blocked aldehyde 18 (70 mg, 0.2 mmol) was refluxed with trifluoroacetic acid (10 ml) for 17 h. It was evaporated to a dark oily compound. The desired product was purified by column chromatography (silica gel, eluted with ethyl acetate). The aldehyde **22** was crystallized from acetone–ether (mp 92–94 °C dec, 27 mg, yield 66%): NMR (acetone-d_6, Me_4Si as internal standard) 135 (4-CH_3), 286 (5-CH_2), 502 (C_6H), 599 (2-CHO); ir ν_{max}^{KBr} 1685 cm^{-1} (C=O); uv $\lambda_{max}^{0.1NHCl}$ 286 nm, $\lambda_{max}^{0.1NNaOH}$ 282 nm. Anal. ($C_8H_9NO_3 \cdot 0.5H_2O$) C, H, N.

Oxime of 22. To an aqueous solution of the aldehyde **22** (30 mg, 0.14 mmol) was added $NH_2OH \cdot HCl$ (15 mg, 0.2 mmol). The mixture, made basic with NaOAc, was heated for 4 h on a steam bath. The reaction mixture was then filtered and the precipitate was crystallized from alcohol (mp 200–202 °C, 25 mg, yield 77%): NMR (D_2O) 140 (4-CH_3), 288 (5-CH_2), 496, 503 (2-CH=N and C_6H). Anal. ($C_8H_{10}N_2O_3$) C, H, N.

Thiosemicarbazone of 22. The aldehyde **22** (25 mg, 0.12 mmol) was warmed with an ethanolic solution (5 ml) of thiosemicarbazide (13 mg, 0.14 mmol) for about 20 min. It was cooled and crystals separated out. The thiosemicarbazone was crystallized from hot methanol (mp 240–243 °C dec, 22 mg, yield 65%): ir ν_{max}^{KBr} 3420, 3310, 3156 cm^{-1} (NH bands). Anal. ($C_9H_{12}N_4O_2S$) C, H, N.

3,α^5-O-Dibenzyl-2-vinyl-4-deoxy-2-norpyridoxol [2-Ethenyl-4-methyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]pyridine (19)]. The aldehyde 18 (174 mg, 0.5 mmol) was condensed with triphenylphosphonium bromide (400 mg, 1 mmol) as described earlier,[5] giving the vinyl compound **19** (153 mg, yield 87%), which was converted to its hydrochloride. Anal. ($C_{23}H_{24}ClNO_2$) C, H.

2-Vinyl-4-deoxy-2-norpyridoxol (6-Ethenyl-5-hydroxy-4-methyl-3-pyridinemethanol) Hydrochloride (20). The blocking groups in **19** were removed in a manner analogous to the conversion of 18 to **22**. The target compound **20** was crystallized from MeOH–ethyl acetate (mp 208–210 °C dec, yield 82%): NMR (D_2O) 143 (4-CH_3), 286 (5-CH_2), 350–390 (2-CH=CH_2), 405–437 (2-CH=CH_2), 489 (C_6H). Anal. ($C_9H_{12}ClNO_2$) C, H, Cl.

2-Vinyl-4-deoxy-2-norpyridoxol 5′-Phosphate [6-Ethenyl-5-hydroxy-4-methyl-3-pyridinemethanol 3-(Dihydrogen phosphate) (21)]. The 2-vinyl compound **20** was phosphorylated with polyphosphoric acid as described earlier,[17] giving the target compound **21**, which was crystallized from water–acetone (mp 245–250 °C dec, yield 62%): ir ν_{max}^{KBr} 1610 (C=C), 1160 cm^{-1} (vs, POC). Anal. ($C_9H_{12}NO_5P \cdot 0.5H_2O$) C, H, N.

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Studies on Vitamin D (Calciferol) and Its Analogues. 10. Side-Chain Analogues of 25-Hydroxyvitamin D_3[1]

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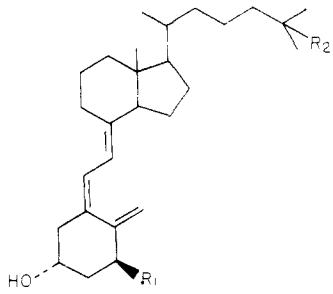
A homologous series of side-chain analogues of 25-hydroxyvitamin D_3 (25-hydroxycholecalciferol) in which the length of the side chain is modified while maintaining its characteristic tertiary hydroxyl moiety has been synthesized. The following five analogues have been prepared and characterized: pentanor-25-OH-D_3 (**2a**), trinor-25-OH-D_3 (**2b**), dinor-25-OH-D_3 (**2c**), nor-25-OH-D_3 (**2d**), and homo-25-OH-D_3 (**2e**). Biological assays in vivo of intestinal calcium absorption and bone calcium mobilization in the chick of the five analogues revealed that the homo analogue **2e** exhibited a significant biological response relative to the –D (–vitamin D_3) control. Compared to the natural vitamin D_3, **2e** is as active in its ability to mobilize bone calcium and is about half as effective in stimulating intestinal calcium transport. The remaining analogues (**2a–d**) exhibited no significant activity in either assay, although the nor analogue **2d** was previously observed to exhibit antimetabolite activity.

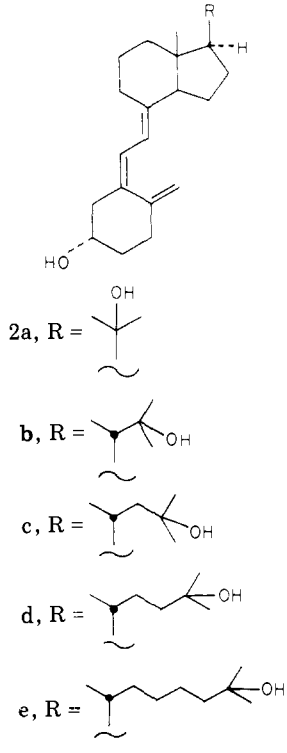
By 1971 it had been shown that cholecalciferol (vitamin D_3, **1a**) must undergo two consecutive obligatory hydroxylations prior to the expression of its biological activities: first, a hydroxylation by a hepatic enzyme system to 25-hydroxyvitamin D_3 (25-OH-D_3, **1b**)[3] and finally hydroxylation of 25-OH-D_3 by a renal enzyme to 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3, **1c**].[4] 1α,25-Dihydroxyvitamin D_3 is the metabolite that is preferentially localized in the chromatin of the intestine[5] and is the most biologically active naturally occurring form of the vitamin

known.[6] Subsequent to the elucidation of this metabolic pathway, it has become increasingly apparent that the presence of the C-25 hydroxyl group is a structural requirement for both the introduction of the 1α-hydroxy moiety by the renal enzyme and for maximal biological activity. The main lines of evidence for this are the following: (i) a rat kidney homogenate is not able to mediate conversion of cholecalciferol to 1α-OH-D_3 (**1d**) under the same conditions that 25-OH-D_3 is metabolized to 1α,25-$(OH)_2$-D_3;[7] (ii) synthetic 1α-OH-D_3[8] is as biologically active as 1α,25-$(OH)_2$-D_3 in vivo[9] but in vitro a large excess (700–900 fold) of 1α-OH-D_3 is required for 50% displacement of 1α,25-$(OH)_2$-D_3 from its intestinal receptor;[10] and (iii) enhanced activity in this in vitro assay[10c] is observed for the 25-hydroxy derivatives of 3-deoxy-1α-hydroxycholecalciferol,[11] dihydrotachysterol$_3$,[12a] or 5,6-*trans*-cholecalciferol.[12b,c]



1a, $R_1 = R_2 = H$
b, $R_1 = H$; $R_2 = OH$
c, $R_1 = R_2 = OH$
d, $R_1 = OH$; $R_2 = H$

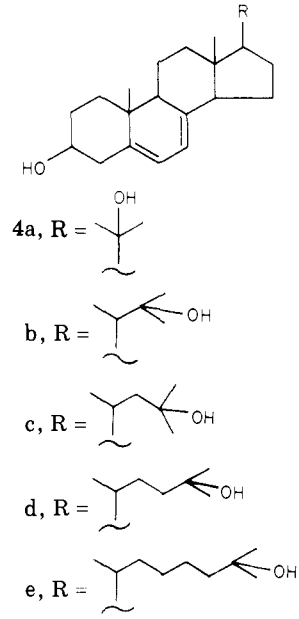
In our continuing efforts to elucidate the minimal and optimal structural features of the vitamin D molecule required for biological activity, we have initiated a program of synthesis of a series of side-chain analogues of 25-OH-D_3. As a result of these efforts, we have synthesized a homologous series of analogues of 25-OH-D_3 (8-carbon side chain) having 3, 5, 6, 7, or 9 carbon atoms and including the terminal tertiary hydroxyl group in their respective side chains (**2a–e**). This study seemed particularly attractive because the nor substance **2d**, previously synthesized by others in only small amounts by a related procedure,[12c] has recently been shown by our laboratories to possess antivitamin D_3 activity.[1c,d] This is the first example of antimetabolite activity among the vitamin D related steroids.[13]



Results and Discussion

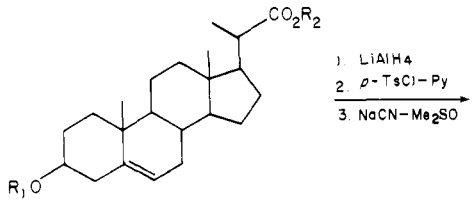
Synthesis. Our general approach to the synthesis of the 25-OH-D_3 analogues consisted of (1) preparation of 3β-acetoxy Δ^5-steroids having appropriate methyl ketone or methyl ester functionalized side chains; (2) introduction of the Δ^7 double bond yielding the corresponding provitamin D ($\Delta^{5,7}$-diene);[14] (3) reaction with methyllithium to produce the provitamin D diol; and (4) irradiation and thermal equilibration of the latter to the vitamin analogue **2**.[14]

Pentanor-25-OH-D_3 (**2a**) was synthesized from pregnenolone acetate (**3a**) in five steps (overall yield, 2.9%). Allylic bromination followed by dehydrobromination of **3a**

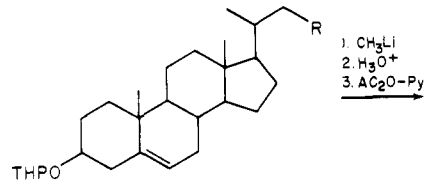


gave the corresponding 5,7-diene (**3b**, 14%). Reaction of **3b** with methyllithium gave the diol **4a** (92%) from which the desired vitamin **2a** (23%) was generated by uv irradiation and then thermal equilibration. The trinor- (**2b**, overall yield 6.6% in five steps) and nor-25-OH-vitamin D_3 (**2d**, overall yield 14% in five steps)[12c] analogues were synthesized analogously from methyl 23,24-dinor-3β-acetoxychol-5-enoate (**5a**) and methyl 3β-acetoxychol-5-enoate (**6a**), respectively.

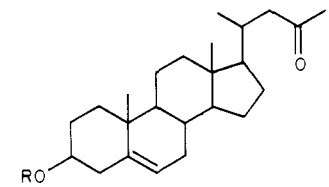
The dinor analogue **2c** was prepared from 23,24-dinorcholenic acid (**7a**) in an overall yield of 1.1% (12 steps). After conversion of **7a** to the methyl ester **7b** (94%) using diazomethane, the 3β-hydroxyl was protected as the tetrahydropyranyloxy ether **7c** (87%). Vigorous reduction of **7c** with lithium aluminum hydride gave the alcohol **8a** (71%) which was converted to the tosylate **8b** (86%). Displacement of the tosylate with sodium cyanide in hot dimethyl sulfoxide gave the corresponding nitrile **8c** (60%). Alkylation of **8c** with methyllithium followed by acid hydrolysis yielded the hydroxy ketone **9a** (81%), which was protected as the acetate **9b** (98%). The latter, after sequential conversion to the corresponding provitamins **9c** and then **4c**, was transformed as above into dinor-25-OH-vitamin D_3 (**2c**). The homovitamin **2e** was synthesized by the same series of reactions from 25-homo-3β-hydroxychol-5-en-25-oic acid (**10b**). The latter was ob-



7a, $R_1 = R_2 = H$
b, $R_1 = H$; $R_2 = CH_3$
c, $R_1 = THP$; $R_2 = CH_3$

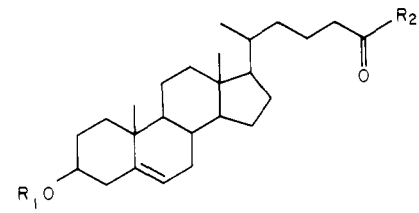


8a, R = OH
b, R = OTs
c, R = C≡N



9a, R = H
b, R = Ac

tained from 27-nor-25-oxycholesteryl acetate (**10a**) by hypobromite oxidation. The overall conversion of **10a** to



10a, R_1 = Ac; $R_2 = CH_3$
b, $R_1 = H$; R_2 = OH

homo-25-OH-vitamin D_3 (**2e**) was accomplished in 4.6% yield in 13 steps.

The provitamins **4a–e** have been fully characterized both by uv and NMR spectroscopy and microanalytic and/or mass spectroscopic means and they all proved to be nicely crystalline but air- and heat-sensitive substances. The corresponding vitamins **2a–e**, however, solidified with great difficulty as amorphous powders. The air sensitivity of the substances coupled with the relatively modest amounts of **2a–e** available precluded extensive efforts toward obtaining crystalline microanalytic samples. The homogeneity of each vitamin analogue was established (particularly before bioassay) by TLC in several chromatographic systems and also by uv, NMR, and mass spectroscopy.[15]

Biological Assay. The biological activity of vitamin D_3 and related compounds is manifested in vivo in a variety of ways, two of which are (a) the mediation of calcium absorption or translocation of calcium from the mucosal to the serosal side of the intestine and (b) an increased resorption of calcium from bone which results in an elevation of serum calcium levels. Each of the 25-OH-D_3 side-chain analogues whose synthesis was described above was tested in rachitic chicks by the method of Hibberd and Norman[16] for its ability to effect these two parameters. Of the five side-chain analogues (**2a–e**), only homo-25-OH-D_3 (**2e**) was found effective in mediating intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) in chicks relative to –D, rachitic control birds. The time courses of these responses are shown in Figure 1 for a 130-nmol dose of the homo analogue for the period which ranged for up to 120 h after dose. In both cases, a maximal response was elicited by 24 h after analogue administration. The potency of homo-25-OH-D_3 relative to vitamin D_3 was determined by administering increasing doses of the different compounds to the test animals and then monitoring the ICA and BCM responses. Figure 2 shows the ICA and BCM dose–response curves for D_3 and 24a-homo-25-OH-D_3 (**2e**). It is clear that the maximal D_3 response at 24 h was 50% greater than the response to an equivalent amount of the homo analogue. The bone responds differently to this analogue than does the intestine. At each dose level of the analogue tested, the BCM response was not significantly different from that observed for the D_3 control. The four nor analogues (**2a–d**) were assayed similarly (ICA and BCM) in a time course (for up to 96 h at a dose level of 32.5 nmol) and a dose–response study (at 24 h for dose levels up to 97.5 nmol). None of the experimental values obtained for the nor analogue treated animals differed significantly from –D control even at the dose level of 97.5 nmol. It is intriguing that although nor-25-OH-D_3 (**2d**) exhibits no biological activity, we have found that in this same assay it is a potent inhibitor of the vitamin D_3 but not the 25-OH-D_3 mediated responses.[1c,d]

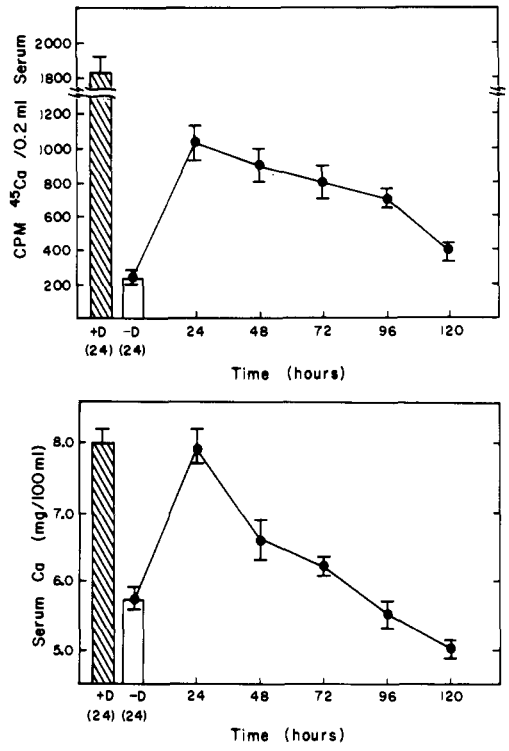


Figure 1. Time courses of intestinal calcium absorption (top) and bone calcium mobilization (bottom) response to homo-25-OH-D_3 (**2e**). Birds were dosed intraperitoneally with 130 nmol of the analogue at the indicated time before assay. Positive (+D) controls received a 6.5 nmol dose of vitamin D_3 24 h prior to assay and –D controls were administered solvent only (0.2 ml of 1,2-propanediol–ethanol, 3/1). Values recorded are the mean ± SEM for 8–10 birds.

Experimental Section

General. All reagents and solvents are analytical reagent grade and were used without further purification unless otherwise indicated. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. A Varian A-60 instrument was used for all nuclear magnetic resonance

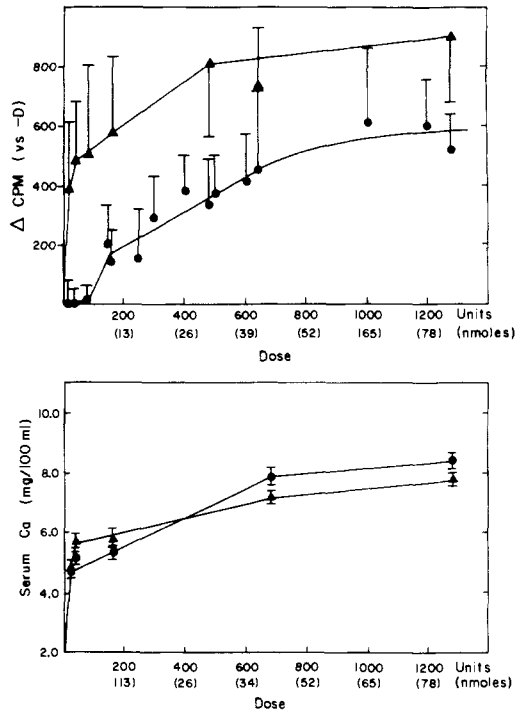


Figure 2. Dose dependence of the intestinal calcium absorption (top) and bone calcium mobilization (bottom) response to D_3 (▲—▲) and homo-25-OH-D_3 (●—●). Birds received an intraperitoneal dose of the appropriate level of compound in 0.2 ml of propanediol-ethanol (3/1) 24 h before assay. Untreated controls received the same amount of solvent. Values recorded are the mean ± SEM for 8–10 birds.

(NMR) spectra. Deuteriochloroform was used as solvent and tetramethylsilane (Me_4Si, τ 10.00) as internal standard. Ultraviolet (uv) spectra were recorded with a Beckman-DB or Cary-14 spectrophotometer and ethanol was used as solvent. Nujol mulls or carbon tetrachloride solutions were used to take infrared (ir) spectra using a Perkin-Elmer 137 or 621 spectrophotometer. Preparative irradiations were carried out at 0 °C with a Hanovia 450-W medium-pressure mercury lamp in a quartz, water-cooled, immersion probe fitted to a 90-ml Pyrex jacket. Mixing was accomplished during the irradiation by bubbling nitrogen into the cold solution. Dry tetrahydrofuran (THF) refers to solvent freshly distilled from lithium aluminum hydride ($LiAlH_4$). LBPE refers to low boiling petroleum ether and silica gel refers to 60–200 mesh Baker analyzed reagent. Microanalyses were performed by C. F. Geiger, Ontario, Calif. Mass spectra (MS) were obtained using a Finnigan 1015 C mass spectrometer with an ionizer setting of 70 eV/300 μA.

3β-Acetoxy-5,7-pregnadien-20-one (3b). To a refluxing solution of pregnenolone acetate **3a** (10.0 g, 0.0279 mol) in 160 ml of benzene–hexane (1:1) was added in one batch 4.05 g (0.0147 mol) of 1,3-dibromo-5,5-dimethylhydantoin (DBDMH). After stirring at reflux for 18.5 min the deep yellow solution was cooled on ice and then filtered free of dimethylhydantoin with cold hexane wash. Following removal of the solvent the semicrystalline oil was dissolved in minimal xylene and added dropwise (~15 min) to rapidly stirred refluxing *s*-collidine (150 ml) under a nitrogen atmosphere. The reaction mixture begins turning brown immediately and a tan precipitate separates as the reaction continues. Refluxing was maintained for 30 min after the final addition of steroid. The reaction vessel was then cooled on ice and the collidinium hydrochloride removed by filtration with ether wash. The filtrate was diluted with ether (1 l.) and washed successively with dilute hydrochloric acid (until the aqueous layer was acidic to litmus paper), saturated aqueous sodium bicarbonate (2 × 250 ml), and water (2 × 250 ml). The solution was dried by filtration through anhydrous sodium sulfate. Concentration under reduced pressure and removal of xylene under high vacuum gave a yellow crystalline solid from which the 5,7-diene, **3b**, was obtained as shiny, colorless plates by direct crystallization from dilute methanol (1.4 g, 14%): mp 166–168 °C (lit.[17] 165–168 °C).

20-Methyl-3β,20-dihydroxy-5,7-pregnadiene (4a). An ethereal solution of methyllithium (9.3 ml, 1.8 M, 0.017 mol) was diluted with 10 ml of dry THF under anhydrous conditions (flamed reaction vessel, constant pressure nitrogen atmosphere). The stirred solution was cooled in an ice bath and a solution of 0.200 g (0.00056 mol) of **3b** in 10 ml of anhydrous THF was added dropwise in 30 min. The reaction was continued for 3 h at 0 °C and then at room temperature overnight. After cooling the solution on ice, excess CH_3Li was decomposed by the dropwise addition of aqueous saturated ammonium chloride (NH_4Cl, saturated) and the reaction mixture poured into NH_4Cl (saturated) and extracted abundantly with chloroform. The combined extracts were washed with H_2O (2 × 50 ml) and dried by filtration over sodium sulfate. Concentration afforded a pale yellow solid which crystallized as stout needles (methanol): 0.155 g (92%); mp, decomposes before melting; uv λ_{max} 293 nm (ϵ 5900), 281 (10 100), 270 (10 100), 270 (9600); NMR τ 4.42 and 4.62 ($H_{6,7}$, AB q, $J_{AB} \simeq 6$ Hz), 6.23–6.72 ($H_{3\alpha}$, br m), 8.68 and 8.78 ($C_{21,22}CH_3$, 2 s), 9.05 ($C_{19}CH_3$, s), 9.22 ($C_{18}CH_3$, s); MS *m/e* 330 (M^+). Due to extensive and rapid oxidation a consistent microanalysis was not obtained despite repeated attempts.

20,21,22,23,24-Pentanor-25-hydroxyvitamin D_3 [20-Methyl-9,10-seco-5,7,10(19)-pregnatriene-3β,20-diol, 2a]. A total of 0.818 g (0.00248 mol) of the diene diol, **4a**, was irradiated in five batches. Each batch was irradiated for 4 min after a 15-min lamp warmup. Solutions of the steroid in ~90 ml of ether were used. After concentration to dryness of the combined crude irradiation mixtures, the pale yellow oil was dissolved in 150 ml of isopropyl ether and heated at reflux overnight under a nitrogen atmosphere. Removal of solvent and chromatography over 50 g of 10% silver nitrate impregnated silica gel afforded 0.187 g (23%) of the vitamin analogue **2a** as a white foam: NMR τ 3.76 and 4.00 ($H_{6,7}$, AB q, $J_{AB} \simeq 11$ Hz), 4.96 and 5.22 (H_{19Z} and H_{19E}, m and d), 5.83–6.33 ($H_{3\alpha}$, br m), 8.70 and 8.80 ($C_{21,22}CH_3$, 2 s), 9.30 ($C_{18}CH_3$, s); uv λ_{max} 265 nm and λ_{min} 227 nm; MS *m/e* 330 (M), 312 (M - H_2O), 136 (base), 118 (base - H_2O).

Methyl 23,24-Dinor-3β-acetoxycholen-5-oate (5a). Esterification of 23,24-dinor-3β-acetoxycholenic acid (**7b**, 4.26 g, 0.011 mol) was carried out with diazomethane.[18] The white crystalline product crystallized from 95% EtOH as small shiny needles (3.45 g, 78%): mp 153–155 °C (lit.[19] 153.5–156.5 °C).

Methyl 23,24-Dinor-3β-acetoxy-5,7-choladien-22-oate (5b). The diester **5a** was converted to **5b** by the general procedure described above for **3a** to **3b**: **5a** (0.528 g, 0.00145 mol) in 1:1 benzene–hexane (20 ml); DBDMH (0.227 g, 0.00080 mol); allylic bromide (minimum volume xylene); *s*-collidine (20 ml). The product was purified by chromatography (20 g of 10% silver nitrate impregnated silica gel; linear gradient of 0–25% ether in LBPE) and then recrystallization from 95% ethanol (0.209 g, 36%): mp 145.5–147 °C. Anal. ($C_{25}H_{36}O_4$) C, H.

22,23,24-Trinor-3β,25-dihydroxy-5,7-cholestadiene (4b). Employing anhydrous conditions, a solution of 0.160 g (0.000399 mol) of **5b** in ~20 ml of dry THF was added dropwise (20 min) to a cold (0 °C) solution of methyllithium (8.9 ml of a 1.8 M etheral solution) in 35 ml of dry THF. The reaction mixture was stirred at 0 °C for 5 h and at ambient temperature overnight. After cooling, the mixture was worked up as in the preparation of **4a**. Extraction with chloroform and chromatography over silica gel gave a white crystalline solid that afforded 0.120 g (88%) of the diol **4b** after crystallization (MeOH): mp, decomposes before melting; uv λ_{max} 292 nm (ϵ 5640), 281 (9940), 270 (9330); NMR τ 4.31 and 4.62 ($H_{6,7}$, AB q, $J_{AB} \simeq 6$ Hz), 6.17–6.83 and 6.50 ($H_{3\alpha}$, br m and $C_{25}OH$, superimposed s), 8.80 and 8.83 ($C_{26,27}CH_3$, 2 s), 8.98 ($C_{19}CH_3$, s), 9.00 ($C_{21}CH_3$, d, $J \simeq 6$ Hz), 9.24 ($C_{18}CH_3$, s). Anal. ($C_{24}H_{38}O_2$) C, H.

22,23,24-Trinor-25-hydroxyvitamin D_3 [22,23,24-Trinor-9,10-seco-5,7,10(19)-cholestatriene-3β,25-diol, 2b]. Four separate batches of **4b** (0.607 g total, 0.00169 mol) in ether solution (90 ml) were each irradiated for 4 min after a 15-min lamp warmup period. Combination of the crude irradiation mixtures and concentration to dryness left a pale yellow oil that was dissolved in 150 ml of isopropyl ether and heated at reflux overnight under a nitrogen atmosphere. Removal of the solvent and chromatography of the oily residue over ~50 g of 10% silver nitrate impregnated silica gel gave 0.127 g (21%) of the vitamin **2b** as

an amorphous solid: NMR (τ) 3.74 and 4.00 ($H_{6,7}$, AB q, $J_{AB} \simeq$ 11 Hz), 4.95 and 5.19 (H_{19Z} and H_{19E}, m and d), 5.83–6.36 ($H_{3\alpha}$, br m), 8.80 and 8.83 ($C_{26,27}CH_3$, 2 s), 9.38 ($C_{18}CH_3$, s); uv λ_{max} 265 nm and λ_{min} 228 nm; MS m/e 358 (M), 340 (M – H_2O), 136 (base), 118 (base – H_2O).

Methyl 23,24-Dinor-3β-hydroxychol-5-en-22-oate (7b). The acid **7a** (6.76 g, 0.0195 mol) was esterified using diazomethane.[18] The product crystallized from 95% ethanol yielding 6.85 g (94%) of **7b**: mp 140.0–141.2 °C (lit.[19] 142–143.7 °C).

Methyl 23,24-Dinor-3β-(2′-tetrahydropyranyloxy)chol-5-en-22-oate (7c). To a slurry of 0.989 g (0.00274 mol) of **7b** in 9 ml of dioxane (distilled from sodium) was added 1.5 ml of dihydropyran and 10 mg of *p*-toluenesulfonic acid monohydrate. The solution was stirred at ambient temperature for 3.5 h and then diluted with 100 ml of aqueous saturated sodium hydrogen carbonate. The aqueous layer was extracted with ether (3 × 50 ml) and the combined extracts were washed with water (2 × 50 ml) and concentrated under vacuum. Recrystallization of the semisolid oil (95% EtOH) afforded 1.0 g (87%) of **7c** as small white needles: mp 133.5–135 °C (lit.[20] 121–123 °C).

23,24-Dinor-22-hydroxy-3β-(2′-tetrahydropyranyloxy)chol-5-ene (8a). The ester **7c** (1.05 g, 0.00236 mol) dissolved in 25 ml of dry THF was added dropwise to a cold, stirred solution of 0.5 g (0.013 mol) of $LiAlH_4$ in 50 ml of dry THF (anhydrous conditions). After the addition (~15 min), the reaction mixture was heated and refluxed for 5 h. Excess $LiAlH_4$ was then decomposed by the careful successive addition of 0.5 ml of water, 0.5 ml of 15% aqueous sodium hydroxide, and 1.5 ml of water. Filtration of the precipitated aluminum salts and concentration of the filtrate to dryness yielded a powdery white solid that crystallized (95% EtOH) as heavy chunks affording 0.696 g (71%) of **8a**: mp 152.3–154 °C (lit.[20] 156.5–158 °C). Anal. ($C_{27}H_{44}O_3$) C, H.

23,24-Dinor-22-tosyloxy-3β-(2′-tetrahydropyranyloxy)chol-5-ene (8b). A solution of 0.110 g (0.264 mmol) of **8a** in 3.0 ml of dry pyridine was cooled on ice and solid *p*-toluenesulfonyl chloride (0.121 g, 0.00063 mol) was added in one batch. The reaction was refrigerated overnight, then diluted with 15 ml of ether, washed with aqueous saturated sodium hydrogen carbonate (2 × 15 ml) and water (15 ml), and finally dried (sodium sulfate). Filtration and concentration left a brown oil which crystallized from 95% EtOH affording 0.130 g (86%) of **8b** as a white powder: mp 145.8–146.8 °C. Anal. ($C_{34}H_{50}O_5S$) C, H, S.

24-Nor-3β-(2′-tetrahydropyranyloxy)-5-cholenonitrile (8c). Under anhydrous conditions, a solution of 0.103 g (0.00018 mol) of **8b** in 6 ml of Me_2SO (distilled from calcium hydride) was heated to 80 °C in an oil bath.[21] In one batch was added sodium cyanide (0.028 g, 0.00054 mol) and the solution was stirred at 80 °C for 9.5 h. The reaction mixture was poured into ~75 ml of saturated aqueous ammonium chloride. After extraction with dichloromethane (3 × 75 ml) the combined extracts were washed with water (2 × 70 ml), dried (sodium sulfate), filtered, and concentrated to dryness leaving a pale yellow solid (0.091 g). Recrystallization afforded 0.046 g (60%) of pure **8c**: mp 169–171 °C dec; ir ν_{max} 2350 cm^{-1} (C≡N). Anal. $C_{28}H_{43}O_2N$) C, H, N.

3β-Hydroxy-23-oxochol-5-ene (9a). The nitrile **8c** (0.557 g, 0.00131 mol) dissolved in ~6 ml of dry THF was added dropwise (90 min) to a stirred solution of methyllithium (6.5 ml of a 2 M ethereal solution) in 2.5 ml of dry THF (0 °C, anhydrous conditions).[21] The solution was stirred at 0 °C for 3 h and after an additional 3 h at room temperature a dioxane–sulfuric acid solution (6 ml of dioxane–3 ml of 3 M H_2SO_4) was added and the reaction mixture heated (60–70 °C) for 2 h with vigorous stirring. The organic layer from the resulting clear orange biphasic solution was separated and the aqueous phase neutralized (10% aqueous sodium hydroxide) and extracted with dichloromethane (2 × 50 ml). The organic layer was washed with water (2 × 50 ml) and the combined organic phases were concentrated to dryness leaving a dark crystalline residue. The residue was chromatographed over 30 g of silica gel by elution with benzene–4% acetone. Recrystallization (95% ethanol) afforded 0.379 g (81%) of hydroxy ketone **9a**: mp 174.5–176 °C; ir ν_{max} 1710 cm^{-1} (C=O). Anal. ($C_{24}H_{38}O_2$) C, H.

3β-Acetoxy-23-oxochol-5-ene (9b). The keto alcohol **9a** (0.350 g, 0.000976 mol) was dissolved in 2.5 ml of dry pyridine and 2.5 ml of acetic anhydride (freshly distilled) was added to the stirred solution. The reaction was continued at ambient temperature overnight. The reaction mixture was poured into 60 ml of ice water and stirred for 2–3 h. The resulting white precipitate was collected by suction filtration and **9b** (0.382 g, 98%) crystallized as shiny flakes (95% ethanol): mp 170.5–171.5 °C. Anal. ($C_{26}H_{40}O_3$) C, H.

3β-Acetoxy-23-oxo-5,7-choladiene (9c). The keto ester **9b** was converted to **9c** by the general procedure described above (**3a** to **3b**): **9b** (0.400 g, 0.00099 mol) in 1:1 benzene–hexane (20 ml); DBDMH (0.157 g, 0.00055 mol); allylic bromide in minimal xylene; *s*-collidine (30 ml). Chromatography (~75 g of 10% $AgNO_3$ impregnated silica gel; LBPE–ether) followed by crystallization (95% ethanol) afforded **9c** (0.090 g, 23%): mp, decomposes before melting; uv λ_{max} 293 nm (ε 6400), 281 (10800), 270 (10300). Anal. ($C_{26}H_{38}O_3$) C, H.

23,24-Dinor-3β,25-dihydroxy-5,7-cholestadiene (4c). A solution of 0.0879 g (0.000221 mol) of **9c** in ~5 ml of dry THF was added dropwise to a stirred, cold (0 °C) solution of methyllithium (3.68 ml of a 1.8 M ethereal solution) in 10 ml of dry THF. After 5 h at 0 °C, the reaction was continued overnight at room temperature. The reaction mixture was poured into 30 ml of saturated aqueous ammonium chloride and extracted thoroughly with chloroform. Concentration to dryness and recrystallization (methanol) yielded 0.075 g (95%) of **4c**: mp, decomposes before melting; uv λ_{max} 293 nm (ε 6100), 281 (9800), 270 (9300); NMR τ 4.43 and 4.60 ($H_{6,7}$, AB q, $J_{AB} \simeq$ 6 Hz), 6.04–6.73 ($H_{3\alpha}$, br m), 6.52 ($C_{25}OH$, s), 8.76 ($C_{26,27}CH_3$, s), 9.04 ($C_{19}CH_3$, s), 9.08 ($C_{21}CH_3$, d, $J \simeq$ 6 Hz), 9.33 ($C_{18}CH_3$, s). Anal. ($C_{25}H_{40}O_2$) C, H.

23,24-Dinor-25-hydroxyvitamin D_3 [23,24-Dinor-9,10-seco-5,7,10(19)-cholestatriene-3β,25-diol, 2c]. Three batches (0.516 g, 0.00138 mol total) of **4c** were irradiated for a total of 4 min each after a 15-min lamp warmup. The pale yellow oil obtained from concentration to dryness of the combined irradiation mixtures was chromatographed over 50 g of 10% silver nitrate impregnated silica gel after thermal equilibration in refluxing isopropyl ether as for **2a**. A total of 0.113 g (22%) of the desired product **2c** was obtained as a white amorphous foam: NMR τ 3.72 and 4.00 ($H_{6,7}$, AB q, $J_{AB} \simeq$ 12 Hz), 4.96 and 5.19 (H_{19Z} and H_{19E}, m and d), 5.80–6.43 ($H_{3\alpha}$, br m), 8.74 ($C_{26,27}CH_3$, s), 9.41 ($C_{18}CH_3$, s); uv λ_{max} 265 nm and λ_{min} 227 nm; MS m/e 372 (M), 354 (M – H_2O), 136 (base), 118 (base – H_2O).

Methyl 3β-Acetoxychol-5-en-24-oate (6a). Esterification of 3β-acetoxychol-5-en-24-oic acid (2.01 g, 4.8 mmol) was carried out with diazomethane.[18] Crystallization of the product (95% ethanol) afforded 1.93 g (94%) of **6a** as small glistening needles: mp 154–156 °C (lit.[22] 154–156 °C).

Methyl 3β-Acetoxy-5,7-choladien-24-oate (6b). The diester **6a** was converted to **6b** by the same procedure described earlier (**3a** to **3b**): **6a** (0.810 g, 0.00188 mol) in 1:1 benzene–hexane (25 ml); DBDMH (0.248 g); crude bromide in minimal xylene; *s*-collidine (20 ml). Chromatography (20 g of 10% $AgNO_3$ impregnated silica gel; LBPE–ether) and then crystallization (95% ethanol) afforded 0.371 g (46%) of **6b**: mp 124–127 °C (lit.[23] 125–127 °C).

24-Nor-3β,25-dihydroxy-5,7-cholestadiene (4d). Under anhydrous conditions, a solution of **6b** (0.112 g, 0.00026 mol) in 10 ml of dry THF was added dropwise to a cold solution (0 °C) of ethereal methyllithium (5.8 ml of a 1.8 M solution) in 10 ml of dry THF. The reaction mixture was stirred for 5 h at 0 °C and then at ambient temperature overnight. The reaction mixture was worked up as in the preparation of **4a** to give a white residue that afforded 0.097 g (97%) of **4d** on crystallization from methanol: mp, decomposes before melting; uv λ_{max} 293 nm (ε 5860), 281 (9700), 270 (9100); NMR τ 4.40 and 4.62 ($H_{6,7}$, AB q, $J_{AB} \simeq$ 6 Hz), 6.13–6.75 ($H_{3\alpha}$, br m), 6.52 ($C_{25}OH$, s), 8.79 ($C_{26,27}CH_3$, s), 9.02 ($C_{21}CH_3$, d, $J \simeq$ 6 Hz), 9.04 ($C_{19}CH_3$, s), 9.35 ($C_{18}CH_3$, s). Anal. ($C_{26}H_{42}O_2 \cdot H_2O$) C, H.

24-Nor-25-hydroxyvitamin D_3 [24-Nor-9,10-seco-5,7,10(19)-cholestatriene-3β,25-diol, 2d]. A total of 0.737 g (0.00191 mol) of **4d** was irradiated in five equal batches. Ether–ethanol (90 ml, 9:1) solutions of the diene diol were irradiated for 4 min each after a lamp warmup of 15 min. The combined crude irradiation mixtures were thermally equilibrated as for **2a** in refluxing isopropyl ether. After concentrating to dryness the pale yellow oily residue was chromatographed over 50 g of 10% silver

nitrate impregnated silica gel affording 0.228 g (31%) of **2d** as a clear oil: NMR τ 3.73 and 4.00 ($H_{6,7}$, AB q, $J_{AB} \simeq 11$ Hz), 4.96 and 5.19 (H_{19Z} and H_{19E}, m and d), 5.80–6.38 ($H_{3\alpha}$, br m), 8.80 ($C_{26,27}CH_3$, s), 9.44 ($C_{18}CH_3$, s); uv λ_{max} 265 nm and λ_{min} 227 nm; MS *m/e* 386 (M), 368 (M – H_2O), 136 (base), 118 (base – H_2O).

25-Homo-3β-hydroxychol-5-en-25-oic Acid (10b). Sodium hydroxide (4.85 g, 0.121 mol) was dissolved in 41.5 ml of water and cooled (ice–salt) to <–5 °C.[24] To the stirred solution was added dropwise (~10 min) 1.10 ml (3.22 g, 0.020 mol) of elemental bromine, at a rate that maintained the temperature below 0 °C. Dioxane (27.5 ml, precooled to 13–14 °C) was added to the hypobromite solution and the mixture kept at 0 °C until used.

The ketone **10a** (5.01 g, 0.0117 mol) was dissolved in 550 ml of dioxane. After diluting with 65 ml of water, enough dioxane was added to keep the steroid in solution. All subsequent steps were performed at <5 °C. To the stirred steroid solution (cooled to 0 °C) was added dropwise the hypobromite solution. A voluminous white precipitate formed within 40 min and the pale yellow green solution completely decolorized after 3–4 h. Stirring was continued for an additional 2 h. A solution of 2.5 g of sodium sulfite in 15 ml of H_2O was added and the reaction mixture stirred for 20 min and then heated at reflux for 1 h. Following acidification (concentrated hydrochloric acid) of the hot (>90 °C) solution it was kept at 5 °C overnight. Collection of the white crystalline product by suction filtration gave 2.99 g of **10b**. Concentration of the mother liquor yielded an additional 1.3 g. Recrystallization from methanol afforded 4.1 g (91%) of pure **10b**: mp 210–212 °C (lit.[25] 210–212 °C).

Methyl 25-Homo-3β-hydroxychol-5-en-25-oate (10c). A total of 6.56 g (0.0169 mol) of **10b** was esterified with diazomethane[18] affording 6.0 g (88%) of **10c** as small needles (methanol): mp 86–87.5 °C (lit.[26] 85.6–87.0 °C).

Methyl 25-Homo-3β-(2′-tetrahydropyranyloxy)chol-5-en-25-oate (10d). To a solution of 5.2 g (0.0129 mol) of **10c** in 65 ml of dioxane (freshly distilled from sodium) was added 5 ml of dihydropyran and ~20 mg of *p*-toluenesulfonic acid monohydrate. After 4.5 h at ambient temperature, the orange solution was diluted with 500 ml of ether and washed with saturated aqueous sodium hydrogen carbonate (2 × 250 ml) and water (2 × 250 ml) and then dried (sodium sulfate). Filtration, concentration to dryness, and crystallization (aqueous ethanol) yielded 5.0 g (81%) of **10d** as small needles: mp 91–93 °C (lit.[26] 92.5–94 °C); mp 149–153 °C (crystallization from acetone).

25-Homo-3β-(2′-tetrahydropyranyloxy)chol-5-en-25-ol (11a). The ester **10d** (1.01 g, 0.00207 mol), dissolved in 20 ml of dry THF, was added dropwise in 40 min to a solution of $LiAlH_4$ (0.324 g, 0.00085 mol) in 40 ml of dry THF (0 °C, nitrogen atmosphere, anhydrous conditions). After heating at reflux for 5 h, the excess $LiAlH_4$ was decomposed (successive additions of 0.32 ml of water, 0.32 ml of 10% aqueous sodium hydroxide, and 0.96 ml of water), and the precipitated aluminum salts were filtered off. Concentration to dryness left **11a** as a white residue (0.930 g, 98%) which, due to its tendency to form gelatinous solvates, could not be recrystallized: mp 132–135 °C.

25-Homo-25-tosyloxy-3β-(2′-tetrahydropyranyloxy)chol-5-ene (11b). To **11a** (0.930 g, 0.00203 mol) dissolved in 2.1 ml of dry pyridine, at 0 °C, was added a solution of 0.780 g (0.00406 mol) of *p*-toluenesulfonyl chloride in 0.5 ml of dry pyridine. The reaction mixture was refrigerated for 5 h and poured into 30 ml of saturated aqueous sodium hydrogen carbonate. Extraction with chloroform left an oily pale yellow residue (1.2 g, 99%) which exhibited an appropriate NMR spectrum but could not be induced to crystallize.

25,26-Dihomo-3β-(2′-tetrahydropyranyloxy)-5-cholenonitrile (11c). The tosylate **11b** (0.458 g, 0.00075 mol) was dissolved in ~10 ml of Me_2SO (distilled from calcium hydride). The solution was heated (under a nitrogen atmosphere) to 70 °C and 0.110 g (0.00225 mol) of sodium cyanide was added in one batch. After stirring 9 h at 70 °C, the dark brown reaction mixture was poured into 30 ml of saturated aqueous ammonium chloride. Extraction with chloroform and concentration to dryness gave a pale yellow crystalline compound that afforded 0.315 g (91%) of **11c** on crystallization (methanol): mp 118.5–120.5 °C; ir ν_{max} 2250 cm^{-1} (C≡N). Anal. ($C_{31}H_{49}O_2N$) C, H, N.

25,26,27-Trihomo-26-oxo-3β-hydroxychol-5-ene (12a). Using anhydrous conditions, 0.116 g (0.000248 mol) of the nitrile **11c** dissolved in ~8 ml of dry THF was added dropwise in 90 min to a stirred solution of methyllithium (1.4 ml of a 1.8 M ethereal solution, 0.00248 mol) in 7.5 ml of dry THF (0 °C). The reaction was continued at 0 °C for an additional 3 h, followed by 1 h at room temperature. Sulfuric acid–dioxane solution (5 ml of 6 N sulfuric acid–10 ml of dioxane) was added to the reaction mixture, which was then heated at 60 °C for 2 h. The separated aqueous layer was neutralized (10% aqueous sodium hydroxide) and extracted with chloroform. The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate (2 × 50 ml) and water (2 × 50 ml) and then dried (sodium sulfate). Filtration and concentration to dryness gave a brown oily residue that afforded 0.080 g (81%) of **12a** as an amorphous solid after chromatography over alumina (elutes in 100% ether): ir ν_{max} 1710 cm^{-1} (C=O).

25,26,27-Trihomo-26-oxo-3β-acetoxychol-5-ene (12b). To a solution of 0.142 g (0.000354 mol) of **12b** in 1.5 ml of dry pyridine was added 1.0 ml of acetic anhydride, and the reaction mixture was left at ambient temperatures overnight. The solution was poured into 60 ml of ice water and stirred for 1.5 h. Collection of the white precipitate by suction filtration and recrystallization (95% EtOH) gave 0.144 g (92%) of **12b**: mp 111.5–112.0 °C. Anal. ($C_{29}H_{46}O_3$) C, H.

25,26,27-Trihomo-26-oxo-3β-acetoxy-5,7-choladiene (12c). The keto ester **12b** was converted to **12c** by the method described earlier (**3a** to **3b**): **12b** (0.443 g, 0.00101 mol) in 1:1 benzene–hexane (5 ml); DBDMH (0.152 g, 0.00053 mol); crude bromide (in 10 ml of xylene); *s*-collidine (35 ml). Concentration to dryness and chromatography over 10% silver nitrate impregnated silica gel afforded 0.213 g (49%) of the diene **12c**: mp 112–112.5 °C, clears 121 °C; NMR τ 4.43 and 4.62 ($H_{6,7}$, AB q, $J_{AB} \simeq 6$ Hz), 4.95–5.65 ($H_{3\alpha}$, br m), 7.88 ($C_{27}CH_3$, s), 7.98 ($AcCH_3$, s), 9.03 ($C_{19}CH_3$, s), 9.04 ($C_{21}CH_3$, d, $J \simeq 6$ Hz), 9.38 ($C_{18}CH_3$, s); uv λ_{max} 293 nm (ϵ 6440), 281 (10900), 270 (10300); ir ν_{max} 1710 cm^{-1} (C=O). Anal. ($C_{29}H_{44}O_3$) C, H.

24a-Homo-3β,25-dihydroxy-5,7-cholestadiene (4e). A solution of 0.181 g (0.000411 mol) of **12c** in ~10 ml of dry THF was added dropwise (15 min) to a cold (0 °C) solution of methyllithium (6.8 ml of a 1.8 M ethereal solution) in 15 ml of dry THF under anhydrous conditions. After 5 h at 0 °C the reaction mixture was left at ambient temperature overnight. The reaction mixture was worked up as in the preparation of **4a** to afford a pale yellow oil. Crystallization from acetone yielded 0.123 g (73%) of **4e** as a white chunky solid (mp 178–181.5 °C dec): NMR τ 4.41 and 4.63 ($H_{6,7}$, AB q, $J_{AB} \simeq 6$ Hz), 6.00–6.70 ($H_{3\alpha}$, br m), 8.80 ($C_{26,27}CH_3$, s), 9.06 ($C_{19}CH_3$, s), 9.38 ($C_{18}CH_3$, s); uv λ_{max} 293, 281, 270 nm; MS *m/e* 414 (M^+). Anal. ($C_{28}H_{46}O_2$) C, H.

24a-Homo-25-hydroxyvitamin D_3 [24a-Homo-3β,25-dihydroxy-9,11-seco-5,7,10(19)-cholestatriene, 2e]. Four equal batches of the provitamin (0.431 g, 0.00104 mol total) in 15% ethanol in ether solution were irradiated for 4 min each. The combined crude irradiation mixtures were concentrated to dryness and thermally equilibrated by refluxing in 30 ml of isopropyl ether–ethanol (9:1) overnight under a nitrogen atmosphere. Purification by repeated chromatography over 10% silver nitrate impregnated silica gel afforded 131 mg (30%) of **2e** (oil): uv λ_{max} 265 nm, λ_{min} 227 nm; NMR τ 3.72 and 3.99 ($H_{6,7}$, AB q, $J_{AB} \simeq$ 11 Hz), 4.96 and 5.20 (H_{19Z} and H_{19E}, m and d), 5.81–6.40 ($H_{3\alpha}$, br m), 8.80 ($C_{26,27}CH_3$, s), 9.43 ($C_{18}CH_3$, s); MS *m/e* 414 (M), 396 (M – H_2O), 136 (base), 118 (base – H_2O).

Biological Studies. The analogues were assayed according to the procedure of Hibberd and Norman[16] for their ability to stimulate intestinal calcium absorption and bone calcium mobilization in vitamin D deficient (rachitic) chicks. White Leghorn cockerels were raised for 3 weeks on a standard rachitogenic, low calcium diet[6b] without vitamin D supplement. Three days before assay the chicks were placed on a zero calcium diet. The vitamin D elicited responses were determined by published procedures[16] as follows. The duodenum of lightly ether anesthetized chicks was surgically exposed and 5 μCi of $^{45}Ca^{2+}$ + 4 mg of nonradioactive $^{40}Ca^{2+}$ carrier were placed into the small intestine. Thirty minutes later the animal was sacrificed by decapitation and the blood collected and allowed to clot. Intestinal calcium absorption was assessed by determining the amount of radioactivity present in a 0.2-ml aliquot of serum. Bone calcium mobilization response was directly quantitated via atomic ab-

sorption spectrometry on appropriately diluted samples of serum. The results were expressed as mg of Ca^{2+}/100 ml of serum. Additional details are given in the captions to Figures 1 and 2.

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References and Notes

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