Conformation−Function Relationship of Vitamin D: Conformational Analysis Predicts Potential Side-Chain Structure

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In previous studies, we have grouped regions in space occupied by the vitamin D side chain into four: A, G, EA, and EG. We showed that the receptor (VDR) affinity of 1α,25-dihydroxyvitamin D_3 derivatives increases, in terms of side-chain region, in the order EG, G, A, and EA. We called this the active space group concept. In the present study, we used this active space group concept to analyze the conformation−activity relationship of about 40 representative potent 1α,25-dihydroxyvitamin D_3 analogues. We initially listed structural modifications in the side chain of potent vitamin D analogues and estimated their potency factor. Possible side-chain conformations of representative analogues were calculated by the molecular mechanics method and plotted on a dot map compared with the regions A, G, EA, and EG. The cell-differentiating potency of the analogues was correlated with our active space group concept with few exceptions. Among potent analogues with a natural configuration at C(20), the side chains of those with a 22-oxa, 22-ene, 16-ene, or a 18-nor modification were located in front of region EA (termed F). The side chains of the most potent 20-epi-22-oxa-24-homovitamin D analogues were concentrated at the left side of the EA region (L-EA). Thus, the side chains of almost all potent analogues were distributed around the EA region, and potency increased in the order A, F, EA, and L-EA.

Introduction

Binding of a ligand to the receptor is the initial and most crucial step in activating gene transcription mediated by a ligand-dependent nuclear transcription factor. How molecular recognition occurs at the interface of a ligand and the receptor is of interest to chemists. Control of this process is also key to developing potent and selective clinical agents.

Vitamin D exerts its function by binding to its nuclear receptor (VDR),[1] which is a member of the nuclear receptor superfamily.[2] In binding to VDR, the three hydroxyl groups of 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$, **1**) play important roles. In particular, those of the 1α- and 25-hydroxyl groups are critical, as their removal diminishes the VDR affinity by 1/500−1/1000.[3]

Over 300 active vitamin D analogues have been synthesized[4] since the discovery of 1,25-$(OH)_2D_3$ (**1**),[5] and some are in clinical use to treat metabolic bone diseases and skin disorders such as psoriasis.[6] Nearly 100 analogues with higher activities than that of the parent natural ligand (**1**) have been identified, and most of them are modified around the side chain.[4] In our initial structure−function studies of vitamin D,[7] we focused our attention on the side chain. Vitamin D side chains are highly flexible as they possess the full carbon structure of cholesterol. Therefore the 25-hydroxyl group, which apparently plays a crucial role in anchoring vitamin D to the VDR, can occupy wide regions of space. The 20-epimer **2** of 1,25-$(OH)_2D_3$ (**1**) is much more potent than the natural vitamin,[8a] although the side chain of these epimers appears to occupy different regions of space. Okamura and Midland[9] analyzed the conformational mobility of the side chain of these epimeric vitamins (**1** and **2**) and relatives by plotting the 25-oxygen position of possible conformations on a dot map. In these studies, they discriminated the contribution of the conformers using the energy window concept. To determine the VDR-bound side-chain conformation, we initially analyzed possible side-chain conformations of 1,25-$(OH)_2D_3$ (**1**) and its 20-epimer **2** by molecular mechanics and grouped the regions occupied by their 25-oxygen into four: A, G, EA, and EG (Figure 1).[7a,b] We then designed and synthesized analogues in which the side-chain mobility was restricted in one of these regions, the diastereomers **3**−**6** at C(20) and C(22) of 22-Me-1,25-$(OH)_2D_3$.[7a,b] The VDR affinity of these conformationally restricted analogues increased in the order **6**, **3**, **4**, and **5** (1/100, 1/60, 1/3, and 20, respectively, relative to 1,25-$(OH)_2D_3$, **1**). In terms of the region, the potency increased in the order EG, G, A, and EA.[7a] From these results, we postulated that 1,25-$(OH)_2D_3$ (**1**) binds to VDR when its side chain is located in the A region[10] and that 20-epi-1,25-$(OH)_2D_3$ (**2**) binds to VDR when its side chain is located in the EA region.

We next studied whether our active space group concept can predict the potency of known vitamin D analogues. In this study, we (1) listed structural modifications in the side chain (including C(17)) of vitamin D that elevate potency, (2) calculated the possible side-chain conformations of these analogues that were displayed on dot maps, and (3) investigated the relationship between the side-chain region and activity. The results not only support our idea of active areas of the side chain but also demonstrate that

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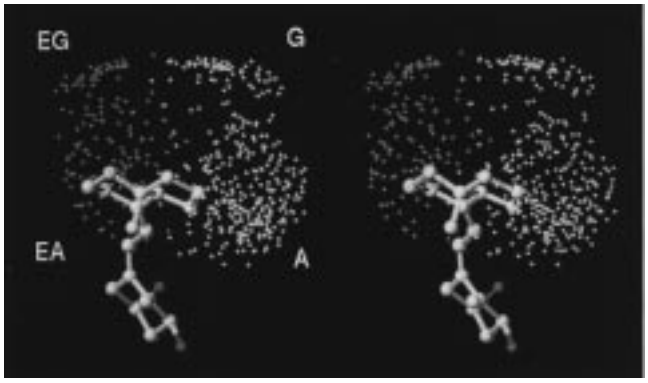


Figure 1. Grouping of the side-chain regions of 1,25-$(OH)_2D_3$ (**1**) and its 20-epimer (**2**) (stereoview). The mobile areas of the side chain of **1** (yellow) and **2** (cyan) are shown in a dot map and are grouped into regions: A and G regions in **1** and EA and EG regions in **2** (see ref 7a).

conformational analysis can predict the potency of vitamin D analogues.

Methods

Potent Vitamin D Analogues and Activation Factor. We compared the potency of vitamin D derivatives in terms of their cell-differentiating activity (throughout this paper, potency indicates cell-differentiating activity unless otherwise noted). Affinity for VDR may be more appropriate for predicting receptor-bound conformation, but IC_{50} values determined by competitive binding assays are not always reproducible for compounds having higher affinity than 1,25-$(OH)_2D_3$ (**1**). This is because attaining equilibrium among the receptor and two such ligands (the sample and radioactive standard) is difficult under assay conditions.[11]

Table 1 lists structural modifications that enhance potency, together with representative compounds and activation factors estimated on the basis of cell-differentiation activity. For simplicity, the analogues were restricted to those derived from 1,25-$(OH)_2D_3$ (**1**). The modifications that increase potency are (1) epimerization at the 20-position,[8] (2) replacement of C(22)-H_2 by oxygen,[12] (3) introduction of double or triple bond(s) to the side chain[13] or the 16-position,[14] (4) removal of the angular C(18) methyl group,[15] (5) elongation of the side chain,[16] (6) methylation of the terminal C(26) and -(27) methyl groups,[16] (7) perfluorination of C(24) methylene[17] or the terminal gem-dimethyl groups,[18] and (8) methylation at C(20).[19] Combinations of these modifications enormously enhance potency as the compounds in Table 2 show. In most cases, the potency of compounds with multimodifications can be estimated by multiplying by the activation factor shown in Table 1.

Conformational Analysis. We analyzed the relationship between the potency of the analogues and the areas occupied by the 25-oxygen. (1) The possible side-chain conformations of each analogue were searched using the molecular mechanics method. (2) These possible conformations were plotted by positioning the 25-oxygen on a dot map where one dot corresponds to one-side chain conformation. (3) The dot map of each analogue was overlaid with those of 1,25-$(OH)_2D_3$ (**1**) and 20-epi-1,25$(OH)_2D_3$ (**2**), and then we analyzed the relationship between the side-chain area and cell-differentiating activity.

The conformations were analyzed using the software SYBYL (version 6.3, Tripos)[20] as reported.[7a,b] Briefly, (1) analogue structures were constructed by modifying the crystallographic vitamin D_3 structure in the SYBYL database; then the global minimum-energy conformation of each analogue was generated by repeating rotations (360° at 60° intervals) of rotatable C−C and C−O bonds in the side chain (grid search in SYBYL) followed by optimization. (2) Using this minimum-energy conformation as the initial structure, all possible side-chain conformations were searched by rotating (360° at 30° intervals) the bonds in the side chain (indicated by numbers in the structures in Chart 1) (systematic search in SYBYL). In the systematic search, the van der Waals bump coefficient was set at 0.95 to eliminate infeasible conformations suffered from van der Waals repulsion. (3) All possible conformations were

Table 1. Side-Chain Modifications That Elevate Vitamin D Potency

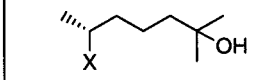
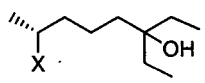
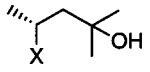
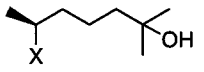
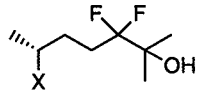
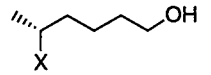
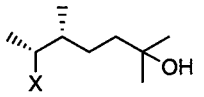
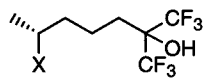
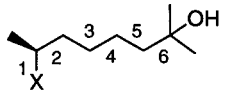
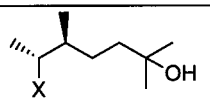
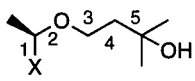
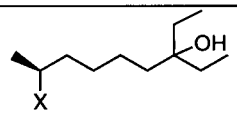
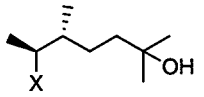
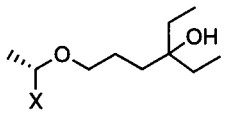
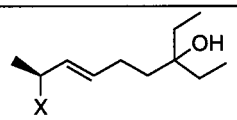
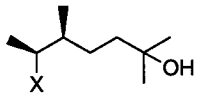
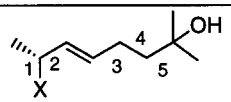
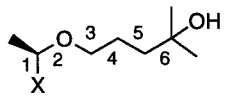
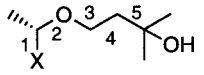
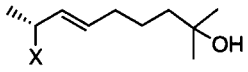
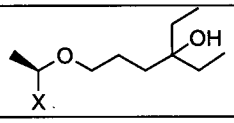
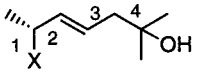
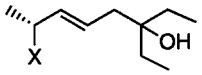
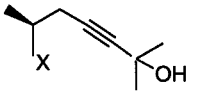
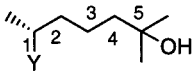
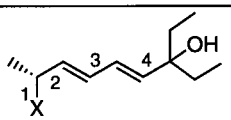
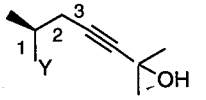
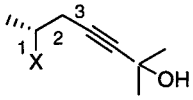
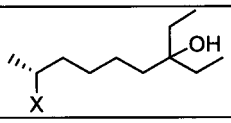
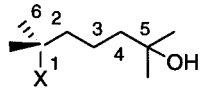
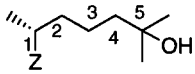
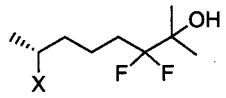
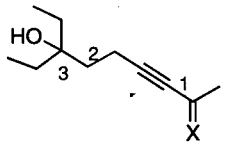
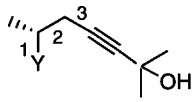
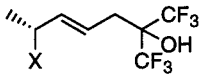
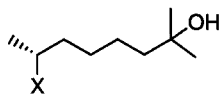
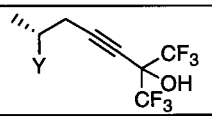
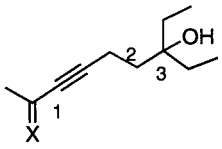
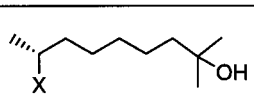
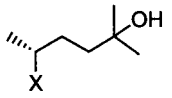
modification	abbrev	activation factor	representative compound
20-epimerization	EP	20−30	20-epi-1,25-$(OH)_2D_3$ (**2**)[8a]
22-oxygen	22O	5−10	22-oxa-1,25-$(OH)_2D_3$ (**7**)[12]
unsaturation [yne (Y) or ene (E)]	22E	1−2	22-ene-1,25-$(OH)_2D_3$ (**8**)[13a]
	23Y	2−3	23-yne-1,25-$(OH)_2D_3$ (**10**)[13a]
	16E	2−5	16-ene-1,25-$(OH)_2D_3$ (**9**)[13a,14a]
18-nor	18N	5−10	18-nor-1,25-$(OH)_2D_3$ (**11**)[15]
elongation [homo (H) or dihomo (DH)]	24H	5−10	24-homo-1,25-$(OH)_2D_3$ (**13**)[16a−c]
	24DH	2−5	24-dihomo-1,25-$(OH)_2D_3$ (**14**)[16c]
	26,27DH	2−5	26,27-dihomo-1,25-$(OH)_2D_3$ (**15**)[16f,g]
fluorination	F2	5−10	24-F_2-1,25-$(OH)_2D_3$ (**16**)[17a,b]
	F6	10	26,27-F_6-1,25-$(OH)_2D_3$ (**17**)[18]
20-methyl	20Me	7	20-Me-1,25-$(OH)_2D_3$ (**38**)[19]

Table 2. Combination of Active Modifications

entry	modification	compound	cell diff[a]
1	24H + 26,27DH	24,26,27-trihomo-1,25-$(OH)_2D_3$ (**24**)[8a]	5 (u)
2	16E + 23Y	16-ene-23-yne-1,25-$(OH)_2D_3$ (**12**)[13a,14a]	10 (h)
3	22E + 24H	22-ene-24-homo-1,25-$(OH)_2D_3$ (**20**)[16a]	10 (h)
4	F2 + 24H	24-F_2-24-homo-1,25-$(OH)_2D_3$ (**25**)[16e,f]	10 (h)
5	22E + 24DH	22-ene-24,24-dihomo-1,25-$(OH)_2D_3$ (**21**)[16c]	10 (h)
6	22O + 24H + 26,27DH	22-oxa-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**19**)[8a]	20 (u)
7	F6 + 22E	26,27-F_6-22-ene-1,25-$(OH)_2D_3$ (**26**)[13a]	30 (h)
8	22E + 26,27DH	22-ene-26,27-dihomo-1,25-$(OH)_2D_3$ (**22**)[13c]	60 (h)
9	22E + 24E + 24H + 26,27DH	22,24-diene-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**23**)[13b]	67 (u)
10	16E + 23Y + F6	16-ene-23-yne-26,27-F_6-1,25-$(OH)_2D_3$ (**27**)[14b]	80 (h)
11	EP + 24H	20-epi-24-homo-1,25-$(OH)_2D_3$ (**31**)[8a]	200 (u)
12	EP + 24H + 26,27DH	20-epi-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**32**)[8a]	200 (u)
13	EP + 22E + 24H + 26,27DH	20-epi-22-ene-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**33**)[8d]	1000 (u)
14	EP + 22O + 24H	20-epi-22-oxa-24-homo-1,25-$(OH)_2D_3$ (**34**)[8a]	1176 (u)
15	EP + 22O + 24H + 26,27DH	20-epi-22-oxa-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**35**)[8a,4d]	20000 (u)

[a] Relative differentiating activity of U 937 (u) or HL-60 (h) cells when the reference value of 1,25-$(OH)_2D_3$ (**1**) is defined as 1.

Chart 1

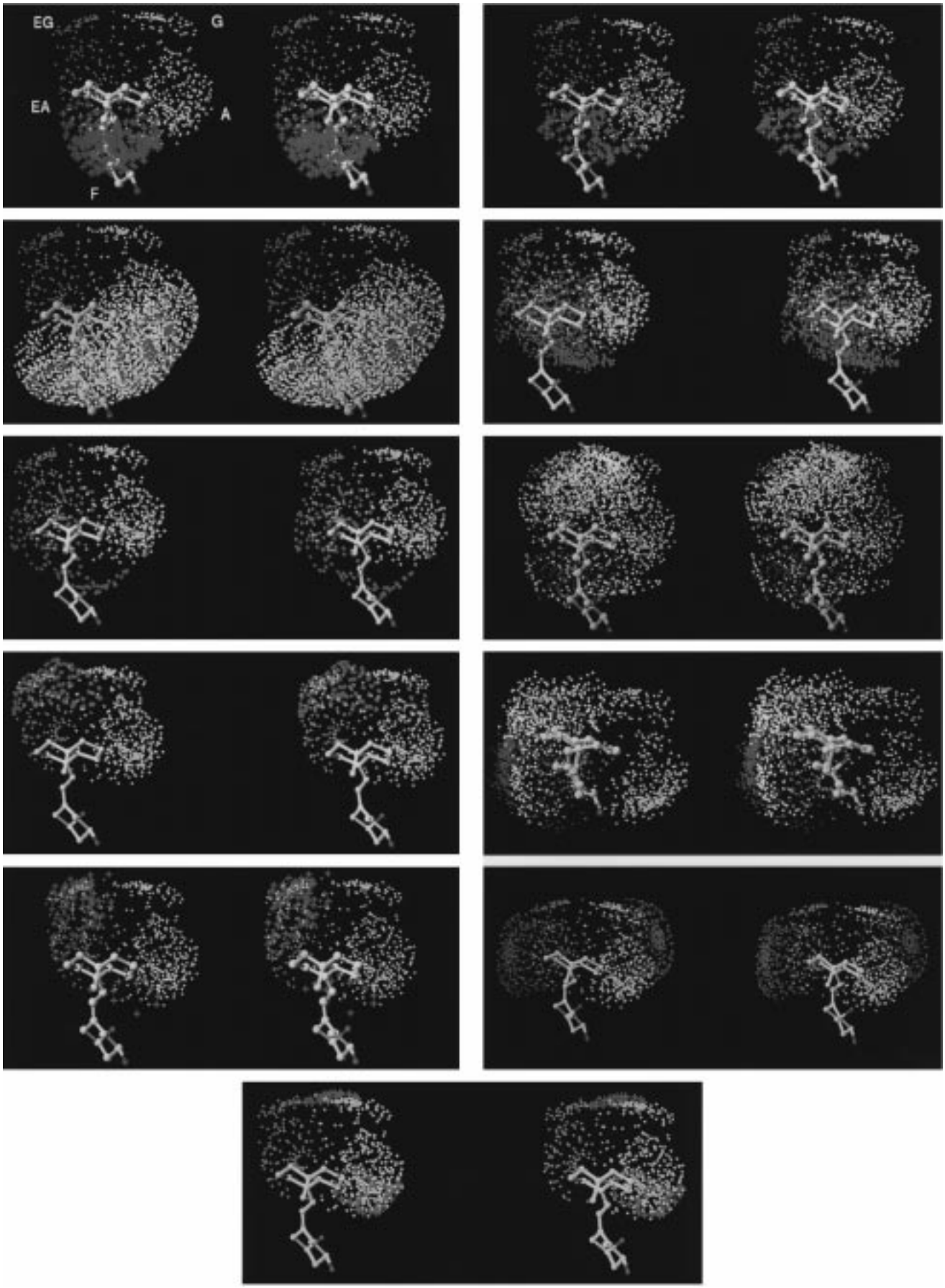
1		15		29	
2		16		30	
3		17		31[a]	
4		18[a]		32	
5		19		33	
6		20[a]		34[a]	
7[a]		21		35	
8[a]		22		36	
9[a]		23[a]		37[a]	
10[a]		24		38[a]	
11[a]		25		39[a]	
12[a]		26			
13		27		40[a]	
14		28		41	

X:

Y:

Z:

[a] The numbered bonds were rotated on the conformational search.


EG
G
EA
A
F

plotted on a dot map (graph sweep in SYBYL) in which the skeleton (the A, seco-B, C, and D ring moieties) is shown with a ball-and-stick model and the terminal oxygen position of each possible conformation is shown as a dot. (4) The mobile areas of the analogue's side chain were compared with the areas A, G, EA, and EG, by superimposing the dot maps of the analogues with those of 1,25-$(OH)_2D_3$ (**1**) and 20-epi-1,25-$(OH)_2D_3$ (**2**) at the skeleton.

Results

Compounds with a Side Chain at the F (Front) Region. We have shown that vitamin D compounds are highly potent when the 25-hydroxyl group is located in the A or EA region.[7a,b] We found a group of compounds in which the 25-oxygen occupies an area in front of the EA region. We call this area F. Compounds which occupy this area have 22-oxa, 16-ene, 22-ene, or 18-nor modifications, such as 22-oxa-1,25-$(OH)_2D_3$ (**7**),[12] 22-ene-1,25-$(OH)_2D_3$ (**8**),[13a] 22,24-diene-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**23**),[13b] 16-ene-1,25-$(OH)_2D_3$ (**9**),[13a,14a] and 18-nor-1,25-$(OH)_2D_3$ (**11**).[15]

22-Oxa-1,25-$(OH)_2D_3$ (7). 22-Oxa-1,25-$(OH)_2D_3$ (**7**) was the first compound in which cell-differentiating and calcemic activities of vitamin D were separated. Compound **7** has about 10-fold higher cell-differentiating activity than 1,25-$(OH)_2D_3$ (**1**), but it has low calcemic activity.[12] It has not been explained in structural terms why replacing $C(22)H_2$ with oxygen dramatically enhances potency. Conformational analysis provided one explanation. Replacing $C(22)H_2$ with oxygen significantly changes the mobility of the 25-oxygen. The 25-oxygen of **7** populates in the F region which lies in front of EA (Figure 2a), whereas the 25-oxygen of 1,25-$(OH)_2D_3$ (**1**) occupies A and G.

The $C(22)H_2$ and 22-oxa compounds differ in their minimum-energy conformation. These two compounds differ in their C(16)−C(17)−C(20)−C(22) torsion angle in the minimum-energy conformation. The $C(22)H_2$ compound adopts a C(16)−C(17)−C(20)−C(22) gauche(+) conformation (Figure 3, yellow), whereas the 22-oxa compound has a gauche(−) conformation (Figure 3, red). In the gauche(−) conformation, $C(22)H_2$ suffers severe steric repulsion from the $C(18)H_3$ group. However in the 22-oxa analogue this repulsion is reduced because the oxygen bears no hydrogen, so it adopts a C(16)−C(17)−C(20)−C(22) gauche(−) conformation. When vitamin D adopts a C(16)−C(17)−C(20)−C(22) gauche(+), the 25-oxygen tends to reside in the A and G regions, whereas when it adopts a gauche(−) conformation the 25-oxygen tends to locate in the F region.

22-Dehydro-1,25-$(OH)_2D_3$ derivatives. Introduction of a π-bond at C(22) similarly reduces the steric repulsion between the C(22) part and the $C(18)H_3$ group. Therefore, the 25-oxygen of 22-ene-1,25-$(OH)_2D_3$ (**8**)[13a] occupies wide areas of the F−A regions (Figure 2b). 22,-24-Diene-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**23**),[13b] which is undergoing clinical trials as a drug to treat cancer, occupies similar regions (Figure 2c, red crosses). Since this compound has an elongated and rigid conjugated diene structure, the area occupied by its 25-oxygen moves further toward the front and the mobile area is restricted compared with the more flexible 22-ene-24-homo-1,25-$(OH)_2D_3$ (**20**) (Figure 2c, white dots).

16-Ene-1,25-$(OH)_2D_3$ Derivatives.[14] The high potency of these vitamin D analogues has been known for a long time, but the reasons were not understood. Introducing a double bond to C(16) also directs the side chain to the front region F. Figure 2d shows that the area occupied by the 25-oxygen of 16-ene-1,25-$(OH)_2D_3$ (**9**) is similar to that occupied by the 22-oxa (Figure 2a) and 22-ene (Figure 2b) analogues. In the minimum-energy conformation, the calculated C(16)−C(17)−C(20)−C(22) torsion angle of 16-ene-1,25-$(OH)_2D_3$ (**9**) was −54°. Introducing a triple bond to C(23) and perfluorination at C(26) and C(27) create analogues with increased potency, **12** (Figure 2e) and **27**.

18-Nor-1,25-$(OH)_2D_3$ (11).[15] Removal of the angular $C(18)H_3$ group eliminates congestion between the methyl group and $C(22)H_2$. The side chain therefore can rotate nearly freely around the 17,20 bond. Thus, when all possible conformations were plotted, the area occupied by the 25-oxygen of **11** extended over a wide range (Figure 2f, white dots). However, when 20% stable conformations are selected, the 25-oxygen is highly concentrated in the F region (Figure 2f, red dots). Thus, we explain that this compound has high potency because its side-chain oxygen is located in the active F region.

Compounds with Side Chains in the EA and Left EA (L-EA) Regions. So far the 20-epimerization has the highest effect on increasing vitamin D potency. The HL-60 differentiating activity is enhanced 25−30-fold.[8] We reasoned that 20-epi-1,25-$(OH)_2D_3$ (**2**) has high potency because its 25-oxygen can occupy the most active EA region.[7a] In the minimum-energy conformation, 20-epi-1,25-$(OH)_2D_3$ analogues adopt the C(16)−C(17)−C(20)−C(22) anti torsion angle (Figure 3, cyan).

20-Epi-16-ene-1,25-$(OH)_2D_3$ Derivatives. The 20-epimerization of 16-ene analogues has the reverse effect:[14d,e] 20-Epi-16-ene-23-yne-1,25-$(OH)_2D_3$ (**37**) is one-half as active as 1,25-$(OH)_2D_3$ (**1**).[14d] Therefore, the 20-epimerization of 16-ene-23-yne **12** decreased potency by 1/20. This difference in potency can be understood by comparing the dot map of 16-ene-23-yne-1,25-$(OH)_2D_3$ (**12**) (Figure 2e) with that of 20-epimer **37** (Figure 2g). While the dots of **12** are found over the active front regions of A, F, and EA, those of **37** are distributed over the inactive rear regions of EG−G.

20-Epi-22-oxa-1,25-$(OH)_2D_3$ derivatives. We examined the effect of replacing $C(22)H_2$ with oxygen in the 20-epivitamin D series. Such replacement signifi-

Figure 2. Side-chain mobile area of potent 1,25-$(OH)_2D_3$ analogues compared with the four regions, A, G, EA, and EG, of **1** (yellow) and **2** (cyan) (stereoviews). Dot maps of each analogue were overlaid with those of **1** and **2**, the skeleton being superimposed: (a, row 1, left) 22-oxa-1,25-$(OH)_2D_3$ (**7**) (red); (b, row 1, right) 22-ene-1,25-$(OH)_2D_3$ (**8**) (red); (c, row 2, left) 22,24-diene-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**23**) (red crosses) and 22-ene-24-homo-1,25-$(OH)_2D_3$ (**20**) (white dots); (d, row 2, right) 16-ene-1,25-$(OH)_2D_3$ (**9**) (red); (e, row 3, left) 16-ene-23-yne-1,25-$(OH)_2D_3$ (**12**) (red); (f, row 3, right) 18-nor-1,25-$(OH)_2D_3$ (**11**) (white dots, all possible conformations; red dots, 20% stable conformations); (g, row 4, left) 20-epi-16-ene-23-yne-1,25-$(OH)_2D_3$ (**37**) (red); (h, row 4, right) 20-epi-24-homo-1,25-$(OH)_2D_3$ (**31**) (white) and 20-epi-22-oxa-24-homo-1,25-$(OH)_2D_3$ (**34**) (red); (i, row 5, left) 20-methyl-1,25-$(OH)_2D_3$ (**38**) (red); (j, row 5, right) (17*E*)- and (17*Z*)-ene-22-yne-24,26,27-trihomo-1,25-$(OH)_2D_3$ (**40** and **39**) (right and left red dots, respectively); (k, row 6) 23-yne-1,25-$(OH)_2D_3$ (**10**) (red).

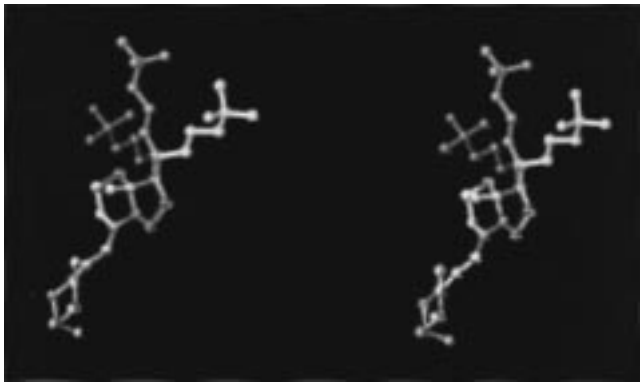


Figure 3. Three staggered conformations at the C(16)−C(17)−C(20)−C(22) torsion angle (stereoview). C(16)−C(17)−C(20)−C(22) gauche(+) (yellow), gauche(−) (red), and anti (cyan) conformations are overlaid.

cantly elevates potency in the 20-epi-24-homovitamin D series. Comparisons of entries 14 and 11 and/or 15 and 12 in Table 2 indicate an activation factor of 5−100 for the 22-oxa modification of 20-epi-24-homovitamins. A difference is also evident in the dot maps: Whereas the dots of the C(22)H_2 compound **31** are distributed over EG and EA (Figure 2h, white dots), EG being more densely populated, those of the 22-oxa analogue **34** are restricted in the left side of EA (L-EA) (Figure 2h, red dots). Their C(17)−C(20)−C(22)−C(23) torsion likely causes these effects. In the minimum-energy conformation, the C(22)H_2 compound **31** adopts a gauche(−) conformation, whereas the 22-oxa analogue **34** adopts an anti torsion angle. The 22-oxygen did not seem to affect the potency of analogues with a side chain of normal length,[8a] although the trends in the dot maps were similar to those of 24-homo analogues (dot map not shown). The potency of 22-oxa compound **18** was reduced by 1/6.6 relative to that of the C(22)H_2 compound **2**. Since oxygen can form hydrogen bonds and the contribution of a hydrogen bond to a protein−ligand complex can vary depending upon its microscopic environment, the hydrogen-bonding network might alter the effect of the 22-oxygen.

20-Methyl-1,25-$(OH)_2D_3$. 20-Methylation of 1,25-$(OH)_2D_3$ elevates the potency:[19] In terms of cell-differentiating activity (HL-60), 20-methyl-1,25-$(OH)_2D_3$ (**38**) is 7.1 times as active as 1,25-$(OH)_2D_3$ (**1**). Conformational analysis explains this effect of the 20-methyl group (Figure 2i). This modification makes the three staggered conformations at the C(16)−C(17)−C(20)−C(22) angle of similar energy, the energy increasing in the order anti, gauche(−), and gauche(+). Thus, the dots of **38** are distributed over the EG, EA, and F regions. The dot map suggests that the potency of **38** is between that of **2** and **7**.

17(20)-Dehydrovitamin D. Introduction of a double bond to the 17, 20 position fixes the side-chain direction. In (*Z*)- and (*E*)-17-ene-22-yne-24,26,27-trihomo-1,25-$(OH)_2D_3$ analogues (**39** and **40**),[13d] the 25-oxygen atoms are located in opposite directions and can never overlap (Figure 2j). The dots of **39** are located in the L-EA region, while those of **40** are distributed over the right side of the G and A regions. The 17*Z*-isomer **39** is 280-fold more potent than the 17*E*-isomer **40** (potency relative to that of **1** is 710 and 2.5, respectively). Thus, the large potency difference between the two geometrical isomers **39** and **40** can be explained by the difference of the direction of their 25-oxygen.

Other Modifications. 1. Elongation of the Side Chain. Elongation of the side chain increases potency 5−10-fold in both 20-normal[16a−c] and 20-epivitamin D[8a] analogues. Side-chain elongation in the 20-normal series expands the area accessible by the 25-oxygen to part of the EA region. This may affect potency. Another and more likely explanation common to both 20-normal and 20-epi compounds is that the 25-oxygen can become closer to the binding site of the ligand-binding domain (LBD) of VDR. If the 25-hydroxyl group of 1,25-$(OH)_2D_3$ (**1**) binds to LBD through a water molecule, that of the homologues can bind directly and tightly to the same binding site.

2. Entropy Effect by Restriction of Conformation. Introducing a triple bond often elevates potency. This function significantly restricts conformational mobility and thus favorably affects entropy in binding to the receptor. Generally, rigid compounds are more potent than flexible compounds with a similar structure.[21] The mobility of the side chains of 23-yne-1,25-$(OH)_2D_3$ (**10**) and 16-ene-23-yne-1,25-$(OH)_2D_3$ (**12**) is restricted compared with that of 1,25-$(OH)_2D_3$ (**1**) and 16-ene-1,25-$(OH)_2D_3$ (**9**), respectively, as shown in Figure 2k,e compared with Figure 2d. In accordance with these figures, the activity of the compounds **10** and **12** is higher than that of the respective compounds **1** and **9**.

Discussion

Since the discovery of active vitamin D_3, several hundred analogues have been synthesized mainly for developing new drugs. Data accumulated from the structure−function studies of the side-chain analogues have revealed nearly 10 modifications that enhance potency (cell differentiation). So far the most activating modification is 20-epimerization. Besides this, 22-oxa, 16-ene, 18-nor, 22-ene, 23-yne, side-chain carbon homologation, perfluorination at the terminal carbons, and 20-methylation also elevate potency. However a systematic theoretical study explaining the relationship between each of these modifications and activity has not been reported.

We have shown, using conformationally restricted analogues (**3**−**6**), that the area occupied by the 25-hydroxyl group of vitamin D derivatives can be correlated to their affinity for VDR.[7a] Furthermore, the transcriptional and cell-differentiating activities of these compounds (**3**−**6**) are demonstrated to be proportional to their VDR affinity.[22] We investigated the relationship between the cell-differentiating activity and the conformations of known potent vitamin D analogues based upon our active spatial area concept. We focused upon likely areas occupied by the biologically important 25-hydroxyl group. These areas were searched starting with the minimum-energy conformation, so they were usually found around the minimum-energy conformation of the compound concerned. We occasionally restricted conformations by energy to show more clearly the areas that were more likely to be occupied (as described above).

Our theory successfully correlated the structure and activity of the analogues shown in Tables 1 and 2.[23] We

Table 3. Spatial Region and Activity

region	VDR affinity[a]	cell diff[b]	representative compound
EG	1/100 (p) 1/250 (b)	ND[c]	22*S*-Me-20-epi-1,25-$(OH)_2D_3$ (**6**)[7a]
G	1/60 (p) 1/50 (b)	1/80 (h)	22*R*-Me-1,25-$(OH)_2D_3$ (**3**)[7a,b]
A + G	1 (p) 1 (b)	1 (h)	1,25-$(OH)_2D_3$ (**1**)
A	1/3 (p) 1/3 (b)	1 (h)	22*S*-Me-1,25-$(OH)_2D_3$ (**4**)[7a,b]
F	ND[c]	10 (h)	22-oxa-1,25-$(OH)_2D_3$ (**7**)[12]
EA + EG	5 (b)	25−30 (u)	20-epi-1,25-$(OH)_2D_3$ (**2**)[8a]
EA	20 (p) 11 (b)	200 (h)	22*R*-Me-20-epi-1,25-$(OH)_2D_3$ (**5**)[7a]
L-EA	ND[c]	1000 (u)	20-epi-22-oxa-24-homo-1,25-$(OH)_2D_3$ (**34**)[8a]

[a] Relative VDR affinity determined in our laboratory using porcine intestinal VDR (p) and bovine thymus VDR (b), the reference value of 1,25-$(OH)_2D_3$ (**1**) being defined as 1. [b] Relative differentiating activity of HL-60 (h) or U 937 (u) cells when the reference value of 1,25-$(OH)_2D_3$ (**1**) is defined as 1. [c] Not determined in our laboratory.

confirmed that the 25-oxygen of highly potent analogues locates around the EA region. We found that the 25-oxygen of potent 20-normal vitamin D analogues, which have 22-oxa, 16-ene, 22-ene, or 18-nor structures, is found in front of the most active EA region (F) (Figure 2a−f). This finding explains why these compounds are highly potent, although they do not have the 20-epi configuration. We argue that 20-epi-22-oxa-24-homo-vitamin D analogues **34** and **35** have high potency because their side chain is located in the left EA region (Figure 2h). Analogues with a fixed 17,20 bond, **39** and **40**, show that the left regions are more active than the right regions (Figure 2j). Also a comparison of 16-ene-23-yne-1,25-$(OH)_2D_3$ (**12**) with its 20-epimer (**37**) indicated that the front regions are more potent than the rear regions (Figure 2e,g). The relationship between the activity and 25-oxygen area is summarized in Table 3.

Modifications that do not significantly affect the conformational mobility of the 25-oxygen are side-chain elongation, methylation of the terminal geminal methyl groups, and perfluorination of the terminal methyls and $C(24)H_2$. Elongation of the side chain increases potency probably because the ligand and the protein can become closer. Conversely, truncating the side chain reduced the potency of 24-nor- (**28**) and 23,24-dinor-1,25-$(OH)_2D_3$ (**29**) to differentiate HL-60 cells by factors of 1/13 and 1/220, respectively, compared with 1,25-$(OH)_2D_3$.[16b] Fluorination and methylation of the terminal positions increase hydrophobicity of the terminal regions and increase the lipophilic contact area, which yields high binding energy.[21,24] Exposing the 25-hydroxyl group by removing the terminal gem-dimethyl groups lowers the cell-differentiating activity of 26,27-dinor-1,25-$(OH)_2D_3$ (**30**) 100-fold relative to 1,25-$(OH)_2D_3$.[16b]

The results of the present study showed that the biological activity of vitamin D derivatives is correlated with the area occupied by the 25-oxygen. Since this area is found around the minimum-energy conformation of vitamin D, these results indicate that the potency of vitamin D derivatives is correlated with their minimum-energy conformations. It is generally accepted that a ligand binds more tightly to the receptor when the ligand adopts a more stable conformation.[11,21] We confirmed that this is also true of vitamin D analogues.

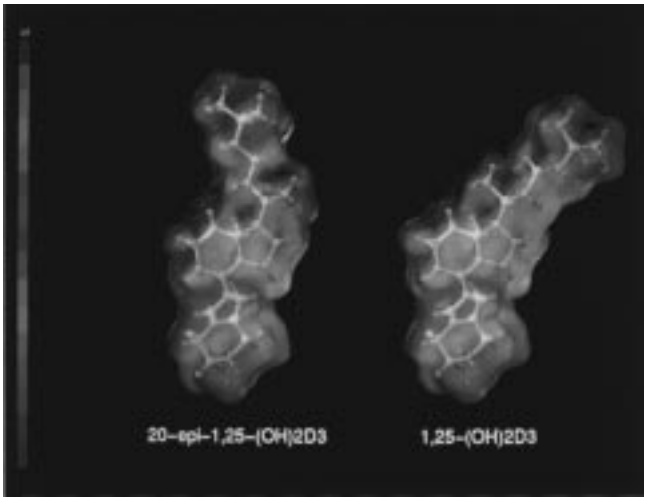


Figure 4. Surface structures of 1,25-$(OH)_2D_3$ (**1**) and 20-epi-1,25-$(OH)_2D_3$ (**2**) in which the side chains are directed to the A and EA regions, respectively. Colors show lipophilic properties, brown and blue being the colors of the most and the least lipophilic nature, respectively.

These studies also allowed us to image a topological feature of the LBD of VDR facing the 25-hydroxyl group of the ligand. Figure 4 shows the surface structures of 1,25-$(OH)_2D_3$ (**1**) and 20-epi-1,25-$(OH)_2D_3$ (**2**) in which the side chains are located in the A and EA regions, respectively (colors show lipophilic properties, brown and blue being the colors of the most and the least lipophilic nature, respectively).

In conclusion, we showed that the conformational search of vitamin D side chains helps predict the potency of vitamin D analogues. A similar approach would be useful for predicting the potency of analogues with seco-B and A ring modifications. These studies may also be used to discriminate the various activities of vitamin D, although this would require further study.

References

(1) Darwish, H. M.; DeLuca, H. F. Recent Advances in the Molecular Biology of Vitamin D Action. *Prog. Nucleic Acid Res. Mol. Biol.* **1996**, *53*, 321−344. 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 Version. *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.

(2) 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.

(3) DeLuca, H. F. The vitamin D system in the regulation of calcium and phosphorus metabolism. *Nutr. Rev.* **1979**, *37*, 161−193. Norman, A. W.; Roth, J.; Orci, L. The Vitamin D Endocrine System: Steroid Metabolism, Hormone Receptors, and Biological Response (Calcium Binding Proteins). *Endocrine Rev.* **1982**, *3*, 331−366.

(4) a) Ikekawa, N. Structures and biological activities of vitamin D metabolites and their analogues. *Med. Res. Rev.* **1987**, *7*, 333−366. (b) DeLuca, H. F.; Ostrem, V. K. Analogues of the hormonal form of vitamin D and their possible use in leukemia. *Prog. Clin. Biol. Res.* **1988**, *259*, 41−55. (c) Dai, H.; Posner, G. H. Synthetic Approaches to Vitamin D. *Synthesis* **1994**, 1383−1398. (d) Bouilion, R.; Okamura, W. H.; Norman, A. W. Structure−Function Relationships in the Vitamin D Endocrine System. *Endocrine Rev.* **1995**, *16*, 200−257. (e) Zhu, G. D.; Okamura, W. H. Synthesis of Vitamin D (Calciferol). *Chem. Rev.* **1995**, *95*, 1877−1952. (f) See also the eight volumes of the consecutive *Proceedings of Vitamin D Workshop*; Walter de Gruyter: Berlin, 1974−1994.

(5) DeLuca, H. F.; Schnoes, H. K. Metabolism and Mechanism of Action of Vitamin D. *Annu. Rev. Biochem.* **1976**, *45*, 631−666. DeLuca, H. F.; Schnoes, H. K. Vitamin D: Recent Advances. *Annu. Rev. Biochem.* **1983**, *52*, 411−439.

(6) DeLuca, H. F. The vitamin D story: a collaborative effort of basic science and clinical medicine. *FASEB J.* **1988**, *2*, 224−236. Reichel, H.; Koeffler, H. P.; Norman, A. W. The role of the vitamin D endocrine system in health and disease. *N. Engl. J. Med.* **1989**, *320*, 980−991. See also the eight volumes of the consecutive *Proceedings of Vitamin D Workshop*; Walter de Gruyter: Berlin, 1974−1994.

(7) (a) Yamamoto, K.; Sun, W.-Y.; Ohta, M.; Hamada, K.; DeLuca, H. F.; Yamada, S. Conformationally Restricted Analogues 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. *J. Med. Chem.* **1996**, *39*, 2727−2737. (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. (d) 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-dihydroxyvitamin D_3: Active Vitamin D_3 Analogues with Restricted Side Chain Conformation. *J. Org. Chem.* **1993**, *58*, 2530−2537.

(8) (a) 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. (b) Binderup, L. Immunological properties of vitamin D analogues and metabolites. *Biochem. Pharmacol.* **1992**, *43*, 1885−1892. (c) Calverley, M.; Binderup, L. Synthesis and biological evaluation of MC 1357, a new 20-epi-23-oxa-1α,25-dihydroxyvitamin D_3 analogue with potent nonclassical effects. *BioMed. Chem. Lett.* **1993**, *3*, 1845−1848. (d) Calverley, M. J.; Binderup, E.; Binderup, L. The 20-Epi modification in the vitamin D series; selective enhancement of "nonclassical" receptor mediated effects. In *Vitamin D: Gene Regulation, Structure−Function Analysis and Clinical Application*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1991; pp 163−164. (e) Gniadecki, R.; Serup, J. Stimulation of epidermal proliferation in mice with 1α,25-dihydroxyvitamin D_3 and receptor-active 20-epi analogues of 1α,25-dihydroxyvitamin D_3. *Biochem. Pharmacol.* **1995**, *49*, 621−624.

(9) Okamura, W. H.; Palenzuela, J. A.; Plumet, J.; Midland, M. M. Vitamin D: Structure−Function Analyses and the Design of Analogues. *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 Analogues. *BioMed. Chem. Lett.* **1993**, *3*, 1799−1804. See also ref 4.

(10) Ikekawa's group obtained the same conclusion using 22-hydroxy- and 22-methoxyvitamin D_3 analogues. Eguchi, T.; Yoshida, M.; Ikekawa, N. Synthesis and Biological Activities of 22-Hydroxy and 22-Methoxy Derivatives of 1α,25-Dihydroxyvitamin D_3: Importance of Side Chain Conformation for Biological Activities. *Bioorg. Chem.* **1989**, *17*, 294−307.

(11) Ajay; Murcko, M. A. Computational Method to Predict Binding Free Energy in Ligand−Receptor Complexes. *J. Med. Chem.* **1995**, *38*, 4953−4967.

(12) (a) Murayama, E.; Miyamoto, K.; Kubodera, N.; Mori, T.; Matsunaga, I. Synthetic studies of vitamin D_3 analogues. VIII. Synthesis of 22-oxavitamin D_3 analogues. *Chem. Pharm. Bull.* **1986**, *34*, 4410−4413. (b) 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. (c) Abe, J.; Nakano, T.; Nishii, Y.; Matsumoto, T.; Ogata, E.; Ikeda, K. A Novel vitamin D_3 Analogue, 22-Oxa-1,25-dihydroxyvitamin D_3, Inhibits the Growth of Human Breast Cancer in Vitro and in Vivo without Causing Hypercalcemia. *Endocrinology* **1991**, *129*, 832−837. (d) Pernalete, N.; Mori, T.; Nishii, Y.; Slatopolsky, E.; Brown, A. J. The Activity of 22-Oxacalcitriol in Osteoblast-Like (ROS 17/2.8) Cells. *Endocrinology* **1991**, *129*, 778−784.

(13) (a) Chen, T. C.; Persons, K.; Uskokovic, M. R.; Horst, R. L.; Holick, M. F. An evaluation of 1,25-dihydroxyvitamin D_3 analogues on the proliferation and differentiation of cultured human keratinocytes, calcium metabolism and the differentiation of human HL-60 cells. *J. Nutr. Biochem.* **1993**, *4*, 49−57. (b) Binderup, E.; Calverley, M. J.; Binderup, L. Synthesis and biological activity of 1α-hydroxylated vitamin D analogues with poly-unsaturated side chains. In *Vitamin D: Gene Regulation, Structure−Function Analysis and Clinical Application*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1991; pp 192−193. (c) Ishizuka, S.; Honda, A.; Mori, Y.; Kurihara, N.; Tatsumi, J.; Anal, K.; Ikeda, K.; Norman, A. W. Effects of vitamin D-binding proteins on biological functions of 1α,25-dihydroxyvitamin D_3 analogues. In *Vitamin D, a Pluripotent Steroid Hormone*: *Structural Studies, Molecular Endocrinology and Clinical Applications*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1994; pp 109−110. (d) Bretting, C.; Hansen, C. M.; Andersen, N. R. Chemistry and biology of 22,23-yne analogues of calcitriol. In *Vitamin D, a Pluripotent Steroid Hormone*: *Structural Studies, Molecular Endocrinology and Clinical Applications*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1994; pp 73−74.

(14) (a) Uskokovic, M. R.; Baggiolini, E.; Shiuey, S. J.; Jacobelli, J.; Hennessy, B.; Kiegiel, J.; Daniewski, A. R.; Pizzolato, G.; Coustney, L. F.; Horst, R. L. The 16-ene-analogues of 1,25-dihydroxycholecalciferol synthesis and biological activity. In *Vitamin D: Gene Regulation, Structure−Function Analysis and Clinical Application*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1991; pp 139−145. (b) Zhou, J. Y.; Norman, A. W.; Akashi, M.; Chen, D. L.; Uskokovic, M. R.; Aurrecoechea, J. M.; Dauben, W. G.; Okamura, W. H.; Koeffler, H. P. Development of a novel 1,25-$(OH)_2$-vitamin D_3 analogue with potent ability to induce HL-60 cell differentiation without modulating calcium metabolism. *Blood* **1991**, *78*, 75−82. (c) Norman, A. W.; Zhou, J.; Henry, H. L.; Uskokovic, M. R.; Koeffler, H. P. Structure−function studies on analogues of 1α,25-dihydroxyvitamin D_3: differential effects on leukemic cell growth, differentiation, and intestinal calcium absorption. *Cancer Res.* **1990**, *50*, 6857−6864. (d) Uskokovic, M. R.; Studzinski, G. P.; Gardner, J. P.; Reddy, S. G.; Campbell, M. J.; Koeffler, H. P. The 16-Ene Vitamin D Analogues. *Curr. Pharm. Des.* **1997**, *3*, 99−123. (e) Daehne, W.; Hansen, C. M.; Hansen, D.; Mathiasen, I. S. Synthesis and biological activity of new side chain analogues of 16-dehydrocalcitriol and its 20-epimer. In *Vitamin D, Chemistry, Biology and Clinical Applications of the Steroid Hormone*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Printing and Reprographics University of California: Riverside, 1997; pp 81−82.

(15) Sicinski, R. R.; Perlman, K. L.; Prahl, J.; Smith, C.; DeLuca, H. F. Synthesis and Biological Activity of 1α,25-Dihydroxy-18-nor-vitamin D_3 and 1α,25-Dihydroxy-18,19-dinor-vitamin D_3. *J. Med. Chem.* **1996**, *39*, 4497−4506.

(16) (a) Ostrem, V. K.; Tanaka, Y.; Prahl, J.; DeLuca, H. F.; Ikekawa, N. 24- and 26-homo-1,25-dihydroxyvitamin D_3: preferential activity in inducing differentiation of human-leukemia cell HL-60 in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2610−2614. (b) Ostrem, V. K.; Lau, W. F.; Lee, S. H.; Perlman, K.; Prahl, J.; Schnoes, H. K.; DeLuca, H. F. Induction of Monocytic Differentiation of HL-60 Cells by 1,25-dihydroxyvitamin D Analogues. *J. Biol. Chem.* **1987**, *262*, 14164−14171. (c) 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. (d) 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-Homologues. *J. Biol. Chem.* **1991**, *266*, 1910−1913. (e) Ikekawa, N.; Eguchi, T.; Hara, N.; Takatsuto, S.; Honda, A.; Mori, T.; Otomo, S. 26,27-Diethyl-1α,25-dihydroxyvitamin D_3 and 24,24-difluoro-24-homo-1α,25-dihydroxyvitamin D_3. *Chem. Pharm. Bull.* **1987**, *35*, 4362−4365. (f) Ikekawa, N. Chemical synthesis of vitamin D analogues with selective biological activities. In *Vitamin D Molecular, Cellular and Clinical Endocrinology*; Norman, A. W., Schaefer, K., Grigoleit, H. G., v Herrath, D., Eds.; Walter de Gruyter: Berlin, 1988; pp 25−33. (g) Honda, A.; Mori, Y.; Otomo, S.; Ishizuka, S.; Ikekawa, N. Effects of novel 26,27-dialkyl analogues of 1α,25-dihydroxyvitamin D_3 on differentiation-inducing activity of human promyelocytic leukemia (HL-60) cells in serum-supplemented or serum-free culture. *Steroid* **1991**, *56*, 142−147.

(17) (a) Yamada, S.; Ohmori, M.; Takayama, H. Synthesis of 24,24-difluoro-1α,25-dihydroxyvitamin D_3. *Chem. Pharm. Bull.* **1979**, *27*, 3196−3198. (b) Shiina, Y.; Abe, E.; Miyaura, C.; Tanaka, H.; Yamada, S.; Ohmori, M.; Nakayama, K.; Takayama, H.; Matsunaga, I.; Nishii, Y.; DeLuca, H. F.; Suda, T. Biological activity of 24,24-difluoro-1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 26,23-lactone in inducing differentiation of human myeloid leukemia cells. *Arch. Biochem. Biophys.* **1983**, *220*, 90−94. (c) Ikekawa, N.; Eguchi, T.; Hara, N.; Takatsuto, S.; Honda, A.; Mori, Y.; Otomo, S. 26,27-Diethyl-1α,25-dihydroxyvitamin D_3 and 24,24-difluoro-24-homo-1α,25-dihydroxyvitamin D_3. *Chem. Pharm. Bull.* **1987**, *35*, 4362−4365.

(18) (a) Inaba, M.; Okuno, S.; Nishizawa, Y.; Yukioka, K.; Otani, S.; Matsui-Yuasa, I.; Morisawa, S.; DeLuca, H. F.; Morii, H. Biological activity of fluorinated vitamin D analogues at C-26 and C-27 on human promyelocytic leukemia cells, HL-60. *Arch. Biochem. Biophys.* **1987**, *258*, 421−425. (b) Yukioka, K.; Otani, S.; Matsui-Yuasa, I.; Goto, H.; Morisawa, S.; Okuno, S.; Inaba, M.; Nishizawa, Y.; Morii, H. Biological activity of 26,26,26,27,

27,27-hexafluorinated analogues of vitamin D_3 in inhibiting interleukin-2 production by peripheral blood mononuclear cells stimulated by phytohemagglutinin. *Arch. Biochem. Biophys.* **1988**, *260*, 45−50.

(19) Danielsson, C.; Nayeri, S.; Wiesinger, H.; Thieroff-Ekerdt, R.; Carlberg, C. Potent Gene Regulatory and Antiproliferative Activities of 20-Methyl Analogues of 1,25-Dihydroxyvitamin D_3. *J. Cell. Biochem.* **1996**, *63*, 199−206. Neef, G.; Kirsch, G.; Schwarz, K.; Wiesinger, H.; Menrad, A.; Fähnrich, M.; Thieroff-Ekerdt, R.; Steinmeyer, A. 20-Methylvitamin D analogues. In *Vitamin D, a Pluripotent Steroid Hormone*: *Structural Studies, Molecular Endocrinology and Clinical Applications*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1994; pp 97−98.

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

(21) Böhm, H. J.; Klebe, G. What Can We Learn from Molecular Recognition in Protein−Ligand Complexes for the Design of New Drugs? *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2588−2614.

(22) Yamamoto, K.; et al. Unpublished results.

(23) There are a few exceptions. Our space group concept failed to explain the high potency of 20-alkoxy-1,25-$(OH)_2D_3$ derivatives (such as **41**): Hansen, K.; Hansen, C. M. Synthesis and biological activity of 20-hydroxylated vitamin D analogues. In *Vitamin D, a Pluripotent Steroid Hormone*: *Structural Studies, Molecular Endocrinology and Clinical Applications*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1994; pp 95−96. The difference in activity between 20-epi-22-oxa-1,-25-$(OH)_2D_3$ (**18**) and 20-epi-1,25-$(OH)_2D_3$ (**2**) cannot be explained solely by conformational properties as described above.

(24) O'Hagan, D.; Rzepa, H. S. Some influences of fluorine in bioorganic chemistry. *J. Chem. Soc., Chem. Commun.* **1997**, 645−652.

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