Synthesis and Biological Activity of 1α,25-Dihydroxy-18-norvitamin D_3 and 1α,25-Dihydroxy-18,19-dinorvitamin D_3

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1α,25-Dihydroxy-18-norvitamin D_3 and 1α,25-dihydroxy-18,19-dinorvitamin D_3 were prepared *via* Wittig−Horner coupling of 25-hydroxy-18-nor Grundmann type ketone with the corresponding A-ring phosphine oxides. Configuration at C-13 in the 18-nor Grundmann type alcohol (C,D-ring synthon), obtained by oxidative degradation of vitamin D_3, was determined by ^{1}H NMR spectroscopy and molecular mechanics calculations. Additional proof of the assigned *trans*-C/D-junction of the key intermediate 18-nor Grundmann type ketone follows from its chiroptical properties (circular dichroism data) and further chemical transformations. 1α,25-Dihydroxy-18-norvitamin D_3 was found more potent than 1α,25-dihydroxyvitamin D_3 in binding to the porcine intestinal vitamin D receptor (5−10×), in differentiation of HL-60 cells (5−10×), and in inhibition of HL-60 proliferation. 1α,25-Dihydroxy-18,19-dinorvitamin D_3 appeared equally active as 1α,25-dihydroxyvitamin D_3 in these activities. *In vivo*, 1α,25-dihydroxy-18-norvitamin D_3 was only slightly less active than 1α,25-dihydroxyvitamin D_3 in intestinal calcium transport and bone calcium mobilization, while 1α,25-dihydroxy-18,19-dinorvitamin D_3 showed activities 10 times lower. These studies imply that deletion of C-18 does not impair activity of analogs of 1α,25-dihydroxyvitamin D_3.

Introduction

It has been well established that metabolism of vitamin D_3 (**1**; Figure 1) involves two subsequent hydroxylations (at C-1 and C-25 positions)[1,2] to the hormonally active form, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **2a**). This natural hormone is known to be a highly potent regulator of calcium homeostasis in animals and humans,[3] and more recently its activity in cellular differentiation has been established.[4] In search of separation of calcemic activity from differentiation activity, many structural analogs have been prepared and tested. A selective activity toward differentiation was found in the 19-nor analog **3a** synthesized in our laboratory.[5] Recently, 1α,25-$(OH)_2D_3$ analogs modified at the C-18 position have been described, i.e., 18-acetoxy,[6] 18-hydroxy,[7] and 18-methyl derivatives as well as 13-vinyl compounds.[8] The last two analogs are potent stimulators of cell differentiation with rather low calciotropic activity *in vitro*.

In our continuing investigation of structure−activity relationships of the vitamin D molecule, we prepared the 18-nor analog **2b** and the 18,19-dinor analog **3b** where the C-13 angular methyl group of **2a** and **3a** has been replaced with hydrogen. Vitamin D analogs lacking the 18-carbon seemed interesting targets because such molecular modification does not change substantially the basic polycyclic carbon skeleton of the vitamin nor introduces an additional substituent which could interfere with vitamin D receptors. However, it can be expected that the absence of an angular 13β-methyl group, and much smaller interaction of the methyl group at C-20 with the 13β-hydrogen, should have some effect on the conformational profile of the side chain. It was, therefore, of interest to biologically evaluate the synthesized 18-norvitamins **2b** and **3b**, by comparing their activities with those of the corresponding 13β-methyl counterparts.

Chemistry

The strategy of our synthesis was based on Lythgoe type Wittig−Horner coupling.[9] Since the corresponding phosphine oxide A-ring synthons **5a,b** are known,[5a,10] we focused our attention on the preparation of an appropriate C,D-fragment (**4b**).

The known 8β-alcohol **7a**[11] (steroidal numbering is used throughout this paper) was efficiently obtained by stereoselective lithium aluminum hydride reduction of Grundmann's ketone **6** prepared from ozonization[12] of **1** (Scheme 1). The axial orientation of the hydroxy group at C-8 in **7a**, sterically fixed in the *trans*-hydrindane system in close proximity to the angular methyl group at C-13, is crucial for a successful intramolecular free radical reaction leading to 18-functionalized compounds. It has been shown that efficiency of abstraction of a hydrogen atom from the angular methyl group in steroids depends strongly on the distance of the oxy radical from the methyl hydrogens. The rate of hydrogen abstraction reaches a maximum at internuclear distances of 2.5−2.7 Å between the oxygen and methyl carbon and decreases rapidly at distances over 3 Å.[13] Our molecular modeling studies[14] show that in the case of 8β-alcohol **7a** the C(18)−O distance is smaller than 3 Å (ca. 2.96 Å) and, therefore, is suitable for the C-18 functionalization process. Intramolecular functionalization at the C-18 position by lead tetraacetate oxidation has been described by Mourino's and Okamura's groups.[6−8] In our approach we used nitrite photolysis (Barton reaction).[15] Thus, Grundmann's alcohol **7a** was easily converted into nitrite **7b** by a *trans*-esterification with *tert*-butyl nitrite.[16] This simple nitrosyl exchange method does not require the

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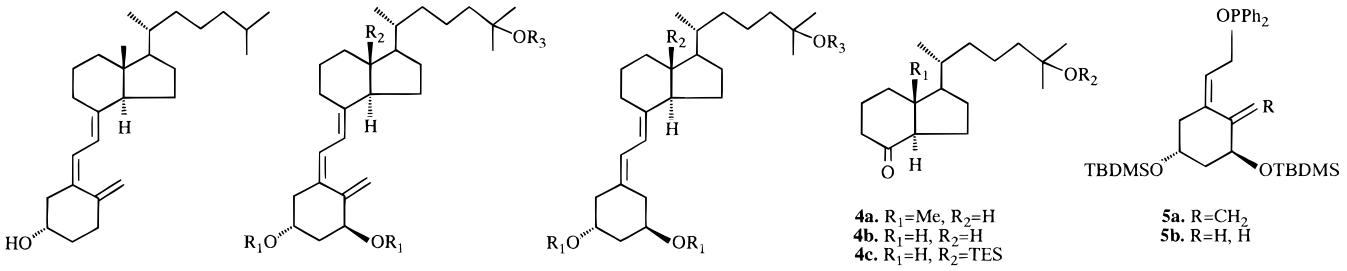


1

2a. R_1=H, R_2=Me, R_3=H
2b. R_1=H, R_2=H, R_3=H
2c. R_1=TBDMS, R_2=H, R_3=TES

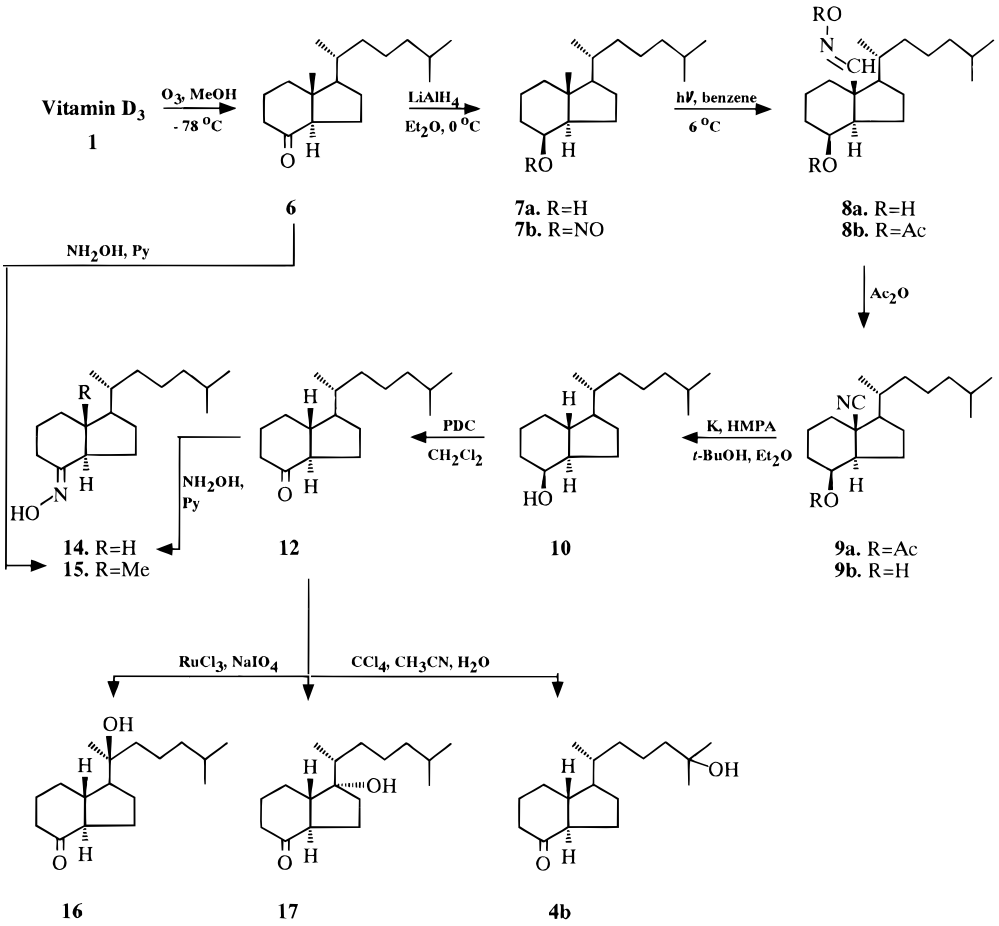
3a. R_1=H, R_2=Me, R_3=H
3b. R_1=H, R_2=H, R_3=H
3c. R_1=TBDMS, R_2=H, R_3=TES

4a. R_1=Me, R_2=H
4b. R_1=H, R_2=H
4c. R_1=H, R_2=TES

5a. R=CH_2
5b. R=H, H

Figure 1. Chemical structure of vitamin D_3, its hydroxylated analogs, and their precursors.

Scheme 1



use of expensive gaseous nitrosyl chloride which is usually recommended for the preparation of steroidal nitrites.[13] Photolysis of 8β-nitrite **7b** in benzene solution under an oxygen-free atmosphere at 10 °C gave the expected 18-oxime **8a** (46% yield from **7a**), produced by intramolecular exchange of the NO of the nitrite moiety with the hydrogen atom attached to C-18, and subsequent rearrangement of the intermediate 18-nitroso compound. Product **8a** was a single isomer; deshielding of the 18-H signal (δ 7.36, s) in its NMR spectrum, typical for *syn*-aldoximes,[17] indicates *E*-configuration. Molecular mechanics calculations confirm that the 18*E*-oxime isomer **8a** is energetically favored (steric energy of the 18*Z*-form is higher by 3.25 kcal/mol). According to the literature, similar *syn*-oxime acetates easily undergo thermal *cis*-elimination of acetic acid.[18] Indeed, boiling in acetic anhydride for 1.5 h was sufficient for a smooth conversion of **8a** into the 18-nitrile **9a** (86% yield); a similar transformation, *via* the intermediate diacetate **8b**, was also accomplished from **8a** with acetic anhydride in pyridine at 60 °C.

One of the best methods for reductive removal of nitrile groups of tertiary alkyl cyanides to give the corresponding hydrocarbons is dissolving metal reduction.[19-21] Compound **9a** was next hydrolyzed under standard conditions to the 8β-hydroxy nitrile **9b** (methanolic KOH, 96% yield). This step was necessary because a decyanation process could otherwise cause a reduction of the 8-acetoxy group to the corresponding alkane (8-unsubstituted derivative).[22] We showed that, contrary to the literature,[19a] the hydroxy function does not need to be alternatively reprotected during the reduction step. Accordingly, a high yield (76%) of the desired 18-nor product **10** was obtained by treatment of hydroxy nitrile **9b** with potassium in hexamethylphosphoric triamide containing *tert*-butyl alcohol as

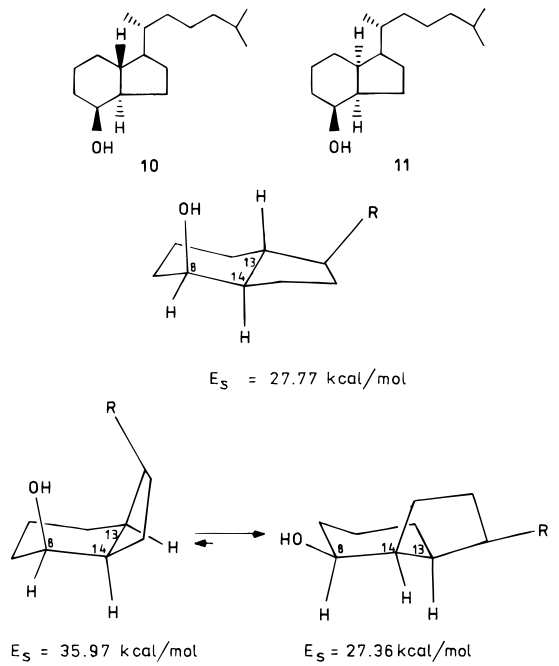


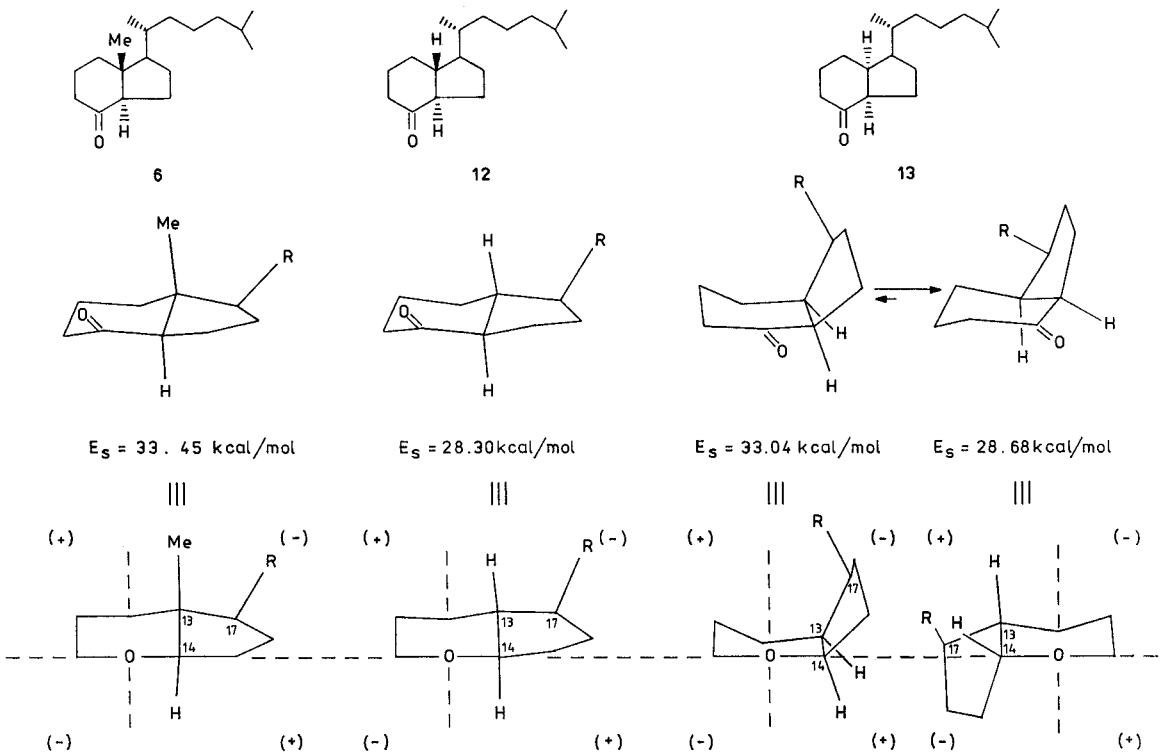
Figure 2. Energy-minimized conformers of 8β-alcohols with *trans* (**10**) and *cis* (**11**) C,D-ring system.

a proton source.[19] This method has recently been used by Mourino et al. for the construction of modified vitamin D side chains.[23] We found that reduction of **9b** using a potassium metal/dicyclohexano-18-crown-6/toluene system[20] also resulted in the removal of the C-13 cyano group and formation of **10** but at a considerably lower yield (50%). Even careful inspection of the reaction mixtures by HPLC did not indicate a presence of the theoretically possible isomer **11** with *cis*-hydrindane skeleton (Figure 2). Since the stereochemistry of dissolving metal reductions so far has not been established and since the latter transformation was crucial for an attempted synthesis of **4b**, assignment of the configuration at C-13 in product **10** was of great importance. The ^{13}C NMR and mass spectra of **10** supported a molecular formula of $C_{17}H_{32}O$. Particularly informative was the signal of 8α-H (δ 3.80, m, $w/2 = 8$ Hz) in the ^{1}H NMR spectrum, apparently indicating an equatorial orientation of the proton. Such an orientation must be expected for the desired 18-nor compound **10** possessing a rigid *trans*-hydrindane system. Molecular mechanics calculations reveal that in the case of an alternative, hypothetical structure **11**, one of its conformers (with an axial 13α-H and equatorial 14α-H), resulting from C/D-ring inversion, would be strongly preferred and the 8α-hydrogen in this isomer could be expected to occupy an axial position (Figure 2).

A similar conformational analysis has also been performed for the 8-keto compound **12** obtained by oxidation of **10** with pyridinium dichromate (80% yield). The force field calculations were carried out for Grundmann's ketone **6**, its 18-nor analog **12**, and the hypothetical isomer **13** with *cis*-C/D-ring junction (Figure 3). The aim of these studies was to establish the preferred geometries and use them for prediction of the corresponding circular dichroism curves. Two facts, however, deserve comment. Application of the octant rule in determining configurations requires a correct assessment of the preferred conformations of the molecule.[24] Contrary to compounds **6** and **12**, the *cis*-hydrindanone **13** is conformationally flexible, and its multiple conformers have to be considered.[11b] Such a detailed conformational analysis would be beyond the scope of this paper. Nevertheless, two forms of **13** which we have listed (differing in an axial or equatorial orientation of the hydrogens at C-13 and C-14; Figure 3) represent the conformations of the lowest steric energy which were found after numerous calculations. Another difficulty was insufficient CD literature data for related hydrindanone analogs, and it forced us to use the corresponding numerical contributions (for ring system and alkyl substituents) derived from analogous compounds of *trans*- and *cis*-1-decalone type. Thus, on the basis of the energy-minimized hydrindanone structures (Figure 3), their "octant" projections, and literature data for the respective 1-decalones (values given for hexane solutions),[25,26] the Cotton effects of the corresponding 8-ketones were calculated. Experimental CD data of the compound obtained from the oxidation of **10**, showing (in hexane) two negative Cotton effects at 190 and 294 nm (Figure 4), are best matched with the values predicted for 18-nor C/D *trans*-hydrindane structure **12**. Ketone **12**, for further characterization purposes, was transformed into the oxime **14** and compared with its homolog **15**, obtained by oximation of **6**. The *E*-configuration of the oximes **14** and **15** followed from the presence of strongly deshielded signals of 9β-H (at δ 3.33 and 3.24, respectively) in their ^{1}H NMR spectra.[17,27] The stereochemistry of the oximes was also confirmed by comparison of their ^{13}C NMR spectra with those of the parent ketones. In both oximes the characteristic shielding effects,[27a] ca. 17 Hz on the secondary α-*syn* carbons C-9 and smaller (ca. 8 Hz) on the tertiary α-*anti* carbons C-14, were observed. The ^{13}C NMR data indicate that in the case of the synthesized compounds **10**, **12**, and **14**, the presence of the hydrogen at C-13 results in a shielding of the neighboring carbon atoms (C-12, C-14, and C-17), as compared with the signals of the corresponding 13β-methyl homologs **6**, **7a**, and **15**. Interestingly, small but consistent upfield shifts were also found in 18-nor compounds for C-21 and C-22 (ca. 0.5 and 2.6 Hz, respectively), whereas a downfield shift was observed for C-23 (ca. 1.5 Hz). These characteristic effects are most likely to result from differences in D-ring conformation and the orientation of the side chain in the two series, those of 13β-methyl compounds and of their 13β-H analogs.

The formation of single products during **6** and **12** oximations supports strongly the ascribed structure of the latter. It is known that reaction of six-membered cyclic ketones with hydroxylamine results in the formation of oximes where the hydroxy group is *anti* to the α-carbon bearing the bulkier equatorial substituent.[27b] Therefore, formation of a single oxime from Grundmann's ketones with C/D *trans*-junction and two isomeric oximes from *cis*-hydrindanone **13** (equatorial hydrogens at C-9 and C-14 in the preferred conformation; Figure 3) must be expected.

For the preparation of 25-hydroxy derivative **4b**, we used the method described by Uskokovic: a catalytic oxidation of Grundmann type ketone with RuO_4,[28] recently described by us in detail.[29] Even though keto compound **12** can be used as a substrate, it was more convenient to carry out the oxidation step on the 8β-alcohol **10**. Careful separation of the reaction mixture



190 nm c.d. band				
	Δε (calc.)	- 5.0	- 3.5	-12	+ 4
	Δε (obs.)	- 2.2	- 1.3		
n → π* c.d. band				
	Δε (calc.)	- 1.25	- 0.8	+ 0.15	-1.8
	Δε (obs.)	- 2.0	-1.3		

Figure 3. Energy-minimized conformers of 8-ketones with *trans* (**6**, **12**) and *cis* (**13**) C,D-ring junction and comparison of $\Delta\epsilon$ estimated from experimental CD data of hydrindanones **6** and **12** with the values of $\Delta\epsilon$ calculated for the corresponding *trans*- and *cis*-1-decalone compounds (refs 25 and 26).

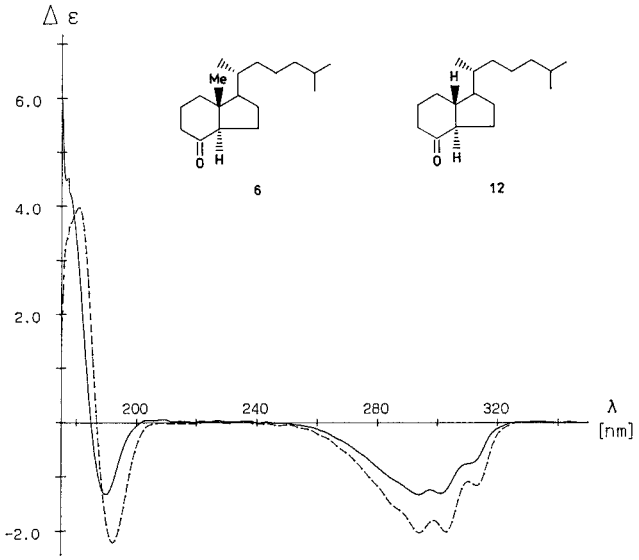


Figure 4. Circular dichroism curves of Grundmann's ketone **6** (—) and its 18-nor analog **12** (– –), showing two negative Cotton effects around 290 and 190 nm.

by flash chromatography and HPLC afforded, in addition to the expected **12** (19%), three compounds: products of C-20, C-17, and C-25 hydroxylation, namely, hydroxy ketones **16**, **17**, and **4b** (12, 4, and 14% yield, respectively). In comparison with an analogous oxidation process of **6**, performed by us previously,[29] a drop in the yield of the desired 25-hydroxylated product is evident. The considerably higher proportion of oxidation at the C-17 and C-20 positions in the 18-nor series reflects a fact that these carbons are no longer hindered by a bulky angular 13β-methyl group. The structures of **4b**, **16**, and **17** were established from their ^{1}H NMR, ^{13}C NMR, and mass spectra; particularly helpful was a comparison with the corresponding spectral data reported in the literature for the 13β-methyl homologs[29] and related side chain-hydroxylated hydrindane analogs.[30]

Silylation of hydroxy ketone **4b** with chlorotriethylsilane and imidazole in DMF afforded the C,D-synthon **4c** suitable for the subsequent Wittig–Horner reaction. Coupling of **4c** with the lithiated phosphine oxide **5a**[10] (Figure 1) gave the expected 18-norvitamin D compound **2c** (49%), which after deprotection with AG 50W-X4 cation exchange resin afforded 1α,25-dihydroxy-18-norvitamin D_3 (**2b**; 76%). Similarly, condensation of **4c** with the lithium phosphinoxy carbanion A-ring fragment **5b**[5a] yielded vitamin **3c** (29%). This, following deprotection, gave the expected 1α,25-dihydroxy-18,19-dinorvitamin D_3 (**3b**; 81%). Not surprisingly, both 18-norvitamins **2b** and **3b** have almost identical chromatographical properties as their homologs **2a** and **3a.** They elute from a Zorbax-Sil HPLC column after their 13β-methyl counterparts. A similar behavior was observed for the hydrindane precursors **4a,b**.

From the studies on 13α-precalciferol analogs, it is apparent that such compounds cannot be converted to

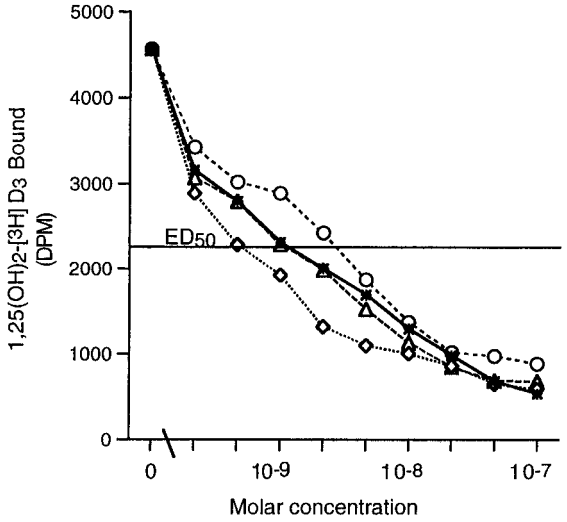


Figure 5. Competitive binding of 1α,25-$(OH)_2D_3$ (**2a**, *), 19-nor-1α,25-$(OH)_2D_3$ (**3a**, ○), and the synthesized analogs [18-nor-1α,25-$(OH)_2D_3$ (**2b**, ◇) and 18,19-dinor-1α,25-$(OH)_2D_3$ (**3b**, ▲)] to the porcine intestinal vitamin D receptor. This experiment was carried out in duplicate on six different occasions with identical results. The horizontal line represents 50% displacement of the radiolabeled 1α,25-$(OH)_2D_3$ by the indicated compound.

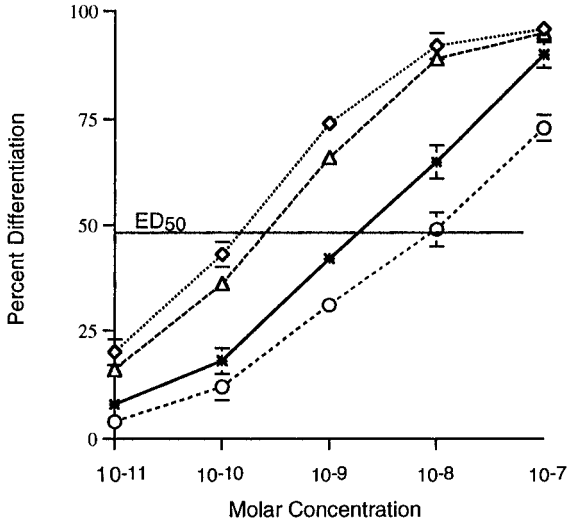


Figure 6. Differentiation activity of 1α,25-$(OH)_2D_3$ (**2a**, *), 19-nor-1α,25-$(OH)_2D_3$ (**3a**, ○), and the synthesized analogs [18-nor-1α,25-$(OH)_2D_3$ (**2b**, ◇) and 18,19-dinor-1α,25-$(OH)_2D_3$ (**3b**, ▲)]. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The results plotted on semilog paper represent mean ± SD of five experiments. Shown as a horizontal line is the ED_{50} as 50% differentiation.

the corresponding vitamin forms.[31] Evidently, in the case of the 13α-isomers as well as of other analogs possessing *cis*-C/D-ring junction,[32] the equilibrium of the thermal previtamin−vitamin interconversion is shifted far to the previtamin side. Thus, formation of 1α,25-dihydroxy-18-norvitamin D_3 (**2b**) and the established fact of its stability, comparable with that of the hormone **2a**, provide additional evidence in favor of the assigned configuration at C-13 in the synthesized 18-norvitamins.

Results and Discussion

We previously reported that 19-nor analogs of vitamin D_3 showed a selective activity profile by retaining their ability to cause cellular differentiation and increase intestinal calcium transport but had markedly reduced the ability to elevate plasma calcium at the expense of bone.[5b,33] We prepared 18-nor and 18,19-dinor analogs of 1α,25-$(OH)_2D_3$ (**2a**) to examine the effect of the removal of the 13β-methyl substituent on biological activities.

The 18-nor-1α,25-$(OH)_2D_3$ (**2b**) is at least as active as and as much as 3 times more active than 1α,25-$(OH)_2D_3$ in binding to the porcine VDR (Figure 5). 18,19-Dinor-1α,25-$(OH)_2D_3$ (**3b**) appeared to equal the activity of either **2a** or 19-nor-1α,25-$(OH)_2D_3$ (**3a**). The 18-nor compound **2b** proved to be about 10 times more active than 1α,25-$(OH)_2D_3$ in eliciting cellular differentiation of HL-60 cells, and the 18,19-dinor compound **3b** is only slightly less active than 18-nor-1α,25-$(OH)_2D_3$ (Figure 6). Thus, removal of the 13-methyl does not diminish and may actually increase biological activity *in vitro* of 1α,25-$(OH)_2D_3$ or 1α,25-dihydroxy-19-norvitamin D_3.

When tested *in vivo* in rats, the 18-nor analogs were found active but not to the extent of the parent 1α,25-$(OH)_2D_3$. Table 1 shows that a 500 pmol dose of 18-nor-1α,25-$(OH)_2D_3$ (**2b**) is less effective than 1α,25-$(OH)_2D_3$ on intestinal calcium transport and bone calcium mobilization (serum calcium increase). This single-point assay suggests the calcemic activity of 18-nor-1α,25-$(OH)_2D_3$ is about one-half that of 1α,25-$(OH)_2D_3$.

Table 1. Intestinal Calcium Transport and Bone Calcium Mobilization Response of Vitamin D-Deficient Rats to a Single Intravenous Injection of 18-Nor-1α,25-$(OH)_2D_3$ (**2b**)[a]

compound	amount (pmol)	Ca transport S/M (mean ± SEM)	serum Ca (mean ± SEM)
none (control)	0	3.0 ± 0.3	3.8 ± 0.1
1α,25-$(OH)_2D_3$	260	5.0 ± 0.3	4.8 ± 0.1
	500	5.7 ± 0.2	5.5 ± 0.2
18-nor-1α,25-$(OH)_2D_3$	260	3.9 ± 0.1	4.4 ± 0.1
	500	4.1 ± 0.3	5.1 ± 0.2

[a] Weanling male rats were maintained on a 0.47% calcium vitamin D-deficient diet for 3 weeks and then switched to a low-calcium (0.02%) vitamin D-deficient diet for 1 week. Each rat was given a single intravenous dose of the indicated compound in 0.1 mL of 95:5 propylene glycol/ethanol. The control group was given the vehicle alone. The determinations were carried out 24 h after the dose, and there were at least 5 rats/group.

When chronic daily doses were given to determine the activity in maintaining intestinal calcium transport and bone calcium mobilization, 18-nor-1α,25-$(OH)_2D_3$ appeared to be approximately one-third as active as 1α,25-$(OH)_2D_3$ in bone calcium mobilization and more than one-fifth as active on intestinal calcium transport (Table 2). These results support the overall calcemic activity of 18-nor-1α,25-$(OH)_2D_3$ as approximately one-third that of 1α,25-$(OH)_2D_3$. The 18,19-dinor analog was found less active than the 18-nor analog (Table 3) in supporting steady-state intestinal calcium transport and bone calcium mobilization. Thus, 1000 pmol/day dose of this analog approximately equaled a 130 pmol/day dose of 1α,25-$(OH)_2D_3$. Thus, this analog has about one-eighth the activity of 1α,25-$(OH)_2D_3$.

Conclusions

These results show that the 18-carbon of 1α,25-$(OH)_2D_3$ is not essential for biological activity. In fact, in binding to the receptor and in causing cellular differentiation, the absence of the 18-methyl increases

Table 2. Support of Intestinal Calcium Transport and Bone Calcium Mobilization by 18-Nor-1α,25-$(OH)_2D_3$ (**2b**) in Vitamin D-Deficient Rats on a Low-Calcium Diet[a]

compound	amount (pmol)	Ca transport S/M (mean ± SEM)	serum Ca (mean ± SEM)
none (control)	0	3.7 ± 0.4^b	4.4 ± 0.2^b
1α,25-$(OH)_2D_3$	260	7.6 ± 0.6^c	5.4 ± 0.2^c
18-nor-1α,25-$(OH)_2D_3$	650	5.4 ± 0.3^{d1}	5.2 ± 0.4^{d1}
	1300	5.5 ± 0.7^{d2}	5.6 ± 0.5^{d2}

[a] Weanling male rats were maintained on a 0.47% Ca diet for 1 week and then switched to a low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, they were dosed daily with the appropriate vitamin D compound for 7 days. All doses were administered intraperitoneally in 0.1 mL of propylene glycol/ethanol (95:5). Controls received the vehicle. Determinations were made 24 h after the last dose. There were at least 5 rats/group. Statistical analysis was done by Student's *t*-test. Statistical data: serosal/mucosal (S/M), *c* from *b*, $p < 0.001$, and *c* from d^1 and d^2, $p < 0.001$; serum calcium, *c* from *b*, $p < 0.001$, and *c* from d^1 and d^2, NS.

Table 3. Support of Intestinal Calcium Transport and Bone Calcium Mobilization by 18,19-Dinor-1α,25-$(OH)_2D_3$ (**3b**) in Vitamin D-Deficient Rats on a Low-Calcium Diet[a]

compound	amount (pmol)	Ca transport S/M (mean ± SEM)	serum Ca (mean ± SEM)
none (control)	0	3.7 ± 0.4^b	4.4 ± 0.2^b
1α,25-$(OH)_2D_3$	130	6.8 ± 0.4^{c1}	5.8 ± 0.2^{c1}
	260	7.6 ± 0.6^{c2}	7.1 ± 0.1^{c2}
18,19-dinor-1α,25-$(OH)_2D_3$	130	3.7 ± 0.2^{d1}	4.8 ± 0.2^{d1}
	260	4.8 ± 0.4^{d2}	4.9 ± 0.4^{d2}
	500	6.2 ± 0.4^{d3}	5.6 ± 0.4^{d3}
	1000	6.9 ± 0.7^{d4}	5.5 ± 0.2^{d4}

[a] The experiment was carried out as described in Table 2 except the compound studied was 18,19-dinor-1α,25-$(OH)_2D_3$ (**3b**). Statistical data: serosal/mucosal (S/M), c^1 and c^2 from *b*, $p < 0.001$, c^1 and c^2 from d^1 and d^2, $p < 0.001$, c^1 from d^3, $p < 0.005$, and d^3 and d^4 from *b*, $p < 0.001$; serum calcium, c^1 from *b* and d^1, $p < 0.001$, c^1 from d^2, d^3, and d^4, NS, and c^2 from *b*, d^1, d^2, d^3, and d^4, $p < 0.001$.

activity. On the other hand, the 18-nor compound was found to be one-third as active as 1α,25-$(OH)_2D_3$ on intestine and bone calcium-mobilizing systems, while 18,19-dinor-1α,25-$(OH)_2D_3$ was found to possess one-eighth the *in vivo* calcemic action of 1α,25-$(OH)_2D_3$. Likely, *in vivo* metabolism or receptor response in transcriptive activity will account for this discrepancy between the *in vivo* and *in vitro* results.

Experimental Section

Chemistry. Ultraviolet (UV) absorption spectra were recorded with a Hitachi Model 60-100 UV−vis spectrometer in ethanol, and circular dichroism (CD) spectra were measured on an AVIV Model 62DS instrument in hexane solutions. Infrared (IR) spectra were recorded on a Nicolet MX-1 FT-IR spectrometer in chloroform solutions. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 500 and 125 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 DS-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. Microanalyses of crystalline compounds were within ±0.4% of the theoretical values. THF was freshly distilled before use from sodium benzophenone ketyl under argon.

The starting Grundmann's ketone **6** was obtained by ozonolysis[12] of commercial vitamin D_3 (**1**). **6**: oil, $[\alpha]^{22}_D$ +6.9° (*c* 1.0, $CHCl_3$); CD $\Delta\epsilon$ (λ_{max}) −1.17 (313), −2.02 (303), −2.03 (294), −1.55 (285), −2.21 (192); IR ($CHCl_3$) 1705 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.639 (3H, s, 18-H_3), 0.867 and 0.872 (3H, 3H, each d, $J = 6.6$ Hz, 26-, 27-H_3), 0.950 (3H, d, $J = 6.1$ Hz, 21-H_3), 2.45 (1H, dd, $J = 11.7$, 7.5 Hz, 14α-H); ^{13}C NMR ($CDCl_3$) δ 12.3 (q, C-18), 18.5 (q, C-21), 18.9 (t, C-15), 22.4 and 22.7 (each q, C-26, C-27), 23.6 (t, C-11), 24.0 (t, C-23), 27.4 (t, C-16), 27.8 (d, C-25), 35.4 (d, C-20), 35.9 (t, C-22), 38.9 (t, C-12), 39.3 (t, C-24), 40.8 (t, C-9), 49.8 (s, C-13), 56.6 (d, C-17), 61.8 (d, C-14), 211.9 (s, C-8); MS *m/z* (relative intensity) 264 (M^+, 39), 249 (24), 221 (29), 151 (30), 125 (100).

Des-A,B-cholestan-8β-yl nitrite (7b). A solution of Grundmann's ketone **6** (2.70 g, 10.2 mmol) in anhydrous ether (90 mL) at 0 °C was added to a slurry of $LiAlH_4$ (3.89 g, 102.5 mmol) in anhydrous ether (270 mL). The reaction mixture was stirred at 0 °C for 1 h, and ethyl acetate (27 mL) followed by cold 10% H_2SO_4 (100 mL) was used to destroy the unreacted $LiAlH_4$ and complete the hydrolysis. The resulting mixture was extracted with ether; the combined extracts were washed with water and brine, dried (Na_2SO_4), and evaporated. The product was purified by flash chromatography on silica. Elution with 10% ethyl acetate in hexane gave the known 8β-alcohol **7a**[11] as a colorless oil (2.42 g, 89%): ^1H NMR ($CDCl_3$) δ 0.865 (6H, br d, $J \sim 6$ Hz, 26-, 27-H_3), 0.891 (3H, d, $J = 6.4$ Hz, 21-H_3), 0.929 (3H, s, 18-H_3), 4.07 (1H, m, $w/2 = 10$ Hz, 8α-H); ^{13}C NMR ($CDCl_3$) δ 13.4 (q, C-18), 17.4 (t, C-11), 18.5 (q, C-21), 22.5 (overlapped C-15 and C-26), 22.7 (q, C-27), 23.8 (t, C-23), 27.1 (t, C-16), 27.9 (d, C-25), 33.5 (t, C-9), 35.2 (d, C-20), 35.9 (t, C-22), 39.5 (t, C-24), 40.4 (t, C-12), 41.8 (s, C-13), 52.6 (d, C-14), 56.7 (d, C-17), 69.3 (d, C-8); MS *m/z* (relative intensity) 266 (M^+, 9), 251 (3), 207 (12), 164 (19), 111 (61), 91 (100).

A solution of alcohol **7a** (533 mg, 2 mmol) in chloroform (10 mL) was treated with *tert*-butyl nitrite (2.2 mL) and stirred at room temperature in the dark for 40 min. Benzene (20 mL) was added, and the solvents were rapidly evaporated under vacuum (temperature of water bath 40 °C). During evaporation of solvents and further high-vacuum drying the nitrite was protected from light. The oily product contained traces of starting alcohol **7a**, but it was suitable for the subsequent reaction. Nitrite **7b**: IR ($CHCl_3$) 1632 (nitrite) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.767 (3H, s, 18-H_3), 0.862 (6H, br d, $J = 6.2$ Hz, 26-, 27-H_3), 0.901 (3H, d, $J = 7.0$ Hz, 21-H_3), 5.76 (1H, narr m, 8α-H).

(18*E*)-18-(Hydroxyimino)-des-A,B-cholestan-8β-ol (8a). The crude nitrite ester **7b** obtained from 2 mmol of 8β-alcohol **7a** was dissolved in anhydrous benzene (140 mL) and irradiated, in an apparatus consisting of a Pyrex vessel with a water-cooled Vycor immersion well and a Hanovia high-pressure mercury arc lamp equipped with Pyrex filter. A slow stream of argon was passed into the vessel, and the temperature of the solution was maintained at 10 °C. After 1 h 40 min of irradiation, TLC showed only traces of unreacted nitrite. The reaction mixture was allowed to stand overnight at room temperature (in order to accomplish an isomerization of the intermediate 18-nitroso compound to the oxime), benzene was evaporated under vacuum, and the oily residue was purified by flash chromatography. Elution with 30% ethyl acetate in hexane afforded pure oxime **8a** (270 mg, 46% from 8β-alcohol **7a**) as a colorless oil: IR ($CHCl_3$) 3590, 3240, 3140 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.865 (6H, d, $J = 6.1$ Hz, 26-, 27-H_3), 0.994 (3H, d, $J = 6.7$ Hz, 21-H_3), 4.04 (1H, m, $w/2 = 9$ Hz, 8α-H), 6.29 (1H, br s, O*H*), 7.36 (1H, s, 18-H), 10.38 (1H, br s, O*H*); MS *m/z* (relative intensity) 295 (M^+, 16), 278 (87), 260 (68), 245 (33), 183 (100); exact mass calcd for $C_{18}H_{33}O_2N$ 295.2511, found 295.2514.

8β-Acetoxy-des-A,B-cholestane-18-nitrile (9a). (a) A solution of the oxime **8a** (120 mg, 0.41 mmol) in acetic anhydride (5 mL) was refluxed for 1.5 h. The reaction mixture was cooled, poured carefully on ice, and extracted with benzene. Extracts were combined, washed with water, $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated. The oily residue was purified by flash chromatography using 10% ethyl acetate in hexane. Pure acetoxy nitrile **9a** (112 mg, 86%) was

obtained as a colorless oil: IR ($CHCl_3$) 2220 (nitrile), 1720, 1240 (acetate) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.864 (6H, d, $J = 6.2$ Hz, 26-, 27-H_3), 1.032 (3H, d, $J = 6.5$ Hz, 21-H_3), 2.13 (3H, s, OAc), 5.20 (1H, m, $w/2 = 8$ Hz, 8α-H); MS m/z (relative intensity) 319 (M^+, 56), 304 (18), 277 (89), 259 (100), 244 (64); exact mass calcd for $C_{20}H_{33}O_2N$ 319.2511, found 319.2506.

(b) Hydroxy oxime **8a** (120 mg, 0.41 mmol) was heated with acetic anhydride (0.3 mL) and pyridine (0.5 mL) for 36 h at 60 °C. The reaction mixture was cooled, poured on ice, and extracted with benzene. Extracts were combined, washed with water, $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated. The oily residue was purified by flash chromatography using 10% ethyl acetate in hexane. Pure acetoxy nitrile **9a** (109 mg, 84%) was obtained as a colorless oil.

Monitoring the reaction with TLC showed a presence of a spot corresponding to diacetate **8b**.

8β-Hydroxy-des-A,B-cholestane-18-nitrile (9b). Acetoxy nitrile **9a** (210 mg, 0.66 mmol) was treated with 10% methanolic KOH (10 mL) at 50 °C for 1.5 h. After concentration under vacuum the reaction mixture was poured into water and extracted with benzene and ether. The organic extracts were combined, washed with brine, dried (Na_2SO_4), and evaporated. The residue was redissolved in hexane/ethyl acetate (7:3) and the solution passed through a silica gel Sep-Pak cartridge. Evaporation of solvents gave pure hydroxy nitrile **9b** (175 mg, 96%) as an oil: IR ($CHCl_3$) 3600 (OH), 2220 (nitrile) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.868 (6H, d, $J = 6.0$ Hz, 26-, 27-H_3), 1.032 (3H, d, $J = 7.1$ Hz, 21-H_3), 4.10 (1H, m, $w/2 = 10$ Hz, 8α-H); MS m/z (relative intensity) 277 (M^+, 37), 262 (28), 244 (18), 234 (26), 220 (32), 206 (87), 121 (100); exact mass calcd for $C_{18}H_{31}ON$ 277.2406, found 277.2406.

Des-A,B-18-norcholestan-8β-ol (10). (a) To a stirred mixture of potassium (55 mg, 1.4 mmol) in hexamethylphosphoric triamide (HMPA; 170 μL) and ether (420 μL) was added a solution of the hydroxy nitrile **9b** (55 mg, 0.2 mmol) in *tert*-butyl alcohol (50 μL) and ether (200 μL) dropwise at 0 °C under argon. The cooling bath was removed, and the brown-yellow solution was stirred at room temperature for 5 h under argon. Unreacted potassium was removed, the mixture was diluted with benzene, and a few drops of 2-propanol were added and water. The organic phase was washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography. Elution with 10% ethyl acetate in hexane gave pure alcohol **10** (38 mg, 76%) as a colorless oil: IR ($CHCl_3$) 3630 and 3470 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.863 and 0.868 (3H, 3H, each d, $J = 6.3$ Hz, 26-, 27-H_3), 0.881 (3H, d, $J = 6.5$ Hz, 21-H_3), 4.05 (1H, m, $w/2 = 8$ Hz, 8α-H); ^1H NMR (C_6D_6) δ 0.901 and 0.907 (3H, 3H, each d, $J = 6.2$ Hz, 26-, 27-H_3), 0.945 (3H, d, $J = 6.5$ Hz, 21-H_3), 3.80 (1H, m, $w/2 = 8$ Hz, 8α-H); ^{13}C NMR ($CDCl_3$) δ 18.1 (q, C-21), 20.3 (t, C-11), 22.5 and 22.7 (each q, C-26, C-27), 24.8 (t, C-15), 25.4 (t, C-23), 25.6 (t, C-16), 27.9 (d, C-25), 31.7 (t, C-12), 33.5 (overlapped C-9 and C-22), 35.1 (d, C-20), 39.3 (t, C-24), 39.6 (d, C-13), 49.8 (d, C-14), 50.7 (d, C-17), 67.9 (d, C-8); MS m/z (relative intensity) 252 (M^+, 1), 234 (3), 219 (2), 121 (100); exact mass calcd for $C_{17}H_{32}O$ 252.2453, found 252.2470.

(b) A lump (ca. 0.25 cm^3) of potassium metal was added to a solution of hydroxy nitrile **9b** (55 mg, 0.2 mmol) and dicyclohexano-18-crown-6 (111 mg, 0.3 mmol) in anhydrous toluene (8 mL). The mixture was stirred under argon at room temperature for 10 h, unreacted potassium was removed, and a few drops of 2-propanol were added and water. The organic phase was washed with water, dried (Na_2SO_4), and evaporated. The residue was subjected to flash chromatography. Elution with 10% ethyl acetate in hexane gave alcohol **10** (30 mg) which was subsequently purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (9:1) solvent system. Pure compound **10** (25 mg, 50%) was eluted at R_V 44 mL as a colorless oil.

Des-A,B-18-norcholestan-8-one (12) and 25-Hydroxy-des-A,B-18-norcholestan-8-one (4b). (a) To a solution of alcohol **10** (5 mg, 20 μmol) in CH_2Cl_2 (2 mL) containing a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) was added pyridinium dichromate (PDC; 25 mg, 66 μmol) at 0 °C with stirring. After 10 min the cooling bath was removed and the mixture was stirred at room temperature for 5 h. The brown mixture was diluted with ether and filtered through a silica Sep-Pak cartridge that was washed with hexane/ethyl acetate (1:1). Evaporation of the solvents gave crude ketone **12** which was further purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (9:1) solvent system. Analytically pure compound **12** (4 mg, 80%) was eluted at R_V 29 mL (Grundmann's ketone **6** was eluted at R_V 31 mL in the same system) as an oil: $[\alpha]^{22}_D$ +16.2° (*c* 0.31, $CHCl_3$); CD $\Delta\epsilon$ (λ_{max}) −0.76 (311), −1.32 (301), −1.34 (294), −0.92 (282), −1.33 (190); IR ($CHCl_3$) 1706 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.866 (6H, d, $J = 6.9$ Hz, 26-, 27-H_3), 0.889 (3H, d, $J = 6.9$ Hz, 21-H_3); ^{13}C NMR ($CDCl_3$) δ 18.0 (q, C-21), 21.5 (t, C-15), 22.5 and 22.7 (each q, C-26, C-27), 25.4 (overlapped C-16 and C-23), 27.8 (t, C-11), 27.9 (d, C-25), 30.6 (t, C-12), 33.2 (t, C-22), 34.8 (d, C-20), 39.3 (t, C-24), 41.5 (t, C-9), 50.8 and 50.9 (each d, C-13, C-17), 58.3 (d, C-14), 212.0 (s, C-8); MS m/z (relative intensity) 250 (M^+, 80), 207 (44), 137 (100); exact mass calcd for $C_{17}H_{30}O$ 250.2297, found 250.2289.

(b) To the stirred solution of ruthenium(III) chloride hydrate (11.5 mg, 0.06 mmol) and $NaIO_4$ (263 mg, 1.23 mmol) in water (1.0 mL) was added a solution of alcohol **10** (85 mg, 0.34 mmol) in CCl_4/CH_3CN (1:1, 1.5 mL). The mixture was vigorously stirred for 72 h at room temperature. A few drops of 2-propanol were added; the mixture was poured into water and extracted with $CCl_4/CHCl_3$ solvent system. The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated to give an oily residue which was subjected to flash chromatography. Elution with 20% ethyl acetate in hexane gave 8-ketone **12** (16 mg, 19%). Subsequent elution with 40% ethyl acetate in hexane afforded impure fractions containing a mixture of hydroxylated compounds **16**, **17**, and **4b** (44 mg, 49%). Subsequent HPLC separation and purification of the combined fractions (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (6:4) solvent system gave the analytically pure oily ketone **16** (10.7 mg, 12%) eluting at R_V 36 mL, the oily ketone **17** (3.7 mg, 4%) eluting at R_V 44 mL, and the keto compound **4b** (12.7 mg, 14%) eluting at R_V 51 mL (25-hydroxy Grundmann's ketone **4a** was eluted at R_V 50 mL in the same system) as an oil crystallizing on standing in the refrigerator.

16: ^1H NMR ($CDCl_3$) δ 0.885 (6H, d, $J = 6.1$ Hz, 26-, 27-H_3), 1.159 (3H, s, 21-H_3); ^{13}C NMR ($CDCl_3$) δ 21.4 (t, C-15), 22.2 (t, C-23), 22.5 and 22.6 (each q, C-26, C-27), 25.0 (t, C-16), 25.7 (q, C-21), 27.8 (t, C-11), 28.0 (d, C-25), 32.0 (t, C-12), 39.6 (t, C-24), 40.1 (t, C-22), 41.3 (t, C-9), 49.4 (d, C-13), 54.4 (d, C-17), 58.5 (d, C-14), 74.5 (s, C-20), 211.7 (s, C-8); MS m/z (relative intensity) 266 (M^+, 10), 181 (28), 163 (46), 138 (100); exact mass calcd for $C_{17}H_{30}O_2$ 266.2246, found 266.2251.

17: ^1H NMR ($CDCl_3$) δ 0.868 (6H, d, $J = 6.1$ Hz, 26-, 27-H_3), 0.974 (3H, d, $J = 6.5$ Hz, 21-H_3), 2.85 (1H, br m, $w/2 = 28$ Hz, 14α-H); MS m/z (relative intensity) 266 (M^+, 30), 248 (2), 153 (100), 135 (73); exact mass calcd for $C_{17}H_{30}O_2$ 266.2246, found 266.2242.

4b: ^1H NMR ($CDCl_3$) δ 0.908 (3H, d, $J = 6.5$ Hz, 21-H_3), 1.216 (6H, s, 26-, 27-H_3); ^{13}C NMR ($CDCl_3$) δ 18.0 (q, C-21), 21.5 (t, C-15), 22.3 (t, C-23), 25.4 (t, C-16), 27.8 (t, C-11), 29.3 (overlapped C-26 and C-27), 30.6 (t, C-12), 33.5 (t, C-22), 34.8 (d, C-20), 41.5 (t, C-9), 44.2 (t, C-24), 50.8 and 50.9 (each d, C-13, C-17), 58.3 (d, C-14), 71.0 (s, C-25), 211.9 (s, C-8); MS m/z (relative intensity) 266 (M^+, <1), 251 (6), 248 (60), 233 (16), 137 (100); exact mass calcd for $C_{17}H_{30}O_2$ 266.2246, found 266.2257. Anal. ($C_{17}H_{30}O_2$) C, H.

(8*E*)-8-(Hydroxyimino)-des-A,B-18-norcholestane (14) and (8*E*)-(Hydroxyimino)-des-A,B-cholestane (15). (a) To a solution of 18-nor Grundmann's ketone **12** (5.5 mg, 0.02 mmol) in anhydrous pyridine (0.12 mL) was added crystalline hydroxylamine hydrochloride (5 mg), and the reaction was allowed to proceed for 16 h at room temperature. TLC (10% ethyl acetate in hexane) showed a presence of a single product (R_f 0.25). The mixture was poured into water and extracted with benzene; the organic layer was washed with saturated $CuSO_4$ and water, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography. Elution with 10% ethyl acetate in hexane gave oily oxime **14** (5.3 mg, 91%): IR ($CHCl_3$) 3586 and 3284 (OH) cm^{-1}; ^1H NMR ($CDCl_3$)

δ 0.864 and 0.867 (3H, 3H, each d, J = 6.6 Hz, 26-, 27-H_3), 0.879 (3H, d, J = 6.7 Hz, 21-H_3), 3.33 (1H, dm, J = 14.4 Hz, 9β-H), 8.19 (1H, br m, O*H*); ^{13}C NMR ($CDCl_3$) δ 18.1 (q, C-21), 22.5 and 22.7 (each q, C-26, C-27), 23.9 (t, C-15), 24.2 (t, C-9), 25.4 (t, C-23), 25.9 (t, C-16), 26.0 (t, C-11), 28.0 (d, C-25), 31.0 (t, C-12), 33.5 (t, C-22), 35.2 (d, C-20), 39.3 (t, C-24), 49.6 (d, C-13), 49.8 (d, C-17), 50.5 (d, C-14), 162.1 (s, C-8); MS m/z (relative intensity) 265 (M^+, 8), 248 (100), 231 (12), 206 (30); exact mass calcd for $C_{17}H_{31}ON$ 265.2405, found 265.2404.

(b) Oximation of Grundmann's ketone **6** (22 mg, 0.08 mmol) with hydroxylamine hydrochloride (20 mg) in pyridine (0.45 mL) according to the above procedure gave the crystalline oxime **15** (22 mg, 95%): R_f 0.27 (10% ethyl acetate in hexane); IR ($CHCl_3$) 3588 and 3286 (OH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.649 (3H, s, 18-H_3), 0.866 and 0.870 (3H, 3H, each d, J = 6.6 Hz, 26-, 27-H_3), 0.935 (3H, d, J = 6.5 Hz, 21-H_3), 2.09 (1H, dd, J = 11.6, 7.5 Hz, 14α-H), 3.24 (1H, dm, J = 13.2 Hz, 9β-H), 9.05 (1H, br m, O*H*); ^{13}C NMR ($CDCl_3$) δ 12.2 (q, C-18), 18.8 (q, C-21), 20.7 (t, C-15), 21.8 (t, C-11), 22.5 and 22.8 (each q, C-26, C-27), 23.8 (overlapped C-9 and C-23), 27.9 (t, C-16), 28.0 (d, C-25), 35.9 (d, C-20), 36.1 (t, C-22), 39.3 (t, C-24), 39.5 (t, C-12), 46.5 (s, C-13), 54.1 (d, C-14), 56.0 (d, C-17), 160.6 (s, C-8); MS m/z (relative intensity) 279 (M^+, 2), 262 (100), 245 (18), 219 (13); exact mass calcd for $C_{18}H_{33}ON$ 279.2562, found 279.2562. Anal. ($C_{18}H_{33}ON$) C, H, N.

25-[(Triethylsilyl)oxy]-des-A,B-18-norcholestan-8-one (4c). A solution of the ketone **4b** (5 mg, 19 μmol) and imidazole (15 mg, 220 μmol) in anhydrous DMF (150 μL) was treated with triethylsilyl chloride (15 μL, 90 μmol). The mixture was stirred at room temperature under argon for 4 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 cartridge in 10% ethyl acetate in hexane and, after evaporation, purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (9:1) solvent system. Pure protected ketone **4c** (3.6 mg, 50%) was eluted at R_V 25 mL as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.559 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.896 (3H, d, J = 7.6 Hz, 21-H_3), 0.939 (9H, t, J = 7.9 Hz, 3 × $SiCH_2CH_3$), 1.183 (6H, s, 26-, 27-H_3).

1α,25-Dihydroxy-18-norvitamin D$_3$ (2b). [2-(1*Z*)-[(3*S*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methylenecyclohexylidene]ethyl]diphenylphosphine oxide (**5a**)[10] (13.9 mg, 24 μmol) was dissolved in anhydrous THF (200 μL) and cooled to −78 °C, and *n*-BuLi (1.5 M in hexanes, 16 μL, 24 μmol) was added under argon with stirring. The mixture turned deep orange. After stirring for 5 min at −78 °C the protected ketone **4c** (1.20 mg, 3 μmol) was added in anhydrous THF (200 + 100 μL). The mixture was stirred under argon at −78 °C for 1 h and at 0 °C for 16 h. Ethyl acetate was added and the organic phase washed with saturated NH_4Cl, 10% $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The residue was passed through a silica Sep-Pak cartridge in 10% ethyl acetate in hexane and, after evaporation, purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using 10% ethyl acetate in hexane. Pure compound **2c** (1.16 mg, 49%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.055, 0.060, and 0.067 (3H, 3H, 6H, each s, 4 × $SiCH_3$), 0.556 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.85−0.88 (21H, 21-H_3, 2 × Si-*t*-Bu), 0.939 (9H, t, J = 7.9 Hz, 3 × $SiCH_2CH_3$), 1.178 (6H, br s, 26-, 27-H_3), 2.21 (1H, dd, J = 12.8, 6.8 Hz, 4β-H), 2.44 (1H, dd, J = 12.8, 3.6 Hz, 4α-H), 2.86 (1H, br d, J = 13.2 Hz, 9β-H), 4.18 (1H, m, 3α-H), 4.38 (1H, m, 1β-H), 4.89 (1H, d, J = 2.4 Hz, 19*Z*-H), 5.19 (1H, br s, 19*E*-H), 6.09 and 6.22 (1H, 1H, each d, J = 11.6 Hz, 7-, 6-H).

Protected vitamin **2c** (1.10 mg) was dissolved in benzene (40 μL), and the resin (AG 50W-X4, 10 mg; prewashed with methanol) in methanol (200 μL) was added. The mixture was stirred at room temperature under argon for 18 h, filtered through a silica Sep-Pak cartridge, and washed with 2-propanol. The solvent was evaporated, and a crude vitamin **2b** was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (7:3) solvent system. Analytically pure compound **2b** (449 μg, 76%) was collected at R_V 31.5 mL [1α,25-$(OH)_2D_3$ (**2a**) was eluted at R_V 31 mL in the same system] as a white solid: UV (in EtOH) λ_{max} 263, λ_{min} 227 nm, A_{max}/A_{min} = 1.9; ^{1}H NMR ($CDCl_3$) δ 0.887 (3H, d, J = 6.6 Hz, 21-H_3), 1.210 (6H, s, 26-, 27-H_3), 2.30 (1H, dd, J = 13.3, 7.2 Hz, 4β-H), 2.61 (1H, dd, J = 13.3, 3.5 Hz, 4α-H), 2.88 (1H, br d, J = 13.4 Hz, 9β-H), 4.22 (1H, m, 3α-H), 4.43 (1H, m, 1β-H), 5.03 (1H, br s, 19*Z*-H), 5.33 (1H, br s, 19*E*-H), 6.09 and 6.38 (1H, 1H, each d, J = 11.4 Hz, 7-, 6-H); MS m/z (relative intensity) 402 (M^+, 11), 384 (74), 366 (44), 348 (14), 152 (33), 134 (100); exact mass calcd for $C_{26}H_{42}O_3$ 402.3134, found 402.3142.

1α,25-Dihydroxy-18,19-dinorvitamin D$_3$ (3b). [2-[(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]cyclohexylidene]ethyl]-diphenylphosphine oxide (**5b**)[5a] (12 mg, 21 μmol) was dissolved in anhydrous THF (200 μL) and cooled to −78 °C, and *n*-BuLi (1.4 M in hexanes, 15 μL, 21 μmol) was added under argon with stirring. The solution turned deep orange. After stirring for 5 min at −78 °C the protected ketone **4c** (3.0 mg, 7.9 μmol) was added in anhydrous THF (200 + 100 μL). The mixture was stirred under argon at −78 °C for 1 h and at 0 °C for 16 h. Ethyl acetate was added and the organic phase washed with saturated NH_4Cl, 10% $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The residue was passed through a silica Sep-Pak cartridge in 10% ethyl acetate in hexane and, after evaporation, purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (9:1) solvent system. Pure protected vitamin **3c** (1.7 mg, 29%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.045 and 0.054 (6H, 6H, each s, 4 × $SiCH_3$), 0.557 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.86−0.87 (21H, 21-H_3, 2 × Si-*t*-Bu), 0.939 (9H, t, J = 7.9 Hz, 3 × $SiCH_2CH_3$), 1.178 (6H, br s, 26-, 27-H_3), 2.84 (1H, br d, J = 13.5 Hz, 9β-H), 4.07 (2H, br m, 1β-, 3α-H), 5.90 and 6.14 (1H, 1H, each d, J = 11.1 Hz, 7-, 6-H).

Protected vitamin **3c** (850 μg, 1.2 μmol) was dissolved in benzene (40 μL), and cation exchange resin (AG 50W-X4, 17 mg; prewashed with methanol) in methanol (200 μL) was added. The mixture was stirred at room temperature under argon for 18 h, filtered through a silica Sep-Pak cartridge, and washed with 2-propanol. The solvent was evaporated, and a crude vitamin **16** was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (7:3) solvent system. Analytically pure compound **3b** (366 μg, 81%) was eluted at R_V 37 mL [1α,25-dihydroxy-19-norvitamin D$_3$ (**3a**) was eluted at R_V 36 mL in the same system] as a white solid: UV (in EtOH) λ_{max} 243, 251.5, 261 nm; ^{1}H NMR ($CDCl_3$) δ 0.879 (3H, d, J = 6.5 Hz, 21-H_3), 1.208 (6H, s, 26-, 27-H_3), 4.07 and 4.11 (1H, 1H, each m, 1β-, 3α-H), 5.94 and 6.30 (1H, 1H, each d, J = 11.2 Hz, 7-, 6-H); MS m/z (relative intensity) 390 (M^+, 39), 372 (62), 354 (23), 259 (42), 231 (84), 175 (25), 133 (53), 121 (64), 69 (100); exact mass calcd for $C_{25}H_{42}O_3$ 390.3134, found 390.3139.

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,[34] 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-OH-D_3 or 1α,25-$(OH)_2D_3$ in their plasma as measured by methods described previously.[35] 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.[34] Statistical analysis was by the Student's *t*-test.[36] Intestinal calcium transport is expressed as 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.[4b] 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 4th 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.[4b] The experiment was repeated five times, and the results are reported as the mean ± SEM.

Measurement of Binding to the Porcine Intestinal Vitamin D Receptor. Porcine intestinal nuclear extract was prepared as described earlier.[37] It was diluted, and 0.1 mg of protein (200 fmol of binding activity) in 100 μL was used in each tube; 10 000 cpm of 1α,25-$(OH)_2$[26,27-^{3}H]D_3 was added in 2.0 μL of ethanol. To this was added either standard radioinert 1α,25-$(OH)_2D_3$ at various concentrations or the indicated analog at various concentrations in 5 μL of ethanol. The mixture was incubated at room temperature for 4 h on a shaker and then 100 μL of hydroxyapatite (50% slurry) added. The sample was vortexed at 5 min intervals for 15 min on ice. The hydroxyapatite was then washed three times by adding 0.5 mL of TE 5% Triton X100, centrifuging at 200*g* for 5 min, and aspirating the supernatant. The radioactivity bound to the hydroxyapatite was determined by liquid scintillation counting in Bio-Safe II scintillation fluid. The values are plotted versus concentration of analog or standard. Each value represents duplicate values. The displacement experiment was carried out on six different occasions with identical results.

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