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# Dramatic Enhancement of Antagonistic Activity on Vitamin D Receptor: A Double Functionalization of 1α-Hydroxyvitamin $D_3$ 26,23-Lactones

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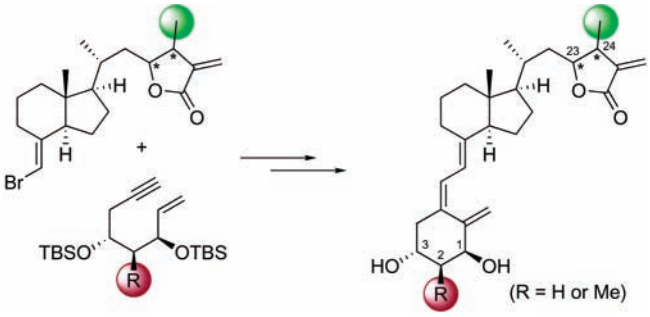
## ABSTRACT




**The synthesis of novel vitamin D receptor antagonists, 24-methyl-1α-hydroxyvitamin $D_3$ 26,23-lactones, is reported. We found that the biological activities of the vitamin $D_3$ lactones were affected by the structure of the lactone part. Furthermore, introduction of a 2α-methyl group into the 24-methylvitamin $D_3$ lactones dramatically enhanced their anti-vitamin D activity.**

The *seco*-steroid hormone 1α,25-dihydroxyvitamin $D_3$ (**1**), which is the potent metabolite of vitamin $D_3$, regulates calcium and phosphorus homeostasis as well as cell differentiation and proliferation of various types of tumor cells.[1,2] Most of the biological responses of **1** are mediated by its specific receptor, vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily and acts as the ligand-dependent gene transcription factor with coactivators.[3,4] Recently, we reported some modification of the C2α position of **1** to exhibit unique biological profiles with superagonistic activity on VDR.[5−7] In particular, introduction of the 2α-methyl (**1a**) showed 4-fold higher

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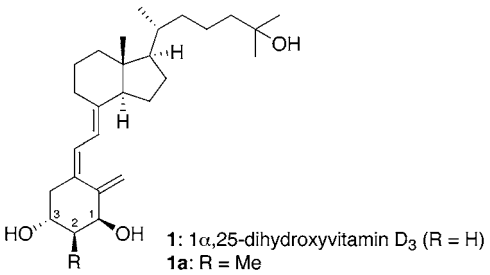


**Figure 1.**

binding affinity to VDR relative to the natural hormone **1** (Figure 1).[5a,b]

Although more than 2000 analogues of **1** have been synthesized over the past few decades,[8] only two different types of antagonists have been described. In 1999, studies on the modification of the side-chain structure based on the 1α,25-dihydroxyvitamin $D_3$ 26,23-lactone metabolite[9] derived from **1** led to the discovery of the TEI-9647 (**2**) and TEI-9648 (**3**) (Figure 2).[10,11] Both vitamin $D_3$ analogues **2**

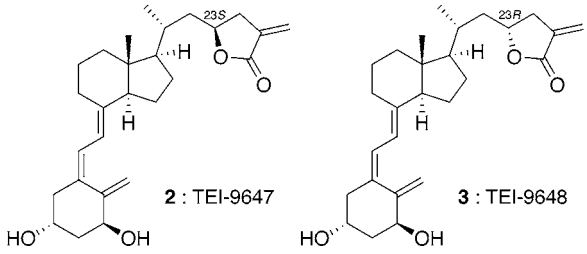


**Figure 2.**

and **3**, which have an α-methylene-γ-butyrolactone part on the side chain, are the first specific antagonists of VDR-mediated genomic action of **1**.[12] Namely, **2** and **3** inhibit differentiation of human leukemia cells (HL-60 cells),[10a] as well as 25-hydroxyvitamin $D_3$ 24-hydroxylase gene expression in human osteosarcoma cells[10b] and in HL-60 cells[10d] induced by **1**. Moreover, TEI-9647 (**2**) shows antagonistic action on the genomic-mediated calcium metabolism regulated by **1** in vivo.[10e] Recently, TEI-9647 (**2**) has received considerable attention because of its possibility to be a potential agent to inhibit the enhanced bone resorption in patients with Paget's disease,[13] which is known as the most flagrant example of disordered bone remodeling and the second most common bone disease after osteoporosis in Anglo-Saxons.[13g]

The unique structures and unprecedented biological profiles of **2** and **3** prompted us to investigate the structure−activity relationships of the vitamin $D_3$ lactones from the standpoint of searching for more potential anti-vitamin D molecules. In particular, we focused on the influence of the structure including the stereochemistry of the lactone moiety on the VDR binding affinity and antagonistic activity, and we planned to introduce a methyl group into the C24 position on the lactone ring of **2** and **3** (**4**−**7**) (Figure 3).

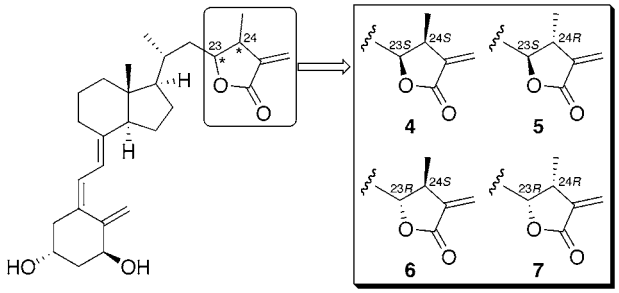


**Figure 3.**

For the synthesis of 24-methylvitamin $D_3$ lactones **4**−**7**, we utilized A-ring/CD-ring coupling methodology.[14] First, we synthesized the CD-ring precursors, which have the 23,24-*syn* lactone moiety, using Cr-mediated *syn*-selective allylation (Scheme 1). According to Oshima's protocol,[15]

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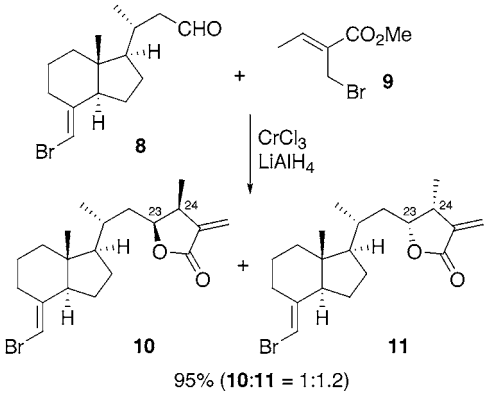
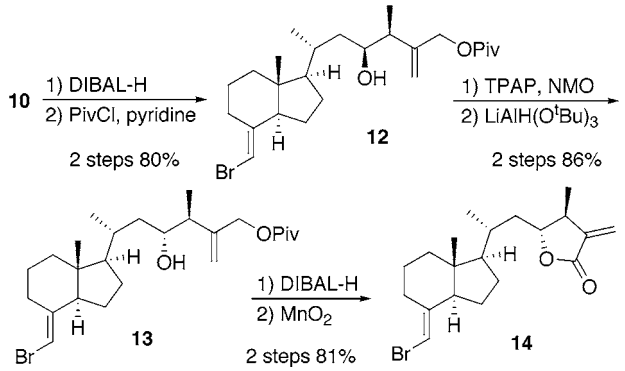
**Scheme 1**




aldehyde **8**, which was obtained from vitamin $D_2$, and allylic bromide **9**[16] were reacted with a low-valent Cr complex prepared from $CrCl_3$ and $LiAlH_4$ to produce two lactone derivatives **10** and **11**, whose stereochemistries on the C23 and C24 positions (based on steroidal numbering) were determined by NOE experiments and modified Mosher's method, in a total 95% yield with a ratio of 1:1.2.[17]

On the other hand, the CD-ring precursor having 23,24-*anti* lactone **14** was synthesized from the 23,24-*syn* lactone **10** (Scheme 2). That is, DIBAL-H reduction of **10** followed

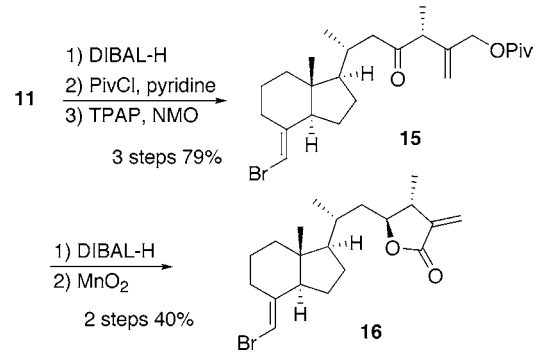
**Scheme 2**




by selective protection of the primary hydroxyl group gave **12**. Inversion of the secondary hydroxyl group of **12** was achieved by TPAP oxidation followed by $LiAlH(OtBu)_3$ reduction, and alcohol **13** was obtained in good yield. Finally, deprotection of the pivaloyl group and subsequent oxidation gave 23,24-*anti* lactone **14**.

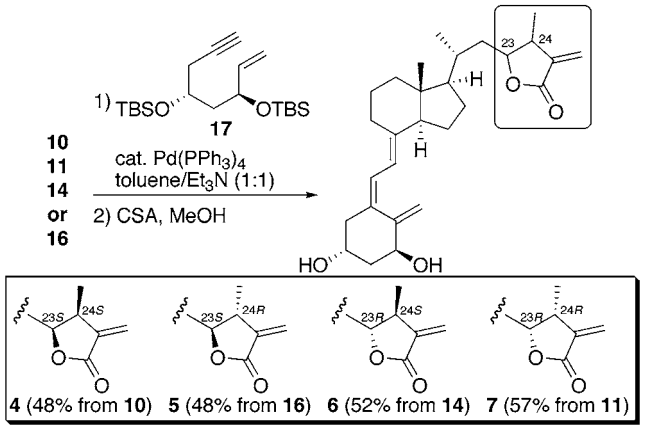
Another *syn* lactone **11** was also transformed into ketone **15** through the same reaction steps. Treatment of **15** with DIBAL-H followed by oxidation produced the CD-ring precursor having an *anti* lactone **16** (Scheme 3).

**Scheme 3**




Construction of the vitamin $D_3$ triene skeleton was accomplished by Pd-catalyzed alkenylative cyclization of enyne **17** with the above CD-ring precursors (**10**, **11**, **14**, or **16**); then, acid-mediated deprotection provided the desired 24-methylvitamin $D_3$-26,23-lactones **4**−**7** (Scheme 4).

**Scheme 4**




The receptor binding affinities and antagonistic activities of the vitamin $D_3$ 26,23-lactones **4**−**7** were evaluated, and both biological activities were markedly affected by the structure of the lactone ring on the side-chain part (Table 1). Binding affinity to the chick intestinal VDR was examined as described previously.[18] The affinity of (23*S*)-lactone analogues increased to be 2.4-fold (**4**) and 1.8-fold (**5**) more potent than that of **2** by introducing the C24 methyl group. In the case of (23*R*)-lactones, introducing the (24*S*)-methyl unit (**6**) into **3** raised the affinity to 1.7 times higher compared with that of **3**, and the affinity of (24*R*)-analogue (**7**) was almost the same as that of **3**. Next, the antagonistic activities of **4**−**7** were assessed by the NBT-reduction method[19] in terms of 50% inhibitory concetration ($IC_{50}$) for

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**Table 1.** Biological Activities of 24-Methylvitamin $D_3$ Lactones **4**−**7**

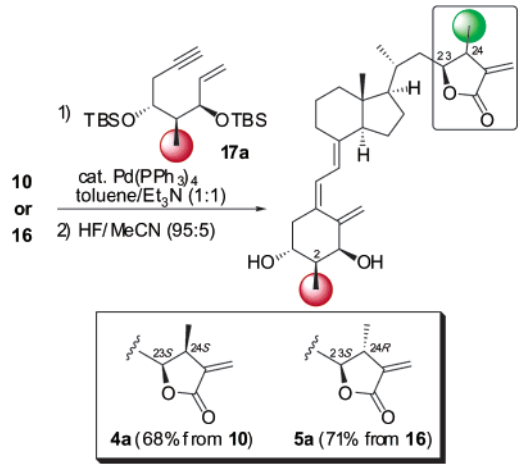
| compd | VDR binding affinity[a] | antagonistic activitiy[b] ($IC_{50}$, nM) |
|---|---|---|
| **1** | 100 | |
| TEI-9647 (**2**) | 12 | 8.3 |
| **4** | 29 | 3.7 |
| **5** | 22 | 3.2 |
| TEI-9648 (**3**) | 7 | 107.0 |
| **6** | 12 | 160.0 |
| **7** | 5 | 51.0 |

[a] The potency of **1** is normalized to 100 (see the Supporting Information). [b] Antagonistic activity was assessed in terms of 50% inhibitory concentration ($IC_{50}$) for differentiation of HL-60 cells induced by 10 nM of **1**.

differentiation of HL-60 cells induced by **1** (10 nM). Introduction of a 24-methyl group into (23*S*)-lactone **2** increased the activity 2.2-fold (**4**) and 2.5-fold (**5**), respectively. While the antagonistic activity of (23*R*)-lactone having the (24*S*)-methyl group (**6**) was the same as that of **3**, (23*R*,24*R*)-24-methylvitamin $D_3$ lactone (**7**) showed three times higher anti-D activity than **3**.

Next, we turned our attention to double functionalization of vitamin $D_3$ lactone analogues. That is, we planned to introduce a 2α-methyl substituent, which was one of our positive motifs for VDR binding affinity,[5a,b,6] into the new 24-methylvitamin $D_3$ lactones. We expected a high increase in VDR binding affinity and striking improvement of the antagonistic activity to VDR through such modifications. On the basis of the above results, we focused on (23*S*)-lactone analogues **4** and **5**, which showed stronger antagonistic

**Scheme 5**




activities than those of **2**, and synthesized the corresponding 2α-methyl derivatives **4a** and **5a** from **10** and **16** with enyne **17a**,[20] respectively (Scheme 5).

Biological evaluation disclosed that modification of the A-ring with the 2α-methyl group dramatically enhanced the antagonistic activities of 24-methylvitamin $D_3$ lactones (Table 2). Namely, (23*S*,24*S*)-2α,24-dimethyl analogue **4a** showed

**Table 2.** Biological Activities of 2α,24-Dimethylvitamin $D_3$ Lactones **4a** and **5a**

| compd | VDR binding affinity[a] | antagonistic activity[b] ($IC_{50}$, nM) |
|---|---|---|
| **2** | 12 | 8.3 |
| **4a** | 63 | 0.19 |
| **5a** | 23 | 0.13 |

[a] The potency of **1** is normalized to 100 (see the Supporting Information). [b] Antagonistic activity was assessed in terms of 50% inhibitory concentration ($IC_{50}$) for differentiation of HL-60 cells induced by 10 nM of **1**.

ca. 5 times stronger VDR binding affinity and 38 times higher antagonistic activity than **2**. Although binding affinity to the VDR of **5** was minimally affected by introducing the 2α-methyl unit (**5a**), it is noteworthy that such modification considerably increased the anti-D activity to ca. 62-fold stronger than that of **2**.

In summary, we have succeeded in the development of highly potent VDR antagonists based on the C24- and C2α-functionalization of 1α-hydroxyvitamin $D_3$ 26,23-lactones. We expect that these analogues with high anti-D activity would contribute to understanding the mechanisms involved in the expression of antagonistic activity on VDR as well as to finding the seeds of new medicines for treating Paget's bone disease. Further synthetic and biological studies are in progress in our group.

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**Supporting Information Available:** Experimental procedure and spectral data for the synthesis of **4**−**8**, **10**−**16**, **4a**, and **5a**. Protocols and charts of vitamin D receptor binding assay and assay for HL-60 cell differentiation to test antagonistic activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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