Conformationally Restricted Analogs of 1α,25-Dihydroxyvitamin D_3 and Its 20-Epimer: Compounds for Study of the Three-Dimensional Structure of Vitamin D Responsible for Binding to the Receptor

Keiko Yamamoto, Wei Yan Sun, Masateru Ohta,† Kazuhiro Hamada, Hector F. DeLuca,‡ and Sachiko Yamada*

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2-3-10 Surugadai Kanda, Chiyoda-ku, Tokyo 101, Japan, Fuji Gotemba Research Laboratories, Chugai Pharmaceutical Company, Ltd., 1-135 Komakado, Gotemba, Shizuoka 412, Japan, and Department of Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin 53706

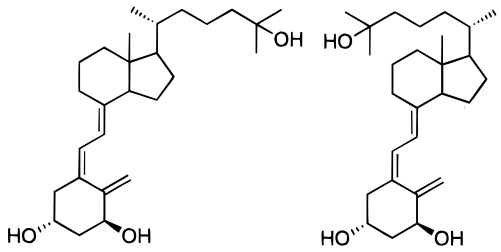
Received January 2, 1996[⊗]

Two proteins play important roles in the expression of vitamin D function: the specific nuclear receptor protein (vitamin D receptor, VDR) and the transport protein (vitamin D binding protein, DBP). This study was conducted to clarify the conformation of vitamin D responsible for binding to those proteins. For the purpose, the side chain mobility of 1,25$(OH)_2D_3$ (**1**) and its 20-epimer, 20-epi-1,25$(OH)_2D_3$ (**2**), was analyzed by a systematic conformational search. The results were depicted as a three-dimensional dot map, which indicates that the side chains of the two vitamins (**1** and **2**) occupy different spatial regions that are separated in two areas. We denoted these areas as A and G for **1** and EA and EG for **2**. Four analogs, the diastereomers at C(20) and C(22) (**3**−**6**) of 22-methylated 1,25$(OH)_2D_3$ whose side chains were confined to occupy G, A, EA, and EG, respectively, were designed. These analogs (**3**−**6**) were synthesized efficiently by a stereoselective conjugate addition of organocuprate to steroidal *E*- and *Z*-22-en-24-ones as the key step. In binding to the VDR the affinities of the analogs (**3**−**6**) relative to 1,25-$(OH)_2D_3$ (**1**) were 1/60, 1/3, 20, and 1/100, respectively. These results indicate that the A region is responsible for binding of **1** to VDR and the EA region for binding of **2**. Only isomer **4** showed significant affinity for DBP, indicating only the A region is responsible for binding to DBP. Thus, **5** showed clear separation of binding affinities for two proteins, VDR and DBP. Having the highest known VDR affinity, (22*R*)-22-methyl-20-epi-1,25$(OH)_2D_3$ (**5**) has potential both as a therapeutic agent and as a tool to study the molecular mechanism of vitamin D-mediated gene transcription.

1α,25-Dihydroxyvitamin D_3 [1,25$(OH)_2D_3$, **1**], the hormonally active form of vitamin D, exhibits control over the expression of various genes whose products are involved in calcium and phosphorus metabolism, cellular differentiation, and regulation of the immune system.[1] These gene expressions are mediated through the nuclear vitamin D receptor (VDR),[2] a member of a nuclear receptor superfamily which behaves as transcription factors that transduce extracellular hormonal signals to target genes that contain specific enhancer sequences referred to as hormone response elements.[3] Though the molecular mechanism of gene expression is not well understood, it is clear that the binding of 1,25$(OH)_2D_3$ (**1**) to the VDR initiates a sequence of events resulting in the activation of transcription. Thus, the VDR−1,25$(OH)_2D_3$ complex is most directly relevant to the ultimate biological event.

The differential activities of synthetic vitamin D analogs provide a useful method for studying how ligand modification alters the mode of the target gene activation. By extensive structure−function studies of analogs of 1,25$(OH)_2D_3$ (**1**), it is now clear that discrimination of the various actions of vitamin D is possible.[4] Many of the analogs that have differential activity are modified on the side chain. For example, 22-oxa-1α,-25-dihydroxyvitamin D_3 (OCT)[4a] and Δ^{22}-26,27-cyclo-1α,24(*S*)-dihydroxyvitamin D_3 (MC903)[4b] have selective activity in inducing cell differentiation. The 20-epi-analogs of 1,25$(OH)_2D_3$ (**1**), such as 20-epi-1α,25-dihydroxyvitamin D_3 [20-epi-1,25$(OH)_2D_3$, **2**] and 20-epi-22-oxa-1α,25-dihydroxy-24,26,27-trihomovitamin D_3 (KH1060), have a remarkably strong activity in inducing cell differentiation and regulating the immune response.[4c] The series of 20-epi-1,25$(OH)_2D_3$ analogs is especially interesting because their affinity to the VDR is a little stronger than that of 1,25$(OH)_2D_3$ (**1**) in spite of the unnatural stereochemistry at C(20).

Chart 1



1 **2**

It is known that steroid hormones and their antagonists, when bound to their receptors, induce distinct conformational changes of these receptors.[5] Therefore, 1,25$(OH)_2D_3$ (**1**) and those analogs with differential activities are expected to cause distinct conformational changes in the VDR. It was reported recently that the sensitivities of VDR to proteolytic digestion differ in the presence of 1,25$(OH)_2D_3$ (**1**) or 20-epi-1,25$(OH)_2D_3$ (**2**), an observation that indicates conformational differences between the two VDR−ligand complexes.[6] We have been continuing the ligand-based molecular studies on the interaction between vitamin D and the VDR.[7] Our

† Chugai Pharmaceutical Co., Ltd.
‡ University of Wisconsin—Madison.
⊗ Abstract published in *Advance ACS Abstracts,* June 1, 1996.

S0022-2623(96)00004-0 CCC: $12.00

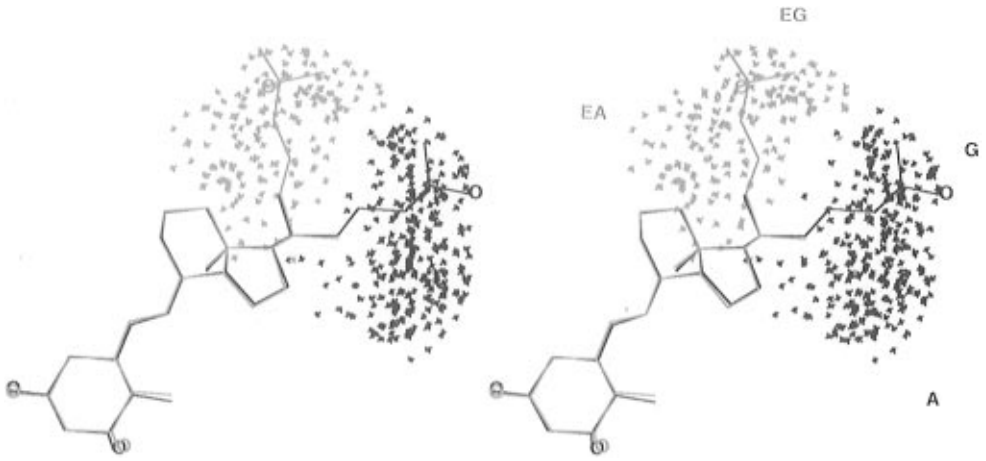


Figure 1. Stereoview of the mobile areas of the side chains of 1,25$(OH)_2D_3$ (**1**) and 20-epi-1,25$(OH)_2D_3$ (**2**): black, 1,25$(OH)_2D_3$ (**1**); light blue, 20-epi-1,25$(OH)_2D_3$ (**2**). The most stable conformations of the two vitamins **1** and **2**, which are represented as wire frames, are superimposed. Dots show the regions where the 25-oxygen of the side chains of these vitamin D compounds can reach. The two distinct spatial regions occupied by the 25-hydroxyl group of **1** are termed A and G, and those regions occupied by that of **2** are EA and EG.

special attention is focused on the conformation of vitamin D itself when it binds to the VDR. Because vitamin D has a great deal of flexibility in the side chain, the seco-B-ring, and the A-ring, a conformation−function study should be an important part of any structure−function of vitamin D.

We report here a conformational analysis of the side chains of 1,25$(OH)_2D_3$ (**1**) and 20-epi-1,25$(OH)_2D_3$ (**2**), visualization of the side chain mobility by computer-generated three-dimensional dot map, synthesis of the four conformation-restricted analogs, and their biological activities. These studies not only suggest the conformations of 1,25$(OH)_2D_3$ (**1**) and 20-epi-1,25-$(OH)_2D_3$ (**2**) that bind to the VDR but also led us to identify an analog that has the strongest VDR binding affinity yet reported.

Chemistry

Conformational Analysis and Design of Conformationally Restricted Analogs. The side chain mobility of 1,25$(OH)_2D_3$ (**1**) and 20-epi-1,25$(OH)_2D_3$ (**2**) was analyzed by molecular mechanics (see details in the Experimental Section). Initial structures of **1** and **2** were constructed by modifying the X-ray crystal structure of 25-hydroxyvitamin D_3[8] (Insight II molecular modeling system)[9] and optimizing the generated structure with the conjugate gradient method using consistent valence force field (CVFF) (Discover).[9] The most stable conformers of **1** and **2** were searched using ROTOR function of Discover. Conformational analysis was performed by SYBYL systematic search,[10] and prior to the analysis CVFF-optimized structures were minimized again with the Tripos force field in the SYBYL program.[10] Each of the 17,20-, 20,22-, 22,23-, 23,24-, and 24,25-bonds in the structures of **1** and **2** was rotated 360° with 30° intervals to yield a total of $(360/30)^5 =$ 248 832 side chain conformers for each vitamin. From these, unfeasible conformers with intramolecular van der Waals repulsion were excluded by applying a van der Waals bump coefficient of 0.95. The most stable conformers and all feasible side chain conformers are shown in stereoview in Figure 1. In the figure the most stable conformers of **1** and **2** are shown as wire figures with their skeletal part (A-ring to D-ring) superimposed, and the feasible conformations of their side chains are shown with dots at the position of their 25-oxygen. The dot map indicates that both 1,25$(OH)_2D_3$ (**1**; black) and 20-epi-1,25$(OH)_2D_3$ (**2**; light blue) have two major densely populated regions for their side chains, which are denoted as A and G for **1** and EA and EG for **2**. The terms A and G refer to anti and gauche, and E refers to epi. We used these terms since the A and G are the regions that the side chain of 1,25$(OH)_2D_3$ (**1**) occupies when its dihedral angles at C(17,20,22,23) are anti and gauche(+), respectively, while EA and EG are those regions for 20-epi-1,25$(OH)_2D_3$ (**2**) when the dihedral angles are anti and gauche(−), respectively.

The mobility of the side chain was expected to be restricted by introducing a substituent at the 22-position. This position being adjacent to the C(20) chiral center next to the steroid skeleton is the key position in determining the side chain conformation. Thus, we designed two pairs of 22-methylated epimers as conformationally restricted analogs for 1,25$(OH)_2D_3$ (**1**) and 20-epi-1,25$(OH)_2D_3$ (**2**): (22*R*)- and (22*S*)-22-methyl-1,-25$(OH)_2D_3$ (**3** and **4**) for **1** and (22*R*)- and (22*S*)-22-methyl-20-epi-1,25$(OH)_2D_3$ (**5** and **6**) for **2**. Conformational analyses of these analogs were performed as described above, and the results are shown in Figure 2. It is clear from Figure 2, top, middle, that the pairs of epimers (**3**/**4** and **5**/**6**) share the regions occupied by 1,-25$(OH)_2D_3$ (**1**) and its 20-epimer (**2**), respectively. Figure 2, bottom, shows that each of the four diastereomers clearly occupies only one of the four spatial regions, G, A, EA, and EG, defined above.

Synthesis of (22*R*)- and (22*S*)-22-Methyl-20-epi-1α,25-dihydroxyvitamin D_3 (5 and 6). 22-Methyl-20-epi-analogs **5** and **6** were synthesized following essentially the method reported for the two 22-methyl-1,25$(OH)_2D_3$ isomers (**3** and **4**)[7a] (Scheme 1). The syntheses started with 1α-hydroxylated C(22) aldehyde **7**.[7a] The aldehyde **7** was isomerized at C(20) by enolization with NaH in aprotic solvents (THF−HMPA) followed by protonation to give a 7:3 mixture of the epimers **9** and **7**, which were separated by column chromatography. The preferential formation of the 20*R*-aldehyde **9** can be explained as follows. According to molecular mechanics calculations,[11] enol **8** adopts three stable conformations (Figure 3). In the conformers A and C, protonation at C(20) occurs preferentially from

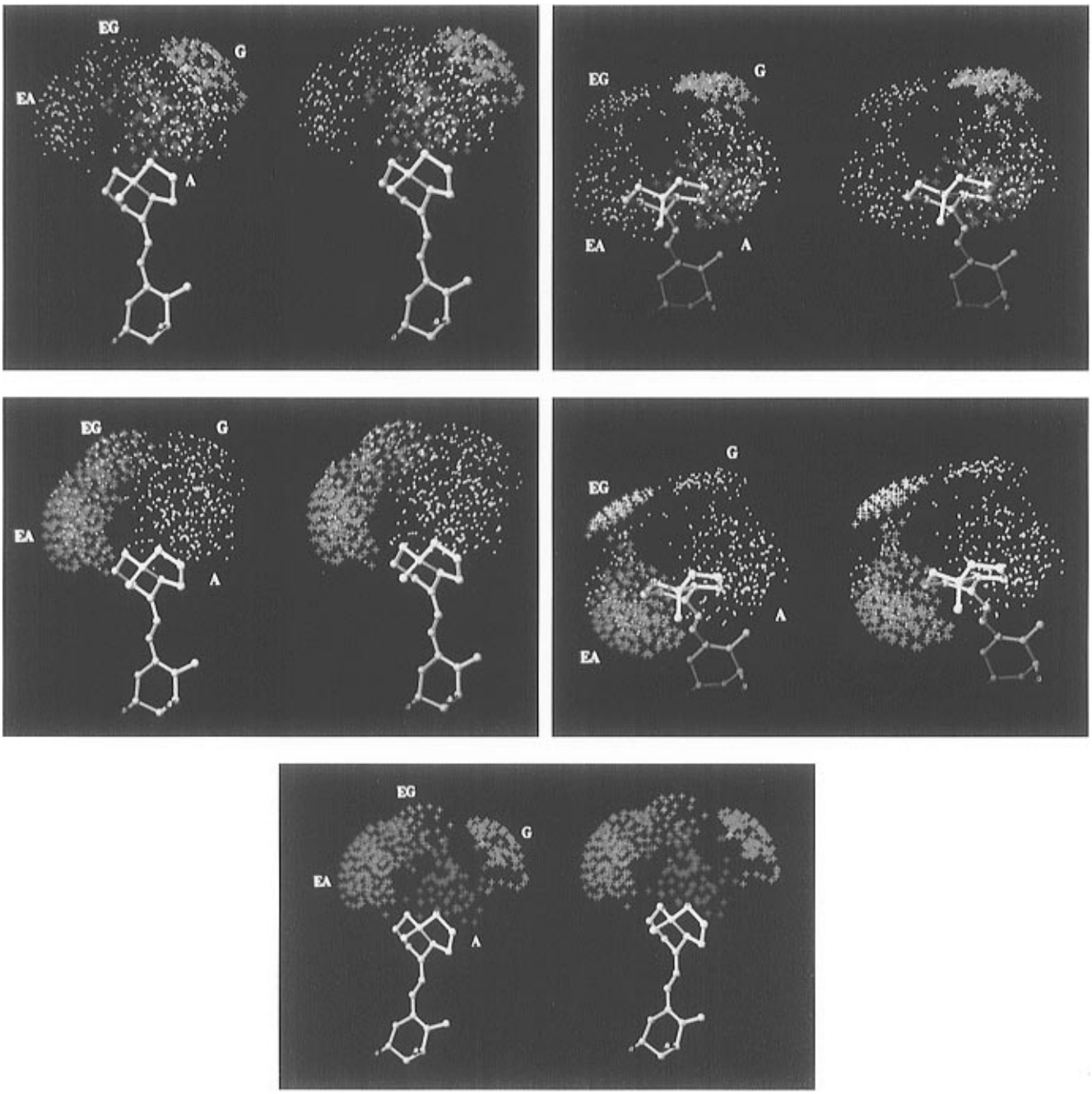
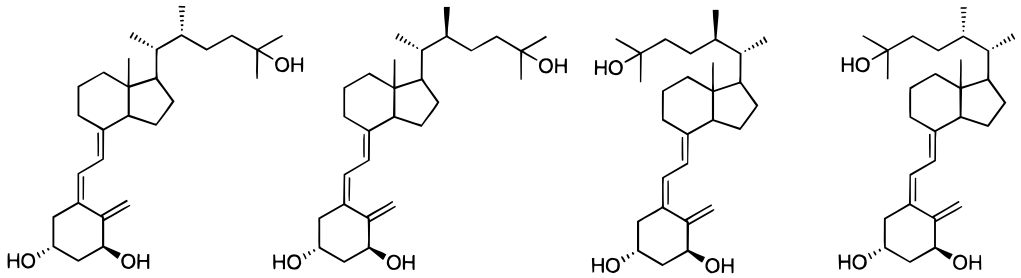


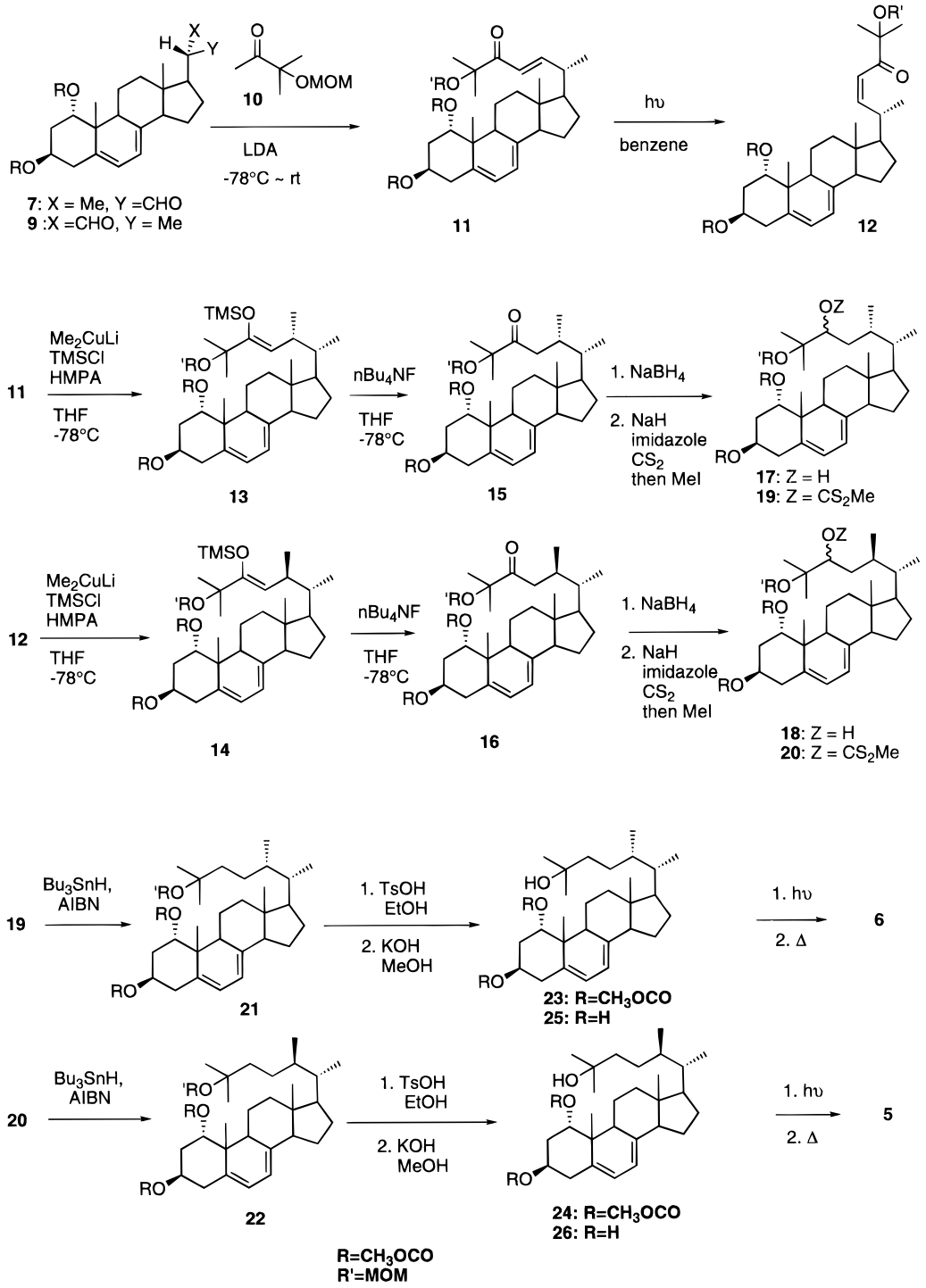
Figure 2. Stereoviews of the side chain mobile areas of 1,25$(OH)_2D_3$ (**1**), 20-epi-1,25$(OH)_2D_3$ (**2**), and their conformationally restricted analogs. (Top) 1,25$(OH)_2D_3$ (**1**), 20-epi-1,25$(OH)_2D_3$ (**2**), and (22*R*)- and (22*S*)-22-methyl-1,25$(OH)_2D_3$ (**3** and **4**); left, side view; right, top view. (Middle) 1,25$(OH)_2D_3$ (**1**), 20-epi-1,25$(OH)_2D_3$ (**2**), and (22*R*)- and (22*S*)-22-methyl-20-epi-1,25$(OH)_2D_3$ (**5** and **6**); left, side view; right, top view. (Bottom) Four diastereomeric 22-methylated analogs **3**−**6**; yellow dots, 1,25$(OH)_2D_3$ (**1**); light-blue dots, 20-epi-1,25$(OH)_2D_3$ (**2**); green crosses, (22*R*)-22-methyl-1,25$(OH)_2D_3$ (**3**); red crosses, (22*S*)-22-methyl-1,25$(OH)_2D_3$ (**4**); blue crosses, (22*R*)-22-methyl-20-epi-1,25$(OH)_2D_3$ (**5**); pink crosses, (22*S*)-22-methyl-20-epi-1,25$(OH)_2D_3$ (**6**). Details of the drawing refer to Figure 1.

Chart 2



the *si* face, which is the sterically less hindered side, whereas in the conformer B it occurs preferentially from the *re* face, which is the sterically less hindered side. The Boltzmann distribution of the three stable conform-

Scheme 1



ers of **8** is consistent with the selectivity of this epimerization reaction.

Aldol condensation of the aldehyde **9** with 3-hydroxy-3-methylbutanone MOM ether (**10**) followed by spontaneous dehydration afforded the *E*-enone **11**. The *Z*-enone **12** was obtained by photochemical isomerization of the *E*-enone **11**, which gave a mixture of *E*- and *Z*-enones **11** and **12** (2:1), from which **12** was isolated readily by column chromatography. Conjugate addition of Me_2CuLi to *E*-enone **11** under thermodynamic conditions (Me_2CuLi, THF, 0 °C) gave 22*S*-ketone **15** in high yield (91%) and high stereoselectivity (97%). Under kinetic conditions (Me_2CuLi, TMSCl, HMPA), the addition proceeded with the same face selectivity (97%) in high yield (90%) at −78°C to give 22*R*-enolsilyl ether **13**. The addition of Me_2CuLi to *Z*-enone **12** was sluggish, and the selectivity was lower. Under the thermodynamic conditions, the reaction gave 22*S*-ketone **15** in low yield (35%) with 86% selectivity. Under kinetic conditions, the face selectivity was reversed and the desired 22*S*-enolsilyl ether **14** was obtained in 41% yield with 75% stereoselectivity.

Since the determination of the configuration at C(22) is of critical importance in the present study, we attempted X-ray analysis using more than 10 derivatives of **13** and **14**. However these attempts were unsuccessful because surprisingly all of the derivatives, which gave single crystals, were shown to be present as a tetramer in a unit cell. Therefore it was nearly impossible to solve their crystal structures.[12] ^1H NMR analysis gave no useful information, since the protons at C(22) of both **13** and **14** overlapped with other allylic

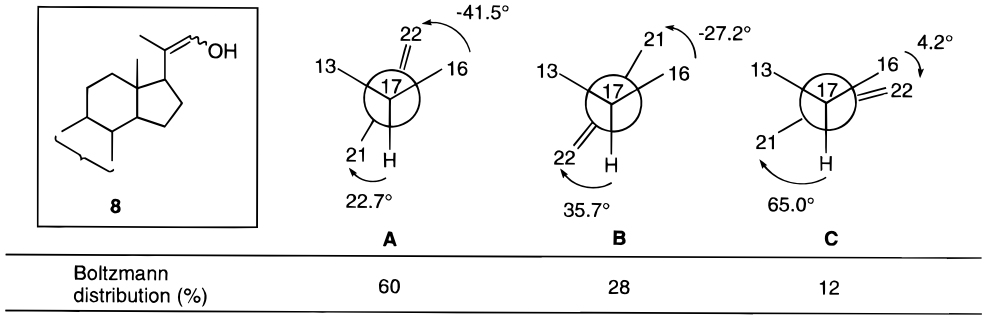


Figure 3. Three stable conformers of enol **8**.

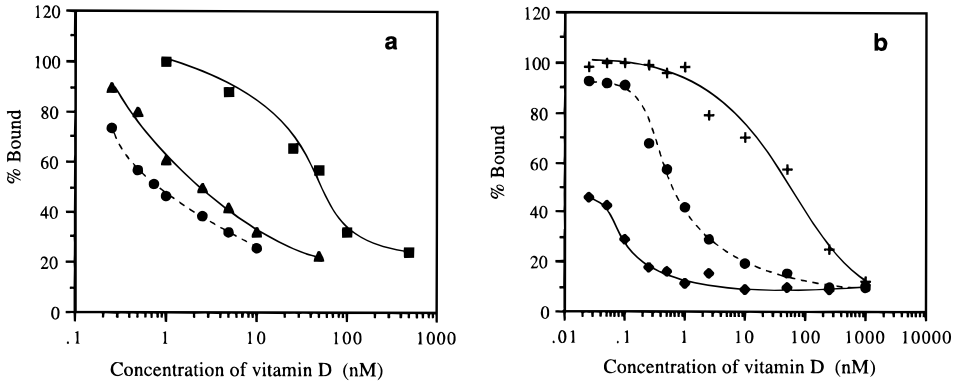


Figure 4. Binding affinity of 22-methyl analogs for 1,25(OH)$_2$D$_3$ receptors (VDR) in porcine intestine: (a) ●, 1,25(OH)$_2$D$_3$ (**1**); ■, (22*R*)-22-methyl-1,25(OH)$_2$D$_3$ (**3**); ▲, (22*S*)-22-methyl-1,25(OH)$_2$D$_3$ (**4**); (b) ●, 1,25(OH)$_2$D$_3$ (**1**); ◆, (22*R*)-22-methyl-20-epi-1,25-(OH)$_2$D$_3$ (**5**); +, (22*S*)-22-methyl-20-epi-1,25(OH)$_2$D$_3$ (**6**).

Table 1. Binding Affinity of 22-Methyl Analogs **3**−**6** for VDR and DBP[a]

	VDR[b]		
	porcine intestine	bovine thymus	DBP
1	1	1	1
3	1/60	1/50	1/220
4	1/3	1/3	2/3
5	20[c]	11[c]	<1/400
6	1/100[c]	1/250[c]	<1/400

[a] The affinities are presented as relative values, the reference value of 1,25(OH)$_2$D$_3$ (**1**) being defined as 1. The relative activity was calculated at 50% displacement except for the VDR affinity of 20-epi-vitamins **5** and **6**. [b] The affinities of **2** relative to **1** reported are 1.2 for chick intestinal VDR[4c] and 5 for bovine thymus VDR.[17] [c] 60% displacement values were used because **5** bound to VDR so strongly that the values at 50% displacement were inaccurate.

protons and their coupling constants could not be exactly determined. The configuration at C(22) was determined on the basis of the mechanism of Me$_2$CuLi addition in comparison with the same reaction with 20*R*-22-en-24-ones.[7a,13,14]

The silyl ethers **13** and **14** were desilylated, and the ketone functions of the 22-methyl-24-ones **15** and **16** were reduced to methylene via the alcohols **17** and **18** as reported. The provitamins **25** and **26** were converted to the target vitamins **6** and **5** by photolysis followed by thermal isomerization.

Biological Testing

The VDR binding affinity of the four diastereomeric 22-methylated analogs (**3**−**6**) in comparison with 1,25-(OH)$_2$D$_3$ (**1**) was examined by a competitive binding assay using the porcine intestinal and the bovine thymus VDRs.[15,16] The results are shown in Figure 4 and summarized in Table 1. The pairs of methylated analogs (**3**/**4** and **5**/**6**) of 1,25(OH)$_2$D$_3$ (**1**) and 20-epi-1,-25(OH)$_2$D$_3$ (**2**), respectively, showed distinct affinities. In the porcine VDR assay, (22*S*)-Me-1,25(OH)$_2$D$_3$ (**4**) was $^1/_3$ as potent as **1**, whereas the 22*R*-isomer **3** was 60 times less potent than **1**. A much more striking difference was found between the pair of 20-epi-analogs **5** and **6**. In the porcine VDR assay, the 22*R*-epimer **5** had 20 times higher affinity than **1**, whereas the 22*S*-isomer **6** was 100 times less potent than **1**. The results with thymus VDR were similar. Thus, the affinity ratio for the pair of conformationally restricted analogs (**3** and **4**) of **1** was about 20, whereas that for the analogs (**5** and **6**) of **2** was about 2000. These results indicate that only the epimers (**4** and **5**) whose side chains occupy the A and EA regions, respectively, have significant affinity for the VDR.

Binding affinity for vitamin D binding protein (DBP) was tested in a competitive binding assay using the vitamin D-deficient rat serum DBP.[16] The affinities of **3** and **4** relative to **1** were 1/220 and 2/3, respectively. Again, epimer **4** mimics the affinity of 1,25(OH)$_2$D$_3$ (**1**), indicating the A region to be important for DBP binding too. In contrast to their affinity profile for VDR, both 22-methylated 20-epi-analogs **5** and **6** showed minimal binding affinity to DBP (<1/400). Thus, both EA and EG regions appear not to be responsible for binding to DBP. This result is consistent with the low affinity of 20-epi-1,25(OH)$_2$D$_3$ (**2**) for DBP.[17]

Discussion

The active form of vitamin D$_3$, 1,25(OH)$_2$D$_3$ (**1**), is the most flexible and longest steroid hormone of all, the distance between the terminal 25-hydroxyl proton and 3β-hydroxyl proton being 17−18 Å. This long hydrophobic molecule is terminated by one hydrophilic hydroxyl group at the end of the chain and by two hydroxyl groups at the end ring. These hydroxyl groups appear to play an important role in anchoring the vitamin to

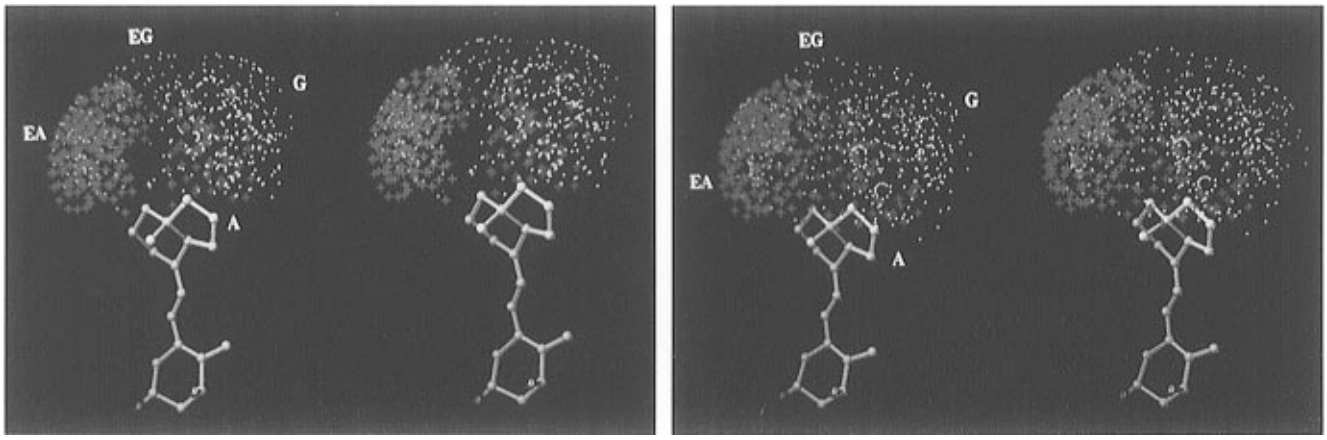


Figure 5. Stereoviews of the hydrogen acceptor model (left) and hydrogen donor model (right) of the two active conformationally restricted analogs **4** and **5** together with their parent vitamins. The dots in the hydrogen acceptor model indicate the position of the 25-oxygen, and those in the hydrogen donor model show the position of hydrogen of the 25-hydroxyl group: yellow dots, $1,25(OH)_2D_3$ (**1**); light-blue dots, 20-epi-$1,25(OH)_2D_3$ (**2**); red crosses, (22*S*)-22-methyl-$1,25(OH)_2D_3$ (**4**); blue crosses, (22*R*)-22-methyl-20-epi-$1,25(OH)_2D_3$ (**5**).

the binding site of the receptor protein. The 1α- and 25-hydroxyl groups are believed to be most important since the removal of these hydroxyl groups reduces the VDR affinity to 1/700 and 1/600, respectively, in comparison with the reduction of the affinity to 1/17 by 3β-hydroxyl group removal. In this paper, we focused on the stereochemical structures of the side chains of $1,25(OH)_2D_3$ (**1**) and 20-epi-$1,25(OH)_2D_3$ (**2**), which are responsible for the binding to VDR as well as to DBP. We showed by molecular mechanics analysis that each 25-hydroxyl group of these vitamins occupies two major spatial regions: A and G for **1** and EA and EG for **2**, as shown in the dot map (Figure 1). The dot map not only indicates the spatial regions which the 25-hydroxyl group of each vitamin can occupy but also provides an image of the VDR cavity in which the side chain resides.[18] We synthesized four diastereomers at C(20) and C(22), 22-methylated $1,25(OH)_2D_3$ analogs **3**−**6**, whose side chains are restricted to only one of the four regions (see Figure 2). Introduction of a methyl group at C(22) causes severe restriction of the side chain mobility because it confines rotation around the 20,22-bond. However, it is expected that the methyl group causes only a minor effect in the shape and hydrophobicity of the parent vitamin D molecule.

The biological activities of analogs **3**−**6** were most intriguing. The difference in the VDR affinities for each pair of analogs was clear-cut. Only one of the 22-epimeric pairs showed strong VDR affinity (Table 1), namely, only the epimers (**4** and **5**) that occupy either the A or EA region bind to VDR with high affinity. It is especially noteworthy that (22*R*)-22-Me-20-epi-$1,25(OH)_2D_3$ (**5**) had about 2000 times higher affinity than its 22*S*-epimer **6** and 20 times higher affinity than $1,25(OH)_2D_3$ (**1**). The results indicate that $1,25(OH)_2D_3$ (**1**) binds to VDR when its side chain is spatially directed to the A region whereas 20-epi-$1,25(OH)_2D_3$ (**2**) binds to VDR when that is spatially directed to the EA region. Interestingly the restriction of the side chain conformation slightly reduced the affinity in the 20-normal series (**4** versus **1**), while it strongly enhanced the affinity in the 20-epi series (**5** versus **2**). It is known that compounds with rigid conformations bind to proteins more effectively than flexible compounds.[19] This can be ascribed to the differences in entropy loss between the flexible and nonflexible compounds when they bind to protein. If a ligand is fixed in a conformation that is preferred by the receptor, the entropy loss of the ligand upon complex formation is less than that of the corresponding conformationally flexible molecule. The strong binding affinity of the conformationally restricted 20-epi-analog **5** in comparison with the parent compound **2** can be explained by this entropy effect, unless the 22-methyl group itself causes a specific favorable effect in binding to VDR. In the 20*R*-series, the conformationally restricted analog **4** showed diminished activity, probably because the 22-methyl group suffers steric repulsion when it binds to VDR.

It is assumed that the vitamin D side chain fits into a characteristic site on a hydrophobic cavity of the VDR and the 25-hydroxyl group forms a hydrogen bond with a polar amino acid at that site. The following three binding models of vitamin D in the VDR are postulated. (1) VDR changes its conformation to adopt both ligands (**4** and **5**). It appears that the role of the ligand in the VDR ligand-binding domain (LBD) is to induce a shift from the initially transcriptionally inactive conformation without a ligand to a more organized and active conformation with a ligand. Investigations on the binding of agonists and antagonists to steroid hormone receptors suggest that the initially proposed hard shell model for the hormone-binding domain should be replaced by the more versatile, but more elusive, induced fit model.[5] Protease clipping experiments of VDR complexes with $1,25(OH)_2D_3$ (**1**) and its 20-epimer **2** showed a possibility of conformational differences between the two complexes.[6] (2) The protein has a single binding site that forms a hydrogen bond with the 25-hydroxyl group. The 25-hydroxyl groups of **4** and **5** bind to this site when they are directed to overlapping areas of the A and EA regions (Figure 5). In Figure 5 the regions occupied by the 25-oxygen and 25-hydroxy hydrogen are compared among $1,25(OH)_2D_3$ (**1**), its 20-epimer **2**, and their active analogs **4** and **5**. In the latter hydrogen donor model, there are some overlapping areas. Therefore, the VDR binding site that forms hydrogen bonding with the 25-hydroxyl group might face these overlapping areas. (3) The 25-hydroxyl groups of the two active compounds (**4** and **5**) bind to different amino acids on the LBD of VDR. In this case there may be another special amino acid that is preferred by the 25-hydroxyl group of the 20-epi-analogs.

It is evident that the DBP binding pocket is able to distinguish the structural difference between 1,25-$(OH)_2D_3$ (**1**) and its 20-epimer **2** because only isomer **4** showed significant affinity for DBP. In the 20-normal series the DBP affinity and VDR affinity are correlated. (22*S*)-22-Me-1,25$(OH)_2D_3$ (**4**) binds strongly to DBP and VDR, whereas the 22*R*-isomer **3** binds weakly to both DBP and VDR. In the 20-epi-series (22*R*)-22-Me-20-epi-1,25$(OH)_2D_3$ (**5**) cannot bind to DBP but binds strongly to VDR, and its 22*S*-isomer **6** cannot bind to DBP and binds weakly to VDR.

In conclusion, we successfully synthesized four conformationally restricted diastereomeric analogs (**3**−**6**) of 1,25$(OH)_2D_3$ (**1**). From biological studies of these analogs, we suggest the side chain conformations of 1,-25$(OH)_2D_3$ (**1**) and its 20-epimer **2** that are responsible for binding to VDR. One of the conformationally restricted analogs, **5**, was found to have the highest VDR binding activity so far known. We expect analog **5** to have potential as a useful therapeutic candidate and be an excellent tool to study the molecular mechanism of vitamin D-mediated gene expression.

Experimental Section

Molecular Modeling and Conformational Analysis. Starting structures of compounds **1**−**6** were constructed by modifying the X-ray structure of 25-hydroxyvitamin D_3 with the Insight II molecular modeling system. These structures were minimized with CVFF using Discover. To find the most stable conformation, conformational analyses were done using the ROTOR function of Discover. In the ROTOR routine, every possible rotatable bond in the side chain except the terminal methyl was rotated with 60° dihedral increment, and each conformation generated was minimized by the conjugate gradient method of Discover. Steric energies of the most stable conformers were 5.55, 5.07, 8.89, 8.79, 8.74, and 7.81 kcal/mol for **1**−**6**, respectively.

Spatial regions occupied by the side chain were searched by the search routine of SYBYL. The CVFF-optimized structures were first minimized with the Tripos force field using MAXMIN2 in SYBYL, and then conformational searches of compounds **1**−**6** were performed. In the systematic search, the five side chain bonds described in the text were rotated with dihedral increments of 30°. Dot maps were created by SYBYL sweep graph routine.

Chemical Synthesis. Melting points were determined by a micromelting point apparatus and are uncorrected. NMR spectra were obtained in $CDCl_3$ on a Bruker ARX 400S or a JEOL JNM-A500 FT NMR spectrometer and are reported relative to $CDCl_3$ (δ 7.26) as an internal standard. IR spectra were obtained on a Jasco Janssen Microsampling FTIR spectrometer. Low- and high-resolution mass spectra were measured on a JEOL JMS-AX505HA spectrometer at 70 eV. UV spectra were recorded on a Hitachi U-3200 spectrophotometer. Tetrahydrofuran (THF) was distilled from benzophenone ketyl prior to use. All air-sensitive reactions were run under argon atmosphere, and reagents were added through septa using oven-dried syringes. The phrase "dried and evaporated" indicates drying over Na_2SO_4 followed by evaporation of the solvents under house vacuum.

(20*R*)-1α,3β-Bis[(methoxycarbonyl)oxy]-23,24-dinor-5,7-choladien-22-al (9). Sodium hydride (60% oil suspension, 520 mg, 13.6 mmol) was added to a solution of 22-aldehyde **7** (5 g, 10.8 mmol) in HMPA−THF (20 mL−50 mL) at −25 °C. The mixture was vigorously stirred at that temperature for 1.5 h and then at 0 °C for 2.5 h. The suspension turned to pink at 0 °C. The mixture was added to ice−water and extracted with AcOEt. The extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel (150 g) with 1% AcOEt−benzene to give 20*R*-aldehyde **9** (3.15g, 63%): mp 186−188 °C; ^{1}H NMR δ 0.62 (3H, s, H-18), 1.00 (3 H, s, H-19), 1.06 (3 H, d, J = 6.8 Hz, H-21), 3.78 and 3.79 (each 3 H, s, OCH_3), 4.82 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.40 and 5.68 (each 1 H, m, H-6 and -7), 9.55 (1 H, d, J = 5.0 Hz, H-22); IR (neat) 2959, 2876, 1749, 1444, 1359, 1280 cm^{-1}; MS m/z 460 (M$^+$, 2), 384 (8), 308 (100), 294 (20), 246 (30), 235 (31), 207 (21), 195 (18), 179 (16), 155 (27), 141 (39). Anal. ($C_{26}H_{36}O_7$) C, H.

(22*E*)-(20*S*)-1α,3β-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-5,7,22-cholestatrien-24-one (11). To a solution of diisopropylamine (168 μL, 1.2 mmol) in THF (2 mL) was added dropwise *n*-BuLi (1.6 M in hexane, 0.75 mL, 1.2 mmol) at −78 °C, and the solution was stirred at that temperature for 20 min. To this LDA solution was added slowly a solution of 3-hydroxy-3-methyl-2-butanone methoxymethyl ether (**10**) (175 mg, 1.2 mmol) in THF (1.5 mL) at −78 °C, and the mixture was stirred for 15 min. A solution of 20*R*-aldehyde **9** (460 mg, 1 mmol) in THF (2 mL) was added slowly to the resulting enolate solution, and the mixture was stirred for 1.5 h at −78 °C. Then the mixture was stirred at −40 to −30 °C for 1 h and at 0 °C for 1 h. The reaction mixture was added to aqueous NH_4Cl, extracted with AcOEt, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (20 g) with 5% AcOEt−benzene to yield 22*E*-en-24-one **11** (480 mg, 82%): ^{1}H NMR δ 0.58 (3 H, s, H-18), 0.97 (3 H, s, H-19), 1.01 (3 H, d, J = 6.6 Hz, H-21), 1.37 and 1.38 (each 3 H, s, H-26 and -27), 3.38 (3 H, s, CH_3-OCH_2), 3.77 and 3.79 (each 3 H, s, OCOCH_3), 4.65 and 4.67 (each 1 H, d, J = 6.9 Hz, OCH_2O), 4.80 (1 H, m, H-1), 4.89 (1 H, m, H-3), 5.38 and 5.67 (each 1 H, m, H-6 and -7), 6.65 (1 H, d, J = 15.6 Hz, H-23), 6.91 (1 H, dd, J = 15.6, 9.9 Hz, H-22); IR (neat) 2959, 2874, 1752, 1695, 1623, 1443, 1289, 1146, 1035 cm^{-1}; MS m/z 588 (M$^+$, 7), 556 (23), 512 (30), 480 (30), 436 (38), 392 (61), 378 (96), 103 (100).

(22*Z*)-(20*S*)-1α,3β-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-5,7,22-cholestatrien-24-one (12). A Pyrex vessel containing a solution of the 22*E*-enone **11** (558 mg, 0.95 mmol) in benzene (40 mL) was charged with high-quality Ar (99.999%). The solution was irradiated externally with a 100-W high-pressure mercury lamp (Shigemi Standard, Tokyo) at room temperature until the ratio of *E*- and *Z*-enones became approximately 2:1 (analyzed by HPLC). The solvent was evaporated, and the residue was chromatographed on silica gel (20 g, 2% AcOEt−benzene) to give 22*Z*-enone **12** (178 mg, 32%), starting 22*E*-enone **11** (335 mg, 60%), and 20-alcohol[20] (28 mg, 5%). **12**: ^{1}H NMR δ 0.51 (3 H, s, H-18), 0.95 (3 H, d, J = 6.6 Hz, H-21), 0.97 (3 H, s, H-19), 1.37 (6 H, s, H-26 and -27), 3.38 (3 H, s, CH_3OCH_2), 3.77 and 3.79 (each 3 H, s, CH_3-OCO), 4.67 and 4.68 (each 1 H, d, J = 7.1 Hz, OCH_2O), 4.82 (1 H, m, H-1), 4.89 (1 H, m, H-3), 5.37 and 5.67 (each 1 H, m, H-6 and -7), 6.12 (1 H, dd, J = 11.6, 10.4 Hz, H-22), 6.50 (1 H, d, J = 11.6 Hz, H-23); IR (neat) 2957, 2874, 1747, 1691, 1612, 1442, 1363, 1271, 1145 cm^{-1}; MS m/z 588 (M$^+$, 12), 556 (25), 512 (57), 480 (37), 436 (51), 392 (49), 378 (40), 249 (100), 180 (85), 103 (85); HRMS calcd for $C_{33}H_{48}O_9$ 588.3299, found 588.3339.

(23*Z*)-(20*S*,22*R*)-22-Methyl-5,7,23-cholestatriene-1α,3β,24,25-tetrol 1,3-Bis(methoxycarbonyl) 25-(Methoxymethyl) 24-(Trimethylsilyl) Tetraether (13). To a suspension of CuI (388 mg, 2.04 mmol) in THF (0.8 mL) was added MeLi (1.4 M in ether, 2.9 mL, 4.06 mmol) at 0 °C. The mixture was stirred for 20 min and then cooled to −78 °C. To this solution were added TMSCl (216 μL, 1.70 mmol), HMPA (296 μL, 1.70 mmol), and 22*E*-en-24-one **11** (200 mg, 0.34 mmol) in THF (0.8 mL) in this order. After stirring for 1 h, triethylamine (2 mL) was added. The mixture was diluted with AcOEt, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (15 g) with 3% AcOEt−benzene to give **14** (6 mg) and **13** (208 mg, 90%) in this order. **13**: ^{1}H NMR δ 0.20 (9 H, s, OSiMe$_3$), 0.55 (3 H, s, H-18), 0.84 (3 H, d, J = 6.9 Hz, H-21), 0.92 (3 H, d, J = 6.9 Hz, 22-Me), 1.01 (3 H, s, H-19), 1.32 and 1.35 (each 3 H, s, H-26 and -27), 3.35 (3 H, s, CH_3OCH_2), 3.77 and 3.78 (each 3 H, s, OCOCH_3), 4.62 and 4.67 (each 1 H, d, J = 7.1 Hz, OCH_2O), 4.75 (1 H, d, J = 9.5 Hz, H-23), 4.85 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.36 and 5.68 (each 1 H, m, H-6 and -7); MS m/z 614 (M$^+$ − CH_3OCH_2OH, 1), 538 (11), 462 (10), 390 (6), 325 (7), 309 (4), 249 (15), 183 (100), 73 (28). **14**: data shown below.

(23*Z*)-(20*S*,22*S*)-22-Methyl-5,7,23-cholestatriene-1α,3*β*,24,25-tetrol 1,3-Bis(methoxycarbonyl) 25-(Methoxymethyl) 24-(Trimethylsilyl) Tetraether (14). To a suspension of CuI (951 mg, 5.0 mmol) in THF (1.4 mL) was added MeLi (1.4 M in ether, 7.13 mL, 10.0 mmol) at 0 °C. The mixture was stirred for 20 min and then cooled to −60 °C. To this solution were added TMSCl (649 μL, 5.12 mmol), HMPA (889 μL, 5.12 mmol), and 22*Z*-en-24-one **12** (367 mg, 0.62 mmol) in THF (2.1 mL) in this order. After stirring for 1 h, triethylamine (2 mL) was added. The mixture was diluted with AcOEt, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (20 g) with 3% AcOEt−benzene to give silyl ether **14** (174 mg, 41%), its 22-epimer **13** (58 mg, 14%), and starting enone **12** (35 mg, 10%) in this order. **14**: ^{1}H NMR δ 0.19 (9 H, s, $OSiMe_3$), 0.61 (3 H, s, H-18), 0.80 (3 H, d, J = 6.1 Hz, H-21), 0.85 (3 H, d, J = 6.8 Hz, 22-Me), 1.01 (3 H, s, H-19), 1.34 and 1.35 (each 3 H, s, H-26 and -27), 3.35 (3 H, s, CH_3OCH_2), 3.78 and 3.80 (each 3 H, s, $OCOCH_3$), 4.61 and 4.67 (each 1 H, d, J = 7.2 Hz, OCH_2O), 4.75 (1 H, d, J = 9.7 Hz, H-23), 4.86 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and -7); IR (neat) 2959, 1747, 1657, 1442, 1251, 1145, 1087, 1037 cm^{-1}; MS *m*/*z* 614 ($M^+ - CH_3OCH_2OH$, 6), 538 (35), 462 (24), 390 (59), 325 (49), 309 (78), 249 (100), 183 (100), 73 (92).

(20*S*,22*S*)-1α,3*β*-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-22-methyl-5,7-cholestadien-24-one (15). To a solution of the silyl ether **13** (226 mg, 0.33 mmol) in THF (2.5 mL) was added *n*-Bu_4NF (1.0 M in THF, 330 μL, 0.33 mmol) at −78 °C, and the mixture was stirred for 30 min. The reaction was quenched with saturated NH_4Cl and the mixture extracted with AcOEt. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (20 g) with 5% AcOEt−benzene to give 24-ketone **15** (182 mg, 90%): ^{1}H NMR δ 0.63 (3 H, s, H-18), 0.78 (3 H, d, J = 6.8 Hz, H-21 or 22-Me), 0.82 (3 H, d, J = 6.6 Hz, 22-Me or H-21), 1.00 (3 H, s, H-19), 1.34 and 1.37 (each 3 H, s, H-26 and -27), 3.39 (3 H, s, CH_3OCH_2), 3.77 and 3.78 (each 3 H, s, $OCOCH_3$), 4.71 (2 H, s, OCH_2O), 4.80 (1 H, m, H-1), 4.89 (1 H, m, H-3), 5.37 and 5.67 (each 1 H, m, H-6 and -7); IR (neat) 2959, 2875, 1748, 1715, 1442, 1372, 1270 cm^{-1}; MS *m*/*z* 604 (M^+, 1), 572 (4), 528 (4), 496 (8), 452 (9), 420 (10), 408 (17), 394 (12), 390 (13), 322 (22), 252 (26), 249 (32), 209 (28), 155 (25), 141 (100), 111 (37), 103 (37), 83 (21).

Me_2CuLi Addition to *E*-Enone 11 under Thermodynamic Conditions. To a suspension of CuI (194 mg, 1.02 mmol) in THF (0.4 mL) was added MeLi (1.4 M in ether, 1.45 mL, 2.03 mmol) at 0 °C. The mixture was stirred for 20 min. To this solution was added *E*-enone **11** (100 mg, 0.17 mmol) in THF (0.4 mL), and the mixture was stirred at 0 °C for 40 min. The reaction was quenched with saturated NH_4Cl and the mixture extracted with AcOEt. The organic extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (7 g) with 5% AcOEt−benzene to give **15** together with a small amount of its 22-epimer **16** (97:3 by HPLC analysis, 93 mg, 91%).

Me_2CuLi Addition to *Z*-Enone 12 under Thermodynamic Conditions. *Z*-Enone **12** (100 mg) was treated with Me_2CuLi under similar conditions to yield **15** together with its 22-epimer **16** (86:14 by HPLC analysis, 36 mg, 35%).

(20*S*,22*R*)-1α,3*β*-Bis[(methoxycarbonyl)oxy]-25-[(methoxymethyl)oxy]-22-methyl-5,7-cholestadien-24-one (16). Deprotection of the silyl ether **14** (200 mg, 0.30 mmol) was performed according to the procedure described for **15** to give 24-ketone **16** in 85% yield: ^{1}H NMR δ 0.68 (3 H, s, H-18), 0.74 (3 H, d, J = 6.1 Hz, H-21 or 22-Me), 0.77 (3 H, d, J = 6.6 Hz, 22-Me or H-21), 1.00 (3 H, s, H-19), 1.34 (6 H, s, H-26 and -27), 3.39 (3 H, s, CH_3OCH_2), 3.78 and 3.79 (each 3 H, s, $OCOCH_3$), 4.71 (2 H, s, OCH_2O), 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 -7); IR (neat) 2959, 2876, 1747, 1714, 1442, 1377, 1282, 1145, 1032 cm^{-1}; MS *m*/*z* 604 (M^+, 5), 572 (6), 528 (22), 496 (28), 484 (9), 452 (41), 420 (31), 408 (46), 394 (31), 390 (22), 322 (42), 249 (44), 209 (43), 197 (27), 155 (32), 141 (100), 111 (42), 103 (71), 83 (22).

(20*S*,22*S*)-22-Methyl-5,7-cholestadiene-1α,3*β*,24,25-tetrol 1,3-Bis(methoxycarbonyl) 25-(Methoxymethyl) Triether (17). To a solution of 24-ketone **15** (300 mg, 0.50 mmol) in CH_2Cl_2 (2 mL) and MeOH (1.5 mL) was added $NaBH_4$ (50 mg, 1.3 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 and water and extracted with CH_2Cl_2. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (15 g) with 15% AcOEt−benzene to give 24-alcohol **17** [241 mg: a less polar isomer (54 mg), a mixture of the less and more polar isomers (176 mg), and a more polar isomer (11 mg), 80%]. The less polar isomer: ^{1}H NMR δ 0.60 (3 H, s, H-18), 0.75 (3 H, d, J = 6.7 Hz, H-21 or 22-Me), 0.90 (3 H, d, J = 6.7 Hz, 22-Me or H-21), 1.00 (3 H, s, H-19), 1.18 and 1.21 (each 3 H, s, H-26 and -27), 3.40 (3 H, s, CH_3OCH_2), 3.77 and 3.79 (each 3 H, s, $OCOCH_3$), 4.72 and 4.77 (each 1 H, d, J = 7.3 Hz, OCH_2O), 4.81 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and -7); IR (neat) 3459, 2960, 2878, 1747, 1443, 1367, 1285 cm^{-1}; MS *m*/*z* 606 (M^+, 1), 574 (4), 530 (2), 498 (31), 422 (100), 392 (33), 251 (50), 209 (54), 197 (49). The more polar isomer: ^{1}H NMR δ 0.60 (3 H, s, H-18), 0.80 (3 H, d, J = 6.9 Hz, H-21 or 22-Me), 1.00 (3 H, d, J = 7.9 Hz, 22-Me or H-21), 1.01 (3 H, s, H-19), 1.18 and 1.21 (each 3 H, s, H-26 and -27), 3.40 (3 H, s, CH_3OCH_2), 3.78 and 3.79 (each 3 H, s, $OCOCH_3$), 4.71 and 4.77 (each 1 H, d, J = 7.4 Hz, OCH_2O), 4.82 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7).

(20*S*,22*R*)-22-Methyl-5,7-cholestadiene-1α,3*β*,24,25-tetrol 1,3-Bis(methoxycarbonyl) 25-(Methoxymethyl) Triether (18). 24-Ketone **16** (150 mg, 0.25 mmol) was reduced with $NaBH_4$ according to the procedure described for **17** to afford 24-alcohol **18** (113 mg, 75%) as a mixture of 24-epimers: ^{1}H NMR δ 1.00 (3 H, s, H-19), 3.77 and 3.79 (each 3 H, s, $OCOCH_3$), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7); IR (neat) 3539, 2961, 2876, 1745, 1442, 1284, 1145, 1035 cm^{-1}; MS *m*/*z* 606 (M^+, 2), 574 (3), 530 (5), 498 (18), 422 (100), 392 (17), 249 (16), 209 (19), 197 (14).

(20*S*,22*S*)-22-Methyl-5,7-cholestadiene-1α,3*β*,24,25-tetrol 1,3-Bis(methoxycarbonyl) 24-(*S*-Methyldithiocarboxy) 25-(Methoxymethyl) Triether (19). To a solution of 24-alcohol **17** (40 mg, 0.066 mmol) and imidazole (0.3 mg) in THF (1.0 mL) was added NaH (60% in oil dispersion, 4 mg, 0.10 mmol) at room temperature. After being stirred for 20 min, carbon disulfide (30 μL, 0.5 mmol) was added all at once. After stirring for 30 min, iodomethane (20 μL, 0.32 mmol) was added slowly. The resulting solution was stirred for 30 min, and then the reaction was quenched with water. The mixture was extracted with AcOEt. The extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel (5 g) with 7% AcOEt−benzene to give 24-dithiocarbonate **19** (32 mg, 70%) as a mixture of 24-epimers: ^{1}H NMR δ 0.56 and 0.58 (3 H (1:1), s, H-18), 0.99 and 1.01 (3 H (1:1), s, H-19), 1.26 (6 H, s, H-26 and -27), 2.575 and 2.578 (3 H (1:1), s, SCH_3), 3.35 and 3.37 (3 H (1:1), s, CH_3OCH_2), 3.77 and 3.78 (each 3 H, s, $OCOCH_3$), 4.68−4.80 (2 H, m, OCH_2O), 4.84 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7), 5.92 (1 H, m, H-24); IR (neat) 2957, 2876, 1743, 1441, 1369, 1251, 1211 cm^{-1}; MS *m*/*z* 664 ($M^+ - CH_3OH$, 7), 544 (3), 512 (6), 468 (14), 436 (13), 422 (54), 392 (98), 374 (72), 249 (100).

(20*S*,22*R*)-22-Methyl-5,7-cholestadiene-1α,3*β*,24,25-tetrol 1,3-Bis(methoxycarbonyl) 24-(*S*-Methyldithiocarboxy) 25-(Methoxymethyl) Triether (20). 24-Alcohol **18** (70 mg, 0.11 mmol) was converted to *S*-methyl dithiocarbonate **20** (37 mg, 46%) under similar conditions: ^{1}H NMR δ 0.56 and 0.59 (3 H (2:1), s, H-18), 0.98 and 1.01 (3 H (2:1), s, H-19), 1.27 (6 H, s, H-26 and -27), 2.55 and 2.56 (3 H (2:1), s, SCH_3), 3.367 and 3.370 (3 H (1:2), s, CH_3OCH_2), 3.78 and 3.80 (each 3 H, s, $OCOCH_3$), 4.68−4.80 (2 H, m, OCH_2O), 4.82 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.37 and 5.68 (each 1 H, m, H-6 and -7), 5.91 (1 H, m, H-24); IR (neat) 2957, 2876, 1745, 1442, 1271, 1215, 1145, 1055 cm^{-1}; MS *m*/*z* 696 (M^+, 0.4), 664 (2), 620 (1), 575 (2), 544 (3), 512 (6), 468 (13), 436 (16), 422 (12), 392 (100), 374 (37), 249 (57).

(20*S*,22*S*)-22-Methyl-5,7-cholestadiene-1α,3*β*,25-triol 1,3-Bis(methoxycarbonyl) 25-(Methoxymethyl) Triether (21). To a solution of dithiocarbonate **20** (32 mg, 0.046 mmol) in dry toluene (1.5 mL) were added azobis(isobutyronitrile) (4 mg, 0.025 mmol) and *n*-Bu_3SnH (80 μL, 0.30 mmol). The mixture

was heated at reflux for 20 min. After being cooled to room temperature, the mixture was concentrated and chromatographed on silica gel (7 g, 7% AcOEt−benzene) to give **21** (22 mg, 81%): ^{1}H NMR δ 0.59 (3 H, s, H-18), 0.75 (3 H, d, J = 6.4 Hz, H-21 or 22-Me), 0.86 (3 H, d, J = 6.8 Hz, 22-Me or H-21), 1.00 (3 H, s, H-19), 1.21 (6 H, s, H-26 and -27), 3.36 (3 H, s, CH_3OCH$_2$), 3.77 and 3.79 (each 3 H, s, OCOCH_3), 4.69 and 4.70 (each 1 H, d, J = 7.2 Hz, OCH_2O), 4.83 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7); IR (neat) 2957, 2874, 1740, 1442, 1252, 1146 cm^{-1}; MS m/z 590 (M$^+$, 0.05), 528 (2), 452 (12), 376 (100), 275 (40), 249 (52).

(20*S*,22*R*)-22-Methyl-5,7-cholestadiene-1α,3*β*,25-triol 1,3-Bis(methoxycarbonyl) 25-(Methoxymethyl) Triether (22). Thiocarbonate **20** (35 mg, 0.05 mmol) was reduced with n-Bu$_3$SnH according to the procedure described for the preparation of **21** to yield **22** (13 mg, 44%): ^{1}H NMR δ 0.61 (3 H, s, H-18), 0.70 (3 H, d, J = 6.6 Hz, H-21 or 22-Me), 0.76 (3 H, d, J = 6.8 Hz, 22-Me or H-21), 1.00 (3 H, s, H-19), 1.21 (6 H, s, H-26 and -27), 3.36 (3 H, s, CH_3OCH$_2$), 3.77 and 3.79 (each 3 H, s, OCOCH_3), 4.70 (2 H, s, OCH_2O), 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 -7); IR (neat) 2959, 2874, 1747, 1442, 1255, 1145, 1091, 1039 cm^{-1}; MS m/z 590 (M$^+$, 0.12), 528 (1), 452 (14), 376 (100), 277 (21), 249 (23).

(20*S*,22*S*)-22-Methyl-5,7-cholestadiene-1α,3*β*,25-triol 1,3-Bis(methoxycarbonyl) Triether (23). A solution of 25-MOM ether **21** (55 mg, 0.093 mmol) and TsOH monohydrate (50 mg, 0.26 mmol) in CHCl$_3$ (1 mL) and 95% EtOH (3 mL) was heated at reflux for 60 min. After dilution with CHCl$_3$, the solution was washed with aqueous NH$_4$Cl and water. The aqueous layer was extracted with CHCl$_3$. The combined organic layer was dried and evaporated. The residue was chromatographed on silica gel (5 g, 25% AcOEt−benzene) to afford 25-alcohol **23** (46 mg, 90%): ^{1}H NMR δ 0.60 (3 H, s, H-18), 0.76 (3 H, d, J = 6.4 Hz, H-21 or 22-Me), 0.86 (3 H, d, J = 6.8 Hz, 22-Me or H-21), 1.00 (3 H, s, H-19), 1.22 (6 H, s, H-26 and -27), 3.77 and 3.80 (each 3 H, s, OCOCH_3), 4.82 (1 H, m, H-1), 4.90 (1 H, m, H-3), 5.38 and 5.69 (each 1 H, m, H-6 and -7); IR (neat) 3466, 2926, 2855, 1740, 1466, 1398, 1383, 1261, 1089, 1035, cm^{-1}; MS m/z 528 (M$^+$ − H$_2$O, 5), 452 (10), 394 (11), 376 (100), 275 (44), 249 (44), 209 (39).

(20*S*,22*S*)-22-Methyl-5,7-cholestadiene-1α,3*β*,25-triol (25). To a solution of 1,3-biscarbonate **23** (12 mg, 0.22 mmol) in CH$_2$Cl$_2$ (0.25 mL) was added a 5% KOH/MeOH solution (1.7 mL), and the mixture was heated at reflux for 60 min. The reaction mixture was concentrated in vacuo and diluted with CHCl$_3$. The mixture was washed with aqueous NH$_4$Cl and water, dried, and evaporated. The residue was chromatographed on silica gel (3 g, 10% EtOH−CHCl$_3$) to give provitamin D **25** (8 mg, 85%): ^{1}H NMR δ 0.61 (3 H, s, H-18), 0.76 (3 H, d, J = 6.5 Hz, H-21 or 22-Me), 0.87 (3 H, d, J = 6.7 Hz, 22-Me or H-21), 0.94 (3 H, s, H-19), 1.21 and 1.22 (each 3 H, s, H-26 and -27), 3.76 (1 H, m, H-1), 4.07 (1 H, m, H-3), 5.37 and 5.71 (each 1 H, m, H-6 and -7); IR (neat) 3377, 2964, 2876, 1456, 1379, 1259, 1217, 1151, 1062 cm^{-1}; MS m/z 430 (M$^+$, 17), 412 (43), 394 (37), 376 (100), 374 (89), 275 (83), 249 (74), 239 (62), 209 (59), 197 (59).

(20*S*,22*R*)-22-Methyl-5,7-cholestadiene-1α,3*β*,25-triol (26). A solution of 25-MOM ether **22** (11 mg, 0.0186 mmol) and TsOH monohydrate (12 mg, 0.062 mmol) in 95% EtOH (2 mL) was heated at reflux for 30 min. After complete hydrolysis of the MOM ether was confirmed, 5% KOH/MeOH (2 mL) was added, and the mixture was heated at reflux for 60 min. The mixture was diluted with AcOEt, washed with aqueous NH$_4$Cl, dried, and evaporated. The residue was chromatographed on silica gel (3 g, 10% EtOH−CHCl$_3$) to yield provitamin D **26** (4.8 mg, 60%): ^{1}H NMR δ 0.63 (3 H, s, H-18), 0.71 (3 H, d, J = 6.6 Hz, H-21 or 22-Me), 0.78 (3 H, d, J = 6.8 Hz, 22-Me or H-21), 0.94 (3 H, s, H-19), 1.22 (6 H, s, H-26 and -27), 3.77 (1 H, m, H-1), 4.07 (1 H, m, H-3), 5.39 and 5.73 (each 1 H, m, H-6 and -7); IR (neat) 3397, 2966, 2934, 2876, 1711, 1456, 1377, 1086 cm^{-1}; MS m/z 430 (M$^+$, 18), 412 (34), 394 (30), 376 (79), 374 (100), 275 (34), 249 (35), 239 (60), 209 (34), 207 (36), 197 (35).

(20*S*,22*S*)-22-Methyl-1α,25-dihydroxyvitamin D$_3$ (6). A solution of the provitamin D **25** (6.75 mg, 0.016 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 **25** was consumed. After removal of the solvent, the residue was chromatographed on Sephadex LH-20 (20 g) with CHCl$_3$/hexane/MeOH (70:30:0.7) to give previtamin D (2.41 mg, 36%). 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 **6** (1.68 mg, 70%). The purity of **6** was proved to be about 100% by two HPLC systems: (1) YMC-Pack ODS-AM, 4.6 × 150 mm, H$_2$O/MeOH (20:80), 1.0 mL/min, retention time, 11.5 min; (2) LiChrospher Si 60, 4.0 × 250 mm, hexane/CHCl$_3$/MeOH (100:25:10), 1.5 mL/min, retention time, 11.0 min. **6**: UV (95% EtOH) λ_{max} 264 nm, λ_{min} 228 nm; ^{1}H NMR δ (CDCl$_3$) 0.53 (3 H, s, H-18), 0.75 (3 H, d, J = 7.0 Hz, H-21 or 22-Me), 0.86 (3 H, d, J = 7.0 Hz, 22-Me or H-21), 1.22 (6 H, s, H-26 and -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 = 11.3 Hz, H-7 and -6); IR (neat) 3371, 2963, 2874, 1452, 1377, 1217, 1149, 1059, 956, 910, 752 cm^{-1}; MS m/z 430 (M$^+$, 2), 412 (19), 394 (82), 376 (100), 251 (36), 249 (23), 209 (22), 197 (22), 155 (36), 141 (21); HRMS calcd for C$_{28}$H$_{46}$O$_3$ 430.3447, found 430.3468.

(20*S*,22*R*)-22-Methyl-1α,25-dihydroxyvitamin D$_3$ (5). Provitamin D **26** (4.80 mg, 0.011 mmol) was converted to the target vitamin D **5** (1.40 mg) according to the procedure for **6** as described above. The purity of **5** was proved to be about 100% by two HPLC systems: (1) YMC-Pack ODS-AM, 4.6 × 150 mm, H$_2$O/MeOH (20:80), 1.0 mL/min, retention time, 16.9 min; (2) LiChrospher Si 60, 4.0 × 250 mm, hexane/CHCl$_3$/MeOH (100:25:10), 1.5 mL/min, retention time, 11.2 min. **5**: UV (95% EtOH) λ_{max} 265 nm, λ_{min} 229 nm; ^{1}H NMR δ (CDCl$_3$) 0.53 (3 H, s, H-18), 0.71 (3 H, d, J = 5.9 Hz, H-21 or 22-Me), 0.78 (3 H, d, J = 7.0 Hz, 22-Me or H-21), 1.21 (6 H, s, H-26 and -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 = 11.3 Hz, H-7 and -6); IR (neat) 3373, 2961, 2874, 1466, 1450, 1379, 1217, 1157, 1113, 1055, 956, 910, 760 cm^{-1}; MS m/z 430 (M$^+$, 3), 412 (11), 394 (30), 376 (100), 251 (27), 249 (19), 209 (18), 197 (17), 155 (29), 141 (19); HRMS calcd for C$_{28}$H$_{46}$O$_3$ 430.3447, found 430.3433.

Competitive Binding Assay: Porcine Intestinal VDR. Porcine intestinal nuclear extract was prepared according to the procedures reported[15] and diluted to 1/30. All these experiments were carried out in triplicate. To an experimental tube were added 100 μL of pig nuclear extract and 5 μL of ethanol containing 1 nM final concentration of [^{3}H]-1,25-(OH)$_2$D$_3$. To this was added 5 μL of ethanol containing either the analog or standard 1,25(OH)$_2$D$_3$. The mixture was vortexed and then placed on a shaker bath at room temperature for 2 h. At the end of this incubation, 50 μL of hydroxyapatite slurry[15] was added and the mixture was vortexed at 10 min intervals for 30 min. The entire operation was carried out at 0 °C or on ice. The hydroxyapatite was sedimented at 600g for 10 min. The supernatant was discarded, and the pellet was washed three times with 5% Triton X-100/TE buffer.[15] The pellet was then transferred to a scintillation vial containing Biosafe scintillation fluid and counted.

Bovine Thymus VDR. Binding to bovine thymus VDR was evaluated according to the procedure reported.[16] These experiments were done in duplicate. 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) was incubated with 0.1 nM [^{3}H]-1,-25(OH)$_2$D$_3$ together with graded amounts of each vitamin D analog for 18 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 1000g for 10 min. The radioactivity of the supernatant was counted.

Rat Serum DBP. Binding to rat serum DBP was evaluated according to the procedure reported.[16] These experiments were done in duplicate. Serum from vitamin D-deficient rats was diluted (×10 000) with 3.5 mM barbiturate buffer (pH 8.6) containing 0.13 M NaCl and used as a source of DBP. The diluted serum (500 μL) was incubated with 0.1 nM [^{3}H]-25-(OH)D$_3$ and graded amounts of each sample for 1 h at 4 °C.

The bound and free [^{3}H]-25(OH)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 measured.

Acknowledgment. We are grateful to Kuraray Co., Ltd. for kindly providing the steroidal C(22)-alcohol.

References

(1) DeLuca, H. F.; Darwish, H. M.; Ross, T. K.; Moss, V. E. Mechanism of Action of 1,25-Dihydroxyvitamin D on Target Gene Expression. *J. Nutr. Sci. Vitaminol.* **1992**, 19−26.

(2) Pike, J. W. Vitamin D_3 Receptors: Structure and Function in Transcription. *Annu. Rev. Nutr.* **1991**, *11*, 189−216. DeLuca, H. F. Mechanism of Action of 1,25-Dihydroxyvitamin D_3: 1990 Verion. *J. Bone Miner. Metab.* **1990**, *8*, 1−9. DeLuca, H. F.; Krisinger, J.; Darwish, H. The Vitamin D System: 1990. *Kidney Int. (Suppl.)* **1990**, *38*, S2−S8. Minghetti, P. P.; Norman, A. W. 1,25$(OH)_2$-Vitamin D_3 Receptors: Gene Regulation and Genetic Circuitry. *FASEB J.* **1988**, *2*, 3043−3053.

(3) Evans, R. M. The Steroid and Thyroid Hormone Receptor Superfamily. *Science* **1988**, *240*, 889−895. Beato, M. Gene Regulation by Steroid Hormones. *Cell* **1989**, *56*, 335−344.

(4) (a) Abe, J.; Morikawa, M.; Miyamoto, K.; Kaiho, S.; Fukushima, M.; Miyaura, C.; Abe, E.; Suda, T.; Nishii, Y. Synthetic Analogues of Vitamin D_3 with an Oxygen Atom in the Side Chain Skeleton. *FEBS. Lett.* **1987**, *226*, 58−62. Abe, J.; Takita, Y.; Nakano, T.; Miyaura, C.; Suda, T.; Nishii, Y. A Synthetic Analogue of Vitamin D_3, 22-Oxa-1α,25-dihydroxyvitamin D_3, Is a Potent Modulator of *In Vivo* Immunoregulating Activity without Inducing Hypercalcemia in Mice. *Endocrinology* **1989**, *124*, 2645−2647. (b) Binderup, L.; Bramm, E. Effects of a Novel Vitamin D Analogue MC903 on Cell Proliferation and Differentiation *In Vitro* and on Calcium Metabolism *In Vivo*. *Biochem. Pharmacol.* **1988**, *37*, 889−895. (c) Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M.; Hansen, K. 20-Epi-vitamin D_3 Analogues: A Novel Class of Potent Regulators of Cell Growth and Immune Responses. *Biochem. Pharmacol.* **1991**, *42*, 1569−1575. (d) Zhou, J. Y.; Norman, A. W.; Lübbert, M.; Collins, E. D.; Uskokovic, M. R.; Koeffler, H. P. Novel Vitamin D Analogs That Modulate Leukemic Cell Growth and Differentiation with Little Effect on Either Intestinal Calcium Absorption or Bone Calcium Mobilization. *Blood* **1989**, *74*, 82−93. (e) Perlman, K.; Kutner, A.; Prahl, J.; Smith, C.; Inaba, M.; Schnoes, H. K.; DeLuca, H. F. 24-Homologated 1,25-Dihydroxyvitamin D_3 Compounds: Separation of Calcium and Cell Differentiation Activities. *Biochemistry* **1990**, *29*, 190−196. (f) Krisinger, J.; Strom, M.; Darwish, H. D.; Perlman, K.; Smith, C.; DeLuca, H. F. Induction of Calbindin-D 9k mRNA but Not Calcium Transport in Rat Intestine by 1,25-Dihydroxyvitamin D_3 24-Homologs. *J. Biol. Chem.* **1991**, *266*, 1910−1913. (g) Bouillon, R.; Okamura, W. H.; Norman, A. W. Structure-Function Relationships in the Vitamin D Endocrine System. *Endocr. Rev.* **1995**, *16*, 200−257.

(5) Allan, G. F.; Leng, X.; Tsai, S. Y.; Weigel, N. L.; Edwards, D. P.; Tsai, M.-J.; O'Malley, B. W. Hormone and Antihormone Induce Distinct Conformational Changes Which Are Central to Steroid Receptor Activation. *J. Biol. Chem.* **1992**, *267*, 19513−19520. Beekman, J. M.; Allan, G. F.; Tsai, S. Y.; Tsai, M.-J.; O'Malley, B. W. Transcriptional Activation by the Estrogen Receptor Requires a Conformational Change in the Ligand Binding Domain. *Mol. Endocrinol.* **1993**, *7*, 1266−1274. Kuil, C. W.; Mulder, E. Mechanism of Antiandrogen Action: Conformational Changes of the Receptor. *Mol. Cell. Endocrinol.* **1994**, *102*, R1−R5.

(6) Peleg, S.; Sastry, M.; Collins, E. D.; Bishop, J. E.; Norman, A. W. Distinct Conformational Changes Induced by 20-Epi Analogues of 1α,25-Dihydroxyvitamin D_3 Are Associated with Enhanced Activation of the Vitamin D Receptor. *J. Biol. Chem.* **1995**, *270*, 10551−10558.

(7) (a) Yamamoto, K.; Takahashi, J.; Hamano, K.; Yamada, S.; Yamaguchi, K.; DeLuca, H. F. Stereoselective Syntheses of (22*R*)- and (22*S*)-22-Methyl-1α,25-dihydroxyviamin D_3: Active Vitamin D_3 Analogs with Restricted Side Chain Conformation. *J. Org. Chem.* **1993**, *58*, 2530−2537. (b) Yamamoto, K.; Ohta, M.; DeLuca, H. F.; Yamada, S. On the Side Chain Conformation of 1α,25-Dihydroxyvitamin D_3 Responsible for Binding to the Receptor. *BioMed. Chem. Lett.* **1995**, *5*, 979−984. (c) Ishida, H.; Shimizu, M.; Yamamoto, K.; Iwasaki, Y.; Yamada, S.; Yamaguchi, K. Syntheses of 1-Alkyl-1,25-dihydroxyvitamin D_3. *J. Org. Chem.* **1995**, *60*, 1828−1833.

(8) Toan, T.; Ryan, R. C.; Simon, G. L.; Calabrese, J. C.; Dahl, L. F.; DeLuca, H. F. Crystal Structure of 25-Hydroxy-vitamin D_3 Monohydrate: A Stereochemical Analysis of Vitamin D Molecules. *J. Chem. Soc., Perkin Trans. 2* **1977**, 393−401.

(9) BIOSYM Technologies, Inc., 9685 Scranton Rd., San Diego, CA 92121-3752.

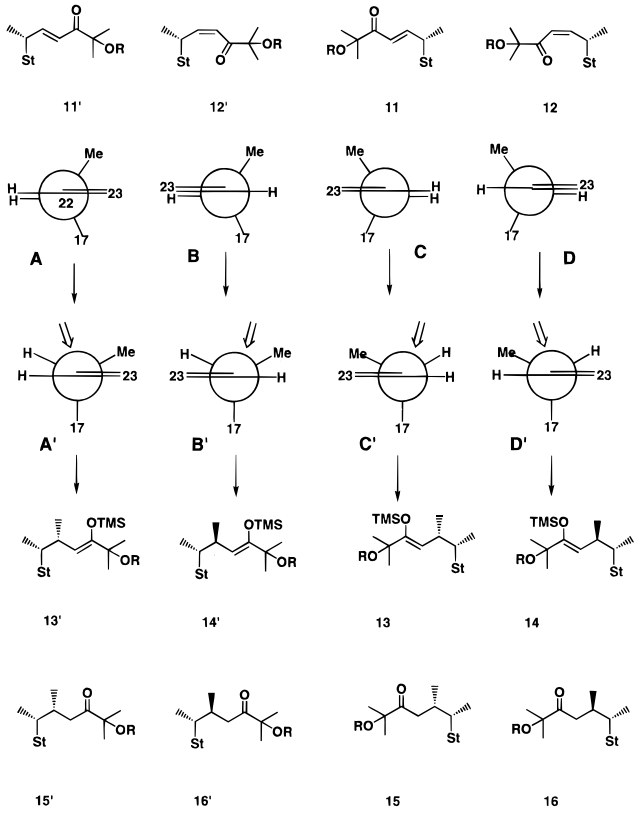
(10) TRIPOS Inc., 1699 South Hanley, Suite 303, St. Louis, MO 63144-2913.

(11) MMX (an enhanced version of MM2) was calculated by using software PCMODEL (Serena Software, Bloomington).

(12) Crystallization of more than 10 derivatives of **13** and **14** was examined in various solvent systems. Among them, four compounds [**15** and its derivatives with deprotected hydroxyl group (**15**, R = CH_3OCO, R′ = H, and **15**, R = R′ = H) and with butyl group in the place of the methyl at C(22)] gave single crystals. However all these crystals contained four molecules in their unit cells. Solution of the crystal structure of compounds with these high formula weights (about 2000) is nearly impossible.

(13) Yamamoto, K.; Yamada, S.; Yamaguchi, K. Highly Diastereoselective Conjugate Addition of Organocuprate to Acyclic *E*- and *Z*-Enones: Reversal Stereoselectivity under Kinetic and Thermodynamic Conditions. *Tetrahedron Lett.* **1992**, *33*, 7521−7524.

(14) We conducted the addition of Me_2CuLi to the four isomeric steroidal 22-en-24-ones: 20*R*,22*E*-22-en-24-one **11′**, 20*R*,22*Z*-22-en-24-one **12′**, 20*S*,22*E*-22-en-24-one **11**, and 20*S*,22*Z*-22-en-24-one **12**. The face selectivity of the additions depended on the conditions and geometry at C(22). Thus under thermodynamic conditions (Me_2CuLi in THF, 0 °C) the pairs of geometrical isomers at C(22) gave the same product with high selectivity regardless of the geometry: Both **11′** and **12′** gave ketone **15′**, and both **11** and **12** yielded **15** in high selectivity. Under kinetic conditions (Me_2CuLi in the presence of TMSCl and HMPA in THF, −78 °C) however, the stereoselectivity depended on the geometry at C(22): **11′** and **12′** gave **13′** and **14′**, respectively, with 97−100% selectivity, and as described in the text **11** and **12** gave **13** and **14**, respectively. The selectivity under kinetic conditions can be explained as follows (see figure below): In kinetically controlled reactions, the conformation at the transition state can be closely related to the stable conformation of the substrate. The most stable conformations of the starting enones calculated by molecular mechanics method (MMX)[11] were A and B for **11′**, B for **12′**, C and D for **11**, and D for **12**. Applying the Felkin−Anh model, we assumed the transition-state conformations of these four enones **11′**, **12′**, **11**, and **12** to be A′, B′, C′, and D′, respectively. The nucleophilic reagent is supposed to approach the enones from the direction shown with the arrows, the angle to the double bond plane being about 100°. Thus **11′**, **12′**, **11**, and **12** are expected to produce **13′**, **14′**, **13**, and **14**, respectively. The configuration at C(22) of **13′** was unequivocally determined to be *S* by X-ray analysis[7a] and then that of **14′** to be *R*. Since the reactivity and the face selectivity under kinetic as well as thermodynamic conditions are parallel in the 20*R*- and 20*S*-enones, the configurations at C(22) of **13** and **14** were assigned with high probability to be *R* and *S*, respectively (those of **15** and **16** to be *S* and *R*, respectively).



(15) Dame, M. C.; Pierce, E. A.; DeLuca, H. F. Identification of the Porcine Intestinal 1,25-Dihydroxyvitamin D_3 Receptor on Sodium Dodecyl Sulfate/Polyacrylamide Gels by Renaturation and Immunoblotting. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7825−

7829. Dame, M. C.; Pierce, E. A.; Prahl, J. M.; Hayes, C. E.; DeLuca, H. F. Monoclonal Antibodies to the Porcine Intestinal Receptor for 1,25-Dihydroxyvitamin D_3: Interaction with Distinct Receptor Domains. *Biochemistry* **1986**, *25*, 4523−4534.

(16) Imae, Y.; Manaka, A.; Yoshida, N.; Ishimi, Y.; Shinki, T.; Abe, E.; Suda, T.; Konno, K.; Takayama, H.; Yamada, S. Biological Activities of 24-Fluoro-1α,25-dihydroxyvitamin D_2 and its 24-Epimer. *Biochim. Biophys. Acta* **1994**, *1213*, 302−308.

(17) Dilworth, F. J.; Calverley, M. J.; Makin, H. L. J.; Jones, G. Increased Biological Activity of 20-Epi-1,25-dihydroxyvitamin D_3 Is Due to Reduced Catabolism and Altered Protein Binding. *Biochem. Pharmacol.* **1994**, *47*, 987−993.

(18) Okamura's group has reported a dot map for models of **1** and its analogs to study the structure−activity relationships; see: Okamura, W. H.; Palenzuela, J. A.; Plumet, J.; Midland, M. M. Vitamin D: Structure-Function Analyses and the Design of Analogs. *J. Cell. Biochem.* **1992**, *49*, 10−18. Midland, M. M.; Plumet, J.; Okamura, W. H. Effect of C20 Stereochemistry on the Conformational Profile of the Side Chains of Vitamin D Analogs. *BioMed. Chem. Lett.* **1993**, *3*, 1799−1804.

(19) (a) Dawson, M. I.; Jong, L.; Hobbs, P. D.; Cameron, J. F.; Chao, W.; Pfahl, M.; Lee, M.; Shroot, B.; Pfahl, M. Conformational Effects on Retinoid Receptor Selectivity. 2. Effects of Retinoid Bridging Group on Retinoid X Receptor Activity and Selectivity. *J. Med. Chem.* **1995**, *38*, 3368−3383. (b) Boehm, M. F.; Zhang, L.; Badea, B. A.; White, S. K.; Mais, D. E.; Berger, E.; Suto, C. M.; Goldman, M. E.; Heyman, R. A. Synthesis and Structure-Activity Relationships of Novel Retinoid X Receptor-Selective Retinoids. *J. Med. Chem.* **1994**, *37*, 2930−2941. (c) Shimamoto, K.; Ishida, M.; Shinozaki, H.; Ohfune, Y. Synthesis of Four Diastereomeric L-2-(Carboxycyclopropyl)glycines. Conformationally Constrained L-Glutamate Analogues. *J. Org. Chem.* **1991**, *56*, 4167−4176.

(20) 20-Alcohol: (22*E*)-(20ξ)-1α,3β-bis[(methoxycarbonyl)oxy]-20-hydroxy-25-[(methoxymethyl)oxy]-5,7,22-cholestatrien-24-one. This compound had been obtained as a byproduct on the photoisomerization of the 20-epimer of **11**.[7a]

JM9600048