Rational Design, Synthesis, and Biological Activity of Novel Conformationally Restricted Vitamin D Analogues, (22*R*)- and (22*S*)-22-Ethyl-1,25-dihydroxy-23,24-didehydro-24a,24b-dihomo-20-epivitamin D_3

Hiroyuki Masuno,[†] Keiko Yamamoto,*[,†] Xinxiang Wang,[†] Mihwa Choi,[†] Hiroshi Ooizumi,[‡] Toshimasa Shinki,[§] and Sachiko Yamada*[,†]

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan, Department of Genetics and Molecular Biology, Kyoto University, Kyoto 606-8507, Japan, and School of Dentistry, Showa University, Tokyo 142-8555, Japan

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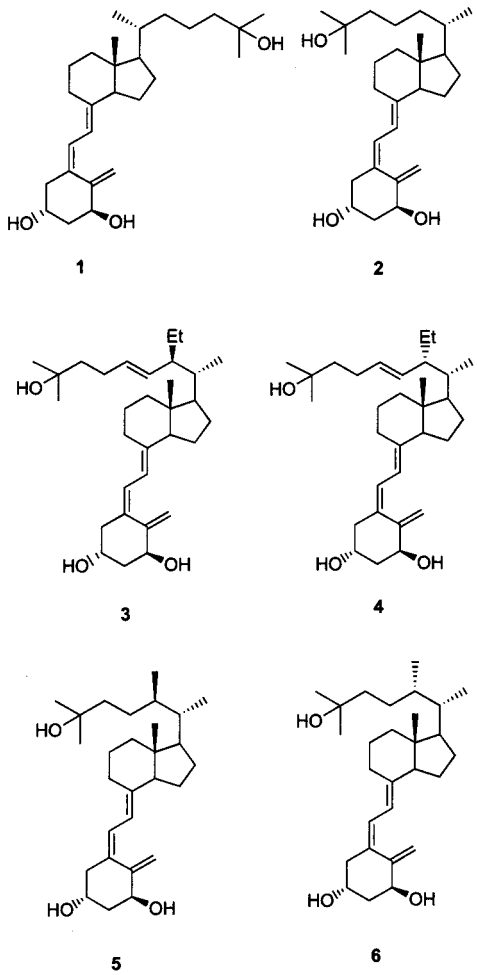
Two new vitamin D analogues, (22*R*)- and (22*S*)-22-ethyl-1,25-dihydroxy-23,24-didehydro-24a,-24b-dihomo-20-epivitamin D_3 (**3** and **4**), were rationally designed on the basis of the active space group concept previously proposed by us. The 22*R* ethyl group of **3** restricts the mobility of the side chain to active space regions, whereas the 22*S* ethyl group of **4** confines the side chain to an inactive region. The double bond at C(23) further restricts the side chain flexibility. These compounds (**3** and **4**) were synthesized using ortho ester Claisen rearrangement as the key step. As expected, the 22*R* isomer **3** has nearly 100 times higher efficacy than 1,25-dihydroxyvitamin D_3 (**1**) in cell differentiation, although its affinity for the vitamin D receptor (VDR) was one-seventh of that of **1**. The 22*S* isomer **4** has significantly lower efficacy than **3**. A docking study in combination with site-directed mutation analysis revealed that two carbon elongated side chain analogue **3** could be fitted in the ligand binding pocket of the VDR by adopting a stable conformation.

Introduction

1α,25-Dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$, **1**] (Chart 1), which was originally recognized as a specific hormone for calcium homeostasis, is now known to be a multifunctional hormone, functioning in inducing cell differentiation, in suppressing cell proliferation, and in regulating the immune response.[1] These biological activities are mediated by the vitamin D receptor (VDR),[2] which is a member of the nuclear receptor (NR) superfamily.[3] Because of their potential value in clinical medicine, nearly a thousand vitamin D analogues have been synthesized and their biological activities have been evaluated.[4] Many of them have already been developed, and some are under development, as clinical agents for treating metabolic bone diseases, skin diseases such as psoriasis, immune disorders, or malignant tumors.[5]

We have been studying the structure−function relationship of vitamin D, focusing on side chain structure and conformation[6] because most active vitamin D analogues are modified in the side chain and we consider that side chain modification is the key to developing potential analogues of vitamin D. On the basis of conformational analysis of the side chain of **1** and its 20-epimer (**2**) and studies using conformationally restricted synthetic vitamin D analogues,[6] we have developed the "active space region" concept of the vitamin D side chain. In this concept, the spatial region that the vitamin D side chain can occupy is divided into

Chart 1



* To whom correspondence should be addressed. Tel.: +81-3-5280-8036. Fax: 03-5280-8005. E-mail: yamada.mr@tmd.ac.jp and yamamoto.mr@tmd.ac.jp.
[†] Tokyo Medical and Dental University.
[‡] Kyoto University.
[§] Showa University.

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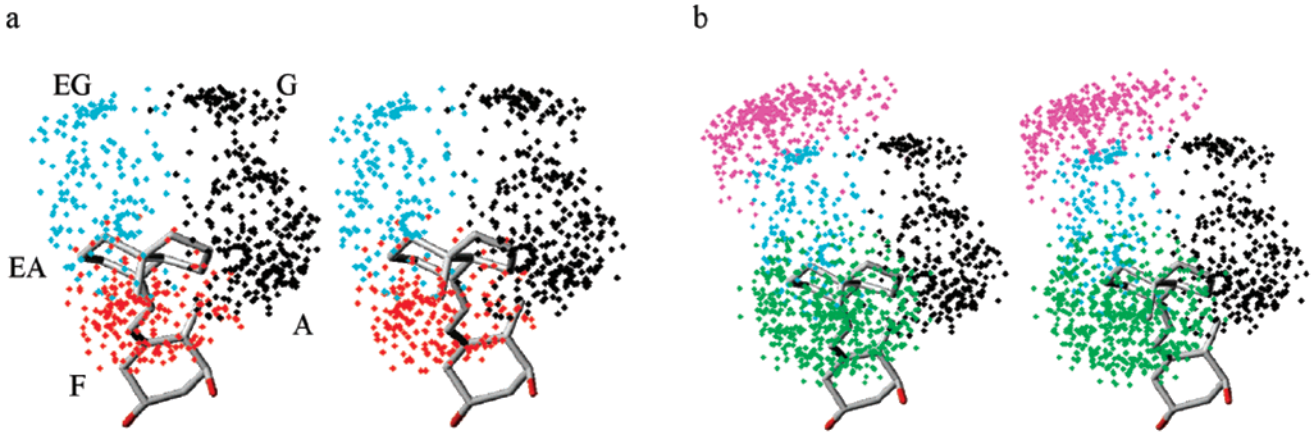


Figure 1. Spatial regions occupied by the side chains of **1**, **2**, 22-oxa-1,25-$(OH)_2D_3$ (OCT), and 22-Et-23-ene-24-dihomo-20-epi-1,25-$(OH)_2D_3$ (**3** and **4**). Dots show the regions where the 25-oxygen of each compound can occupy. Black, **1**; cyan, **2**; red, OCT; green, **3**; magenta, **4**. (a) Dot map of **1**, **2**, and OCT. (b) Dot map of **1**−**4**.

four regions (A, G, EA, and EG); the front A and EA regions were suggested to be important for high potency of vitamin D (Figure 1a). Furthermore, by applying this theory to most of the known active vitamin D analogues, we found a new active region F occupied by the side chains of vitamin D analogues having 22-oxa, 22,23-didehydro, 18-nor, or 16-ene modification (Figure 1a, red dots).[7] Altogether, in terms of the space region, the orders of activity were found as follows: affinity for the VDR, EA > A > F > G > EG; affinity for vitamin D binding protein, A ≫ G, EA, EG; target gene transactivation, EA > F > A > EG ≥ G; cell differentiation, EA > F > A > EG ≥ G; bone calcium mobilization, EA > G ≥ A > F ≥ EG; and intestinal calcium absorption, EA = A ≥ G ≫ EG.[8]

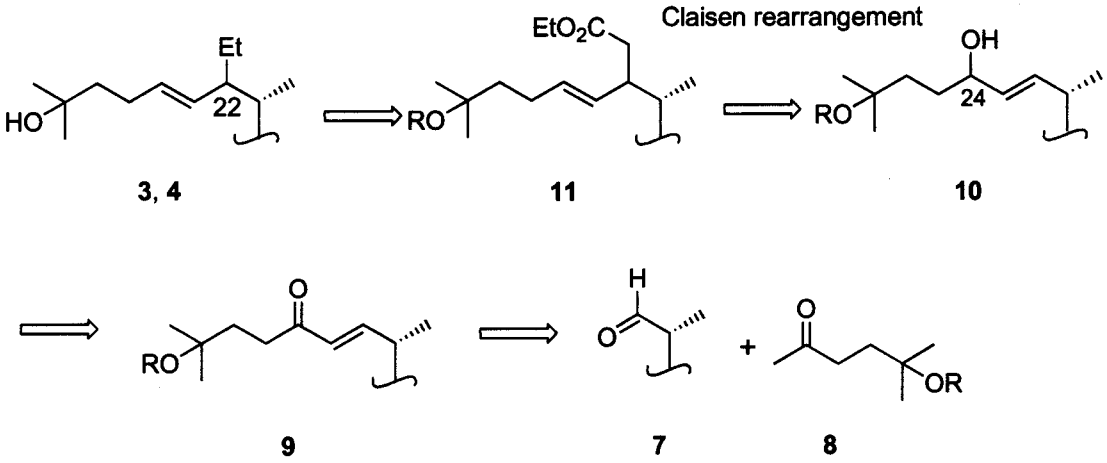
From these studies, we learned that introduction of an alkyl group, specifically a methyl or ethyl group, at C(22) is effective in restricting the side chain conformation and inducing high activity. In this paper, to examine further the effect of the double bond at C(23) and the length of the side chain, we designed and synthesized two new 22-substituted vitamin D analogues, (22*R*)- and (22*S*)-22-ethyl-1,25-dihydroxy-23,24-didehydro-24-dihomo-20-epivitamin D_3 [22-Et-23-ene-24-dihomo-20-epi-1,25-$(OH)_2D_3$, **3** and **4**] and evaluated their biological activities. As predicted, the 22*R* isomer **3** has higher biological potency than **1**, although its affinity for the VDR is lower (one-seventh) than that of **1**.

Results

Design and Conformational Analysis of 3 and 4. The two side chain analogues of vitamin D, **3** and **4**, were designed with regard to the following characteristics: (i) the ethyl group at C(22) directs the side chain to the EA (**3**) or EG (**4**) regions; (ii) the double bond at C(23) further restricts the flexibility of the side chain and has some beneficial effect on potency; (iii) the two methylene elongation increases the appropriate hydrophobic interaction with the receptor and has some effect in the separation of activities.[9] We analyzed the side chain conformation of the two compounds **3** and **4** by systematic search in SYBYL.[6b] The initial conformations (structures having global minimum energy) of **3** and **4** for the conformational analysis were obtained by modification of the crystal structure of 25-hydroxyvitamin D_3[10] followed by force field energy minimization. The dihedral angles at C(16,17,20,22), C(17,20,22,23), C(20,-22,23,24), and C(23,24,24a,24b) of the global minimum conformation are as follows: −175.6, 173.0, −88.0, and 112.9°, respectively, for **3**, and 178.5, −53.6, 136.2, and 102.4°, respectively, for **4**. In these compounds with a 23,24-double bond, the side chain direction is determined by the configuration at C(22): the double bond points to the same direction as the hydrogen at C(22). The results of the conformational analysis are shown as a dot map (Figure 1b). In the dot map, the global minimum conformations of **3** and **4** are drawn with a stick model, with the side chain parts omitted for clarity, and superimposed. The positions of the 25-oxygen atom of all possible side chain conformers of **3** (green) and **4** (magenta) were plotted as dots and overlaid with the dot map of **1** (black) and 20-epi-1,25-$(OH)_2D_3$ (**2**) (cyan).[6] Compound **3** occupies areas EA to F. The double bond at C(23) acts to direct the side chain of **3** to the front region. Compound **4** occupies the area behind the EG region (Figure 1b). This analysis predicts compound **3** to be highly potent but **4** to have low potency.

Stereoselective Synthesis of 3 and 4. The key step in the synthesis of compounds **3** and **4** is the introduction of an ethyl group at C(22). We have reported a highly stereoselective method for introducing an alkyl group at C(22): stereoselective conjugate addition of organocuprates to steroidal 22-en-24-ones and 22-en-24-oates in the presence of TMSCl and hexamethylphosphoramide.[11] Using the method, we successfully synthesized four diastereomers at C(20) and C(22) of 22-methyl-1,25-$(OH)_2D_3$.[6] A drawback of this method is that organocuprates do not efficiently add to 20-epi-22Z-en-24-one; therefore, the stereoselectivity is not high. In searching for a better method, we examined ortho ester Claisen rearrangement of 20-epi-22-en-24-ols (**10**). By Claisen rearrangement of the allylic alcohols (**10**), the 22-substituent and 23,24-double bond can be introduced stereoselectively at the same time. Because the Claisen rearrangement is known to proceed stereoselectively,[12] the stereochemistry at C(22) of the 22-alkyl derivatives can be readily deduced if the stereochemistry at C(24) of the starting allylic alcohols (**10**) is known. The chirality at a carbon atom bearing a secondary hydroxyl group can be readily determined by a method using ^1H nuclear magnetic resonance (NMR).[13] Thus,

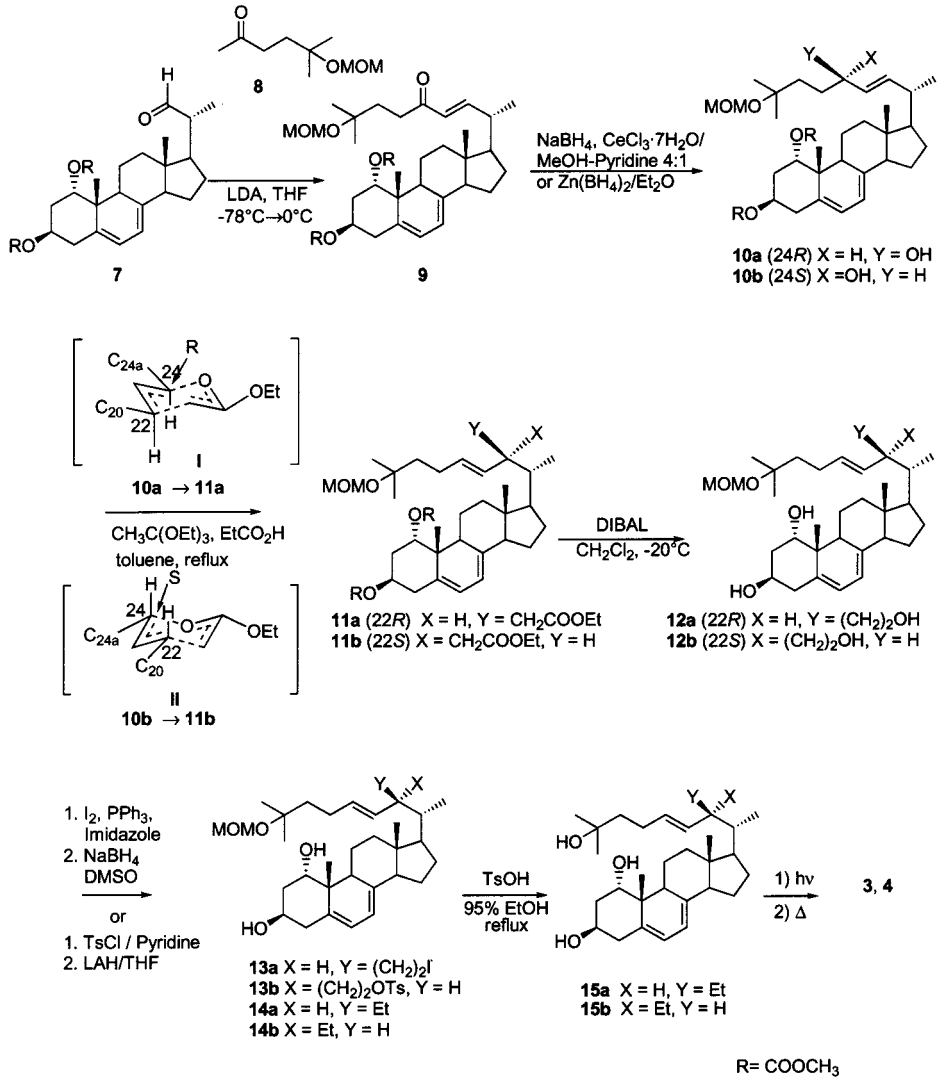
Scheme 1

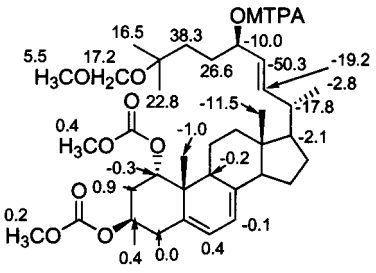


we planned the synthesis of the two compounds (**3** and **4**) as shown in Scheme 1. The allylic alcohol (**10**) can be derived from 22-en-24-one (**9**), which is, in turn, a product of aldol condensation of the C(22)-aldehyde **7** with a side chain fragment, 5-methyl-5-hydroxy-2-hexanone methoxymethyl (MOM) ether (**8**).

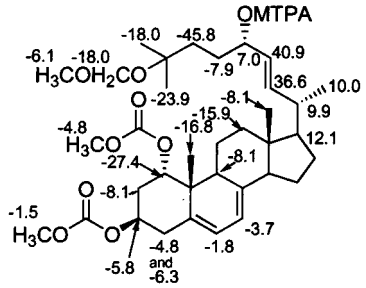
The side chain fragment **8** was readily synthesized from γ-valerolactone. 22-Aldehyde **7** was treated with ketone **8** in the presence of LDA in tetrahydrofuran (THF; −78 °C, then room temperature) (Scheme 2). Aldol condensation and subsequent dehydration occurred in one pot to give the 22-en-24-one **9** (61%). Reduction of the enone **9** with $NaBH_4$ in combination with $CeCl_3$ (MeOH/pyridine) gave the 24*R*- and 24*S*-alcohols **10a**,**b** in a 28:72 ratio (88%). In trying to improve the yield of the 24*R* isomer (**10a**), which is

Scheme 2





16
(MTPA esters from **10a**)



17
(MTPA esters from **10b**)

Figure 2. Determination of stereochemistry at C-24 of **10a**,**b** by modified Mosher's method. The difference of chemical shift values between *R*- and *S*-MTPA esters is shown in Hertz units.

expected to give the final product (**3**) with higher biological potency, we examined $Zn(BH_4)_2$ reduction. However, this reduction also gave the two isomers in a similar ratio (**10a**:**10b**, 43:57, 74%).

The stereochemistry of the epimeric 24-alcohols was determined by modified Mosher's method.[13] A portion of the epimeric alcohols **10a**,**b** was separated,[14] and each isomer was converted to its MTPA ester. Chemical shift differences in the ^{1}H NMR spectra of the two MTPA esters were determined, and the results are summarized in Figure 2. From these results, we assigned the major isomer **10b** as 24*S* and the minor isomer **10a** as 24*R*.

A mixture of the epimeric 24-alcohols (**10a**,**b**) was subjected to ortho ester Claisen rearrangement[12] by treatment with triethyl orthoacetate in the presence of a catalytic amount of propionic acid in refluxing toluene to give the desired rearranged products (**11a**,**b**) in good yield (74%). In the Claisen rearrangement of the pure epimers (**10a**,**b**), the reaction proceeded stereoselectively, **10a** giving exclusively **11a** and **10b** giving exclusively **11b**. The configuration at C(22) of **11a**,**b** was deduced on the basis of the accepted mechanism of Claisen rearrangement: the 3,3-sigmatropic rearrangement proceeds stereoselectively via a six-membered ring transition state. We assumed the reaction of **10a** to proceed via I, giving 22*R*-**11a**, and that of **10b** via II, giving 22*S*-**11b** (Scheme 2).

Reduction of **11** with DIBAL gave alcohol **12** in which the methoxycarbonyl protecting groups of the A-ring hydroxyl groups have been removed. The 22-epimers **12a**,**b** were easily separated by simple column chromatography. The hydroxyethyl group was converted to an ethyl group. The primary alcohol **12a** was selectively transformed to iodide **13a** (75%), which was reduced by $NaBH_4$ in dimethyl sulfoxide (DMSO) to give **14a** (71%). The alcohol **12b** was converted to tosylate **13b** (55%), and the tosylate (**13b**) was reduced by LAH in THF to afford **14b** (41%). The MOM protecting group was removed by treatment with *p*-toluenesulfonic acid, and the resulting provitamins (**15a**,**b**) were converted to vitamin D (**3** and **4**) by photolysis followed by thermal isomerization.

Table 1. Biological Activities of Compounds **3** and **4**[a]

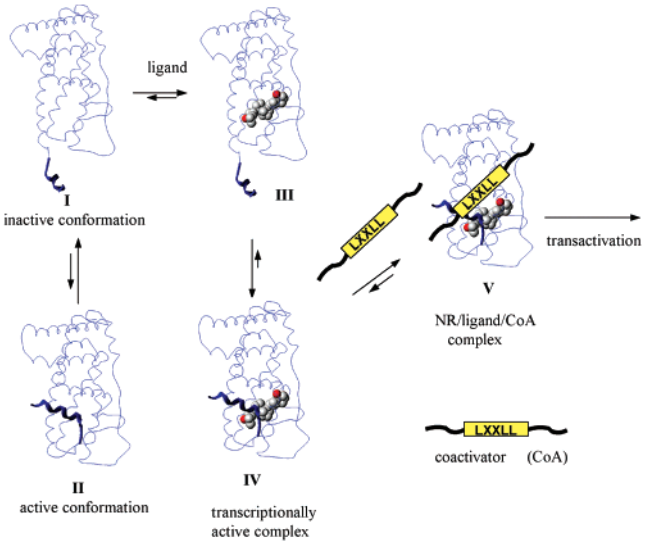
compds	VDR[b]	transcription[c]	differentiation[c,d]	calcemic[e]
$1,25(OH)_2D_3$	100	100	100	100
3 (22*R*)	14	2500	9800	140
4 (22*S*)	0.2	33	77	38
5 (22*R*)[f]	1100	10 000	12 000	220
6 (22*S*)[f]	0.4	3	8	5

[a] Activities are presented as percent effect of **1**. [b] The relative activities were calculated at 50% displacement. [c] The relative activities were calculated at ED_{50}. [d] HL-60 cell differentiation was determined by a NBT assay. [e] Calcemic activity was measured by the rise in serum calcium. [f] Biological activities have been reported previously.[8a]

Biological Evaluation. The biological activities of compounds **3** and **4** were evaluated as follows: affinity for the VDR, transactivation of a target gene,[15] induction of HL-60 cell differentiation,[16] and elevation of serum calcium in vitamin D and Ca deficient rats.[17] The results are summarized in Table 1 and compared with the activities of the 22-epimeric pair of 22-methyl-20-epi-1,25-$(OH)_2D_3$ (**5** and **6**)[8a] and of **1**. Isomer **3**, with the 22*R* configuration, showed much higher potency than isomer **4**, with the 22*S* configuration, in all activities examined. The results are in good agreement with our prediction based on the active space region concept (Figure 1b) and previous results.[7] Particularly, it is interesting that compound **3** is nearly 100 times more potent than **1** in inducing cell differentiation, although its affinity for the VDR is one-seventh of that of the natural hormone (**1**). The 22*R* isomer **3** had lower affinity for the VDR than did the natural hormone, probably because its bulky and flexible side chain made it difficult to enter the hydrophobic pocket of the VDR. However, it is likely that this compound has a stronger hydrophobic interaction with the amino acid residues lining the ligand binding pocket (LBP) once it enters the LBP. This may explain why compound **3** has higher biological activity than **1**, regardless of its lower affinity for the VDR. The affinity of a ligand for the VDR and its transcriptional and cell differentiating activities are not always parallel. We can understand this discrepancy by considering the mechanism of transactivation by NRs (Scheme 3).

On the basis of generally accepted mechanism of transactivation regulated by NRs,[18] we postulate a schematic transactivation pathway shown in Scheme 3. (i) In ligand free apo NR, the protein is in dynamic equilibrium between the two major conformations, inactive conformation I and active conformation without ligand II; (ii) the ligand enters the LBP of the inactive conformation I to form III; (iii) binding of the ligand significantly stabilizes the active conformation IV; (iv) the LXXLL motif of a coactivator binds to the AF-2 (activation function 2) surface of the stabilized complex IV to form an NR/ligand/coactivator ternary complex V.

Scheme 3. Mechanism of Transactivation by the NR[a]



[a] The ligand is depicted as a space-filling model.

Binding of the coactivator is thought to be essential for the commitment of gene transcription. Affinity of ligands for the VDR is determined by competitive binding between radioactive **1** and a substrate for the receptor in the equilibrium state (II ⇆ I ⇆ III ⇆ IV); therefore, K_D = ([I] + [II])[ligand]/([III] + [IV]). However, in transactivation, kinetics from IV to V should be important. Then, the potency may be proportional to the product of the concentrations of IV and CoA; therefore, potency ∝ [IV][CoA]. This difference explains why the transactivation potency of the substrate does not necessarily parallel its binding potency for the VDR.

As described below in the docking model, the elongated side chain of compound **3** can be packed tightly inside the LBP. The 22*S* isomer **4** still has significant cell differentiating and calcemic activities, although its affinity for the VDR is rather low, 1/500 of that of **1**. The activity ratios of the 22*R* and 22*S* isomer pair (**3** and **4**) (for example, 70 in VDR affinity and 127 in cell differentiation) are much lower than those found for the 22-epimeric pair of 22-methylated compounds **5** and **6** (for example, 2750 in VDR affinity and 1500 in cell differentiation). The higher efficacy of **4** relative to **6** may also be explained by tight hydrophobic interaction of the bulky and long side chain with the hydrophobic residues inside the LBP.

Docking of Analogues 3 and 4 to VDR-LBD. Crystal structures of three VDR-LBD deletion mutant (Δ165−215)/ligand complexes have been reported as follows: complexes with **1**,[19] **2**,[20] and 26,27-dimethyl-24-homo-20-epi-22-oxa-1,25-$(OH)_2D_3$ (KH1060).[20] The amino acid residues lining the LBP change their conformation only slightly in forming complexes with these ligands with distinct side chain structures. The only differences observed between the complex with natural hormone (**1**) and those with the 20-epi analogues (**2** and KH1060) are χ1 and χ2 (the torsional angles of the side chain) of I271 and L313. The long and bulky side chain of KH1060 is packed tightly in the same space of the VDR-LBP adopting a near-eclipsed conformation (24.8°) at C(20)-O(22)-C(23)-C(24).

We examined docking of **3** and **4** in the LBP of the crystal structure of VDR-LBD (Δ165−215). We docked these compounds manually into the hydrophobic pocket of VDR as follows. (i) We overlaid the A to CD ring part of the ligands (**3** and **4**) with that of **1** in the crystal structure of the VDR. (ii) The side chain conformation of the ligand was changed, avoiding collision between the ligand and the amino acid residues lining the LBP and placing the 25-OH group of the ligand within a hydrogen-bonding distance from H305 and H397, which have been shown to form hydrogen bonds with the 25-OH of the natural ligand. The ligand in the docking models thus constructed was energy-minimized by keeping the protein part fixed. The resulting docking models are shown in Figure 3. In this model, compound **3** adopts a compact conformation with near local minimum energy (Figure 3a); the dihedral angles are C(16,-17,20,22), −169.8°; C(17,20,22,23), 157.4°; C(20,22,23,-24), −151.7°; C(23,24,24a,24b), 91.7°; C(24,24a,24b,25), −66.9°; C(24a,24b,25,O), 61.9°; and C(20,22,22a,22b), 170.5°.

It is interesting to note that regardless of the two carbon elongation, the 25-OH group of **3** can form a hydrogen bond with H397 as does the 25-OH group of the natural hormone (**1**). We confirmed this by evaluating the transactivation potency of one point mutants, H397A and H305A. We have previously shown that of the two residues H397 and H305 within hydrogen-bonding distance from the 25-OH, H397 plays a major role whereas H305 has a supporting role in anchoring the ligand to the LBP, because one point mutant H397A completely abolished the transactivation potency of VDR

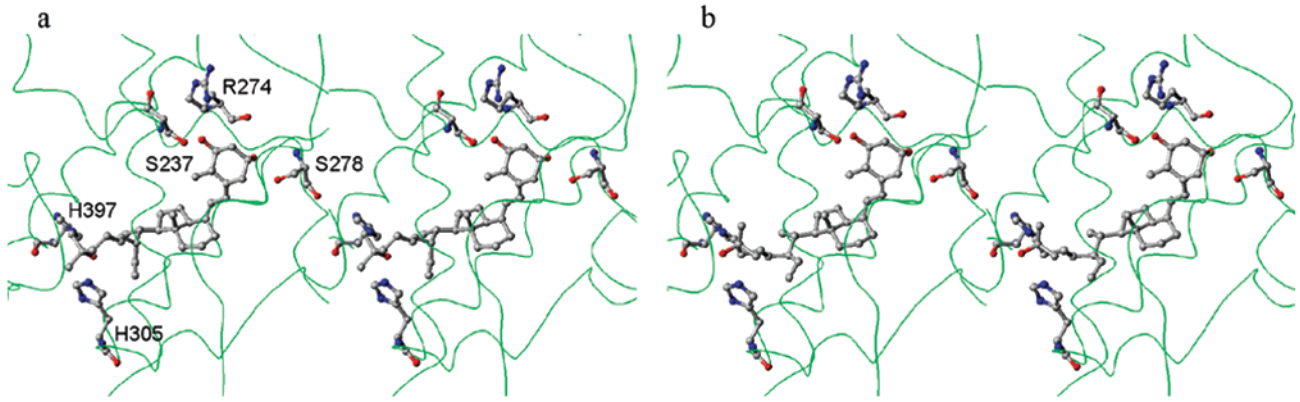


Figure 3. Complex models of the VDR-LBD with (a) 22*R*-Et-23-ene-24-dihomo-20-epi-1,25-$(OH)_2D_3$ **3** and (b) the 22*S* isomer **4** (stereoview).

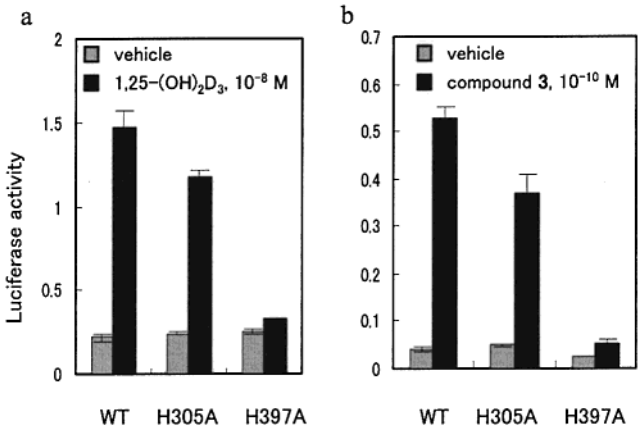


Figure 4. Transcriptional activity of WT and mutant (H305A and H397A) hVDRs. COS-7 cells were cotransfected with WT or mutant hVDR expression vectors, SPP × 3-TK-Luc as a reporter plasmid and pRL-CMV vector as an internal control. Before harvesting, cells were treated with 10^{-8} M **1**, 10^{-10} M **3**, or ethanol vehicle for 18 h. Transactivation was determined by luciferase activity and normalized to the internal control.

induced by **1** but mutant H305A did not significantly reduce the potency (80%, Figure 4a).[21] Similar results were obtained with the dihomoanalogue **3**: H397A abolished the transactivation potency of VDR induced by **3**, whereas H305A reduced that potency to only about 70% (Figure 4b). In the docking model of **3**, tight hydrophobic interactions are observed at C(23), C(24), and C(24a) with V234, at the 22-ethyl group with V300 and H305, and at C(21) with I268. These lines of evidence explain the high potency of compound **3**. On the other hand**,** the 22*S* isomer **4** (Figure 3b) is forced to adopt a considerably unstable conformation in the docking model shown.

Discussion

It is generally known in the vitamin D field that analogues with the unnatural 20*S* configuration have a much higher potency than the corresponding compounds with the natural 20*R* configuration. However, as we reported, 22*S* methylation of **2** drastically reduces potency, whereas 22*R* methylation of the same compound significantly elevates potency.[6b,8a] In terms of affinity for the VDR, 22*R*-methyl-20-epi-1,25-$(OH)_2D_3$ (**5**) has 11-fold higher potency, and the 22*S* methyl analogue (**6**) has 1/250 lower potency than **1**.[8a] This comprises the base of our active space region theory described above: the 25-oxygen of the active 22*R* methyl analogue **5** occupies the EA region, whereas that of the inactive 22*S* analogue **6** occupies the EG region. The active space group concept in our structure−function theory of vitamin D is based exclusively on the ligand structure and is not correlated with the structure of the VDR-LBP. We recently modeled the VDR-LBD harboring **1** and were able to explain this active space group region concept on the basis of the ligand-docking properties of the VDR.[22] Compound **1** is placed in the VDR-LBP with the side chain directed toward the front region, and the biologically important 25-OH group is situated at the place where the A, F, and EA regions overlap (Figure 1). The conformation of the ligand in which the side chain is directed toward the G and EG regions suffers steric congestion with helix 11 of the VDR-LBD. Our model including the docking properties of the natural ligand coincided well with the crystal structure of the VDR-LBD deletion mutant (Δ165−215) reported at nearly the same time.[19]

In this study, we successfully designed a biologically potent vitamin D side chain analogue **3** on the basis of the active space group concept. From the biological activities and VDR docking studies of **3** and **4**, the following conclusions were drawn. Introduction of *R* and *S* ethyl groups at C(22) has inverse effects on potency, in accordance with our theory. However, as compared with a similar compound without the 22-ethyl and 23-ene modifications [24-homo-20-epi-1,25-$(OH)_2D_3$, EB1231],[23] the affinity of **3** for the VDR is considerably reduced (to approximately one-eighth). Regardless of its reduced affinity for the VDR, 22*R* isomer **3** is 100 times more potent than the natural hormone in cell differentiating activity. We explained this discrepancy using schematically presented NR transactivation mechanism (Scheme 3). We postulated that the increased hydrophobic interaction between the amino acid residues lining the LBP and the elongated side chain have a role to stabilize the active conformation IV harboring the ligand and in turn to drive the complex further to ternary coactivator complex V. Our postulation was supported by ligand docking study and mutational analysis. The compound **3** is docked in the LBD with a compact form where the ethyl group and the elongated side chain have tight contacts with the LBP amino acid residues. We assume that the double bond at the side chain enables the compact packing of **3**. Furthermore, we demonstrated that the 25-OH group of **3** still can form a hydrogen bond with H397, as the 25-OH of the natural hormone does. Altogether, we conclude that two carbon elongation is a suitable modification to harbor vitamin D ligand tightly in the LBD. The activity spectrum of **3** is similar to that of the highly potent analogue KH1060. Detailed biological studies of this potential analogue **3** are now under way.

Experimental Section

Computational analysis and modeling were performed using the program SYBYL with ADVANCED COMPUTATION and BIOPOLYMER (Tripos inc.). ^{1}H NMR spectra were recorded in $CDCl_3$ at 400 MHz, and chemical shifts are reported as δ units relative to tetramethylsilane or solvent signal as an internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ at 100 MHz, and chemical shifts are reported as δ units relative to solvent signal as an internal standard. High- and low-resolution mass spectra were obtained on a JEOL JMS-AX505HA spectrometer at 70 eV. Relative intensities are given in parentheses in low mass. IR spectra were recorded on a JASCO Janssen FTIR spectrometer. UV spectra were recorded on a BECKMAN DU7500 spectrophotometer. All air and moisture sensitive reactions were carried out under an argon atmosphere. The phrase "dried and evaporated" indicates drying over $MgSO_4$ followed by evaporation of the solvents under house vacuum.

(22*E*)-(*1S,3R,20S*)-1,3-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-24a,24b-dihomo-5,7,22-cholestatrien-24-one (9). To a solution of diisopropylamine (122 μL, 0.87 mmol) in dry THF (1 mL) was added dropwise *n*-BuLi (1.6 M in hexane, 410 μL, 0.65 mmol) at −78 °C, and the solution was stirred at that temperature for 15 min. To this LDA solution was added a solution of the ketone **8** (114 mg, 0.65 mmol) in dry THF (1 mL) at −78 °C, and the mixture was stirred for 15 min. To a solution of 22-aldehyde **7** (104 mg, 0.22 mmol) in dry THF (1 mL) was added the resulting

enolate solution at −78 °C. Then, the mixture was stirred at −78 °C for 1 h, at −20 °C for 1 h, and at 0 °C for 2.5 h. The reaction mixture was quenched with aqueous NH_4Cl and extracted with AcOEt. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (30 g) with 5−30% AcOEt−hexane to give enone **9** (85 mg, 61%). ^{1}H NMR: δ 0.58 (s, 3 H, H-18), 0.98 (s, 3 H, H-19), 1.01 (d, 3 H, J = 6.6 Hz, H-21), 1.24 (s, 6 H, H-26 and −27), 3.36 (s, 3 H, CH_3OCH$_2$), 3.77 and 3.79 (s, each 3 H, CH_3OCO), 4.69 (s, 2 H, CH$_3$OCH_2), 4.80 (m, 1 H, H-1), 4.90 (m, 1 H, H-3), 5.37 and 5.67 (m, each 1 H, H-6 and −7), 6.07 (d, 1 H, J = 15.8 Hz, H-23), 6.74 (dd, 1 H, J = 15.8, 9.8 Hz, H-22). IR (neat): 2957, 1746, 1443, 1254 cm^{-1}. MS *m*/*z*: 584 (M$^+$ − MeOH, 7), 554 (62), 478 (72), 402(90), 368 (21), 278 (40), 256 (57), 178 (84), 125 (59), 69 (100). HRMS calcd for $C_{34}H_{48}O_8$ (M$^+$ − MeOH), 584.3349; found, 584.3373.

(22*E*)-(1*S*,3*R*,20*S*)-24a,24b-Dihomo-5,7,22-cholestatriene-1,3,24,25-tetrol 1,3-bis(methyl carbonate) 25-Methoxymethyl Ether (10). Reduction with $NaBH_4$−$CeCl_3$. To a solution of enone **9** (454 mg, 0.73 mmol) in pyridine (0.6 mL) and methanol (2.4 mL) were added $CeCl_3$·7H_2O (412 mg, 1.10 mmol) and $NaBH_4$ (42 mg, 1.10 mmol) at 0 °C, and the mixture was stirred for 1 h. Then, $CeCl_3$·7H_2O (276 mg, 0.74 mmol) and $NaBH_4$ (28 mg, 0.74 mmol) were added again, and the mixture was stirred for 3 h. The reaction mixture was diluted with AcOEt, washed with aqueous HCl and water, dried, and evaporated. The residue was chromatographed on silica gel (15 g) with 5−20% AcOEt−benzene to yield alcohol **10** (400 mg, 88%, **10a**:**10b** = 28:72) as a mixture of 24-epimers.

Reduction with $Zn(BH_4)_2$. A solution of $ZnCl_2$ (0.5 g, 3.7 mmol) in ether (6 mL) was refluxed until all $ZnCl_2$ was dissolved. The solution was cooled to room temperature. $NaBH_4$ (0.35 g, 9.2 mmol) was added, and the mixture was stirred overnight. A supernatant of the reaction mixture was collected and used as a $Zn(BH_4)_2$ solution at the following reaction. To a solution of enone **9** (505 mg, 0.82 mmol) in ether (0.5 mL) was added $Zn(BH_4)_2$ solution in ether (6 mL) at 0 °C, and the mixture was stirred at that temperature for 1.5 h. The reaction mixture was poured into water and extracted with AcOEt. The aqueous layer was extracted again with AcOEt after acidification. The combined organic layer was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (20 g) with 20% AcOEt−hexane to yield 24-alcohol **10** (376 mg, 74%, **10a**:**10b** = 43:57) as a mixture of 24-epimers. A part of the mixture was separated by column chromatography (10% AcOEt−hexane) to give pure epimers **10a**,**b**.

10a. ^{1}H NMR: δ 0.59 (s, 3 H, H-18), 0.95 (d, 3 H, J = 6.5 Hz, H-21), 0.98 (s, 3 H, H-19), 1.23 (s, 6 H, H-26 and −27), 3.37 (s, 3 H, −OCH_3), 3.77 and 3.78 (s, each 3 H, −COOCH_3), 4.04 (m 1 H, H-24), 4.71 (s, 2 H, OCH_2O), 4.81 (m 1 H, H-1), 4.89 (m, 1 H, H-3), 5.40 (dd, 1 H, J = 15.3, 6.9 Hz, H-23), 5.54 (dd, 1 H, J = 15.3, 9.1 Hz, H-22), 5.37 and 5.68 (m, each 1 H, H-6 and −7). IR (neat): 3478, 2955, 1746, 1443 cm^{-1}. MS *m*/*z*: 618 (M$^+$, 0.2), 586 (0.6), 556 (3), 510 (5), 480 (24), 404 (100), 249 (40), 125 (75). HRMS calcd for $C_{33}H_{48}O_7$ (M$^+$ − MOMOH), 556.3400; found, 556.3409.

10b. ^{1}H NMR: δ 0.60 (s, 3 H, H-18), 0.94 (d, 3 H, J = 6.5 Hz, H-21), 0.99 (s, 3 H, H-19), 1.23 (s, 6 H, H-26 and −27), 3.37 (s, 3 H, −OCH_3), 3.77 and 3.78 (s, each 3 H, −COOCH_3), 4.04 (m, 1 H, H-24), 4.71 (s, 2 H, OCH_2O), 4.81 (m, 1 H, H-1), 4.89 (m, 1 H, H-3), 5.39 (dd, 1 H, J = 15.4, 6.3 Hz, H −23), 5.53 (dd, 1 H, J = 15.4, 9.0 Hz, H-22), 5.36 and 5.68 (m, each 1 H, H-6 and −7). IR (neat): 3457, 2957, 1746, 1443 cm^{-1}. MS *m*/*z*: 618 (M$^+$, 0.4), 586 (1), 556 (3), 510 (6), 480 (26), 404 (100), 386 (31), 249 (45), 125 (70). HRMS calcd for $C_{34}H_{50}O_8$ (M$^+$ − MeOH), 586.3506; found, 586.3510.

(23*E*)-(1*S*,3*R*,20*S*)-22-(Ethoxycarbonyl)methyl-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol 1,3-Bis(methyl carbonate) 25-Methoxymethyl Ether (11). To a solution of 24-alcohol (**10**) (506 mg, 0.82 mmol, **10a**:**10b** = 42:58) in toluene (4 mL) were added triethyl orthoacetate (4.5 mL, 24.5 mmol) and 1% propionic acid−toluene (300 μL, 0.041 mmol), and the mixture was refluxed for 3 h. The reaction mixture was diluted with benzene, washed with aqueous $NaHCO_3$ and water, dried, and evaporated. The residue was chromatographed on silica gel (30 g) with 2−30% AcOEt−benzene to give the Claisen rearrangement product (**11**) (416 mg, 74%, **11a**:**11b** = 37:63) as a mixture of 22-isomers.

Reaction of 24-Epimer 10a. 24*R*-alcohol **10a** (52 mg, 0.084 mmol), triethyl orthoacetate (460 μL, 2.5 mmol), and propionic acid (0.3 μL, 4.0 μmol) in toluene (0.5 mL) were treated according to the procedure described above to yield rearrangement product **11** (31 mg, 54%, **11a**:**11b** = 94:6). **11a**: ^{1}H NMR: δ 0.65 (s, 3 H, H-18), 0.77 (d, 3 H, J = 6.8 Hz, H-21), 1.02 (s, 3 H, H-19), 1.21 (s, 6 H, H-26 and −27), 1.23 (t, 3 H, J = 7.1 Hz, CH_3CH$_2$), 3.36 (s, 3 H, −OCH_3), 3.77 and 3.79 (s, each 3 H, −COOCH_3), 4.10 (q, 2 H, J = 7.1 Hz, CH$_3$CH_2), 4.69 (s, 2 H, OCH_2O), 4.83 (m, 1 H, H-1), 4.90 (m, 1 H, H-3), 5.39 (m, 2 H, H-23 and −24), 5.37 and 5.68 (m, each 1 H, H-6 and −7). ^{13}C NMR: δ 12.0, 13.9, 14.3, 16.0, 20.4, 22.9, 26.3, 27.2, 27.3, 31.7, 34.0, 35.6, 37.7, 38.0, 39.4, 41.2, 41.6, 41.7, 43.0, 52.3, 54.4, 54.6, 54.8, 55.1, 60.1, 72.2, 76.0, 78.4, 91.0, 115.5, 122.1, 131.0, 131.8, 133.9, 141.0, 155.0, 173.3. MS *m*/*z*: 656 (M$^+$ − MeOH, 3), 626 (5), 550 (36), 474 (100), 279 (48), 249 (45), 209 (32). HRMS calcd for $C_{38}H_{56}O_9$ (M$^+$ − MeOH), 656.3924; found, 656.3950.

Reaction of 24-Epimer 10b. 24*S*-alcohol **10b** (41 mg, 0.066 mmol), triethyl orthoacetate (364 μL, 1.99 mmol), and propionic acid (0.25 μL, 3.3 μmol) in toluene were treated according to the procedure described above for Claisen rearrangement to yield **11** (26 mg, 57%, **11a**:**11b** = 2:98). **11b**: ^{1}H NMR: δ 0.63 (s, 3 H, H-18), 0.76 (d, 3 H, J = 6.7 Hz, H-21), 1.01 (s, 3 H, H-19), 1.22 (s, 6 H, H-26 and −27), 1.24 (t, 3 H, J = 7.0 Hz, CH_3CH$_2$-), 3.36 (s, 3 H, CH$_2$OCH_3), 3.77 and 3.80 (s, each 3 H, OCOCH_3), 4.11 (m, 2 H, CH$_3$CH_2O), 4.70 (s, 2 H, OCH_2O), 4.84 (m, 1 H, H-1), 4.90 (m, 1 H, H-3), 5.39 (m, 2 H, H-22 and −23), 5.38 and 5.68 (m, each 1 H, H-6 and −7). IR (neat): 2961, 2878, 1753, 1443 cm^{-1}. MS *m*/*z*: 656 (M$^+$ − MeOH, 3), 626 (5), 550 (29), 474 (100). HRMS calcd for $C_{38}H_{56}O_9$ (M$^+$ − MeOH), 656.3924; found, 656.3918.

(23*E*)-(1*S*,3*R*,20*S*)-22-(2-Hydroxyethyl)-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol 25-Methoxymethyl Ether (12). To a solution of ester **11** (118 mg, 0.17 mmol, **11a**:**11b** = 27:73) in dry CH_2Cl_2 (1 mL) was added DIBAL (1 M in hexane, 2.0 mL, 2.0 mmol) at −20 °C, and the mixture was stirred at that temperature for 1 h. The reaction mixture was quenched with aqueous NH_4Cl and stirred at 0 °C for 20 min. The mixture was extracted with AcOEt. The extracts were washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (6 g) with 5% EtOH−CH_2Cl_2 to give 22*S* isomer **12b** (60 mg, 66%) and 22*R* isomer **12a** (23 mg, 26%) in this order.

12b. ^{1}H NMR: δ 0.63 (s, 3 H, H-18), 0.80 (d, 3 H, J = 5.8 Hz, H-21), 0.92 (s, 3 H, H-19), 1.22 (s, 6 H, H-26 and −27), 3.37 (s, 3 H, CH_3OCH$_2$), 3.63 (m, 2 H, CH_2OH), 3.78 (m, 1 H, H-1), 4.06 (m, 1 H, H-3), 4.70 (s, 2 H, OCH_2O), 5.33−5.44 (s, 3 H, H-7, −22 and −23) 5.73 (m, 1 H, H-6). ^{13}C NMR: δ 12.6, 13.4, 16.2, 20.9, 22.7, 26.2, 26.9, 27.6, 36,7, 37.5, 38.0, 38.5, 39.1, 39.4, 40.0, 41.2, 42.0, 42.3, 43.4, 52.8, 54.3, 55.1, 61.6, 65.4, 72.8, 76.1, 91.0, 115.2, 122.1, 130.2, 132.3, 135.9, 141.3. IR (neat): 3378, 2934, 2878, 1450, 1367 cm^{-1}. MS *m*/*z*: 530 (M$^+$, 3), 498 (12), 468 (48), 450 (72), 432 (46), 267 (100). HRMS calcd for $C_{32}H_{50}O_4$ (M$^+$ − MeOH), 498.7309; found, 498.3696.

12a. ^{1}H NMR: δ 0.63 (s, 3 H, H-18), 0.80 (d, 3 H, J = 5.9 Hz, H-21), 0.92 (s, 3 H, H-19), 1.22 (s, 6 H, H-26 and −27), 3.37 (s, 3 H, CH_3OCH$_2$), 3.50 and 3.62 (m, each 1 H, CH_2OH), 3.78 (m, 1 H, H-1), 4.20 (m, 1 H, H-3), 4.70 (s, 2 H, OCH_2O), 5.40 (m, 3 H, H-7, −22 and −23), 5.61 (m, 1 H, H-6). ^{13}C NMR: δ 11.9, 13.7, 16.2, 20.8, 22.6, 26.3, 27.2, 37.7, 38.3, 41.0, 41.4, 41.8, 42.6, 52.3, 55.1, 73.7, 76.0, 91.0, 114.9, 122.0, 130.6, 133.6, 136.3, 141.3. IR (neat): 3360, 2932, 1460, 1368 cm^{-1}. MS *m*/*z*: 530 (M$^+$, 3), 498 (10), 468 (43), 450 (48), 432 (8), 394 (15), 267 (100).

(23*E*)-(1*S*,3*R*,20*S*,22*R*)-22-(2-Iodoethyl)-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol 25-Methoxymethyl Ether (13a). To a solution of 22*R* isomer **12a** (20 mg, 0.037 mmol), triphenylphosphine (20 mg, 0.076 mmol), and imidazole (7 mg,

0.10 mmol) in THF (0.4 mL) was added iodine (14 mg, 0.055 mmol) at 0 °C and stirred at the temperature for 1 h. The reaction mixture was quenched with aqueous $Na_2S_2O_5$ and extracted with AcOEt. The extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel (3 g) with 2% EtOH/CH_2Cl_2 to give iodide **13a** (18 mg, 75%) and starting compound **12a** (2.6 mg, 13%). **13a**: ^{1}H NMR: δ 0.63 (s, 3 H, H-18), 0.80 (d, 3 H, J = 6.8 Hz, H-21), 0.95 (s, 3 H, H-19), 1.22 (s, 6 H, H-26 and −27), 2.95 and 3.22 (m, each 1 H, CH_2I), 3.37 (s, 3 H, −OCH_3), 3.78 (m, 1 H, H-1), 4.07 (m, 1 H, H-3), 4.71 (s, 2 H, OCH_2O), 5.26 (dd, 1 H, J = 15.2, 8.8 Hz, H-23), 5.49 (dt, 1 H, J = 15.2, 6.6 Hz, H-24), 5.38 and 5.73 (m, each 1 H, H-6 and −7). ^{13}C NMR: δ 5.6, 12.2, 14.2, 16.3, 20.8, 22.9, 26.3, 26.8, 27.3, 33.0, 38.0, 38.6, 38.7, 39.7, 40.0, 41.8, 42.3, 43.3, 47.0, 52.1, 54.4, 55.1, 65.5, 72.9, 76.0, 91.0, 115.3, 122.0, 131.6, 132.2, 136.0, 141.0. MS *m*/*z*: 640 (M^+, 6), 578 (59), 560 (100), 504 (41), 267 (64).

(23*E*)-(*1S,3R,20S,22S*)-22-[2-(4-Methylbenzene)sulfonyloxy]ethyl-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol 25-Methoxymethyl Ether (13b). To a solution of 22*S* isomer **12b** (13 mg, 0.024 mmol) in pyridine (0.25 mL) was added *p*-toluenesulfonyl chloride (7 mg, 0.037 mmol) at 0 °C, and the mixture was stirred at that temperature for 2 h. *p*-Toluenesulfonyl chloride (14 mg, 0.073 mmol) was added again, and the mixture was stirred for 24 h. The reaction mixture was quenched with water and extracted with AcOEt. The extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel (2.5 g) with 5% EtOH−CH_2Cl_2 to give tosylate **13b** (9.3 mg, 55%). ^{1}H NMR: δ 0.57 (s, 3 H, H-18), 0.73 (d, 3 H, J = 6.7 Hz, H-21), 0.96 (s, 3 H, H-19), 1.22 (s, 6 H, H-26 and −27), 2.45 (s, 3 H, CH_3−Ph), 3.37 (s, 3 H, CH_3O), 3.79 (m, 1 H, H-1), 3.98 and 4.06 (m, each 1 H, CH_2OTs), 4.06 (m, 1 H, H-3), 4.71 (s, 2 H, OCH_2O), 5.19 (m, 2 H, H-22 and −23), 5.37 and 5.73 (m, each 1 H, H-6 and −7), 7.34 and 7.78 (d, each 2 H, J = 8.2 Hz, H-aromatic).

(23*E*)-(*1S,3R,20S,22R*)-22-Ethyl-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol 25-Methoxymethyl Ether (14a). To a solution of iodide **13a** (21 mg, 0.032 mmol) in DMSO (0.4 mL) was added $NaBH_4$ (6 mg, 0.16 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with water and extracted with AcOEt. The extracts were washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (3 g) with 2% EtOH/CH_2Cl_2 to yield 22-ethyl derivative **14a** (12 mg, 71%). **14a**: ^{1}H NMR: δ 0.64 (s, 3 H, H-18), 0.77 (d, 3 H, J = 6.3 Hz, H-21), 0.80 (t, 3 H, J = 7.3 Hz, CH_3CH$_2$), 0.95 (s, 3 H, H-19), 1.23 (s, 6 H, H-26 and −27), 3.37 (s, 3 H, CH_3O), 3.77 (m, 1 H, H-1), 4.07 (m, 1 H, H-3), 4.71 (s, 2 H, OCH_2O), 5.30−5.45 (m, 3 H, H-7, −23 and −24), 5.73 (dd, 1 H, J = 5.6, 2.1 Hz, H-6). MS *m*/*z*: 514 (M^+, 4), 482 (14), 465 (12), 452 (74), 434 (100).

(23*E*)-(*1S,3R,20S,22S*)-22-Ethyl-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol 25-Methoxymethyl Ether (14b). A suspension of LAH (7 mg, 0.18 mmol) in dry THF (0.3 mL) was refluxed for 15 min and cooled to room temperature. To this suspension was added a solution of tosylate **13b** (6.3 mg, 0.009 mmol) in dry THF (0.1 mL), and the mixture was refluxed for 20 min. The mixture was cooled and quenched with THF−H_2O (1:1). The mixture was diluted with aqueous HCl and extracted with AcOEt. The extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel (2.5 mg) with 5% EtOH−CH_2Cl_2 to yield 22-ethyl derivative **14b** (2.1 mg, 44%). **14b**: ^{1}H NMR: δ 0.62 (s, 3 H, H-18), 0.75 (d, 3H, J = 6.9 Hz, H-21), 0.84 (t, 3 H, J = 7.4 Hz, CH_3CH$_2$), 0.95 (s, 3 H, H-19), 1.23 (s, 6 H, H-26 and −27), 3.37 (s, 3 H, CH_3O), 3.78 (m, 1 H, H-1), 4.07 (m, 1 H, H-3), 4.72 (s, 2 H, OCH_2O), 5.25−5.39 (m, 3 H, H-7, −22 and −23), 5.74 (m, 1 H, H-6). IR (neat): 3380, 2932, 1723, 1451, 1379, 1210, 1146, 1090, 1042, 970, 916 cm^{-1}. MS *m*/*z*: 514 (M^+, 2), 496 (5), 482 (8), 464 (12), 452 (72), 434 (100). HRMS calcd for $C_{33}H_{52}O_3$ ($M^+ - H_2O$), 496.3916; found, 496.3937.

(23*E*)-(*1S,3R,20S,22R*)-22-Ethyl-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol (15a). To a solution of MOM ether **14a** (8 mg, 0.015 mmol) in 95% EtOH (0.3 mL) was added TsOH·H_2O (6.8 mg, 0.036 mmol), and the mixture was refluxed for 15 min. The reaction mixture was diluted with Et_3N and then evaporated. The residue was diluted with AcOEt, washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (3 g) with 3% EtOH−CH_2Cl_2 to yield provitamin D **15a** (6.6 mg, 90%). UV (95% EtOH): λ_{max} 293, 282, 271 nm. ^{1}H NMR: δ 0.64 (s, 3 H, H-18), 0.77 (d, 3 H, J = 6.3 Hz, H-21), 0.80 (t, 3 H, J = 7.3 Hz, CH$_2$CH_3), 0.95 (s, 3 H, H-19), 1.23 (s, 6 H, H-26 and −27), 3.77 (m, 1 H, H-1), 4.07 (m, 1 H, H-3), 5.31−5.45 (m, 3 H, H-7, −23 and −24), 5.73 (dd, 1 H, J = 5.6, 2.3 Hz, H-6). ^{13}C NMR: δ 12.1, 12.3, 13.9, 16.2, 20.8, 20.9, 22.9, 27.3, 27.8, 29.3, 38.1, 38.5, 38.6, 40.0, 40.5, 42.3, 43.2, 43.6, 47.0, 52.2, 54.6, 65.5, 72.9, 115.2, 122.1, 130.5, 134.4, 135.8, 141.3. MS *m*/*z*: 470 (M^+, 11), 452, (100), 434 (32). HRMS calcd for $C_{31}H_{50}O_3$, 470.3760; found, 470.3765.

(23*E*)-(*1S,3R,20S,22S*)-22-Ethyl-24a,24b-dihomo-5,7,23-cholestatriene-1,3,25-triol (15b). MOM ether **14b** (5.9 mg, 0.011 mmol) was treated with TsOH·H_2O (6.7 mg, 0.035 mmol) according to the procedure described for 22*R* isomer **14a** to yield provitamin D **15b** (3.4 mg, 63%). UV (95% EtOH): λ_{max} 294, 282, 271 nm. ^{1}H NMR: δ 0.62 (s, 3 H, H-18), 0.75 (d, 3 H, J = 6.8 Hz, H-21), 0.84 (t, 3 H, J = 7.4 Hz, CH_3CH$_2$), 0.95 (s, 3 H, H-19), 1.23 (s, 6 H, H-26 and −27), 3.77 (m, 1 H, H-1), 4.06 (m, 1 H, H-3), 5.28−5.42 (m, 3 H, H-7, −22 and −23), 5.73 (m, 1 H, H-6). MS *m*/*z*: 470 (M^+, 19), 452 (100), 434 (42), 297 (26), 279 (36), 227 (26). HRMS calcd for $C_{31}H_{50}O_3$, 470.3760; found, 470.3782.

(5*Z*,7*E*,23*E*)-(*1S,3R,20S,22R*)-22-Ethyl-24a,24b-dihomo-9,10-seco-5,7,10(19),23-cholestatetraene-1,3,25-triol (3). A solution of the provitamin D **15a** (16.8 mg, 0.036 mmol) in benzene/EtOH (150:20, 170 mL) was flushed with argon for 20 min and then irradiated at 0 °C under argon with a 100 W high-pressure mercury lamp (Shigemi Standard, Tokyo) through a Vycor filter until most of the starting material **15a** was consumed. After the solvent was removed, the residue was chromatographed on Sephadex LH-20 (20 g) with $CHCl_3$/hexane/MeOH (70:30:0.7) to give previtamin D (7.31 mg, 44%). The previtamin D in 95% EtOH (5 mL) was stored in the dark at room temperature. After 14 days, the solution was evaporated, and the residue was chromatographed on Sephadex LH-20 (20 g) with $CHCl_3$/hexane/MeOH (70:30:1) to give vitamin D **3** (3.97 mg, 54%). The purity of **3** was proved to be about 100% by two HPLC systems: 1, YMC-Pack ODS-AM, 4.6 mm × 150 mm; H_2O/MeOH (15:85), 1.0 mL/min, retention time, 11.3 min. 2, LiChrospher Si 60, 4.0 mm × 250 mm; hexane/$CHCl_3$/MeOH (100:25:10), 1.0 mL/min, retention time, 16.5 min. **3**: UV (95% EtOH): λ_{max} 264 nm. ^{1}H NMR: δ 0.54 (s, 3 H, H-18), 0.76 (d, 3 H, J = 6.4 Hz, H-21), 0.81 (t, 3 H, J = 7.3 Hz, CH_3CH$_2$), 1.22 (s, 6 H, H-26 and −27), 4.23 (m, 1 H, H-3), 4.43 (m, 1 H, H-1), 5.00 and 5.33 (s, each 1 H, H-19), 5.28−5.44 (m, 2 H, H-23 and −24), 6.02 and 6.38 (d, each 1 H, J = 11.2 Hz, H-7 and −6). ^{13}C NMR: δ 12.2, 12.4, 14.1, 21.1, 22.2, 23.6, 26.6, 27.8, 29.1, 29.3, 39.6, 40.2, 42.8, 43.6, 45.3, 46.1, 47.3, 52.8, 56.2, 66.8, 70.8, 71.1, 111.8, 117.0, 125.0, 130.5, 132.9, 134.3, 143.1, 147.6. IR (neat): 3387, 2959, 2930, 2874 cm^{-1}. MS *m*/*z*: 470 (M^+, 15), 452 (100), 434 (84), 416 (44), 297 (34), 279 (38), 134 (59). HRMS calcd for $C_{31}H_{50}O_3$, 470.3760; found, 470.3751.

(5*Z*,7*E*,23*E*)-(*1S,3R,20S,22S*)-22-Ethyl-24a,24b-dihomo-9,10-seco-5,7,10(19),23-cholestatetraene-1,3,25-triol (4). Provitamin D **15b** (3.17 mg, 6.74 × 10^{-3} mmol) was converted to target vitamin D **4** (0.84 mg, 26%) according to the procedure for **3**. The purity of **4** was proved to be about 100% by two HPLC systems: 1, YMC-Pack ODS-AM, 4.6 mm × 150 mm; H_2O/MeOH (15:85), 1.0 mL/min, retention time, 10.3 min. 2, LiChrospher Si 60, 4.0 mm × 250 mm; hexane/$CHCl_3$/MeOH (100:25:10), 1.0 mL/min, retention time, 16.2 min. **4**: UV (95% EtOH): λ_{max} 263 nm. ^{1} H NMR: δ 0.53 (s, 3 H, H-18), 0.74 (d, 3 H, J = 6.8 Hz, H-21), 0.83 (t, 3 H, J = 7.4 Hz, CH_3CH$_2$), 1.24 (s, 6 H, H-26 and −27), 4.23 (m, 1 H, H-3), 4.43 (m, 1 H, H-1), 5.00 and 5.33 (s, each 1 H, H-19), 5.28−5.42 (m, 2 H, H-23 and −24), 6.02 and 6.38 (d, each 1 H, J = 11.3 Hz, H-7 and −6). IR (neat): 3382, 2959, 2928, 2874 cm^{-1}. MS *m*/*z*: 470 (M^+, 1), 452 (12), 434 (41), 416 (100), 297 (12), 279 (45). HRMS calcd for $C_{31}H_{50}O_3$, 470.3760; found, 470.3768.

(22*E*)-(1*S*,3*R*,20*S*,24*R*)-24a,24b-Dihomo-5,7,22-cholestatriene-1,3,24,25-tetrol 1,3-Bis(methyl carbonate) 25-Methoxymethyl Ether 24-[(*R*)-Methoxy(trifluoromethyl)phenylacetate] (16a). To a solution of 24-alcohol **10a** (9.2 mg, 0.015 mmol) in dichloromethane (0.2 mL) was added triethylamine (3.1 μL, 0.022 mmol), 4-(dimethylamino)pyridine (10.3 mg, 0.84 mmol), and (*S*)-methoxy(trifluoromethyl)phenylacetyl chloride (5.6 μL, 0.030 mmol) at 0 °C. The solution was stirred at that temperature for 20 min. The reaction mixture was poured into ice and water and extracted with dichloromethane. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (2.5 g) with 20% AcOEt−hexane to give *R*-MTPA ester **16a** (9.7 mg, 78%). ^{1}H NMR: δ 0.57 (s, 3 H, H-18), 0.93 (d, 3 H, J = 6.9 Hz, H-21), 0.97 (s, 3 H, H-19), 1.14 and 1.16 (s, each 3 H, H-26 and −27), 3.32 (s, 3 H, CH_3OCH$_2$-), 3.54 (s, 3 H, CH_3O-), 3.77 and 3.79 (s, each 3 H, CH_3OCO-), 4.62 (s, 2 H, OCH_2O), 4.79 (m, 1 H, H-1), 4.89 (m, 1 H, H-3), 5.36−5.48 (m, 3 H, H-7, −23 and −24), 5.67 (dd, 1 H, J = 2.2, 5.7 Hz. H-6), 5.79 (dd, 1 H, J = 14.9, 9.4 Hz, H-22), 7.34−7.53 (m, 5 H, Ph-*H*). ^{19}F NMR: δ −71.6.

(22*E*)-(1*S*,3*R*,20*S*,24*R*)-24a,24b-Dihomo-5,7,22-cholestatriene-1,3,24,25-tetrol 1,3-Bis(methyl carbonate) 25-Methoxymethyl Ether 24-[(*S*)-Methoxy(trifluoromethyl)phenylacetate] (16b). 10a (10.9 mg, 0.018 mmol) was treated with (*R*)-methoxy(trifluoromethyl)phenylacetyl chloride according to the procedure described above to give *S*-MTPA ester **16b** (4.5 mg, 31%). ^{1}H NMR: δ 0.54 (s, 3 H, H-18), 0.92 (d, 3 H, J = 6.6 Hz, H-21), 0.97 (s, 3 H, H-19), 1.19 (s, 6 H, H-26 and −27), 3.33 (s, 3 H, CH_3OCH$_2$-), 3.55 (s, 3 H, CH_3O-), 3.77 and 3.79 (s, each 3 H, CH_3OCO-), 4.66 (s, 2 H, OCH_2O), 4.79 (m, 1 H, H-1), 4.89 (m, 1 H, H-3), 5.26 (dd, 1 H, J = 15.3, 7.9 Hz, H-23), 5.36−5.45 (m, 2 H, H-7 and −24), 5.68 (m, 1 H, H-6), 5.74 (dd, 1 H, J = 15.3, 9.4 Hz, H-22), 7.34−7.52 (m, 5 H, Ph-*H*).

(22*E*)-(1*S*,3*R*,20*S*,24*S*)-24a,24b-Dihomo-5,7,22-cholestatriene-1,3,24,25-tetrol 1,3-Bis(methyl carbonate) 25-Methoxymethyl Ether 24-[(*R*)-Methoxy(trifluoromethyl)phenylacetate] (17a). 24*S*-alcohol **10b** (20.2 mg, 0.033 mmol) was treated with (*S*)-methoxy(trifluoromethyl)phenylacetyl chloride according to the procedure described above to give **17a** (25.1 mg, 92%). ^{1}H NMR: δ 0.56 (s, 3 H, H-18), 0.92 (d, 2 H, J = 6.6 Hz, H-21), 0.94 (s, 3 H, H-19), 1.19 (s, 6 H, H-26 and −27), 3.33 (s, 3 H, CH_3OCH$_2$), 3.54 (s, 3 H, CH$_3$O), 3.77 and 3.78 (s, each 3 H, CH_3OCOO), 4.66 (s, 2 H, OCH_2O), 4.76 (m, 1 H, H-1), 4.89 (m, 1 H, H-3), 5.28−5.40 (m, 3 H, H-7, −23 and −24), 5.62−5.68 (m, 2 H, H-6 and −22), 7.35−7.43 (m, 3 H, Ph-H), 7.50−7.54 (m, 2 H, Ph-H). ^{13}C NMR: δ 12.0 (C-18), 15.9 (C-19), 20.0 (C-11), 21.2 (C-21), 22.8 (C-15), 26.2 and 26.3 (C-26 and −27), 27.6 (C-12), 29.0 (C-24a), 31.7 (C-2), 35.6 (C-4), 37.2 (C-24b), 37.7 (C-9), 37.8 (C-16), 40.4 (C-20), 41.2 (C-10), 42.8 (C-13), 54.2 (C-14), 54.6, 54.8, 55.1 and 55.3 (*C*H$_3$O-), 55.7 (C-17), 72.2 (C-3), 75.5 (C-25), 78.3 (C-24), 78.4 (C-1), 91.0 (O*C*H$_2$O), 115.4 (C-7), 122.0 (C-6), 124.9 (C-23), 127.5, 128.3, 129.5 and 132.4 (Phenyl), 133.8 and 141.0 (C-5 and −8), 142.1 (C-22), 154.9 and 155.0 (O*C*OO-), 165.7 (carbonyl of MTPA). ^{19}F NMR: δ −71.8.

(22*E*)-(1*S*,3*R*,20*S*,24*S*)-24a,24b-Dihomo-5,7,22-cholestatriene-1,3,24,25-tetrol 1,3-Bis(methyl carbonate) 25-Methoxymethyl Ether 24-[(*S*)-Methoxy(trifluoromethyl)phenylacetate] (17b). 24*S*-alcohol **10b** (17.5 mg, 0.028 mmol) was treated with (*R*)-methoxy(trifluoromethyl)phenylacetyl chloride according to the procedure described above to give MTPA ester **17b** (18.7 mg, 79%). ^{1}H NMR: δ 0.54 (s, 3 H, H-18), 0.90 (s, 3 H, H-19), 0.94 (d, J = 6.6 Hz, H-21), 1.13 and 1.14 (s, each 3 H, H-26 and −27), 3.32 (s, 3 H, CH_3OCH$_2$), 3.54 (s, 3 H, CH_3O), 3.76 and 3.77 (s, each 3 H, CH_3OCOO), 4.61 (s, 2 H, OCH_2O), 4.69 (m, 1 H, H-1), 4.87 (m, 1 H, H-3), 5.34−5.43 (m, 3 H, H-7, −23 and −24), 5.67 (m, 1 H, H-6), 5.74 (m, 1 H, H-22), 7.34−7.40 (m, 3 H, Ph-H), 7.51−7.55 (m, 2 H, Ph-H). ^{13}C NMR: δ 12.10 (C-18), 16.0 (C-19), 20.0 (C-11), 21.2 (C-21), 22.8 (C-15), 26.1 and 26.4 (C-26 and 27), 27.6 (C-12), 28.9 (C-24a), 31.7 (C-2), 35.6 (C-4), 37.0 (C-24b), 37.7 (C-9), 37.8 (C-16), 40.4 (C-20), 41.1 (C-10), 42.8 (C-13), 54.2 (C-14), 54.6, 54.8, 55.1 and 55.3 (*C*H$_3$O-), 55.6 (C-17), 72.2 (C-3), 75.4 (C-25), 78.3 (C-24), 90.9 (O*C*H$_2$O), 115.4 (C-7), 122.0 (C-6), 125.0 (C-23), 127.4, 128.3, 129.5 and 132.4 (Phenyl), 133.8 and 141.0 (C-5 and −8), 142.9 (C-22), 154.8 and 154.9 (O*C*OO-), 165.8 (carbonyl of MTPA). ^{19}F NMR: δ −71.9.

Competitive Binding Assay, Bovine Thymus VDR. Binding to bovine thymus VDR was evaluated according to the procedure reported.[6b,16] Bovine thymus VDR was purchased from Yamasa Biochemical (Choshi, Chiba, Japan) and dissolved in 0.05 M phosphate buffer (pH 7.4) containing 0.3 M KCl and 5 mM dithiothreitol just before use. The receptor solution (500 μL) in an assay tube was incubated with 0.1 nM [^{3}H]-1,25-(OH)$_2$D$_3$ together with graded amounts of each vitamin D analogue for 19 h at 4 °C. The bound and free [^{3}H]-1,25-(OH)$_2$D$_3$ were separated by treating with dextran-coated charcoal for 20 min at 4 °C. The assay tubes were centrifuged at 1000*g* for 10 min. The radioactivity of the supernatant was counted. These experiments were done in duplicate.

Transcriptional Activity. 10T1/2 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% resin-charcoal-stripped fetal bovine serum at 37 °C in 5% CO_2. Transfection was performed via the calcium−phosphate precipitation method as described[15] with 200 ng of reporter plasmid (SPP × 3-TK-LUC), 350 ng of pCMX-β-GAL as internal control, and 200 ng of carrier plasmid pUC18. The cells were transfected for 12 h, and after the DNA precipitates were washed, they were incubated for an additional 48 h with the ligand (10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, and 10^{-11} M). Cells were harvested and were assayed for luciferase and β-galactosidase activities. All points were duplicated.

Differentiation of HL-60 Cells. Human promyelocytic leukemia cells (HL-60) were cultured in RPMI 1640 medium (GIBCO) supplemented with 10% heat-inactivated fetal calf serum in a humidified atmosphere of 5% CO_2 in air at 37 °C. For the evaluation of cell differentiation, HL-60 cells (5×10^6) were incubated with each vitamin D compound (10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-12} M) for 3 days. The differentiation activity was determined by an NBT reduction assay method as previously reported.[16]

In Vivo Bone Calcium Mobilization.[8a,16,17] Weanling male rats were purchased from Shizuoka Laboratories Animal Center and were fed a vitamin D-deficient low calcium diet (Teklad, Madison, WI; 0.03% calcium and 0.6% phosphorus) for 3 weeks. At the end of the 3 week feeding period, they received a single intravenous dose of either vehicle (ethanol, 50 μL) or 1 μg of active vitamin D analogue dissolved in the vehicle. Serum calcium was measured at 0, 6, 12, 24, and 48 h after dosing. Serum calcium concentration was determined by colorimetric assay using *o*-cresolphthalein complexone (Calcium C-test Wako, Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions.

Transactivation Assay of Mutant VDR. Transactivation potency of mutant VDRs was tested according to the procedure previously reported.[21] COS-7 cells transfected with a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPP × 3-TK-Luc), a wild-type (WT) or mutant hVDR expression plasmid (pCMX-hVDR), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) were treated with either 10^{-8} M **1**, 10^{-10} M **3**, or ethanol vehicle and cultured for 18 h. Cells were harvested, and luciferase activity was measured with a luciferase assay kit (Toyo Ink, Inc., Japan) as reported.[21] All experiments were done in triplicate.

Supporting Information Available: Experimental synthesis of compound **8** and experimental details in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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