Synthesis and Biological Activity of 2-Hydroxy and 2-Alkoxy Analogs of 1α,25-Dihydroxy-19-norvitamin D_3

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Received January 31, 1994®

1α,2α,25-Trihydroxy-19-norvitamin D_3, 1α,2β,25-trihydroxy-19-norvitamin D_3, and their alkoxy analogs were efficiently prepared in a convergent synthesis, starting with (−)-quinic acid and a Windaus–Grundmann type ketone. Configurations of the A-ring fragment substituents were determined by ^1H,^1H COSY 2D spectra and ^1H NOE difference spectroscopy. The new analogs exhibited selective activity in stimulating intestinal calcium transport while having little or no activity in mobilizing bone calcium. They also showed HL-60-differentiating activity equal to or 10 times lower than that of 1α,25-dihydroxyvitamin D_3.

Introduction

Vitamin D_3 is synthesized in the skin from 7-dehydrocholesterol and then hydroxylated to 25-hydroxyvitamin D_3[1] in the liver and subsequently in the kidney to the most active hormonal form of vitamin D_3,[2] namely, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, 1; Figure 1). Discovery of 1α,25-$(OH)_2D_3$ has stimulated considerable interest in establishing its role in physiology. The basic function of the natural hormone 1 is to increase the serum calcium and phosphorus concentrations to the supersaturated levels necessary for bone mineralization. It has been established that 1α,25-$(OH)_2D_3$ stimulates intestinal calcium absorption and mobilizes calcium from bone.[3,4] Recently, an important function of 1 in monocytic differentiation of human promyelocytic leukemia cells (HL-60) has been discovered,[5] and numerous vitamin D analogs have been synthesized and tested for their potential anticancer activity. These studies showed the crucial role of the hydroxy groups at C-1 and C-25 and indicated the structural alterations of the vitamin D molecule which result in interesting separation of calcemic and cell differentiation activities.[6-8] In our continuing investigation of structure–activity relationships of the vitamin D molecule, we prepared the 19-nor analog, **2**, of 1α,25-$(OH)_2D_3$, in which the A-ring exocyclic methylene group (carbon 19) has been replaced by two hydrogen atoms.[9] Analog **2** showed a selective activity profile, combining high potency in inducing differentiation of malignant cells with very low or no bone calcification activity.

Recently 2β-hydroxy and alkoxy analogs of 1α,25-$(OH)_2D_3$ have been described and are being examined as potential drugs for osteoporosis (e.g., ED-71, **3**).[10] However, the new analog **3** is extremely potent and produces hypercalcemia, and the applied synthesis provided only one of the epimers (2β) at C-2. Because the 19-nor derivatization reduces the mobilization of calcium from bone, we considered that a 19-nor derivative of ED-71 might retain potent bone formation activity without hypercalcemia resulting from bone calcium mobilization. We prepared both 2α- and 2β-hydroxy analogs of 19-nor-1α,25-$(OH)_2D_3$ (**4a,b**) and their 2-alkoxy derivatives. In this paper, we describe an efficient synthesis of these 2-substituted 19-nor analogs of calcitriol, based on the Wittig–Horner coupling approach, pioneered by Lythgoe,[11] and successfully used by us in the preparation of **2**.[12] Since an alkylation of the 2-hydroxy group was performed at the end of the synthesis, numerous analogs with different alkoxy substituents at the 2-position were conveniently obtained. These 19-nor derivatives afford potent intestinal calcium transport activity while having little ability to mobilize bone calcium. The desired dichotomy between calcemic and cellular differentiation activity was also observed for some synthesized vitamins.

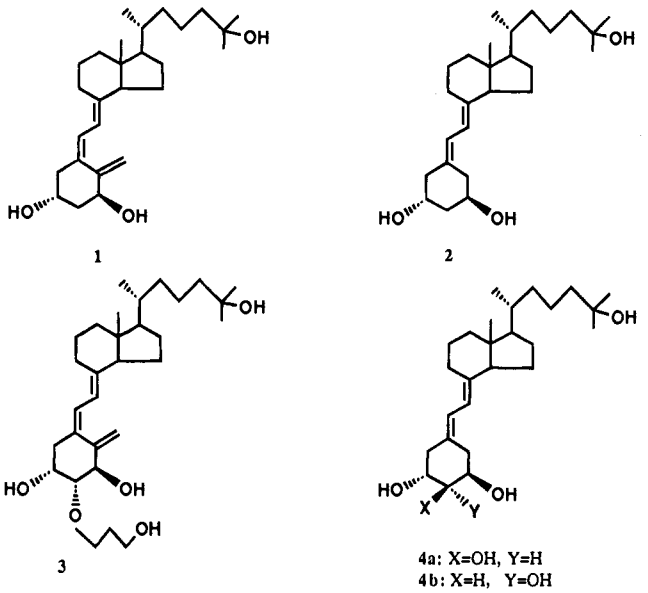

Figure 1. Chemical structure of 1α,25-$(OH)_2D_3$ and its analogs.

Chemistry

The cyclohexanone derivative **5** (Scheme 1) was prepared from commercially available (1*R*,3*R*,4*S*,5*R*)-(−)-quinic acid.[12,13] The 4′-hydroxyl group of **5** was protected as the trimethylsilyl ether, by treatment with *N*-(trimethylsilyl)imidazole to give **6** (95%). Peterson reaction of the trisilyl ether **6** with methyl (trimethyl-

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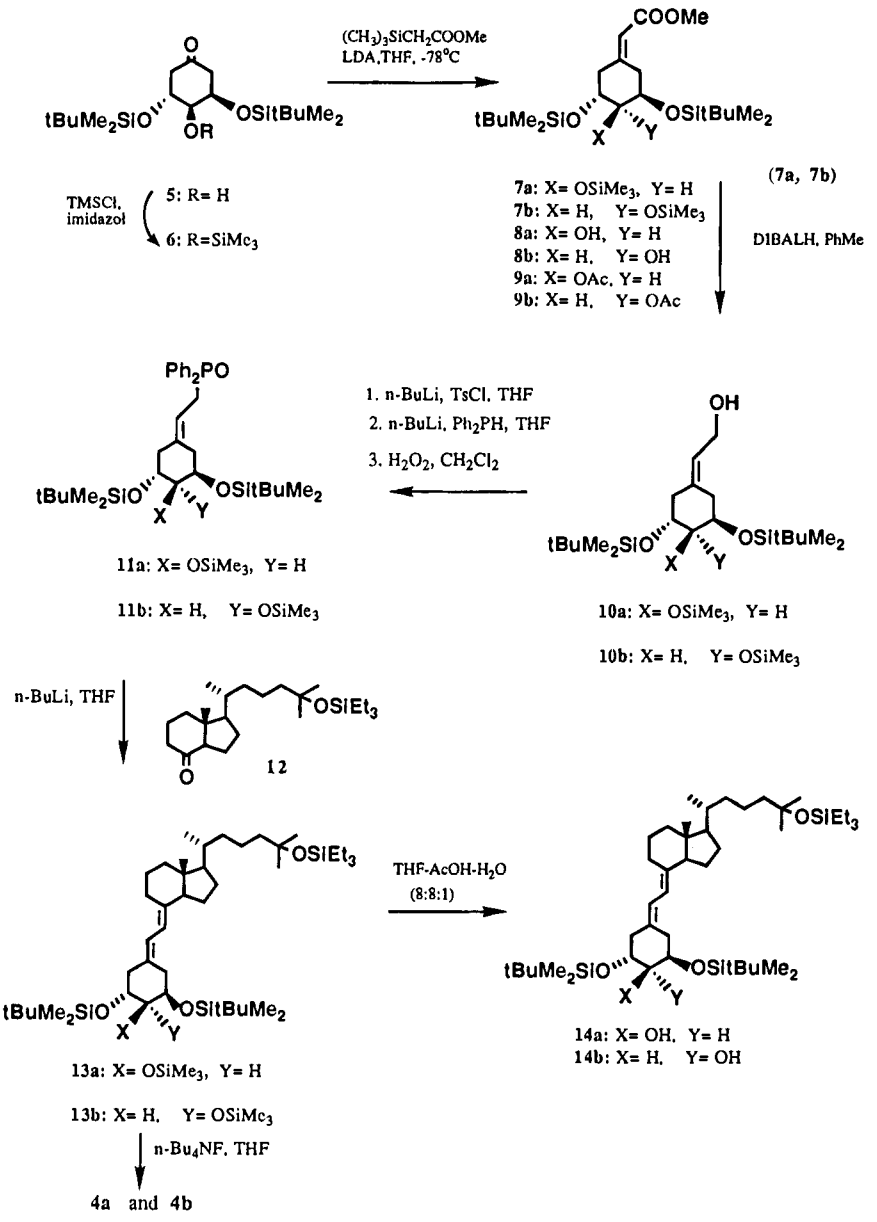
® Abstract published in *Advance ACS Abstracts,* October 1, 1994.

0022-2623/94/1837-3730$04.50/0

Scheme 1



silyl)acetate gave, in excellent yield (91%), a 3:1 mixture of the two isomeric allylic esters **7a,b**. The isomeric mixture was the result of the newly created axial chirality of the methyl 2-(4′-hydroxycyclohexylidene)-ethanoate system. Allylic esters **7a,b**, after separation by HPLC, were selectively deprotected with THF/acetic acid/water (5:5:1) to give, in good yield, the 4'-hydroxy compounds **8a,b** (83% and 80% yield, respectively). We therefore anticipated that the 4′-trimethylsilyl protecting group would give the needed chemoselectivity at this very hindered hydroxyl functionality in subsequent steps.

Analysis of ^{1}H NMR spectra (in $CDCl_3$) of alcohols **8a,b** revealed that both isomers assume the conformation in which the 4′-hydroxy group is axially oriented (Figure 2). Magnitudes of the degenerate couplings of the proton at C′-4 (resonating at ca. δ 3.6) to the protons at C′-3 and C′-5 were correlated with the Karplus relation, assuming that larger trans vicinal coupling of the 4′-H (4.8 and 4.5 Hz for **8a,b**, respectively) represents averaged axial−axial and equatorial−equatorial values (analogical limiting values for cyclohexanol protons are $J_{ax,ax}$ = 11.1 Hz and $J_{eq,eq}$ = 2.7 Hz).[14] Accordingly, the ratios were established to be 75:25 and 79:21 in favor of the OH-axial conformer for **8a,b**, respectively. Alcohols **8a,b** were converted to the corresponding acetates, **9a,b** (Ac_2O, pyridine), in an attempt to find derivatives with well-resolved ^{1}H NMR spectra. As expected, the chemical shifts of the 4′-H protons are considerably changed upon acetylation (downfield shift of ca. 1.2 ppm). Magnitudes of their coupling constants to the neighboring protons (7.4 and 2.4 Hz for **9a**, 7.5 and 2.5 Hz for **9b**) were correlated with limiting values for cyclohexanol acetate.[14] These calculations indicate that conformational equilibria are 57:43 and 55:45 for **9a,b**, respectively, in favor of the conformers that have axially oriented 4′-OAc. However, alcohols **8a,b** proved to be the most suitable for spectroscopic structure elucidation studies. Their ^{1}H NMR spectra, taken in deuteriobenzene, are characterized by good proton dispersion and lack of peak broadening due to slow exchange between two chair forms.

Configurations at C′-4 in both epimers were unequivocally assigned by NOE measurements and COSY spec-

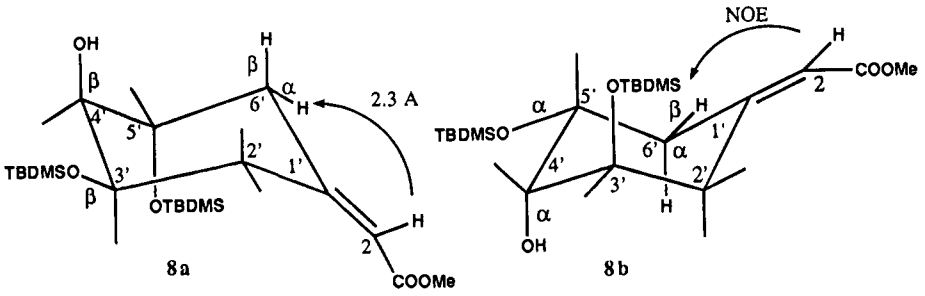


Figure 2. Preferred conformations of compounds **8a,b**.

tra. Thus, in a ^{1}H NOE difference spectroscopy experiment, **8a** showed, upon irradiation of the vinyl proton signal at δ 5.83, a 4.8% enhancement of the signal at δ 1.94 and a 1.4% enhancement at δ 2.57. These resonances have therefore been assigned to the 6′α-H and its partner, 6′β-H, respectively. From further analysis of these signals, it was possible to identify all of the remaining protons by considering the magnitudes of their coupling constants and the corresponding connectivities established by a ^{1}H,^{1}H COSY 2D spectrum. The magnitudes of the coupling constants of the protons at the oxygen-bearing carbons C-3′, C-4′, and C-5′ are consistent with them being arranged in an axial–equatorial–equatorial (α, α, β) sequence around a cyclohexane ring in the preferred chair conformation. Similarly, irradiation of vinylic 2-H (δ 5.86) in the epimeric compound **8b** resulted in a 4.6% enhancement at δ 2.03 (6′β-H) and 1.0% at δ 2.35 (6′α-H). The latter proton was found to be trans diaxially coupled to 5′β-H (δ 4.07, J = 8.6 Hz). A coupling of 3.2 Hz in the 5′β-H multiplet was attributed to an axial–equatorial coupling to the methine proton at C′-4, establishing their cis relationship and, therefore, the α-configuration of the 4′-hydroxy group. The connectivities of the corresponding protons were also confirmed by analysis of the ^{1}H,^{1}H COSY spectrum of **8b**, which delineated the coupling network.

Cyclohexylidene esters **7a,b** were reduced to the allylic alcohols **10a,b** which were easily separated by preparative HPLC (61%).[15] These were in turn transformed to the desired A-ring phosphine oxides **11a,b** by in situ tosylation and conversion into the corresponding phosphines followed by oxidation with hydrogen peroxide (ca. 60% overall yield).

The synthesis of the C,D-ring synthon, namely, the Windaus–Grundmann ketone with the appropriate protected side chain hydroxyl, is well documented in the literature.[16] We chose a recently described procedure, i.e., ozonolysis of commercial vitamin D_3 followed by ruthenium tetraoxide oxidation.[17] The resulting 25-hydroxy group (steroidal numbering) was protected as a triethylsilyl ether in order to give chemoselectivity from the 2-OTMS group of the corresponding 19-norvitamin compounds.

With the required synthons on hand, the final convergent formation of **4a,b** was accomplished as described in our synthesis of the parent 2-desoxy analog **2**.[12] Wittig–Horner coupling of the lithium phosphinoxy carbanion prepared from **11a** and *n*-BuLi in anhydrous THF with the protected hydroxy ketone **12** gave the expected 19-norvitamin compound **13a** (54%). This, after deprotection with tetrabutylammonium fluoride, gave crystalline 1α,2α,25-trihydroxy-19-norvitamin D_3 (**4a**; 60%). The synthesis of epimeric **4b** from **11b** was accomplished as described for **4a**.

For the synthesis of the 2α-alkoxy derivatives of 19-nor-1α,25-$(OH)_2D_3$, the trimethylsilyl protecting group in **13a** was selectively hydrolyzed under carefully controlled conditions, i.e., treatment with a mixture of THF/acetic acid/water (8:8:1) at room temperature for 4.5 h. The resulting mixture of partially hydrolyzed compounds was separated by HPLC to give 19-nor-2α-hydroxyvitamin **14a** (38%). As alkylating agent, we chose bromo ether **15** (Scheme 2), prepared by silylation of the parent bromo alcohol. The ether **16a** was obtained by treating **14a** with sodium hydride and the protected bromo compound **15** at room temperature for 48 h in the presence of 18–crown-6 in anhydrous DMF. Deprotection of **16a** with tetrabutylammonium fluoride gave the expected **17a** (20–25% overall yield from **14a**). The 2β-(3′-hydroxypropoxy) analog **17b** was prepared in the same manner from **13b**. To prove the versatility of our synthetic approach for the preparation of different 2–alkoxy analogs of **4a,b**, we treated **14a** with benzyl bromide under similar conditions as above to obtain **18**. This, following deprotection, gave the 2α-benzyloxy analog (**19**) of 19-nor-1α,25-$(OH)_2D_3$.

Results and Discussion

We previously reported that elimination of the 19-methylene carbon from 1α,25-$(OH)_2D_3$ resulted in a vitamin D compound that retained its ability to cause cellular differentiation and increase intestinal calcium transport but which lacked the ability to elevate plasma calcium at the expense of bone, i.e., bone calcium mobilization.[9] Chemists at Chugai in Japan have produced 2β-(3′-hydroxypropoxy)-1α,25-$(OH)_2D_3$ (**3**) that retains potent activity and biological lifetime, exceeding that of the native hormone **1**.[10] We endeavored to instill into the 2β-(3′-hydroxypropoxy) derivative selective activity by eliminating the 19–methylene carbon. The series of reactions started with quinic acid, ultimately providing the 2α- and 2β-hydroxy A-ring derivatives which, upon conversion to the appropriate phosphine oxides, could be condensed with the C,D-ring unit to give the 19-norvitamin D compounds. By selective removal of the silyl group, the 2–alcohols could be alkylated to the appropriate derivatives.

These compounds were then tested in the vitamin D-deficient rat to determine if they are able to support intestinal absorption of calcium and mobilization of calcium from bone. Our initial experiments were a study of the time course of intestinal calcium transport and bone calcium mobilization (serum calcium) in response to a single dose of the analogs. All compounds showed the same course with peak intestinal activity

Scheme 2

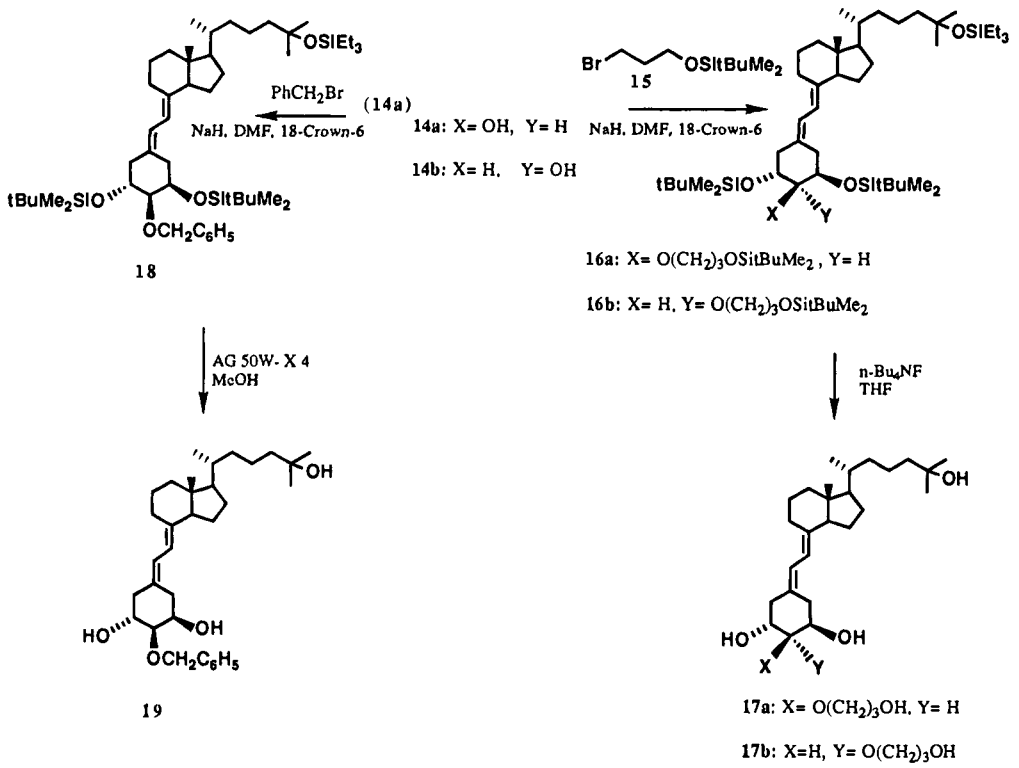


Table 1. Intestinal Calcium Transport and Bone Calcium-Mobilizing Activity of 1α,25-Dihydroxy-19-norvitamin D$_3$ Compounds[a]

compound	compd no.	amount (pmol/d/7 days)	intestinal Ca transport *S/M* ratio (av ± SEM)	bone calcium mobilization serum Ca (av ± SEM)
none (control)		0	2.1 ± 0.18[b]	4.3 ± 0.14[b]
1α,25-(OH)$_2$D$_3$	1	130	8.0 ± 0.80[c]	5.6 ± 0.20[c]
19-nor-1α,2α,25-(OH)$_3$D$_3$	**4a**	130	5.3 ± 0.26[d1]	4.1 ± 0.30[d1]
		325	5.0 ± 0.14[d2]	4.1 ± 0.10[d2]
none (control)		0	3.0 ± 0.80[b]	4.3 ± 0.23[b]
1α,25-(OH)$_2$D$_3$	1	65	8.3 ± 0.32[c]	5.2 ± 0.16[c1]
		325	10.5 ± 1.20[c2]	6.8 ± 0.24[c2]
19-nor-1α,2β,25-(OH)$_3$D$_3$	**4b**	65	5.8 ± 0.022[d1]	4.2 ± 0.19[d1]
		325	10.8 ± 0.39[d2]	4.2 ± 0.28[d2]
none (control)		0	2.5 ± 0.15[b]	4.3 ± 0.13[b]
1α,25-(OH)$_2$D$_3$	1	130	7.1 ± 0.49[c1]	5.7 ± 0.23[c1]
		325	7.8 ± 0.60[c2]	7.1 ± 0.30[c2]
19-nor-1α,25-(OH)$_2$-2α-(3′-hydroxypropoxy)D$_3$	**17a**	130	3.5 ± 0.23[d1]	4.9 ± 0.11[d1]
		325	4.3 ± 0.33[d2]	5.8 ± 0.20[d2]
none (control)		0	3.8 ± 0.38[b]	4.0 ± 0.11[b]
1α,25-(OH)$_2$D$_3$	1	260	10.5 ± 1.10[c]	5.9 ± 0.24[c]
19-nor-1α,25-(OH)$_2$-2β-(3′-hydroxypropoxy)D$_3$	**17b**	260	5.7 ± 0.43[d]	4.7 ± 0.15[d]
19-nor-1α,25-(OH)$_2$-2α-(benzyloxy)D$_3$	**19**	260	11.9 ± 1.00[e]	4.6 ± 0.10[e]

[a] Holtzman weanling rats were maintained on a 0.47% Ca and 3% P diet for 1 week and then switched to a low-calcium diet (0.02% Ca) for an additional 3 weeks. During the fourth week, all animals were dosed with the appropriate compounds via the peritonal cavity. All doses were suspended in propylene glycol/ethanol (95:5) and administered daily for 7 days. Controls received the vehicle. At 7 days, the rats were killed after 24 h after the last dose and the determinations were done. There were at least six rats per group. Statistical analysis was done by Student's *t*-test. Statistical data: serosal/mucosal (*S/M*), panel 1, all from *b*, $p < 0.001$; panel 2, all from *b*, $p < 0.001$; panel 3, all from *b*, $p < 0.001$; panel 4, *c* and *d* from *b*, $p < 0.001$, and *e* from *b*, $p < 0.005$; serum calcium, panel 1, *c* from *b*, $p < 0.001$, and d^1 and d^2 from *b*, NS; panel 2, c^1 and c^2 from *b*, $p < 0.001$, and d^1 and d^2 from *b*, NS; panel 3, c^1 and c^2 from *b*, $p < 0.001$, d^1 from *b*, $p < 0.005$, and d^2 from *b*, $p < 0.001$; panel 4, *c* from *b*, $p < 0.001$, and *d* and *e* from *b*, $p < 0.005$.

at 24 h and peak bone activity at 12 h analogous to 1,25-(OH)$_2$D$_3$. Table 1 provides the results of daily injections of the compounds at the doses shown. Two of the 2-substituted 19-nor compounds had potent intestinal calcium transport activity equal to that of the native hormone, 1α,25-(OH)$_2$D$_3$. These compounds were 1α,2β,25-trihydroxy-19-norvitamin D$_3$ (**4b**) and 1α,25-dihydroxy-2α-(benzyloxy)-19-norvitamin D$_3$ (**19**). Of great interest is that the former compound possessed no ability to mobilize calcium from bone. This compound is, therefore, of considerable interest as a possible agent for the treatment of osteoporosis, since it does not support the mobilization of calcium from bone, while being extremely potent on intestinal calcium absorption. It is of some interest that the 19-nor derivative of ED-71 or 2β-(3′-hydroxypropoxy)-1α,25-(OH)$_2$D$_3$, i.e., compound **17b**, compound **17a** which is the 2α-(3′-hydroxypropoxy) derivative, and compound **4a** which is the 2α-

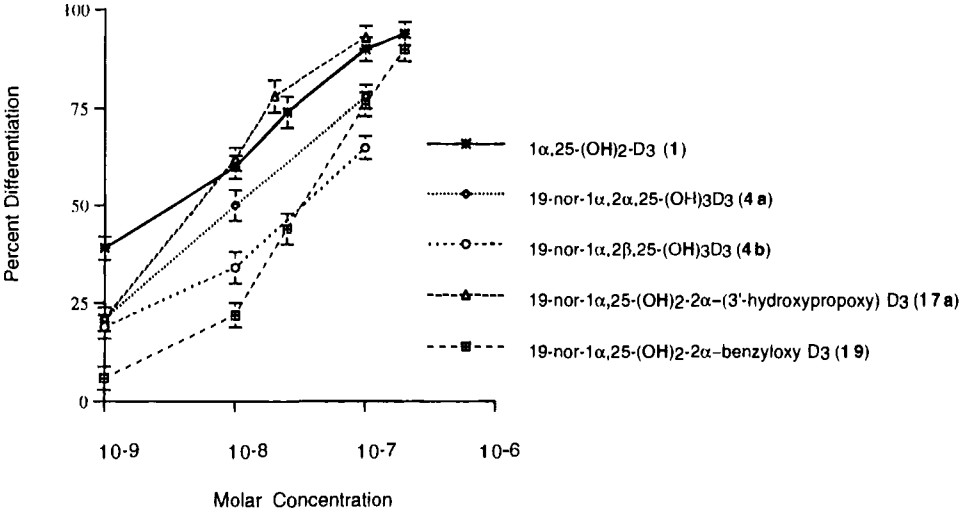


Figure 3. Differentiation activity of 1α,25-$(OH)_2D_3$ and the synthesized analogs. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The results plotted on semilog paper represent mean ± SD of two to four experiments.

hydroxy derivative possessed intestinal calcium transport activity but much less than that of the native hormone or compounds **4b** or **19**. All compounds with an alkoxy (aryloxy) substituent at C-2, i.e., vitamins **17a,b** and **19** showed some bone calcium-mobilizing activity. When provided at a higher dose, the 2α-(3′-hydroxypropoxy) analog **17a** elicited a substantial activation of bone calcium mobilization but less than the native hormone 1.

The above results indicate that removing the 19-carbon from the ED-71 or 2β-(3′-hydroxypropoxy) derivative of 1α,25-$(OH)_2D_3$ markedly reduces its biopotency in both intestine and bone. At the present time, we have no explanation why compound **17a** possesses unique bone calcium-mobilizing activity in this group of 2-substituted derivatives of the 19-nor series. An interesting but unexplained result was the finding that the 2α-benzyloxy derivative was one of the more potent of the 19-nor derivatives tested, whereas the 2α-hydroxy derivative had weak intestinal and no bone calcium-mobilizing activity. The mere substitution of the benzyloxy group apparently provided a marked increase in biopotency in the intestine.

In the next assay, the synthesized compounds were studied with regard to their activity in inducing differentiation of human promyelocyte HL-60 cells to monocytes. Cellular differentiation ability of 1α,25-$(OH)_2D_3$ was measured and compared with the activities of the two 19-nor compounds which showed the weakest calcium mobilization responses, i.e., 2α-hydroxy and 2α-(3′-hydroxypropoxy) derivatives **4a** and **17a**, and, additionally, with the analogs **4b** and **19**. As shown in Figure 3, 1α,25-dihydroxy-2β-(3′-hydroxypropoxy)-19-norvitamin D_3 (**17a**) has the same activity as 1α,25-$(OH)_2D_3$ and, therefore, is as potent as the earlier researched **2**. Our previous studies showed that the native hormone 1 and its 19-nor analog, **2**, have equal differentiation activity.[9a] Presence of a 2α-hydroxy group resulted in diminished activity of the analog **4a** but still approximated that of 1α,25-$(OH)_2D_3$. 2β-Hydroxy and 2α-benzyloxy analogs **4b** and **19** are approximately 10 times less active than 1α,25-$(OH)_2D_3$ in causing differentiation of HL-60 cells.

Conclusions

The results of calcium mobilization assays indicate that the 2β-hydroxy derivative of 19-nor-1α,25-$(OH)_2D_3$ and the 2α-benzyloxy derivatives of this compound (**4b** and **19**) are both worthy of continued investigation as possible agents for restoring bone mass. Studies on the ability of the synthesized analogs to induce HL-60 cell differentiation indicated another interesting pair of vitamins, namely, 2α-hydroxy compound **4a** and its 2-(3′-hydroxypropyl) derivative, **17a**. Because both compounds **4a** and **17a** have selective activity profiles combining high potency in inducing differentiation of malignant cells with relatively weak calcium-mobilizing activity, these analogs show promise in the treatment of cancer.

Experimental Section

Chemistry. Ultraviolet (UV) absorption spectra were recorded with a Hitachi Model 60–100 UV–vis spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz with a Bruker AM-500 FT spectrometer in the solvent noted. Chemical shifts (δ) are reported downfield from internal Me_4Si (δ 0.00). Low- and high-resolution mass spectra were recorded at 70 eV on a Kratos MS-50 TC instrument equipped with a Kratos DS-55 data system. High-resolution data were obtained by peak matching. Samples were introduced into the ion source maintained at 120–250 °C via a direct insertion probe. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model 6 UK Universal injector, a Model 486 tunable absorbance detector, and a differential R 401 refractometer. THF was freshly distilled before use from sodium benzophenone ketyl under argon.

The synthesis of starting ketone **5** was accomplished as described previously.[12] **5**: ^1H NMR ($CDCl_3$) δ 0.06 and 0.08 (6H and 6H, each s, 4 × SiMe), 0.85 and 0.90 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.25 (1H, m), 2.45 (1H, dd, J = 14.1, 5.2 Hz), 2.59 (1H, dd, J = 13.1, 10.7 Hz), 2.77 (1H, dd, J = 14.1, 3.4 Hz), 3.80 (1H, m), 4.28 (2H, m); MS m/z (rel intensity) 317 (62), 231 (16), 185 (76), 143 (100).

(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-[(trimethylsilyl)oxy]cyclohexanone (6). *N*-(Trimethylsilyl)-imidazole (2.52 mL, 26.67 mmol) was added to a solution of the keto alcohol **5** (1.56 g, 4.17 mmol) in methylene chloride (38 mL). The solution was stirred for 20 h. Water (1 mL) was added and the solution stirred for 30 min. Brine and methylene chloride were added, and the mixture was extracted with

methylene chloride. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. The residue was further purified by column chromatography on silica gel with 10% ethyl acetate in hexane to give **6** (1.76 g, 95% yield): ^{1}H NMR ($CDCl_3$) δ 0.05, 0.06, and 0.07 (6H, 3H, and 3H, each s, 4 × SiMe), 0.16 (9H, s, $SiMe_3$), 0.86 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.18 (1H, br d, $J \simeq 14.5$ Hz), 2.37 (1H, dd, J = 13.9, 4.2 Hz), 2.74 (2H, m), 3.81 (1H, narrow m), 4.03 (1H, ~q, J = 4.2 Hz), 4.24 (1H, br m); MS *m*/*z* (rel intensity) 431 (5), 389 (100), 299 (45), 257 (28).

[(a*S,3′*R*,5′*R*)- and [(a*R**,3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[(trimethylsilyl)oxy]cyclohexylidene]acetic Acid Methyl Ester (7a,b).** *n*-BuLi (1.3 M in hexanes, 2.3 mL, 3.0 mmol) was added to a solution of diisopropylamine (0.42 mL, 3.0 mmol) in anhydrous THF (2.0 mL) under argon at −78 °C with stirring, and methyl (trimethylsilyl)acetate (0.49 mL, 3.0 mmol) was then added. After 15 min, the protected keto compound **6** (0.629 g, 1.4 mmol) in anhydrous THF (2.0 + 1.0 mL) was added. The solution was stirred for 2 h at −78 °C. The reaction was quenched with saturated ammonium chloride solution and the mixture extracted with ether. The combined ether fractions were washed with brine and water, dried ($MgSO_4$), filtered, and evaporated. The product was further purified by Sep-Pak filtration (5% ethyl acetate in hexane) to give a mixture of the two stereoisomeric allyl esters **7a,b** (0.69 g, 98%). For analytical purposes, the two isomers were separated by HPLC (1% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column).

7a, peak II (major): ^{1}H NMR ($CDCl_3$) δ 0.04, 0.05, and 0.08 (3H, 3H, and 6H, each s, 4 × SiMe), 0.13 (9H, s, $SiMe_3$), 0.86 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.00 (1H, dd, J = 13.5, 4.7 Hz, 6′α-H), 2.60 (1H, br d, $J \simeq 13.5$ Hz, 6′β-H), 2.74 and 3.28 (1H and 1H, each br m, 2′-H_2), 3.62 (1H, narrow m, 4′α-H), 3.68 (3H, s, OMe), 3.86 (1H, ~q, $J \simeq 4$ Hz, 5′β-H), 3.95 (1H, dt, J = 9.5, 2.5 Hz, 3′α-H), 5.63 (1H, s, 2-H).

7b, peak I (minor): ^{1}H NMR ($CDCl_3$) δ 0.04, 0.05, and 0.06 (3H, 3H, and 6H, each s, 4 × SiMe), 0.13 (9H, s, $SiMe_3$), 0.84 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.12 (1H, dd, J = 12.7, 3.8 Hz, 6′β-H), 2.57 (1H, br t, $J \simeq 12$ Hz, 6′α-H), 2.62 and 3.35 (1H and 1H, each br d, $J \simeq 13$ Hz, 2′-H_2), 3.65 (1H, narrow m, 4′β-H), 3.66 (3H, s, OMe), 3.86 (1H, ~q, $J \simeq 4$ Hz, 3′α-H), 3.99 (1H, dt, J = 9.9, 3.7 Hz, 5′β-H), 5.70 (1H, s, 2-H).

[(a*S,3′*R*,5′*R*)- and [(a*R**,3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-hydroxycyclohexylidene]acetic Acid Methyl Ester (8a,b).** Allylic ester **7a** (50 mg, 0.1 mmol) was dissolved in AcOH/THF/H_2O (5:5:1, 500 μL), and the reaction was allowed to proceed for 40 h at room temperature. Ether was added and the mixture carefully treated with saturated $NaHCO_3$ solution. The ether layer was separated, washed with water, dried (Na_2SO_4), filtered, and evaporated. The product was purified by HPLC (5% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column, 63 mL) to give the pure crystalline alcohol **8a** (35.8 mg, 83%). Hydrolysis of **7b** (7 mg, 0.014 mmol) in THF/AcOH/H_2O (100 μL) as described for **7a** gave, after workup and HPLC purification (5% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column, 66 mL), the pure crystalline **8b** (4.8 mg, 80%).

8a: ^{1}H NMR ($CDCl_3$) δ 0.058, 0.062, 0.099, and 0.116 (each 3H, each s, 4 × MeSi), 0.86 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.07 (1H, dd, J = 13.5, 5.1 Hz, 6′α-H), 2.42 (1H, d, J = 2.1 Hz, O*H*), 2.59 (1H, ddd, J = 13.5, 3.3, 1.4 Hz, 6′β-H), 2.64 (1H, dd, J = 13.3, 9.3 Hz, 2′β-H), 3.39 (1H, dd, J = 13.3, 4.3 Hz, 2′α-H), 3.61 (1H, narrow m; after D_2O dd, J = 4.8, 3.0 Hz, 4′α-H), 3.69 (3H, s, OMe), 4.04 (2H, br m, 3′α- and 5′β-H), 5.67 (1H, br s, 2-H); ^{1}H NMR (C_6D_6) δ −0.020, 0.010, 0.141, and 0.174 (each 3H, each s, 4 × MeSi), 0.91 and 0.94 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.94 (1H, dd, J = 13.3, 5.8 Hz, 6′α-H), 2.31 (1H, d, J = 2.7 Hz, O*H*), 2.57 (1H, dd, J = 13.3, 3.5 Hz, 6′β-H), 3.05 (1H, dd, J = 13.3, 8.6 Hz, 2′β-H), 3.37 (3H, s, OMe), 3.44 (1H, dd, J = 13.3, 3.8 Hz, 2′α-H), 3.63 (1H, narrow m; after D_2O dd, J = 5.2, 3.0 Hz, 4′α-H), 4.08 (1H, ~dt, J = 3.5, 5.5 Hz, 5′β-H), 4.13 (1H, ddd, J = 8.6, 3.8, 3.0 Hz, 3′α-H), 5.83 (1H, br s, 2-H); MS *m*/*z* (rel intensity) no M^+, 415 (M^+ − Me, 4), 373 (M^+ − *t*-Bu, 100).

8b: ^{1}H NMR ($CDCl_3$) δ 0.058, 0.062, 0.082, and 0.087 (each 3H, each s, 4 × MeSi), 0.84 and 0.90 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.21 (1H, dd, J = 13.0, 4.5 Hz, 6′β-H), 2.42 (1H, d, J = 1.7 Hz, O*H*), 2.44 (1H, ddd, J = 13.0, 9.7, 1.3 Hz, 6′α-H), 2.68 (1H, ddd, J = 14.0, 2.9, 1.4 Hz, 2′α-H), 3.35 (1H, dd, J = 14.0, 4.8 Hz, 2′β-H), 3.63 (1H, narrow m; after D_2O dd, J = 4.5, 3.1 Hz, 4′β-H), 3.67 (3H, s, OMe), 4.05 (1H, ddd, J = 9.7, 4.5, 3.1 Hz, 5′β-H), 4.07 (1 H, ~dt, $J \simeq 3$, 4.6 Hz, 3′α-H), 5.73 (1H, br s, 2-H); ^{1}H NMR (C_6D_6) δ 0.009, 0.043, 0.079, and 0.156 (each 3H, each s, 4 × MeSi), 0.90 and 0.94 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.03 (1H, dd, J = 13.0, 4.1 Hz, 6′β-H), 2.30 (1H, d, J = 2.1 Hz, O*H*), 2.35 (1H, ddd, J = 13.0, 8.6, 1.1 Hz, 6′α-H), 3.21 (1H, ddd, J = 13.4, 3.6, 1.2 Hz, 2′α-H), 3.39 (3H, s, OMe), 3.42 (1H, dd, J = 13.4, 5.7 Hz, 2′β-H), 3.66 (1H, narrow m; after D_2O dd, J = 5.7, 3.2 Hz, 4′β-H), 4.07 (1H, ddd, J = 8.6, 4.1, 3.2 Hz, 5′β-H), 4.18 (1H, dt, J = 3.6, 5.7 Hz, 3′α-H), 5.86 (1H, br s, 2-H); MS *m*/*z* (rel intensity) no M^+, 415 (M^+ − Me, 1), 373 (M^+ − *t*-Bu, 43).

[(a*S,3′*R*,5′*R*)- and [(a*R**,3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-acetoxycyclohexylidene]acetic Acid Methyl Ester (9a,b).** Alcohol **8a** (1 mg) was treated with acetic anhydride (50 μL) in anhydrous pyridine (100 μL) for 22 h at room temperature. The reaction was quenched with ice and saturated $NaHCO_3$ solution and extracted with benzene. The organic layer was washed with saturated $NaHCO_3$, water, and saturated $CuSO_4$ solution, dried (Na_2SO_4), filtered, and evaporated. HPLC purification (5% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column, 56 mL) gave analytically pure, crystalline **9a**. Compound **9b** was prepared in the same way as **9a**; HPLC purification (5% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column, 58 mL) gave **9b** as a colorless oil.

9a: ^{1}H NMR ($CDCl_3$) δ 0.02, 0.03, 0.06, and 0.07 (each 3H, each s, 4 × SiMe), 0.84 and 0.87 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.09 (3H, s, OAc), 2.25 (1H, dd, J = 13.4, 9.1 Hz, 6′α-H), 2.48 (1H, br d, J = 14.4 Hz, 2′α-H), 2.50 (1H, dd, J = 13.4, 4.8 Hz, 6′β-H), 3.58 (1H, dd, J = 14.4, 5.3 Hz, 2′β-H), 3.67 (3H, s, OMe), 4.07 (1H, ~dt, J = 5, 8.3 Hz, 5′β-H), 4.18 (1 H, m, 3′α-H), 4.78 (1H, dd, J = 7.4, 2.4 Hz, 4′α-H), 5.77 (1H, s, 2-H); MS *m*/*z* (rel intensity) no M^+, 457 (M^+ − Me, 22), 415 (M^+ − *t*-Bu, 67), 281 (100).

9b: ^{1}H NMR ($CDCl_3$) δ 0.02, 0.03, 0.07, and 0.09 (each 3H, each s, 4 × SiMe), 0.86 and 0.87 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.09 (3H, s, OAc), 2.36 (2H, m, 6′-H_2), 2.58 (1H, ~dd, $J \simeq 14$, 8 Hz, 2′β-H), 3.49 (1H, ~dd, $J \simeq 14$, 4 Hz, 2′α-H), 3.69 (3H, s, OMe), 4.04 (1H, ~dt, $J \simeq 4$, 7.7 Hz, 3′α-H), 4.18 (1 H, m, 5′β-H), 4.82 (1H, dd, J = 7.5, 2.5 Hz, 4′β-H), 5.69 (1H, s, 2-H); MS *m*/*z* (rel intensity) no M^+, 457 (M^+ − Me, 4), 415 (M^+ − *t*-Bu, 67), 281 (100).

2-[(a*S,3′*R*,5′*R*)- and 2-[(a*R**,3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[(trimethylsilyl)oxy]cyclohexylidene]ethanol (10a,b).** A solution of a mixture of the esters **7a,b** (410 mg, 0.82 mmol) in anhydrous toluene (8 mL) was treated at −78 °C under argon with diisobutylaluminum hydride (1.5 M in toluene, 7 mL, 10.5 mmol). After the addition, stirring was continued for 1 h at −78 °C. The reaction was then quenched by the addition of 2 N potassium sodium tartrate, the organic phase was separated, and the aqueous phase was extraced with ethyl acetate. The combined organic phases were washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by fast filtration through a silica gel column, using 20% ethyl acetate in hexane as eluent, to give alcohols **10a,b** (352 mg, 91%) which were separated by HPLC (10% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column). First the minor alcohol was eluted (**10b**; 42 mg) followed by the major product (**10a**; 188 mg, 61% recovery).

10a, peak II (major): ^{1}H NMR ($CDCl_3$) δ 0.04, 0.05, 0.06, 0.07 (3H, each s, 4 × SiMe), 0.13 (9H, s, $SiMe_3$), 0.87 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.93 (1H, dd, J = 13.5, 5.5 Hz, 6′α-H), 2.24 (1H, br d, J = 12.6 Hz, 2′α-H), 2.38 (1H, dd, J = 12.6, 9.2 Hz, 2′β-H), 2.50 (1H, dd, J = 13.5, 3.5 Hz, 6′β-H), 3.57 (1H, narrow m, 4′α-H), 3.80 (1H, dt, J = 3.5, 5.5 Hz, 5′β-H), 3.89 (1H, ddd, J = 9.2, 3.0, 2.6 Hz, 3′α-H), 4.13 (2H, m, 1-H_2), 5.45 (1H, t, J = 6.9 Hz, 2-H).

10b, peak I (minor): ^{1}H NMR ($CDCl_3$) δ 0.06, 0.07 (6H and 6H, each s, 2 × $SiMe_2$), 0.13 (9H, s, $SiMe_3$), 0.87 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.06 (1H, dd, J = 12.3, 3.9 Hz,

6′β-H), 2.23 (1H, dd, J = 13.6, 4.0 Hz, one of 2′-H_2), 2.38 (1H, br d, J = 13.6 Hz, one of 2′-H_2), 2.45 (1H, br t, $J \simeq 11$ Hz, 6′α-H), 3.62 (1H, dd, 4.0, 2.0 Hz, 4′β-H), 3.82 (1H, ~q, $J \simeq 4$ Hz, 3′α-H), 3.89 (1H, ddd, $J \simeq 10$, 4, 2 Hz, 5′β-H), 4.03 and 4.12 (1H and 1H, each br m, 1-H_2), 5.57 (1H, t, J = 7.1 Hz, 2-H).

[2-[(a*S,3′*R*,5′*R*)- and [2-[(a*R**,3′*R*,5′*R*)-3′5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[(trimethylsilyl)oxy]cyclohexylidene]ethyl]diphenylphosphine Oxide (11a,b).** To the allyl alcohol **10a** (265 mg, 0.56 mmol) dissolved in anhydrous THF (5.0 mL) was added n-BuLi (1.6 M in hexanes, 0.35 mL, 0.56 mmol) under argon. Recrystallized tosyl chloride (106 mg, 0.56 mmol) was dissolved in anhydrous THF (1.0 mL) and added to the allyl alcohol−BuLi solution under argon at 0 °C. The solution was stirred at 0 °C for 5 min and set aside at 0 °C. In another dry flask, with air replaced by argon, n-BuLi (1.6 M in hexanes, 350 μL, 0.56 mmol) was added to diphenylphosphine (100 μL, 0.56 mmol) in anhydrous THF (200 μL) at 0 °C with stirring. To the orange solution was syphoned under argon pressure at 0 °C the tetrahydrofuran solution of the allylic tosylate. The resulting mixture was stirred an additional 30 min at 0 °C and the reaction quenched by the addition of water. Solvents were evaporated under reduced pressure, and the residue was dissolved in dichloromethane (5 mL) and stirred with 10% hydrogen peroxide (20 mL) at 0 °C for 1 h. The dichloromethane layer was separated, washed with cold aqueous sodium sulfite, water, and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in 20% 2-propanol in hexane, passed through a silica Sep-Pak, and purified by HPLC (20% 2-propanol in hexane, Zorbax Sil 9.4 mm × 25 cm column) to give the crystalline phosphine oxide **11a** (220 mg, 60%).

11a: UV (EtOH) λ_{max} 258, 265, 272 nm; ^{1}H NMR ($CDCl_3$) δ −0.02, 0.00, 0.01, 0.03 (3H, each s, 4 × SiMe), 0.09 (9H, s, $SiMe_3$), 0.83 and 0.87 (9H and 9H, each s, 2 × Si-t-Bu), 1.86 (1H, br d, $J \simeq 13.5$ Hz, one of 6′-H_2), 1.99 and 2.08 (1H and 1H, each m, 2′-H_2), 2.42 (1H, br d, $J \simeq 13.5$ Hz, one of 6′-H_2), 3.10 (2H, m, 1-H_2), 3.51 (1H, narrow m, 4′α-H), 3.72 (1H, dt, J = 3.7, 5.2 Hz, 5′β-H), 3.81 (1H, ddd, J = 8.8, 4.2, 2.4 Hz, 3′α-H), 5.24 (1H, q, J = 6.9 Hz, 2-H), 7.46, 7.52, and 7.71 (4H, 2H, and 4H, each m, Ar-H); MS m/z (rel intensity) 658 (M^+, 1), 643 (3), 601 (100), 526 (12), 469 (43); exact mass calcd for $C_{35}H_{59}O_4Si_3P$ 658.3459, found 658.3453. The isomeric phosphine oxide **11b** was prepared in the same way as **11a** from the corresponding **10b**.

11b: ^{1}H NMR ($CDCl_3$) δ 0.02 (12H, s, 4 × SiMe), 0.09 (9H, s, $SiMe_3$), 0.85 (18H, s, 2 × Si-t-Bu), 1.88 (1H, br d, $J \simeq 14$ Hz, one of 2′-H_2), 2.01 (1H, br d, $J \simeq 12$ Hz, 6′β-H), 2.06 (1H, br d, $J \simeq 14$ Hz, one of 2′-H_2), 2.34 (1H, br m, 6′α-H), 3.02 and 3.14 (1H and 1H, each br m, 1-H_2), 3.51 (1H, narrow m, 4′β-H), 3.71 (1H, ~q, J = 4.4 Hz, 3′α-H), 3.84 (1H, m, 5′β-H), 5.27 (1H, m, 2-H), 7.46, 7.52, and 7.71 (4H, 2H, and 4H, each m, Ar-H).

25-(Triethylsilyl)oxy Windaus−Grundmann Ketone 12. The C,D-ring fragment for the 19-norvitamin D derivative was prepared by ozonolysis of commercial vitamin D_3 followed by RuO_4 oxidation[17] to give the 25−hydroxy Grundmann ketone.[16] The ketone (30 mg, 0.1 mmol) and imidazole (28 mg, 0.41 mmol) in anhydrous DMF (500 μL) were treated with triethylsilyl chloride (40 μL, 0.24 mmol), and the mixture was stirred at room temperature for 2 h. Ethyl acetate was added and water, and the organic layer was separated. The ethyl acetate layer was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was passed through a silica Sep-Pak in 10% ethyl acetate in hexane and, after evaporation, purified by HPLC (10% ethyl acetate in hexane, Zorbax Sil 9.4 mm × 25 cm column) to give the pure protected hydroxy ketone **12** (31 mg, 79%): ^{1}H NMR ($CDCl_3$) δ 0.56 (6H, q, J = 8.0 Hz, 3 × Si-CH_2), 0.64 (3H, s, 18-H_3), 0.94 (9H, t, J = 8.0 Hz, 3 × $SiCH_2CH_3$), 0.95 (3H, d, J = 6.5 Hz, 21-H_3), 1.19 (6H, br s, 26- and 27-H_3), 2.45 (1H, dd, J = 11.7, 7.4 Hz, 14α-H).

1α,2α,25-Trihydroxy-19-norvitamin D_3 (4a). Phosphine oxide **11a** (16.9 mg, 25.7 μmol) was dissolved in anhydrous THF (200 μL) and cooled to 0 °C and n-BuLi (1.3 M in hexanes, 20 μL, 26 μmol) added under argon with stirring. The solution turned deep orange. The mixture was cooled to −78 °C and protected hydroxy ketone **12** (7.5 mg, 21 μmol) added in anhydrous THF (200 + 100 μL). The mixture was stirred under argon at −78 °C for 1 h (at that time the solution became colorless) and at room temperature for 18 h. Ethyl acetate was added, and the organic phase was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in 10% ethyl acetate in hexane, passed through a silica Sep-Pak, and washed with 40 mL of the same to give the 19-norvitamin derivative **13a**. The Sep-Pak was then washed with 20% 2-propanol in hexane to recover some unchanged diphenylphosphine oxide (5 mg). HPLC of the vitamin D product (10% ethyl acetate in hexane, Zorbax Sil 9.4 mm × 25 cm column) gave the pure protected 19-nor compound **13a** (8.2 mg, 54%): ^{1}H NMR ($CDCl_3$) δ 0.04, 0.05, and 0.06 (3H, 3H, and 6 H, each s, 4 × SiMe), 0.12 (9H, s, $SiMe_3$), 0.55 (3H, s, 18-H_3), 0.56 (6H, q, J = 7.4 Hz, 3 × Si-CH_2), 0.87 and 0.88 (9H and 9H, each s, 2 × Si-t-Bu), 0.92 (3H, d, J = 6.1 Hz, 21-H_3), 0.95 (9H, t, J = 7.4 Hz, 3 × $SiCH_2CH_3$), 1.19 (6H, br s, 26- and 27-H_3), 2.79 (1H, br d, J = 12.6 Hz, 9β-H), 3.53 (1H, m, 2β-H), 3.80 (1 H, m, 3α-H), 3.88 (1 H, m, 1β-H), 5.81 and 6.10 (1H and 1H, each d, J = 11.4 Hz, 6- and 7-H); MS m/z (rel intensity) 834 (M^+, 12), 805 (3), 702 (100), 645 (18), 599 (45); exact mass calcd for $C_{47}H_{94}O_4Si_4$ 834.6229, found 834.6241.

All of **13a** was dissolved in anhydrous THF (1.0 mL) and treated with tetrabutylammonium fluoride (1.0 M in THF, 150 μL). The mixture was stirred under argon at room temperature for 16 h and extracted with ethyl acetate. The organic phase was washed with 10% $NaHCO_3$ solution and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in 1:1 2-propanol/hexane, filtered through a silica Sep-Pak, and purified by HPLC (30% 2-propanol in hexane, Zorbax-Sil 9.4 mm × 25 cm column) to give pure 1α, 2α,25-trihydroxy-19-norvitamin D_3 (**4a**; 1.48 mg): UV (in EtOH) λ_{max} 243, 251.5, 261 nm; ^{1}H NMR ($CDCl_3$) δ 0.55 (3H, s, 18-H_3), 0.94 (3H, d, J = 6.5 Hz, 21-H_3), 1.22 (6H, s, 26- and 27-H_3), 2.62 (1H, dd, J = 13.0, 4.2 Hz), 2.79 (1H, br d, J = 12.9 Hz), 2.90 (1H, dd, J = 14.3, 4.2 Hz), 3.25 (1H, br m), 3.53 (1H, dd, J = 8.4, 2.6 Hz), 3.79 (1 H, br m), 4.10 (1 H, m), 5.81 and 6.38 (1 H and 1 H, each d, J = 11.0 Hz, 6- and 7-H); MS m/z (rel intensity) 420 (M^+, 100), 402 (56), 387 (18), 291 (58), 245 (53), 95 (78), 59 (90); exact mass calcd for $C_{26}H_{44}O_4$ 420.3240, found 420.3238.

1α,2β,25-Trihydroxy-19-norvitamin D_3 (4b). Vitamin **4b** was prepared in the same way as **4a**, except from the corresponding **11b**. **4b**: UV (in EtOH) λ_{max} 243, 251.5, 261 nm; ^{1}H NMR ($CDCl_3$) δ 0.55 (3H, s, 18-H_3), 0.94 (3H, d, J = 6.8 Hz, 21-H_3), 1.22 (6H, s, 26- and 27-H_3), 2.79 (1H, br d, J = 13.0 Hz), 3.08 (1H, dd, J = 13.2, 4.6 Hz), 3.36 (2H, br m), 3.49 (1H, m), 3.68 (1 H, br m), 4.08 (1 H, m), 5.84 and 6.29 (1H and 1H, each d, J = 11.3 Hz, 6- and 7-H); MS m/z (rel intensity) 420 (M^+, 99), 402 (59), 387 (50), 291 (44), 245 (49), 95 (94), 59 (100); exact mass calcd for $C_{26}H_{44}O_4$ 420.3240, found 420.3239.

1α-[(*tert*-Butyldimethylsilyl)oxy]-2α-hydroxy-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-Butyldimethylsilyl Ether (14a). Protected compound **13a** (10 mg) was stirred for 4.5 h at room temperature with a mixture of THF/AcOH/H_2O (8:8:1, 3 mL). Ethyl acetate was added, and the organic layer was washed with ice cold water, ice cold 10% $NaHCO_3$ solution until neutral, water, and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by HPLC (5% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column) to give (in order of peak elutions) unchanged starting material (1.2 mg, 12%), the expected 2-hydroxy compound **14a** (3.5 mg, 38%), and the 2α,25-dihydroxy compound (0.75 mg, 10%). **14a**: ^{1}H NMR ($CDCl_3$) δ 0.06, 0.07, 0.08, and 0.10 (each 3H, each s, 4 × SiMe), 0.55 (3H, s, 18-H_3), 0.56 (6H, q, J = 7.8 Hz, 3 × Si-CH_2), 0.87 and 0.88 (9H and 9H , each s, 2 × Si-t-Bu), 0.93 (3H, d, J = 6.4 Hz, 21-H_3), 0.95 (9H, t, J = 7.8 Hz, 3 × $SiCH_2CH_3$), 1.19 (6H, br s, 26- and 27-H_3), 2.27 (1H, d, J = 3.3 Hz, OH), 2.79 (1H, dd, J = 12.3, 4.2 Hz, 9β-H), 3.51 (1H, dt, $J \simeq 6$, 3 Hz; after D_2O dd, J = 5.8, 2.7 Hz, 2β-H), 3.91 (1H, dt, J = 4.3, 5.8 Hz, 3α-H), 4.00 (1H, ~dt, $J \simeq 7.5$, 3 Hz, 1β-H), 5.80 and 6.16 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H).

1α-[(*tert*-Butyldimethylsilyl)oxy]-2β-hydroxy-25-[(triethylsilyl)oxy]-19-norvitamin D$_3$ *tert*-Butyldimethylsilyl Ether (14b). Compound **14b** was prepared in the same way as **14a** except from **13b**. Vitamin **14b** was subjected to HPLC (5% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column); after purification, the compound gave a single peak on HPLC and was judged 99% pure. **14b**: ^{1}H NMR ($CDCl_3$) δ 0.06, 0.07, 0.08, and 0.10 (each 3H, each s, 4 × SiMe), 0.54 (3H, s, 18-H$_3$), 0.56 (6H, q, J = 8.0 Hz, 3 × Si-CH_2), 0.86 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.93 (3H, d, J = 6 Hz, 21-H$_3$), 0.94 (9H, t, J = 8.0 Hz, 3 × $SiCH_2CH_3$), 1.19 (6H, br s, 26- and 27-H$_3$), 2.81 (1H, br d, $J \simeq$ 13 Hz, 9β-H), 3.59 (1H, narrow m; after D_2O dd, J = 3.6, 3.3 Hz, 2α-H), 4.00 (2H, m, 1β- and 3α-H), 5.80 and 6.19 (1H and 1H, each, d, J = 11.0 Hz, 6- and 7-H).

3-Bromo-1-[(*tert*-butyldimethylsilyl)oxy]propane (15). 3-Bromo-1-propanol (1.4 g, 1 mmol) was dissolved in anhydrous DMF (5 mL), and imidazole (3.0 g) followed by *tert*-butyldimethylsilyl chloride (3.3 g) were added at 0 °C with stirring. The mixture was stirred at room temperature for 2 h, ether was then added, and the ether phase was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in hexane and passed through a small silica gel column to give pure **15** (2.03 g, 80%): ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s, $SiMe_2$), 0.90 (9H, s, Si-*t*-Bu), 2.03 (2H, ~quint, $J \simeq$ 6 Hz, CH_2), 3.51 (2H, t, J = 6.2 Hz, CH_2O), 3.73 (2H, t, J = 5.8 Hz, CH_2Br).

2α-(3′-Hydroxypropoxy)-1α,25-dihydroxy-19-norvitamin D$_3$ (17a). Hydroxy compound **14a** (1.6 mg, 2 μmol) was dissolved in anhydrous DMF (200 μL), and sodium hydride (as 60% oil dispersion, 3 mg) followed by 18-crown-6 (3 mg) and the bromo compound **15** (5 μL) were added. The mixture was stirred under an argon atmosphere at room temperature for 48 h, and then it was extracted with ethyl acetate, washed with water, dried ($MgSO_4$), filtered, and evaporated. The residue was passed through a silica Sep-Pak in ethyl acetate and purified by HPLC (5% ethyl acetate in hexane, Zorbax Sil 10 mm × 25 cm column) to give **16a**: ^{1}H NMR ($CDCl_3$) δ 0.04, 0.05, 0.06, and 0.07 (6H, 6H, 3H, and 3H, each s, 6 × SiMe), 0.55 (3H, s, 18-H$_3$), 0.56 (6H, q, J = 7.5 Hz, 3 × $SiCH_2$), 0.87, 0.88, and 0.89 (9H, 9H, and 9H, each s, 3 × Si-*t*-Bu), 0.93 (3H, d, J = 6 Hz, 21-H$_3$), 0.95 (9H, t, J = 7.5 Hz, 3 × $SiCH_2CH_3$), 1.19 (6H, br s, 26- and 27-H$_3$), 2.79 (1H, br d, $J \simeq$ 14 Hz), 3.12 (1H, m), 3.4–4.1 (at least 7H, complex m), 5.80 and 6.12 (1H and 1H, each d, J = 11 Hz, 6- and 7-H).

Compound **16a** was dissolved in anhydrous THF (1 mL), treated with tetrabutylammonium fluoride (1.0 M in THF, 0.5 mL), and stirred under an argon atmosphere for 20 h. The mixture was extracted with ethyl acetate, washed with water, 10% $NaHCO_3$ solution, and brine, dried ($MgSO_4$), filtered, and evaporated. The residue was passed through a silica Sep-Pak in 1:1 2-propanol/hexane and purified by HPLC (40% 2-propanol in hexane, Zorbax Sil 10 mm × 25 cm column) to give the expected product, **17a** (202 μg, overall yield from **14a** 21%), and some **4a** (20 μg). **17a**: UV (in EtOH) λ_{max} 243, 251.5, 261 nm; ^{1}H NMR ($CDCl_3$) δ 0.55 (3H, s, 18-H$_3$), 0.93 (3H, d, J = 6.8 Hz, 21-H$_3$), 1.22 (6H, s, 26- and 27-H$_3$), 3.3–4.2 (at least 7H, complex m), 5.83 and 6.34 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H); MS m/z (rel intensity) 478 (M^+, 5), 460 (6), 442 (2), 402 (4), 384 (3), 245 (15), 184 (20), 142 (100), 95 (50), 59 (38); exact mass calcd for $C_{29}H_{50}O_5$ 478.3658, found 478.3659.

2β-(3′-Hydroxypropoxy)-1α,25-dihydroxy-19-norvitamin D$_3$ (17b). Vitamin **17b** was prepared in the same way as **17a** except from the corresponding **14b**. After purification by HPLC (40% 2-propanol in hexane, Zorbax Sil 10 mm × 25 cm column), the compound gave a single peak and was judged to be 99% pure. **17b**: UV (in EtOH) λ_{max} 243, 251.5, 261 nm; ^{1}H NMR ($CDCl_3$) δ 0.54 (3H, s, 18-H$_3$), 0.94 (3H, d, J = 6.5 Hz, 21-H$_3$), 1.22 (6H, s, 26- and 27-H$_3$), 2.79 (1H, br d, $J \simeq$ 12 Hz, 9β-H), 3.0–4.3 (at least 7H, complex m), 5.84 and 6.29 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H); MS m/z (rel intensity) 478 (M^+, 6), 460 (12), 442 (7), 420 (15), 402 (11), 366 (9), 348 (8), 245 (34), 181 (9), 95 (80), 69 (100), 59 (68); exact mass calcd for $C_{29}H_{50}O_5$ 478.3658, found 478.3659.

2α-(Benzyloxy)-1α,25-dihydroxy-19-norvitamin D$_3$ (19). Alcohol **14a** (1.6 mg, 2 μmol) was dissolved in anhydrous DMF (200 μL), and sodium hydride (as 60% oil dispersion, 3 mg) followed by 18-crown-6 (3 mg) and a benzyl bromide benzene solution (prepared from 120 μL benzyl bromide in 1 mL benzene, 6 μL) were added. The mixture was stirred under an argon atmosphere for 48 h. The mixture was then extracted with ethyl acetate, washed with water, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in ethyl acetate, passed through a silica Sep-Pak, and evaporated to give crude **18** (860 μg) which was without purification deprotected. All of **18** was dissolved in methanol (200 μL), and methanol-washed AG 50W-X4 cation exchange resin (10 mg) was added. The mixture was stirred under an argon atmosphere at room temperature for 18 h, filtered through a silica Sep-Pak, and washed with 2-propanol. The solvent was evaporated under reduced pressure and the residue purified by HPLC (30% 2-propanol in hexane, Zorbax Sil 10 mm × 25 cm column) to give the 2α-benzyloxy compound **19** (170 μg): UV (in EtOH) λ_{max} 243, 251.5, 261 nm; ^{1}H NMR ($CDCl_3$) δ 0.55 (3H, s, 18-H$_3$), 0.93 (3H, d, J = 6.7 Hz, 21-H$_3$), 1.22 (6H, s, 26- and 27-H$_3$), 2.79 (2H, m), 3.45 (1H, dd, J = 7.3, 3.0 Hz, 2β-H), 3.97 (1H, m, 3α-H), 4.11 (1 H, m, 1β-H), 4.65 and 4.72 (1H and 1H, each d, J = 11.8 Hz, O-CH_2′), 5.83 and 6.33 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H), 7.2–7.4 (5H, br m, Ar-H); MS m/z (rel intensity) 510 (M^+, 11), 492 (8), 474 (2), 401 (8), 91 (100); exact mass calcd for $C_{33}H_{50}O_4$ 510.3709, found 510.3703.

Biological Studies. Measurement of Intestinal Calcium Transport and Bone Calcium Mobilization. Weanling male rats from the low-vitamin D colony were purchased from the Sprague–Dawley Co. (Indianapolis, IN) and fed the vitamin D-deficient diet,[18] containing 0.47% calcium and 0.3% phosphorus, for 1 week. They were then switched to the reduced calcium diet (0.02% Ca) for an additional 2 weeks. These animals have no detectable levels of 25-OHD$_3$ or 1α,25-(OH)$_2$D$_3$ in their plasma as measured by methods described previously.[19] For this first experiment, the indicated rats received a single intravenous dose of the indicated compound in 0.05 mL of ethanol (data not shown). In the other experiment, the rats were given the indicated doses of compounds in 0.1 mL of 95:5 1,2-propanediol/ethanol by intraperitoneal injection each day for 7 days. In the first experiment, the rats were euthanized at various times after the dose (data not shown). In the second experiment, they were sacrificed 24 h after the last dose. The rats were sacrificed under ether anesthesia by decapitation. Serum and intestines were collected and used immediately to determine intestinal calcium transport and serum calcium concentration. Calcium was determined using the Calcette automatic calcium titrator (Precision Systems, Inc., Natick, MA) and intestinal calcium transport by the everted intestinal sac method using the proximal 10 cm of intestine as described earlier.[18] Statistical analysis was by the Student's t-test.[20] Intestinal calcium transport is expressed as the serosal:mucosal ratio of calcium in the sac to the calcium in the final incubation medium or S/M. Bone calcium mobilization represents the rise in serum calcium of the rats maintained on a very low calcium diet. In that measurement, the rise in serum calcium must arise from bone and hence is a determination of bone calcium mobilization.

Measurement of Cellular Differentiation. Human leukemia HL-60 cells, originally obtained from ATTC, were plated at 2 × 10^5 cells/plate and incubated in Eagle's modified medium as described previously.[7] The compounds tested were added in the indicated concentrations in 0.05 mL of ethanol so that the ethanol concentration never exceeded 1%. The incubation was carried out for 4 days, and at the end of the fourth day, superoxide production was measured by nitro blue tetrazolium (NBT) reduction. The number of cells containing intracellular black-blue formazan deposits was determined by light microscopy using a hemacytometer. At least 200 cells were counted in duplicate per determination. Percentage differentiation represents the percentage of cells providing NBT reduction appearance. The results were plotted on semilog paper, and relative differentiation activities of the analogs were determined by comparison of the compound

concentrations capable of inducing 50% maturation according to the assay. This method is described in detail elsewhere.[7]

Acknowledgment. We are indebted to Dr. Hans Reich for many discussions and helpful suggestions. We acknowledge the assistance of Rowland Randall in recording the mass spectra. The work was supported in part by program project Grant No. DK-14881 from the National Institutes of Health, the National Foundation for Cancer Research, and the Wisconsin Alumni Research Foundation.

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