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Synthesis and Biological Activity of 22-Iodo- and (*E*)-20(22)-Dehydro Analogues of 1α,25-Dihydroxyvitamin D_3

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Abstract—Construction of 25-hydroxy-steroidal side chain substituted with iodine at C-22 was elaborated on a model PTAD-protected steroidal 5,7-diene and applied to a synthesis of (22*R*)- and (22*S*)-22-iodo-1α,25-dihydroxyvitamin D_3. Configuration at C-22 in the iodinated vitamins, obtained by nucleophilic substitution of the corresponding 22*S*-tosylates with sodium iodide, was determined by comparison of their iodine-displacement processes and cyclizations leading to isomeric five-membered (22,25)-epoxy-1α-hydroxyvitamin D_3 compounds. Also, 20(22)-dehydrosteroids have been obtained and their structures established by ^{1}H NMR spectroscopy. When compared to the natural hormone, (*E*)-20(22)-dehydro-1α,25-dihydroxyvitamin D_3 was found 4 times less potent in binding to the porcine intestinal vitamin D receptor (VDR) and 2 times less effective in differentiation of HL-60 cells. 22-Iodinated vitamin D analogues showed somewhat lower in vitro activity, whereas (22,25)-epoxy analogues were inactive. Interestingly, it was established that (22*S*)-22-iodo-1α,25-dihydroxyvitamin D_3 was 3 times more potent than its (22*R*)-isomer in binding to VDR and four times more effective in HL-60 cell differentiation assay. The restricted mobility of the side chain of both 22-iodinated vitamin D compounds was analyzed by a systematic conformational search indicating different spatial regions occupied by their 25-oxygen atoms. Preliminary data on the in vivo calcemic activity of the synthesized vitamin D analogues indicate that (*E*)-20(22)-dehydro-1α,25-dihydroxyvitamin D_3 and 22-iodo-1α,25-dihydroxyvitamin D_3 isomers were ca. ten times less potent than the natural hormone 1α,25-$(OH)_2D_3$ both in intestinal calcium transport and bone calcium mobilization.

Introduction

Metabolism of vitamins D has been widely studied in our and many other laboratories during the past three decades.[1,2] 1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol) is the most potent known metabolite in the vitamin D_3 series for the regulation of calcium and phosphate homeostasis.[3–6] The extensive metabolic studies have stimulated many efforts to prepare radiolabeled vitamin D_3 derivatives.[7,8] The availability of such radioactive compounds is of critical importance for numerous biomedical experiments. Therefore, over the past several years an effort has been made in our laboratory to synthesize tritium labeled vitamin D_2 and D_3 metabolites of still higher specific activity.[9–15] Recently, we have turned our attention to the vitamin D analogues containing radionuclids which can be detected by gamma spectrometry, namely, the radioiodinated derivatives of the natural hormone, 1α,25-$(OH)_2D_3$.

It was established that the nuclear receptor (VDR) for 1α,25-$(OH)_2D_3$ exists in more than 30 tissues and cancer cell lines.[16] It has become clear that important studies could be made possible if hormone analogues containing a γ-emitting isotope were available. Such vitamins would have marked advantages over the presently used tritium labeled ones. Since the specific activity of ^{125}I (ca. 2200 Ci/mmol) is much higher than that of ^{3}H (ca. 29 Ci/mmol) it would allow receptors to be detected with greater sensitivity. It was, therefore, of interest to obtain such an iodo vitamin D compound which could have similar tissue distribution properties as native hormone and, in general, behave similarly to this natural steroid in most biochemical analyses. As a consequence, our goal was to synthesize an iodinated analogue possessing considerable ability to bind to the intestinal nuclear 1α,25-$(OH)_2D_3$ receptor. It is well known that the natural hormone is characterized by a very high affinity for the VDR. Broad structure-function studies clearly demonstrated that deletion of the hydroxyl groups and almost all alterations of its carbon

Key words: Vitamin D_3 analogues; receptor binding; molecular modeling/mechanics; antiproliferative agents.

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skeleton resulted in considerable diminished affinities of the synthesized analogues for the VDR binding domains.[8,17–19] Such analogues are usually characterized by low calcemic activity and decreased potency of inducing cell differentiation.[20] Therefore, we have decided to synthesize a compound being a simple iodo derivative of the natural hormone. Thus, we attempted to establish such a position in the *seco*-B-steroidal carbon skeleton of the 1α,25-$(OH)_2D_3$ where the introduced iodine atom would not considerably alter either the basic hormone structure or its conformation. Additionally, the position would have to be relatively easily accessible during the synthesis and assure the resistance of the formed iodo derivative to deiodination processes. Taking into account the above requirements we excluded cyclohexane A-ring and hydrindane CD fragment from our consideration and focused on the steroidal side chain at C-17. Finally, we have decided to introduce the iodine into the side chain at the 22-position.

Nucleophilic substitution at C-22 appears to be the most popular method for construction of the 25-hydroxycholesterol side chain whereas Wittig reaction of steroidal 22-aldehydes is often used in the synthesis of 22,23-unsaturated analogues.[21] C-22 Aldehydes, those containing intact ABCD rings[22] and those possessing ring-B secosteroidal system,[23] are readily available compounds. Thus, introduction of iodine at C-22 seemed to be a reasonable choice for our studies, especially considering the literature data concerning similarly modified vitamin D compounds. Although the presence of a secondary 22-hydroxy or methoxy group resulted in considerably diminished biological activity of vitamin D analogues,[24–26] the corresponding derivatives of 1α,25-$(OH)_2D_3$ possessing a bulky hydrophobic 22-methyl substituents showed (especially 22*S*-isomer) significant affinity for intestinal VDR.[27] It was, therefore, expected that introduction of the iodine at the 22-position of the side chain might also provide biologically active vitamins. This paper describes the synthesis, conformational analysis and biological potency of epimeric 22-iodo-1α,25-$(OH)_2D_3$ compounds.

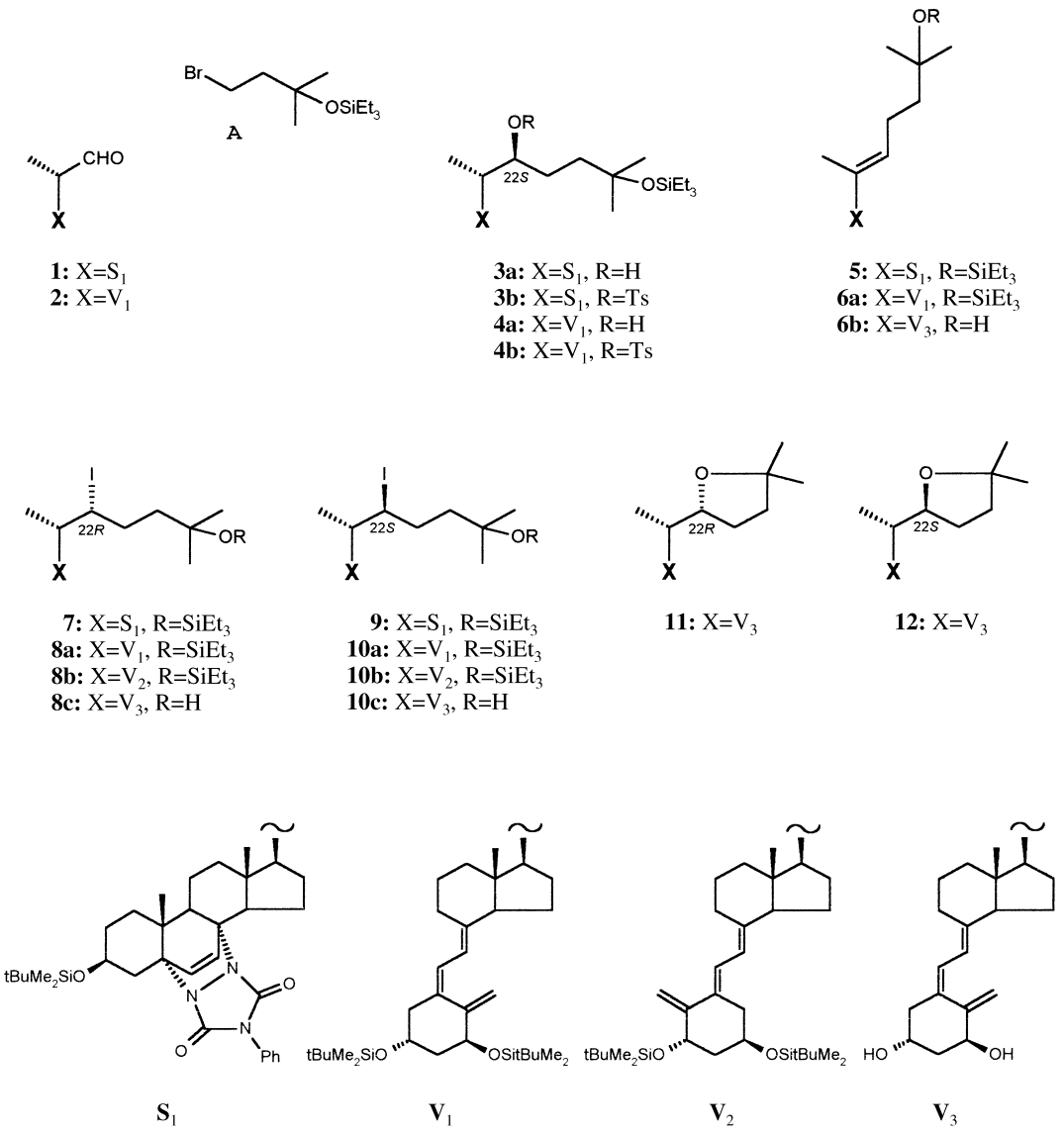
Results

Synthesis

We decided to test the synthetic pathway leading to 22-iodo vitamin D derivatives **8c** and **10c** (Scheme 1) using much less expensive model compounds derived from ergosterol. Thus, we used as a starting material C-22 aldehyde **1**, obtained in high yield by ozonolysis of a TBDMS-ether of PTAD-diene protected ergosterol,[28] and subjected it to a reaction with Grignard reagent, prepared from bromo compound **A**.[29] Since it is well known that steroidal (20*S*)-aldehydes, combined with organometallic reagents, form predominantly products which can be predicted by Cram's rule,[21] it was not surprising that we isolated the corresponding (22*S*)-hydroxy compound **3a** in 83% yield. Even careful HPLC analysis did not indicate the presence of the epimeric (22*R*)-alcohol in the reaction mixture. Treatment of **3a** with *p*-toluenesulphonyl chloride afforded quantitatively a tosylate **3b** which served as convenient model compound for the nucleophilic substitution process. The reaction of **3b** with sodium iodide was carried out in acetone:2-butanone (1:1) solvent system and required prolonged heating at 45°C for completion. After 48 h the mixture of products was carefully separated by HPLC and, in addition to the main 20(22)-olefinic product **5** (50%), two iodine-containing compounds **7** and **9** were also isolated in 13 and 26% yield, respectively. The presence of both epimeric 22-iodo derivatives could be easily explained taking into consideration the high reactivity of the aliphatic iodo compounds for nucleophilic substitution, including the halogen–halogen exchange processes. Classical examples of such 'double substitution' provide the reported formation of a racemic mixture of 2-iodooctanes on reaction of (*R*)-2-octyl mesylate with potassium iodide[30] and isolation of 3β-iodocholestane as a product of the reaction of 3β-cholestanyl tosylate with sodium iodide.[31]

HPLC analysis of the reaction mixture of tosylate **3b** with NaI indicated that a ratio of **7**:**9** was changed from approx. 1:1 to 1:2 after 24 and 48 h, respectively. That the interconversion of both isomers occurred in the process was further confirmed when individual iodo compounds **7** and **9** were subjected to the reaction conditions with NaI, analogous to those applied to the tosylate **3b**. HPLC analysis revealed that **9** was partially (20%) converted into isomeric **7** after 24 h and the composition of the products did not change considerably upon further heating, reaching 21% of **7** after 100 h. In the case of reaction of iodo compound **7** with NaI, however, the ratio 1:1.9 of **7** and **9** was established after 24 h, whereas 23% of **7** remained in the equilibrium mixture after 100 h. These observations clearly indicated the primary and secondary reaction products, and therefore, allowed us to ascribe the 22*R*- and 22*S*-configurations for the formed iodo compounds **7** and **9**, respectively. The assignment of the side-chain double bond configuration in the product **5** was based on its ^1H NMR spectrum, especially on the chemical shifts of the 18- and 21-methyl signals typical of (*E*)-20(22)-dehydrosteroids.[32–35] Compound **5** could also be obtained, albeit in a low yield (35%), by prolonged heating of the tosylate **3b** in pyridine.

An analogous reaction sequence was performed starting from the known[23] vitamin D 22-aldehyde **2**. Thus, the aldehyde was converted to the respective (22*S*)-hydroxy compound **4a** in 72% yield. Its tosyl derivative **4b** was subjected to the nucleophilic substitution with NaI, similar as in the case of the PTAD-adduct **3b**. In view of the known susceptibility of 5*Z*-vitamin D compounds to undergo an iodine-catalyzed isomerization to their 5*E*-isomers,[36,37] the reaction was carried out in the presence of mercury and in the dark. Monitoring of the reaction course by HPLC indicated that a primary substitution product **8a** was slowly transformed into the isomeric iodide **10a**. After 80 h at 45°C, preparative HPLC led to isolation of the main reaction product, namely, 20(22)-dehydrovitamin **6a** (32%) and iodo compounds **8a** (8%) and **10a** (20%). Both 22-iodovitamins, when protected



Scheme 1.

from light, can be stored for a prolonged time in a freezer (ca. −20°C) with neither 5,6-*E*/*Z* isomerization nor epimerization at C-22. However, both processes, if desired, can be performed easily. Thus, the isomerization of **8a** and **10a** to the respective 5*E*-compounds **8b** and **10b**, can be accomplished by the well-known procedure using iodine as a catalyst.[36] In order to achieve the interconversion between both C-22 epimers, each compound **8a** and **10a** can be subjected to the analogous nucleophilic substitution reaction with sodium iodide as described above for the tosylate **4b**. In both cases the similar equilibrium mixture can be reached consisting of epimeric (22*S*)- and (22*R*)-iodo compounds **10a** and **8a** in a ratio of about 3–4:1.

Deprotection of hydroxyl groups in the synthesized compounds **6a**, **8a** and **10a**, providing the corresponding 1α,25-dihydroxyvitamins **6b**, **8c** and **10c**, can be effectively accomplished using AG 50W-X4 ion exchange resin in methanol. In the case of iodo vitamins it was necessary to add mercury to the reaction mixture, carry out the hydrolysis in the dark and shorten the reaction time (ca. 10 h). Elongation of the time of the hydrolysis of vitamins **8a** and **10a** resulted in diminished yields of the desired vitamins **8c** and **10c**, and a steady built-up of less polar products (i.e. cyclic ethers **12** and **11**, respectively). These conversions, although undesired, were useful for assignment of configuration in the respective C(22)-epimeric iodo compounds by their correlation with the starting tosylate **4b**. Since the tosylate **4b**, when subjected to the analogous hydrolysis conditions, formed the tetrahydrofuran derivative **11** as the only isolable product, it was reasonable to ascribe to this compound a structure of (22*R*)-22,25-epoxy-1α-hydroxyvitamin D_3. Consequently, configurations at C-22 in the iodo vitamins **10** and the tosylate **4b** must be the same (22*S*).

Biological evaluation and conformational analysis

An initial examination of biological potency of the synthesized vitamins was undertaken by determining their

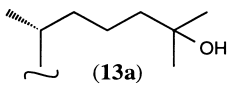
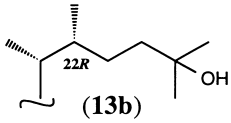
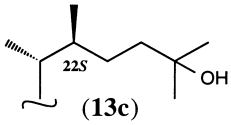
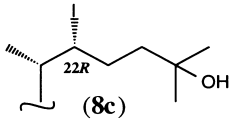
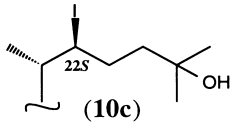
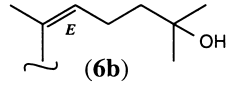
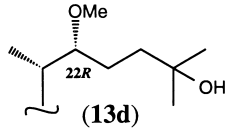
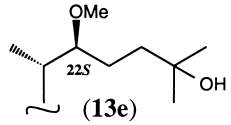
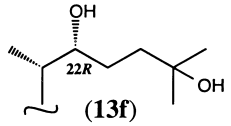
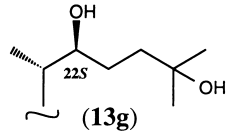
ability to displace tritiated 1α,25-$(OH)_2D_3$ from the porcine intestinal vitamin D receptor. The results shown in Table 1 demonstrate that (*E*)-20(22)-dehydrocalcitriol (**6b**) is 4 times less effective in binding to the receptor than the parent hormone 1α,25-$(OH)_2D_3$ (**13a**, Fig. 1), whereas iodo vitamins **8c** and **10c** show, respectively, 1/60 and 1/20 of the activity of the natural hormone. Cyclic ethers **11** and **12** show very little activity in this regard (data not shown). Cellular activity of the synthesized vitamins was established by studying the differentiation of HL-60 cells into monocytes. Also in this system side-chain olefin **6b** was the most active, followed by 22*S*- and 22*R*-vitamins **10c** and **8c**, being, respectively, 3 and 12 times less active than 1α,25-$(OH)_2D_3$.

It was interesting to compare biological activities of the synthesized iodo vitamins **10c** and **8c** with recent results of similar studies performed by Yamada's group on closely related 22-methylated vitamin D analogues **13b,c**.[27,38] These 22*S*- and 22*R*-methyl derivatives were 3 and 60 times, respectively, less active than the parent hormone **13a** in the porcine VDR assay. Measurements of cellular differentiation showed that 22*S*-isomer had similar potency as 1α,25-$(OH)_2D_3$, whereas its 22*R*-epimer was 80 times less potent. The observed differences in the receptor binding between 22-methylated analogues were related, through interesting conformational and molecular mechanics studies,[38,39] to the corresponding spatial regions A (for 22*S*-isomer) and G (for 22*R*-isomer) occupied by their 25-oxygen atoms.

Comparison of activity data listed above indicated that the binding potency of iodo- and methyl-derivatives was identical among 22*R*-substituted compounds, whereas in the case of 22*S*-isomers the methyl-substituted analogue showed significantly increased activity. In the latter case, the observed discrepancy can be the result of an increased electronegativity of an iodine atom (2.66 versus 2.55 for carbon). However, considering also substantial difference between size of iodine atom and methyl group, in terms of space-filling capacity (van der Waals radii 2.15 and 2.0, respectively), it is obvious that such bulky substituent can severely restrict the rotation around the C(20)–C(22) bond and, therefore, influence mobility and conformation of the C(17)-side chain. These considerations prompted us to perform conformational analysis of model 8-methylene compounds **14a,b,c** with 25-hydroxy- and 25-hydroxy-22-iodo-substituted side chains and compare the results with those of the corresponding 22-methyl counterparts.

We carried out MM^+ force field calculations of compounds **14a–c** by using the HyperChem molecular modeling program and performed a conformational search with its extension modules from ChemPlus program. These molecular mechanics studies were carried out according to protocol described in detail in Experimental. The distribution of the side-chain conformers resulted from this conformational study is presented in Table 2 together with steric energy (E_s) values of the corresponding global minimum structures. These most stable conformers and the respective positions of the

Table 1. VDR Binding affinities[a] and HL-60 differentiating activities[b] of 1α,25-$(OH)_2D_3$ and its 22-substituted analogues

Side chain analogue of 1α,25-$(OH)_2D_3$	VDR	HL-60
 (**13a**)	1	1
 (**13b**)	1/60[e]	1/80[f]
 (**13c**)	1/3[e]	1[f]
 (**8c**)	1/60[c]	1/12[d]
 (**10c**)	1/20[c]	1/3[d]
 (**6b**)	1/4[c]	1/2[d]
 (**13d**)	1/95[g]	1/170[h]
 (**13e**)	1/35[g]	1/4[h]
 (**13f**)		< 1/400[h]
 (**13g**)	< 1/1000[c,i]	1/13[h]

[a] Competitive binding of 1α,25-$(OH)_2D_3$ and the synthesized vitamin D analogues to the porcine intestinal vitamin D receptor (VDR). The experiments were carried out in triplicate on two different occasions. The values of VDR binding affinities are related to that of 1α,25-$(OH)_2D_3$ for which the value is set at 1.0.

[b] Induction of differentiation of HL-60 promyelocytes to monocytes by 1α,25-$(OH)_2D_3$ and the synthesized vitamin D analogues. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated three times. The values of HL-60 differentiation activity are related to that produced by 1α,25-$(OH)_2D_3$ for which the value is set at 1.0.

[c] Relative binding affinities to the porcine intestinal VDR determined in our laboratory.

[d] Determined in our laboratory.

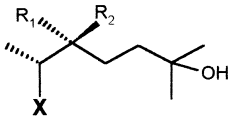
[e] Relative binding affinities to the porcine intestinal cytosol receptor.[39]

[f] Literature data.[38]

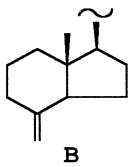
[g] Relative binding affinities to the chick intestinal cytosol receptor.[25]

[h] Literature data.[25]

[i] Compound prepared by deprotection of hydroxyl groups in **3a** with AG 50W-X4 resin in methanol (80% yield).



13a: X=V, R_1=R_2=H
13b: X=V, R_1=Me, R_2=H
13c: X=V, R_1=H, R_2=Me
13d: X=V, R_1=OMe, R_2=H
13e: X=V, R_1=H, R_2=OMe
13f: X=V, R_1=OH, R_2=H
13g: X=V, R_1=H, R_2=OH
14a: X=B, R_1=R_2=H
14b: X=B, R_1=I, R_2=H
14c: X=B, R_1=H, R_2=I



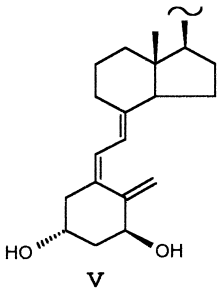
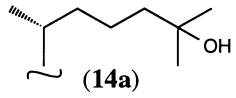
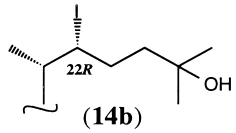
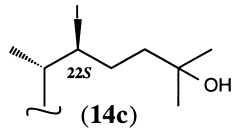


Figure 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol) and its 22-substituted analogues.

25-oxygen in the other lowest energy structures are shown in Figure 2. Figure 3 shows the result of superimposition of all conformers of **14a–c**, falling into 1 kcal/mol energy window, on the carbon skeleton of the global minimum structure of **14a**. It is immediately apparent from the above figures that location in space of the 25-oxygen, crucial for anchoring of the vitamin D side chain to the receptor, is different for the compounds studied. Thus, in the case of compound **14a**, the 25-hydroxy group, situated in the side chain that is characteristic of the natural hormone, occupies two spatial regions A and G (as defined by Yamada).[39] Due to restricted conformational mobility, the location in space of the 25-oxygens belonging to the side chain of 22*R*- and 22*S*-iodo compounds **14b** and **14c** is limited to single regions G and A, respectively. Detailed conformation-activity studies of Yamada et al.[38,39] indicated the relation between the receptor (VDR) affinity of the vitamin D analogue and the spatial region occupied by its side-chain 25-hydroxy group and showed that region A is more important than G. Thus, the observed difference in receptor binding properties of the iodo vitamins **8c** and **10c** can be explained, at least to some extent, by Yamada's "active space group concept".

As might be expected from X-ray crystallography of several steroids,[40] for the low energy conformers generated during our conformational search, only the gauche(+) conformation in relation to the dihedral angle C(16–17–20–22) was found. Thus, the largest substituent at C-20 (i.e. the remaining side chain fragment) is directed towards ring D and 'outside the molecule'. However, the conformation around the next two C–C bonds of the side chain is influenced by the presence of C(22)-substituents. X-ray data of the 25-OH-D_3[41] revealed that only the *anti* isomer, with respect to C(17–20–22–23) torsion angle, exists in the crystalline form. Our calculations, however, indicate that an alternative gauche(+) form is a minimum energy conformation for 25-hydroxy-8-methylene derivative **14a**; its most stable conformer was found to have ca. 0.2 kcal/mol lower steric energy than the lowest energy structure with the anti conformation.[42] In the remaining 9 least energy side-chain conformers of **14a**, falling into 1 kcal/mol energy window, both *gauche*(+) and *anti* conformations were present. However, the corresponding least energy side-chain conformers of the 22*R*-iodo compound **14b** adopted only one conformation in respect to the C(17–20–22–23) and C(20–22–23–24) torsion angles, gauche(+) and anti, respectively. In the case of the lowest energy conformers (0–1 kcal/mol energy window) of the 22*S*-iodo isomer **14c**, the *anti* conformation was the only preferred one in respect to the C(17–20–22–23) bond, whereas *anti* and *gauche*(+)

Table 2. Distribution of the side-chain conformers of model compounds **14a,b,c** resulted from conformational search

Model 8-methylene compound	Number of conformers (energy window in kcal/mol)					The lowest energy (*Es*) conformer (kcal/mol)
	1st step	2nd step		3rd step		
	(0–6)	(0–1)	(0–4)	(0–1)	(0–3)	
 (**14a**)	11	11	78	11	99	33.09[a]/33.31[b]
 (**14b**)	10	8	41	10	48	33.81[a]
 (**14c**)	9	15	49	15	41	33.65[b]

[a] *gauche*(+) Conformation in respect to the C(17–20–22–23) torsion angle.
[b] *anti* Conformation in respect to the C(17–20–22–23) torsion angle.

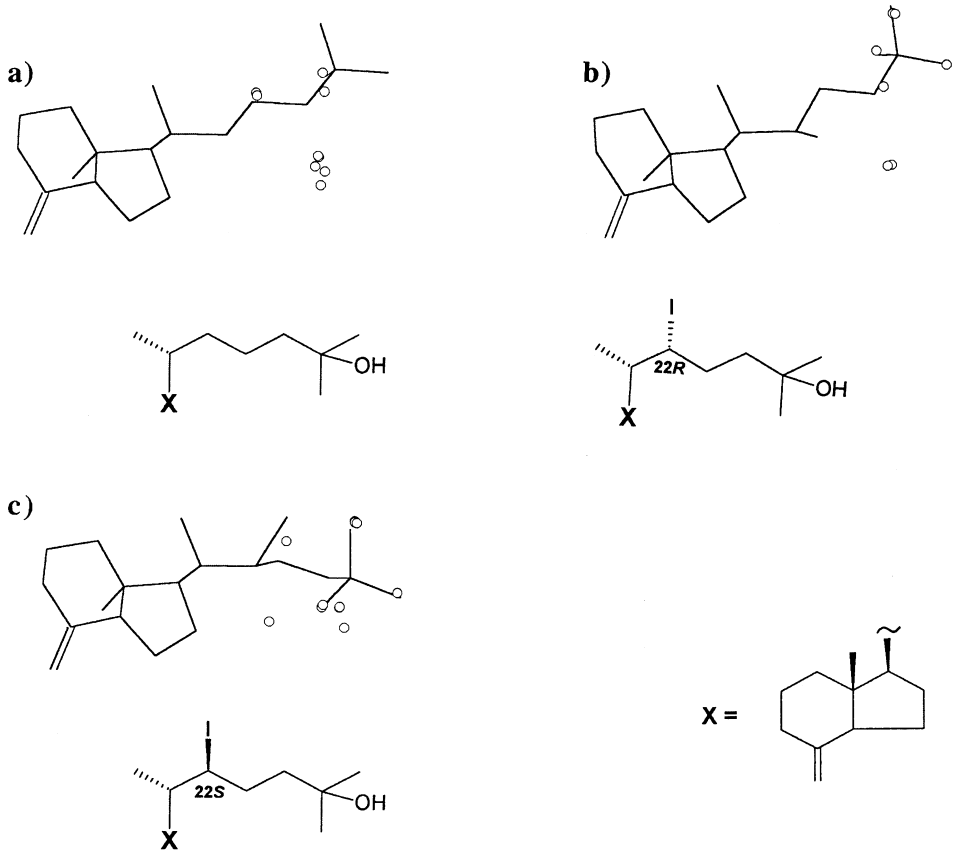


Figure 2. Stereoplot of the global energy minimum conformations (wireframe drawings) of the model 8-methylene compound **14a** with 25-hydroxyvitamin D_3 side chain (a), its 22*R*-iodo substituted analogue **14b** (b), and isomeric compound **14c** (c). The circles show the location of 25-oxygen atoms in the other higher energy conformers (energy window 1 kcal/mol). All hydrogen atoms are omitted.

forms were found for the neighboring C(20–22–23–24) bond.

Discussion

The side chain of the vitamin D_3 is a highly flexible fragment of this molecule. That the tertiary hydroxyl group, situated at its terminal carbon atom, plays an important role in anchoring the whole vitamin D compound to VDR is evident: removal of this substituent reduces the binding affinity by three orders of magnitude.[8] Therefore, vitamin D side chain has been a preferred target of many synthetic efforts and structure–activity studies.[7,17–19] During last few years interesting conformational analyses of 25-hydroxylated side chain have been performed by Okamura's[43,44] and Yamada's[38,39] groups. They introduced a concept of three-dimensional 'dot maps' showing the possible locations in space of 25-oxygen in the native hormone and its different side-chain analogues. They also attempted to correlate the receptor (VDR) affinity and cell differentiation potency of such analogues with the corresponding spatial regions that can be reached by their terminal 25-hydroxy groups. Stimulated by these findings we envisaged the conformational analysis of the 22-iodovitamins, whose synthesis is presented in this study. Results of our conformational search described above allow us to hypothesize that side chain of 22*R*-substituted vitamin D analogues does not fit well to the hydrophobic cavity of VDR, and it is likely that in the formed complex, the 22*R*-group attached to the ligand does not interact with the receptor. On the contrary, the side chain modified with 22*S*-substituent fits better to the receptor, the 22*S*-group being probably in the proximity of some hydrophobic center. Therefore, increasing electronegativity of the atom bonded to C-22 should result in enhancing repulsion between ligand and binding domain. In agreement with this postulate are literature data on 22*R*- and 22*S*-methoxy analogues of 1α,25-$(OH)_2D_3$ (**13d** and **13e**),[25] reported to be 95 and 35 times less potent, respectively, in binding to the chick intestinal vitamin D receptor than the parent hormone (Table 1). Not surprisingly, an analogue bearing a more polar substituent at C-22, that is, 1α,22*S*,25-$(OH)_3D_3$ (**13g**) showed even more significantly (by three orders of magnitude!) reduced affinity for VDR. The analogous relationship between potency of 22*S*-functionalized vitamins and electronegativity of 22-substituent can be observed in the HL-60 cell differentiation assay; in the case of 22*R*-compounds such clear-cut tendency does not exist. Interestingly, the literature data indicate that 22-hydroxy and 22-methoxy analogues of 1α,25-$(OH)_3D_3$ were essentially devoid of calcemic activity,[25] whereas 22-iodovitamins synthesized by us, especially 22*R*-isomer **8c**, were effective both in intestinal calcium

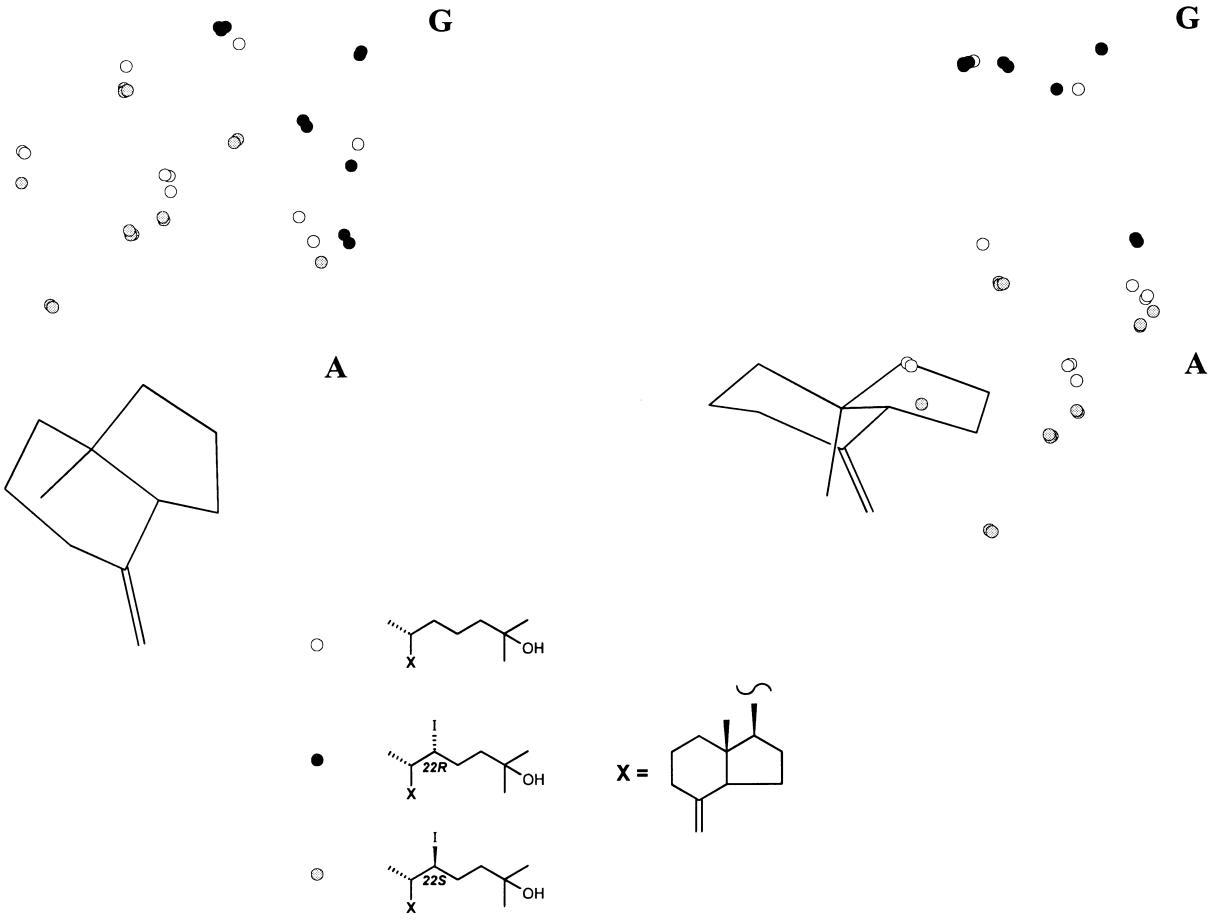


Figure 3. Two different stereoviews ilustrating results of conformational analysis of the side chain of model CD-ring compounds **14a–c**. Energy-minimized conformations of these compounds (energy window 1 kcal/mol) were overlaid on the global minimum conformer of **14a** as described in Experimental. The circles show the location of 25-oxygen atoms in the corresponding conformers. They are indicated two distinct spatial regions A and G defined by Yamada.[39] Side-chain carbons and all hydrogen atoms are omitted for clarity.

Table 3. Support of intestinal calcium transport and bone calcium mobilization by (*E*)-20(22)-dehydro- and 22-iodo-analogues of 1α,25-$(OH)_2D_3$ in vitamin D-deficient rats on a low-calcium diet[a]

Compound	Compound no.	Amount (μg/d)	Ca transport S/M (mean ± SEM)	Serum Ca (mean ± SEM)
None (control)		0	4.0 ± 0.2^b	4.1 ± 0.1^b
1α,25-$(OH)_2D_3$	**13a**	0.1	11.2 ± 1.2^c	5.9 ± 0.3^c
(*E*)-20(22)-dehydro-1α,25-$(OH)_2D_3$	**6b**	1.0	12.4 ± 0.7^d	5.2 ± 0.2^d
None (control)		0	3.8 ± 0.4^b	4.0 ± 0.1^b
1α,25-$(OH)_2D_3$	**13a**	0.1	10.5 ± 1.1^c	5.9 ± 0.2^c
(22*R*)-22-iodo-1α,25-$(OH)_2D_3$	**8c**	1.1	10.7 ± 0.7^d	6.2 ± 0.2^d
(22*S*)-22-iodo-1α,25-$(OH)_2D_3$	**10c**	1.1	13.2 ± 1.5^e	4.5 ± 0.1^e

[a] Weanling male rats were maintained on a 0.47% Ca diet for 1 week and then switched to a low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, they were dosed daily with the appropriate vitamin D compound for 7 consecutive days. All doses were administered intraperitoneally in 0.1 mL propylene glycol:ethanol (95:5). Controls received the vehicle. Determinations were made 24 h after the last dose. There were 5–6 rats per group. Statistical analysis was done by Student's *t*-test. Statistical data: serosal/mucosal (S/M), panel 1, b from c and d, $p < 0.001$; panel 2, b from c,d, and e, $p < 0.001$; serum calcium, panel 1, b from c, $p < 0.001$, b from d, $p = 0.05$; panel 2, b from c and d, $p < 0.001$, b from e, NS.

transport and bone calcium mobilization. Preliminary biological in vivo tests performed in our laboratory showed that both 22-iodovitamins, as well as *E*-20(22)-dehydro analogue do not differ considerably in their calcemic potency (Table 3). Thus, all these analogues have about one-tenth the activity of 1α,25-$(OH)_3D_3$.

Conclusion

A convenient method of the synthesis of 22-iodinated steroids from their easily available C(20)-aldehyde precursors have been elaborated. The introduction of iodine atom can be achieved by nucleophilic substitution of the

corresponding 22*S*-tosylates with sodium iodide. This reaction provides a convenient means for preparing radioiodinated derivatives if isotopically labeled iodides (e.g. NH_4 ^{123}I, Na ^{125}I, Na ^{131}I, etc.) are employed. Due to susceptibility of aliphatic iodo derivatives to nucleophilic substitution, such radioiodinated compounds can also be prepared by iodine–iodine isotope exchange process. The synthesized vitamins, especially (22*S*)-22-iodo-1α,25-$(OH)_2D_3$ (**10c**), retain substantial binding affinity for the vitamin D receptor and have the ability to differentiate HL-60 cells. Also, the isomer **10c** shows high in vivo calcium transport activity with little or no bone calcium mobilization activity. Therefore, the isotopically labeled compound **10c** has good potential for development as a new tracer in various known binding assays and for other experimental research studies. The observed differences in VDR binding affinity of both isomeric 22-iodovitamins **8c** and **10c** were attributed, after molecular modeling studies and conformational analysis, to the different spatial regions occupied by their 25-oxygens.

Experimental

Chemistry. Ultraviolet (UV) absorption spectra were recorded with a Hitachi Model 60-100 UV–vis spectrometer in the solvent noted. ^1H nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz with a Bruker AM-500 FT spectrometer in deuteriochloroform. Chemical shifts, (δ) are reported downfield from internal Me_4Si (δ 0.00). Low- and high-resolution mass spectra were recorded at 70 eV on a Kratos DS-50 TC instrument equipped with a Kratos DS-55 data system. High-resolution data were obtained by peak matching. Samples were introduced into the ion source maintained at 120–250°C via a direct insertion probe. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model 6 UK Universal injector, and a Model 486 tunable absorbance detector. Analytical and preparative separations were done using the same Zorbax-Sil (Dupont, Wilmington, PA) 6.2 mm×25 cm column (4 mL/min, 1500 psi).

Microanalyses of crystalline compounds were within ±0.4% of the theoretical values. THF was freshly distilled before use from sodium benzophenone ketyl under argon.

The starting (20*S*)-22-aldehyde **1**[22] was obtained by ozonolysis of PTAD adduct of 7-dehydrocholesterol TBDMS ether.[28] The synthesis of starting vitamin D (20*S*)-22-aldehyde **2** was described by us previously.[23]

1. ^1H NMR δ 0.084 and 0.105 (3H and 3H, each s, 2×$SiCH_3$), 0.842 (3H, s, 18-H_3), 0.885 (9H, s, Si-*t*-Bu), 0.973 (3H, s, 19-H_3), 1.146 (3H, d, J=6.8 Hz, 21-H_3), 3.13 (1H, dd, J=14.4, 4.9 Hz, 9α-H), 4.39 (1H, m, 3α-H), 6.22 and 6.36 (1H and 1H, each d, J=8.3 Hz, 6- and 7-H), 7.3–7.5 (5H, br m, Ar-H), 9.58 (1H, d, J=3.3 Hz, 22-H).

Reaction of C-22 aldehydes 1 and 2 with Grignard reagent derived from bromo compound A. (a) A solution of 4-bromo-2-methyl-2-(triethylsilyloxy)butane **A** (281 mg, 1 mmol) in anhydrous ether (0.5 mL, containing a catalytic quantity of iodine) was added dropwise to a stirred mixture of magnesium powder (29 mg, 1.2 mmol; ~50 mesh, Aldrich) in anhydrous ether (0.5 mL) under argon at room temperature with occasional warming it up to 35°C. After addition was complete the mixture was stirred for 1 h at room temperature and for 30 min at 40°C. Then it was cooled to 0°C and a solution of a PTAD-protected diene (20*S*)-C-22 aldehyde **1** (123 mg, 0.2 mmol) in anhydrous THF (0.5 mL, cooled to 0°C) was added dropwise. After the mixture was stirred for 20 min at 0°C and 1 h at room temperature it was quenched with aqueous solution of NH_4Cl (2 mL) and diluted with benzene (20 mL). The organic layer was separated, washed with brine and diluted $NaHCO_3$, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue using 20% ethyl acetate in hexane as an eluent yielded pure (22*S*)-alcohol **3a** (135 mg, 83%) as a foam: ^1H NMR δ 0.084 and 0.106 (3H and 3H, each s, 2×$SiCH_3$), 0.578 (6H, q, J=8.0 Hz, 3×$SiCH_2$), 0.813 (3H, s, 18-H_3), 0.885 (9H, s, Si-*t*-Bu), 0.936 (3H, d, J=7.7 Hz, 21-H_3), 0.944 (9H, t, J=8.0 Hz, 3×$SiCH_2C\mathit{H}_3$), 0.968 (3H, s, 19-H_3), 1.222 (6H, br s, 26- and 27-H_3), 3.12 (1H, dd, J=14.2, 5.1 Hz, 9α-H), 3.65 (1H, m, 22-H), 4.40 (1H, br m, 3α-H), 6.20 and 6.38 (1H and 1H, each d, J=8.2 Hz, 6- and 7-H), 7.3–7.5 (5H, br m, Ar-H); MS m/z (rel intensity) 819 (M^+, 19), 762 (48), 644 (M^+–RDA, 74), 497 (61), 119 (PhNCO, 100); exact mass calcd for $C_{47}H_{77}N_3O_5Si_2$ 819.5402, found 819.5407. Anal. calcd for $C_{47}H_{77}N_3O_5Si_2$: C, 68.82; H, 9.47; N, 5.13. Found: C, 69.08; H, 9.51; N, 5.10.

(b) A solution of the Grignard reagent was prepared from bromo compound **A** (94 mg, 0.33 mmol) and magnesium powder (9.7 mg, 0.4 mmol) in anhydrous ether (0.5 mL) as described in the preceding procedure. Then it was cooled to 0°C and a solution of (20*S*)-C-22 vitamin D aldehyde **2** (32 mg, 0.056 mmol) in anhydrous ether (0.3 mL, cooled to 0°C) was added dropwise. After the mixture was stirred for 20 min at 0°C and 75 min at room temperature it was quenched with aqueous solution of NH_4Cl (2 mL) and diluted with 4:1 (v/v) benzene:ether (20 mL). The organic layer was separated, washed with brine and diluted $NaHCO_3$, dried (Na_2SO_4) and evaporated. TLC and HPLC control indicated formation of only one (22*S*) of the two possible isomers. Pure (22*S*)-hydroxyvitamin D derivative **4a** was obtained as a colorless oil (31 mg, 72%) by preparative HPLC using 3.5% ethyl acetate in hexane as an eluent; peak at 30 mL was collected. **4a**: UV (EtOH) λ_{max} 264 nm, λ_{min} 225 nm, A264/A225=1.6; ^1H NMR δ 0.062 (12H, br s, 4×$SiCH_3$), 0.546 (3H, s, 18-H_3), 0.583 (6H, q, J=8 Hz, 3×$SiCH_2$), 0.887 (18H, s, 2×Si-*t*-Bu), 0.920 (3H, d, J=6.4 Hz, 21-H_3), 0.947 (9H, t, J=8 Hz, 3×$SiCH_2C\mathit{H}_3$), 1.221 and 1.225 (3H and 3H, each s, 26- and 27-H_3), 2.83 (1H, br d, J=12.5 Hz, 9β-H), 3.63 (1H, m, 22-H), 4.19 (1H, m, 3α-H), 4.37 (1 H, m, 1β-H), 4.87 and 5.18 (1H and 1H, each s, 19-H_2), 6.02 (1H, d, J=11.2 Hz, 7-H), 6.24 (1H, d, J=11.2 Hz, 6-H); MS m/z (rel intensity) 774 (M^+, 15), 642 (43), 75 (100);

exact mass calcd for $C_{45}H_{86}O_4Si_3$ 774.5834, found 774.5850.

Reaction of 22-hydroxy compounds 3a and 4a with *p*-toluenesulfonyl chloride. (a) To a solution of alcohol **3a** (75 mg, 0.093 mmol) in dry pyridine (200 μL) was added freshly recrystallized *p*-toluenesulfonyl chloride (49 mg, 0.26 mmol) and the reaction was allowed to proceed for 64 h at 4°C. The reaction mixture was poured into ice/saturated $NaHCO_3$ with stirring. After 40 min of stirring the aqueous suspension was extracted with 1:1 (v/v) benzene:ether (3×10 mL). The combined extracts were washed with saturated $NaHCO_3$, water, saturated $CuSO_4$, water again, dried (Na_2SO_4) and evaporated. The oily yellowish residue (quantitative yield) was pure enough to be used for the subsequent synthetic step. An analytical sample of the tosylate **3b** was obtained after HPLC purification using 10% ethyl acetate in hexane (R_v 16 mL): ^{1}H NMR δ 0.089 and 0.108 (3H and 3H, each s, 2×SiCH$_3$), 0.537 (6H, q, J=7.9 Hz, 3×SiCH$_2$), 0.723 (3H, s, 18-H$_3$), 0.887 (9H, s, Si-*t*-Bu), 0.921 (9H, t, J=7.9 Hz, 3×SiCH$_2$C*H*$_3$, 0.94 (3H, d, J ~7 Hz, 21-H$_3$), 0.943 (3H, s, 19-H$_3$), 1.123 and 1.157 (3H and 3H, each s, 26- and 27-H$_3$), 2.43 (3H, s, Ar-CH$_3$), 3.13 (1H, dd, J=14.2, 5.0 Hz, 9α-H), 4.39 (1H, br m, 3α-H), 4.49 (1H, t, J=7.0 Hz, 22-H), 6.18 and 6.30 (2H, each d, J=8.3 Hz, 6- and 7-H), 7.3–7.5 (7H, br m, Ar-H), 7.79 (2H, d, J=8.2 Hz, Ar-H).

(b) 22-Hydroxyvitamin D (**4a**) (28 mg, 0.036 mmol) was treated with *p*-toluenesulfonyl chloride (20 mg, 0.10 mmol) in dry pyridine (100 μL) as described above for compound **3a**. The crude oily product was purified by preparative HPLC using 2% ethyl acetate in hexane as an eluent. Pure tosylate **4b** (R_v 20 mL) was obtained as a colorless oil. **4b**: UV (hexane) λ_{max} 264 and 223 nm, λ_{min} 238 nm; ^{1}H NMR δ 0.059 and 0.067 (6H and 6H, each s, 4×SiCH$_3$), 0.479 (3H, s, 18-H$_3$), 0.528 (6H, q, J=8 Hz, 3×SiCH$_2$), 0.877 (18H, s, 2×Si-*t*-Bu), 0.915 (9H, t, J=8 Hz, 3×SiCH$_2$C*H*$_3$), 0.929 (3H, d, J=6.0 Hz, 21-H$_3$), 1.103 and 1.141 (3H and 3H, each s, 26- and 27-H$_3$), 2.43 (3H, s, Ar-CH$_3$), 2.80 (1H, br d, J=12.3 Hz, 9β-H), 4.19 (1H, m, 3α-H), 4.38 (1H, m, 1β-H), 4.58 (1H, t, J=7.1 Hz, 22-H), 4.86 and 5.19 (1H and 1H, each s, 19-H$_2$), 5.99 and 6.22 (1H and 1H, each d, J=11.2 Hz, 7- and 6-H), 7.32 and 7.80 (2H and 2H, each d, J=8 Hz, Ar-H); MS m/z (rel intensity) 928 (M$^+$, 1), 796 (2), 756 (3), 664 (3), 624 (47), 492 (27), 173 (100); exact mass calcd for $C_{52}H_{92}O_6Si_3S$ 928.5922, found 928.5894.

Reaction of 22-tosyloxy compounds 3b and 4b with sodium iodide. (a) To a stirred solution of tosylate **3b** (4.9 mg, 5 mmol) in 1:1 (v/v) acetone:2-butanone (200 μL) was added calcium carbonate (1 mg, 10 μmol) followed by sodium iodide (3.7 mg, 25 μmol). The resultant mixture was stirred and heated for 48 h at 45°C under argon. The reaction mixture was poured into water (10 mL) and extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with 1% $Na_2S_2O_3$ and water, dried (Na_2SO_4) and evaporated. The residue was separated by preparative HPLC using 5% ethyl acetate in hexane as an eluent. The 20(22)-dehydro compound **5** was collected at R_v 18 mL and repurified by HPLC using 3% ethyl acetate in hexane to give an analytically pure material (2.0 mg, 50%) as a foam. Pure isomeric 22-iodo compounds **7** (0.6 mg, 13%) and **9** (1.2 mg, 26%) were collected at R_v 23 mL and 28 mL, respectively. Some unreacted tosylate (0.6 mg, 12%) was also isolated at R_v 90 mL.

5. ^{1}H NMR δ 0.087 and 0.109 (3H and 3H, each s, 2×SiCH$_3$), 0.571 (6H, q, J=8 Hz, 3×SiCH$_2$), 0.658 (3H, s, 18-H$_3$), 0.888 (9H, s, Si-*t*-Bu), 0.950 (9H, t, J=8 Hz, 3×SiCH$_2$C*H*$_3$), 0.964 (3H, s, 19-H$_3$), 1.212 (6H, s, 26- and 27-H$_3$), 1.639 (3H, s, 21-H$_3$), 3.13 (1H, dd, J=14.1, 5.1 Hz, 9α-H), 4.40 (1H, br m, 3α-H), 5.23 (1H, t, J=7.0 Hz, 22-H), 6.19 and 6.36 (1H and 1H, each d, J=8.3 Hz, 6- and 7-H), 7.3–7.5 (5H, br m, Ar-H); MS m/z (rel intensity) 801 (M$^+$, <1), 626 (M$^+$–RDA, 100), 479 (77), 119 (PhNCO, 58); exact mass calcd for $C_{39}H_{70}O_2Si_2$ (M$^+$–RDA) 626.4915, found 626.4909.

7. ^{1}H NMR δ 0.082 and 0.104 (3H and 3H, each s, 2×SiCH$_3$), 0.568 (6H, q, J=7.6 Hz, 3×SiCH$_2$, 0.789 (3H, s, 18-H$_3$), 0.885 (9H, s, Si-*t*-Bu), 0.947 (9H, t, J=8 Hz, 3×SiCH$_2$C*H*$_3$), 0.964 (3H, s, 19-H$_3$), 1.167 (3H, d, J=7.0 Hz, 21-H$_3$), 1.189 and 1.194 (3H and 3H, each s, 26- and 27-H$_3$), 3.12 (1H, dd, J=14.0, 5.0 Hz, 9α-H), 4.39 (1H, br m, 3α-H), 4.46 (1H, d, J=11.5 Hz, 22-H), 6.21 and 6.36 (1H and 1H, each d, J=8.2 Hz, 6- and 7-H), 7.3–7.5 (5H, br m, Ar-H); MS m/z (rel intensity) 929 (M$^+$, <1), 872 (M$^+$–*t*-Bu, 1), 754 (M$^+$–RDA, 46), 607 (40), 173 (100), 119 (PhNCO, 46); exact mass calcd for $C_{39}H_{71}IO_2Si_2$ (M$^+$–RDA) 754.4037, found 754.4036.

9. ^{1}H NMR δ 0.083 and 0.106 (3H and 3H, each s, 2×SiCH$_3$), 0.568 (6H, q, J=7.6 Hz, 3×SiCH$_2$), 0.870 (3H, s, 18-H$_3$), 0.885 (9H, s, Si-*t*-Bu), 0.943 (9H, t, J=7.9 Hz, 3×SiCH$_2$C*H*$_3$), 0.969 (3H, s, 19-H$_3$), 0.975 (3H, d, J=6.9 Hz, 21-H$_3$), 1.191 and 1.212 (3H and 3H, each s, 26- and 27-H$_3$), 3.12 (1H, dd, J=14.2, 5.1 Hz, 9α-H), 4.16 (1H, m, w/2=18 Hz, 22-H), 4.40 (1H, br m, 3α-H), 6.21 and 6.37 (1H and 1H, each d, J=8.2 Hz, 6- and 7-H), 7.3–7.5 (5H, br m, Ar-H); MS m/z (rel intensity) 929 (M$^+$, 1), 872 (M$^+$–*t*-Bu, 2), 754 (M$^+$–RDA, 80), 607 (58), 173 (100), 119 (PhNCO, 38); exact mass calcd for $C_{39}H_{71}IO_2Si_2$ (M$^+$–RDA) 754.4037, found 754.4029.

(b) To a stirred solution of vitamin D tosylate (**4b**) (4.6 μg, 5 μmol) in 1:1 (v/v) acetone:2-butanone (200 mL) was added a drop of mercury (220 mg) and calcium carbonate (1 mg, 10 μmol) followed by sodium iodide (3.7 mg, 25 μmol). The resultant mixture was stirred and heated for 80 h at 45°C in the dark under argon, by which time no starting material remained. The reaction mixture was poured into water (10 mL) and extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with 1% $Na_2S_2O_3$ and water, dried (Na_2SO_4) and evaporated. The workup of the reaction mixture and following chromatographic separations were done using subdued light in the laboratory. The mixture of products was separated by preparative HPLC using 0.1% ethyl acetate in hexane

as an eluent. The 20(22)-dehydrovitamin (**6a**) was collected at R_v 37 mL and repurified by HPLC in the same solvent system to give an analytically pure material (1.2 mg, 32%). The peaks of 22-iodo vitamins **10a** and **8a** partially overlapped (R_v 48 and 53 mL, respectively) but rechromatography (or recycling of both peaks) afforded pure 22*R*-iodocompound **8a** (0.4 mg, 8%) and its 22*S*-isomer **10a** (0.9 mg, 20%).

6a. UV (hexane) λ_{max} 265.0 nm, λ_{min} 228.5 nm (A265/A228 = 1.7); ^{1}H NMR δ 0.063 and 0.072 (6H and 6H, each s, 4×SiCH$_3$), 0.403 (3H, s, 18-H$_3$), 0.567 (6H, q, J = 8 Hz, 3×SiCH$_2$), 0.884 (18H, s, 2×Si-*t*-Bu), 0.946 (9H, t, J = 8 Hz, 3×SiCH$_2$C*H*$_3$), 1.264 (6H, br s, 26- and 27-H$_3$), 1.630 (3H, s, 21-H$_3$), 2.84 (1H, br d, J = 12.8 Hz, 9β-H), 4.19 (1H, m, 3α-H), 4.38 (1H, m, 1β-H), 4.87 (1H, s, one of 19-H$_2$), 5.19 (2H, m, one of 19-H$_2$ and 22-H), 6.02 and 6.24 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS *m*/*z* (rel intensity) 756 (M^+, 12), 624 (31), 248 (47), 117 (100); exact mass calcd for $C_{45}H_{84}O_3Si_3$ 756.5728, found 756.5707.

8a. UV (hexane) λ_{max} 264.0 nm, λ_{min} 227.0 nm (A264/A227 = 1.8); ^{1}H NMR δ 0.064 (12H, br s, 4×SiCH$_3$), 0.517 (3H, s, 18-H$_3$), 0.568 (6H, q, J = 8 Hz, 3×SiCH$_2$), 0.875 (18*H*, br s, 2×Si-*t*-Bu), 0.950 (9H, t, J = 8 Hz, 3× SiCH$_2$CH$_3$), 1.158 (3H, d, J = 6.8 Hz, 21-H$_3$), 1.179 (6H, br s, 26- and 27-H$_3$), 2.83 (1H, br d, J ~13 Hz, 9β-H), 4.19 (1H, m, 3α-H), 4.37 (1H, m, 1β-H), 4.45 (1H, d, J = 11 Hz, 22-H), 4.86 and 5.18 (1H and 1H, each s, 19-H$_2$), 6.02 and 6.23 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS *m*/*z* (rel intensity) 884 (M^+, 20), 752 (54), 624 (24), 248 (100); exact mass calcd for $C_{45}H_{85}O_3Si_3I$ 884.4851, found 884.4899.

10a. UV (hexane) λ_{max} 264.0 nm, λ_{min} 227.0 nm (A264/A227 = 1.6); ^{1}H NMR δ 0.058 and 0.064 (6H and 6H, each s, 4×SiCH$_3$), 0.569 (6H, q, J = 8 Hz, 3×SiCH$_2$), 0.597 (3H, s, 18-H$_3$), 0.875 (18H, br s, 2×Si-*t*-Bu), 0.944 (9H, t, J = 8 Hz, 3×SiCH$_2$C*H*$_3$),0.964 (3H, d, J = 6 Hz, 21-H$_3$), 1.194 and 1.211 (3H and 3H, each s, 26- and 27-H$_3$), 2.82 (1H, br d, J ~13 Hz, 9β-H), 4.19 (2H, m, 3α- and 22-H), 4.37 (1H, m, 1β-H), 4.86 and 5.18 (1H and 1H, each s, 19-H$_2$), 6.02 and 6.23 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS *m*/*z* (rel intensity) 884 (M^+, 21), 752 (62), 624 (22), 248 (100); exact mass calcd for $C_{45}H_{85}O_3Si_3I$ 884.4851, found 884.4875.

Elimination of *p*-toluenesulfonate ester 3b in pyridine. A solution of tosylate (**3b**) (1 mg, 1 μmol) in a dry pyridine (200 μL) was heated under argon for 48 h at 70°C. Solvent was evaporated, the residue taken up in ethyl acetate and the solution was washed with saturated $CuSO_4$, water and saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated. HPLC separation of the residue using 3% ethyl acetate in hexane yielded the olefin **5** (0.29 mg, 35%; collected at 52 mL).

5,6-Double bond isomerization of 22-iodo vitamin D compounds 8a and 10a. Treatment of compound **8a** in ether with a catalytic amount of iodine (2% of the amount of **8a**), while keeping the solution under diffuse daylight for 1 h, resulted in *cis* to *trans* isomerization, and after HPLC separation using 0.1% ethyl acetate in hexane the 5,6-*trans* isomer **8b** was obtained (R_v 70 mL). Similarly, compound **10a**, upon treatment with iodine under the above conditions, was isomerized to **10b**, which can be obtained in pure form after HPLC (conditions as above) separation (R_v 60 mL).

8b. ^{1}H NMR δ 0.532 (3H, s, 18-H$_3$), 1.167 (3H, d, J = 6 Hz, 21-H$_3$), 1.185 (6H, br s, 26- and 27-H$_3$), 2.88 (1H, br d, J ~13 Hz, 9β-H), 4.22 (1H, m, 3α-H), 4.46 (1H, d, J = 11 Hz, 22-H), 4.54 (1H, m, 1β-H), 4.95 and 4.99 (1H and 1H, each s, 19-H$_2$), 5.83 and 6.46 (1H and 1H, each d, J = 11 Hz, 7- and 6-H).

10b. ^{1}H NMR δ 0.609 (3H, s, 18-H$_3$), 0.967 (3H, d, J = 6 Hz, 21-H$_3$), 1.201 and 1.216 (3H and 3H, each s, 26- and 27-H$_3$), 2.88 (1H, br d, J = 13 Hz, 9β-H), ca. 4.2 (2H, m, 3α- and 22-H), 4.57 (1H, m, 1β-H), 4.95 and 4.99 (1H and 1H, each s, 19-H$_2$), 5.83 and 6.47 (1H and 1H, each d, J = 11 Hz, 7- and 6-H).

Hydrolysis of tosylate 4b and 20(22)-dehydrovitamin 6a. (a) To a solution of vitamin D tosylate (**4b**) (300 μg) in benzene (20 μL) was added AG 50W-X4 ion exchange resin (5 mg, prewashed with methanol) in anhydrous methanol (50 μL). The mixture was vigorously stirred at room temperature for 13 h under argon, and it was diluted with 1:1 (v/v) ether:ethyl acetate (1 mL). The solution was decanted and transferred to a separatory funnel and the resin was washed with 1:1 ether:ethyl acetate (2×2 mL). The combined organic phase was washed with 5 mL portions of brine and saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated. Purification of the product by HPLC using 1:1 (v/v) ethyl acetate:hexane as an eluent provided an oily (22*R*,25)-epoxy vitamin **11** (84 μg, 63%) collected at R_v 34 mL. **11**: UV (EtOH) λ_{max} 264.5 nm, λ_{min} 227.5 nm (A264/A227 = 1.8); ^{1}H NMR δ 0.553 (3H, s, 18-H$_3$), 0.915 (3H, d, J = 6.3 Hz, 21-H$_3$), 1.230 and 1.240 (3H and 3H, each s, 26- and 27-H$_3$), 2.84 (1H, br d, J = 12.9 Hz, 9β-H), 4.05 (1H, m, w/2 = 16 Hz, 22-H), 4.23 (1H, m, 3α-H), 4.43 (1H, m, 1β-H), 5.00 and 5.32 (1H and 1H, each s, 19-H$_2$), 6.02 and 6.38 (1H and 1H, each d, J = 11.0 Hz, 7- and 6-H); MS *m*/*z* (rel intensity) 414 (M^+, 8), 396 (M^+–H_2O, 11), 378 (M^+–2H_2O, 4), 152 (13), 134 (42), 99 (100); exact mass calcd for $C_{27}H_{42}O_3$ 414.3134, found 414.3132.

(b) Deprotection of hydroxyl groups in (*E*)-20(22)-dehydrocompound **6a** (0.93 mg) was performed under conditions identical to those used for the conversion of **4b** into **11**. Crude product was purified by HPLC using 1:1 (v/v) ethyl acetate:hexane as an eluent. Crystalline 20(22)-dehydrocalcitriol **6b** (337 μg, 66%) was eluted at R_V 59 mL. **6b**: UV (EtOH) λ_{max} 265.0 nm, λ_{min} 228.5 nm (A265/A228 = 1.8); ^{1}H NMR δ 0.424 (3H, s, 18-H$_3$), 1.231 (6H, br s, 26- and 27-H$_3$), 1.651 (3H, s, 21-H$_3$), 2.84 (1H, br d, J = 12.2 Hz, 9β-H), 4.23 (1H, m, 3α-H), 4.44 (1H, m, 1β-H), 5.00 (1H, s, one of 19-H$_2$), 5.23 (1H, t, J = 7.1 Hz, 22-H), 5.33 (1H, s, one of 19-H$_2$), 6.03 and 6.38 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS *m*/*z* (rel intensity) 414 (M^+, 8), 396 (M^+–H_2O, 15), 378 (M^+–2H_2O, 10), 152 (37), 134 (100); exact mass calcd for $C_{27}H_{42}O_3$ 414.3134, found 414.3138.

Deprotection of hydroxyl groups in 22-iodovitamins 8a and 10a. (a) To a solution of protected compound **8a** (1 mg) in anhydrous benzene (50 μL) was added AG 50W-X4 ion exchange resin (20 mg, prewashed with methanol) as a slurry in anhydrous methanol (200 μL). A drop of mercury (400 mg) was added and the resultant mixture was vigorously stirred at room temperature for 10 h in the dark under argon. The mixture was diluted with 1:1 (v/v) ether:ethyl acetate (1 mL), the solution was decanted and transferred to a separatory funnel and the resin was washed with 1:1 ether:ethyl acetate (2×2 mL). The combined organic phase was washed with 5 mL portions of brine, 1% $Na_2S_2O_3$, saturated $NaHCO_3$, and brine again, dried (Na_2SO_4) and evaporated (temp. below 35°C). The work up of the reaction mixture and following chromatographic separations were done using subdued light in the laboratory. Preparative HPLC using 1:1 (v/v) ethyl acetate:hexane as an eluent provided small amount (< 50 μg) of (22*S*,25)-epoxy-1α-hydroxyvitamin D_3 (**12**) and 0.33 mg (52%) of (22*R*)-iodo-1α,25-dihydroxyvitamin D_3 (**8c**) eluted at R_v 28 and 63 mL, respectively.

8c. UV (EtOH) λ_{max} 264 nm, λ_{min} 228 nm, A264/A228 = 1.7; ^{1}H NMR δ 0.535 (3H, s, 18-H_3), 1.172 (3H, d, J = 6.5 Hz, 21-H_3), 1.224 (6H, br s, 26- and 27-H_3), 2.82 (1H, br d, J = 13 Hz, 9β-H), 4.24 (1H, m, 3α-H), 4.45 (1H, m, 1β-H), 4.47 (1H, d, J = 11.6 Hz, 22-H), 5.00 and 5.33 (1H and 1H, each s, 19-H_2), 6.02 and 6.37 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (rel intensity) 542 (M^+, 8), 524 (M^+–H_2O, 64), 506 (M^+–2 H_2O, 19), 414 (M^+–HI, 10), 396 (M^+–HI–H_2O, 62), 378 (M^+–HI–2 H_2O, 9), 99 (100); exact mass calcd for $C_{27}H_{43}IO_3$ 542.2257, found 542.2248; calcd for $C_{27}H_{41}IO_2$ (M^+–H_2O) 524.2151, found 524.2136.

12. ^{1}H NMR δ 0.533 (3H, s, 18-H_3), 0.910 (3H, d, J = 6.8 Hz, 21-H_3), 1.191 and 1.236 (3H and 3H, each s, 26- and 27-H_3), 2.82 (1H, br d, J = 13.3 Hz, 9β-H), 4.08 (1H, t, J = 7.0 Hz, 22-H), 4.22 (1H, m, 3α-H), 4.42 (1H, m, 1β-H), 5.00 and 5.32 (1H and 1H, each s, 19-H_2), 6.01 and 6.38 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (rel intensity) 414 (M^+, 33), 396 (M^+–H_2O, 49), 378 (M^+–2 H_2O, 21), 152 (18), 134 (46), 99 (100); exact mass calcd for $C_{27}H_{42}O_3$ 414.3134, found 414.3129.

(b) The isomeric 22-iodo vitamin **10a** (1 mg) subjected to hydrolysis and HPLC separation as described above for compound **8a** gave small amount (ca. 50 μg) of (22*R*,25)-epoxy-1α-hydroxyvitamin D_3 (**11**) and 0.3 mg (49%) of (22*S*)-iodo-1α,25-dihydroxyvitamin D_3 (**10c**), eluted at R_v 34 and 79 mL, respectively.

10c. UV (EtOH) λ_{max} 264 nm, λ_{min} 227 nm, A264/A227 = 2.0; ^{1}H NMR δ 0.607 (3H, s, 18-H_3), 0.975 (3H, d, J = 5.9 Hz, 21-H_3), 1.23 and 1.24 (3H and 3H, each s, 26- and 27-H_3), 2.83 (1H, br d, J = 13 Hz, 9β-H), 4.21 (2H, m, 3α- and 22-H), 4.44 (1H, m, 1β-H), 5.00 and 5.33 (1H and 1H, each s, 19-H_2), 6.02 and 6.38 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS m/z (rel intensity) 542 (M^+, 2), 524 (M^+–H_2O, 31), 506 (M^+–2 H_2O, 17), 414 (M^+–HI, 8), 396 (M^+–HI–H_2O, 54), 378 (M^+–HI–2 H_2O, 21), 99 (100); exact mass calcd for $C_{27}H_{41}IO_2$ (M^+–H_2O) 524.2151, found 524.2145.

Molecular modeling: conformational search. The calculation of optimized geometries and steric energies (E_s) were carried out using the algorithm from the MM^+ HyperChem (release 4.0) software package (Autodesk, Inc., 1994). MM^+ is an all-atom force field based on the MM2 functional form. Molecular mechanics studies on the three model 8-methylene compounds **14a–c** were performed according to the protocol described below.

1. Initial geometry of each compound was obtained by steric energy minimization performed by HyperChem program.
2. This energy-minimized structure was used for the generation of other conformers with the aid of Conformational Search module of ChemPlus (release 1.5) program (Hypercube, Inc., 1995) and the following three-step procedure. At the first step, each of the 17,20- and 20,22-bonds in the examined structure was subjected to rotations with 15° intervals. All resulting conformers were energy-minimized and compared with the previously generated ones. Carbons 20, 23, 24, as well as iodine and oxygen atoms were chosen for comparison in an RMS fit. Structures were considered to be duplicates when: (a) their steric energy difference was smaller than 0.05 kcal/mol, (b) their rotated torsions differed less than 5°, and (c) RMS error was found within 0.25 Å. The least energy structures (energy window 6 kcal/mol) obtained after 50 iterations, were subjected to the second step of conformational search, where rotations (15°) of the 22,23-, 23,24-, and 24,25-bonds were applied. After 300 iterations the lowest energy conformers (energy window 4 kcal/mol) were subjected to the third step, consisting of rotations (15°) of the 17,20-, 20,22-, 22,23-, 23,24- and 24,25-bonds.
3. Conformers, resulting from the additional 650 iterations and falling into the energy window 1.0 kcal/mol, were chosen for a construction of the three-dimensional dot maps indicating the position of their oxygen atom at C-25. Since the CD-hydrindane part of the molecules is very rigid and does not undergo any significant conformational changes it was possible to superimpose all conformers on the global minimum structure using the carbons 13, 14, 17, and 20. For this purpose RMS Fit module of ChemPlus was used and a special PC computer program that deleted all atoms from the conformer being overlaid, except its oxygen at C-25, which was then attached with a single O–O bond to its counterpart belonging to the basic, lowest energy conformer. Where all conformers of each compound **14a–c** were overlaid in such manner, oxygen–oxygen bonds were deleted from the resulted 'polioxide' structure. The global minimum conformers of all model compounds and positions of the 25-oxygen in the other lowest energy conformers are shown in Figure 2.
4. In the manner analogous to that described above, all conformers of the model compounds **14a–c**, falling

into 1 kcal/mol energy window, were overlaid on the carbon skeleton of the global minimum conformer of **14a** (steric energy 33.09 kcal/mol). The result of such superimposition is shown in Figure 3.

Biological studies 1. Measurement of binding to the porcine intestinal vitamin D receptor. Porcine intestinal nuclear extract was prepared as described earlier.[46] It was diluted, and 0.1 mg of protein (200 fmol of binding activity) in 100 μL was used in each tube; 10,000 cpm of 1α,25-$(OH)_2$[26,27-^{3}H]D_3 was added in 2.0 μl of ethanol. To this was added either standard radioinert 1α,25-$(OH)_2D_3$ at various concentrations or the indicated analogue at various concentrations in 5 μL of ethanol. The mixture was incubated at room temperature for 4 h on a shaker and then 100 μL of hydroxyapatite (50% slurry) added. The sample was vortexed at 5 min intervals for 15 min on ice. The hydroxyapatite was then washed 3 times by adding 0.5 mL TE 5% Triton×100, centrifuging at 200 *g* for 5 min, and aspirating the supernatant. The radioactivity bound to the hydroxyapatite was determined by liquid scintillation counting in Bio-Safe II scintillation fluid. The displacement experiments were carried out in triplicate on two different occasions with identical results. The relative VDR binding affinities of the analogues were determined by comparison of the compound concentrations required for 50% displacement of the radiolabeled 1α,25-$(OH)_2D_3$ from the receptor protein.

Measurement of cellular differentiation. Human leukemia HL-60 cells, originally obtained from ATTC, were plated at 2×10^5 cells/plate, incubated in Eagle's modified medium as described previously.[45] The compounds tested were added in the indicated concentrations in 0.05 mL of ethanol so that the ethanol concentration never exceeded 1%. The incubation was carried out for 4 days and at the end of the fourth day, superoxide production was measured by nitro blue tetrazolium (NBT) reduction. The number of cells containing intracellular black-blue formazan deposits was determined by light microscopy using a hemacytometer. At least 200 cells were counted in duplicate per determination. Percentage differentiation represents percentage cells providing NBT reduction appearance. The experiment was repeated 3 times. The relative differentiation activities of the analogues were determined by comparison of the compound concentrations capable of inducing 50% maturation according to the assay. This method is described in detail elsewhere.[46]

Measurement of intestinal calcium transport and bone calcium mobilization. Weanling male rats from the low vitamin D colony were purchased from the Sprague–Dawley Co. (Indianapolis, IN) and fed the vitamin D-deficient diet,[47] containing 0.47% calcium and 0.3% phosphorus, for 1 week. They were then switched to the reduced calcium diet (0.02% Ca) for an additional 2 weeks. These animals have no detectable levels of 25-OH-D_3 or 1α,25-$(OH)_2D_3$ in their plasma as measured by methods described previously.[48] The rats were given the indicated doses of compounds in 0.1 mL of (95:5) 1,2-propanediol:ethanol by intraperitoneal injection each day for 7 days and they were sacrificed 24 h after the last dose. The rats were sacrificed under ether anesthesia by decapitation; their blood and intestines were collected and used immediately to determine intestinal calcium transport and serum calcium concentration. Calcium was determined using the Calcette automatic calcium titrator (Precision Systems, Inc., Natick, MA) and intestinal calcium transport by the everted intestinal sac method using the proximal 10 cm of intestine as described earlier.[47] Statistical analysis was by the Student's *t*-test.[49] Intestinal calcium transport is expressed as serosal:mucosal ratio of calcium in the sac to the calcium in the final incubation medium, or S/M. Bone calcium mobilization represents the rise in serum calcium of the rats maintained on a very low calcium diet. In that measurement, the rise in serum calcium must arise from bone and hence is a determination of bone calcium mobilization.

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