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Synthesis and biological evaluation of 6-methyl analog of 1α,25-dihydroxyvitamin D_3 ☆

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ABSTRACT

Among more than 3000 analogs of 1α,25-dihydroxyvitamin D_3 synthesized to date, only a few were characterized by structural modifications in the seco-B-ring. The compounds alkylated at C-6 seemed to be interesting targets for synthetic efforts. Such vitamin D analogs easily undergo thermal conversion to their previtamin forms. The results of molecular modeling indicate that significant deviation from planarity must be present in their molecules associated with the interaction of the 6-alkyl substituent and hydrogens from the C-ring. The synthesis of the analog of 1α,25-$(OH)_2D_3$, being characterized by the presence of the 6-methyl group, is reported here, together with the results of preliminary testing of its biological potency. This 6-alkylated compound was efficiently prepared using a novel stereoconvergent strategy in which the ring A and the triene unit of the vitamin D skeleton are constructed by a one-pot Pd-catalyzed tandem cyclization-Negishi coupling process.

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1. Introduction

The most active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, **1**; Fig. 1) is a potent calcium and phosphorus-regulating hormone playing an important role in bone homeostasis in animals and humans. Also, in addition to this classical role, the natural hormone elicits both immunomodulation, cell differentiation and proliferation inhibition activities in numerous malignant cells and keratinocytes [1,2]. 1α,25-Dihydroxyvitamin D_3 expresses these functions by binding to the vitamin D receptor (VDR), a ligand-regulated transcription factor [3–5]. Numerous analogs of the native hormone **1** have been prepared and some of them have been used clinically to treat bone disorders such as osteoporosis and the skin disorder-psoriasis [6]. Although more than 3000 different structural analogs of the natural hormone have been obtained and tested to date [7], very few of them were characterized by substitution of the intercyclic C(5)=C(6)–C(7)=C(8) diene moiety. 6-Fluorovitamin D_3 (**3**) was synthesized by Dauben et al. [8] and this compound has been shown to antagonize the activity of 1α,25-$(OH)_2D_3$, especially intestinal calcium absorption, *in vivo* in chicken [9]. The synthesis of 6-methylvitamin D_3 (**4**) was reported by Sheves and Mazur [10] using a 6-oxo-3,5-cyclovitamin D precursor; the same compound was also obtained by Yamada et al. [11] by reductive thermal desulfonylation of the 6-methylated vitamin D_3–sulfur dioxide adduct. Recently, 1α-hydroxy-6-methylvitamin D_3 (**5**) was synthesized by a novel approach involving a Pd-catalyzed carbocyclization–Negishi cross-coupling cascade [12].

Compounds alkylated at C-6 seem to be interesting targets for synthetic and biological studies. Such vitamin D analogs easily undergo thermal conversion to their previtamin forms [10] and a significant deviation from planarity must be present in their diene system, connecting the ring A to the C,D-hydrindane fragment. In a continuing effort to develop 1α,25-dihydroxyvitamin D_3 analogs having biological profiles suitable for pharmaceutical uses, we have synthesized 1α,25-dihydroxy-6-methylvitamin D_3 (**2**).

2. Materials and methods

2.1. Preparation of 1α,25-dihydroxy-6-methylvitamin D_3 (2)

The synthesis of the 6-methyl substituted vitamin D analog **2** and its intermediates was performed at the Department of Biochemistry, University of Wisconsin-Madison, at the Department of Chemistry, University of Warsaw and at Departamento de Quimica Organica, Universidad de Santiago de Compostela, according to the synthetic route presented in Scheme 1. Spectroscopic and analytical properties of the prepared compounds were consistent with their structures. Full details of this synthesis will be reported elsewhere.

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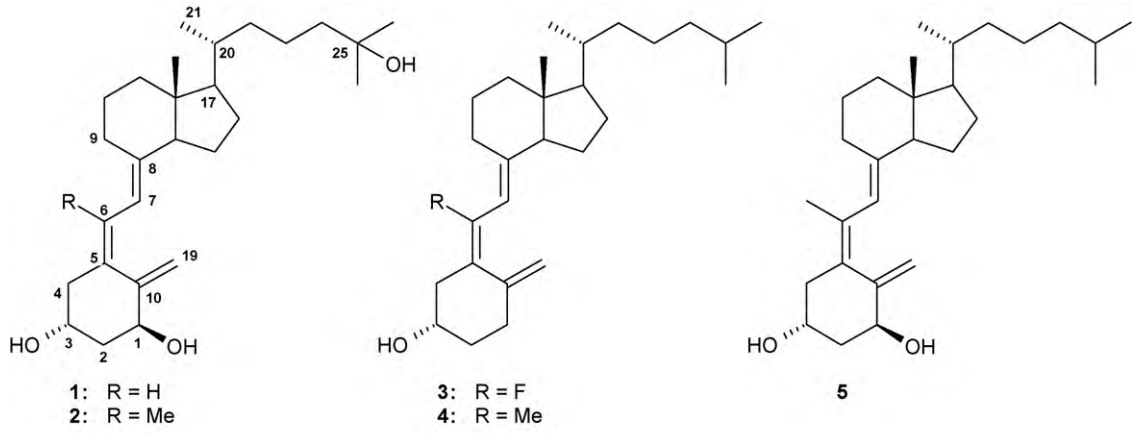
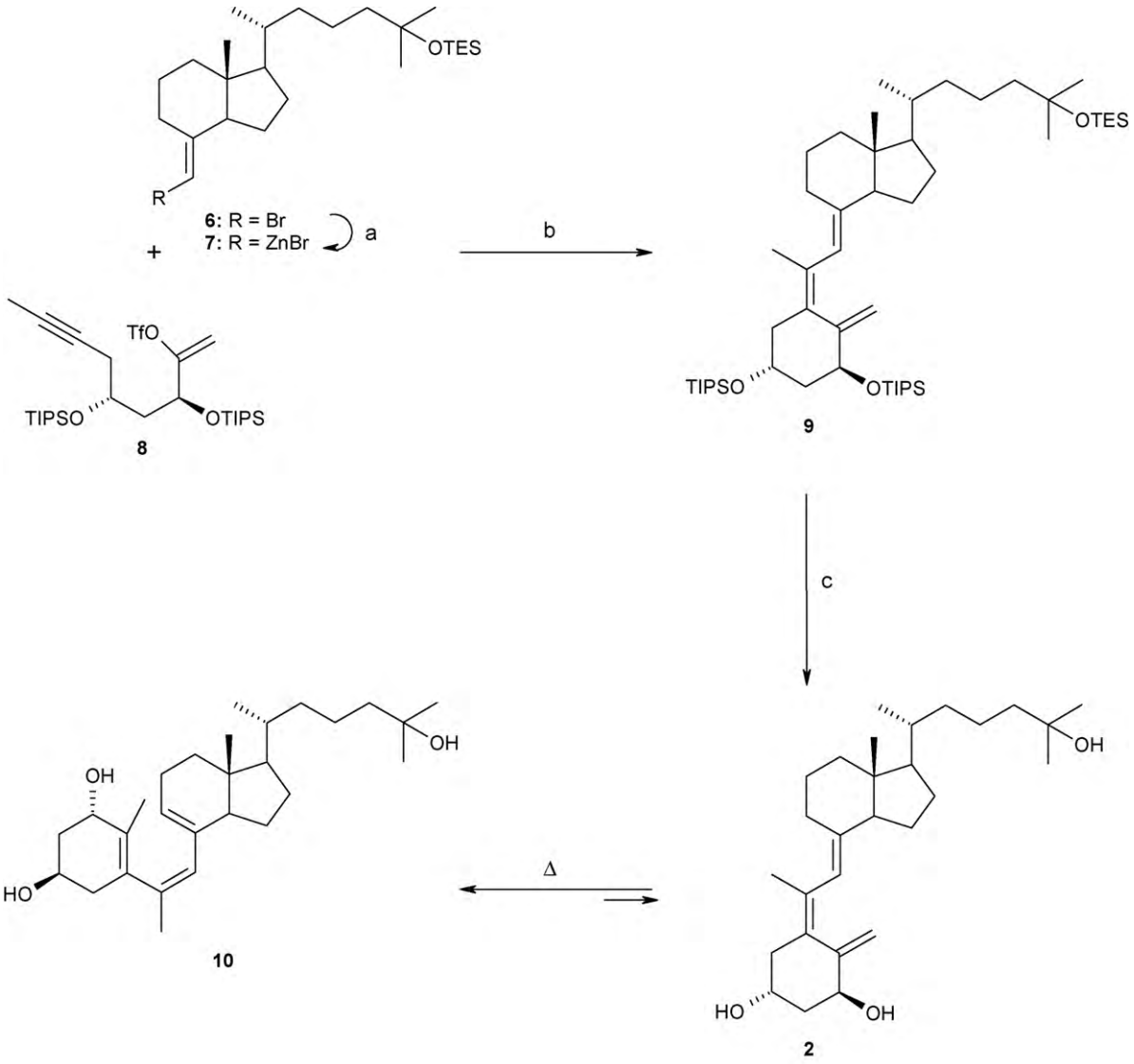


Fig. 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and its analogs.



Scheme 1. (a) t-BuLi, $ZnBr_2$, THF, −78 to 0 °C and (b) **8**, $(PPh_3)_4Pd$, Et_3N, THF, −40 °C to rt, 88% (over two steps); (c) HF × py, Et_3N, CH_2Cl_2, MeCN (1:1:4), 86%.

2.2. In vitro studies

2.2.1. Measurement of binding to the rat recombinant vitamin D receptor

The procedure for obtaining the purified rat recombinant vitamin D receptor used in the binding studies will be reported in detail elsewhere. Competition binding assays were performed using 1α,25-$(OH)_2$[26,27-^{3}H]D_3, as previously described [13]. The experiments were carried out two times, each time in triplicate.

2.2.2. Measurement of cellular differentiation

Human leukemia HL-60 cells (obtained from ATTC) were plated at 1.2×10^5 cells per plate and incubated. Eighteen hours after plating, the test compounds were added and, after 4 days, superoxide production was measured by nitro blue tetrazolium (NBT) reduction. This method is described in detail elsewhere [14]. Two separate experiments were conducted.

2.2.3. Transcriptional assay

Transcription activity was measured in ROS 17/2.8 (bone) cells that were stably transfected with a 24-hydroxylase (24OHase) gene promoter upstream of a luciferase reporter gene [15]. Cells were given a range of doses. Sixteen hours after dosing, the cells were harvested and luciferase activities were measured using a luminometer. Each experiment was performed two times, each time in duplicate.

2.3. In vivo studies

Bone calcium mobilization and intestinal calcium transport. Male, weanling Sprague–Dawley rats were purchased from Harlan (Indianapolis, IN). The animals were group housed and placed on Diet 11 (0.47% Ca.) + AEK oil for 1 week followed by Diet 11 (0.02% Ca.) + AEK oil for 3 weeks. The rats were then switched to a diet containing 0.47% Ca. [13] for 1 week followed by 2 weeks on a diet containing 0.02% Ca. Dose administration began during the last week on 0.02% Ca. diet. Four consecutive intraperitoneal doses were given approximately 24 h apart. Twenty-four hours after the last dose, blood was collected from the severed neck and the concentration of serum calcium was determined as a measure of bone calcium mobilization. The first 10 cm of the intestine was also collected for the intestinal calcium transport analysis using the everted gut sac method [16].

2.4. Molecular modeling

The calculation of the optimized geometry of vitamin D compound **2** was carried out using the PC MODEL (release 9.0) molecular

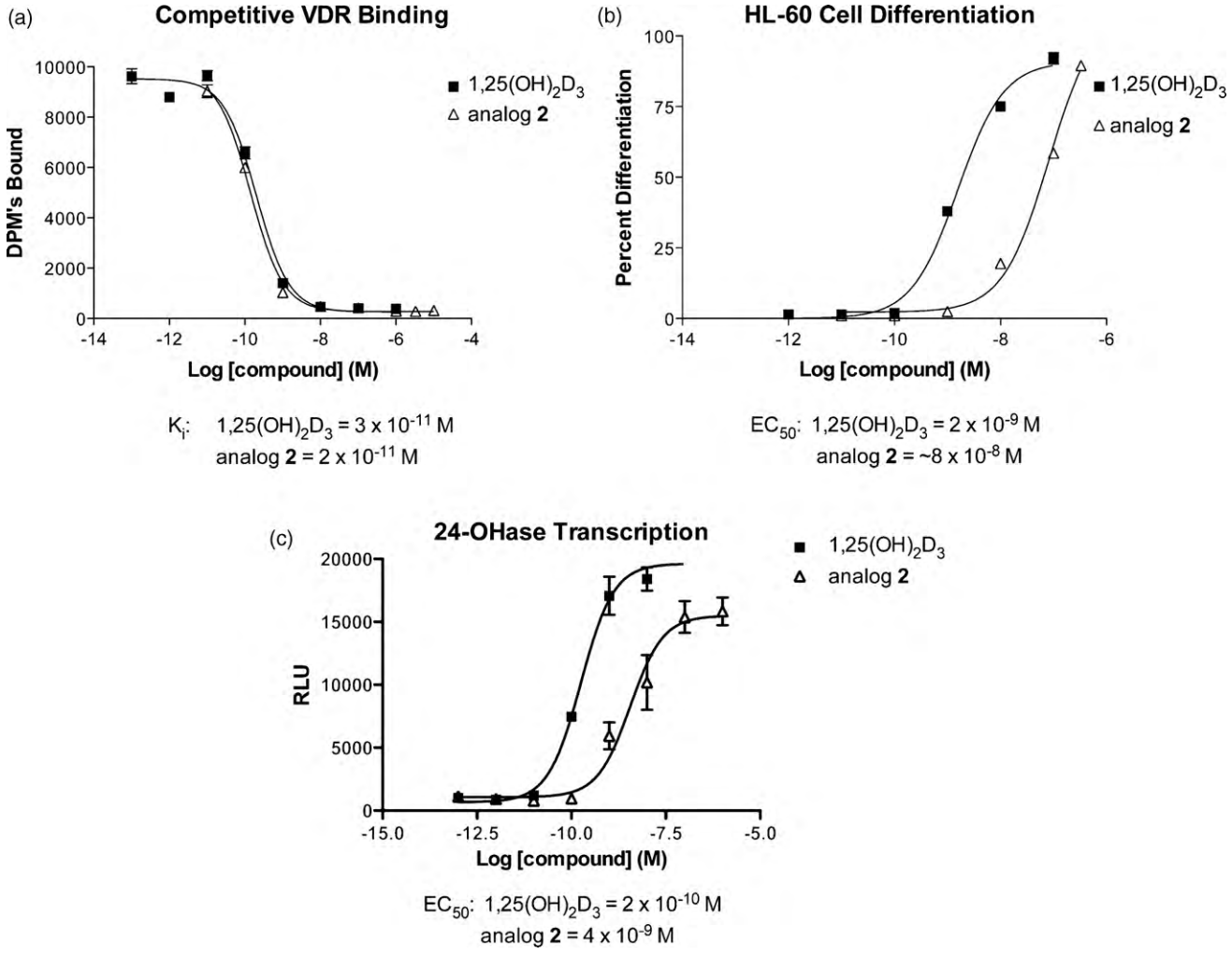


Fig. 2. (a) Competitive binding of 1α,25-$(OH)_2D_3$ and the synthesized analog **2** to the rat recombinant vitamin D receptor. This experiment was carried out three times, each time in duplicate. (b) Differentiation activity of 1α,25-$(OH)_2D_3$ and the analog **2**. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). (c) The transcriptional activity of 1α,25-$(OH)_2D_3$ and the analog **2**. The transcriptional assay was carried out with rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. Each experiment was performed twice, each time in duplicate.

modeling software (Serena Software) and it was performed in the MMX mode. The force field MMX is an enhanced version of MM2, with the pi-VESCF routines taken from MMP1.

3. Results and discussion

3.1. Chemical synthesis of **5**

Both C,D- and A-ring fragments required for the synthesis of the vitamin D compound **2**, *i.e.* , the alkenyl bromide **6** [17] (Scheme 1) and the vinyl triflate **8** [12] were first prepared according to described methods. Then, metalation of bromide **6** with tert-butyllithium and subsequent transmetalation with $ZnBr_2$ furnished the intermediate organozinc derivative **7**. The coupling reaction of this C,D-ring portion with the A-ring acyclic enyne fragment **8** was carried out in the presence of tetrakis(triphenylphosphine)palladium(0) as a catalyst. Removal of the silyl protecting groups in the obtained 6-methylvitamin **9** was performed under acidic conditions using the hydrofluoric acid-pyridine complex. The final 1α,25-dihydroxy-6-methylvitamin D_3 (**2**) was purified by HPLC. Although the vitamin **2** very easily undergoes a thermal isomerization to its previtamin D form **10**, it can be stored for a prolonged time in a freezer.

3.2. Biological evaluation of the analog **2**

The synthesized vitamin D analog **2** described in this paper was examined for its affinity to the full-length recombinant rat vitamin D receptor. It has been established that this analog is very similar to the natural hormone **1** in this assay (Fig. 2a). However, in the remaining two *in vitro* tests performed for the 6-methylated compound **2**, *i.e.*, in eliciting the cellular differentiation of HL-60 cells and in the reporter cell assay, it has been found to be forty (Fig. 2b) and twenty times (Fig. 2c), respectively, less potent than the parent hormone **1**. This lower potency of the analog **2** relative to the native hormone was also observed *in vivo* when analyzing the ability of vitamins to release calcium from bone stores and to promote active calcium transport in the gut. Thus, the 6-alkylated compound **2** is ca. four hundred times less potent in the bone tissue (Fig. 3a) and three orders of magnitude less potent in the intestinal tissue (Fig. 3b) compared to 1α,25-$(OH)_2D_3$.

3.3. Conclusion

Recently, Moras' group reported the X-ray crystal structure of the ligand binding domain (LBD) of the hVDR complexed with the native hormone [18]. Later, many other crystal structures of the LBD–VDR bound to different vitamin D compounds were solved and it became clear that VDR bound (at least in the crystalline state) the vitamin D ligands having their intercyclic C(5)=C(6)–C(7)=C(8) diene moiety in the s-trans conformation, exhibiting a torsion angle of ca. −149°.

Molecular modeling studies of 6-methyl-1α,25-$(OH)_2D_3$ supported that its diene fragment C(5)=C(6)–C(7)=C(8) is significantly distorted from planarity. The calculations of optimized geometry and steric energies, carried out using the PC MODEL software, revealed that the value of its C(5–8) torsion angle was −143.5° for the lowest energy conformation. An alternative low-energy conformer of **2**, with a positive value of the intercyclic diene torsion angle (157.1°), is characterized by a steric energy higher by ca. 4.2 kcal/mol. These findings are obviously explained by the interaction of the 6-alkyl substituent and hydrogens from the C-ring (at C-9). Such a deviation of the 5,7-diene moiety from the planar geometry can be of importance when a vitamin D analog forms a

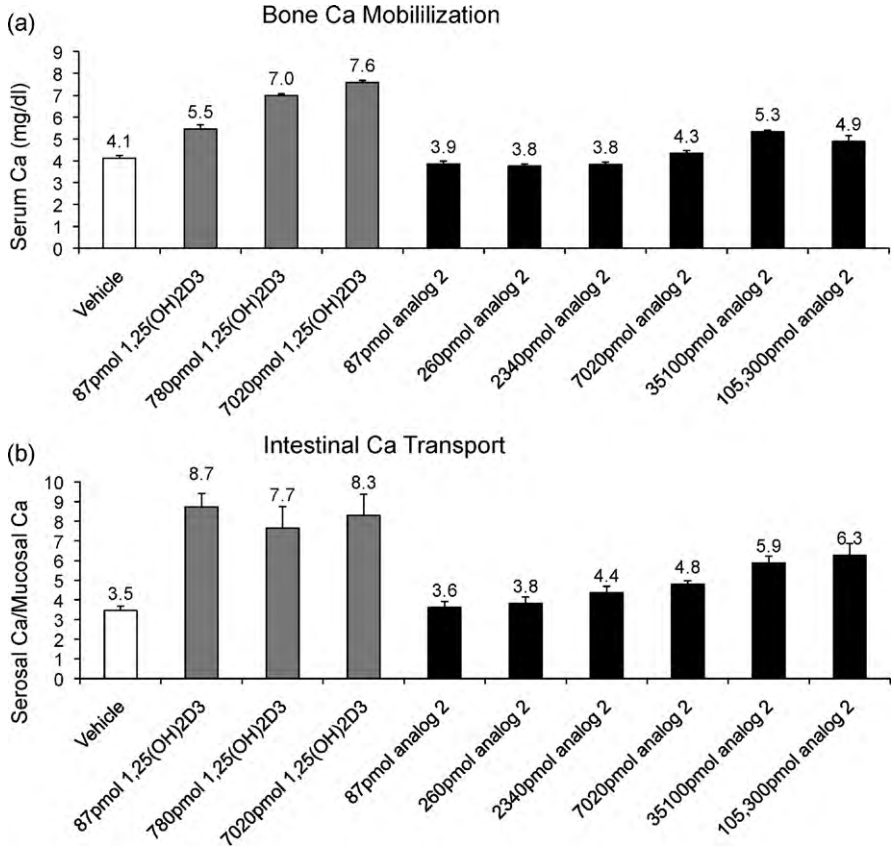


Fig. 3. (a) Bone calcium mobilization of 1α,25-$(OH)_2D_3$ and the synthesized analog **2**. (b) Intestinal calcium transport activity of 1α,25-$(OH)_2D_3$ and the synthesized analog **2**.

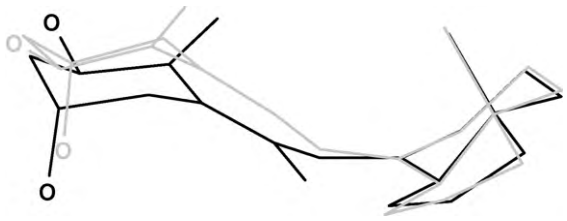


Fig. 4. Overlaid structures of the analog **2** in the preferred conformation (black) and the conformer of 1α,25-$(OH)_2D_3$ (grey) found in its crystalline complex with hVDRmt [18]. Side chains and all hydrogen atoms are omitted for clarity.

complex with VDR. A minimum energy conformation of the analog **2** closely resembles that found in the crystalline complex of the natural hormone and VDR (Fig. 4). The distances between the respective oxygen atoms in both structures are smaller than 1 Å.

The results of testing the ability of the synthesized analog **2** to bind vitamin D receptor fully confirmed our expectations. A presence of bulky 6-methyl substituent did not diminish the affinity of compound to the receptor. Because the synthesized analog binds VDR very effectively but has a markedly lower potency in biological activities downstream from receptor binding, it is possible that this compound could act as a dominant negative inhibitor and be useful as an antidote for vitamin D intoxication.

Acknowledgements

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