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# Synthesis of Some Side-Chain Homologues of 1α,25-Dihydroxyvitamin $D_3$ and Investigation of Their Biological Activities

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In order to determine the structure of a new metabolite of 25-hydroxy-24-epivitamin $D_2$, 1,25-dihydroxy-22-dehydro-24-homo- and 26-homo-vitamin $D_3$ were synthesized. The saturated analogues at the C-22 position of these homovitamin $D_3$ compounds were also synthesized. For the construction of the side chain of these homo-analogues, the orthoester Claisen rearrangement of the allylic alcohol was employed.

Assay of binding of these compounds with the chick intestinal cytosol receptor protein for 1,25-$(OH)_2D_3$ was carried out by the displacement method. The results indicated that these homovitamin $D_3$ analogues are as active as 1,25-$(OH)_2D_3$. The bone calcium-mobilizing activities of these compounds were equivalent to that of 1,25-$(OH)_2D_3$.

**Keywords**——vitamin D analogue; bone calcium mobilization; 1,25-$(OH)_2D_3$ chick intestinal receptor; 1,25-dihydroxy-22-dehydro-24-homovitamin $D_3$; 1,25-dihydroxy-22-dehydro-26-homovitamin $D_3$; 1,25-dihydroxy-24-homovitamin $D_3$; 1,25-dihydroxy-26-homovitamin $D_3$

## Introduction

Vitamin $D_3$ undergoes metabolic hydroxylation to produce a number of metabolites, among which 1,25-dihydroxyvitamin $D_3$ (1,25-$(OH)_2D_3$), a hormonal form of vitamin $D_3$, exhibits the highest biological activity of all vitmain $D_3$ metabolites.[2)] In recent years, a great amount of synthetic effort has been directed toward the synthesis of vitamin $D_3$ metabolites and fluorine-substituted vitamin $D_3$ analogues in order to investigate the biological importance of the metabolism of vitamin $D_3$ and to obtain new analogues with high biological activity or without the full spectrum of vitamin $D_3$ activities.[2)]

During the course of our investigation into the metabolism of 25-hydroxy-24-epivitamin $D_2$, we isolated a new biologically active metabolite.[3)] The mass spectrum (MS) of the metabolite suggested that it could be either 1,25-dihydroxy-2-dehydro-24-homovitamin $D_3$ (1,25-$(OH)_2$-$\Delta^{22}$-24-homo-$D_3$) (**8**) or 1,25-dihydroxy-22-dehydro-26-homovitamin $D_3$ (1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$) (**18**). In order to determine the structure of the new metabolite, we chemically synthesized both compounds. We have also synthesized their C-22 saturated analogues, 1,25-$(OH)_2$-24-homo-$D_3$ (**11**) and 1,25-$(OH)_2$-26-homo-$D_3$ (**21**). The synthetic procedures and the results of preliminary studies of the biological activities of these compounds are reported in this paper.

### Syntheses

For construction of the side-chain skeletons of these homovitamin $D_3$ analogues, we employed to orthoester Claisen rearrangement of the allylic alcohol **2** as a key reaction. Coupling of the known 22-aldehyde **1**[4,5)] with vinylmagnesium bromide gave an epimeric mixture of the allylic alcohol **2** in 94% yield, and this was used as such in the ensuing orthoester Claisen rearrangement. The 22*E*-dehydro-24-homo-ester **3** was obtained in 93% yield by treatment of **2** with an excess of triethyl orthoacetate in the presence of a catalytic amount of propionic acid in refluxing xylene. The ester **3** was treated with methylmagnesium bromide and subsequently hydrolyzed with 6 M HCl at 50 °C to provide the triol **5** in 80% yield.

Transformation of the triol **5** into 1,25-(OH)$_2$-$\Delta^{22}$-24-homo-D$_3$ (**8**) was carried out as follows. The 1,3-diacetate **6**, obtained by acetylation of **5**, was brominated at the C-7 position with *N*-bromosuccinimide in refluxing carbon tetrachloride and then dehydrobrominated

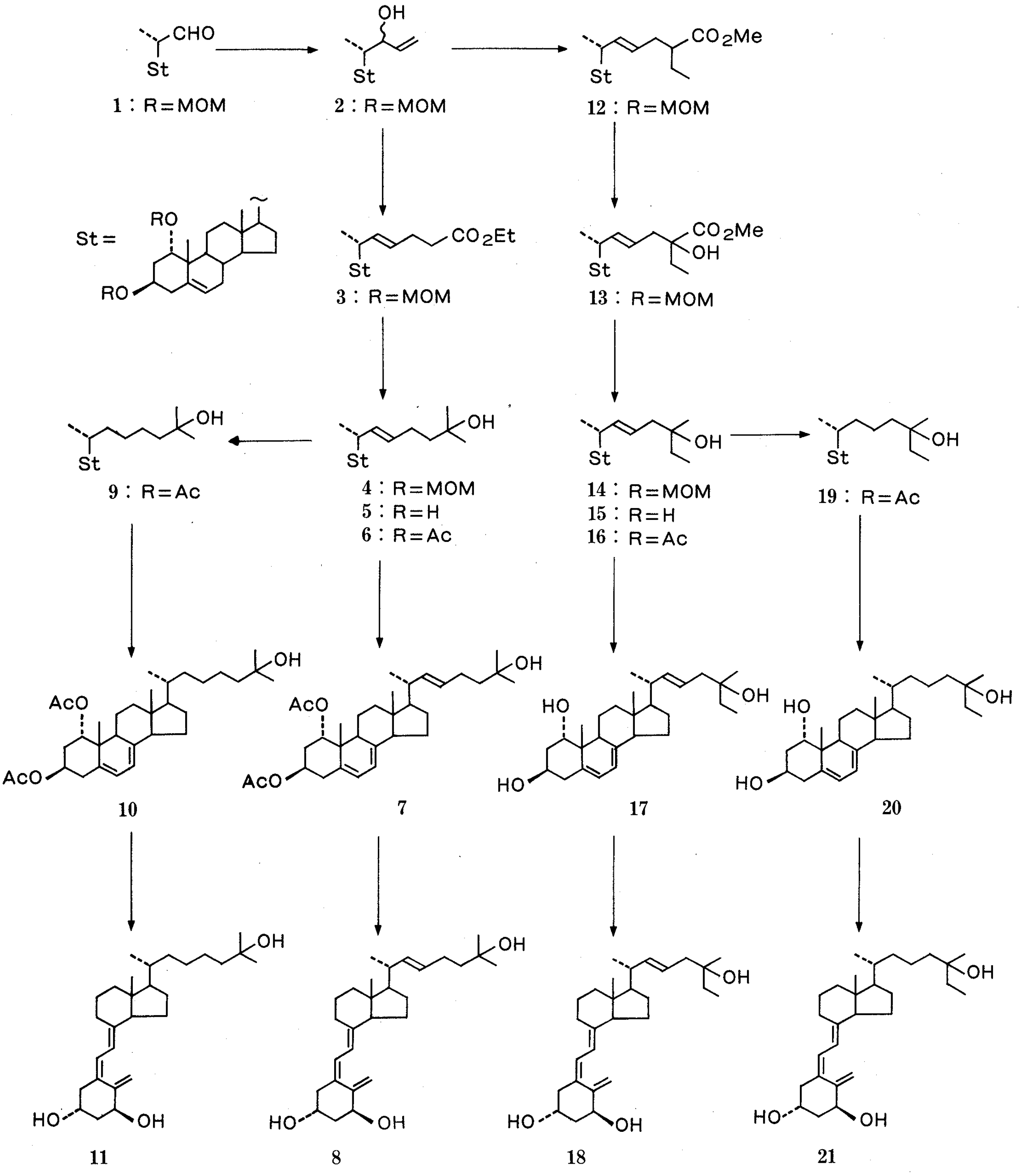

Chart 1

with tetra-*n*-butylammonium fluoride. Purification by preparative thin layer chromatography (TLC) gave the 5,7,22-triene **7** in 24% yield. Compound **7** was irradiated with ultraviolet (UV) light through a Vycor filter in a mixed solvent (benzene–ethanol, 2 : 1) at 0 °C for 5 min and the resulting mixture containing previtamin was refluxed for 1 h. The vitamin $D_3$ diacetate, isolated in 25% yield, was saponified with 5% KOH/MeOH to provide 1,25-$(OH)_2$-$\Delta^{22}$-24-homo-$D_3$ (**8**) in 90% yield. Its structure was supported by the UV, proton nuclear magnetic resonance ($^1$H-NMR), and mass spectra.

For the synthesis of 1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$ (**18**), the allylic alcohol **2** was treated with trimethyl ortho-*n*-butyrate in place of triethyl orthoacetate to give the 22*E*-dehydro-26-homo-ester **12** in 97% yield. Introduction of a hydroxyl group at the C-25 position was achieved in 67% yield by treatment of the enolate of the ester **12** with oxygen followed by reduction with triethylphosphite.[6] The hydroxy ester **13**, obtained as a mixture of C-25 epimers, was converted into the carbinol **14** by successive reactions of $LiAlH_4$ reduction, mesylation, and then $LiAlH_4$ reduction again. Removal of the protecting group provided the triol **15**, which was transformed into 1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$ (**18**) as described for **8**. The structure of **18** was established by measurement of the UV, $^1$H-NMR, and mass spectra.

The C-22 saturated vitamin $D_3$ homologues **11** and **21** were prepared as follows. Selective hydrogenation at the C-22 double bond of **6** and **16** was achieved in ethyl acetate by using a 10% Pd–C catalyst under a hydrogen atmosphere in 92% and 85% yields, respectively. The resulting 5-ene compounds, **9** and **19**, were converted *via* the 5,7-dienes, **10** and **20**, into 1,25-$(OH)_2$-24-homo-$D_3$ (**11**) and 1,25-$(OH)_2$-26-homo-$D_3$ (**21**), respectively, as described for **8**.

It was found that the UV absorption and mass spectra of the synthetic 1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$ (**18**) are identical with those of the new biologically active metabolite of 25-hydroxy-24-epivitamin $D_2$. The synthetic analogue comigrated with the metabolite on both normal- and reversed-phase high-performance liquid chromatography (HPLC). The details

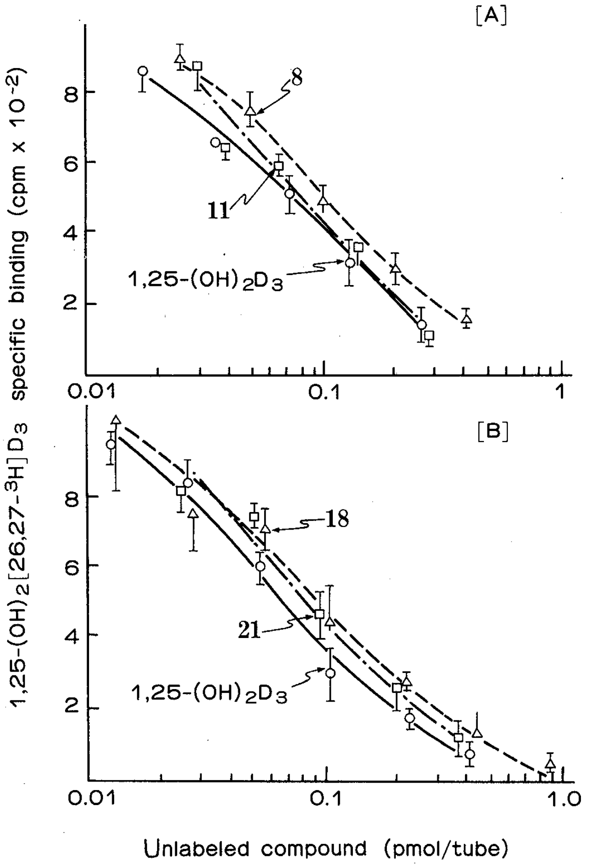

Fig. 1. Displacement of 1,25-$(OH)_2$-[26,27-$^3$H]-$D_3$ from the Chick Intestinal Cytosol Receptor Protein for 1,25-$(OH)_2D_3$ by 1,25-$(OH)_2$-$\Delta^{22}$-24-homo-$D_3$ (**8**), 1,25-$(OH)_2$-24-homo-$D_3$ (**11**), 1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$ (**18**), and 1,25-$(OH)_2$-26-homo-$D_3$ (**21**)

will be published elsewhere.[3)]

**Biological Activities**

The biological activities of side-chain analogues of 25-hydroxyvitamin $D_3$, including 24-homo derivatives, have been investigated by Okamura *et al.*[7,8)] They reported that lengthening or particularly shortening the side-chain of 25-hydroxyvitamin $D_3$ by as little as one methylene group dramatically depressed the vitamin $D_3$ activities. Since the biological activities of the side-chain homologues of 1,25-$(OH)_2D_3$ have not been reported, the 24- and 26-homologues synthesized as described above were assayed using intestinal receptor for 1,25-$(OH)_2D_3$. Displacement studies were carried out as described by Shepard *et al.*[9)] The results show that the 24-homo analogues, despite the C-22 double bond, are as active as 1,25-$(OH)_2D_3$ in interacting with the chick intestinal cytosol receptor protein (Fig. 1A). The 26-homologues are also equally active, as shown in Fig. 1B. It is, therefore, apparent that the addition of one carbon, either on the 26-terminal methyl or within the side chain, does not modify the interaction with the receptor protein. These compounds are approximately equal in biological activity to 1,25-$(OH)_2D_3$, suggesting a considerable tolerance of the receptor site for an enlarged side chain.

The effects of the homologues on calcium metabolism *in vivo* were assessed by measuring their bone calcium-mobilizing activity in vitamin D-deficient rats. As shown in Tables I and II, the results demonstrated that 1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$ (**18**) and 1,25-$(OH)_2$-26-homo-$D_3$ (**21**) were as active as 1,25$(OH)_2D_3$. On the other hand, 1,25-$(OH)_2$-$\Delta^{22}$-24-homo-$D_3$ (**8**) and 1,25-$(OH)_2$-24-homo-$D_3$ (**11**) were slightly less active than 1,25-$(OH)_2D_3$. It is noteworthy that synthetic 1,25-$(OH)_2$-26,27-bishomovitamin $D_3$ exhibited more potent activity than 1,25-$(OH)_2D_3$.[5)] The extremely high potency of those homologues for inducing

Table I. Increase of Serum Calcium Concentration in Response to 1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$ (**18**), 1,25-$(OH)_2$-26-homo-$D_3$ (**21**), and 1,25-$(OH)_2D_3$

| Compound administered | Amount administered (pmol/rat) | Serum calcium concentration (mg/100 ml) |
|---|---|---|
| Ethanol | | $3.4 \pm 0.3^{a)}$ |
| 1,25-$(OH)_2$-$\Delta^{22}$-26-homo-$D_3$ (**18**) | 650 | $4.6 \pm 0.3^{b)}$ |
| 1,25-$(OH)_2$-26-homo-$D_3$ (**21**) | 650 | $4.6 \pm 0.2^{b)}$ |
| 1,25-$(OH)_2D_3$ | 650 | $4.5 \pm 0.2^{b)}$ |

Standard deviation of the mean. Significantly different: *b*) from *a*), $p < 0.001$.

Table II. Increase of Serum Calcium Concentration in Response to 1,25-$(OH)_2$-$\Delta^{22}$-24-homo-$D_3$ (**8**), 1,25-$(OH)_2$-24-homo-$D_3$, and 1,25-$(OH)_2D_3$

| | Compound administered | Amount administered (pmol/rat) | Serum calcium concentration (mg/100 ml) |
|---|---|---|---|
| Exp. I | Ethanol | | $4.2 \pm 0.1^{a)}$ |
| | 1,25-$(OH)_2$-$\Delta^{22}$-24-homo-$D_3$ (**8**) | 650 | $5.0 \pm 0.5^{b)}$ |
| | 1,25-$(OH)_2D_3$ | 325 | $5.0 \pm 0.5^{b)}$ |
| Exp. II | Ethanol | | $3.6 \pm 0.3^{c)}$ |
| | 1,25-$(OH)_2$-24-homo-$D_3$ (**11**) | 650 | $4.4 \pm 0.2^{d)}$ |
| | 1,25-$(OH)_2D_3$ | 650 | $4.9 \pm 0.2^{d)}$ |

Standard deviation of the mean. Significantly different: *b*) from *a*), and *d*) from *c*), $p < 0.001$.

the differentiation of leukemic cells to normal macrophages will be reported in our forthcoming paper.[10)]

## Experimental

**General Directions**——Melting points were determined with a hot-stage microscope and are uncorrected. $^1$H-NMR spectra were taken with a Hitachi R-24A (60 MHz) in $CDCl_3$ with $Me_4Si$ as an internal standard, unless otherwise noted. Mass spectra were obtained with a Shimadzu QP-1000 mass spectrometer at 70 eV. UV spectra were obtained in ethanol solution with a Shimadzu UV-200 double-beam spectrophotometer. Column chromatography was done on silica gel (E. Merck, Kieselgel 60, 70—230 mesh). Preparative thin layer chromatography was carried out on precoated plates of silica gel (E. Merck, Kieselgel 60 $F_{254}$, 0.25 mm thickness). The usual work-up refers to dilution with water, extraction with an organic solvent indicated in parenthesis, washing the extract to neutrality, drying over anhydrous magnesium sulfate, filtration, and removal of the solvent under reduced pressure. The following abbreviations are used; THP, tetrahydropyranyl; THF, tetrahydrofuran; ether, diethyl ether; MeOH, methanol.

**1α,3β-Bis(methoxymethoxy)chola-5,23-dien-22-ol (2)**——A solution of vinyl bromide in THF (50%, 0.42 ml, 2.98 mmol) was added to magnesium (70 mg, 2.92 mmol) in THF (3 ml). The mixture was stirred at room temperature under argon for 30 min. To the resulting Grignard reagent, the aldehyde **1** (595 mg, 1.37 mmol) in THF (6 ml) was added at room temperature. The mixture was stirred at room temperature for 1 h, and the usual work-up (ether) gave a crude product, which was applied to a column of silica gel (30 g). Elution with hexane–ethyl acetate (3 : 1) gave the allylic alcohol **2** (595 mg, 94%) as an amorphous solid. $^1$H-NMR $\delta$: 0.70 (3H, s, 18-$H_3$), 1.02 (3H, s, 19-$H_3$), 3.35 (3H, s, –O–$CH_3$), 3.38 (3H, s, –O–$CH_3$), 3.69 (1H, m, 1β-H), 4.20 (1H, m, 22-H), 4.64 (2H, ABq, $J=7$ Hz, $\Delta AB=11$ Hz, 1α-O–$CG_2$–O–), 4.65 (2H, s, 3β-O–$CH_2$–O–), 5.52 (1H, m, 6-H), 4.90—6.0 (3H, m, 23-H and 24-$H_2$). MS *m/z* (%): 430 ($M^+ -$MeOH, 1.6), 400 (15), 368 (8.2), 340 (100), 338 (77), 283 (20), 253 (5.2). (Found: $M^+ -$MeOH, 430.3103. $C_{27}H_{42}O_4$ requires $M^+ -$MeOH, 430.3085).

**(22*E*)-1α,3β-Bis(methoxymethoxy)-27-norcholesta-5,22-dien-26-oic Acid Ethyl Ester (3)**——A solution of the allylic alcohol **2** (590 mg, 1.28 mmol), triethyl orthoacetate (1.0 ml, 5.46 mmol), propionic acid (4 drops), and xylene (8 ml) was refluxed under argon for 2 h. Removal of the solvent under reduced pressure gave the residue, which was applied to a column of silica gel (30 g). Elution with hexane–ethyl acetate (4 : 1) gave the ester **3** (630 mg, 93%) as an oil. $^1$H-NMR $\delta$: 0.68 (3H, s, 18-$H_3$), 0.97 (3H, d, $J=6$ Hz, 21-$H_3$), 1.03 (3H, s, 19-$H_3$), 1.24 (3H, t, $J=7$ Hz, –$CO_2CH_2CH_3$), 3.35 (3H, s, –O–$CH_3$), 3.39 (3H, s, –O–$CH_3$), 3.70 (1H, m, 1β-H), 4.11 (2H, q, $J=7$ Hz, –$CO_2CH_2CH_3$), 4.64 (2H, ABq, $J=7$ Hz, $\Delta AB=11$ Hz, 1α-O–$CH_2$–O–), 4.65 (2H, s, 3β-O–$CH_2$–O–), 5.29 (2H, m, 22-H and 23-H), 5.52 (1H, m, 6-H). MS *m/z* (%): 500 ($M^+ -$MeOH, 2.0), 470 (15), 455 (3.8), 438 (7.8), 410 (100), 408 (63), 255 (28), 253 (13), 155 (79). (Found: $M^+ -$MeOH, 500.3499. $C_{31}H_{48}O_5$ requires $M^+ -$MeOH, 500.3503).

**(22*E*)-1α,3β-Bis(methoxymethoxy)-24-homocholesta-5,22-dien-25-ol (4)**——Methylmagnesium bromide (1 M solution in THF; 4.5 ml, 4.5 mmol) was added to a solution of the ester **3** (605 mg, 1.14 mmol) in THF (6 ml) at room temperature. The mixture was stirred at room temperature for 1 h. The usual work-up (ether) gave a crude product, which was applied to a column of silica gel (30 g). Elution with hexane–ethyl acetate (3 : 1) gave the alcohol **4** (538 mg, 93%) as an oil. $^1$H-NMR $\delta$: 0.68 (3H, s, 18-$H_3$), 0.97 (3H, d, $J=6$ Hz, 21-$H_3$), 1.01 (3H, s, 19-$H_3$), 1.21 (6H, s, 26-$H_3$ and 27-$H_3$), 3.33 (3H, s, –O–$CH_3$), 3.38 (3H, s, –O–$CH_3$), 3.70 (1H, m, 1β-H), 4.64 (2H, ABq, $J=7$ Hz, $\Delta AB=11$ Hz, 1α-O–$CH_2$–O–), 4.65 (2H, s, 3β-O–$CH_2$–O–), 5.29 (2H, m, 22-H and 23-H), 5.50 (1H, m, 6-H). MS *m/z* (%): 486 ($M^+ -$MeOH, 2.2), 436 (21), 396 (100), 394 (84), 255 (30), 253 (13), 123 (58). (Found: $M^+ -$MeOH, 486.3717. $C_{31}H_{50}O_4$ requires $M^+ -$MeOH, 486.3711).

**(22*E*)-24-Homocholesta-5,22-diene-1α,3β,25-triol (5)**——A solution of the dimethoxymethyl ether **4** (540 mg, 1.04 mmol) in THF (15 ml) was treated with 6 M HCl (3 ml) at 50 °C for 2.5 h. The usual work-up (ethyl acetate) gave a crude product, which was applied to a column of silica gel (20 g). Elution with hexane–ethyl acetate (1 : 1) gave the triol **5** (428 mg, 95%) as crystals, mp 164—166 °C (hexane–ethyl acetate). $^1$H-NMR $\delta$: 0.68 (3H, s, 18-$H_3$), 0.95 (3H, d, $J=6$ Hz, 21-$H_3$), 1.00 (3H, s, 19-$H_3$), 1.20 (6H, s, 26-$H_3$ and 27-$H_3$), 3.80 (1H, m, 1β-H), 3.92 (1H, m, 3α-H), 5.30 (2H, m, 22-H and 23-H), 5.53 (1H, m, 6-H). MS *m/z* (%): 430 ($M^+$, 15), 412 (96), 396 (12), 394 (30), 379 (12), 289 (38), 271 (39), 253 (49), 123 (100). (Found: C, 78.03; H, 10.66. $C_{28}H_{46}O_3$ requires C, 78.09; H, 10.77).

**(22*E*)-1α,3β-Diacetoxy-24-homocholesta-5,22-dien-25-ol (6)**——A solution of the triol **5** (395 mg, 0.919 mmol) in pyridine (2 ml) was treated with acetic anhydride (1 ml) at room temperature for 16 h. The usual work-up (ethyl acetate) gave a crude product, which was applied to a column of silica gel (20 g). Elution with hexane–ethyl acetate (2 : 1) gave the diacetate **6** (361 mg, 77%) as an oil. $^1$H-NMR $\delta$: 0.67 (3H, s, 18-$H_3$), 0.97 (3H, d, $J=6$ Hz, 21-$H_3$), 1.07 (3H, s, 19-$H_3$), 1.21 (6H, s, 26-$H_3$ and 27-$H_3$), 2.01 (3H, s, acetyl), 2.04 (3H, s, acetyl), 4.98 (1H, m, 3α-H), 5.05 (1H, m, 1β-H), 5.31 (2H, m, 22-H and 23-H), 5.52 (1H, m, 6-H).

**(22*E*)-1α,3β-Diacetoxy-24-homocholesta-5,7,22-trien-25-ol (7)**——A solution of the 5,22-diene **6** (51 mg, 0.0992 mmol) and *N*-bromosuccinimide (21 mg, 0.118 mmol) in carbon tetrachloride (3 ml) was refluxed under argon for 20 min. After the mixture had been cooled to 0 °C, the resulting precipitate was filtered off. The filtrate was concentrated below 40 °C to leave the residue, which was taken up in THF (5 ml) and treated with a catalytic amount of tetra-*n*-butylammonium bromide at room temperature for 50 min. Then, the mixture was treated with a solution of tetra-*n*-butylammonium fluoride in THF (3.5 ml, 3.5 mmol) at room temperature for 30 min. The usual work-up (ethyl acetate) gave a crude product, which was submitted to preparative TLC (hexane–ethyl acetate, 4 : 1, developed five times). The band of *Rf* 0.48 was scraped off and the product was extracted with ethyl acetate. Removal of the solvent provided the 5,7,22-triene **7** (12.5 mg, 24%). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 293, 282, 271.

**(22*E*)-1α,25-Dihydroxy-22-dehydro-24-homovitamin D$_3$ (8)**——A solution of the 5,7,22-triene **7** (7.3 mg, 0.0143 mmol) in benzene (90 ml) and ethanol (40 ml) was irradiated with a medium-pressure mercury lamp through a Vycor filter at 0 °C under argon for 5 min. The reaction mixture was refluxed under argon for 1 h. Removal of the solvent under reduced pressure gave a crude product, which was submitted to preparative TLC (hexane–ethyl acetate, 4 : 1, developed five times). The band of *Rf* 0.38 was scraped off and the product was extracted with ethyl acetate. Removal of the solvent gave the vitamin D$_3$ diacetate (1.8 mg, 25%). The band of *Rf* 0.43 was scraped off and the product was extracted with ethyl acetate.

The vitamin D$_3$ diacetate (1.1 mg, 2.14 μmol) in THF (4 ml) was treated with 5% KOH/MeOH (1 ml) at room temperature for 20 min. The usual work-up (ethyl acetate) gave a crude product, which was submitted to preparative TLC (hexane–ethyl acetate, 1 : 2, developed three times). The band of *Rf* 0.43 was scraped off and the product was extracted with ethyl acetate. Removal of the solvent gave the vitamin D$_3$ analogue **8** (1.4 mg, 90%). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 265, $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 228, $^1$H-NMR (360 MHz) δ: 0.55 (3H, s, 18-H$_3$), 1.02 (3H, d, *J*=6.6 Hz, 21-H$_3$), 1.22 (6H, s, 26-H$_3$ and 27-H$_3$), 2.32 (1H, dd, *J*=13.2, 6.7 Hz), 2.60 (1H, dd, *J*=13.0, 3.0 Hz), 2.83 (1H, dd, *J*=12.0, 3.0 Hz), 4.23 (1H, m, $W_{1/2}$=18.4 Hz, 3α-H), 4.43 (1H, m, $W_{1/2}$=16.9 Hz, 1β-H), 5.00 (1H, br s, $W_{1/2}$=3.2 Hz, 19-H), 5.30 (1H, dd, *J*=15.0, 7.1 Hz, 23-H or 22-H), 5.33 (1H, br s, $W_{1/2}$=3.2 Hz, 19-H), 5.37 (1H, dd, *J*=15.0, 5.8 Hz, 22-H or 23-H), 6.01 (1H, d, *J*=11.0 Hz, 7-H), 6.32 (1H, d, *J*=11.0 Hz, 6-H). MS *m/z* (%): 428 (M$^+$, 3.9), 410 (82), 392 (100), 374 (20), 287 (4.8), 269 (21), 251 (38), 152 (10), 134 (30), 123 (54), 59 (4.2). (Found: M$^+$, 428.3315. $C_{28}H_{44}O_3$ requires M$^+$, 428.3392).

**1α,3β-Diacetoxy-24-homocholest-5-en-25-ol (9)**——A mixture of the 5,22-diene **6** (40 mg, 0.0778 mmol) and 10% Pd–C (4 ml) in ethyl acetate (2 ml) was stirred at room temperature under hydrogen for 3 h. The Pd catalyst was filtered off and the filtrate was concentrated to leave the residue, which was applied to a column of silica gel (5 g). Elution with hexane–ethyl acetate (4 : 1) gave the 5-ene **9** (37 mg, 92%) as an oil. $^1$H-NMR δ: 0.66 (3H, s, 18-H$_3$), 1.08 (3H, s, 19-H$_3$), 1.20 (6H, s, 26-H$_3$ and 27-H$_3$), 2.02 (3H, s, acetyl), 2.05 (3H, s, acetyl), 4.97 (1H, m, 3α-H), 5.07 (1H, m, 1β-H), 5.53 (1H, m, 6-H). MS *m/z* (%): 396 (M$^+$ −2AcOH, 55), 381 (9.0), 378 (5.2), 363 (5.4), 253 (5.2), 118 (100). (Found: M$^+$ −2AcOH, 396.3390. $C_{28}H_{44}O$ requires M$^+$ −2AcOH, 396.3394).

**1α,3β-Diacetoxy-24-homocholesta-5,7-dien-25-ol (10)**——The 5-ene **9** (19 mg, 0.037 mmol) was converted, as described for **7**, to the 5,7-diene **10** (5.8 mg, 31%). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 283, 282, 271.

**1α,25-Dihydroxy-24-homovitamin D$_3$ (11)**——The 5,7-diene **10** (5.8 mg, 0.0113 mmol) was converted, as described for **8**, to the vitamin D$_3$ analogue **11** (1.1 mg, 23%). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 265, $\lambda_{\min}^{\mathrm{EtOH}}$ nm: 228. MS *m/z* (%): 430 (M$^+$, 5.5), 412 (51), 394 (100), 376 (18), 287 (3.8), 269 (9.4), 251 (21), 152 (9.0), 134 (28), 59 (7.1). (Found: M$^+$, 430.3441. $C_{28}H_{46}O_3$ requires M$^+$, 430.3449).

**(22*E*,25ξ)-1α,3β-Bis(methoxymethoxy)-26-homocholesta-5,22-dien-27-oic Acid Methyl Ester (12)**——A solution of the allylic alcohol **2** (390 mg, 0.844 mmol), trimethyl ortho-*n*-butyrate (0.7 ml) and propionic acid (3 drops) in toluene (6 ml) was refluxed under argon for 2 h. Removal of the solvent under reduced pressure gave a crude product, which was applied to a column of silica gel (20 g). Elution with hexane–ethyl acetate (5 : 1) gave the ester **12** (466 mg, 97%) as an oil. $^1$H-NMR δ: 0.68 (3H, s, 18-H$_3$), 0.88 (3H, t, *J*=7 Hz, $-CH_2CH_3$), 0.98 (3H, d, *J*=6 Hz, 21-H$_3$), 1.03 (3H, s, 19-H$_3$), 3.38 (3H, s, $-OCH_3$), 3.43 (3H, s, $-OCH_3$), 3.68 (3H, s, $-CO_2CH_3$), 3.76 (1H, m, 1β-H), 4.68 (2H, s, 3β-O–CH$_2$–O–), 4.69 (2H, ABq, *J*=7 Hz, ΔAB=11 Hz, 1α-O–CH$_2$–O–), 5.27 (2H, m, 22-H and 23-H), 5.56 (1H, m, 6-H). MS *m/z* (%): 514 (M$^+$ −MeOH, 2.0), 484 (14), 452 (7.2), 424 (100), 422 (65), 283 (4.2), 253 (9.4), 169 (55). (Found: M$^+$ −MeOH, 514.3636. $C_{32}H_{50}O_5$ requires M$^+$ −MeOH, 514.3660).

**(22*E*,25ξ)-1α,3β-Bis(methoxymethoxy)-25-hydroxy-26-homocholesta-5,22-dien-27-oic Acid Methyl Ester (13)**——The ester **12** (437 mg, 0.800 mmol) in THF (5 ml) was added to a solution of lithium diisopropylamine (prepared with diisopropylamine (0.13 ml, 0.929 mmol), 1.56 M *n*-BuLi (0.59 ml) and THF (2 ml) and the mixture was stirred under argon at −78 °C for 30 min. Oxygen was bubbled into this solution and then triethylphosphite (0.14 ml, 0.817 mmol) was added. The usual work-up (ether) gave a crude product, which was applied to a column of silica gel (25 g). Etlution with hexane–ethyl acetate (5 : 1) provided the hydroxy ester **13** (303 mg, 67%) as an oil. $^1$H-NMR δ: 0.68 (3H, s, 18-H$_3$), 0.85 (3H, t, *J*=7 Hz, $-CH_2CH_3$), 0.98 (3H, d, *J*=6 Hz, 21-H$_3$), 1.02 (3H, s, 19-H$_3$), 3.08 (1H, br s, $W_{1/2}$=3 Hz, –OH), 3.38 (3H, s, $-OCH_3$), 3.42 (3H, s, $-OCH_3$), 3.76 (3H, s, $-CO_2CH_3$), 4.68 (2H, s, 3β-O–CH$_2$–O–), 4.68 (2H, ABq, *J*=7 Hz, ΔAB=22 Hz, 1α-O–CH$_2$–O–), 5.32 (2H, m, 22-H and 23-H), 5.55 (1H, m, 6-H). MS *m/z* (%): 530 (M$^+$ −MeOH, 2.0), 500 (15), 468 (7.0), 440 (100), 438 (64), 283 (5.2), 253 (11), 185 (8.2), 167 (13), 117 (9.4). (Found: M$^+$ −MeOH, 530.3605. $C_{32}H_{50}O_6$ requires M$^+$ −MeOH, 540.3609).

**(22*E*,25ξ)-1α,3β-Bis(methoxymethoxy)-26-homocholesta-5,22-dien-25-ol (14)**——Lithium aluminum hydride (20 mg, 0.526 mmol) was added to a solution of the hydroxyester **13** (294 mg, 0.539 mmol) in THF (5ml), and this mixture was stirred at room temperature for 30 min. The usual work-up (ether for extraction) gave a crude diol. This was treated with methanesulfonyl chloride (0.04 ml, 0.517 mmol) and pyridine (1.5 ml) at room temperature for 30 min. The usual work-up (ether for extraction) gave a crude mesylate. Lithium aluminum hydride (20 mg, 0.526 mmol) was added to a solution of the crude mesylate in THF (5 ml), and the mixture was refluxed for 30 min. The usual work-up (ether) gave a crude product, which was applied to a column of silica gel (20 g). Elution with hexane–ethyl acetate (5 : 1) provided the alcohol **14** (190 mg, 70%) as an oil. $^1$H-NMR $\delta$: 0.71 (3H, s, 18-$H_3$), 0.90 (3H, t, $J=7$ Hz, -$CH_2CH_3$), 1.03 (3H, s, 19-$H_3$), 1.12 (3H, s, 27-$H_3$), 3.36 (3H, s, -$OCH_3$), 3.40 (3H, s, -$OCH_3$), 3.74 (1H, m, 1$\beta$-H), 4.66 (2H, s, 3$\beta$-O-$CH_2$-O-), 4.67 (2H, ABq, $J=7$ Hz, $\Delta$AB = 11 Hz, 1$\alpha$-O-$CH_2$-O-), 5.35 (2H, m, 22-H and 23-H), 5.54 (1H, m, 6-H).

**(22*E*,25$\xi$)-26-Homocholesta-5,22-diene-1$\alpha$,3$\beta$,25-triol (15)**——The dimethoxymethyl ether **14** (181 mg, 0.349 mmol) was converted, as described for **4**, to the triol **15** (147 mg, 98%), mp 85—87 °C (hexane–dichloromethane). $^1$H-NMR $\delta$: 0.69 (3H, s, 18-$H_3$), 0.89 (3H, t, $J=7$ Hz, -$CH_2CH_3$), 1.02 (3H, s, 19-$H_3$), 1.13 (3H, s, 27-$H_3$), 3.85 (1H, m, 1$\beta$-H), 3.98 (1H, m, 3$\alpha$-H), 5.40 (2H, m, 22-H and 23-H), 5.60 (1H, m, 6-H). MS *m/z* (%): 430 ($M^+$, 0.2%), 412 (1.2), 358 (36), 340 (46), 322 (5.8), 290 (6.0), 272 (31), 254 (13), 73 (100). (Found: C, 77.82; H, 10.59. $C_{28}H_{46}O_3$ requires C, 78.09; H, 10.77).

**(22*E*,25$\xi$)-1$\alpha$,3$\beta$-Diacetoxy-26-homocholesta-5,22-dien-25-ol (16)**——The triol **15** (100 mg, 0.233 mmol) was converted, as described for **5**, to the diacetate **16** (101 mg, 85%) as an amorphous solid. $^1$H-NMR $\delta$: 0.68 (3H, s, 18-$H_3$), 0.88 (3H, t, $J=7$ Hz, -$CH_2CH_3$), 0.98 (3H, d, $J=6$ Hz, 21-$H_3$), 1.08 (3H, s, 19-$H_3$), 1.12 (3H, s, 27-$H_3$), 2.03 (3H, s, acetyl), 2.06 (3H, s, acetyl), 4.98 (1H, m, 3$\alpha$-H), 5.06 (1H, m, 6-H). MS *m/z* (%): 394 ($M^+ - 2AcOH$, 14), 322 (96), 254 (84), 118 (100), 73 (95). (Found: $M^+ - 2AcOH$, 394.3222. $C_{28}H_{42}O$ requires $M^+ - 2AcOH$, 394.3238).

**(24*E*,25$\xi$)-26-Homocholesta-5,7,22-triene-1$\alpha$,3$\beta$,25-triol (17)**——A solution of the 5,22-diene **16** (38 mg, 0.0739 mmol) and *N*-bromosuccinimide (19 mg, 0.107 mmol) in carbon tetrachloride (3 ml) was refluxed under argon for 20 min. The mixture was cooled to 0 °C, and the resulting precipitate was filtered off. The filtrate was concentrated below 40 °C to leave a residue. The THF (5 ml) solution of this residue was treated with a catalytic amount of tetra-*n*-butylammonium bromide at room temperature for 50 min. Then, the mixture was treated with a solution of tetra-*n*-butylammonium fluoride in THF (0.3 ml, 0.3 mmol) at room temperature for 30 min. The usual work-up (ethyl acetate) gave a crude triene. This triene in THF (5 ml) was treated with 5% KOH–MeOH (4 ml) at room temperature for 14 h. The usual work-up (ethyl acetate) gave a crude product, which was submitted to preparative TLC (benzene–ethyl acetate, 1 : 1, developed six times). The band of *Rf* 0.45 was scraped off and the product was extracted with ethyl acetate. Removal of the solvent provided the 5,7,22-triene **17** (8.7 mg, 40%). UV $\lambda_{max}^{EtOH}$ nm: 293, 282, 271.

**(22*E*,25$\xi$)-1$\alpha$,25-Dihydroxy-22-dehydro-26-homovitamin $D_3$ (18)**——A solution of the triene **17** (4.4 mg, 0.0103 mmol) in benzene (90 ml) and ethanol (40 ml) was irradiated with a medium pressure mercury lamp through a Vycor filter at 0 °C under argon for 2.5 min. The reaction mixture was refluxed under argon for 1 h. Removal of the solvent under reduced pressure gave a crude product, which was submitted to preparative TLC (benzene–ethyl acetate, 1 : 1, developed six times). The band of *Rf* 0.49 was scraped off and the product was extracted with ethyl acetate. Removal of the solvent provided the vitamin $D_3$ analogue **18** (0.91 mg, 21%). UV $\lambda_{max}^{EtOH}$ nm: 265, $\lambda_{min}^{EtOH}$ nm: 228. $^1$H-NMR (400 MHz) $\delta$: 0.56 (3H, s, 18-$H_3$), 0.91 (3H, t, $J=7.6$ Hz, -$CH_2C\underline{H}_3$), 1.04 (3H, d, $J=6.8$ Hz, 21-$H_3$), 1.13 (3H, s, 27-$H_3$), 4.23 (1H, m, $W_{1/2}=18.4$ Hz, 3$\alpha$-H), 4.43 (1H, m, $W_{1/2}=16.9$ Hz, 1$\beta$-H), 5.00 (1H, br s, $W_{1/2}=3.2$ Hz, 19-H), 5.32 (1H, br s, $W_{1/2}=3.2$ Hz, 19-H), 5.37 (2H, m, 22-H and 23-H), 6.02 (1H, d, $J=11.5$ Hz, 7-H), 6.38 (1H, d, $J=11.5$ Hz, 6-H). MS *m/z* (%): 428 ($M^+$, 15), 410 (25), 392 (20), 374 (1.0), 338 (6.8), 320 (4.4), 287 (9.0), 269 (15), 251 (14), 141 (8.6), 134 (99), 123 (8.2), 73 (100). (Found: $M^+$, 428.3284. $C_{28}H_{44}O_3$ requires $M^+$, 428.3292).

**(25$\xi$)-1$\alpha$,3$\beta$-Diacetoxy-26-homocholest-5-en-25-ol (19)**——The 5,22-diene **16** (35 mg, 0.0681 mmol) was converted, as described for **9**, to the 5-ene **19** (30 mg, 85%) as an amorphous solid. $^1$H-NMR $\delta$: 0.66 (3H, s, 18-$H_3$), 0.88 (3H, t, $J=7$ Hz, -$CH_2CH_3$), 1.08 (3H, s, 19-$H_3$), 1.12 (3H, s, 27-$H_3$), 2.02 (3H, s, acetyl), 2.04 (3H, s, acetyl), 4.97 (1H, m, 3$\alpha$-H), 5.04 (1H, m, 1$\beta$-H), 5.51 (1H, m, 6-H). MS *m/z* (%): 396 ($M^+ - 2AcOH$, 52), 378 (12), 363 (5.8), 253 (5.2), 118 (100). (Found: $M^+ - 2AcOH$, 396.3380. $C_{28}H_{44}O$ requires $M^+ - 2AcOH$, 396.3394).

**(25$\xi$)-26-Homocholesta-5,7-diene-1$\alpha$,3$\beta$,25-triol (20)**——The 5-ene **19** (22 mg, 0.0426 mmol) was converted, as described for **17**, to the 5,7-diene **20** (6.7 mg, 37%). UV $\lambda_{max}^{EtOH}$ nm: 293, 282, 271.

**(25$\xi$)-1$\alpha$,25-Dihydroxy-26-homovitamin $D_3$ (21)**——The diene **20** (4.8 mg, 0.0112 mmol) was converted, as described for **18** to the vitamin $D_3$ analogue **21** (1.3 mg, 27%). UV $\lambda_{max}^{EtOH}$ nm: 265, $\lambda_{min}^{EtOH}$ nm: 228. MS *m/z* (%): 430 ($M^+$, 2.9), 412 (9.7), 394 (8.8), 379 (2.3), 376 (0.9), 287 (2.0), 269 (4.1), 251 (5.9), 152 (30), 134 (100), 116 (17), 73 (89), 55 (69). (Found: $M^+$, 430.3452. $C_{28}H_{46}O_3$ requires $M^+$, 430.3449).

**Measurement of Biological Activity**——Determination of Serum Calcium in Rats: Weanling male rats were purchased from Holzman Co. (Madison, WI), and a low-calcium, vitamin D-deficient diet for 3 weeks. They were then subcutaneously given a test compound dissolved in 0.1 ml of 95% ethanol–propylene glycol (5 : 95) daily for 7 d. Each group consisted of 7 rats. Serum was obtained by centrifugation of clotted blood. Calcium was determined in the presence of 0.1% lanthanum chloride by means of a Perkin–Elmer atomic absorption spectrometer.

Displacement of 1,25-$(OH)_2$-[26,27-$^3$*H*]-$D_3$ from Chick Intestinal Cytosol Receptor Protein for 1,25-$(OH)_2D_3$: Graded amounts of a test compound were dissolved in 0.05 ml of 95% ethanol. Triplicate determination of displacement of 1,25-$(OH)_2$-[26,27-$^3$H]-$D_3$ from the receptor protein by unlabeled compounds was carried out as described by Shepard *et al.*[9] Authentic 1,25-$(OH)_2D_3$ was a generous gift from Hoffmann-La Roche Co., Nutley, N.J.

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