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New C15-Substituted Active Vitamin D_3

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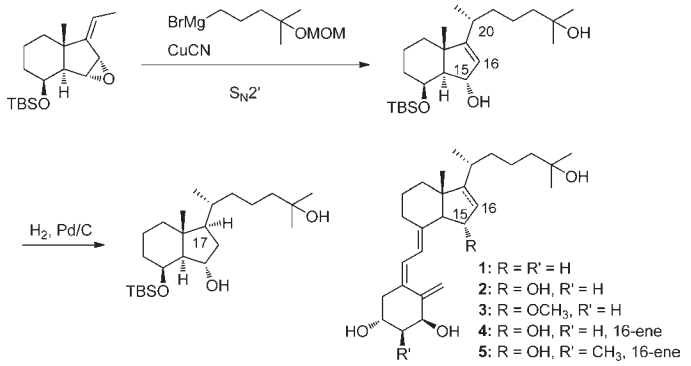
ABSTRACT



C15-Substituted 1α,25-dihydroxyvitamin D_3 analogs were synthesized for the first time to investigate the effects of the modified CD-ring on biological activity concerning the agonistic positioning of helix-3 and helix-12 of the vitamin D receptor (VDR). X-ray cocrystallographic analysis proved that 0.6 Å shifts of the CD-ring and shrinking of the side chain were necessary to maintain the position of the 25-hydroxy group for proper interaction with helix-12. The 15-hydroxy-16-ene derivative showed higher binding affinity for hVDR than the natural hormone.

The physiologically active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$, **1**], regulates cellular growth, differentiation or apoptosis, and immunomodulation, in addition to its classical role in serum calcium/phosphorus homeostasis as well as bone metabolism in the human body.[1] Hormonal vitamin D mediates most of its actions by binding to the specific nuclear vitamin D receptor (VDR) in the target tissues. VDR belongs to the nuclear receptor superfamily acting as a ligand-dependent transcription factor. When 1α,25$(OH)_2D_3$ binds to the VDR, the protein undergoes a conformational change that allows it to interact with coactivators to form an active transcriptional complex with its heterodimeric partner, the retinoid X receptor (RXR); that is, the liganded VDR-RXR heterodimer binds to vitamin D response elements (VDRE) in the promoter regions of target genes with high affinity and modulates gene expression after recruiting coactivators in a wide variety of cells.[2] During the 1α,25$(OH)_2D_3$ signaling process, the stabilization of the ligand binding domain (LBD) of the VDR by moving helix 12 (H12) to the agonistic position is the most important step. In the presence of 1α,25$(OH)_2D_3$, H12 of the human VDR occupies the optimum position for charge clamp formation between the Glu420 residue as the minus charge on H12 and the Lys246 residue as the plus charge on helix 3 (H3). The correct positioning of H12 and the

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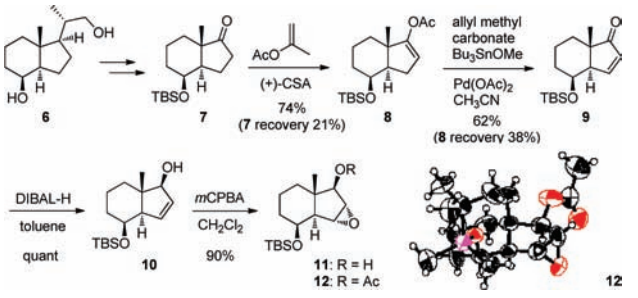
subsequent precise charge clamp formation are critical for effective VDR–coactivator interaction.[3]

Moras' X-ray crystallographic analysis of the engineered human VDR LBD-1α,25(OH)$_2$D$_3$ complex showed that H3 covered the CD-ring of the ligand molecule.[4] H3 contacts with H12 in the agonistic position through both hydrophobic and polar interactions. We thought that if H3 was pushed upward by a newly introduced functional group at the C15 position of the ligand CD-ring, the position of H3 would move and affect the agonistic location of H12, and the new ligand with C15-substitution would exhibit a unique biological profile. First, 1α,15α,25-trihydroxyvitamin D$_3$ (**2**) and 15α-methoxy-1α,25(OH)$_2$D$_3$ (**3**) were designed, synthesized, and biologically evaluated.

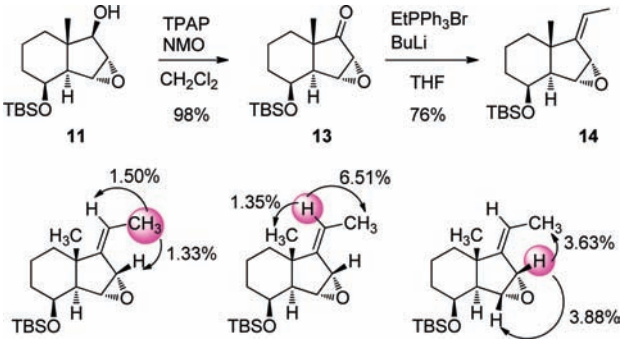
The CD-ring parts were synthesized from an Inhoffen-Lythgoe diol (**6**), which was converted to the known ketone (**7**).[5] Vinyl acetate **8** from ketone **7** via transacetylation with isopropenyl acetate was treated with allyl methyl carbonate in the presence of catalysts Pd(OAc)$_2$ and tributyltin methoxide to give α,β-unsaturated ketone **9** in good yield with recovery of **8**.[6] Reduction of enone **9** with DIBAL-H gave allyl alcohol **10** stereoselectively and subsequent mCPBA epoxidation afforded 15α,16α-epoxyhydrindan derivative **11** (steroidal numbering) exclusively, whose stereochemistry was determined by X-ray crystallographic analysis of its acetate **12** (Scheme 1).[7,8]

Scheme 1. Epoxyhydrindan (**11**) Synthesis and ORTEP Drawing of X-ray Molecular Structure of Its Acetate **12**



TPAP oxidation of **11** afforded α-epoxy ketone **13**,[9] and subsequent Wittig reaction gave ethylidene **14**[10] as a single isomer with *Z*-configration determined by NOE experiments (Scheme 2), although it was reported that 15β,16β-epoxy-17-keto sterols afforded only an *E*-ethylidene epoxide by the corresponding Wittig reaction.[11]

Scheme 2. (*Z*)-Ethylidene Synthesis and NOE Experiments of **14**



1,4-Addition of the magnesium cyanocuprate derivative of 5-bromo-2-methyl-2-pentanol MOM ether[12] to ethylidene epoxide **14** afforded the 15α-hydroxy-16-ene CD ring **15** with 20*R* (natural) and 20*S* side chains in a ratio of 11:1 (Scheme 3). The stereochemistry at C20 of the major isomer **15a** results from attack of the alkyl cuprate on the β-face of the *Z*-alkene **14** in an S$_N$2′ manner.[11c] The Δ^{16} double bond of the major isomer **15a** was hydrogenated using Pd/C to give **16** with 17*R* (natural) and 17*S* configuration in a ratio of 14:1. To confirm the stereochemistry at C17 and C20 of the major isomer **16a**, the 15α-hydroxy group of **16a** was reduced by Barton–McCombie radical deoxygenation[13] followed by oxidation at the C8-hydroxy group to give ketone **19**, whose spectral properties were identical to the known ketone[14] derived from vitamin D$_3$ (Scheme 3).

The secondary hydroxy group at C15 of **16a** was methoxymethylated (**20**) or methylated (**21**) followed by C8-OH oxidation using TPAP/NMO after deprotection of its TBS group to give C8-ketone. The resulting ketones **22** and **23** were coupled with A-ring phosphine oxide **24**[15] by a Wittig–Horner reaction to afford the coupling products **25** (13%) and **26** (9%) with the eliminated Δ^{14}-CD-ring (**27**, 4–17%) and recovered **22** (62%) and **23** (78%), respectively.[16] The subsequent deprotection and HPLC

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(7) Crystallographic data: empirical formula = $C_{18}H_{32}O_4Si$; formula weight = 340.53; crystal system = orthorhombic; space group = $P2_12_12_1$ (#19); a = 8.03971(19) Å; b = 11.3007(3) Å; c = 22.2692(6) Å; V = 2023.26(9) Å^3; Z = 4; D_{calc} = 1.118 g/cm^3; crystal dimensions = 0.20 × 0.15 × 1.00 mm^3; data collection temp = 296.0 ± 1 K; radiation = Cu Kα (λ = 1.54187 Å); μ (Cu Kα) = 11.53 cm^{-1}; number of reflections measured = 20 529; number of unique reflections = 3674 (R_{int} = 0.079); Residuals: $R_1(I > 2.00\sigma(I))$ = 0.0801; Residuals: $_wR_2$ = 0.0926.

(8) The direct epoxidation of enone **9** with *m*-CPBA was unsucessful, since the Baeyer–Villiger reaction gave a tetrahydroqoumarin derivative.

(9) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625–1627.

(10) Ethylidene epoxide **14** was unstable, and it was used within 24 h for the next step.

(11) (a) Takahashi, T.; Ootake, A.; Tsuji, J.; Tachibana, K. *Tetrahedron* **1985**, *41*, 5747–5754. (b) Marino, J. P.; Abe, H. *J. Am. Chem. Soc.* **1981**, *103*, 2907–2909. (c) Moon, S.; Stuhmiller, L. M.; Chadha, R. K.; McMorris, T. C. *Tetrahedron* **1990**, *46*, 2287–2306.

(12) Larsen, S. D.; Spilman, C. H.; Yagi, Y.; Dinh, D. M.; Hart, K. L.; Hess, G. F. *J. Med. Chem.* **1994**, *37*, 2343–2351.

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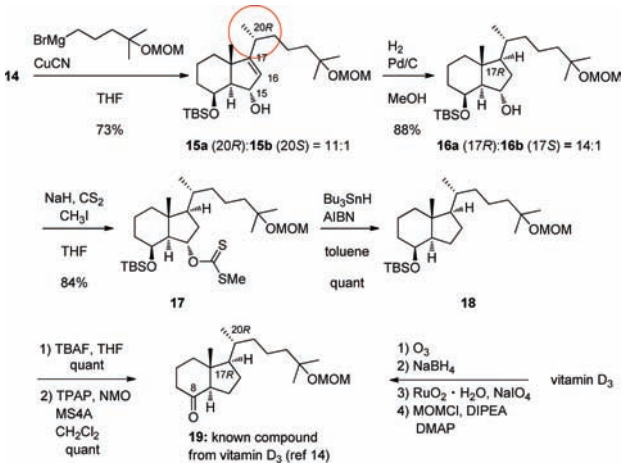
(14) (a) Torneiro, M.; Fall, Y.; Castedo, L.; Mouriño, A. *Tetrahedron* **1997**, *53*, 10851–10870. (b) Ono, K.; Yoshida, A.; Saito, N.; Fujishima, T.; Honzawa, S.; Suhara, Y.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, T.; Kittaka, A. *J. Org. Chem.* **2003**, *68*, 7407–7415.

(15) (a) Hatakeyama, S.; Irie, H.; Shintani, T.; Noguchi, Y.; Yamada, H.; Nishizawa, M. *Tetrahedron* **1994**, *50*, 13369–13376. (b) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098–3108. (c) Miles, W. H.; Connell, K. B.; Ulas, G.; Tuson, H. H.; Dethoff, E. A.; Mehta, V.; Thrall, A. J. *J. Org. Chem.* **2010**, *75*, 6820–6829.

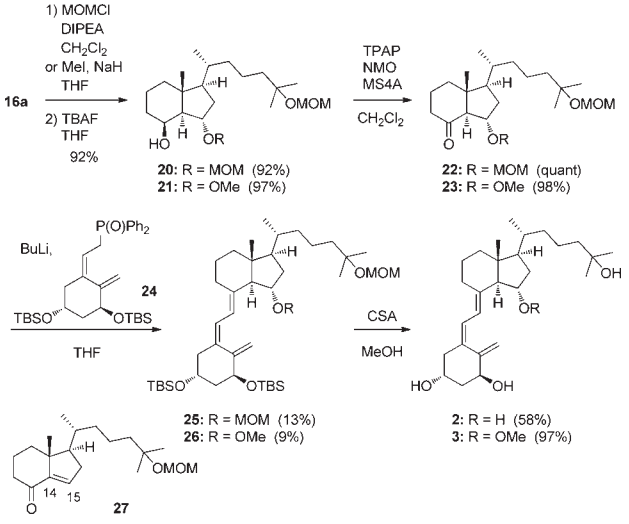
(16) The coupling yield of the 15α-substituted CD-ring (**22**,**23**) was low; however, the 15β-OMOM counterpart gave the corresponding triene product in 49% yield under the same reaction conditions.

Scheme 3. Introduction of Side Chain to Ethylidene Epoxide **14** via S_N2' Type 1,4-Addition



purification gave the desired C15-modified analogs **2** and **3** (Scheme 4).

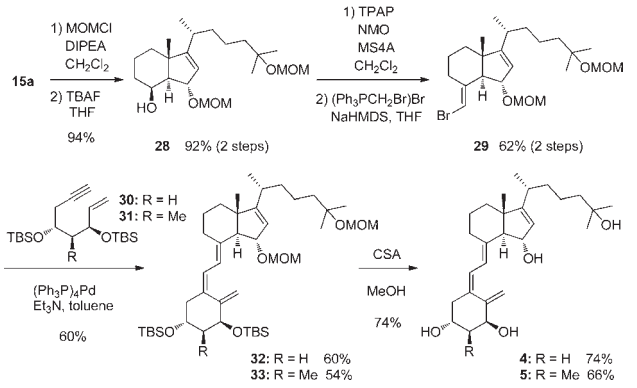
Scheme 4. Wittig–Horner Coupling Reaction



In our synthetic route, the new C15-substituted 16-ene-active vitamin D_3 analogs were also available from **15a**. It is known that 1α,25-dihydroxy-16-ene-vitamin D_3 shows higher binding affinity for VDR, lower affinity for the vitamin D binding protein in serum, and greater resistance in an active form to catabolism as compared with 1α,25-dihydroxyvitamin D_3 (**1**);[17] therefore, synthesis of 16-ene analogs was suggested as a candidate drug in the 1990s.[17e] We therefore revisited 16-ene-vitamin D_3 analog synthesis. The Wittig–Horner coupling yield of the 15α-substituted CD-ring was low, and here, we utilized the Trost Pd-mediated coupling reaction between CD-ring bromoolefin **29** and enyne **30** as shown in Scheme 5.[18] The free secondary hydroxy group of **15a** was protected by MOM followed by deprotection of the TBS group and TPAP/NMO oxidation to give C8-ketone, which was converted to bromoolefin **29** in good yield. The coupling reaction proceeded in moderate yield to give 16-ene analog **32**, which was deprotected under mild acidic conditions to afford the desired molecule **4**. Since we were interested in the synergetic effects of the 2α-methyl group in the A-ring and 16-ene modification in the CD-ring on biological activity, compound **5** was also synthesized using enyne **31**.[19,20]

We tested the preliminary biological activity of the new compounds **2**–**5**: (1) binding affinity for VDR was 13% (for **2**), 1.5% (for **3**), 65% (for **4**), and 278% (for **5**) of that of the natural hormone, 1α,25$(OH)_2D_3$. (2) EC_{50} values of transactivation activity of an osteocalcin promoter in HOS cells were 0.12, 0.12, 0.08, and 0.12 nM, respectively, while

Scheme 5. Trost Coupling Reaction



a value of 0.09 nM was observed in the case of 1α,25$(OH)_2$-D_3. 16-Ene analogs **4** and **5** showed comparable and even stronger affinity for VDR and transactivation activity than 1α,25$(OH)_2D_3$ (Table 1).

Next, we studied the crystal structure of the complex between truncated hVDR LBD and the new ligand **3** to see new interactions of the 15α-OCH_3 group and amino acid residues (Figure 1).[21] The hVDR LBD-**3** complex was

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(20) For the effects of 2α-functionalization on VDR binding and biological activities, see: (a) Saito, N.; Honzawa, S.; Kittaka, A. *Curr. Top. Med. Chem.* **2006**, *6*, 1273–1288. (b) Hourai, S.; Fujishima, T.; Kittaka, A.; Suhara, Y.; Takayama, H.; Rochel, N.; Moras, D. *J. Med. Chem.* **2006**, *49*, 5199–5205.

(21) The Protein Data Bank accession number for the coordinates of the structures of the vitamin D receptor complex with **3** reported in this article is 3AX8.

Table 1. VDR Binding Affinity and Osteocalcin Promoter Transactivation Activity in HOS Cells

compound	relative VDR binding affinity (%)	osteocalcin transactivation activity in HOS/SF cells (EC_{50}, nM)
1α,25(OH)$_2$D$_3$ (**1**)	100[a,b]	0.09
2	13[a]	0.12
3	1.5[a]	0.12
4	65[b]	0.08
5	278[b]	0.12

[a] Chick intestinal VDR. [b] Human VDR.

superposed to hVDR LBD-**1** with an rmsd of 0.308 Å on all Cα atoms. In this study, the methyl group of 15α-OCH_3 made a new CH···O interaction at a 3.1 Å distance with a Ser275Oγ atom, which had never been found in the reported hVDR LBD-ligand complexes so far. The 25-OH group of **1** formed hydrogen bonds to His305 and His397 in the active hVDR-ligand complex, and the 25-OH group of **3** was located at the same position as in **1** in the LBD of the hVDR for the stable complex formation, and it caused the CD-ring of **3** to push upward in 0.6 Å and the C17 side chain was shrunk to keep the original position of the 25-OH group that formed hydrogen bonds with His305 and His397. The H3 and H12 of this study took similar positions with those in the hVDR LBD-natural ligand complex.[4] However, the interaction between the C15-substituted group and Ser275Oγ could be used positively to design new profile ligands, which would affect the dynamic process of a ligand−receptor complex formation including cofactor recruiting steps to show unique biological profiles.[22]

In summary, we synthesized C15-modified active vitamin D_3 derivatives for the first time and tested their preliminary biological activities. C16-Ene analogs showed greater activity than the natural hormone. We are studying further modifications at the C15 position to understand the structural function on both the ligands and the receptor.

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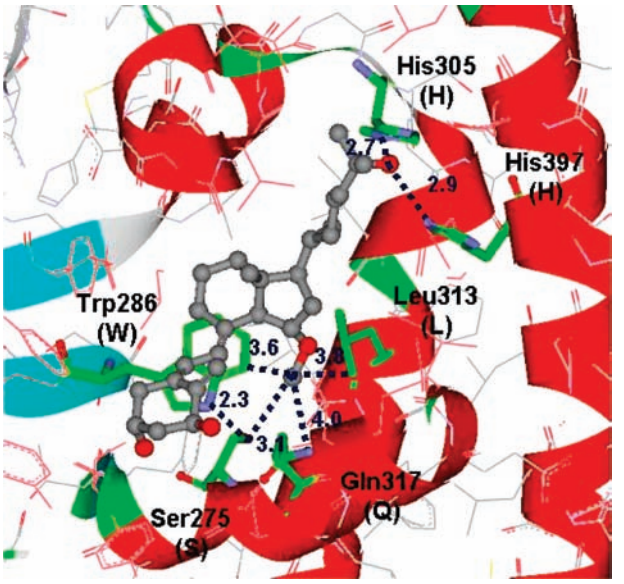

Figure 1. X-ray studies on hVDR-**3** complex.

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Supporting Information Available. Detailed experimental procedures and spectral data for all new compounds and X-ray structural data of **12**. This material is available free of charge via the Internet at http://pubs.acs.org.