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Removal of the 26-Methyl Group from 19-nor-1α,25-Dihydroxyvitamin D_3 Markedly Reduces in Vivo Calcemic Activity without Altering in Vitro VDR Binding, HL-60 Cell Differentiation, and Transcription

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Twelve new analogues of 19-nor-1α,25-dihydroxyvitamin D_3 (**5**−**16**) were prepared by convergent syntheses, employing the Wittig−Horner reaction. The necessary Grundmann type ketones (**45**−**48**), possessing fixed configurations of the hydroxyl group at C-25, were obtained by a multistep procedure from commercial vitamin D_2 and enantiomers of 1,3-butanediol (**23** and **24**). We have examined the influence of removal of one of the methyl groups located at C-25 on the biological in vitro and in vivo activity. The in vivo tests showed that the synthesized vitamin D compounds (**5**−**16**) exhibit reduced calcemic activity both in bone and in the intestine. However, in vitro potency of 2-methylene and 2α-methyl compounds (**5**−**10**, **13**, and **14**) remained similar or enhanced as compared to that of 1α,25-$(OH)_2D_3$.

Introduction

1α,25-Dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$, calcitriol, **1**; Figure 1], the active form of vitamin D_3, is one of the primary biological regulators of calcium and phosphorus homeostasis in humans and animals.[1−4] Its important biological effects include the inhibition of proliferation and increased differentiation of various malignant cells,[5−8] as well as suppression of the autoimmune disease.[9] Such a broad range of diverse functions suggests enormous therapeutic potential of vitamin D compounds.[10] However, the application of these compounds has been limited by the danger of hypercalcemia. Structural modifications of the vitamin D molecule have led to a search for new analogues that exhibit reduced calcemic potency and selective actions.[11] In 2007, we reported the biological properties of two 2-methylene-19-nor analogues (**2** and **3**) in which the side chains were modified at C-25.[12,13] Removal of the 25-hydroxy group from compound **4**[14] led to reduced activity in vitro, but in vivo calcemic potency remained unchanged likely because of in vivo 25-hydroxylation. Methyl replacement of the 25-hydroxy group in **4** reduced the overall potency but retained bone selectivity (compound **3**). These results suggest that a possible approach to reducing calcemic activity might be the elimination of the methyl groups surrounding C-25. We now report several new analogues produced by removing the 26-methyl group from the 2-carbon-substituted 19-nor-1α,25-dihydroxyvitamin D_3 and its 20S isomer.

Results and Discussion

Chemistry. The synthetic strategy for new analogues (**5**−**16**) was based on the Lythgoe type Horner−Wittig olefination reaction,[15] which was successfully utilized by us earlier for preparation of the vitamin D compounds (**2** and **3**).[12] This approach required using new Grundmann type ketones (**45**−**48**) (Scheme 1), which we intended to prepare from the known alcohols (**17** and **18**) and commercially available enantiomers of 1,3-butanediols (**23** and **24**) (Scheme 2). To obtain C,D ring ketones (**45**−**48**), we employed the Wittig reaction[16] to attach new side chains to the C,D ring aldehydes (**19** and **20**). Commercial (*S*)- and (*R*)-1,3-butanediols (**23** and **24**), respectively, appeared to be suitable starting materials for construction of the side chains, possessing fixed configurations of the hydroxyl group at C-25 (steroidal numbering). Selective tosylation of primary hydroxy groups of diols (**23** and **24**), followed by a triethylsilyl protection of secondary hydroxy groups, provided corresponding tosylates **25** and **26** (Scheme 2). These compounds (**25** and **26**) were converted into primary iodides **27** and **28**, which were treated with triphenylphosphine in acetonitrile under reflux. The triethylsilyl ethers appeared to be unstable under these reaction conditions, which led to the generation of hydroxyphosphonium iodides **21** and **22**. Despite the loss of the protective group, we used the salts **21** and **22**, based on the efficient application of the analogous salt in the synthesis of the Windaus−Grundmann ketone as described by Mourino.[17]

The C,D ring aldehydes **19** and **20** were obtained by the oxidation of the corresponding alcohols **17** and **18**, which were previously prepared in our laboratory from commercial vitamin D_2.[12] The Wittig reaction between compounds **19** and **20** and ylides, generated from the hydroxyphosphonium iodides **21** and **22** by *n*-butyllithium, provided only olefinic products (**29**−**32**) with the *E*-geometry of the introduced double bond. The catalytic hydrogenation of the compounds (**29**−**32**) furnished the tertiary alcohols **33**−**36**, which were protected as *tert*-butyldimethylsilyl ethers (**37**−**40**). The removal of the benzoyl group under basic conditions gave the secondary alcohols **41**−**44**, which were subjected to catalytic oxidation with tetrapropylammonium perruthenate[18] in the presence of 4-methylmorpholine *N*-oxide and afforded the expected ketones **45**−**48**. The Horner−Wittig reaction between

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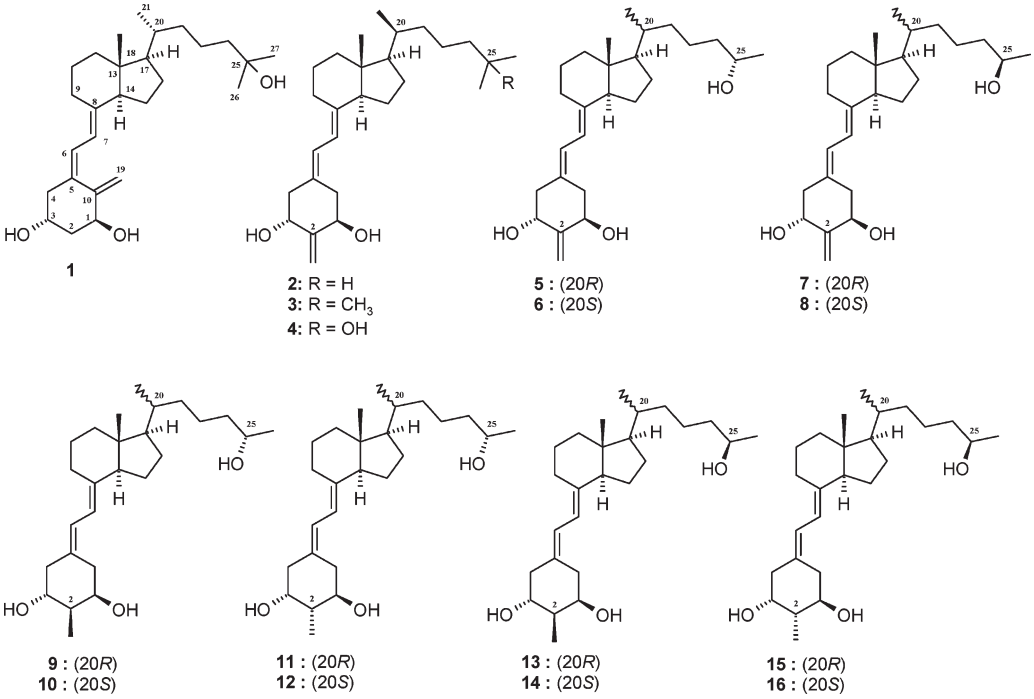
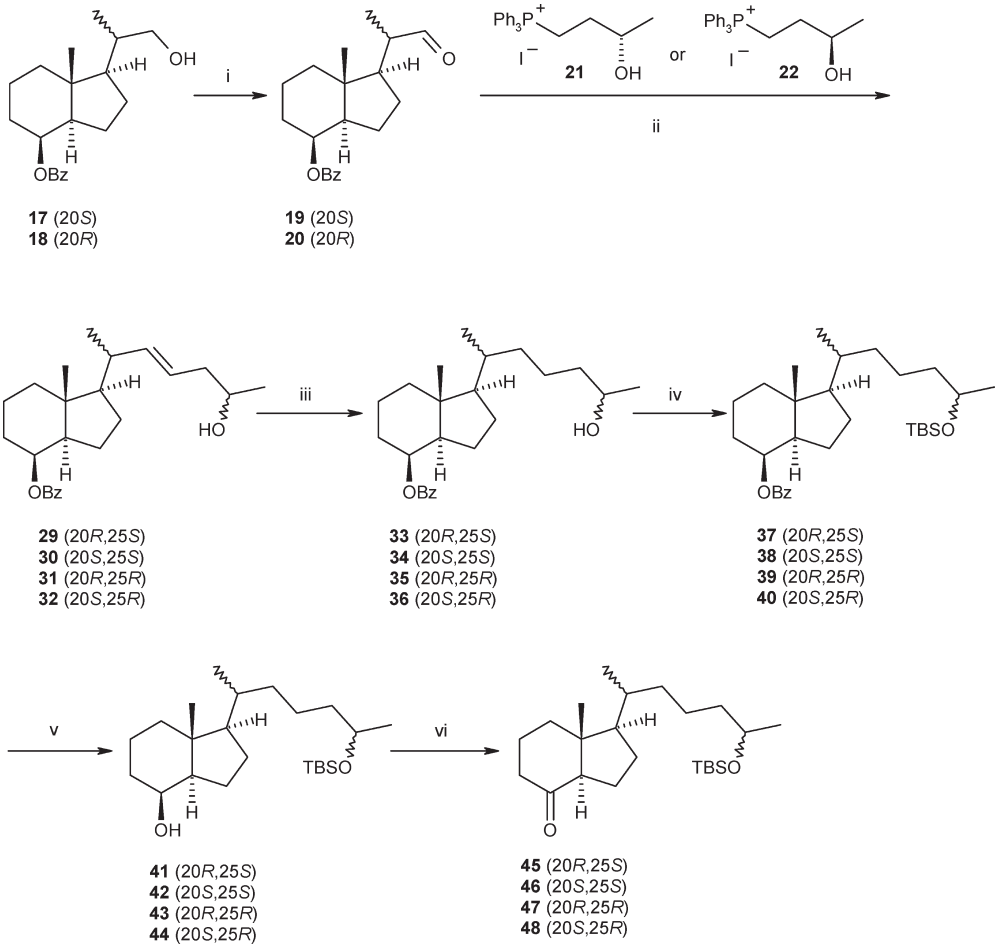


Figure 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and 19-nor analogues (**2**−**16**).

Scheme 1. Synthesis of the Grundmann Ketones **45**−**48**[a]



[a] Reagents: (i) SO_3·pyridine, Et_3N, DMSO, CH_2Cl_2. (ii) (1) **21** or **22**, *n*-BuLi, THF; (2) **19** or **20**, THF. (iii) H_2, Pd/C, MeOH. (iv) TBSOTf, 2, 6-lutidine, CH_2Cl_2. (v) (1) KOH, EtOH; (2) aqueous HCl. (vi) TPAP, NMO, molecular sieves 4 Å, CH_2Cl_2.

Scheme 2. Synthesis of the Phosphonium Iodides **21** and **22**[a]

23 (*S*)
24 (*R*)

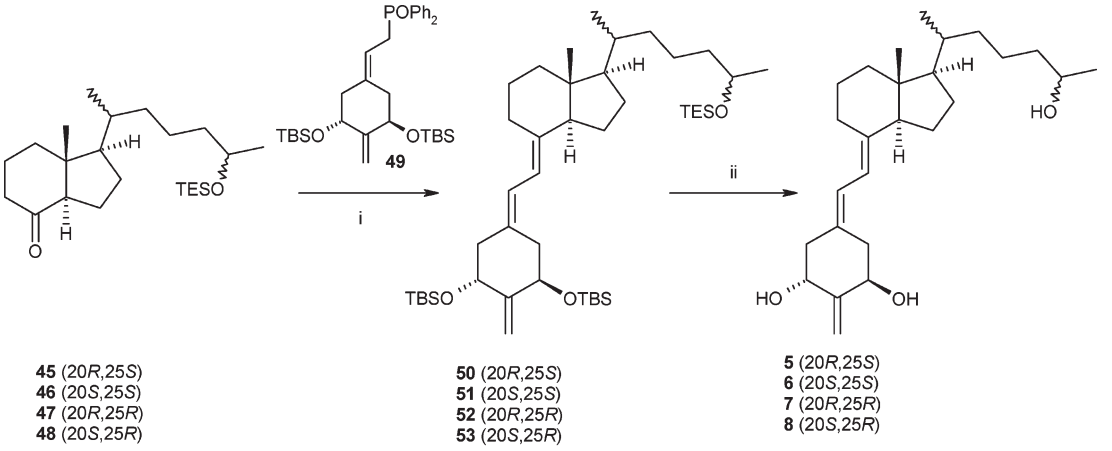
25 (*S*)
26 (*R*)

27 (*S*)
28 (*R*)

21 (*S*)
22 (*R*)

[a] Reagents: (i) (1) TsCl, DMAP, Et_3N, CH_2Cl_2; (2) TESOTf, 2,6-lutidine, CH_2Cl_2. (ii) KI, acetone. (iii) Ph_3P, MeCN.

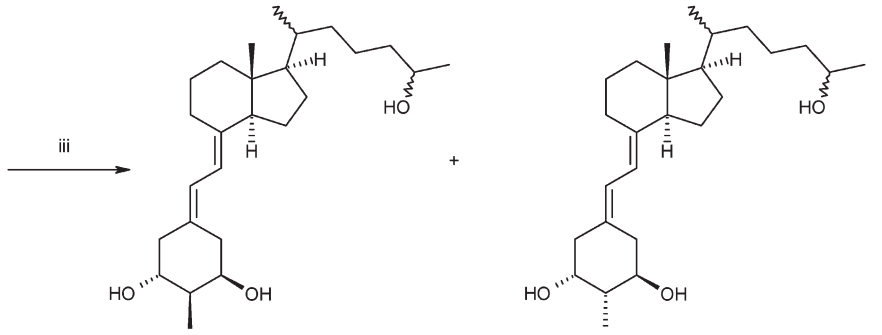
Scheme 3. Syntheses of the Vitamin D Analogues **5**−**16**[a]



45 (20*R*,25*S*)
46 (20*S*,25*S*)
47 (20*R*,25*R*)
48 (20*S*,25*R*)

50 (20*R*,25*S*)
51 (20*S*,25*S*)
52 (20*R*,25*R*)
53 (20*S*,25*R*)

5 (20*R*,25*S*)
6 (20*S*,25*S*)
7 (20*R*,25*R*)
8 (20*S*,25*R*)



9 (20*R*,25*S*)
10 (20*S*,25*S*)
13 (20*R*,25*R*)
14 (20*S*,25*R*)

11 (20*R*,25*S*)
12 (20*S*,25*S*)
15 (20*R*,25*R*)
16 (20*S*,25*R*)

[a] Reagents: (i) (1) **49**, PhLi, THF; (2) **45**, **46**, **47**, or **48**, THF. (ii) aqueous HF, THF, MeCN. (iii) H_2, $(Ph_3P)_3RhCl$, PhH.

the corresponding C,D fragments (**45**−**48**) and the anion, generated from the phosphine oxide **49** by phenyllithium, produced the protected vitamin D compounds **50**−**53** (Scheme 3). The silyl-protective groups were cleaved in the presence of hydrofluoric acid, and after the final purification by high-performance liquid chromatography (HPLC), the target vitamin D analogues **5**−**8** were obtained. The X-ray analysis of a single-crystal of the compound **5** (Figure 2) confirmed the stereochemistry at C-25 as (25*S*). The homogeneous catalytic hydrogenation of 2-methylene compounds **5**−**8**, in the presence of tris(triphenylphosphine)rhodium(I) chloride, provided approximately an equimolar mixture of 2-methyl-19-norvitamins, which were easily separated by HPLC.

Biological Evaluation

A standard set of in vitro assays was conducted with all 12 compounds (**5**−**16**) to ascertain receptor binding, cell differentiation, and gene transcription activity. Table 1 shows the results of the competitive VDR binding assays as compared to the natural hormone **1** and to 2-methylene-19-nor-(20*S*)-1α,25-dihydroxyvitamin D_3 (**4**). No remarkable differences in binding to the receptor were noted except with (20*R*)-2β-methyl compounds **11** and **15**. The VDR binding affinities of these analogues are approximately one log less than those of the native hormone **1** or analogues possessing 2-methylene (**4**−**8**) or 2α-methyl (**9**, **10**, **13**, and **14**) groups.

The results of the HL-60 cell differentiation assays are depicted in Table 1. In these assays, cells were given one dose of the indicated amounts, and after 4 days, the extent of differentiation was assessed by incubating the cells with a substrate (nitro blue tetrazolium), which is reduced by the cells if they have differentiated into monocytes. Although (25*R*) analogues demonstrate slightly more activity than (25*S*) compounds, the configuration of the 25-hydroxy group exerts

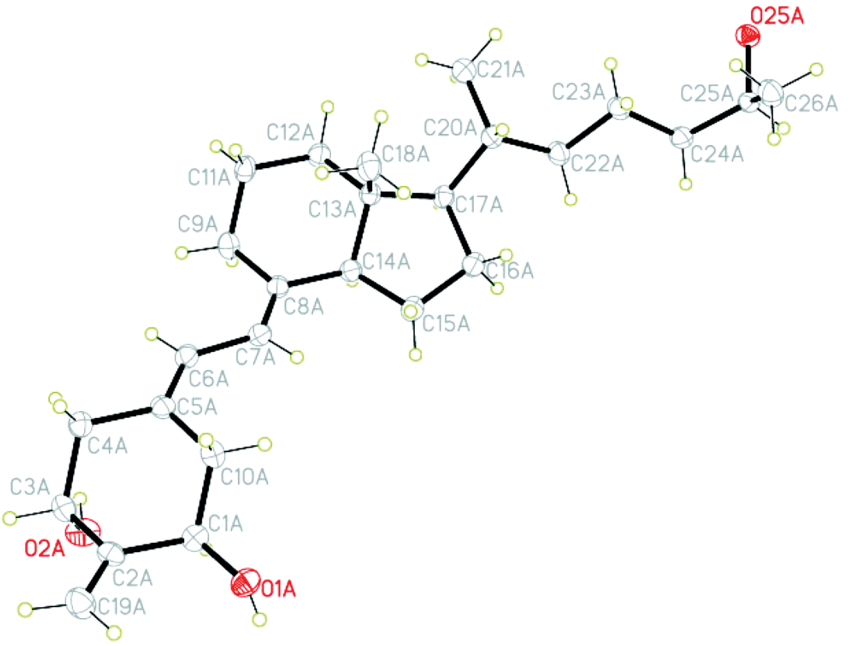
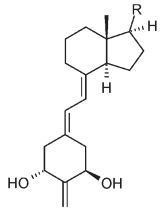
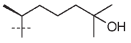
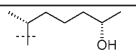
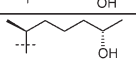
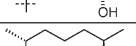
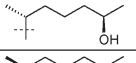
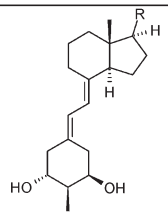
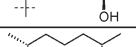
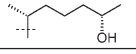
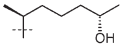
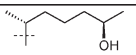
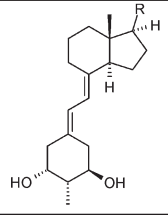
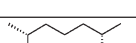
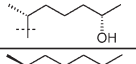
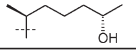
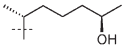


Figure 2. X-ray crystal structure of (20*R*,25*S*)-2-methylene-19,26-dinor-1α,25-dihydroxyvitamin D_3 (**5**).

Table 1. VDR Binding Properties,[a] HL-60 Differentiating Activities,[b] and Transcriptional Activities[c] of the Vitamin D Analogues (**5**−**16**)

Compound	Structure of a side chain (R)	Compd No.	VDR binding		HL-60 differentiation		24-OHase transcription	
			K_i	ratio	EC_{50}	ratio	EC_{50}	ratio
1α,25-$(OH)_2D_3$	-	**1**	1×10^{-10} M	1	2×10^{-9} M	1	2×10^{-10} M	1
		**4**	1×10^{-10} M	1	8×10^{-11} M	0.04	7×10^{-12} M	0.035
		**5**	1×10^{-10} M	1	2×10^{-9} M	1	4×10^{-10} M	2
		**6**	6×10^{-11} M	0.6	6×10^{-10} M	0.3	6×10^{-11} M	0.3
		**7**	1×10^{-10} M	1	1×10^{-9} M	0.5	2×10^{-10} M	1
		**8**	9×10^{-11} M	0.9	9×10^{-11} M	0.045	1×10^{-11} M	0.05
		**9**	2×10^{-10} M	2	5×10^{-9} M	2.5	6×10^{-10} M	3
		**10**	1×10^{-10} M	1	4×10^{-10} M	0.2	8×10^{-11} M	0.4
		**13**	3×10^{-10} M	3	2×10^{-9} M	1	4×10^{-10} M	2
		**14**	8×10^{-11} M	0.8	1×10^{-10} M	0.05	2×10^{-11} M	0.1
		**11**	5×10^{-9} M	50	1×10^{-7} M	50	3×10^{-8} M	150
		**12**	5×10^{-10} M	5	2×10^{-8} M	10	7×10^{-9} M	35
		**15**	2×10^{-9} M	20	6×10^{-8} M	30	2×10^{-8} M	100
		**16**	4×10^{-10} M	4	6×10^{-9} M	3	1×10^{-9} M	5

[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 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 analogue K_i to the K_i for 1α,25-$(OH)_2D_3$. [b] Induction of differentiation of HL-60 promyelocytes to monocytes by 1α,25-$(OH)_2D_3$ and the synthesized vitamin D analogues. The differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The EC_{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 analogue EC_{50} to the EC_{50} for 1α,25-$(OH)_2D_3$. [c] Transcriptional assay in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. The EC_{50} values are derived from dose−response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. The luciferase activity ratio is the average ratio of the EC_{50} for the analogue to the EC_{50} for 1α,25-$(OH)_2D_3$. All of the experiments were carried out in duplicate on at least two different occasions.

a small impact on the overall potency in cell differentiation. Two (20*S*,25*R*) compounds, **8** and **14**, possessing 2-methylene or 2α-methyl group, respectively, have differentiation activity 20 times higher than 1,25$(OH)_2D_3$ **1** but approximately 0.8

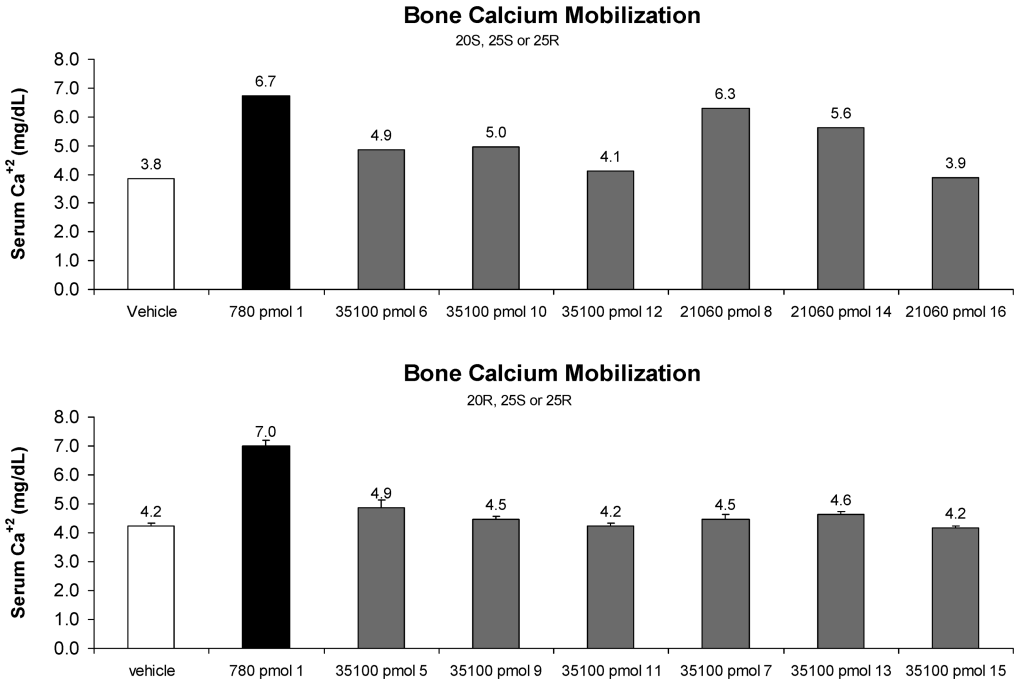


Figure 3. Serum calcium levels of vitamin D-deficient rats on a 0.02% calcium diet and given vehicle or vehicle plus the indicated compound each day for 4 days as an intraperitoneal injection. Serum was harvested 24 h after the last dose. Note: Dose ranging studies were performed with these compounds, but only the highest dose levels tested are shown to highlight the differences and make it easier for the reader.

times lower than parent compound **4**. Four (20*R*) compounds (**5**, **7**, **9**, and **13**) and two (20*S*,25*S*)-analogues (**6** and **10**) exhibit the next highest activity in this assay, which is approximately similar to that observed with the native hormone **1**. Not surprisingly, the derivatives **11**, **12**, **15**, and **16**, containing a 2β-methyl substituent, have the lowest activities, 3−35 times lower than 1,25$(OH)_2D_3$ **1**.

The pattern of potencies in in vitro transcription assays is shown in Table 1. Similar to that observed in the HL-60 cell differentiation assays, (25*R*) compounds demonstrate higher transcriptional potency than in the case of (25*S*) analogues. This difference is more noticeable in the case of the (20*S*) compounds. In this cell-based assay, two (20*S*,25*R*) compounds **8** and **14** express the highest transcriptional potency. The activity of the other 2-methylene and 2α-methyl analogues (**5**−**7**, **9**−**13**) is similar to that observed with natural hormone **1**. Consistent with the other two in vitro assays, the derivatives possessing a 2β-methyl group **11**, **12**, **15**, and **16** possess the lowest transcriptional activities.

In vivo biological activities are shown in Figure 3 and Table 2. Both intestinal calcium transport and bone calcium mobilization were measured in the same rats made vitamin D-deficient prior to administering any of the experimental compounds. A rise in blood calcium levels reflects the ability of an analogue to stimuate the mobilization of bone calcium because the animals are fed a diet essentially devoid of calcium. Analogue activity in the intestine was assessed ex vivo by analyzing calcium transport in the everted gut sac preparation. Figure 3 highlights the fact that all of the analogues have low bone mobilizing activity. The most potent compound **8** is at least 25 times (21060 pmol/780 pmol) less active than the native hormone **1** in vivo; yet, analogue **8** is much more potent than 1,25-$(OH)_2D_3$ **1** in causing cellular differentiation and stimulating in vitro 24-OHase transcription. The order of bone calcium mobilizing activity of the 19,26-dinor compounds (**5**−**16**) parallels that observed in vitro, among them 2-methylene and 2α-methyl analogues of (20*S*) configuration **6**, **8**, **10**, and **14** being the most potent. It is worth noting that (25*R*) diastereoisomers are more potent on bone.

Table 2 summarizes the results obtained from the intestinal calcium transport assays. In contrast to the bone, the (20*S*,25*R*) compound **8** has similar activity to the native hormone **1** or the parent analogue **4** in the intestine. The remainder of the compounds has reduced intestinal calcium transport activity, among them 2β-methyl compounds **11**, **15**, and **16** have the lowest in particular when coupled with a (20*R*) configuration.

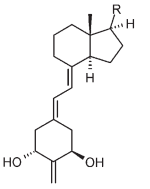
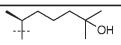
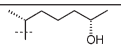
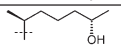
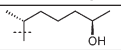
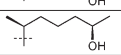
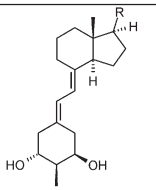
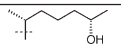
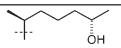
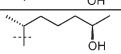
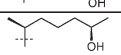
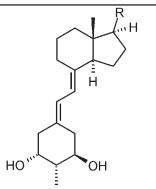
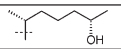
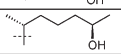
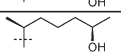
Conclusions

The present study clearly shows that removal of one methyl from C-25 of the 19-norvitamin D_3 analogues (**5**−**16**) markedly reduces the calcemic activity derived from either bone or intestine. On the other hand, the lack of the same methyl group has little impact on receptor binding, HL-60 differentiation, and in vitro transcription. The exceptions are the 2β-methyl analogues that are virtually devoid of any activity. In the case of the 20*S* compounds analogues (**6**, **8**, **10**, and **14**), the in vitro activity is increased by removal of the methyl at C-25 accompanied by a 25*R* configuration. Thus, the most potent of the 19,26-dinorvitamin D series are (20*S*,25*R*)-2-methylene and 2α-methyl analogues **8** and **14**, respectively. In general, 25*R*-hydroxy analogues exhibit higher potency, measured both in vitro and in vivo, than 25*S* diastereoisomers. The greatly reduced calcemic activities of the presented compounds (**5**−**10**, **13**, and **14**) coupled with their ability to promote differentiation make them drug candidates for treatment of secondary hyperparathyroidism of renal failure and, possibly, certain cancers.

Experimental Section

Chemistry. Melting points (uncorrected) were determined on a Thomas-Hoover capillary melting point apparatus. Optical rotations were measured in chloroform or methanol using a

Table 2. Summary of Intestinal Ca Transport

Compound	Structure of a side chain (R)	Compd No.	Dose level (pmol/rat/day)	Intestinal Ca transport (increase in S/M ratio* compared to Vehicle)
1α,25-$(OH)_2D_3$	-	**1**	260	3.2
		**4**	260	4.0
		**5**	260	1.5
		**6**	260	1.4
		**7**	260	1.6
		**8**	260	2.8
		**9**	35100	2.4
		**10**	7020	3.0
		**13**	35100	3.0
		**14**	7020	3.2
		**11**	35100	1.4
		**15**	35100	1.6
		**16**	21060	1.6

[a] S/M ratio = the amount of calcium found in the serosal compartment divided by the amount of calcium present in the mucosal compartment.

Perkin-Elmer model 343 polarimeter at 22 °C. Ultraviolet (UV) absorption spectra were recorded with a Perkin-Elmer Lambda 3B UV−vis spectrophotometer in ethanol or hexane. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in deuteriochloroform, acetone-d_6, or methanol-d_4 at 400 and 500 MHz with Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers. ^{13}C NMR spectra were recorded in deuteriochloroform, acetone-d_6, or methanol-d_4 at 100 and 125 MHz with the same Bruker Instruments. Chemical shifts (δ) in parts per million are quoted relative to internal Me_4Si (δ 0.00). Numbers in parentheses following the chemical shifts in the ^{13}C NMR spectra refer to the number of attached hydrogens as revealed by DEPT experiments. ^{31}P NMR spectra were recorded at 162 MHz with Bruker Instruments DMX-400 Avance console spectrometer in methanol-d_4. Chemical shifts (δ) in parts per million are quoted relative to external H_3PO_4 (δ 0.00). Electron impact (EI) mass spectra were obtained with a Micromass AutoSpec (Beverly, MA) instrument. HPLC was performed on a Waters Associates liquid chromatograph equipped with a model 6000A solvent delivery system, model U6K Universal injector, and model 486 tunable absorbance detector. The known alcohols **17** and **18** were prepared according to the published procedure.[12] THF was freshly distilled before use from sodium benzophenone ketyl under argon. A designation "(volume + volume)", which appears in general procedures, refers to an original volume plus a rinse volume.

All final vitamin D analogues synthesized by us gave single sharp peaks on HPLC, and they were judged at least 99.5% pure. Two HPLC systems (straight- and reversed-phase) were employed as indicated in Table 3 in the Supporting Information.

General Procedure for the Synthesis of Compounds 19 and 20. To a solution of the alcohol **17** or **18** (1 equiv), triethylamine (5 equiv) in anhydrous methylene chloride, and anhydrous DMSO, the sulfur trioxide pyridine complex (6 equiv) was added at 0 °C. The reaction mixture was stirred under argon at 0 °C for 1 h, and then, it was concentrated under reduced pressure. The crude mixture was diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (2 → 5% ethyl acetate/hexane) to give the aldehyde **19** or **20**.

General Procedure for the Synthesis of Compounds 25 and 26. To a stirred solution of 1,3-butanediol **23** or **24** (1 equiv), DMAP (0.02 equiv), and triethylamine (3 equiv) in anhydrous methylene chloride, *p*-toluenesulfonyl chloride (1.2 equiv) was added at 0 °C. The reaction mixture was stirred at 4 °C for 22 h. It was diluted with methylene chloride, washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica (20 → 50% ethyl acetate/hexane) to give a tosylate.

To a stirred solution of tosylate (1 equiv) and 2,6-lutidine (1.1 equiv) in anhydrous methylene chloride, triethylsilyl trifluoromethanesulfonate (1 equiv) was added at −50 °C. The reaction mixture was allowed to warm to room temperature (4 h), and stirring was continued for an additional 20 h. It was diluted with methylene chloride, washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica (3% ethyl acetate/hexane) to give the product **25** or **26**.

General Procedure for the Synthesis of Compounds 27 and 28. To a stirred solution of the tosylate **25** or **26** (1 equiv) in anhydrous acetone (50 mL), potassium iodide (5.5−6.3 equiv) was added. The reaction mixture was refluxed for 10 h. Water (30 mL) was added to dissolve salts, and the mixture was extracted with ethyl acetate. Combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica (3% ethyl acetate/hexane) to give the iodide **27** or **28**.

General Procedure for the Synthesis of Compounds 21 and 22. To a stirred solution of the iodide **27** or **28** (1 equiv) in anhydrous acetonitrile (50 mL), triphenylphosphine (3 equiv) was added. The reaction mixture was refluxed for 2 days. The solvent was evaporated under reduced pressure to give the solid. Ethyl acetate (50 mL) was added, the mixture was stirred at room temperature for 4 h, and the solvent was removed by filtration. The solid was stirred again with ethyl acetate for 1 h, and the solvent was removed. After it was dried, the pure phosphonium iodide **21** or **22** was obtained. An alternative to this procedure is to dissolve the solid mixture of the phosphonium iodide and triphenylphosphine in methylene chloride and reprecipitate the salt by adding diethyl ether.

General Procedure for the Synthesis of Compounds 29−32. To a stirred suspension of the phosphonium salt **21** or **22** (3 equiv) in anhydrous THF (5 mL), *n*-butyllithium (4.5−6 equiv) was added at −20 °C. The solution was stirred at −20 °C for 1 h, and it turned deep orange. A precooled solution of aldehyde **19** or **20** (1 equiv) in anhydrous THF (1 + 1 mL) was added, and the reaction mixture was stirred at −20 °C for 4 h and at room temperature for 18 h. The reaction was quenched with water, and the mixture was extracted with ethyl acetate. Combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica (5 → 10% ethyl acetate/hexane) to give the product **29**, **30**, **31**, or **32**.

General Procedure for the Synthesis of Compounds 33−36. To a solution of the olefin **29**, **30**, **31**, or **32** in methanol (6 mL), 10% palladium on activated carbon (7 mg) was added, and the mixture was hydrogenated overnight. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica (5 → 10% ethyl acetate/hexane) to give the product **33**, **34**, **35**, or **36**.

General Procedure for the Synthesis of Compounds 37−40. To a stirred solution of the alcohol **33**, **34**, **35**, or **36** (1 equiv) and 2,6-lutidine (3.6−4 equiv) in anhydrous methylene chloride (3 mL), *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.8−2 equiv) was added at −20 °C. The reaction mixture was stirred at 0 °C for 1 h. It was quenched with water and extracted with methylene chloride. Combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (3% ethyl acetate/hexane) to give the product **37**, **38**, **39**, or **40**.

General Procedure for the Synthesis of Compounds 41−44. To a stirred solution of the benzoate **37**, **38**, **39**, or **40** in anhydrous ethanol (10 mL), a solution of sodium hydroxide in anhydrous ethanol (2.5 M, 2 mL) was added. The reaction mixture was refluxed for 18 h. It was cooled to room temperature, neutralized with 5% aqueous solution of HCl, and extracted with methylene chloride. Combined organic phases were washed with a saturated aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (5 → 10% ethyl acetate/hexane) to give the alcohol **41**, **42**, **43**, or **44**.

General Procedure for the Synthesis of Compounds 45−48. To a stirred solution of 4-methylmorpholine *N*-oxide (3.2−3.6 equiv) in anhydrous methylene chloride (0.5 mL), pulverized molecular sieves A4 (ca. 60 mg) were added, and the mixture was stirred for 15 min. Then tetrapropylammonium perruthenate (0.13−0.22 equiv) was added, followed by a solution of the alcohol **41**, **42**, **43**, or **44** (1 equiv) in anhydrous methylene chloride (300 + 300 μL). The resulting dark mixture was stirred at room temperature for 1 h, and then, it was filtered through a silica Sep-Pak, which was further washed with methylene chloride. After evaporation of the solvent, the ketone **45**, **46**, **47**, or **48** was obtained.

General Procedure for the Synthesis of Compounds 50−53. To a stirred solution of the phosphine oxide **49** (2.5−3.8 equiv) in anhydrous THF (500 μL), a solution of phenyllithium (3.3−4.6 equiv) was added at −20 °C under argon. The mixture was stirred for 30 min and then cooled to −78 °C. A precooled solution of the Grundmann's type ketone **45**, **46**, **47**, or **48** (1 equiv) in anhydrous THF (200 + 100 μL) was added via cannula, and the reaction mixture was stirred for 4 h at −78 °C. Then, the reaction mixture was stirred at 4 °C for 19 h. Ethyl acetate (20 mL) was added, and the organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (0 → 2% ethyl acetate/hexane) to give the protected vitamin D compound **50**, **51**, **52**, or **53**.

General Procedure for the Synthesis of Compounds 5−8. To a solution of the protected vitamin **50**, **51**, **52**, or **53** in THF (5 mL) and acetonitrile (4 mL), a solution of aqueous 48% HF in acetonitrile (1:9 ratio, 2 mL) was added at 0 °C, and the resulting mixture was stirred at room temperature for 6 h. The reaction was quenched with a saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. Combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (10 → 30% ethyl acetate/hexane) to give the crude products. Final purifications of the vitamin D compounds were performed by straight phase HPLC (15% 2-propanol/hexane; 4 mL/min; 9.4 mm × 25 cm Zorbax Sil column), followed by reversed-phase HPLC (15% water/methanol; 3 mL/min; 9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column) to give the analytically pure 19,26-dinorvitamin D analogues **5**, **6**, **7**, or **8**.

General Procedure for the Synthesis of Compounds 9−16. Tris(triphenylphosphine)rhodium(I) chloride (1.0−1.5 equiv) was added to dry benzene (5 mL) presaturated with hydrogen (15 min). The mixture was stirred at room temperature until a homogeneous solution was formed (ca. 25 min). A solution of the analogue **5**, **6**, **7**, or **8** (1 equiv) in dry benzene (3 + 1 mL) was added, and the reaction was allowed to proceed under a continuous stream of hydrogen for 4 h. The solvent was removed under reduced pressure, and the residue was redissolved in hexane/ethyl acetate (1:1) and applied on a Waters silica Sep-Pak cartridge (2 g). A mixture of 2-methyl vitamins was eluted with the same solvent system. A mixture of compounds was further purified by HPLC (9.4 mm × 250 mm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (85:15) solvent system. The separation of 2α-methyl analogues **9**, **10**, **13**, or **14**, from the 2β-methyl ones **11**, **12**, **15**, or **16**, was achieved by reversed-phase HPLC (9.4 mm × 250 mm Zorbax RX-C18 column, 3 mL/min) using a methanol/water (85:15) solvent system.

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 were performed as previously described.[19] Briefly, weanling rats were made vitamin D-deficient by housing under lighting conditions that block vitamin D production in the skin. In addition, the animals were fed a diet devoid of vitamin D and alternating levels of calcium. Experimental compounds were administered intraperitoneally once per day for four consecutive days. Twenty-four hours after the last dose was given, the blood was collected, and everted gut sacs were prepared. Calcium was measured in the blood and two different intestinal compartments using atomic absorption spectrometry. Each study was comprised of at least 5−6 animals/experimental group and was controlled with a vehicle group (5% ethanol:95% propylene glycol) and one or more positive control groups [$1,25(OH)_2D_3$].

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Supporting Information Available: Purity criteria, spectral and X-ray data of the synthesized compounds; representative

figures with either the competitive binding curves or the dose−response curves derived from the binding, cellular differentiation, and transcriptional assays of the vitamin D analogues (**5**−**16**). This material is available free of charge via the Internet at http://pubs.acs.org.

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