New 2-Alkylidene 1α,25-Dihydroxy-19-norvitamin D_3 Analogues of High Intestinal Activity: Synthesis and Biological Evaluation of 2-(3′-Alkoxypropylidene) and 2-(3′-Hydroxypropylidene) Derivatives

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In a search for novel vitamin D compounds of potential therapeutic value, *E*- and *Z*-isomers of 1α,25-dihydroxy-2-(3′-hydroxypropylidene)-19-norvitamin D_3, as well as a derivative of the former compound possessing a 3′-(methoxymethoxy)propylidene substituent at C-2, were efficiently prepared. All vitamins were obtained in convergent syntheses, starting with (−)-quinic acid and the protected 25-hydroxy Grundmann ketones. Quinic acid was converted into keto lactone **11**, and a substituted hydroxypropylidene group was attached by Wittig reaction yielding pairs of isomeric compounds **12**, **13** and **14**, **15**. These olefinic products were then transformed into phosphine oxides **32**−**34** which were subjected to Lythgoe type Wittig−Horner coupling with C,D-fragments **35a** and **35b**. An alternative route was also elaborated that comprised Julia coupling of sulfones **39a** and **39b** with the cyclohexanone derivative **23**. The binding of all synthesized vitamins to the full-length rat recombinant vitamin D receptor (VDR) is either similar to or within one log of 1α,25$(OH)_2D_3$. The in vivo tests have revealed that the calcemic activity of all analogues in the *E*-series (**5a**, **6a**, **6b**) is considerably higher than that of the native hormone.

Introduction

It is well-known that 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **1**; Figure 1), the hormonally active form of vitamin D_3, exhibits a multitude of biological activities.[1,2] It has been established that the hormone 1α,25-$(OH)_2D_3$ controls the expression of numerous genes whose products are involved in calcium and phosphorus homeostasis as well as in cellular differentiation.[3] The vitamin D receptor (VDR),[4] which belongs to the nuclear receptor superfamily, plays a crucial role in these genomic biological actions.[5] Upon binding to vitamin D, VDR undergoes heterodimerization with retinoic X receptor (RXR) and coactivators, and then the resulting complex binds to a vitamin D response element (VDRE) inducing transcription.[6] In view of such an important role of the vitamin D−VDR complex we became interested in designing a vitamin D analogue that could not only bind effectively to VDR, but also introduce additional interactions that would alter the complex.

In 1998 we reported the synthesis of vitamin D analogues **2a**,**b** which had the A-ring exocyclic methylene unit attached to C-2, instead of C-10 as in the natural hormone **1**.[7] These analogues are characterized by VDR binding affinity comparable to 1α,25-$(OH)_2D_3$ but exhibit a significantly higher (and selective) ability to mobilize calcium from bone, in particular 20*S*-isomer **2b** (2MD). Recently, 2-ethylidene analogues have been prepared in our laboratory, and it has been determined that *E*-isomers **3a**,**b** are two times more active than the hormone **1** in binding to VDR.[8] Moreover, in vivo studies have shown that these analogues have higher intestinal activity than **1** and they exhibit similar (**3a**) or significantly increased (**3b**) ability to mobilize calcium from bone. The isomeric *Z* compounds **4a**,**b** are less potent, showing no activity in bone. Conformational analysis and spectral data of 2-ethylidene-19-norvitamin D analogues prove that their A-ring conformational equilibrium is significantly shifted, due to a strong $A^{(1,3)}$-strain interaction,[9,10] to the β-chair (*E*-isomers, Figure 2a) or α-chair form (*Z*-isomers, Figure 2b).

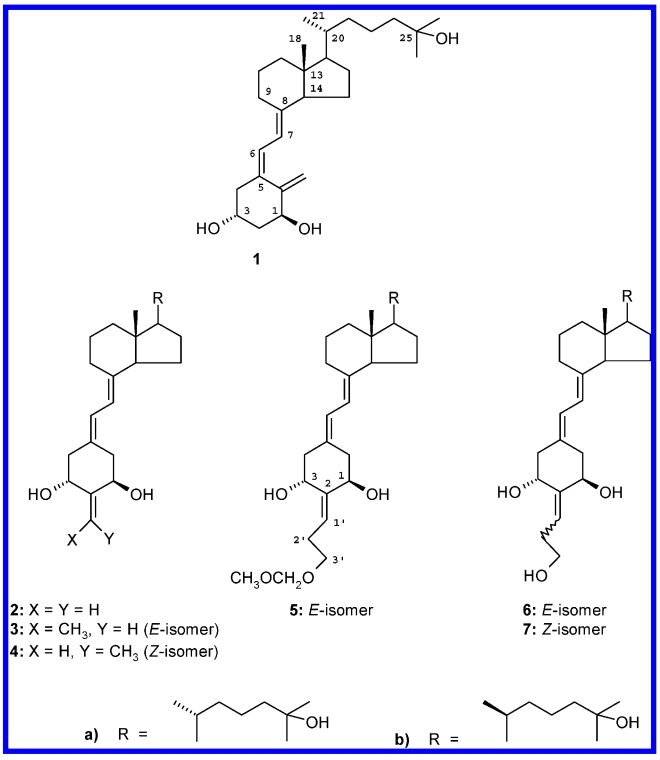

Figure 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and analogues.

Previously we noted very high biological activity of the 2α-(3′-hydroxypropyl) and 2α-(ω-hydroxyalkoxy) derivatives of the hormone **1**[11] as well as crystallographic data indicating that the VDR binding pocket can accommodate rather bulky fragments

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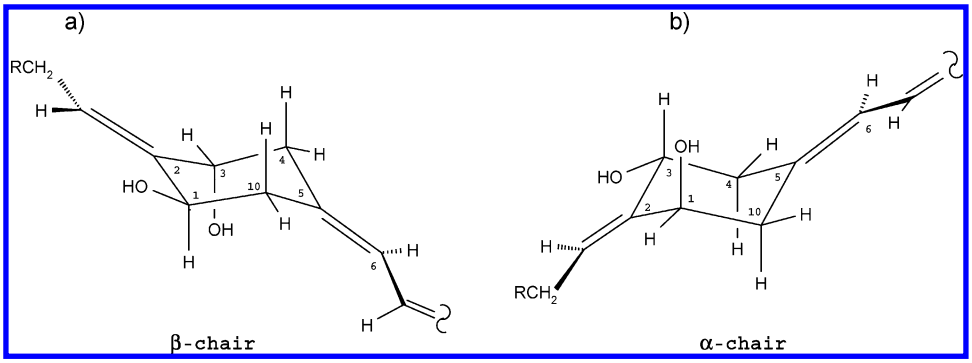
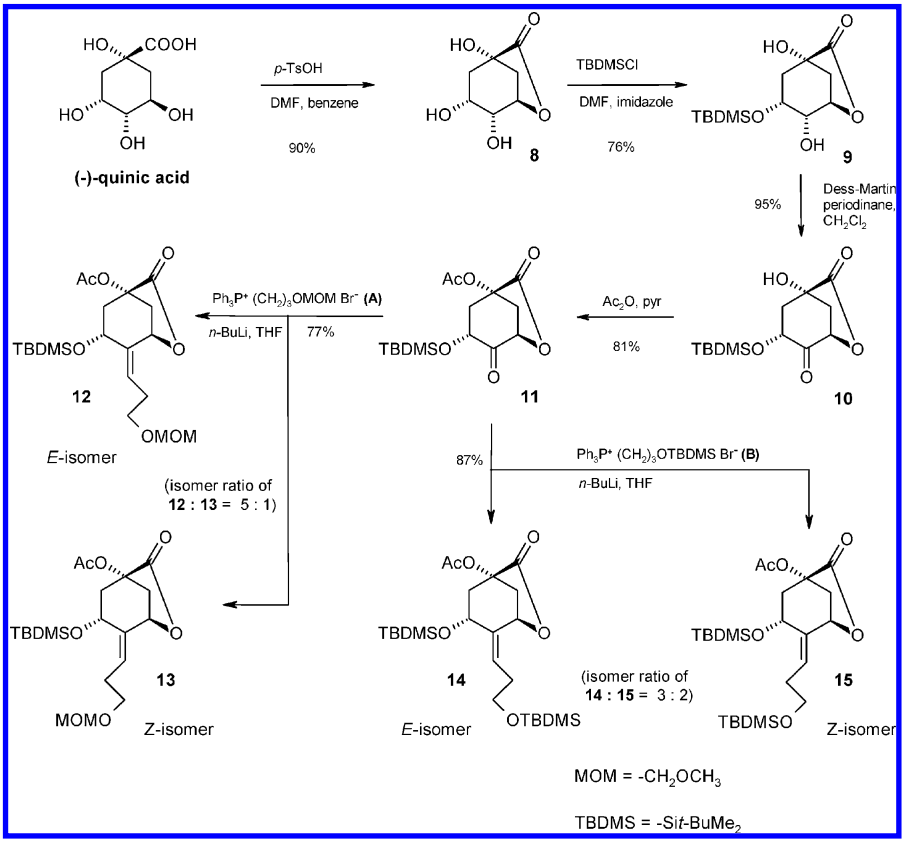


Figure 2. Preferred A-ring conformations of 2-alkylidene-1α-hydroxy-19-norvitamin D analogues: *E*-isomers (a) and *Z*-isomers (b).

Scheme 1

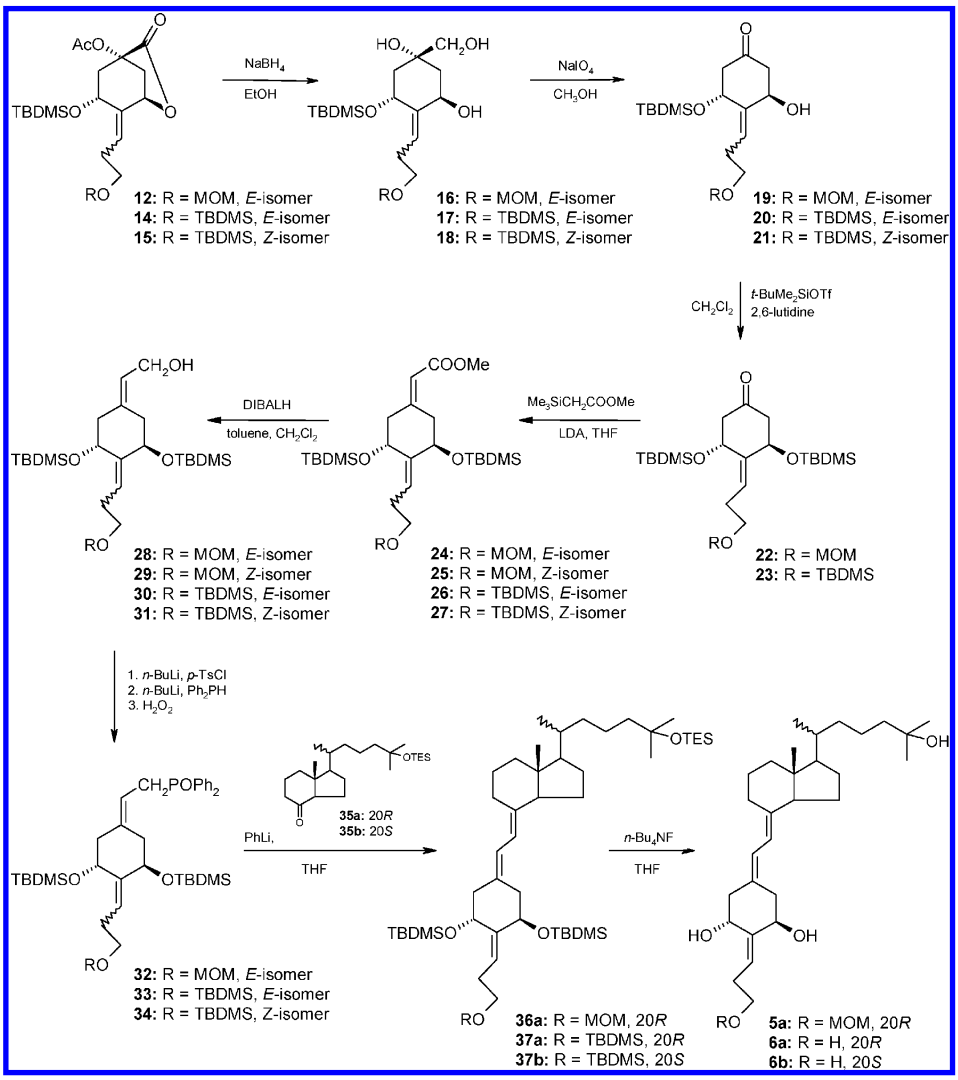


attached to C-2.[12] These facts encouraged us to synthesize 2-(3′-hydroxypropylidene) derivatives of 19-nor-1α,25-$(OH)_2D_3$ (structures **6** and **7**) as well as compounds possessing 2-(3′-alkoxypropylidene) substituents terminated with an alkoxyl group, containing an additional oxygen atom capable of forming hydrogen bonds with the VDR, i.e., the analogues substituted at C-2 with $=CHCH_2CH_2O(CH_2)_nOR$ moiety, where $n = 1$, 2, 3, etc. Our choice fell on a relatively simple vitamin D structure **5** with the (methoxy)methoxyl unit attached to the terminal C′-3 atom of the propylidene moiety at C-2. In view of preliminary results of molecular modeling of the free ligand **5a** and its complex with rVDR,[13] such an analogue seemed to be an interesting synthetic target. The strategy of our synthesis was based on the Wittig−Horner coupling[14] of the readily available protected 25-hydroxy Grundmann ketones **35a**[15] and **35b**[7] with the corresponding phosphine oxides of structures **32**−**34**.

Results and Discussion

Chemistry. As a starting compound for the synthesis of the A-ring fragments we chose a known bicyclic trihydroxy lactone **8**[16] (Scheme 1) easily prepared from the commercially available (1*R*,3*R*,4*S*,5*R*)-quinic acid.[17] The next step was selective protection of the equatorial hydroxyl group at C-3 as the *tert*-butyldimethylsilyl (TBDMS) ether. Vandewalle described the reaction of **8** with TBDMSCl and separation of the isomeric silyl ethers by preparative HPLC.[18] We established that the lower temperature of the reaction and small excess (1.15 molar) of the silylating agent resulted in considerably better chemoselectivity of the process and higher yields of the desired 3-OTBDMS derivative **9** that can be isolated by crystallization and column chromatography. The axial 4-hydroxyl was subsequently oxidized with Dess−Martin periodinane reagent, and the remaining tertiary hydroxyl in the formed **10** was acetylated. Esterification of the hydroxyl at C-1 was advantageous, because the presence of a free 1-hydroxyl group resulted in significantly diminished yields of the following Wittig reactions. According to our expectations, reaction of ketone **11** with the ylide generated from phosphonium bromide **A** and *n*-butyllithium proceeded smoothly, providing two isomeric olefinic products **12** and **13** (5:1 ratio) in 77% yield. Configuration of the propylidene unit at C-4 in

Scheme 2

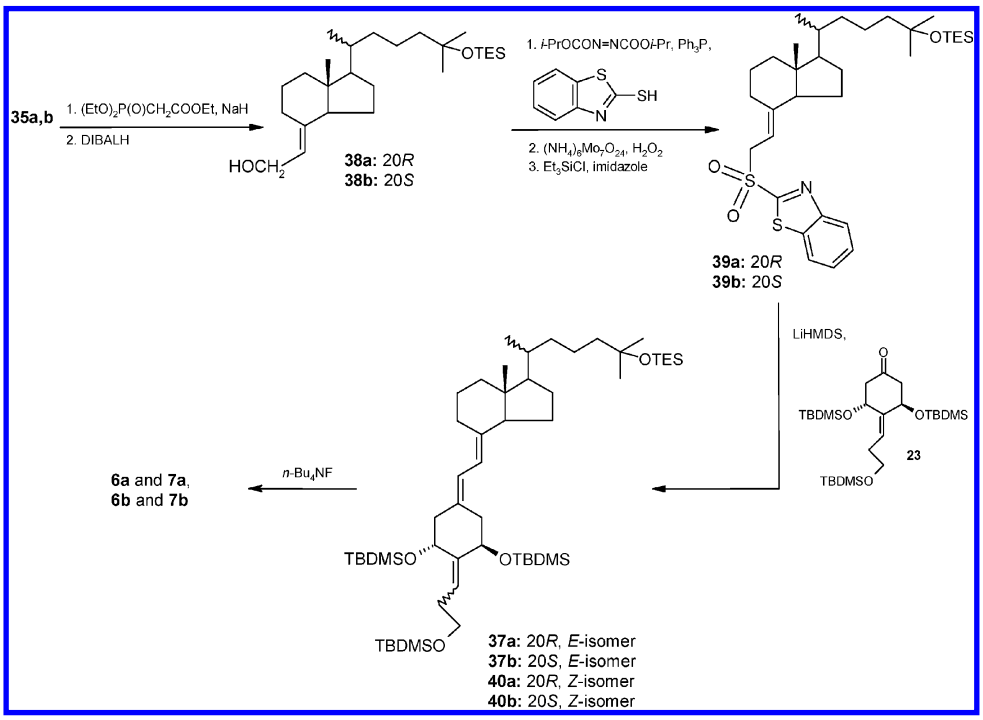


compounds **12** and **13** was unambiguously determined by ^{1}H NOE difference spectroscopy experiments (see Supporting Information). Wittig reaction of **11** with the ylide generated from phosphonium salt **B** proved to be even more efficient giving the olefinic products **14** and **15** (3:2 ratio) in 87% yield. Therefore, we have also decided to test an alternative way to the final compounds **6** and **7** using intermediates possessing a TBDMS group as a protection of the terminal hydroxyl in the A-ring hydroxypropylidene moiety.

Both compounds **12** and **13** can be easily converted to the cyclohexanone derivative **22** (Scheme 2). However, since chromatographical separation of these geometrical isomers was relatively easy, we only used compound **12** for this purpose, whereas the isomer **13** served as a starting compound for the synthesis of a different vitamin D analogue (unpublished results). Thus, sodium borohydride reduction of **12** followed by periodate oxidation of the resulting triol **16** gave hydroxy ketone **19** that was subsequently silylated to the desired protected ketone **22**. Peterson olefination of **22** resulted in the mixture of α,*β*-unsaturated esters **24** and **25** (7:1 ratio) in 86% yield. ^{1}H NOE difference spectroscopy and analysis of proton couplings allowed us to unequivocally establish the configurations of the obtained products (see Supporting Information), which could be separated by HPLC. However, it was more convenient to first perform DIBALH reduction and then column chromatography separation of the allylic alcohols **28** and **29**. The next three steps were executed with the prevailing *E*-geometrical isomer, providing the desired A-ring fragment, the phosphine oxide **32**. A synthetic sequence analogous to the conversion of **12** to **32** was used for the transformation of isomeric compounds **14** and **15** into the corresponding phosphine oxides **33** and **34**. Since a separation of the isomeric *E*- and *Z*-compounds was rather difficult, all synthetic steps were performed with the mixture of isomers.

The anion generated from **32** and phenyllithium reacted with the protected 25-hydroxy Grundmann ketone **35a** to give the 19-norvitamin D compound **36a** in 48% yield. Silyl protecting groups were removed by treatment with tetrabutylammonium fluoride and the final 2-[3′-(methoxymethoxy)propylidene] derivative of 19-nor-1α,25-$(OH)_2D_3$ (**5a**) was obtained in 71% yield. Comparison of its ^{1}H NMR spectrum with that of the respective 2-ethylidene-substituted analogue **3a**[8] indicated their close similarity. Half-widths of the multiplets derived from the methine protons at C-1 (δ 4.44, w/2 = 20 Hz) and C-3 (δ 4.84, w/2 = 10 Hz), found in the spectrum of **5a**, indicated that its 1α- and 3*β*-hydroxy groups occupy, respectively, equatorial and axial orientation**.** Conformational equilibrium of the A ring is in this case significantly shifted to the *β*-chair form (Figure 2a) because in the alternative α-chair conformation strong interaction exists between the propylidene group and 3*β*-hydroxyl. Obviously, it was expected that ketal derivative **5a** could be hydrolyzed in appropriate conditions to yield the parent 3′-hydroxypropylidene compound **6a**. Unfortunately, all attempts

Scheme 3



($MgBr_2$ and *n*-BuSH, $LiBF_4$, AlI_3, camphor-10-sulfonic acid in MeOH) to deprotect the terminal 3′-hydroxyl of the propylidene unit in **5a** failed. Wittig−Horner reaction between the mixture of phosphine oxides **33** and **34** and the protected Grundmann ketones **35a** and **35b** provided 19-norvitamin D compounds **37a** and **37b** in 41% and 47% yield, respectively. Only traces of the corresponding *Z*-isomers were detected in the reaction mixtures. Such reaction outcome could be expected because our previous studies on the synthesis of 2-ethylidene vitamin D analogues **3** and **4** indicated low reactivity of the phosphine oxides in the Z-series.[8] Deprotection of the four hydroxy goups in the obtained products **37a**,**b** gave the desired 2-(3′-hydroxypropylidene)-substituted 19-norvitamin D_3 analogues **6a**,**b**. Similarly as in the case of **5a**, their A-rings exist preferentially in the β-chair conformation.

Promising results of preliminary biological tests performed with *E*-geometrical isomers **5a** and **6a**,**b** encouraged us to also synthesize their counterparts in the *Z*-series. To achieve this goal an alternative synthesis was chosen, employing the modified Julia olefination[19] as a crucial step for providing the 19-norvitamin D skeleton. Such an attempt, based on the Kittaka approach,[20] involved preparation of the isomeric sulfones **39a**,**b** (Scheme 3), as the C,D-fragments, and their coupling with the A-ring cyclohexanone derivative **23**. Thus, both Grundmann ketones **35a**,**b** were coupled with the anion of triethyl phosphonoacetate and the prepared esters reduced with DIBALH to give the allylic alcohols **38a**,**b**. They were, in turn, subjected to Mitsunobu reaction with 2-mercaptobenzothiazole and the formed sulfides easily converted to the allylic sulfones **39a**,**b**. According to our expectations, Julia coupling of the anions, generated from these compounds with LiHMDS, and the protected ketone **23** provided the corresponding pairs of *E*- and *Z*-isomeric products: **37a**, **40a** and **37b**, **40b**. Each pair of the vitamin D compounds was initially purified by column chromatography, then treated with tetrabutylammonium fluoride, and the final isomeric vitamins **6a**, **7a** and **6b**, **7b** were separated by reversed-phase HPLC. Analysis of ^{1}H NMR spectra confirmed that in the 2-(3′-hydroxypropylidene)-substituted 19-norvitamin D_3 compounds **7a**,**b** their A-ring conformational equilibrium is strongly biased toward the α-chair form (Figure 2b).

Biological Evaluation. The synthesized derivative of 19-nor-1α,25-$(OH)_2D_3$ with a 3′-(methoxymethoxy)propylidene moiety at C-2 (**5a**) was tested for its ability to bind the full-length recombinant rat vitamin D receptor. The competitive binding analysis shows (Table 1) that **5a** exhibits ca. 6 times lower affinity for the receptor than 1α,25-$(OH)_2D_3$ (**1**). Thus, despite the fact that an alkoxyalkylidene chain consisting of seven atoms is attached to C-2, this analogue still retains a significant binding ability that is most likely due to the presence of two additional oxygen atoms. In the case of the closely related *E*-isomer **6a**, and its analogue **6b** with the unnatural configuration at C-20, the presence of a free terminal hydroxy group in the 2-propylidene substituent results in increasing affinity to receptor, essentially identical to that of the hormone **1**. Compounds in the *Z*-series (**7a**,**b**) are ca. 3−8 times less active than 1α,25-$(OH)_2D_3$ in binding to VDR.

High binding potency of the synthesized *E*-isomers is not surprising when the results of the docking experiments, performed for analogue **6a** and full length rVDR-LBD,[8] are taken into account. It turned out that this analogue, regardless of its initial positioning in the binding pocket, is finally oriented (Figure 3) quite similar as the natural hormone **1** in its crystalline complex with the VDRmt.[12] Thus, the plane of the intercyclic diene moiety of **6a** is approximately parallel to the Trp 282 rings, whereas the 1α- and 3β- and 25-hydroxy groups create strong hydrogen bonds with Ser 274, Arg 270, and His 301, respectively. The terminal hydroxyl from the ligand's 2-propylidene fragment contacts Arg 270 and, additionally, Asp 144.

Studies on the cellular activity of the synthesized compounds to induce the differentiation of human promyelocyte HL-60 cells into monocytes have indicated that they are almost equivalent to hormone **1**, with the exception of the analogue **6b** being 10 times more active (Table 1).

The ability of the vitamin D analogues in the *E*-series to induce transcription of vitamin D-responsive genes was exam-

Table 1. VDR Binding Properties,[a] HL-60 Differentiating Activities,[b] and Transcriptional Activities of the Vitamin D Compounds **5a**, **6a**, **6b**, **7a**, and **7b**

		VDR binding		HL-60 differentiation		24-OHase transcription	
compound	compd no.	K_i (M)	binding ratio	ED_{50} (M)	activity ratio	ED_{50} (M)	activity ratio
1α,25-$(OH)_2D_3$	**1**	1.1×10^{-10}	1	2.4×10^{-9}	1	2.6×10^{-10}	1
2-(3′-MOMO-propylidene)-19-nor-1α,25-$(OH)_2D_3$	**5a**	5.0×10^{-10}	6.6	4.7×10^{-9}	1.5	3.8×10^{-9}	15
2-(3′-hydroxypropylidene)-19-nor-1α,25-$(OH)_2D_3$ (*E*-isomer)	**6a**	1.3×10^{-10}	1.3	1.3×10^{-9}	0.6	4.2×10^{-11}	0.2
2-(3′-hydroxypropylidene)-19-nor-(20*S*)-1α,25-$(OH)_2D_3$ (*E*-isomer)	**6b**	6.2×10^{-11}	0.9	4.0×10^{-10}	0.1	6.5×10^{-12}	0.02
2-(3′-hydroxypropylidene)-19-nor-1α,25-$(OH)_2D_3$ (*Z*-isomer)	**7a**	6.1×10^{-10}	7.7	3.0×10^{-9}	1.7		
2-(3′-hydroxypropylidene)-19-nor-(20*S*)-1α,25-$(OH)_2D_3$ (*Z*-isomer)	**7b**	1.5×10^{-10}	2.8	1.4×10^{-9}	0.8		

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

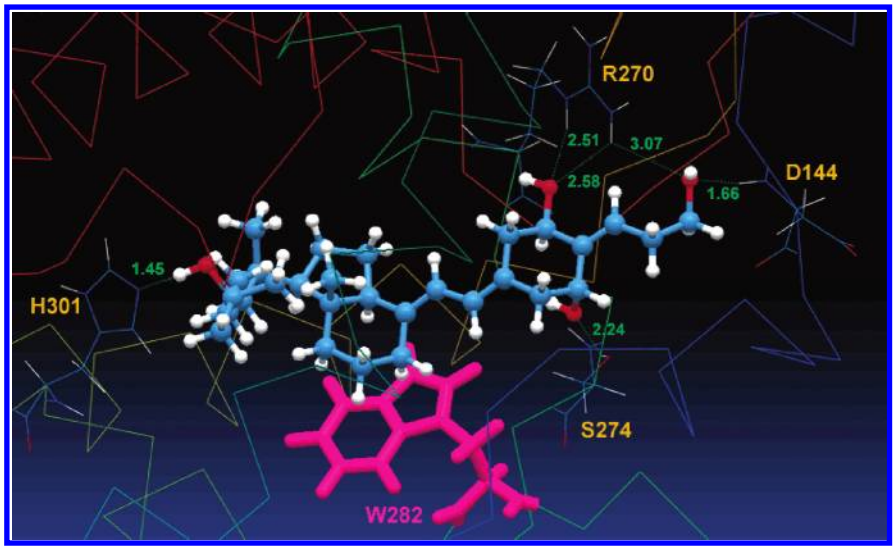


Figure 3. View of the three-dimensional structure of ligand binding cavity of the rat VDR with the docked vitamin D analogue **6a** (FlexiDock, TRIPOS). The four amino acids (Asp 144, Ser 274, Arg 270, and His 301) forming the shortest hydrogen bonds (the Å distances are marked in green) with the ligand are depicted. Also the Trp 282 residue is shown (pink).

ined using the 24-hydroxylase (CYP-24) luciferase reporter gene system and it was established that compound **5a** has 15 times lower activity with respect to 1α,25-$(OH)_2D_3$ (Table 1). However, analogues **6a** and **6b** are characterized by significantly higher transcriptional potencies, 5 and 50 times higher, respectively, in comparison with **1**.

Considering the results of the in vitro tests, a relatively low calcemic activity might be expected for the analogue **5a**. However, tests in vivo in mice indicate that the potency of compound **5a** in raising serum calcium is higher than that of the parent hormone **1** (Figure 4). Taking into account a relatively weak transcriptional activity of **5a** and its significant potency in vivo, it can be suggested that the analogue must be first metabolized in the living organism, most likely to the parent 3′-hydroxypropylidene derivative **6a**. This idea is supported by studies done in the mouse and the rat. A single oral dose of **5a** raises serum calcium 3 days later to the same level as **6a** (Figure 5). In addition, **5a** causes similar bone calcium mobilization and intestinal calcium transport activity as **6a** in D-deficient rats (Figure 6). The distinct activity differences observed in vitro between **6a** and **6b** were not observed in vivo (Figures 5 and 6). However, the *Z*-isomers are at least 10 times less active than their *E*-counterparts which is consistent with the results obtained in vitro (Figure 5). Finally, it is important to note that all three analogues **5a**, **6a**, and **6b**, while more potent than the native hormone in both bone calcium mobilization as well as intestinal calcium transport, have selective intestinal activity. Note that 100 pmol/day of compound **2b** (Figure 7) gave an increase in intestinal calcium transport equivalent to that induced by 5.7 pmol of **6a** or **6b**. On the other hand, compound **2b** was shown previously to have approximately equal activity on intestine as **1** but was found 30 times more active on bone.[21]

Conclusions

The structures of all synthesized compounds were mainly established by different NMR methods. NOE difference spectroscopy and heteronuclear correlation NMR spectra (HMQC, HMBC) were used for the complete assignments of ^1H and ^{13}C signal groups. Analysis of the observed vicinal proton coupling constants, confirmed by spin decoupling experiments, and their

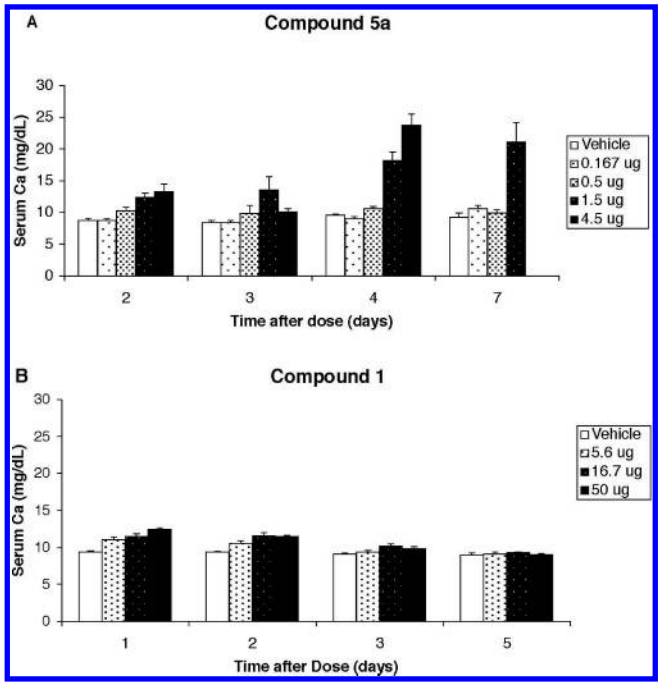


Figure 4. Serum calcium changes in response to a single oral dose of the analogue **5a** or native hormone in CD-1 mice. Six to seven week old female mice were given one oral dose of **5a** or 1α,25-$(OH)_2D_3$ (**1**), and blood was collected at several timepoints following dose administration for calcium concentration determination in the serum.

correlation with the couplings obtained by force field calculations allowed us to successfully assign both stereochemistries of the compounds and their preferred solution conformations.

Synthesis of 1α,25-dihydroxy-19-norvitamin D_3 analogues substituted at C-2 with (3′-methoxymethoxy)propylidene (**5a**), and 3′-hydroxypropylidene moiety (**6a**,**b** and **7a**,**b**) allowed detailed biological testing of these compounds. Biological evaluation of the analogue **5a** revealed that it is highly potent in vivo. This result was unexpected because of its relatively low transcriptional activity observed in vitro compared to the native hormone. One explanation for the differences observed in vitro compared to in vivo, is that **5a** is metabolized in the living organism to a more potent derivative and the most likely metabolite is generated by cleavage of the terminal methoxymethoxy group in the 2-alkylidene substituent resulting in 2-(3′-hydroxypropylidene) compound **6a**. This is further supported by the delay of serum calcium response noted in Figure 4 for **5a**. This explanation is also supported by the results obtained with analogue **6a** and other literature reports indicating that ether moieties are readily cleaved in the body.[22] Both analogues **6a** and **6b** showed increased potency in vitro compared to 1α,25$(OH)_2D_3$, but in vivo, these compounds were no more potent than **5a**. In vitro the analogue with the 20 carbon in the unnatural configuration (**6b**) exhibited higher potency than **6a** which has the natural 20*R*-configuration. This result was expected as several other compounds have been shown to exhibit enhanced potency when the 20-carbon is switched to the unnatural configuration.[23] Another structure/activity pattern that has been reported before is that *Z*-isomers of 2-alkylidene vitamin D analogues have less activity than their counterparts in the *E*-configuration.[8] This pattern was repeated with this series of compounds as well in that **7a** and **7b** were less active than **6a** and **6b** both in vitro and in vivo. Besides the unique in vitro/in vivo activity associated with **5a**, this series of compounds show an interesting biological profile in that compounds **5a**, **6a** and **6b** have intestinal selective activity which could be useful in shedding light on the design of new compounds with intestine or bone only activity. In addition, these compounds might have some utility in the therapeutic treatment of intestinal diseases.

The results of the tests presented above support our previous findings concerning 2-ethylidene 19-norvitamins **3a**,**b** and **4a**,**b**.[8] It has been found that introduction of alkylidene, hydroxyalkylidene, or alkoxyalkylidene substituents at position 2 in the 19-nor-1α,25-$(OH)_2D_3$ molecule results in the creation of vitamin D analogues characterized by strong affinities to VDR and highly elevated in vivo potencies. Such high potent in vivo activity is limited to *E*-geometric isomers possessing β-chair conformation of the cyclohexane ring A.

Experimental Section

Chemistry. Melting points (uncorrected) were determined on a Thomas-Hoover capillary melting-point apparatus. Ultraviolet (UV) absorption spectra were recorded with a Perkin-Elmer Lambda 3B UV−Vis spectrophotometer in ethanol. ^1H nuclear magnetic resonance (NMR) spectra were recorded at 400 and 500 MHz with a Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers in deuteriochloroform. ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 125 MHz with a Bruker Instruments DMX-500 Avance console in deuteriochloroform. Chemical shifts (δ) are reported downfield from internal Me_4Si (δ 0.00). Electron impact (EI) mass spectra were obtained with a Micromass AutoSpec (Beverly, MA) instrument. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model U6K Universal injector, and a Model 486 tunable absorbance detector. THF was freshly distilled before use from sodium benzophenone ketyl under argon.

The starting bicyclic lactone **8** was obtained from commercial (−)-quinic acid (90% yield) according to the published procedure.[17]

(1*R*,3*R*,4*S*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1,4-dihydroxy-6-oxa-bicyclo[3.2.1]octan-7-one (9). To a stirred solution of lactone **8** (1.80 g, 10.34 mmol) and imidazole (2.63 g, 38.2 mmol) in anhydrous DMF (14 mL) was added *tert*-butyldimethylsilyl chloride (1.80 g, 11.9 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min and 1 h at room temperature, poured into water and extracted with ethyl acetate and ether. The organic layer was washed several times with water, dried ($MgSO_4$), and evaporated to give a colorless crystalline residue that was crystallized from hexane/ethyl acetate to give 2.12 g of pure ether **9**. The mother liquors were evaporated and purified by flash chromatography. Elution with hexane/ethyl acetate (8:2) gave additional quantity of monosilylated (3-OTBDMS) derivative **9** (0.14 g, overall yield 76%) and some quantity of crystalline isomeric (4-OTBDMS) ether (0.10 g, 3%).

(1*R*,3*R*,5*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-6-oxa-bicyclo[3.2.1]octane-4,7-dione (10). To a stirred suspension of Dess−Martin periodinane reagent (6.60 g, 15.5 mmol) in anhydrous CH_2Cl_2 (100 mL) was added 3-silyloxy compound **9** (3.86 g, 13.4 mmol). The mixture was stirred at room temperature for 18 h, poured into water and extracted with ethyl acetate. The organic layer was washed several times with water, dried ($MgSO_4$), and evaporated to give an oily residue which slowly crystallized on cooling (3.67 g, 95%). TLC indicated high purity of the obtained ketone **10** which could be used in the next step without further purification. The analytical sample was obtained by recrystallization from hexane.

(1*R*,3*R*,5*R*)-1-Acetoxy-3-[(*tert*-butyldimethylsilyl)oxy]-6-oxa-bicyclo[3.2.1]octane-4,7-dione (11). A solution of a crude hydroxy ketone **10** (1.64 g, 5.8 mmol) in anhydrous pyridine (12 mL) and acetic anhydride (5.5 mL) was stirred for 3 h at room temperature. It was poured into water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$, saturated $CuSO_4$, and water, dried ($MgSO_4$), and evaporated to give an oily residue which was dissolved in hexane/ethyl acetate (8:2) and filtered through short path of silica gel. Evaporation of solvents gave pure crystalline acetate **11** (1.51 g, 81%). An analytical sample was obtained by recrystallization from hexane/ethyl acetate.

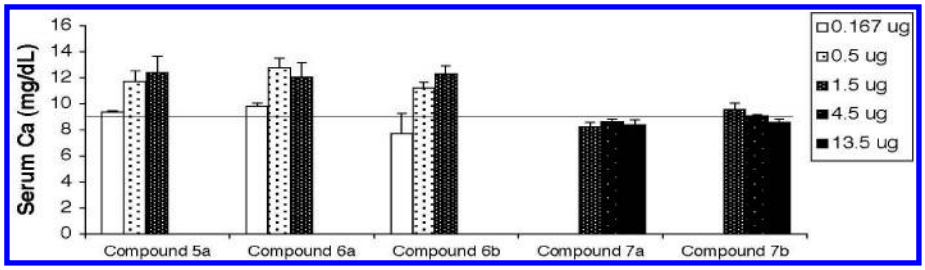


Figure 5. Serum calcium changes in response to a single oral dose of **5a**, **6a**, **6b**, **7a** and **7b** in CD-1 mice. Six to seven week old female mice were given one oral dose of the designated compounds, and blood was collected for serum calcium analysis 3 days following dose administration.

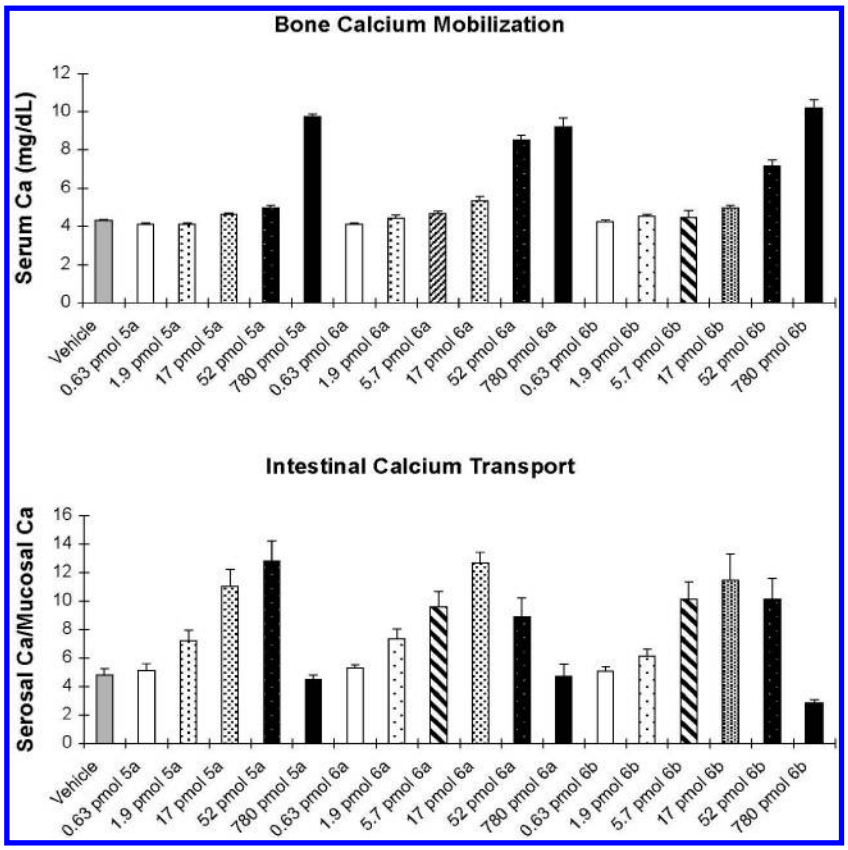


Figure 6. Bone calcium mobilization and intestinal calcium transport activity of **5a**, **6a**, and **6b**. Rats were made vitamin D-deficient and placed on a diet nearly devoid of calcium. Blood and duodenum were collected 24 h following the last of 4 consecutive daily ip doses.

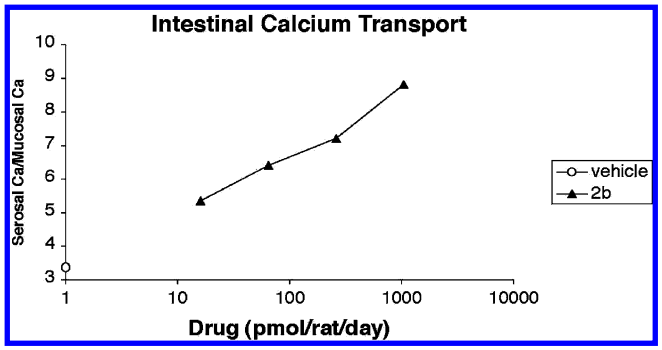


Figure 7. Dose-dependent intestinal calcium transport activity of **2b**. Rats were made vitamin D-deficient and placed on a diet nearly devoid of calcium. Duodena were collected for gut sac preparation 24 h following the last of four consecutive daily ip doses.

[3-(Methoxymethoxy)propyl]triphenylphosphonium Bromide (A). To a solution of bromomethyl methyl ether (1.3 mL, 16 mmol) and *N*,*N*-diisopropylethylamine (4.5 mL, 27.7 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C was added 3-bromo-1-propanol (1.0 mL, 11 mmol), and the mixture was stirred at 0 °C for 1 h and at room temperature for 20 h. The reaction mixture was poured into 1 N HCl (150 mL), the organic phase was separated and the water phase extracted with CH_2Cl_2. The combined organic phases were washed with water and diluted in $NaHCO_3$, dried ($MgSO_4$), and evaporated to give a yellowish oil. The residue was purified by flash chromatography. Elution with hexane/ethyl acetate (95:5) afforded pure oily 1-bromo-3-(methoxymethoxy)propane (1.12 g, 55%).

To a solution of 1-bromo-3-(methoxymethoxy)propane (0.46 g, 2.5 mmol) in anhydrous toluene (1.5 mL) was added triphenylphoshine (0.71 g, 2.7 mmol) under argon with stirring. The mixture was heated at 100 °C for 20 h and cooled to room temperature. The liquid was decanted and the solid residue was ground with a spatula, filtered, and washed several times with ether. After drying overnight in a vacuum desiccator, colorless crystals of phosphonium salt **A** (0.98 g, 88%) could be used in the Wittig reaction without further purification.

[3-[(*tert*-Butyldimethylsilyl)oxy]propyl]triphenylphosphonium Bromide (B). To a solution of 1-bromo-3-[(*tert*-butyldimethylsilyl)oxy]propane (Aldrich, 2.18 g, 8.56 mmol) in anhydrous benzene (1.6 mL) was added triphenylphoshine (2.64 g, 10.2 mmol) under argon with stirring. The mixture was heated at 85 °C for 18 h and cooled to room temperature. The liquid was decanted and the solid residue was ground with a spatula, filtered, and washed several times with ether. Colorless crystals of phosphonium salt **B**

(3.7 g) were purified by silica column chromatography. Pure salt **B** (3.04 g, 69%) was eluted with chloroform/methanol (96:4).

[(4*E*)- and (4*Z*)-(1*R*,3*R*,5*R*)-1-Acetoxy-3-[(*tert*-butyldimethylsilyl)oxy]-6-oxa-4-[3′-(methoxymethoxy)propylidene]bicyclo-[3.2.1]octan-7-one (12 and 13). To the phoshonium bromide **A** (420 mg, 0.94 mmol) in anhydrous THF (5 mL) at 0 °C was added dropwise *n*-BuLi (1.6 M in hexanes, 1.12 mL, 1.8 mmol) under argon with stirring. After 5 min another portion of **A** was added (420 mg, 0.94 mmol) and the solution was stirred at 0 °C for 10 min and then at room temperature for 20 min. The orange-red mixture was cooled to −78 °C and siphoned in 2 equal portions (30 min interval) to a solution of keto lactone **11** (300 mg, 0.91 mmol) in anhydrous THF (8 mL). The reaction mixture was stirred at −78 °C and stopped by addition of brine cont. 1% HCl (3 h after addition of the first portion of the Wittig reagent). Ethyl acetate (9 mL), benzene (6 mL), ether (3 mL), sat. $NaHCO_3$ (3 mL), and water (3 mL) were added, and the mixture was vigorously stirred at room temperature for 18 h. Then the organic phase was separated, washed with brine, dried ($MgSO_4$), and evaporated. The oily residue (consisting mainly of isomeric **12** and **13** in the ratio of ca. 5:1) was separated by flash chromatography on silica. Elution with hexane/ethyl acetate (85:15) resulted in partial separation of products: 29 mg of **13**, mixture of **12** and **13** (ca. 3.5:1, 85 mg), and pure **12** (176 mg). Rechromatography of the mixed fractions resulted in almost complete separation of the products (total yield of both isomers 77%).

[(4*E*)- and (*Z*)-(1*R*,3*R*,5*R*)-1-Acetoxy-3-[(*tert*-butyldimethylsilyl)oxy]-6-oxa-4-[3′-((*tert*-butyldimethylsilyl)oxy)propylidene]-bicyclo[3.2.1]octan-7-one (14 and 15). To the phosphonium bromide **B** (1.55 g, 3.04 mmol) in anhydrous THF (42 mL) at − 20 °C was added dropwise *n*-BuLi (2.0 M in cyclohexane, 1.50 mL, 3.00 mmol) under argon with stirring. The solution was stirred at − 20 °C for 15 min. The orange-red mixture was cooled to −45 °C and siphoned during 15 min to a solution of keto lactone **11** (700 mg, 2.13 mmol) in anhydrous THF (24 mL). The reaction mixture was stirred at −40 °C for 2 h and stopped by addition of brine cont. 1% HCl. Ethyl acetate (30 mL), benzene (20 mL), ether (10 mL), saturated $NaHCO_3$ (10 mL), and water (10 mL) were added, and the mixture was vigorously stirred at room temperature for 18 h. Then the organic phase was separated, washed with brine, dried ($MgSO_4$), and evaporated. The residue (consisting mainly of isomeric **14** and **15** in the ratio of ca. 3:2) was purified by flash chromatography on silica. Elution with hexane/ethyl acetate (9:1) gave the mixture of products **14** and **15** (905 mg, 87%). Analytical samples of both isomers were obtained after HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) separation using a hexane/ethyl acetate (9:1) solvent system. Pure oily compounds **14** and **15** were eluted at R_V 28 mL and 29 mL, respectively.

[(4*E*)-(1′*R*,3′*R*,5′*R*)-3′-[(*tert*-Butyldimethylsilyl)oxy]-1′,5′-dihydroxy-4′-[3″-(methoxymethoxy)propylidene]cyclohexyl]methanol (16). To a stirred solution of compound **12** (165 mg, 0.40 mmol) in anhydrous ethanol (5 mL) at 0 °C was added $NaBH_4$ (151 mg, 4.0 mmol), and the mixture was stirred at 0 °C for 1 h, then for 10 h at 6 °C, and for 2 h at room temperature. The saturated NH_4Cl was added, and the mixture was poured into brine and extracted several times with ether and methylene chloride. The extracts were washed with brine, combined, dried ($MgSO_4$), and evaporated. The oily residue was purified by flash chromatography. Elution with hexane/ethyl acetate (2:8) gave pure triol **16** as a colorless oil (115 mg, 79%).

[(*E*)- and (*Z*)-(1′*R*,3′*R*,5′*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1′,5-dihydroxy-4′-[3″-[((*tert*-butyldimethylsilyl)oxy)propylidene]-cyclohexyl]methanol (17 and 18). Triols **17** and **18** were obtained by $NaBH_4$ reduction of compounds **14** and **15**, performed analogously to the process described above for **12** except the reaction was carried out for 21 h at room temperature. Products were purified by column chromatography. Elution with hexane/ethyl acetate (4:6) gave a semicrystalline mixture of triols **17** and **18** in 98% yield.

(4*E*)-(3*R*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-5-hydroxy-4-[3′-(methoxymethoxy)propylidene]cyclohexanone (19). Sodium periodate−saturated water (1.2 mL) was added to a solution of the triol **16** (79 mg, 0.21 mmol) in methanol (5 mL) at 0 °C. The solution was stirred at 0 °C for 1 h, poured into brine, and extracted with ethyl acetate and ether. The extract was washed with brine, dried ($MgSO_4$), and evaporated. An oily residue was redissolved in hexane/CH_2Cl_2 and applied on a Sep-Pak cartridge. Pure hydroxy ketone **19** (64 mg, 88%) was eluted with hexane/ethyl acetate (7:3) as an oil slowly crystallizing in the refrigerator.

[(4*E*)- and (4*Z*)-(3*R*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-5-hydroxy-4-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]]cyclohexanone (20 and 21). Sodium periodate cleavage of the triols **17** and **18** was performed analogously to the process described above for **16**. The mixture of hydroxy ketones **20** and **21** (88% yield) was eluted from a Sep-Pak cartridge with hexane/ethyl acetate (8:2) as an oil slowly crystallizing in the refrigerator.

[(3*R*,5*R*)-3,5-Bis[(*tert*-Butyldimethylsilyl)oxy]-4-[3′-(methoxymethoxy)propylidene]cyclohexanone (22). To a solution of hydroxy ketone **19** (40 mg, 117 μmol) in anhydrous CH_2Cl_2 (0.4 mL) at −50 °C was added 2,6-lutidine (32 μL, 274 μmol) and *tert*-butyldimethylsilyl triflate (56 μL, 240 μmol). The mixture was stirred for 5 min at −50 °C, then it was allowed to warm to −15 °C and stirred at this temperature for an additional 30 min. Benzene and water were added, and the mixture was poured into water and extracted with benzene. The extract was washed with saturated $CuSO_4$ and water, dried ($MgSO_4$), and evaporated. The oily residue was redissolved in hexane and purified by flash chromatography on silica. Elution with hexane/ethyl acetate (95:5) gave pure protected ketone **22** as a colorless oil (30 mg, 57%; 66% based on recovered substrate) and unreacted **19** (6 mg).

[(3*R*,5*R*)-3,5-Bis[(*tert*-Butyldimethylsilyl)oxy]-4-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]cyclohexanone (23). Silylation of hydroxy ketones **20** and **21** was performed analogously to the process described above for **19** except the reaction was carried out for 50 min at −50 °C. The product was purified by flash chromatography on silica. Elution with hexane/ethyl acetate (95:5) gave pure protected ketone **23** as a colorless oil (18 mg, 64%; 74% based on recovered substrates) and a mixture of unreacted **20** and **21** (3 mg).

[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[3″-(methoxymethoxy)propylidene]cyclohexylidene]acetic Acid Methyl Esters (24 and 25). To a solution of diisopropylamine (25 μL, 0.18 mmol) in anhydrous THF (0.15 mL) was added *n*-BuLi (2.5 M in hexanes, 72 μL, 0.18 mmol) under argon at −78 °C with stirring. Methyl(trimethylsilyl)acetate (30 μL, 0.18 mmol) was then added. After 15 min, the ketone **22** (38.4 mg, 84 μmol) in anhydrous THF (0.2 mL) was added. The solution was stirred at −78 °C for an additional 2 h, and the reaction mixture was quenched with wet ether, poured into brine and extracted with ether and benzene. The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. An oily residue was redissolved in hexane and applied on a Sep-Pak cartridge. Pure allylic esters **24** and **25** (37.2 mg, 86%; isomer ratio of **24**:**25** = ca. 7:1) were eluted with hexane/ethyl acetate (97:3). Separation of the products was achieved by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using the hexane/ethyl acetate (95:5) solvent system. Pure compounds **24** and **25** were eluted at R_V 41 mL and 44 mL, respectively, as colorless oils.

[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[3″-[((*tert*-butyldimethylsilyl)oxy)propylidene]cyclohexylidene]acetic Acid Methyl Esters (26 and 27). Peterson olefination of protected ketone **23** was performed analogously to the process described above for **22**. The mixture of allylic esters **26** and **27** (60 mg, 68%; isomer ratio of **26**:**27** = ca. 6:1) was eluted from a Sep-Pak cartridge with hexane/ethyl acetate (98.5:1.5).

2-[(3′*R*,5′*R*)-3′,5′-bis[(*tert*-butyldimethylsilyl)oxy]-4′-[3″-(methoxymethoxy)propylidene]cyclohexylidene]ethanol (28 and 29). Diisobutylaluminum hydride (1.0 M in toluene, 0.35 mL, 0.35 mmol) was slowly added to a stirred solution of the allylic esters **24** and **25** (7:1, 37.2 mg, 74 μmol) in toluene/methylene chloride (2:1, 1.5 mL) at −78 °C under argon. Stirring was continued at −78 °C for 1 h, the mixture was quenched by addition of potassium

sodium tartrate (2 N, 2 mL), aq. HCl (2 N, 2 mL) and H_2O (24 mL), and then diluted with ether and benzene. The organic layer was washed with diluted $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography. Elution with hexane/ethyl acetate (95:5) resulted in partial separation of allylic alcohols: pure major product **28** (16 mg), mixture of both isomers (15 mg) and pure minor *Z*-isomer **29** (3 mg; total yield 97%). Rechromatography of the mixed fractions resulted in almost complete separation of the products.

2-[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[3″-[((*tert*-butyldimethylsilyl)oxy)propylidene]cyclohexylidene]ethanol (30 and 31). DIBALH reduction of allylic esters **26** and **27** was performed analogously to the process described above for **24** and **25**. The products were purified by flash chromatography. Elution with hexane/ethyl acetate (95:5) yielded a mixture of allylic alcohols **30** and **31** (86% yield). Analytical samples of both isomers were obtained after HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/ethyl acetate (9:1) solvent system. Pure oily compounds **30** and **31** were eluted at R_V 28 mL and 29 mL, respectively.

[2-[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[3″-(methoxymethoxy)propylidene]cyclohexylidene]ethyl]diphenylphosphine Oxide (32). To the allylic alcohol **28** (16 mg, 33 μmol) in anhydrous THF (0.4 mL) was added *n*-BuLi (2.5 M in hexanes, 13 μL, 33 μmol) under argon at 0 °C with stirring. Freshly recrystallized tosyl chloride (6.7 mg, 35 μmol) was dissolved in anhydrous THF (90 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, 64 μL, 0.16 mmol) was added to Ph_2PH (30 μL, 0.16 mmol) in anhydrous THF (200 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. 1/4 of the solution was added). The resulting mixture was stirred an additional 40 min at 0 °C and quenched by addition of H_2O (20 μL). Solvents were evaporated under reduced pressure, and the residue was redissolved in methylene chloride (0.5 mL) and stirred with 10% H_2O_2 (0.25 mL) at 0 °C for 1 h. The organic layer was separated, washed with cold aq. sodium sulfite and H_2O, dried ($MgSO_4$), and evaporated. The residue was subjected to flash chromatography. Elution with hexane/ethyl acetate (85:15) gave an unchanged allylic alcohol (2 mg). Subsequent elution with benzene/ethyl acetate (7:3) provided pure phosphine oxide **32** (15.5 mg, 68%; 78% based on recovered starting material) as a colorless oil. An analytical sample was obtained after HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) purification using a hexane/2-propanol (9:1) solvent system (R_V 41 mL).

[2-[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[3″-[((*tert*-butyldimethylsilyl)oxy)propylidene]cyclohexylidene]ethyl]-diphenylphosphine Oxides (33 and 34). The phosphine oxides **33** and **34** were obtained from the mixture of allylic alcohols **30** and **31** analogously as described above for compound **32**. The crude reaction products were subjected to flash chromatography. Elution with hexane/ethyl acetate (95:5) gave unchanged allylic alcohols. Subsequent elution with hexane/ethyl acetate (7:3) resulted in a mixture of isomeric phosphine oxides **33** and **34** (49% yield; 81% yield based on recovered substrates **30** and **31**).

1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-(methoxymethoxy)propylidene]-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-Butyldimethylsilyl Ether (36a). To a solution of phosphine oxide **32** (15.5 mg, 23 μmol) in anhydrous THF (0.25 mL) at −78 °C was slowly added phenyllithium (1.8 M in cyclohexane/ether, 13 μL, 23 μmol) under argon with stirring. The solution turned deep orange. The mixture was stirred at −78 °C for 20 min and a precooled (−78 °C) solution of protected hydroxy ketone **35a** (19 mg, 48 μmol; prepared according to a published procedure)[15] in anhydrous THF (0.25 mL) was slowly added. The mixture was stirred under argon at −78 °C for 3 h and at 6 °C for 16 h. Ethyl acetate and water were 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 (98:2, 10 mL) to give 19-norvitamin derivative **36a** (9.5 mg, 48%). The Sep-Pak was then washed with hexane/ethyl acetate (96:4, 10 mL) to recover some unchanged C,D-ring ketone **35a** (10 mg), and with ethyl acetate (10 mL) to recover diphenylphosphine oxide **32** (1 mg).

1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-Butyldimethylsilyl Ether (37a). Protected vitamin **37a** was obtained by the Wittig−Horner coupling of the corresponding phosphine oxides **33** and **34** (ca. 6:1) and protected hydroxy ketone **35a**, performed analogously to the process described above for coupling of **32** and **35a**. The crude mixture resulting from the reaction was separated by column chromatography on silica. Elution with hexane/ethyl acetate (99.5:0.5) yielded 19-norvitamin derivative **37a** (41%, 47% based on recovered substrates). The column was then washed with hexane/ethyl acetate (96:4) to recover some unchanged C,D-ring ketone **35a**, and with ethyl acetate to recover unreacted diphenylphosphine oxides. An analytical sample of the product was obtained by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) purification using a hexane/ethyl acetate (99.8:0.2) solvent system**.** Pure protected vitamin **37a** was eluted at R_V 28 mL as a colorless oil.

(20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-[3′-[((*tert*-butyldimethylsilyl)oxy)propylidene]-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-Butyldimethylsilyl Ether (37b). Silylated 19-norvitamin D_3 compound **37b** was obtained by Wittig−Horner coupling of protected 25-hydroxy Grundmann ketone **35b** (prepared according to a published procedure)[7] with the phosphine oxides **33** and **34** performed analogously to the process described above for the preparation of (20*R*)-isomer **37a**. The protected vitamin was purified on a silica column, using a hexane/ethyl acetate (99.5:0.5) solvent system, and it was obtained in ca. 47% yield. An analytical sample of the protected vitamin **37b** was obtained by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) purification using a hexane/ethyl acetate (99.7:0.3) solvent system**.** Pure compound **37b** was eluted at R_V 25 mL as a colorless oil.

1α,25-Dihydroxy-2-[3′-(methoxymethoxy)propylidene]-19-norvitamin D_3 (5a). To a solution of the protected 19-norvitamin D_3 **36a** (3.0 mg, 3.5 μmol) in anhydrous THF (200 μL) was added tetrabutylammonium fluoride (1.0 M in THF, 210 μL, 210 μmol). The mixture was stirred under argon at room temperature for 18 h, poured into brine and extracted with ethyl acetate. Organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (75:25) solvent system. Analytically pure 19-norvitamin **5a** (1.27 mg, 71%) was collected at R_V 26 mL. The compound gave also a single peak on reversed-phase HPLC (6.2 mm × 25 cm Zorbax-ODS column, 2 mL/min) using a methanol/water (8:2) solvent system: it was collected at R_V 35 mL.

1α,25-Dihydroxy-2-[3′-hydroxypropylidene]-19-norvitamin D_3 (6a). Treatment of the protected vitamin **37a** with tetrabutylammonium fluoride, performed as described for **36a**, gave a vitamin D compound that was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. Analytically pure *E*-isomer **6a** (97%) was collected at R_V 37.5 mL. The compound gave also a single peak on reversed-phase HPLC (6.2 mm × 25 cm Zorbax-ODS column, 2 mL/min) using a methanol/water (8:2) solvent system: it was collected at R_V 23 mL.

(20*S*)-1α,25-Dihydroxy-2-[3′-hydroxypropylidene]-19-norvitamin D_3 (6b). Vitamin **6b** was obtained by hydrolysis of the silyl protecting groups in the 19-norvitamin derivative **37b** performed analogously to the process described above for the preparation of (20*R*)-isomers **5a** and **6a**. The product was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. Pure 19-norvitamin **6b** (95% yield) was collected at R_V 36.5 mL. The compound gave also a single peak on reversed-phase HPLC (6.2 mm × 25 cm Zorbax-ODS

column, 2 mL/min) using a methanol/water (8:2) solvent system: it was collected at R_V 18 mL.

2-[(1*R*,3a*S*,7a*R*)-7a-Methyl-[(*R*)-6-[(triethylsilyl)oxy]-6-methylheptan-2-yl]-octahydro-inden-(4*E*)-ylidene]ethanol (38a). To a suspension of NaH (49 mg, 2.04 mmol) in anhydrous THF (1.2 mL) was added $(EtO)_2P(O)CH_2COOEt$ (500 μL, 2.53 mmol) at 0 °C. The mixture was stirred at room temperature for 10 min and lithium chloride (13 mg, 0.30 mmol) was then added. The stirring was continued for 1 h and cooled to 0 °C, and a solution of the protected hydroxy ketone **35a** (100 mg, 0.25 mmol) in THF (0.6 mL) was added. After stirring at room temperature for 32 h, the reaction mixture was diluted with ethyl acetate and poured into saturated ammonium chloride. The organic phase was separated, washed with brine, dried (Na_2SO_4) and evaporated. The residue was separated by flash chromatography. Elution with hexane/ethyl acetate (99:1) afforded pure oily [(1*R*,3a*S*,7a*R*)-7a-methyl-1-[(*R*)-6-[(triethylsilyl)oxy]-6-methylheptan-2-yl]-octahydro-inden-(4*E*)-ylidene]acetic acid ethyl ester (82 mg, 70%).

Diisobutylaluminum hydride (1 M in toluene, 200 μL, 0.2 mmol) was added to a stirred solution of allylic ester (29 mg, 62 μmol) in anhydrous toluene (0.5 mL) at −78 °C under argon. The mixture was stirred at −78 °C for 1 h, and the reaction was quenched by addition of potassium sodium tartrate (2 N, 1 mL), aq HCl (2 N, 1 mL), and water (12 mL). The mixture was poured into brine and extracted with ethyl acetate and ether. The combined extracts were washed with diluted $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was redissolved in hexane and applied on a silica gel Sep-Pak. Elution with hexane/ethyl acetate (95:5, 20 mL, and 9:1, 10 mL) gave allylic alcohol **38a** (23 mg, 87%) as a colorless oil.

2-[(1*R*,3a*S*,7a*R*)-7a-Methyl-[(*S*)-6-[(triethylsilyl)oxy]-6-methylheptan-2-yl]-octahydro-inden-(4*E*)-ylidene]ethanol (38b). Transformation of the protected Grundmann ketone **35b** into allylic alcohol **38b** was achieved using an analogous two-step reaction sequence as described above for 20*R*-isomer **35a**. Thus, the allylic ester, prepared in 68% yield by Wittig reaction of the ketone **35b** and triethyl phosphonoacetate, was reduced with DIBALH to give the alcohol **38b** in 98% yield.

(1*R*,3a*S*,7a*R*)-4-[2-(Benzothiazole-2-sulfonyl)-(4*E*)-ethylidene]-7a-methyl-1-[(*R*)-6-[(triethylsilyl)oxy]-6-methylheptan-2-yl]-octahydro-indene (39a). To a solution of 2-mercaptobenzotriazole (12.5 mg, 75 μmol) and Ph_3P (19.5 mg, 75 μmol) in dry methylene chloride (150 μL) at 0 °C was added a solution of allylic alcohol **38a** (21 mg, 50 μmoL) in methylene chloride (150 μL) followed by DIAD (14 μL, 50 μmol). The mixture was stirred at 0 °C for 1 h, and the solvents were evaporated in vacuo. The residue was dissolved in ethanol (300 mL) and cooled to 0 °C, and 30% H_2O_2 (30 μL) was added, followed by $(NH_4)_6Mo_7O_{24}$ Ï $4H_2O$ (12.3 mg, 10 μmol). The mixture was stirred at room temperature for 3 h, poured into cold saturated Na_2SO_3, and extracted with ethyl acetate. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was dissolved in a small volume of benzene/hexane (1:1) and applied on a silica Sep-Pak. Elution with hexane/ethyl acetate (9:1, 20 mL and 85:15, 20 mL) and removal of the solvents gave an oily product (33 mg) that was dissolved in anhydrous DMF (300 μL). Imidazole (18 mg, 0.26 mmol) was added followed by triethylsilyl chloride (50 μL, 0.29 mmol), and the mixture was stirred at room temperature for 3 h. Ethyl acetate was added and water, and the organic layer was separated, washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/ethyl acetate (9:1) solvent system. Analytically pure sulfone **39a** (22.8 mg, 76%) was collected at R_V 24 mL.

(1*R*,3a*S*,7a*R*)-4-[2-(Benzothiazole-2-sulfonyl)-(4*E*)-ethylidene]-7a-methyl-1-[(*S*)-6-[(triethylsilyl)oxy]-6-methylheptan-2-yl]-octahydro-indene (39b). Conversion of the allylic alcohol **38b** into sulfone **39b** involved an analogous reaction sequence as that described above for 20*R*-isomers **38a** and **39a**. The final product was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/ethyl acetate (9:1) solvent system. Analytically pure sulfone **39b** (80%) was collected at R_V 23 mL.

1α,25-Dihydroxy-2-[3′-hydroxypropylidene]-19-norvitamin D_3 (6a and 7a). To a solution of sulfone **39a** (26.6 mg, 44 μmol) in dry THF (150 μL) was added LiHMDS (1 M in THF, 41 μL, 41 μmol) at −78 °C under argon. The solution turned deep red. The mixture was stirred at −78 °C for 1 h, and a solution of the ketone **23** (17.0 mg, 32.1 μmol) in THF (150 μL) was added. The stirring was continued at −78 °C for 2 h, and the reaction mixture was allowed to warm slowly to −50 °C. After stirring for an additional 50 min at −50 °C, it was poured into saturated NH_4Cl and extracted with ethyl acetate and hexane. The extract was washed with brine, dried (Na_2SO_4) and evaporated. The yellow oily residue was chromatographed on silica gel column. Elution with hexane provided a mixture of protected vitamins **37a** and **40a** (1.3:1, 16 mg, 40%, 77% based on recovered starting material). Further elution with hexane/ethyl acetate (97:3) gave unreacted sulfone **39a** (13 mg).

Treatment of the obtained mixture of protected vitamin D compounds **37a** and **40a** with tetrabutylammonium fluoride, performed as described for **36a**, gave a mixture of vitamin D analogues **6a** and **7a** which was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. A pure mixture of 19-norvitamins **6a** and **7a** (1.3:1, 95% yield) was collected at R_V 37.5 mL. Separation of both isomers was easily achieved by reversed-phase HPLC (6.2 mm × 25 cm Zorbax-ODS column, 2 mL/min) using a methanol/water (8:2) solvent system. Analytically pure *E*-isomer **6a** was collected at R_V 23 mL and *Z*-isomer **7a** at R_V 29 mL.

(20*S*)-1α,25-Dihydroxy-2-[3′-hydroxypropylidene]-19-norvitamin D_3 (6b and 7b). Julia coupling between sulfone **39b** and ketone **23** followed by chromatographical separation of the products was carried out as described above for the 20*R*-isomer **39a**. Elution with hexane provided a mixture of protected vitamins **37b** and **40b** (1.3:1, 42%, 78% based on recovered starting material).

Treatment of the obtained mixture of protected vitamin D compounds **37b** and **40b** with tetrabutylammonium fluoride, performed as described for **36a**, gave a mixture of vitamin D analogues **6b** and **7b** which was purified by HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. A pure mixture of 19-norvitamins **6b** and **7b** (1.3:1, 96% yield) was collected at R_V 36.5 mL. Separation of both isomers was easily achieved by reversed-phase HPLC (6.2 mm × 25 cm Zorbax-ODS column, 2 mL/min) using a methanol/water (8:2) solvent system. Analytically pure *E*-isomer **6b** was collected at R_V 18 mL and *Z*-isomer **7b** at R_V 28 mL.

Biological Studies. 1. Measurement of Intestinal Calcium Transport and Bone Calcium Mobilization. Male, weanling Sprague−Dawley rats were purchased from Harlan (Indianapolis, IN), housed in D-deficient lighting conditions and fed a D-deficient diet.[24] The first week the animals were fed Diet 11 (0.47% Ca) + AEK oil. During the next 3 weeks, the rats were fed the same diet with the calcium carbonate removed (0.02% Ca). The rats were then switched back to a diet containing 0.47% Ca for one week before returning to a diet containing 0.02% Ca for two more weeks. Dose administration began during the last week on the 0.02% calcium diet. Four consecutive intraperitoneal doses in 0.1 mL of (95:5) 1,2-propanediol/ethanol were given approximately 24 h apart. Twenty-four hours after the last dose, blood was collected from the severed neck and the concentration of serum calcium determined as a measure of bone calcium mobilization. The first 10 cm of the intestine was also collected for intestinal calcium transport analysis using the everted gut sac method as previously described except radioactive tracer was not used.[25]

Calcium was measured in the presence of 0.1% lanthanum chloride by means of a Perkin-Elmer atomic absorption spectrometer Model 3110. Intestinal calcium transport is expressed as the serosal:mucosal ratio of calcium in the sac to the calcium in the final incubation medium. Bone calcium mobilization represents the rise in serum calcium of the rats maintained on a very low calcium diet.

2. Mouse Studies. 6−7 week old female CD-1 mice were purchased from Harlan (Indianapolis, IN). The animals were group

housed and fed a purified diet containing 0.47% calcium.[24] After a 5−7 day acclimation period, the animals were given a single dose of the designated analogues by gastric gavage. The compounds were formulated in Neobee oil and ethanol (5%) so that the dose could be delivered in a volume of 4 mL/kg body weight. Blood was collected for serum calcium concentration analyses at various timepoints following dose delivery. Serum calcium was analyzed as described above.

3. Measurement of Binding to the Full-Length Rat Recombinant Vitamin D Receptor (VDR). Purified full-length rat recombinant receptor was prepared as described earlier with a few modifications.[25] The entire coding region for the rat VDR was inserted into the p29 plasmid including the flexible insertion region (residues 165−211). During the purification of the full-length receptor, the eluate from the metal affinity column was dialyzed against the same buffer but at a pH of 8.0 instead of 7.0 and 50 mM sodium phosphate was used instead of 20 mM. The size of the SP−Sepharose Fast Flow column was slightly different, 1.5 × 17 cm, and the salt gradient used for elution of the VDR from this column linearly increased from 0 to 0.8 M phosphate buffer over a total volume of 300 mL. Fractions judged pure by SDS−PAGE were combined and dialyzed against 25 mM EPPS at pH 8.5, containing 50 mM NaCl and 0.02% NaN_3. Following dialysis, the protein was concentrated by ultracentrifugation to approximately 1.4 mg/mL. Aliquots of the purified protein were flash-frozen in liquid nitrogen and stored at −80 °C until use. On the day of each binding assay, the protein was diluted in $TEDK_{50}$ (50 mM Tris, 1.5 mM EDTA, pH 7.4, 5 mM DTT, 150 mM KCl) with 0.1% Chaps detergent. The receptor protein and ligand concentration were optimized such that no more than 20% of the added radiolabeled ligand was bound to the receptor. Unlabeled ligands were dissolved in ethanol and the concentrations determined using UV spectrophotometry ($1\alpha,25(OH)_2D_3$: molar extinction coefficient ϵ = 18 200 and λ_{max} = 265 nm; the tested 19-norvitamin D compounds: ϵ = 42 000 and λ_{max} = 252 nm). Radiolabeled ligand (^{3}H-$1\alpha,25(OH)_2D_3$, ∼159 Ci/mmol) was added in ethanol at a final concentration of 1 nM. Radiolabeled and unlabeled ligands were added to 100 μL of the diluted protein at a final ethanol concentration of ≤10%, mixed, and incubated overnight on ice to reach binding equilibrium. The following day, 100 μL of hydroxylapatite slurry (50%) was added to each tube and mixed at 10-min intervals for 30 min. The hydroxylapatite was collected by centrifugation and then washed three times with Tris-EDTA buffer (50 mM Tris, 1.5 mM EDTA, pH 7.4) containing 0.5% Titron X-100. After the final wash, the pellets were transferred to scintillation vials containing 4 mL of Biosafe II scintillation cocktail, mixed, and placed in a scintillation counter. Total binding was determined from the tubes containing only radiolabeled ligand. The displacement experiments were carried out in duplicate on two to three different occasions.

4. Measurement of Cellular Differentiation. Human promyelocytic leukemia (HL-60) cells were grown in RPMI-1640 medium containing 10% fetal bovine serum at 37 °C in the presence of 5% CO_2. HL-60 cells were plated at 1.2 × 10^5 cells/plate. Eighteen hours after plating, cells in duplicate were treated with the compound tested so that the final concentration of ethanol was less than 0.2%. Four days later, the cells were harvested and a nitro blue tetrazolium (NBT) reduction assay was performed. The percentage of differentiated cells was determined by counting a total of 200 cells and recording the number that contained intracellular black-blue formazan deposits.[26] The experiment was repeated 2 to 3 times, and the results are reported as the mean. Verification of differentiation to monocytic cells was determined by measuring phagocytic activity (data not shown).

5. Transcriptional Assay. Transcription activity was measured in ROS 17/2.8 (bone) cells that were stably transfected with a 24-hydroxylase (24OHase) gene promoter upstream of a luciferase reporter gene.[27] Cells were given a range of doses. Sixteen hours after dosing, the cells were harvested and luciferase activities were measured using a luminometer. Each experiment was performed in duplicate two to three separate times.

Molecular Modeling. Molecular mechanism studies were used to establish the energy-minimized structures of the most important synthetic intermediates. The calculation of optimized geometries and steric energies was carried out using the algorithm from the MM^+ HyperChem (release 5.0) software package (Autodesk, Inc.). MM^+ is an all-atom force field based on the MM2 functional form. The procedure used for generation of the respective conformers of the alkoxypropylidene substituent and finding the global minimum structures was analogous to that described previously by us for the vitamin D side chain conformers [28] and involved the Conformational Search module of the ChemPlus (release 1.5) program (Hypercube, Inc.). The couplings observed in the ^{1}H NMR spectra of the synthesized compounds were compared to those calculated using the PC MODEL (release 6.0) molecular modeling software (Serena Software); molecular modeling was performed in the MMX mode. The force field MMX is an enhanced version of MM2, with the pi-VESCF routines taken from MMP1.

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Supporting Information Available: Purity criteria for the synthesized compounds; spectral data of the synthesized compounds; figures with preferred, energy-minimized conformations of the synthetic intermediates **12**, **13**, **19**, **22**, **24**, **25**; figures with either the competitive binding curves or dose−response curves derived from the binding, cellular differentiation, and transcriptional assays of the vitamin D analogues **5a, 6a**,**b, 7a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) DeLuca, H. F.; Burmester, J.; Darwish, H.; Krisinger, J. Molecular mechanism of the action of 1,25-dihydroxyvitamin D_3. In *Comprehensive Medicinal Chemistry*; Hansch, C.; Sammes, P. G.; Taylor, J. B.; Eds.; Pergamon Press: Oxford, 1991; Vol. 3, pp 1129−1143.

(2) Norman, A. W.; Bouillon, R.; Thomasset, M., Eds. *Vitamin D, a pluripotent steroid hormone: Structural studies, molecular endocrinology and clinical applications*; Walter de Gruyter: Berlin, 1994.

(3) Jones, G.; Strugnell, S. A.; DeLuca, H. F. Current understanding of the molecular action of vitamin D. *Physiol. Rev.* **1998**, *78*, 1193−1231.

(4) (a) Chawla, A.; Repa, J. J.; Evans, R. M.; Mangelsdorf, B. J. *Science* **2001**, *294*, 1866−1870. (b) Malloy, P. J.; Pike, J. W.; Feldman, D. The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. *Endocr. Rev.* **1999**, *20*, 156−188.

(5) Evans, R. M. The steroid and thyroid hormone receptor superfamily. *Science* **1988**, *240*, 889−895.

(6) Feldman, D.; Glorieux, F. H.; Pike, J. W. *Vitamin D*; Academic Press: New York, 1997.

(7) Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. 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. *J. Med. Chem.* **1998**, *41*, 4662−4674.

(8) Sicinski, R. R.; Rotkiewicz, P.; Kolinski, A.; Sicinska, W.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. 2-Ethyl and 2-ethylidene analogues of 1α, 25-dihydroxy-19-norvitamin D_3: Synthesis, conformational analysis, biological activities, and docking to the modeled rVDR ligand binding domain. *J. Med. Chem.* **2002**, *45*, 3366−3380.

(9) (a) Johnson, F. Allylic strain in six-membered rings. *Chem. Rev.* **1968**, *68*, 375−413. (b) Johnson, F.; Malhotra, S. K. Steric interference in allylic and pseudo-allylic systems. I. Two stereochemical theorems. *J. Am. Chem. Soc.* **1965**, *87*, 5492−5493.

(10) Nasipuri, D. *Stereochemistry of organic compounds*; Wiley Eastern: New Delhi, 1991; pp 270−271.

(11) (a) Saito, N.; Suhara, Y.; Kurihara, M.; Fujishima, T.; Honzawa, S.; Takayanagi, H.; Kozono, T.; Matsumoto, M.; Ohmori, M.; Miyata, N.; Takayama, H.; Kittaka, A. Design and efficient synthesis of 2α-(ω-hydroxyalkoxy)-1α,25-dihydroxyvitamin D_3 analogues, including 2-*epi*-ED-71 and their 20-epimers with HL-60 cell differentiation activity. *J. Org. Chem.* **2004**, *69*, 7463−7471. (b) Suhara, Y.; Nihei,

K.; Kurihara, M.; Kittaka, A.; Yamaguchi, K.; Fujishima, T.; Konno, K.; Miyata, N.; Takayama, H. Efficient and versatile synthesis of novel 2α-substituted 1α, 25-dihydroxyvitamin D_3 analogues and their docking to vitamin D receptors. *J. Org. Chem.* **2001**, *66*, 8760−8771. (c) Suhara, Y.; Nihei, K.; Tanigawa, H.; Fujishima, T.; Konno, K.; Nakagawa, K.; Okano, T.; Takayama, H. Syntheses and biological evaluation of novel 2α-substituted 1α,25-dihydroxy-19-norvitamin D_3 analogues. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1129−1132.

(12) (a) Vanhooke, J. L.; Benning, M. M.; Bauer, C. B.; Pike, J. W.; DeLuca, H. F. Molecular structure of the rat vitamin D receptor ligand binding domain complexed with 2-carbon-substituted vitamin D_3 hormone analogues and a LXLL-containing coactivator peptide. *Biochemistry* **2004**, *43*, 4101−4110. (b) Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D. The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Mol. Cell* **2000**, *5*, 173−179.

(13) Glebocka, A.; Sicinski, R. R.; DeLuca, H. F. New derivative of 1α, 25-dihydroxy-19-norvitamin D_3 with 3′-alkoxypropylidene moiety at C-2: Synthesis, biological activity and conformational analysis. *J. Steroid Biochem. Mol. Biol.* **2004**, *89*−*90*, 25−30.

(14) (a) Lythgoe, B. Synthetic approaches to vitamin D and its relatives. *Chem. Soc. Rev.* **1981**, 449−475. (b) Lythgoe, B.; Moran, T. A.; Nambudiry, M. E. N.; Ruston, S. Allylic phosphine oxides as precursors of conjugated dienes of defined geometry. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2386-2390.

(15) Sicinski, R. R.; Perlman, K. L.; DeLuca, H. F. Synthesis and biological activity of 2-hydroxy and 2-alkoxy analogues of 1α,25-dihydroxy-19-norvitamin D_3. *J. Med. Chem.* **1994**, *37*, 3730−3738.

(16) Elliott, J. D.; Hetmanski, M.; Stoodley, R. J.; Palfreyman, M. N. Studies related to cyclopentanoid natural products. 1. Preparation of (4*RS*) and (4*R*)-4-hydroxy-2-hydroxymethylcyclopent-2-en-1-one − a versatile synthetic intermediate. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1782−1789.

(17) Hanessian, S.; Pan, J.; Carnell, A.; Bouchard, H.; Lesage, L. Total synthesis of (−)-reserpine using the chiron approach. *J. Org. Chem.* **1997**, *62*, 465−473.

(18) Huang, P.-Q.; Sabbe, K.; Pottie, M.; Vandewalle, M. A novel synthesis of 19-nor 1α,25-dihydroxyvitamin D_3 and related analogues. *Tetrahedron Lett.* **1995**, *36*, 8299−8302.

(19) (a) Bellingham, R.; Jarowicki, K.; Kocienski, P.; Martin, V. Synthetic approaches to rapamycin: synthesis of a C10−C26 fragment via a one-pot Julia olefination reaction. *Synthesis* **1996**, 285−296. (b) Baudin, J. B.; Hareau, G.; Julia, S. A.; Ruel, O. A direct synthesis of olefins by reaction of carbonyl compounds with lithio derivatives of 2-[alkyl- or (2-alkenyl)- or benzyl-sulfonyl]-benzothiazoles. *Tetrahedron Lett.* **1991**, *32*, 1175−1178.

(20) (a) Yoshida, A.; Ono, K.; Suhara, Y.; Saito, N.; Takayama, H.; Kittaka, A. Efficient and convergent coupling route for the short-step synthesis of enantiopure 2α- and 2β-alkylated 1α,25-dihydroxy-19-norvitamin D_3 analogues. *Synlett* **2003**, *8*, 1175−1179. (b) Ono, K.; Yoshida, A.; Saito, M.; Fuishima, T.; Honzawa, S.; Suhara, Y.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A.; Efficient synthesis of 2-modified 1α,25-dihydroxy-19-norvitamin D_3 with Julia olefination: High potency in induction of differentiation on HL-60 cells. *J. Org. Chem.* **2003**, *68*, 7407−7415.

(21) Shevde, N. K.; Plum, L. A.; Clagett-Dame, M.; Yamamoto, H.; Pike, J. W.; DeLuca, H. F. A novel potent analogue of 1α,25-dihydroxyvitamin D_3 selectively induces bone formation. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, 99, 13487−13491.

(22) Magyar, K.; Lengyel, J.; Bolehovszky, A.; Grézal, G.; Klebovich, I. Studies of the side chain cleavage of deramciclane in rats with radiolabeled compounds. *Eur. J. Pharm. Sci.* **2002**, 15, 217−223.

(23) Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M.; Hansen, K. 20-Epi-vitamin D_3 analogues: A novel class of potent regulators of cell growth and immune responses. *Biochem. Pharmacol.* **1991**, *42*, 1569−1575.

(24) Suda, T.; DeLuca, H. F.; Tanaka, Y. Biological activity of 25-hydroxyergocalciferol in rats. *J. Nutr.* **1970**, *100*, 1049−1052.

(25) Martin, D. L.; DeLuca, H. F. Influence of sodium on calcium transport by the rat small intestine. *Am. J. Physiol.* **1969**, *216*, 1351−1359.

(26) Ostrem, V. K.; Lau, W. F.; Lee, S. H.; Perlman, K.; Prahl, J.; Schnoes, H. K.; DeLuca, H. F.; Ikekawa, N. Induction of monocytic differentiation of HL-60 cells by 1,25-dihydroxyvitamin D analogues. *J. Biol. Chem.* **1987**, *262*, 14164−14171.

(27) Arbour, N, C.; Ross, T. K.; Zierold, C.; Prahl, J, M.; DeLuca, H. F. A highly sensitive method for large-scale measurements of 1,25-dihydroxyvitamin D. *Anal. Biochem.* **1998**, *255*, 148−154.

(28) Sicinski, R. R.; DeLuca, H. F. Synthesis and biological activity of 22-iodo- and (*E*)-20(22)-dehydro analogues of 1α,25-dihydroxyvitamin D_3. *Bioorg. Med. Chem.* **1999**, *7*, 2877−2889.

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