New 1α,25-Dihydroxy-19-norvitamin D_3 Compounds of High Biological Activity: Synthesis and Biological Evaluation of 2-Hydroxymethyl, 2-Methyl, and 2-Methylene Analogues

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New highly active isomers of the natural hormone 1α,25-dihydroxyvitamin D_3 possessing an exomethylene group at the 2-position were prepared in a convergent manner, starting with (−)-quinic acid and the corresponding (20*R*)- and (20*S*)-25-hydroxy Grundmann ketones. These 2-methylene-19-norvitamins were efficiently converted to the 2-methyl and 2-hydroxymethyl derivatives, some of which exhibited pronounced in vivo biological activity. Configurations of the A-ring substituents were determined by ^{1}H NOE difference spectroscopy as well as by spin decoupling experiments. It was established that the bulky methyl and hydroxymethyl substituents at C-2, due to their large conformational free energies, occupy mainly equatorial positions. Additionally, hydroxylation of the C(10)−C(19) double bond in 1α,25-$(OH)_2D_3$ was performed, resulting in 1α,19,25-trihydroxy-10,19-dihydrovitamin D_3 derivatives in which the hydroxymethyl substituent at C-10, for steric reasons, is forced to occupy an axial position. In consequence, the vitamin D_3 analogues were synthesized in which the 1α-hydroxy group, required for biological activity, is almost exclusively axially or equatorially oriented because of stabilization of the single A-ring chair conformations. The relative ability of the synthesized analogues to bind the porcine intestinal vitamin D receptor was assessed and compared with that of the natural hormone. It was established that vitamins possessing the axial orientation of the 1α-hydroxy substituent exhibit a significantly increased receptor binding affinity. Compounds with a 2-methylene substituent showed selective calcemic activity profiles, being extremely effective on bone calcium mobilization. 2α-Methyl-substituted vitamins proved to be much more active in vivo than the corresponding epimers with 2*β*-configuration. All of the 2-substituted vitamins exhibited pronounced HL-60 differentiating activity, those 2α-substituted in the 20*S*-series being especially potent. The present studies imply that the axial orientation of the 1α-hydroxy group is necessary for biological activity of vitamin D compounds.

Introduction

The discovery of the hormonally active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **1**; Figure 1), has stimulated great interest in establishing its physiological role.[1,2] It is now well-known that **1** not only regulates calcium homeostasis and phosphorus metabolism in animals and humans,[3] but it also affects the human immune system, inhibits cell proliferation, and promotes cellular differentiation.[4] The hormone has been successfully used in the treatment of calcium and bone disorders[5] and the skin disorder psoriasis.[6] Since large doses of **1** can cause hypercalcemia, there is a considerable interest in academia and the pharmaceutical industry to prepare noncalcemic calcitriol analogues that still retain antiproliferative activity. Such a desired selective activity toward differentiation was found in the 19-nor analogue **2**, synthesized in our laboratory.[7] Prompted by our result and literature data concerning interesting biological potency of 2-substituted calcitriol derivatives,[8] exhibiting strong binding affinities to vitamin D-binding protein (DBP), we have recently prepared 2α- and 2*β*-hydroxy analogues of **2**.[9] The synthesized vitamins **3** and **4**, as well as their 2-alkoxy derivatives, showed selective activity in stimulating intestinal calcium transport while having little or no activity in bone calcium mobilization. Interestingly, they also exhibited a high HL-60 differentiating activity. Furthermore, some other 2-substituted (with hydroxyalkyl and fluoroalkyl groups) calcitriol analogues have recently been synthesized and reported to exhibit strong affinities to DBP.[10] All these studies indicate that binding sites in vitamin D receptors and other binding proteins can accommodate different substituents at C-2, present in the synthesized vitamin D analogues.

In a continuing investigation of the structure−activity relationship of the vitamin D molecule, we decided to prepare 19-norvitamin D compound analogues with 2-methyl and 2-hydroxymethyl substituents in an effort to alter the orientation of the 1α-hydroxyl functions. As a convenient precursor of such analogues, we prepared the 2-methylene compound, which by itself seemed to be an interesting synthetic target. It is commonly accepted that the presence of a 1α-hydroxy group in the vitamin D molecule is the dominant factor in receptor binding[11] and it is required for the expression of

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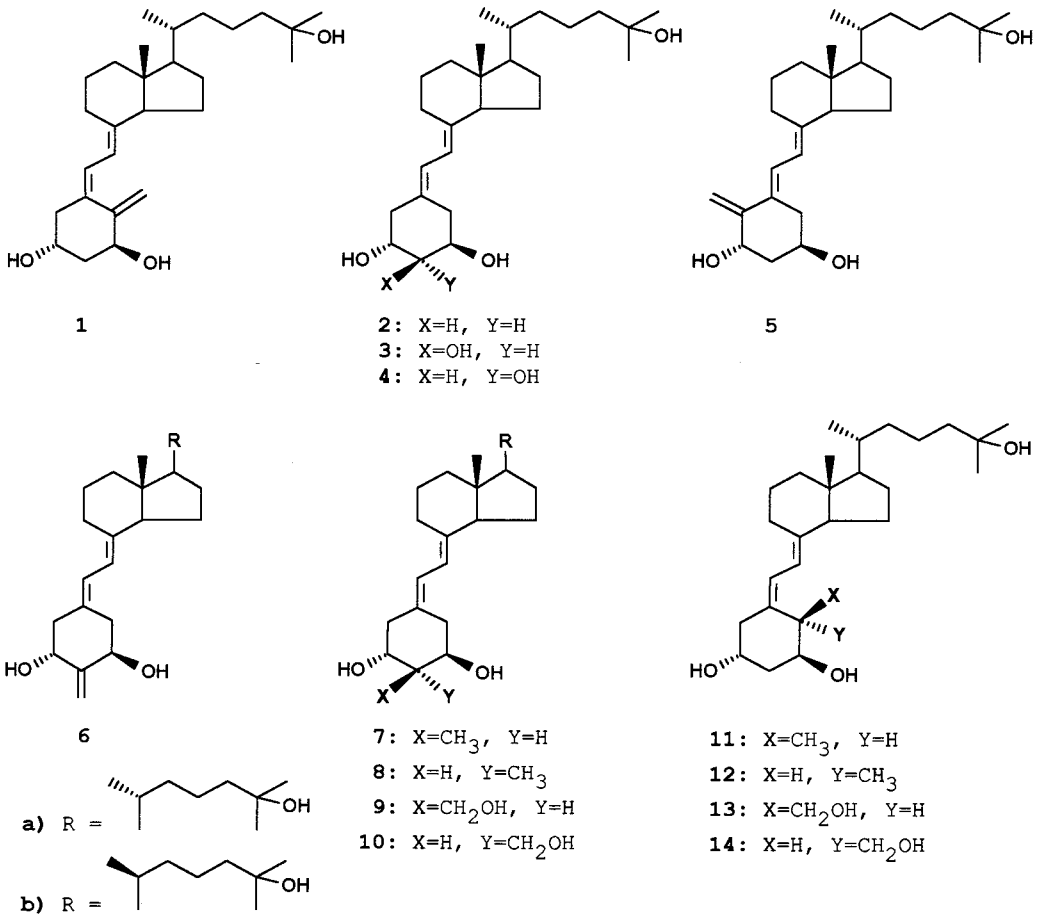


Figure 1. Chemical structure of 1α,25-dihydroxyvitamin D$_3$ (calcitriol) and its analogues.

biological activity. An analogue of the natural hormone **1**, 1α,25-dihydroxy-(5*E*)-vitamin D$_3$ (**5**), in which the exocyclic methylene group is transposed, in comparison with **1**, from the right to the left side of ring A exhibits only ca. 8-fold lower binding to the receptor protein than the hormone **1**.[11d] Both isomers **1** and **5**, in a purely formal sense, can be considered as A-ring isomers having the exocyclic methylene unit in the two alternative "ortho" positions in relation to the C(5)−C(6) double bond. However, the third isomer is also possible, and it was therefore of interest to synthesize compound **6a**, characterized by the transposition of the methylene unit to the "para" position, i.e., at C-2. The relatively small 2-methylene substituent should not interfere with binding to the vitamin D receptor, but it changes the character of both (1α and 3*β*) cyclohexanediol A-ring hydroxyls. Both are now in the allylic positions, similarly as the 1α-hydroxy group, crucial for biological activity, in the natural hormone **1**.

This paper describes our synthetic route to the isomeric (at C-20) 1α,25-dihydroxy-2-methylene-19-norvitamin D$_3$ compounds **6a**,**b** and their conversion to the respective 2-methyl and 2-hydroxymethyl derivatives **7a**,**b**−**10a**,**b**. The calcemic and cellular differentiation activities of these new 19-norvitamins were tested, and a strong influence on biological potency of their A-ring conformations was observed, strongly suggesting that the 1α-hydroxyl must be in the axial orientation for maximal biological activity. Further, the (20*S*)-2α-methyl-substituted compound **7b** and 2-methylene derivatives **6a,b** show strong preferential activity on bone.

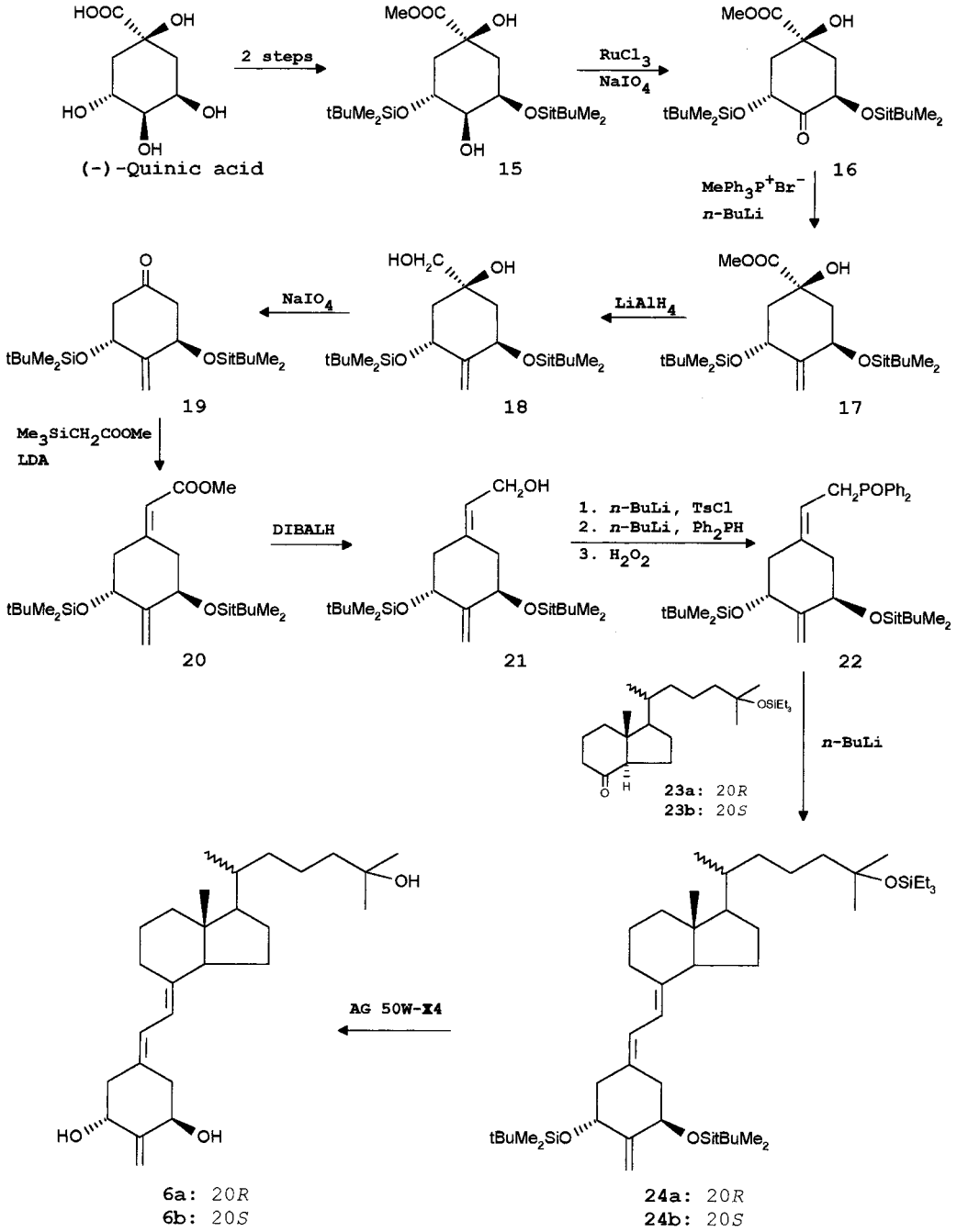
Additionally, two vitamin D analogues (**13** and **14**) with hydroxymethyl substituents at C-10 were prepared, being 19-hydroxy derivatives of 1α,25-dihydroxy-10,19-dihydrovitamin D$_3$ compounds **11** and **12**, synthesized by us previously.[12]

Results and Discussion

Chemistry. The strategy of our synthesis of 2-substituted 19-norvitamins was based on the Wittig−Horner coupling, pioneered by the Lythgoe group[13] and successfully used by us in the recent preparation of **3** and **4**. Since the corresponding C,D-ring ketones were available,[9,14] we focused our attention on the synthesis of the phosphine oxide A-ring synthon.

The secondary hydroxy group of the methyl quinicate derivative **15** (Scheme 1), easily prepared from commercial (1*R*,3*R*,4*S*,5*R*)-(−)-quinic acid,[7a] was oxidized with ruthenium tetroxide in a catalytic process employing RuCl$_3$ and NaIO$_4$. The use of such a strong oxidant was necessary for an effective oxidation of this very hindered alcohol. Other, more commonly used reagents can also be employed (e.g., pyridinium dichromate), but the reaction usually requires much longer time for completion. The next step of the synthesis comprises the Wittig reaction of the sterically hindered 4-keto compound **16** with an ylide prepared from methyltriphenylphosphonium bromide and *n*-butyllithium. Our numerous attempts did not succeed in improving the yield (24%) of the desired 4-methylene product **17**. Also, attempted reaction of **16** with the PO-ylide obtained from methyldiphenylphosphine oxide upon deprotona-

Scheme 1

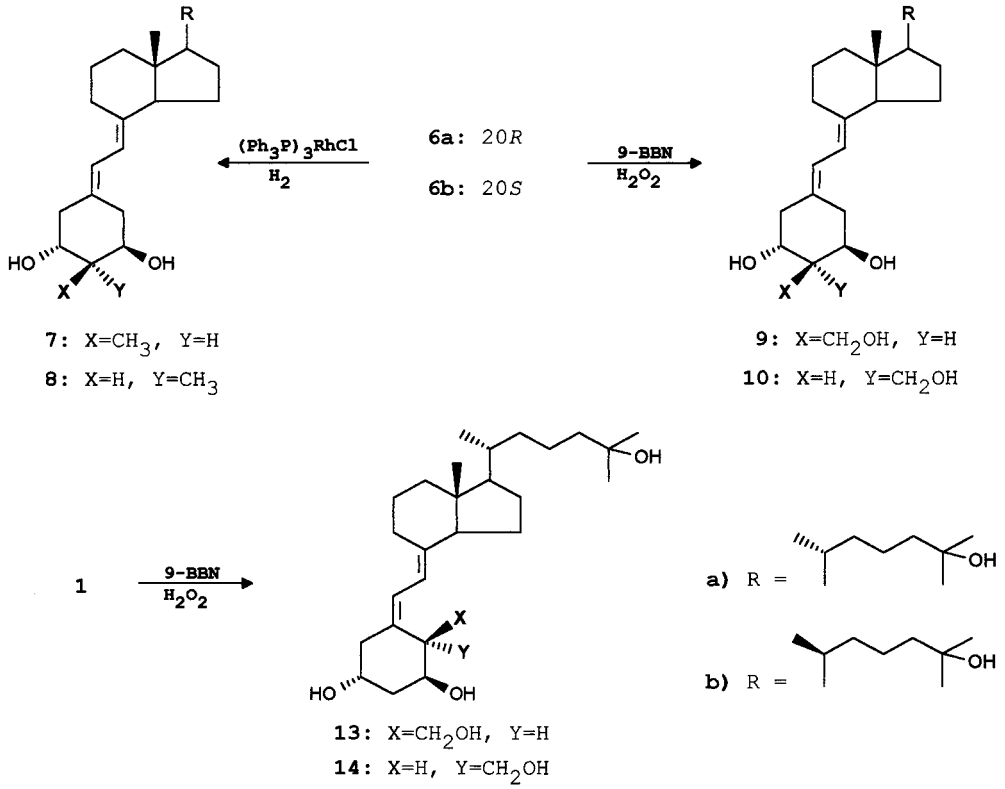


tion with *n*-butyllithium, the reagent recommended for methylenation of sterically hindered ketones,[15] resulted in formation of a complex mixture of products. Rather unexpectedly, the following reduction of a carbomethoxy group in **17** with diisobutylaluminum hydride (DIBALH) proceeded as poorly as in the case of the previous synthetic step; however, the use of lithium aluminum hydride as a reducing agent doubled the yield of **18**. Without any further problems, this vicinal diol was cleaved by sodium periodate, and the resulting cyclohexanone derivative **19** was smoothly converted to the unsaturated ester **20** by the Peterson olefination with methyl (trimethylsilyl)acetate. On the basis of literature precedent[13c] and our experience,[7a,9] compound **20** was reduced with DIBALH to the allylic alcohol **21** which was in turn tosylated in situ with *n*-butyllithium and *p*-toluenesulfonyl chloride, converted into the corresponding phosphine by a reaction with diphenylphosphine lithium salt, and oxidized with hydrogen peroxide to the final A-ring phosphine oxide **22**.

The Wittig−Horner coupling of the conjugate base of **22**, generated upon deprotonation with *n*-butyllithium, with the protected 25-hydroxy Grundmann's ketone **23a**, prepared according to the published procedure,[9] produced the expected protected 19-norvitamin D compound **24a** in a high yield. This, after quantitative deprotection with AG 50W-X4 cation-exchange resin, afforded 1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (**6a**).

In our previous paper we demonstrated that 10,19-dihydro derivatives **11** and **12** can be efficiently obtained by selective reduction of **1**; similar selectivity was observed in the case of the isomer **5** with a (5*E*,7*E*)-5,7,-10(19)-triene system.[12] It turned out that the selective

Scheme 2



hydrogenation of the A-ring exomethylene unit is possible also with compound **6a**, where it is located in the "para" position in relation to the C(5)−C(6) double bond. Thus, homogeneous catalytic hydrogenation of **6a** performed in the presence of tris(triphenylphosphine)-rhodium(I) chloride [Wilkinson's catalyst, $(Ph_3P)_3RhCl$] provided efficiently an equimolar mixture of 2-methyl-19-norvitamins **7a** and **8a** (Scheme 2), easily separated by HPLC. A similar chemoselectivity was also observed in hydroboration reactions. We employed for this purpose 9-borabicyclo[3.3.1]nonane (9-BBN) as a reagent and reaction conditions analogous as those used by Okamura for hydroboration of simple vitamin D compounds.[16] Since this literature precedent concerned hydroboration of 1-desoxy compounds, namely, 5*E*- and 5*Z*-isomers of vitamins D_2 and D_3, we first tested the process using **1** as a model compound. The formed organoborane intermediate (**1**/9-BBN adduct) was subsequently oxidized with basic hydrogen peroxide. The best yield of the corresponding 10α- and 10*β*-hydroxymethyl products **13** and **14**, separated by flash chromatography, achieved by us amounted to 12% and 9%, respectively. Such hydroboration−oxidation conditions allowed us also to hydroxylate exclusively the C(2)=CH_2 unit in the vitamin **6a**, leaving the intercyclic C(5)=C(6)−C(7)=C(8) diene moiety unaffected. The isolated epimeric mixture of 2-hydroxymethyl derivatives **9a** and **10a** (ca. 1:2, 35% yield) was purified and separated by straight- and reversed-phase HPLC.

One of the interesting modifications in the vitamin D side chain is the C-20 epimerization. It is well-known that vitamin D analogues with "unnatural" stereochemistry of the methyl group at C-20 exhibit considerably increased calcemic activity.[17] Thus, condensation of the protected C,D-ring synthon **23b**, prepared by triethylsilylation of the parent (20*S*)-25-hydroxy Grundmann's ketone,[14] with the lithiated phosphine oxide **22** provided 19-norvitamin **24b**, which after hydrolysis of the hydroxy-protecting groups gave (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (**6b**). This compound, like its 20*R*-counterpart **6a**, was subjected to homogeneous hydrogenation with Wilkinson's catalyst leading to 2-methyl analogues (**7b**, **8b**) and underwent hydroboration with 9-BBN yielding 2-hydroxymethyl derivatives (**9b**, **10b**). In accord with the literature data concerning similar systems,[18] all of the synthesized vitamin D analogues with the 20*S*-configuration (**6b**−**10b**) show in their ^{1}H NMR spectra signals of the 20-methyl group which are shifted by ca. 0.083 ppm upfield with respect to those of the corresponding 20*R*-isomers of "natural" series. Stereochemistry at C-2 and C-10 in the synthesized vitamin D compounds was tentatively assigned on the basis of a simple conformational analysis, molecular modeling studies, and especially 500-MHz ^{1}H NMR spectroscopy.

It has been commonly accepted[19] that vitamin D compounds in solutions exist as a mixture of two rapidly equilibrating A-ring chair conformers abbreviated as α- and *β*-forms (Figure 2a). Such conformational equilibria have been found for vitamin D_3, 25-hydroxyvitamin D_3 (25-OH-D_3), 1α-hydroxyvitamin D_3 (1α-OH-D_3), and the natural hormone 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, **1**)[20] as well as some other A-ring-substituted vitamin D derivatives.[16,21] Considering the conformational free energy (*A* value)[22] of a methyl substituent (1.7 kcal/mol)[23] and a value of syn-clinal interaction between methyl and hydroxy group (0.35 kcal/mol),[24] a significant free energy advantage (1.35 kcal/mol) can be easily computed for the corresponding conformers of the synthesized 19-norvitamins **7** and **8**, those possessing an equatorial methyl group at C-2. It can be also predicted that the A-ring conformational equilibrium for

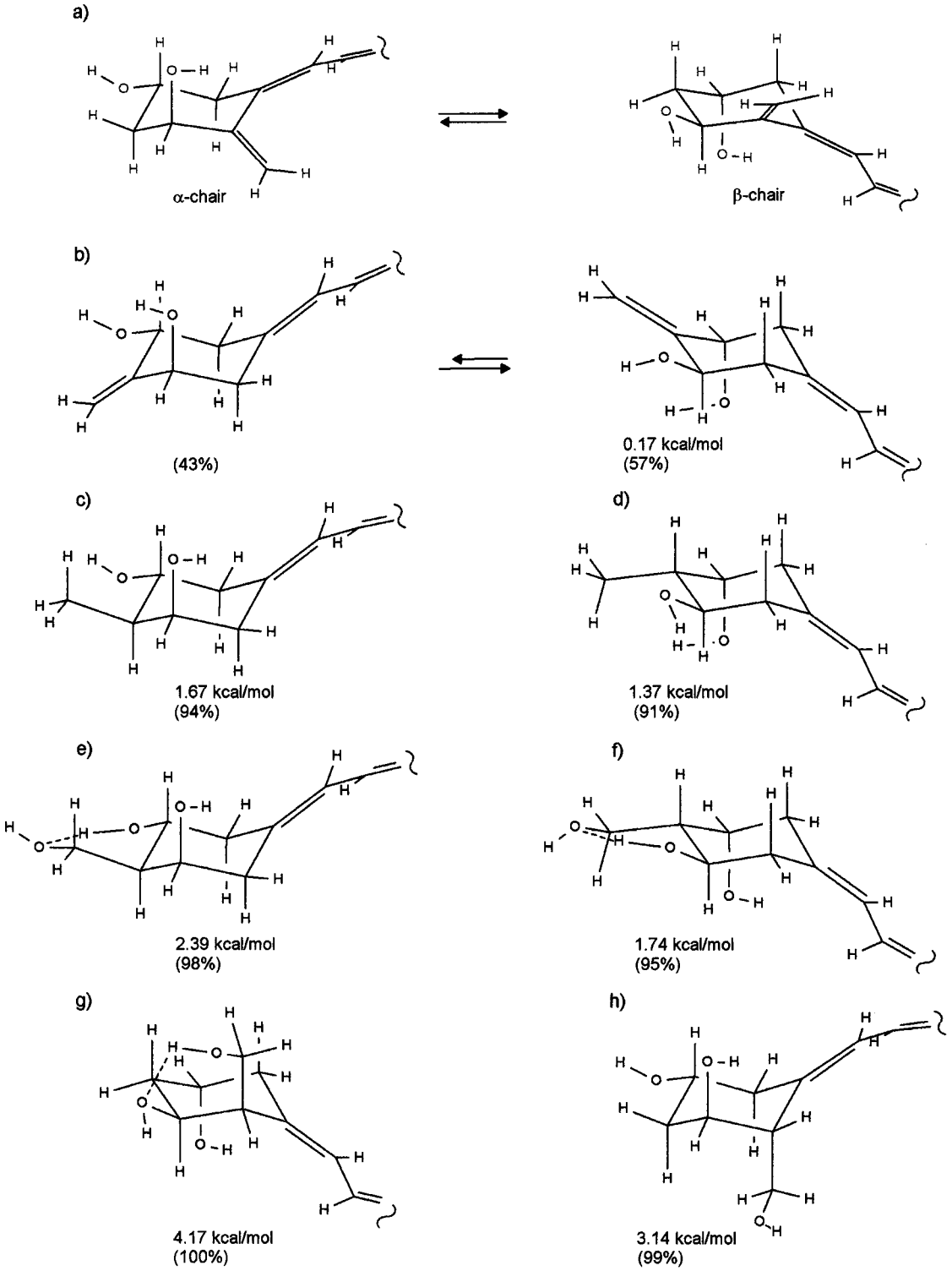


Figure 2. Conformational equilibrium in ring A of 1α-hydroxyvitamin D analogues (a) and 2-methylene-19-norvitamins **6** (b) and the preferred, energy-minimized A-ring conformations of the synthesized analogues: **7** (c), **8** (d), **9** (e), **10** (f), **13** (g), and **14** (h). Hydrogen bonds are represented by dotted lines. The steric energy differences between the preferred conformers and their partners with the inverted chair forms (calculated for model compounds lacking a side chain) are given. The corresponding percentage populations (in parentheses) of conformers are given for room temperature (25 °C).

the vitamins **9** and **10**, which have bulkier hydroxymethyl substituents, should be even more biased toward the conformers with the equatorial 2-CH_2OH group.[25]

It has been established that in 10,19-dihydrovitamin D compounds an additional strong interaction (4.84 kcal/mol),[12] usually termed as the $A^{(1,3)}$-strain,[26,27] takes place between an equatorial 10-methyl and C(7)-H group. Therefore, in the synthesized vitamins **13** and **14**, a large energy gap of at least 3 kcal/mol can be expected in favor of the A-ring chair conformer with an axial 10-hydroxymethyl substituent, even if we allow for some free energy decrease caused by the formation of a hydrogen bond between the equatorial CH_2OH group and 1α-hydroxyl in the isomer **14** (where the presence of two axial substituents at C-1 and C-10 in the inverted chair form precludes hydrogen bond forma-

tion). Thus, it can be predicted that the large conformational free energy of the 2-methyl or 2-hydroxymethyl substituent in 19-norvitamins **7**, **8** and **9**, **10**, respectively, and the strong interaction between the 10-hydroxymethyl and C(7)-H group in the 10(19)-dihydro analogues **13** and **14** destabilize one A-ring chair conformation and favor an alternate inverted chair form. A careful analysis of ^{1}H NMR spectra (in $CDCl_3$) of the synthesized vitamins (exemplified for **6a**, **7a**, and **13**) confirmed these predictions. The proportion of the two rapidly equilibrated chair forms of ring A of 2-methylene-19-norvitamin **6a** (Figure 2b) can be established by the analysis of a multiplet pattern of the methylene protons at C-4 and C-10, namely, from the magnitudes of their degenerate couplings to the methine protons at C-3 and C-1, respectively. The corresponding signals of the equatorial protons at C-4 and C-10 were assigned by ^{1}H NOE difference spectroscopy, involving vinylic 6- and 7-H, whereas the connectivity of the protons was established by spin decoupling experiments. The observed trans-vicinal coupling (6.0 Hz) of the 4β-proton resonating at δ 2.33 and the analogous trans-coupling (8.4 Hz) of the 10α-proton resonating at δ 2.29 represent averaged axial-axial and equatorial−equatorial values. From these data and the vicinal coupling constants reported for cyclohexanol protons ($J_{ax,ax}$ = 11.1 Hz, $J_{eq,eq}$ = 2.7 Hz),[28] the conformational equilibrium for **6a** was established to be ca. 6:4 in favor of the conformer that has an equatorial 1α-OH. In the case of vitamin **7a**, a large half-width (24 Hz) of the 3α-H multiplet at δ 3.61 supports its axial disposition and indicates that two axial−axial couplings must be involved, namely, those with the protons at C-2 and C-4. Therefore, the 2α-configuration of the equatorial methyl substituent in **7a** has been unequivocally assigned. Moreover, the magnitudes of the vicinal coupling constants of the 4α- and 4β-H (4.3 and ca. 12 Hz, respectively)[28] indicate that compound **7a** exists in the single A-ring conformation with an axial 3α-H. In the ^{1}H NMR spectrum of the vitamin **13**, the multiplet pattern of 1β-H, resonating at δ 4.29 (∼dt, J = 12.2, 4.3 Hz), clearly indicates its axial orientation. The cis-relationship between this proton and the equatorial proton at C-10, supported by the vicinal coupling constant (4.3 Hz), establishes the 10α-orientation of the hydroxymethyl group.

Molecular modeling was also employed to establish the A-ring conformations of the synthesized compounds. Force field calculations[29] were carried out for model compounds, analogues of vitamins **6**−**10**, **13**, and **14** lacking the steroidal side chain at C-17. The calculated A-ring conformational populations (Figure 2c−h) were in excellent agreement with our previous conformational considerations and fully supported the strong bias toward conformers with the equatorial configuration of C(2)-substituents in vitamins **7**−**10** and preponderance of the forms with an axial orientation of the hydroxymethyl groups at C-10 in the corresponding derivatives **13** and **14**. The results of our molecular modeling studies indicate that in all but one (**14**) of the vitamins possessing an A-ring hydroxymethyl group, the latter substituent is involved in hydrogen bond formation with the neighboring secondary hydroxyl.

Biological Evaluation. In 1974, it was proposed that the calcium regulation ability of vitamins D is limited to the compounds that can assume an A-ring chair conformation in which the 1α-hydroxy group occupies the equatorial orientation.[30] Such conformation, according to this hypothesis, has the proper geometry for binding to the protein receptor, a step which is necessary to induce the biological response leading to the calcium transport and calcium mobilization in the body. However, our recent results of biological testing of 1α,25-dihydroxy-10,19-dihydrovitamin D$_3$ compounds did not support the idea that the most equatorially favored 1α-hydroxyl would be the most biologically active one. On the contrary, 1α,25-dihydroxy-10(*S*),19-dihydrovitamin D$_3$ (**12**), the analogue strongly biased toward the A-ring chair conformer possessing the axially oriented 1α-hydroxy group, provided the greatest in vivo biological response and showed very significant activity on intestinal calcium transport.[12] While this work was in progress, a communication appeared on the synthesis and biological activities of 2α- and 2β-methyl-1α,25-dihydroxyvitamin D$_3$ analogues.[31] The Japanese authors conclude that the introduction of a methyl substituent at C-2 shifts either way, to a certain extent, the equilibrium between the A-ring chair conformers, and the biological potency of the synthesized vitamins is highly dependent on the configuration of the 2-methyl group.

Recently, we have reported that 1α,25-dihydroxy-19-norvitamin D$_3$ derivatives, substituted at C-2 with hydroxy (**3** and **4**) or alkoxy groups, showed selective activity profiles by retaining their ability to cause cellular differentiation and to increase intestinal calcium transport while having markedly reduced the ability to elevate plasma calcium at the expense of bone.[9] However, the complexity of their ^{1}H NMR spectra precluded any establishing of the A-ring conformational equilibria in these vitamins. Nevertheless, the conformational analysis of some intermediates[9] as well as a low *A* value of the hydroxy substituent (0.52 kcal/mol)[23] seems to indicate that the free energy difference between the equilibrating A-ring chair forms of the 2-hydroxy(alkoxy)-substituted 19-norvitamins should not be remarkable.

In a continuing effort to explore the 19-nor class of pharmacologically important vitamin D compounds, the analogues which are characterized by the transposition of the A-ring exocyclic methylene group, present in the normal vitamin D skeleton at C-10, to carbon 2, i.e., 2-methylene-19-norvitamin D compounds **6a**,**b**, have been synthesized and tested. 2-Methyl (**7a,b** and **8a,b**) and 2-hydroxymethyl (**9a,b** and **10a,b**) analogues of 19-nor-1α,25-(OH)$_2$D$_3$ (**2**) were easily obtained from the 2-methylene precursors, and analogously 1α,19,25-trihydroxy-10,19-dihydrovitamin D$_3$ isomers (**13**, **14**) were prepared, all of them possessing the strongly preferred, single chair conformation of ring A. It was, therefore, possible to examine the effect of the orientation of the 1α-substituent on biological activities of the compounds synthesized.

The synthesized vitamins were tested for their ability to bind the porcine intestinal vitamin D receptor. A comparison between the natural hormone **1** and analogue **6a** indicates that the transposition of the exocyclic methylene group from C-10 to C-2 does not result in a change of the binding affinity (Table 1). Similarly,

Table 1. VDR Binding Properties[a] and HL-60 Differentiating Activities[b] of 2-Substituted Analogues of 1α,25-Dihydroxy-19-norvitamin D_3 and Their 20*S*-Isomers

		VDR binding		HL-60 differentiation	
compound	compd no.	ED_{50} (M)	binding ratio	ED_{50} (M)	activity ratio
1α,25-$(OH)_2D_3$	**1**	8.0×10^{-11}	1	4.0×10^{-9}	1
2-methylene-19-nor-1α,25-$(OH)_2D_3$	**6a**	1.2×10^{-10}	1.5	4.2×10^{-9}	1
2-methylene-19-nor-(20*S*)-1α,25-$(OH)_2D_3$	**6b**	1.0×10^{-10}	1.3	1.5×10^{-10}	0.04
1α,25-$(OH)_2D_3$	**1**	9.0×10^{-11}	1	4.0×10^{-9}	1
2α-methyl-19-nor-1α,25-$(OH)_2D_3$	**7a**	4.2×10^{-10}	4.6	8.0×10^{-11}	0.02
2α-methyl-19-nor-(20*S*)-1α,25-$(OH)_2D_3$	**7b**	4.0×10^{-10}	4.4	7.0×10^{-11}	0.02
2β-methyl-19-nor-1α,25-$(OH)_2D_3$	**8a**	3.5×10^{-9}	39	8.0×10^{-9}	2.0
2β-methyl-19-nor-(20*S*)-1α,25-$(OH)_2D_3$	**8b**	5.0×10^{-10}	5.5	7.0×10^{-10}	0.17
1α,25-$(OH)_2D_3$	**1**	6.0×10^{-11}	1	4.0×10^{-9}	1
2α-(hydroxymethyl)-19-nor-1α,25-$(OH)_2D_3$	**9a**	8.0×10^{-10}	13	2.0×10^{-8}	5.0
2α-(hydroxymethyl)-19-nor-(20*S*)-1α,25-$(OH)_2D_3$	**9b**	8.0×10^{-11}	1.3	2.0×10^{-9}	0.5
2β-(hydroxymethyl)-19-nor-1α,25-$(OH)_2D_3$	**10a**	7.0×10^{-9}	117	1.0×10^{-7}	25
2β-(hydroxymethyl)-19-nor-(20*S*)-1α,25-$(OH)_2D_3$	**10b**	5.0×10^{-10}	8.3	4.5×10^{-9}	1.1

[a] Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues to the porcine intestinal vitamin D receptor. The experiments were carried out in triplicate on two different occasions. The ED_{50} values are derived from dose−response curves and represent the analogue concentration required for 50% displacement of the radiolabeled 1α,25-$(OH)_2D_3$ from the receptor protein. Binding ratio is the ratio of the analogue average ED_{50} to the ED_{50} for 1α,25-$(OH)_2D_3$. [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 three times. The ED_{50} values are derived from dose−response curves and represent the analogue concentration capable of inducing 50% maturation. Differentiation activity radio is the ratio of the analogue average ED_{50} to the ED_{50} for 1α,25-$(OH)_2D_3$.

2-methyl-substituted 19-norvitamins **7a**,**b** and **8b** were only 4−5 times less active than 1α,25-$(OH)_2D_3$, while the 2β-methyl isomer in the 20*R*-series (**8a**) was 39-fold less effective. The 2α-(hydroxymethyl)vitamin D analogue with the "unnatural" configuration at C-20 (**9b**) was almost equivalent to the hormone **1** with respect to receptor binding, and the isomeric **10b** proved to be less potent (6−8 times) than these compounds. The corresponding 2α-hydroxymethyl analogue possessing the "natural" 20*R*-configuration (**9a**) was 10-fold less effective than the 20*S*-compound **9b**, whereas the 2β-isomer **10a** was 90 times less potent. The foregoing results of the competitive binding analysis show that vitamins with the axial orientation of the 1α-hydroxy group exhibit a significantly enhanced affinity for the receptor. Both isomeric 1α,19,25-trihydroxy-10,19-dihydrovitamin D_3 compounds **13** and **14** were 1000 times less potent than the natural hormone **1** in displacement of the radiolabeled 1α,25-$(OH)_2D_3$ from the receptor protein (data not shown).

Attention is drawn to the fact that both 2-methylene-19-norvitamins **6a**,**b**, when tested in vivo in rats, exhibited an extremely high ability to mobilize calcium from bone, while having no intestinal calcium transport activity (Table 2). It was not surprising that the 1α,-19,25-trihydroxy-10,19-dihydrovitamin D_3 compounds **13** and **14** were devoid of biological activity (data not shown), in view of our previous results indicating low potency of the parent 1α,25-dihydroxy-10,19-dihydrovitamin D_3 isomers.[12] However, vitamins possessing a hydroxymethyl substituent at C-2 turned out to be inactive, including those in the 20*S*-series (**9b**, **10b**). Thus, on the basis of the results described above, it was impossible to draw any conclusions regarding the conformation−activity relationship. On the contrary, the in vivo biological testing of 2-methyl-substituted 19-nor-1α,25-$(OH)_2D_3$ analogues appeared to be much more interesting and informative. Table 2 shows that a 260-pmol dose of 2α-methyl vitamin **7a** is equal to or only slightly less effective on intestinal calcium transport and bone calcium mobilization than hormone **1**. The overall calcemic activity of its analogue **7b** from the 20*S*-series proved to be appreciably higher than that of 1α,25-$(OH)_2D_3$. However, no elevation of serum calcium or bone calcium mobilization was found for any of the 2β-methyl analogues **8a**,**b** at this dose level. The foregoing activity of the vitamin D analogues strongly depends on the conformation of the A-ring. However, in any case, these results do not support the suggestion that the equatorially favored 1α-hydroxyl is required for calcemic activity, because analogues with such an orientation of the 1α-OH group show reduced biopotency in both intestine and bone. Thus, the results indicate a much greater biological activity of the vitamins possessing the axial 1α-hydroxy substituent.

In the next assay, the cellular activity of the synthesized compounds was established by studying their ability to induce differentiation of human promyelocyte HL-60 cells into monocytes. Interestingly, the transposition of the A-ring exocyclic methylene group to C-2 did not change the cellular differentiation ability of the analogue **6a** with respect to the parent hormone **1** (Table 1). It was found that, with an exception of an equally active **10b**, all of the synthesized vitamin D analogues with the "unnatural" 20*S*-configuration were more potent than 1α,25-$(OH)_2D_3$. Moreover, the same relationship between cellular activity and conformation of the vitamin D compounds was established as in the case of receptor binding analysis and in vivo studies: i.e., 2α-substituted vitamin D analogues were considerably more active than their 2β-substituted counterparts with the equatorially oriented 1α-hydroxy group. Thus, 2α-methyl vitamins **7a**,**b** proved to be 100 and 10 times, respectively, more active than their corresponding 2β-isomers **8a**,**b** in the cultures of HL-60 in vitro, whereas in the case of 2-hydroxymethyl derivatives (**9a**,**b** versus **10a**,**b**) these differences were smaller. Since vitamins with a 2β-methyl substituent (**8a**,**b**) and both 2-hydroxymethyl analogues in the 20*S*-series (**9b**, **10b**) have selective activity profiles combining high potency in cellular differentiation and lack of calcemic activity, such compounds are potentially useful as therapeutic

Table 2. Support of Intestinal Calcium Transport and Bone Calcium Mobilization by 2-Substituted Analogues of 1α,25-Dihydroxy-19-norvitamin D$_3$ in Vitamin D-Deficient Rats on a Low-Calcium Diet[a]

compound	compd no.	amount (pmol)	Ca transport S/M (mean ± SEM)	serum Ca (mean ± SEM)
none (control)		0	5.5 ± 0.2^{b}	5.1 ± 0.2^{b}
1α,25-(OH)$_2$D$_3$	**1**	260	6.2 ± 0.4^{c}	7.2 ± 0.5^{c}
2-methylene-19-nor-1α,25-(OH)$_2$D$_3$	**6a**	130	5.3 ± 0.4^{d1}	9.9 ± 0.2^{d1}
		260	4.9 ± 0.6^{d2}	9.6 ± 0.3^{d2}
2-methylene-19-nor-(20*S*)-1α,25-(OH)$_2$D$_3$	**6b**	130	5.8 ± 0.8^{e1}	13.8 ± 0.5^{e1}
		260	4.6 ± 0.7^{e2}	14.4 ± 0.6^{e2}
none (control)		0	2.3 ± 0.4^{b}	3.9 ± 0.1^{b}
1α,25-(OH)$_2$D$_3$	**1**	260	5.6 ± 0.6^{c}	6.1 ± 0.2^{c}
2α-methyl-19-nor-1α,25-(OH)$_2$D$_3$	**7a**	130	4.3 ± 0.6^{d1}	4.8 ± 0.1^{d1}
		260	5.3 ± 0.6^{d2}	5.8 ± 0.3^{d2}
2β-methyl-19-nor-1α,25-(OH)$_2$D$_3$	**8a**	130	4.4 ± 0.2^{e1}	4.1 ± 0.1^{e1}
		260	3.0 ± 0.4^{e2}	3.8 ± 0.1^{e2}
none (control)		0	2.9 ± 0.3^{b}	4.2 ± 0.1^{b}
1α,25-(OH)$_2$D$_3$	**1**	260	4.6 ± 0.2^{c}	6.6 ± 0.4^{c}
2α-methyl-19-nor-(20*S*)-1α,25-(OH)$_2$D$_3$	**7b**	130	12.9 ± 1.9^{d1}	8.3 ± 0.7^{d1}
		260	8.4 ± 1.1^{d2}	10.3 ± 0.1^{d2}
2β-methyl-19-nor-(20*S*)-1α,25-(OH)$_2$D$_3$	**8b**	130	2.9 ± 0.3^{e1}	4.4 ± 0.1^{e1}
		260	3.8 ± 0.1^{e2}	4.4 ± 0.1^{e2}
none (control)		0	4.0 ± 0.3^{b}	3.8 ± 0.1^{b}
1α,25-(OH)$_2$D$_3$	**1**	260	6.6 ± 0.5^{c}	5.2 ± 0.1^{c}
2α-(hydroxymethyl)-19-nor-(20*S*)-1α,25-(OH)$_2$D$_3$	**9b**	130	5.0 ± 0.3^{d1}	4.0 ± 0.1^{d1}
		260	5.8 ± 0.4^{d2}	3.9 ± 0.1^{d2}
2β-(hydroxymethyl)-19-nor-(20*S*)-1α,25-(OH)$_2$D$_3$	**10b**	130	3.5 ± 0.7^{e1}	3.6 ± 0.1^{e1}
		260	3.5 ± 0.3^{e2}	3.5 ± 0.2^{e2}

[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 consecutive 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 6 rats/group. Statistical analysis was done by Student's *t*-test. Statistical data: serosal/mucosal (S/M) *m*, panel 1, *b* from *c*, $p < 0.05$, *c* from d^1, d^2, and e^2, $p < 0.025$, *b* from d^1, d^2, e^1, and e^2, NS; panel 2, *b* from *c*, $p < 0.001$, *b* from d^1, e^1, *and* e^2, NS, *b* from d^2, $p < 0.005$; panel 3, *b* from *c*, $p = 0.05$, *b* from d^1 and d^2, $p < 0.001$, *b* from e^1 and e^2, NS; panel 4, *b* from *c*, $p < 0.001$, *b* from d^1, $p < 0.01$, *b* from d^2, $p < 0.001$, *b* from e^1 and e^2, NS; serum calcium *m*, panel 1, *b* from *c*, $p < 0.005$, *b* from d^1, d^2, e^1, and e^2, $p < 0.001$; panel 2, *b* from *c*, $p < 0.005$, *b* from d^1, e^1, and e^2, NS, *b* from d^2, $p < 0.025$; panel 3, *b* from *c*, $p = 0.05$, *b* from d^1 and d^2, $p < 0.001$, *b* from e^1 and e^2, NS; panel 4, *b* from *c*, $p = 0.001$, *b* from all others, NS.

agents for the treatment of cancer. As in the case of the bioassays described previously, compounds **13** and **14** were found to be totally inactive also in the HL-60 system (data not shown).

Conclusions

These results as well as our previous studies on 19-nor analogues of the hormone 1α,25-(OH)$_2$D$_3$ indicate that variation of substituents on C-2 in the parent 19-nor-1α,25-dihydroxyvitamin D$_3$ can change completely (and selectively) the biological potency of the analogues. Thus, introduction of a 2β-hydroxy group into ring A of **2**, which by itself has no calcemic activity,[7b] yielded a vitamin D compound of high intestinal calcium transport activity and no ability to mobilize calcium from bone.[9] An exomethylene substituent at C-2 has an opposite effect, providing the analogue that very effectively increased bone calcium mobilization (serum calcium) while being inactive in intestine. The present results suggest that the 2-methylene- and 2α-methyl-substituted 19-nor-1α,25-dihydroxyvitamin D$_3$ compounds in the 20*S*-series have very strong and preferential activity on bone, making them candidates for treatment of bone diseases.

The present study allows us also to conclude that the axial orientation of the 1α-hydroxyl group in the vitamin D molecule is essential for its biological potency.

Experimental Section

Chemistry. Ultraviolet (UV) absorption spectra were recorded with a Hitachi Model 60-100 UV−vis spectrometer in the solvent noted. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz with a Bruker AM-500 FT spectrometer in deuteriochloroform. Chemical shifts (δ) are reported downfield from internal Me$_4$Si (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. 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 methyl quinicate derivative **15** was obtained from commercial (−)-quinic acid as described previously.[7] **15**: mp 82−82.5 °C (from hexane); ^{1}H NMR ($CDCl_3$) δ 0.098, 0.110, 0.142, and 0.159 (each 3H, each s, 4 × SiC*H*$_3$), 0.896 and 0.911 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.820 (1H, dd, J = 13.1, 10.3 Hz), 2.02 (1H, ddd, J = 14.3, 4.3, 2.4 Hz), 2.09 (1H, dd, J = 14.3, 2.8 Hz), 2.19 (1H, ddd, J = 13.1, 4.4, 2.4 Hz), 2.31 (1H, d, J = 2.8 Hz, O*H*), 3.42 (1H, m; after D$_2$O dd, J = 8.6, 2.6 Hz), 3.77 (3H, s), 4.12 (1H, m), 4.37 (1H, m), 4.53 (1H, br s, O*H*).

(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-4-oxocyclohexanecarboxylic Acid Methyl Ester (16). To a stirred mixture of ruthenium(III) chloride hydrate (434 mg, 2.1 mmol) and sodium periodate (10.8 g, 50.6 mmol) in water (42 mL) was added a solution of methyl quinicate **15** (6.09 g, 14 mmol) in CCl_4/CH_3CN (1:1, 64 mL). Vigorous stirring was continued for 8 h. Few drops of 2-propanol were added; the mixture was poured into water and extracted with chloroform. The organic layer was washed with water, dried ($MgSO_4$), and evaporated to give a dark oily residue (ca. 5 g) which was purified by flash chromatography. Elution with hexane/ethyl

acetate (8:2) gave pure, oily 4-ketone **16** (3.4 g, 56%): ^{1}H NMR ($CDCl_3$) δ 0.054, 0.091, 0.127, and 0.132 (each 3H, each s, 4 × $SiCH_3$), 0.908 and 0.913 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.22 (1H, dd, J = 13.2, 11.7 Hz), 2.28 (1H, ~ dt, J = 14.9, 3.6 Hz), 2.37 (1H, dd, J = 14.9, 3.2 Hz), 2.55 (1H, ddd, J = 13.2, 6.4, 3.4 Hz), 3.79 (3H, s), 4.41 (1H, t, J ~ 3.5 Hz), 4.64 (1H, s, O*H*), 5.04 (1H, dd, J = 11.7, 6.4 Hz); MS *m/z* (relative intensity) no M^+, 375 (M^+ − *t*-Bu, 32), 357 (M^+ − *t*-Bu − H_2O, 47), 243 (31), 225 (57), 73 (100).

(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-4-methylenecyclohexanecarboxylic Acid Methyl Ester (17). To the methyltriphenylphoshonium bromide (2.813 g, 7.88 mmol) in anhydrous THF (32 mL) at 0 °C was added dropwise *n*-BuLi (2.5 M in hexanes, 6.0 mL, 15 mmol) under argon with stirring. Another portion of $MePh_3P^+Br^-$ (2.813 g, 7.88 mmol) was then added, and the solution was stirred at 0 °C for 10 min and at room temperature for 40 min. The orange-red mixture was again cooled to 0 °C, and a solution of 4-ketone **16** (1.558 g, 3.6 mmol) in anhydrous THF (16 + 2 mL) was siphoned to reaction flask during 20 min. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 3 h. The mixture was then carefully poured into brine containing 1% HCl and extracted with ethyl acetate and benzene. Organic extract was washed with diluted $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give an orange oily residue (ca. 2.6 g) which was purified by flash chromatography. Elution with hexane/ethyl acetate (9:1) gave pure 4-methylene compound **17** as a colorless oil (368 mg, 24%): ^{1}H NMR ($CDCl_3$) δ 0.078, 0.083, 0.092, and 0.115 (each 3H, each s, 4 × $SiCH_3$), 0.889 and 0.920 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.811 (1H, dd, J = 12.6, 11.2 Hz), 2.10 (2H, m), 2.31 (1H, dd, J = 12.6, 5.1 Hz), 3.76 (3H, s), 4.69 (1H, t, J = 3.1 Hz), 4.78 (1H, m), 4.96 (2H, m; after D_2O 1H, br s), 5.17 (1H, t, J = 1.9 Hz); MS *m/z* (relative intensity) no M^+, 373 (M^+ − *t*-Bu, 57), 355 (M^+ − *t*-Bu − H_2O, 13), 341 (19), 313 (25), 241 (33), 223 (37), 209 (56), 73 (100).

[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-1′-hydroxy-4′-methylenecyclohexyl]methanol (18). (a) To a stirred solution of the ester **17** (90 mg, 0.21 mmol) in anhydrous THF (8 mL) was added lithium aluminum hydride (60 mg, 1.6 mmol) at 0 °C under argon. The cooling bath was removed after 1 h, and the stirring was continued at 6 °C for 12 h and at room temperature for 6 h. The excess of the reagent was decomposed with saturated aqueous Na_2SO_4, and the mixture was extracted with ethyl acetate and ether, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue with hexane/ethyl acetate (9:1) afforded unreacted substrate (12 mg) and a pure, crystalline diol **18** (35 mg, 48% based on recovered ester **17**): ^{1}H NMR ($CDCl_3$ + D_2O) δ 0.079, 0.091, 0.100, and 0.121 (each 3H, each s, 4 × $SiCH_3$), 0.895 and 0.927 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.339 (1H, t, J ~ 12 Hz), 1.510 (1H, dd, J = 14.3, 2.7 Hz), 2.10 (2H, m), 3.29 and 3.40 (1H and 1H, each d, J = 11.0 Hz), 4.66 (1H, t, J ~ 2.8 Hz), 4.78 (1H, m), 4.92 (1H, t, J = 1.7 Hz), 5.13 (1H, t, J = 2.0 Hz); MS *m/z* (relative intensity) no M^+, 345 (M^+ − *t*-Bu, 8), 327 (M^+ − *t*-Bu − H_2O, 22), 213 (28), 195 (11), 73 (100). Anal. ($C_{20}H_{42}O_4Si_2$) C, H.

(b) Diisobutylaluminum hydride (1.5 M in toluene, 2.0 mL, 3 mmol) was added to a solution of the ester **17** (215 mg, 0.5 mmol) in anhydrous ether (3 mL) at −78 °C under argon. The mixture was stirred at −78 °C for 3 h and at −24 °C for 1.5 h, diluted with ether (10 mL), and quenched by the slow addition of 2 N potassium sodium tartrate. The solution was warmed to room temperature, stirred for 15 min, and then poured into brine and extracted with ethyl acetate and ether. Organic extracts were washed with diluted (ca. 1%) HCl and brine, dried ($MgSO_4$), and evaporated. The crystalline residue was purified by flash chromatography. Elution with hexane/ethyl acetate (9:1) gave crystalline diol **18** (43 mg, 24%).

(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methylenecyclohexanone (19). Sodium periodate-saturated water (2.2 mL) was added to a solution of the diol **18** (146 mg, 0.36 mmol) in methanol (9 mL) at 0 °C. The solution was stirred at 0 °C for 1 h, poured into brine, and extracted with ether and benzene. The extract was washed with brine, dried ($MgSO_4$), and evaporated. An oily residue was dissolved in hexane (1 mL) and applied on a Sep-Pak cartridge. Pure 4-methylenecyclohexanone derivative **19** (110 mg, 82%) was eluted with hexane/ethyl acetate (95:5) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.050 and 0.069 (6H and 6H, each s, 4 × $SiCH_3$), 0.881 (18H, s, 2 × Si-*t*-Bu), 2.45 (2H, ddd, J = 14.2, 6.9, 1.4 Hz), 2.64 (2H, ddd, J = 14.2, 4.6, 1.4 Hz), 4.69 (2H, dd, J = 6.9, 4.6 Hz), 5.16 (2H, s); MS *m/z* (relative intensity) no M^+, 355 (M^+ − Me, 3), 313 (M^+ − *t*-Bu, 100), 73 (76).

[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-methylenecyclohexylidene]acetic Acid Methyl Ester (20). To a solution of diisopropylamine (37 μL, 0.28 mmol) in anhydrous THF (200 μL) was added *n*-BuLi (2.5 M in hexanes, 113 μL, 0.28 mmol) under argon at −78 °C with stirring, and methyl (trimethylsilyl)acetate (46 μL, 0.28 mmol) was then added. After 15 min, the keto compound **19** (49 mg, 0.132 mmol) in anhydrous THF (200 + 80 μL) was added dropwise. The solution was stirred at −78 °C for 2 h, and the reaction mixture was quenched with saturated NH_4Cl, poured into brine and extracted with ether and benzene. The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was dissolved in hexane (1 mL) and applied on a Sep-Pak cartridge. Elution with hexane and hexane/ethyl acetate (98:2) gave a pure allylic ester **20** (50 mg, 89%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.039, 0.064, and 0.076 (6H, 3H, and 3H, each s, 4 × $SiCH_3$), 0.864 and 0.884 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.26 (1H, dd, J = 12.8, 7.4 Hz), 2.47 (1H, dd, J = 12.8, 4.2 Hz), 2.98 (1H, dd, J = 13.3, 4.0 Hz), 3.06 (1H, dd, J = 13.3, 6.6 Hz), 3.69 (3H, s), 4.48 (2H, m), 4.99 (2H, s), 5.74 (1H, s); MS *m/z* (relative intensity) 426 (M^+, 2), 441 (M^+ − Me, 4), 369 (M^+ − *t*-Bu, 100), 263 (69).

2-[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-methylenecyclohexylidene]ethanol (21). Diisobutylaluminum hydride (1.5 M in toluene, 1.6 mL, 2.4 mmol) was slowly added to a stirred solution of the allylic ester **20** (143 mg, 0.33 mmol) in toluene/methylene chloride (2:1, 5.7 mL) at −78 °C under argon. Stirring was continued at −78 °C for 1 h and at −46 °C (cyclohexanone/dry ice bath) for 25 min. The mixture was quenched by the slow addition of potassium sodium tartrate (2 N, 3 mL), aqueous HCl (2 N, 3 mL), and H_2O (12 mL) and then diluted with methylene chloride (12 mL) and extracted with ether and benzene. The organic layers were washed with diluted (ca. 1%) HCl and brine, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography. Elution with hexane/ethyl acetate (9:1) gave crystalline allylic alcohol **21** (130 mg, 97%): ^{1}H NMR ($CDCl_3$) δ 0.038, 0.050, and 0.075 (3H, 3H, and 6H, each s, 4 × $SiCH_3$), 0.876 and 0.904 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.12 (1H, dd, J = 12.3, 8.8 Hz), 2.23 (1H, dd, J = 13.3, 2.7 Hz), 2.45 (1H, dd, J = 12.3, 4.8 Hz), 2.51 (1H, dd, J = 13.3, 5.4 Hz), 4.04 (1H, m; after D_2O dd, J = 12.0, 7.0 Hz), 4.17 (1H, m; after D_2O dd, J = 12.0, 7.4 Hz), 4.38 (1H, m), 4.49 (1H, m), 4.95 (1H, br s), 5.05 (1H, t, J = 1.7 Hz), 5.69 (1H, ~t, J = 7.2 Hz); MS *m/z* (relative intensity) 398 (M^+, 2), 383 (M^+ − Me, 2), 365 (M^+ − Me − H_2O, 4), 341 (M^+ − *t*-Bu, 78), 323 (M^+ − *t*-Bu − H_2O, 10), 73 (100). Anal. ($C_{21}H_{42}O_3Si_2$) C, H.

[2-[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-methylenecyclohexylidene]ethyl]diphenylphosphine Oxide (22). To the allylic alcohol **21** (105 mg, 0.263 mmol) in anhydrous THF (2.4 mL) was added *n*-BuLi (2.5 M in hexanes, 105 μL, 0.263 mmol) under argon at 0 °C. Freshly recrystallized tosyl chloride (50.4 mg, 0.264 mmol) was dissolved in anhydrous THF (480 μL) and added to the allylic alcohol−BuLi solution. The mixture was stirred at 0 °C for 5 min and set aside at 0 °C. In another dry flask with air replaced by argon, *n*-BuLi (2.5 M in hexanes, 210 μL, 0.525 mmol) was added to Ph_2PH (93 μL, 0.534 mmol) in anhydrous THF (750 μL) at 0 °C with stirring. The red solution was siphoned under argon pressure to the solution of tosylate until the orange color persisted (ca. one-half of the solution was added). The resulting mixture was stirred for an additional 30 min at 0 °C and quenched by addition of H_2O (30 μL). Solvents were evaporated under reduced pressure, and the residue was

redissolved in methylene chloride (2.4 mL) and stirred with 10% H_2O_2 at 0 °C for 1 h. The organic layer was separated, washed with cold aqueous sodium sulfite and H_2O, dried ($MgSO_4$), and evaporated. The residue was subjected to flash chromatography. Elution with benzene/ethyl acetate (6:4) gave semicrystalline phosphine oxide **22** (134 mg, 87%): ^{1}H NMR ($CDCl_3$) δ 0.002, 0.011, and 0.019 (3H, 3H, and 6H, each s, 4 × $SiCH_3$), 0.855 and 0.860 (9H and 9H, each s, 2 × Si-*t*-Bu), 2.0−2.1 (3H, br m), 2.34 (1H, m), 3.08 (1H, m), 3.19 (1H, m), 4.34 (2H, m), 4.90 and 4.94 (1H and 1H, each s,), 5.35 (1H, ∼q, J = 7.4 Hz), 7.46 (4H, m), 7.52 (2H, m), 7.72 (4H, m); MS *m*/*z* (relative intensity) no M^+, 581 ($M^+ - 1$, 1), 567 ($M^+ -$ Me, 3), 525 ($M^+ - t$-Bu, 100), 450 (10), 393 (48). Anal. ($C_{33}H_{51}O_3PSi_2$) C, H.

1α,25-Dihydroxy-2-methylene-19-norvitamin D$_3$ (6a). To a solution of phosphine oxide **22** (33.1 mg, 56.8 μmol) in anhydrous THF (450 μL) at 0 °C was slowly added *n*-BuLi (2.5 M in hexanes, 23 μL, 57.5 μmol) under argon with stirring. The solution turned deep orange. The mixture was cooled to −78 °C, and a precooled (−78 °C) solution of protected hydroxy ketone **23a** (9.0 mg, 22.8 μmol; prepared according to a published procedure[9]) in anhydrous THF (200 + 100 μL) was slowly added. The mixture was stirred under argon at −78 °C for 1 h and at 0 °C for 18 h. Ethyl acetate was added, and the organic phase was washed with brine, dried ($MgSO_4$), and evaporated. The residue was dissolved in hexane, applied on a silica Sep-Pak cartridge, and washed with hexane/ethyl acetate (99.5:0.5, 20 mL) to give 19-norvitamin derivative **24a** (13.5 mg, 78%). The Sep-Pak was then washed with hexane/ethyl acetate (96:4, 10 mL) to recover some unchanged C,D-ring ketone **23a** (2 mg) and with ethyl acetate (10 mL) to recover diphenylphosphine oxide (20 mg). For analytical purpose a sample of protected vitamin **24a** was further purified by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.9:0.1) solvent system. Pure compound **24a** was eluted at R_V 26 mL as a colorless oil: UV (in hexane) λ_{max} 244, 253, 263 nm; ^{1}H NMR ($CDCl_3$) δ 0.025, 0.049, 0.066, and 0.080 (each 3H, each s, 4 × $SiCH_3$), 0.546 (3H, s, 18-H_3), 0.565 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.864 and 0.896 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.931 (3H, d, J = 6.0 Hz, 21-H_3), 0.947 (9H, t, J = 7.9 Hz, 3 × $SiCH_2CH_3$), 1.188 (6H, br s, 26- and 27-H_3), 2.00 (2H, m), 2.18 (1H, dd, J = 12.5, 8.5 Hz, 4β-H), 2.33 (1H, dd, J = 13.1, 2.9 Hz, 10β-H), 2.46 (1H, dd, J = 12.5, 4.5 Hz, 4α-H), 2.52 (1H, dd, J = 13.1, 5.8 Hz, 10α-H), 2.82 (1H, br d, J = 12 Hz, 9β-H), 4.43 (2H, m, 1β- and 3α-H), 4.92 and 4.97 (1H and 1H, each s, =CH_2), 5.84 and 6.22 (1H and 1H, each d, J = 11.0 Hz, 7- and 6-H); MS *m*/*z* (relative intensity) 758 (M^+, 17), 729 ($M^+ -$ Et, 6), 701 ($M^+ - t$-Bu, 4), 626 (100), 494 (23), 366 (50), 73 (92).

Protected vitamin **24a** (4.3 mg) was dissolved in benzene (150 μL), and the resin (AG 50W-X4, 60 mg; prewashed with methanol) in methanol (800 μL) was added. The mixture was stirred at room temperature under argon for 17 h, diluted with ethyl acetate/ether (1:1, 4 mL), and decanted. The resin was washed with ether (8 mL), and the combined organic phases were washed with brine and saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was purified by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system. Analytically pure 2-methylene-19-norvitamin **6a** (2.3 mg, 97%) was collected at R_V 29 mL (1α,25-dihydroxyvitamin D_3 was eluted at R_V 52 mL in the same system) as a white solid: UV (in EtOH) λ_{max} 243.5, 252, 262.5 nm; ^{1}H NMR ($CDCl_3$) δ 0.552 (3H, s, 18-H_3), 0.941 (3H, d, J = 6.4 Hz, 21-H_3), 1.222 (6H, s, 26- and 27-H_3), 2.01 (2H, m), 2.29 (1H, dd, J = 13.3, 8.4 Hz, 10α-H), 2.33 (1H, dd, J = 13.4, 6.0 Hz, 4β-H), 2.58 (1H, dd, J = 13.4, 3.9 Hz, 4α-H), 2.82 (1H, br d, J = 12 Hz, 9β-H), 2.86 (1H, dd, J = 13.3, 4.6 Hz, 10β-H), 4.49 (2H, m, 1β- and 3α-H), 5.10 and 5.11 (1H and 1H, each s, =CH_2), 5.89 and 6.37 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS *m*/*z* (relative intensity) 416 (M^+, 83), 398 (25), 384 (31), 380 (14), 351 (20), 313 (100); exact mass calcd for $C_{27}H_{44}O_3$ 416.3290, found 416.3279.

(20*S*)-25-[(Triethylsilyl)oxy]-des-A,B-cholestan-8-one (23b). A solution of (20*S*)-25-hydroxy Grundmann's ketone[14] (56 mg, 0.2 mmol) and imidazole (65 mg, 0.95 mmol) in anhydrous DMF (1.2 mL) was treated with triethylsilyl chloride (95 μL, 0.56 mmol), and 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 ($MgSO_4$), and evaporated. The residue was passed through a silica Sep-Pak cartridge in hexane/ethyl acetate (9:1) 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 hydroxy ketone **23b** (55 mg, 70%) was eluted at R_V 35 mL as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.566 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.638 (3H, s, 18-H_3), 0.859 (3H, d, J = 6.0 Hz, 21-H_3), 0.947 (9H, t, J = 7.9 Hz, 3 × $SiCH_2CH_3$), 1.196 (6H, s, 26- and 27-H_3), 2.45 (1H, dd, J = 11.4, 7.5 Hz, 14α-H).

(20*S*)-1α,25-Dihydroxy-2-methylene-19-norvitamin D$_3$ (6b). Vitamin **6b** was prepared in the same way as **6a**, except from the corresponding **23b**. The protected compound **24b** was isolated by flash chromatography in 79% yield. For analytical purposes a sample of vitamin **24b** was further purified by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.9:0.1) solvent system.

Protected vitamin **24b** was hydrolyzed as described for **24a**, and the product was purified by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system. Analytically pure 2-methylene-19-norvitamin **6b** (99% yield) was collected at R_V 28 mL as a white solid: UV (in EtOH) λ_{max} 243.5, 252.5, 262.5 nm; ^{1}H NMR ($CDCl_3$) δ 0.551 (3H, s, 18-H_3), 0.858 (3H, d, J = 6.6 Hz, 21-H_3), 1.215 (6H, s, 26- and 27-H_3), 1.95−2.04 (2H, m), 2.30 (1H, dd, J = 13.3, 8.4 Hz, 10α-H), 2.33 (1H, dd, J = 13.4, 6.3 Hz, 4β-H), 2.58 (1H, dd, J = 13.4, 3.7 Hz, 4α-H), 2.83 (1H, br d, J = 13.7 Hz, 9β-H), 2.85 (1H, dd, J = 13.3, 4.5 Hz, 10β-H), 4.49 (2H, m, 1β- and 3α-H), 5.09 and 5.11 (1H and 1H, each s, =CH_2), 5.89 and 6.36 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS *m*/*z* (relative intensity) 416 (M^+, 100), 398 (26), 380 (13), 366 (21), 313 (31); exact mass calcd for $C_{27}H_{44}O_3$ 416.3290, found 416.3275.

1α,25-Dihydroxy-2α- and 1α,25-Dihydroxy-2β-methyl-19-norvitamin D$_3$ (7a and 8a). Tris(triphenylphosphine)-rhodium(I) chloride (23 mg, 25 μmol) was added to dry benzene (25 mL) presaturated with hydrogen. The mixture was stirred at room temperature until a homogeneous solution was formed (ca. 45 min). A solution of vitamin **6a** (10 mg, 24 μmol) in dry benzene (5 mL) was then added, and the reaction was allowed to proceed under a continuous stream of hydrogen for 3 h. Benzene was removed under vacuum; the residue was redissolved in hexane/ethyl acetate (1:1, 4 mL) and applied on Waters silica Sep-Pak column (vac 12 cm^3). A mixture of 2-methyl vitamins was eluted with the same solvent system (20 mL). The compounds were further purified by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (85:15) solvent system. The mixture (ca. 1:1) of 2-methyl-19-norvitamins **7a** and **8a** (8.0 mg, 80%) gave a single peak at R_V 17 mL. Separation of both epimers was achieved by reversed-phase HPLC (10-mm × 25-cm Zorbax-ODS column, 4 mL/min) using methanol/water (85:15) solvent system. 2β-Methyl vitamin **8a** (3.5 mg, 35%) was collected at R_V 41 mL and its 2α-epimer **7a** (3.4 mg, 34%) at R_V 46 mL.

(20*S*)-1α,25-Dihydroxy-2α- and (20*S*)-1α,25-Dihydroxy-2β-methyl-19-norvitamin D$_3$ (7b and 8b). Vitamins **7b** and **8b** were obtained by hydrogenation of **6b**, performed analogously to the process described above for 20*R*-isomer **6a**. The hydrogenated compounds were first purified by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (85:15) solvent system. The mixture of 2-methyl-19-norvitamins **7b** and **8b** (ca. 1:1, 45%) gave a single peak at R_V 16.5 mL. Separation of both epimers was achieved by reversed-phase HPLC (10-mm × 25-cm Zorbax-ODS column, 4 mL/min) using methanol/water (85:15) solvent system. 2β-Methyl vitamin **8b** (16%) was collected at R_V 36 mL and its 2α-epimer **7b** (20%) at R_V 45 mL.

1α,25-Dihydroxy-2α- and 1α,25-Dihydroxy-2β-(hydroxymethyl)-19-norvitamin D$_3$ (9a and 10a). 9-Borabicyclo-

[3.3.1]nonane (0.5 M in THF, 60 μL, 30 μmol) was added to a solution of vitamin **6a** (1.25 mg, 3 μmol) in anhydrous THF (50 μL) at room temperature (evolution of hydrogen was observed). After 3 h of stirring, the mixture was quenched with methanol (20 μL), stirred for 15 min at room temperature, cooled to 0 °C, and treated successively with 6 M NaOH (10 μL, 60 μmol) and 30% H_2O_2 (10 μL). The mixture was heated for 1 h at 55 °C and cooled, benzene and brine were added, and the organic phase was separated, dried, and evaporated. The crystalline residue was dissolved in ether (0.5 mL) and kept in a freezer overnight. The ether solution was carefully removed from the precipitated crystals of cyclooctanediol and evaporated. Separation of the residue was achieved by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (85:15) solvent system. Traces of unreacted substrate **6a** were eluted at R_V 16 mL, whereas isomeric 2-hydroxymethyl vitamins **9a** and **10a** were collected at R_V 33 and 40 mL, respectively. Further purification of both products by reversed-phase HPLC (10-mm × 25-cm Zorbax-ODS column, 4 mL/min) using methanol/water (9:1) solvent system afforded analytically pure vitamin **9a** (0.14 mg, 11%) and its 2β-isomer **10a** (0.31 mg, 24%) collected at R_V 26 and 23 mL, respectively.

(20*S*)-1α,25-Dihydroxy-2α- and (20*S*)-1α,25-Dihydroxy-2β-(hydroxymethyl)-19-norvitamin D_3 (9b and 10b). The hydroboration of 20*S*-vitamin **6b** and subsequent oxidation of the organoborane adduct were performed using the procedure analogous to that described above for 20*R*-epimer **6a**. The reaction products were separated by HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (87.5:12.5) solvent system, and the isomeric 2-hydroxymethyl vitamins **9b** and **10b** were collected at R_V 40 and 47 mL, respectively. Further purification of both products by reversed-phase HPLC (10-mm × 25-cm Zorbax-ODS column, 4 mL/min) using methanol/water (9:1) solvent system afforded analytically pure vitamin **9b** (9%) and its 2β-isomer **10b** (26%) collected at R_V 25 and 22 mL, respectively.

(10*S*)- and (10*R*)-1α,19,25-Trihydroxy-10,19-dihydrovitamin D_3 (13 and 14). The hydroboration of 1α,25-$(OH)_2D_3$ (**1**) and subsequent oxidation of the borane adduct were performed using the procedure analogous to that described above for vitamin **6a**. The reaction products were subjected to flash chromatography. Elution with ethyl acetate resulted in separation of both isomers: vitamins **13** and **14** were gradually eluted from the column. Further purification of products by straight-phase HPLC (6.2-mm × 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (8:2) solvent system (both isomers collected at R_V ca. 33 mL) and reversed-phase HPLC (10-mm × 25-cm Zorbax-Sil column, 4 mL/min) using methanol/water (9:1) solvent system (the isomers eluted at R_V ca. 19 mL) provided the analytically pure, crystalline vitamins **13** (12%) and **14** (9%).

Biological Studies. 1. Measurement of Intestinal Calcium Transport and Bone Calcium Mobilization. Twenty-day-old 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,[32] 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.[33] 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; their blood and intestines were collected and used immediately to determine calcium transport activity and serum calcium concentration. Calcium was measured in the presence of 0.1% lanthanium chloride by means of a Perkin-Elmer atomic absorption spectrometer model 3110. Intestinal calcium transport was determined by the everted intestinal sac method using the proximal 10 cm of intestine as described earlier.[32] Statistical analysis was by the Student's *t*-test.[34] 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.

2. 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.[4d] The compounds tested were added in the indicated concentrations in 0.05 mL of ethanol so that the ethanol concentration never exceeded 1%. The incubation was carried out for 4 days, and at the end of the fourth day, superoxide production was measured by nitro blue tetrazolium (NBT) reduction. The number of cells containing intracellular black-blue formazan deposits was determined by light microscopy using a hemacytometer. At least 200 cells were counted in duplicate per determination. Percentage differentiation represents percentage cells providing NBT reduction appearance. The results were plotted on semilog paper, and relative differentiation activities of the analogues were determined by comparison of the compound concentrations capable of inducing 50% maturation according to the assay. This method is described in detail elsewhere.[4d] The experiment was repeated three times, and the results are reported as the mean ± SD.

3. Measurement of Binding to the Porcine Intestinal Vitamin D Receptor. Porcine intestinal nuclear extract was prepared as described earlier.[35] 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 analogue 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 ×100, 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 analogue or standard. Each value represents triplicate values. The displacement experiments were carried out in triplicate on two different occasions.

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Supporting Information Available: Spectral data of the vitamin D compounds **7a**,**b**−**10a**,**b**, **13**, **14**, and **24b**; figures with the competitive binding curves derived from the binding assay of the vitamin D analogues **6a**,**b**−**10a**,**b**; and figures with dose−response curves derived from the cellular differentiation assay performed for the same compounds (12 pages). Ordering information is given on any current masthead page.

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