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A New Synthesis of 24-Fluoro-1α,25-dihydroxyvitamin D_2 and Its 24-Epimer, and Determination of the C-24 Configuration

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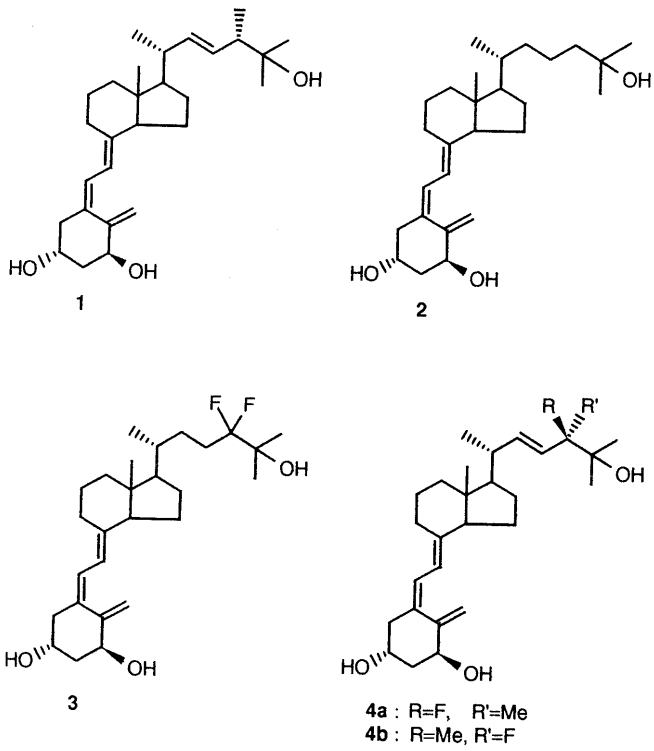
A new synthesis of 24-fluoro-1α,25-dihydroxyvitamin D_2 (4a) and its 24-epimer (4b) is described. Starting with 1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]-24-norchol-5,7-dien-23-al (5), a mixture of 4a and 4b was obtained in 3% overall yield in 6 steps. Reversed-phase HPLC cleanly separated the mixture into the two C-24 epimers. The X-ray crystallographic analysis of the 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) adduct 11b, which was derived from the ester 6, unambiguously determined the configuration at C-24 of this compound. Based on the X-ray analysis, the configuration at C-24 of 4a and 4b was unequivocally determined.

Keywords 1α,25-dihydroxyvitamin D_2, 1α,25-dihydroxyvitamin D_3, 24,24-difluoro-1α,25-dihydroxyvitamin D_3, 24-fluoro-1α,25-dihydroxyvitamin D_2, vitamin D_2 analog, X-ray crystallographic analysis

Vitamin D_2 and D_3 undergo sequential hydroxylations at C-25 in liver and at C-1 in kidney to form 1α,25-dihydroxyvitamin D_2 (**1**) and 1α,25-dihydroxyvitamin D_3 (**2**), respectively, which mediate the hormonal activity on regulation of calcium and phosphorus homeostasis.[1] Both **1** and **2**, the biologically active forms of vitamin D, are further hydroxylated at C-24, and then the compounds are further metabolized in somewhat different ways because of the difference in the side-chain structure.[2] Since the biological activity of **1** is similar to that of **2**,[3,4] **1** and its analogs could be potential therapeutic agents, as in the case of **2**. However, few synthetic studies on **1** and its analogs have been performed,[5,6] whereas a number of analogs of **2** have been synthesized and their biological activity investigated.[7]

The extensive synthetic studies on vitamin D_3 demonstrated that incorporation of fluorine atom(s) into the side-chain alters the activity. Among such compounds, 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (**3**), which we first synthesized,[8-10] showed much higher potency than the parent compound (**2**) in several *in vivo* vitamin D-responsive systems,[11,12] which could be due to blockade of 24-hydroxylation in the metabolic pathway. These results prompted us to synthesize 24-fluoro-1α,25-dihydroxyvitamin D_2 (**4a**), the counterpart of **3** for **1**. Chart 1 outlines



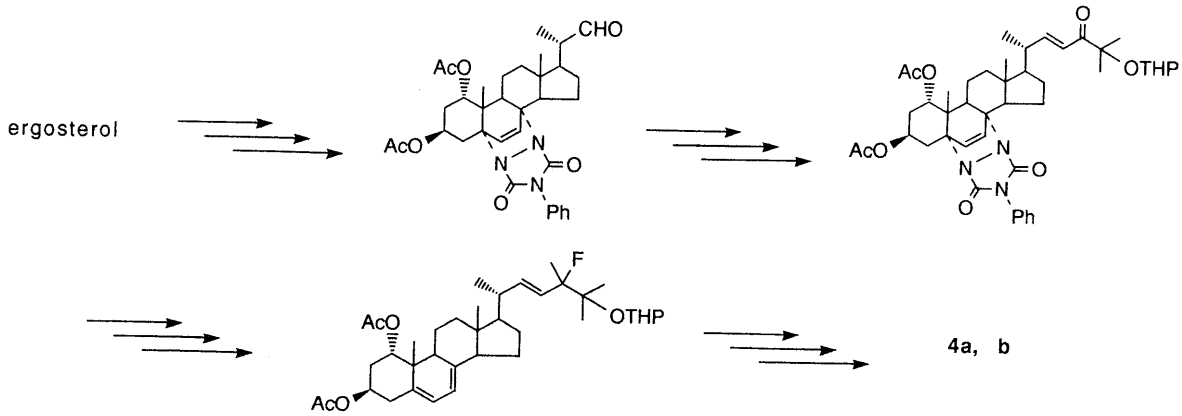
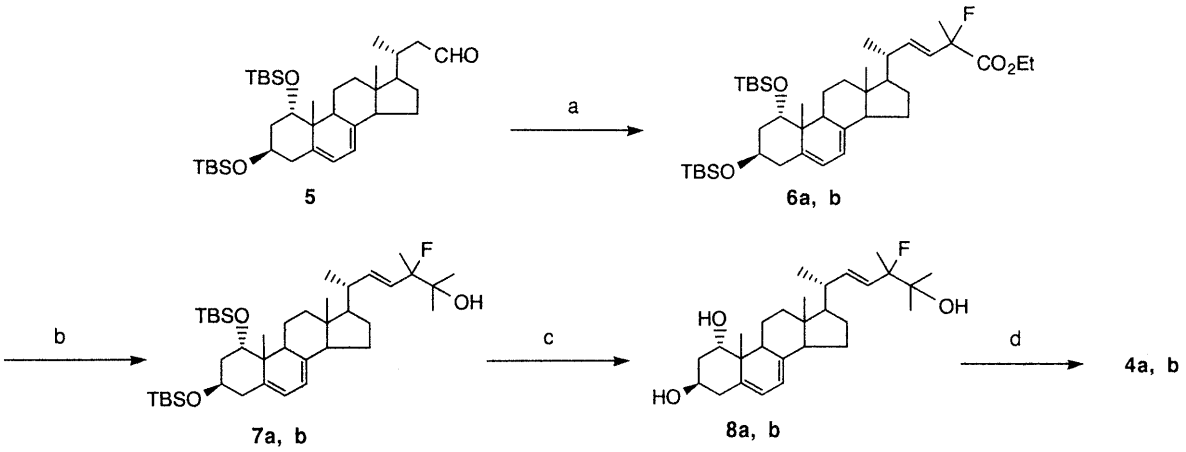


Chart 1

the first synthesis of **4a**, which we reported previously.[13] Compound **4a** was obtained along with the C-24 epimer **4b**. Although reversed-phase HPLC cleanly separated the epimers, the stereochemistry at C-24 of each compound remained unknown. We report here an alternative and efficient synthesis of **4a** and **4b**, and determination of the C-24 configuration by means of X-ray crystallographic analysis.[14]

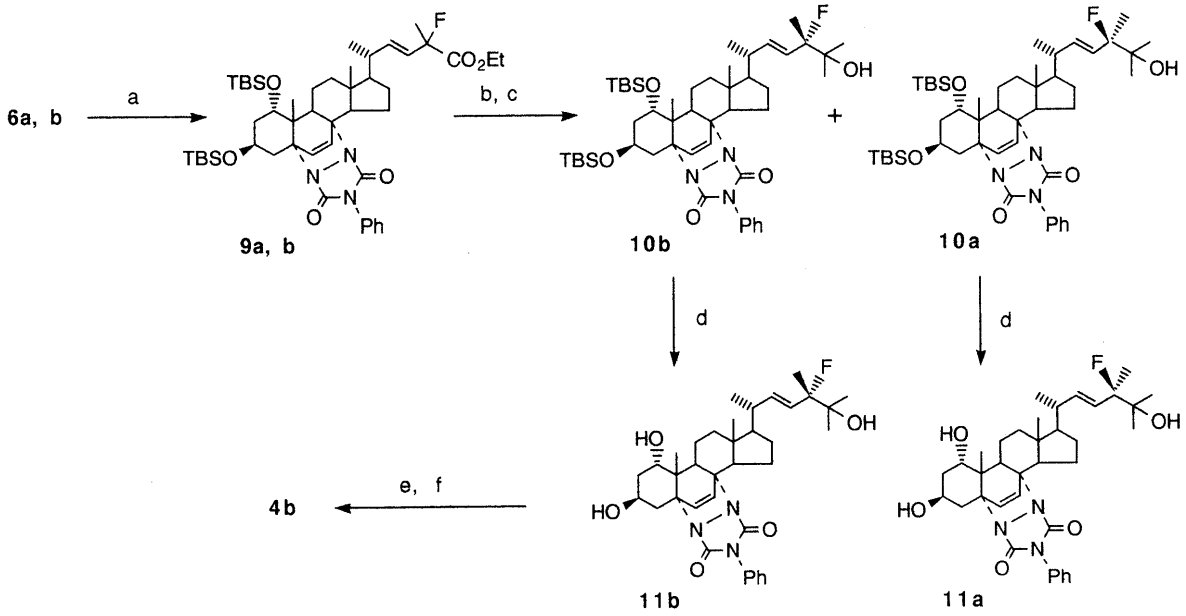
In order to determine the C-24 configuration, we decided to separate the C-24 epimers at some stage of the synthesis and to subject the pure isomer to X-ray crystallographic analysis, because preparation of the stereochemically pure side-chain moiety with established configuration could be difficult. The aldehyde **5**, an intermediate for the synthesis of **3**, would serve as a starting material for this purpose, and it should make the synthesis more effective than that reported previously.

The new synthesis of **4a** and **4b** is shown in Chart 2. Condensation of the aldehyde **5**, obtained from 1α-hydroxydehydroepiandrosterone in good yield,[10] with ethyl 2-fluoropropionate[15] and lithium diisopropylamide (LDA) gave the adduct, which was subsequently dehydrated by treatment with trifluoromethanesulfonic anhydride (Tf_2O)/4-dimethylaminopyridine (DMAP), triethylamine (Et_3N) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the ester **6** in 65% overall yield. The 400 MHz ^{1}H-NMR of **6** clearly showed the *trans* configuration of the olefin formed ($J=15.7$ Hz) and the epimeric ratio at C-24 as approximately 55:45. The ester group of **6** was then methylated by methyllithium (MeLi) to give **7** in 52% yield, and this product, on treatment with tetrabutylammonium fluoride (TBAF), afforded the provitamin **8** in 75% yield. Finally, photolytic and subsequent thermal isomerization of the provitamin **8** in a usual manner yielded the vitamin D_2 analogs **4a** and **4b**, which were identical to those obtained previously.[13] Thus, the method described here generated



a, 1) CH_3CHFCO_2Et/LDA 2) Tf_2O/DMAP/Et_3N 3) DBU ; b, MeLi ; c, n-Bu_4NF ; d, 1) $h\nu$ 2) reflux/EtOH.

Chart 2



a, PTAD ; b, MeLi ; c, recycling chromatography ; d, n-Bu_4NF ; e, K_2CO_3, f, 1) $h\nu$ 2) Δ.

Chart 3

the vitamin D_2 analogs **4a** and **4b** in 3% overall yield in 6 steps, providing a more efficient synthesis of these compounds.

In order to determine the C-24 configuration, attempts were made to separate the epimers of **6**, **7** and **8**. However, all attempts were unsuccessful; all the compounds were chromatographically homogeneous even with HPLC under all conditions used. The epimers of the analogs **4a** and **4b** were cleanly separated from each other by reversed-phase HPLC as reported previously, but neither of the isomers was crystalline, being amorphous solids instead. Therefore, the intermediates were derivatized as described in Chart 3. Cycloaddition of the ester **6** with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) afforded the adduct **9** in 77% yield. The adduct gave two peaks on HPLC, but only on an analytical scale. On a preparative scale, only a poorly resolved peak was obtained. Recycling HPLC could not improve the resolution. Further derivatization of **9** to **10** with MeLi in 53% yield solved the problem. On analytical HPLC, clear base line separation was obtained for **10a, b**. A recycling preparative HPLC afforded pure **10a** and **10b**. Since these compounds were not crystalline, they were further treated with TBAF to give the triols **11a** and **11b**, respectively, in 70—75% yield. Of these, **11b** was crystalline, being suitable for X-ray crystallographic analysis. The X-ray diffraction experiment was undertaken at 113 K, because the isotropic thermal parameters of the terminal functional group including the fluorine atom were quite large at room temperature. Figure 1 shows the ORTEP drawing. From the results, the C-24 configuration of **11b** was unambiguously determined to be *S*, in other words, the unnatural form. The PTAD moiety of **11b** was removed by treatment with potassium carbonate (K_2CO_3)/dimethylsulfoxide (DMSO) to give the provitamin in 76% yield, and this was isomerized as described above to the vitamin D_2 analog **4b**. Thus-obtained **4b** was identical with the less polar isomer of **4** obtained from **8**, proving the compound to have 24*S* configuration. Accordingly, the other isomer was **4a**, the natural form of the vitamin D_2 with 24*R* configuration. Thus, the configuration at C-24 of each compound was unequivocally determined on the basis of the X-ray crystallographic analysis of **11b**.

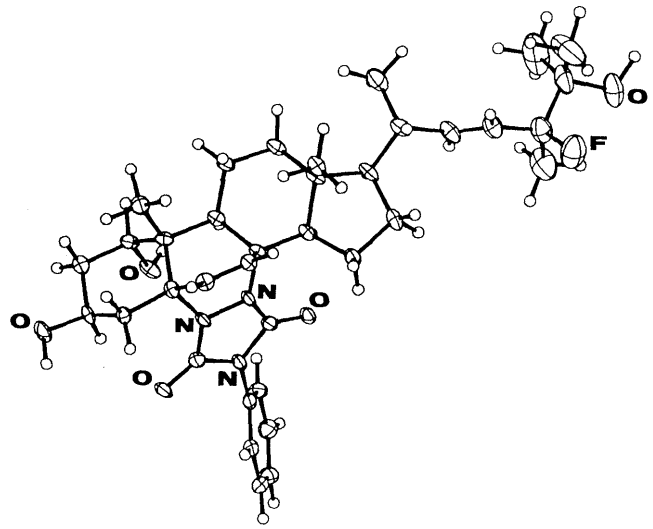


Fig. 1

In contrast to the 24-F_2-vitamin D_3 analog **3**, the biological activity of **4a** and **4b** was equal to or less than that of **1**. Both **4a** and **4b** showed comparable activity to **1** in *in vitro* differentiation of HL-60 cells and bone-resorption assay. As regards elevation of serum calcium and intestinal calcium transport, **4a** showed equal activity to **1**, whereas **4b** was several times less active than **1**.[16]

Experimental

Melting points are uncorrected. Spectral data were recorded on the following instruments: ^{1}H-NMR, JEOL JSX-400; MS, JMS-D 300; IR, JASCO FT/IR-8000; optical rotations, JASCO DIP-370. Tetramethylsilane (TMS) was used as an internal standard for ^{1}H-NMR. Wakogel C-300 (Wako Pure Chemical Industries Ltd.) and Kieselgel 60 F_{254} (Merck) were used for silica gel flash chromatography and preparative TLC, respectively. HPLC separations were performed on a Waters LC equipped with a Model 600E system controller, U6K pump and 490E multiwavelength detector (Waters Associates). Recycling preparative HPLC was performed on a Model LC-908 (Japan Analytical Industry Co., Ltd.). Crystallographic data were collected in a Rigaku AFC-5 diffractometer.

Ethyl (22*E*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24-fluoro-24-methylhomocholesta-5,7,22-trien-25-oate (6) Ethyl 2-fluoropropionate (108 μl, 0.9 mmol, 6 eq) was added to a cooled (−78 °C) solution of LDA/THF [prepared from diisopropylamine (108 μl, 0.72 mmol, 4.8 eq) and 1.3 M *n*-butyllithium (558 μl, 0.72 mmol, 4.8 eq) in THF (14 ml) at 0 °C for 30 min] under an argon atmosphere and the mixture was stirred at −78 °C. After 30 min, **5** (90 mg, 0.15 mmol) in THF (4 ml) was added and the mixture was stirred at −78 °C for 30 min. The reaction was quenched with aqueous NH_4Cl, then the mixture was 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 (10 g, AcOEt/hexane, 1 : 20—1 : 15) to give the adducts (100 mg) as a colorless oil.

A cooled (0 °C) solution of the adducts (100 mg, 0.14 mmol) in CH_2Cl_2 (10 ml) was successively treated with DMAP (71 mg, 0.57 mmol, 4 eq), Et_3N (100 μl, 0.7 mmol, 5 eq) and Tf_2O (97 μl, 0.57 mmol, 4 eq) under an argon atmosphere and the mixture was stirred at 0 °C for 1 h, and at room temperature for 1 h, then DBU (216 μl, 2.1 mmol, 15 eq) was added and the mixture was stirred at room temperature overnight. The mixture was poured into water and extracted with CH_2Cl_2. The combined extracts were successively washed with saturated $CuSO_4$, aqueous NH_4Cl and brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (10 g, AcOEt/hexane, 0—1 : 50) to give **6** (69 mg, 65% overall yield) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.05 (3H, s), 0.06 (6H, s), 0.11 (3H, s), 0.63 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 0.90 (3H, s), 1.06 (3H, d, J=6.7), 1.30 (3H, t, J=7.3), 1.62 (3H, d, J=21.4), 3.69—3.71 (1H, m), 4.0—4.09 (1H, m), 4.29 (2H, q, J=7.3), 5.31 (1H, d, J=4.3), 5.52—5.63 (2H, m), 5.71—5.79 (1H, m). IR ($CHCl_3$): 1740 cm^{-1}. MS *m/z*: 688 (M^+), 668 (M−HF), 631 (M−*tert*-Bu). HR-MS *m/z*: 688.4686 (M^+) Calcd for $C_{40}H_{69}FO_4Si_2$ 688.4715.

(22*E*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24-fluoro-5,7,22-ergostatrien-25-ol (7) A cooled (−78 °C) solution of the ester **6** (21 mg, 0.03 mmol) in THF (8 ml) was treated with 1.5 M MeLi/hexane (81 μl, 0.12 mmol, 4 eq) under an argon atmosphere and the mixture was stirred at −78 °C for 1 h, then gradually warmed to −50 °C over 1 h. The reaction was quenched with aqueous NH_4Cl and the mixture was extracted with AcOEt. The combined extracts were dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel preparative TLC (AcOEt/hexane, 1 : 7) to give **7** (11 mg, 52%) as a colorless amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.05 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.11 (3H, s), 0.64 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 0.90 (3H, s), 1.06 (1.6H, d, J=6.4), 1.07 (1.4H, d, J=6.7), 1.20 (3H, s), 1.22 (3H, s), 1.42 (1.6H, d, J=22.6), 1.43 (1.4H, d, J=22.9), 3.70 (1H, d, J=2.1), 4.0—4.1 (1H, m), 5.30—5.34 (1H, m), 5.50—5.65 (3H, m). MS *m/z*: 654 (M−HF), 636 (M−HF−H_2O). HR-MS *m/z*: 654.4857 Calcd for $C_{40}H_{69}FO_3Si_2$ 654.4860.

(22*E*)-24-Fluoro-5,7,22-ergostatriene-1α,3β,25-triol (8) A mixture of the alcohol **7** (5.4 mg, 8 μmol) and 1 M TBAF/THF (108 μl, 108 μmol, 13.5 eq) was heated at 75 °C for 2 h under an argon atmosphere. 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 preparative TLC (EtOH/$CHCl_3$,

1 : 10) to give **7** (2.7 mg, 75%) as a colorless amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s), 0.96 (3H, s), 1.07 (1.6H, d, J=6.7), 1.08 (1.4H, d, J=6.7), 1.21 (3H, s), 1.22 (3H, s), 1.43 (1.6H, d, J=22.6), 1.44 (1.4H, d, J=22.9), 3.78 (1H, s), 4.07 (1H, tt, J=4.6, 12.4), 5.37—5.40 (1H, m), 5.51—5.65 (2H, m), 5.73—5.74 (1H, m). IR ($CHCl_3$): 3399, 1622, 1028 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 262 (sh), 271 (6080), 282 (10100), 294 (9540). MS m/z: 426 (M−HF), 408 (M−HF−H_2O). HR-MS m/z: 426.3117 (M−HF) Calcd for $C_{28}H_{42}O_3$ 426.3132.

24-Fluoro-1α,25-dihydroxyvitamin D_2 (4a) and Its 24-Epimer (4b) A solution of the provitamin **8** (1.8 mg, 4 μmol) in 1 : 9 THF–ether (20 ml) was cooled to 0 °C and deoxygenated by bubbling argon through the solution for 45 min. The solution was irradiated with a high-pressure mercury lamp fitted with a Vycol filter for 5 min at 0 °C. The solvent was evaporated at below 25 °C and the residue was dissolved in EtOH (5 ml). The solution was refluxed for 2 h under argon, then evaporated. The crude product was purified by HPLC (Lichrosorb RP-18, 10 × 250 mm, H_2O–MeCN 55 : 45, 4 ml/min) to give the vitamin D_2 analogs **4a** (t_R 17.6 min) and **4b** (t_R 18.2 min) (200 μg each, 11%) as a white solid.

(24*R*)-More Polar Isomer (**4a**): $[\alpha]_D$ +0.4° (c=0.01, EtOH). ^{1}H-NMR ($CDCl_3$) δ: 0.57 (3H, s), 1.06 (3H, d, J=6.4), 1.23 (3H, s), 1.26 (3H, s), 1.43 (3H, d, J=22.8), 2.32 (1H, dd, J=6.4, 13.8), 2.60 (1H, dd, J=3.2, 13.8), 2.83 (1H, dd, J=3.9, 12.1), 4.19—4.26 (1H, m), 4.42—4.46 (1H, m), 5.00 (1H, s), 5.32 (1H, t, J=1.5), 5.55 (1H, dd, J=7.2, 15.3), 5.60 (1H, t, J=15.3), 6.02 (1H, d, J=11.1), 6.38 (1H, d, J=11.1). IR ($CHCl_3$): 3420, 1576, 1541, 1047 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 264 (18800). MS m/z: 426 (M−HF), 408 (M−HF−H_2O), 390 (M−HF−2H_2O), 372 (M−HF−3H_2O). HR-MS m/z: 426.3127 (M−HF) Calcd for $C_{28}H_{42}O_3$ 426.3132.

(24*S*)-Less Polar Isomer (**4b**): $[\alpha]_D$ +1.6° (c=0.03, EtOH). ^{1}H-NMR ($CDCl_3$) δ; 0.57 (3H, s), 1.05 (3H, d, J=6.7), 1.23 (3H, s), 1.26 (3H, s), 1.43 (3H, d, J=22.8), 2.32 (1H, dd, J=6.4, 13.9), 2.60 (1H, dd, J=3.1, 13.9), 2.83 (1H, dd, J=3.8, 12.1), 4.19—4.26 (1H, m), 4.42—4.45 (1H, m), 5.00 (1H, s), 5.32 (1H, t, J=1.6), 5.54 (1H, t, J=16.0), 5.61 (1H, dd, J=8.3, 16.0), 6.01 (1H, d, J=11.3), 6.38 (1H, d, J=11.3). IR ($CHCl_3$): 3420, 1576, 1541, 1047 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 264 (18900). MS m/z: 426 (M−HF), 408 (M−HF−H_2O), 390 (M−HF−2H_2O), 372 (M−HF−3H_2O). HR-MS m/z: 426.3167 (M−HF) Calcd for $C_{28}H_{42}O_3$ 426.3132.

Ethyl (22*E*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1,2-diyl)-24-fluoro-24-methylhomocholesta-6,22-dien-25-oate (9) PTAD was added to a stirred solution of the diene **6** (310 mg, 0.45 mmol) in AcOEt (10 ml) until the red color of PTAD developed, then the mixture was stirred at room temperature for 5 min and the solvent was evaporated. The crude product was purified by silica gel flash chromatography (35 g, AcOEt/Hex, 1 : 20—1 : 7) to give **9** (299 mg, 77%) as a pale yellow amorphous solid: ^{1}H-NMR ($CDCl_3$) δ: 0.06 (3H, s), 0.08 (3H, s), 0.09 (3H, s), 0.13 (3H, s), 0.81 (1.5H, s), 0.82 (1.5H, s), 0.87 (9H, s), 0.89 (9H, s), 0.92 (3H, s), 1.06 (3H, d, J=6.7), 1.28 (3H, t, J=7.0), 1.61 (3H, d, J=21.2), 3.23 (1H, dd, J=4.2, 13.7), 3.84 (1H, s), 4.22 (2H, q, J=7.0), 4.77 (1H, tt, J=4.4, 11.6), 5.54—5.63 (1H, m), 5.71—5.80 (1H, m), 6.22 (1H, d, J=8.0), 6.35 (1H, d, J=8.0), 7.24—7.28 (1H, m), 7.36—7.40 (2H, m), 7.44—7.62 (2H, m). IR ($CHCl_3$): 1748, 1690, 1550 cm^{-1}. MS m/z: 863 (M^+), 806 (M−*tert*-Bu), 688 (M−PTAD). HR-MS m/z: 688.4718 (M−PTAD) Calcd for $C_{40}H_{69}FO_4Si_2$ 688.4715.

(22*E*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidine-1,2-diyl)-24-fluoro-6,22-ergostadien-25-ol (10a and 10b) A cooled (−78 °C) solution of the ester **9** (125 mg, 0.14 mmol) in THF (5 ml) was treated with 1.5 M MeLi/hexane (0.29 ml, 0.44 mmol, 3 eq) under argon and the mixture was stirred at −78 °C for 30 min, then at −40 °C for 5 min. The reaction was quenched with aqueous NH_4Cl and the mixture was 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 (25 g, AcOEt/hexane, 1 : 15) to give **10** (66 mg, 53%) as a pale yellow amorphous solid.

A mixture of the diastereoisomers (127 mg) was subjected to a recycling preparative HPLC (JAIGEL SIL S-043-10, 20 × 250 mm × 2, AcOEt/Hex, 15 : 85) to yield pure **10a** (38 mg) and **10b** (48 mg) as amorphous solids.

(24*R*)-Less Polar Isomer (**10a**): $[\alpha]_D$ −16.7° (c=0.36, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (3H, s), 0.08 (3H, s), 0.10 (3H, s), 0.13 (3H, s), 0.83 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 0.92 (3H, s), 1.08 (3H, d, J=6.7), 1.19 (3H, s), 1.20 (3H, s), 1.41 (3H, d, J=22.6), 3.24 (1H, dd, 4.6, 13.6), 3.85 (3H, s), 4.77 (1H, tt, J=4.1, 12.2), 5.58 (1H, dd, J=8.0, 16.0), 5.62 (1H, d, J=16.0), 6.22 (1H, d, J=8.2), 6.36 (1H, d, J=8.2), 7.26 (1H, t, J=8.2), 7.38 (2H, t, J=8.2), 7.45 (2H, d, J=8.2). IR ($CHCl_3$): 1750, 1698, 1550 cm^{-1}. UV $\lambda_{max}^{CHCl_2}$: nm (ε) 240 (4640). MS m/z: 811 (M−HF−H_2O), 754 (M−HF−H_2O−*tert*-Bu); HR-MS m/z: 754.4454 (M−HF−H_2O−*tert*-Bu) Calcd for $C_{44}H_{67}O_5N_3Si_2$ 754.4432.

(24*S*)-More Polar Isomer (**10b**): $[\alpha]_D$ −16.7° (c=0.42, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (3H, s), 0.08 (3H, s), 0.10 (3H, s), 0.13 (3H, s), 0.83 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 0.92 (3H, s), 1.07 (3H, d, J=6.7), 1.19 (3H, s), 1.20 (3H, s), 1.41 (3H, d, J=22.6), 3.24 (1H, dd, 4.6, 13.6), 3.85 (3H, s), 4.77 (1H, tt, J=4.1, 12.2), 5.55 (1H, t, J=15.8), 5.61 (1H, dd, J=8.4, 15.8), 6.22 (1H, d, J=8.2), 6.36 (1H, d, J=8.2), 7.26 (1H, t, J=8.2), 7.38 (2H, t, J=8.2), 7.45 (2H, d, J=8.2). IR ($CHCl_3$): 1750, 1698 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 240 (4640). MS m/z: 811 (M−HF−H_2O), 754 (M−HF−H_2O−*tert*-Bu). HR-MS m/z: 754.4463 (M−HF−H_2O−*tert*-Bu) Calcd for $C_{44}H_{67}O_5N_3Si_2$ 754.4432.

(22*E*,24*R*)-5α,8α-(3,5-Dioxo-4-phenyl-1,2,4-triazolidine-1,2-diyl)-24-fluoro-6,22-ergostadiene-1α,3β,25-triol (11a) A mixture of the silyl ether **10a** (27 mg, 0.03 mmol) in THF (9 ml) and 1 M TBAF/THF (0.57 ml, 0.57 mmol, 18 eq) was heated at 75 °C for 3.5 h, then 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 preparative TLC (EtOH/$CHCl_3$, 1 : 10) to give **11a** (15 mg, 75%) as colorless needles: mp 221—222 °C (EtOH–ether); $[\alpha]_D$ −77.4° (c=0.79, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s), 0.94 (3H, s), 1.07 (3H, d, J=6.4), 1.19 (3H, s), 1.20 (3H, s), 1.41 (3H, d, J=22.9), 3.16 (1H, dd, J=4.6, 14.1), 3.89 (1H, s), 4.90 (1H, tt, J=4.4, 11.6), 5.57 (1H, dd, 8.0, 15.7), 5.62 (1H, t, J=15.7), 6.27 (1H, d, J=8.0), 6.41 (1H, d, J=8.0), 7.28—7.41 (5H, m). IR ($CHCl_3$): 3420, 1747, 1682, 1520 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 240 (3980). MS m/z: 446 (M−PTAD), 426 (M−PTAD−HF), 408 (M−PTAD−HF−H_2O), 390 (M−PTAD−HF−2H_2O). HR-MS m/z: 426.3163 (M−PTAD−HF) Calcd for $C_{28}H_{42}O_3$ 426.3132.

(22*E*,24*S*)-5α,8α-(3,5-Dioxo-4-phenyl-1,2,4-triazolidine-1,2-diyl)-24-fluoro-6,22-ergostadiene-1α,3β,25-triol (11b) A mixture of the silyl ether **10b** (31 mg, 0.04 mmol) in THF (10 ml) and 1 M *n*-BuN_4F/THF (0.65 ml, 0.65 mmol, 18 eq) was heated at 75° for 3.5 h, then 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 preparative TLC (EtOH/$CHCl_3$, 1 : 10) to give **11b** (17 mg, 76%) as colorless needles: mp 220.5—221 °C (EtOH–ether); $[\alpha]_D$ −57.2° (c=0.74, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s), 0.94 (3H, s), 1.06 (3H, d, J=6.4), 1.19 (3H, s), 1.20 (3H, s), 1.42 (3H, d, J=22.6), 3.16 (1H, dd, J=4.6, 14.1), 3.89 (1H, s), 4.90 (1H, tt, J=4.4, 11.6), 5.55 (1H, t, J=15.9), 5.61 (1H, dd, J=7.9, 15.9), 6.27 (1H, d, J=8.0), 6.41 (1H, d, J=8.0), 7.28—7.41 (5H, m). IR ($CHCl_3$): 3420, 1747, 1682, 1520 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 240 (3980). MS m/z: 446 (M−PTAD), 426 (M−PTAD−HF), 408 (M−PTAD−HF−H_2O), 390 (M−PTAD−HF−2H_2O). HR-MS m/z: 426.3157 (M−PTAD−HF) Calcd for $C_{28}H_{42}O_3$ 426.3132.

(24*S*)-24-Fluoro-1α,25-dihydroxyvitamin D_2 (4b) from 11b A mixture of the PTAD adduct **11b** (8.6 mg, 13 μmol) and K_2CO_3 (23 mg, 167 μmol, 12 eq) in DMSO (1.5 ml) was heated at 110—115 °C for 22 h. 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 preparative TLC (AcOEt) to give the provitamin (4.6 mg, 74%) as a colorless amorphous solid. $[\alpha]_D$ −11.2° (c=0.34, EtOH). ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s), 0.96 (3H, s), 1.07 (3H, s), 1.21 (3H, s), 1.22 (3H, s), 1.43 (3H, d, J=22.6), 3.78 (1H, s), 4.07 (1H, tt, J=4.6, 12.4), 5.38 (1H, dt, J=5.6, 2.7), 5.55 (1H, t, J=15.9), 5.62 (1H, dd, J=8.5, 15.9), 5.73 (1H, dd, J=2.4, 5.6). IR ($CHCl_3$): 3399, 1622, 1028 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 262 (sh), 271 (6080), 282 (10100), 294 (9540). MS m/z: 426 (M−HF), 408 (M−HF−H_2O). HR-MS m/z: 426.3117 (M−HF) Calcd for $C_{28}H_{42}O_3$ 426.3132.

A solution of the provitamin (4.5 mg, 10 μmol) in 1 : 9 THF–ether (40 ml) was cooled to 0 °C and deoxygenated by bubbling argon through the solution for 45 min. The solution was irradiated with a high-pressure mercury lamp fitted with a Vycol filter for 5 min at 0 °C. The solvent was evaporated at below 25 °C and the residue was dissolved in EtOH (10 ml). The solution was refluxed for 1 h under argon, then evaporated. The crude product was purified by HPLC (Lichrosorb RP-18, 10 × 250 mm, H_2O–MeCN 55 : 45, 4 ml/min) to give **4b** (0.5 mg, 11%) as a white solid. This was identical with **4b** obtained from **8** (^{1}H-NMR, IR, UV, MS and HPLC comparisons).

X-Ray Crystallographic Analysis of 11b A crystal with dimensions of 0.04 × 0.05 × 0.58 mm was obtained by recrystallization from ethanol/ether. The observed cell parameters are as follows: $C_{36}H_{48}FN_3O_5$, M_r=621.79, monoclinic, $P2_1$, a=12.791(1), b=11.507(4), c=11.007(1) Å, β=93.99(1) Å, V=1616.2(6) Å^3, Z=2, D_χ=1.278 Mgm^{-3}, λ(Cu$K_{\alpha 1}$)=1.54050 Å, μ=0.684 mm^{-1}, F(000)=668, T=113 K. The structure was solved by the direct method, and refined by full matrix least-squares calculations assuming anisotropic temperature factors for nonhydrogen atoms and

isotropic ones for hydrogen atoms. $R=0.047$, $R_w=0.042$ for 2353 reflections above $3\sigma(F)$. Further X-ray crystallographic data including bond lengths and angles, H-atom coordinates, anisotropic thermal parameters and structure factors (F_o–F_c tables) for this compound (13 pages) have been deposited as supplementary material.

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