An Alternative and Efficient Synthesis of 24,24-Difluoro-1α,25-dihydroxyvitamin D_3

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An alternative synthesis of the title compound 2, a highly potent analog of 1α,25-dihydroxyvitamin D_3 (1), is described. Starting with 1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]androst-5-ene (8), 2 was obtained in 3.8% total yield through 10 steps. This method provides compound 2 in much higher yield than that reported previously.

Keywords 1α,25-dihydroxyvitamin D_3; vitamin D_3 biologically active form; 24,24-difluoro-1α,25-dihydroxyvitamin D_3; vitamin D_3 analog; synthesis

1α,25-Dihydroxyvitamin D_3 (**1**), the biologically active form of vitamin D_3, functions as a hormone regulating calcium and phosphorous homeostasis.[1] The recent discovery that **1** shows a variety of physiological activities such as induction of cell differentiation and proliferation has prompted renewed interest in biological roles of vitamin D_3, synthesis of its analogs, and possible use as a therapeutic agent.[2]

During the investigations of vitamin D_3 metabolism, we synthesized 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (**2**), an analog in which 24-hydroxylation could be prevented.[3] Ikekawa *et al.* also produced compound **2** by enzymatic 25-hydroxylation of 24,24-difluoro-1α-hydroxyvitamin D_3.[4] Physiological studies demonstrated **2** to be a highly potent vitamin D_3 analog, about 4—5 times more active than the parent compound **1** in terms of intestinal calcium absorption in the chick.[5] In the rat, **2** has a potency of approximately 5—10 times that of **1** in several *in vivo* vitamin D-responsive systems, including intestinal calcium transport, bone calcium mobilization, calcification of epiphyseal plate cartilage, and elevation of plasma calcium and phosphorus concentrations.[6] Moreover, in inducing phagocytosis and C3 rosette formation of HL-60 cells, **2** is 4—7 times more potent than **1**, though both compounds bind equally well to the cytosol receptor.[7] Thus, the fluoro analog **2** is among the most potent vitamin D analogs so far known, together with 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3 (**3**),[8] and may be of therapeutic use.

The medical importance necessitates a practical synthesis of **2** to obtain sufficient material for detailed investigation of the biological properties. We describe here an alternative synthesis of **2**, which provides compound **2** in much higher yield than that reported previously.

In the previous method, the analog **2** was synthesized from lithocholic acid (**4**) through 20 steps in 0.07% overall yield, as shown in Chart 1; the side chain was constructed in the early stage, then the 1α-hydroxyl and diene moieties were introduced to give the provitamin **5**, and finally photolytic and thermal isomerization of **5** in a usual way afforded **2**.[3] The problem encountered in this method was the laborious procedures for 1α-hydroxylation, diene formation and incorporation of fluorine atoms, which resulted in low overall yield. The new synthesis started with 1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]androst-5-ene (**8**)[9] (Chart 2), which is readily available from 1α-hydroxydehydroepiandrosterone (**9**),[10] because it has the hydroxyl functionality at C-1 with proper stereochemistry. Condensation of aldehyde **6** with ethyl bromodifluoroacetate by means of the Reformatsky reaction would facilitate fluorine incorporation. The aldehyde **6** could be accessible from **8** through the alcohol **7** in conventional ways. Thus,

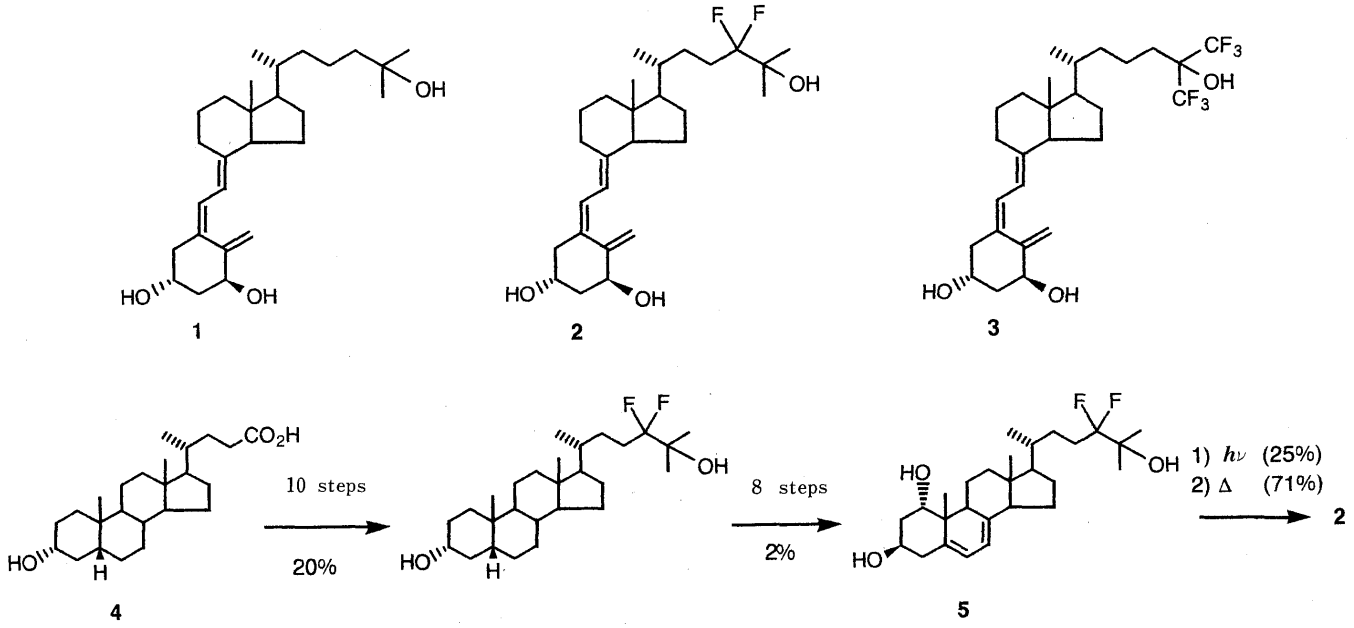
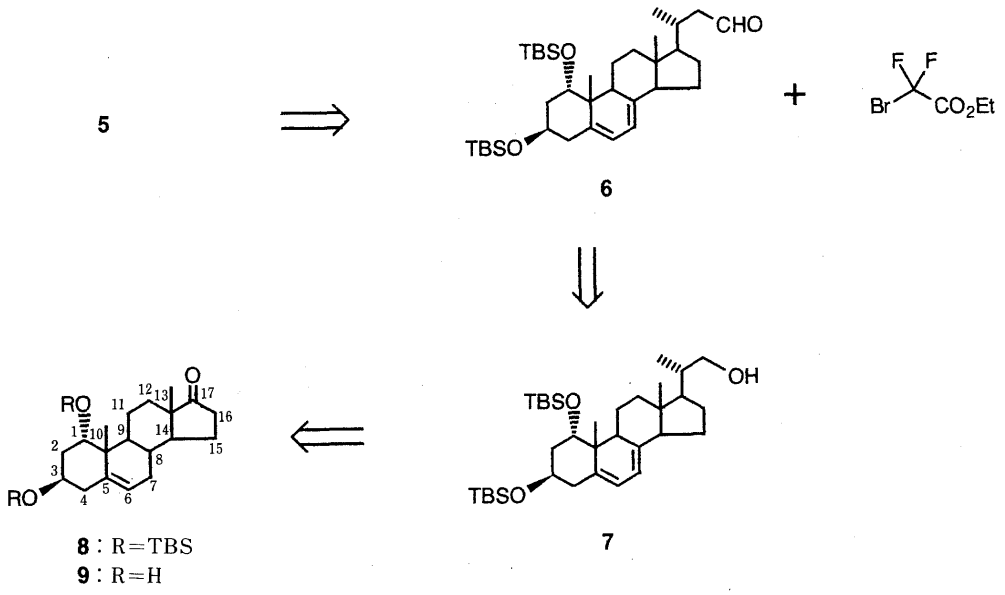


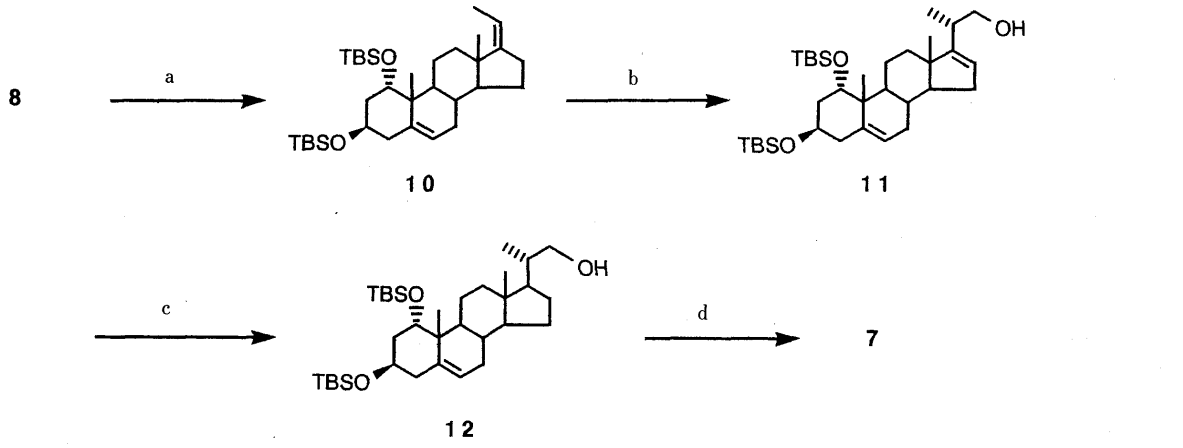
Chart 1



8 : R=TBS
9 : R=H

TBS=*tert*-butyl dimethylsilyl

Chart 2



a : $Et\overset{+}{P}Ph_3\overset{-}{Br}$-*tert*-BuOK b : $HCHO/BF_3 \cdot Et_2O$ c : H_2/Pt-C d : 1) NBS 2) *n*-Bu_4NBr 3) *n*-Bu_4NF

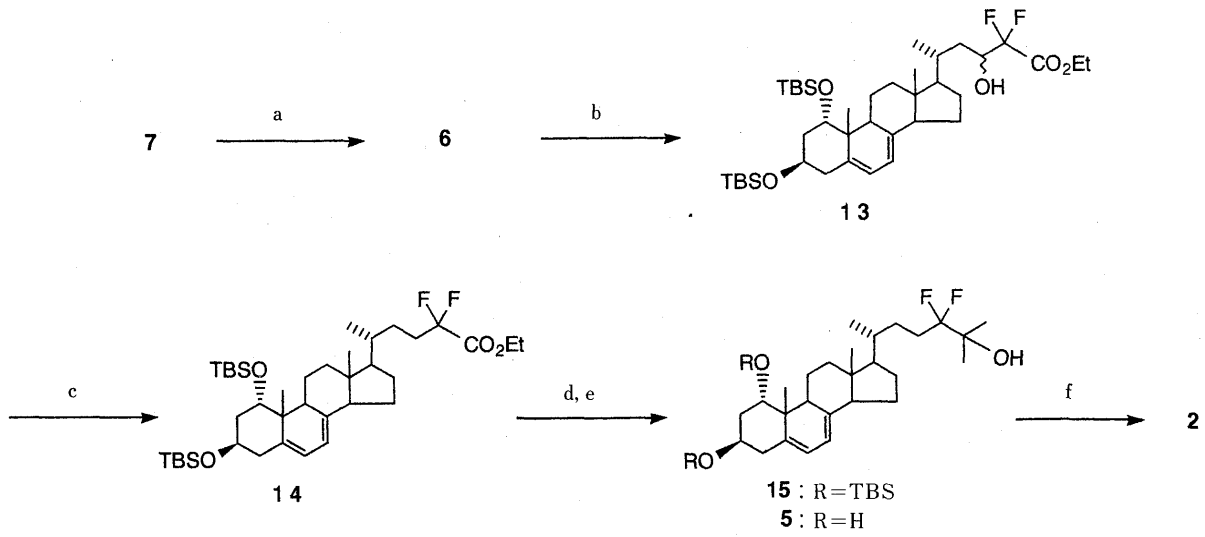
Chart 3

the entire process should be straightforward.

The side chain with natural configuration at C-17 and C-20 was introduced by the method developed by Uskokovic *et al.*[11] (Chart 3). The Wittig reaction of **8** with ethylidene triphenylphosphorane stereoselectively gave the *Z*-olefin **10** with a trace of the isomeric olefin (*E*:*Z*=1:20) in quantitative yield. Due to the difficulty in separating the stereoisomers, the mixture of the isomers was used in the next step. The ene reaction of thus-obtained olefins with paraformaldehyde in the presence of a catalytic amount of borontrifluoride etherate afforded the alcohol **11** stereospecifically in 89% yield. The epimer at C-20, derived from the *E*-olefin, was cleanly separated by column chromatography at this stage. Catalytic hydrogenation of **11** over 5% Pt–C reduced only the Δ^{16}-double bond stereoselectively to give the mono-olefin **12** in quantitative yield.

Formation of the 5,7-diene moiety was best achieved by the improved procedure reported by Rappoldt *et al.*[12]; successive treatment of **12** with *N*-bromosuccinimide (NBS), *n*-Bu_4NBr and *n*-Bu_4NF produced the diene **7** in 41% yield. The classical procedure and the new method using sulfur chemistry[13] gave only poor results. It is noteworthy that this diene moiety is stable enough to allow further elaboration, as described before.[9]

Fluorine incorporation into the side chain was performed by the procedure of Kumar *et al.*[14] with some modification (Chart 4). The C_1 elongated aldehyde **6** was obtained from **7** through the sequence of reactions of tosylation, cyanide formation and diisobutylaluminum hydride (DIBAL-H) reduction in 73% overall yield. The Reformatsky reaction of **6** with ethyl bromodifluoroacetate and activated zinc[15] gave the alcohol **13** (1:1 epimeric ratio at C-23) in 64% yield, and this was reductively deoxygenated by successive treatment with methyl chlorooxalate and *n*-Bu_3SnH[16] to afford the fluoro-ester **14** in 80% yield. This oxalate ester procedure is superior to that of Kumar using the thiocarbonyl derivative in terms of easy handling and high reactivity. The ester **14** was further treated with methylmagnesiumbromide, affording **15** in 98% yield, followed



a : 1) TsCl-DMAP 2) NaCN 3) DIBAL-H b : BrF_2CCO_2Et-Zn c : 1) $ClCOCO_2Me$-DMAP 2) n-Bu_3SnH/AIBN d : MeMgBr e : n-Bu_4NF f : 1) $h\nu$ 2) reflux/EtOH

Chart 4

by deprotection with n-Bu_4NF to produce the provitamin **5** in 80% yield. Finally, isomerization of provitamin to previtamin by photolysis and subsequent thermolysis without purification provided the vitamin D_3 analog **2** in 32% yield.

Thus, 24,24-difluoro-1α,25-dihydroxyvitamin D_3 **2** was synthesized from **8** in a total yield of 3.8% through 10 steps. The efficiency and much higher overall yield as compared with the previous method made it possible to obtain the highly potent vitamin D_3 analog **2** in large quantities, which should be advantageous for further biological investigations and possible therapeutic application. In addition, the intermediates **6**, **7** and **12** could be useful precursors for a variety of vitamin D analogs altered in the side chain. For example, we have synthesized 24-fluoro-1α,25-dihydroxyvitamin D_2 from **6**.[17] Further studies along this line are in progress.

Experimental

Melting points are uncorrected. Spectral data were recorded on the following instruments: proton nuclear magnetic resonance (^{1}H-NMR), JEOL JSX-400; mass spectra (MS), JMS-D 300; infrared (IR), JASCO FT/IR-8000; optical rotations, JASCO DIP-370. Tetramethylsilane (TMS) was used as an internal standard for ^{1}H-NMR.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]androst-5-en-17-one (8) Although this compound was reported to be prepared by a single step in high yield,[9] in our hands, the reported procedure did not give a satisfactory yield. Thus, we modified the procedure as described below. A solution of 1α-hydroxydehydroepiandrosterone[10] (5.0 g, 16.4 mmol), TBSCl (2.81 g, 18.0 mmol, 1.1 eq) and imidazole (2.46 g, 36.1 mmol, 2.2 eq) in dimethylformamide (DMF) (50 ml) was stirred at room temperature under argon for 30 min. The mixture was poured into water and extracted with ether. The combined extracts were washed twice with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (150 g, 1—4% AcOEt–PhH) to give the mono-silyl ether (6.12 g, 89%) as colorless needles: mp 214—215 °C (tetrahydrofuran (THF)–MeOH); $[\alpha]_D$ +0.5° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.01 (6H, s), 0.82 (3H, s), 0.83 (9H, s), 0.99 (3H, s), 3.76 (1H, dd, J=1.5, 3.4 Hz), 3.84 (1H, tt, J=5.5, 10.8 Hz), 5.53 (1H, d, J=5.5 Hz). MS m/z: 418 (M^+), 400 ($M-H_2O$), 361 ($M-$ *tert*-Bu), 343 ($M-$*tert*-Bu$-H_2O$). IR ($CHCl_3$): 3640, 1732 cm^{-1}. HR-MS m/z: 418.2932 (M^+). Calcd for $C_{25}H_{42}O_3Si$ 418.2901.

The obtained mono-silyl ether (6.12 g, 14.6 mmol) was dissolved in CH_2Cl_2 (100 ml), and 2,6-lutidine (9.18 ml, 78.8 mmol, 5.4 eq) and TBSOTf (10.3 ml, 43.8 mmol, 3.0 eq) were added. The mixture was stirred under argon at room temperature for 30 min, then 1 N HCl (60 ml) was added and the whole was stirred at room temperature for 15 min. The mixture was poured into water and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude crystals were recrystallized from THF–MeOH to give the di-silyl ether **8** (6.79 g, 88%) as colorless needles. The mother liquor was evaporated and the residue was purified by silica gel flash chromatography (50 g, AcOEt–hexane, 1:80—1:50) to afford **8** (0.79 g, 10%, total of 7.58 g, 98%); mp 155—156 °C (THF–MeOH); $[\alpha]_D$ +53.5° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.01 (3H, s), 0.04 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 0.89 (3H, s), 0.99 (3H, s), 3.78 (1H, dd, J=1.5, 3.4 Hz), 3.99 (1H, tt, J=5.5, 10.8 Hz), 5.48 (1H, d, J=5.8 Hz). IR ($CHCl_3$): 1732 cm^{-1}. MS m/z: 532 (M^+), 517 ($M-$Me), 475 ($M-$*tert*-Bu). HR-MS m/z: 532.3747 (M^+), Calcd for $C_{31}H_{56}O_3Si_2$ 532.3765.

(17*Z*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]pregna-5,17(20)-diene (10) A mixture of **8** (6.0 g, 11.3 mmol), (ethyl)triphenyl-phosphonium bromide (12.5 g, 33.8 mmol, 3.0 eq) and *tert*-BuOK (3.42 g, 30.4 mmol, 2.7 eq) in THF (90 ml) was allowed to reflux under argon for 4 h. After being cooled, the mixture was filtered and the filtrate was evaporated. The crude product was purified by silica gel flash chromatography (120 g, AcOEt–hexane, 1:90) to give **10** (6.12 g, quantitative) as a white solid; $[\alpha]_D$ +19.0° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.03 (3H, s), 0.04 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.88 (18H, s), 0.89 (3H, s), 0.98 (3H, s), 1.66 (3H, d, J=7.3 Hz), 3.78 (1H, dd, J=1.5, 3.6 Hz), 3.99 (1H, tt, J=5.2, 10.8 Hz), 5.14 (1H, tq, J=2.5, 7.0 Hz), 5.46 (1H, d, J=5.5 Hz). IR ($CHCl_3$): 1470, 1255, 1078 cm^{-1}. MS m/z: 544 (M^+), 529 ($M-$Me), 487 ($M-$*tert*-Bu). HR-MS m/z: 544.4122 (M^+), Calcd for $C_{33}H_{60}O_2Si_2$ 544.4129.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-23,24-bisnorchol-15,16-dien-22-ol (11) A suspension of **10** (6.12 g, 11.3 mmol) and paraformaldehyde (2.12 g, 56.3 mmol, 5.0 eq) in CH_2Cl_2 (612 ml) was treated with $BF_3 \cdot Et_2O$ (0.14 ml, 1.1 mmol, 0.1 eq) and the mixture stirred under argon at room temperature for 5 min. The reaction mixture was poured into water and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (120 g, AcOEt–hexane, 1:20) to give **11** (5.54 g, 89%) as white needles: mp 174.5—175.5 °C (MeOH); $[\alpha]_D$ +5.3° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.01 (3H, s), 0.02 (3H, s), 0.03 (3H, s), 0.05 (3H, s), 0.80 (3H, s), 0.85 (9H, s), 0.86 (9H, s), 0.98 (3H, s), 1.01 (3H, d, J=7.0 Hz), 3.43 (1H, dt, J=10.3, 5.8 Hz), 3.54 (1H, ddd, J=10.3, 5.8, 5.1 Hz), 3.75 (1H, dd, J=1.5, 3.6 Hz), 3.96 (1H, tt, J=10.3, 5.2 Hz), 5.42 (1H, dd, J=1.5, 1.2 Hz), 5.45 (1H, d, J=5.5 Hz). IR ($CHCl_3$): 3620 cm^{-1}. MS m/z: 574 (M^+), 517 ($M-$*tert*-Bu), 499 ($M-$*tert*-Bu$-H_2O$). HR-MS m/z: 574.4252 (M^+), Calcd for $C_{34}H_{62}O_3Si_2$ 574.4234.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-23,24-bisnorchol-5-en-22-ol

(12) A mixture of **11** (5.50 g, 9.6 mmol) and 5% Pt–C (2.75 g) in EtOH (550 ml) was stirred overnight under hydrogen at room temperature. The mixture was filtered through Celite and the filtrate was evaporated. The crude product was purified by silica gel flash chromatography (100 g, AcOEt–hexane, 1 : 15) to give **12** (5.22 g, 95%) as white needles: mp 179.5—180.5 °C (ether–MeOH); $[\alpha]_D$ +5.2° (c=0.46, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.02 (3H, s), 0.04 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.70 (3H, s), 0.87 (18H, s), 0.96 (3H, s), 1.05 (3H, d, J=6.5 Hz), 3.38 (1H, dt, J=10.4, 6.4 Hz), 3.63 (1H, ddd, J=2.1, 5.5, 10.4 Hz), 3.77 (1H, dd, J=1.5, 3.6 Hz), 3.99 (1H, tt, J=5.3, 11.2 Hz), 5.45 (1H, d, J=5.8 Hz). IR ($CHCl_3$): 3630 cm^{-1}. MS m/z: 576 (M^+), 561 (M−Me), 558 (M−H_2O), 543 (M−Me−H_2O), 519 (M−*tert*-Bu), 501 (M−*tert*-Bu−H_2O). HR-MS m/z: 576.4407 (M^+). Calcd for $C_{34}H_{64}O_3Si_2$ 576.4391.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-23,24-bisnorchol-5,7-dien-22-ol (7) A mixture of **12** (1.5 g, 2.6 mmol), NBS (900 mg, 5.9 mmol, 1.9 eq) and $NaHCO_3$ (1.5 g, 17.9 mmol, 6.8 eq) in *n*-hexane (150 ml) was allowed to reflux for 1 h. The mixture was filtered, then the filtrate washed successively with saturated $NaHCO_3$ and water, dried over $MgSO_4$, and evaporated. The residue was dissolved in THF (30 ml), then *n*-Bu_4NBr (84 mg, 0.26 mmol, 0.1 eq) was added to the solution and the mixture was stirred under argon at 0 °C for 15 min. Then 1.0 M *n*-Bu_4NF/THF (18.3 ml, 18.3 mmol, 7 eq) was added and the whole was stirred under argon at 0 °C for 3.5 h. The reaction mixture was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (60 g, 5% AcOEt–hexane) to give the diene **7** (614 mg, 41%) as white needles: mp 179—180.5 °C (ether–MeOH); $[\alpha]_D$ −0.3° (c=0.078, $CHCl_3$). UV $\lambda_{max}^{CHCl_3}$ nm (ε): 273 (9600), 283 (10300), 295 (6800). ^{1}H-NMR ($CDCl_3$) δ: 0.05 (6H, s), 0.06 (6H, s), 0.65 (3H, s), 0.88 (18H, s), 0.91 (3H, s), 3.40 (1H, dd, J=6.7, 10.4 Hz), 3.65 (1H, dd, J=3.4, 10.4 Hz), 3.70 (1H, dd, J=1.5, 3.6 Hz), 4.03 (1H, tt, J=6.5, 12.1 Hz), 5.32 (1H, dt, J=5.8, 2.8 Hz), 5.58 (1H, d, J=5.8 Hz). IR ($CHCl_3$): 3650 cm^{-1}. MS m/z: 574 (M^+), 556 (M−H_2O), 517 (M−*tert*-Bu). HR-MS m/z: 574.4258. Calcd for $C_{39}H_{62}O_3Si_2$ 574.4234.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24-norchol-5,7-dien-23-al (6) A solution of **7** (758 mg, 1.3 mmol), TsCl (377 mg, 2.0 mmol, 1.5 eq) and 4-dimethylaminopyridine (DMAP, 403 mg, 3.3 mmol, 2.5 eq) in CH_2Cl_2 (60 ml) was allowed to stand overnight at room temperature. The mixture was poured into water and extracted with ether. The combined extracts were successively washed with saturated $CuSO_4$, water and brine, dried over $MgSO_4$ and evaporated to give the tosylate of **7** as white solid: ^{1}H-NMR ($CDCl_3$) δ: 2.23 (3H, s), 7.37 (2H, d, J=8.4 Hz), 7.79 (2H, d, J=8.4 Hz). MS m/z: 728 (M^+).

A mixture of the tosylate and NaCN (194 mg, 4.0 mmol, 3 eq), in DMF (15 ml) was heated at 80 °C for 1.5 h. After being cooled, the mixture was poured into water and extracted with ether. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated to give the 22-cyanide as a white solid. IR ($CHCl_3$): 2215 cm^{-1}. MS m/z: 583 (M^+).

A 1.76 M DIBAL-H/hexane (1.88 ml, 3.3 mmol, 2.5 eq) solution was added to a stirred solution of the cyanide in ether (50 ml) at 0 °C, and the mixture was stirred under argon at 0 °C for 15 min. The mixture was poured into a suspension of silica gel (50 g), in wet ether (100 ml) at 0 °C and the mixture stirred at 0 °C for 15 min. The mixture was filtered through Celite and the filtrate dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (30 g, AcOEt–hexane, 1 : 80) to give the aldehyde **6** (565 mg, 73%) as white needles: mp 165—167 °C (THF–MeOH); $[\alpha]_D$ −7.3° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.05 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.10 (3H, s), 0.67 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 0.90 (3H, s), 1.04 (3H, d, J=6.4 Hz), 3.70 (1H, dd, J=1.5, 3.6 Hz), 4.03 (1H, ddt, J=3.9, 6.6, 11.8 Hz), 5.32 (1H, dt, J=5.5, 2.5 Hz), 5.58 (1H, d, J=5.5 Hz), 9.76 (1H, dd, J=1.2, 3.3 Hz). IR ($CHCl_3$): 1710 cm^{-1}. MS m/z: 586 (M^+), 529 (M−*tert*-Bu). HR-MS m/z: 586.4193 (M^+), Calcd for $C_{35}H_{62}O_3Si_2$ 586.4234.

Ethyl 24,24-Difluoro-1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]homochol-5,7-dien-25-oate (14) Ethyl bromodifluoroacetate (0.25 ml, 20 mmol, 4.0 eq) was added to a suspension of Zn dust (131 mg, 2.0 mmol, 4.0 eq) in THF (2 ml) and the mixture allowed to reflux for 2 min. The aldehyde **6** (291 mg, 0.5 mmol) in THF (2 ml) was added to the resultant cloudy solution and the mixture was allowed to reflux for 15 min. The mixture was poured into 1 M $KHSO_4$ and extracted with AcOEt. The combined extracts were successively washed with 1 M $KHSO_4$ and brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (30 g, 2% AcOEt–PhH) to give a mixture of diastereoisomers of the alcohol **13** (223 mg, 64%) as a colorless oil. ^{1}H-NMR ($CHCl_3$) δ: 1.37 (3H, t, J=7.3 Hz), 4.35, 4.36 (2H, q, J=7.3 Hz). IR ($CHCl_3$): 3593, 1765 cm^{-1}. HR-MS m/z: 710.4588 (M^+), Calcd for $C_{39}H_{68}O_5Si_2F_2$ 710.4570.

A mixture of the alcohol **13** (223 mg, 0.3 mmol), DMAP (115 mg, 0.9 mmol, 3.0 eq) and methyl chlorooxalate (0.09 ml, 0.9 mmol, 3.0 eq) in CH_2Cl_2 (8 ml) was allowed to stand at room temperature for 1 h. The mixture was poured into water and extracted with ether. The combined extracts were successively washed with saturated $CuSO_4$, water and brine, dried over $MgSO_4$ and evaporated to give the oxalyl ester.

A solution of the oxalyl ester and azobisisobutyronitrile (AIBN, 26 mg, 0.15 mmol, 0.5 eq) in toluene (10 ml) was treated with *n*-Bu_3SnH (0.04 ml, 0.45 mmol, 1.5 eq) and the mixture allowed to reflux for 1 h. After being cooled, the mixture was poured into water and extracted with ether. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (10 g, 2% AcOEt–hexane) to give the fluoro-ester **14** (174 mg, 80%) as a colorless oil; $[\alpha]_D$ −0.5° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.05 (3H, s), 0.06 (3H, s), 0.10 (3H, s), 0.61 (3H, s), 0.88 (18H, s), 0.92 (3H, s), 0.96 (3H, d, J=6.4 Hz), 1.37 (3H, t, J=7.3 Hz), 3.70 (1H, dd, J=1.5, 3.6 Hz), 4.04 (1H, tt, J=5.3, 10.4 Hz), 4.33 (2H, q, J=7.3 Hz), 5.31 (1H, dt, J=5.3, 3.1 Hz), 5.58 (1H, d, J=5.3 Hz), IR ($CHCl_3$): 1763 cm^{-1}. MS m/z: 694 (M^+). HR-MS m/z: 694.4605 (M^+), Calcd for $C_{39}H_{68}O_4Si_2F_2$ 694.4621.

24,24-Difluoro-1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]cholesta-5,7-dien-25-ol (15) A solution of the difluoro ester **14** (163 mg, 0.235 mmol) in ether (15 ml) was treated with 3.0 M MeMgBr/ether (1 ml, 3.0 mmol, 12.8 eq) at 0 °C, and the mixture stirred at 0 °C for 30 min. The reaction mixture was poured into water and extrated with ether. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (7 g, 1—2% AcOEt–hexane) to give the alcohol **15** (157 mg, 98%) as white needles: mp 175—176 °C (THF–MeOH); $[\alpha]_D$ −1.1° (c=0.88, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.05 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.10 (3H, s), 0.62 (3H, s), 0.88 (18H, s), 0.90 (3H, s), 0.96 (3H, d, J=6.4 Hz), 1.30 (6H, s), 3.70 (1H, dd, J=1.5, 3.6 Hz), 4.05 (1H, tt, J=5.3, 10.4 Hz), 5.32 (1H, dt, J=5.3, 2.6 Hz), 5.57 (1H, d, J=5.3 Hz). IR ($CHCl_3$): 3595 cm^{-1}. MS m/z: 680 (M^+), 665 (M−Me), 660 (M−HF), 642 (M−HF−H_2O), 623 (M−*tert*-Bu). HR-MS m/z: 680.4814 (M^+). Calcd for $C_{39}H_{70}O_3Si_2F_2$ 680.4828.

24,24-Difluorocholesta-5,7-dien-1α,3β,25-triol (5) A solution of the alcohol **15** (69 mg, 0.1 mmol) in THF (2.5 ml) was added 1.0 M *n*-Bu_4NF/THF (1.22 ml, 1.22 mmol, 1.2 eq) and the mixture was stirred overnight under argon in the dark at room temperature. The mixture was heated at 70 °C for 1 h in the dark. After being cooled, the mixture was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (8 g, 3% MeOH–$CHCl_3$) to give the provitamin **5** (45 mg, 98%) as white needles: mp 188—189 °C (ether–hexane); $[\alpha]_D$ −33.4° (c=0.65, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 262 (7300), 271 (10200), 282 (10800), 293 (6500). ^{1}H-NMR ($CDCl_3$) δ: 0.64 (3H, s), 0.95 (3H, s), 0.97 (3H, d, J=6.4 Hz), 1.31 (6H, s), 3.77 (1H, dd, J=1.5, 3.6 Hz), 4.09 (1H, tt, J=5.3, 10.4 Hz), 5.40 (1H, dt, J=5.2, 2.6 Hz), 5.73 (1H, dd, J=5.2, 2.0 Hz). IR ($CHCl_3$): 3605 cm^{-1}. MS m/z: 452 (M^+), 434 (M−H_2O), 432 (M−HF), 416 (M−2H_2O), 414 (M−HF−H_2O). HR-MS m/z: 452.3081 (M^+). Calcd for $C_{27}H_{42}O_3F_2$ 452.3100.

(5*E*,7*Z*)-24,24-Difluoro-9,10-*seco*-5,7,10(19)-cholestatriene-1α,3β,25-triol (24,24-Difluoro-1α,25-dihydroxyvitamin D_3) (2) A solution of provitamin **5** (10.5 mg, 0.02 mmol) in ether (100 ml) was cooled to 0 °C and deoxygenated by bubbling argon through the solution for 40 min. The solution was irradiated with a high-pressure mercury lamp and Vycol filter for 6 min at 0 °C. The solvent was evaporated below at 25 °C and the residue was dissolved in EtOH (100 ml). The solution was allowed to reflux for 1 h, then evaporated. The crude product was purified by high performance liquid chromatography (HPLC) (Lichrosorb Si-60, 25 × 250 mm, 7% iso-PrOH–CH_2Cl_2) to give the vitamin D_3 analog **2** (3.4 mg, 32%) as a white solid; $[\alpha]_D$ +10.0° (c=0.25, EtOH). UV λ_{max}^{EtOH} nm (ε): 265 (18100). ^{1}H-NMR ($CDCl_3$) δ: 0.55 (3H, s), 0.94 (3H, d, J=6.4 Hz), 1.30 (6H, s), 4.24 (1H, m), 4.42 (1H, m), 5.00 (1H, s), 5.32 (1H, s), 6.02 (1H, d, J= 11.3), 6.38 (1H, d, J=11.3 Hz). IR ($CHCl_3$): 3690 cm^{-1}. MS m/z: 452 (M^+), 434 (M−H_2O), 416 (M−2H_2O), 287, 269, 251, 152, 134. HR-MS m/z: 452.3083 (M^+). Calcd for $C_{27}H_{42}O_3F_2$ 452.3100.

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