# 22-Alkyl-20-*epi*-1α,25-dihydroxyvitamin $D_3$ Compounds of Superagonistic Activity: Syntheses, Biological Activities and Interaction with the Receptor

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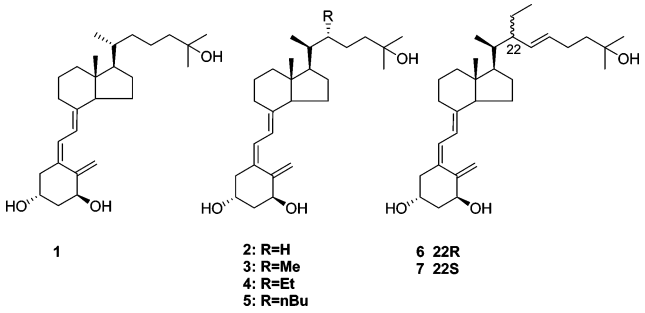
We previously reported that 22*R*-methyl-20-*epi*-1,25-$(OH)_2D_3$ (**3**) possesses strong binding affinity for the vitamin D receptor (VDR) and shows superagonistic biological activities. To examine the effect of the length of an alkyl substituent at C(22) and to extend our compound library, we successfully synthesized 22*R*-ethyl-20-*epi*-1,25-$(OH)_2D_3$ (**4**) and 22*R*-butyl-20-*epi*-1,25-$(OH)_2D_3$ (**5**). Surprisingly, 22-ethyl analogue **4** showed stronger VDR binding affinity and transactivation potency than the superagonist of methyl analogue **3**, but its calcemic activity in vivo was weaker than that of both the methyl analogue **3** and the natural hormone (**1**), while 22-butyl analogue **5** showed activities comparable to those of the hormone (**1**). A study of the docking of these new analogues to the VDR-LBD and alanine scanning mutational analysis demonstrated that 22-methyl and 22-ethyl substituents enhance the favorable hydrophobic interactions with residues lining the ligand binding pocket of the VDR, and that 22-butyl analogue **5** binds to the VDR by an induced fit mechanism.

## Introduction

1α,25-Dihydroxyvitamin $D_3$ [1,25-$(OH)_2D_3$, **1**] was originally recognized as a specific hormone for calcium homeostasis, and it is now known to be a multifunctional hormone, operating in the induction of cell differentiation, in the suppression of cell proliferation and in regulation of the immune response.[1] These biological activities are mediated mostly through the vitamin D receptor (VDR), which is a specific nuclear receptor.[2] More than 3000 vitamin D analogues have been synthesized because of their potential value in clinical medicine.[3] 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.[4]

We have been studying the structure−function relationships of vitamin D, focusing on side-chain structure and conformation.[5] On the basis of conformational analysis of the side chain of 1,25-$(OH)_2D_3$ (**1**) and its 20-epimer **2** and of studies using conformationally restricted synthetic vitamin D analogues,[6−8] we have developed an 'active space region' concept of vitamin D in which the spatial region potentially occupiable by the side chain is divisible into four regions (A, G, EA, and EG). Furthermore, by applying this theory to most of the known active vitamin D analogues, we have found another active region, F, occupied by the side chains of vitamin D analogues having 22-oxa, 22,23-didehydro, 18-nor, or 16-ene modification.[5] In addition, to examine the effects 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 $D_3$ (**6** and **7**).[9] In accordance with the active space region concept, the 22*R*-isomer **6** showed higher biological activities than 1,25-$(OH)_2D_3$ (**1**). As part of our study on the structure−function relationships of vitamin D, we have reported the design and synthesis of four diastereomers at C(20) and C(22) of 22-methyl-1,25-$(OH)_2D_3$ whose side chains are restricted to separate regions.[6,8] Surprisingly, one of these four diastereomers, 22*R*-methyl-20-*epi*-1,25-$(OH)_2D_3$ (**3**), showed strong binding affinity for the VDR and demonstrated superagonistic biological activities.[10] In this paper we report the syntheses of 22*R*-ethyl-20-*epi*-1,25-$(OH)_2D_3$ (**4**) and 22*R*-butyl-20-*epi*-1,25-$(OH)_2D_3$ (**5**) to investigate the effect of the length of the alkyl substituent at C(22) and to extend our compound library. Their biological activities such as VDR binding affinity, transactivation potency, differentiation of HL-60 cells, and in vivo Ca activity are also examined. We also describe the docking study of these new analogues to the VDR-LBD and alanine scanning mutational analysis.

**Chart 1.** Structures of 1, 25-$(OH)_2D_3$ and Its Analogues




**Synthesis.** We synthesized 22*R*-ethyl analogue **4** and 22*R*-butyl analogue **5** using a procedure similar to that described previously for the syntheses of the corresponding methyl analogue **3**.[6,8] Diastereofacial selective conjugate addition of $R_2$-CuLi to the α,β-unsaturated ketone, which we had developed previously,[11,12] was applied as a key step of the synthesis. 20-epi-22*Z*-Enone **8** was derived from the corresponding 22*E*-enone by photochemical *E*−*Z* isomerization.[8] Introduction of an alkyl group to the 22-position was performed with high stereoselectivity by diastereofacial selective conjugate addition of $R_2CuLi$ to 22Z-enone **8**. Under kinetic conditions ($Et_2CuLi$ or n-$Bu_2$-CuLi, TMSCl, HMPA, THF, −78 °C), the alkyl group added to the si-face of Z-enone **8** with more than 97% diastereofacial

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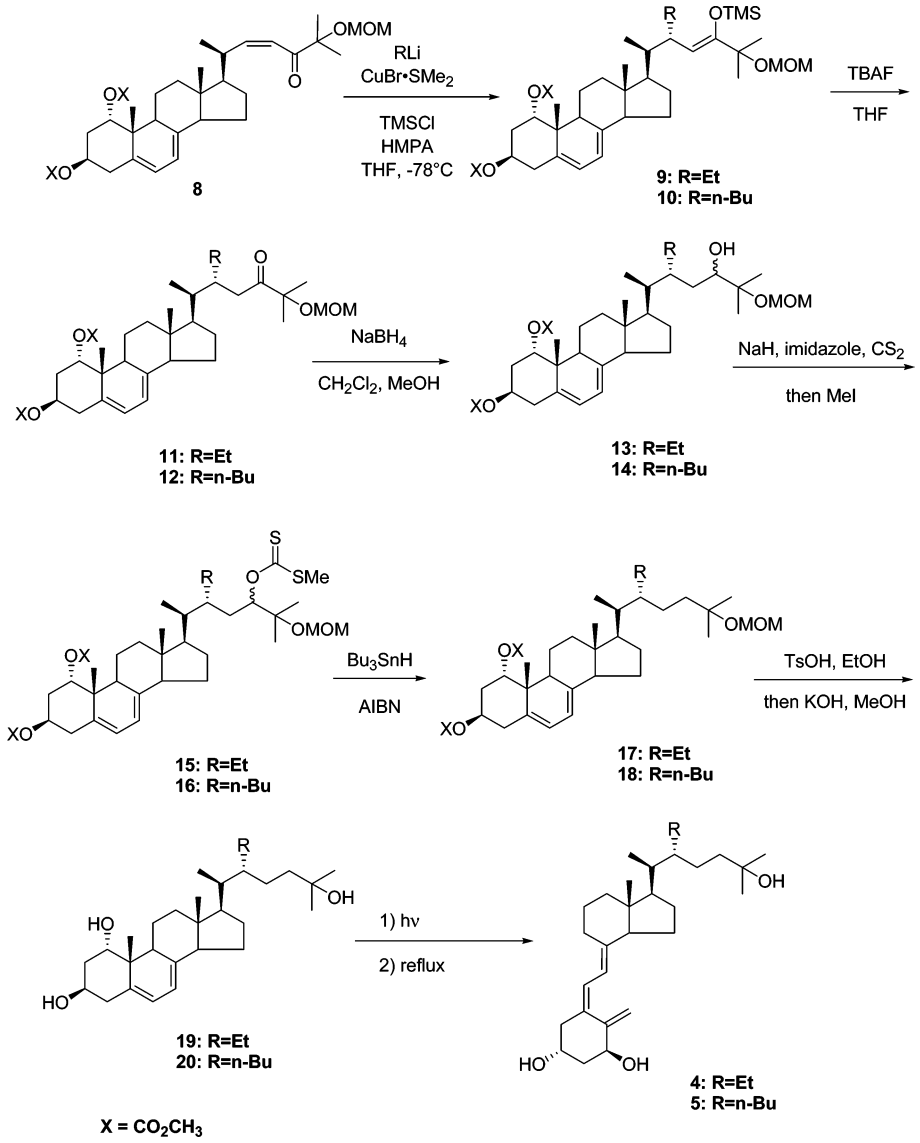
**Scheme 1.** Synthetic Scheme of 22-Alkyl Analogues **4** and **5**




selectivity to afford the desired 22S-enolsilyl ethers **9** and **10** at the yields of 41% and 73%, respectively.[12] An alternative method for introduction of an alkyl group to the 22-position was reported by the Mourino and Fall group,[13,14] although it was nonstereoselective reaction to afford a mixture of diastereomers. Silyl ethers **9** and **10** were deprotected by n-$Bu_4NF$ to give ketone **11** (96%) and **12** (69%), respectively. 24-Ketones **11** and **12** were treated with $NaBH_4$ to give the reduced compounds **13** and **14**, which were treated with NaH and $CS_2$ in the presence of imidazole and then with MeI to give dithiocarbonates **15** and **16**. They were then treated with $Bu_3SnH$ in the presence of AIBN to give the reduced compounds **17** and **18**. Removal of the protecting three hydroxyl groups by acid hydrolysis followed by basic hydrolysis gave provitamin D (**19** and **20**). Finally, these provitamins were converted to the desired 22*R*-alkyl vitamin D analogues **4** and **5** by high-pressure mercury lamp irradiation, followed by thermal isomerization.

**Biological Activities.** Biological activities in vitro are summarized in Table 1. VDR binding affinity was evaluated by using bovine thymus VDR. In this assay, 22*R*-ethyl analogue **4** showed stronger affinity ($EC_{50}$, $1.8 \times 10^{-11}$ M) than not only the natural hormone **1** ($7.1 \times 10^{-11}$ M) but also 22*R*-methyl analogue **3** ($3.1 \times 10^{-11}$ M), indicating that the 22*R*-ethyl analogue **4** possesses the strongest affinity for the VDR among the known 22-alkyl analogues. On the other hand, 22*R*-butyl analogue **5** showed more than ten times weaker affinity ($8.9 \times 10^{-10}$ M) than the natural hormone (**1**). Thus, affinity for the VDR increases in the order 22*R*-butyl analogue **5** < 1,25-$(OH)_2D_3$ (**1**) < 20-epi analogue **2** < 22*R*-methyl analogue **3** < 22*R*-ethyl analogue **4**.

The ability of 22-alkyl analogues (**3**−**5**) to induce transcription of the vitamin D responsive gene was tested using rat osteopontin luciferase reporter gene assay system. In this assay, 22-ethyl analogue **4** ($EC_{50}$, $2 \times 10^{-12}$ M) showed 250 times more potent activity than the hormone (**1**) ($5 \times 10^{-10}$ M). This fact is consistent with the strong transactivation potency of 22-methyl analogue **3** ($3 \times 10^{-12}$ M). Furthermore, 22-butyl analogue **5** ($2 \times 10^{-10}$ M) showed 2.5 times more potent activity than 1,25-$(OH)_2D_3$ (**1**), even though the VDR affinity of this analogue is weaker than that of the hormone (**1**). It is well-known that receptor affinity and transactivation potency are not proportional to each other.[15] These enhanced transactivation potencies could be explained by newly generated hydrophobic interactions between the residues lining the ligand binding pocket (LBP) of the VDR and the 22-alkyl substituent of the ligands. This explanation is consistent with the conclusion obtained from exhaustive alanine scanning mutational analysis

**Table 1.** Biological Activities of 22-Alkyl Analogues

| compd | VDR binding,[a] $EC_{50}$ (M) | Transactivation,[b] $EC_{50}$ (M) | HL-60 differentiation,[c] $EC_{50}$ (M) |
|---|---|---|---|
| 1,25-$(OH)_2D_3$ (**1**) | $7.1 \times 10^{-11}$ | $5 \times 10^{-10}$ | $2.15 \times 10^{-9}$ |
| 22-methyl-20-*epi*-1,25-$(OH)_2D_3$ (**3**) | $3.1 \times 10^{-11}$ | $3 \times 10^{-12}$ | $1.80 \times 10^{-11}$ |
| 22-ethyl-20-*epi*-1,25-$(OH)_2D_3$ (**4**) | $1.8 \times 10^{-11}$ | $2 \times 10^{-12}$ | $5.70 \times 10^{-11}$ |
| 22-butyl-20-*epi*-1,25-$(OH)_2D_3$ (**5**) | $8.9 \times 10^{-10}$ | $2 \times 10^{-10}$ | $1.05 \times 10^{-9}$ |

[a] Competitive binding of 1,25-$(OH)_2D_3$ (**1**) and 22-alkyl analogues (**3**−**5**) to the bovine thymus vitamin D receptor. The $EC_{50}$ values are derived from dose−response curves and represent the analogue concentration required for 50% displacement of the radiolabeled 1,25-$(OH)_2D_3$ from the receptor protein. The experiments were carried out in duplicate. [b] Transactivation was evaluated by dual luciferase assay using a full-length human VDR expression plasmid (pCMX-hVDR), a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPPx3-TK-Luc), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) in COS7 cells as described previously.[16−19] The $EC_{50}$ values are derived from done−response curves and represent the analogue concentration capable of inducing 50% maximal transactivation response. All experiments were carried out in triplicate. [c] Induction of differentiation of HL-60 promyelocytes to monocytes by 1,25-$(OH)_2D_3$ (**1**) and 22-alkyl analogues (**3**−**5**). Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The $EC_{50}$ values are derived from done−response curves and represent the analogue concentration capable of inducing 50% maturation. The experiments were carried out in duplicate.

of the VDR, from which we have concluded that hydrophobic interactions are more effective for transactivation than for VDR binding.[16]

In the differentiation of human promyelocyte HL-60 cells into monocytes, all of the three 22-alkyl analogues (**3**, **4**, and **5**) showed $EC_{50}$ values of $1.8 \times 10^{-11}$ M, $5.7 \times 10^{-11}$ M, and $1.05 \times 10^{-9}$ M, respectively. As expected, 22-methyl and 22-ethyl analogues (**3** and **4**) showed potent activity (120 and 38 times stronger than the natural hormone, respectively), which is in good agreement with their VDR affinity and transactivation potency. It was noteworthy that, in spite of its weaker VDR affinity, 22-butyl analogue **5** showed higher potency than 1,25-$(OH)_2D_3$ (**1**) for both transactivation and cell differentiation.

We have performed and reported exhaustive alanine scanning mutational analysis using various ligands to investigate the mutual interactions between the VDR and its ligands.[16−19] We applied this analysis to 22-alkyl analogues (**3**−**5**). Transactivation was examined using the 22-methyl and 22-ethyl analogues (**3** and **4**) at $10^{-10}$ M and the 22-butyl analogue (**5**) at $10^{-8}$ M. The results are shown in Figure 1 in comparison with the natural hormone (**1**) and the parent molecule (**2**). 22-Methyl analogue **3** and 22-ethyl analogue **4** showed quite similar transactivation patterns, whereas 22-butyl analogue **5** showed a distinct pattern.

In vivo calcemic activity was evaluated by bone calcium mobilization (BCM) and intestinal calcium transport (ICA) in vitamin D-deficient rats using potent analogues **3** and **4** in comparison with the hormone (**1**) (Table 2). It was noteworthy that 22-methyl analogue **3** showed high potency for in vivo BCM but not for in vivo ICA, whereas 22-ethyl analogue **4** showed lower potency than the hormone (**1**) for both in vivo BCM and ICA. It is interesting that both analogues showed relatively weak calcemic activities in spite of their strong differentiation-inducing activity. The result suggested that 22-ethyl analogue **4** might be a potentially useful vitamin D drug having weak calcemic activity in vivo.

## Discussion

As shown in Figure 2a,b the molecular shape of 22-methyl analogue **3** is similar to that of 20-*epi*-1,25-$(OH)_2D_3$ (**2**), but the 22-methyl group newly confers lipophilicity (Figure 2b). It is easy to predict that this increased lipophilicity would enhance hydrophobic interaction with the VDR. 22-Alkyl analogues were docked into the VDR-LBD based on the mode of docking of their parent molecule **2** observed in the X-ray crystal structure of the 20-*epi*-1,25-$(OH)_2D_3$ (**2**)/VDR-LBD complex.[20] The model of the 22-methyl analogue **3**/VDR-LBD complex indicates newly generated favorable hydrophobic interaction between the 22-methyl group and the $\beta$- and $\gamma$-carbons of Val300 at helix 6 (Figure 2d). The model of docking of 22-ethyl analogue **4** into the VDR-LBD also indicates the enhancement of favorable hydrophobic interaction with the $\delta$-carbon of Leu309 in addition to Val300 (Figure 2e). These increased hydrophobic interactions must explain why the 22-methyl and 22-ethyl analogues (**3** and **4**) show strong transactivation potency. Results of our mutational analysis supported these close contacts of ligands with the VDR. Thus, the 22-methyl and -ethyl analogues (**3** and **4**) strongly activate even the alanine mutant of Val300, in contrast to both the hormone (**1**) and the parent compound **2** (Figure 1). Elimination of the dimethyl group of Val300 creates a cleft in the pocket of the VDR and reduces the hydrophobicity of the pocket surface, but the mutant V300A still functions when stimulated with 22-alkyl analogues **3** and **4**. Therefore, it is reasonable to assume that the alkyl substituent at C(22) of ligands fills the cleft and compensates for the reduced hydrophobicity of the LBP.

In comparison with the 22-methyl and -ethyl analogues (**3** and **4**), the transactivation potency of 22-butyl analogue **5** is about 1/100. However, analogue **5** also has the ability to activate the V300A mutant. As shown in the docking model (Figure 2f), the terminal of the butyl substituent forms favorable hydrophobic interactions with the $\gamma$- and $\delta$-carbons of Leu309. The experimental result that only 22-butyl analogue **5** showed significant activity for L309A (Figure 1) suggests that the butyl group points toward Leu309 at the beginning of helix 7. This agrees with the fact that an additional cavity of the LBP has been observed in the crystal structure of the VDR-LBD/Gemini complex by Moras's group.[21] Gemini is an interesting ligand with two identical side chains at C(20).[22−24] Taken together, we think that 22-butyl analogue **5** binds to the VDR by an induced fit mechanism in which the butyl group induces a rearrangement at the beginning of helix 7, leading to the formation of a new pocket like that in the VDR-LBD/Gemini complex.

It was also noteworthy that all three 22-alkyl analogues (**3**−**5**) have the ability to activate Y147A, unlike the hormone (**1**) and the parent compound **2**. We have observed and reported that KH1060[25] and 2MD[26,27] also activate Y147A.[16] We think that since 22-alkyl analogues and KH1060 have some steric repulsion between their bulky side chain and the VDR residue, mutation of Tyr147 to a small residue such as alanine would release the complex from the steric repulsion. In the case of 2MD, the exomethylene group at C(2) also exhibits steric repulsion with the residues including Tyr147 on the loop connected to the end of helix 1, so that 2MD can activate Y147A for the same reason, i.e., release from steric repulsion. These results coincide with our previous finding that the number of alanine mutants enhancing the transactivation potency increases with increasing ligand volume.[16]

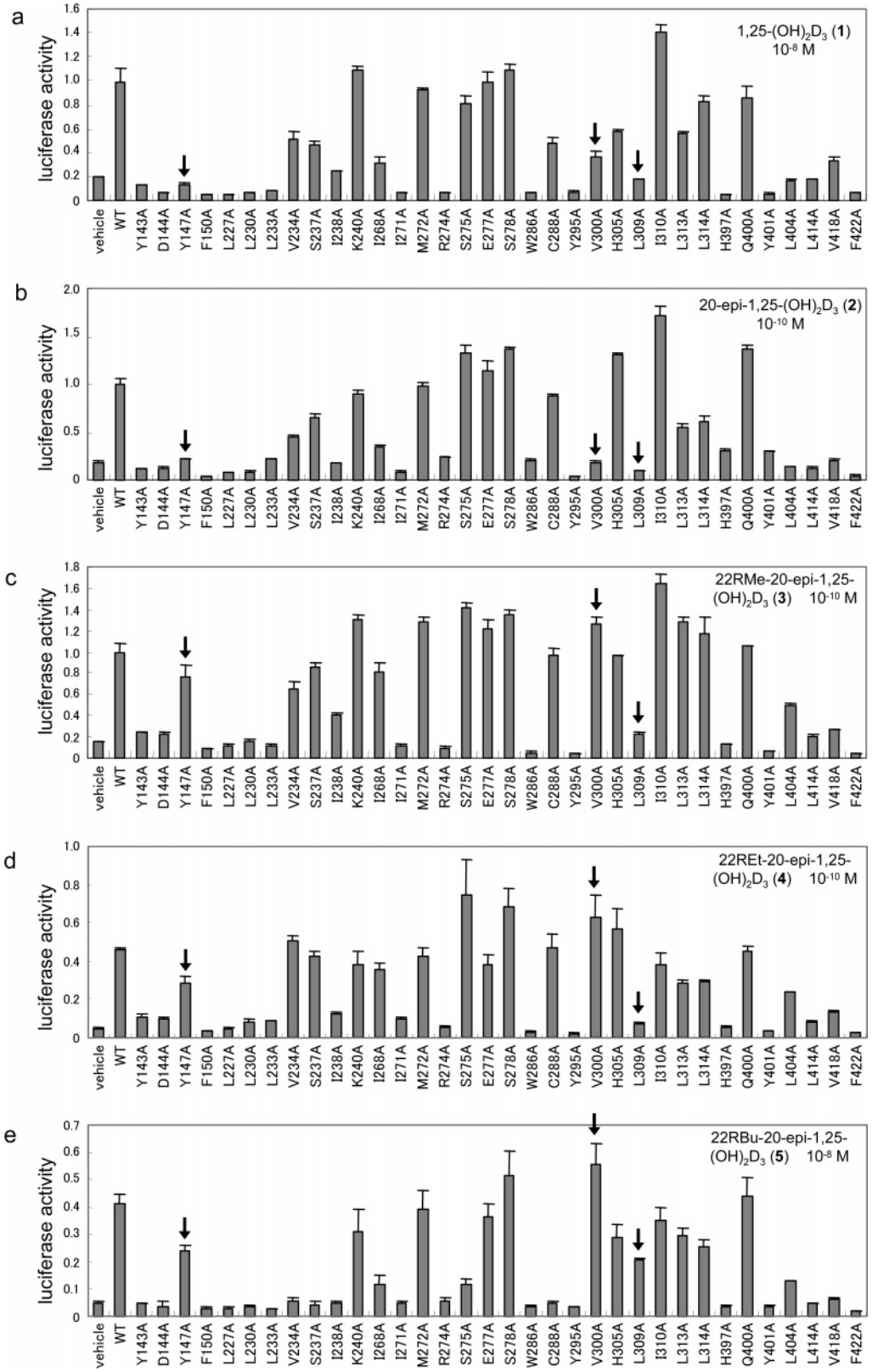



**Figure 1.** Transcriptional activities of one-point alanine mutants of 34 residues lining the ligand binding pocket of the full-length human VDR. The activities were evaluated by dual luciferase assay using a full-length wild-type or mutant human VDR expression plasmid (pCMX-hVDR), a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPPx3-TK-Luc), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) in COS7 cells as described previously.[16−19] a. 1,25-$(OH)_2D_3$ (**1**) ($10^{-8}$ M), b. 20-*epi*-1,25-$(OH)_2D_3$ (**2**) ($10^{-10}$ M), c. 22*R*Me-20-*epi*-1,25-$(OH)_2D_3$ (**3**) ($10^{-10}$ M), d. 22*R*Et-20-*epi*-1,25-$(OH)_2D_3$ (**4**) ($10^{-10}$ M), e. 22*R*Bu-20-*epi*-1,25-$(OH)_2D_3$ (**5**) ($10^{-8}$ M).

From these results, we conclude that introduction of an appropriate alkyl group into vitamin D compounds enhances their affinity for the VDR. Takayama's group has similarly reported that 2α-methyl-1,25-$(OH)_2D_3$ has four times higher affinity for the VDR than the natural hormone (**1**).[28] However, 22-alkyl analogues with high VDR affinity (**3** and **4**) enhance transactivation and cell differentiation exponentially, whereas in the case of 2α-methyl-1,25-$(OH)_2D_3$, which is an A-ring

**Table 2.** Intestinal Calcium Transport and Bone Calcium Mobilization by 22-Alkyl Analogues in Vitamin D Deficient Rats on a Low-Calcium Diet[a]

| compound | dose (pmol/day/7 days) | intestinal Ca transport (S/M)[b] (mean ± SEM)[c] | bone Ca mobilization (serum Ca mg/100 mL) (mean ± SEM)[c] |
|---|---|---|---|
| deficient controls | 0 (vehicle only) | 3.4 ± 0.3 | 3.4 ± 0.0 |
| 1,25-$(OH)_2D_3$ (**1**) | 100 | 7.7 ± 0.8 | 4.6 ± 0.0 |
| | 325 | 12.7 ± 1.0 | 5.5 ± 0.2 |
| 22-methyl-20-*epi*-1,25-$(OH)_2D_3$ (**3**) | 100 | 7.0 ± 0.5 | 5.0 ± 0.3 |
| | 325 | 8.2 ± 1.1 | 8.1 ± 1.0 |
| | 650 | 12.2 ± 1.0 | 8.4 ± 0.4 |
| 22-ethyl-20-*epi*-1,25-$(OH)_2D_3$ (**4**) | 100 | 5.7 ± 0.5 | 3.9 ± 0.2 |
| | 325 | 6.3 ± 0.5 | 5.0 ± 0.3 |
| | 650 | 8.5 ± 1.3 | 6.2 ± 0.6 |

[a] Rats were maintained on a 0.47% Ca diet for 1 week and then switched to a low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, they were dosed daily with the appropriate vitamin D compound for 7 consecutive days. All doses were administered intraperitoneally in 0.1 mL of propylene glycol/ethanol (95:5). Controls received the vehicle. Determinations were made 24 h after the last dose. There were five rats per group. [b] S/M means serosal/mucosal. [c] The values represent the mean ± SEM.

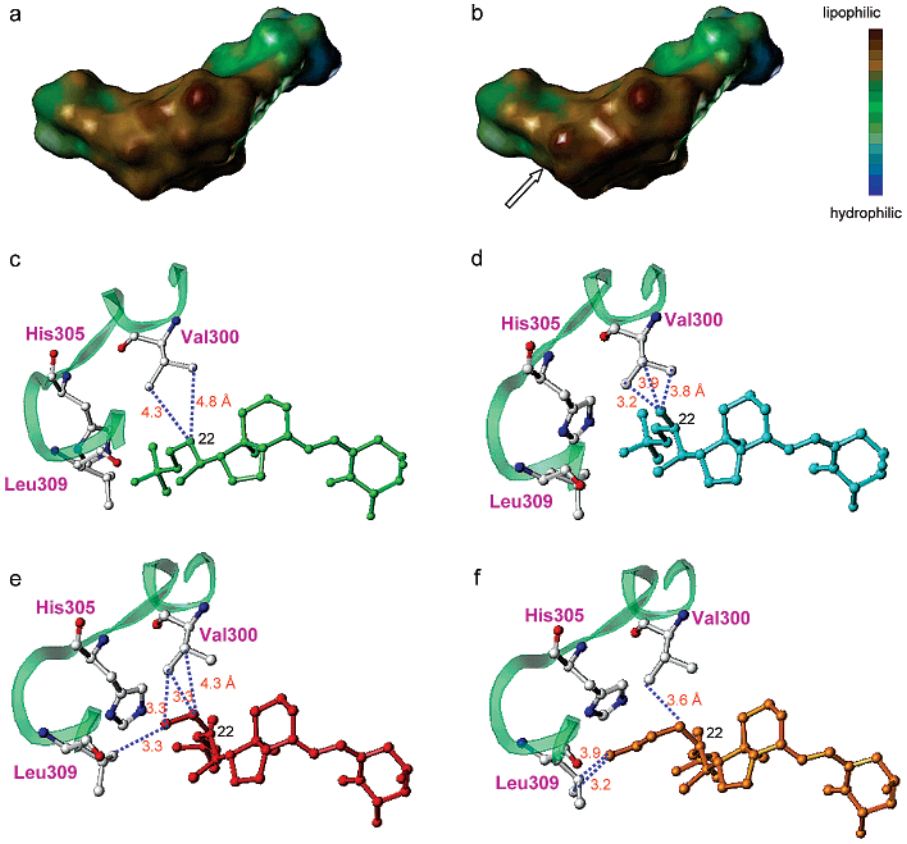



**Figure 2.** Connolly surface of 20-*epi*-1,25-$(OH)_2D_3$ (**2**) (a) and 22*R*Me-20-*epi*-1,25-$(OH)_2D_3$ (**3**) (b); and complexes of the VDR-LBD with 20-*epi*-1,25-$(OH)_2D_3$ (**2**) (c), 22*R*Me-20-*epi*-1,25-$(OH)_2D_3$ (**3**) (d), 22*R*Et-20-*epi*-1,25-$(OH)_2D_3$ (**4**) (e), and 22*R*Bu-20-*epi*-1,25-$(OH)_2D_3$ (**5**) (f). The complex model of methyl analogue **3**/VDR-LBD indicates newly generated favorable hydrophobic interaction between the 22-methyl group and the Val300 on helix 6 (d). Docking model of ethyl analogue **4** into the VDR-LBD also indicates the enhancement of the favorable hydrophobic interaction with Leu309 in addition to Val300 (e). In the complex of butyl analogue **5** and the VDR-LBD, terminal of the butyl substituent favorably interacts with Leu309 (f).

modified analogue, these activities parallel the VDR affinity.[29] This suggests that suitable hydrophobic interaction in the LBP region surrounded by helicies 3, 6, 7, 11, and 12 is important to strengthen biological activities such as transactivation and cell differentiation. Close interactions with the ligand in this region of the LBP seem to reinforce the stability of the AF2 surface of the VDR where coactivators bind.

In summary, we have found that 22-ethyl analogue **4** has superagonistic activity similar to 22-methyl analogue **3**, while 22-butyl analogue **5** shows comparable activity to 1,25-$(OH)_2D_3$ (**1**). 22-Ethyl analogue **4** showed weak calcemic activity in vivo, suggesting that it might possess biological activity separate from calcemic activity. Studies of docking into the VDR and mutational analysis using these analogues demonstrated that 22-methyl and 22-ethyl substituents interact favorably with the LBP residues, and that the 22-butyl substituent is able to fit into an additional cavity of the ligand binding pocket observed in the crystal structure of the VDR-LBD/Gemini complex.[21,30] If **5** binds to the VDR by an induced fit mechanism, the surface structure of the VDR would be distinct from that bound with the hormone (**1**). Therefore, **5** might show cofactor recruitment differing from that of the hormone. If this is the case, then 22-butyl analogue **5** might function as a selective VDR modulator (SVDRM) like the well-known selective estrogen receptor modulator, SERM.

## Conclusions

We synthesized 22-ethyl analogue **4** and 22-butyl analogue **5** and evaluated their biological activities. Ethyl analogue **4** showed strong VDR binding, transactivation, and cell dif-

ferentiation potencies, but its calcemic activity in vivo was weaker than that of the natural hormone (**1**), while 22-butyl analogue **5** showed activities comparable to those of the hormone (**1**). The docking study of these analogues to the VDR-LBD and alanine scanning mutational analysis demonstrated that the 22-ethyl substituent enhances the favorable hydrophobic interactions with the residues lining the LBP of the VDR, and that 22-butyl analogue **5** binds to the VDR by an induced fit mechanism.

## Experimental Section

$^1$H NMR spectra were recorded in $CDCl_3$ at 400 MHz, and chemical shifts are reported as $\delta$ units relative to tetramethylsilane or 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 argon atmosphere.

**(23*Z*)-(20*S*,22*S*)-1α,3β-Bis(methoxycarbonyloxy)-22-ethyl-25-[(methoxymethyl)oxy]-24-trimethylsilyloxycholesta-5,7,23-triene (9)**. To a suspension of CuBr·$Me_2S$ (114 mg, 0.556 mmol) in THF (1 mL) was added ethyl lithium (1.21 M in THF, 0.92 mL) at −40 °C. The mixture was stirred for 0.5 h and then cooled to −78 °C. To this solution were added TMSCl (88.2 μL, 0.695 mmol), HMPA (121 μL, 0.695 mmol), and the enone **8** (40 mg, 0.0695 mmol) in THF (0.5 mL) in this order. The mixture was stirred at this temperature for 40 min and then quenched with water. The mixture was extracted with ethyl acetate. The extracts were washed with water, dried over $Na_2SO_4$, and evaporated. The residue was chromatographed on silica gel (7 g) with 5% ethyl acetate−benzene to give the desired product **9** (19.5 mg, 41%). $^1$H NMR $\delta$ 0.19 [9 H, s, OSi(C*H*$_3$)$_3$], 0.61 (3 H, s, H-18), 0.80 (3 H, d, $J$ = 8.0 Hz, H-21), 0.85 (3 H, m, $CH_2CH_3$) 1.01 (3 H, s, H-19), 1.35 and 1.36 (each 3 H, s, H-26 and H-27), 3.35 (3 H, s, $OCH_2OCH_3$), 3.77 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.61 (1 H, d, $J$ = 10.0 Hz, H-23), 4.65 (2 H, dd, $J$ = 10.7, 7.3 Hz, O*CH*$_2$O), 4.85 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.67 (each 1 H, m, H-6 and H-7); IR (neat) 2950, 2860, 1740, 1440, 1245, 1140, 1030 cm$^{-1}$; MS *m*/*z* 628 ($M^+ - CH_3OCH_2OH$, 1), 552 (3), 476 (2), 465 (1), 401 (6), 325 (52), 249 (34), 197 (62), 155 (23), 125 (56), 73 (100).

**(23*Z*)-(20*S*,22*S*)-1α,3β-Bis(methoxycarbonyloxy)-22-butyl-25-[(methoxymethyl)oxy]-24-trimethylsilyloxycholesta-5,7,23-triene (10)**. This compound was obtained from enone **8** by the same procedure using n-BuLi instead of EtLi as described for **9** (yield 73%). $^1$H NMR $\delta$ 0.19 [9 H, s, OSi(C*H*$_3$)$_3$], 0.60 (3 H, s, H-18), 0.80 (3 H, d, $J$ = 6.7 Hz, H-21), 0.89 (3 H, t, $J$ = 6.9 Hz, $CH_3$ of Bu), 1.01 (3 H, s, H-19), 1.35 and 1.36 (each 3 H, s, H-26 and H-27), 3.36 (3 H, s, $OCH_2OCH_3$), 3.78 and 3.81 (each 3 H, s, OCOC*H*$_3$), 4.62 (1 H, d, $J$ = 10.3 Hz, H-23), 4.63 and 4.67 (each 1 H, d, $J$ = 7.1 Hz, O*CH*$_2$O), 4.85 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and H-7); IR (neat) 2954, 2929, 2849, 1746, 1250 cm$^{-1}$; MS *m*/*z* 656 ($M^+ - CH_3OCH_2OH$, 1), 580 (3), 504 (6), 432 (20), 348 (13), 309 (23), 249 (22), 225 (100), 209 (12), 153 (7), 73 (22); HRMS calcd for $C_{36}H_{56}O_4Si$ ($M^+ - CH_3OCH_2OH - CH_3OCO_2H$) 580.3948, found 580.3954.

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-ethyl-25-[(methoxymethyl)oxy]-24-oxocholesta-5,7-diene (11).** To a solution of TMS ether **9** (175 mg, 0.253 mmol) in THF (2.5 mL) was added n-$Bu_4NF$ (1 M in THF, 304 μL, 0.304 mmol) at − 35 °C, and the mixture was stirred for 15 min at that temperature. The reaction was quenched with 10% $NH_4Cl$ and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and evaporated. The residue was chromatographed on silica gel (8 g) with 20% ethyl acetate−hexane to give the desired product **11** (150 mg, 96%). $^1$H NMR $\delta$ 0.70 (3 H, s, H-18), 0.73 (3 H, d, $J$ = 6.6 Hz, H-21), 0.87 (3 H, d, $J$ = 6.8 Hz, $CH_2CH_3$), 1.01 (3 H, s, H-19), 1.34 and 1.37 (each 3 H, s, H-26 and H-27), 3.40 (3 H, s, $OCH_2OCH_3$), 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.73 (2 H, s, O*CH*$_2$O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and H-7); IR (neat) 2950, 2870, 1740, 1710, 1440, 1380, 1340, 1260, 1145, 1030 cm$^{-1}$; MS *m*/*z* 586 ($M^+ - CH_3OH$, 0.7), 542 (2), 510 (3), 497 (2), 466 (6), 336 (11), 326 (9), 249 (18), 224 (11), 210 (22), 155 (27), 141 (17), 103 (100).

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-butyl-25-[(methoxymethyl)oxy]-24-oxocholesta-5,7-diene (12).** This compound was obtained from **10** by the same procedure as described for **11** (yield 69%). $^1$H NMR $\delta$ 0.69 (3 H, s, H-18), 0.73 (3 H, d, $J$ = 6.1 Hz, H-21), 0.90 (3 H, t, $J$ = 6.7 Hz, $CH_3$ of Bu), 1.01 (3 H, s, H-19), 1.34 and 1.37 (each 3 H, s, H-26 and H-27), 3.40 (3 H, s, $OCH_2OCH_3$), 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.72 (2 H, s, O*CH*$_2$O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and H-7); IR (neat) 2954, 2932, 2871, 1746, 1267, 1251 cm$^{-1}$; MS *m*/*z* 614 ($M^+ - CH_3OH$, 0.8), 538 (5), 494 (9), 462 (15), 432 (26), 348 (16), 249 (32), 209 (30), 183 (100), 153 (25), 69 (23); HRMS calcd for $C_{34}H_{50}O_5$ ($M^+ - CH_3OH - CH_3OCO_2H$) 538.3658, found 538.3658.

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-ethyl-24-hydroxy-25-[(methoxymethyl)oxy]cholesta-5,7-diene (13)**. $NaBH_4$ (23.2 mg, 0.614 mmol) was added to a solution of ketone **11** (146 mg, 0.236 mmol) in $CH_2Cl_2$ (1 mL) and THF (0.8 mL), and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with $CH_2Cl_2$, washed with water, dried over $Na_2SO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) with 30% ethyl acetate−hexane to afford the alcohol **13** as 1:1 diastereo-mixture at C(24) (133 mg, 91%). $^1$H NMR $\delta$ 0.67 (3 H, s, H-18), 0.69 and 0.78 [3 H (1:1), each d, $J$ = 6.6 Hz, H-21], 0.91 (3 H, m, $CH_2CH_3$), 1.00 and 1.01 [3 H (1:1), each s, H-19], 1.185, 1.193, 1.22 and 1.23 [6 H (1:1:1:1), each s, H-26 and H-27], 3.40 (3 H, s, $OCH_2OCH_3$), 3.49 and 3.52 [1 H (1:1), each m, H-24], 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.76 (2 H, m, O*CH*$_2$O), 4.84 (1 H, m, H-1), 4.93 (1 H, m, H-3), 5.38 and 5.67 (each 1 H, m, H-6 and H-7); IR (neat) 3550, 2950, 2860, 1750, 1440, 1380, 1340, 1260, 1140, 1035 cm$^{-1}$; MS *m*/*z* 544 ($M^+ - CH_3OCOOH$, 5), 512 (8), 468 (8), 436 (40), 407 (32), 365 (39), 278 (28), 249 (40), 224 (23), 209 (56), 155 (53), 141 (45), 103 (79), 72 (87), 55 (100).

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-butyl-24-hydroxy-25-[(methoxymethyl)oxy]cholesta-5,7-diene (14).** This compound was obtained from **12** by the same procedure as described for **13** (yield 87%). $^1$H NMR $\delta$ 0.66 (3 H, s, H-18), 0.69 and 0.78 [3 H (1:1), d, $J$ = 6.7 Hz, H-21], 0.91 (3 H, t, $J$ = 6.7 Hz, $CH_3$ of Bu), 1.00 and 1.01 [3 H (1:1), s, H-19], 1.18, 1.19, 1.21, 1.23[6 H (1:1:1:1), s, H-26 and -27], 3.389, 3.393 [3 H (1:1), s, $OCH_2OCH_3$], 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.706, 4.713 [1 H (1:1), d, $J$ = 7.3 Hz, O*CH*$_2$O], 4.77 (1 H, d, $J$ = 7.3 Hz, O*CH*$_2$O), 4.83 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and H-7); IR (neat) 3446, 2954, 1747, 1268, 1037 cm$^{-1}$; MS *m*/*z* 572 ($M^+ - CH_3OCOOH$, 2), 540 (7), 496 (5), 464 (100), 434 (23), 277 (17), 249 (35), 209 (42), 197 (29), 155 (27), 141 (17); HRMS calcd for $C_{34}H_{52}O_5$ ($M^+ - CH_3OH - CH_3OCO_2H$) 540.3815, found 540.3813.

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-ethyl-24-[(methylthio)thiocarbonyloxy]-25-[(methoxymethyl)oxy]cholesta-5,7-diene (15).** To a solution of **13** (9.7 mg, 0.0156 mmol) and imidazole (0.5 mg, 0.0078 mmol) in THF (0.8 mL) was added NaH (60 wt % in oil, 9.4 mg, 0.234 mmol) at ambient temperature, and the mixture was stirred for 1.5 h. Then carbon disulfide (18.8 μL, 0.312 mmol) was added to this solution, and the mixture was stirred for 16 h at ambient temperature. To this solution was added MeI (12.1 μL, 0.195 mmol), and the mixture was stirred for 2 h. The mixture was diluted with ethyl acetate, washed with water and brine, dried over $Na_2SO_4$, and evaporated. The residue was chromatographed on thin-layer silica gel plate (30% ethyl aceate−hexane) to afford **15** (7.0 mg, 63%). $^1$H NMR $\delta$ 0.56 and 0.60 [3 H (1:1), each s, H-18], 0.75 (3 H, d, $J$ = 6.4 Hz, H-21), 0.89 (3 H, m, $CH_2CH_3$), 1.00 and 1.01 [3 H (1:1), each s, H-19], 1.28 (6 H, s, H-26 and H-27), 2.54 and 2.55 [3 H (1:1), each s, SC*H*$_3$], 3.37 (3 H, s, $OCH_2OCH_3$), 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.68 and 4.70 (each 1 H, d, $J$ = 7.3 Hz, O*CH*$_2$O), 4.84 (1 H, m, H-1),

4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and H-7), 5.83 (0.5 H, dd, $J = 10.0$, 2.9 Hz, H-24), 5.95 (0.5 H, d, $J = 9.3$ Hz, H-24); IR (neat) 2950, 2875, 1740, 1440, 1380, 1340, 1260, 1140, 1050 $cm^{-1}$; MS *m*/*z* 678 ($M^+ - CH_3OH$, 1), 590 (3), 540 (2), 528 (3), 497 (3), 406 (27), 325 (57), 249 (40), 209 (47), 155 (54), 91 (56), 69 (100).

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-butyl-24-[(methylthio)thiocarbonyloxy]-25-[(methoxymethyl)oxy]cholesta-5,7-diene (16)**. This compound was obtained from **14** by the same procedure as described for **15** (yield 37%). $^1$H NMR $\delta$ 0.55 and 0.60 [3 H (2:1), s, H-18], 0.75 (3 H, d, $J = 6.8$ Hz, H-21), 0.92 (3 H, t, $J = 6.4$ Hz, *CH*$_3$ of Bu), 1.00 and 1.01 [3 H (2:1), s, H-19], 1.268, 1.273 (each 3 H, s, H-26 and H-27), 2.54 and 2.55 [3 H (2:1), s, SC*H*$_3$], 3.37 (3 H, s, $OCH_2OCH_3$), 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.70, 4.78 (each 1 H, d, $J = 7.4$ Hz, OC*H*$_2$O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.36 and 5.68 (each 1 H, m, H-6 and H-7), 5.84 (1/3 H, dd, $J = 8.5$, 3.3 Hz, H-24), 5.93 (2/3 H, d, $J = 10.0$ Hz, H-24); IR (neat) 2954, 2870, 1746, 1268, 1217, 1053 $cm^{-1}$; MS *m*/*z* 676 ($M^+ - CH_3OCH_2OH$, 0.5), 554 (3), 478 (80), 464 (100), 434 (35), 277 (40), 249 (72), 209 (82), 197 (58), 155 (47), 141 (42), 69 (29).

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-ethyl-25-[(methoxymethyl)oxy]cholesta-5,7-diene (17)**. A mixture of **15** (23.5 mg, 0.0330 mmol), $Bu_3SnH$ (53.0 μL, 0.198 mmol), and AIBN (2.7 mg, 0.0165 mmol) in toluene (1 mL) was refluxed for 0.5 h, cooled to room temperature and evaporated. The residue was chromatographed on silica gel (5 g) with 30% ethyl acetate−hexane to afford **17** (18.0 mg, 90%). $^1$H NMR $\delta$ 0.61 (3 H, s, H-18), 0.70 (3 H, d, $J = 6.4$ Hz, H-21), 0.91 (3 H, m, $CH_2CH_3$), 1.01 (3 H, s, H-19), 1.22 (6 H, s, H-26 and H-27), 3.37 (3 H, s, $OCH_2OCH_3$), 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.71 (2 H, s, OC*H*$_2$O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and H-7); IR (neat) 2950, 2860, 1745, 1440, 1380, 1340, 1270, 1140, 1040 $cm^{-1}$; MS *m*/*z* 542 ($M^+ - CH_3OCH_2OH$, 7), 466 (39), 390 (100), 277 (58), 249 (32), 209 (58), 197 (32), 155 (38), 141 (36), 103 (56), 69 (82), 55 (91).

**(20*S*,22*R*)-1α,3β-Bis(methoxycarbonyloxy)-22-butyl-25-[(methoxymethyl)oxy]cholesta-5,7-diene (18)**. This compound was obtained from **16** by the same procedure as described for **17** (yield 89%).$^1$H NMR $\delta$ 0.61 (3 H, s, H-18), 0.70 (3 H, d, $J = 6.7$ Hz, H-21), 0.91 (3 H, t, $J = 6.7$ Hz, *CH*$_3$ of Bu), 1.01 (3 H, s, H-19), 1.22 (6 H, s, H-26 and H-27), 3.37 (3 H, s, $OCH_2OCH_3$), 3.78 and 3.80 (each 3 H, s, OCOC*H*$_3$), 4.71 (2 H, s, OC*H*$_2$O), 4.83 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.68 (each 1 H, m, H-6 and H-7); IR (neat) 2950, 1750, 1654, 1253, 1044 $cm^{-1}$; MS *m*/*z* 632 ($M^+$, 0.3), 570 (3), 494 (21), 418 (100), 403 (19), 277 (33), 249 (30), 209 (34), 197 (22), 155 (19), 69 (15); HRMS calcd for $C_{35}H_{54}O_6$ ($M^+ - CH_3OCH_2OH$) 570.3921, found 570.3909.

**(20*S*,22*R*)-22-Ethyl-1α,3β,25-trihydroxycholesta-5,7-diene (19).** A solution of **17** (12.1 mg, 0.0200 mmol) and TsOH monohydrate (19.0 mg, 0.100 mmol) in 95% EtOH (2 mL) was refluxed for 0.5 h. Then, 5%-KOH/MeOH (2 mL) was added to this solution, and the mixture was refluxed for 1 h. The reaction mixture was poured into the saturated aqueous $NH_4Cl$, extracted with ethyl acetate, washed with water, dried over $Na_2SO_4$, and evaporated. The residue was chromatographed on thin-layer silica gel plate with 12% EtOH−$CH_2Cl_2$ to give **19** (8.19 mg, 92%). $^1$H NMR $\delta$ 0.63 (3 H, s, H-18), 0.71 (3 H, d, $J = 6.4$ Hz, H-21), 0.91 (3 H, m, $CH_2CH_3$), 0.94 (3 H, s, H-19), 1.23 (6 H, s, H-26 and H-27), 3.77 (1 H, m, H-1), 4.06 (1 H, m, H-3), 5.38 and 5.72 (each 1 H, m, H-6 and H-7); IR (neat) 3380, 2950, 2930, 2860, 1460, 1380, 1150, 1055 $cm^{-1}$; MS *m*/*z* 444 ($M^+$, 13), 426 (21), 408 (15), 393 (10), 295 (15), 251 (13), 227 (26), 171 (27), 157 (31), 69 (88), 59 (97), 55 (100); UV (EtOH) $\lambda_{max}$ 271, 282, 294 nm.

**(20*S*,22*R*)-22-Butyl-1α,3β,25-trihydroxycholesta-5,7-diene (20)**. This compound was obtained from **18** by the same procedure as described for **19** (yield 74%). $^1$H NMR $\delta$ 0.63 (3 H, s, H-18), 0.71 (3 H, d, $J = 6.1$ Hz, H-21), 0.90 (3 H, t, $J = 7.0$ Hz, *CH*$_3$ of Bu), 0.95 (3 H, s, H-19), 1.22 (6 H, s, H-26 and H-27), 3.78 (1 H, m, H-1), 4.07 (1 H, m, H-3), 5.39 and 5.75 (each 1 H, m, H-6 and H-7).

**(20*S*,22*R*)-22-Ethyl-1α,25-dihydroxyvitamin $D_3$ (4).** A solution of provitamin D **19** (28.7 mg, 0.0645 mmol) in EtOH (99.5%, 200 mL) was bubbled with argon at 0 °C for 10 min and then irradiated with 400 W high-pressure mercury lamp through a vycor filter for 3 min. Then the solution was refluxed for 1.5 h and evaporated. The residue was chromatographed on Sephadex LH20 (20 g) with $CHCl_3$/hexane/MeOH (70:30:0.7) to afford **4** (2.02 mg, 7%). The purity of **4** was proved to be about 98% by two HPLC systems: 1, YMC-Pack ODS-AM, 4.6 × 150 mm; $H_2O$/MeOH (20:80), 1.0 mL/min, retention time, 20.9 min 2, LiChrospher Si 60, 4.0 × 250 mm; hexane/$CHCl_3$/MeOH (100:25:8), 1.5 mL/min, retention time, 14.7 min $^1$H NMR $\delta$ 0.54 (3 H, s, H-18), 0.70 (3 H, d, $J = 6.3$ Hz, H-21), 0.90 (3 H, t, $J = 6.3$ Hz, $CH_2CH_3$), 1.22 (6 H, s, H-26 and H-27), 4.23 (1 H, m, H-3), 4.43 (1 H, m, H-1), 5.00 and 5.33 (each 1 H, m, H-19), 6.02 and 6.38 (each 1 H, d, $J = 11.2$ Hz, H-7, H-6, respectively); IR (neat) 3400, 2950, 2920, 2860, 1460, 1380, 1210, 1160, 1050 $cm^{-1}$; MS *m*/*z* 444 ($M^+$, 3), 426 (6), 408 (5), 393 (3), 313 (3), 302 (1), 152 (26), 134 (100), 105 (29); UV (EtOH) $\lambda_{max}$ 264 nm, $\lambda_{min}$ 227 nm.

**(20*S*,22*R*)-22-Butyl-1α,25-dihydroxyvitamin $D_3$ (5).** This compound was obtained from **20** by the same procedure as described for **4** (yield 10%). The purity of **5** was proved to be 98% by two HPLC systems: 1, YMC-Pack ODS-AM, 4.6 × 150 mm; $H_2O$/MeOH (20:80), 1.5 mL/min, retention time, 29.8 min 2, LiChrospher Si 60, 4.0 × 250 mm; hexane/$CHCl_3$/MeOH (100:25:8), 1.5 mL/min, retention time, 13.3 min $^1$H NMR ($CD_3OD$) $\delta$ 0.57 (3 H, s, H-18), 0.73 (3 H, d, $J = 6.9$ Hz, H-21), 0.92 (3 H, t, $J = 6.9$ Hz, *CH*$_3$ of Bu), 1.17 (6 H, s, H-26 and H-27), 4.12 (1 H, m, H-3), 4.35 (1 H, m, H-1), 4.90 and 5.29 (each 1 H, m, H-19), 6.08 and 6.32 (each 1 H, d, $J = 10.9$ Hz, H-7 and H-6, respectively); IR (neat) $cm^{-1}$; MS *m*/*z* 472 ($M^+$, 100), 454 (9), 436 (10); UV (EtOH) $\lambda_{max}$ 264 nm, $\lambda_{min}$ 227 nm.

**Competitive Binding Assay, Bovine Thymus VDR.** Binding to bovine thymus VDR was evaluated according to the procedure reported.[8] 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 0.072 nM [$^3$H]-1,25-$(OH)_2D_3$ together with graded amounts of each vitamin D analogue (0.001−20 nM) or vehicle for 19 h at 4 °C. The bound and free [$^3$H]-1,-25-$(OH)_2D_3$ 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. Nonspecific binding was subtracted. These experiments were done in duplicate.

**Transfection and Transactivation Assay.** COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS). Cells were seeded on 24-well plates at a density of $2 \times 10^4$ per well. After 24 h, the cells were transfected with a reporter plasmid containing three copies of the mouse osteopontin VDRE (5′-GGTTCAcgaGGTTCA, SPPx3-TK-Luc), a wild-type or mutant hVDR expression plasmid (pCMX-hVDR), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) by the lipofection method as described previously.[16,18,19] After 4 h-incubation, the medium was replaced with fresh DMEM containing 5% charcoal-treated FCS (HyClone, UT). The next day, the cells were treated with either the ligand or ethanol vehicle and cultured for 24 h. Cells in each well were harvested with a cell lysis buffer, and the luciferase activity was measured with a luciferase assay kit (Toyo Ink, Inc., Japan). Transactivation measured by the luciferase activity was normalized with the internal control. All experiments were done in triplicate.

**Differentiation of HL-60 Cells.** Human promyelocytic leukemia cells (HL-60) were plated at $2 \times 10^5$ cells/plate and were cultured in Eagle's modified medium as described previously.[26,31] The cells were incubated with each vitamin D compound ($10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$, $10^{-11}$, and $10^{-12}$ M) for 4 days. The differentiation activity was determined by an NBT reduction assay method as previously reported.[26,31] The experiments were done in duplicate.

***In Vivo* Intestinal Calcium Transport and Bone Calcium Mobilization.** Rats were maintained on a 0.47% Ca diet for 1 week and then switched to a low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, test compounds were given intraperitoneally at 130, 325, and 650 pmol/day for 7 days. All doses were administered in 0.1 mL of propylene glycol/ethanol (95:5). Controls received the vehicle. Determinations were made 24 h after the last dose as described previously.[26,31] There were five rats per group. Intestinal calcium transport is expressed as serosal:mucosal ratio of calcium in the sac to the calcium in the final incubation medium. Bone calcium mobilization represents the rise in serum calcium of the rats maintained on a very-low-calcium diet. In that measurement, the rise in serum calcium must arise from bone and hence is a determination of bone calcium mobilization.

**Graphical Manipulations and Ligand Docking.** Graphical manipulations were performed using SYBYL 7.1 (Tripos, St. Louis). The atomic coordinates of the crystal structure of hVDR-LBD (Δ165−215) complexed with 20-*epi*-1,25-$(OH)_2D_3$ (**2**) were retrieved from Protein Data Bank (PDB) (entry 1IE9).[20] 22-Alkyl vitamin D analogues (**3**−**5**) were docked into the ligand-binding pocket manually by superposition with the parent molecule (**2**). The ligand and the amino acid residues within 6.0 Å from the ligand were minimized on Tripos force field until energy gradient was lower than 0.05 kcal/(mol)(Å).

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