Further Synthetic and Biological Studies on Vitamin D Hormone Antagonists Based on C24-Alkylation and C2α-Functionalization of 25-Dehydro-1α-hydroxyvitamin D_3-26,23-lactones

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An efficient synthesis and the biological evaluation of 80 novel analogs of 25-dehydro-1α-hydroxyvitamin D_3-26,23*S*-lactone **2** (TEI-9647) and its 23*R* epimer (**3**) in which the lactone ring was systematically functionalized by introduction of a C_1 to C_4 primary alkyl group at the C24 position (5 sets of 4 diastereomers), together with their C2α-methyl, 3-hydroxypropyl, and 3-hydroxypropoxy-substituted derivatives were described. The triene structure of the vitamin D_3 was constructed using palladium-catalyzed alkenylative cyclization of the A-ring precursor enyne with the CD-ring counterpart bromoolefin having the C24-alkylated lactone moiety on the side chain. The CD-ring precursors having 23,24-*cis* lactones were prepared by using a chromium-mediated *syn*-selective allylation−lactonization process, and the 23,24-*trans* lactone derivatives were derived from these via inversion of the C23 stereochemistry. The biological evaluation revealed that both binding affinity for chick vitamin D hormone receptor and antagonistic activity (inhibition of vitamin D hormone induced HL-60 cell differentiation) were affected by the orientation and chain-length of the primary alkyl group on the lactone ring. Furthermore, the C2α-functionalization of the C24-alkylated vitamin D_3 lactones dramatically enhanced their biological activities. The most potent compound to emerge, (23*S*,24*S*)-2α-(3-hydroxypropoxy)-24-propyl exhibited almost 1000-fold stronger antagonistic activity (IC_{50} = 7.4 pM) than **2** (IC_{50} = 6.3 nM).

Introduction

The *seco*-steroidal hormone 1α,25-dihydroxyvitamin D_3 (Figure 1, **1**) is the most potent natural metabolite of vitamin D_3 and shows a broad spectrum of biological activities. The most prominent physiological role of **1** is the regulation of calcium and phosphorus metabolism as well as bone remodeling via its action in the bone, intestine, and kidney. Moreover, **1** affects the proliferation and differentiation of various types of tumor cells and also regulates immune reactions.[1,2] The natural hormone **1** exerts its biological effects through the interaction with a vitamin D receptor (VDR[a]), which is a member of the nuclear receptor superfamily and acts as a ligand-dependent gene transcription factor with coactivators.[3,4] The first step in the VDR-mediated transactivation is a ligand-binding process to the ligand-binding domain (LBD) of the apo form of VDR. Next, the ligand−VDR complex changes conformation into a transcriptionally active holo form, which binds to the coactivators to activate the target gene.[5] During conformational change, helix 12, which is the most C-terminal α-helix of VDR and has the site for interaction with other proteins such as coactivators, is important and controls whether the function of a ligand is agonism or antagonism.[6,7]

The major reason for therapeutic limitation of **1** is calcemic and phosphatemic activities. Thus, **1** can cause serious side effects such as hypercalcemia and hyperphosphatemia at superphysiological levels. Therefore, to find the new vitamin D analogs, which are more efficacious, safer, and more selective than the natural **1**, numerous analogs of **1** have been developed. Although more than two thousands vitamin D analogs have been synthesized over the past few decades, most of the synthetic studies of the vitamin D_3 analogs have involved side-chain modification.[2,8] On the other hand, we have developed the first systematic synthesis of novel analogs of vitamin D_3 based on the structural modification of the A-ring core to investigate A-ring conformation and structure−activity relationships.[9−14] During the course of our studies, we found out some functionalization of C2α position on the A-ring increased the binding affinity for VDR with potent agonistic activity. Namely, introduction of methyl (**1a**),[9] 3-hydroxypropyl (**1b**),[10] and 3-hydroxypropoxy (**1c**)[11] groups into the C2α position showed 2- to 4-fold higher binding affinity relative to the natural hormone **1**. The molecular modeling of the three analogs (**1a**−**c**) based on Moras' X-ray crystallographic analysis of VDR−**1** complex[15] showed the analogs (**1a**−**c**) fit well to the cavity of the LBD of the VDR and each C2α substituent on the A-ring interacted with some amino acid residues of the LBD.[10b,11,16] That is, the C2α methyl group of **1a** interacts with some hydrophobic amino acid residues Leu233, Tyr236, and Phe150 in the LBD.[16] In the case of **1b** and **1c**, each C2α terminal

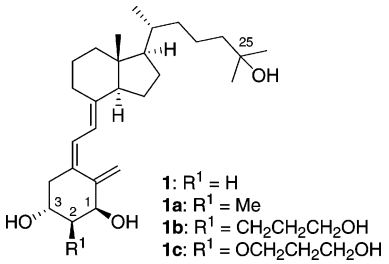

Figure 1. Structures of 1α,25-dihydroxyvitamin D_3 (**1**) and its representative C2α-modified analogs (**1a**−**c**).

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[a] VDR, vitamin D receptor; LBD, ligand-binding domain; NBT, nitro blue tetrazolium; FCS, foetal calf serum; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PBS, phosphate-buffered saline.

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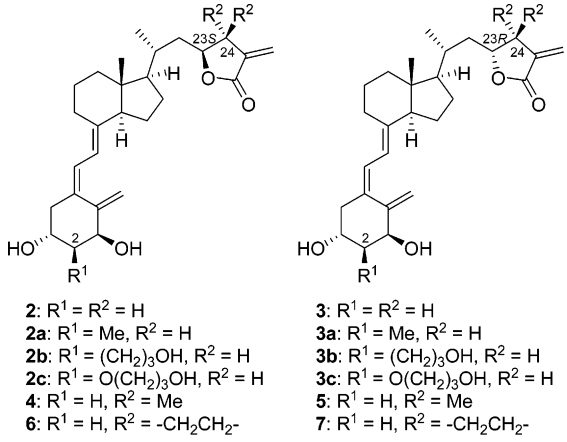


Figure 2. Structures of 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones (**2** and **3**), their C2α-functionalized analogs (**2a**−**c** and **3a**−**c**), and 24,24-disubstituted vitamin D_3-26,23-lactones (**4**−**7**).

hydroxy group forms a new hydrogen bond between the hydroxy group and Arg274.[10b,11] Such hydrophilic or hydrophobic interaction between the C2α side chain and the amino acid residues in the LBD could improve the binding affinity for the VDR.

In 1999, the first vitamin D antagonists, 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones, **2** (TEI-9647) and its (23*R*)-epimer **3** (TEI-9648), were discovered during the course of studies on the side-chain modification of the 1α,25-dihydroxyvitamin D_3-26,23-lactone metabolite[17] derived from **1** (Figure 2).[18−20] Both vitamin D_3 analogs **2** and **3** are the first specific antagonists of VDR-mediated genomic action of **1**.[21] Namely, **2** and **3** inhibit the differentiation of human leukemia cells (HL-60 cells) induced by **1**.[18a] Moreover, **2** suppresses the gene expression of 25-hydroxyvitamin D_3-24-hydroxylase in human osteosarcoma cells[18b] and in HL-60 cells[18d] induced by **1**. Furthermore, **2** antagonizes the genomic-mediated calcium metabolism regulated by **1** in vivo in rat.[18e] Vitamin D antagonists have received considerable attention because of their possibility to be potential agents for some diseases caused by the hypersensitivity of the VDR to 1α,25-dihydroxyvitamin D_3 (**1**), such as Paget's disease of bone,[22] which is the most flagrant example of disordered bone remodeling and the second most common bone disease after osteoporosis in Anglo-Saxons.[22a] Recent studies on Paget's disease suggested a specific increase in osteoclasts sensitivity to the differentiation activity of **1** as the principal mechanism for abnormal bone formation.[22,23] With this background, we set out to conduct an investigation of the structure−activity relationships of the vitamin D_3 lactones from the standpoint of searching for more potential antivitamin D molecules, and we found some pertinent modifications of **2** and **3** that resulted in an enhancement of their activities.[24] That is, introduction of the above three motifs, that is, the methyl, the 3-hydroxypropyl, or the 3-hydroxypropoxy groups into the C2α position of **2** and **3**, increased the antagonistic activity up to 30-fold in the case of **2b**.[24a,25] We also synthesized the 24,24-dimethylvitamin D_3 lactones (**4** and **5**) and 24,24-ethanovitamin D_3 lactones (**6** and **7**), which do not have an extra chiral center on the lactone ring, to examine the effects of the C24 substituents on the biological activity.[24b,c] The biological evaluation of the 24,24-disubstituted analogs (**4**−**7**) revealed that both the VDR binding affinity and the antagonistic activity were affected by the substituents and the stereochemistry on the C23 position. Namely, both the (23*S*)- and (23*R*)-24,24-dimethylvitamin D_3 lactones (**4** and **5**) had enhanced biological activities compared to the corresponding **2** and **3**, respectively.[24b] On the other hand, both the VDR binding affinity and the antagonistic activity of the (23*S*)-24,24-ethanovitamin D_3 analogs (**6**) were improved, however, the biological activities of its stereoisomer (**7**) were weaker than those of the original **3**.[24c]

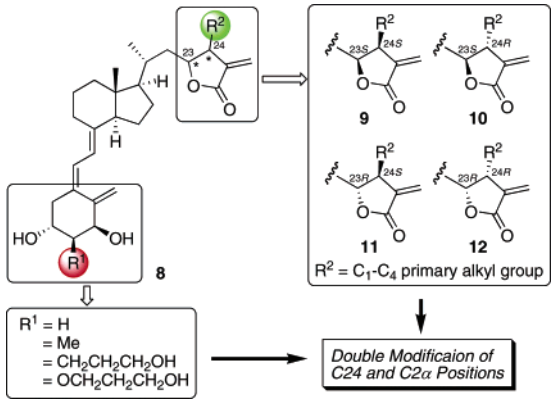
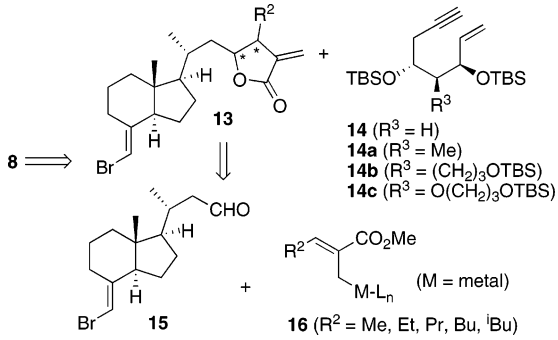


Figure 3. Plan for the functionalization of 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones.

Scheme 1. Retrosynthesis



Our previous results as above indicated that the functionalization of the C2α and C24 positions can be effective in the enhancement of the biological activity of the vitamin D_3 lactones, and we decided to investigate the further structure-activity relationships of the vitamin D_3 lactones along the C2α and C24-functionalization strategy for the creation of more potent vitamin D antagonists applicable to the treatment of Paget's disease. Now we designed C24 monoalkylated vitamin D_3 26,23-lactones and their C2α-modified derivatives (Figure 3, **8**). That is, we planned the systematic introduction of the primary alkyl group (C_1 to C_4 unit) into the C24 position on the lactone ring (**9**−**12**) to examine the influence of both the alkyl chain length and the stereochemistry of the lactone moiety on the biological activities. Moreover, we expected both the VDR binding affinity and the antagonistic activity of the C24 alkylated lactones (**9**−**12**) to be enhanced by introducing the above three motifs, that is, 2α-methyl, 2α-(3-hydroxypropyl), and 2α-(3-hydroxypropoxy) groups as in above-mentioned our previous results.[24b,c] Here, we describe our detailed results of the systematic structural evolution of the vitamin D_3-26,23-lactones based on the C24-monoalkylation and C2α-modification strategy.[26]

Results

Our synthetic plan of functionalized vitamin D_3-26,23-lactones is shown in Scheme 1. The triene skeleton of the desired vitamin D_3 lactone analogs (**8**) would be constructed by Trost's Pd-catalyzed alkenylative cyclization[27] of A-ring precursor enynes (**14** or **14a**−**c**) with the CD-ring bromoolefin counterpart

Scheme 2. Synthesis of 23,24-*cis*-Substituted Lactone Derivatives

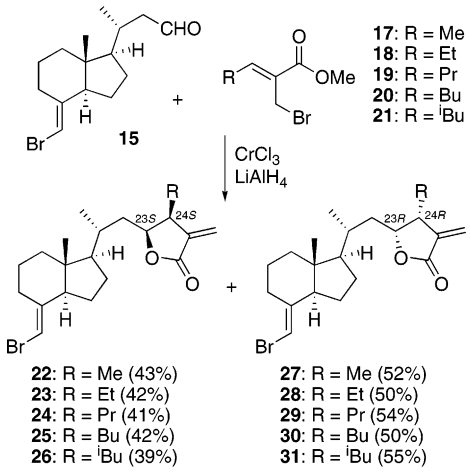
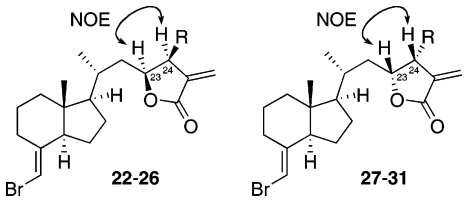


Table 1. NOE Experiments on **22**−**26** and **27**−**31**



compd	23-H to 24-H (%)	24-H to 23-H (%)
22	6.09	5.50
23	7.24	7.47
24	8.22	7.28
25	8.29	5.63
26	8.00	8.03
27	8.35	6.98
28	8.78	4.19
29	9.55	8.01
30	9.63	7.98
31	9.59	8.11

having a C24-alkylated α-methylene-γ-lactone side chain (**13**). The alkyl chain substituted lactone ring of **13** could be synthesized through the stereoselective allylation of the aldehyde **15** by allylic metal species **16**.

1. Synthesis and Biological Evaluation of 24-Alkylated Vitamin D_3-26,23-lactones. 1.1. Preparation of CD-Ring Precursors. We synthesized the CD-ring counterparts, which have 23,24-*cis* lactone moiety, using Oshima's chromium-promoted *syn*-selective allylation (Scheme 2).[28] The aldehyde **15**, which was prepared via oxidative degradation of vitamin D_2,[24a] reacted with allylic bromides (**17**−**21**) in the presence of low-valent Cr complex generated from $CrCl_3$ and $LiAlH_4$ to produce the corresponding lactone derivatives **22**−**26** and **27**−**31**, respectively. Some of the lactone derivatives were separated by recycle HPLC (see Supporting Information).

The stereochemistries on the C23 and C24 positions (based on the steroidal numbering) of each lactone derivative (**22**−**26** and **27**−**31**) were determined by the combination of NOE experiments and modified Mosher's method reported by Kusumi.[29] First of all, NOE experiments were carried out to determine the relative stereochemistries on C23 and C24 positions on the 5-membered lactone ring, and the NOEs between 23-H and 24-H were observed as shown in Table 1. Therefore, the stereochemistries of substituents on the lactone ring of all compounds (**22**−**26** and **27**−**31**) were determined to be *cis*-orientation.

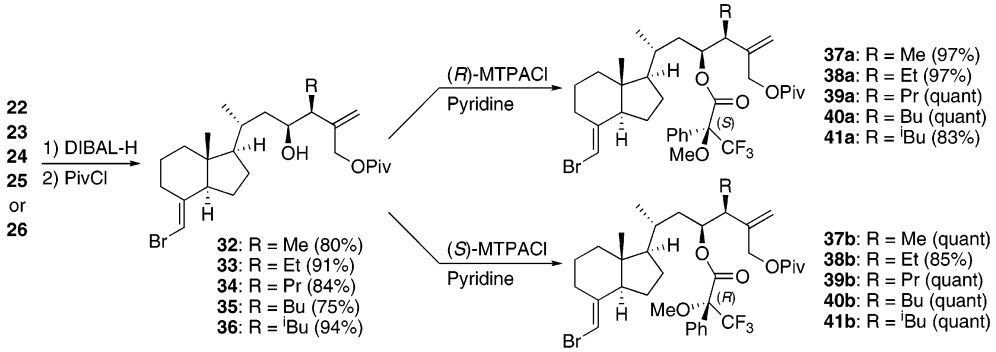
Next, the lactone derivatives (**22**−**26**) were reduced to the corresponding diols followed by pivaloylation of the primary alcohols to give **32**−**36**, which reacted with (*R*)- or (*S*)-MTPA chloride to give the corresponding (*S*)-MTPA esters (**37a**−**41a**) and (*R*)-MTPA esters (**37b**−**41b**), respectively (Scheme 3). The values of $\Delta\delta = \delta_{(S)-\text{MTPA ester}} - \delta_{(R)-\text{MTPA ester}}$ in the 600 MHz ^{1}H NMR spectra of **37**−**41** were calculated as shown in Figure 4. These data were considered by applying the modified Mosher's method, and the configuration at the C23 position of **37**−**41** was determined to be 23*S*. From these results and NOE experiments shown in Table 1, the absolute configuration at the C24 position of **37**−**41** was determined to be 24*S*.

On the other hand, the lactone derivatives (**27**−**31**) were also transformed into the corresponding (*S*)-MTPA esters (**47a**−**51a**) and (*R*)-MTPA esters (**47b**−**51b**) in good yields (Scheme 4). Similar to the lactones (**22**−**26**), the values of $\Delta\delta = \delta_{(S)-\text{MTPA ester}} - \delta_{(R)-\text{MTPA ester}}$ in the ^{1}H NMR spectra of **47**−**51** were calculated, and the data were considered by applying the modified Mosher's method. As the result, the absolute configuration at the C23 position of **47**−**51** was determined to be 23*R*. From these results and NOE experiments shown in Table 1, the stereochemistry at the C24 position of **47**−**51** was determined to be 24*R* (Figure 5).

The CD-ring precursors (**57**−**61**) having 23,24-*trans* lactone were synthesized from 23,24-*cis* lactones (**22**−**26**; Scheme 5). The secondary alcohol derivatives (**32**−**36**), which were obtained by the above DIBAL-H reduction of the corresponding lactones (**22**−**26**), were oxidized by TPAP-NMO to produce the corresponding ketone derivatives (**52**−**56**). The ketones (**52**−**56**) were treated with LiAlH(O*t*-Bu)$_3$, followed by deprotection of pivaloyl groups using DIBAL-H to give the diols (for stereoselectivity at C23, see Supporting Information No. 2). The resulting diols were oxidized to produce the desired (23*R*,24*S*)-lactone derivatives, respectively (**57**−**61**).

The other 23,24-*trans* lactones (**67**−**71**) were similarly derived from the corresponding (23*R*,23*R*)-alcohol derivatives

Scheme 3. Transformation of **22**−**26** into the Corresponding (*S*)- or (*R*)-MTPA Esters (**37a**−**41a** and **37b**−**41b**)



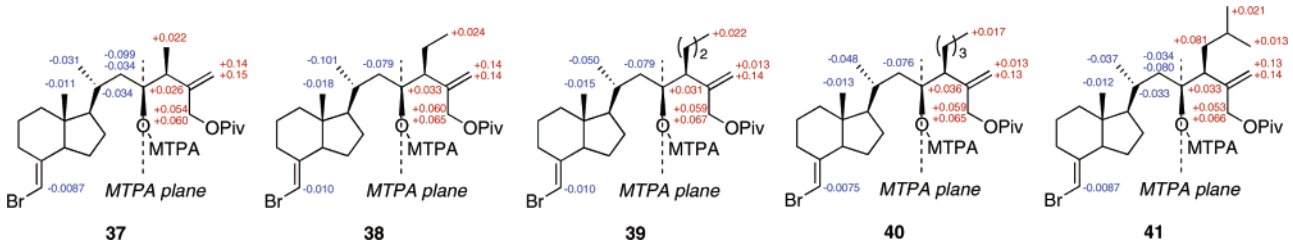


Figure 4. Determination of stereochemistry at the C23 position of **37**−**41** by modified Mosher's method.

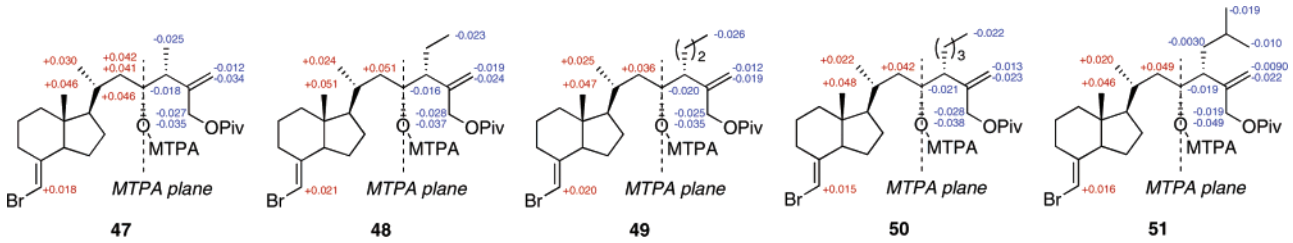
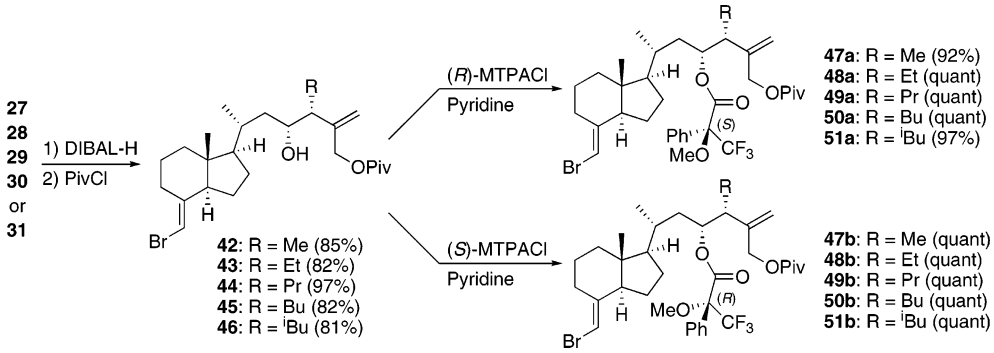


Figure 5. Determination of stereochemistry at the C23 position of **47**−**51** by modified Mosher's method.

Scheme 4. Transformation of **27**−**31** into the Corresponding (*S*)- or (*R*)-MTPA Esters (**37a**−**41a** and **37b**−**41b**)

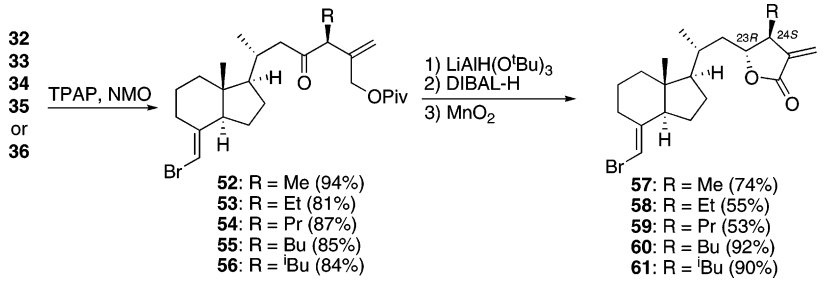


(**42**−**46**; Scheme 6). The alcohol (**42**−**46**) were oxidized by using TPAP-NMO to give the corresponding ketones (**62**−**66**). Then, the ketones were transformed into the (23*S*,24*R*)-lactones (**67**−**71**) through reduction of the C23-keto group, followed by oxidative lactonization process (method A for **69**−**71**, method B for **67** and **68**).

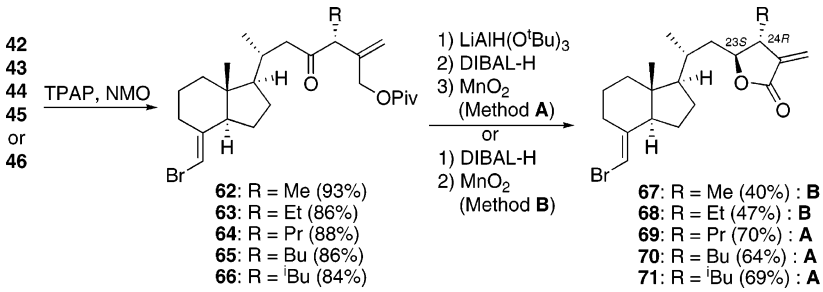
1.2. Synthesis of 24-Alkylated Vitamin D_3-26,23-lactones. Construction of vitamin D_3 triene skeleton was achieved by Pd-catalyzed coupling reaction of CD-ring precursors (**22**−**31**, **57**−**61**, or **67**−**71**) with A-ring enyne (**14**) and then deprotection of the silyl groups under acidic conditions gave the corresponding 24-alkylated vitamin D_3-26,23-lactones (**72**−**91**), respectively (Scheme 7).

1.3. Biological Activities of 24-Alkylated Vitamin D_3-26,-23-lactones. Biological evaluation of the C24-alkylated vitamin D_3 lactones (**72**−**91**) showed that both VDR binding affinity

Scheme 5. Preparation of (23*R*,24*S*)-Lactones from (23*S*,24*S*)-Lactones



Scheme 6. Synthesis of (23*S*,24*R*)-Lactones from (23*R*,24*R*)-Lactones.



Scheme 7. Synthesis of 24-Alkylated Vitamin D_3-26,23-lactones

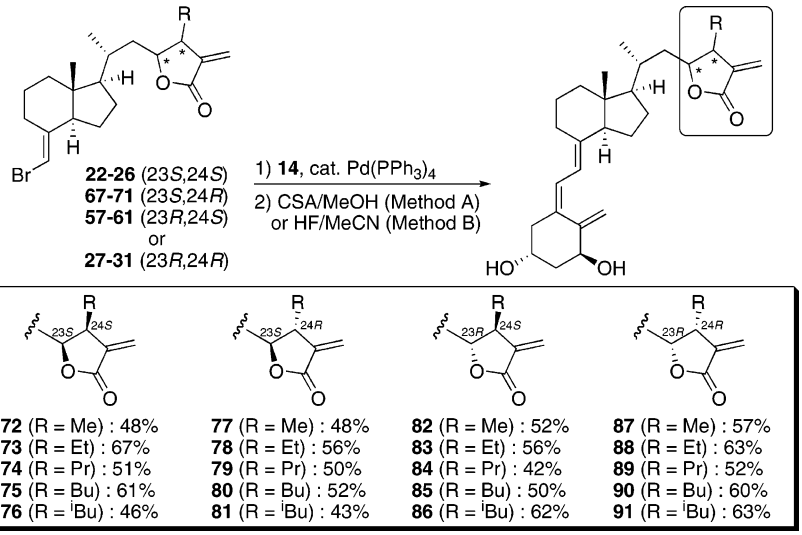


Table 2. Biological Activities of C24-Alkylated Vitamin D_3 Lactones (**72**−**91**)

compd	VDR binding affinity[a]	relative antagonistic activity[b]	compd	VDR binding affinity[a]	relative antagonistic activity[b]
2	12.3	100	**3**	7.2	7
4[c]	37.0	1169	**5**[c]	17.5	16
6[d]	166.7	284	**7**[d]	0.72	<0.3
72	28.6	220	**82**	11.9	7
73	30.3	345	**83**	6.5	6
74	43.5	86	**84**	4.4	8
75	45.5	179	**85**	7.5	4
76	27.8	174	**86**	0.97	5
77	21.7	250	**87**	4.9	18
78	19.2	70	**88**	7.1	13
79	8.6	60	**89**	8.8	38
80	16.7	58	**90**	5.2	17
81	3.0	40	**91**	1.5	9

[a] The potency of the natural hormone **1** is normalized to 100. [b] The potency of **2** (IC_{50} = 6.0∼11.0 nM) is normalized to 100 (see Experimental Section). [c] See ref 24b. [d] See ref 24c.

and antagonistic activity were markedly affected by the structure of the lactone ring, including length of the alkyl chain and the stereochemistries on C23 and C24 positions (Table 2). We also show the data of 24,24-disubstituted vitamin D_3 lactones (**4**−**7**) for comparison.[24b,c] The binding affinity for chick intestinal VDR was examined as described previously,[30] and **2** and **3** showed 8 (12.3%) and 14 (7.2%) times weaker potencies than that of the natural hormone **1**. The VDR binding affinity of the (23*S*,24*S*)-24-alkylated vitamin D_3 lactones (**72**−**76**) increased to 2.3- ∼ 3.7-fold more potent than that of **2**. As the carbon number of the alkyl group increased, the binding affinity was also getting higher except for 24-isobutyl analog (**76**). In the case of the (23*S*,24*R*)-series (**77**−**81**), the binding affinity for VDR seemed to be relatively affected by the alkyl chain length. That is, the analogs, which have methyl (**77**), ethyl (**78**), and butyl (**80**) groups, showed slightly higher binding affinity than **2**, and the VDR binding affinities of 24-propyl (**79**) and 4-isobutyl (**81**) substituted analogs of **2** decreased to 1.4 and 4.1 times lower than that of **2**. On the other hand, the VDR binding affinity of the (23*R*)-vitamin D_3 lactone was little affected by introduced alkyl groups (**82**−**91**) without regard to the stereochemistry of the C24 position.

Next, the antagonistic activities of **72**−**91** were assessed by the NBT-reduction method[31] in terms of IC_{50} for differentiation of HL-60 cells induced by 10 nM of the natural hormone **1**, and their activities relative to **2** were calculated based on the IC_{50} values. The antagonistic activities of (23*S*,24*S*)-isomers (**72**, **73**, **75**, and **76**) were enhanced to be 2.2-, 3.5-, 1.8-, and 1.7-fold higher potencies than that of **2**, respectively. In this (23*S*,24*S*)-series, only the vitamin D_3 lactone having the propyl group (**74**) showed weaker activity than **2**. In the case of the (23*S*,24*R*)-series (**77**−**81**), the antagonistic activity was increased by the introduction of the methyl group into the lactone ring (**77**) to 2.5-times higher than that of **2**. The other analogs (**78**−**81**) showed the weaker potency than **2**. On the other hand, the antagonistic activities of (23*R*,24*S*)-isomers (**82**−**86**) were little affected by C24-alkylation, whereas (23*R*,24*R*)-lactones (**87**−**91**) showed stronger antagonistic activities than **3**. Especially (23*R*,24*R*)-24-propylvitamin D_3 lactone (**89**), which exerted 5.4-fold higher antagonistic activity than **3**. Among these (23*R*,24*R*)-isomers, the antagonistic activity of 24-isobutyl analog (**91**) was almost same as that of **3**.

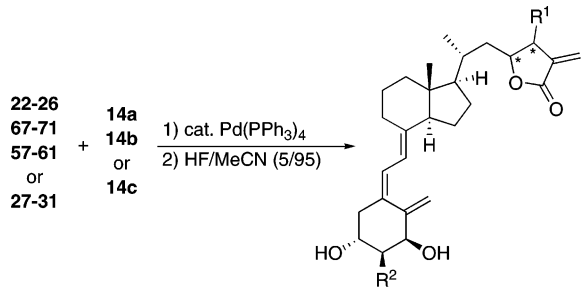
2. Double Modification of C2α- and C24-Positions of Vitamin D_3 Lactones. 2.1. Synthesis of 2α,24-double modified vitamin D_3-26,23-lactones. Next, we focused on the C2α-modification of the twenty 24-alkylvitamin D_3 lactones (**72**−**91**). According to our results, C2α-modification of the 24,24-disubstituted vitamin D_3 lactone analogs effectively enhanced both binding affinity for VDR and antagonistic activity.[24b,c] Therefore, we expected that introducing the three motifs, for example, methyl, 3-hydroxypropyl, and 3-hydroxypropoxy groups into the C2α-position of **72**−**91** would increase the receptor binding affinity and improve the antagonistic activity. The C2α-modified 24-alkylvitamin D_3 lactones (2α-methyl, **72a**−**91a**; 2α-(3-hydroxypropyl), **72b**−**91b**; and 2α-(3-hydroxypropoxy), **72c**−**91c**) were similarly synthesized from the corresponding CD-ring unit **22**−**26**, **67**−**71**, **57**−**61**, and **27**−**31** with the A-ring counterpart **14a**,[32] **14b**[24b] and **14c**,[11b] respectively (Scheme 8). The coupling yields are summarized in Table 3.

2.2. Biological Activities of Double Modified Vitamin D_3 Lactone Derivatives. The biological activities of C2α and C24 double modified analogs are shown in Table 4 (23*S*-series) and Table 5 (23*R*-series), including the previous data of C2α-functionalized vitamin D_3 lactones (**2a**−**c** and **3a**−**c**) for comparison.[24a] The biological evaluation of the (23*S*)-series (**72a**−**81a**, **72b**−**81b**, and **72c**−**81c**) demonstrated that the C2α-modification was remarkably effective in improving the biological activities of (23*S*)-24-alkylvitamin D_3-26,23-lactones

Table 3. Synthetic Yields of C2α-Modified C24-Alkylvitamin D_3 Lactones (**72a**−**91a**, **72b**−**91b**, and **72c**−**91c**)

(23*S*)-series				(23*R*)-series			
compd	R^1	R^2	yield (%)	compd	R^1	R^2	yield (%)
72a	(24*S*)-Me	Me	68	**82a**	(24*S*)-Me	Me	53
72b		$(CH_2)_3OH$	61	**82b**		$(CH_2)_3OH$	26
72c		$O(CH_2)_3OH$	41	**82c**		$O(CH_2)_3OH$	52
73a	(24*S*)-Et	Me	56	**83a**	(24*S*)-Et	Me	67
73b		$(CH_2)_3OH$	62	**83b**		$(CH_2)_3OH$	53
73c		$O(CH_2)_3OH$	61	**83c**		$O(CH_2)_3OH$	61
74a	(24*S*)-Pr	Me	59	**84a**	(24*S*)-Pr	Me	54
74b		$(CH_2)_3OH$	51	**84b**		$(CH_2)_3OH$	56
74c		$O(CH_2)_3OH$	54	**84c**		$O(CH_2)_3OH$	53
75a	(24*S*)-Bu	Me	57	**85a**	(24*S*)-Bu	Me	50
75b		$(CH_2)_3OH$	52	**85b**		$(CH_2)_3OH$	40
75c		$O(CH_2)_3OH$	56	**85c**		$O(CH_2)_3OH$	44
76a	(24*S*)-*i*-Bu	Me	49	**86a**	(24*S*)-*i*-Bu	Me	49
76b		$(CH_2)_3OH$	59	**86b**		$(CH_2)_3OH$	44
76c		$O(CH_2)_3OH$	56	**86c**		$O(CH_2)_3OH$	50
77a	(24*R*)-Me	Me	71	**87a**	(24*R*)-Me	Me	55
77b		$(CH_2)_3OH$	54	**87b**		$(CH_2)_3OH$	66
77c		$O(CH_2)_3OH$	54	**87c**		$O(CH_2)_3OH$	51
78a	(24*R*)-Et	Me	53	**88a**	(24*R*)-Et	Me	63
78b		$(CH_2)_3OH$	62	**88b**		$(CH_2)_3OH$	54
78c		$O(CH_2)_3OH$	68	**88c**		$O(CH_2)_3OH$	58
79a	(24*R*)-Pr	Me	59	**89a**	(24*R*)-Pr	Me	48
79b		$(CH_2)_3OH$	48	**89b**		$(CH_2)_3OH$	43
79c		$O(CH_2)_3OH$	57	**89c**		$O(CH_2)_3OH$	50
80a	(24*R*)-Bu	Me	51	**90a**	(24*R*)-Bu	Me	66
80b		$(CH_2)_3OH$	54	**90b**		$(CH_2)_3OH$	52
80c		$O(CH_2)_3OH$	51	**90c**		$O(CH_2)_3OH$	57
81a	(24*R*)-*i*-Bu	Me	54	**91a**	(24*R*)-*i*-Bu	Me	53
81b		$(CH_2)_3OH$	57	**91b**		$(CH_2)_3OH$	49
81c		$O(CH_2)_3OH$	43	**91c**		$O(CH_2)_3OH$	47

Scheme 8. Synthesis of 2α-Modified 24-Monoalkylvitamin D_3-26,23-lactones (**72a**−**91a**, **72b**−**91b**, and **72c**−**91c**).



(**72**−**76** and **77**−**81**; Table 4). Namely, the binding affinity of (24*S*)-series (**72a**−**76a**, **72b**−**76b**, and **72c**−**76c**) significantly increased to 1.2−6.8 times higher as compared to **2**. On the other hand, the (24*R*)-series (**77a**−**81a**, **77b**−**81b**, and **77c**−**81c**) showed lower VDR binding affinity relative to the corresponding (24*S*)-isomers. However, the affinity for VDR of some analogs (**77a**−**c**, **78a**, **79b**, and **80a**−**c**) were slightly improved by the C2α-functionalization up to 3.4 times higher than that of **2**. The antagonistic activity of the all (23*S*)-type analogs (**72**−**81**) was enhanced by introducing C2α-substituents. That is, all of the (24*S*)-analogs (**72a**−**76a**, **72b**−**76b**, and **72c**−**76c**) showed high antagonistic activity, at least more than 5.2-fold higher potency (**72b**). In particular, (23*S*,24*S*)-2α-(3-hydroxypropoxy)-24-propylvitamin D_3 lactone (**74c**) exerted 851-fold stronger antagonistic activity (IC_{50} = 7.4 pM) than **2** (IC_{50} = 6.3 nM). On the other hand, C2α-functionalization of (24*R*)-series (**77a**−**81a**, **77b**−**81b**, and **77c**−**81c**) also exhibited improvement of the antagonistic activity than the corresponding C2α-nonsubstituted analogs (**77**−**81**). Especially, the antagonistic activity of (23*S*,-24*R*)-2-(3-hydroxypropoxy)-24-propylvitamin D_3 lactone (**79c**) was increased to 121 times stronger (IC_{50} = 52 pM) than that of **2** (IC_{50} = 6.3 nM).

The biological activities of C2α-modified (23*R*)-type analogs (**82a**−**91a**, **82b**−**91b**, and **82c**−**91c**) were summarized in Table 5. In most of the C2α-modified analogs, enhancement of the binding affinity for VDR was observed. Especially, the (23*R*,24*S*)-2α,24-dimethyl analog (**82b**) showed 5.3 times higher VDR binding affinity than that of **3** and 3.2 times higher VDR binding affinity than the 2α-nonsubstituted analog (**82**). It was also demonstrated that the antagonistic activity of (23*R*)-24-alkylvitamin D_3 lactones (**82**−**91**) was improved by the C2α-functionalization. However, **82b**, **85a**, and **86a** showed almost same or lower antagonistic activity relative to the corresponding C2α-nonsubstituted analogs (**82**, **85**, and **86**). In the (24*S*)-series, the analogs **82a**, **84b**, **84c**, **85b**, and **85c** showed more than 10-fold higher antagonistic activity than **3**. In particular, the antagonistic activity of (23*R*,24*S*)-2α-(3-hydroxypropyl)-24-propylvitamin D_3 (**84b**) was enhanced to 82 times higher than that of **3**. In the case of the (24*R*)-isomers, **87a**, **88a**, **89a**−**c**, and **90a** exhibited more than 10 times greater antagonistic activity than **3**, and the most potent (23*R*,24*R*)-analog was 2α,24-dimethylvitamin D_3 lactone (**87a**), which showed 68-fold stronger antagonistic activity than **3** and 26-fold higher antagonistic activity than the corresponding C2α-nonsubstituted analog **87**.

Discussion

Plausible Mechanism of VDR Antagonism by Vitamin D_3 Lactones. The mechanism of VDR antagonism by **2** is not clear yet. However, it was found that the complex of **2** and VDR changed into an unusual transcriptionally inactive form, when **2** binds to the LBD of a VDR.[33] Recently, it was revealed that the two cysteines, Cys403 and Cys410, play an important role in the VDR antagonism of **2**.[34] Furthermore, the *exo*-methylene lactone structure is indispensable for the antagonistic action of the vitamin D_3 lactones.[35] Based on these results, we consider that the nucleophilic thiol groups of the cysteines could attack

Table 4. Biological Activities of 2α-Modified (23*S*)-24-Alkylvitamin D_3 Lactones (**72a**−**81a**, **72b**−**81b**, and **72c**−**81c**)

compd	VDR binding affinity[a]	relative antagonistic activity[b]	compd	VDR binding affinity[a]	relative antagonistic activity[b]
2a[c]	15.6	1019			
2b[c]	18.2	2989			
2c[c]	16.4	1160			
72a	62.5	3752	**77a**	22.7	6191
72b	83.3	517	**77b**	32.3	1512
72c	50.0	1968	**77c**	41.7	1743
73a	41.6	1288	**78a**	25.0	248
73b	34.5	442	**78b**	5.64	866
73c	15.2	600	**78c**	3.13	158
74a	41.6	1537	**79a**	8.62	2032
74b	58.8	4200	**79b**	17.9	913
74c	37.0	85135	**79c**	9.43	12115
75a	58.8	2913	**80a**	21.7	216
75b	66.7	1158	**80b**	16.4	122
75c	50.0	1660	**80c**	20.0	205
76a	41.6	1219	**81a**	4.06	229
76b	40.0	989	**81b**	12.7	388
76c	30.3	3444	**81c**	9.00	291

[a] The potency of the natural hormone **1** is normalized to 100. [b] The potency of **2** (IC_{50} = 6.0∼11.0 nM) is normalized to 100 (see Experimental Section). [c] See ref 24a.

Table 5. Biological Activities of 2α-Modified (23*R*)-24-alkylvitamin D_3 Lactones (**82a**−**91a**, **82b**−**91b**, and **82c**−**91c**)

compd	VDR binding affinity[a]	relative antagonistic activity[b]	compd	VDR binding affinity[a]	relative antagonistic activity[b]
3a[c]	37.0	38			
3b[c]	33.3	100			
3c[c]	22.7	66			
82a	11.9	85	**87a**	4.90	476
82b	38.5	8	**87b**	33.3	30
82c	33.3	12	**87c**	13.2	41
83a	6.54	18	**88a**	7.09	74
83b	15.4	37	**88b**	20.8	18
83c	7.04	25	**88c**	7.46	27
84a	4.39	12	**89a**	8.77	170
84b	10.6	573	**89b**	30.3	117
84c	6.41	124	**89c**	13.5	97
85a	7.46	1	**90a**	5.18	102
85b	15.4	303	**90b**	16.7	46
85c	8.00	88	**90c**	11.6	37
86a	0.97	4	**91a**	1.52	50
86b	5.24	32	**91b**	14.1	31
86c	3.79	42	**91c**	6.13	25

[a] The potency of the natural hormone **1** is normalized to 100. [b] The potency of **2** (IC_{50} = 6.0∼11.0 nM) is normalized to 100 (see Experimental Section). [c] See ref 24a.

the α-methylene-γ-lactone of **2** and its analogs *via* 1,4-addition to give the corresponding cysteine adduct.[36] Such interaction between the ligand and the LBD might prevent correct positioning of helix 12 to activate the target genes. Therefore, it is conceivable that the vitamin D antagonists, whose *exo*-methylene moiety is located at a more favorable position to react with Cys403 and/or Cys410 after binding, show stronger vitamin D antagonistic activity. The novel vitamin D_3 lactones we synthesized, which showed more potent antagonistic activity, might be situated in a preferable position of the *exo*-methylene group toward the cysteine residues after binding to the LBD of the VDR and vice versa. This hypothesis on mechanism of antagonism, right positioning of functional groups in the LBD of the VDR, is different from that of **92** (ZK-159222)[19] and **93** (DLAMs)[20b] (Figure 6), and would be one reason why the potency of antagonistic activity of **2** and its analogs does not correlate to the VDR binding affinity.

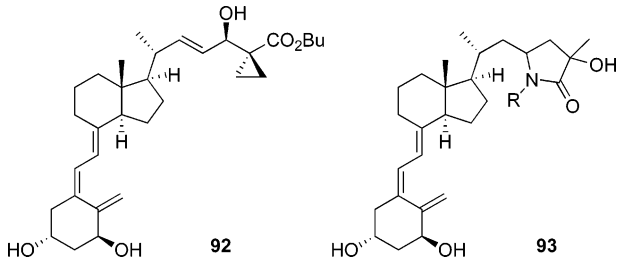


Figure 6. Structures of VDR antagonists **92** and **93** with presumably different antagonism on VDR.

Conclusions

We have systematically synthesized novel vitamin D antagonists, 1α-hydroxyvitamin D_3-26,23-lactone analogs that have 24-methyl, 24-ethyl, 24-propyl, 24-butyl, and 24-isobutyl groups on the lactone ring, to investigate the structure−activity relationships on the lactone ring core. As the results demonstrated, both binding affinity for VDR and antagonistic activity were affected by the orientation and chain-length of the alkyl group on the lactone ring. Furthermore, it was also found that the C2α-functionalization of the C24-alkylated vitamin D_3 lactones remarkably enhanced their biological activities. In particular, (23*S*,24*S*)-2α-(3-hydroxypropoxy)-24-propylvitamin D_3-26,23-lactone exhibited approximately 850-fold stronger antagonistic activity (IC_{50} = 7.4 pM) than **2** (IC_{50} = 6.3 nM).

We expected that the analogs with potent anti-D activity would contribute to understanding the mechanisms involved in the expression of antagonistic activity on VDR, for example, based on X-ray crystallographic data if available,[37] as well as to finding the seeds of new medicines for treating Paget's disease.

Experimental Section

General Procedure for the Synthesis of Vitamin D_3 Lactones: Method A. To a solution of an A-ring precursor and the CD-ring precursor in toluene were added Et_3N and $Pd(PPh_3)_4$ (30 mol % to the CD-ring precursor), and the mixture was stirred at 110 °C. After the mixture was concentrated, the residue was roughly purified by flash column chromatography on silica gel to give a crude vitamin D_3. The crude product was dissolved in MeOH. To the MeOH solution was added (+)-10-camphorsulfonic acid (CSA) at 0 °C, and the mixture was stirred at room temperature. To the mixture was added saturated $NaHCO_3$ aq solution, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aq solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel or preparative thin-layer chromatography on silica gel to give the vitamin D_3 lactone derivative. Further purification for biological assays was conducted by reverse-phase recycle HPLC (YMC-PacK ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 95:5−85:15).

General Procedure for the Synthesis of Vitamin D_3 Lactones: Method B. To a solution of an A-ring precursor (1.5 equiv to a CD-ring precursor) and the CD-ring precursor in toluene were added Et_3N and $Pd(PPh_3)_4$ (30 mol % to the CD-ring precursor), and the mixture was stirred at 110 °C. After the mixture was concentrated, the residue was roughly purified by flash column chromatography on silica gel to give a crude vitamin D_3. The crude product was dissolved in MeCN. To the solution was added a 10% solution of HF (46% aqueous solution, commercially available) in MeCN at 0 °C, and the mixture was stirred at room temperature. To the mixture was added saturated $NaHCO_3$ aq solution, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aq solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel or preparative thin-layer chromatography on

silica gel to give the vitamin D_3 lactone derivative. Further purification for biological assays was conducted by reversed-phase recycle HPLC (YMC-Pack ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 95:5−85:15).

(23*S*,24*S*)-25-Dehydro-1α-hydroxy-24-methylvitamin D_3-26,-23-lactone (72). According to the general procedure (method A), a crude product, which was obtained from **22** (34.9 mg, 91.5 μmol), **14** (44.0 mg, 0.119 mmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (31.9 mg, 27.6 μmol) in toluene (3 mL) at 110 °C for 1.5 h, was treated with CSA (42.1 mg, 0.181 mmol) in MeOH (2 mL) for 1.5 h. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/3) to give **72** (19.7 mg, 48% in 2 steps) as a colorless oil. $[\alpha]^{18}_D$ −17.0 (*c* 0.52, $CHCl_3$); IR (neat) 3395, 1755, 1638, 1269, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 1.13 (d, *J* = 7.1 Hz, 3H), 1.20−1.75 (m, 13H), 1.87−1.95 (m, 2H), 1.96−2.08 (m, 3H), 2.31 (dd, *J* = 13.4, 6.6 Hz, 1H), 2.59 (dd, *J* = 13.4, 3.4 Hz, 1H), 2.82 (dd, *J* = 12.5, 4.4 Hz, 1H), 3.11 (dddq, *J* = 2.2, 2.2, 6.8, 7.1 Hz, 1H), 4.22 (m, 1H), 4.43 (m, 1H), 4.59 (ddd, *J* = 8.2, 6.8, 5.3 Hz, 1H), 4.99 (dd, *J* = 1.5, 1.5 Hz, 1H), 5.32 (dd, *J* = 1.5, 1.5 Hz, 1H), 5.53 (d, *J* = 2.2 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.18 (d, *J* = 2.2 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 14.7, 19.9, 22.4, 23.6, 27.8, 29.1, 34.5, 36.4, 38.2, 40.8, 42.9, 45.3, 46.0, 56.2, 56.9, 66.8, 70.8, 80.3, 111.6, 117.1, 120.4, 124.3, 132.9, 141.3, 142.6, 147.5, 170.2; EI-LRMS *m*/*z* 440 (M^+), 422, 404, 251, 105; EI-HRMS calcd for $C_{28}H_{40}O_4$, 440.2987; found, 440.2932.

(23*S*,24*S*)-25-Dehydro-24-ethyl-1α-hydroxyvitamin D_3-26,23-lactone (73). According to the general procedure (method B), a crude product, which was obtained from **23** (19 mg, 49 μmol), **14** (24.0 mg, 65.1 μmol), Et_3N (3.0 mL), and $Pd(PPh_3)_4$ (15.0 mg, 12.9 μmol) in toluene (0.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 3 h. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/1) to give **73** (13.0 mg, 67% in 2 steps) as a colorless oil. $[\alpha]^{23}_D$ −20.6 (*c* 1.00, $CHCl_3$); IR (neat) 3366, 2924, 1761, 1653, 1061 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H), 1.05 (d, *J* = 6.3 Hz, 3H), 1.25−1.77 (m, 15H), 1.88−2.05 (m, 5H), 2.31 (dd, *J* = 13.3, 6.6 Hz, 1H), 2.59 (dd, *J* = 13.3, 3.3 Hz, 1H), 2.78−2.84 (m, 2H), 4.23 (m, 1H), 4.43 (dd, *J* = 7.7, 4.3 Hz, 1H), 4.58 (m, 1H), 4.99 (s, 1H), 5.32 (s, 1H), 5.51 (d, *J* = 1.8 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.20 (d, *J* = 1.8 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.9, 12.0, 19.8, 20.2, 22.3, 23.6, 27.8, 29.1, 34.6, 35.9, 40.4, 42.9, 45.2, 45.3, 46.0, 56.2, 56.9, 66.8, 70.8, 80.5, 111.7, 117.1, 121.1, 124.8, 133.0, 139.4, 142.7, 147.6, 170.6; EI-LRMS *m*/*z* 454 (M^+), 436, 418, 322, 249; EI-HRMS calcd for $C_{29}H_{42}O_4$, 454.3083; found, 454.3088.

(23*S*,24*S*)-25-Dehydro-1α-hydroxy-24-propylvitamin D_3-26,-23-lactone (74). According to the general procedure (method B), a crude product, which was obtained from **24** (26.9 mg, 65.7 μmol), **14** (36.3 mg, 98.5 μmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (22.8 mg, 19.7 μmol) in toluene (1.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (4 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **74** (15.8 mg, 51% in 2 steps) as a colorless amorphous solid. $[\alpha]^{18}_D$ −14.8 (*c* 1.22, $CHCl_3$); IR (film, $CHCl_3$) 3376, 1759, 1661, 1643, 1348, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.95 (t, *J* = 7.0 Hz, 3H), 1.06 (d, *J* = 6.6 Hz, 3H), 1.20−1.80 (m, 17H), 1.88−2.08 (m, 5H), 2.32 (dd, *J* = 13.4, 6.6 Hz, 1H), 2.60 (dd, *J* = 13.4, 3.4 Hz, 1H), 2.83 (m, 1H), 2.91 (m, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 4.58 (ddd, *J* = 8.6, 6.2, 4.9 Hz, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.50 (d, *J* = 1.8 Hz, 1H), 6.02 (d, *J* = 11.2 Hz, 1H), 6.19 (d, *J* = 1.8 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.9, 14.0, 19.4, 19.8, 22.3, 23.5, 27.8, 29.0, 29.2, 34.6, 36.0, 40.4, 42.9, 43.4, 45.2, 46.0, 56.1, 56.8, 66.8, 70.8, 80.6, 111.7, 117.2, 120.9, 124.9, 133.0, 139.7, 142.8, 147.6, 170.7; EI-LRMS *m*/*z* 468 (M^+), 450, 432, 417, 263, 251, 209, 195, 155, 141; EI-HRMS calcd for $C_{30}H_{44}O_4$, 468.3240; found, 468.3233.

(23*S*,24*S*)-25-Dehydro-24-butyl-1α-hydroxyvitamin D_3-26,23-lactone (75). According to the general procedure (method B), a crude product, which was obtained from **25** (40.0 mg, 94.5 μmol), **14** (52.2 mg, 0.142 mol), Et_3N (3 mL), and $Pd(PPh_3)_4$ (32.8 mg, 28.4 μmol) in toluene (3 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **75** (28.0 mg, 61% in 2 steps) as a colorless amorphous solid. $[\alpha]^{22}_D$ −13.3 (*c* 1.07, $CHCl_3$); IR (film, $CHCl_3$) 3368, 1759, 1663, 1647, 1348, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.92 (t, *J* = 7.1 Hz, 3H), 1.06 (d, *J* = 6.3 Hz, 3H), 1.20−1.78 (m, 19H), 1.88−2.08 (m, 5H), 2.32 (dd, *J* = 13.5, 6.4 Hz, 1H), 2.60 (dd, *J* = 13.5, 3.4 Hz, 1H), 2.83 (m, 1H), 2.89 (m, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 4.57 (ddd, *J* = 8.5, 6.3, 5.1 Hz, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.50 (d, *J* = 2.0 Hz, 1H), 6.02 (d, *J* = 11.2 Hz, 1H), 6.19 (d, *J* = 2.0 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.9, 13.9, 19.7, 22.2, 22.6, 23.5, 26.8, 27.8, 28.4, 29.0, 34.5, 35.9, 40.3, 42.8, 43.6, 45.2, 45.9, 56.1, 56.8, 66.8, 70.8, 80.6, 111.7, 117.2, 120.9, 124.8, 133.1, 139.7, 142.7, 147.6, 170.7; EI-LRMS *m*/*z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3396; found, 482.3487.

(23*S*,24*S*)-25-Dehydro-1α-hydroxy-24-isobutylvitamin D_3-26,-23-lactone (76). According to the general procedure (method B), a crude product, which was obtained from **26** (15.7 mg, 37.1 μmol), **14** (20.5 mg, 55.6 μmol), Et_3N (2 mL), and $Pd(PPh_3)_4$ (12.9 mg, 11.2 μmol) in toluene (2 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **76** (8.3 mg, 46% in 2 steps) as a colorless amorphous solid. $[\alpha]^{26}_D$ −11.4 (*c* 0.64, $CHCl_3$); IR (neat) 3384, 1763, 1663, 1644, 1346, 1269, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.95 (d, *J* = 6.3 Hz, 6H), 1.06 (d, *J* = 6.4 Hz, 3H), 1.20−1.75 (m, 16H), 1.85−2.10 (m, 5H), 2.32 (dd, *J* = 13.4, 6.6 Hz, 1H), 2.60 (dd, *J* = 13.4, 2.8 Hz, 1H), 2.83 (m, 1H), 3.02 (m, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 4.58 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.48 (d, *J* = 1.7 Hz, 1H), 6.02 (d, *J* = 11.2 Hz, 1H), 6.19 (d, *J* = 1.7 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.9, 19.8, 21.9, 22.3, 23.1, 24.4, 27.8, 29.0, 34.6, 36.0, 36.1, 40.4, 41.5, 42.9, 45.2, 46.0, 46.1, 56.1, 56.8, 66.9, 70.8, 80.6, 111.8, 117.1, 120.6, 124.9, 133.0, 140.0, 142.8, 147.6, 170.7; EI-LRMS *m*/*z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3396; found, 482.3401.

(23*S*,24*R*)-25-Dehydro-1α-hydroxy-24-methylvitamin D_3-26,-23-lactone (77). According to the general procedure (method A), a crude product, which was obtained from **67** (14.0 mg, 36.7 μmol), **14** (17.8 mg, 48.3 μmol), Et_3N (1 mL), and $Pd(PPh_3)_4$ (13.0 mg, 11.2 μmol) in toluene (2 mL) at 110 °C for 1.5 h, was treated with CSA (20.0 mg, 86.1 μmol) in MeOH (1.5 mL) for 1.5 h. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/2) to give **77** (7.8 mg, 48% in 2 steps) as an amorphous solid. $[\alpha]^{23}_D$ +19.7 (*c* 0.30, $CHCl_3$); IR (neat) 3400, 1759, 1630 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 1.06 (d, *J* = 5.9 Hz, 3H), 1.28 (d, *J* = 6.8 Hz, 3H), 1.25−1.80 (m, 13H), 1.85−2.10 (m, 5H), 2.32 (dd, *J* = 13.6, 6.4 Hz, 1H), 2.55−2.70 (m, 2H), 2.83 (m, 1H), 4.07 (dt, *J* = 5.9, 6.4 Hz, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.53 (d, *J* = 2.9 Hz, 1H), 6.01 (d, *J* = 11.1 Hz, 1H), 6.22 (d, *J* = 2.9 Hz, 1H), 6.37 (d, *J* = 11.1 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 17.2, 19.5, 22.3, 23.5, 27.9, 29.0, 34.5, 40.4, 40.7, 41.5, 42.9, 45.2, 45.9, 56.2, 56.5, 66.9, 70.8, 84.2, 111.8, 117.2, 120.8, 124.9, 133.0, 140.8, 142.8, 147.6, 170.4; EI-LRMS *m*/*z* 440 (M^+), 422, 404, 251, 105; EI-HRMS calcd for $C_{28}H_{40}O_4$, 440.2927; found, 440.2929.

(23*S*,24*R*)-25-Dehydro-24-ethyl-1α-hydroxyvitamin D_3-26,23-lactone (78). According to the general procedure (method B), a crude product, which was obtained from **68** (14.0 mg, 35.4 μmol), **14** (20.0 mg, 54.2 μmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (12.0 mg, 10.4 μmol) in toluene (0.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 3 h. After the usual

work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 2/3) to give **78** (9.0 mg, 56% in 2 steps) as a colorless oil. $[\alpha]^{22}_D$ +34.8 (*c* 0.69, $CHCl_3$); IR (neat) 3441, 2936, 1757, 1649, 1059 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 1.05 (d, *J* = 5.9 Hz, 3H), 1.18−1.69 (m, 16H), 1.86−2.05 (m, 4H), 2.31 (dd, *J* = 13.3, 6.5 Hz, 1H), 2.54−2.61 (m, 2H), 2.82 (m, 1H), 4.24 (m, 2H), 4.43 (m, 1H), 4.99 (s, 1H), 5.32 (s, 1H), 5.59 (d, *J* = 2.3 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.27 (d, *J* = 2.3 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.7, 12.0, 19.7, 22.3, 23.6, 26.8, 28.0, 29.1, 32.2, 40.4, 42.5, 42.9, 45.3, 45.9, 46.4, 56.2, 56.5, 66.8, 70.8, 82.1, 111.7, 117.1, 122.2, 124.8, 133.0, 138.8, 142.7, 147.8, 170.4; EI-LRMS *m*/*z* 454 (M^+), 436, 418, 322, 249; EI-HRMS calcd for $C_{29}H_{42}O_4$, 454.3083; found, 454.3086.

(23*S*,24*R*)-25-Dehydro-1α-hydroxy-24-propylvitamin D_3-26,-23-lactone (79). According to the general procedure (method B), a crude product, which was obtained from **69** (36.0 mg, 87.9 μmol), **14** (48.6 mg, 0.132 mmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (30.5 mg, 26.4 μmol) in toluene (1.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (4 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **79** (20.5 mg, 50% in 2 steps) as a colorless amorphous solid. $[\alpha]^{15}_D$ +32.7 (*c* 1.15, $CHCl_3$); IR (film, $CHCl_3$) 3387, 1761, 1661, 1647, 1601, 1348, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.96 (t, *J* = 7.2 Hz, 3H), 1.06 (d, *J* = 5.9 Hz, 3H), 1.15−1.73 (m, 17H), 1.85−2.08 (m, 5H), 2.32 (dd, *J* = 13.4, 6.4 Hz, 1H), 1.56−2.65 (m, 2H), 2.82 (m, 1H), 4.18−4.30 (m, 2H), 4.43 (m, 1H), 4.99 (s, 1H), 5.32 (dd, *J* = 1.7, 1.5 Hz, 1H), 5.58 (d, *J* = 2.3 Hz, 1H), 6.01 (d, *J* = 11.4 Hz, 1H), 6.25 (d, *J* = 2.3 Hz, 1H), 6.37 (d, *J* = 11.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.9, 14.0, 19.5, 19.6, 22.2, 23.5, 27.9, 29.0, 34.2, 36.4, 40.3, 42.4, 42.9, 44.9, 45.2, 45.9, 56.2, 56.5, 66.8, 70.7, 82.5, 111.7, 117.2, 122.2, 124.9, 133.1, 139.2, 142.7, 147.6, 170.5; EI-LRMS *m*/*z* 468 (M^+), 450, 432, 417, 263, 251, 209, 195, 155, 141; EI-HRMS calcd for $C_{30}H_{44}O_4$, 468.3240; found, 468.3222.

(23*S*,24*R*)-25-Dehydro-24-butyl-1α-hydroxyvitamin D_3-26,23-lactone (80). According to the general procedure (method B), a crude product, which was obtained from **70** (34.7 mg, 81.9 μmol), **14** (45.3 mg, 0.123 mol), Et_3N (3 mL), and $Pd(PPh_3)_4$ (28.4 mg, 24.6 μmol) in toluene (3.0 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 3 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **80** (20.5 mg, 52% in 2 steps) as a colorless amorphous solid. $[\alpha]^{20}_D$ +29.2 (*c* 1.62, $CHCl_3$); IR (film, $CHCl_3$) 3403, 1759, 1647, 1663, 1348, 1053 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.92 (t, *J* = 6.6 Hz, 3H), 1.06 (d, *J* = 5.9 Hz, 3H), 1.15−1.75 (m, 19H), 1.85−2.10 (m, 5H), 2.32 (dd, *J* = 13.3, 6.5 Hz, 1H), 2.55−2.65 (m, 2H), 2.83 (m, 1H), 4.18−4.30 (m, 2H), 4.43 (m, 1H), 4.99 (s, 1H), 5.32 (s, 1H), 5.58 (d, *J* = 2.1 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.23 (d, *J* = 2.1 Hz, 1H), 6.26 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.9, 13.9, 10.6, 22.2, 22.6, 23.5, 27.9, 28.4, 29.0, 33.8, 34.2, 40.3, 42.4, 42.8, 45.0, 45.2, 45.9, 56.2, 56.5, 66.8, 70.7, 82.5, 111.7, 117.2, 122.2, 124.9, 133.1, 139.2, 142.7, 147.6, 170.5; EI-LRMS *m*/*z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3396; found, 482.3399.

(23*S*,24*R*)-25-Dehydro-1α-hydroxy-24-isobutylvitamin D_3-26,-23-lactone (81). According to the general procedure (method B), a crude product, which was obtained from **71** (21.2 mg, 50.0 μmol), **14** (27.7 mg, 75.1 μmol), Et_3N (2 mL), and $Pd(PPh_3)_4$ (17.3 mg, 15.0 μmol) in toluene (2 mL) at 110 °C for 1.5 h, was treated with a 5% solution of HF in MeCN (2 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **81** (10.3 mg, 43% in 2 steps) as a colorless amorphous solid. $[\alpha]^{26}_D$ +28.0 (*c* 0.79, $CHCl_3$); IR (neat) 3389, 1761, 1657, 1269, 1140, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.97 (d, *J* = 6.6 Hz, 3H), 1.06 (d, *J* = 6.1 Hz, 3H), 1.10−1.75 (m, 16H), 1.85−2.10 (m, 5H), 2.32 (dd, *J* = 13.4, 6.6 Hz, 1H), 2.60 (dd, *J* = 13.4, 3.6 Hz, 1H), 2.67 (m, 1H), 2.83 (m, 1H), 4.15−4.25 (m, 2H), 4.43 (m, 1H), 4.99 (s, 1H), 5.32 (s, 1H), 5.57 (d, *J* = 2.3 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.24 (d, *J* = 2.3 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.9, 19.6, 22.2, 22.3, 22.7, 23.5, 25.1, 27.9, 29.0, 34.3, 40.3, 42.3, 42.9, 43.0, 44.0, 45.2, 45.9, 56.2, 56.5, 66.8, 70.8, 83.0, 111.7, 117.2, 122.1, 124.9, 133.1, 139.7, 142.8, 147.6, 170.5; EI-LRMS *m*/*z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3396; found, 482.3397.

(23*R*,24*S*)-25-Dehydro-1α-hydroxy-24-methylvitamin D_3-26,-23-lactone (82). According to the general procedure (method A), a crude product, which was obtained from **57** (18.8 mg, 49.3 μmol), **14** (27.3 mg, 74.0 μmol), Et_3N (1 mL), and $Pd(PPh_3)_4$ (17.1 mg, 14.8 μmol) in toluene (2 mL) at 110 °C for 1.5 h, was treated with CSA (27.0 mg, 0.116 mmol) in MeOH (1.5 mL) for 1.5 h. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/2) to give **82** (7.8 mg, 52% in 2 steps) as an amorphous solid. $[\alpha]^{18}_D$ +81.3 (*c* 0.27, $CHCl_3$); IR (neat) 3383, 1765, 1643, 1247, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 1.01 (d, *J* = 6.4 Hz, 3H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.20−1.38 (m, 4H), 1.40−2.10 (m, 14H), 2.31 (dd, *J* = 13.4, 6.4 Hz, 1H), 2.55−2.65 (m, 2H), 2.82 (dd, *J* = 12.2, 3.9 Hz, 1H), 4.07 (ddd, *J* = 10.5, 7.3, 2.0 Hz, 1H), 4.22 (m, 1H), 4.42 (dd, *J* = 7.6, 4.4 Hz, 1H), 4.99 (s, 1H), 5.32 (dd, *J* = 1.7, 1.4 Hz, 1H), 5.52 (d, *J* = 2.9 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.21 (d, *J* = 2.9 Hz, 1H), 6.36 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 16.3, 18.8, 22.4, 23.6, 27.7, 29.1, 33.1, 40.6, 40.9, 41.6, 43.0, 45.3, 46.1, 56.4, 56.9, 66.9, 70.8, 82.5, 111.7, 117.1, 120.4, 124.7, 133.0, 140.8, 142.5, 147.4, 170.2; EI-LRMS *m*/*z* 440 (M^+), 422, 404, 251, 105; EI-HRMS calcd for $C_{28}H_{44}O_4$, 440.2927; found, 440.2920

(23*R*,24*S*)-25-Dehydro-24-ethyl-1α-hydroxyvitamin D_3-26,23-lactone (83). According to the general procedure (method B), a crude product, which was obtained from **58** (14.0 mg, 35.4 μmol), **14** (20.0 mg, 54.2 μmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (12.0 mg, 10.4 μmol) in toluene (0.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 3 h. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 2/3) to give **83** (9.0 mg, 56% in 2 steps) as a colorless oil. $[\alpha]^{24}_D$ +50.7 (*c* 0.38, $CHCl_3$); IR (neat) 3366, 2946, 1751, 1630, 1061 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.98 (t, *J* = 7.4 Hz, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 1.23−1.85 (m, 16H), 1.86−2.05 (m, 4H), 2.31 (dd, *J* = 13.3, 6.5 Hz, 1H), 2.51 (m, 1H), 2.60 (dd, *J* = 13.5, 3.4 Hz, 1H), 2.83 (dd, *J* = 12.2, 3.9 Hz, 1H), 4.23 (m, 1H), 4.29 (ddd, *J* = 11.0, 4.9, 2.0 Hz, 1H), 4.43 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.5 (d, *J* = 2.6 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.27 (d, *J* = 2.6 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.9, 12.1, 18.6, 22.3, 23.6, 26.4, 27.6, 29.1, 33.1, 40.6, 42.9, 43.3, 45.3, 46.0, 46.6, 56.4, 56.9, 66.9, 70.8, 80.6, 111.7, 117.2, 122.0, 124.8, 133.0, 139.1, 142.7, 147.5, 170.4; EI-LRMS *m*/*z* 454 (M^+), 436, 418, 322, 249; EI-HRMS calcd for $C_{29}H_{42}O_4$, 454.3083; found, 454.3080.

(23*R*,24*S*)-25-Dehydro-1α-hydroxy-24-propylvitamin D_3-26,-23-lactone (84). According to the general procedure (method B), a crude product, which was obtained from **59** (21.3 mg, 52.0 μmol), **14** (28.8 mg, 78.1 μmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (18.0 mg, 15.6 μmol) in toluene (1.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (4 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **84** (10.3 mg, 42% in 2 steps) as a colorless amorphous solid. $[\alpha]^{15}_D$ +57.0 (*c* 0.623, $CHCl_3$); IR (film, $CHCl_3$) 3387, 1759, 1661, 1644, 1634, 1609, 1350, 1053 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 0.96 (t, *J* = 7.3 Hz, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 1.20−2.10 (m, 22H), 2.31 (dd, *J* = 13.4, 6.4 Hz, 1H), 2.53−2.63 (m, 2H), 2.83 (m, 1H), 4.20−4.35 (m, 2H), 4.43 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.57 (d, *J* = 2.6 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.25 (d, *J* = 2.6 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 14.1, 18.5, 19.8, 22.3, 23.5, 27.6, 29.0, 33.0, 35.9, 40.5, 42.8, 43.2, 45.1, 45.2, 46.0, 56.3, 56.9, 66.8, 70.8, 81.0, 111.8, 117.2, 121.9, 124.9, 133.1, 139.6, 142.8, 147.6, 170.5;

EI-LRMS *m/z* 468 (M^+), 450, 432, 417, 263, 251, 209, 195, 155, 141; EI-HRMS calcd for $C_{30}H_{44}O_4$, 468.3239; found, 468.3228.

(23*R*,24*S*)-25-Dehydro-24-butyl-1α-hydroxyvitamin D$_3$-26,23-lactone (85). According to the general procedure (method B), a crude product, which was obtained from **60** (40.0 mg, 94.5 μmol), **14** (52.2 mg, 0.145 mol), Et_3N (3 mL), and $Pd(PPh_3)_4$ (28.4 mg, 24.6 μmol) in toluene (3.0 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **85** (22.9 mg, 52% in 2 steps) as a colorless amorphous solid. $[\alpha]^{20}_D$ +57.2 (*c* 1.08, $CHCl_3$); IR (film, $CHCl_3$) 3387, 1759, 1659, 1647, 1269, 1053 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 0.93 (t, *J* = 7.0 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 1.20−2.08 (m, 24H), 2.31 (dd, *J* = 13.6, 6.4 Hz, 1H), 2.55 (m, 1H), 2.60 (dd, *J* = 13.6, 3.1 Hz, 1H), 2.83 (m, 1H), 4.23 (m, 1H), 4.27 (ddd, *J* = 11.1, 4.9, 2.1 Hz, 1H), 4.43 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.57 (d, *J* = 2.6 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.26 (d, *J* = 2.6 Hz, 1H), 6.37 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 13.9, 18.5, 22.2, 22.7 (2C), 23.5, 27.6, 28.6, 29.0, 33.0, 33.4, 40.5, 42.8, 43.1, 45.2, 46.0, 56.3, 56.9, 66.8, 70.8, 81.0, 111.8, 117.2, 121.9, 124.8, 133.1, 139.6, 142.7, 147.6, 170.5; EI-LRMS *m/z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3396; found, 482.3398.

(23*R*,24*S*)-25-Dehydro-1α-hydroxy-24-isobutylvitamin D$_3$-26,-23-lactone (86). According to the general procedure (method B), a crude product, which was obtained from **61** (20.4 mg, 48.2 μmol), **14** (26.6 mg, 72.1 μmol), Et_3N (2 mL), and $Pd(PPh_3)_4$ (16.7 mg, 14.5 μmol) in toluene (2 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **86** (14.4 mg, 62% in 2 steps) as a colorless amorphous solid. $[\alpha]^{24}_D$ +49.3 (*c* 1.11, $CHCl_3$); IR (neat) 3385, 1761, 1659, 1381, 1143, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 1.20−1.35 (m, 4H), 1.40−2.08 (m, 17H), 2.31 (dd, *J* = 13.3, 6.5 Hz, 1H), 2.60 (d, *J* = 13.3, 2.9 Hz, 1H), 2.63 (m, 1H), 2.83 (m, 1H), 4.18−4.30 (m, 2H), 4.43 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.56 (d, *J* = 2.3 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.24 (d, *J* = 2.3 Hz, 1H), 6.36 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 18.5, 22.2, 22.3, 22.8, 23.5, 25.2, 27.6, 29.0, 33.0, 40.5, 42.8, 43.0, 43.1, 43.5, 45.2, 46.0, 56.3, 56.9, 66.8, 70.8, 81.4, 112.0, 117.2, 121.8, 124.8, 133.1, 140.0, 142.7, 147.6, 170.5; EI-LRMS *m/z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3396; found, 482.3797.

(23*R*,24*R*)-25-Dehydro-1α-hydroxy-24-methylvitamin D$_3$-26,-23-lactone (87). According to the general procedure (method A), a crude product, which was obtained from **27** (36.5 mg, 95.7 μmol), **14** (45.9 mg, 0.124 mmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (33.2 mg, 28.7 μmol) in toluene (3 mL) at 110 °C for 1.5 h, was treated with CSA (48.8 mg, 0.201 mmol) in MeOH (1.5 mL) for 45 min. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/2) to give **87** (24.1 mg, 57% in 2 steps) as an amorphous solid. $[\alpha]^{23}_D$ +113.9 (*c* 0.38, $CHCl_3$); IR (neat) 3420, 1757, 1658 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 1.02 (d, *J* = 6.4 Hz, 3H), 1.08 (m, 1H), 1.13 (d, *J* = 7.3 Hz, 3H), 1.15−1.35 (m, 3H), 1.40−2.10 (m, 14H), 2.31 (dd, *J* = 13.4, 6.6 Hz, 1H), 2.59 (dd, *J* = 13.4, 3.3 Hz, 1H), 2.83 (dd, *J* = 12.1, 3.8 Hz, 1H), 3.16 (dq, *J* = 7.8, 7.3 Hz, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 4.67 (ddd, *J* = 11.8, 7.8, 2.0 Hz, 1H), 4.99 (s, 1H), 5.33 (s, 1H), 5.52 (d, *J* = 2.7 Hz, 1H), 6.01 (d, *J* = 11.3 Hz, 1H), 6.21 (d, *J* = 2.7 Hz, 1H), 6.36 (d, *J* = 11.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 13.9, 18.6, 22.4, 23.6, 27.7, 29.1, 32.6, 37.0, 37.8, 40.6, 42.9, 45.3, 46.1, 56.4, 57.1, 66.8, 70.7, 78.4, 111.7, 117.1, 120.5, 124.7, 133.0, 140.7, 142.5, 147.4, 170.2; EI-LRMS *m/z* 440 (M^+), 422, 404, 378, 289, 209,105; EI-HRMS calcd for $C_{28}H_{40}O_4$, 440.2927; found, 440.2935.

(23*R*,24*R*)-25-Dehydro-24-ethyl-1α-hydroxyvitamin D$_3$-26,23-lactone (88). According to the general procedure (method B), a crude product, which was obtained from **28** (18.0 mg, 45.7 μmol), **14** (25.0 mg, 67.9 μmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (16.0 mg, 13.8 μmol) in toluene (0.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 3 h. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/1) to give **88** (13.0 mg, 63% in 2 steps) as a colorless oil. $[\alpha]^{22}_D$ +75.7 (*c* 0.92, $CHCl_3$); IR (neat) 3416, 2934, 1755, 1649, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.98 (t, *J* = 7.3 Hz, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 1.12 (ddd, *J* = 14.0, 10.9, 1.8 Hz, 1H), 1.25−2.04 (m, 19H), 2.31 (dd, *J* = 13.5, 6.3 Hz, 1H), 2.59 (dd, *J* = 13.5, 2.8 Hz, 1H), 2.80−2.90 (m, 2H), 4.23 (m, 1H), 4.43 (*J* = 7.8, 4.2 Hz, 1H), 4.66 (ddd, *J* = 11.5, 7.0, 1.5 Hz, 1H), 4.99 (s, 1H), 5.33 (s, 1H), 5.52 (d, *J* = 2.6 Hz, 1H), 6.01 (d, *J* = 11.4 Hz, 1H), 6.21 (d, *J* = 2.6 Hz, 1H), 6.36 (d, *J* = 11.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 12.1, 18.6, 20.5, 22.3, 23.6, 27.6, 29.1, 32.7, 36.4, 40.6, 42.9, 45.1, 45.3, 46.0, 56.4, 57.1, 66.8, 70.8, 78.3, 111.7, 117.2, 120.9, 124.8, 133.0, 139.3, 142.6, 147.5, 170.6; EI-LRMS *m/z* 454 (M^+), 436, 418, 322, 249; EI-HRMS calcd for $C_{29}H_{42}O_4$, 454.3083; found, 454.3083.

(23*R*,24*R*)-25-Dehydro-1α-hydroxy-24-propylvitamin D$_3$-26,-23-lactone (89). According to the general procedure (method B), a crude product, which was obtained from **29** (25.5 mg, 62.3 μmol), **14** (34.5 mg, 93.6 μmol), Et_3N (1.5 mL), and $Pd(PPh_3)_4$ (21.6 mg, 18.7 μmol) in toluene (1.5 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (4 mL) for 3 h. After the usual work up, the crude product was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 2/3) to give **89** (13.0 mg, 52% in 2 steps) as a colorless amorphous solid. $[\alpha]^{18}_D$ +108.6 (*c* 1.16, $CHCl_3$); IR (film, $CHCl_3$) 3382, 1757, 1663, 1645, 1346, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 0.96 (t, *J* = 7.1 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 1.11 (ddd, *J* = 14.0, 10.6, 1.8 Hz, 1H), 1.20−2.08 (m, 21H), 2.31 (dd, *J* = 13.4, 6.4 Hz, 1H), 2.60 (dd, *J* = 13.4, 3.3 Hz, 1H), 2.83 (m, 1H), 2.98 (m, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 4.66 (ddd, *J* = 11.6, 7.2, 1.6 Hz, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.50 (d, *J* = 2.4 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.21 (d, *J* = 2.4 Hz, 1H), 6.36 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 14.0, 18.5, 19.9, 22.2, 23.5, 27.6, 29.0, 29.5, 32.6, 36.5, 40.5, 42.8, 43.2, 45.2, 46.0, 56.4, 57.0, 66.8, 70.7, 78.3, 111.8, 117.2, 120.8, 124.8, 133.1, 139.5, 142.7, 147.6, 170.7; EI-LRMS *m/z* 468 (M^+), 450, 432, 417, 263, 251, 209, 195, 155, 141; EI-HRMS calcd for $C_{30}H_{44}O_4$, 468.3240; found, 468.3231.

(23*R*,24*R*)-25-Dehydro-24-butyl-1α-hydroxyvitamin D$_3$-26,-23-lactone (90). According to the general procedure (method B), a crude product, which was obtained from **30** (52.4 mg, 0.124 mmol), **14** (68.4 mg, 0.186 mmol), Et_3N (3 mL), and $Pd(PPh_3)_4$ (43.0 mg, 37.2 μmol) in toluene (3.0 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 30 min. After the usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 2/3) to give **90** (20.5 mg, 52% in 2 steps) as a colorless amorphous solid. $[\alpha]^{21}_D$ +58.4 (*c* 0.59, $CHCl_3$); IR (film, $CHCl_3$) 3351, 1758, 1658, 1643, 1379 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3H), 0.93 (t, *J* = 7.1 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 1.11 (ddd, *J* = 14.1, 10.8, 1.8 Hz, 1H), 1.20−2.08 (m, 23H), 2.31 (dd, *J* = 13.4, 6.4 H, 1H), 2.59 (dd, *J* = 13.4, 3.4 Hz, 1H), 2.83 (m, 1H), 2.96 (m, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 4.66 (ddd, *J* = 11.6, 7.1, 1.6 Hz, 1H), 4.99 (s, 1H), 5.33 (s, 1H), 5.51 (d, *J* = 2.6 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.21 (d, *J* = 2.6 Hz, 1H), 6.36 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 13.9, 18.5, 22.2, 22.6, 23.5. 27.0, 27.5, 28.8, 29.0, 32.6, 36.4, 40.5, 42.8, 43.4, 45.2, 46.0, 56.3, 57.0, 66.8, 70.7, 78.4, 111.8, 117.2, 120.8, 124.8, 133.2, 139.5, 142.7, 147.6, 170.7; EI-LRMS *m/z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3397; found, 482.3387.

(23*R*,24*R*)-25-Dehydro-1α-hydroxy-24-isobutylvitamin D$_3$-26,23-lactone (91). According to the general procedure (method B), a crude product, which was obtained from **31** (17.3 mg, 40.9 μmol), **14** (22.6 mg, 61.3 μmol), Et_3N (2 mL), and $Pd(PPh_3)_4$ (14.2 mg, 12.3 μmol) in toluene (2 mL) at 110 °C for 1 h, was treated with a 5% solution of HF in MeCN (2 mL) for 1 h. After the usual work up, the crude product was purified by preparative thin-layer

chromatography on silica gel (hexane/AcOEt = 2/3) to give **91** (12.5 mg, 63% in 2 steps) as a colorless amorphous solid. $[\alpha]^{25}_{D}$ +85.5 (*c* 0.95, $CHCl_3$); IR (neat) 3378, 1759, 1662, 1346, 1269, 1053 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.4 Hz, 3H), 1.08 (m, 1H), 1.15−2.10 (m, 20H), 2.39 (dd, *J* = 13.3, 6.5 Hz, 1H), 2.59 (dd, *J* = 13.3, 2.9 Hz, 1H), 2.84 (m, 1H), 3.21 (m, 1H), 4.23 (br s, 1H), 4.43 (br s, 1H), 4.66 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 5.48 (d, *J* = 2.2 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.20 (d, *J* = 2.2 Hz, 1H), 6.36 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 18.5, 22.2, 22.5, 22.7, 23.5, 24.9, 27.6, 29.0, 32.6, 36.2, 36.7, 40.5, 41.2, 42.8, 45.2, 46.0, 56.4, 57.0, 66.8, 70.7, 78.3, 111.8, 117.2, 120.6, 124.8, 133.1, 139.6, 142.7, 147.6, 170.6; EI-LRMS *m/z* 482 (M^+), 464, 446, 251, 153; EI-HRMS calcd for $C_{31}H_{46}O_4$, 482.3396; found, 482.3394.

Vitamin D Receptor (VDR) Binding Assay. [26,27-Methyl-^{3}H]-1α,25-dihydroxyvitamin D_3 (specific activity 6.623 TBq/mmol, 15 000 dpm, 15.7 pg) and various amounts of 1α,25-dihydroxyvitamin D_3 and the analog to be tested were dissolved in 50 μL of absolute ethanol in 12 × 75-mm polypropylene tubes. The chick intestinal VDR (0.2 mg) and 1 mg of gelatin in 1 mL of phosphate buffer solution (25 nM KH_2PO_4, 0.1 M KCl, and 1 mM dithiothreitol, pH 7.4) were added to each tube in an ice bath. The assay tubes were incubated in a shaking water bath for 1 h at 25 °C and then chilled in an ice bath. One milliliter of 40% polypropylene glycol 6000 in distilled water was added to each tube, which was mixed vigorously and centrifuged at 2260 × *g* for 60 min at 4 °C. After the supernatant was decanted, the bottom of the tube containing the pellet was cut off into a scintillation vial containing 10 mL of dioxane-based scintillation fluid, and the radioactivity was measured with a Beckman liquid scintillation counter (model LS6500). The relative potency of the analog was calculated from the concentration needed to displace 50% of [26,27-methyl-^{3}H]-1α,25-dihydroxyvitamin D_3 from the receptor compared with the activity of 1α,25-dihydroxyvitamin D_3 (assigned a 100% value).

Assay for HL-60 Cell Differentiation. Nitro blue tetrazolium (NBT)-reducing activity was used as a cell differentiation marker. HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FCS. Exponentially proliferating cells were collected, suspended in fresh medium, and seeded in culture plates (Falcon, Becton Dickinson and Company, Franklin Lakes, NJ). The cell concentration at seeding was adjusted to 2×10^4 cells/mL, and the seeding volume was 1 mL/well. An ethanol solution of 1α,25-dihydroxyvitamin D_3 (final concentration: 10^{-8} M) and an analog (final concentration: 3×10^{-12} to 10^{-6} M) was added to the culture medium at 0.1% volume, and the culture was continued for 96 h at 37 °C in a humidified atmosphere of 5% CO_2/air without a change of medium. The same amount of vehicle was added to the control culture. The NBT-reducing assay was performed according to the method of Collins.[31] Briefly, cells were collected, washed with PBS, and suspended in serum-free medium. NBT/TPA solution (dissolved in PBS) was added. Final concentrations of NBT and TPA were 0.1% and 100 ng/mL, respectively. Then, the cell suspensions were incubated at 37 °C for 25 min. After incubation, cells were collected by centrifugation and resuspended in FCS. Cytospin smears were prepared, and the counter-staining of nuclei was done with Kemechrot solution. At least 500 cells per preparation were observed. The IC_{50} values (nM) of **2** at the each experiment using the vitamin D_3 lactone analogs (**72**−**91**, **72a**−**91a**, **72b**−**91b**, and **72c**−**91c**) were as follows: 6.0 (for **74**, **79**, **84**, and **89**), 6.1 (for **72c**, **77c**, **82c**, and **87c**), 6.2 (for **72b**, **77b**, **82b**, and **87b**), 6.3 (for **74a**−**c**, **79a**−**c**, **84a**−**c**, and **89a**−**c**), 6.7 (for **73a**, **75a**, **78a**, **80a**, **83a**, **85a**, **88a**, and **90a**), 6.8 ± 1.13 (for **72a** and **77a**), 7.7 ± 1.38 (for **72** and **77**), 8.1 (for **82a** and **87a**), 8.2 ± 0.85 (for **75**, **80**, **85**, and **90**), 8.4 (for **73b**,**c**, **78b**,**c**, **83b**,**c**, and **88b**,**c**), 8.6 ± 0.28 (for **73**, **78**, **83**, and **88**), 8.8 (for **75b**,**c**, **80b**,**c**, **85b**,**c**, and **90b**,**c**), 9.2 (for **87**), 9.3 (for **81**, **76b**,**c**, **81b**,**c**, **86b**,**c**, and **91b**,**c**), 9.5 (for **76**), 10.2 ± 1.20 (for **86** and **91**), 10.5 ± 0.71 (for **76a** and **91a**), 11.0 (for **82**, **81a**, and **86a**). The charts of assay for HL-60 cell differentiation to test antagonistic activity are shown in the Supporting Information.

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Supporting Information Available: General experimental details, synthetic procedures and spectral data of 2α-substituted vitamin D_3 lactone analogs (**72a**−**91a**, **72b**−**91b**, and **72c**−**92c**), details for the synthesis and structural elucidation of the CD-ring precursors (**22**−**31**, **57**−**61**, and **67**−**71**) and spectral data of all new compounds, charts of vitamin D receptor binding assay and assay for HL-60 cell differentiation to test antagonistic activity for all vitamin D_3 lactone analogs (**72**−**91**, **72a**−**91a**, **72b**−**91b**, and **72c**−**92c**). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) *Vitamin D 2nd Edition*, Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic Press: New York, 2005. (b) Ettinger, R. A.; DeLuca, H. F. The Vitamin D Endocrine System and Its Therapeutic Potential. *Adv. Drug Res.* **1996**, *28*, 269−312.

(2) Bouillon, R.; Okamura, W. H.; Norman, A. W. Structure-Function Relationships in the Vitamin D Endocrine System. *Endocr. Rev.* **1995**, *16*, 200−257.

(3) (a) Evans, R. M. The Steroid and Thyroid Hormone Receptor Superfamily. *Science* **1988**, *240*, 889−895. (b) Umezono, K.; Murakami, K. K.; Thompson, C. C.; Evans, R. M. Direct Repeats as Selective Response Elements for the Thyroid Hormone, Retinoic acid, and Vitamin D_3 Receptors. *Cell* **1991**, *65*, 1255−1266. (c) Chambon, P. The Nuclear Receptor Superfamily: A Personal Retrospect on the First Two Decades. *Mol. Endocrinol.* **2005**, *19*, 1418−1428.

(4) Takeyama, K.; Masuhiro, Y.; Fuse, H.; Endoh, H.; Murayama, A.; Kitanaka, S.; Suzawa, M.; Yanagisawa, J.; Kato, S. Selective Interaction of Vitamin D Receptor with Transcriptional Coactivators by a Vitamin D Analog. *Mol. Cell Biol.* **1999**, *19*, 1049−1055.

(5) For a description of the VDR-generated transactivation mechanism, see: Masuno, H.; Yamamoto, K.; Wang, X.; Choi, M.; Ooizumi, H.; Shinki, T.; Yamada, S. Rational Design, Synthesis, and Biological Activity of Novel Conformationally Restricted Vitamin D Analogues, (22*R*)- and (22*S*)-22-Ethyl-1,25-dihydroxy-23,24-didehydro-24a,24b-dihomo-20-epivitamin D_3. *J. Med. Chem.* **2002**, *45*, 1825−1834.

(6) For a recent review on the function of VDR in response to **1** and the other synthetic ligands, see: Carlberg, C. Current Understanding of the Function of the Nuclear Vitamin D Receptor in Response to Its Natural and Synthetic Ligands. In *Vitamin D Analogs in Cancer Prevention and Therapy, Recent Results in Cancer Research*. 164; Reichrath, J., Friedrich, M., Tilgen, M., Eds.; Springer: Berlin, 2003; pp 29−42.

(7) Carlberg, C. Ligand-Mediated Conformational Changes of the VDR are Required for Gene Transactivation. *J. Steroid Biochem. Mol. Biol.* **2004**, *89*−*90*, 227−232.

(8) For selected papers on the synthesis of vitamin D analogs, see: (a) Uskokovic, M. R., Eds. *Bioorg. Med. Chem. Lett*; Symposia-in-Print **1993**, *3*, 1777−1896. (b) Jones, G.; Calverley, M. J. A Dialogue on Analogues Newer Vitamin-D Drugs for Use in Bone Disease, Psoriasis, and Cancer. *Trends Endocrinol. Metab.* **1993**, *4*, 297−303. (c) Zhu, G. D.; Okamura, W. H. Synthesis of Vitamin D (Calciferol). *Chem. Rev.* **1995**, *95*, 1877−1952.

(9) (a) Konno, K.; Maki, S.; Fujishima, T.; Liu, Z.; Miura, D.; Chokki, M.; Takayama, H. A Novel and Practical Route to A-Ring Enyne Synthon for 1α,25-Dihydroxyvitamin D_3 Analogs: Synthesis of A-Ring Diastereomers of 1α,25-Dihydroxyvitamin D_3 and 2-Methyl-1,25-dihydroxyvitamin D_3. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 151−156. (b) Konno, K.; Fujishima, T.; Maki, S.; Liu, Z.; Miura, D.; Chokki, M.; Ishizuka, S.; Yamaguchi, K.; Kan, Y.; Kurihara, M.; Miyata, N.; Smith, C.; DeLuca, H. F.; Takayama, H. Synthesis,

Biological Evaluation, and Conformational Analysis of A-Ring Diastereomers of 2-Methyl-1,25-dihydroxyvitamin D_3 and Their 20-Epimers: Unique Activity Profiles Depending on the Stereochemistry of the A-Ring and at C-20. *J. Med. Chem.* **2000**, *43*, 4247−4265.

(10) (a) Suhara, Y.; Nihei, K.-i.; Tanigawa, H.; Fujishima, T.; Konno, K.; Nakagawa, K.; Okano, T.; Takayama, H. Syntheses and Biological Evaluation of Novel 2α-Substituted 1α,25-Dihydroxyvitamin D_3 Analogues. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1129−1132. (b) Suhara, Y.; Nihei, K.-i.; Kurihara, M.; Kittaka, A.; Yamaguchi, K.; Fujishima, T.; Konno, K.; Miyata, N.; Takayama, H. Efficient and Versatile Synthesis of Novel 2α-Substituted 1α,25-Dihydroxyvitamin D_3 Analogues and Their Docking to Vitamin D Receptor. *J. Org. Chem.* **2001**, *66*, 8760−8771.

(11) (a) Kittaka, A.; Suhara, Y.; Takayanagi, H.; Fujishima, T.; Kurihara, M.; Takayama, H. A Concise and Efficient Route to 2α-(ω-Hydroxyalkoxy)-1α,25-dihydroxyvitamin D_3: Remarkably High Affinity to Vitamin D Receptor. *Org. Lett.* **2000**, *2*, 2619−2622. (b) Saito, N.; Suhara, Y.; Kurihara, M.; Fujishima, T.; Honzawa, S.; Takayanagi, H.; Kozono, T.; Matsumoto, M.; Ohmori, M.; Miyata, N.; Takayama, H.; Kittaka, A. Design and Efficient Synthesis of 2α-(ω-Hydroxyalkoxy)-1α,25-dihydroxyvitamin D_3 Analogues Including 2-*epi*-ED-71 and Their 20-Epimers with HL-60 Cell Differentiation Activity. *J. Org. Chem.* **2004**, *69*, 7463−7471.

(12) For our account, see: Takayama, H.; Kittaka, A.; Fujishima, T.; Suhara, Y. Design, Synthesis, and Biological Studies of the A-ring-Modified 1,25-Dihydroxyvitamin D_3 Analogs. *Vitamin D Analogs in Cancer Prevention and Therapy, Recent Results in Cancer Research*. 164; Reichrath, J.; Friedrich, M.; Tilgen, W., Eds.; Springer-Verlag: Berlin, Heidelberg, 2003; pp 289−317.

(13) This concept was also applicable to 1α,25-dihydroxy-19-norvitamin D_3, see: (a) Yoshida, A.; Ono, K.; Suhara, Y.; Saito, N.; Takayama, H.; Kittaka, A. Efficient and Convergent Coupling Route for the Short-Step Synthesis of Enantiopure 2α- and 2β-Alkylated 1α,25-Dihydroxy-19-norvitamin D_3 Analogues. *Synlett* **2003**, 1175−1179. (b) Ono, K.; Yoshida, A.; Saito, N.; Fujishima, T.; Honzawa, S.; Suhara, Y.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. Efficient Synthesis of 2-Modified 1α,25-Dihydroxy-19-norvitamin D_3 with Julia Olefination: High Potency in Induction of Differentiation on HL-60 Cells. *J. Org. Chem.* **2003**, *68*, 7407−7415.

(14) For the pioneering works on A-ring modification with C2α-functionalization, see: (a) Posner, G. H.; Johnson, N. Stereocontrolled Total Synthesis of Calcitriol Derivatives: 1,25-Dihydroxy-2-(4′-hydroxybutyl)vitamin D_3 Analogs of an Osteoporosis Drug. *J. Org. Chem.* **1994**, *59*, 7855−7861. (b) Posner, G. H.; Cho, C.-G.; Anjeh, T. E. N.; Johnson, N.; Horst, R. L.; Kobayashi, T.; Okano, T.; Tsugawa, N. 2-Fluoroalkyl A-Ring Analogs of 1,25-Dihydroxyvitamin D_3. Stereocontrolled Total Synthesis via Intramolecular and Intermolecular Diels-Alder Cycloadditions. Preliminary Biological Testing. *J. Org. Chem.* **1995**, *60*, 4617−4628.

(15) Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D. The Crystal Structure of the Nuclear Receptor for Vitamin D Bound to Its Natural Ligand. *Mol. Cell* **2000**, *5*, 173−179.

(16) (a) Fujishima, T.; Konno, K.; Nakagawa, K.; Tanaka, M.; Okano, T.; Kurihara, M.; Miyata, N.; Takayama, H. Synthesis and Biological Evaluation of all A-Ring Stereoisomers of 5,6-*trans*-2-methyl-1,25-dihydroxyvitamin D_3 and their 20-Epimers: Possible Binding Modes of Potent A-Ring Analogues to Vitamin D Receptor. *Chem. Biol.* **2001**, *8*, 1011−1024. See also refs 10b and 11.

(17) (a) Ishizuka, S.; Ishimoto, S.; Norman, A. W. Isolation and Identification of 1α,25-Dihydroxy-24-oxovitamin D_3, 1α,25-Dihydroxyvitamin D_3 26,23-Lactone, and 1α,24(*S*),25-Trihydroxyvitamin D_3: In vivo Metabolites of 1α,25-Dihydroxyvitamin D_3. *Biochemistry* **1984**, *23*, 1473−1478. (b) Ishizuka, S.; Ohba, T.; Norman, A. W. 1α,25$(OH)_2D_3$-26,23-lactone is a Major Metabolite of 1α,25$(OH)_2D_3$ under Physiological Conditions. *Vitamin D: Molecular, Cellular and Clinical Endocrinology*; Norman, A. W., Schaefer, K., Grigoleit, H. G., von Herrath, D., Eds.; Walter de Gruyter: Berlin, 1988; pp 143−144.

(18) (a) Miura, D.; Manabe, K.; Ozono, K.; Saito, M.; Gao, Q.; Norman, A. W.; Ishizuka, S. Antagonistic Action of Novel 1α,25-Dihydroxyvitamin D_3-26,23-lactone Analogs on Differentiation of Human Leukemia Cells (HL-60) Induced by 1α,25-Dihydroxyvitamin D_3. *J. Biol. Chem.* **1999**, *274*, 16392−16399. (b) Ozono, K.; Saito, M.; Miura, D.; Michigami, T.; Nakajima, S.; Ishizuka, S. Analysis of the Molecular Mechanism for the Antagonistic Action of a Novel 1α,25-Dihydroxyvitamin D_3 Analogue toward Vitamin D Receptor Function. *J. Biol. Chem.* **1999**, *274*, 32376−32381. (c) Miura, D.; Manabe, K.; Gao, Q.; Norman, A. W.; Ishizuka, S. 1α,25-Dihydroxyvitamin D_3-26,23-lactone Analogs Antagonize Differentiation of Human Leukemia Cells (HL-60 cells) but not of Human Acute Promyelocytic Leukemia Cells (NB4 cells). *FEBS Lett.* **1999**, *460*, 297−302. (d) Ishizuka, S.; Miura, D.; Eguchi, H.; Ozono, K.; Chokki, M.; Kamimura, T.; Norman, A. W. Antagonistic Action of Novel 1α,25-Dihydroxyvitamin D_3-26,23-lactone Analogs on 25-Hydroxyvitamin-D_3-24-hydroxylase Gene Expression Induced by 1α,25-Dihydroxyvitamin D_3 in Human Promyelocytic Leukemia (HL-60) Cells. *Arch. Biochem. Biophys.* **2000**, *380*, 92−102. (e) Ishizuka, S.; Miura, D.; Ozono, K.; Chokki, M.; Mimura, H.; Norman, A. W. Antagonistic Actions in Vivo of (23*S*)-25-Dehydro-1α-Hydroxyvitamin D_3-26,23-lactone on Calcium Metabolism Induced by 1α,-25-Dihydroxyvitamin D_3. *Endocrinology* **2001**, *142*, 59−67. (f) Ishizuka, S.; Miura, D.; Ozono, K.; Saito, M.; Eguchi, H.; Chokki, M.; Norman, A. W. (23*S*)- and (23*R*)-25-Dehydro-1α-hydroxyvitamin D_3-26,23-lactone Function as Antagonists of Vitamin D Receptor-Mediated Genomic Actions of 1α,25-Dihydroxyvitamin D_3. *Steroids* **2001**, *66*, 227−237.

(19) For the other type of vitamin D antagonist, 25-carboxylic esters ZK159222 and ZK168281, see: (a) Herdick, M.; Steinmeyer, A.; Carlberg, C. Antagonistic Action of a 25-Carboxylic Ester Analogue of 1α,25-Dihydroxyvitamin D_3 Is Mediated by a Lack of Ligand-induced Vitamin D Receptor Interaction with Coactivators. *J. Biol. Chem.* **2000**, *275*, 16506−16512. (b) Bury, Y.; Steinmeyer, A.; Carlberg, C. Structure Activity Relationship of Carboxylic Ester Antagonists of the Vitamin D_3 Receptor. *Mol. Pharmacol.* **2000**, *58*, 1067−1074. (c) Herdick, M.; Steinmeyer, A.; Carlberg, C. Carboxylic Ester Antagonists of 1α,25-Dihydroxyvitamin D_3 Show Cell-Specific Actions. *Chem. Biol.* **2000**, *7*, 885−894. (d) Väisänen, S.; Peräkylä, M.; Kärkkäinen, J. I.; Steinmeyer, A.; Carlberg, C. Critical Role of Helix 12 of the Vitamin D_3 Receptor for the Partial Agonism of Carboxylic Ester Antagonists. *J. Mol. Biol.* **2002**, *315*, 229−238.

(20) Recently, the "third" type of vitamin D antagonist DLAM was reported, see: (a) Kato, Y.; Nakano, Y.; Sano, H.; Tanatani, A.; Kobayashi, H.; Shimazawa, R.; Koshino, H.; Hashimoto, Y.; Nagasawa, K. Synthesis of 1α,25-Dihydroxyvitamin D_3-26,23-lactams (DLAMs), a Novel Series of 1α,25-Dihydroxyvitamin D_3 Antagonist. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2579−2583. (b) Nakano, Y.; Kato, Y; Imai, K.; Ochiai, E.; Namekawa, J.-i.; Ishizuka, S.; Takenouchi, K.; Tanatani, A.; Hashimoto, Y.; Nagasawa, K. Practical Synthesis and Evaluation of the Biological Activities of 1α,25-Dihydroxyvitamin D_3 Antagonists, 1α,25-Dihydroxyvitamin D_3-26,23-lactams. Designed on the Basis of the Helix 12-Folding Inhibition Hypothesis. *J. Med. Chem.* **2006**, *49*, 2398−2406.

(21) (a) Carlberg, C. Molecular Basis of the Selective Activity of Vitamin D Analogues. *J. Cell. Biochem.* **2003**, *88*, 274−281. (b) Toell, A.; Gonzalez, M. M.; Ruf, D.; Steinmeyer, A.; Ishizuka, S.; Carlberg, C. Different Molecular Mechanisms of Vitamin D_3 Receptor Antagonists. *Mol. Pharmacol.* **2001**, *59*, 1478−1485.

(22) Recent reports on Paget's bone disease, see: (a) Reddy, S. V.; Kurihara, N.; Menaa, C.; Roodman, G. D. Paget's Disease of Bone: a Disease of the Osteoclast. *Rev. Endocr. Metab. Disord.* **2001**, *2*, 195−201. (b) Reddy, S. V. Etiology of Paget's Disease and Osteoclast Abnormalities. *J. Cell. Biochem.* **2004**, *93*, 688−696. (c) Roodman, G. D.; Windle, J. J. Paget Disease of Bone. *J. Clin. Invest.* **2005**, *115*, 200−208.

(23) TEI-9647 (**2**) inhibits osteoclast formation induced by the natural hormone **1** from pagetic bone marrow cells: (a) Ishizuka, S.; Kurihara, N.; Miura, D.; Takenouchi, K.; Cornish, J.; Cundy, T.; Reddy, S. V.; Roodman, G. D. Vitamin D Antagonist, TEI-9647, Inhibits Osteoclast Formation Induced by 1α,25-Dihydroxyvitamin D_3 from Pagetic Bone Marrow Cells. *J. Steroid Biochem. Mol. Biol.* **2004**, *89*−*90*, 331−334. (b) Ishizuka, S.; Kurihara, N.; Reddy, S. V.; Cornish, J.; Cundy, T.; Roodman, G. D. (23*S*)-25-Dehydro-1α-Hydroxyvitamin D_3-26,23-lactone, a Vitamin D Receptor Antagonist that Inhibits Osteoclast Formation and Bone Resorption in Bone Marrow Cultures from Patients with Paget's Disease. *Endocrinology* **2005**, *146*, 2023−2030.

(24) (a) Saito, N.; Matsunaga, T.; Fujishima, T.; Anzai, M.; Saito, H.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takayama, H.; Kittaka, A. Remarkable Effect of 2α-Modification on the VDR Antagonistic Activity of 1α-Hydroxyvitamin D_3-26,23-lactones. *Org. Biomol. Chem.* **2003**, *1*, 4396−4402. (b) Saito, N.; Masuda, M.; Matsunaga, T.; Saito, H.; Anzai, M.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takimoto-Kamimura, M.; Kittaka, A. 24,24-Dimethylvitamin D_3-26,23-lactones and Their 2α-Functionalized Analogues as Highly Potent VDR Antagonists. *Tetrahedron* **2004**, *60*, 7951−7961. (c) Saito, N.; Masuda, M.; Saito, H.; Takenouchi, K.; Ishizuka, S.; Namekawa, J.-i.; Takimoto-Kamimura, M.; Kittaka, A. Synthesis of 24,24-Ethanovitamin D_3 Lactones Using Ruthenium-Catalyzed Intermolecular Enyne Metathesis: Potent Vitamin D Receptor Antagonists. *Synthesis* **2005**, 2533−2543.

(25) Introduction of the C2α methyl group into the other type of vitamin D antagonist ZK159222 was also reported by our group. Fujishima, T.; Kojima, Y.; Azumaya, I.; Kittaka, A.; Takayama, H. Design and Synthesis of Potent Vitamin D Receptor Antagonists with A-Ring Modifications: Remarkable Effects of 2α-Methyl Introduction on Antagonistic Activity. *Bioorg. Med. Chem.* **2003**, *11*, 3621−3631.

(26) A part of this work has been communicated: Saito, N.; Saito, H.; Anzai, M.; Yoshida, A.; Fujishima, T.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takayama, H.; Kittaka, A. Dramatic Enhancement of Antagonistic Activity on Vitamin D Receptor: A Double Functionalization of 1α-Hydroxyvitamin D_3-26,23-lactones. *Org. Lett.* **2003**, *5*, 4859−4862.

(27) Trost, B. M.; Dumas, J.; Villa, M. New Strategies for the Synthesis of Vitamin D Metabolites via Pd-Catalyzed Reactions. *J. Am. Chem. Soc.* **1992**, *114*, 9836−9845.

(28) Okuda, Y.; Nakatsukasa, S.; Oshima, K.; Nozaki, H. A Facile and Stereoselective Synthesis of α-Methylene-γ-butyrolactone by Means of Chromium(II) Reagent. *Chem. Lett.* **1985**, 481−484.

(29) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-field FT NMR Application of Mosher's Method. The Absolute Configurations of Marine Terpenoids. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(30) (a) Eisman, J. A.; Hamstra, A. J.; Kream, B. E.; DeLuca, H. F. A Sensitive, Precise, and Convenient Method for Determination of 1,25-Dihydroxyvitamin D in Human Plasma. *Arch. Biochem. Biophys.* **1976**, *176*, 235−243. (b) Inaba, M.; DeLuca, H. F. Stabilization of 1,25-Dihydroxyvitamin D_3 Receptor in the Human Leukemia Cell Line, HL-60, with Diisopropylfluorophosphate. *Biochim. Biophys. Acta* **1989**, *1010*, 20−27.

(31) Collins, S. J.; Ruscetti, F. W.; Gallagher, R. E.; Gallo, R. C. Normal Functional Characteristics of Cultured Human Promyelocytic Leukemia Cells (HL-60) After Induction of Differentiation by Dimethylsulfoxide. *J. Exp. Med.* **1979**, *149*, 969−974.

(32) (a) Suhara, Y.; Kittaka, A.; Kishimoto, S.; Calverley, M. J.; Fujishima, T.; Saito, N.; Sugiura, T.; Waku, K.; Takayama, H. Synthesis and Testing of 2α-Modified 1α,25-Dihydroxyvitamin D_3 Analogues with a Double Side Chain: Marked Cell Differentiation Activity. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3255−3258. (b) Honzawa, S.; Yamamoto, Y.; Hirasaka, K.; Takayama, H.; Kittaka, A. Synthesis of A-Ring Synthon of 2α-Substituted Vitamin D_3 Analogues Utilizing Grignard Reaction towards Methyl 2,3-Anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside. *Heterocycles* **2003**, *61*, 327−338. (c) Honzawa, S.; Suhara, Y.; Nihei, K.-i.; Saito, N.; Kishimoto, S.; Fujishima, T.; Kurihara, M.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. Concise Synthesis and Biological Activities of 2α-Alkyl- and 2α-(ω-Hydroxyalkyl)-20-*epi*-1α,25-dihydroxyvitamin D_3. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3503−3506.

(33) Bula, C. M.; Bishiop, J. E.; Ishizuka, S.; Norman, A. W. 25-Dehydro-1α-Hydroxyvitamin D_3-26,23*S*-lactone Antagonizes the Nuclear Vitamin D Receptor by Mediating a Unique Noncovalent Conformational Change. *Mol. Endocrinol.* **2000**, *14*, 1788−1796.

(34) (a) Ochiai, E.; Miura, D.; Eguchi, H.; Takenouchi, K.; Harada, Y.; Azuma, Y.; Kamimura, T.; Ishizuka, S. Antagonistic Actions of TEI-9647 Depend on the Primary Structure of AF-2 Domain of Vitamin D Receptors. *J. Bone Miner. Res.* **2003**, *18* (suppl. 2), S103. (b) Ochiai, E.; Miura, D.; Eguchi, H.; Ohara, S.; Takenouchi, K.; Azuma, Y.; Kamimura, T.; Norman, A. W.; Ishizuka, S. Molecular Mechanism of the Vitamin D Antagonistic Actions of (23*S*)-25-Dehydro-1α-Hydroxyvitamin D_3-26,23-lactone Depends on the Primary Structure of the Carboxyl-Terminal Region of the Vitamin D Receptor. *Mol. Endocrinol.* **2005**, *19*, 1147−1157. (c) Peräkylä, M.; Molnár, F.; Carlberg, C. A Structural Basis for the Species-Specific Antagonism of 26,23-Lactones on Vitamin D Signaling. *Chem. Biol.* **2004**, *11*, 1147−1156.

(35) Takenouchi, K.; Sogawa, R.; Manabe, K.; Saitoh, H.; Gao, Q.; Miura, D.; Ishizuka, S. Synthesis and Structure-Activity Relationships of TEI-9647 Derivatives as Vitamin D_3 Antagonists. *J. Steroid Biochem. Mol. Biol.* **2004**, *89*−*90*, 31−34.

(36) Some biologically active natural products having an α-methylene-γ-lactone structure react with the thiol group of cysteine to give the corresponding cysteine adduct: Kupchan, S. M.; Fessler, D. C.; Eakin, M. A.; Giacobbe, T. J. Reactions of Alpha Methylene Lactone Tumor Inhibitors with Model Biological Nucleophile. *Science* **1970**, *168*, 376−377.

(37) Recently, X-ray crystallographic analyses of VDR−(**1a**−**c**) complexes were reported, see: Hourai, S.; Fujishima, T.; Kittaka, A.; Suhara, Y.; Takayama, H.; Rochel, N.; Moras, D. Probing a Water Channel near the A-Ring of Receptor-Bound 1α,25-Dihydroxyvitamin D3 with Selected 2α-Substituted Analogues. *J. Med. Chem.* **2006**, *49*, 5199−5205.

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