Low-Calcemic, Highly Antiproliferative, 1-Difluoromethyl Hybrid Analogs of the Natural Hormone 1α,25-Dihydroxyvitamin D_3: Design, Synthesis, and Preliminary Biological Evaluation

Sara Peleg,[†] Kimberly S. Petersen,[‡] Byung Chul Suh,[‡] Patrick Dolan,[§] Elin S. Agoston,[§] Thomas W. Kensler,[§] and Gary H. Posner*[,‡]

Department of Endocrine Neoplasia and Hormonal Disorders, The University of Texas, M.D. Anderson Cancer Center, Houston, Texas 77030, Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, Baltimore, Maryland 21218, and Division of Environmental Health Sciences, Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

Received August 17, 2006

Replacing the 1α-OH group of the natural hormone 1α,25-dihydroxyvitamin D_3 (calcitriol) by a 1α-CHF_2 group and incorporating a potentiating side chain produced two new hybrid analogs **6** and **7**. Both of these two hybrid analogs are as transcriptionally active as calcitriol and are strongly antiproliferative in vitro but are low-calcemic in vivo.

Introduction

The natural hormone **1** (1α,25-dihydroxyvitamin D_3 or calcitriol) is essential for maintaining our good health. However, medicinal use of supraphysiological amounts of this hormone in humans often causes toxic hypercalcemia by inducing excessive increases in intestinal calcium absorption and bone resorption.[1,2] Many calcitriol analogs have been designed and synthesized with the goal of maximizing therapeutic potential while minimizing undesirable calcemic activity.[3,4] Some of these synthetic analogs are currently used as safe and efficacious drugs for chemotherapy of patients having osteoporosis, psoriasis, or renal dysfunction.[4] During the past decade, our laboratories have developed several strategies to design analogs that have minimal calcemic activity but possess significant vitamin D receptor (VDR[a])-mediated transcriptional activity and antiproliferative activity.[5,6] One successful approach has been the synthesis of hybrid analogs containing modifications in the 1-α position combined with modifications in the side chain. Analog **2** with a 1-α-hydroxymethyl group alone was a weak deltanoid with poor biological activities in vitro and in vivo.[7] However, when a "potentiating" side chain was also incorporated, the biological activities of the hybrid compounds were partially or completely restored, but with minimal calcemic activity in rodents.[8−10] Our most successful hybrid analog in this group is **4** (QW-1624F_2-2),[11,12] in which unsaturation at C16 was combined with homologation at C26 and C27 and in which two fluorine atoms were incorporated at C24 to prevent 24-hydroxylation and further catabolism by the cytochrome P450 enzyme CYP24. This rationally designed, low calcemic, highly antiproliferative analog inhibited mouse skin carcinogenesis when applied topically[13,14] and diminished significantly the growth of human neuroblastoma in mouse xenografts when administered systemically,[15] without inducing hypercalcemia in either study. Recently, we have adopted a different strategy to design hybrid analogs by substituting a hydroxyl group with a difluoromethyl group, which we hypothesized would be a hydrogen-bonding surrogate for the natural 1-hydroxyl or 25-hydroxyl groups of calcitriol.[16] When a difluoromethyl group was substituted for the 1-hydroxyl group, the resulting compound analog **5** (BCS-1αCHF_2)[17] had only weak antiproliferative activity in murine keratinocytes, poor transcriptional activity through the human VDR (hVDR), and a significant decrease in affinity for the hVDR. A virtual docking of this analog into the binding pocket of hVDR indeed predicted that the bulkier difluoromethyl group at C1 (1-CHF_2) would form weaker hydrogen bonds than the natural 1-α hydroxyl group.[17] To determine if biological activities of analogs with this new type of modification in the C-1 position can be as effectively restored by incorporating potentiating side chains as were biological activities of analogs with the 1-hydroxymethyl group,[8] we have now designed and prepared new hybrid analog **6** (KSP−BCS-1αCHF_2-1624F_2-2) carrying 1-CHF_2 and 16-ene-24,24-difluoro structural units and also new hybrid analog **7** (KSP-BCS-1α-CHF_2-20-epi-22-oxa-bishomo-26-OH) carrying a 1-CHF_2 group and a KH-1060-type 20-epi-22-oxa-27,28-bishomo-26-OH side chain. These new 1-CHF_2 hybrid analogs **6** and **7** are as transcriptionally active as calcitriol and are strongly antiproliferative in vitro but are low-calcemic in vivo.

Results and Discussion

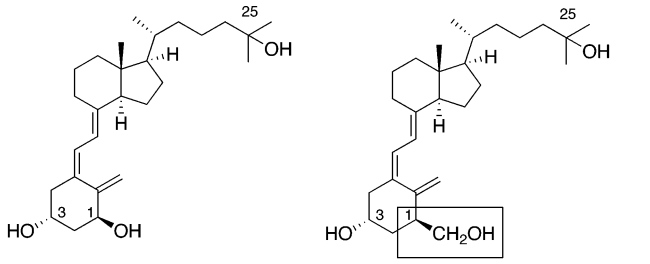
Chemistry: Syntheses of hybrid analogs **6** and **7** are outlined in Scheme 1, starting with the recently reported A-ring 1-CHF_2 7-phosphine oxide synthon (±)-**8**.[17] Lythgoe-type coupling[18] of racemic phosphine oxide **8** with enantiomerically pure C,D-ring C-8 ketone **9** led smoothly after desilylation to a mixture of diastereomers from which the major diasteromer analog (+)-**6** was isolated via preparative HPLC. Similarly, coupling with C,D-ring C-8 ketone **10** led easily after desilylation and HPLC purification to analog (−)-**7**. Assignment of the A-ring stereochemistry in these pairs of diastereomers was done as before[9,11,12] based on characteristic proton NMR data for the exocyclic 19-methylene hydrogen atoms. Diastereomer (+)-**6** has C-19a at 5.22 δ (vs at 5.25 δ for its 1-β-3-α A-ring diastereomer) and has C-19b at 5.07 δ (vs at 5.12 δ). Likewise, diastereomer (−)-**7** has C-19a at 5.21 δ (vs at 5.23 δ for its 1-β-3-α A-ring diastereomer) and has C-19b at 5.01 δ (vs 5.08 δ).

Biology: Our standard in vitro murine keratinocyte assay[11] indicates that both the new hybrid analogs **6** and **7** are more

* To whom correspondence should be addressed. Phone: 410-516-4670. Fax: 410-516-8420. E-mail: ghp@jhu.edu.
[†] The University of Texas.
[‡] Department of Chemistry, The Johns Hopkins University.
[§] Division of Environmental Health Sciences, The Johns Hopkins University.

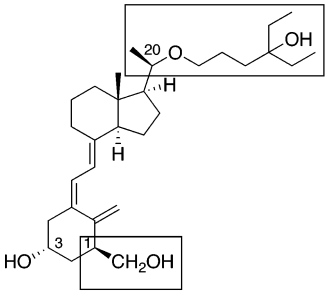
[a] Abbreviations: VDR, vitamin D receptor; hVDR, human vitamin D receptor; TBAF, tetrabutylammonium fluoride.

10.1021/jm0609925 CCC: $33.50
Published on Web 11/17/2006

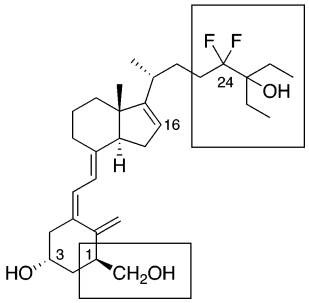


1

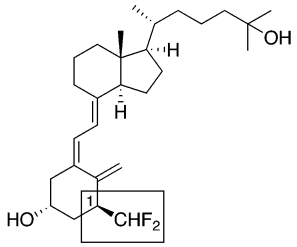
2



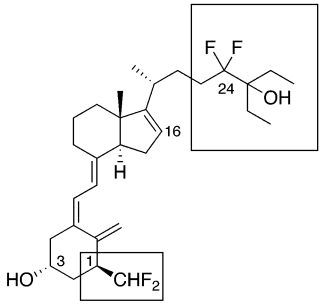
3 (1α-CH_2OH-KH)
(MCW-YB)[19]



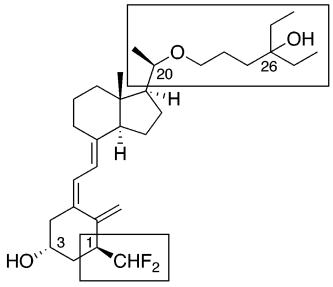
4



5



6



7

antiproliferative than calcitriol, even at 1 nM concentrations (Figure 1). Our standard measurements of urine calcium in rats in vivo[11] show that the compound **4**-like analog **6** is at least six times less calciuric than calcitriol, whereas the KH-1060-like analog **7** requires 20 times higher concentration than calcitriol to reach the same calciuric levels (Figure 2). Comparing the calciuric activities of our new 1-CHF_2 hybrid analog **6** and our very similar 1-hydroxymethyl hybrid analog **4** reveals that the 1-CHF_2 substitution is less calcemia-ablating than the 1-CH_2OH substitution, because **4** is approximately 100 times less calciuric than calcitriol.[12] Comparing the calciuric activities of our new KH-1060-like analog **7** and our very similar 1-hydroxymethyl hybrid analog **3** also reveals that the 1-CHF_2 substitution is less calcemia-ablating than the 1-CH_2OH substitution, because analog **3** is >100 times less calciuric than calcitriol.[19]

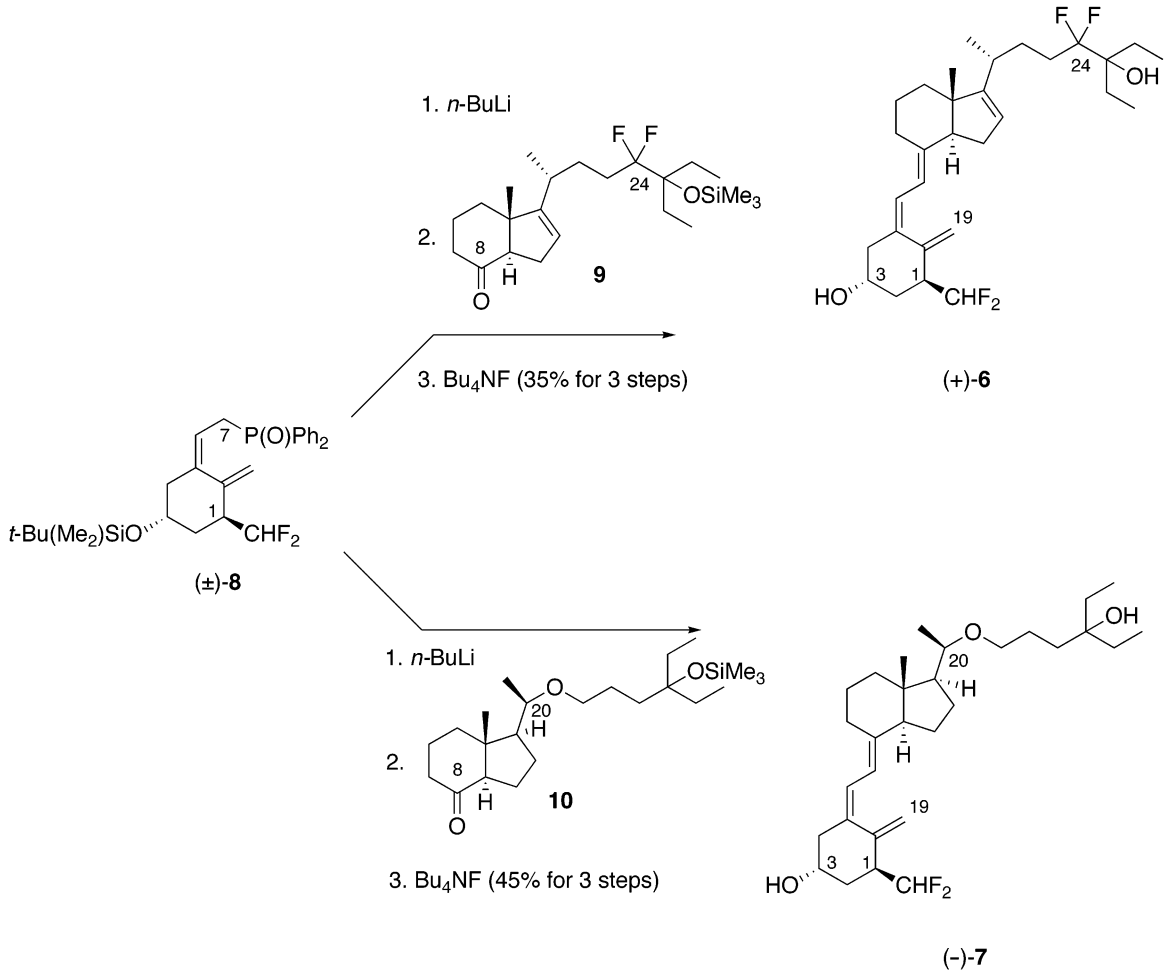
We assessed transcriptional activity of these compounds in CV1 cells cotransfected with hVDR expression plasmid and a transgene containing the minimal osteocalcin VDRE attached to the thymidine kinase promoter and the growth hormone reporter.[20] Using this assay, we found that the ED_{50} for transcriptional potency of the hybrid analog **7** containing a KH-1060 type side chain was 3 nM, while the hybrid analog **6** containing the side chain features of analog **4** had an ED_{50} of 0.6 nM (Table 1). Testing the binding to the hVDR in a cell-free system by using a protease assay with ^{35}S-labeled, in vitro-translated hVDR as a substrate,[20] revealed that the KH-1060 side chain has increased the ability of analog **7** to stabilize hVDR conformation by 10-fold relative to the 1-CHF_2 analog **5** with natural side chain. The side chain of analog **4** has increased the ability of analog **6** to stabilize VDR conformation by more than a 100-fold. These cell-free assays suggested that the increase in transcriptional and antiproliferative activities of the new hybrid analogs in cultured cells was partially (analog **7**) or primarily (analog **6**) due to direct improvement in their mode of interaction with the VDR and due not only to their pharmacokinetic properties.

To obtain a comprehensive assessment of the similarities and differences of the two sets of hybrid analogs, we summarized our earlier findings with the 1-hydroxymethyl analogs and compared them with the present evaluation of the 1-CHF_2 analogs. This comparison (shown in Table 1) reveals that the 1-CHF_2 substitution (analog **5**) had a more deleterious effect on affinity for the hVDR than did the 1-α hydroxymethyl substitution (analog **2**). However, in either case, the large decrease in affinity was not proportional to the larger decrease in ability of these analogs to stabilize hVDR conformation or to induce transcriptional activity of hVDR in cultured cells. Because we have found that the ability of agonists to stabilize the conformation of VDR in vitro correlates directly with their ability to induce transcription and interaction of p160 transcription coactivators with VDR (activation function 2, or AF-2),[10,22,23] we hypothesize that binding of analogs with either one of these A-ring substitutions to hVDR primarily impairs AF-2 functions of the receptor.[23]

In assessing the mechanisms by which the potentiating side chains restore the diminished activities of the A-ring modified analogs, we observed that they are distinct. Transcriptional and antiproliferative activities of hybrid analogs containing the KH-1060-like side chain were improved without an increase in their affinity for VDR and either without a change (1-α-hydroxymethyl analog **3**)[8] or with only a moderate improvement (1-CHF_2 analog **7**) in their ability to stabilize hVDR conformation in cell-free assays. This discrepancy between biochemical activities of 20-epi side chain analogs (without or with A ring modification) in cell-free assays and their ability to act as superagonists in cultured cells has been attributed to their pharmacokinetic properties (slow catabolism, more efficient uptake by the cultured cells),[24] to their significantly slower dissociation rate from the VDR,[20] and to their superior ability to stabilize the VDR against proteasome degradation in living cells.[25]

In contrast, the rescued functions of hybrid analogs with the side-chain modifications of analog **4** in cultured cells is consistent with dramatic improvement in the ability of such analogs to stabilize VDR conformation in cell-free assays, which occurs without a significant change in their affinity for the VDR.[10] These findings indicate that the side chain of analog **4** restores AF-2 function of the VDR independently of cellular factors and post-translational modifications that may be other-

Scheme 1



wise required for the potentiating activity of 20-epi side chains. These comparisons also suggest that side chains of vitamin D analogs have autonomous properties that are independent of the A ring structure and can be used as building blocks of new analogs with predictable properties in vitro and in cultured cells. In contrast, the dramatic difference in calcemic activity of the QW-1624-2 analog with the 1-α hydroxymethyl group (**4**, 1% of calcitriol)[12] and the QW-1624-2-like analog with the 1-CHF_2 group (**6**, 16% of calcitriol) as well as the difference in calcemic activity of the 1-hydroxymethyl analog with KH-1060 side chain (**3**, <1% of calcitriol)[9] and its 1-CHF_2-KH-1060-like analog (**7**, 5% of calcitriol) suggests that the outcome of these A-ring substitutions in vivo is distinct, although our studies in culture or in vitro did not reveal differences in their ability to induce responses through the VDR. This is not unexpected because calcemic activity of vitamin D analogs depends not only on their VDR-mediated transcriptional activities but also on chemical and biochemical properties that determine their clearance rate, including affinity for vitamin D binding protein and for catabolic enzymes.[26] Therefore, we speculate that the A-ring modifications used here have similar effects on the VDR functions but distinct effects on proteins that regulate clearance of vitamin D metabolites and analogs in vivo.

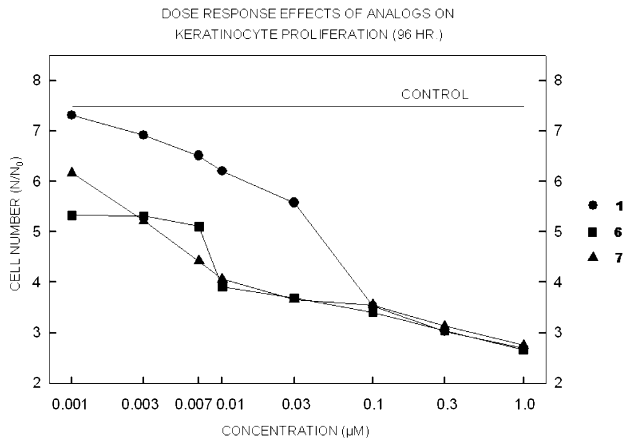


Figure 1.

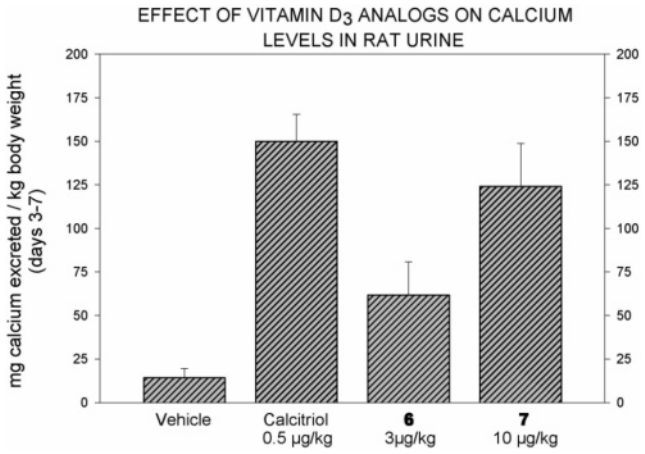


Figure 2.

Table 1. Comparing Biological Activities of 1-CH_2OH and 1-CHF2 Analogs[a]

compd	binding (hVDR)	protease assay (hVDR)	transcription (hVDR)	growth inhibition	calciuric index[b]
1	0.7 nM	0.5 nM	0.7 nM	7−40 nM	1 (0.5 μg/kg)
2	7 nM	1000 nM	175 nM	175 nM	N/A[c]
3	320 nM	>1000 nM	10 nM	7 nM	1/>100
4	3 nM	1 nM	0.5 nM	<1 nM	1/100
5	30 nM	500 nM	390 nM	150 nM	N/A[c]
6	50 nM	4 nM	0.6 nM	2 nM	1/6
7	19.5 nM	100 nM	3 nM	7 nM	1/20

[a] Values shown are the dose of analog (in nM) for competition of 50% (IC_{50}) of ^{3}H-1,25-dihydroxy vitamin D_3 binding to hVDR (by HAP binding assays),[20] the ED_{50} for stabilizing ^{35}S-hVDR against trypsin digestion (protease assay),[20] the ED_{50} for inducing transcriptional activity through hVDR in CV-1 cells (transcription),[20] and the ED_{50} for inhibition of murine keratinocytes proliferation (growth inhibition). In all these assays, ED_{50} was determined by comparison with maximal activity induced by calcitriol (which was defined as 100%). Data for analogs **2**, **3**, and **4** were obtained from references 7, 11, 12, 19, and 21. [b] The calciuric index was determined by measuring urinary calcium excretion during days 3−7 in female Sprague−Dawley rats treated by gavage daily with the analogs. Excretion rates are compared to levels of calcium excretion produced by daily treatment with 0.5 μg/kg body weight of calcitriol. [c] N/A = not available.

Experimental Section

All air- and moisture-sensitive reactions were carried out in flame-dried or oven-dried (at 120 °C) glassware under an inert atmosphere of argon. All reactive liquids were transferred by syringe or cannula and were added into the flask through a rubber septum. All other solvents and reagents were used as received unless otherwise stated. Melting points were obtained on Mel-Temp metal block apparatus and are not corrected. ^{1}H, ^{13}C, and ^{19}F NMR spectra were obtained on a Bruker 400 MHz spectrometer. All NMR spectra were obtained in a solution in $CDCl_3$ or acetone-d_6. Chemical shifts (δ) are reported in parts per million (ppm). Multiplicities of signals in the ^{1}H spectra are reported as follows, s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), and so on. Infrared spectra were obtained on a Perkin-Elmer 1600 FR-IR spectrometer as liquid films or as a thin layer with NaCl cells. Intensities were reported as s (strong 67−100%), m (medium 34−66%), and w (weak 0−33%), with the following notations: br (broadened), sh (shoulder), and so on. UV-vis absorbance measurements were made on a Hewlett-Packard 8453 diode array spectrophotometer. Optical rotations were recorded on JASCO, P-1100 model polarimeter (Japan Spectroscopic Co., Ltd.) with sodium D line at the temperatures, as indicated in the experimental for the specific compounds. Analytical thin layer chromatography (TLC) was performed on Merck silica gel plates (Merck Kieselgel, 60, 0.25-mm thickness) with F_{254} indicator. Compounds were visualized under UV lamp and/or by developing with iodine, vanillin, (*p*)-anisaldehyde, $KMnO_4$, or phosphomolybdic acid, followed by heating with a heat gun. High-pressure liquid chromatography (HPLC) was performed on a Rainin HPLX system equipped with two 25-mL pump heads and a Rainin Dynamax UV-C dualbeam variable wavelength detector set at 254 using a Phenomenex Luna 5 μ C18 250 × 10 mm column. FAB mass spectra were obtained using a VG70S double focusing magnetic sector mass spectrometer (VG Analytical, Manchester, U.K., now Micromass/Waters) equipped with a Xe gas FAB gun (8kV @ 1.2mA), an off-axis electron multiplier, and an MSS data system (MasCom, Bremen, Germany) at Johns Hopkins University. The resolution of the instrument was set at 10 000 (100 ppm peak width). Samples were mixed with *m*-nitrobenzyl-alcohol matrix deposited on the target of a direct insertion probe for introduction into the source. Nominal mass scan spectra were acquired with a mass scan range of 10−950 amu using a magnet scan rate of 25 s/dec. For accurate mass measurements, a narrower mass scan range was employed, with the matrix containing 10% PEG mass calibrant.

The purity of analogs **6** and **7** was ≥96% based on HPLC analysis.

Analog 6. Racemic 1-CHF_2 phosphine oxide **8**[17] and CD-ring ketone **9**[11] were separately azeotropically dried with anhydrous benzene (4 × 5 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mmHg) for at least 48 h prior to use.

A flame-dried 10-mL recovery flask equipped with a magnetic stir bar and an Ar balloon was charged with phosphine oxide (±)-**8**[17] (40 mg, 0.08 mmol). The reagent was dissolved in 2.0 mL freshly distilled THF and cooled to −78 °C. To this solution *n*-BuLi (45 μL, 0.07 mmol, 1.60 M solution in hexanes) was added dropwise over several minutes during which time a deep red color developed and persisted. This mixture was allowed to stir at −78 °C for an additional 10 min. Meanwhile, a flame-dried 10-mL flask containing CD-ring ketone **9**[11] (17.0 mg, 0.04 mmol) was dissolved in 0.75 mL of freshly distilled THF and cooled to −78 °C. The solution of CD-ring ketone was transferred dropwise into the flask containing the phosphine oxide anion at −78 °C via cannula over several minutes. After the addition was complete, the deep red color persisted, and the mixture was allowed to stir at −78 °C for 6 h. Upon observation of a light yellow color, the reaction was quenched with 5 mL of pH 7 buffer and allowed to warm to room temperature. The mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give crude product that was purified by column chromatography (5% ethyl acetate in hexanes with 1% NEt_3), affording 12.0 mg of product in a 44% yield. The protected analog was dissolved in THF (2 mL), and TBAF (1.6 M in THF, 91 μL) was added. After stirring overnight, the reaction was quenched with H_2O and extracted with CH_2Cl_2 (3 × 5 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give the crude product that was purified by column chromatography (50−75% ethyl acetate in hexanes with 1% NEt_3) to afford 9 mg of a 3:1 mixture of diastereomers (35% yield for two steps). Separation of diastereomers by HPLC (Phenomenex Luna 5 μ C18 250 × 10 mm eluted with 73:27 CH_3CN/H_20, 2.5 mL/min, t_R = 84.0 min) afforded 3.0 mg of (+)-**6** as an oil. $[\alpha]^{24}_D$ +54.2 (*c* 0.125, $CHCl_3$); IR (neat, cm^{-1}) 3400 (br s), 2924 (m), 2861 (m), 2361 (s), 2339 (s), 1456 (w), 1373 (w), 1061 (w), 673 (w), 650 (w); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.31 (d, 1H, J = 11.2 Hz), 6.05 (d, 1H, J = 11.2 Hz), 5.78 (dt, 1H, J = 5.6, 56.4 Hz), 5.34 (s, 1H), 5.22 (s, 1H), 5.07 (s, 1H), 4.07 (m, 1H), 2.86 (m, 2H), 2.62 (m, 1H), 2.41−1.22 (m, 18 H) 1.06 (d, 3H, J = 6.8 Hz), 0.97−0.82 (m, 10H) 0.66 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.92, 142.76, 140.99, 133.48, 123.99, 120.91, 116.90, 115.93, 77.72, 66.66, 58.38, 50.01, 45.67, 45.56, 35.24, 34.17, 32.60, 29.68, 29.24, 28.76, 27.18, 25.34, 23.56, 21.64 16.64, 14.09, 7.55; ^{19}F NMR ($CDCl_3$, 280 MHz) δ −111.51 (t, 2F, J = 19.6 Hz), −119.48 (ddd 1F, J = 277, 56, 14 Hz), −124.09 (ddd, 1F, J = 277,56, 14 Hz); HRMS calcd for $C_{30}H_{44}F_4O_4$ [M+], 512.3277; found, 512.3263. UV (MeOH) λ_{max} 263 nm (ϵ 10 568).

Analog 7. Racemic 1-CHF_2 phosphine oxide **8**[17] and CD-ring ketone **10**,[19] were separately azeotropically dried with anhydrous benzene (4 × 5 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mmHg) for at least 48 h prior to use.

A flame-dried 10-mL recovery flask equipped with a magnetic stir bar and an Ar balloon was charged with phosphine oxide (±)-**8**[17] (45 mg, 0.09 mmol). The reagent was dissolved in 2.0 mL freshly distilled THF and cooled to −78 °C. To this solution *n*-BuLi (63 μL, 0.10 mmol, 1.60 M solution in hexanes) was added dropwise over several minutes during which time a deep red color developed and persisted. This mixture was allowed to stir at −78 °C for an additional 10 min. Meanwhile, a flame-dried 10-mL flask containing CD-ring ketone **10**[19] (14.0 mg, 0.035 mmol) was dissolved in 0.75 mL of freshly distilled THF and cooled to −78 °C. The solution of CD-ring ketone was transferred dropwise into the flask containing the phosphine oxide anion at −78 °C via cannula over several minutes. After the addition was complete, the deep red color persisted and the mixture was allowed to stir at −78 °C for 8 h. Upon observation of a light yellow color, the reaction was quenched with 5 mL of pH 7 buffer and allowed to warm to room temperature. The mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with water

and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give crude product that was purified by column chromatography (5% ethyl acetate in hexanes with 1% NEt_3), affording 14.0 mg of product in a 56% yield. The protected analog was dissolved in THF (2 mL), and TBAF (1.6 M in THF, 91 μL) was added. After stirring overnight, the reaction was quenched with H_2O and extracted with CH_2Cl_2 (3 × 5 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give the crude product that was purified by column chromatography (50−75% ethyl acetate in hexanes with 1% NEt_3) to afford 7 mg of a 4:1 mixture of diastereomers (45% yield for two steps). Separation of diastereomers by HPLC (Phenomenex Luna 5 μ C18 250 × 10 mm eluted with 75:25 CH_3CN/H_20, 2.5 mL/min, t_R = 52.8 min) afforded 4.0 mg of (−)-**7** as an oil. $[\alpha]^{25}_D$ −13.55 (*c* 0.20, $CHCl_3$); IR (neat, cm^{-1}) 3402 (br s), 2968 (s), 2924 (s), 2850 (m), 1672 (w), 1459 (w), 1372 (w), 1262 (w), 1056 (m), 799 (w); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.32 (d, 1H, J = 11.2 Hz), 5.94 (d, 1H, J = 11.2 Hz), 5.76 (dt, 1H, 5.6, 56.8 Hz), 5.21 (s, 1H), 5.05 (s, 1H), 4.07 (m, 1H), 3.56 (m, 1H), 3.24 (m, 2H), 2.86 (m, 2H), 2.62 (dd, 1H, J = 12. 8, 3.6 Hz), 2.31−1.98 (m, 5H), 1.84 (m, 2H), 1.78−1.12 (m, 17 H), 1.08 (d, 3H, J = 6.0 Hz), 0.85 (dt 6H, J = 2.4, 7.6 Hz), 0.53 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.52, 141.00, 133.24, 124.06, 116.95, 115.99, 78.28, 74.12, 68.83, 66.70, 56.81, 55.80, 45.85, 45.76 (t, J = 21.00 Hz), 45.73, 40.35, 35.67, 34.29, 31.15, 30.85, 29.71, 29.16, 25.14, 24.24, 23.55, 22.34, 18.28, 12.52, 7.86, 7.83; ^{19}F NMR ($CDCl_3$, 280 MHz) δ −119.14 (ddd 1F, J = 277, 56, 14 Hz), −123.85 (ddd, 1F, J = 277,56, 14 Hz); HRMS calcd for $C_{30}H_{48}F_2O_3$ [M+], 494.3572; found, 494.3562. UV (MeOH) λ_{max} 263 nm (ϵ 10 409).

Acknowledgment. We thank the NIH for financial support (Grant CA 93547 to G.H.P.) and Prof. Dr. Klaus Müller (Hoffman-LaRoche, Basel) for a stimulating discussion about the CHF_2 group.

Supporting Information Available: ^1H and ^{13}C spectra as well as HPLC traces for compounds **6** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Feldman, D.; Pike, J.W.; Glorieux, F. *Vitamin D* 2nd Ed., Academic Press: San Diego, CA, 2005.
(2) Holick, M.F. *Vitamin D: Molecular Biology, Physiology, and Clinical Applications*; Humana: Totawa, NJ, 1999.
(3) Bouillon, R.; Okamura, W. H.; Norman, A. W. Structure−function relationships in the vitamin D endocrine system. *Endocrinol. Rev.* **1995**, *16*, 200−257.
(4) Posner, G. H.; Kahraman, M. Organic chemistry of vitamin D analogues. *Eur. J. Org. Chem.* **2003**, 3889−3895.
(5) Posner, G. H.; Halford, B. A.; Peleg, S.; Dolan, P.; Kensler, T. W. Conceptually new low-calcemic oxime analogues of the hormone 1α,25-dihydroxyvitamin D_3: Synthesis and biological testing. *J. Med. Chem.* **2002**, *45*, 1723−1730.
(6) Posner, G. H.; Wang, Q.; Han, G.; Lee, J. K.; Crawford, K.; Zand, S.; Brem, H.; Peleg, S.; Dolan, P.; Kensler, T. W. Conceptually new sulfone analogues of the hormone 1α,25-dihydroxyvitamin D_3: Synthesis and preliminary biological evaluation. *J. Med. Chem.* **1999**, *42*, 3425−3435.
(7) Posner, G. H.; Nelson, T. D.; Guyton, K. Z.; Kensler, T. W. New vitamin D_3 derivatives with unexpected antiproliferative activity: 1-(hydroxymethyl)-25-hydroxyvitamin D_3 homologs. *J. Med. Chem.* **1992**, *35*, 3280−3287.
(8) Peleg, S.; Liu, Y.-Y.; Reddy, S.; Horst, R. L.; White, M. C.; Posner, G. H. A 20-epi side chain restores growth-regulatory and transcriptional activities of an A ring-modified hybrid analog of 1α,25-dihydroxyvitamin D_3 without increasing its affinity to the vitamin D receptor. *J. Cell. Biochem.* **1996**, *63*, 149−161.
(9) White, M. C.; Burke, M. D.; Peleg, S.; Brem, H.; Posner, G. H. Conformationally restricted hybrid analogues of the hormone 1-α,25-dihydroxyvitamin D_3: design, synthesis, and biological evaluation. *Bioorg. Med. Chem.* **2001**, *9*, 1691−1699.
(10) Peleg, S.; Nguyen, C.; Woodard, B. T.; Lee, J. K.; Posner, G. H. Differential use of transcription activation function 2 domain of the vitamin D receptor by 1,25-dihydroxyvitamin D_3 and it's A ring-modified analogs. *Mol. Endocrinol.* **1998**, *12*, 525−535.
(11) Posner, G. H.; Lee, J. K.; Wang, Q.; Peleg, S.; Burke, M.; Brem, H.; Dolan, P.; Kensler, T. W. Noncalcemic, antiproliferative, transcriptionally active, 24-fluorinated hybrid analogues of the hormone 1α,25-dihydroxyvitamin D_3. Synthesis and preliminary biological evaluation. *J. Med. Chem.* **1998**, *41*, 3008−3014.
(12) Posner, G. H.; Jeon, H. B.; Sarjeant, A.; Riccio, E. S.; Doppalapudi, R. S.; Kapetanovic, I. M.; Saha, U.; Dolan, P.; Kensler, T. W. Low-calcemic, efficacious, 1α,25-dihydroxyvitamin D_3 analog QW-1624F_2-2: calcemic dose-response determination, preclinical genotoxicity testing, and revision of A-ring stereochemistry. *Steroids* **2004**, *69*, 757−762.
(13) Kensler, T. W.; Dolan, P. M.; Gange, S. J.; Lee, J.-K.; Wang, Q.; Posner, G. H. Conceptually new deltanoids (vitamin D analogs) inhibit multistage skin tumorigenesis. *Carcinogenesis* **2000**, *21*, 1341−1345.
(14) Dixon, K. M.; Deo, S. S.; Wong, G.; Slater, M.; Norman, A. W.; Bishop, J. E.; Posner, G. H.; Ishizuka, S.; Halliday, G. M.; Reeve, V. E.; Mason, R. S. Skin cancer prevention: A possible role of 1,25-dihydroxyvitamin D3 and its analogs. *J. Steroid Biochem. Mol. Biol.* **2005**, *97*, 137−143.
(15) Reddy, C. D.; Patti, R.; Guttapalli, A.; Maris, J. M.; Yanamandra, N.; Rachamallu, A.; Sutton, L. N.; Phillips, P. C.; Posner, G. H. Anticancer effects of the novel 1α,25-dihydroxyvitamin D3 hybrid analog QW1624F2-2 in human neuroblastoma. *J. Cell. Biochem.* **2006**, *97*, 198−206.
(16) Erickson, J. A.; McLoughlin, J. I. Hydrogen bond donor properties of the difluoromethyl group. *J. Org. Chem.* **1995**, *60*, 1626−1631.
(17) Posner, G. H.; Suh, B. C.; Petersen, K. S.; Dolan, P.; Agoston, E. S.; Kensler, T. W.; Koh, J. T.; Peleg, S. Difluoromethyl analogs of the natural hormone 1α,25-dihydroxyvitamin D_3: Design, synthesis, and preliminary biological evaluation. *J. Steroid Biochem. Mol. Biol.* **2006**, *98*, in press.
(18) Kabat, M. M.; Burger, W.; Guggino, S.; Hennessy, B.; Iacobelli, J. A.; Takeuchi, K.; Uskokovic, M. R. Total synthesis of 25-hydroxy-16,23*E*-diene vitamin D_3 and 1α,25-dihydroxy-16,23*E*-diene vitamin D_3: Separation of genomic and nongenomic vitamin D activities. *Bioorg. Med. Chem.* **1998**, *6*, 2051−2059.
(19) Posner, G. H.; Lee, J. K.; White, M. C.; Hutchings, R. H.; Dai, H.; Kachinski, J. L.; Dolan, P.; Kensler, T. W. Antiproliferative hybrid analogs of the hormone 1α,25-dihydroxyvitamin D_3: Design, synthesis, and preliminary biological evaluation. *J. Org. Chem.* **1997**, *62*, 3299−3314.
(20) Liu, Y. Y.; Collins, E. D.; Norman, A. W.; Peleg, S. Differential interaction of 1-α,25-dihydroxyvitamin D_3 analogues and their 20-epi homologues with the vitamin D receptor. *J. Biol. Chem.* **1997**, *272*, 3336−3345.
(21) Posner, G. H.; Lee, J. K.; Li, Z.; Takeuchi, K.; Guggino, S. E.; Dolan, P.; Kensler, T. W. 1α,25-Dihydroxyvitamin D_3 hybrid analogs with structural changes at both the A-ring and the C,D-ring side-chain. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2163−2168.
(22) Gardezi, S. A.; Nguyen, C.; Malloy, P. J.; Posner, G. H.; Feldman, D.; Peleg, S. A rationale for treatment of hereditary vitamin D-resistant rickets with analogs of 1-α,25-dihydroxyvitamin D_3. *J. Biol. Chem.* **2001**, *276*, 29148−29156.
(23) MacDonald, P. N.; Baudino, T. A.; Tokumaru, H.; Dowd, D. R.; Zhang, C. Vitamin D receptor and nuclear receptor coactivators: crucial interactions in vitamin D-mediated transcription. *Steroids* **2001**, *66*, 171−176.
(24) Dilworth, F. J.; Calverley, M. J.; Makin, H. L.; Jones, G. Increased biological activity of 20-epi-1,25-dihydroxyvitamin D_3 is due to reduced catabolism and altered protein binding. *Biochem. Pharmacol.* **1994**, *47*, 987−993.
(25) Jaaskelainen, T.; Ryhanen, S.; Mahonen, A.; DeLuca, H. F.; Maenpaa, P. H. Mechanism of action of superactive vitamin D analogs through regulated receptor degradation. *J. Cell. Biochem.* **2000**, *76*, 548−558.
(26) Masuda, S.; Jones, G. Vitamin D analogs-drug design based on proteins involved in vitamin D signal transduction. *Curr. Drug Targets: Immune Endocr. Metabol. Disord.* **2003**, *3*, 43−66.

JM0609925