New Vitamin D₃ Derivatives with Unexpected Antiproliferative Activity: 1-(Hydroxymethyl)-25-hydroxyvitamin D₃ Homologs[†]

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Surprisingly, both of the synthetic 1-(hydroxymethyl)-25-hydroxyvitamin D₃ diastereomers (–)-2 and (+)-3 retained the antiproliferative activity of natural calcitriol in murine keratinocytes. Preliminary studies indicated, however, that both of these synthetic diastereomers were less than 0.1% as effective as calcitriol for binding to the 1,25-(OH)₂-D₃ receptor and that they were less than 0.1% as potent as calcitriol for calbindin-D₂₈ₖ induction in organ-cultured embryonic chick duodenum. 1-(Hydroxymethyl)-25-hydroxyvitamin D₃ homologs (–)-2 and (+)-3 were synthesized in a convergent manner by combining enantiomerically pure C,D-ring ketone 12 with highly enantiomerically enriched A-ring phosphine oxides (–)-11a and (+)-11b. These A-ring chirons were prepared starting from thermal [2 + 4] cycloaddition of 3-bromo-2-pyrone and acrolein.

In addition to regulating phosphorus metabolism and intestinal calcium absorption (ICA) as well as bone calcium mobilization (BCM), the hormonally active metabolite 1α,25-dihydroxyvitamin D₃ (calcitriol, 1) potently promotes cell differentiation and inhibits cell proliferation.[1] Calcitriol also affects the human immune system.[2] Calcitriol and some synthetic vitamin D₃ derivatives have been used recently in practical, clinical chemotherapy of such diverse human illnesses as osteoporosis, cancer, immunodeficiency syndromes, and the skin disorders dermatitis and psoriasis.[3] A major international objective among researchers in academia[4] and in the pharmaceutical industry[5] still is to prepare vitamin D₃ analogs as new drugs in which calcitropic activity is effectively separated from cell growth regulation. Toward this goal, well over 200 analogs of calcitriol have been synthesized and evaluated. Among these, all of the leading drug candidates have in common in ring-A the 1α-hydroxyl substituent characteristic of calcitriol; they differ mostly in the side chain attached to ring-D of the steriod framework. Indeed, it is now commonly accepted that *the 1α-hydroxyl group is required for desirable biological activity.*[6]

Various calcitriol analogs lacking the 1α-hydroxyl group have been prepared and have been found to be much less biologically active than calcitriol; examples include 1β-hydroxyl,[7] 1α-fluoro,[8] and 1-unfunctionalized (i.e., 25-hydroxyvitamin D₃).[9] A recent computer search of the literature showed no known 1-(hydroxyalkyl) derivative of calcitriol.

As part of our ongoing research program using Diels–Alder cycloadditions of heteroaromatic dienes to prepare valuable and versatile unsaturated, bridged, bicyclic lactones and lactams,[4a,10] we have now synthesized 1-(hydroxymethyl)-25-hydroxyvitamin D₃ analogs (–)-2 and (+)-3. *Quite unexpectedly, the 1-(hydroxymethyl)-25-hydroxyvitamin D₃ homologs (–)-2 and (+)-3 are very similar to calcitriol in inhibiting growth of murine keratinocytes.* Herein are reported the chemical syntheses of these new 1-hydroxymethyl homologs of calcitriol and their preliminary in vitro biological evaluation.

Chemistry

Utilizing recently developed synthetic methodology,[10,11] we have prepared ring-A phosphine oxide 11 for Horner–Wittig coupling with C,D-ring ketone 12 in a convergent
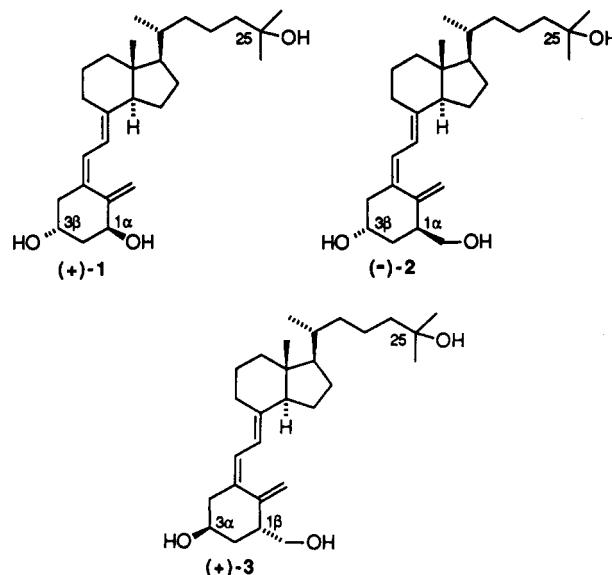
approach to the vitamin D₃ family that was pioneered by the Lythgoe group.[12] Thus, easily prepared, ambiphilic 3-bromo-2-pyrone (4) underwent smooth, regiospecific, and stereoselective Diels–Alder cycloaddition with acrolein under sufficiently mild thermal conditions (70–90 °C) to allow isolation on gram scale of the desired, unsaturated, bridged, bicyclic lactone adduct;[11a] because this bicyclic aldehyde was unstable to chromatography, it was immediately reduced and then O-silylated to give chromatographically stable, crystalline, bicyclic, primary alcohol

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(1) (a) Tanaka, H.; Abe, E.; Miyaura, C.; Kuribayashi, T.; Konno, K.; Nishii, Y.; Suda, T. 1α,25-Dihydroxycholecalciferol and a human myeloid leukaemia cell line (HL-60). *Biochem. J.* **1982**, *204*, 713–719. (b) Abe, E.; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. Differentiation of mouse myeloid leukemia cells induced by 1α,25-dihydroxyvitamin D₃. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4990–4994. (c) For a review, see Cancela, L.; Theofan, G.; Norman, A. W. The pleiotropic vitamin D hormone. In *Hormones and Their Actions*; Cooke, B. A., King, R. J. B., Van der Moler, H. J., Eds.; Elsevier: New York, 1988; Part I, Chapt. 15, pp 269–289.
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derivative **5** in 46% overall yield. Reductive cleavage of the bridgehead carbon–bromine bond was achieved in high yield under neutral radical conditions using tributyltin hydride and azobis(isobutyronitrile) (AIBN);[13] the halogen-free bicyclic lactone product is the synthetic equivalent of the product derived from 2-pyrone itself cycloadding to acrolein, a *Diels–Alder reaction that requires high pressures and that cannot be accomplished simply by heating because of loss of CO_2 from the lactone bridge.*[11a] Basic methanolysis of the lactone bridge and in situ conjugation of the carbon–carbon double bond gave conjugated cyclohexene ester alcohol **6**. Resolution of this alcohol **6** was achieved via formation and separation by preparative HPLC and preparative TLC of diastereomeric esters **7a** and **7b**, derived from enantiomerically pure α-methoxyphenylacetic acid (Scheme I). Analytical HPLC indicated purified diastereomer **7a** to have a diastereomeric excess (de) of 98.8% and **7b** of 96.5%. Methanolysis of diastereomic esters **7a** and **7b** separately gave back the original alcohol **6** as a pair of enantiomers, **6a** and **6b**; each enantiomer was carried on separately.

The absolute stereochemistry of enantiomer **6b** (and therefore also **6a**) was assigned by chemical correlation with a closely related compound of established absolute configuration,[14] as outlined in Scheme II.

O-Silylation of alcohols **6** gave bis-silyl ethers **8**, and then reduction of the conjugated methyl ester functionality produced allylic alcohols **9**. The [3,3] sigmatropic rearrangement using our newly-developed sulfinyl orthoester allowed efficient, one-flask, regiospecific formation of two-carbon-extended conjugated dienoate esters **10**;[15] this mixture of geometric isomers was photochemically isomerized into the desired (Z)-**10**.[11] On the basis of literature precedent[16] and on our own experience,[4a,11b] dienoate esters **10** were reduced, chlorinated, converted into the corresponding phosphines, and finally oxidized to give ring-A phosphine oxides **11** as two enantiomers (**11a** and **11b**) having almost equal but opposite specific rotations of approximately 54°.

Lythgoe-type coupling[12] of 80–100 mg of ring-A phosphine oxides **11a** and **11b** with enantiomerically pure ring-C,D chiron **12** was followed immediately by fluoride-promoted desilylation to form (−)-1α-(hydroxy-

methyl)-25-hydroxyvitamin D₃ [(−)-**2**] and (+)-1β-(hydroxymethyl)-3α,25-dihydroxy analog (+)-**3** in good yields (Scheme III). Two aspects of this coupling are worthy of emphasis. First, a systematic study of bases used to deprotonate phosphine oxides like **11** (e.g., MeLi, MeLi·TMEDA, n-BuLi, PhLi, LDA) showed PhLi to be best as determined by the yield of the coupled triene product.

(3) (a) Koeffler, H. P.; Amatruda, T.; Ikekawa, N.; Kobayashi, Y.; DeLuca, H. F. Induction of macrophage differentiation of human normal and leukemic myeloid stem cells by 1,25-dihydroxyvitamin D₃ and its fluorinated analogues. *Cancer Res.* **1984**, *44*, 5624–5628. (b) MacLaughlin, J. A.; Gange, W.; Taylor, D.; Smith, E.; Holick, M. F. Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D₃. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5409–5412. (c) Kragballe, K. Treatment of psoriasis by the topical application of the novel cholecalciferol analogue calcipotriol (MC 903). *Arch. Dermatol.* **1989**, *125*, 1647–1652. (d) Zhou, J. Y.; Norman, A. W.; Chen, D. L.; Sun, G. W.; Uskokovič, M.; Koeffler, H. P. 1,25-Dihydroxy-16-ene-23-yne-vitamin D₃ prolongs survival time of leukemic mice. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3929–3932 and references therein. (e) Norman, A. W.; Zhou, J. Y.; Henry, H. L.; Uskokovič, M. R.; Koeffler, H. P. Structure-function studies on analogues of 1α,25-dihydroxyvitamin D₃: differential effects on leukemic cell growth, differentiation, and intestinal calcium absorption. *Cancer Res.* **1990**, *50*, 6857–6864. For reviews see (f) DeLuca, H. F. The vitamin D story: a collaborative effort of basic science and clinical medicine. *FASEB J.* **1988**, *2*, 224–236. (g) Reichel, H.; Norman, A. W. Systemic effects of vitamin D. *Annu. Rev. Med.* **1989**, *40*, 71–78. (h) Reichel, H.; Koeffler, H. P.; Norman, A. W. The role of the vitamin D endocrine system in health and disease. *N. Engl. J. Med.* **1989**, *320*, 980–991.

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(5) For recent examples, see (a) Calverley, M. J.; Binderup, E. T. Preparation of Vitamin D Analogs. PCT Int. Pat. Appl. WO 91 00,271; *Chem. Abstr.* **1991**, *115*, 28990q. (b) Hansen, K. Preparation of 20-aralkoxy vitamin D derivatives as drugs. PCT Int. Pat. Appl. WO 91 09,841; *Chem. Abstr.* **1991**, *115*, 159516n. (c) Neef, G.; Kirsch, G.; Steinmeyer, A.; Schwarz, K.; Braeutigam, M.; Thieroff-Ekerdt, R.; Rach, P. Preparation of vitamin D analogs as antihyperproliferatives with reduced risk of hypercalcemia. Eur. Pat. Appl. EP 441,467; *Chem. Abstr.* **1991**, *115*, 232611w. (d) Kubodera, N.; Watanabe, H. Preparation of 22-oxavitamin D derivatives as antitumor agents. Jpn. Kokai Tokkyo Koho JP 03,188,061; *Chem. Abstr.* **1991**, *115*, 280378x. (e) Takayama, H.; Yamada, S.; Manaka, A.; Kasama, T. Preparation of vitamin D₂ fluoro derivatives. Jpn. Kokai Tokkyo Koho, JP 03 68,528; *Chem. Abstr.* **1991**, *115*, 136498u. (f) Tsuji, M.; Tachibana, Y.; Yokoyama, S.; Ikekawa, N. Preparation of 1α,25-dihydroxyvitamin D₃ analogs via photoisomerization of 5,7-ergostadiene-1α,3β,25-triols. Eur. Pat. Appl. EP 390,097; *Chem. Abstr.* **1991**, *114*, 122,842u.
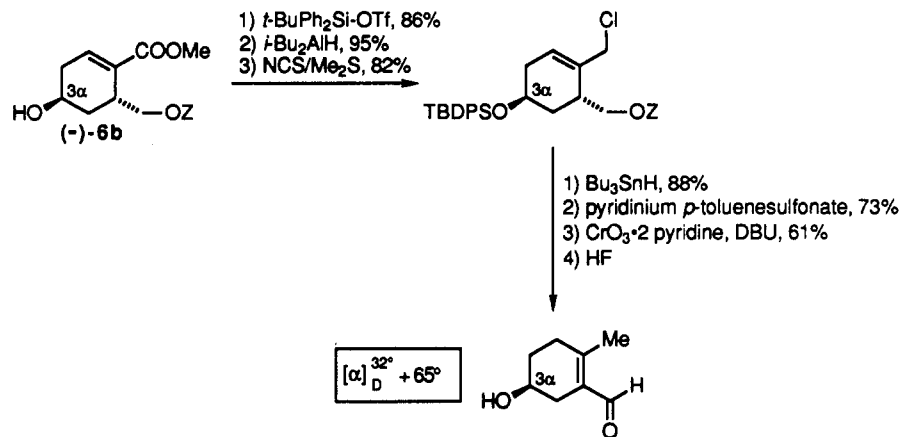
Scheme I
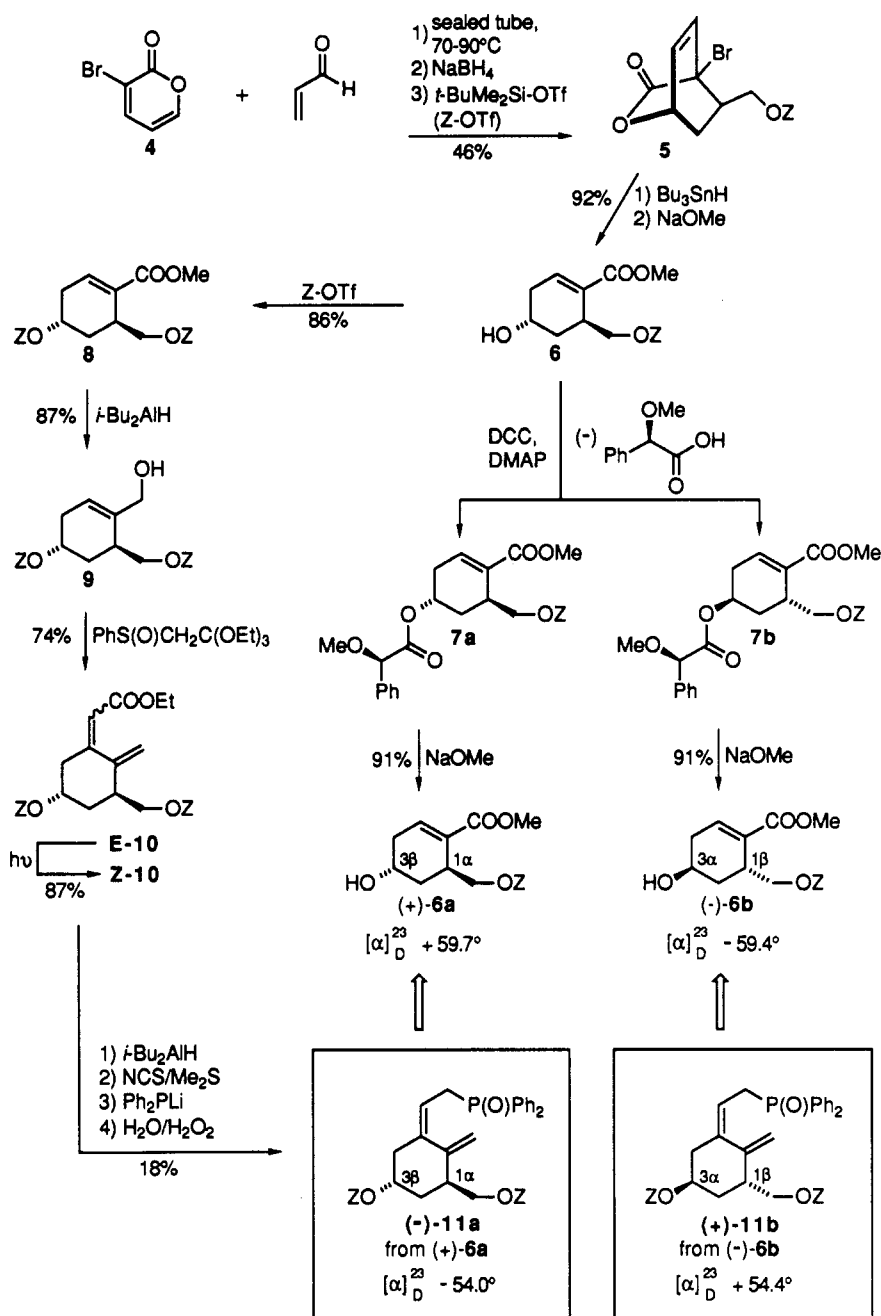
Second, the scale of the coupling reaction was critical to its success; whereas coupling using 80–100 mg of ring-A phosphine oxide proceeded routinely in good yields, coupling on 10–20 mg scale proceeded very poorly. An enormous amount of effort was devoted to make these small-scale couplings work; however, all attempts failed, including scrupulous drying of the gaseous nitrogen or argon gas used as the atmosphere above the reaction

mixture, scrupulous drying of solvents and reagents, use of molecular sieves, and azeotroping off any adventitious water by adding and removing benzene from the A and the C,D-ring units repeatedly.[4d] This previously unreported effect of the scale of the coupling reaction on its effec-

(6) (a) Kobayashi, Y.; Taguchi, T. Fluorinated Vitamin D₃ Analogs. Syntheses and biological activities. In *biomedical aspects of fluorine chemistry*; Filler, R., Kobayashi, Y., Eds.; Kodansha LTD/Elsevier Biomedical: New York, 1982; pp 33–49. (b) Ikekawa, N. Structures and biological activities of vitamin D metabolites and their analogs. *Med. Res. Rev.* 1987, 7, 333–336. (c) de Costa, B. R.; Holick, S. A.; Holick, M. F. Synthesis of 19-acetylthio-3-β-acetoxy-9,10-secochola-1-(10),5Z,7E-triene, and 1-β-acetylthio-3-β-acetoxy-9,10-secochola-5Z,7E,10(19)-triene. An approach to 1β- and 19-thiolation of the vitamin D skeleton. *J. Chem. Soc., Chem. Commun.* 1989, 325–326.

(7) (a) Holick, S. A.; Holick, M. F.; MacLaughlin, J. A. Chemical synthesis of [1β-³H]1α,25-dihydroxyvitamin D₃ and [1α-³H]-1β,25-dihydroxyvitamin D₃: biological activity of 1β,25-dihydroxyvitamin D₃. *Biochem. Biophys. Res. Commun.* 1980, 97, 1031–1037. (b) Chen, T. C.; Tian, X.; Holick, M. F. 1α,25-dihydroxyvitamin D₃: a novel agent for wound healing. *Vitamin D Gene Regulation, Structure–Function Analysis, and Clinical Application*, Proceedings of the Eighth Workshop on Vitamin D, July 5–10, 1991, Paris, France; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: New York, 1991; pp 435–436.

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Scheme II

Scheme III
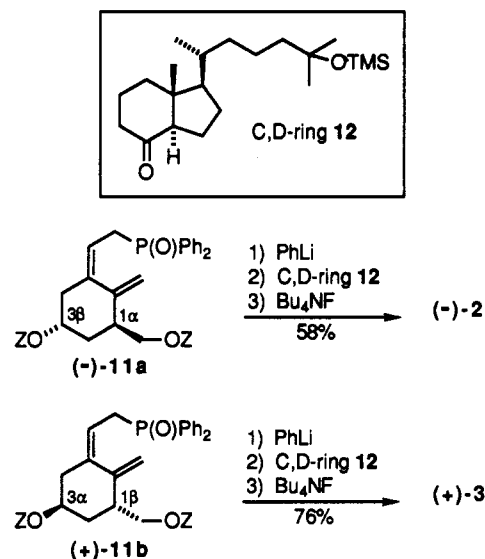
tiveness was a crucial observation for us, and now will be useful for others working in this field.

Surprisingly, some of the physical properties of 1-(hydroxymethyl)-25-hydroxyvitamin D₃ diastereomers (-)-2 and (+)-3 are significantly different. For example, whereas 1α-(hydroxymethyl) diastereomer (-)-2 was easily crystallized, 1β-(hydroxymethyl) diastereomer (+)-3 was very difficult to crystallize; eventually, this difference in crystallinity might be of great practical advantage if a mixture of diastereomers (-)-2 and (+)-3, produced from *racemic* ring-A phosphine oxide 11 and enantiomerically pure

ring-C,D chiron 12, could be induced to yield crystals of only diastereomer (-)-2. Also, 1α-(hydroxymethyl) diastereomer (-)-2 was unexpectedly poorly soluble in such organic solvents as methylene chloride, chlorofom, and methanol. Nevertheless, both hydroxymethyl diastereomers (-)-2 and (+)-3 had extremely similar UV and high field ¹H and ¹³C NMR spectra as well as extremely similar chromatographic properties.

Biology

Calcitriol and its 1-(hydroxymethyl) analogs (-)-2 and (+)-3 were equipotent at inhibiting growth of PE cells. Figure 1 shows the antiproliferative effects of the three compounds as demonstrated by reduction in cell number over time as compared to control plates. While the control cells continued in the exponential phase of cell growth from 24 h onward, this rapid rate of cell proliferation was significantly blunted by treatment with calcitriol or its 1-(hydroxymethyl) analogs. Further, the treated cell populations had reached a plateau by 72 h, days before the control cells would become confluent and senescent. Thus, all three vitamin D₃ compounds were active in inhibiting cell growth and division. The activity of these compounds was due to cytostatic rather than cytotoxic effects, as cell viability was unchanged in the treated groups as determined by dye exclusion assay.

Calcitriol and the 1-(hydroxymethyl) diastereomers also significantly inhibited the effects of TPA (12-*O*-tetradecanoylphorbol-13-acetate) on the activity of ornithine
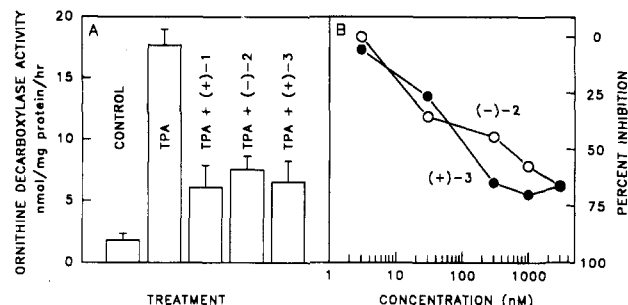
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(10) (a) Posner, G. H.; Nelson, T. D.; Kinter, C. M.; Johnson, N. Diels-Alder cycloadditions using nucleophilic 3-*p*-tolylthio-2-pyrone. Regiocontrolled and stereocontrolled synthesis of unsaturated, bridged, bicyclic lactones. *J. Org. Chem.* In press. (b) Posner, G. H.; Vinader, V.; Afarinkia, K. Diels-Alder cycloadditions using nucleophilic 2-pyridones: regiocontrolled and stereocontrolled synthesis of unsaturated, bridged, bicyclic lactams. *J. Org. Chem.* In press.

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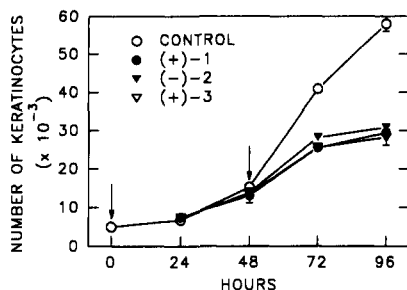
Figure 1. Growth inhibition of keratinocyte cell line PE by calcitriol and 1-(hydroxymethyl) homologs at 3 μM. Values represent the mean from 12 wells $\pm$ SD. Arrows indicate administration of fresh medium into which the compounds dissolved in DMSO had been added. Control cells were treated with DMSO alone (0.1% in culture medium). The treated values are significantly different from the solvent control at 72 and 96 h ($p < 0.001$, Student's t-test).

Figure 2. Inhibition of TPA-induced ornithine decarboxylase activity by pretreatment with calcitriol and 1-(hydroxymethyl) homologs. Panel A shows inhibition of TPA-stimulated response by pretreatment of cells for 15 min with 1 μM of the compounds. Values represent the mean $\pm$SD for three measurements. Pretreatment with calcitriol or its synthetic analogs resulted in a statistically significant reduction in TPA-induced ODC activity ($p < 0.001$, Student's t-test). Panel B shows a dose-response curve for the inhibition of TPA-induced ODC activity with the 1-(hydroxymethyl)vitamin D_3 diastereomers (-)-2 and (+)-3. Treatments are described in the Experimental Section.

decarboxylase (ODC). ODC catalyzes the initial and rate-limiting step in the polyamine biosynthetic pathway; while the function of polyamines is not fully understood, they are essential for growth, differentiation, and replication. This enzyme can be induced rapidly and dramatically by many growth stimuli, including the tumor promoter TPA.[17] The ability of TPA to induce ODC is associated with its proliferative and tumor-promoting properties.[18] A variety of agents have been shown to inhibit TPA effects on ODC induction as well as TPA-stimulated tumor promotion, including calcitriol,[19a,b] antiinflammatory steroids and vitamin A analogs,[19c] as well as free radical scavenging compounds.[19d] Similarly, Figure 2 shows the effects of vitamin D_3 and its 1-(hydroxymethyl) analogs on the TPA-stimulated ODC activity in vitro. The potencies of the three compounds as inhibitors of the effects of TPA on this enzyme were not significantly different from each other. Panel B of Figure 2 illustrates the similar dose-response characteristics of the 1-(hydroxymethyl) vitamin D_3 diastereomers.

Preliminary competitive studies indicated that both (-)-2 and (+)-3 were less than 0.1% as effective as calcitriol for binding to the 1,25-$(OH)_2D_3$ receptor.[20] Preliminary studies indicated also that both (-)-2 and (+)-3 were less

than 0.1% as potent as calcitriol for calbindin-D_{28K} induction in organ-cultured embryonic chick duodenum.[21]

Conclusion

These results indicate for the first time that replacing the 1α-hydroxyl group in calcitriol by the homologous 1-(hydroxymethyl) group does not diminish the antiproliferative activity characteristic of calcitriol in murine keratinocytes. Other small structural changes at the 1-position, therefore, might lead also to biologically active analogs. Further, this is the first demonstration that changing the stereochemistry of a 1-substituent (i.e., 1α → 1β, 2 → 3) does *not* necessarily change antiproliferative activity. Although it is now clear that these 1-(hydroxymethyl) analogs do not show their biological activity by binding to the calcitriol receptor, the mechanism for their keratinocyte antiproliferative activity is still unclear. We are actively evaluating other biological properties of these hydroxymethyl analogs (-)-2 and (+)-3 as well as preparing related vitamin D_3 derivatives to determine structure-activity relationships and to maximize separation of an antiproliferative activity from calcemic activity.

Experimental Section

Chemistry. General. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from benzophenone ketyl prior to use. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride immediately prior to use. Commercially available anhydrous solvents were used in other instances. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and, unless otherwise specified, were used as received without further purification. FT-IR spectra were determined using a Perkin-Elmer Model 1600 FT-IR spectrophotometer. The ^1H NMR spectra were recorded on a Varian XL-400 spectrometer and a Bruker AMX-300 spectrometer operating at 400 MHz and 300 MHz, respectively. The ^{13}C NMR spectra were recorded on the same instruments operating at 100 and 75 MHz, respectively. High resolution mass

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spectra were obtained on a two sector high resolution VG-70S mass spectrometer run at 70 eV. Melting points are uncorrected. Preparative HPLC work was performed on a Waters Delta Prep 3000 system with a PrepPAK-500 silica (55–105 μm) column (30-cm bed × 4.7 cm i.d.). Analytical HPLC separations were performed on a Rainin HPXL system with a Dynamax-60A 8-μm silica column (25-cm bed × 4.6 mm i.d.). Purity of products was judged to be ≥95% on the basis of their chromatographic homogeneity. Yields for enantiomerically enriched and racemic compounds were comparable in all cases for a given transformation. Optical rotation concentrations (c) are given in 9 g/100 mL.

Bromobicyclic Lactone 5. A 25-mL hydrolysis tube was charged with 1.43 g (8.2 mmol, 1.0 equiv) of 3-bromo-2-pyrone (4),[11a] 3.69 g (65.7 mmol, 8.0 equiv) of acrolein, 23.0 mg of barium carbonate, and 10 mL of CH_2Cl_2. This was sealed under nitrogen and warmed to 70–90 °C for 91 h with constant stirring. Examination of an aliquot of the reaction mixture by 400-MHz ^{1}H NMR indicated that complete formation of a single bicycloadduct had occurred. A stream of nitrogen was then blown over the reaction mixture to remove the acrolein. After holding this under high vacuum, the crude product was diluted with CH_2Cl_2/Et_2O (ca. 1:1) and passed through a plug of Celite. The solvent was evaporated to give 3.32 g of a yellow oil which was dissolved in 50 mL of ethanol and 20 mL of diglyme and cooled to −78 °C (dry ice/acetone) under argon. To this, a solution of 476 mg (12.6 mmol, 1.5 equiv) of NaBH₄ in 8 mL of EtOH was added. After stirring for 30 min the mixture was diluted with CH_2Cl_2, and then 4 mL of saturated aqueous ammonium chloride was added. After warming to room temperature, this mixture was dried over MgSO₄, filtered through a plug of Celite, and purified by column chromatography (silica gel, 20–50% EtOAc/hexane) to afford 1.42 g of a yellow oil which was immediately dissolved in 20 mL of anhydrous CH_2Cl_2 under argon and cooled to 0 °C. To this, 0.75 mL (6.4 mmol, 1.05 equiv) of 2,6-lutidine was added followed by the addition of 1.5 mL (6.5 mmol, 1.07 eq.) of *tert*-butyldimethylsilyl trifluoromethanesulfonate. This was stirred for 30 min, warmed to room temperature, diluted with CH_2Cl_2, washed with water, the organic portion dried over MgSO₄, and the solvent evaporated. Purification by silica gel column chromatography (10–20% EtOAc/hexane) afforded 1.32 g (3.8 mmol, 46%) of the bicycloadduct 5 as a white solid (R_f = 0.7, 50% EtOAc/hexane): mp 100.5–102 °C; ^{1}H NMR (CDCl₃) δ 6.37–6.40 (m, 1 H), 6.33 (dd, J = 5 Hz, 1 H), 5.18–5.22 (m, 1 H), 3.96 (dd, J = 10.1, 3.5 Hz, 1 H), 3.65 (dd, J = 10.1, 7.1 Hz, 1 H), 2.43–2.49 (m, 1 H), 2.31–2.37 (m, 1 H), 1.91 (ddd, J = 13.2, 3.9, 1.3 Hz, 1 H), 0.86 (s, 9 H), 0.05 (s, 3 H), 0.04 (s, 3 H); ^{13}C NMR (CDCl₃) δ 169.0, 136.4, 130.4, 73.5, 64.3, 62.1, 41.1, 31.2, 25.7 (3C), 18.1, −5.4, −5.5; FT-IR (CHCl₃) 1763 cm⁻¹; HRMS, m/z (M⁺ − t-Bu) calcd for C₁₄H₂₃O₃SiBr 288.9896, found 288.9901.

Hydroxy α,β-Unsaturated Ester 6 (from 5). To a 100-mL flame-dried round-bottomed flask, 3.32 g (9.56 mmol, 1.0 equiv) of bicycloadduct 5, 2.6 mL (9.65 mmol, 1.02 equiv) of tributyltin hydride, ca. 0.2 g of azobis(isobutyronitrile) (AIBN), and 20.0 mL of anhydrous benzene were added and refluxed (placed in a preheated oil bath) for 2 h under Ar. This was cooled to room temperature and then diluted with wet Et₂O, and then 1.5 mL of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was added and the mixture stirred for 5 min at which time the white precipitate was removed by filtration through a plug of silica gel with Et₂O. The solvent was evaporated and the resulting oil placed in a 100-mL flame-dried round-bottomed flask under argon. The oil was dissolved in 20 mL of anhydrous THF and cooled to −35 °C. To this, 2.0 mL of a freshly prepared sodium methoxide solution (31 mg of sodium in 5.0 mL of anhydrous MeOH) was added and stirred at −35 °C for 10 h and then at 25 °C for 1 h. The reaction mixture was diluted with CH_2Cl_2, quenched with saturated aqueous ammonium chloride, dried over MgSO₄, filtered, and the solvent evaporated. Purification by silica gel chromatography afforded 2.65 g (8.82 mmol, 92%) of methyl ester 6 as a colorless oil (R_f = 0.3, 25% EtOAc/hexane): ^{1}H NMR (CDCl₃) δ 6.94 (ddd, J = 5, 3, 1 Hz, 1 H), 4.20–4.12 (m, 1 H), 3.72 (s, 3 H), 3.74–3.71 (m, 1 H), 3.50 (dd, J = 10.0, 8.0 Hz, 1 H), 2.90 (bs, 1 H), 2.60 (dtdd, J = 19.2, 6, 1.6, 1 Hz, 1 H), 2.23 (dddd, J = 12.4, 4, 2.8, 1.6 Hz, 1 H), 2.09 (dddd, J = 19.2, 8.8, 3.0, 2.0 Hz, 1 H), 1.65 (bs, 1-OH, this signal disappears upon D₂O quench), 1.57 (ddd, J = 12.4, 11.2,

6 Hz, 1 H), 0.87 (s, 9 H), 0.03 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR (CD₂Cl₂) δ 167.4, 139.9, 130.5, 65.1, 63.6, 51.8, 38.1, 35.6, 33.8, 26.1 (3C), 18.5, −5.3, −5.4; FT-IR (thin film) 3412, 1716 cm⁻¹; HRMS, m/z (M⁺ − t-Bu) calcd for C₁₅H₂₈O₄Si 243.1053, found 243.1059.

Hydroxy α,β-Unsaturated Ester 6 (from 7). A round-bottomed flask was charged with 0.632 g (1.41 mmol) of the diester 7a which was dissolved in 10 mL of THF and 10 mL of methanol and then cooled to 0 °C. To this, 0.20 mL of a freshly prepared sodium methoxide stock solution (32.1 mg of sodium in 5.0 mL of methanol) was added and rapidly stirred for 1 h and then warmed to room temperature. Rapid stirring was maintained, and the progress of the reaction was monitored by TLC. Periodic addition of sodium methoxide stock solution was made until the reaction was complete (ca. 8 h). Most of the solvent was evaporated, and the mixture was diluted with Et₂O and passed through a two-in. plug of silica gel. Purification by silica gel column chromatography (25–75% EtOAc/hexane) gave 0.386 g (1.28 mmol, 91%) of the hydroxy ester (+)-6a as a colorless oil: $[\alpha]^{23}_D$ +59.7° (c = 8.2, CH_2Cl_2, de 98.8%). The same procedure was used for the conversion of 0.900 g (2.01 mmol) of the diester 7b into 0.548 g (1.82 mmol, 91%) of the hydroxy ester (−)-6b as a colorless oil: $[\alpha]^{23}_D$ −59.4° (c = 8.5, CH_2Cl_2, de 96.5%).

α-Methoxyphenylacetic Esters 7a and 7b. To a flame-dried 250 mL round-bottomed flask 3.11 g (10.4 mmol) of hydroxy ester 6, 2.06 g (12.4 mmol, 1.2 equiv) of (R)-(−)-α-methoxyphenyl acetic acid, 2.45 g (11.9 mmol, 1.15 equiv) of 1,3-dicyclohexylcarbodiimide, and 0.15 g (1.2 mmol, 0.1 equiv) of 4-(dimethylamino)-pyridine were dissolved in 150 mL of anhydrous Et₂O under argon. This reaction mixture was stirred at room temperature for 12 h. The white precipitate was then removed by filtration, the organic layer was washed twice with water and dried over MgSO₄, and the solvent was removed by rotary evaporation to leave a very light yellow oil. All impurities were removed from the diastereomeric ester 7a and 7b by silica gel column chromatography (0–20% EtOAc/hexane). The diastereomers where then separated by preparative normal-phase HPLC (4.5% EtOAc/hexane, 30 mL/min) and by preparative thick-layer chromatography (PTLC, multiple elutions with 15% EtOAc/hexane, 1500-μm plates). On a preparative scale the diastereomers overlapped on both HPLC and PTLC; therefore, fractions were cut and repurified by numerous injections (ca. eight) and applications, respectively. The diastereomeric excess (de) of fractions was deduced by analytical normal-phase HPLC (7a: t_R = 13.4; 7b: t_R = 15.1, 1.0 mL/min, 10% EtOAc/hexane). A 1.09-g (2.43 mmol, 46%) sample of 7a (de 98.5%) and a 0.90-g (2.01 mmol, 38%) sample of 7b (de 96.5%) were obtained (yields were based on a possible 5.2-mmol yield for each diastereomer). A 1.22-g (2.72 mmol, 26%) mixture of 7a and 7b was not adequately separated so as to be used in the subsequent synthetic transformations. 7a: ^{1}H NMR (CDCl₃) δ 7.44–7.32 (m, 5 H), 6.80 (ddd, J = 4.7, 3.35, 1.1 Hz, 1 H), 5.34–5.24 (m, 1 H), 4.75 (s, 1 H), 3.72 (s, 3 H), 3.69 (d, J = 3.4 Hz, 1 H), 3.57 (dd, J = 10, 7.2 Hz, 1 H), 3.41 (s, 3 H), 2.90 (bs, 1 H), 2.57–2.51 (m, 1 H), 2.20–2.15 (m, 1 H), 1.95 (dddd, J = 19.1, 8.1, 3.35, 1.9 Hz, 1 H), 1.72 (ddd, J = 12.8, 11.2, 6.0 Hz, 1 H), 0.85 (s, 9 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR (CDCl₃) δ 169.9, 166.5, 137.9, 136.1, 130.0, 128.4, 128.3 (2 C), 126.9 (2 C), 82.4, 67.6, 64.3, 57.1, 51.3, 36.7, 30.9, 29.7, 25.7 (3C), 18.0, −5.7, −5.8; FT-IR (thin film) 1749, 1716 cm⁻¹; HRMS m/z (M⁺ − t-Bu) calcd for C₂₄H₃₆O₆Si 391.1577, found 391.1580. 7b: ^{1}H NMR (CDCl₃) δ 7.43–7.31 (m, 5 H), 6.88 (ddd, J = ~4.75, 3.3, 1 Hz, 1 H), 5.29–5.21 (m, 1 H), 4.73 (s, 1 H), 3.71 (s, 3 H), 3.64 (dd, J = 9.9, 3.5 Hz, 1 H), 3.52 (dd, J = 9.9, 7.1 Hz, 1 H), 3.40 (s, 3 H), 2.77 (bs, 1 H), 2.67 (dddd, J = 19, 6, ~4.75, 1 Hz, 1 H), 2.16 (dddd, J = 19, 8, 3.3, 2 Hz, 1 H), 2.00 (m, 1 H), 1.59 (12.8, 11.0, 6, 1 H), 0.81 (s, 9 H), −0.03 (s, 3 H), −0.07 (s, 3 H); ^{13}C NMR (CDCl₃) δ 170.1, 166.7, 138.1, 136.2, 130.3, 128.6, 128.5 (2 C), 127.0 (2 C), 82.5, 67.8, 64.3, 57.2, 51.5, 36.7, 31.4, 29.6, 25.7 (3 C), 18.1, −5.6, −5.7; FT-IR (thin film) 1749, 1716 cm⁻¹; HRMS m/z (M⁺ − t-Bu) calcd for C₂₄H₃₆O₆Si 391.1577, found 391.1576.

Bis-Silyloxy α,β-Unsaturated Ester 8. In a 50-mL flame-dried round-bottomed flask 202.5 mg (0.67 mmol, 1.0 equiv) of hydroxy ester 6 was dissolved in 15 mL of anhydrous CH_2Cl_2 under argon. To this, 0.100 mL (0.84 mmol, 1.25 equiv) of 2,6-lutidine was added and stirred for 3 min followed by the addition of 0.195 mL (0.84 mmol, 1.25 equiv) of *tert*-butyldimethylsilyl trifluoromethanesulfonate. After 30 min, the solvent was evaporated and

purification by silica gel column chromatography (5–10% EtOAc/hexane) gave 240.4 (0.58 mmol, 86%) of the silyloxy ester 8 as a colorless oil (R_f = 0.6, 10% EtOAc/hexane). ^{1}H NMR (CDCl$_3$) δ 6.92 (ddd, J = 5.2, 2.8, 1 Hz, 1 H), 4.15 (m, 1 H), 3.72–3.69 (m, 1 H), 3.71 (s, 3 H), 3.52 (dd, J = 9, 8 Hz, 1 H), 2.76 (bs, 1 H), 2.47 (dtd, J = 19.2, ca. 5.2, 1 Hz, 1 H), 2.17–2.12 (m, 1 H), 2.13–2.05 (dddd, J = 19.2, 9, 2.8, 2.0 Hz, 1 H), 1.58–1.51 (ddd, J = 12.8, 11.2, 2.0 Hz, 1 H), 0.88 (s, 9 H), 0.87 (s, 9 H), 0.07 (s, 3 H), 0.06 (s, 3 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR (CD$_2$Cl$_2$) δ 167.4, 140.3, 130.4, 65.3, 64.6, 51.7, 38.4, 36.5, 34.6, 26.1 (6 C), 18.6, 18.5, –4.4 to –5.3 (4 C); FT-IR (thin film) 1716 cm^{-1}; HRMS m/z (M$^+$ – t-Bu) calcd for C$_{21}$H$_{42}$O$_4$Si$_2$ 357.1917, found 357.1922. (–)-8 (from (–)-6b): $[\alpha]^{23}_D$ –46.7° (c = 9.4, CH$_2$Cl$_2$, de 96.5%). (+)-8 (from (+)-6a): $[\alpha]^{23}_D$ +47.1° (c = 10.0, CH$_2$Cl$_2$, de 98.8%).

Dienoates (E)-10 and (Z)-10. A flame-dried 50-mL round-bottomed flask was charged with 240.4 mg (0.58 mmol, 1.0 equiv) of the silyloxy ester 8, dissolved in 4.0 mL of anhydrous toluene, and cooled to –78 °C under argon. To this was added 1.3 mL (1.2 mmol, 2.2 equiv) of diisobutylaluminum hydride (DIBAL-H) (1.0 M in hexane) and it was stirred at –78 °C for 30 min and then at 25 °C for 90 min. This was quenched with 5 drops of 2 N sodium potassium tartrate and 15 mL of water, and diluted with CH$_2$Cl$_2$. This was separated and the organic portion was dried over MgSO$_4$. Purification by silica gel column chromatography (10–25% EtOAc/hexane) gave 194.2 mg (0.050 mmol, 87%) of the allylic alcohol 9 as a colorless oil (R_f = 0.5, 25% EtOAc/hexane) which was immediately used in the preparation of (E)-10 and (Z)-10. A 25-mL hydrolysis tube was charged with 184.7 mg (0.48 mmol, 1.0 equiv) of the allylic alcohol 9, a total of 427 mg (1.5 mmol, 3.1 equiv) of 1-(phenylsulfinyl)-2,2,2-triethoxyethane, 3 mg of 2,4,6-trimethylbenzoic acid, and 9 mL of anhydrous CH$_2$Cl$_2$. This was sealed under nitrogen and warmed to 135–145 °C for a total of 12.5 h. After cooling the reaction mixture, the solvent was evaporated and purification by PTLC (3 × 1000 μm, 3% EtOAc/hexane) gave 141.6 mg (0.31 mmol, 65%) of (E)-10 and 19.9 mg (0.04 mmol, 9%) of (Z)-10 as oils. Shorter reaction times lead to increased Z/E ratios. (E)-10: ^{1}H NMR (CDCl$_3$) δ 5.84 (t, J = 1.4 Hz, 1 H), 5.11 (s, 1 H), 4.81 (t, J = 1.4 Hz, 1 H); ^{13}C NMR (CDCl$_3$) δ 166.4, 158.0, 149.6, 115.4, 111.4, 66.7, 65.2, 59.6, 42.2, 38.4, 36.5, 25.8 (3 C), 25.7 (3 C), 18.1, 18.0, 14.3, –4.89, –4.94, –5.48, –5.53; FT-IR (thin film) 1716 cm^{-1}; HRMS m/z (M$^+$ – t-Bu) calcd for C$_{24}$H$_{46}$O$_4$Si$_2$ 397.2230, found 397.2235. (Z)-10: ^{1}H NMR (CDCl$_3$) δ 5.58 (t, J = 1 Hz, 1 H), 4.96–4.93 (m, 2 H), 4.15–4.04 (m, 3 H), 3.71 (dd, J = 10, 5.0 Hz, 1 H), 3.52 (t, J = 10 Hz, 1 H), 2.75–2.68 (m, 1 H), 2.44 (ddt, J = 12.4, 4.0, 1 Hz, 1 H), 2.26 (ddd, J = 12.4, 8.0, 1.6 Hz, 1 H), 2.03 (dddd, J = 13, 5.6, 4.0, 1.6 Hz, 1 H), 1.71 (ddd, J = 13, 4, 1 Hz, 1 H), 1.23 (t, J = 7.2 Hz, 3 H), 0.089 (s, 9 H), 0.087 (s, 9 H), 0.06 (s, 6 H), 0.043 (s, 3 H), 0.040 (s, 3 H); ^{13}C NMR (CDCl$_3$) δ 166.3, 154.2, 145.6, 116.4, 112.3, 67.5, 64.2, 60.0, 47.2, 44.0, 36.9, 25.84 (3 C), 25.75 (3 C), 18.2, 18.0, 14.0, –4.73, –4.80, –5.42, –5.50; FT-IR (CDCl$_3$) 1718 cm^{-1}; HRMS m/z (M$^+$ – t-Bu) calcd for C$_{24}$H$_{46}$O$_4$Si$_2$ 397.2230, found 397.2231. (–)-(E)-10 (from (+)-8: $[\alpha]^{23}_D$ –38.0° (c = 9.4, CHCl$_3$, de 98.5%). (+)-(E)-10 (from (–)-8): $[\alpha]^{23}_D$ +37.2° (c = 5.1, CHCl$_3$, de 96.5%). (+)-(Z)-10 (from (–)-8): $[\alpha]^{23}_D$ +42.8° (c = 8.6, CHCl$_3$, de 96.5%).

Photoisomerization to Dienoate (Z)-10. A borosilicate test tube was charged with 141.1 mg (0.31 mmol) of dienoate (E)-10, 9.3 mg of 9-fluorenone, and 9.0 mL of *tert*-butyl methyl ether. The tube was sealed with a rubber septum, placed in a solution of 2 M sodium orthovanadate, and irradiated with a medium pressure mercury arc lamp for 16 h. This was purified by PTLC (1 × 1000 μm, 1 × 1500 μm, 3% EtOAc/hexane) to give 132.3 mg of an inseparable mixture of (Z)-10 and 9-fluorenone [therefore, the yield of (Z)-10 would be 123.0 mg (0.27 mmol, 87%); that is, 132.3 mg of starting material minus 9.3 mg of fluorenone].

Phosphine Oxide 11. A flame-dried round-bottomed flask was charged with 123.0 mg (0.27 mmol, 1.0 equiv containing 9.3 mg of 9-fluorenone) of (Z)-10 and 1.5 mL of anhydrous toluene under argon and then cooled to 0 °C. To this was added 0.60 mL (0.60 mmol, 2.2 equiv) of diisobutylaluminum hydride (DIBAL-H) (1 M in hexane) and it was stirred at 0 °C for 35 min and then warmed to 25 °C. An additional 0.06 mL (0.06 mmol, 0.2 equiv) of DIBAL-H was added and stirred for 2 h. The reaction mixture was quenched with 0.5 mL of 2 N sodium potassium tartrate, diluted with CH$_2$Cl$_2$, separated, and the organic portion dried over MgSO$_4$. Purification by PTLC [2 × 1000 μm (2 elutions) 10%

EtOAc/hexane and then 15% EtOAc/hexane] gave 56.8 mg (0.14 mmol, 51%) of the allylic alcohol as an oil. A flame-dried 25-mL round-bottomed flask was charged with 90 mg (0.67 mmol, 4.8 equiv) of N-chlorosuccinimide and dissolved in 1.5 mL of anhydrous CH$_2$Cl$_2$ and then cooled to 0 °C under argon. To this was added 0.052 mL (0.71 mmol, 5.1 equiv) of dimethyl sulfide. The white precipitate that immediately formed was stirred at 0 °C for 10 min and then at –20 °C (dry ice/ethylene glycol) for 10 min. To this was added a solution of the freshly prepared allylic alcohol in 1.5 mL of anhydrous CH$_2$Cl$_2$ via cannula (the flask containing the alcohol solution was rinsed with 0.5 mL of anhydrous CH$_2$Cl$_2$ and this was also transfered to the reaction mixture via cannula). This was stirred at –20 °C for 15 min and then at 25 °C for 50 min. The reaction mixture was quenched with H$_2$O, diluted with CH$_2$Cl$_2$, and separated, the organic portion was dried over MgSO$_4$ and filtered, and the solvent was evaporated. This was passed through a column of florisil with 10% EtOAc/hexane to give 46.7 mg (0.11 mmol, 79%) of the allylic chloride. This was then dissolved in 2.0 mL of anhydrous THF in a flame-dried 50-mL round-bottomed flask under argon and to this a freshly prepared THF solution of lithium diphenylphosphide (Ph$_2$PLi, this deep orange reactant was prepared by the equimolar addition of n-butyllithium to diphenylphosphine) was added slowly until a yellow color persisted. This was then quenched with 0.5 mL of water, and the THF was evaporated. It was diluted with 10 mL of CH$_2$Cl$_2$, and 6 drops of 30% hydrogen peroxide were added and then rapidly stirred for 10 min. This was diluted with CH$_2$Cl$_2$, dried over MgSO$_4$, and filtered, and the solvent was evaporated. Purification by silica gel column chromatography (5–50% EtOAc/hexane) afforded 29.3 mg (0.049 mmol, 45%) (18% from (Z)-10) of the phosphine oxide 11 as a white solid after removal from benzene: R_f = 0.3, 50% EtOAc/hexane); mp 118–122 °C; ^{1}H NMR (C$_6$D$_6$) δ 7.83–7.78 (m, 4 H), 7.05–7.03 (m, 6 H0, 5.46 (ddt, J = 14.0, 7.6, 1.2 Hz, 1 H), 5.42 (d, J = 2 Hz, 1 H), 4.99 (dd, J = 2, 1.2 Hz, 1 H), 3.95–3.90 (m, 1 H), 3.69 (dd, J = 10.0, 6.4 Hz, 1 H), 3.55 (dd, J = 10.0, 8.8 Hz, 1 H), 3.32–3.12 (m, 2 H), 2.70–2.63 (m, 1 H), 2.40–2.33 (m, 1 H), 2.26–2.19 (m, 1 H), 1.94–1.87 (m, 1 H), 1.83 (ddd, J = 13, 7.6, 4.8 Hz, 1 H), 0.98 (s, 9 H), 0.95 (s, 9 H), 0.071 (s, 3 H), 0.065 (s, 3 H), 0.049 (s, 3 H), 0.014 (s, 3 H); ^{13}C NMR (C$_6$D$_6$) δ 145.4 (d, J = 2.5 Hz), 142.0 (d, J = 12.2 Hz), 132.8 (d, J = 98.0 Hz) 132.7 (d, J = 98.2 Hz), 131.62 (d, J = 2.5 Hz), 131.58 (d, J = 2.6), 130.93 (d, J = 9.2 Hz), 130.88 (d, J = 9.2 Hz), 128.42 (d, J = 11.7 Hz), 128.40 (d, J = 11.6), 114.0 (d, J = 7.8 Hz), 112.6, 67.32, 67.30, 64.1, 46.7, 44.1, 37.4, 31.2 (d, J = 70.9 Hz), 25.8 (3 C), 25.7 (3 C), 18.0 (2 C), –4.8, –4.9, –5.4, (2 C); IR (CHCl$_3$) 3020, 2956, 2930, 2857, 1680, 1472, 1463, 1438, 1255, 1100 cm^{-1}; MS m/z (EI) 596 (M$^+$, 3), 540 (43), 539 (100), 407 (58), 332 (22), 202 (27), 201 (25), 75 (30), 73 (86); HRMS m/z (M$^+$) calcd for C$_{34}$H$_{53}$O$_3$Si$_2$P 596.3271, found 596.3277. (–)-11a (from (–)-(Z)-10): $[\alpha]^{23.5}_D$ –54.0° (c = 6.1, CH$_2$Cl$_2$, de 98.5%). (+)-11b (from (+)-(Z)-10): $[\alpha]^{23}_D$ +54.4° (c = 9.6, CH$_2$Cl$_2$, de 96.5%).

1α-(Hydroxymethyl)-25-hydroxyvitamin D$_3$ [(–)-2]. A flame-dried 10-mL round-bottomed flask was charged with 79.7 mg (0.13 mmol, 1.9 equiv) of the phosphine oxide (–)-11a which was dissolved in 1.0 mL of freshly distilled anhydrous THF and cooled to –78 °C under argon. Phosphine oxide (–)-11a was azeotropically dried with benzene and held under high vacuum for 24 h immediately prior to use. To this was added 0.091 mL (0.138 mmol, 2.0 equiv) of PhLi (1.52 M in Et$_2$O) dropwise over a 5-min period. A deep orange-red color persisted after the second drop of the PhLi solution was added. This was allowed to stir an additional 8 min at –78 °C at which time a precooled (–78 °C) solution consisting of 24.3 mg (0.069 mmol, 1.0 equiv) of the C,D-ring ketone 12 in 0.5 mL of freshly distilled anhydrous THF was added dropwise via cannula. The C,D-ring ketone 12 was also azeotropically dried with benzene and held under high vacuum immediately prior to use. The flask containing the C,D-ring 12 was rinsed with 0.4 mL of THF, and this was also slowly added to the reaction mixture via cannula. This deep orange-red solution was stirred in the dark at –78 °C for 2.5 h and then warmed to –65 °C over 30 min. At this temperature the reaction mixture turned to a light yellow. This was immediately quenched with 0.3 mL of 2 N sodium potassium tartrate followed by the addition of dilute aqueous potassium carbonate. After warming to room temperature, the reaction was diluted with CH$_2$Cl$_2$ and separated,

the organic portion was dried over MgSO$_4$ and filtered. Purification by silica gel column chromatography (5–10% EtOAc/hexane) afforded 37.9 mg (0.049 mmol, 69%) of the crude coupled product. This was immediately placed in a flame-dried 10-mL round-bottomed flask and dissolved in 3.0 mL of freshly distilled anhydrous THF under argon. To this was added 0.17 mL (0.17 mmol, 3.5 equiv) of tetrabutylammonium fluoride (1 M in THF), and it was stirred at 25 °C in the dark for 14 h. The solvent was evaporated and the crude product passed through a column of silica gel with 5–10% MeOH/Et$_2$O and then purified by PTLC (3 × 1000 μm, 8% methanol,Et$_2$O) to afford 17.2 mg (0.039 mmol, 83%)[58% from (–)-11a] of 1α-(hydroxymethyl)-25-hydroxyvitamin D$_3$ [(–)-2]. This compound was only sparingly soluble in organic solvents (e.g. MeOH, CHCl$_3$, CH$_2$Cl$_2$): ^{1}H NMR (CDCl$_3$) δ 6.32 (d, J = 11.2 Hz, 1 H), 5.95 (d, J = 11.2 Hz, 1 H), 5.18 (d, J = 2 Hz, 1 H), 5.02 (d, J = 2 Hz, 1 H), 0.93 (d, J = 6.4 Hz, 3 H), 0.54 (s, 3 H); ^{13}C NMR (CD$_3$OD) δ 147.7, 142.6, 136.7, 124.0, 119.0, 114.1, 71.5, 67.4, 64.7, 58.0, 57.6, 47.4, 47.0, 46.5, 45.3, 41.9, 37.8, 37.6, 37.5, 30.0, 29.3, 29.1, 28.7, 24.7, 23.3, 22.0, 19.4, 12.3; UV (MeOH) λ_{max} 264 nm; [α]^{23}$_D$ –64° (c = 0.09, CH$_2$Cl$_2$); HRMS m/z (M$^+$) calcd for C$_{28}$H$_{46}$O$_3$ 430.3447, found 430.3449.

1β-(Hydroxymethyl)-3β-norhydroxy-3α,25-dihydroxyvitamin D$_3$ [(+)-3]. This procedure was similar to the one used for the preparation of vitamin 2. The amounts of reagents utilized were as follows: phosphine oxide (+)-11b, 101.3 mg (0.17 mmol, 2.7 equiv); PhLi (1.52 M in Et$_2$O) 0.135 mL (0.21 mmol, 3.3 equiv); C,D ring 12 22.3 mg (0.063 mmol, 1.0 equiv). This afforded 21.1 mg (0.049 mmol, 76%) of the vitamin (+)-3 as an off white solid: ^{1}H NMR (CDCl$_3$) δ 6.31 (d, J = 11.3 Hz, 1 H), 5.94 (d, J = 11.3 Hz, 1 H), 5.15 (dd, J = 2.1, 1.0 Hz, 1 H), 4.99 (d, J = 2 Hz, 1 H), 4.03–3.97 (m, 1 H), 3.63–3.55 (m, 2 H), 2.83–2.78 (m 1 H), 2.65–2.57 (m, 1 H), 2.30–2.24 (m, 1 H), 0.93 (d, J = 9.8 Hz, 3 H), 0.5 (s, 3 H); ^{13}C NMR (CDCl$_3$) δ 145.4, 143.3, 134.1, 123.7, 117.0, 113.9, 71.1, 67.2, 64.4, 56.5, 56.3, 46.3, 45.9, 44.5, 44.4, 40.5, 37.5, 36.4, 36.1, 29.4, 29.2, 29.1, 27.7, 23.6, 22.3, 20.8, 18.8, 11.9; UV (MeOH) λ_{max} 265 nm; [α]^{23}$_D$ +24° (c = 0.74, CH$_2$Cl$_2$); HRMS m/z (M$^+$) calcd for C$_{28}$H$_{46}$O$_3$ 430.3447, found 430.3453.

Biology. In Vitro Testing Materials. Murine keratinocyte cell line PE was kindly provided by Dr. James E. Strickland, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute. Chosen for its particular sensitivity to the induction of ornithine decarboxylase (ODC) activity by the extensively characterized tumor promoter TPA, cell line PE was derived from a papilloma-induced in female SENCAR mice by a standard skin initiation/promotion protocol.[22] PE cell culture medium consisted of Eagle's minimal essential medium without calcium chloride (Whittaker Bioproducts, Walkersville, MA) supplemented with 8% chelexed fetal calf serum and 1% antibiotic-antimycotic (Gibco BRL) and the addition of CaCl$_2$ to 0.05 mM Ca^{++}.

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Sigma Chemical Co. (St. Louis, MO), and TPA was supplied by L.C. Services (Woburn, MA). L-[^{14}C]ornithine (56 μCi/mol) was from Amersham/Searle Corp. (Arlington Heights, IL). Chemical solvents used in all assays of biological activity were of the highest grade commercially available.

Growth Inhibition. Growth curves for PE cells treated with calcitriol and its 1-(hydroxymethyl) homologs were generated by assay for the reduction of the tetrazolium-based compound MTT.[23] A mitochondrial dehydrogenase reduces MTT to a blue formazan

product with an absorbance maximum of 505 nm in DMSO; the number of viable cells can thus be determined spectrophotometrically. PE cells were seeded at a density of 5 000 cells/well in 50 μL of medium into 96-well microtiter plates. Twelve hours later, the medium was removed, and cells were treated with 100 μL of fresh medium into which the appropriate amount of calcitriol or analog dissolved in dimethyl sulfoxide (DMSO) had been added, with the concentration of DMSO held constant at 0.1%. The plates were fed once at 48 h, with the readdition of the vitamin D$_3$ analogs at this time. At 24-h intervals following the initial treatment of the cells with compounds, 0.1 mg (50 μL of a 2 mg/mL solution) of MTT was added to each well. After 4 h, the MTT was removed and DMSO added to dissolve the blue formazan dye. Using a microtiter plate reader, the A_{505} was then determined and cell number calculated from blank-subtracted absorbance values. Results from the MTT assay for the inhibition of cell growth were independently confirmed by treating 100-cm^2 dishes of cells in an analogous manner for 96 h, whereupon the cells were harvested by trypsinization and counted. Further, the viability of the cells treated with calcitriol or analogs was determined to be identical to control cells at 96 h by trypan blue exclusion.

Inhibition of TPA-Induced ODC Activity. The 100-cm^2 dishes of PE cells were treated with calcitriol or analogs dissolved in DMSO by direct addition into the culture medium. Fifteen minutes later, the plates were treated with 100 ng/mL TPA dissolved in ethanol. For both additions, the solvent concentration was held constant at 0.1%, and control values represent the results from plates treated with these solvents. Three plates were used for each experimental group. Following incubation for 4 h after addition of TPA, the medium was removed and the dishes washed with ice cold phosphate-buffered saline (PBS). The excess PBS was then removed, and the dishes were rinsed with an ice cold solution of pyridoxal phosphate in PBS (50 μg/mL). The excess liquid was removed, and the dishes were frozen at –80 °C. The dishes were scraped into Eppendorf tubes while still partially frozen and the cells further lysed by freeze-thawing for generation of the 12000g cytosol. Cytosolic ODC activity was determined in triplicate by measuring the release of ^{14}CO$_2$ from L-[^{14}C]-ornithine using an Eppendorf microvessel assay as previously described.[24]

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