Studies on Vitamin D (Calciferol) and Its Analogues. 15. 24-Nor-1α,25-dihydroxyvitamin D_3 and 24-Nor-25-hydroxy-5,6-*trans*-vitamin D_3[1]

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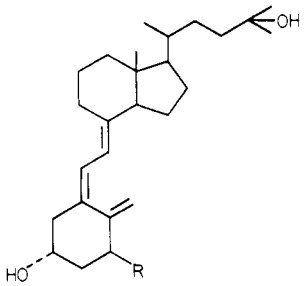
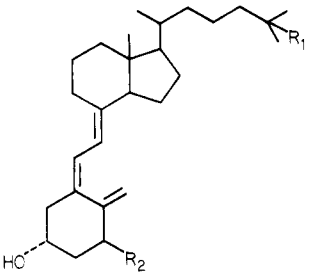
As part of our continuing structure–function study of the vitamin D_3 (**2a**) endocrine system and particularly the key metabolites 25-hydroxyvitamin D_3 (**2b**) and 1α,25-dihydroxyvitamin D_3 (**2c**), the analogues 24-nor-1α,25-dihydroxyvitamin D_3 (**1b**), 24-nor-25-hydroxy-5,6-*trans*-vitamin D_3 (**3**), and 24a-homo-25-hydroxy-5,6-*trans*-vitamin D_3 (**4**) were chemically synthesized and biologically evaluated. Analogues **3** and **4** were synthesized in one step by iodine-catalyzed isomerization of 24-nor-25-hydroxyvitamin D_3 (**1a**) and 24a-homo-25-hydroxyvitamin D_3 (**5**), respectively. Analogue **1b** was synthesized in ten steps (2% overall yield) by the procedure of Barton from 24-nor-25-hydroxycholesterylbenzoate (**6**). Analogue **1b** had readily detectable biological activity in vivo in the rachitic chick while **3** was without detectable activity. This suggests that our previous observations, that not only did analogue **1a** have no detectable biological activity but also functioned as an antagonist or antivitamin in the metabolic conversion of **2a** to **2b**, may be due to **1a**'s inability to be 1α-hydroxylated in vivo. Also, quantitation of the relative stimulation of intestinal calcium absorption, in vivo, as well as relative binding in vitro with the chick intestinal cytosol–chromatin receptor system for metabolite **2c** suggests the following. For 25-hydroxy analogues both in the 5,6-cis series (with a 1α-hydroxyl) as well as the 5,6-trans series (with a pseudo-1α-hydroxyl), shortening (as in **1b** and **3**) or lengthening (as in **4**) the side chain by one methylene group greatly reduces biological activity.

The analogue 24-nor-25-hydroxyvitamin D_3 (**1a**),[3] which differs from the natural steroidal prohormone 25-hydroxyvitamin D_3 (**2b**) only in that it lacks a single



1a, R = H
b, R = OH



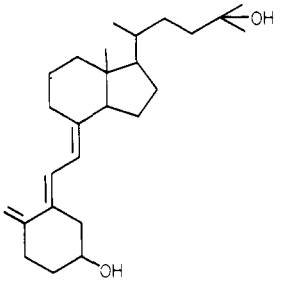
2a, $R_1 = R_2 = H$
b, R_1 = OH; $R_2 = H$
c, $R_1 = R_2$ = OH

side-chain methylene group, was recently biologically evaluated (in vivo, chicks) for its ability to elicit the classic vitamin D mediated responses[4] of stimulation of intestinal calcium absorption (ICA) and bone calcium mobilization (BCM). Although **1a** proved to be devoid of activity in these assays, it was observed to inhibit the normal responses produced by a physiological dose of vitamin D_3 (**2a**)[3a] but not that of **2b** or 1α,25-dihydroxyvitamin D_3 (**2c**).[5] This inhibitory effect on **2a** represents the first known example of *antivitamin* or antagonist activity in the vitamin D field.[6] Intriguing questions center around why 24-nor-25-hydroxyvitamin D_3 (**1a**) is devoid of biological activity and how it functions as an antivitamin. It is the purpose of this paper to describe a pharmacological approach to these questions wherein the synthesis and biological activities of putative metabolites or metabolite equivalents of **1a** are described.

In order to establish whether the biological inactivity (ICA and BCM) and antivitamin D_3 activity of **1a** are due to its direct biological action or whether it is first metabolized to 24-nor-1α,25-dihydroxyvitamin D_3 (**1b**), just as **2b** must be metabolized by the kidney to **2c** before it can function physiologically,[7] it seemed eminently logical to synthesize and biologically evaluate the 5,6-trans **3**[8] as well as the 1α-OH derivative **1b**. These goals have been achieved and the results are described below.

Results and Discussion

Synthesis. The iodine-catalyzed isomerization[9] of 24-nor-25-OH-D_3 (**1a**)[3] to 24-nor-25-OH-5,6-*trans*-D_3 (**3**)



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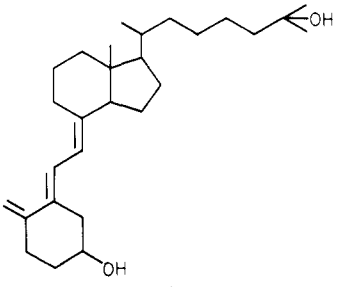
proceeded as expected. The product was purified chromatographically and characterized spectroscopically. For comparison purposes, the corresponding 24a-homo-25-hydroxy-5,6-*trans*-vitamin D_3 (**4**) was prepared by anal-



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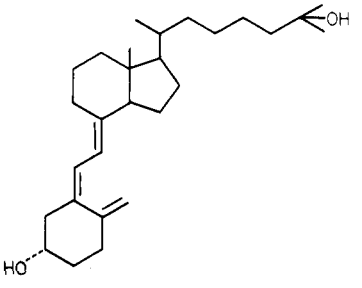

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ogous iodine catalysis from the previously reported 24a-homo-5,6-cis isomer **5**.[3] Both **3** and **4** exhibited the UV λ_{max} 273/λ_{min} 233 nm characteristic of 5,6-trans vitamins[9] as well as the unique A-ring fragment (by cleavage across the C_7–C_8 bond) in the mass spectrum characteristic of 5,6-trans as well as 5,6-cis vitamins.[10] Both **3** and **4** were rigorously purified to TLC homogeneity immediately before biological studies.

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Table I.[a] Stimulation of Intestinal Calcium Absorption (ICA) and Bone Calcium Mobilization (BCM) by 24-Nor-1α,25-$(OH)_2$-D_3

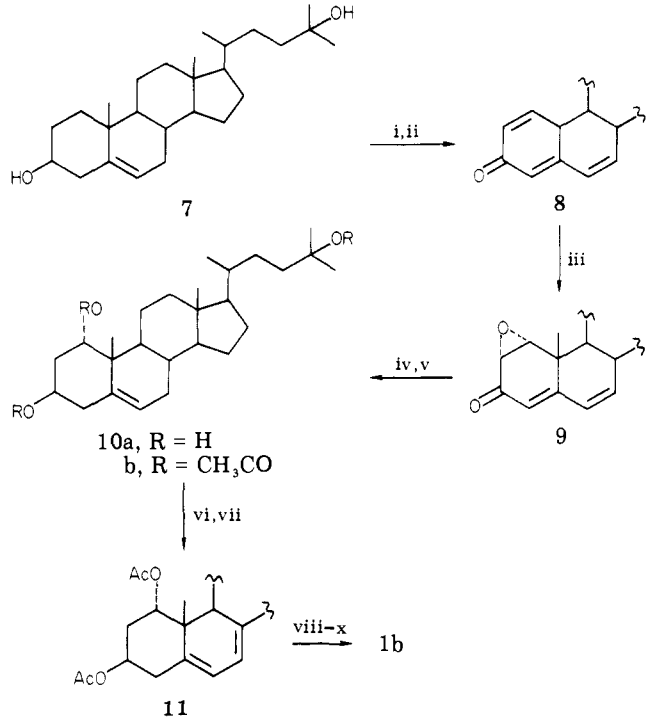
compd	administered dose, nmol	time of assay after dosing, h	BCM[b] (mg of Ca^{2+}/ 100 mL of serum ± SEM)	ICA[b,c] (plasma $^{45}Ca^{2+}$, cpm/ 0.2 mL ± SEM)	rel enhancement over control (ICA)
control (−D_3)			5.0 ± 0.6	520 ± 160	(1.0)
D_3	3.3	24	6.3 ± 0.8+	2430 ± 540*	4.6
1α,25-$(OH)_2$-D_3	0.60	12	6.6 ± 0.8+	1630 ± 530*	3.1
	3.0	12	6.1 ± 0.8	1320 ± 530*	2.5
24-nor-1α,25-$(OH)_2$-D_3	0.62	14	4.8 ± 0.6	420 ± 160	0.8
	3.1	14	4.4 ± 0.1	560 ± 80	1.1
	15.5	14	5.1 ± 0.1	680 ± 200	1.3
	62	14	5.0 ± 0.3	930 ± 240+	1.8
	310	14	6.5 ± 0.6	1530 ± 440*	2.9
	62	14	5.0 ± 0.3	930 ± 240+	1.8
	62	18	5.6 ± 1.0	1470 ± 700*	2.8
	62	24	5.4 ± 1.0	770 ± 280	1.5
	62	36	4.3 ± 0.9	590 ± 310	1.1

[a] The steroids were administered intraperitoneally in 0.2 mL of 1,2-propanediol–ethanol (1:1). At the indicated time an assay of ICA and BCM was carried out (Experimental Section and ref 4). [b] Each number (ICA and BCM) is the average ± SEM for groups of six to ten birds all from a single population of birds raised under identical conditions. [c] For the groups marked with an asterisk (*) $p < 0.01$ or with a plus (+) $p < 0.05$ compared with the -D controls (ICA).

With the availability of large quantities of 24-nor-25-hydroxycholesteryl-3β-benzoate (**6**)[11] we used the efficient Barton procedure[12] for synthesizing 1α,25-$(OH)_2$-24-nor-D_3 as depicted in Scheme I. The overall yield was 2.0% for the ten steps (average yield, 68%). Vitamin **1b** was proven to be unusually susceptible to conversion to a less polar substance (λ_{max} 230 nm) of unknown constitution. Thus, it was necessary to purify **1b** by preparative thin-layer chromatography (TLC) immediately before determining its spectral properties or utilizing it for biological assays. It was stored as a dilute solution under argon at dry ice temperature and used within 1 day after purification. The purified **1b** was TLC homogeneous in several systems and its spectral properties (NMR, UV, and MS) were in accord with the assigned structure. Its mass spectrum also reveals the characteristic peaks at *m/e* 152 and 134, due to the A-ring part by cleavage across the C_7–C_8 bond.[10]

Biological Assay. Vitamin D_3 and related compounds manifest their biological activity in vivo in a number of ways. Two of the more classic physiologic responses include intestinal calcium absorption (ICA) and bone calcium mobilization (BCM). In the former, the calciferol stimulates the translocation of calcium from the mucosal to the serosal side of the intestine. In the latter, the calciferol, by effecting the resorption of calcium from bone, causes an elevation of serum calcium levels. The new analogues (**1b** and **3**) resulting from the synthetic studies described herein were each assayed in vivo in rachitic chicks by the method of Hibberd and Norman.[4] The assay provides a good measure of the effectiveness of calciferol analogues in mediating the ICA and BCM responses. Shown in Table I is a time-course and dose–response evaluation of the ICA and BCM properties of the newly synthesized 24-nor-1α,25-$(OH)_2$-D_3 (**1b**). For comparative purposes a saturating dose of D_3 (3.3 nmol) and 1α,25-$(OH)_2$-D_3 (0.6 nmol) was given; we have previously established that these represent minimal saturating doses of D_3[13] and 1α,25-$(OH)_2$-D_3[13c] and that the time course for maximal response is 24–36[13a] and 12–14 h,[14] respectively. Thus, as is shown in Table I, when a large (62 nmol) dose of 24-nor-1α,-25-$(OH)_2$-D_3 (**1b**) is given intraperitoneally, the maximum ICA response occurred between 14 and 18 h. This time of maximum response is comparable to the ICA time courses for other 1α-hydroxylated analogues in the chick, e.g., 1α-OH-D_3 (14 h)[15] and 3-deoxy-1α,25-$(OH)_2$-D_3 (15 h).[16] Having determined that 14 h was an appropriate time for evaluation of 24-nor-1α,25-$(OH)_2$-D_3 (**1b**), a dose–response study over the range of 0.62–310 nmol was carried out. At a dose of 310 nmol of this analogue, a maximum stimulation of ICA occurred with only a marginally significant BCM response ($p < 0.1$). These data suggest that 24-nor-1α,25-$(OH)_2$-D_3 (**1a**) is relatively more active in the ICA than the BCM system; we have previously reported other examples of differing structural specificity of these two systems.[4,17] Also an approximate relative dose ratio for maximal ICA response can be estimated for 24-nor-

Scheme I. Synthetic Scheme for 1b[a]



[a] Reactions: i, KOH-CH_3OH (97%) on **6** (3β-benzoate of **7**); ii, DDQ (57%); iii, H_2O_2-NaOH (79%); iv, Li/NH_3-THF-NH_4Cl (79%); v, Ac_2O-C_5H_5N (81%); vi, NBS-$(C_6H_5COO)_2$-hexanes; vii, *s*-collidine (56% from **10b**); viii, *hν*, ether; ix, 85–90 °C, 2.25 h; x, $LiAlH_4$-ether (13% from **11**).

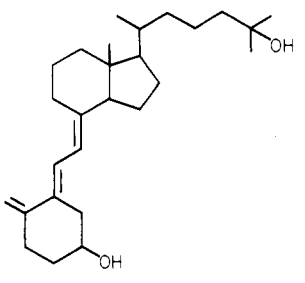
Table II. Relative Competitive Index (RCI) Values for Analogues of 1α,25-$(OH)_2$-D_3

compd	RCI[a]
1α,25-$(OH)_2$-D_3 (**2c**)	100
24-nor-1α,25-$(OH)_2$-D_3 (**1b**)	46 ± 4
25-OH-5,6-*t*-D_3 (**12**)	0.58 ± 0.19
24-nor-25-OH-5,6-*t*-D_3 (**3**)	0.10 ± 0.04
24a-homo-25-OH-5,6-*t*-D_3 (**4**)	0.066 ± 0.015

[a] The uncertainties are standard deviations. See the Experimental Section for details.

1α,25-$(OH)_2$-D_3/1α,25-$(OH)_2$-D_3; this is calculated to be 310/0.6 or 5200/1. Thus the new analogue **1b** is only approximately 0.02% as active as 1α,25-$(OH)_2$-D_3. This emphasizes the critical importance of the normal 8-carbon isooctyl side chain terminated by a tertiary hydroxyl for ICA activity. These results further suggest that our previous reports of no biological activity for 24-nor-25-OH-D_3 (**1a**) might be due to a lack of further metabolism since, as shown in the current study, the presence of a 1α-hydroxyl group does produce a biologically active molecule.

In separate experiments (data not presented) where a large 163-nmol dose of 24-nor-25-OH-5,6-*trans*-D_3 was given intraperitoneally, there was no stimulation between 5 and 36 h of either ICA or BCM. In the same experiment 25-OH-D_3, 1α,25-$(OH)_2$-D_3, and 25-OH-5,6-*trans*-D_3 all stimulated both ICA and BCM. As shown by comparison of the bioassay results given in Table I with the intestinal cytosol–chromatin assay results given in Table II, it is apparent that an analogue with a 24-nor-25-OH side chain, either with (Table I) a 1α-hydroxy group or with a pseudo-1α-hydroxyl group as in **3**, is not efficiently delivered to the intestine or bone. While 1α,25-$(OH)_2$-D_3 (Table I) and 25-OH-5,6-*trans*-D_3 (**12**) both were capable



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of significantly stimulating an ICA response, neither 24-nor-1α,25-$(OH)_2$-D_3 (discussed above) nor particularly 24-nor-25-OH-5,6-*trans*-D_3 (**3**), even at a high 163-nmol dose, could marginally stimulate ICA or BCM. It may be concluded that the effective dose ratio for ICA of 24-nor-25-OH-5,6-*trans*-D_3/1α,25-$(OH)_2$-D_3 is less than 163/0.6 or 272/1; i.e., the analogue is less than 0.4% and almost surely less than 0.02% as active as the natural hormone 1α,25-$(OH)_2$-D_3 when assayed in vivo.

Yet as can be seen (Table II) when 24-nor-1α,25-$(OH)_2$-D_3 (**1b**) is evaluated in the chick intestinal cytosol–chromatin assay, rather than being only 0.02% as effective as 1α,25-$(OH)_2$-D_3 (Table I), it was about 40% as effective in competing with [^{3}H]-1α,25-$(OH)_2$-D_3. Thus under in vitro analysis, where there is no "delivery problem", this analogue is a quite effective competitor.

Also shown are the RCI values for the 5,6-trans derivatives of 25-OH-D_3 (**12**), 24-nor- and 24a-homo-25-D_3. As we have reported previously,[18] 5,6-trans analogues, which also have 25-hydroxyl groups, still are not effective competitors with 1α,25-$(OH)_2$-D_3 even though they have the critical pseudo-1α-hydroxyl. This is apparently more a reflection of the orientation of the 10,19-methylene in the 5,6-trans geometry rather than the absence of a 3β-hydroxy.[18] By comparison of the RCI values for the standard 25-hydroxyl-containing 8-carbon side chain (RCI = 0.58) with that of a similar side chain which has been shortened (RCI = 0.10) or lengthened (RCI = 0.07) by only one methylene group, it is apparent that these subtle changes effect an 80–90% reduction in an ability to compete. It must be concluded that the chick intestinal cytosol–chromatin receptor system is remarkably sensitive to the length of the 25-hydroxyl containing side chain of its endogeneous steroid hormone 1α,25-$(OH)_2$-D_3 (**2c**).

In summary, we have established that it is likely that the biological inactivity of 24-nor-25-OH-D_3 (**1a**) previously reported[3,19] may be due to its inability to be 1α-hydroxylated since the new steroid 24-nor-1α,25-$(OH)_2$-D_3 (**1b**), whose synthesis is reported in this communication, had detectable ICA activities. This possibility can only be directly evaluated by synthesis of radioactive 24-nor-25-OH-D_3 and analysis of its metabolic fate. Also we have presented new evidence from both in vivo as well as in vitro analysis that the chick vitamin D endocrine system has a remarkably high specificity with regard to its 25-hydroxylated side chain.

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 (unless otherwise indicated) for nuclear magnetic resonance 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-GT spectrophotometer and 95% ethanol was used as solvent. Chloroform solutions were used to take infrared (IR) spectra using a Perkin-Elmer 137 spectrophotometer. Dry tetrahydrofuran (THF) or dry ether 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. Alumina refers to Woelm activity III material of neutral grade. Microanalyses were performed by Elek Microanalytical Labs, Torrance, Calif. Mass spectra (MS) were obtained using a Finnigan 1015 C mass spectrometer with an ionizer setting of 70 eV/300 μA.

24-Nor-25-hydroxy-5,6-*trans*-vitamin D_3 (3). To a solution of 24-nor-25-hydroxyvitamin D_3 (**1a**, 5 mg)[3] in LBPE (25 mL) was added the dilute iodine *stock solution* (1 mL of stock diluted to 25 mL with LBPE) (*stock solution*: freshly prepared, 10 mg of iodine in 100 mL of LBPE). The reaction was allowed to proceed at room temperature for 1 h (nitrogen, diffuse fluorescent room lights). The solution was vigorously washed with 1% aqueous sodium sulfite and then water. After drying (sodium sulfate), the solution was concentrated under vacuum to afford a residue which exhibited λ_{max} 270 nm; TLC (80:20 ether–LBPE) showed the presence of nearly equal amounts of two components (**1a**/**3**, R_f 0.24/0.17). Preparative TLC effected separation of pure **3** and recovered **1a** contaminated by some **3**. The isolated yields (by UV assuming ϵ_{273} = 22700) ranged from 12 to 20% for reactions carried on a 3–5-mg scale and from 6 to 10% on a 1-mg scale. The homogeneity of the product was established by TLC (silica gel G and 10% $AgNO_3$–silica gel G using ether, 80:20 ether–LBPE, or 40:60 acetone–benzene). Starting material and the corresponding isotachysterol were absent (TLC, UV). Synthesis, purification, and administration of steroid for biological assays were performed on the same day. Moreover, duplicate bioassays were performed on two or more independently synthesized and purified samples. Synthesis and purification of **3** was also carried out immediately before spectral determination: UV[9] λ_{max} 273 nm,

λ_{min} 233 nm; MS m/e (rel intensity) 386 (M, 3), 368 (M − H_2O, 2), 136 (48), 118 (100), and 59 (48).[10]

24a-Homo-25-hydroxy-5,6-*trans*-vitamin D_3 (4). The 5,6-cis isomer **5** reported earlier[3] was isomerized as described in the preceding section except that the isomerization was run at a tenfold greater dilution. One-milligram scale reactions afforded ~20% yields (UV determination assuming $\epsilon_{273} = 22700$)[9] of TLC homogeneous material (R_f 0.62; **5**, R_f 0.58; the corresponding isotachysterol had λ_{max} 289 nm, R_f 0.51). A freshly synthesized and purified sample exhibited UV[9] λ_{max} 273 nm, λ_{min} 233 nm; MS m/e (rel intensity) 414 (M, 2), 136 (90), 118 (100), and 59 (91).[10]

24-Nor-25-hydroxycholesterol (7). The starting material 7 was obtained by saponification of 24-nor-25-hydroxycholesteryl-3β-benzoate (**6**).[11] Conditions: benzoate **6**, 5 g in 10% KOH–methanol (250 mL) and THF (50 mL) for 3 h (room temperature, nitrogen). Workup and crystallization (methanol) afforded 3.84 g (97%, mp 179–180 °C) of pure **7**.

24-Nor-25-hydroxy-1,4,6-cholestatrien-3-one (8). A mixture of **7** (2.15 g, 5.52 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 4.5 g, 18.8 mmol) in *p*-dioxane (60 mL, freshly distilled from sodium) was refluxed (11 h) under anhydrous conditions. The cooled mixture was filtered and the solid washed with acetone. The combined filtrate and washings were combined and concentrated to a residue which was passed through a short column of alumina (acetone). The residue upon chromatography (dry column alumina, 100 g, 10% acetone–benzene) afforded material which was crystallized (acetone–LBPE). The purified trienone **8** (1.2 g, 57%) exhibited the following: mp 150 °C; NMR τ 2.90 and 3.80 (H_1 and H_2, AB q, J_{AB} ~ 10.0 Hz, H_2 further split, 1.5 Hz), 3.8–4.1 ($H_{4,6,7}$, 3 H, m), 8.80 ($C_{19,26,27}$ $3CH_3$, s), and 9.19 ($C_{18}CH_3$, s); IR ν_{max} 3570, 3420, 1630, 1600 cm^{-1}; UV λ_{max} (ε) 226 (12000), 260 (9400), 304 nm (12900). Anal. ($C_{26}H_{38}O_2$) C, H.

24-Nor-25-hydroxy-1α,2α-oxido-4,6-cholestadien-3-one (9). A 25% aqueous sodium hydroxide solution (5 mL) was added to an ice cooled solution of trienone **8** (1.56 g, 4.1 mmol) in *p*-dioxane (120 mL, freshly distilled from sodium). After adding 30% hydrogen peroxide solution (28 mL) and stirring 10 h at room temperature, the reaction mixture was poured at once into ice water (300 mL). Workup (ether; sodium sulfate drying) and crystallization (isopropyl ether–methanol) afforded 1.29 g (79%) of pure **9**: mp 148–150 °C; NMR τ 3.88 (H_4, s), 4.3–4.4 ($H_{6,7}$, m), 6.39 and 6.56 ($H_{2\beta}$ and $H_{1\beta}$, AB q, J_{AB} ~ 4 Hz; $H_{1\beta}$ further split into d, J ~ 1.5 Hz), 8.78 ($C_{26,27}$ $2CH_3$, s), 8.81 ($C_{19}CH_3$, s), and 9.20 ($C_{18}CH_3$, s); IR ν_{max} 3570, 3510, 1680, 1640 cm^{-1}; UV λ_{max} 296 nm (ε 20200). Anal. ($C_{26}H_{38}O_3$) C, H.

24-Nor-1α,25-dihydroxycholesterol (10a). A solution of lithium (1.67 g, 0.241 mol) in ammonia (~110 mL) and dry THF (84 mL) was prepared under nitrogen at −78 °C. The epoxydienone **9** (1.26 g, 3.16 mmol) in dry THF (25 mL) was added dropwise (1 h) with stirring to the lithium solution at −78 °C. The bath was removed and stirring continued at refluxing ammonia temperature for 3 h. Dry ammonium chloride (14.5 g) was added in small portions over 2.25 h, a saturated aqueous ammonium chloride solution was added over 20 min, and then the ammonia was allowed to evaporate by stirring overnight at room temperature (nitrogen). After adding H_2O–THF to dissolve the precipitated salts, the mixture was extracted thoroughly with chloroform (5 × 100 mL). After drying and evaporating the solvent, chromatography (silica gel, 35 g, chloroform and 10–20% acetone–benzene) of the residue afforded, after vacuum drying, the triol **10a** (1.02 g, 80%) sufficiently pure for the next step: NMR τ 4.4 (H_6, m), ~6.0 ($H_{3\beta}$, br, W ~ 20 Hz), 6.15 ($H_{1\beta}$, br, W ~ 8 Hz), 8.80 ($C_{26,27}$ $2CH_3$, s), 8.96 ($C_{19}CH_3$, s), and 9.32 ($C_{18}CH_3$, s). Recrystallization (ethyl acetate–ethanol) afforded a sample with mp 211–212 °C. Anal. ($C_{26}H_{44}O_3$) C, H.

24-Nor-1α,3β,25-triacetoxycholest-5-ene (10b). A mixture of triol **10a** (534 mg, 1.2 mmol), acetic anhydride (0.2 mL, freshly distilled), and pyridine (6.5 mL, freshly distilled) was refluxed for 40 h under nitrogen. At 13, 26, and 39 h, additional acetic anydride (0.1 mL each time) was added. After quenching by adding ice pellets and allowing to stand (2 h), the reaction mixture was filtered and then worked up with ether in the usual way. An essentially quantitative yield of vacuum-dried crude triacetate **10b** was obtained. Crystallization (2-propanol) afforded 567 mg (81%) of **10b** as colorless needles: mp 117–119 °C; NMR τ 4.4 (H_6, br), ~4.9 ($H_{1\beta}$, br, W ~ 8 Hz), ~4.9–5.2 ($H_{3\alpha}$, br, W ~ 20 Hz), 7.94, 7.99, 8.03 (3OAc, s), 8.59 ($C_{26,27}$ $2CH_3$, s) 8.90 ($C_{19}CH_3$, s), and 9.32 ($C_{18}CH_3$, s); MS m/e (rel intensity) 410 (M − 2HOAc, 6), 118 (100).

24-Nor-1α,3β,25-triacetoxy-5,7-cholestadiene (11). All operations (under nitrogen) were conducted with minimal exposure to air. To a refluxing solution of **10b** (100 mg, 0.19 mmol) and dibenzoyl peroxide (10 mg) in hexanes (4 mL; purified) was added *N*-bromosuccinimide (40 mg, 0.22 mmol) at once. After 20 min at reflux, the mixture was ice cooled and filtered and then the precipitate thoroughly washed with hexanes (4 × 4 mL). The combined filtrate and washings were stripped (room temperature) and then the resulting residue in xylene (6 mL, purified) was added dropwise (10 min) to refluxing *s*-collidine (6 mL). After 25 min, the cooled mixture was filtered into ether–water and the ether phase was washed with cold 5% hydrochloric acid (until *s*-collidine was absent), saturated sodium bicarbonate, and water. After drying, filtering, and concentrating (high vacuum), the crude residue was chromatographed (10% $AgNO_3$–silica gel, 35 g, gradient between LBPE and 30% ether–LBPE) to afford TLC pure provitamin triacetate (56 mg, 56% yield) **11**. Crystallization (isopropyl ether) afforded 40 mg of material with mp 145–146.5 °C: NMR (Bruker WH-FT-90/D-18, 90 MHz) τ 4.31 and 4.59 ($H_{6,7}$, AB q, J_{AB} ~ 5.6 Hz), ~5.0 ($H_{1\beta}$, W ~ 8 Hz, overlapped with $H_{3\alpha}$, $W \gtrsim 30$ Hz), 7.92, 7.97, 8.05 (3Ac, s), 8.52 ($C_{26,27}$ $2CH_3$, s), 8.99 ($C_{19}CH_3$, s), and 9.39 ($C_{18}CH_3$, s); UV λ_{max} (ε) 265 (8400), 274 (11900), 284 (13000), and 296 nm (7600); MS (20 eV) m/e (rel intensity) 528 (M, 2.1), 468 (M − HOAc, 11), 408 (M − 2HOAc, 37), 348 (M − 3HOAc, 63), 155 (100).

24-Nor-1α,25-dihydroxyvitamin D_3 (1b). A total of 90 mg of provitamin triacetate **10b** in ether (ice cooling, nitrogen purging) was irradiated in six equal batches (15 mg of **10b** per 100 mL of ether) by the method previously described.[3] The combined photolysis residue was chromatographed (10% $AgNO_3$–silica gel, 35 g, gradient between 5% ether–LBPE and 30% ether–LBPE). Fractions enriched in provitamin were irradiated again in two batches and the resulting residue was chromatographed (10% $AgNO_3$–silica gel, 14 g, solvents as above). Fractions from the two chromatographies enriched in λ_{max} 260 nm, λ_{min} 236 nm material (previtamin triacetate) were pooled, concentrated, and then heated (85–90 °C, 2.5 h) in isooctane (18 mL, freshly distilled and deoxygenated). Upon concentration, the resulting residue (vacuum dried) was dissolved in dry ether (20 mL) under nitrogen. Lithium aluminum hydride (40 mg) was added under nitrogen to the ether solution and the mixture was refluxed for 1 h. After the addition of ice pellets to quench the reaction, the product was worked up by the usual protocol. The vacuum-dried residual product was chromatographed twice (silica gel, 40 g each time; gradient between 50% ether–LBPE and ether and then between 1–5% methanol–ether). The amount of desired **1b** (12.1 mg) was determined from its UV spectrum (λ_{max} 266 nm, λ_{min} 230 nm in ether) assuming ϵ_{266} 18300: NMR (Bruker WT-FT-90/D-18, 90 MHz) τ 3.61 (H_6, d, J ~ 11.5 Hz), 4.00 (H_7, d, J ~ 11.5 Hz), 4.67 (H_{19Z}, br t, J ~ 1.5 Hz), 5.00 (H_{19E}, br t, J ~ 1.3 Hz), 5.58 ($H_{1\beta}$, br t, J ~ 6.3 Hz), 5.78 ($H_{3\alpha}$, br m), 8.81 ($C_{26,27}$ $2CH_3$, s), and 9.47 ($C_{18}CH_3$, s); MS (20 eV) m/e (rel intensity) 402 (M, 1), 384 (M − H_2O, 23), 366 (M − $2H_2O$, 5), 152 (39), 134 (100).

Vitamin **1b** proved to be unusually labile. For this reason, samples were purified (preparative TLC, silica gel G, ethyl acetate) immediately before bioassay or recording spectra. Samples were stored in ether solution under argon at dry ice temperatures for no more than 1 day. The vitamin tended to deteriorate to a less polar material (appeared as a single spot by TLC) with λ_{max} 230 nm in its UV spectrum.

Bioassay (in Vivo). The analogues 24-nor-25-OH-5,6-*trans*-D_3 (**3**) and 24-nor-1α,25-dihydroxyvitamin D_3 (**1b**) were assayed in vitamin D deficient (rachitic) chicks for their ability to stimulate intestinal calcium absorption (ICA) and bone calcium mobilization (BCM). White Leghorn cockerels were raised for 4 weeks on a standard rachitogenic, low calcium diet[20] without vitamin D supplement. Three days before assay the chicks were placed on a zero calcium diet. The ICA/BCM responses were determined by the procedure of Hibberd and Norman[4] as follows. The duodenum of lightly ether anesthesized chicks was exposed surgically and 5 μCi of $^{45}Ca^{2+}$ + 4 mg of $^{40}Ca^{2+}$ carrier were

introduced in the small intestine. After 30 min the animal was sacrificed by decapitation and the blood collected and allowed to clot. The ICA response was reflected in the amount of measured radioactivity per 0.2-mL aliquot of serum. The BCM response was quantitated directly by atomic absorption spectrometry (Perkin-Elmer Model 303 atomic absorption spectrometer) on appropriately diluted samples of serum (expressed as mg of Ca^{2+}/100 mL of serum). Additional details and the results are given in Table I.

Bioassay (in Vitro). The ability of 24-nor-1α,25-dihydroxyvitamin D_3 (1b), 24-nor-25-hydroxy-5,6-*trans*-vitamin D_3 (**3**), 24a-homo-25-hydroxy-5,6-*trans*-vitamin D_3 (**4**), and 25-hydroxy-5,6-*trans*-vitamin D_3 (**12**) to interact with the intestinal receptor system in the chick for 1α,25-dihydroxyvitamin D_3 (**2c**) was assessed, in vitro, by employing a competitive binding assay described previously in detail.[18] This assay is based on the decrease in radioactivity bound to a finite number of chromatin acceptor sites in the presence of increasing concentrations of a nonradioactive competitor and a constant saturating concentration of radioactive **2c** which occurs in a reconstituted cytosol–chromatin receptor system. The assay mixture consisted of nonradioactive competitor, tritiated **2c** (2×10^{-8} M), and chick intestinal chromatin (375 μg of DNA) dispersed in a 0.5 mL of cytosol fluid. After incubating these components (30 min, 25 °C), the chromatin was reisolated by centrifugation. The chromatin fraction was washed three times (0.5% Triton X-100 in 0.01 M Tris–HCl, pH 8.5) to remove loosely bound and free steroid. The radioactivity bound to the chromatin was determined to 2% error at a tritium efficiency ranging from 50 to 55% in a Beckman liquid scintillation counter. The results are expressed as the relative competitive index (RCI).[18b,22] The RCI value is a linear scale where **2c**, the natural hormone, is defined as having an RCI of 100. The method of calculation has been previously described in detail.[18,21,22] See Table II for the results.

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References and Notes

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