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Synthesis of 26,27-Dialkyl Analogues of 1α,25-Dihydroxyvitamin D_3

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1α,25-Dihydroxy-26,27-dimethylvitamin D_3 (**5**), 1α,25-dihydroxy-26,27-diethylvitamin D_3 (**6**), 25-hydroxy-26,27-dimethylvitamin D_3 (**7**), and 1α-hydroxy-26,27-dimethylvitamin D_3 (**8**), as dialkyl analogues of 1α,25-dihydroxyvitamin D_3 (**1**), were synthesized starting from 3β-hydroxycholenic acid (**9**). When tested for ability to increase serum calcium concentration in rats, **5** was slightly less active than **1**, whereas **6** was much less active. Compound **8** was less active than 1α-hydroxyvitamin D_3. In test for ability to induce cell differentiation of HL-60, **5** was 2.5 times and **6** was 12.5 times more active than **1**.

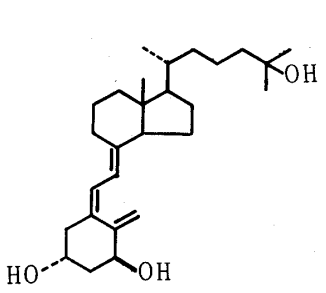
Keywords——vitamin D_3 analogue; 1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxy-26,27-dimethylvitamin D_3; 1α,25-dihydroxy-26,27-diethylvitamin D_3; 25-hydroxy-26,27-dimethylvitamin D_3; 1α-hydroxy-26,27-dimethylvitamin D_3; serum calcium concentration; cell differentiation

It is now well established that 1α,25-dihydroxyvitamin D_3 (**1**) is a hormonal metabolite of vitamin D_3, which mediates calcium and phosphorus metabolism.[2] Since its discovery, much effort has been made to synthesize many analogues of vitamin D_3 and its metabolites with the aim of increasing and separating the biological activities.[3] We previously reported the synthesis and biological activities of 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (**2**)[4] and 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3 (**3**),[5] as fluorinated analogues of 1α,25-dihydroxyvitamin D_3. These fluorinated compounds were 5—10 times more active than **1** in various vitamin D bioassays including the ability to increase serum calcium concentration in rats. It has been postulated that hydroxylation at the C-24 or C-26 position, which is a step in deactivation of the active form of vitamin D_3 (**1**), is blocked by fluorine substitution. We envisaged that such a hydroxylation could also be blocked by substitution with methyl groups, and prepared 1α,25-dihydroxy-24,24-dimethyl-22*E*-dehydrovitamin D_3 (**4**)[6] and 1α,25-dihydroxy-26,27-dimethylvitamin D_3 (**5**)[7] to test for vitamin D activities. However, both compounds were slightly less active than **1** in increasing serum calcium concentration in vitamin D-deficient rats.[8]

A potent alkylated analogue of **1** was discovered in our independent study on the metabolism of 24-*epi*-25-hydroxyvitamin D_2.[9] The new metabolite, 1α,25-dihydroxy-26-methyl-22*E*-vitamin D_2 (26-homo-Δ^{22}-1α,25-dihydroxyvitamin D_3) was more active than **1** in the assay described above.

Recently it has been reported that the active form of vitamin D_3 (**1**) possesses the ability to induce differentiation of malignant cells.[10] In tests of many analogues of **1** for activities, a parallelism was usually observed between the binding affinity to the receptor of **1** and the induction of differentiation.[11] There is an important possibility that **1** or one of its analogues might be effective in causing differentiation of malignant cells *in vivo*. However, it seems that **1** would induce severe hypercalcemia at the concentrations required to induce myeloid differentiation *in vivo*. Consequently, a compound having a strong effect on cell differentiation

without showing calcium-regulating activity would be very desirable for a possible therapeutic use. It is therefore of interest to investigate the biological activities of 26,27-dialkyl analogues of **1**. Thus, we prepared 1α,25-dihydroxy-26,27-dimethylvitamin D_3 (**5**) and 1α,25-dihydroxy-26,27-diethylvitamin D_3 (**6**), and as deshydroxy compounds of **5** we also synthesized 25-hydroxy-26,27-dimethylvitamin D_3 (**7**) and 1α-hydroxy-26,27-dimethylvitamin D_3 (**8**). Here we report the synthesis of these compounds **5**—**8** in full detail. The results of preliminary biological tests of these compounds are briefly described. Although the synthesis of **5** was previously reported by our group,[7] we describe herein an alternative, more convenient synthesis of **5**.



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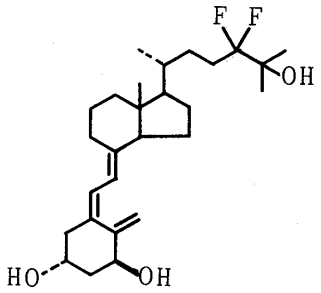

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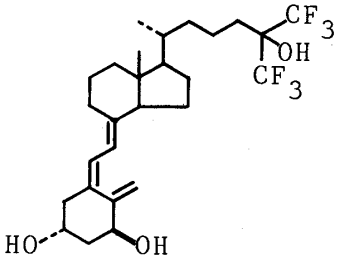

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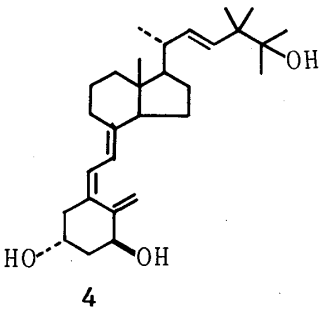

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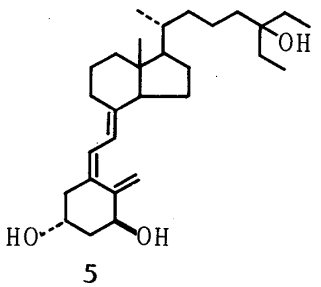

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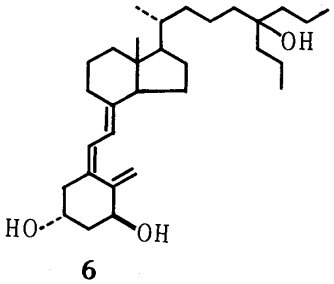

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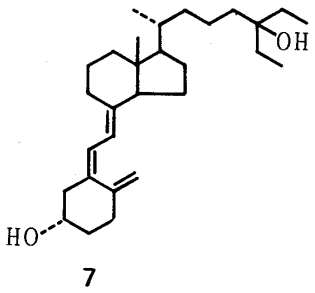

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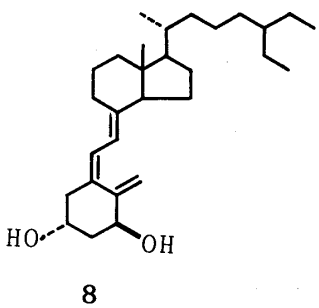

8

Our synthetic plan for the 1α-hydroxy compounds **5**—**7** is as follows: the 1α-hydroxy group is first introduced into a commercially available steroid, the side chain is next constructed, and finally conversion into vitamin D form is performed. According to this plan, we chose 3β-hydroxycholenic acid (**9**) as a starting material.

3β-Hydroxycholenic acid (**9**) was converted into the triol derivative **12** through the same procedure as used for the transformation of bisnorcholenic acid to the corresponding triol derivative.[6,7] Thus, the seven-step sequence of reactions afforded **12** in 15% yield: reduction

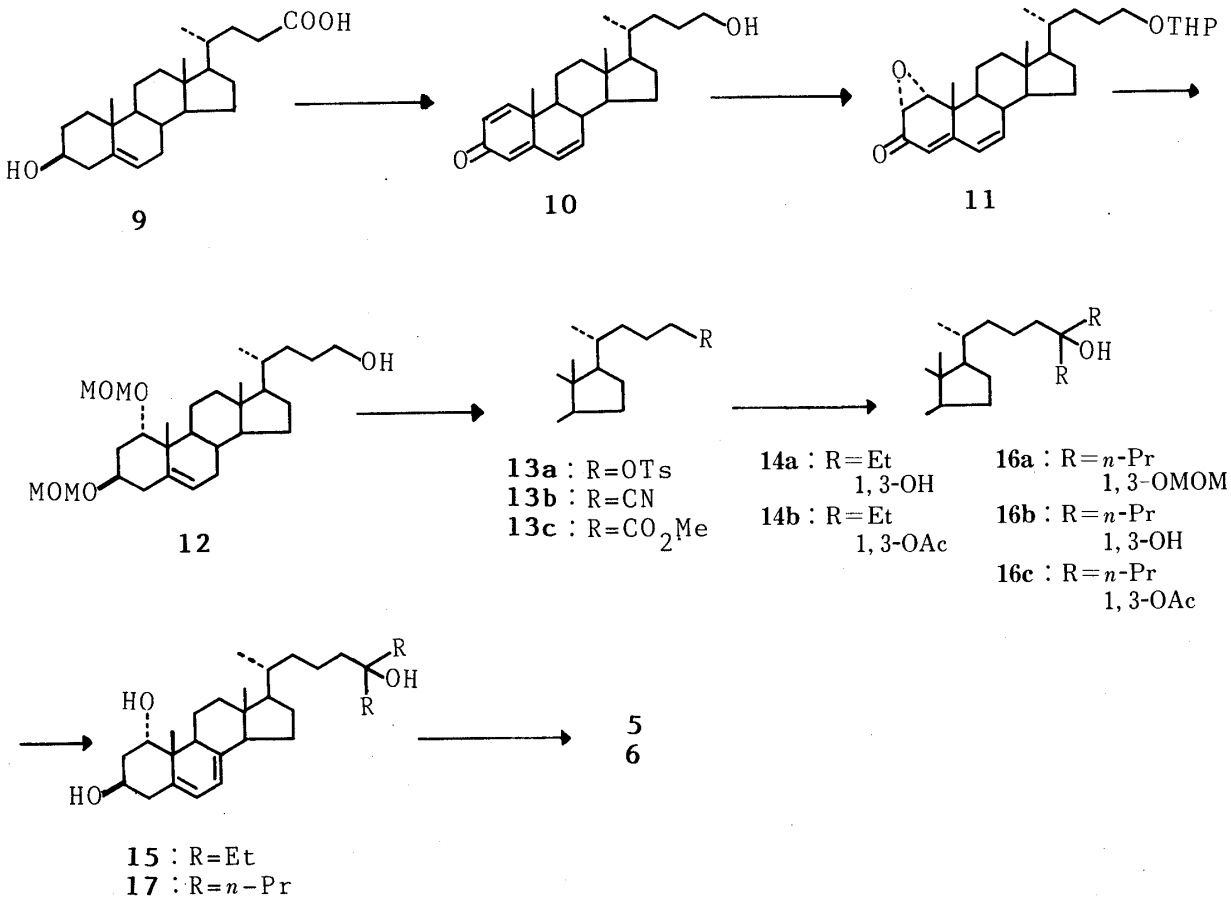

Chart 1

with lithium aluminium hydride, oxidation with dichlorodicyanobenzoquinone (leading to **10**), protection of the hydroxy group as the tetrahydropyranyl (THP) ether, epoxidation with alkaline H_2O_2 (leading to **11**), Birch reduction,[12] protection of the 1α- and 3β-hydroxy groups with methoxymethyl ether, and deprotection of the THP group under mild acidic conditions. The compound **12** was converted into the cyanide **13b** *via* the tosylate **13a** (tosyl chloride–pyridine, NaCN in dimethylsulfoxide at 90—100 °C) in 62% yield. Alkaline hydrolysis of **13b** followed by esterification with diazomethane provided the methyl ester **13c** in 87% yield. Grignard reaction of **13c** with ethylmagnesium bromide and removal of the methoxymethyl group with 6 N HCl in aqueous tetrahydrofuran (THF) at 50 °C gave 1α,25-dihydroxy-26,27-dimethylcholesterol (**14a**) in 91% yield. The corresponding acetate **14b** was transformed into vitamin D form. Thus, bromination of **14a** with *N*-bromosuccinimide, dehydrobromination with tetra-*n*-butylammonium fluoride[13] and alkaline hydrolysis gave the 5,7-diene **15** in 26% yield. Irradiation of **15** with a medium-pressure mercury lamp through a Vycor filter in benzene and ethanol at 0 °C under an argon atmosphere and subsequent thermal isomerization under reflux provided **5** in 23% yield.

We next synthesized the 26,27-diethyl analogue **6** from the same intermediate **13c**. The methyl ester **13c** was treated with propylmagnesium bromide to give the 25-ol **16a** in 81% yield. Deprotection of the methoxymethyl groups of **16a** was not successful under usual acidic conditions (a trace amount of concentrated HCl in methanol[14] or 6 N HCl–THF at 50 °C[15]) due to elimination of the 25-hydroxy group, but was achieved under milder acidic conditions (6 N HCl–THF, room temperature, 72 h), affording the 1,3,25-triol **16b** in 82% yield. The corresponding diacetate **16c** was transformed into **6** in the same manner as described for **14b**.

The synthesis of 25-hydroxy-26,27-dimethylvitamin D_3 (**7**), an analogue of 25-hy-

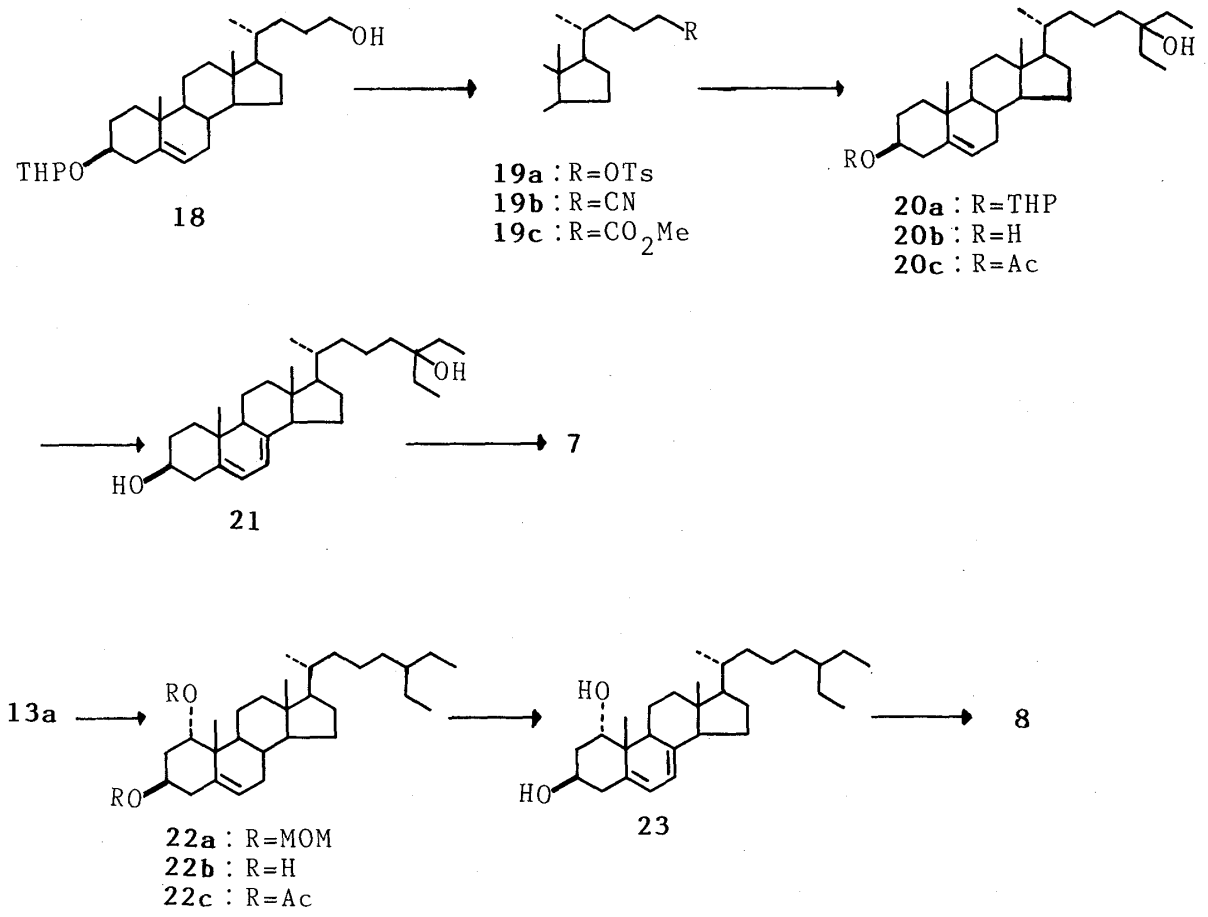

Chart 2

droxyvitamin D_3, was started with the known 24-alcohol **18**,[16] which is easily available from cholenic acid. The construction of the side chain was carried out by essentially the same procedure as used for **12**. Thus, compound **18** was converted into the 3,25-diol **20b** in 17% total yield in 6 steps: tosylation with *p*-toluenesulfonyl chloride in pyridine, substitution of the tosyl group by a cyano group, alkaline hydrolysis of the cyano group, methylation with diazomethane, Grignard reaction with ethylmagnesium bromide and deprotection of the THP group under acidic conditions. The compound **20b** was converted into the acetate **20c**, which was then transformed into **7** in the same manner as described above.

The last target, 1α-hydroxy-26,27-dimethylvitamin D_3 (**8**), an analogue of 1α-hydroxyvitamin D_3, was synthesized from 24-tosylate **13a**. Reaction of **13a** with 3-pentylmagnesium bromide in the presence of Li_2CuCl_4[17] provided the coupling product **22a** in 93% yield. Conversion of the protecting groups of **22a** gave the diacetate **22c** in high yield. The diacetate **22c** was transformed into **8** as described above.

All vitamin D_3 analogues **5**—**8** synthesized in the present work exhibited ultraviolet (UV) absorption characteristic of vitamin D_3 form (λ_{max} 264.5—265 nm, λ_{min} 228 nm). The structures of these compounds were also supported by their mass spectra, *e.g.* molecular ion peak and fragment ion peaks at *m/z* 152 and 134 (fission of C-7/C-8 bond) (in the case of **7**, 136 and 118), and proton nuclear magnetic resonance (^{1}H-NMR) spectra.

Biological Activities

In tests for ability to increase serum calcium concentration in vitamin D-deficient rats,[18] the dimethyl compound **5** was slightly less active than **1**. When **5** was administered to the rats at a dosage of 500 pmol/kg, serum calcium concentration was increased to 6.11 ± 0.15 mg/dl

(control group, 4.75 ± 0.08 mg/dl), whereas in the case of **1**, the level of calcium concentration was 6.39 ± 0.17 mg/dl at the same dosage. The diethyl compound **6** was much less active, since **6** showed no significant increase of calcium concentration at a dosage of 1000 pmol/kg in the same experiment.

However, strong activities of **5** and **6** were observed for cell growth inhibition and cell differentiation of human promyelocytic leukemia cells, HL-60.[19] Thus, **5** was about 2.5 times and **6** was about 10 times more active than **1**.[20] The compound **8** was less active than 1α-hydroxyvitamin D_3 in bone calcium mobilization. Details of the biological activities of **5**—**8** will be reported elsewhere.

We have reported that 24-homo- and 26-homo-1α,25-dihydroxyvitamin D_3 and their Δ^{22} analogues were about ten-fold more potent than **1** in inducing differentiation of HL-60 cells *in vitro*.[21] However, in the mobilization of bone calcium, the 24-homo analogues were significantly less active than **1**, whereas the 26-homo analogues were more active, as described above. In *in vivo* experiments, vitamin D activities were decreased as larger alkyl groups were introduced. The binding affinities of the dialkylated analogues to the vitamin D receptor may be decreased by their steric hindrance at the 25-position. It can be concluded that the distance between the two hydroxy groups at the 1α- and 25-positions may be critical for the vitamin D activity. Tight binding between the substance and vitamin D receptor is also important.

It should be noted that introduction of alkyl groups at the C-26/27 terminal and side chain elongation as shown in 24-homo analogues enhance the activity to induce cell differentiation. Such a modification in the steroid side chain could be promising for drug design, as evidenced by compound **6**, for the separation of vitamin D actions, *e.g.* calcium regulation *vs.* cell differentiation.[22]

Experimental

Melting points were determined on a hot stage with a microscope and are uncorrected. UV spectra were obtained in ethanol solution with a Shimadzu UV-200 double-beam spectrometer. ^{1}H-NMR spectra were taken on a Hitachi R-24A (60 MHz), JEOL PS-100 (100 MHz), Varian XL-200 (200 MHz) or JEOL GX-400 (400 MHz) spectrometer in $CDCl_3$ solution using tetramethylsilane as an internal standard. Electron impact mass spectra (MS) were obtained with a Shimadzu LKB-9000S or Hitachi M-80 spectrometer at 20 or 70 eV. Infrared (IR) spectra were taken with a JASCO DS-701G spectrometer. Preparative thin layer chromatography (p-TLC) was performed on 20 × 20 cm glass plates coated with a 0.25 mm layer of Kieselgel 60 F_{254} (E. Merck). Column chromatography was carried out on Kieselgel 60 (E. Merck, 70—230 mesh). THF refers to tetrahydrofuran, and THP to tetrahydropyran. Extractive work-up refers to dilution with water (or the indicated solution), extraction with the given organic solvent, washing of the extract to neutrality, drying over $MgSO_4$, filtration, and removal of the solvent under reduced pressure.

24-Hydroxychola-1,4,6-trien-3-one (10)——A mixture of **9** (20 g) and lithium aluminium hydride (4 g) in THF (500 ml) was stirred at 60 °C for 18 h. Ethyl acetate and water were carefully added to destroy the excess reagent. Filtration to remove insoluble materials and evaporation of the solvent gave a crude 3,24-diol. Dichlorodicyanobenzoquinone (43.5 g) was added to the diol dissolved in dioxane (450 ml) and the mixture was refluxed for 15 h. After cooling to room temperature, the precipitates were filtered off and washed several times with dioxane. The filtrate and washings were combined and concentrated to dryness. The residue was applied to a column of alumina. Elution with dichloromethane gave a crude product. Further purification by column chromatography on silica gel with hexane–ethyl acetate (1 : 1) as an eluent gave **10** (7.9 g, 42%), oil. ^{1}H-NMR δ: 0.78 (3H, s, 18-H_3), 0.92 (3H, d, J=6 Hz, 21-H_3), 1.19 (3H, s, 19-H_3), 3.75 (2H, t, J=6 Hz, 24-H_2), 5.85—6.35 (4H, m, 2-, 4-, 6- and 7-H), 7.05 (1H, d, J=11 Hz, 1-H). MS m/z: 354 (M^+), 339, 205, 128.

1α,2α-Epoxy-24-tetrahydropyranyloxychola-4,6-dien-3-one (11)——A solution of **10** (7.5 g), dihydropyran (5 ml) and a catalytic amount of *p*-toluenesulfonic acid in dichloromethane (150 ml) was stirred at room temperature for 30 min. Addition of saturated aqueous $NaHCO_3$ solution and extractive (ethyl acetate) work-up gave a crude THP ether. A solution of 10% NaOH in methanol (2.5 ml) and 30% H_2O_2 (15 ml) were added to the THP ether dissolved in methanol (200 ml), and the mixture was stirred at room temperature for 1.5 h. Extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with hexane–ethyl acetate (5 : 1) as an eluent to afford **11** (6.9 g, 72%), oil. UV λ_{max} nm (ε): 290 (22000). ^{1}H-NMR δ: 0.77 (3H, s, 18-H_3), 0.93 (3H, d, J=5.6 Hz, 21-H_3), 1.16 (3H, s, 19-H_3), 3.38 (1H, dd, J=4.4, 1.5 Hz, 2-H), 3.55 (1H, d, J=4.4 Hz, 1-H), 4.54 (1H, s, 2′-H of THP), 5.60 (1H, d, J=1.6 Hz, 4-H), 6.05 (2H, s, 6- and 7-H). MS m/z: 454 (M^+), 370, 353, 337, 85.

1α,3β-Bis(methoxymethoxy)chol-5-en-24-ol (12)——Lithium (9.5 g) was added in small portions to liquid ammonia (300 ml) at −78 °C under argon during 30 min. The mixture was stirred for 1 h at −78 °C, then **11** (3.8 g) in dry THF (300 ml) was added dropwise at −78 °C during 1.5 h. Anhydrous NH_4Cl (300 g) was added to this reaction mixture in small portions at −78 °C during 2 h. The cooling bath was removed and most of the ammonia was evaporated off by bubbling argon into the mixture. Careful addition of water and extractive (ethyl acetate) work-up gave a crude product. A mixture of the product, chloromethyl methyl ether (2.2 ml) and *N,N*-diethylcyclohexylamine (6.73 ml) in dioxane (20 ml) was stirred at 50 °C for 6 h and then at 80 °C for 2 h. Extractive (ethyl acetate) work-up gave a crude bismethoxymethoxy ether. A mixture of the ether and 2 N HCl (3 ml) in THF (10 ml) and methanol (10 ml) was stirred at room temperature overnight. Addition of saturated aqueous $NaHCO_3$ solution and extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with hexane–ethyl acetate (2 : 1) as an eluent. Recrystallization from ether–petroleum ether gave **12** (912 mg, 23%) as colorless plates, mp 88—89 °C. ^{1}H-NMR δ: 0.69 (3H, s, 18-H_3), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 3.36 (3H, s, OCH_3), 3.40 (3H, s, OCH_3), 3.62 (2H, m, 24-H_2), 3.75 (1H, m, 1-H), 3.86 (1H, m, 3-H), 4.60 and 4.76 (2H, each d, J=7 Hz, OCH_2O), 4.68 (2H, s, OCH_2O), 5.67 (1H, m, 6-H). IR (KBr): 3450 cm^{-1}. *Anal.* Calcd for $C_{28}H_{48}O_5$: C, 72.37; H, 10.41. Found: C, 72.42; H, 10.12.

1α,3β-Bis(methoxymethoxy)-24-*p*-toluenesulfonyloxychol-5-ene (13a)——A mixture of **12** (833.4 mg) and *p*-toluenesulfonyl chloride (445 mg) in pyridine (5 ml) was stirred at 0 °C for 30 min and allowed to stand in a refrigerator (*ca.* 2 °C) for 2 d. Extractive (ethyl acetate) work-up gave a crude product which was chromatographed over silica gel with hexane–ethyl acetate (5 : 1) as an eluent to give **13a** (813 mg, 73%), oil. ^{1}H-NMR δ: 0.65 (3H, s, 18-H_3), 0.88 (3H, d, J=6 Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 2.45 (3H, s, CH_3Ph), 3.36 (3H, s, OCH_3), 3.40 (3H, s, OCH_3), 3.75 (1H, m, 1-H), 3.86 (1H, m, 3-H), 4.01 (2H, t, J=6 Hz, 24-H_2), 4.60 and 4.76 (2H, each d, J=7 Hz, OCH_2O), 4.68 (2H, s, OCH_2O), 5.57 (1H, m, 6-H), 7.36 and 7.81 (2H × 2, each d, J=9 Hz, Ar-H). IR (neat): 1590, 1465 cm^{-1}. *Anal.* Calcd for $C_{35}H_{54}O_7S$: C, 67.93; H, 8.79. Found: C, 67.97; H, 8.52.

1α,3β-Bis(methoxymethoxy)chol-5-en-24-yl Cyanide (13b)——A mixture of **13a** (258.4 mg) and NaCN (30.7 mg) in dimethylsulfoxide (10 ml) was heated under nitrogen at 90—100 °C for 4 h. Extractive (ethyl acetate) work-up gave a crude product which was chromatographed over silica gel with benzene–ethyl acetate (25 : 1) as an eluent. Recrystallization from ether–hexane gave **13b** (168 mg, 85%) as colorless needles, mp 92—95 °C. ^{1}H-NMR δ: 0.69 (3H, s, 18-H_3), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 2.32 (2H, t, J=7 Hz, 24-H_2), 3.36 (3H, s, OCH_3), 3.40 (3H, s, OCH_3), 3.75 (1H, m, 1-H), 3.86 (1H, m, 3-H), 4.59 and 4.76 (2H, each d, J=7 Hz, OCH_2O), 4.68 (2H, s, OCH_2O), 5.56 (1H, m, 6-H). IR (KBr): 2240 cm^{-1}. *Anal.* Calcd for $C_{29}H_{47}NO_4$: C, 73.53; H, 10.00; N, 2.96. Found: C, 73.27; H, 9.71; N, 3.04.

1α,3β-Bis(methoxymethoxy)-26,27-bisnorcholest-5-en-25-oic Acid Methyl Ester (13c)——A mixture of **13b** (174 mg) in 30% aqueous KOH solution (2 ml) and ethanol (20 ml) was refluxed under nitrogen for 2 d. Acidification with 2 N HCl and extractive (chloroform) work-up gave a crude product which was dissolved in a small amount of methanol. An excess of ethereal diazomethane was added and the mixture was left to stand at room temperature for 1 h. Removal of the solvent and column chromatography with benzene–ethyl acetate (20 : 1) as an eluent gave **13c** (162 mg, 87%), oil. ^{1}H-NMR δ: 0.70 (3H, s, 18-H_3), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.04 (3H, s, 19-H_3), 3.37 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.68 (3H, s, CO_2CH_3), 3.76 (1H, br s, 1-H), 3.88 (1H, m, 3-H), 4.60 and 4.77 (2H, each d, J=7 Hz, OCH_2O), 4.69 (2H, s, OCH_2O), 5.57 (1H, m, 6-H). IR (neat): 1730 cm^{-1}. MS *m/z*: 444 ($M^+ - CH_3OCH_2OH$), 412, 384, 382, 255.

26,27-Dimethylcholest-5-ene-1α,3β,25-triol (14a)——Ethylmagnesium bromide (0.2 ml, 3 M solution in THF) was added to a solution of **13c** (80 mg) in THF (4 ml). The mixture was stirred at room temperature for 1 h. Addition of saturated aqueous NH_4Cl solution and extractive (ether) work-up gave a crude product, which was dissolved in THF (2 ml), and 6 N HCl (0.4 ml) was added. The mixture was heated at 50 °C for 2 h. Extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with hexane–ethyl acetate (1 : 1) as an eluent to afford **14a** (50 mg, 91%), mp 115—116 °C. ^{1}H-NMR δ: 0.70 (3H, s, 18-H_3), 0.85 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.90 (3H, d, J=6 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 3.80 (1H, m, 1-H), 3.95 (1H, m, 3-H), 5.56 (1H, m, 6-H).

1α,3β-Diacetoxy-26,27-dimethylcholest-5-en-25-ol (14b)——A solution of **14a** (40 mg) and acetic anhydride (0.1 ml) in pyridine (0.5 ml) was stirred at room temperature for 16 h. Water was added and the mixture was stirred for 30 min. Extractive (ether) work-up gave a crude product, which was chromatographed over silica gel with hexane–ethyl acetate (2 : 1) as an eluent to afford **14b** (44 mg, 93%), oil. ^{1}H-NMR δ: 0.65 (3H, s, 18-H_3), 0.86 (6H, t, J=7 Hz, 26- and 27-CH_3), 1.07 (3H, s, 19-H_3), 2.01 (3H, s, acetyl), 2.03 (3H, s, acetyl), 4.97 (1H, m, 3-H), 5.03 (1H, m, 1-H), 5.48 (1H, m, 6-H).

1α,25-Dihydroxy-26,-27-dimethylvitamin D_3 (5)——A solution of **14b** (16 mg) and *N*-bromosuccinimide (7 mg) in carbon tetrachloride (2 ml) was refluxed under argon for 20 min. After cooling with ice-water, the insoluble materials were filtered off and washed with carbon tetrachloride. The filtrate and washings were combined and evaporated to dryness. The residue was dissolved in THF (5 ml) and a catalytic amount of $(n\text{-Bu})_4NBr$ was added. The mixture was stirred under argon at room temperature. After 50 min, a solution of $(n\text{-Bu})_4NF$ in THF (0.12 ml, 1 M solution) was added and stirring was continued for 30 min. Extractive (ethyl acetate) work-up gave a crude product. This was dissolved in THF (2 ml), and a solution of 5% KOH in methanol (2 ml) was added. The mixture was stirred at room

temperature for 14 h. Extractive (ethyl acetate) work-up gave a crude product, which was purified by p-TLC with benzene–ethyl acetate (1 : 1, developed five times) as a developing solvent to afford **15** (3.5 mg, 26%). UV λ_{max} nm: 294, 282, 272. A solution of **15** (3.5 mg) in benzene (90 ml) and ethanol (40 ml) was irradiated with an ultraviolet lamp (Hanovia 654A; 200W) through a Vycor filter under argon with ice-cooling for 2.5 min. Then, the solution was refluxed for 1 h. After removal of the solvent, the residue was purified by p-TLC with benzene–ethyl acetate (1 : 1, developed three times) as a developing solvent to give **5** (0.80 mg, 23%). UV λ_{max} nm: 265, λ_{min} nm: 228. ^{1}H-NMR δ: 0.55 (3H, s, 18-H_3), 0.86 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.93 (3H, d, J=6 Hz, 21-H_3), 1.45 (4H, q, J=7 Hz, 26- and 27-CH_2), 4.22 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.01 (1H, br s, 19*Z*-H), 5.33 (1H, br s, 19*E*-H), 6.03 (1H, d, J=11 Hz, 7-H), 6.38 (1H, d, J=11 Hz, 6-H). MS *m/z*: 444 (M^+), 408, 390, 375, 269, 251, 157, 152, 134, 116, 87. High-resolution MS Calcd for $C_{29}H_{48}O_3$: 444.3605. Found: 444.3597.

1α,3β-Bis(methoxymethoxy)-26,27-diethylcholest-5-en-25-ol (16a)——*n*-Propyl bromide (0.76 ml) was added to a suspension of magnesium (204.4 mg) in THF (5 ml) under nitrogen. The mixture was stirred at room temperature for 20 min and cooled to 0 °C. A solution of **13c** (142 mg) in THF (5 ml) was added and the resulting mixture was stirred under nitrogen at 0 °C for 30 min and then at room temperature for 4.5 h. Addition of saturated aqueous NH_4Cl solution and extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with benzene–ethyl acetate (25 : 1) as an eluent to afford **16a** (127 mg, 81%), oil. ^{1}H-NMR δ: 0.69 (3H, s, 18-H_3), 0.92 (6H, t, J=6 Hz, 26- and 27-$CH_2C\underline{H}_3$), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 3.36 (3H, s, OCH_3), 3.41 (3H, s, OCH_3), 3.76 (1H, br s, 1-H), 3.87 (1H, m, 3-H), 4.60 and 4.76 (2H, each d, J=7 Hz, OCH_2O), 4.68 (2H, s, OCH_2O), 5.57 (1H, m, 6-H). IR (neat): 3450 cm^{-1}. MS *m/z*: 500 ($M^+ - CH_3OCH_2OH$), 482, 468, 456, 440, 438, 422, 115.

26,27-Diethylcholest-5-ene-1α,3β,25-triol (16b)——A solution of **16a** (31.4 mg) and 6 N HCl (1 ml) in THF (5 ml) was stirred at room temperature for 72 h. Addition of saturated aqueous $NaHCO_3$ solution and extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with hexane–ethyl acetate (5 : 1—3 : 1) as an eluent. Recrystallization from acetone–hexane gave **16b** (21.6 mg, 82%) as a colorless powder, mp 76—79 °C. ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.92 (6H, t, J=6.3 Hz, 26- and 27-$CH_2C\underline{H}_3$), 0.93 (3H, d, J=6.8 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 3.85 (1H, br s, 1-H), 3.99 (1H, m, 3-H), 5.60 (1H, m, 6-H). IR (KBr): 3350 cm^{-1}. MS *m/z*: 474 (M^+), 456, 438, 420, 395, 289, 271, 253. *Anal.* Calcd for $C_{31}H_{54}O_3$: C, 78.42; H, 11.47. Found: C, 78.16; H, 11.58.

1α,3β-Diacetoxy-26,27-diethylcholest-5-en-25-ol (16c)——The triol **16b** (21.6 mg) was converted into **16c** (19 mg, 74%) in the same manner as described for **14a** except that solvents for extraction and chromatography were ethyl acetate and hexane–ethyl acetate (10 : 1), respectively. **16c**: oil. ^{1}H-NMR δ: 0.66 (3H, s, 18-H_3), 0.92 (6H, t, J=7.1 Hz, 26- and 27-$CH_2C\underline{H}_3$), 0.92 (3H, d, J=6.2 Hz, 21-H_3), 2.00 (3H, s, acetyl), 2.05 (3H, s, acetyl), 4.92 (1H, m, 3-H), 5.06 (1H, br s, 1-H), 5.53 (1H, m, 6-H). IR (neat): 1735 cm^{-1}. MS *m/z*: 348 ($M^+ - 2CH_3COOH$), 420, 395, 324, 302, 253, 118.

26,27-Diethyl-1α,25-dihydroxyvitamin D_3 (6)——The diacetate **16c** (18 mg) was converted into **17** (2.89 mg, 19%) in the same manner as described for **14b** except that the reaction time with *N*-bromosuccinimide was 20 min and the solvent for p-TLC was benzene–ethyl acetate (2 : 1, developed six times). **17**: UV λ_{max} nm: 294, 282, 272. The 5,7-diene **17** (2.89 mg) was converted into **6** (0.47 mg, 16%) in the same manner as described for **15**. **6**: UV λ_{max} nm: 264.5, λ_{min} nm: 228. ^{1}H-NMR δ: 0.54 (3H, s, 18-H_3), 0.88 (6H, t, J=6.7 Hz, 26- and 27-$CH_2C\underline{H}_3$), 0.93 (3H, d, J=5.7 Hz, 21-H_3), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.01 (1H, br s, 19*Z*-H), 5.33 (1H, br s, 19*E*-H), 6.02 (1H, d, J=11 Hz, 7-H), 6.38 (1H, d, J=11 Hz, 6-H). MS *m/z*: 472 (M^+), 454, 436, 410, 393, 269, 251, 152, 134, 115. High-resolution MS Calcd for $C_{31}H_{52}O_3$: 472.3919. Found: 472.3903.

3β-Tetrahydropyranyloxy-24-*p*-toluenesulfonyloxychol-5-ene (19a)——Compound **18** (5 g) was converted into **19a** in the same manner as described for **12** except that the solvent for chromatography was benzene–ethyl acetate (100 : 1). Recrystallization from acetone–hexane afforded **19a** (4.17 g, 62%) as colorless needles, mp 108—110 °C. ^{1}H-NMR δ: 0.64 (3H, s, 18-H_3), 0.88 (3H, d, J=6 Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 2.26 (3H, s, $C\underline{H}_3$Ph), 3.51 (2H, m, 6′-H_2 of THP), 3.92 (1H, m, 3-H), 4.02 (2H, t, J=8 Hz, 24-H_2), 4.73 (1H, m, 2′-H of THP), 5.36 (1H, m, 6-H), 7.37 and 7.82 (2H×2, each d, J=7 Hz, Ar-H). IR (KBr): 2920, 1590, 1460, 1375 cm^{-1}. *Anal.* Calcd for $C_{36}H_{54}O_5S$: C, 72.20; H, 9.09. Found: C, 72.28; H, 8.87.

3β-Tetrahydropyranyloxychol-5-en-24-yl Cyanide (19b)——Compound **19a** (1.31 g) was converted into **19b** (662.5 mg, 65%) in the same manner as described for **13a** except that the solvents for chromatography and recrystallization were benzene and acetone–hexane, respectively. **19b**: colorless needles, mp 139—140 °C. ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.97 (3H, d, J=6 Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 3.52 (2H, m, 6′-H_2 of THP), 3.92 (1H, m, 3-H), 4.72 (1H, m, 2′-H of THP), 5.36 (1H, m, 6-H). IR (KBr): 2220 cm^{-1}. *Anal.* Calcd for $C_{30}H_{47}NO_2$: C, 79.42; H, 10.44; N, 3.09. Found: C, 79.56; H, 10.22; N, 3.14.

3β-Tetrahydropyranyloxy-26,27-dinorcholest-5-en-25-oic Acid Methyl Ester (19c)——Compound **19b** (718 mg) was converted into **19c** (579 mg, 75%) in the same manner as described for **13b** except that the reaction time with diazomethane was 2 h and the solvents for chromatography and recrystallization were benzene and methanol, respectively. **19c**: colorless needles, mp 158—160 °C. ^{1}H-NMR δ: 0.67 (3H, s, 18-H_3), 0.93 (3H, d, J=6 Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 3.51 (2H, m, 6′-H_2 of THP), 3.67 (3H, s, CO_2CH_3), 3.91 (1H, m, 3-H), 4.72 (1H, m, 2′-H of THP), 5.35 (1H, m, 6-H), IR (KBr): 1740 cm^{-1}. *Anal.* Calcd for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.53; H, 10.06.

3β-Tetrahydropyranyloxy-26,27-dimethylcholest-5-en-25-ol (20a)——Ethylmagnesium bromide (2 ml, 3 M so-

lution in THF) was added to a solution of **19c** (562 mg) in THF (10 ml) at 0 °C. The mixture was stirred at 0 °C for 2 h. Addition of saturated aqueous NH_4Cl solution and extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with benzene as an eluent. Recrystallization from acetone–hexane afforded **20a** (456 mg, 77%) as a colorless powder: mp 122—123 °C. ^{1}H-NMR δ: 0.67 (3H, s, 18-H_3), 0.85 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.93 (3H, d, J=6 Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 1.46 (q, J=7 Hz, 26- and 27-H_2), 3.51 (2H, m, 6′-H_2 of THP), 3.91 (1H, m, 3-H), 4.72 (1H, m, 2′-H of THP), 5.35 (1H, m, 6-H). IR (KBr): 3440 cm^{-1}. *Anal.* Calcd for $C_{34}H_{58}O_3$: C, 79.32; H, 11.36. Found: C, 79.61; H, 11.50.

26,27-Dimethylcholest-5-ene-3β,25-diol (20b)——A solution of **20a** (495.9 mg) and 2 N HCl (5 drops) in THF (10 ml) and methanol (10 ml) was stirred at room temperature for 2 h. Addition of saturated aqueous $NaHCO_3$ solution and extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with benzene–ethyl acetate (50 : 1) as an eluent. Recrystallization from acetone–hexane afforded **20b** (308 mg, 74%) as a colorless powder, mp 145.5—147.5 °C. ^{1}H-NMR δ: 0.66 (3H, s, 18-H_3), 0.86 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 1.47 (q, J=7 Hz, 26- and 27-H_2), 3.54 (1H, m, 3-H), 5.37 (1H, m, 6-H). IR (KBr): 3340 cm^{-1}. *Anal.* Calcd for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.97; H, 11.84.

3β-Acetoxy-26,27-dimethylcholest-5-en-25-ol (20c)——The diol **20b** (258.2 mg) was converted into **20c** in the same manner as described for **14a** except that solvents for extraction and chromatography were ethyl acetate and hexane–ethyl acetate (5 : 1), respectively. Recrystallization from aqueous ethanol afforded **20c** (208 mg, 73%) as an amorphous powder, mp 104—107 °C. ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.87 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.95 (3H, d, J=6 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 1.48 (q, J=7 Hz, 26- and 27-H_2), 2.04 (3H, s, acetyl), 4.63 (1H, m, 3-H), 5.40 (1H, m, 6-H). IR (KBr): 3380, 1730 cm^{-1}. *Anal.* Calcd for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 78.42; H, 11.09.

25-Hydroxy-26,27-dimethylvitamin D_3 (7)——The acetate **20c** (52.2 mg) was converted into **21** (7.60 mg, 16%) in the same manner as described for **14b** except that the solvent for p-TLC was hexane–ethyl acetate (3 : 1, developed five times). **21**: UV λ_{max} nm: 294, 282, 272. The diene **21** (7.60 mg) was converted into **7** (1.43 mg, 19%) in the same manner as described for **17** except that the solvent for p-TLC was benzene–ethyl acetate (1 : 1, developed three times). **7**: UV λ_{max} nm 265, λ_{min} nm: 228. ^{1}H-NMR δ: 0.54 (3H, s, 18-H_3), 0.858 and 0.860 (6H, each t, J=7.6 Hz, 26- and 27-CH_3), 0.97 (3H, d, J=6.6 Hz, 21-H_3), 1.47 (q, J=7.6 Hz, 26- and 27-H_2), 3.95 (1H, m, 3-H), 4.82 (1H, br s, 19*E*-H), 5.05 (1H, br s, 19*Z*-H), 6.03 (1H, d, J=11 Hz, 7-H), 6.24 (1H, d, J=11 Hz, 6-H). MS *m/z*: 428 (M^+), 410, 395, 271, 253, 136, 118, 87. High-resolution MS Calcd for $C_{29}H_{48}O_2$: 428.3656. Found: 428.3642.

1α,3β-Bis(methoxymethoxy)-26,27-dimethylcholest-5-ene (22a)——3-Pentyl bromide (2.92 ml) was added to a suspension of magnesium (572 mg) in THF (10 ml) under nitrogen. The mixture was stirred at room temperature for 30 min and cooled to 0 °C. A solution of Li_2CuCl_4 in THF [3.24 ml, prepared from LiCl (85 mg) and $CuCl_2$ (135 mg) in THF (10 ml)] and then a solution of **20a** (485.4 mg) in THF (5 ml) were added. The mixture was stirred under nitrogen at 0 °C for 1 h. Addition of saturated aqueous NH_4Cl solution and extractive (ethyl acetate) work-up gave a crude product, which was chromatographed with hexane–ethyl acetate (50 : 1—25 : 1) as an eluent to afford **22a** (378 mg, 93%), oil. ^{1}H-NMR δ: 0.69 (3H, s, 18-H_3), 0.89 (6H, t, J=7.4 Hz, 26- and 27-CH_3), 0.91 (3H, d, J=6.4 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 3.75 (1H, m, 1-H), 3.85 (1H, m, 3-H), 4.59 and 4.75 (2H, each d, J=6.9 Hz, OCH_2O), 4.67 (2H, s, OCH_2O), 5.56 (1H, m, 6-H). IR (neat): 2930, 1460, 1380 cm^{-1}. *Anal.* Calcd for $C_{33}H_{58}O_4$: C, 76.40; H, 11.27. Found: C, 76.53; H, 10.90.

26,27-Dimethylcholest-5-ene-1α,3β-diol (22b)——A solution of **22a** (358 mg) and concentrated HCl (4 drops) in methanol (20 ml) was refluxed for 3 h. Extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with hexane–ethyl acetate (3 : 1) as an eluting solvent. Recrystallization from methanol afforded **22b** (155 mg, 52%) as colorless needles, mp 142—144 °C. ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.83 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.92 (3H, d, J=6 Hz, 21-H_3), 1.04 (3H, s, 19-H_3), 3.86 (1H, m, 1-H), 4.01 (1H, m, 3-H), 5.62 (1H, m, 6-H). IR (KBr): 3380 cm^{-1}. *Anal.* Calcd for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.55; H, 11.33.

1α,3β-Diacetoxy-26,27-dimethylcholest-5-ene (22c)——A solution of **22b** (214 mg), acetic anhydride (0.4 ml) and 4-dimethylaminopyridine (6 mg) in pyridine was stirred at room temperature for 4 h. Extractive (ethyl acetate) work-up gave a crude product, which was chromatographed over silica gel with hexane–ethyl acetate (3 : 1) as an eluting solvent to afford **22c** (244 mg, 95%) as an oil. ^{1}H-NMR δ: 0.66 (3H, s, 18-H_3), 0.83 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.90 (3H, d, J=6 Hz, 21-H_3), 1.08 (3H, s, 19-H_3), 2.03 (3H, s, acetyl), 2.05 (3H, s, acetyl), 4.92 (1H, m, 3-H), 5.06 (1H, m, 1-H), 5.53 (1H, m, 6-H). IR (neat): 1735 cm^{-1}. *Anal.* Calcd for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57. Found: C, 76.77; H, 10.33.

1α-Hydroxy-26,27-dimethylvitamin D_3 (8)——The acetate **22c** (65.5 mg) was converted into **23** (6.55 mg, 12%) in the same manner as described for **20c** except that the reaction time with *N*-bromosuccinimide was 30 min. **23**: UV λ_{max} nm: 294, 282, 272. The diene **23** (6.5 mg) was converted into **8** (0.74 mg, 11%) in the same manner as described for **17**. **8**: UV λ_{max} nm: 265, λ_{min} nm: 228. ^{1}H-NMR δ: 0.54 (3H, s, 18-H_3), 0.83 (6H, t, J=7 Hz, 26- and 27-CH_3), 0.92 (3H, d, J=6.1 Hz, 21-H_3), 4.24 (1H, m, 3-H), 4.36 (1H, m, 1-H), 5.01 (1H, br s, 19*Z*-H), 5.33 (1H, br s, 19*E*-H), 6.03 (1H, d, J=11 Hz, 7-H), 6.39 (1H, d, J=11 Hz, 7-H). MS *m/z*: 428 (M^+), 410, 392, 287, 269, 251, 152, 134. High-resolution MS Calcd for $C_{29}H_{48}O_3$: 428.3656. Found: 428.3630.

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