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STEREOSELECTIVE SYNTHESIS OF *(25R)*-26-FLUORO-5-CHOLESTEN-3β,25-DIOL 3-ACETATE FROM METHYL 3β-HYDROXY-5-CHOLENOATE

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SUMMARY

A stereoselective synthesis of *(25R)*-26-fluoro-5-cholesten-3β,25-diol 3-acetate (**1**) from methyl 3β-hydroxy-5-cholenoate (**2**) is presented. Formation of the asymmetric center at C-25 was accomplished by application of the Sharpless epoxidation methodology from 6β-methoxy-3α,5-cyclo-5α-cholest-25(27)-en-26-ol (**7**). Substitution of the C-27 hydroxyl group of *(25R)*-6β-methoxy-3α,5-cyclo-25,26-epoxy-5α-cholestan-27-ol (**8**) by a fluorine atom, *via* mesylate derivative, and subsequent reduction of the oxirane ring with $LiAlH_4$ afforded *(25R)*-6β-methoxy-26-fluoro-3α,5-cyclo-5α-cholestan-25-ol (**16**). Deprotection of the 3β-hydroxy-5(6)-en system in **16** with BF_3·Et_2O/acetic acid yielded the title compound **1**.

INTRODUCTION

The active hormonal form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 {1α,25-$(OH)_2D_3$}, is a powerful regulator of calcium metabolism in humans and plays a role in the regulation of replication of several human and animal cancer lines *in vitro* [1-8]. The effect of 1α,25-$(OH)_2D_3$ on cellular replication *in vitro* has been also confirmed *in vivo* [9-10]. 1α,25-$(OH)_2D_3$ could be useful as an anti-tumor agent except that doses required to achieve differentiation and/or to inhibit proliferation cause hypercalcemia in man [11]. Therefore much effort has been devoted [12] to synthesizing vitamin D_3 analogues with separated biological activities. As a result, the substitution of a hydrogen atom by fluorine in the vitamin D_3 side chain (at C-23, C-24, C-25, C-26, and C-27) [13-19] became an

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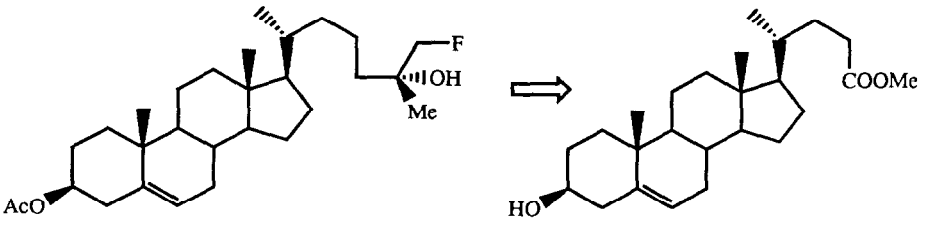
effective tool for activity modification. Among several compounds having a strong effect on cell differentiation, but a weak or almost no regulating effect on calcium, two containing fluorine atoms in the side chain, {24-homo-24,24-difluoro-1α,25-$(OH)_2D_3$ and 26,26,26,27,27,27-hexafluoro-1α-OH-D_3} have been reported [12,20].

In our laboratory studies have been carried out [21,22] on the synthesis of 25-hydroxycholesterol precursors of vitamin D_3 with a fluorine atom(s) located at the metabolic positions [23,24] in the side chain. In the previous paper [21] we presented an effective method of preparation of *(25R,S)*-26-fluoro-5-cholesten-3β,25-diol 3-acetate and its C-27 C_1-C_3 alkyl homologues using methyl 3β-hydroxy-5-cholenoate as a substrate. The fluorine atom was introduced at C-26 by application of a general methodology elaborated by us [25] which involves formation of α-fluoroketones *via* allene oxides. Now we would like to report the stereoselective synthesis of *(25R)*-26-fluoro-5-cholesten-3β,25-diol 3-acetate (**1**) (**Scheme 1**) from methyl 3β-hydroxy-5-cholenoate (**2**).



1 2

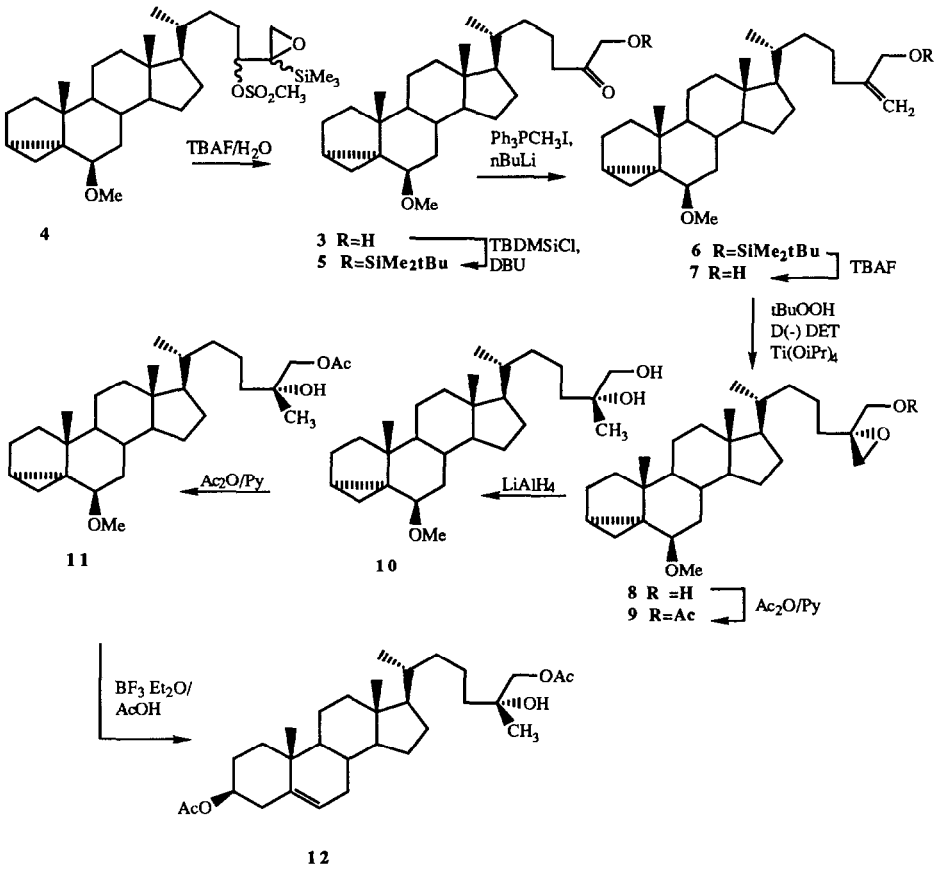
Scheme 1.

RESULTS

Our plan required transformation of the carbonyl group of hydroxyketone **3** [21] (**Scheme 2**) into a methylene group and subsequent stereospecific introduction of an epoxide function. The compound obtained in such a way, bearing hydroxyl and epoxide moieties, was regarded as a precursor of the target molecule with the desired substituents: fluorine (C-26), hydroxyl (C-25), and methyl groups (C-27).

Similar to that described [21], the starting compound, hydroxyketone **3**, was obtained in 84% yield by treatment of silyl epoxide **4** [21] with

tetrabutylammonium fluoride trihydrate (TBAF·3H_2O, 1 eq.) and water (10 eq.). The hydroxyl group of **3** was protected as the tert-butyldimethylsilyl ether (tert-BuMe_2SiCl, DBU, CH_2Cl_2, 93% yield) to afford **5**. The reaction of **5** with methyltriphenylphosphonium iodide (Ph_3PCH_3I) and n-BuLi in THF gave compound **6** which subsequently was deprotected with TBAF to yield allylic alcohol **7** {^{1}H NMR: δ, 4.07 (2H, d, J=6.1 Hz, 26-H), 4.87 and 5.01 (2H, 2d, J=1.3 Hz, 27-H)}. Compound **7** was targeted as the progener for the stereoselective introduction of the C-25,26 epoxide moiety. This



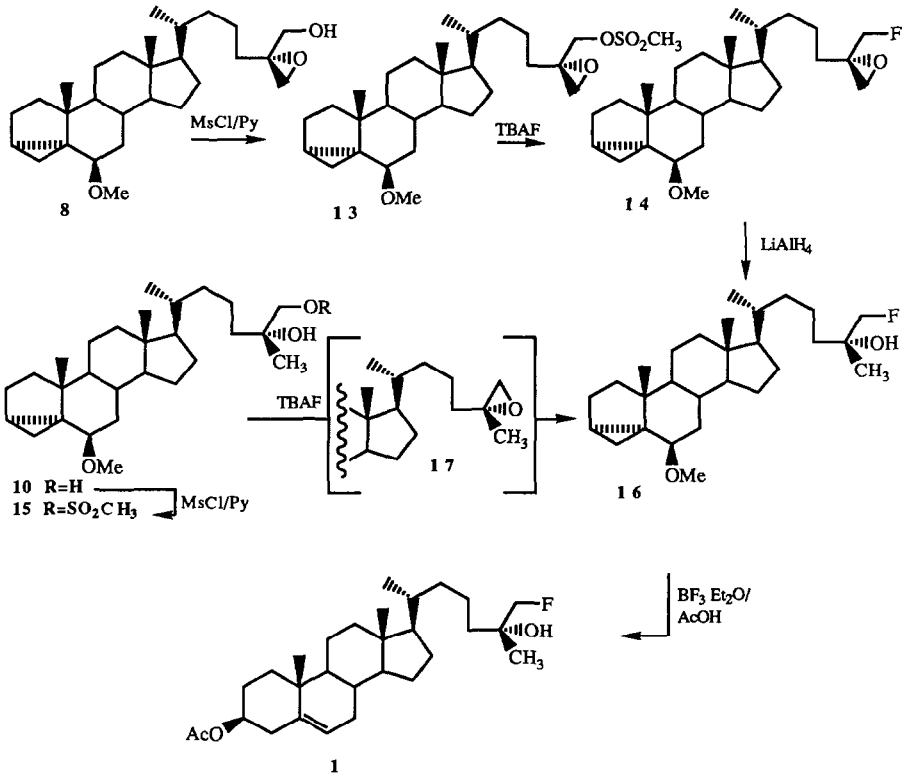
Scheme 2.

goal was accomplished by application of a Sharpless asymmetric epoxidation reaction [26,27] using tert-butyl hydroperoxide (tert-BuOOH) in the presence of titanium tetraisopropoxide {$Ti(O^iPr)_4$} and *(S,S)*-(-)-diethyl tartrate in CH_2Cl_2 at -25° C for 24 h thereby affording epoxide **8** in 82% yield. The selectivity of the Sharpless process was estimated as 95:5 from the appropriate acetate **9** by the ^1H NMR (500 MHz). One group of proton signals (C-27, AB quartets) in **9** was separated from the other: the main epimer at δ 4.03 and 4.26 ppm, the minor epimer at δ 4.01 and 4.28 ppm. The configuration at C-25 of the major diastereomer **8** could be assigned as *R* by the Sharpless prediction model [27-29] and was confirmed by conversion into the known *(25R)*-5-cholesten-3β,25,26-triol 3,26-diacetate [30] in the following sequence of reactions: a) reduction of the epoxide function of **8** by $LiAlH_4$ to afford diol **10**, b) acetylation of primary hydroxyl group (Ac_2O, Py) to give acetate **11**, c) deprotection of C-5,6 double bond (AcOH, $BF_3{\cdot}Et_2O$) [31] to produce diacetate **12**, {mp 151-154° C, (MeOH), lit. [30] mp 154-155° C}. All analytical and spectral properties of compound **12** were in full agreement to those of *(25R)*-5-cholesten-3β,25,26-triol 3,26-diacetate synthesized by the Roche group [30].

Stereoselective preparation of hydroxyepoxide **8** was the crucial step of the synthesis assuming that the primary hydroxyl group could be substituted by fluorine and the epoxide function could be regiospecifically opened by a hydride ion to yield 26-fluoro-25-hydroxycholesterol, without epimerization at C-25. Bearing this in mind, we converted **8** into mesylate **13** (MsCl, Py, 87%) (**Scheme 3**), which subsequently was treated with anhydrous TBAF in THF to afford fluoroepoxide **14** {83% yield, ^1H NMR: δ, 4.37 and 4.47 ppm, dq_{AB}, J(HH)=10.3 Hz, J(HF)=47.6 Hz, 26-H}. The reaction of mesylate **13** with TBAF did not cause epimerization at C-25 in **14**. This process should be considered as a one pot opening of the epoxide by fluoride followed by formation of a new epoxide ring (from the C-25 hydroxyl group and the neighboring methanesulphonyl function). Verification that **14** had not epimerized was proven by transformation of **10** into mesylate **15** followed by reaction with anhydrous TBAF. The product **16** (obtained *via* epoxide **17**) showed the same analytical and spectral properties as those of compound **16** obtained in the main reaction sequence: **13** → **14** → **16** *(vide infra)*.

Stereoselective synthesis of fluoroepoxide **14** gave easy access to *(25R)*-26-fluoro-25-hydroxycholesterol **1**. Reduction of the epoxide function of **14** with $LiAlH_4$ in Et_2O at reflux afforded 26-fluoro-6β-

methoxy-3α,5-cyclo-5α-cholestan-25-ol (**16**) in 89% yield. Compound **16** consisted of a mixture (95:5) of *25R*- and *25S*- epimers. ^{1}H NMR signals of the C-26 protons of the main *25R*-epimer were observed at δ 4.214 and 4.232 ppm {dq_{AB}, J(HH)=19.1 Hz, J(HF)=47.8 Hz}, whereas in the minor *25S*-compound **16** at δ 4.214 and 4.230 ppm {dq_{AB}, J(HH)=16.2 Hz, J(HF)=47.8 Hz}. The pure *25R* epimer **16** was obtained by recrystallization of crude product. The synthesis of the title compound, *(25R)*-26-fluoro-5-cholesten-3β,25-diol 3-acetate (**1**), was completed by the deprotection [31] of **16** with acetic acid and BF_3·Et_2O in ethyl ether (86% yield) to liberate C-5,6 double bond.



Scheme 3.

In summary, *(25R)*-26-fluoro-5-cholesten-3β-,25-diol 3-acetate (**1**) was stereoselectively obtained from methyl 3β-hydroxy-5-cholenoate (**2**). It should be pointed out that, by opening of the epoxide function of **14** with various nucleophiles, a wide range of *(25R)*-26-fluoro-25-hydroxy-27-substituted cholesterols may be obtained. One can assume that, according to the presented methodology, application of *(R,R)*-(+)-diethyl tartrate to the stereoselective formation of the epoxide moiety should give access to *(25S)*-26-fluoro-25-hydroxycholesterol.

EXPERIMENTAL

Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. The spectra were recorded using the following units: IR spectra - Beckman 4240 or Unicam SP 200, ^1H NMR spectra - Bruker AM 500 (in $CDCl_3$ solution), mass spectra - Finnigan MAT 8200. Chemical shifts were reported in δ units, downfield from $MeSi_4$. Column chromatography was performed on Kieselgel 60 (70-320 mesh) - Merck, and TLC on aluminium sheets Kieselgel 60 - Merck. Organic extracts were dried over anhydrous $MgSO_4$ and were evaporated under reduced pressure on a rotary evaporator. Yields refer to homogeneous products (TLC).

6β-Methoxy-26-hydroxy-27-nor-3α,5-cyclo-5α-cholestan-25-one **(3)**

A solution of compound **4** [21] (350 mg, 0.62 mmol), TBAF $3H_2O$ (195 mg, 0.62 mmol), and water (111 μl, 6.2 mmol) in THF (8 ml) was stirred at room temperature for 3 h. Then the product was extracted with Et_2O and the organic phase was washed with water and dried. After evaporation of solvent, the residue was chromatographed on silica gel with hexane-Et_2O (85:15) to give 216 mg (84%) of hydroxyketone **3** [21].

6β-Methoxy-3α,5-cyclo-26-hydroxy-27-nor-5α-cholestan-25-one Dimethyltertbutylsilyl Ether **(5)**

A cold solution (0° C) of alcohol **3** (200 mg, 0.48 mmol), DBU (88 mg, 0.58 mmol) and TBDMSiCl (88 mg, 0.58 mmol) in CH_2Cl_2 (3 ml) was stirred at 0° C for 0.5 h following by 3 h at room temperature. The solvent was evaporated *in vacuo* and the residue was chromatographed on silica gel with hexane-Et_2O (98:2) to give 228 mg (89%) of silyl ether **5** as a colorless oil.

IR (film): 1725 (C=O) cm^{-1};

^1H NMR (200 MHz): δ, 0.09 (6H, s, $SiMe_2$) 0.43 (1H, dd, J_1=4.9 Hz, J_2=7.9 Hz,

cyclopropyl-H), 0.65 (1H, t, J=4.8 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.93 (9H, s, SitBu), 0.94 (3H, d, J=6.5 Hz, 21-H), 1.02 (3H, s, 19-H), 2.46 (2H, m, 24-H), 2.77 (1H, t, J=2.8 Hz, 6-H), 3.32 (3H, s, OMe), 4.16 (2H, s, 26-H);

Anal. Calcd for $C_{33}H_{58}O_3Si$: C, 74.66, H, 11.01. Found: C, 74.92 , H, 11.26.

6β-Methoxy-3α,5-cyclo-5α-cholest-25(27)-en-26-ol (**7**)

To a solution of ylide, prepared from Ph_3PCH_3I (305 mg, 0.75 mmol) and n-BuLi (0.47 ml, 1.6M solution in hexane, 0.75 mmol) in THF (10 ml) at -20° C under argon, a solution of ketone **5** (200 mg, 0.38 mmol) in THF (2 ml) was slowly added by a syringe. The reaction mixture was stirred at -20° C for 2 h whereupon the temperature was slowly raised (*ca* 1 h) to 20° C. Then TBAF•$3H_2O$ (123 mg, 0.45 mmol) in THF (2 ml) was added. Stirring was continued for 1 h when water was added (30 ml) and the product was extracted with Et_2O. The organic phase was washed 3 times with water and dried. After evaporation of solvent the residue was chromatographed on silica gel with hexane-Et_2O (85:15) to give 131 mg (84%) of allylic alcohol **7**, mp 120.5-123° C (hexane-Et_2O).

IR (film): 3460 (OH) cm^{-1};

^1H NMR: δ, 0.43 (1H, dd, J_1=4.9 Hz, J_2=7.9 Hz, cyclopropyl-H), 0.65 (1H, t, J=4.8 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.92 (3H, d, J=6.6 Hz, 21-H), 1.02 (3H, s, 19-H), 2.76 (1H, t, J=2.8 Hz, 6-H), 3.32 (3H, s, OMe), 4.07 (2H, d, J=6.1 Hz, 26-H), 4.87 and 5.01 (2H, 2d, J=1.3 Hz, 27-H);

Anal. Calcd for $C_{28}H_{46}O_2$: C, 81.10, H, 11.18. Found: C, 80.8, H, 11.35.

(25R)-6β-Methoxy-3α,5-cyclo-25,26-epoxy-5α-cholestan-27-ol (**8**)

A solution of titanium tetraisopropoxide (62 μl, 0.24 mmol) and *(S,S)*-(-)-diethyl tartarate (42 μl, 0.24 mmol), was stirred in CH_2Cl_2 at -25° C for 5 min. Then successively compound **7** (100 mg, 0.24 mmol) and tert-BuOOH (135 μl, 3.6 M solution in toluene, 0.48 mmol) were slowly added. The reaction mixture was stirred at -25° C for 24 h whereupon water was added and the product was extracted with Et_2O. The organic phase was washed successively with 5% HCl, H_2O, and $NaHCO_3$ and then it was dried. After evaporation of solvent the oily residue was chromatographed on silica gel with hexane-Et_2O (85:15) to give 85 mg (82%) of epoxide **8**, mp 78-83° C.

IR($CHCl_3$): 3260 (OH) cm^{-1};

^1H NMR: δ, 0.42 (1H, dd, J_1=5.2 Hz, J_2=8.0 Hz, cyclopropyl-H), 0.65 (1H, t, J=4.9 Hz, cyclopropyl-H), 0.70 (3H, s, 18-H), 0.91 (3H, d, J=6.6 Hz, 21-H),

1.01 (3H, s, 19-H), 2.66 (1H, d, J=4.7 Hz, 26-H), 2.76 (1H, t, J=2.7 Hz, 6-H), 2.88 (1H, d, J=4.8 Hz, 26-H), 3.32 (3H, s, OMe), 3.65 and 3.78 (2H, q_{AB}, J=12.3 Hz, 27-H);
Anal. Calcd for $C_{28}H_{46}O_3$: C, 78.09, H, 10.77. Found: C, 78.16, H, 10.91.

(25R)-6β-Methoxy-3α,5-cyclo-25,26-epoxy-5α-cholestan-27-ol 27-Acetate (**9**)

Alcohol **8** (5 mg, 0.01 mmol) was acetylated with acetic anhydride (0.5 ml) in pyridine (0.5 ml) at room temperature for 24 h. Standard work-up afforded 5 mg (91%) of acetate **9**, mp 95-97° C (hexane-Et_2O).
IR (KBr): 1750 (C=O) cm^{-1};
^1H NMR: δ, 0.43 (1H, dd, J_1=5.0 Hz, J_2=8.0 Hz, cyclopropyl-H), 0.65 (1H, t, J=4.9 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.91 (1H, d, J=6.6 Hz, 21-H), 2.10 (3H, s, $OCOCH_3$), 2.69 (1H, d, J=4.7 Hz, 26-H), 2.75 (1H, d, J=4.6 Hz, 26-H), 2.77 (1H, t, J=2.6 Hz, 6-H), 4.03 and 4.26 (2H, q_{AB}, J=12.0 Hz, 27-H);
Anal. Calcd for $C_{30}H_{48}O_4$: C, 76.23, H, 10.24. Found: C, 76.17, H, 10.41.

(25R)-6β-Methoxy-3α,5-cyclo-5α-cholestan-25,26-diol (**10**)

A solution of epoxide **8** (5 mg, 0.01 mmol) and $LiAlH_4$ (2 mg, 0.05 mmol) in Et_2O (2 ml) was heated at reflux for 1 h. Then water (100 μl) was added and the organic layer was passed through a short column with anhydrous $MgSO_4$. After evaporation of solvent 5 mg (97%) of diol **10** was obtained, mp 152-155° C (hexane-Et_2O).
IR ($CHCl_3$): 3440 and 3620 (OH) cm^{-1};
^1H NMR: δ, 0.43 (1H, dd, J_1=5.1 Hz, J_2=8.0 Hz, cyclopropyl-H), 0.65 (1H, t, J=4.8 Hz, cyclopropyl-H), 0.72 (3H, s, 18-H), 0.92 (3H, d, J=6.6 Hz, 21-H), 1.02 (3H, s, 19-H), 1.18 (3H, s, 27-H), 2.77 (1H, t, J=2.7 Hz, 6-H), 3.32 (3H, s, OMe), 3.42 and 3.48 (2H, q_{AB}, J=10.8 Hz, 26-H);
Anal. Calcd for $C_{28}H_{48}O_3$: C, 77.73, H, 11.18. Found: C, 77.94, H, 10.98.

(25R)-6β-Methoxy-3α,5-cyclo-5α-cholestan-25,26-diol 26-Acetate (**11**)

Diol **10** (8 mg, 0.02 mmol) was acetylated with acetic anhydride (0.3 ml) in pyridine (0.5 ml) at room temperature for 6 h. Standard work-up afforded 8 mg (91%) of acetate **11**, mp 119-123° C (hexane-Et_2O).
IR(film): 1745 (C=O), 3450 (OH) cm^{-1};
^1H NMR: δ, 0.43 (1H, dd, J_1=5.2 Hz, J_2=8.0 Hz, cyclopropyl-H), 0.65 (1H, t, J=4.9 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.92 (3H, d, J=6.5 Hz, 21-H), 1.02 (3H, s, 19-H), 1.20 (3H, s, 27-H), 2.11 (3H, s, $OCOCH_3$), 2.76 (1H, t, J=2.5 Hz, 6-H), 3.32 (3H, s, OMe), 3.96 and 4.00 (2H, q_{AB}, J=11.3 Hz, 26-H);
Anal. Calcd for $C_{30}H_{50}O_4$: C, 75.91, H, 10.62. Found: C, 75.95, H, 10.59.

(25R)-5-Cholesten-3β,25,26-triol 3,26-Diacetate (**12**)

A solution of compound **11** (6 mg, 0.013 mmol), AcOH (0.3 ml) and $BF_3 \cdot Et_2O$ (0.3 ml) in Et_2O (2 ml) was stirred at room temperature for 1 h. Then the solution was diluted with Et_2O, washed with sodium bicarbonate and dried. After evaporation of solvent the residue was chromatographed on silica gel with hexane-Et_2O (85:15) to give 5 mg (79%) of diacetate **12**, mp 151-154° C (MeOH), {lit. [12] mp 154-155° C}.

IR(film): 1730 and 1745 (C=O) cm^{-1};

^1H NMR: δ, 0.67 (3H, s, 18-H), 0.92 (3H, d, J=6.5 Hz, 21-H), 1.01 (3H, s, 19-H), 2.00 (3H, s, 27-H), 2.03 (3H, s, 3-$OCOCH_3$), 2.11 (3H, s, 26-$OCOCH_3$), 3.96 and 3.99 (2H, q_{AB}, J=11.3 Hz, 26-H), 5.34 (1H, m, 3-H);

Anal. Calcd for $C_{31}H_{50}O_5$: C, 74.07, H, 10.03. Found: C, 74.13,H, 10.08.

(25R)-6β-Methoxy-25,26-epoxy-3α,5-cyclo-5α-cholestan-27-ol 27-Methanesulphonate (**13**)

A solution of alcohol **8** (80 mg, 0.19 mmol), MsCl (22 μl, 0.30 mmol) in pyridine (2 ml) was stirred at 0° C for 1 h and then 3 h at room temperature. The product was extracted with Et_2O and the solution was washed succesively with H_2O, 5% HCl, and $NaHCO_3$ whereupon it was dried. Evaporation of solvent afforded an oily residue which was chromatographed on silica gel with hexane-Et_2O (4:1) to give 82 mg (87%) of mesylate **13**, mp 82.5-84° C (hexane-Et_2O).

^1H NMR: δ, 0.43 (1H, dd, J_1=5.2 Hz, J_2=7.8 Hz, cyclopropyl-H), 0.65 (1H, t, J=4.2 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.92 (3H, d, J=6.6 Hz, 21-H), 1.02 (3H, s, 19-H), 2.75 (1H, d, J=4.5 Hz, 26-H), 2.77 (1H, t, J=2.8 Hz, 6-H), 2.80 (1H, d, J=4.1 Hz, 26-H), 3.07 (3H, s, OSO_2CH_3), 3.32 (3H, s, OMe), 4.16 and 4.38 (2H, q_{AB}, J=11.5 Hz, 27-H);

Anal. Calcd for $C_{29}H_{48}O_5S$: C, 68.47, H, 9.50. Found: C, 68.37, H, 9.62.

(25R)-6β-Methoxy-25,26-epoxy-27-fluoro-3α,5-cyclo-5α-cholestan (**14**)

A solution of mesylate **13** (60 mg, 0.12 mmol) and anhydrous TBAF (149 mg, 0.5 mmol) in THF (3 mL) was stirred at room temperature for 4 h. Then Et_2O (20 ml) was added and solution was washed 3 times with water (3x20 ml) whereupon it was dried. After evaporation of solvent the residue was chromatographed on silica gel with hexane-Et_2O (95:5) to give 42 mg (83%) of **14**, mp 46-55° C.

^1H NMR: δ, 0.43 (1H, dd, J_1=5.1 Hz, J_2=8.0 Hz, cyclopropyl-H), 0.65 (1H, dd, J_1=4.0 Hz, J_2=5.0 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.92 (3H, d, J=6.6 Hz, 21-H), 1.02 (3H, s, 19-H), 2.72 (1H, d, J=4.4 Hz, 26-H), 2.76 (2H, m, 6-H and 26-H), 3.32 (3H, s, OMe), 4.37 and 4.47 {2H, dq_{AB}, J(HH)=10.3 Hz, J(HF)=47.6 Hz, 26-H};

Anal. Calcd for $C_{28}H_{45}O_2F$: C, 77.73, H, 10.48. Found: C, 77.68, H, 10.31.

(25R)-6β-Methoxy-3α,5-cyclo-5α-cholestan-25,26-diol 26-Methanesulphonate (**15**)

A solution of diol **10** (5 mg, 0.012 mmol), MsCl (10 μl, 0.13 mmol) in pyridine (1 ml) was stirred at room temperature for 5 h. Standard work-up afforded 5 mg (85%) of mesylate **15**, oil.

^{1}H NMR: δ, 0.43 (1H, dd, J_1=5.2 Hz, J_2=7.9 Hz, cyclopropyl-H), 0.64 (1H, t, J=4.9 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.92 (3H, d, J=6.6 Hz, 21-H), 1.02 (3H, s, 19-H), 1.25 (3H, s, 27-H), 2.76 (1H, t, J=2.7 Hz, 6-H), 3.07 (3H, s, OSO_2CH_3), 3.32 (3H, s, OMe), 4.06 and 4.10 (2H, q_{AB}, J=10.1 Hz, 26-H);

Anal. Calcd for $C_{29}H_{50}O_5S$: C, 68.20, H, 9.87. Found: C, 68.32, H, 10.01.

(25R)-6β-Methoxy-26-fluoro-3α,5-cyclo-5α-cholestan-25-ol (**16**)

A solution of epoxide **14** (48 mg, 0.11 mmol) and $LiAlH_4$ (8 mg, 0.21 mmol) in Et_2O (2 ml) was stirred at reflux for 0.5 h. Then water (100 μl) was added and the solution was passed through a short column with anhydrous $MgSO_4$. Evaporation of solvent afforded 43 mg (89%) of **16**, mp 168-172.5° C (hexane-Et_2O).

IR (KBr): 3450 (OH) cm^{-1};

^{1}H NMR: δ, 0.43 (1H, dd, J_1=5.2 Hz, J_2=7.9 Hz, cyclopropyl-H), 0.65 (1H, dd, J_1=4.1 Hz, J_2=4.5 Hz, cyclopropyl-H), 0.72 (3H, s, 18-H), 0.93 (3H, d, J=6.6 Hz, 21-H), 1.02 (3H, s, 19-H), 2,77 (1H, t, J=2.5 Hz, 6-H), 3.32 (3H, s, OMe), 4.214 and 4.232 {2H, dq_{AB}, J(HH)=19.1 Hz, J(HF)=47.8 Hz, 26-H};

Anal. Calcd for $C_{28}H_{47}O_2F$: C, 77.37, H, 10.90. Found: C, 77.31, H, 10.82.

(25R)-6β-Methoxy-25,26-epoxy-3α,5-cyclo-5α-cholestan (**17**)

A solution of mesylate **15** (3 mg, 0.006 mmol), TBAF (10 mg, 0.04 mmol) in THF (1 ml) was stirred at reflux for 5 h. Then Et_2O was added and solution was washed 3 times with water and dried. Evaporation of solvent afforded oily residue which was chromatographed on silica gel using following eluates:

i) hexane-Et_2O (95:5) to give 1 mg (41%) of **17**, oil.

^{1}H NMR: δ, 0.43 (1H, dd, J_1=5.5 Hz, J_2=8.0 Hz, cyclopropyl-H), 0.65 (1H, t, J=4.0 Hz, cyclopropyl-H), 0.71 (3H, s, 18-H), 0.92 (3H, d, J=6.6 Hz, 21-H), 1.01 (3H, s, 19-H), 1.31 (3H, s, 27-H), 2.57 and 2.61 (2H, q_{AB}, J=4.9 Hz, 26-H), 2.76 (1H, t, J=2.9 Hz, 6-H), 3.22 (3H, s, OMe);

Anal. Calcd for $C_{28}H_{46}O_2$: C, 81.10, H, 11.18. Found: C, 81.23, H, 11.09.

ii) hexane-Et_2O (90:10) to give 1 mg (39%) of **16**.

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