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A New and Convenient Synthesis of 1α,25-Dihydroxyvitamin D_2 and Its 24*R*-Epimer

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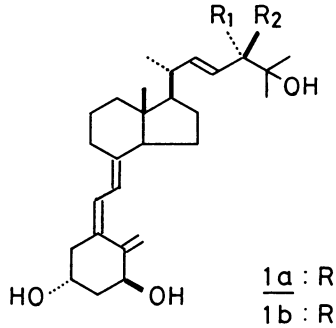
1α, 25-Dihydroxyvitamin D_2 (**1a**) was synthesized by irradiation and subsequent thermal isomerization of (22*E*)-5,7,22-ergostatriene-1α,3β,25-triol (**16a**). The triol **16a** was obtained via 22-oxo-5α,8α-(4-phenyl-3,5-dioxo-1,2,4-triazolidine-1,2-diyl)-23,24-dinor-6-cholene-1α,3β-diyl diacetate (**12**) starting from (22*E*)-5,7,22-ergostatriene-1α,3β-diyl diacetate (**10**), a precursor of 1α-hydroxyvitamin D_2. Introduction of the new side chain with the desired stereochemistry was carried out selectively by the reductive elimination of the β-hydroxy sulfone derived from the C-22 aldehyde (**12**) and an optically active sulfone, prepared via (*S*)-2,3-dimethyl-1,3-butanediol from methyl (*S*)-3-hydroxy-2-methylpropionate. Similarly a C-24 epimer of **1a** was synthesized.

Although the metabolism of vitamin D_2 (ercalciol) has not been extensively investigated as in the case of vitamin D_3 (calciol), it has been shown that vitamin D_2 is hydroxylated at C-25 position in the liver to 25-hydroxyvitamin D_2 (ercalcidiol) and subsequently at the C-1α position in the kidney to 1α,25-dihydroxyvitamin D_2 (ercalcitriol) (**1a**), a most potent metabolite.[1,2] Because it has been reported that 1α-hydroxyvitamin D_2 ((1*S*)-hydroxyercalciol)[3,4] is less toxic than the corresponding 1α-hydroxyvitamin D_3 ((1*S*)-hydroxycalciol), in spite of the almost equal potency of vitamin D activity,[5-7] much attention has been paid to the 1α-hydroxylated vitamin D_2. However, 1α,25-dihydroxyvitamin D_2 (**1a**), an "active form" of vitamin D_2, seems to be more potent than 1α-hydroxyvitamin D_2 for vitamin D activity, because **1a** is thought to elicit its physiological activity after being hydroxylated at C-25 position as described above. These observations prompted us to investigate the synthesis of **1a** and its 24*R*-epimer (**1b**).

DeLuca et al. reported the synthesis of **1a** and/or **1b**[8,9] by the selective hydroxylation at C-1α position of cyclovitamin D derivatives. However, this method



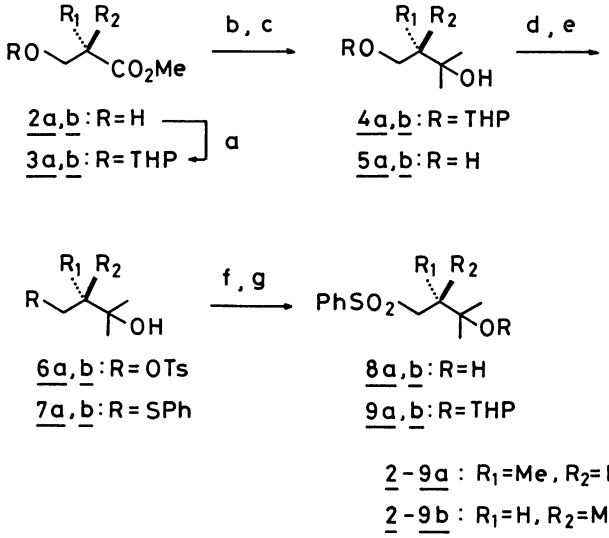
1a : R_1=Me, R_2=H
1b : R_1=H, R_2=Me

gave 1β-hydroxylated and transvitamin D derivatives as by-products, which were difficult to separate from the 1α-hydroxylated compound. Although Baggiolini et al. reported the synthesis of **1a**,[10] their procedure is complicated.

It is advantageous to use the steroidal block as an intermediate for the preparation of 1α-hydroxylated vitamin D derivatives, because 1α-hydroxylated provitamin D derivatives are easily and selectively converted to the corresponding 1α-hydroxylated vitamin D derivatives.

Previously, we reported the convenient synthesis of (22*E*)-5,7,22-ergostatriene-1α,3β-diyl diacetate (**10**),[11] a precursor of 1α-hydroxyvitamin D_2. To our best knowledge, no one has used this **10** in the synthesis of **1a** and **1b**. The C-22 aldehyde (**12**), available readily from **10**, seemed to be a good key-intermediate for the preparation of **1a** and **1b**. In the present paper we wish to describe a new and convenient synthesis of **1a** and **1b** using **10** as the starting material.

The side-chain fragments, namely optically active sulfones **9a**[12] for **1a** and **9b**[9] for **1b**, were synthesized respectively starting from methyl (*S*)- and (*R*)-3-hydroxy-2-methylpropionate (**2a** and **2b**) as follows (Scheme 1). Although it would be possible to convert **2a** directly to the diol (**5a**) by the Grignard reaction, this method gave a poor yield of **5a** due to its solubility in water. Thus, the hydroxyl group of **2a** was protected as a tetrahydropyranyl (THP) ether[13] to give **3a**.



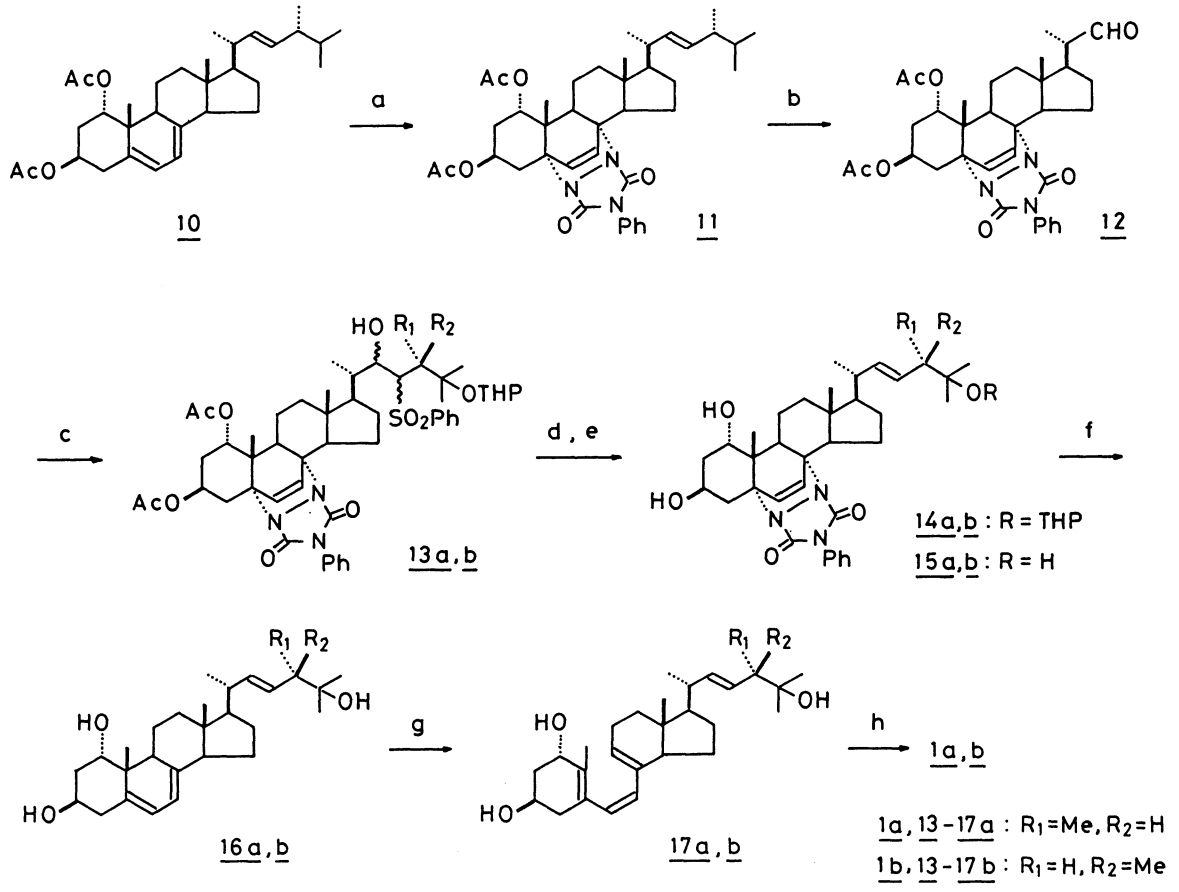
Scheme 1. a) DHP, *p*-TsOH; b) MeMgI/Et_2O; c) *p*-TsOH/MeOH; d) *p*-TsCl, Py; e) PhSH, *t*-BuOK/DMF; f) Na_2WO_4-H_2O_2; g) DHP, PPTS.

The Grignard reaction of **3a** using methylmagnesium iodide in ether afforded **4a**, which was subsequently subjected to deprotection of the THP group to provide the diol (**5a**) after distillation in 84% yield from **2a**. Tosylation of the primary hydroxyl group of **5a** in the usual manner afforded the tosylate (**6a**), which was reacted with benzenethiol in the presence of potassium *t*-butoxide in DMF to give the phenyl sulfide (**7a**). Oxidation of **7a** with Na_2WO_4-H_2O_2 yielded the crystalline hydroxy sulfone (**8a**) in 92% yield. Conventional protection of the hydroxyl group of **8a** as a THP ether gave **9a** in 92% yield. Similarly, **2b** was converted to the corresponding sulfone (**9b**) via the hydroxy sulfone (**8b**).

The next task was to construct the (22*E*)-olefin moiety leading to **1a** and **1b** (Scheme 2). The 5,7-diene of (22*E*)-5,7,22-ergostatriene-1α,3β-diyl diacetate (**10**), our starting material, was protected as a Diels-Alder adduct with 4-phenyl-3*H*-1,2,4-triazole-3,5(4*H*)-dione (PTAD)[14] to give **11** in 87% yield. Selective cleavage of the side-chain double bond of **11** was achieved by ozonolysis at −65 °C in 1% pyridine-CH_2Cl_2 solution and successive reductive workup of the resulting ozonide with dimethyl sulfide[14,15] to give the C-22 aldehyde (**12**) after recrystallization in 49% yield. The ^1H NMR spectrum of **12** exhibited only a doublet at δ 9.55 for the aldehyde proton (22-H), while the ^1H NMR spectrum of C(20) epimeric mixture, obtained by treatment of **12** with alumina (grade II—III) in $CHCl_3$,[14] showed a pair of doublets at δ 9.55 and 9.48. This indicates that the aldehyde obtained (**12**) was a single isomer.

Addition of the aldehyde (**12**) to the carbanion generated from the sulfone (**9a**) by butyllithium in THF[16-18] gave the β-hydroxy sulfone (**13a**), which was used in the next step without further purification. Reductive elimination of the β-hydroxy sulfone (**13a**) with sodium amalgam in buffered MeOH (Na_2HPO_4)[16-18] afforded the olefinic product (**14a**) in 32% yield from **12**. This olefination reaction is known to give predominantly or exclusively an (*E*)-olefin.[16-19] Removal of the THP group of **14a** by pyridinium *p*-toluenesulfonate (PPTS) in EtOH afforded the triol (**15a**), which was treated with $LiAlH_4$ in refluxing THF for removal of the triazoledione protective group[14] to give (22*E*)-5,7,22-ergostatriene-1α,3β,25-triol (**16a**) in 54% yield from **14a**.

Irradiation[3,4] of the 5,7-diene-1α,3β,25-triol (**16a**) with a high-pressure mercury lamp using aq. 1.5% KNO_3 solution as a filter gave the previtamin D (**17a**). Thermal-isomerization[3,4] of **17a** in refluxing EtOH and purification by preparative HPLC furnished



Scheme 2. a) 4-Phenyl-3*H*-1,2,4-triazole-3,5(4*H*)-dione; b) O_3/1% Py-CH_2Cl_2, then Me_2S; c) **9a** or **9b**, *n*-BuLi/THF; d) Na-Hg/MeOH (Na_2HPO_4); e) PPTS/EtOH; f) $LiAlH_4$/THF; g) *hν*; h) reflux/EtOH.

1α,25-dihydroxyvitamin D_2 (**1a**) in 22% yield from **16a**. The physical and spectral data of **1a** were in accord with those reported earlier.[8,10]

Similarly, (24*R*,22*E*)-5,7,22-ergostatriene-1α,3β, 25-triol (**16b**) was prepared from the aldehyde (**12**) and the sulfone (**9b**) in place of **9a** in four steps in 15% yield from **12**. Irradiation of **16b** and purification by preparative HPLC gave the previtamin D (**17b**), which was thermally isomerized to afford the crystalline (24*R*)-1α,25-dihydroxyvitamin D_2 (**1b**) in 12% yield from **16b** after purification by preparative HPLC.

Since (22*E*)-5,7,22-ergostatriene-1α,3β,25-triol is obtainable readily from (22*E*)-5,7,22-ergostatriene-1α,3β-diyl diacetate (**10**), the present study provides an efficient method for the preparation of 1α,25-dihydroxyvitamin D_2. The synthetic method described here will be applicable to other 1α-hydroxylated vitamin D_2 or D_3 derivatives.

Experimental

All the melting points and boiling points are uncorrected. IR spectra were measured on a Jasco IR-810 spectrometer. ^{1}H NMR spectra were recorded with TMS as an internal standard and $CDCl_3$ as a solvent at 200 MHz on a JEOL JNM-FX 200 spectrometer unless otherwise stated. Optical rotations were measured with $CHCl_3$ as a solvent on a Jasco DIP-4 polarimeter or a Jasco DIP-370 polarimeter unless otherwise stated. Mass spectra were recorded on a Hitachi M-80 spectrometer at 70 eV. UV spectra were measured on a Hitachi 320 spectrometer. Merck Kieselgel 60 (Art 7734, 70—230 mesh) or Merck Kieselgel 60 (Art 9385, 230—400 mesh) were used for SiO_2 column chromatography. TLC analyses were performed on a Merck Kieselgel 60 F-254 (Art 5715, 0.25 mm).

(*S*)-2,3-Dimethyl-1,3-butanediol (5a). A solution of methyl (*S*)-3-hydroxy-2-methylpropionate (**2a**) (8.09 g, 68.6 mmol; Sigma Chemical Co.), dihydropyran (8.63 g, 0.10 mol), and *p*-TsOH·H_2O (0.10 g) in dry ether (50 ml) was stirred for 1 h. The mixture was washed with sat. $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give 13.8 g of crude **3a**. Its IR spectrum was identical with that reported previously.[13] This was employed in the next step without further purification.

A Grignard reagent was prepared from methyl iodide (29.2 g, 0.21 mol) and magnesium (5.00 g, 0.21 mol) in dry ether (30 ml). To this Grignard solution, a solution of crude **3a** (13.8 g) in dry ether (30 ml) was added dropwise for 1 h at a rate sufficient to maintain a gentle reflux. The mixture was stirred for 2 h at reflux temperature. Then to the ice-cooled reaction mixture was carefully added dropwise a cold sat. NH_4Cl solution. The ether layer was separated and the aq. layer was extracted with ether. The combined ether solution was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give 13.5 g of crude **4a**: IR (film) 3460, 1460, 1390, 1205, 1180, 1125, 1080, 1060, 980 cm^{-1}. This was employed in the next step without further purification.

A solution of crude **4a** (13.5 g) and *p*-TsOH·H_2O (0.65 g, 3.4 mmol) in MeOH (150 ml) was stirred for 1 h at room temperature. The mixture was neutralized with K_2CO_3 and filtered through Celite. After removal of MeOH in vacuo from the filtrate, the residue was dissolved in ether. The ether solution was dried ($MgSO_4$), and concentrated in vacuo. The residue was distilled to give 6.28 g (84% from **2a**) of **5a**: bp 83—85 °C/0.22 mmHg (1 mmHg≈133.322 Pa); n_D^{23} 1.4439; $[\alpha]_D^{23}$+1.7° (*c* 5.12)[lit,[12] $[\alpha]_D^{22}$—2.5° (0.18%, $CHCl_3$)]; IR (film) 3350, 1470, 1385, 1370, 1175, 1160, 1030 cm^{-1}; ^{1}H NMR δ=0.84 (3H, d, *J*=7.1 Hz), 1.18 (3H, s), 1.25 (3H, s), 1.81 (3H, m), 3.70 (1H, m), 3.91 (1H, s), 4.17 (1H, t, *J*=4.6 Hz). Found: *m/z* 118.1000. Calcd for $C_6H_{14}O_2$: M, 118.0994.

(*S*)-3-Hydroxy-2,3-dimethylbutyl *p*-Toluenesulfonate (6a). To a stirred and ice-cooled solution of **5a** (6.00 g, 50.8 mmol) in dry pyridine (24 ml) was added *p*-toluenesulfonyl chloride (11.64 g, 61.1 mmol) and the mixture was stirred for 2 h at that temperature. The mixture was poured into ice water and extracted with ether. The ether solution was washed with water, sat. $CuSO_4$ solution, sat. $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give 13.5 g of crude **6a**: IR (film) 3450, 1600, 1500, 1470, 1360, 1190, 1175, 1100, 965 cm^{-1}; ^{1}H NMR δ=0.96 (3H, d, *J*=7.1 Hz), 1.12 (3H, s), 1.19 (3H, s), 1.54 (1H, s), 1.80 (1H, m), 2.45 (3H, s), 3.92 (1H, dd, *J*=9.5 and 9.8 Hz), 4.23 (1H, dd, *J*=9.5 and 4.4 Hz), 7.35 (2H, d, *J*=8.3 Hz), 7.80 (2H, d, *J*=8.3 Hz). This was employed in the next step without further purification.

(*R*)-2,3-Dimethyl-4-phenylthio-2-butanol (7a). To a stirred solution of potassium *t*-butoxide (5.89 g, 53.4 mmol) and benzenethiol (5.87 g, 53.4 mmol) in dry DMF (60 ml) was added crude **6a** (13.5 g) and the mixture was stirred for 4 h at room temperature under N_2. The mixture was poured into water and extracted with benzene. The benzene solution was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (100 g) eluting with hexane-EtOAc (9:1) to give 10.13 g of **7a**: IR (film) 3420, 3060, 1590, 1485, 1440, 1375, 1140, 1095, 1030 cm^{-1}; ^{1}H NMR δ=0.96 (3H, d, *J*=6.8 Hz), 1.18 (3H, s), 1.23 (3H, s), 1.54 (1H, s), 1.80 (1H, m), 2.55 (1H, dd, *J*=10.1 and 12.7 Hz), 3.40 (1H, dd, *J*=10.1 and 2.9 Hz), 7.15—7.48 (5H, m). This was employed in the next step without further purification.

(*R*)-2,3-Dimethyl-4-phenylsulfonyl-2-butanol (8a). WO_3 (55 mg) in water (18 ml) was dissolved by adding 50% NaOH solution to pH 11, the solution was then adjusted to pH 5.6 by adding AcOH. To this solution was added **7a** (10.13 g) in EtOH (10 ml) and then 30% H_2O_2 (3.4 ml). The mixture was stirred for 10 min at 60°C. To the mixture was added 30% H_2O_2 (3.4 ml) and the mixture was stirred for a further 30 min at 75°C. After cooling, $Na_2S_2O_3$ was added to the reaction mixture. The mixture was next extracted with benzene. The benzene solution was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (200 g) eluting with hexane-EtOAc (9:1—4:1) to give 11.32 g (92% from **5a**) of **8a**, which crystallized on standing. Recrystallization from ether gave **8a** as prisms: mp 68.4—69.1 °C; $[\alpha]_D^{25}$—33.7° (*c* 1.26) [lit,[12] $[\alpha]_D^{22}$—22.4° (0.6%, $CHCl_3$)]; IR (KBr) 3500, 3070, 1585, 1450, 1370, 1300, 1155, 1140, 1110, 1085 cm^{-1}; ^{1}H NMR δ=1.03 (3H, s), 1.12 (3H, d, *J*=6.8 Hz), 1.22 (3H, s), 1.88 (1H, s), 2.15 (1H, m), 2.82 (1H, dd, *J*=13.9 and 9.3 Hz), 3.60 (1H, dd, *J*=13.9 and 2 Hz), 7.53—7.95 (5H, m). Found: C, 59.49; H, 7.48; S, 13.25%. Calcd for $C_{12}H_{18}O_3S$: C, 59.48; H, 7.49; S, 13.23%.

(3*R*)-2,3-Dimethyl-4-phenylsulfonyl-2-butanol THP Ether

(**9a**). A solution of **8a** (5.31 g, 21.9 mmol), dihydropyran (2.76 g, 32.9 mmol) and PPTS (0.55 g, 2.2 mmol) in dry CH_2Cl_2 (60 ml) was stirred for 4 h at room temperature. The mixture was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (150 g) eluting with hexane-EtOAc (4 : 1) to give 6.58 g (92%) of **9a**: IR (film) 3060, 1585, 1450, 1395, 1305, 1200, 1150, 1110, 1090, 1085, 1025 cm^{-1}; ^1H NMR δ=1.04 and 1.06 (3H, pair of s), 1.13 (3H, m), 1.18 and 1.19 (3H, pair of s), 1.3—1.8 (6H, m), 2.19 (1H, m), 2.88 (1H, m), 3.42 (1H, m), 3.65 (1H, m), 3.82 (1H, m), 4.73 (1H, m), 7.51—7.94 (5H, m).

(*R*)-2,3-Dimethyl-1,3-butanediol (5b). In the same manner as described for **5a**, methyl (*R*)-3-hydroxy-2-methylpropionate (**2b**) (9.28 g, 78.6 mmol; Sigma Chemical Co.) was converted in three steps to 7.61 g (82%) of **5b**: bp 83—84°C/0.25 mmHg; n_D^{23} 1.4437; $[\alpha]_D^{23}$—1.6° (*c* 5.29). Its IR and ^1H NMR spectra were identical with those of **5a**.

(*S*)-2,3-Dimethyl-4-phenylsulfonyl-2-butanol (8b). In the same manner as described for **8a**, **5b** (6.58 g, 55.8 mmol) was converted in three steps to 12.10 g (90%) of **8b**: mp 68.2—69.0 °C (from ether, prisms); $[\alpha]_D^{23}$+34.1° (*c* 1.09) [lit,[9] a thick, colorless oil; $[\alpha]_D^{22}$+27.9° (*c* 1.3, $CHCl_3$)]. Its IR and ^1H NMR spectra were identical with those of **8a**. Found: C, 59.35; H, 7.48; S, 13.29%. Calcd for $C_{12}H_{18}O_3S$: C, 59.48; H, 7.49; S, 13.23%.

(3*S*)-2,3-Dimethyl-4-phenylsulfonyl-2-butanol THP Ether (9b). In the same manner as described for **9a**, **8b** (6.23 g, 25.7 mmol) was converted to 7.89 g (94%) of **9b**. Its IR and ^1H NMR spectra were identical with those of **9a**.

(22*E*)-5α,8α-(4-Phenyl-3,5-dioxo-1,2,4-triazolidine-1,2-diyl)-6,22-ergostadiene-1α,3β-diyl Diacetate (11). To a stirred solution of (22*E*)-5,7,22-ergostatriene-1α,3β-diyl diacetate (**10**) (5.62 g, 11.3 mmol) in $CHCl_3$ (70 ml) was added dropwise a solution of 4-phenyl-3*H*-1,2,4-triazole-3,5(4*H*)-dione (PTAD) (2.26 g, 13 mmol) in acetone (40 ml) at room temperature until a faint pink coloration persisted, and the mixture was concentrated in vacuo. The residue was chromatographed over SiO_2 (80 g) eluting with hexane-EtOAc (2 : 1) to give 6.61 g (87%) of **11** as a foam: $[\alpha]_D^{25}$—139° (*c* 1.09); IR (KBr) 1750, 1700, 1600, 1505, 1395, 1240, 1030 cm^{-1}; ^1H NMR δ=0.79 and 0.82 (6H, each d, *J*=3.7 Hz, 26-H_3 and 27-H_3), 0.84 (3H, s, 18-H_3), 0.89 (3H, d, *J*=6.8 Hz, 28-H_3), 1.02 (3H, d, *J*=6.6 Hz, 21-H_3), 1.06 (3H, s, 19-H_3), 2.01 (3H, s, Ac), 2.03(3H, s, Ac), 3.25 (1H, dd, *J*=13.7 and 5.6 Hz, 9-H), 5.11 (1H, m, 1-H), 5.20 (2H, m, 22-H and 23-H), 5.89 (1H, m, 3-H), 6.33 and 6.45 (2H, ABq, *J*=8.3 Hz, 6-H and 7-H), 7.24—7.51 (5H, m, Ph); MS *m/z* (rel intensity) 671(M^+; 0.3), 496(0.4), 436(8), 376(100), 251(28), 209(23), 155(34).

22-Oxo-5α,8α-(4-phenyl-3,5-dioxo-1,2,4-triazolidine-1,2-diyl)-23,24-dinor-6-cholene-1α,3β-diyl Diacetate (12). Ozone (flow rate 0.07 mmol min^{-1}) was bubbled into a solution of **11** (10.00 g, 14.9 mmol) in 1% pyridine-CH_2Cl_2 (400 ml) with stirring for 4.5 h at −65 °C. After the excess ozone was driven out by bubbling Ar, dimethyl sulfide (20 ml) was added dropwise to the mixture at −65 °C, and the mixture was stirred for 1 h at −65 °C. After the cooling bath was removed, stirring was continued for a further 1 h. The mixture was washed with 2% HCl and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (130 g) eluting with hexane-EtOAc (1 : 1) to give 5.99 g of **12.** This was recrystallized from benzene to give 4.40 g (49%) of **12** as needles: mp 191—193 °C; $[\alpha]_D^{25}$—131° (*c* 1.06); IR (KBr) 2720, 1740, 1685, 1605, 1505, 1405, 1370, 1250, 1230, 1035 cm^{-1}; ^1H NMR δ=0.87 (3H, s, 18-H_3), 1.07 (3H, s, 19-H_3), 1.14 (3H, d, *J*=6.8 Hz, 21-H_3), 2.01 (3H, s, Ac), 2.04 (3H, s, Ac), 3.26 (1H, dd, *J*=14.2 and 5.4 Hz, 9-H), 5.12 (1H, m, 1-H), 5.88 (1H, m, 3-H), 6.36 and 6.44 (2H, ABq, *J*=8.3 Hz, 6-H and 7-H), 7.26—7.51 (5H, m, Ph), 9.55 (1H, d, *J*=3.4 Hz, 22-H); MS *m/z* (rel intensity) 603(M^+; 0.3), 428(0.3), 368(11), 308(100), 235(20), 177(20), 141(57). Found: C, 67.57; H, 6.84; N, 6.99%. Calcd for $C_{34}H_{41}N_3O_7$: C, 67.64; H, 6.84; N, 6.96%.

(22*E*)-5α,8α-(4-Phenyl-3,5-dioxo-1,2,4-triazolidine-1,2-diyl)-25-tetrahydropyranyloxy-6,22-ergostadiene-1α, 3β-diol (14a). Butyllithium (1.5 mol dm^{-3} in hexane, 3.5 ml, 5.2 mmol) was added to a stirred solution of **9a** (1.70 g, 5.2 mmol) in dry THF (60 ml) at −60—−70°C under Ar. After stirring for 30 min, a solution of **12** (2.40 g, 4.0 mmol) in dry THF (25 ml) was added and the mixture was stirred for 30 min at −60—−70°C. Sat. NH_4Cl solution (1 ml) was added dropwise to the mixture at that temperature, and the mixture was allowed to rise to room temperature. The mixture was poured into sat. NH_4Cl solution and extracted with EtOAc. The EtOAc solution was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give crude **13a.** This was employed in the next step without further purification.

To a solution of crude **13a** in MeOH saturated with Na_2HPO_4 (330 ml) sodium amalgam (5%, 18.3 g) was added and the mixture was stirred for 15 h at 0 °C, followed by a further 3 h at room temperature. The supernatant was concentrated in vacuo, and the residue was poured into water and extracted with EtOAc. The EtOAc solution was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (65 g) eluting with hexane-EtOAc (1 : 4) to give 0.87 g (32% from **12**) of **14a** as a crystalline material: IR (KBr) 3420, 1745, 1685, 1600, 1505, 1405, 1315, 1130, 1025, 980 cm^{-1}; ^1H NMR δ=3.48 (1H, m, 6′-H), 3.69 (1H, m, 1-H), 3.95 (1H, m, 6′-H), 4.78 (1H, m, 2′-H), 4.85 (1H, m, 3-H), 5.15—5.42 (2H, m, 22-H and 23-H), 6.18 and 6.34 (2H, ABq, *J*=8.3 Hz, 6-H and 7-H), 7.2—7.4 (5H, m, Ph); MS *m/z* (rel intensity) 512 (M^+—PTAD; 0.3), 428(3), 410(22), 324(3), 251(5), 177(22), 119(28), 85(100), 59(94). This was employed in the next step without further purification.

(22*E*)-5,7,22-Ergostatriene-1α,3β,25-triol (16a). A solution of **14a** (0.84 g, 1.2 mmol) and PPTS (15 mg, 0.06 mmol) in 95% EtOH (6 ml) was stirred for 1 h at 50 °C. After removal of EtOH in vacuo from the mixture, the residue was extracted with EtOAc. The EtOAc solution was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give crude **15a.** This was employed in the next step without further purification. An analytical sample was prepared by recrystallization from ether-EtOH to give **15a** as needles: mp 209—212 °C; $[\alpha]_D^{25}$—86.4° (*c* 0.22); IR (KBr) 3530, 3470, 1745, 1680, 1505, 1415, 1150, 1035 cm^{-1}; ^1H NMR δ=0.84 (3H, s, 18-H_3), 0.93 (3H, s, 19-H_3), 0.98 (3H, d, *J*=7.1 Hz, 28-H_3), 1.05 (3H, d, *J*=6.6 Hz, 21-H_3), 1.13 and 1.16 (6H, each s, 26-H_3 and 27-H_3), 1.21 (t, *J*=7.1 Hz, Et_2O), 3.15 (1H, dd, *J*=14 and 7.1 Hz, 9-H), 3.48 (q, *J*=7.1 Hz, Et_2O), 3.85 (1H, m, 1-H), 4.90 (1H, m, 3-H), 6.26 and 6.41 (2H, ABq, *J*=8.3 Hz, 6-H and 7-H), 7.30—7.45 (5H, m, Ph); MS *m/z* (rel intensity) 428(M^+—PTAD; 18), 350(15), 324(25), 251(15), 177(90), 119(100). Found: C, 71.06; H, 8.28; N, 6.81%. Calcd for $C_{36}H_{49}N_3O_5 \cdot 1/4\ Et_2O$: C, 71.41; H, 8.34; N, 6.75%.

A mixture of crude **15a** and $LiAlH_4$ (0.73 g) in dry THF (80 ml) was stirred for 1.5 h at reflux temperature. The excess $LiAlH_4$ was destroyed by the successive addition of water (0.7 ml), 10% NaOH solution (0.7 ml), water (2.1 ml) to the stirred and ice-cooled mixture. The mixture was stirred for a further 30 min at room temperature, dried ($MgSO_4$), and filtered through Celite. The filtrate was concentrated in vacuo to give crude crystalline **16a**. This was recrystallized from EtOH to give 0.28 g (54% from **14a**) of **16a** as needles: mp 222—224 °C; $[\alpha]_D^{25}$—55° (*c* 0.12, MeOH); IR (KBr) 3520, 3350, 1655, 1610, 1465, 1370, 1135, 1070, 975 cm^{-1}; ^1H NMR δ=0.64 (3H, s, 18-H_3), 0.95 (3H, s, 19-H_3), 1.00 (3H, d, *J*=7.1 Hz, 28-H_3), 1.05 (3H, d, *J*=6.8 Hz, 21-H_3), 1.13 and 1.17 (6H, each s, 26-H_3 and 27-H_3), 3.77 (1H, m, 1-H), 4.04 (1H, m, 3-H), 5.35 (3H, m, 7-H, 22-H and 23-H), 5.73 (1H, m, 6-H); MS *m/z* (rel intensity) 428(M^+; 4), 353(2), 312(2), 251(5), 225(5), 145(8), 81(15), 59(100); UV (EtOH) 282nm (ε 11900). Found: C, 77.97; H, 10.40%. Calcd for $C_{28}H_{44}O_3$: C, 78.48; H, 10.35%.

1α,25-Dihydroxyvitamin D_2 (1a). A solution of **16a** (100 mg, 0.23 mmol) in ether-THF (19:1, 1000 ml) was irradiated for 3 min under Ar at water-cooled temperature with a high-pressure mercury lamp (Ushio, UM-452) using 1.5% KNO_3 solution as a filter. The mixture was next concentrated in vacuo. A solution of the residue containing the previtamin D (**17a**) in EtOH (30 ml) was stirred for 1 h at reflux temperature under Ar. The mixture was concentrated in vacuo. The residue was chromatographed on HPLC (Merck, LiChrosorb® Si60 (7 μm), 25×250 mm) eluting with 4% MeOH-CH_2Cl_2 (8.0 ml min^{-1}) to give 22 mg (22% from **16a**) of crystalline **1a**. This was recrystallized from hexane-ether to give **1a** as needles: mp 168—170 °C; $[\alpha]_D^{25}$+48° (*c* 0.07, EtOH) [lit,[10] mp 169—170 °C; $[\alpha]_D^{25}$+47.2° (*c* 0.2, EtOH)]; IR (KBr) 3400, 1640, 1460, 1380, 1370, 1350, 1300, 1265, 1220, 1140, 1055, 975 cm^{-1}; ^1H NMR δ=0.56 (3H, s, 18-H_3), 1.00 (3H, d, *J*=6.8 Hz, 28-H_3), 1.04 (3H, d, *J*=6.8 Hz, 21-H_3), 1.13 and 1.17 (6H, each s, 26-H_3 and 27-H_3), 4.23 (1H, m, 3-H), 4.42 (1H, m, 1-H), 5.00 (1H, narrow m, 19-H), 5.32 (3H, m, 19-H, 22-H and 23-H), 6.01 (1H, d, *J*=10.6 Hz, 7-H), 6.38 (1H, d, *J*=10.6 Hz, 6-H); MS *m/z* (rel intensity) 428(M^+; 5) 410(9), 392(12), 352(6), 269(10), 197(15), 152(23), 134(100); UV (EtOH) 265 nm (ε 16400). Found: *m/z* 428.3271. Calcd for $C_{28}H_{44}O_3$: M, 428.3292.

(24*R*,22*E*)-5α,8α-(4-Phenyl-3,5-dioxo-1,2,4-triazolidine-1,2-diyl)-25-tetrahydropyranyloxy-6,22-ergostadiene-1α,3β-diol (14b). In the same manner as described for **14a**, **12** (1.50 g, 2.5 mmol) and **9b** (1.62 g, 5.0 mmol) was converted in two steps to 0.46 g (27%) of **14b**: IR (KBr) 3430, 1750, 1690, 1605, 1505, 1410, 1310, 1135, 1030, 985 cm^{-1}; ^1H NMR δ=3.50 (1H, m, 6′-H), 3.71 (1H, m, 1-H), 3.90 (1H, m, 6′-H), 4.75 (1H, m, 2′-H), 4.85 (1H, m, 3-H), 5.15—5.40 (2H, m, 22-H and 23-H), 6.19 and 6.35 (2H, ABq, *J*=8.3 Hz, 6-H and 7-H), 7.2—7.4 (5H, m, Ph); MS *m/z* (rel intensity) 428 (M^+—PTAD—DHP; 10), 410 (7), 350 (12), 177 (40), 119 (100).

(24*R*,22*E*)-5α,8α-(4-Phenyl-3,5-dioxo-1,2,4-triazolidine-1,2-diyl)-6,22-ergostadiene-1α,3β,25-triol (15b). This was prepared in the same manner as described for **15a**, and recrystallization from ether-EtOH gave an analytical sample as needles: mp 212—215 °C; $[\alpha]_D^{23}$—95.6° (*c* 0.25); IR (KBr) 3530, 3450, 1745, 1680, 1505, 1420, 1155, 1035 cm^{-1}; ^1H NMR δ=0.83 (3H, s, 18-H_3), 0.92 (3H, s, 19-H_3), 0.98 (3H, d, *J*=7.1 Hz, 28-H_3), 1.04 (3H, d, *J*=6.4 Hz, 21-H_3), 1.12 and 1.15 (6H, each s, 26-H_3 and 27-H_3), 1.21 (t, *J*=7.1 Hz, Et_2O), 3.14 (1H, dd, *J*=13.7 and 4.4 Hz, 9-H), 3.48 (q, *J*=7.1 Hz, Et_2O), 3.83 (1H, m, 1-H), 4.90 (1H, m, 3-H), 5.32 (2H, m, 22-H and 23-H), 6.25 and 6.40 (2H, ABq, *J*=8.1 Hz, 6-H and 7-H), 7.30—7.45 (5H, m, Ph); MS *m/z* (rel intensity) 428 (M^+—PTAD; 22), 350 (16), 324 (48), 251 (20), 177 (75), 119 (100). Found: C, 70.63; H, 8.34; N, 6.62%. Calcd for $C_{36}H_{49}N_3O_5 \cdot 1/4\ Et_2O$: C, 71.41; H, 8.34; N, 6.75%.

(24*R*,22*E*)-5,7,22-Ergostatriene-1α,3β,25-triol (16b). In the same manner as described for **16a**, **14b** (0.87 g, 1.3 mmol) was converted in two steps to 0.31 g (57%) of **16b**: mp 214—217 °C (from EtOH, needles); $[\alpha]_D^{24}$—16.2° (*c* 0.14, MeOH); IR (KBr) 3510, 3360, 1660, 1610, 1465, 1385, 1145, 1075, 970 cm^{-1}; ^1H NMR δ=0.65 (3H, s, 18-H_3), 0.95 (3H, s, 19-H_3), 0.99 (3H, d, *J*=6.8 Hz, 28-H_3), 1.05 (3H, d, *J*=6.8 Hz, 21-H_3), 1.13 and 1.17 (6H, each s, 26-H_3 and 27-H_3), 3.77 (1H, m, 1-H), 4.08 (1H, m, 3-H), 5.35 (3H, m, 7-H, 22-H and 23-H), 5.74 (1H, m, 6-H); MS *m/z* (rel intensity) 428 (M^+; 42), 251 (60), 225 (62), 157 (100), 145 (80); UV (EtOH) 282 nm (ε 11700). Found: C, 78.24; H, 10.37%. Cacld for $C_{28}H_{44}O_3$: C, 78.48; H, 10.35%.

(24*R*)-1α,25-Dihydroxyvitamin D_2 (1b). A solution of **16b** (100 mg, 0.23 mmol) in ether-THF (19:1, 1000 ml) was irradiated for 3 min under Ar at water-cooled temperature with a high pressure mercury lamp (Ushio, UM-452) using 1.5% KNO_3 solution as a filter. The mixture was next concentrated in vacuo. The residue was chromatographed over HPLC (Merck, LiChrosorb® Si60 (7 μm), 25×250 mm) eluting with 5% MeOH-CH_2Cl_2 (8.0 ml min^{-1}) to give 28 mg (28%) of the previtamin D (**17b**): ^1H NMR δ=0.72 (3H, s, 18-H_3), 0.99 (3H, d, *J*=6.8 Hz, 28-H_3), 1.05 (3H, d, *J*=6.6 Hz, 21-H_3), 1.13 and 1.17 (6H, each s, 26-H_3 and 27-H_3), 1.76 (3H, s, 19-H_3), 4.05 (1H, m, 3-H), 4.19 (1H, m, 1-H), 5.31 (2H, m, 22-H and 23-H), 5.51 (1H, m, 9-H), 5.76 and 5.92 (2H, ABq, *J*=12.9 Hz, 6-H and 7-H).

A solution of **17b** (28 mg) in EtOH (15 ml) was stirred for 1 h at reflux temperature. The mixture was concentrated in vacuo. The residue was chromatographed on HPLC (Merck, LiChrosorb® Si60 (7 μm), 25×250 mm) eluting with 5% MeOH-CH_2Cl_2 (7.0 ml min^{-1}) to give 11.8 mg (42%: 12% from **16b**) of **1b**. This was recrystallized from hexane-CH_2Cl_2 to give **1b** as rods: mp 150—152 °C [lit,[9] a colorless oil]; $[\alpha]_D^{23}$+74° (*c* 0.16, EtOH); ^1H NMR δ=0.56 (3H, s, 18-H_3), 0.99 (3H, d, *J*=6.8 Hz, 28-H_3), 1.03 (3H, d, *J*=6.6 Hz, 21-H_3), 1.12 and 1.17 (6H, each s, 26-H_3 and 27-H_3), 4.22 (1H, m, 3-H), 4.42 (1H, m, 1-H), 4.99 (1H, narrow m, 19-H), 5.32 (3H, m, 19-H, 22-H and 23-H), 6.01 (1H, d, *J*=11.2 Hz, 7-H), 6.37 (1H, d, *J*=11.2 Hz, 6-H); MS *m/z* (rel intensity) 428 (M^+; 6), 410 (7), 392 (10), 352 (5), 334 (16), 269 (9), 251 (10), 134 (100), 105 (46); UV (EtOH) 265 nm (ε 16900).

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19) The (*E*)-geometry was finally confirmed by the single-crystal X-ray analysis of **16a**. This will be reported elsewhere.