H16α, 1H, m); 4.15 (H3α, 1H, m); 2.52 (H7β, 1H, d, $J = 12.0$ Hz); 2.02 (OAc, 3H, s); 1.02 (H19, 3H, s); 0.97 (H21, 3H, d, $J = 6.6$ Hz); 0.90 (H18, 3H, s); 0.86 (H26, H27, 6H, d, $J = 6.6$ Hz);. ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 202.0 (C6, s); 170.1 (OAc); 146.1 (C5, s); 132.4 (C4, d); 74.2 (C16, d); 66.8 (C3, d); 59.4; 54.1; 51.2; 42.7; 42.0; 39.2; 38.2; 35.7; 34.6; 34.4; 33.6; 29.7; 28.7; 27.5; 22.9; 22.6; 22.3; 22.1; 20.8; 19.3; 17.7; 13.6; 12.2. LREIMS (70 eV, *m/z* %): 458 (M^+, 3); 83 (100). (+)-LRFABMS, *m/z* (%): 459 ($[M+H]^+$, 67); 133 (100).

4.40. 3β,16β-Diacetoxy-6*E*-hydroximinocholest-4-ene (44)

3β,16β-Diacetoxycholest-4-en-6-one (**42**, 12 mg, 0.1 mmol) was treated with hydroxylamine hydrochloride (20 mg,

0.015 mmol) in a similar way as compound **11** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate, 8:2) to give 3β,16β-diacetoxy-6*E*-hydroximinocholest-4-ene (**44**, 6 mg, 60%): white solid; $[\alpha]_D$ +27.5 ($CHCl_3$, $c = 0.05$); ^1H NMR (500 MHz, $CDCl_3$) δ_H: 5.70 (H4, 1H, s); 5.26 (H16α, 1H, m); 5.22 (H3α, 1H, m); 3.31 (H7β, 1H, dd, $J = 12.5$, 4.4 Hz); 2.05 (OAc, 3H, s); 2.01 (OAc, 3H, s); 1.01 (H19, 3H, s); 0.96 (H21, 3H, d, $J = 6.5$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.5$ Hz); 0.87 (H18, 3H, s). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 170.9 (OAc); 170.6 (OAc); 161.7 (C6, s); 143.7 (C5, s, s); 123.4 (C4, d); 74.9 (C16, d); 74.8 (C3, d); 69.6; 59.8; 54.4; 52.3; 42.8; 42.6; 39.7; 39.3; 37.8; 35.7; 34.6; 34.0; 33.2; 29.8; 27.9; 24.2; 22.7; 22.5; 21.3; 21.2; 20.6; 18.6; 18.2; 12.6. LREIMS (70 eV, *m/z* %): 515 (M^+, 15); 456 (M^+-CH_3COO, 38); 308 (100). (+)-HRESIMS, *m/z* (%): 538.3510 $[M+Na]^+$ (calcd for $C_{31}H_{49}NNaO_5$, 538.3503); 516.3685 $[M+H]^+$ (calcd for $C_{31}H_{50}NNaO_5$, 516.3684).

4.41. 16β-Acetoxy-3β-hydroxy-6*E*-hydroximinocholest-4-ene (45)

16β-Acetoxy-3β-hydroxycholest-4-en-6-one (**43**, 12 mg, 0.1 mmol) was treated with hydroxylamine hydrochloride (15 mg, 0.01 mmol) in a similar way as compound **11** to give a residue which was purified by RP-HPLC [Sharlau C18, flow rate 1 mL/min, retention time 62 min, MeOH:H_2O (9:1)] to give 16β-acetoxy-3β-hydroxy-6*E*-hydroximinocholest-4-ene (**45**, 2 mg, 16%): white solid; $[\alpha]_D$ +72.6 ($CHCl_3$, $c = 0.05$); ^1H NMR (500 MHz, $CDCl_3$) δ_H: 5.82 (H4, 1H, s); 5.21 (H16α, 1H, m); 4.19 (H3α, 1H, m); 3.32 (H7β, 1H, dd, $J = 11.5$, 4.2 Hz); 2.02 (OAc, 3H, s); 1.0 (H19, 3H, s); 0.96 (H21, 3H, d, $J = 6.6$ Hz); 0.89 (H18, 3H, s); 0.86 (H26, H27, 6H, d, $J = 6.6$ Hz);. ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 170.6 (OAc); 164.4 (C6, s); 142.4 (C5, s); 127.5 (C4, d); 74.9 (C16, d); 74.6 (C3, d); 21.2 (OAc); 20.1 (q); 18.5 (q); 12.6 (q). LREIMS (70 eV, *m/z* %): 473 (M^+, 5); 91 (100).

4.42. 3β-Acetoxy-5α-fluoro-6β-hydroxycholestane (47)

A solution of 3β-acetoxy-5β,6β-epoxycholestane[5] (**46**, 2.5 g, 5.6 mmol) in dry Et_2O (40 mL) was treated with BF_3–Et_2O (2.8 mL) and the mixture was stirred at 0 °C for 5 h. The reaction mixture was poured into 30 mL of $NaHCO_3$ and extracted with ether (2 × 20 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β-acetoxy-5α-fluoro-6β-hydroxycholestane (**47**, 2.25 g, 80%): white solid; $[\alpha]_D$ −6.3 (CH_2Cl_2, $c = 0.5$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.09 (H3α, 1H, m); 3.74 (H6α, 1H, dt, $J = 4.5$, 2.3 Hz); 2.02 (OAc, 3H, s); 1.15 (H19, 3H, s); 0.94 (H21, 3H, d, $J = 6.5$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.2$ Hz); 0.67 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 170.4 (OAc); 98.6 (C5, $^1J_{CF} = 166.9$ Hz); 73.0 (C6, $^2J_{CF} = 35.3$ Hz); 72.6; 70.4 (C3, $^3J_{CF} = 3.5$ Hz); 63.5; 56.2; 55.6; 45.8; 42.6; 39.5 (C10, $^2J_{CF} = 14.1$ Hz); 38.4; 38.1; 35.7; 35.6; 35.2; 34.9; 32.0; 31.9; 29.6; 28.1; 27.9; 26.4; 24.1; 21.3; 20.8; 18.7; 16.4 (C19, $^3J_{CF} = 5.2$ Hz); 14.2 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −156.4. LREIMS (70 eV, *m/z* %): 464 (M^+, 18); 446 (M^+-OH, 4); 404 (M^+-CH_3COOH, 6); 309 (100).

4.43. 3β-Acetoxy-5α-fluorocholestan-6-one (48)

A solution of 3β-acetoxy-5α-fluoro-6β-hydroxycholestane (**47**, 2.5 g, 6.5 mmol) in dry CH_2Cl_2 (20 mL) was treated with Dess–Martin reagent (2.5 g, 5.5 mmol) in dry CH_2Cl_2 (20 mL) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into 50 mL of $Na_2S_2O_3{\cdot}5H_2O$ and extracted with ether (2 × 20 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β-acetoxy-5α-fluorocholestan-6-one (**48**, 2.25 g, 90%): white solid; $[\alpha]_D$ +0.3 (CH_2Cl_2, $c = 0.25$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.03 (H3α, 1H, m); 2.59 (H7β, 1H, dd, $J = 12.6$, 12.3 Hz); 2.02 (OAc, 3H, s); 1.08 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.1$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.67 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 207.2 (C6, $J_{CF} = 26.8$ Hz); 170.3 (OAc); 100.8 (C5, $J_{CF} = 174.8$ Hz); 87.6; 69.6 (C3, $J_{CF} = 2.1$ Hz); 69.5; 63.4; 56.2 (C14); 56.0 (C17); 45.4; 43.2 (C10, $J_{CF} = 12.7$ Hz); 42.6; 39.4; 37.8; 36.0; 35.6; 30.4; 30.3; 29.9; 27.9; 26.0; 23.8; 23.7; 22.7; 22.5; 21.2; 18.5; 13.6 (C19 $J_{CF} = 5.1$ Hz); 11.9 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −156.8. LREIMS (70 eV, *m/z* %): 462 (M^+, 16); 402 (M^+-CH_3COOH, 36); 384 ($M^+ - CH_3COOH - H_2O$, 7); 93 (100).

4.44. 5α-Fluoro-3β-hydroxycholestan-6-one (49)

K_2CO_3 (0.45 g, 3.26 mmol) was added to a solution of 3β-acetoxy-5α-fluorocholestan-6-one (**48**, 2.15 g, 4.8 mmol) in MeOH (50 mL). The reaction mixture was stirred at room temperature for 6 h. The reaction mixture was poured into 50 mL NH_4Cl, extracted with CH_2Cl_2 (2 × 50 mL), dried, and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate 9:1) to give 5α-fluoro-3β-hydroxycholestan-6-one (**49**, 1.8 g, 84%): white solid; $[\alpha]_D$ −6.8 [$c = 0.5$, CH_2Cl_2]; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 3.92 (H3α, 1H, m); 2.58 (H7β, 1H, dd, $J = 12.6$, 12.3 Hz); 0.92 (H19, 3H, s); 0.87 (H21, 3H, d, $J = 6.1$ Hz); 0.83 (H26, H27, 6H, d, $J = 6.3$ Hz); 0.62 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 207.5 (C6, $J_{CF} = 26.8$ Hz); 101.2 (C5, $J_{CF} = 174.5$ Hz); 66.8 (C3, $J_{CF} = 1$ Hz); 56.2 (C14); 56.0 (C17); 43.1 (C10, $J_{CF} = 15.1$ Hz); 42.8; 42.6; 42.5; 39.4; 37.8; 36.0; 35.6; 34.0; 33.6; 30.6; 29.8; 23.8; 23.7; 23.0; 22.8; 22.5; 21.3; 18.6; 13.8; 13.7 (C19, $J_{CF} = 5.2$ Hz); 11.9 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −157.2. EIMS (70 eV, *m/z* %): 420 (M^+, 38); 421 (M^+-H, 11); 403 (M^+-OH, 3); 265 (100).

4.45. 5α-Fluoro-3β-hydroxy-6*E*-hydroximinocholestane (50)

5α-Fluoro-3β-hydroxycholestan-6-one (**49**, 0.1 g, 0.24 mmol) was treated with hydroxylamine hydrochloride (0.12 g, 1.7 mmol) in a similar way as compound **11** to give a

residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate 8:2) to afford 5α-fluoro-3β-hydroxy-6*E*-hydroximinocholestane (**50**, 96 mg, 96%): white solid; $[\alpha]_D$ −18.8 (CH_2Cl_2, $c = 1.0$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 8.63 (NOH, 1H, br s); 4.01 (H3α, 1H, m); 3.18 (H7β, 1H, br d, $J = 11.2$ Hz); 1.26 (H19, 3H, s); 0.90 (H21, 3H, d, $J = 6.4$ Hz); 0.86 (H26, H27, 6H, d, $J = 5.9$ Hz); 0.65 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 158.2 (C6, $J_{CF} = 26.1$ Hz); 99.4 (C5, $J_{CF} = 166.5$ Hz); 67.3 (C3); 63.5; 56.0; 45.2 (C10, $J_{CF} = 13.2$ Hz); 42.2; 39.6; 39.5; 38.4; 37.5; 36.0; 35.7; 34.7; 28.1; 25.6; 23.9; 23.8; 23.7; 22.9; 22.7; 22.5; 21.2; 20.6; 18.6; 14.4 (C19, $J_{CF} = 5.4$ Hz); 12.0 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −158.7. (+)-LRFABMS, *m/z* (%): 458 ($[M+Na]^+$, 100); 436 ($[M+H]^+$, 100). (+)-HRESIMS: *m/z* 436.3573 $[M+H]^+$ (calcd for $C_{27}H_{47}FNO_2$, 436.3585).

4.46. 5α-Fluoro-6*E*-hydroximinocholestan-3-one (51)

Method A. A solution of 5α-fluoro-3β-hydroxy-6*E*-hydroximinocholestane (**50**, 0.1 g, 0.23 mmol) in 5 mL of pyridine was added dropwise to the chromium trioxide/pyridine complex prepared by the addition of CrO_3 (0.3 g, 3 mmol) to 5 mL of pyridine at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then diluted with 10 mL of ethyl acetate. The resulting precipitate was filtered off and the filtrate was washed (10% HCl, 10% $NaHCO_3$, and brine), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate 8:2) and then purified by normal phase HPLC [Sharlau column, flow rate 1 mL/min, retention time 24 min, hexanes/ethyl acetate (1:1)] to give 5α-fluoro-6*E*-hydroximinocholestan-3-one (**51**, 75 mg, 76%). *Method B.* A solution of 5α-fluoro-3β-hydroxy-6*E*-hydroximinocholestane (**50**, 0.1 g, 0.23 mmol) in C_6H_6 (6 mL) was treated with Jones' reagent (1.5 mL) and the mixture was stirred at 0 °C for 5 h. The reaction mixture was poured into 10 mL of MeOH and extracted with ether (2 × 20 mL). The combined extracts were washed (NaCl, $NaHCO_3$, and water). The organic layer was dried and concentrated. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate 8:2) and was then purified by HPLC using the same conditions as above to give 5α-fluoro-6*E*-hydroximinocholestan-3-one (**51**, 56 mg, 56%): white solid; $[\alpha]_D$ +115.4 (CH_2Cl_2, $c = 0.1$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.51 (OH, br s); 3.22 (H7β, 1H, br d, $J = 15.6$ Hz); 2.99 (H4, 1H, d, $J = 16.6$ Hz); 2.58 (H2, 2H, m); 1.04 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.2$ Hz); 0.87 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.69 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 209.2 (C3); 157.0 (C6, $J_{CF} = 25.4$ Hz); 100.5 (C5, $J_{CF} = 167.8$ Hz); 56.0 (C14); 55.9 (C17); 45.8 (C9, $J_{CF} = 3.1$ Hz); 43.9 (C13); 43.6 (C10, $J_{CF} = 13.4$ Hz); 43.1 (C24); 39.4 (C12); 36.9 (C22); 36.5 (C20); 36.0 (C1); 35.0 (C2); 34.7 (C8); 31.4 (C4, $J_{CF} = 19.4$ Hz); 30.4 (C7); 30.1 (C16); 27.9 (C25); 25.6 (C15); 23.7 (C23); 22.7 (C26); 22.5 (C27); 21.3 (C11); 18.5 (C21); 14.0 (C19, $J_{CF} = 5.3$ Hz); 12.0 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −158.2. (+)-LRFABMS, *m/z* (%): 456 ($[M+Na]^+$, 11); 414 ($[M-FH+H]^+$, 100). (+)-HRESIMS: *m/z* 414.3368 $[M-FH+H]^+$ (calcd for $C_{27}H_{44}NO_2$, 414.3367).

4.47. 5α-Fluoro-3β-mesyloxylcholestan-6-one (52)

A solution of 5α-fluoro-3β-hydroxycholestan-6-one (**49**, 0.25 g, 0.6 mmol) in 10 mL of dry pyridine was treated with 0.6 mL mesylsulfonyl chloride (MsCl) and the mixture was stirred at 0 °C for 4 h. The reaction mixture was poured into 5% aqueous $NaHCO_3$ (30 mL) and was left to stand for 1 h. The solid was filtered off, washed with water, dried, and subjected to chromatography (silica gel, hexanes/ethyl acetate 9:1) to give 5α-fluoro-3β-mesyloxylcholestan-6-one (**52**, 0.25 g, 99%): white solid; $[\alpha]_D$ −10.2 (CH_2Cl_2, $c = 0.5$); ^1H NMR (200 MHz, $CDCl_3$) δ_H: 4.91 (H3α, 1H, m); 3.01 ($MeSO_3^-$, 3H, s); 2.57 (H7β, 1H, m); 0.95 (H19, 3H, s); 0.90 (H21, 3H, d, $J = 6.71$ Hz); 0.85 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.65 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 206.3 (C6, $J_{CF} = 26.1$ Hz); 100.8 (C5, $J_{CF} = 175.6$ Hz); 77.9 (C3, $J_{CF} = 1$ Hz); 56.1 (C14); 56.0 (C17); 45.4; 45.3; 43.1 (C9, $J_{CF} = 14.2$ Hz); 42.7; 42.5; 42.4; 39.4; 38.6 ($MeSO_3^-$); 37.7; 36.0; 35.4; 31.7 (C4, $J_{CF} = 19.8$ Hz); 30.4; 27.8; 27.5; 23.8; 23.7; 22.2 (C21); 21.4 (C27); 21.2 (C11); 18.9 (C26); 13.7 (C19, $J_{CF} = 5.6$ Hz); 11.9 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −159.6. (+)-LRFABMS, *m/z* (%): 499 ($[M+Na]^+$, 6).

4.48. 5α-Fluorocholest-2-en-6-one (53a)

A solution of 5α-fluoro-3β-mesyloxylcholestan-6-one (**52**, 0.25 g, 0.5 mmol) in 8 mL of dry DMF was treated with LiBr (0.18 g, 2 mmol) under argon and the solution was heated under reflux in the dark with stirring for 3 h. The reaction mixture was poured into 100 mL of water and extracted with ethyl acetate (2 × 20 mL). The combined extracts were washed (5% HCl and 5% $NaHCO_3$), dried, and the solvent evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, hexanes/ethyl acetate 9:1) to give 0.22 g of 5α-fluorocholest-2-en-6-one (**53a**, 0.146 g, 60%), 3β-bromo-5α-fluoro-cholestan-6-one (**53b**, 0.073 g, 30%), cholest-2,4-dienone[5] (**53d**, 20 mg, 6%), and 5α-fluorocholest-3-en-6-one (**53c**, 10 mg, 3%). *5α-Fluorocholest-2-en-6-one* (**53a**): white solid; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 5.62 (H2, H3, 2H, m); 2.67 (H7β, 1H, m); 0.92 (H21, 3H, d, $J = 6.1$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.72 (H19, 3H, s); 0.62 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 207.6 (C6, $J = 26.1$ Hz); 124.4 (C2); 121.4 (C3); 100.7 (C5, $J_{CF} = 177.3$ Hz); 56.4 (C17); 56.0 (C14); 45.9; 45.6; 43.1; 42.9; 39.4; 37.9; 36.0; 34.7; 34.6; 32.9; 29.6; 27.6; 27.0; 26.7 (C4, $J_{CF} = 23.5$ Hz); 23.8; 22.7; 22.5; 21.7; 18.4; 14.0 (C19, $J_{CF} = 6.5$ Hz), 12.0 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −158.2. LREIMS (70 eV, *m/z* %): 402 (M^+, 4); 383 (M^+-F, 2); 84 (100). *3β-Bromo-5α-fluoro-cholestan-6-one* (**53b**): white solid; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 4.20 (H3, 1H, m); 2.67 (H7β, 1H, m); 0.92 (H21, 3H, d, $J = 6.1$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.4$ Hz); 0.71 (H19, 3H, s); 0.59 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 206.1 (C6, $J_{CF} = 25.7$ Hz); 121.3 (C2); 120.2 (C3); 99.2 (C5, $J_{CF} = 170.4$ Hz). 56.8 (C17); 56.2 (C14); 45.9; 45.4; 44.1; 43.9; 40.4; 38.9;

37.0; 35.7; 35.0; 34.9; 32.6; 29.6; 28.0; 27.2; 25.8; 23.7; 21.5; 20.7; 18.9; 14.5 (C19); 13.2 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −158.2. LREIMS (70 eV, *m/z* %): 482/484 (M^+, 7); 84 (100). *5α-Fluorocholest-3-en-6-one* (**53c**): white solid; $[\alpha]_D$ +6.5 (CH_2Cl_2, $c = 0.3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 5.32 (H3, H4, 2H, m); 2.62 (H7β, 1H, m); 0.91 (H21, 3H, d, $J = 5.7$ Hz); 0.86 (H26, H27, 6H, d, $J = 6.4$ Hz); 1.18 (H19, 3H, s); 0.66 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 214.2 (C6, s); 132.5 (C4, d); 124.6 (C3, d); 99.3 (C5, s); 56.2; 55.6; 45.7; 45.1; 43.8; 42.4; 39.8; 37.2; 36.6; 34.2; 34.8; 32.5; 28.5; 27.1; 27.0; 26.7; 23.4; 21.7; 21.5; 21.0; 17.4; 15.0; 11.8. EIMS (70 eV, *m/z* %): 402 (M^+, 4); 383 (M^+-F, 2); 83 (100).

4.49. 2,3-Dihydroxy-5α-fluorocholestan-6-one (54)

N-Methylmorpholine *N*-oxide (4 g, 34 mmol) and tetrabutylammonium hydrogen sulfate (2 g, 6 mmol) were dissolved in a mixture of 15 mL of THF (free of peroxides), 20 mL of *t*-BuOH, and 4 mL of water. To the resulting solution was added osmium tetroxide (0.1 g, 0.4 mmol) followed by the dropwise addition of a solution of 5α-fluorocholest-2-en-6-one (**53a**, 0.4 g, 1 mmol) in 10 mL of THF. The mixture was stirred at 0 °C in the dark under an argon atmosphere for 80 h. The reaction mixture was poured into 5% Na_2SO_3 (100 mL), stirred for 1 h, and extracted with CH_2Cl_2 (2 × 30 mL). The combined extracts were evaporated and the residue was redissolved in 50 mL of ethyl acetate, washed (5 M H_2SO_4, saturated $NaHCO_3$, and saturated brine), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue subjected to chromatography (silica gel, hexane/ethyl acetate, 7:3, 1:1) to give 2α,3α-dihydroxy-5α-fluorocholestan-6-one (**54a**, 50 mg, 15%) and 2β,3β-dihydroxy-5α-fluorocholestan-6-one (**54 b**, 50 mg, 15%). Compound **54a**: white solid; $[\alpha]_D$ +9.2 (CH_2Cl_2, $c = 0.1$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 4.04 (H3β, 1H, dd, $J = 6.0$, 3.0 Hz); 3.75 (H2β, 1H, m); 2.63 (H7β, 1H, m), 0.92 (H19, 3H, s); 0.87 (H21, 3H, d, $J = 6.4$ Hz); 0.85 (H26, H27, 6H, d, $J = 6.5$ Hz); 0.63 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 206.7 (C6, $J_{CF} = 26.1$ Hz); 101.7 (C5, $J_{CF} = 170.6$ Hz); 68.3 (C2); 67.2 (C3); 56.2 (C14); 55.9 (C17); 45.4 (C9, $J_{CF} = 3.9$ Hz); 43.0; 42.8 (C10, $J_{CF} = 24.8$ Hz); 39.4; 39.3; 37.4; 36.0; 35.6; 35.0; 30.3; 29.9; 27.9; 27.7; 23.8; 22.7; 22.5; 21.2; 18.5; 14.4 (C19, $J_{CF} = 5.1$ Hz); 14.3; 11.9 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −154.9. LREIMS (70 eV, *m/z* %): 436 (M^+, 13); 418 (M^+-H_2O, 10); 402 (M^+-2 OH, 10); 401 (M^+-OH-F, 18); 383 (M^+-2 OH−F, 10); 95 (100). Compound **54b**: white solid; $[\alpha]_D$ +10.2 (CH_2Cl_2, $c = 0.1$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 4.06 (H3α, 1H, br s); 3.97 (H2α, 1H, m); 2.62 (H7β, 2H, m); 1.03 (H19, 3H, s); 0.92 (H21, 3H, d, $J = 6.2$ Hz); 0.85 (H26, H27, 6H, d, $J = 6.3$ Hz); 0.64 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 207.3 (C6, $J_{CF} = 26.8$ Hz); 101.5 (C5, $J_{CF} = 173.7$ Hz); 68.6 (C2); 67.9 (C3); 63.4 (C14); 56.1 (C17); 46.0 (C9, $J_{CF} = 3.7$ Hz); 45.2; 42.4 (C10, $J_{CF} = 25.2$ Hz); 39.8; 39.3; 37.8; 37.0; 36.6; 35.5; 30.3; 29.5; 28.9; 27.9; 25.8; 24.3; 22.8; 22.2; 21.7; 18.5; 15.8 (C19, $J_{CF} = 5.2$ Hz); 12.0 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −160.1. LREIMS (70 eV, *m/z* %): 436 (M^+, 4); 400 ($M^+-2\ H_2O$, 1); 84 (100).

4.50. 5α-Fluoro-6*E*-hydroximino-2α,3α-cholestanediol (55a)

2α,3α-Dihydroxy-5α-fluorocholestan-6-one (**54a**, 0.1 g, 0.2 mmol) was treated with hydroxylamine hydrochloride (0.12 g, 1.7 mmol) in a similar way as compound **11** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate 7:3) to afford 5α-fluoro-6*E*-hydroximino-2α,3α-cholestanediol (**55a**, 88 mg, 88%): white solid; $[\alpha]_D$ −17.2 (CH_2Cl_2, $c = 0.1$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 8.04 (OH, br s); 4.04 (H3β, 1H, br s); 3.77 (H2β, 1H, br d, $J = 4.0$ Hz); 3.21 (H7β, 1H, dd, $J = 5.8$, 2.0 Hz), 0.92 (H21, 3H, d, $J = 6.8$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.5$ Hz); 0.87 (H19, 3H, s); 0.68 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 157.7 (C6, $J_{CF} = 26.3$ Hz); 100.7 (C5, $J_{CF} = 161.25$ Hz); 68.9 (C2); 67.4 (C3); 56.6; 55.3; 53.2 (C14); 46.8; 45.3 (C17); 42.8 (C9, $J_{CF} = 3.2$ Hz); 43.2; 40.9 (C10, $J_{CF} = 24.1$ Hz); 39.8; 39.5; 36.1; 35.7; 33.9; 31.9; 29.7; 28.1; 25.5; 23.9; 22.8; 21.3; 18.9; 16.2 (C19, $J_{CF} = 5$ Hz); 13.1 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −158.7. (+)-LRFABMS, *m/z* (%): 474 ($[M+Na]^+$, 21); 452 ($[M+H]^+$, 100).

4.51. 5α-Fluoro-6*E*-hydroximino-2β,3β-cholestanediol (55b)

2β,3β-Dihydroxy-5α-fluorocholestan-6-one (**54b**, 0.1 g, 0.2 mmol) was treated with hydroxylamine hydrochloride (0.12 g, 1.7 mmol) in a similar way as compound **11** to give a residue which was subjected to chromatography (silica gel, hexanes/ethyl acetate 8:2) to afford 5α-fluoro-6*E*-hydroximino-2β,3β-cholestanediol (**55b**, 90 mg, 90%): white solid; $[\alpha]_D$ −32.6 (CH_2Cl_2, $c = 0.1$); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 8.12 (OH, br s); 4.12 (H3α, H2α, 2H, br s); 3.22 (H7β 1H, dd, $J = 4.0$, 12.0 Hz); 1.08 (H19, 3H, s); 0.91 (H21, 3H, d, $J = 6.4$ Hz); 0.88 (H26, H27, 6H, d, $J = 6.5$ Hz); 0.68 (H18, 3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 158.4 (C6, $J_{CF} = 26.9$ Hz); 99.1 (C5, $J_{CF} = 166.4$ Hz); 68.9 (C2); 68.1 (C3); 58.4; 56.7; 56.1 (C14); 46.1 (C17); 43.0 (C9, $J_{CF} = 2.9$ Hz); 42.9 (C10, $J_{CF} = 22$ Hz); 39.5; 39.4; 36.1; 31.9; 29.7; 29.5; 28.1; 27.9; 23.8; 22.6; 22.5; 21.7; 18.4; 16.3; 15.3 (C19, $J_{CF} = 4.7$ Hz); 13.1; 12.8 (C18). ^{19}F NMR ($CDCl_3$) δ_F: −160.1. EIMS (70 eV, *m/z* %): 451 (M^+, 4); 433 (M^+-H_2O, 2); 402 ($M^+-NOH-H_2O$, 4); 84 (100). (+)-LRFABMS, *m/z* (%): 452 ($[M+H]^+$, 68). 432 ($[M-F]^+$, 100). (+)-HRESIMS: *m/z* 452.3516 $[M+H]^+$ (calcd for $C_{27}H_{47}FNO_3$, 452.3535); 474.3334 $[M+Na]^+$ (calcd for $C_{27}H_{46}FNNaO_3$, 474.3354).

4.52. Biological materials: inhibition of cell growth by cell counting

This form of assay employs 96-well microplates. The tumor cell lines employed are: Human lung carcinoma A-549 (ATCC CCL-185), colon adenocarcinoma HCT-116 (ATCC CCL-247), Human Caucasian glioblastoma multiforme T98G (ECACC 92090213), and human pancreatic adenocarcinoma PSN1 (ECACC 94060601). These were cultured in RPMI medium containing glutamine (2 mM), penicillin (50 IU/mL), streptomycin (50 μg/mL), supplemented with 5% FBS (A-549 and

HCT-116) or 10% FBS (PSN1), T98G was maintained in RPMI 1640, 2 mM glutamine, penicillin (50 IU/mL), streptomycin (50 μg/mL), 1 mM Pyruvate supplemented with amino acids and 10% FBS.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was used, essentially as described.[26] The assay is dependent on the reduction of MTT by mitochondrial dehydrogenases of viable cells to a blue formazan product, which can be measured spectophotometrically. For each experiment the cells were harvested from subconfluent cultures using trypsin and resuspended in fresh medium before planting. The tumor cells were incubated in each well with serial dilutions of the tested compounds in 200 μL of complete medium. A separate set of wells was seeded as a growth control to ensure that cells remained in the exponential growth phase. After 2 days of incubation at 37 °C and 5% CO_2 in an atmosphere with 98% humidity, MTT (5 mg/mL in PBS) was added to each well and the plate was incubated for a further 2 h (37 °C). The resulting formazan was dissolved in DMSO and read at 490 nm. All determinations were carried out in triplicate. The IC_{50} value was calculated as the concentration of drug yielding 50% cell survival by comparing the OD in wells with drug to the OD in the control wells.

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