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Synthesis and Biological Activity of 2-(3′-Hydroxypropylidene)-1α-hydroxy-19-norvitamin D Analogues with Shortened Alkyl Side Chains

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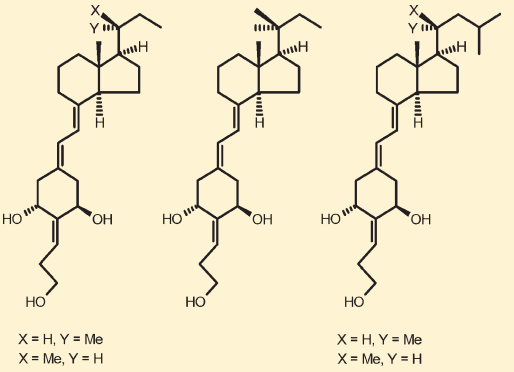
Supporting Information

ABSTRACT: As a continuation of our efforts directed to vitamin D compounds of promising biological properties, 19-norvitamins **9**−**13**, possessing a 3′-hydroxypropylidene fragment attached to C-2 and shortened 17β-alkyl chains, were synthesized. A new synthetic pathway providing the CD-ring ketones **20**−**24** is described starting from the epimeric aldehydes **25** and **26**. The hydrindanones **20**−**24** were subjected to the Wittig−Horner reaction with the phosphine oxide **14**, and the vitamin D compounds **9**−**13** were obtained after hydroxyl deprotection. In comparison to 1α,25-$(OH)_2D_3$ (**1**), the prepared analogues, except for the 20*R*-compound **12**, were only ca. 3 times less potent in binding to VDR. Compounds **9**−**11** and **13** exhibited HL-60 cellular activity 5−20 times lower and transcriptional activity ca. 10 times decreased related to those for the hormone **1**. When tested in vivo, all the analogues showed no ability to mobilize calcium from bone, and intestinal calcium transport activity was observed only at high doses of the vitamins **10**, **12**, and **13**.



INTRODUCTION

Since the isolation and structural characterization of the most active vitamin D_3 metabolite, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **1**; Figure 1) representing its hormonal form,[1] the biological functions of this compound have been extensively studied.[2] It has been established that calcitriol, in addition to its classical action as the regulator of calcium and phosphorus homeostasis, also plays a pivotal role in cellular differentiation, inhibition of cell proliferation, and immunomodulation.[3] Such a broad array of biological activities of the natural hormone **1** encouraged many research groups to undertake the synthesis of vitamin D analogues characterized by a selective biological profile. This great synthetic effort resulted in the preparation of more than 3000 calcitriol analogues.[4] There were two main goals of introducing structural modifications: to search for the vitamin D compounds with increased calcemic potency (superagonists) or to search for noncalcemic compounds, which could be useful in treating specific diseases (cancer, psoriasis, multiple sclerosis, etc.) without causing undesired side effects related to hypercalcemia.

In 1998, we discovered an important modification of the vitamin D carbon skeleton, which led to the compounds with interesting biological properties. This structural change consists of the removal of the exomethylene group from C-10 and its shift to C-2.[5] Thus, the 2-methylene-1α,25-dihydroxy-19-norvitamin D_3 analogue **2** bound to the vitamin D receptor (VDR) equally well as the natural hormone **1** but showed an enhanced ability to mobilize calcium from bone. The isomeric (20*S*)-compound (**3**, 2MD) exerted even stronger activity in bone, exhibiting the unique ability to induce bone formation in vitro and in vivo.[5,6] During the following years, several vitamin D analogues characterized by such A-ring modification were synthesized, and many of them showed promising biological activities.[7−17]

Seven years ago, a set of noncalcemic 2-methylene-19-nor-1α-hydroxyvitamin D analogues **4**−**6** with shortened side chains were described.[18] Interestingly, it was established that these compounds, substituted at C-17 with a branched (or even unbranched) alkyl group, retained some valuable genomic functions related to those of 2MD but lacked its calcemic activity. Therefore, these analogues show promise of being used as anticancer agents or, if they have an ability to decrease the PTH level, for the treatment of secondary hyperthyroidism. Continuing our structure−activity studies in the vitamin D area, we also prepared 19-nor-2-(3′-hydroxypropylidene) vitamin D compounds and found that their *E*-geometrical isomers **7** and **8** were more potent in the in vitro and in vivo tests.[19] Taking into account these findings, we decided to synthesize different hybrid compounds combining both structural modifications of the vitamin D molecule: shortened alkyl side chains and the presence of the ring A of 19-norvitamins substituted at C-2 with a 3′-hydroxypropylidene group.[20] This article describes the preparation of the vitamin D analogues **9**−**13** and the results of their biological testing.

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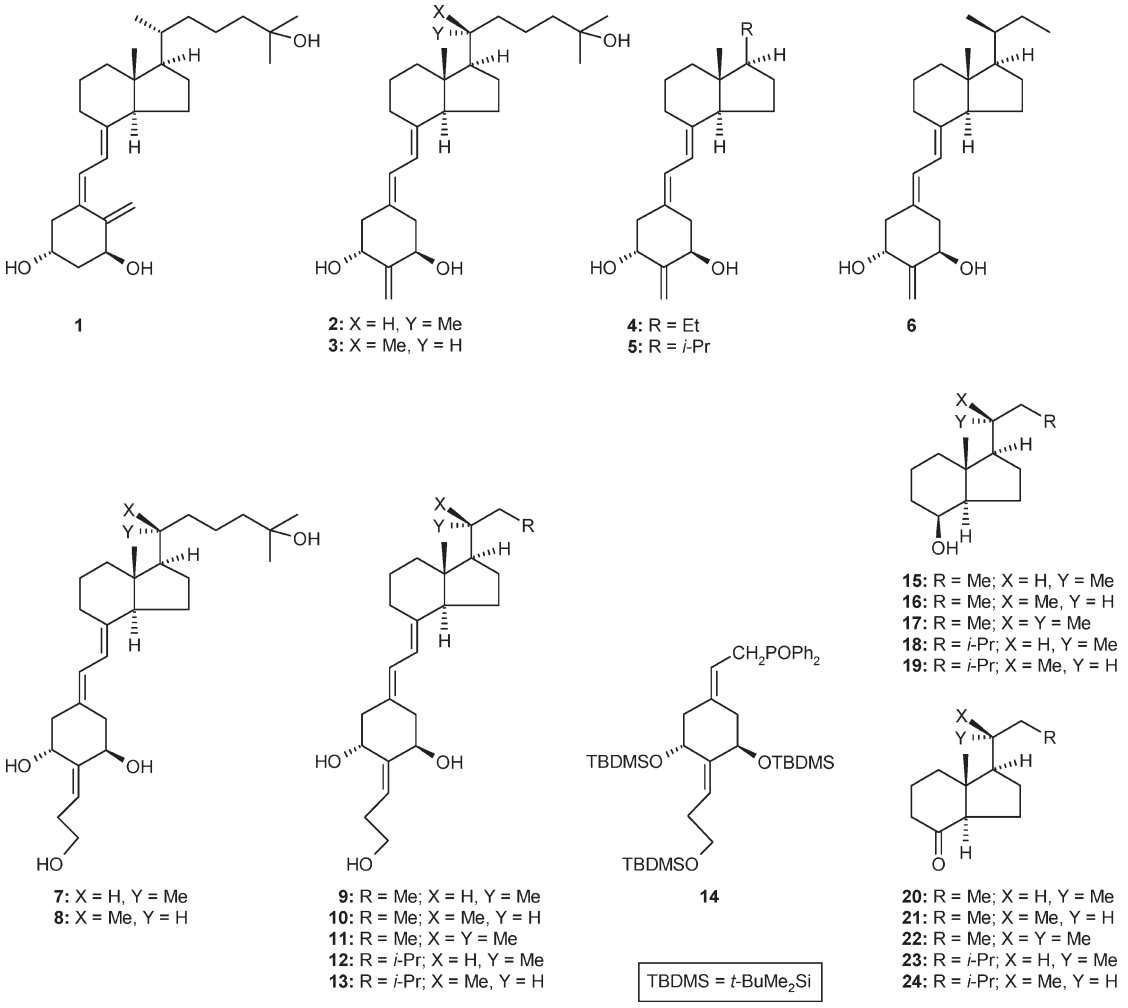

Figure 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**), its analogues, and the building blocks for the synthesis of vitamins **9**−**13**.

RESULTS AND DISCUSSION

Chemistry. Availability of the phosphine oxide **14**, synthesized previously in our laboratory,[19] allowed us to focus on the preparation of the corresponding CD-ring ketones **20**−**24**, which can be condensed with an anion of **14** in a Wittig−Horner process. Considering the hydrindanols **15**−**19** as the immediate precursors of the desired Grundmann ketone analogues, we have decided to explore the usefulness of the 20-cyano compound as a crucial synthetic precursor. Its α-alkylation process seemed to be a convenient method of introducing the desired alkyl fragment at C-20, and the subsequent alkali metal reduction should be suitable for the removal of the cyano group.

Preparation of the nitrile **28** (Scheme 1) was easily accomplished from the known bicyclic aldehyde **25** obtained,[21] together with its epimer **26**,[22] by the degradation of vitamin D_2. Thus, conversion of **25** into the oxime **27** followed by acetolysis provided the nitrile compound **28** in the overall 81% yield. Both aldehydes **25** and **26** proved also to be suitable substrates for the preparation of hydrindanols **15** and **16**. The Wittig methylenation of the aldehydes (carried out separately for each isomer) and the following hydrogenation of the formed olefins **29** and **30** gave the benzoates **31** and **32**, which after hydrolysis provided alcohols **15** and **16** in the overall yield ca. 50%.

It turned out that alkylation of the nitrile **28** proceeded quite efficiently. Thus, treatment of its anion, generated using LDA, with ethyl or isobutyl bromide resulted in the formation of single substitution products **33** and **34** (Scheme 2) in 80% and 74% yield, respectively. A lower yield was obtained when silylated 2-bromoethanol was used as an alkylating agent for the preparation of **35**. Interestingly, in all cases, the nucleophilic substitution turned out to be a highly diastereoselective process, resulting in the products having the *S*-configuration at the newly formed stereogenic center. Therefore, it might be suggested that alkyl bromide reacts with a rotamer of the nitrile **28** anion possessing the α-methyl substituent directed to C-7 and the CN group facing C-2. The approach of the alkylating agent from the *si*-face of this anion is incomparably easier than from the opposite side, significantly hindered by an angular methyl group. Such a stereochemical outcome of this alkylation reaction was supported by X-ray crystal structure analysis of the compound obtained by alkaline hydrolysis of the benzyloxy group in **34**. The ORTEP drawing of the hydroxy nitrile **37** is shown in Figure 2.

The benzoyloxy nitrile **33** proved to be a convenient precursor of **17**. Its hydrolysis to the hydroxy nitrile **36** and the following DIBALH reduction gave the hydroxy aldehyde **38** (64%; direct reduction **33** → **38** proved to be less efficient). After conversion of the formyl substituent in **38** to the *p*-tosylhydrazone derivative

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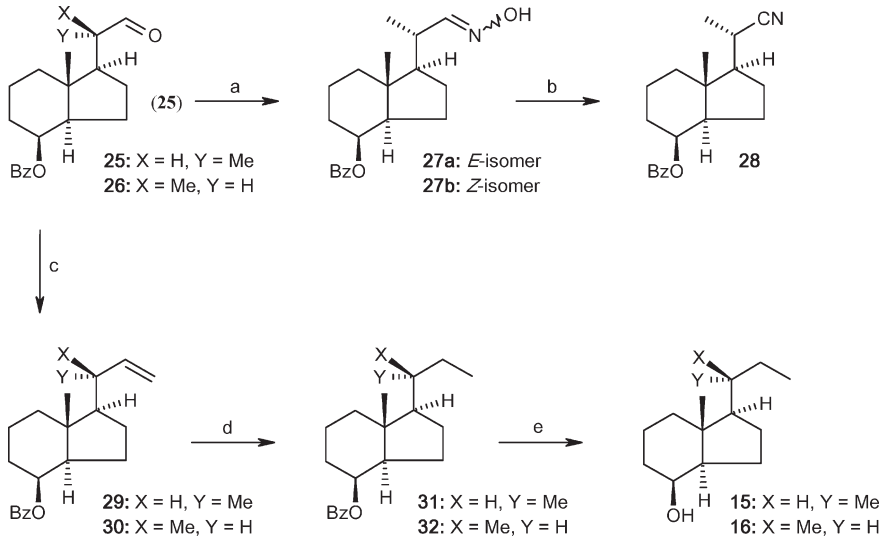
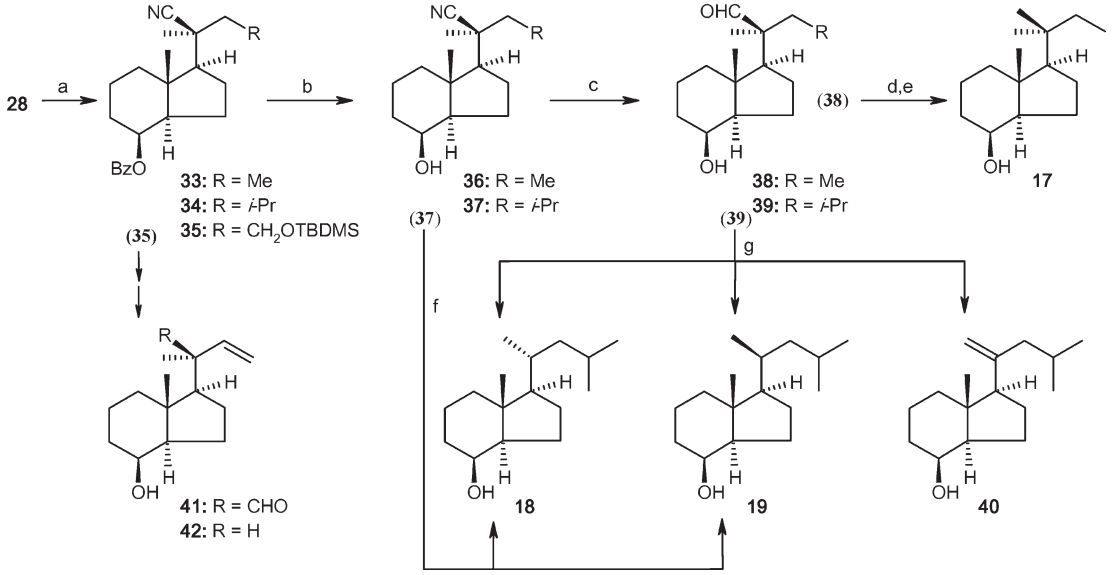
Scheme 1[a]



[a] (a) $NH_2OH \times HCl$, py, 89%; (b) Ac_2O, 91%; (c) $Ph_3P^+ CH_3 Br^-$, *n*-BuLi, THF; (d) H_2/Pd, AcOEt; (e) KOH, MeOH.

Scheme 2[a]



[a] (a) RCH_2Br, LDA, THF; (b) KOH, MeOH; (c) DIBALH, CH_2Cl_2; (d) *p*-$TsNHNH_2$, MeOH, 67%; (e) $NaBH_3CN$, DMF, 65%; (f) K, HMPA, *t*-BuOH, Et_2O, 84%; (g) hν.

and its subsequent reduction with sodium cyanoborohydride to methyl, the desired hydridanol **17** was obtained in 44% overall yield.

For subsequent removal of the cyano group in the prepared tertiary nitriles, we decided to test two synthetic pathways: the reduction with dissolving metal and the less frequently used strategy, involving conversion of the nitrile to the formyl group and the photolysis of the formed aldehyde. The first procedure rules out the presence of the benzoyloxy group in the nitrile molecule because it could be reduced by alkali metal to the hydrocarbon.[23] Thus, the reduction of the hydroxy nitrile **37** with a branched side chain was performed by its treatment with potassium in ether and hexamethylphosphoric triamide.[24] The reaction resulted in the efficient formation of two epimeric products **18** and **19** (in 84% yield) possessing alkyl side chains. However, no stereoselectivity was found in the examined nitrile removal process; both epimers were formed in virtually identical quantities.

Pursuing the second synthetic strategy, aldehyde **39**, prepared by DIBALH reduction of hydroxy nitrile **37**, was subjected to photolysis in hexane solution under an oxygen-free atmosphere at 0 °C, and the obtained complex mixture of products was separated by column chromatography and HPLC. According to our expectations, the UV irradiation resulted mainly in the formation of the desired compounds **18** and **19**. However, the photochemical removal of carbon oxide from aldehyde **39** occurred

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nonstereoselectively, and the yield of these photoproducts (20%, ca. 1:1) was disappointing. Similar decarbonylation processes of 10β-formyl steroids (19-aldehydes) in 5α- and 5β-series, as reported in the literature, were much more efficient.[25] The mechanism of such a photochemical process is well-known and involves radical cleavage of the bond between the carbonyl group and α-carbon atom (Norrish type I reaction)[26] in the excited aldehyde or ketone, with the following extrusion of carbon monoxide and recombination of the radicals (or hydrogen incorporation from the solvent). Rather to our surprise, among the isolated and characterized products of the examined photolysis of **39**, the olefinic compound **40** (12%) was also present, resulting from the extrusion of formaldehyde molecule. Most likely, in this case, an alternative chain scission process operates (Norrish type III reaction),[26,27] involving abstraction of the β-hydrogen by the oxygen atom of the excited aldehyde **39** and cleavage of the resulting diradical to give the olefin **40** and hydroxycarbene,[28] which further photochemically rearranges to formaldehyde.[29] Although theoretically possible,[30] such photodissociation processes were very rarely observed during the photolysis of aliphatic aldehydes.[31,32] In our case, the observed preferential formation of the least substituted olefin **40** can be explained by conformational analysis of the aldehyde **39**, possessing a formyl group attached to the tertiary carbon. Assuming that the preferred ground-state conformations of the aldehyde can be correlated with an outcome of its intramolecular photoreactions, a preference for hydrogen abstraction from the 2-methyl group emerges from molecular modeling (HyperChem release 7.0) indicating that a side-chain rotamer of **39**, characterized by the synperiplanar orientation of the carbonyl group with respect to C(2)-CH_3, is energetically favored.

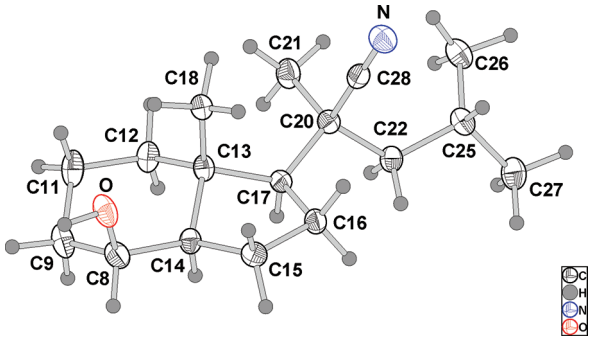
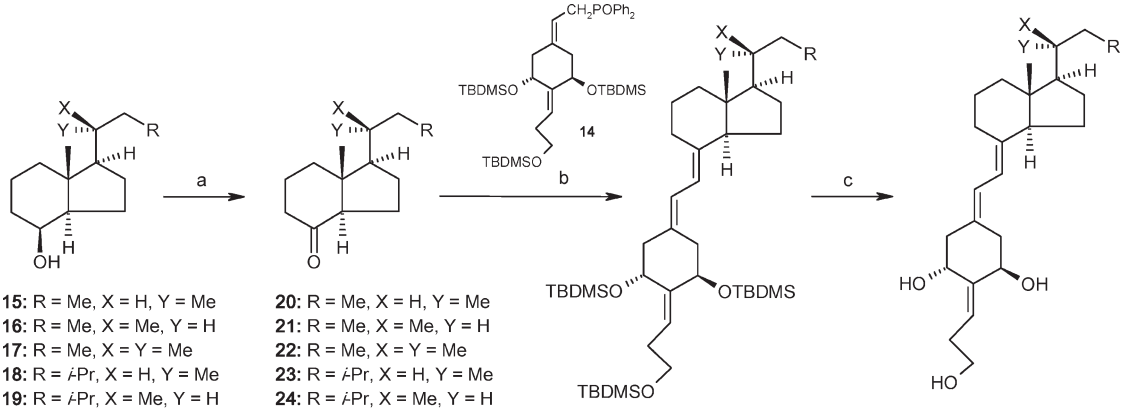


Figure 2. ORTEP derived from the single-crystal X-ray analysis of hydroxy nitrile **37** (one of the two independent structures, slightly differing in their side chain conformation, which were found in the asymmetric part of the unit cell).

These results prompted us to examine also the stereochemical course of the photochemically induced decarbonylation reaction occurring with the β,γ-unsaturated aldehyde **41**, and this model compound was easily synthesized from **35** (unpublished results). Its irradiation with UV light resulted in smooth decarbonylation leading to the hydrindanol **42** (59%) with an unsaturated side chain. Even careful examination of the reaction mixture by HPLC did not indicate the presence of the epimeric product. These results are in agreement with the data reported in the literature.[25,33] The results of the photolysis of **41** presented here could also be explained by molecular modeling. As in the case of **39**, the least-energy side-chain rotamer of **41** has a synperiplanar orientation of the carbonyl group with respect to C(2)-CH_3. Assuming that such an excited rotamer then undergoes an α-cleavage (Norrish type I) process, the aldehyde hydrogen is suitably oriented in this biradical intermediate for a selective incorporation at the α-carbon upon the extrusion of carbon monoxide. Although such a stereoselective photodecarbonylation process occurring in a conformationally labile system is of interest, its application to the construction of the steroidal alkylidene side chains would be limited because of the alternative use of the Wittig reactions of 20-aldehydes.

Successful preparation of hydrindanols **15**−**19** allowed us to accomplish the last steps of the synthesis of the desired vitamin D analogues. Oxidation of these alcohols with tetrapropylammonium perruthenate furnished the respective Grundmann ketones **20**−**24** (Scheme 3), and the obtained CD-ring fragments were coupled with the lithium phosphinoxy carbanion generated from the phosphine oxide **14**, to give the protected vitamin D compounds **43**−**47**. These, in turn, after treatment with tetrabutylammonium

Scheme 3[a]



15: R = Me, X = H, Y = Me
16: R = Me, X = Me, Y = H
17: R = Me, X = Y = Me
18: R = *i*-Pr, X = H, Y = Me
19: R = *i*-Pr, X = Me, Y = H

20: R = Me, X = H, Y = Me
21: R = Me, X = Me, Y = H
22: R = Me, X = Y = Me
23: R = *i*-Pr, X = H, Y = Me
24: R = *i*-Pr, X = Me, Y = H

43: R = Me, X = H, Y = Me
44: R = Me, X = Me, Y = H
45: R = Me, X = Y = Me
46: R = *i*-Pr, X = H, Y = Me
47: R = *i*-Pr, X = Me, Y = H

9: R = Me, X = H, Y = Me
10: R = Me, X = Me, Y = H
11: R = Me, X = Y = Me
12: R = *i*-Pr, X = H, Y = Me
13: R = *i*-Pr, X = Me, Y = H

[a] (a) NMO, TPAP, CH_2Cl_2; (b) **14**, PhLi, THF; (c) *n*-Bu_4NF, THF.

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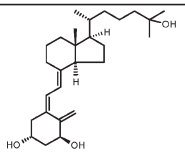
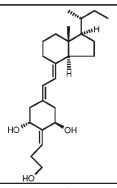
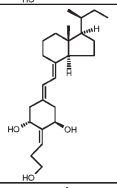
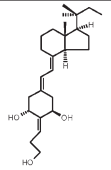
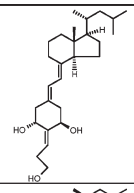
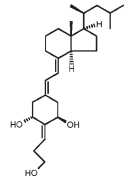
Table 1. VDR Binding Properties,[a] HL-60 Differentiating Activities,[b] and Transcriptional Activities[c] of the Vitamin D Analogues 9−13

		VDR binding		HL-60 differentiation		24-OHase transcription	
Compound	Compd No.	K_i (nM)	Ratio	ED_{50} (nM)	ratio	ED_{50} (nM)	ratio
	**1**	0.1	1	1	1	0.2	1
	**9**	0.3	0.3	8	0.13	2	0.1
	**10**	0.3	0.3	5	0.2	2	0.1
	**11**	0.3	0.3	7	0.14	2	0.1
	**12**	3	0.03	600	0.002	100	0.002
	**13**	0.4	0.25	20	0.05	8	0.025

[a] Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues to the full-length recombinant rat vitamin D receptor. The experiments were carried out in duplicate on two different occasions. The K_i values are derived from dose−response curves and represent the inhibition constant when radiolabeled 1α,25-$(OH)_2D_3$ is present at 1 nM and a K_d of 0.2 nM is used. The binding ratio is the average ratio of the 1α,25-$(OH)_2D_3$ K_i to the K_i for the analogue. [b] Induction of differentiation of HL-60 promyelocytes to monocytes by 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated in duplicate two times. The ED_{50} values are derived from dose−response curves and represent the analogue concentration capable of inducing 50% maturation. The differentiation activity ratio is the average ratio of the 1α,25-$(OH)_2D_3$ ED_{50} to the ED_{50} for the analogue. [c] Transcriptional assay in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. The ED_{50} values are derived from dose−response curves and represent the analogue concentration capable of increasing the luciferase activity 50%. The luciferase activity ratio is the average ratio of the 1α,25-$(OH)_2D_3$ ED_{50} to the ED_{50} for the analogue.

fluoride, afforded the final 2-(3′-hydroxypropylidene)-1α-hydroxy-19-norvitamin D compounds **9**−**13**.

Biological Evaluation. Biological activity of the side chain modifications in the synthesized 2-(3′-hydroxypropylidene)-19-norvitamin D analogues **9**−**13** were determined using both in vitro and in vivo methods. Deletion of 2 or even 4 carbons from the vitamin D_3 side chain and an absence of 25-hydroxyl, commonly considered as crucial for the VDR binding, had little effect on the affinity for the receptor. Thus, all tested vitamins, with a notable exception for analogue **12**, bound equally well to the full length

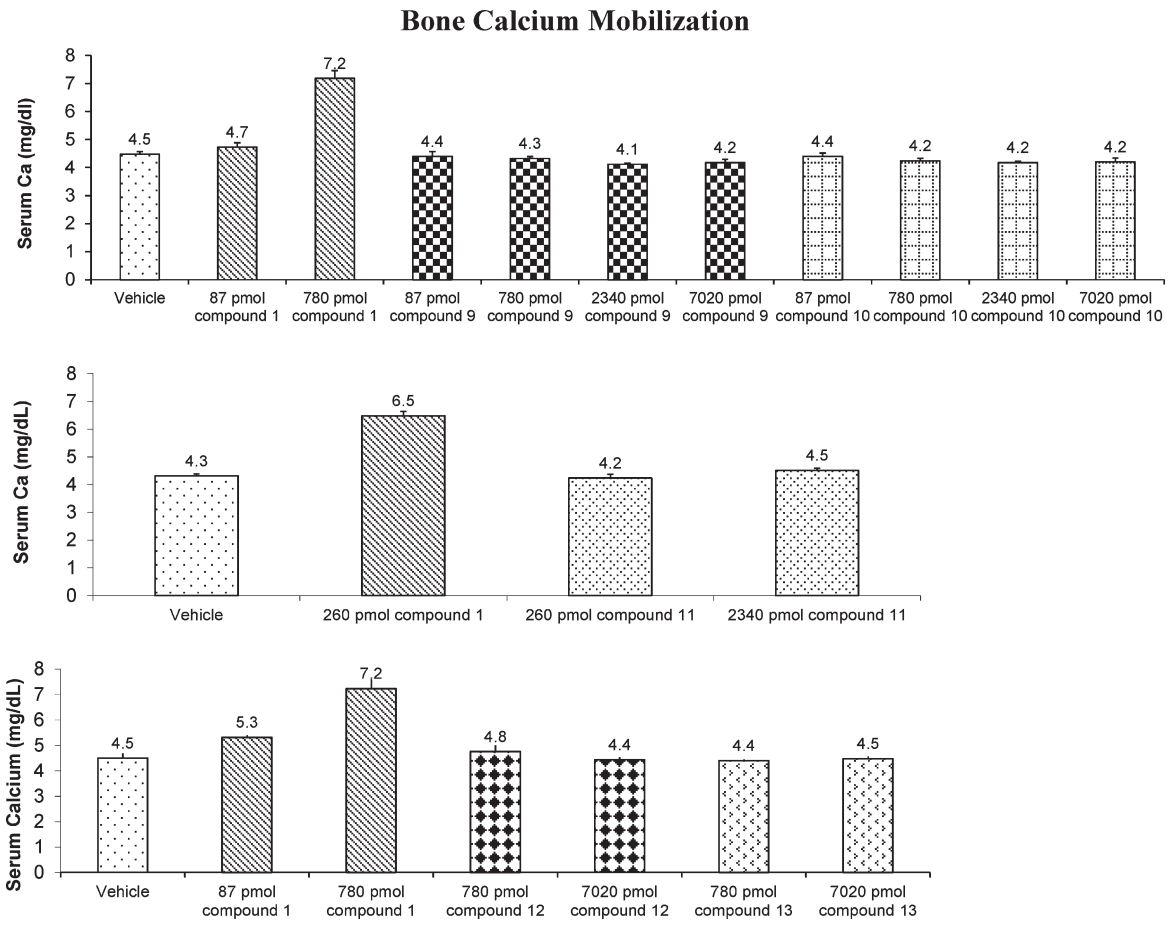


Figure 3. Bone calcium mobilization activity of calcitriol (**1**) and the synthesized analogues **9**−**13**.

recombinant rat vitamin D receptor, being only ca. 3 times less active than 1α,25-$(OH)_2D_3$ (Table 1). It was established that compounds **9**, **10**, and **11** were also approximately equal in their ability to elicit cellular differentiation of human promyelocytic HL-60 cells into monocytes showing only 5−8 times lower activity than the natural hormone **1**. Not surprisingly, these vitamins tested in the transcription assay showed a similar activity in inducing transcription of a vitamin D-responsive gene, i.e., the 24-hydroxylase (CYP-24) promoter driving the luciferase reporter gene system. The 20*R*-compound **12**, possessing an *iso*-butyl fragment attached to C-20, showed the reduction of receptor affinity by 20-fold. Moreover, its cell differentiation and transcriptional activities were decreased 600- and 500-fold, respectively. Except for this analogue, all analogues prepared had significant in vitro activity. On the basis of their in vitro activity, it was expected that some in vivo activity would be found. Surprisingly, none of the synthesized compounds **9**−**13** showed an ability to mobilize calcium from bone, even at high doses (Figure 3). Similarly, low doses of the tested compounds failed to support intestinal calcium transport (Figure 4). Of considerable importance was finding that compound **9** and its homologue **11**, substituted at C-20 with two methyl groups, showed little or no intestinal calcium transport activity. However, compounds **10** and **12** did have some intestinal calcium transport activity at high doses. Vitamins differing in their configurations at C-20 (**9** versus **10** and **12** versus **13**) revealed that 20*S*-epimers are characterized by higher intestinal activity. Also, the elongation of 17β-alkyl substituent seems to enhance the biological effect in vivo. We previously prepared 2-(3′-hydroxypropylidene)-1α,25-dihydroxyvitamin D_3 and showed it to be more potent than 1α,25-$(OH)_2D_3$ on both intestine and bone.[19] The absence of a full chain in this series clearly eliminates intestinal and bone activities in vivo, despite their similarity in in-vitro measurements.

Discussion. The presented results show that an α-alkylation process of the 22-nitrile can provide the desired extension of the steroidal 17β-substituent by the attachment of the different (even branched) alkyl fragments. Such a side-chain construction strategy can be used if both series of epimeric compounds, differing in configuration at C-20, are of interest. Although isomer separation is necessary, this synthetic path is comparable with other methods, for instance, those involving equilibration of 22-aldehydes and their reduction and separation of epimeric 22-alcohols.

All vitamin D analogues described in this work represent a new class of hybrid compounds with structural modifications combining the presence of a long 3′-hydroxypropylidene substituent at C-2 and an abbreviated 17β-side chain. The synthesized compounds have significantly diminished calcemic activity, but, at the same time, they are characterized by very good binding ability to VDR and possess high cell differentiation and transcriptional activities. These properties suggest that they might be useful in suppressing cancer growth or some other disease where calcemic activity is not desired.

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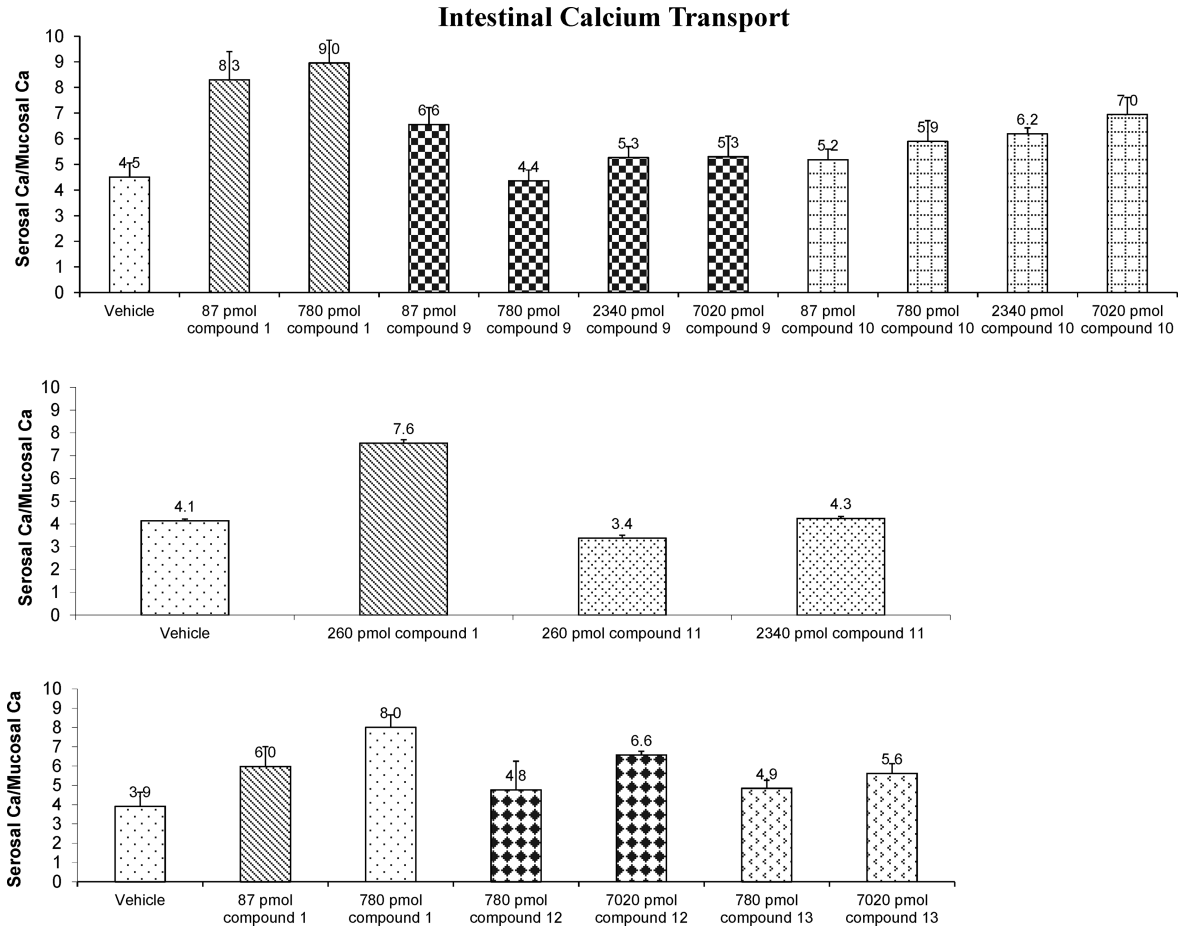

Figure 4. Intestinal calcium transport activity of calcitriol (**1**) and the synthesized analogues **9**−**13**.

CONCLUSIONS

The vitamin D side-chain modification consisting in significant shortening to the small branched alkyl fragments containing no hydroxyls can be very beneficial for the biological properties of the analogues, making them possible candidates for therapeutic use. Such truncation of the normal side chain present, for example, in higly calcemic compounds as 2MD or 2-(3′-hydroxypropylidene)-19-norvitamins **7** and **8**, can result in noncalcemic compounds still retaining other genomic activities.

A significant potency of the synthesized vitamin D analogues **9** and **11**, possessing the shortened 17β-side chain, in displacement of the radiolabeled 1α,25-$(OH)_2D_3$ from the receptor protein, as well as their high cellular and transcriptional potency combined with lack of ability to raise serum calcium concentration by either a bone mobilization or intestinal transport, suggests that both compounds could be effective in psoriasis or as anticancer agents.

EXPERIMENTAL SECTION

Chemistry. Melting points (uncorrected) were determined on a Thomas-Hoover capillary melting-point apparatus. Ultraviolet (UV) absorption spectra were recorded with a Perkin-Elmer Lambda 3B UV−vis spectrophotometer in ethanol. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 400 and 500 MHz with Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers in deuteriochloroform. Chemical shifts (δ) are reported downfield to internal Me_4Si (δ 0.00). Electron impact (EI) mass spectra were obtained with a Micromass AutoSpec (Beverly, MA) instrument. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model U6K Universal injector, and a Model 486 tunable absorbance detector. THF was freshly distilled prior to use from sodium benzophenone ketyl under argon.

Preparation of the phosphine oxide **14** from commercial (−)-quinic acid was reported by us previously.[19] The starting 22-aldehydes **25** and **26** were obtained from vitamin D_2[21,22] according to the published procedures. The aldehyde **41** was prepared from **35** (Supporting Information).

The purity of final compounds was determined by HPLC, and they were judged to be at least 99% pure. Two HPLC systems (straight phase, hexane/2-propanol, and reversed-phase, water/methanol) were employed as indicated in Table 1 (Supporting Information). The purity and identity of the synthesized vitamins were additionally confirmed by inspection of their ^{1}H NMR and high-resolution mass spectra.

Compound **41**: ^{1}H NMR (400 MHz, $CDCl_3$) δ, 0.989 (3H, s, 7a′-CH_3), 1.240 (3H, s, 2-CH_3), 4.10 (1H, narr m, 4′α-H), 5.04 (1H, dd, J = 17.6, 0.9 Hz, 4-H_Z), 5.22 (1H, dd, J = 10.9, 0.9 Hz, 4-H_E), 6.07 (1H, dd, J = 17.6, 10.9 Hz, 3-H), 9.51 (1H, s, CHO).

(*E*)- and (*Z*)-Benzoic Acid (1*R*,3a*R*,4*S*,7a*R*)-1-[(2′-Hydroxyimino-1′-methyl)ethyl]-7a-methyl-octahydro-inden-4-yl Esters (27a,b). To a solution of aldehyde **25** (284 mg, 0.90 mmol) in anhydrous pyridine (5 mL) was added $NH_2OH \times HCl$ (210 mg). The mixture was stirred at room temperature for 20 h, poured into water, and extracted using ethyl acetate. The combined organic phases were separated, washed with saturated $NaHCO_3$, water, and saturated $CuSO_4$, dried ($MgSO_4$), and evaporated. The oily residue was purified using

column chromatography on silica. Elution with hexane/ethyl acetate (9:1) gave pure isomeric oximes, the less polar **27a** (167 mg), and the more polar **27b** (105 mg; total yield 89%).

Benzoic Acid (1*R*,3a*R*,4*S*,7a*R*)-1-[(*R*)-1′-Cyano-ethyl]-7a-methyl-octahydro-inden-4-yl Ester (28). The solution of oximes **27a**,**b** (1.6:1; 248 mg, 0.75 mmol) in acetic anhydride (8 mL) was refluxed for 1.5 h. The reaction mixture was cooled, poured carefully on ice, and extracted with toluene. The extracts were combined, washed with water, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The residue was applied on a silica Sep-Pak (5 g). Elution with hexane/ethyl acetate (95:5) gave the pure, semicrystalline nitrile **28** (212 mg, 91%).

Benzoic Acid (1*R*,3a*R*,4*S*,7a*R*)-7a-Methyl-1-[(*R*)-1′-methyl-prop-2′-enyl]-octahydro-inden-4-yl Ester (29). To the methyltriphenylphoshonium bromide (31 mg, 87 μmol) in anhydrous THF (0.5 mL) at 0 °C was added dropwise *n*-BuLi (2.65 M in hexanes, 64 μL, 0.170 mmol) under argon with stirring. After 5 min, another portion of $Ph_3P^+CH_3\ Br^-$ was added (31 mg, 87 μmol), and the solution was stirred at 0 °C for 10 min and then at room temperature for 20 min. The orange-red mixture was cooled to −78 °C and siphoned to a solution of aldehyde **25** (33 mg, 0.109 mmol) in anhydrous THF (0.1 mL). The reaction mixture was stirred at −78 °C and stopped by the addition of brine containing 1% HCl 3 h after the addition of the first portion of the Wittig reagent. Ethyl acetate (3 mL), benzene (2 mL), ether (1 mL), saturated $NaHCO_3$ (1 mL), and water (1 mL) were added, and the mixture was vigorously stirred at room temperature for 18 h. Then, the organic phase was separated, washed with brine, dried ($MgSO_4$), and evaporated. The oily residue was filtered through a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (99:1) resulted in the pure olefinic product **29** (19 mg, 68%).

Benzoic Acid (1*R*,3a*R*,4*S*,7a*R*)-7a-Methyl-1-[(*S*)-1′-methyl-prop-2′-enyl]-octahydro-inden-4-yl Ester (30). Wittig reaction of aldehyde **26**, performed as described above for **25**, gave an olefinic product that was purified on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (98:2) provided pure compound **30** (73%).

Benzoic Acid (1*R*,3a*R*,4*S*,7a*R*)-1-[(*R*)-1′-Methyl-propyl]-7a-methyl-octahydro-inden-4-yl Ester (31). To a solution of olefin **29** (45 mg, 0.146 mmol) in ethyl acetate (5.5 mL) was added Pd/C (10%, 27 mg), and the resultant suspension was stirred under constant flow of hydrogen at room temperature for 19 h. Then, the suspension was filtered, the filtrate was evaporated and applied to a silica Sep-Pak cartridge (2 g). Elution with hexane/ethyl acetate (96:4) gave the pure, oily ester **31** (40 mg, 87%).

Benzoic Acid (1*R*,3a*R*,4*S*,7a*R*)-1-[(*S*)-1′-Methyl-propyl]-7a-methyl-octahydro-inden-4-yl Ester (32). Hydrogenation of olefin **30** was performed as described for **29**. The crude product was purified on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (97:3) yielded the pure, oily ester **32** (86%).

(1*R*,3a*R*,4*S*,7a*R*)-1-[(*R*)-1′-Methyl-propyl]-7a-methyl-octahydro-inden-4-ol (15). Solution of the ester **31** (40 mg, 0.129 mmol) in 10% methanolic KOH (2 mL) was heated at 50 °C for 24 h, poured into water, and extracted with ethyl acetate. The organic phase was washed with water and then dried ($MgSO_4$) and evaporated. The oily residue was applied on a silica Sep-Pak (2 g) and eluted with hexane/ethyl acetate (96:4) to give the pure product **15** (22 mg, 81%).

(1*R*,3a*R*,4*S*,7a*R*)-1-[(*S*)-1′-Methyl-propyl]-7a-methyl-octahydro-inden-4-ol (16). Alkaline hydrolysis of the ester **32**, performed as described for **31**, gave the crude hydrindanol that was purified on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (97:3) furnished the pure alcohol **16** (80%).

Benzoic Acid (1*S*,3a*R*,4*S*,7a*R*)-1-[(*S*)-1′-Cyano-1′-methyl-propyl]-7a-methyl-octahydro-inden-4-yl Ester (33). *n*-Butyllithium (1.6 M in hexanes, 1.0 mL, 1.6 mmol) was added at 0 °C to the flask containing diisopropylamine (262 μL, 1.54 mmol) and THF (2 mL). The solution was stirred at 0 °C for 20 min, cooled to −78 °C, and siphoned to the solution of **28** (430 mg, 1.31 mmol) in THF (1.5 mL). The resulting yellow mixture was stirred for 30 min, then HMPA (600 μL) was added, and stirring was continued for another 15 min. Then, CH_3CH_2Br (310 μL, 4.08 mmol) was added, and the solution was stirred at −78 °C for 40 min. Saturated NH_4Cl was added, and the mixture was extracted with ethyl acetate. The combined organic phases were washed with water, dried ($MgSO_4$), and evaporated. The residue was subjected to column chromatography on silica. Elution with hexane/ethyl acetate (95:5) gave the pure compound **33** (280 mg, 60%; 80% based on recovered substrate). Further elution with hexane/ethyl acetate (95:5) gave unreacted **28** (107 mg).

Benzoic Acid (1*S*,3a*R*,4*S*,7a*R*)-1-[(*S*)-1′-Cyano-1′,3′-dimethyl-butyl]-7a-methyl-octahydro-inden-4-yl Ester (34). Alkylation of the nitrile **28** with $(CH_3)_2CHCH_2Br$ was performed analogously as described above for the preparation of **33**, except that after the addition of *iso*-butyl bromide, the reaction mixture was allowed to warm up to −40 °C during 1 h. The crude product was applied to a silica Sep-Pak (2 g) and eluted with hexane/ethyl acetate (98:2) to give a pure semicrystalline compound **34** (60 mg, 66%; 74% based on recovered substrate); further elution with hexane/ethyl acetate (97:3) gave unreacted **28** (8.5 mg).

Benzoic Acid (1*S*,3a*R*,4*S*,7a*R*)-1-[(*S*)-3′-[(*tert*-Butyldimethyl-silyl)oxy]-1′-cyano-1′-methyl-propyl]-7a-methyl-octahydro-inden-4-yl Ester (35). Alkylation of the nitrile **28** with $BrCH_2$-$CH_2OTBDMS$ was performed analogously as described above for the preparation of **33**. The crude product was applied to a silica Sep-Pak (5 g) and eluted with hexane/ethyl acetate (96:4) to give the compound **35** as an oil (15 mg, 45%).

(*S*)-2-[(1′*S*,3a′*R*,4′*S*,7a′*R*)-4′-Hydroxy-7a′-methyl-octahydro-inden-1′-yl]-2-methyl-butylnitrile (36). Alkaline hydrolysis of the benzoyloxy nitrile **33**, performed as described for **31**, gave the crude product that was purified on a silica Sep-Pak (2 g) and eluted with hexane/ethyl acetate (8:2) to furnish the pure hydroxy nitrile **36** (179 mg, 99%).

(*S*)-2-[(1′*S*,3a′*R*,4′*S*,7a′*R*)-4′-Hydroxy-7a′-methyl-octahydro-inden-1′-yl]-2,4-dimethyl-pentanenitrile (37). The benzoyloxy nitrile **34** was treated with 10% methanolic KOH as described above for **33**. The crude product was purified on a silica Sep-Pak (2 g) and eluted with hexane/ethyl acetate (95:5) to afford the pure hydroxy nitrile **37** (92%).

(*S*)-2-[(1′*S*,3a′*R*,4′*S*,7a′*R*)-4′-Hydroxy-7a′-methyl-octahydro-inden-1′-yl]-2-methyl-butyraldehyde (38). To the solution of nitrile **36** (172 mg, 0.773 mmol) in anhydrous methylene chloride (3.3 mL) was slowly added a solution of diisobutylaluminum hydride (1.5 M in toluene; 1.66 mL, 2.3 mmol) at −78 °C. The solution was stirred for 1 h, then it was allowed to warm up to −30 °C during 1 h 30 min and was cooled to −78 °C again. The reaction was quenched by the addition of brine containing 5% HCl, and it was extracted with ethyl acetate. The combined organic layers were washed with $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. The remaining residue was purified on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (8:2) gave the pure hydroxy aldehyde **38** (112 mg, 65%).

(*S*)-2-[(1′*S*,3a′*R*,4′*S*,7a′*R*)-4′-Hydroxy-7a′-methyl-octahydro-inden-1′-yl]-2,4-dimethyl-pentanal (39). Reduction of nitrile **37** performed as described for **36** gave the crude product that was purified on a silica Sep-Pak (2 g) and eluted with hexane/ethyl acetate (95:5) to afford the pure hydroxy aldehyde **39** (60%).

(1*R*,3a*R*,4*S*,7a*R*)-1-(1′,1′-Dimethyl-propyl)-7a-methyl-octahydro-inden-4-ol (17). A solution of aldehyde **38** (10 mg, 0.42 μmol) and *p*-toluenesulfonyl hydrazide (31 mg, 0.168 mmol) in dry methanol (0.5 mL) was stirred with molecular sieves of 4 Å at 55 °C for 19 h. Then, the reaction mixture was cooled, poured into water, and extracted with toluene. The combined organic phases were washed with water, dried

($MgSO_4$), evaporated, and applied on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (85:15) gave *p*-tosylhydrazone (12 mg, 67%) slightly contaminated with *p*-$TsNHNH_2$. This crude tosylhydrazone was dissolved in DMF (0.15 mL), and *p*-TsOH (2 mg, evaporated twice with benzene) was added followed by $NaBH_3CN$ (8 mg, 0.126 mmol). The mixture was stirred at 100 °C for 19 h, then it was cooled, poured into water, and extracted with hexane and ethyl acetate. The combined organic phases were washed with water, dried ($MgSO_4$), and evaporated. The remaining oily residue was applied on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (98:2) gave pure hydrindanol **17** (4 mg, 65%).

(1*R*,3a*R*,4*S*,7a*R*)-1-[(*R*)-1′,3′-Dimethyl-butyl]- and (1*R*,3a*R*,4*S*,7a*R*)-1-[(*S*)-1′,3′-Dimethyl-butyl]-7a-methyl-octahydro-inden-4-ol (18 and 19). (a) A solution of the hydroxy nitrile **37** (49 mg, 0.186 mmol) in *t*-BuOH (50 μL) and ether (200 μL) was added dropwise to a blue mixture of potassium (55 mg, 1.4 mmol) in hexamethylphosphoric triamide (HMPA; 170 μL) and ether (420 μL) at 0 °C under argon with stirring. A cooling bath was removed and stirring continued for 4 h at room temperature. The reaction mixture was diluted using benzene, unreacted potassium was removed, and a few drops of 2-propanol were added. The organic phase was washed using water, dried ($MgSO_4$), and evaporated. The residue was applied to a silica Sep-Pak (2 g) and eluted with hexane/ethyl acetate (95:5) to give a 1:1 mixture of epimeric alcohols **18** and **19** (37 mg, 84%).

(b) A solution of the hydroxy aldehyde **39** (20 mg, 0.084 mmol) in hexane (350 mL) was irradiated with a 350 W Hanau S 81 mercury arc lamp in an apparatus consisting of a Pyrex vessel with a water-cooled Vycor immersion well. A slow stream of argon was passed into the vessel, and the temperature of the solution was maintained at 0 °C. The solution was irradiated for 70 min and allowed to warm up to room temperature. Then, the solution was concentrated under vacuum and the residue applied on a silica column. Elution with hexane/ethyl acetate (95:5) gave a mixture of three compounds (5 mg). Separation of the products was achieved by HPLC (10 mm × 25 cm, Zorbax-Sil column, 4 mL/min) using a hexane/ethyl acetate (88:12) solvent system. A 1:1 mixture of the epimeric alcohols **18** and **19** (2.8 mg, 15%) was collected at R_v 30 mL, and the olefinic product **40** (1.7 mg, 9%) was collected at R_v 34 mL.

(1*R*,3a*R*,4*S*,7a*R*)-7a-Methyl-1-[(*R*)-1′-methyl-allyl)-octahydro-inden-4-ol (42). A solution of hydroxy aldehyde **41** in hexane was irradiated for 25 min with a 350 W Hanau S 81 mercury arc lamp in an analogous manner as was described above for **38**. The solution was concentrated under vacuum, and the residue was applied to a silica Sep-Pak (2 g) and eluted with hexane/ethyl acetate (97:3) to afford unsaturated alcohol **42** (60%).

(1*R*,3a*R*,4*S*,7a*R*)-1-[(*R*)-1′-Methyl-propyl]-7a-methyl-octahydro-inden-4-one (20). To a solution of NMO (23 mg) in methylene chloride (0.9 mL) were added molecular sieves of 4 Å (120 mg), and the mixture was stirred at room temperature for 15 min. Then, a solution of hydrindanol **15** (21 mg, 0.10 mmol) in methylene chloride (0.15 mL) was added followed by tetrapropylammonium perruthenate (TPAP; 2.5 mg). The resulted dark mixture was stirred for 30 min and applied on a silica Sep-Pak (2 g). Elution using hexane/ethyl acetate (95:5) gave pure ketone **20** (18.5 mg, 88%).

(1*R*,3a*R*,4*S*,7a*R*)-1-[(*S*)-1′-methyl-propyl]-7a-methyl-octahydro-inden-4-one (21). Oxidation of hydrindanol **16** with TPAP was performed in a manner analogous to that for the isomeric **15**. The crude hydrindanone was purified on silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (96:4) gave pure ketone **21** (82%).

(1*R*,3a*R*,7a*R*)-1-(1′,1′-Dimethyl-propyl)-7a-methyl-octahydro-inden-4-one (22). Oxidation of hydrindanol **17** with TPAP was performed in a manner analogous to that for the isomeric **15**. The crude hydrindanone was purified on silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (96:4) gave pure ketone **22** (59%).

(1*R*,3a*R*,7a*R*)-1-[(*R*)-1′,3′-Dimethyl-butyl]- and (1*R*,3a*R*,7a*R*)-1-[(*S*)-1′,3′-Dimethyl-butyl]-7a-methyl-octahydro-inden-4-one (23 and 24). The mixture of alcohols **18** and **19** (1:1) was oxidized with TPAP as described above for **15**. The crude products were purified on a silica Sep-Pak (2 g). Elution with methylene chloride gave a 1:1 mixture of epimeric ketones **23** and **24** (91%). Separation of isomers was achieved by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/ethyl acetate (95:5) solvent system. The (20*S*)-ketone **24** was collected at R_V 39 mL and the *R*-isomer **23** at R_V 40 mL.

1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]-19,24,25,26,27-pentanorvitamin D_3 *tert*-Butyldimethylsilyl Ether (43). To a solution of phosphine oxide **14** (11.5 mg, 15.6 μmol) in anhydrous THF (0.30 mL) at −78 °C, phenyllithium (1.8 M in butyl ether, 9 μL, 16 μmol) was slowly added under argon with stirring. The solution turned deep orange. The mixture was stirred at −78 °C for 20 min. A precooled (−78 °C) solution of ketone **20** (19 mg, 91 μmol) in anhydrous THF (0.10 mL) was slowly added. The mixture was stirred under argon at −78 °C for 2 h and at 6 °C for 16 h. Ethyl acetate and water were added, and the organic phase was washed with brine, dried with $MgSO_4$, and evaporated. The residue was dissolved in hexane, applied on a silica column, and washed with hexane/ethyl acetate (98.5:1.5) to produce silylated vitamin **43** (1.44 mg, 13%; 20% based on recovered **14**). The column was then washed with hexane/ethyl acetate (95:5) to recover a portion of unchanged C,D-ring ketone **20** (7 mg), and hexane/ethyl acetate (6:4) was used to recover diphenylphosphine oxide **14** (4.2 mg).

(20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]-19,24,25,26,27-pentanorvitamin D_3 *tert*-Butyldimethylsilyl Ether (44). Silylated 19-norvitamin D compound **44** was obtained by Wittig−Horner coupling of hydrindanone **21** with the phosphine oxide **14** performed analogously to the process described above for the preparation of **43**. After separation of the reaction mixture by column chromatography on silica, using hexane/ethyl acetate (98.5:1.5) as an eluent, the protected vitamin **44** was obtained (19%; 30% based on recovered **14**).

1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]-20-methyl-19,24,25,26,27-pentanorvitamin D_3 *tert*-Butyldimethylsilyl Ether (45). Silylated 19-norvitamin D compound **45** was obtained by Wittig−Horner coupling of hydrindanone **22** with the phosphine oxide **14** performed analogously to the process described above for the preparation of **43**. After separation of the reaction mixture by column chromatography on silica, using hexane/ethyl acetate (99.5:0.5) as an eluent, the protected vitamin **45** was obtained (48%).

1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]-19,23,24-trinorvitamin D_3 *tert*-Butyldimethylsilyl Ether (46). Silylated 19-norvitamin D compound **46** was obtained by Wittig−Horner coupling of hydrindanone **23** with the phosphine oxide **14** performed analogously to the process described above for the preparation of **43**. After separation of the reaction mixture by column chromatography on silica, using hexane/ethyl acetate (98.5:1.5) as an eluent, the protected vitamin **46** was obtained (50%; 83% based on recovered **23**).

(20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]-19,23,24-trinorvitamin D_3 *tert*-Butyldimethylsilyl Ether (47). Silylated 19-norvitamin D compound **47** was obtained by Wittig−Horner coupling of hydrindanone **24** with the phosphine oxide **14** performed analogously to the process described above for the preparation of **43**. After separation of the reaction mixture by column chromatography on silica, using hexane/ethyl acetate (98.5:1.5) as an eluent, the protected vitamin **47** was obtained (53%; 62% based on recovered **14**).

1α-Hydroxy-2-(3′-hydroxypropylidene)-19,24,25,26,27-pentanorvitamin D_3 (9). To a solution of the protected vitamin **43**

(1.4 mg, 1.91 μmol) in anhydrous THF (1.3 mL), tetrabutylammonium fluoride (1.0 M in THF, 86 μL, 86 μmol) and triethylamine (16 μL) were added. The mixture was stirred under argon at room temperature for 18 h, poured into brine, and extracted using ethyl acetate and diethyl ether. The organic extracts were washed with brine, dried using $MgSO_4$, and evaporated. The residue was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. Pure 19-norvitamin **9** (0.56 mg, 72%) was collected at R_V 25.5 mL. On a reversed-phase HPLC (9.4 mm × 25 cm Eclipse XDB-C18 column, 4 mL/min) using a methanol/water (95:5) solvent system, compound **9** was collected at R_V 42 mL.

(20*S*)-1α-Hydroxy-2-(3′-hydroxypropylidene)-19,24,25,26,27-pentanorvitamin D_3 (10). Treatment of the protected vitamin **44** with tetrabutylammonium fluoride, performed as described for **43**, gave a vitamin D compound that was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. Pure 19-norvitamin **10** (53%) was collected at R_V 25.5 mL. On a reversed-phase HPLC (9.4 mm × 25 cm Eclipse XDB-C18 column, 4 mL/min) using a methanol/water (95:5) solvent system, vitamin **10** was collected at R_V 42 mL.

1α-Hydroxy-2-(3′-hydroxypropylidene)-20-methyl-19,24,25,26,27-pentanorvitamin D_3 (11). Treatment of the protected vitamin **45** with tetrabutylammonium fluoride, performed as described for **43**, gave a vitamin D compound that was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (7:3) solvent system. Pure 19-norvitamin **11** (75%) was collected at R_V 24.5 mL. On a reversed-phase HPLC (9.4 mm × 25 cm Eclipse XDB-C18 column, 4 mL/min) using a methanol/water (95:5) solvent system, vitamin **11** was collected at R_V 27 mL.

1α-Hydroxy-2-(3′-hydroxypropylidene)-19,23,24-trinorvitamin D_3 (12). Treatment of the protected vitamin **46** with tetrabutylammonium fluoride, performed as described for **43**, gave a vitamin D compound that was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (7:3) solvent system. Pure 19-norvitamin **12** (98%) was collected at R_V 24 mL. On a reversed-phase HPLC (9.4 mm × 25 cm Eclipse XDB-C18 column, 4 mL/min), using a methanol/water (95:5) solvent system, vitamin **12** was collected at R_V 31.5 mL.

(20*S*)-1α-Hydroxy-2-(3′-hydroxypropylidene)-19,23,24-trinorvitamin D_3 (13). Treatment of the protected vitamin **47** with tetrabutylammonium fluoride, performed as described for **43**, gave a vitamin D compound that was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (7:3) solvent system. Pure 19-norvitamin **13** (98%) was collected at R_V 24 mL. On a reversed-phase HPLC (9.4 mm × 25 cm Eclipse XDB-C18 column, 4 mL/min) using a methanol/water (95:5) solvent system, vitamin **13** was collected at R_V 30 mL.

Biological Studies. *In Vitro Studies.* VDR binding, HL-60 differentiation, and 24-hydroxylase transcription assays were performed as previously described.[19]

In Vivo Studies. Bone Calcium Mobilization and Intestinal Calcium Transport. Male weanling Sprague−Dawley rats were purchased from Harlan (Indianapolis, IN). The animals were group housed and placed on Diet 11 (0.47% Ca) + AEK oil for one week followed by Diet 11 (0.02% Ca) + AEK oil for 3 weeks. The rats were then switched to a diet containing 0.47% Ca^{34} for one week followed by two weeks on a diet containing 0.02% Ca. Dose administration began during the last week on the 0.02% Ca diet. Four consecutive intraperitoneal doses were given approximately 24 h apart. Twenty four hours after the last dose, blood was collected from the severed neck, and the concentration of serum calcium was determined as a measure of bone calcium mobilization. The first 10 cm of the intestine was also collected for the intestinal calcium transport analysis using the everted gut sac method.[19]

Crystallographic Studies. *Crystal Data (For Compound **37**).* $C_{17}H_{29}NO$, M = 263.41, T = 100(2) K, orthorhombic, space group $P2_12_12_1$, Z = 8, a = 6.2985(5), b = 21.9702(16), c = 22.7730(14) Å, $\alpha\beta\gamma$ = 90°, V = 3151.3(4) $Å^3$, D_x = 1.110 $g \cdot cm^{-3}$.

Structure Determination. The data were collected using the Kuma KM4CCD κ-axis diffractometer with graphite-monochromated MoKα radiation. The crystal was positioned at 65 mm from the KM4CCD camera. Frames (1204) were measured at 1.0° intervals with a counting time of 20 s. The data were corrected for Lorentz and polarization effects. No absorption correction was applied. Data collection, cell refinement, and data reduction were carried out with the Kuma Diffraction programs CrysAlis CCD and CrysAlis RED.[35]

The structure was solved by direct methods[36] and refined using SHELXL.[37] The refinement was based on F^2 for all reflections except those with very negative intensities. Weighted R factors wR and all goodness-of-fit S values were based on F^2. Conventional R factors were based on F with F set to zero for negative F^2. The $F_0^2 > 2s(F_0^2)$ criterion was applied only for the R factors calculation and was not relevant to the choice of reflections for the refinement. The R factors based on F^2 are about twice as large as those based on F. All hydrogen atoms were located from a differential map and refined isotropically. Scattering factors were taken from Tables 6.1.1.4 and 4.2.4.2 from the International Tables for Crystallography.[38]

Crystallographic data for the structure reported in this paper has been deposited at the Cambridge Crystallographic Data Centre with deposition number CCDC-838768.

■ ASSOCIATED CONTENT

Supporting Information. Purity criteria of the vitamin D analogues **9**−**13**, their ^1H NMR spectra and spectral data of the all synthesized compounds, experimental procedures for the chemical synthesis of compound **41**, and figures with dose−response curves derived from cellular differentiation assay of the vitamin D analogues **9**−**13**. CCDC-838768 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

$1\alpha,25\text{-}(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; VDR, vitamin D receptor

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