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The First Locked Side-Chain Analogues of Calcitriol (1α,25-Dihydroxyvitamin D_3) Induce Vitamin D Receptor Transcriptional Activity

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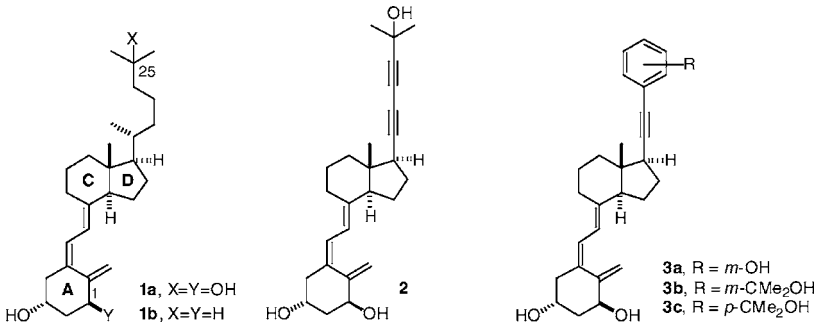
ABSTRACT



We describe the synthesis of the first locked side-chain analogues of the natural hormone 1α,25-$(OH)_2$-D_3 and their effects on gene transcription in human colon cancer cells. Analogue 2 was more potent than 1α,25-$(OH)_2$-D_3 at inducing vitamin D receptor (VDR) transcriptional activity. Analogues 3a and 3b show potency similar to that of 1α,25-$(OH)_2$-D_3, whereas 3c was less active. The novel analogues efficiently bind VDR in vivo to induce transcription from a consensus vitamin D responsive element (VDRE).

It is now known that 1α,25-dihydroxyvitamin D_3 [**1a**, 1α,-25-$(OH)_2$-D_3, calcitriol], the hormonally active form of vitamin D_3 (**1b**, cholecalciferol), exerts a wide range of genomic biological actions through a multistep mechanism that includes binding to the nuclear vitamin D receptor (VDR),[1] heterodimerization of the VDR with retinoid X receptor (RXR), and binding of the resulting complex to specific DNA sequences, named vitamin D responsive element (VDRE), to induce transcription.[2,3] The hormone 1α,25-$(OH)_2$-D_3, besides its important role in calcium homeostasis, also promotes cell differentiation and inhibits cell proliferation of various tumor cells, a fact that suggests its possible use in the treatment of cancer. Unfortunately, the therapeutic value of 1α,25-$(OH)_2$-D_3 as an antitumor agent finds serious limitations due to its potent calcemic side effects.[2,4] Recent interest in the development of an analogue of 1α,25-$(OH)_2$-D_3 with selective properties for treatment of cancer and dermatological diseases has led to an increased activity in the vitamin D field.[5,6]

Prior to Moras' X-ray study of a mutant VDR complexed to 1α,25-$(OH)_2$-D_3, incomplete understanding of the con-

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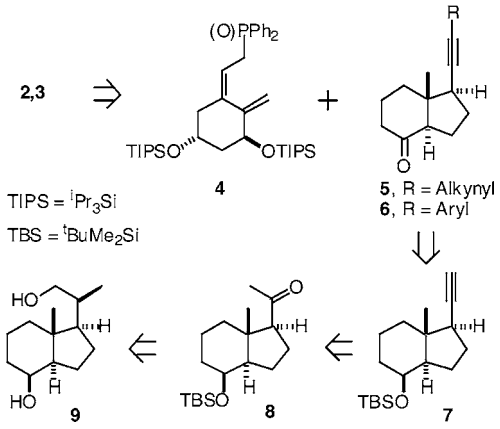
formation that the side chain of 1α,25-$(OH)_2$-D_3 adopts in the binding pocket of the vitamin D receptor (VDR) led to the synthesis of numerous side-chain-modified analogues, of which only a few have been identified as promising for the treatment of certain cancers and psoriasis.[7,8] To study indirectly the location of the C25 hydroxyl group of 1α,25-$(OH)_2$-D_3 in its bioactive conformation or conformations, we recently reported the synthesis and conformational analysis of side-chain analogues of 1α,25-$(OH)_2$-D_3 that incorporate conformationally locked units in the form of a double bond, a cyclopropane ring, an aromatic ring, or an additional five-membered ring.[9,10] The fact that some of these analogues improved the biological profile of the natural hormone for potential therapeutic applications has now led us to synthesize novel analogues of 1α,25-$(OH)_2$-D_3 with side chains with higher degrees of rotational restriction in order to define the topography of the side-chain hydroxyl group that is required to induce gene transcription. Conformational and docking studies of a series of analogues using Moras' X-ray crystal structure[11] led us to design the locked side-chain analogues **2** and **3**, which incorporate two triple bonds or a triple bond and an aromatic unit in their respective side chains. Here we describe the synthesis of these four novel analogues of 1α,25-$(OH)_2$-D_3 and report preliminary data on their biological behavior.

The synthesis of analogues **2** and **3** follows the mild convergent Wittig−Horner approach originally developed by Lythgoe and later improved by the Hoffmann La Roche group (Scheme 1).[5,12] In this route, **5** or **6** is coupled with

Scheme 1. Retrosynthetic Analysis of Analogues **2** and **3**

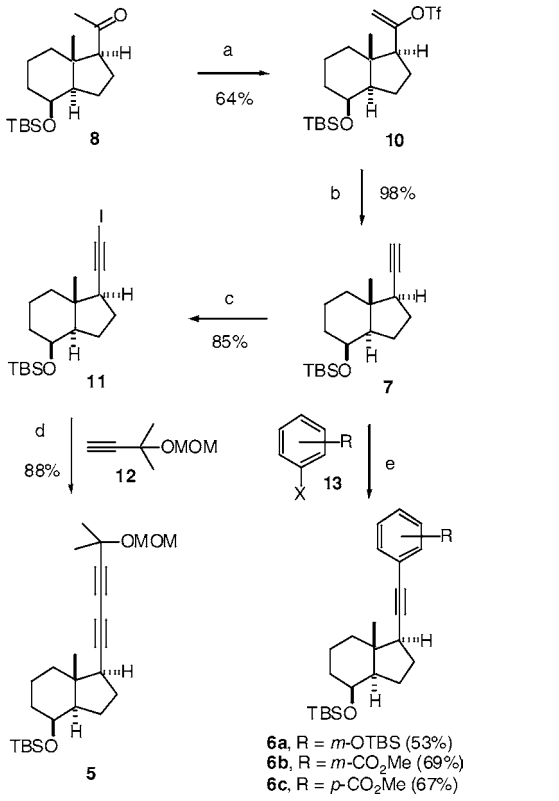


the anion of phosphine oxide **4** to provide, after deprotection, the desired 1α,25-dihhydroxyvitamin D_3 analogues **2** or **3**.

The upper fragments **5** and **6** are prepared from alkyne **7** under metal-catalyzed couplings. Methyl ketone **8**, which is readily prepared by degradation of Inhoffen−Lythgoe diol (**9**) as shown in earlier work,[13] serves for the preparation of the key alkyne **7**.

The preparation of the upper fragments **5** and **6** required for the convergent synthesis of the target compounds **2** and **3** is shown in Scheme 2. Treatment of ketone **8** with LDA

Scheme 2 [a]



[a] Reagents and reaction conditions: (a) LDA; *N*-(5-chloro-2-pyridyl)-triflimide, THF −78 °C. (b) LDA, THF, rt. (c) ^{n}HexLi; I_2, THF, −78 °C. (d) **12** (1.3 equiv), CuI (10%), pyrrolidine. (e) **13** (2 equiv), Et_3N (4 equiv), $(PPh_3)_2PdCl_2$ (10%), DMF, 80 °C. **13a** (X = *m*-Br, R = OTBS), **13b** (X = *m*-OSO_2CF_3, R = CO_2Me), **13c** (X = *p*-OSO_2CF_3, R = CO_2Me).

and reaction of the resulting kinetic enolate with *N*-(5-chloro-2-pyridyl)-triflimide[14] gave vinyl triflate **10** (64%), which

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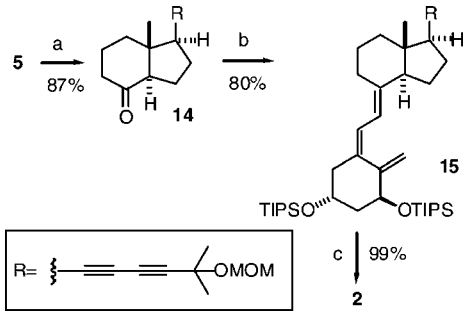
was subjected to elimination (LDA) to deliver the key alkyne **7** in good yield (98%). With the common intermediate **7** in hand, we first accomplished the synthesis of the upper fragment **5** precursor of analogue **2**. Metalation of **7** (HexLi)[15] and subsequent reaction of the resulting lithium acetylide with iodine provided the iodo alkyne **11** (85%). Coupling between **11** and propargylic ether **12** in the presence of copper(I) iodide[16] and pyrrolidine gave the desired diyne **5** in good yield (88%) (five steps from **8**, 47%). The aromatic upper fragments **6** required for the synthesis of analogues **3** were prepared from alkyne **7** using Pd-catalyzed alkynylation under the Sonogashira reaction.[17] Thus, heating of a mixture of **7** and 1-bromo-3-*tert*-butyldimethylsilyloxybenzene (**13a**) in DMF at 80 °C in the presence of Et_3N and $(PPh_3)_2PdCl_2$ provided **6a** (53%). Alkynes **6b** (69%) and **6c** (67%) were prepared in a similar manner using methyl 3-[[(trifluoromethyl)sulfonyl)]oxy]-benzoate (**13b**) and methyl 4-[[(trifluoromethyl)sulfonyl)]-oxy]benzoate (**13c**) as the aromatic partners (Scheme 2).

Ketone **14** required for the synthesis of analogue **2** was prepared from diyne **5** by sequential desilylation (HF) and oxidation with pyridinium dichromate (two steps, 87%) (Scheme 3). Ketone **14** was coupled at −78 °C with the anion

Scheme 3 [a]



[a] Reagents and conditions: (a) HF (45%), CH_3CN, rt; PDC (2 equiv), CH_2Cl_2, rt. (b) Anion of **4**, THF, −78 °C. (c) $^nBu_4NF$ (3 equiv), THF, rt; AG50 WX4, MeOH, rt.

of phosphine oxide **4**[18] to form stereoselectively the corresponding protected analogue **15**. Sequential deprotection of **15** ($^nBu_4NF$; AG50 WX4) provided the requisite analogue **2** (80% from **14**).

Analogues **3** were prepared in a similar way from the upper fragments **6** (Scheme 4). Desilylation and oxidation of **6** provided ketones **16**. Wittig−Horner coupling of **16a** with the anion of phosphine oxide **4** led, after desilylation ($^nBu_4NF$), the desired analogue **3a** (four steps from **6a**, 36%).

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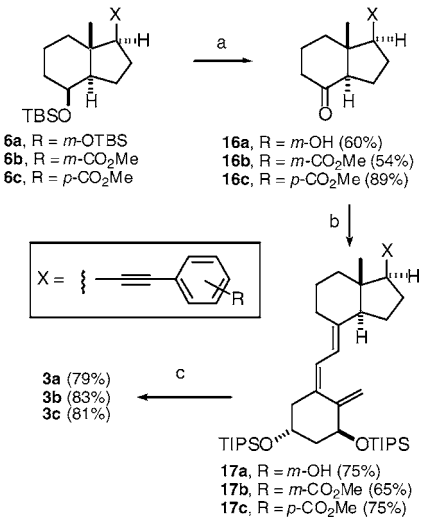
Scheme 4 [a]



[a] Reagents and reaction conditions: (a) HF (45%), CH_3CN, rt; PDC (2 equiv), CH_2Cl_2, rt. (b) Anion of **4**, −78 °C. (c) **17a**: $^nBu_4NF$ (3 equiv), THF, rt. **17b**−**c**: MeLi (3 equiv), THF, 0°C; $^nBu_4NF$.

Desilylation and subsequent oxidation of **6b** and **6c** delivered ketones **16b** and **16c**, which were coupled as above to provide esters **17b** and **17c**, respectively.

Treatment of **17b** and **17c** with methyllithium followed by desilylation ($^nBu_4NF$) led to the respective desired analogues **3b** and **3c** (29 and 54% overall yields from **6b** and **6c**, respectively).

Finally, the biological activity of the four novel locked vitamin D analogues was assayed in the human SW480-ADH colon cancer cell line. These cells express endogenous VDR and respond to 1α,25-$(OH)_2$-D_3 addition by inhibition of proliferation and differentiation to an epithelial phenotype.[19]

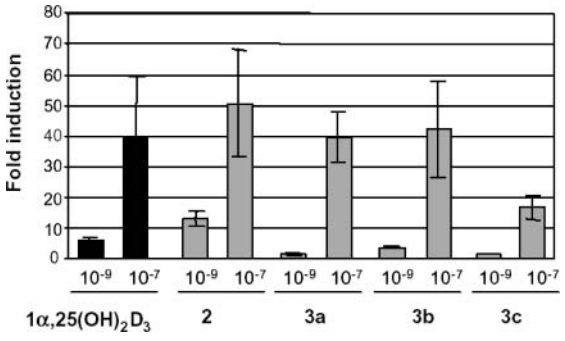


Figure 1. SW480-ADH colon cancer cells were transfected with the 4xVDRE-DR3.tk-luc construct containing the luciferase gene under the control of four copies of direct repeat 3 (DR3) VDRE. A β-galactosidase expression vector (pRSV-LacZ) was also transfected as an internal control. After 72 h of incubation in the presence or absence of the indicated concentrations of 1α,25-$(OH)_2$-D_3, luciferase and β-galactosidase activities in total cell extracts were measured. Mean values and standard deviations of the mean obtained in three experiments using triplicates are shown.

The ability of the four derivatives to active VDR was examined in transactivation experiments using cells that were transfected with a plasmid encoding the luciferase reporter gene under the control of a vitamin D response element (VDRE). Treatment with 10^{-7} M or 10^{-9} M 1α,25-$(OH)_2$-D_3 for 48 h led to 39- and 7-fold increases, respectively, in luciferase expression over vehicle-treated cells (Figure 1). Remarkably, analogue **2** was more potent that calcitriol, causing higher increases in VDR transactivating activity (50- and 12.5-fold at 10^{-7} or 10^{-9} M, respectively). Analogues **3a** and **3b** showed potency similar to that of calcitriol at 10^{-7} M (around 40-fold activation) but lower potency at 10^{-9} M, whereas analogue **3c** was less active (18-fold activation at 10^{-7} M). These results show that our compounds efficiently bind VDR in vivo, inducing its ability to activate transcription from a consensus VDRE.

In summary, we have developed the first locked side-chain analogues of 1α,25-$(OH)_2D_3$. It is noteworthy that the novel analogues **2** and **3a**−**c** lead to significant transcription activation in comparison to 1α,25-$(OH)_2$-D_3, giving important structural information with regard to the bioactive conformation of the natural hormone. Further biological results, the clinical potential of the novel analogues, and the development of new analogues in this series 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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