New 1α,25-Dihydroxy-19-norvitamin D_3 Compounds Constrained in a Single A-Ring Conformation: Synthesis of the Analogues by Ring-Closing Metathesis Route and Their Biological Evaluation

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Vitamin D compounds possessing A rings prohibited from flipping to the alternative chair form (i.e., analogues **2** and **26**) were synthesized. The bicyclic fragment **22** consisting of the fused cyclohexane and dihydropyran rings was constructed via the ring-closing metathesis route. Also, a homologous synthon **23** with an attached dihydropyran ring was successfully synthesized using this strategy. The carbonyl deprotection in **22** yielded cyclohexanone **5** that was subjected to Julia coupling with the anion of the phenylthiazoline sulfone **25**. In the resulting isomeric 19-norvitamins **2** and **26**, their A rings can exist only in the α- and β-conformation. The analogue **26** was 300 times more active in binding to the vitamin D receptor protein, 30 times more effective in causing HL-60 differentiation, and 10 times more active in transcription. These results confirm that the β-chair form of the vitamin D ring A is necessary for the binding to the receptor.

Introduction

The biologically most active vitamin D metabolite, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$,[a] calcitriol, **1**; Figure 1), plays a crucial role in calcium and phosphorus homeostasis.[1,2] It has also been established that it inhibits proliferation and causes differentiation of numerous tumor cells and keratinocytes and also controls immune regulation.[3,4] Such a broad array of biological activities of the natural hormone 1α,25-$(OH)_2D_3$ is mediated through the vitamin D receptor (VDR),[5] an endocrine member of the nuclear receptor superfamily.[6] It has been confirmed that formation of a complex between vitamin D hormone **1** and the VDR results in conformational changes of the protein.[7] These, in turn, influence the binding of VDR to its responsive elements together with the retinoid X receptor (RXR) and subsequent recruitment of several coactivator proteins resulting in the regulation of transcription of a target gene. Upon binding of the final complex to the vitamin D response element (VDRE), transcription is induced.[8]

The importance of the hydroxyl groups, located in the vitamin D molecule at C-1 (1α-OH) and C-25 for the receptor binding, was established long ago.[9] Further studies indicate that some vitamin D analogues lacking the 25-hydroxyl can also bind effectively to the VDR;[10] however, the pivotal role of a 1α-hydroxyl group has remained clear. Taking into consideration these findings and a fast conformational equilibrium of the cyclohexane derivatives, an important question is what A-ring form of the 1α-hydroxylated vitamin D compound is needed for receptor binding? More than 3 decades ago Okamura proposed that equatorial orientation of the 1α-hydroxy substituent (Figure 2a, β-chair form) is necessary for the calcium regulation activity.[11] The first crystal structure of hVDR ligand binding domain bound to the natural hormone **1**, reported by Moras in 2000,[12] and the following reports on the crystallographic data of the complexes formed between other VDRs and different ligands strongly supported Okamura's hypothesis.[13,14] Since all these ligands possessed the chairlike A rings in which equilibration between both α- and β-forms was not prohibited (and therefore theoretically posssible in the solution), we decided to synthesize a vitamin D analogue with the A ring "frozen" in a single chair conformation. We have recently described the preparation of such 19-norvitamin D compound **3** possessing the propylidene substituent at C-2 connected to a 3β-oxygen atom.[15] The synthesized analogue **3** was characterized, in comparison to the natural hormone, by very low binding and transciptional activity. To our surprise, the in vivo tests showed remarkable potency of this compound. Therefore, we decided to extend our studies to similar analogues by exploring metathesis as a route to the bicyclic fragments, consisting of the vitamin D carbocyclic A ring fused with oxygen-containing heterocycles. As the final vitamin D targets, we have chosen the analogue **2** with a dihydrofuran ring and the previously synthesized compound **3**.

The strategy of our synthesis was based on Julia coupling[16,17] of the protected sulfone **4**, prepared by us previously[15] and comprising a C,D-ring fragment, with bicylic ketones **5** and **6** which we planned to obtain by ring-closing metathesis (RCM)[18] of the corresponding diolefins **7**−**10**. We expected that these dienes, in turn, could be prepared from the commercially available (1*R*,3*R*,4*S*,5*R*)-(−)-quinic acid.

Results and Discussion

Chemistry. Our attempts to synthesize the dienes **7** and **8** are summarized in Scheme 1. As a starting compound, we have chosen the bicyclic lactone **11** obtained in four steps from the quinic acid as described previously.[19] Its keto function was protected as cyclic thioacetal, and the formed intermediate **12** was reduced with $NaBH_4$. In the obtained triol **13**, the vicinal diol system was protected as an acetonide in the acid-catalyzed reaction with 2,2-dimethoxypropane. The subsequent Williamson's reaction of the anion of alcohol **14** with an allyl bromide proceeded smoothly providing the desired tricyclic allylic ether **15**. Then we intended to deprotect the keto group and introduce

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[a] Abbreviations: 1α,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; VDR, vitamin D receptor; RXR, retinoic X receptor; hVDRE, human vitamin D response element; RCM, ring-closing metathesis.

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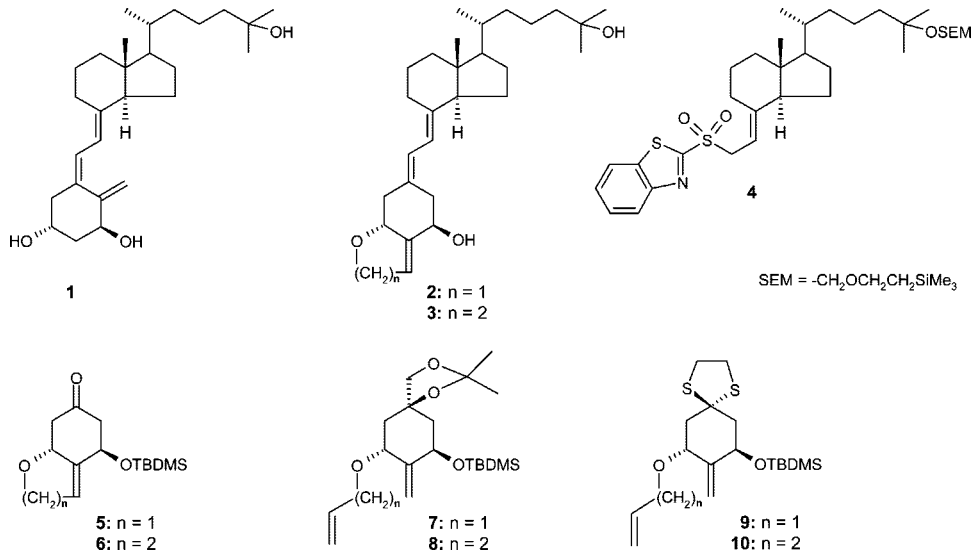


Figure 1. Chemical structure of 1α,25-$(OH)_2D_3$ (calcitriol, **1**), its analogues with the frozen A-ring conformation (**2** and **3**), and the building blocks for their synthesis.

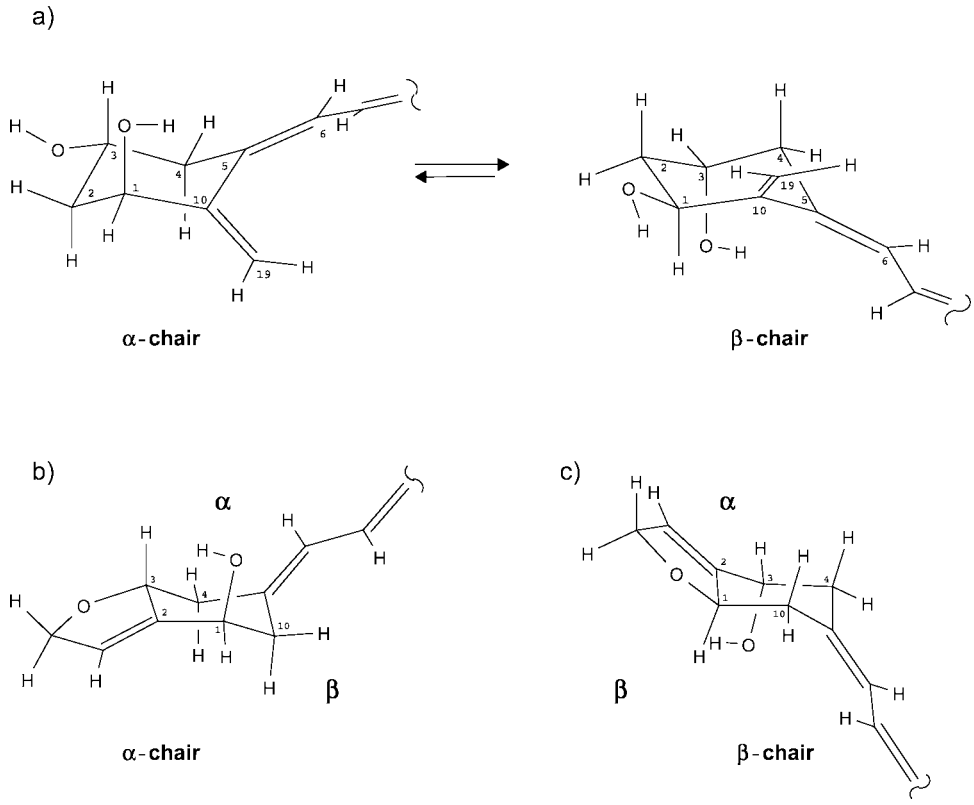
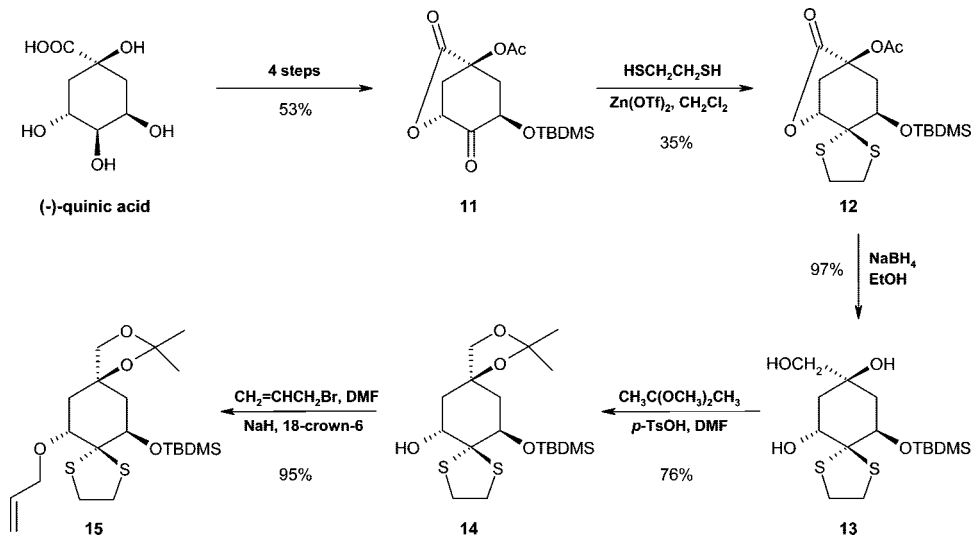


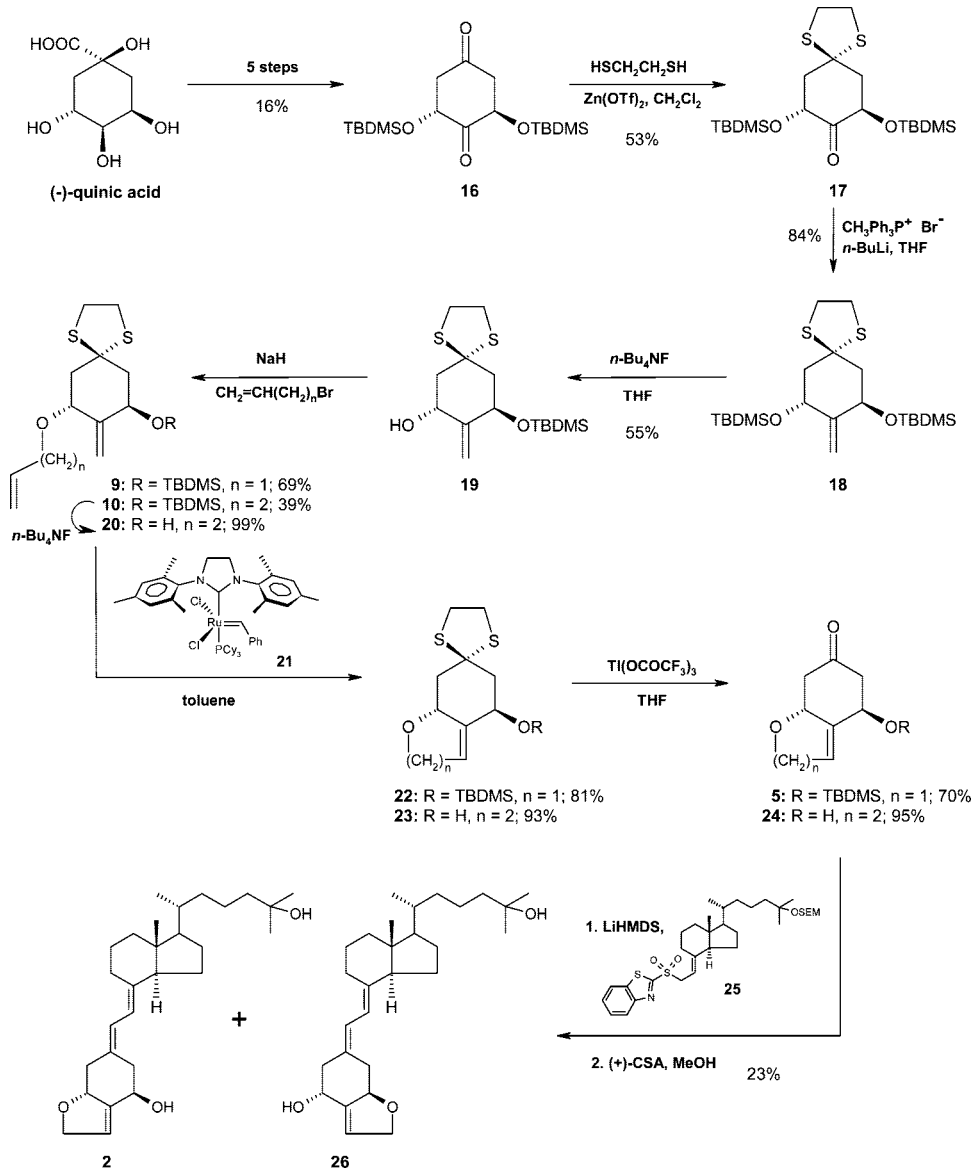
Figure 2. Conformational equilibrium in the A ring of 1α-hydroxyvitamin D analogues (a) and the A-ring conformations of the synthesized vitamin D analogues **4** (b) and **5** (c).

the exomethylene substituent by Wittig reaction. Unfortunately, all our attempts to remove the protecting group proved to be unsuccessful. Thus, for example, the cyclic thioacetal moiety in **15** resisted prolonged (24 h) treatment with oxidizing agents, such as $Hg(ClO_4)_2$, $Tl(OCOCF_3)_3$, NBS, and J_2 (in DMSO), whereas the more drastic conditions of acidic hydrolysis (*p*-TsOH in refluxing acetone) resulted in the compound decomposition. We have therefore directed our efforts to the synthesis of the dienes **9** and **10**. As a starting compound, diketone **16** (Scheme 2) was used, which was prepared by us previously in five steps from the quinic acid.[20] A significant difference in the steric crowding of the carbonyl groups in **16** allowed us to selectively protect one of them and to obtain the desired thioacetal **17**. The subsequent Wittig olefination provided the exomethylene compound **18**. In this bicylic compound characterized (similarly as its precursors **16** and **17**) by the *C*2 symmetry, one silyl protecting group was removed and the obtained single product **19** was alkylated with the corresponding alkyl bromides: allylic and homoallylic. Both unsaturated ethers **9** and **10** were formed, and not surprisingly the yield of the latter one was significantly lower. These products of the Williamson's reaction were next subjected to the RCM reactions carried out in the presence of 10 mol % of the Grubb's second generation catalyst **21**. It turned out that a closure of the five-

Scheme 1



Scheme 2



membered ether ring proceeded very smoothly and the expected RCM product **22** was isolated in 81% yield. However, the analogous reaction of the homoallylic ether **10** failed; only the unreacted substrate was recovered. Since the steric hindrance

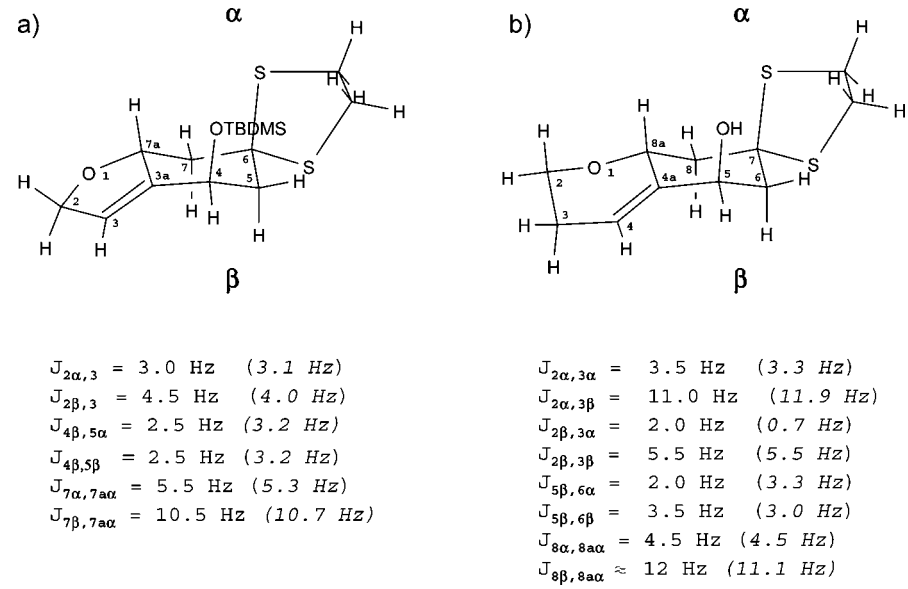


Figure 3. Preferred, energy-minimized (PC MODEL) conformations of the tricyclic compounds **22** (a) and **23** (b) obtained by ring-closing metathesis. The most informative $^1H-^1H$ coupling constants are given. Values of the calculated couplings are enclosed in parentheses.

caused by a bulky OTBDMS group could be responsible for failure of this reaction, the silyl protecting group was removed and the metathesis reaction of hydroxydiene **20** was performed. Contrary to previous findings, the desired RCM product **23**, possessing a six-membered ether ring, was obtained in very high yield (91%). The structures of the synthesized tricyclic ethers were confirmed by molecular modeling and by spectral data analysis. A particularly informative comparison was made between the coupling constant values observed in their ^1H NMR spectra with the respective values of proton couplings calculated by the PC MODEL program (release 9.0) for the energy-minimized conformers (Figure 3). Thus, the vicinal *J* values between 7a- and 7β-H (in **22**) as well as between 8a- and 8β-H (in **23**) unequivocally proved their diaxial arrangement, whereas the small vicinal couplings of 4β-H (in **22**) and 5β-H (in **23**) supported their equatorial disposition and, consequently, axial orientation of OH and OTBDMS substituents of the cyclohexane ring.

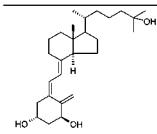
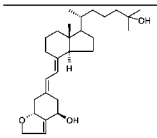
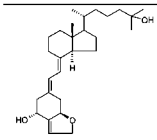
Treatment of both compounds **22** and **23** with thalium triacetate resulted in removal of the thioacetal protecting group and formation of the bicyclic ketones **5** and **24**. The latter proved to be identical in all respects with the previously synthesized compound; its silyl derivative **6** was used by us as a building block in the synthesis of the vitamin D analogue **3**.[15]

The Julia olefination reaction, carried out with the ketone **5** and the anion of the sulfone **25**, was followed by the acidic deprotection of the hydroxyls. The resulting 19-norvitamin D compounds **2** and **26** were separated and purified by repeated HPLC. As expected, ^1H NMR spectra of the isomers **2** and **26** were similar to those of the previously obtained vitamins with fused dihydropyran rings (**3** and its isomeric form with 3β-OH).[15] As it was shown before, the chemical shifts of the protons from the vitamin D intercyclic diene (6- and 7-H) were indicative of the α- or β-chair form of the ring A.[20] Thus, in the spectrum of analogue **2**, the signals of olefinic protons at C-6 and C-7, located at 6.48 and 5.85 ppm, respectively, a narrow signal of the methine proton at C-1 (δ 4.76), and a broad multiplet of 3α-H (δ 4.90, $w/2$ = 19 Hz) fully support the presence of the α-chair form (Figure 2b) with an axially oriented 1α-hydroxyl. In the case of the isomer **26**, the chemical shifts of the olefinic protons at C-6 and C-7 (6.36 and 5.90 ppm) and the shape of the multiplets of 1β- and 3α-H confirm its existence in an alternative β-chair form.

Biological Evaluation. The synthesized vitamin D analogues **2** and **26** were tested for their ability to bind the VDR (Table 1). The 1α,25-$(OH)_2D_3$ compound **2** showed a significantly reduced affinity, decreased 600 times compared to the natural hormone **1**. In a sharp contrast, the isomeric vitamin **26**, possessing a methylene bridge between 1α-oxygen and a terminal carbon of the exocyclic double bond, was only one-half as potent as **1** in displacement of the radiolabeled 1α,25-$(OH)_2D_3$ from the receptor protein. A similar tendency was also observed in other in vitro tests. Thus, when cellular activity of the examined compounds was assessed by studying their ability to induce differentiation of human promyelocytic HL-60 cells into monocytes, the isomer **26** was only 10-fold less active than the vitamin D hormone wheras compound **2** was found to be 300 times less active. Also, vitamins **2** and **26** proved to be 30 and 300 times less potent, respectively, than 1α,25-$(OH)_2D_3$ in their ability to induce transcription of the 24-hydroxylase (CYP-24) luciferase reporter gene system. These results are in total agreement with our previous results that demonstrate that the α-chair form with an axially oriented 1α-hydroxyl exerts a highly detrimental effect on the affinity of the vitamin D compound for the receptor protein and its transcriptional activity.

Considering these results of the in vitro tests performed for the synthesized analogues, one can expect that the compound **26** would be only moderately active in vivo wheras its isomer **2** should be devoid of any calcemic activity. In a sharp contrast to these predictions, the in vivo studies confirmed calcemic potency of both tested compounds. The activities of the analogues in mobilization of calcium from bone were tested in vitamin D deficient rats. The study reported in Figure 4 shows that analogue **2** is approximately 10 times less potent than 1α,25-$(OH)_2D_3$ as the administration of 7020 pmol/day of analogue **2** resulted in a serum calcium level of 6.1 mg/dL, which is comparable to the change in serum calcium observed in animals given 780 pmol/day of 1α,25-$(OH)_2D_3$. The isomeric analogue **26** has a relatively low activity in mobilizing calcium from bone, as its dose of 7020 pmol/day does not significantly alter serum calcium levels. Intestinal calcium transport was evaluated in the same group of animals using the everted gut sac method.

Table 1. VDR Binding Properties,[a] HL-60 Differentiating Activities,[b] and Transcriptional Activities[c] of the Vitamin D Analogues **2** and **26**

		VDR binding		HL-60 differentiation		24-OHase transcription	
compound	compd no.	K_i (M)	ratio	ED_{50} (M)	ratio	ED_{50} (M)	ratio
	**1**	1×10^{-10} M	1	3×10^{-9} M	1	2×10^{-10} M	1
	**2**	6×10^{-8} M	600	1×10^{-6} M	330	6×10^{-8} M	300
	**26**	2×10^{-10} M	2	3×10^{-8} M	10	6×10^{-9} M	30

[a] Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues to the full-length recombinant rat VDR. The experiments were carried out in duplicate on two different occasions. The K_i values are derived from dose−response curves and represent the inhibition constant when radiolabeled 1α,25-$(OH)_2D_3$ is present at 1 nM and a K_d of 0.2 nM is used. The binding ratio is the average ratio of the 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 in duplicate two times. The ED_{50} values are derived from dose−response curves and represent the analogue concentration capable of inducing 50% maturation. The differentiation activity ratio is the average ratio of the 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-$(OH)_2D_3$.

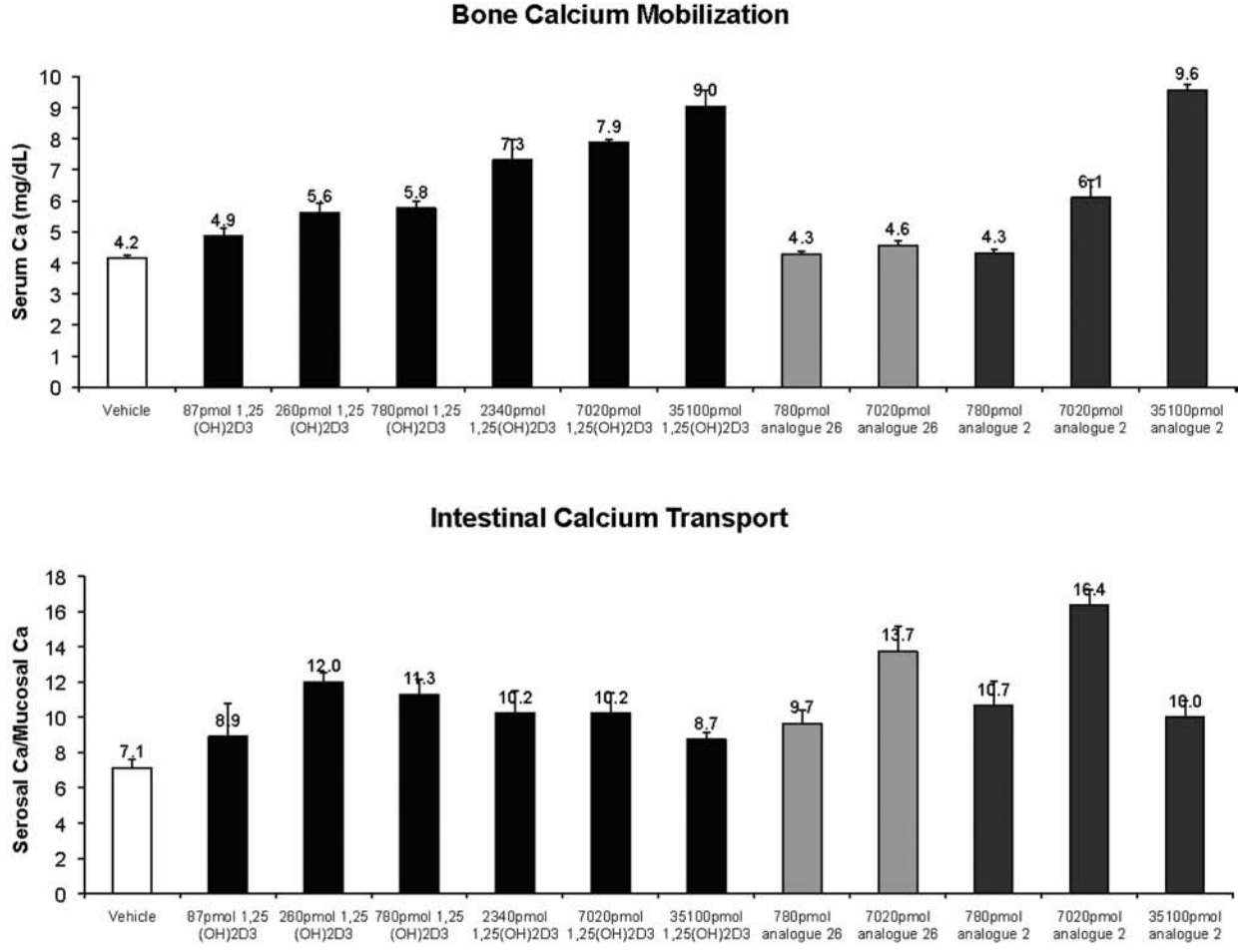


Figure 4. Bone calcium mobilization and intestinal calcium transport activity of the calcitriol (**1**) and the synthesized analogues **2** and **26**.

The results shown in Figure 5 indicate that both synthesized compounds **2** and **26** have a significant intestinal calcium transport activity but both are less potent than the natural hormone **1**. On the basis of the evidence presented above, an interesting conclusion can be drawn: the significantly less active in vitro analogue **2** is characterized by a pronounced calcemic in vivo activity, approaching that of 1α,25-$(OH)_2D_3$. The explanation of this phenomenon can be based on the assumption that, in vivo, both analogues **2** and **26** undergo some metabolic changes resulting presumably in the cleavage of the heterocyclic rings.[21] Since an analogous in vivo activity was observed for the previously synthesized compound **3**, one can further hypothesize that a cleavage of the dihydrofuran/dihydropyran rings in analogues **2** and **3** could result in the vitamin D compounds with the A rings capable of assuming the β-conformation with an equatorial 1α-hydroxyl group required for effective VDR binding.[9]

Discussion

A development of spectroscopic methods allowed studies on the A-ring conformational α-chair−β-chair equilibrium existing in the different vitamin D compounds;[22] especially useful were NMR techniques enabling researchers to calculate the proportions of rapidly equilibrating conformers. An interesting aspect of these studies involved establishing the possible dependence of the biological activity of the vitamin D compound on the conformation of its cyclohexane A ring. Since the publication of Okamura's hypothesis predicting that the β-chair form is responsible for the calcemic activity of vitamin D analogues,[11] many research groups attempted to verify it and solve this conformation−activity problem.[23] The results of our investigation of 10,19-dihydrovitamin D compounds[24] and the subsequent studies on 2-substituted vitamin D analogues carried out in Japanese[25] and our laboratories[26] suggested that the opposite α-form of the ring A can be associated with enhanced biological potency. However, during the past 8 years, a few research groups succeeded in crystallization of the complexes of different vitamin D compounds with the VDR ligand binding domain;[12−14] it turned out that in all cases the bound ligands have their A rings in the β-chair conformation. Trying to exclude the theoretical possibility that the A-ring conformation of the complexed ligand might be different in the crystalline state than that present in the physiological milieu, we decided to synthesize a vitamin D compound "frozen" in the single α-chair A-ring conformation. Very recently, we successfully synthesized analogue **3** and established its structure and conformation by spectroscopic methods.[15] Such structural modification of the 19-nor-1α,25-$(OH)_2D_3$, involving the presence of an additional six-membered ring and a "flattening bond" system,[27] restrained the A ring from interconversion to the alternative chair form and "froze" it in the conformation possessing an axial 1α-hydroxyl. As a method of construction of an additional six-membered heterocyclic ring, we applied the intramolecular nucleophilic substitution of the tosylate by the alkoxide anion. The obtained compound **3** was completely devoid of VDR binding ability, and it was unable to induce transcription of a vitamin D responsive gene. However, surprisingly, this analogue exerted a significant in vivo activity in mice, increasing serum calcium level similarly as 1α,25-$(OH)_2D_3$. These results encouraged us to continue the studies on such modification of the vitamin D skeleton and to explore an alternative synthetic route leading to the analogues with their A rings "frozen" in a single conformation. We decided to obtain the bicyclic fragments containing the modified A rings of the vitamin D analogues using a relatively mild but very powerful method of metathetic transformation. Thus, we first focused our efforts on the preparation of cyclohexanediol derivatives **7**−**10** possessing at the two adjacent carbons an exocyclic methylene group and an unsaturated ether substituent with a terminal double bond. After unsuccessful attempts to obtain compound **7**, we succeded in synthesizing the dienes **9** and **10** which turned out to be suitable substrates (in the latter compound, a silyl group had to be removed) for the ring-closing metathesis process. The structures and conformations of the RCM products were established by NMR spectroscopy. The presented method of construction of the vitamin D analogues, posssessing an additional ring attached to ring A, can be undoubtedly extended to the preparation of compounds characterized by various modifications in the "lower" fragment of the vitamin D molecule, i.e., different sizes of the both rings, presence of additional substituents, etc. It must be emphasized that in both obtained vitamins **2** and **26**, their cyclohexane A rings, whose exocylic double bonds belong to the fused five-membered rings,[27] are completely prevented from flipping and they can only exist in a single chair conformation.

A drastic difference in the VDR affinity of analogues **2** and **26** strongly supports the hypothesis that only compounds with an A-ring β-chair form containing an equatorial 1α-hydroxyl can effectively bind the receptor protein. It is highy possible that the metabolic trasformations of the synthesized vitamin D analogues **2** and **26**, most likely involving the cleavage of the dihydrofuran ring, are responsible for a disparity between their in vitro and in vivo activities.

Conclusions

Two new vitamin D analogues **2** and **26** were synthesized with their A rings prevented from interconversion to the alternative chair conformers. Since the analogue **26** binds the VDR almost as well as the natural hormone, it is obvious that the presence of a small dihydrofuran ring does not interfere with binding to the receptor protein. Therefore, the fact that a binding potency of the isomeric vitamin **2**, possessing a free 1α-hydroxyl group, is decreased 600 times (!) compared to 1α,25-$(OH)_2D_3$ provides definite proof that an α-form of the cyclohexane A ring cannot accommodate the binding pocket of the receptor. Thus, whenever the equilibrum between both forms of the ligand is possible, only the β-form should be considered as the one participating in the formation of the vitamin D complex with its receptor.

Experimental Section

Chemistry. Melting points (uncorrected) were determined on a Thomas-Hoover capillary melting-point apparatus. Both final vitamin D analogues synthesized by us gave single sharp peaks on HPLC, and they were judged at least 99% pure. Two HPLC systems (straight- and reversed-phase) were employed as indicated in Table 2 (Supporting Information). Ultraviolet (UV) absorption spectra were recorded with a Perkin-Elmer Lambda 3B UV−vis spectrophotometer in ethanol. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 400 and 500 MHz with Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers in deuteriochloroform. ^{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 keto compounds **11** and **16** were obtained from commercial (−)-quinic acid according to the published procedures.[19,20]

(1*R*,3*R*,5*R*)-1-Acetoxy-3-[(*tert*-butyldimethylsilyl)oxy]-4,4-ethylenedithio-6-oxabicyclo[3.2.1]octan-7-one (12). To a stirred solution of 1,2-ethanedithiol (385 μL, 702 mg, 4.66 mmol) and $Zn(OTf)_2$ (930 mg, 2.33 mmol) in anhydrous methylene chloride (3.3 mL), a solution of **11** (610 mg, 1.86 mmol) in anhydrous methylene chloride (3.5 mL) was transferred at 0 °C under argon. The mixture was stirred at room temperature for 26 h, poured into brine, and extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$, water, 5% HCl, water again, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (85:15) afforded the oily thioacetal **12** (266 mg, 35%).

(1′*R*,3′*R*,5′*R*)-3′-[(*tert*-Butyldimethylsilyl)oxy]-1′,5′-dihydroxy-4,4-ethylenedithiomethanol (13). To a stirred solution of the lactone **12** (266 mg, 637 μmol) in anhydrous ethyl alcohol (6 mL), $NaBH_4$ (235 mg, 6.36 mmol) was added at 0 °C. The mixture was stirred for 24 h, poured into saturated NH_4Cl, and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (6:4) afforded an oily triol **13** (233 mg, 97%).

(5*R*,7*R*,9*R*)-7-[(*tert*-Butyldimethylsilyl)oxy]-2,2-dimethyl-1,3-dioxa-8,8-ethylenedithio-9-hydroxyspiro[4.5]decanol-9 (14). To a stirred solution of **13** (114 mg, 0.325 mmol) in anhydrous DMF (1.2 mL), 2,2-dimethoxypropane (148 μL, 126.5 mg, 1.215 mmol) and *p*-toluenosulfonic acid (7.6 mg, 0.040 mmol) were added at room temperature. The mixture was stirred for 1.5 h, poured into water, and extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$, water, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (9:1) afforded the compound **14** (97 mg, 76%).

(5*R*,7*R*,9*R*)-9-Allyloxy-7-[(*tert*-butyldimethylsilyl)oxy]-2,2-dimethyl-1,3-dioxa-8,8-ethylenedithiospiro[4.5]decane (15). Sodium hydride (60% suspension in oil; 7.6 mg, 0.190 mmol) was washed with anhydrous hexane (1 mL) under argon, 18-crown-6 (7.6 mg, 29 μmol) was added, and then a solution of alcohol **14** (23 mg, 59 μmol) in anhydrous DMF (0.55 mL) was transferred to the reaction flask via cannula. After 5 min of stirring, allyl bromide (78.3 mg, 80 μL, 0.648 mmol) was added. The mixture was stirred at room temperature for 1.5 h, water was added, and the mixture was extracted with ethyl acetate. The organic phase was separated, washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (98:2) gave the oily allyl ether **15** (24 mg, 95%).

(2*R*,6*R*)-2,6-Bis[(*tert*-butyldimethylsilyl)oxy]-4,4-ethylenedithiocyclohexanone (17). To a stirred solution of 1,2-ethanedithiol (0.25 mL, 3.0 mmol) and $Zn(OTf)_2$ (646 mg, 1.78 mmol) in anhydrous methylene chloride (7.2 mL), a solution of **16** (964 mg, 2.24 mmol) in anhydrous methylene chloride (9.6 mL) was transferred at 0 °C under argon. The mixture was stirred at 0 °C for 1 h and at room temperature for 1.5 h, and then it was poured into brine and extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$, water, 5% HCl, water again, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (96:4) afforded the oily ketone **17** (729 mg, 53%).

(1*R*,3*R*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-5,5-ethylenedithio-2-methylenecyclohexane (18). To the methyltriphenylphosphonium bromide (59 mg, 0.167 mmol) in anhydrous THF (0.8 mL), *n*-BuLi (1.6 M in cyclohexane, 105 μL, 0.167 mmol) was added at 0 °C under argon with stirring. The orange-red mixture was cooled to −78 °C and transferred via cannula to a stirred solution of the ketone **17** (30 mg, 0.067 mmol) in anhydrous THF (0.5 mL). The mixture was stirred at −78 °C for 2 h, and the reaction was quenched by the addition of brine containing 1% HCl. Saturated $NaHCO_3$ (3 mL), water (3 mL), diethyl ether (3 mL), and ethyl acetate (6 mL) were added, and the mixture was vigorously stirred at room temperature. After 16 h the layers were separated, and the organic phase was washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (99:1) gave the oily compound **18** (25 mg, 84%).

(1*R*,3*R*)-3-Allyloxy-1-[(*tert*-butyldimethylsilyl)oxy]-5,5-ethylenedithio-2-methylenecyclohexane (9). Sodium hydride (60% suspension in oil; 8.5 mg, 0.213 mmol) was washed with anhydrous hexane (1 mL) under argon, 18-crown-6 (8.5 mg, 32 μmol) was added, and then a solution of cyclohexanol **19** (22 mg, 66 μmol) in anhydrous DMF (0.63 mL) was transferred via cannula to the reaction flask. After the mixture was stirred for 5 min, allyl bromide (87.8 mg, 89 μL, 0.726 mmol) was added. The mixture was stirred at room temperature for 1.5 h, water was added, and the mixture was extracted with ethyl acetate. The organic phase was separated, washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (99:1) gave the oily compound **9** (17 mg, 69%).

(1*R*,3*R*)-3-(But-3′-enyloxy)-1-[(*tert*-butyldimethylsilyl)oxy]-5,5-ethylenedithio-2-methylenecyclohexane (10). Sodium hydride (60% suspension in oil; 32 mg, 0.64 mmol) was washed with anhydrous hexane (1 mL) under argon, and a solution of alcohol **19** (16 mg, 48.1 μmol) in anhydrous xylene (0.2 mL) was transferred via cannula to the reaction flask. After the mixture was stirred for 10 min, 4-bromo-1-buten (0.24 mmol, 32 mg, 24 μL) was added. The mixture was refluxed for 5 h, cooled to room temperature, and poured into the water. The mixture was extracted with ethyl acetate. The organic phase was separated, washed with water, dried ($MgSO_4$), and evaporated. The oily residue was dissolved in hexane, applied on a silica Sep-Pak cartridge (2 g), and washed with hexane/ethyl acetate (98:2) to give the oily product **10** (7.2 mg, 39%). Further elution with hexane/ethyl acetate (95:5) provided the unreacted substrate **19** (3.5 mg).

(1*R*,3*R*)-3-(But-3′-enyloxy)-5,5-ethylenedithio-2-methylenecyclohexanol (20). A solution of compound **10** (17 mg, 44 μmol) in dry THF (0.25 mL) was treated with tetrabutylammonium fluoride (1 M in THF, 85 μL, 85 μmol). The mixture was stirred at room temperature for 2 h, poured into brine, and extracted with ethyl acetate. The organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The oily residue was purfied on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (94:6) gave the alcohol **20** (12 mg, 100%).

(4*R*,7a*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]-6,6-ethylenedithio-2,4,5,6,7,7a-hexahydrobenzofuran (22). To a stirred solution of compound **9** (12 mg, 0.032 mmol) in anhydrous toluene (8 mL) at 80 °C under argon, Grubb's second generation catalyst **21** (2.7 mg, 3 μmol) was added, and the mixture was stirred at 80 °C for 40 min. Then it was cooled to room temperature, applied on a silica Sep-Pak (0.5 g) to remove the catalyst, and eluted with anhydrous toluene. The residue was purified by column chromatography on silica. Elution with hexane/ethyl acetate (99:1) gave the oily compound **22** (9 mg, 81%).

(5*R*,8a*R*)-7,7-Ethylenedithio-2,3,5,6,8,8a-hexahydro-2*H*-chromen-5-ol (23). The metathesis reaction of diene **20** (6 mg, 2.2 μmol) in anhydrous toluene (1 + 5 mL) was performed in the presence of Grubb's second generation catalyst **21** (2 mg, 2.4 μmol) as described above for compound **9**. After being stirred for 40 min at 80 °C under argon, the mixture was cooled and applied on a silica Sep-Pak (0.5 g). Elution with hexane/ethyl acetate (7:3) yielded the product **23** (5 mg, 93%).

(4*R*,7a*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]-4,5,7,7a-tetrahydro-2*H*-benzofuran-6-one (5). Thallium trifluoroacetate (39 mg, 60 mmol) was added at room temperature to a solution of **22** (20 mg, 58 mmol) in anhydrous THF (1 mL). After the mixture was stirred for 40 min, saturated Na_2SO_3 was added. The mixture was poured into brine and extracted with ethyl acetate. The organic phase was washed with brine, dried ($MgSO_4$), and evaporated to give a colorless residue which was purified by column chromatography on silica. Elution with hexane/ethyl acetate (95:5) gave the ketone

5 (6 mg, 38%; 70% based on the recovered substrate). Then the column was washed with hexane/ethyl acetate (9:1) to afford the unreacted thioacetal **22** (9 mg).

(5*R*,8a*R*)-5-Hydroxy-2,3,5,6,8,8a-hexahydrochromen-7-one (24). The deprotection of the carbonyl group in the thioacetal **23** (2 mg, 8 μmol) in anhydrous THF (0.15 mL) was performed in the presence of thallium trifluoroacetate (5 mg, 9 μmol) as described above for the compound **22**. After the mixture was stirred for 20 min, saturated $Na_2S_2O_3$ was added. The mixture was poured into brine and extracted with ethyl acetate. Organic phase was washed with brine, dried ($MgSO_4$), and evaporated to give a colorless residue which was purified on a silica Sep-Pak (0.5 g). Elution with hexane/ethyl acetate (1:1) gave the ketone **24** (1.3 mg, 95%), identical in all respects with compound prepared by us previously.[15]

1α,25-Dihydroxy- and 25-Hydroxy-19-norvitamin D_3 Analogues (2 and 26). To a solution of sulfone **25** (30.0 mg, 48 μmol) in dry THF (200 μL), LiHMDS (1 M in THF, 48 μL, 48 μmol) was added at −78 °C under argon. The solution turned deep-red. The mixture was stirred at −78 °C for 20 min, and a solution of the ketone **5** (5.4 mg, 20 μmol) in THF (100 + 80 μL) was added. The stirring was continued at −78 °C for 1.5 h, and the reaction mixture was allowed to warm to −10 °C during ∼1.5 h. Then it was poured into saturated NH_4Cl and extracted with ether. The extract was washed with brine, dried (Na_2SO_4), and evaporated. The yellow oily residue was applied on a silica Sep-Pak, eluted with hexane/ethyl acetate (98:2), concentrated under vacuum, dissolved in anhydrous methanol (1 mL), and treated with (+)-10-camphorosulfonic acid (15 mg, 64.5 μmol). The solution was stirred at room temperature under argon for 19 h, poured into brine, and extracted with ethyl acetate. The extract was washed with diluted $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was separated by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (87:13) solvent system. Isomeric 19-norvitamins **2** (1.4 mg, 17%) and **26** (0.5 mg, 6%) were collected at R_V = 30 mL and R_V = 35 mL, respectively. Final purification of both isomers was achieved by reversed-phase HPLC (9.4 mm × 25 cm Zorbax-ODS column, 4 mL/min) using methanol/water (95:5) solvent system: 25-hydroxyvitamin D analogue **26** was collected at R_V = 19 mL and isomeric 1α,25-$(OH)_2D_3$ analogue **2** at R_V = 23 mL.

Biological Studies. 1. In Vitro Studies. VDR binding, HL-60 differentiation and 24-hydroxylase transcription assays were performed as previously described.[19]

2. In Vivo Studies. Bone Calcium Mobilization and Intestinal Calcium Transport. Male, weanling Sprague−Dawley rats were purchased from Harlan (Indianapolis, IN). The animals were group-housed and placed on diet 11 (0.47% Ca) + AEK oil for 1 week followed by diet 11 (0.02% Ca) + AEK oil for 3 weeks. The rats were then switched to a diet containing 0.47% Ca[28] for 1 week followed by 2 weeks on a diet containing 0.02% Ca. Dose administration began during the last week on 0.02% Ca diet. Four consecutive intraperitoneal doses were given approximately 24 h apart. Twenty-four hours after the last dose, blood was collected from the severed neck and the concentration of serum calcium determined as a measure of bone calcium mobilization. The first 10 cm of the intestine was also collected for the intestinal calcium transport analysis using the everted gut sac method.[19]

Molecular Modeling. Molecular mechanism studies were used to establish the energy-minimized structures of the RCM products and the final vitamin D analogues. The calculation of optimized geometries and steric energies was carried out using the algorithm from the MM^+ HyperChem (release 7.0) software package (Autodesk, Inc.). MM^+ is an all-atom force field based on the MM2 functional form. The couplings observed in the ^1H NMR spectra of the synthesized compounds were compared to those calculated using PC MODEL (release 9.0) 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 and spectral data of the synthesized compounds; 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 **2** and **26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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