Chem. Pharm. Bull.
30(12)4297—4303(1982)

Studies on Organic Fluorine Compounds. XXXIX.[1] Studies on Steroids. LXXIX.[2] Synthesis of 1**α**,25-Dihydroxy-26,26,26,27,27,27-hexafluorovitamin D_3

Yoshiro Kobayashi,*[,a] Takeo Taguchi,[a] Satoshi Mitsuhashi,[a]
Tadashi Eguchi,[b] Etsuo Ohshima,[b] and Nobuo Ikekawa*[,b]

Tokyo College of Pharmacy,[a] Horinouchi, Hachioji, Tokyo 192-03, Japan and
Department of Chemistry, Tokyo Institute of Technology,[b]
Meguro-ku, Tokyo 152, Japan

(Received June 7, 1982)

1α,25-Dihydroxy-26,26,26,27,27,27-hexafluorocholesterol was synthesized by two procedures. Cholenic acid was converted to the 1α-hydroxy-24-sulfone derivative and hexafluoroacetone was reacted with the lithiated 24-sulfone. Subsequent removal of the sulfonyl group afforded the hexafluoro-1α,25-dihydroxy compound. An alternative process consists of construction of the hexafluoro-25-hydroxy part on the side chain followed by the introduction of a hydroxy group at the 1α-position. The hexafluoro derivative was converted to the vitamin D form, 1α,25-dihydroxyhexafluorovitamin D_3, *via* the corresponding 5,7-diene.

Keywords——vitamin D_3; fluoride substitution; 26-hydroxylation; hexafluoroacetone; 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3

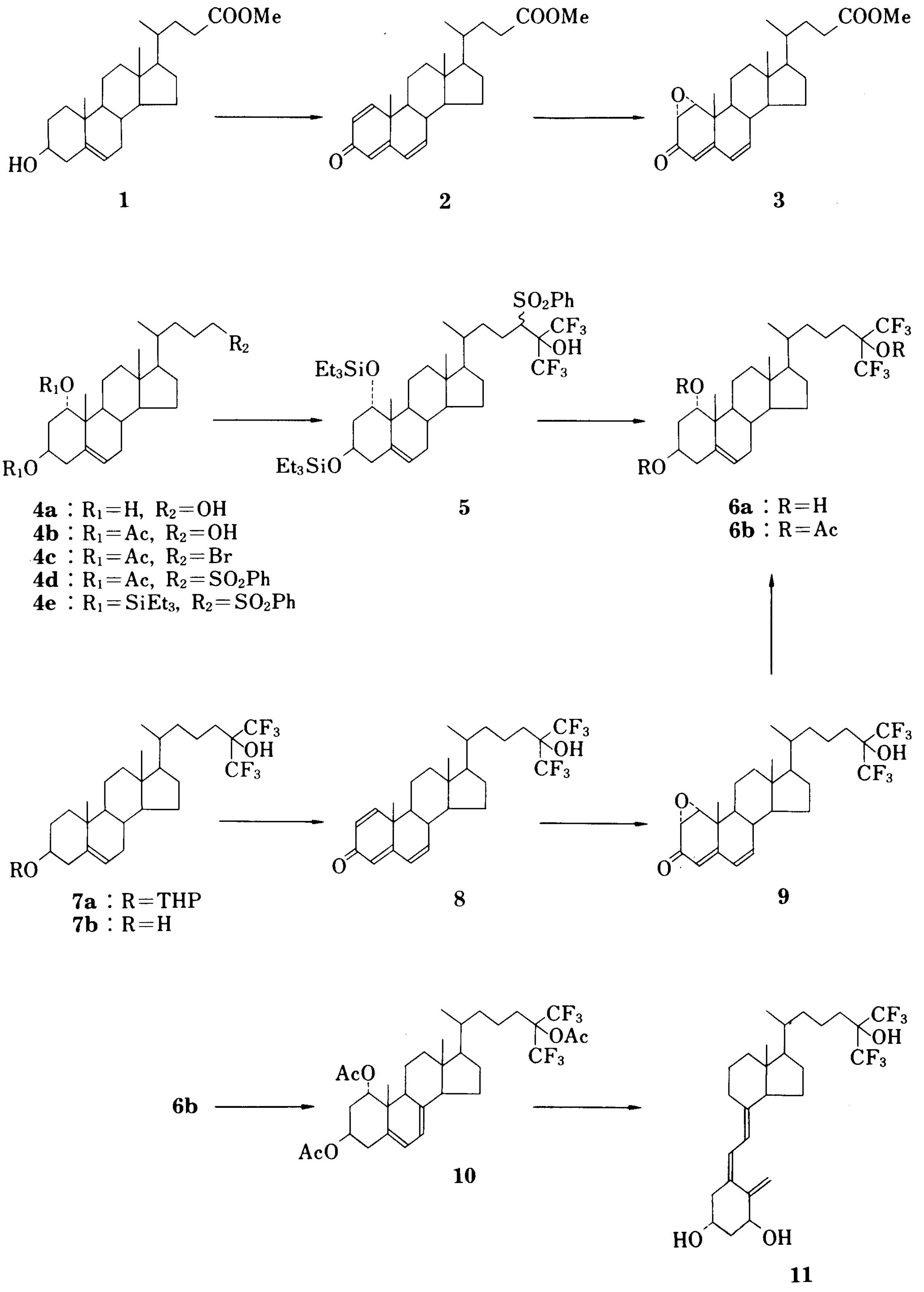
Since the discovery of 1α,25-dihydroxyvitamin $D_3(1,25\text{-}(OH)_2D_3)$[3] as the most potent metabolite of vitamin D_3 in mediating intestinal calcium absorption and bone mineral resorption, there has been much interest in the preparation of structural analogs of the metabolites in order to obtain more active compounds or to separate the original activities.[4]

Fluoride substitution has significant effects on the biological activities of many compounds.[5] Mechanisms by which fluoride substitution is postulated to alter biological activity include electronic inductive effects, steric effects, and obstructive halogenation. Recently a number of fluoro derivatives of vitamin D_3, including 25-fluoro,[6-8] 24-hydroxy-25-fluoro,[9-11] 1α-hydroxy-25-fluoro[12,13] and 1α,24-dihydroxy-25-fluorovitamin D_3,[10,11] have been synthesized and tested for biological activity.

Another series of fluorine-substituted compounds, 24,24-difluoro-25-hydoxy-(24-F_2-25-OH-D_3),[14] 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (24-F_2-1,25$(OH)_2D_3$)[15] and 26,26,26,-27,27,27-hexafluoro-25-hydroxyvitamin D_3 (26,27-F_6-25-OH-D_3),[16] has been synthesized in our laboratory based on the idea that 24- or 26-hydroxylation of 25-hydroxyvitamin D_3 can be postulated as the inactivation step in vitamin D_3 metabolism. These analogs blocked at the 24- or 26-position have very interesting biological activities. 24-F_2-25-OH-D_3 has almost identical activity with 25-OH-D_3 *in vivo*.[17] 24-F_2-1,25-$(OH)_2D_3$ was found to be approximately four times more potent than 1,25-$(OH)_2D_3$ in an *in vitro* test of calcium binding protein induction.[18,19] Furthermore, 26,27-F_6-25-OH-D_3 was found to be 40 times more potent than 25-OH-D_3 in terms of bone resorbing activity on fetal rat forelimb bones *in vitro*.[20] Thus, further potent activity can be expected for 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3 (26,27-F_6-1,25-$(OH)_2D_3$), the synthesis of which is described in this paper.

The commercially available cholenic acid was converted to the methyl ester (**1**), which was oxidized with dichlorodicyanobenzoquinone (DDQ) to give the trienone (**2**) in 55% yield. Treatment of the trienone (**2**) with alkaline hydrogen peroxide afforded the 1α,2α-epoxide (**3**), which was reduced with lithium metal and ammonium chloride in liquid ammonia–tetrahydrofuran to yield the 1α,3β,24-triol (**4a**) in 65% yield. Tritylation of the 24-alcohol group followed by acetylation and hydrolysis of the trityl group gave the 1,3-diacetate (**4b**)

(72%) together with 21% of the 1-acetoxy-3,24-diol. When the tosylate of **4b** was treated with lithium bromide, the 24-bromide (**4c**) was obtained in 38% overall yield from **4a**. The direct bromination of the primary alcohol[21] in **4a** with triphenylphosphine and *N*-bromosuccinimide provided a 30% yield of the bromide (**4c**) after acetylation. Treatment of the bromide (**4c**) with sodium benzenesulfinate (3.5 eq) in dimethylformamide at 70—80°C afforded



1 **2** **3**

4a : $R_1=H$, $R_2=OH$
4b : $R_1=Ac$, $R_2=OH$
4c : $R_1=Ac$, $R_2=Br$
4d : $R_1=Ac$, $R_2=SO_2Ph$
4e : $R_1=SiEt_3$, $R_2=SO_2Ph$

5

6a : R=H
6b : R=Ac

7a : R=THP
7b : R=H

8 **9**

10 **11**

the sulfone (**4d**) in high yield. After conversion of the acetyl protecting groups to the bis-triethylsilyl ether (**4e**), lithiation of the sulfone with 1.2 eq of lithium diisopropylamide in tetrahydrofuran followed by introduction of an excess of hexafluoroacetone gas at −78°C gave the adduct (**5**) in 74% yield as a stereoisomeric mixture. Acid hydrolysis and desulfonylation of **5** with sodium amalgam in the presence of Na_2HPO_4 gave the hexafluoride (**6a**) in 72% yield.

Since we found that the hexafluoro-25-hydroxy moiety remained unaffected under the Birch reduction conditions, we carried out an alternative procedure for the synthesis of the hexafluoro-1,3,25-triol (**6a**). It consisted of construction of the side chain followed by introduction of a hydoxyl group at the 1α-position. The 26,27-hexafluoro-3,25-diol 3-tetrahydropyranyl ether (**7a**) prepared by the method of our previous paper[16] was hydrolyzed with *p*-toluenesulfonic acid to give the 3-ol (**7b**). Oxidation of **7b** with DDQ gave the 1,4,6-trien-3-one (**8**) in 55% yield. Treatment of the trienone (**8**) with alkaline hydrogen peroxide gave the 1α,2α-epoxide (**9**), which was reduced with lithium metal and ammonium chloride in liquid ammonia to yield the 1α-hydroxy compound (**6a**) in 63% yield.

After acetylation, the triacetate (**6b**) was treated with *N*-bromosuccinimide and then with *s*-collidine to afford the 5,7-diene (**10**). The 5,7-diene (**10**) was irradiated with a medium-pressure mercury lamp in benzene–ethanol to give the 26,27-F_6-1,25-$(OH)_2D_3$ triacetate, which was then hydrolyzed to the corresponding 26,27-F_6-1,25-$(OH)_2D_3$ (**11**). The yields of two procedures for the synthesis of compound (**6a**) were about the same. The biological activity of 26,27-F_6-1,25-$(OH)_2D_3$ will be reported elsewhere.

Experimental

Melting points were determined with a hot-stage microscope and are uncorrected. ^{1}H-Nuclear magnetic resonance (NMR) spectra were run on a JEOL PS-100 spectrometer for solutions in $CDCl_3$ unless otherwise stated, with Me_4Si as an internal reference. ^{19}F-NMR spectra were recorded on a Varian EM-360L in $CDCl_3$ solution, with benzotrifloride as an internal reference (a plus sign indicates high field.). Mass spectra (MS) were determined with a Shimadzu-LKB-9000 or a Hitachi RMU-7L instrument at 70 eV. Infrared (IR) spectra were taken with a Model 260-10 Hitachi infrared spectrophotometer. Ultraviolet (UV) spectra were obtained in ethanol solution with a Shimadzu UV-200 double beam spectrophotometer. Column chromatography was effected using silica gel (Merck, 70—230 mesh). Thin layer chromatography was carried out on precoated plates of silica gel (Merck, silica gel 60 F_{254}). THF refers to tetrahydrofuran, THP to tetrahydropyran, and TsOH to *p*-toluenesulfonic acid.

25,26,27-Trisnorcholesta-1,4,6-trien-3-on-24-oic Acid Methyl Ester (**2**)——A solution of the ester (**1**) (110 g, 0.28 mol) and dichlorodicyanobenzoquinone (212 g, 3.3 eq) in dioxane (850 ml) was refluxed for 14 h under nitrogen, then cooled. The resulting precipitate was filtered off and washed with several portions of CH_2Cl_2. The filtrate was evaporated to dryness and the residue was chromatographed on alumina (2 kg). Elution with benzene–ethyl acetate (50: 1) gave the trienone (**2**) (60 g, 55%), mp 134—136°C (from methanol). Optical rotatory dispersion (ORD) ($c=1.00$, chloroform) $[\alpha]_D^{30}$: −42.5°. UV λ_{max} nm (ε): 299 (13000), 258 (9200), 223 (12000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1655. ^{1}H-NMR δ: 0.80 (3H, s, 18-H_3), 0.93 (3H, d, $J=6$ Hz, 21-H_3), 1.20 (3H, s, 19-H_3), 3.68 (3H, s, –$CO_2\underline{Me}$), 5.90—6.30 (4H, m, 2-, 4-, 6- and 7-H), 7.05 (1H, d, $J=$ 10 Hz. 1-H). High-resolution MS Calcd for $C_{25}H_{34}O_3$: 382.2505. Found: 382.2494.

25,26,27-Trisnor-1*α*,2*α*-epoxycholesta-4,6-dien-3-on-24-oic Acid Methyl Ester (**3**)——To a solution of the trienone (**2**) (30 g, 0.0785 mol) in methanol (550 ml) was added a mixture of 5% NaOH–MeOH (15 ml) and 30% H_2O_2 (42 ml). The reaction mixture was allowed to stand at room temperature for 18 h, then extracted with ethyl acetate. The extract was washed with brine, dried over Na_2SO_4 and concentrated. Chromatography on silica gel (400 g), eluting with benzene–ethyl acetate (100: 1), gave the epoxide (**3**) (24.8 g, 80%), mp 166—168°C (from methanol). ORD ($c=1.00$, chloroform) $[\alpha]_D^{27}$: +187.3°. UV λ_{max} nm (ε): 290 (22000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1660. ^{1}H-NMR δ: 0.78 (3H, s, 18-H_3), 0.95 (3H, d, $J=6$ Hz, 21-H_3), 1.18 (1H, s, 19-H_3), 3.42 (1H, dd, $J=4$ and 1.5 Hz, 1β-H), 3.58 (1H, d, $J=4$ Hz, 2β-H), 3.67 (3H, s, –$CO_2\underline{Me}$), 5.61 (1H, d, $J=$ 1.5 Hz, 4-H), 6.04 (2H, br s, 6- and 7-H). *Anal.* Calcd for $C_{25}H_{34}O_4$: C, 75.34; H, 8.60. Found: C, 75.27; H, 8.61.

25,26,27-Trisnorcholest-5-ene-1*α*,3*β*,24-triol (**4a**)——A four-necked flask was fitted with a sealed mechanical stirrer, a dropping funnel, a cold-finger filled with dry ice and an inlet connected to an anhydrous ammonia source. Nitrogen was swept through the system and then anhydrous ammonia (1000 ml) was trapped in the flask with a cooling bath (dry ice-methanol). Lithium wire (30.5 g) was cut into short pieces and added during 30 min. The mixture was stirred for 1 h, then the epoxide (3) (21.7 g, 0.054 mol) in dry THF (1000 ml) was added dropwise during 1.5 h. Next, anhydrous NH_4Cl (350 g) was added portionwise during 2 h. The mixture turned white and pasty. The cooling bath was removed and most of the ammonia was removed in a stream of nitrogen. Water was carefully added and the mixture was extracted with ethyl acetate. The extract was washed with dil. HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to dryness. Chromatography on silica gel (350 g), eluting with benzene–acetone (3: 1), gave the triol **4a** (13.9 g, 65%), mp 210—211°C (from methanol–acetone). ORD ($c=1.00$, methanol) $[\alpha]_D^{26}$: $-17.4°$. IR ν_{max}^{KBr} cm^{-1}: 3400. ^{1}H-NMR (py-d_5+$CDCl_3$, 1: 1) δ: 0.67 (3H, s, 18-H_3), 1.02 (3H, s, 21-H_3), 3.69 (2H, m, 24-H_2), 3.95 (1H, m, 1β-H), 4.40 (1H, m, 3α-H), 5.50 (1H, m, 6-H). High-resolution MS Calcd for $C_{24}H_{30}O_3$: 376.2974. Found: 376.2956.

25,26,27-Trisnorcholest-5-ene-1α,3β,24-triol 1,3-Diacetate (**4b**)——A solution of the triol (**4a**) (1.0 g) and trityl chloride (2.2 g) in pyridine (10 ml) was stirred overnight at room temperature. To this reaction mixture, acetic anhydride (2 ml) and a catalytic amount of 4-dimethylaminopyridine were added and the mixture was stirred at 40°C for 4 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic phase was washed with 2 N HCl, sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. The residue obtained by evaporation of the solvent was treated with a catalytic amount of TsOH in aqueous dioxane (50 ml) at 90—100°C for 3 h. The mixture was extracted with ethyl acetate and the extract was washed with 2 N HCl, sat. $NaHCO_3$ and brine, then dried over $MgSO_4$. After removal of the solvent, the crude product was chromatographed on silica gel (50 g). The fraction eluted with benzene–ethyl acetate (10: 1) gave the desired diacetate (**4b**) (880 mg, 72%) as an amorphous powder, ORD ($c=1.05$, chloroform) $[\alpha]_D^{26}$: $-16.4°$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1730, 1250. ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.92 (3H, d, $J=6$ Hz, 21-H_3), 1.10 (3H, s, 19-H_3), 2.02, 2.06 (6H, each s, acetyl), 3.59 (2H, t, $J=6$ Hz, 24-H_2), 4.90 (1H, m, 3α-H), 5.04 (1H, m, 1β-H), 5.51 (1H, m, 6H), High-resolution MS Calcd for $C_{24}H_{36}O$ ($M^+-2AcOH$): 340.2765. Found: 340.2765. The fraction eluted with benzene–ethyl acetate (1: 1) gave the 1-acetoxy-3,24-diol (230 mg, 21%) as an amorphous powder.

25,26,27-Trisnorcholest-24-bromo-5-ene-1α,3β-diol Diacetate (**4c**)——To a solution of the diacetate (**4b**) (177 mg) in pyridine (3 ml), tosyl chloride (96.8 mg) was added at 0°C and the mixture was stirred overnight at 0°C. Several pieces of ice were added and the whole was stirred for 1 h, then extracted with ethyl acetate. The organic phase was washed with 2 N HCl, sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the solvent gave the tosylate as a colorless oil (149 mg). To a solution of this tosylate (149 mg) in dimethylformamide (5 ml) was added LiBr (42.2 mg) and the mixture was refluxed under an argon atmosphere for 2 h. The reaction mixture was cooled, then water and ethyl acetate were added. The organic phase was washed consecutively with 2 N HCl, sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. The residue obtained by evaporation of the solvent was purified by column chromatography on silica gel (4 g). Elution with benzene gave the bromide (**4c**) (107 mg, 53%), mp 127—129°C (from hexane). ORD ($c=1.00$, chloroform) $[\alpha]_D^{26}$: $-16.9°$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1250. ^{1}H-NMR δ: 0.69 (3H, s, 18-H_3), 0.93 (3H, d, $J=6$ Hz, 21-H_3), 1.10 (3H, s, 19-H_3), 2.03, 2.06 (6H, each s, acetyl), 3.49 (2H, m, 24-H_2), 4.90 (1H, m, 3β-H), 5.03 (1H, m, 1-H), 5.50 (1H, m, 6-H). *Anal.* Calcd for $C_{28}H_{43}BrO_4$: C, 64.23; H, 8.28; Br, 15.26. Found: C, 64.50; H, 8.20; Br, 15.08.

25,26,27-Trisnorcholest-24-bromo-5-ene-1α,3β-diol Diacetate (4c) from 4a——Triphenylphosphine (56mg) was added in small portions to a cooled (ice bath) and stirred mixture of the triol (**4a**) (40 mg), *N*-bromosuccinimide (38 mg) and dimethylformamide (3 ml). The solution was stirred at 0°C for 15 min and then at 50°C for 15 min. Excess reagent was destroyed by addition of methanol and the solvent was removed under reduced pressure. Ether was added and the organic layer was washed with brine. Evaporation of the solvent yielded the crude bromide, which was acetylated with pyridine (2 ml), acetic anhydride (0.1 ml) and a catalytic amount of 4-dimethylaminopyridine at room temperature overnight. The crude product was chromatographed on silica gel to give 16.5 mg of pure diacetate (**4c**) (30% yield).

25,26,27-Trisnor-1α,3β-diacetoxycholest-5-en-24-yl Phenyl Sulfone (**4d**)——To a solution of the bromide (**4c**) (164 mg) in dimethylformamide (8 ml) was added $PhSO_2Na$ (260 mg) and the suspension was stirred at 70—80°C for 4 h. The reaction mixture was cooled and diluted with ether. The ethereal solution was washed with 2 N HCl, sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. The residue obtained by evaporation of the solvent was chromatographed on silica gel (20 g). Elution with benzene gave the sulfone (**4d**) (169 mg, 92%) as an amorphous powder, ORD ($c=0.66$, chloroform) $[\alpha]_D^{28}$: $+16.9°$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1310, 1250, 1150. ^{1}H-NMR δ: 0.64 (3H, s, 18-H_3), 0.86 (3H, d, $J=6$ Hz, 21-H_3), 1.08 (3H, s, 19-H_3), 2.02, 2.05 (6H, each s, acetyl), 3.04 (2H, t, $J=7$ Hz, 24-H_2), 4.91 (1H, m, 3α-H), 5.04 (1H, m, 1β-H), 5.50 (1H, m, 6-H), 7.50—8.10 (5H, m, aromatic). MS *m/z*: 464 ($M^+-2AcOH$), 345, 251, 211. High-resolution MS Calcd for $C_{30}H_{40}O_2S$ ($M^+-2AcOH$): 464.2746. Found: 464.2742.

25,26,27-Trisnor-1α,3β-bistriethylsiloxycholest-5-en-24-yl Phenyl Sulfone (**4e**)——A solution of the diacetate (**4d**) (85.6 mg) in 5% KOH–methanol (6 ml) and THF (4 ml) was stirred at room temperature for 18 h. After dilution with ethyl acetate, the whole was washed with 2 N HCl, sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. The residue obtained by evaporation of the solvent was purified by column chromatography on silica gel (16 g). Elution with hexane–ethyl acetate (1: 1) gave the desired diol (62.4 mg, 85%), mp 110—111°C (from methylene chloride–hexane). The diol (75 mg, 150 μmol) was treated with triethylsilyl chloride

(0.3 ml) and triethylamine (0.5 ml) in pyridine (3 ml) at room temperature for 15 h. The reaction mixture was poured into ice-water and extracted with ether. The organic layer was successively washed with 0.5 N HCl and brine, then dried over $MgSO_4$. The extract was subjected to column chromatography on silica gel. Elution with benzene gave the desired sulfone (**4e**) (61.8 mg, 57%) as an oil. ^{1}H-NMR δ: 0.44—0.70 (15H, m, 18-H_3 and $SiC\underline{H}_2CH_3$), 3.07 (2H, t, $J=7$ Hz, 24-H_2), 3.83 (1H, m, 1β-H), 4.00 (1H, m, 3α-H), 5.50 (1H, m, 6-H), 7.50—8.10 (5H, m, aromatic). MS *m/z*: 728, 596 ($M^+ - HOSiEt_3$), 567, 464, 301.

1α,3β,25-Trihydroxy-26,26,26,27,27,27-hexafluorocholest-5-en-24-yl Phenyl Sulfone 1,3-Bis(triethylsilyl) Ether (5)——To a solution of diisopropylamine (28 µl, 200 µmol) in THF (2 ml) was added *n*-BuLi (190 µmol) at −78°C under an argon atmosphere and the resulting solution was stirred for 5 min. To this LDA solution was added the sulfone (**4e**) (58 mg, 80 µmol) in THF (3 ml) and the reaction mixture was stirred for 20 min at 0°C. This solution was recooled to −78°C and treated with an excess of hexafluoroacetone gas for 3 min at the same temperature. The reaction mixture was quenched by addition of NH_4Cl solution and extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$ and then purified by silica gel column chromatography. The fraction eluted with benzene afforded the adduct (**5**) (53 mg, 74%) as a stereoisomeric mixture. ^{1}H-NMR δ: 3.53 (1H, m, 24-H), 3.81 (1H, m, 1β-H), 3.98 (1H, m, 3α-H), 5.47 (1H, m, 6-H), 6.80 (1H, br, 25-OH), 7.67—7.87 and 8.00—8.13 (5H, m, aromatic). ^{19}F-NMR ($CDCl_3$) δ: +7.8, +10.8.

1α,25-Dihydroxy-26,26,26,27,27,27-hexafluorocholesterol (6a)——A solution of the adduct (**5**) (45 mg) in a mixture of dimethoxyethane (1 ml), methanol (1 ml) and 1 N HCl (1 ml) was stirred for 1 h at room temperature. The reaction mixture was diluted with brine and extracted with ethyl acetate. After the organic extract had been concentrated *in vacuo*, the residue was chromatographed on silica gel (CH_2Cl_2–ethyl acetate 2: 3) to give the triol of **5** (34 mg, 99%). ^{1}H-NMR δ: 0.60 (3H, s, 18-H_3), 0.77 (3H, d, $J=6$ Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 1.70 (2H, each m, 1α-OH and 3β-OH), 3.50 (1H, m, 24-H), 3.83 (1H, m, 1β-H), 4.00 (1H, m, 3α-H), 5.60 (1H, m, 6-H), 6.77 (1H, br s, 25-OH), 7.43—7.87 and 8.00—8.10 (5H, m, aromatic). ^{19}F-NMR ($CDCl_3$) δ: +7.7, +10.8. To a mixture of the triol (31 mg) and Na_2HPO_4 (50 mg) in THF (2 ml) and methanol (2 ml) was added 5% Na(Hg) (800 mg), and the whole was stirred for 45 min at room temperature. Then a further 300 mg of 5% Na(Hg) was added and the reaction mixture was stirred for 1.5 h at room temperature. The reaction mixture was diluted with brine and extracted with ethyl acetate. The extract was chromatographed on silica gel (CH_2Cl_2–ethyl acetate 1: 2) to give the desired hexafluoride (**6a**) (17.6 mg, 72%), mp 201—202°C (from chloroform).

26,26,26,27,27,27-Hexafluoro-25-hydroxycholesterol (7b)——After treatment of the THP ether (**7a**) (354 mg) with TsOH (10 mg) in a mixture of CH_2Cl_2 (15 ml) and methanol (8 ml) at room temperature for 2 h, $NaHCO_3$ solution was added to the reaction mixture, which was then extracted with CH_2Cl_2. The extract was recrystallized from benzene–cyclohexane to give the alcohol (**7b**) (212 mg), mp 180—181°C. ^{1}H-NMR (acetone-d_6–D_2O) δ: 0.71 (3H, s, 18-H_3), 0.95 (3H, d, $J=6$ Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 3.40 (1H, m, 3-H), 5.32 (1H, m, 6-H). MS *m/z*: 510 (M^+), 495, 492, 477, 255, 213. *Anal.* Calcd for $C_{27}H_{40}F_6O_2$: C, 63.51; H, 7.90; F, 22.33. Found: C, 63.72; H, 7.84; F, 22.54.

26,26,26,27,27,27-Hexafluoro-25-hydroxycholest-1,4,6-trien-3-one (8)——A mixture of the diol (**7b**) (893 mg) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.2 g) in dioxane (50 ml) was stirred for 15 h at 80—90°C and then refluxed for 4 h. After cooling to room temperature, the precipitate was filtered off and the filtrate was diluted with ether. The ether extract was washed successively with 1 N KOH and brine, then purified by silica gel column chromatography (ethyl acetate–*n*-hexane, 1: 2) to give the trienone (**8**) (490 mg, 55%), mp 166—168°C (from ethyl acetate–cyclohexane). IR ν_{max}^{KBr} cm^{-1}: 3180, 1650, 1595. ^{1}H-NMR δ: 0.72 (3H, s, 18-H_3), 0.95 (3H, d, $J=6$ Hz, 21-H_3), 1.18 (3H, s, 19-H_3), 5.88—6.33 (4H, m, 2-, 4-, 6- and 7-H), 7.04 (1H, d, $J=10$ Hz, 1H). MS *m/z*: 504 (M^+), 489. *Anal.* Calcd for $C_{27}H_{34}F_6O_2$: C, 64.27; H, 6.79; F, 22.59. Found: C, 64.17; H, 6.81; F, 22.34.

26,26,26,27,27,27-Hexafluoro-25-hydroxy-1α,2α-epoxycholest-4,6-dien-3-one (9)——To a solution of NaOH (27 mg) and 30% H_2O_2 (1 ml) in degassed methanol (20 ml) was added a THF solution (10 ml) of the trienone (**8**) (497 mg). The reaction mixture was stirred for 20 h at room temperature, then diluted with brine and extracted with ether. The ethereal extract was subjected to silica gel column chromatography (ethyl acetate–*n*-hexane, 1: 4) to give the epoxide (**9**) (499 mg, 97%), mp 181—184°C (from ethyl acetate–cyclohexane). ^{1}H-NMR δ: 0.70 (3H, s, 18-H_3), 0.95 (3H, d, $J=6$ Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 3.45 (1H, m, 2-H), 3.62 (1H, d, $J=6$ Hz, 1-H), 4.10 (1H, s, -OH), 5.62 (1H, br s, 4-H), 6.04 (2H, s, 6-H and 7-H). MS *m/z*: 520 (M^+), 505, 503. *Anal.* Calcd for $C_{27}H_{34}F_6O_3$: C, 62.29; H, 6.58; F, 21.90. Found: C, 62.21; H, 6.57; F, 22.07.

1α,25-Dihydroxy-26,26,26,27,27,27-hexafluorocholesterol (6a)——To a solution of lithium (1.2 g) in liquid ammonia (80 ml) (distilled over Na) was added the epoxide (**9**) (443 mg) in THF (70 ml) dropwise during 1 h under dry ice-acetone bath cooling, then the reaction mixture was stirred for 1 h at reflux. The reaction mixture was recooled in the dry ice–acetone bath, then solid NH_4Cl (12 g) was added in small portions during 1 h. The whole was refluxed for 3 h. After bubbling argon gas through the reaction mixture to remove NH_3, water was added and the mixture was extracted with ethyl acetate. The extract was subjected to silica gel column chromatography. The fraction eluted with *n*-hexane–ethyl acetate (1: 2) afforded the triol (**6a**) (274 mg, 65%), mp 201—202°C (from chloroform). ^{1}H-NMR ($CDCl_3$–acetone-d_6) δ: 0.69 (3H, s, 18-H_3), 0.93 (3H, d, $J=6$ Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 3.83 (1H, m, 1-H), 4.00 (1H, m, 3-H), 5.53 (1H, m, 6-H). High-resolution MS Calcd for $C_{27}H_{40}F_6O_3$: 526.2879. Found: 526.2878.

1α,25-Dihydroxy-26,26,26,27,27,27-hexafluorocholesterol Triacetate (6b)——A solution of the triol (**6a**) (216 mg) and a catalytic amount (*ca.* 20 mg) of 4-dimethylaminopyridine in acetic anhydride (1.5 ml) and pyridine (3 ml) was stirred for 20 h at room temperature. After the reaction mixture had been concentrated *in vacuo*, the residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 10: 1) to give the triacetate (**6b**) (263 mg, 98%), which was dried at 70°C (5 mmHg) for 20 h to yield a glass. ^{1}H-NMR δ: 0.66 (3H, s, 18-H_3), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.10 (3H, s, 19-H_3), 2.03, 2.06, 2.16 (9H, each s, acetyl), 4.98 (1H, m, 3-H), 5.06 (1H, m, 1-H), 5.53 (1H, m, 6-H). MS *m/z*: 592 (M^+-AcOH), 532 ($M^+-2AcOH$), 517, 413, 253.

26,26,26,27,27,27-Hexafluoro-1α,3β,25-triacetoxycholest-5,7-diene (10)——*N*-Bromosuccinimide (14 mg) was added to a refluxing solution of 35 mg of the triacetate (**6b**) in 2 ml of CCl_4 and the reaction mixture was refluxed for a further 20 min under an argon atmosphere. After cooling in an ice-water bath, the resulting precipitate was filtered off. The filtrate was evaporated to dryness below 40°C. The residue in xylene (1 ml) was added dropwise to a refluxing solution of xylene (1.5 ml) and *s*-collidine (0.5 ml) and refluxing was continued under an argon atmosphere for 20 min. The reaction mixture was extracted with ethyl acetate. The extract was washed with 2 N HCl, sat. $NaHCO_3$ and brine, then dried over $MgSO_4$. After removal of the solvent, the residue was treated with a catalytic amount of TsOH in acetone (10 ml) at room temperature for 16 h under argon in the dark. The mixture was extracted with ethyl acetate and the extract was washed with sat. $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent gave a crude 5,7-diene, which was purified by preparative TLC developed twice with *n*-hexane–ethyl acetate (10: 1). The band of *Rf* 0.26 was scraped off and eluted with ethyl acetate. Removal of the solvent gave the 5,7-diene (**10**) (8.8 mg, 25%), UV λ_{max} nm: 294, 282, 272.

1α,25-Dihydroxy-26,26,26,27,27,27-hexafluorovitamin D_3 (11)——A solution of the 5,7-diene (**10**) (8.8 mg) in benzene (90 ml) and ethanol (40 ml) was irradiated with a medium-pressure mercury lamp through a Vycor filter for 2.5 min with ice-cooling under argon. Then, the mixture was refluxed for 1 h under argon. Evaporation of the solvent gave a crude vitamin D derivative, which was subjected to preparative TLC (developed twice, hexane–ethyl acetate 10: 1). The band of *Rf* 0.36 was scraped off and eluted with ethyl acetate. Removal of the solvent gave the desired triacetate (1.6 mg), UV λ_{max} nm: 264.5, λ_{min} nm: 228.

A solution of the triacetate (1.6 mg) in 5% KOH–methanol (2 ml) and THF (2 ml) was stirred at room temperature under argon in the dark for 14 h. The reaction mixture was acidified with 2 N HCl and extracted twice with ethyl acetate. The extract was washed with sat. $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent gave crude **11** (1.13 mg, 90%), which was further purified by HPLC on a Zorbax Sil column (15 cm × 4.6 mm i.d.) at a flow rate of 3 ml with 1% methanol in CH_2Cl_2 as the solvent. The retention time of **11** was 6 min; UV λ_{max} nm: 264.5, λ_{min} nm: 228. MS *m/z*: 524 (M^+), 506, 488, 473, 462, 383, 287, 269, 251, 152, 134.

Acknowledgement This work was supported by a U.S.-Japan Co-operative research grant from the Japan Society for the Promotion of Science.

References

1) Part XXXVIII: Y. Kobayashi, T. Taguchi, T. Morikawa, T. Takase, and H. Takanashi, *J. Org. Chem.*, **47**, 3232 (1982).
2) Part LXXVIII: Y. Kobayashi, T. Taguchi, T. Terada, J. Oshida, M. Morisaki, and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 85.
3) H.F. DeLuca and H.K. Schnoes, *Annu. Rev. Biochem.*, **45**, 631 (1976).
4) N. Ikekawa and T. Takeshita, *J. Org. Syn. Chem. Japan*, **37**, 755, 809 (1979).
5) M.B. Chenoweth and L.P. McCarty, *Pharmacol. Rev.*, **15**, 673 (1963).
6) B.L. Onisko, H.K. Schnoes, and H.F. DeLuca, *Tetrahedron Lett.*, **1977**, 1107.
7) S.S. Yong, C.P. Dorn, and H. Jones, *Tetrahedron Lett.*, **1977**, 2315.
8) B.L. Onisko, H.K. Schnoes, H.F. DeLuca, and R.S. Glover, *Biochem. J.*, **182**, 1 (1979).
9) J.L. Napoli, W.S. Mellon, M.A. Fivizzani, H.K. Schnoes, and H.F. DeLuca, *J. Biol. Chem.*, **254**, 2017 (1979).
10) J.L. Napoli, W.S. Mellon, H.K. Schnoes, and H.F. DeLuca, *Arch. Biochem. Biophys.*, **197**, 193 (1979).
11) J.J. Partridge, S.J. Shiuey, A. Boris, J.P. Mallon, and M.R. Uskokovic, "Vitamin D, Basic Research and Its Clinical Application," ed. by A. Norman, Walter de Gruyter, Berlin, 1979, p.37.
12) J.L. Napoli, M.A. Fivizzani, A.H. Hamstra, H.K. Schnoes, H.F. DeLuca, and P.H. Stern, *Steroids*, **32**, 453 (1978).
13) J.L. Napoli, W.S. Mellon, M.A. Fivizzani, H.K. Schnoes, and H.F. DeLuca, *Biochemistry*, **17**, 2389 (1978).
14) Y. Kobayashi, T. Taguchi, T. Terada, J. Oshida, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, **1979**, 2023.
15) Y. Tanaka, H.F. DeLuca, H.K. Schnoes, N. Ikekawa, and Y. Kobayashi, *Arch. Biochem. Biophys.*, **199**,

473 (1980).

16) Y. Kobayashi, T. Taguchi, N. Kanuma, N. Ikekawa, and J. Oshida, *J. Chem. Soc., Chem. Commun.*, **1980**, 459.

17) Y. Tanaka, H.F. DeLuca, Y. Kobayashi, T. Taguchi, N. Ikekawa, and M. Morisaki, *J. Biol. Chem.*, **254**, 7163 (1979).

18) R.A. Corradino, H.F. DeLuca, Y. Tanaka, N. Ikekawa, and Y. Kobayashi, *Biochem. Biophys. Res. Commun.*, **96**, 1800 (1980).

19) R.A. Corradino, N. Ikekawa, and H.F. DeLuca, *Arch. Biochem. Biophys.*, **208**, 273 (1981).

20) P.H. Stern, Y. Tanaka, H.F. DeLuca, N. Ikekawa, and Y. Kobayashi, *Mol. Pharmacol.*, **20**, 460 (1981).

21) T. Arunachalam, P.J. Mackoul, N.M. Green, and E. Caspi, *J. Org. Chem.*, **46**, 2966 (1981).