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# Functionalization at C-12 of 1α,25-Dihydroxyvitamin $D_3$ Strongly Modulates the Affinity for the Vitamin D Receptor (VDR)

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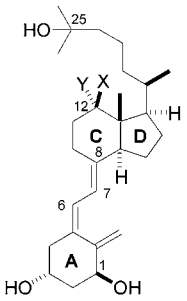
## ABSTRACT




| | VDR relative affinities |
|---|---|
| **1a**, X=Y=H | **100%** |
| **1b**, X=H, Y=OH | **1%** |
| **1c**, X=Y=$CH_2$ | **50%** |
| **1d**, X=$CH_3$, Y=H | **440%** |

**The first synthesis of analogues of the natural hormone 1α,25-dihydroxyvitamin $D_3$ (1a) with substituents at C-12 is reported. The following are the relative affinities of the novel compounds for the vitamin D receptor (VDR) compared to that of 1a (100%): 1α,12α,25-$(OH)_3$-$D_3$ (1b, 1%), 1α,25-$(OH)_2$-12-methylene-$D_3$ (1c, 50%), and 1α,25-$(OH)_2$-12β-methyl-$D_3$ (1d, 440%).**

The steroid hormone 1α,25-dihydroxyvitamin $D_3$ [1α,25-$(OH)_2$-$D_3$, **1a**] is the bioactive metabolite of vitamin $D_3$. It plays an important role in the regulation of mineral metabolism and also promotes cell differentiation and inhibits the proliferation of various types of tumor cells, a fact that suggests its possible use in the treatment of cancer and other hyperproliferation diseases. Unfortunately, the therapeutic value of 1α,25-$(OH)_2$-$D_3$ as an antitumor agent is severely limited by its potent calcemic activity.[1] Efforts aimed at developing vitamin D analogues with strong cell-differentiating ability and low calcemic action have led to the synthesis of more than 3000 analogues of 1α,25-$(OH)_2$-$D_3$, and some of them show acceptable therapeutic profiles.[2] Most of these synthetic analogues have structural modifications at the side chain[2] and A-ring,[3] but only a few examples are known with modifications in the CD-rings[4] and triene system.[5]

The hormone 1α,25-$(OH)_2$-$D_3$ and the majority of its analogues exert their biological effects by binding with high affinity to the nuclear vitamin D receptor (VDR). Activation of the molecular switch of nuclear 1α,25-$(OH)_2$-$D_3$ signaling, a process that starts with the formation of the complex composed of the ligand-activated vitamin D receptor (VDR),

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(3) (a) Sicinski, R. R.; Rotkiewicz, P.; Kolinski, A.; Sicinska, W.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.* **2002**, *45*, 3366. (b) Suhara, Y.; Nihei, K.; Kurihara, M.; Kittaka, A.; Yamaguchi, K.; Fujishima, T.; Konno, K.; Miyata, N.; Takayama, H. *J. Org. Chem.* **2001**, *66*, 8760.

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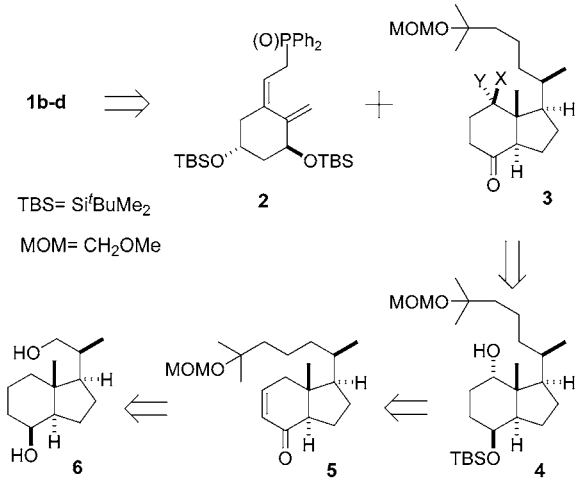
the retinoid X receptor (RXR), and a 1α,25-$(OH)_2$-$D_3$ response element (VDRE),[6] leads to the regulation of the expression of various target genes, some of them involved in the regulation of cell growth, differentiation, and proliferation. Therefore, the ability of a vitamin D analogue (or mimic) to bind to the VDR is a prerequisite for the development of anticancer drugs that activate the vitamin D genomic mode of action. We recently embarked on a research program aimed at studying the structural requirements of the as yet unexplored C-12 position of the hormone 1α,25-$(OH)_2$-$D_3$ for binding to the VDR. Here we describe the synthesis and VDR-binding properties of the first 1α,25-$(OH)_2$-$D_3$ analogues bearing substituents at C-12, namely, 1α,12α,25-$(OH)_3$-$D_3$ (**1b**), 1α,25-$(OH)_2$-12-methylene-$D_3$ (**1c**), and 1α,25-$(OH)_2$-12$\beta$-methyl-$D_3$ (**1d**).[7]

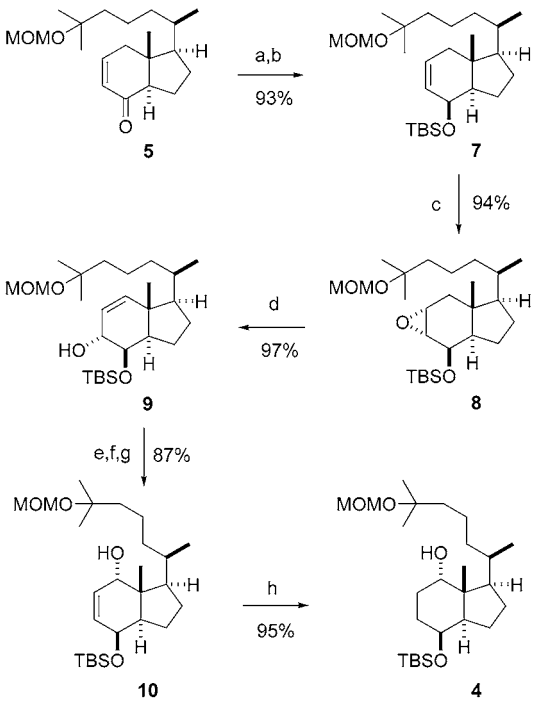
The synthetic plan for the preparation of **1b**−**d** involves the construction of the corresponding vitamin D triene units by the convergent Wittig−Horner approach (coupling between the anion of phosphine oxide **2** and ketones **3**), and the preparation of the common intermediate **4** from Inhoffen−Lythgoe diol (**6**).[8] This synthetic strategy offers a simple solution for the introduction of the 7,8-double bond of target vitamin D analogues but represents a challenge for the functionalization at C-12 (Scheme 1).

**Scheme 1.** Retrosynthetic Analysis of Analogues **1b**−**d**




The α,$\beta$-unsaturated ketone **5** (Scheme 2) was prepared from Inhoffen−Lythgoe diol (**6**) as shown in earlier work.[9] Stereoselective reduction of ketone **5** with diisobutylaluminum hydride and protection of the resulting alcohol with *tert*-butyldimethylsilyl chloride provided **7**. Epoxidation of the double bond of **7** from the less hindered α face using *m*-chloroperbenzoic acid gave epoxide **8**. Attempts to open the epoxide ring with diethylaluminum-2,2,4,4-tetramethylpiperidine[10] failed. However, treatment of epoxide **8** with freshly prepared lithium diethylamide in the presence of hexamethylphosphoramide[11] provided the desired allylic alcohol **9**. Conversion of **9** into **10** was accomplished by a three-step sequence: (i) epoxidation with *m*-chloroperbenzoic acid, (ii) mesylation, and (iii) reaction with sodium-naphthalene.[12] The stereochemistry of the resulting allylic alcohol **10** at C-12 was determined at the epoxyalcohol stage by NMR NOESY experiments. Catalytic hydrogenation of the double bond of **10** afforded the precursor **4** of the desired vitamin D analogues **1b**−**d** (70% overall yield from ketone **5**, eight steps).

**Scheme 2**[a]




[a] Reagents and conditions: (a) $^i$Bu$_2$AlH, THF, −78 °C, 30 min (94%). (b) TBSCl, imidazole, DMF (99%). (c) *m*-CPBA, $CH_2Cl_2$ (94%). (d) $LiNEt_2$ (prepared from $HNEt_2$ and $^n$BuLi, −78 °C to room temperature, $Et_2O$, 15 min), $Et_2O$, HMPA, rt, overnight (97%). (e) *m*-CPBA, $CH_2Cl_2$ (99%). (f) MsCl, $Et_3N$, $CH_2Cl_2$ (99%). (g) Na-naphthalene, THF, rt, 2 h (89%). (h) $H_2$, 5% Pd/C, EtOAc (95%).

With the alcohol **4** in hand, we decided to prepare the upper ketone fragments **3** required for the convergent synthesis of **1b**−**d**. Conversion of **4** into **3a** (Scheme 3) began with desilylation ($^n$Bu$_4$NF, 89%), followed by selective oxidation of the C-8-OH group (PDC, 70%) and subsequent protective silylation of C-12-OH (TMSCl, 86%). The ketone **3b** was obtained in four steps from alcohol **4**. Oxidation of alcohol **4** (PDC, 95%), followed by olefination of the

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(9) Torneiro, M.; Fall, Y.; Castedo, L.; Mouriño, A. *Tetrahedron* **1997**, *53*, 10851.
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(11) Fiaud, J. C.; Legros, J. Y. *J. Org. Chem.* **1987**, *52*, 1907.
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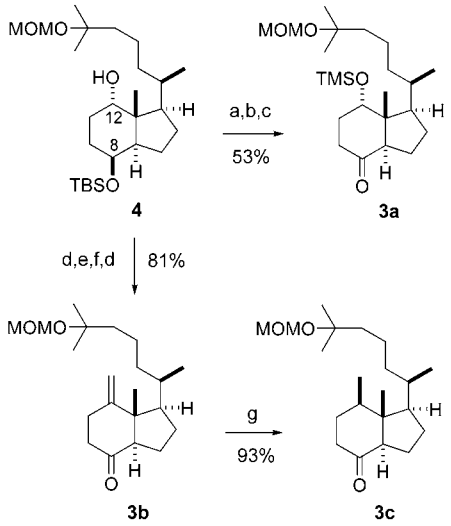
**Scheme 3**[b]



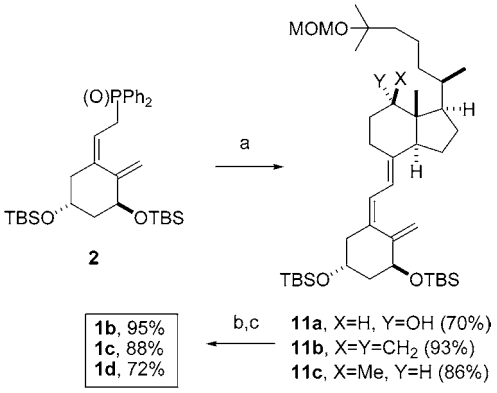

[b] Reagents and conditions: (a) $^{n}Bu_4NF$, THF (89%). (b) Pyridinium dichromate (PDC), $CH_2Cl_2$ (70%). (c) $Me_3SiCl$, $Et_3N$, $CH_2Cl_2$ (86%). (d) PDC, $CH_2Cl_2$ (95%). (e) $Ph_3PMeBr$, $KO^{t}Bu$, toluene (98%). (f) $^{n}Bu_4NF$, THF (92%). (g) $H_2$, 5% Pd/C, EtOAc (93%).

resulting C-12 ketone ($Ph_3P{=}CH_2$, 98%) and successive desilylation ($^{n}Bu_4NF$, 92%) and oxidation (PDC, 95%), gave the C-8 ketone **3b** (81% yield from **4**). The ketone **3c** was prepared by catalytic hydrogenation of **3b** (5% Pd/C, 93%). NOESY studies established the C-12-Me of **3c** as having $\beta$ stereochemistry.

Formation of the vitamin D triene units of protected analogues **11** (Scheme 4) proceeded under mild conditions by the coupling reaction between the anion of phosphine oxide **2** with the respective upper fragment **3**. Finally, deprotection of the hydroxyl groups of **11** ($^{n}Bu_4NF$, THF; AG50W-X4, MeOH) provided the desired 1α,25-$(OH)_2$-$D_3$ analogues **1b**−**d**.

The receptor binding affinities of the novel vitamin D analogues **1b**−**d** were determined by in vitro competitive binding assays (RCI assay)[13] using calf thymus vitamin D receptor. The following are the relative binding affinities of these compounds in comparison with that of the natural hormone 1α,25-$(OH)_2$-$D_3$ (100%): **1b** (1%), **1c** (50%), and

**Scheme 4**[c]




[c] Reagents and conditions: (a) $^{n}BuLi$, THF, **3**, −78 °C. (b) $^{n}Bu_4NF$, THF. (c) AG50W-X4, MeOH.

**1d** (440%). Noteworthy is the binding affinity of **1d**, which is 4.4 times higher than that of the natural hormone 1α,25-$(OH)_2$-$D_3$.

In summary, we have developed the first synthesis of C-12-substituted analogues of the natural hormone 1α,25-$(OH)_2$-$D_3$. The presence of different substituents at this position dramatically changes the affinity of the resulting analogues for the vitamin D receptor. The high receptor binding affinity of analogue **1d** has encouraged us to develop new analogues in this series. The biological results and clinical potential of the new compounds will be reported in due course.

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**Supporting Information Available:** Experimental procedures and spectral data ($^{1}H$ and $^{13}C$ NMR). This material is available free of charge via the Internet at http://pubs.acs.org.

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