A New Synthesis of 24-Fluoro-1 α ,25-dihydroxyvitamin D₂ and Its 24-Epimer, and Determination of the C-24 Configuration

Takaaki HAYASHI,^a Katsuji OJIMA,^a Katsuhiro KONNO,^a Akihiko MANAKA,^b Kentaro YAMAGUCHI,^c Sachiko YAMADA^d and Hiroaki TAKAYAMA^{*,a}

Faculty of Pharmaceutical Sciences, Teikyo University,^a Sagamiko, Kanagawa 199-01, Japan, Research Laboratories of Biological Science, Kodama Ltd.,^b 957 Wanagaya, Matsudo, Chiba 271, Japan, School of Pharmaceutical Sciences, Showa University,^c 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan and Institute for Medical and Dental Engineering, Tokyo Medical and Dental University,^d 2-3-10 Surugadai Kanda, Chiyoda-ku, Tokyo 101, Japan. Received April 13, 1992

A new synthesis of 24-fluoro-1 α ,25-dihydroxyvitamin D₂ (4a) and its 24-epimer (4b) is described. Starting with 1 α ,3 β -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 1 α ,25-dihydroxyvitamin D₂, 1 α ,25-dihydroxyvitamin D₃, 24,24-difluoro-1 α ,25-dihydroxyvitamin D₃, 24-fluoro-1 α ,25-dihydroxyvitamin D₂, vitamin D₂ analog, X-ray crystallographic analysis

Vitamin D₂ and D₃ undergo sequential hydroxylations at C-25 in liver and at C-1 in kidney to form 1 α ,25-dihydroxyvitamin D₂ (1) and 1 α ,25-dihydroxyvitamin D₃ (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 D₃ demonstrated that incorporation of fluorine atom(s) into the side-chain alters the activity. Among such compounds, 24,24-difluoro-1 α ,25-dihydroxyvitamin D₃ (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-1 α ,25-dihydroxyvitamin D₂ (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 1 α -hydroxydehydroepiandrosterone in good yield,¹⁰⁾ with ethyl 2-fluoropropionate¹⁵⁾ and lithium diisopropylamide (LDA) gave the adduct, which was subsequently dehydrated by treatment with trifluoromethanesulfonic anhydride (Tf₂O)/4-dimethylaminopyridine (DMAP), triethylamine (Et₃N) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the ester **6** in 65% overall yield. The 400 MHz ¹H-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 D₂ analogs **4a** and **4b**, which were identical to those obtained previously.¹³⁾ Thus, the method described here generated

a, 1) CH₃CHFCO₂Et / LDA 2) Tf₂O/DMAP/Et₃N 3) DBU ; b, MeLi ; c, *n*-Bu₄NF ; d, 1) *h* ν 2) reflux/EtOH.

Chart 2

a, PTAD ; b, MeLi ; c, recycling chromatography ; d, *n*-Bu₄NF ; e, K₂CO₃ ; f, 1) *h* ν 2) Δ .

Chart 3

the vitamin D₂ 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 (K₂CO₃)/dimethylsulfoxide (DMSO) to give the provitamin in 76% yield, and this was isomerized as described above to the vitamin D₂ 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 D₂ 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-F₂-vitamin D₃ 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: ¹H-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 ¹H-NMR. Wakogel C-300 (Wako Pure Chemical Industries Ltd.) and Kieselgel 60 F₂₅₄ (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*)-1*α*,3*β*-Bis[(*tert*-butyldimethylsilyl)oxy]-24-fluoro-24-methylhomocholesta-5,7,22-trien-25-oate (6**)** Ethyl 2-fluoropropionate (108 μ l, 0.9 mmol, 6 eq) was added to a cooled (-78°C) solution of LDA/THF [prepared from diisopropylamine (108 μ l, 0.72 mmol, 4.8 eq) and 1.3 M *n*-butyllithium (558 μ l, 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 NH₄Cl, then the mixture was extracted with AcOEt. The combined extracts were washed with brine, dried over MgSO₄ 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 CH₂Cl₂ (10 ml) was successively treated with DMAP (71 mg, 0.57 mmol, 4 eq), Et₃N (100 μ l, 0.7 mmol, 5 eq) and Tf₂O (97 μ l, 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 μ l, 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 CH₂Cl₂. The combined extracts were successively washed with saturated CuSO₄, aqueous NH₄Cl and brine, dried over MgSO₄ 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. ¹H-NMR (CDCl₃) 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 (CHCl₃): 1740 cm⁻¹. MS *m/z*: 688 (M⁺), 668 (M–HF), 631 (M–*tert*-Bu). HR-MS *m/z*: 688.4686 (M⁺) Calcd for C₄₀H₆₉FO₄Si₂ 688.4715.

(22*E*)-1*α*,3*β*-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 μ l, 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 NH₄Cl and the mixture was extracted with AcOEt. The combined extracts were dried over MgSO₄ 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. ¹H-NMR (CDCl₃) 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–H₂O). HR-MS *m/z*: 654.4857 Calcd for C₄₀H₆₉FO₃Si₂ 654.4860.

(22*E*)-24-Fluoro-5,7,22-ergostatriene-1*α*,3*β*,25-triol (8**)** A mixture of the alcohol **7** (5.4 mg, 8 μ mol) and 1 M TBAF/THF (108 μ l, 108 μ mol, 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 MgSO₄ and evaporated. The crude product was purified by silica gel preparative TLC (EtOH/CHCl₃,

1:10) to give **7** (2.7 mg, 75%) as a colorless amorphous solid. $^1\text{H-NMR}$ (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, t, $J=4.6, 12.4$), 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 $\lambda_{\text{max}}^{\text{EtOH}}$ 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 $\text{C}_{28}\text{H}_{42}\text{O}_3$ 426.3132.

24-Fluoro-1 α ,25-dihydroxyvitamin D₂ (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 Vycor 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 $\times$ 250 mm, H_2O –MeCN 55:45, 4 ml/min) to give the vitamin D₂ analogs **4a** (t_R 17.6 min) and **4b** (t_R 18.2 min) (200 μg each, 11%) as a white solid.

(24R)-More Polar Isomer (**4a**): $[\alpha]_D + 0.4^\circ$ ($c=0.01$, EtOH). $^1\text{H-NMR}$ (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 $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 264 (18800). MS m/z : 426 (M–HF), 408 (M–HF– H_2O), 390 (M–HF– $2\text{H}_2\text{O}$), 372 (M–HF– $3\text{H}_2\text{O}$). HR-MS m/z : 426.3127 (M–HF) Calcd for $\text{C}_{28}\text{H}_{42}\text{O}_3$ 426.3132.

(24S)-Less Polar Isomer (**4b**): $[\alpha]_D + 1.6^\circ$ ($c=0.03$, EtOH). $^1\text{H-NMR}$ (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 $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 264 (18900). MS m/z : 426 (M–HF), 408 (M–HF– H_2O), 390 (M–HF– $2\text{H}_2\text{O}$), 372 (M–HF– $3\text{H}_2\text{O}$). HR-MS m/z : 426.3167 (M–HF) Calcd for $\text{C}_{28}\text{H}_{42}\text{O}_3$ 426.3132.

Ethyl (22E)-1 α ,3 β -Bis[(*tert*-butyldimethylsilyloxy)-5 α ,8 α -(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: $^1\text{H-NMR}$ (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 $\text{C}_{40}\text{H}_{69}\text{FO}_4\text{Si}_2$ 688.4715.

(22E)-1 α ,3 β -Bis[(*tert*-butyldimethylsilyloxy)-5 α ,8 α -(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 $\times$ 250 mm $\times$ 2, AcOEt/Hex, 15:85) to yield pure **10a** (38 mg) and **10b** (48 mg) as amorphous solids.

(24R)-Less Polar Isomer (**10a**): $[\alpha]_D - 16.7^\circ$ ($c=0.36$, CHCl_3). $^1\text{H-NMR}$ (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 $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (ϵ): 240 (4640). MS m/z : 811 (M–HF– H_2O), 754 (M–HF– H_2O –*tert*-Bu); HR-MS m/z : 754.4454 (M–HF– H_2O –*tert*-Bu) Calcd for $\text{C}_{44}\text{H}_{67}\text{O}_5\text{N}_3\text{Si}_2$ 754.4432.

(24S)-More Polar Isomer (**10b**): $[\alpha]_D - 16.7^\circ$ ($c=0.42$, CHCl_3). $^1\text{H-NMR}$ (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 $\lambda_{\text{max}}^{\text{CHCl}_3}$ 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 $\text{C}_{44}\text{H}_{67}\text{O}_5\text{N}_3\text{Si}_2$ 754.4432.

(22E,24R)-5 α ,8 α -(3,5-Dioxo-4-phenyl-1,2,4-triazolidine-1,2-diyl)-24-fluoro-6,22-ergostadiene-1 α ,3 β ,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); $[\alpha]_D - 77.4^\circ$ ($c=0.79$, CHCl_3). $^1\text{H-NMR}$ (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 $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (ϵ): 240 (3980). MS m/z : 446 (M–PTAD), 426 (M–PTAD–HF), 408 (M–PTAD–HF– H_2O), 390 (M–PTAD–HF– $2\text{H}_2\text{O}$). HR-MS m/z : 426.3163 (M–PTAD–HF) Calcd for $\text{C}_{28}\text{H}_{42}\text{O}_3$ 426.3132.

(22E,24S)-5 α ,8 α -(3,5-Dioxo-4-phenyl-1,2,4-triazolidine-1,2-diyl)-24-fluoro-6,22-ergostadiene-1 α ,3 β ,25-triol (11b) A mixture of the silyl ether **10b** (31 mg, 0.04 mmol) in THF (10 ml) and 1 M *n*-Bu₄F/THF (0.65 ml, 0.65 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 **11b** (17 mg, 76%) as colorless needles: mp 220.5–221°C (EtOH–ether); $[\alpha]_D - 57.2^\circ$ ($c=0.74$, CHCl_3). $^1\text{H-NMR}$ (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 $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (ϵ): 240 (3980). MS m/z : 446 (M–PTAD), 426 (M–PTAD–HF), 408 (M–PTAD–HF– H_2O), 390 (M–PTAD–HF– $2\text{H}_2\text{O}$). HR-MS m/z : 426.3157 (M–PTAD–HF) Calcd for $\text{C}_{28}\text{H}_{42}\text{O}_3$ 426.3132.

(24S)-24-Fluoro-1 α ,25-dihydroxyvitamin D₂ (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. $[\alpha]_D - 11.2^\circ$ ($c=0.34$, EtOH). $^1\text{H-NMR}$ (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 $\lambda_{\text{max}}^{\text{EtOH}}$ 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 $\text{C}_{28}\text{H}_{42}\text{O}_3$ 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 Vycor 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 $\times$ 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** ($^1\text{H-NMR}$, IR, UV, MS and HPLC comparisons).

X-Ray Crystallographic Analysis of 11b A crystal with dimensions of 0.04 $\times$ 0.05 $\times$ 0.58 mm was obtained by recrystallization from ethanol/ether. The observed cell parameters are as follows: $\text{C}_{36}\text{H}_{48}\text{FN}_3\text{O}_5$, $M_r=621.79$, monoclinic, $P2_1$, $a=12.791(1)$, $b=11.507(4)$, $c=11.007(1)$ Å, $\beta=93.99(1)^\circ$, $V=1616.2(6)$ Å³, $Z=2$, $D_x=1.278$ Mgm^{-3} , $\lambda(\text{CuK}\alpha)=1.54050$ Å, $\mu=0.684$ 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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