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Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 89–92
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

2,2-Functionalized analogues of 1α,25-dihydroxyvitamin D_3, the potent inducers of cell differentiation☆

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Abstract

All four possible A-ring stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 (**4**) were designed and convergently synthesized. Nine-step conversion of methyl hydroxypivalate **6** provided the desired A-ring enyne synthon (**13a,b**) in good overall yield. Cross-coupling reaction of the A-ring synthon **13a,b** with the CD-ring portion in the presence of palladium catalyst, followed by deprotection, gave the vitamin analogues (**4a–d**). We also synthesized four stereoisomers of 2,2-ethano-1,25-dihydroxyvitamin D_3 (**5**), as novel spiro-ring analogues having cyclopropane fused at the C2 position. Biological potencies of the synthesized compounds were assessed in terms of the vitamin D receptor (VDR) binding affinity, as well as the HL-60 cell differentiation-inducing activity. The 2,2-ethano analogue **5a** showed a comparable activity to the natural hormone **1**, while the 2,2-dimethyl analogue **4a** exhibited one-third of the activity of **1** in cell differentiation, with the reduced VDR binding affinity.
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Keywords: Vitamins; Hormones; Receptors; Chemical synthesis

1. Introduction

Cholecalciferol, known as vitamin D_3, is metabolized via 25-hydroxyvitamin D_3 to afford the hormonally active form, 1α,25-dihydroxyvitamin D_3 (**1**), formation of which is strictly regulated. The broad spectrum of biological activities of **1** is considered to be mediated by a ligand-inducible transcriptional factor, vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily. The specific interaction of ligands with the ligand-binding domain (LBD) of VDR has been a major focus of attention, since the X-ray crystal structure of deletion mutant VDR complexed with the natural ligand **1** was solved in 2000 [1]. Insight into the structure-function relationships of a variety of ligands is essential to understand how the subtype-free, singular VDR can deliver the diverse biological activities of **1**, as well as to allow the development of potential therapeutic agents with selective activity profiles for the treatment of cancer or osteoporosis.

Modification of **1** in the A-ring, which possesses two critical hydroxyl groups at C1 and C3, has become of interest in recent years, because the other three A-ring stereoisomers of **1** have proven to exhibit unique activity profiles, being different from the natural hormone **1** [2,3]. Our study of all eight possible A-ring stereoisomers of 2-methyl-1,25-dihydroxyvitamin D_3 revealed that introduction of a simple methyl group into **1** yields analogues with distinct activity profiles [4–6]. The eight 2-methyl analogues, which differ in stereochemistry of the methyl group on C2, and the hydroxyl groups on C1 and C3, exhibited cell differentiation- or apoptosis-inducing activity towards HL-60 cells depending on the A-ring structure [7]. In addition, some of our synthesized 2α-substituted analogues of **1** exhibited remarkably high affinity for VDR [4–10].

The X-ray crystal structure of VDR complexed with **1** indicated the presence of an extra space in the vicinity of the A-ring, suggesting that substituents of synthetic A-ring

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

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doi:10.1016/j.jsbmb.2004.03.053

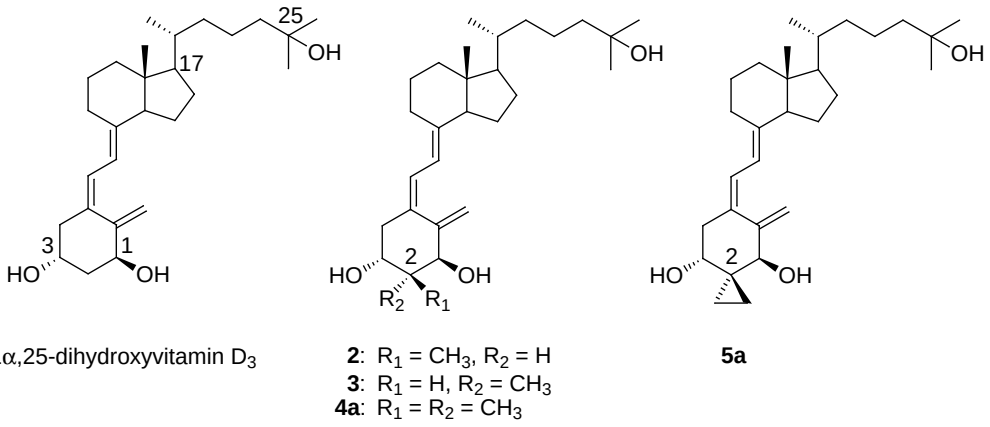
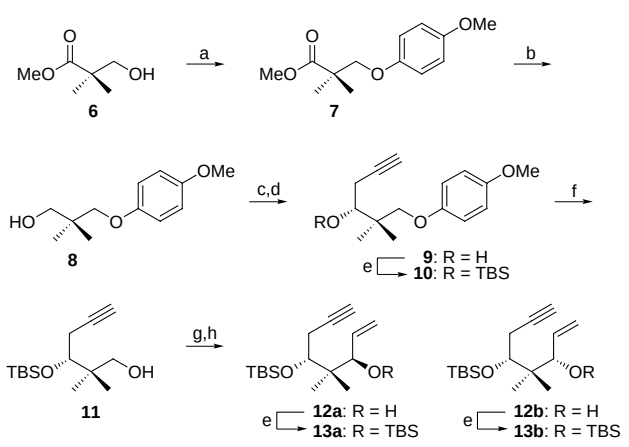


Fig. 1. Structures of 1α,25-dihydroxyvitamin D_3 (**1**), its 2-methyl-substituted analogues (**2,3,4a**) and the spiro-ring analogue (**5a**) having a cyclopropane fused at the C2 position.

analogues could occupy this additional space. Our study of the A-ring analogues of **1** revealed that 2α-methyl-1α,25-dihydroxyvitamin D_3 (**2**) was a 4-fold better binder to VDR, whereas its 2-epimer, 2β-methyl-1α,25-dihydroxyvitamin D_3 (**3**) showed one-eighth of the affinity of **1** [4,5]. In view of these important results, we have now synthesized all four possible A-ring stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 (**4a–d**) as A-ring analogues having methyl substituents projecting in both directions in the cavity, to investigate how the second methyl group affects the activity profiles of the parent compounds. We also designed novel spiro-ring analogues having cycloalkane fused at the C2 position, as exemplified by 2,2-ethano-1α,25-dihydroxyvitamin D_3 (**5a**) in Fig. 1, to study the effects of the additional ring structure on the biological activity, as well as on the A-ring conformation.



Scheme 1. Reagents and conditions: (a) 4-methoxyphenol, DEAD, Ph_3P/THF, 98%; (b) $LiAlH_4$/THF; 97%; (c) PDC, 4 Å MS/CH_2Cl_2, 89%; (d) allenylmagnesium bromide/ether, 68%; (e) TBSOTf, 2,6-lutidine/CH_2Cl_2, 81% for **10**, quant. for **13a,b**; (f) CAN/CH_3CN, H_2O, 77%; (g) TPAP, NMO, 4 Å MS/CH_2Cl_2, 69%; (h) vinylmagnesium bromide/toluene, 60%.

2. Results and discussion

Synthesis was carried out by using a convergent method pioneered by Trost et al. [11]. The synthetic route to the 2,2-disubstituted A-ring precursors (**13a,b**) is shown in Scheme 1 [12]. Nine-step conversion of methyl hydroxypivalate (**6**) provided the A-ring synthon of 1,3-*anti*- and 1,3-*syn*-diol derivatives (**13a,b**). The A-ring precursors of the cycloalkane analogues were also accessible by using similar procedures. Coupling reaction of the protected A-ring enynes **13a,b** with the CD-ring portion [11] in the presence of the tetrakis(triphenylphosphine)palladium,

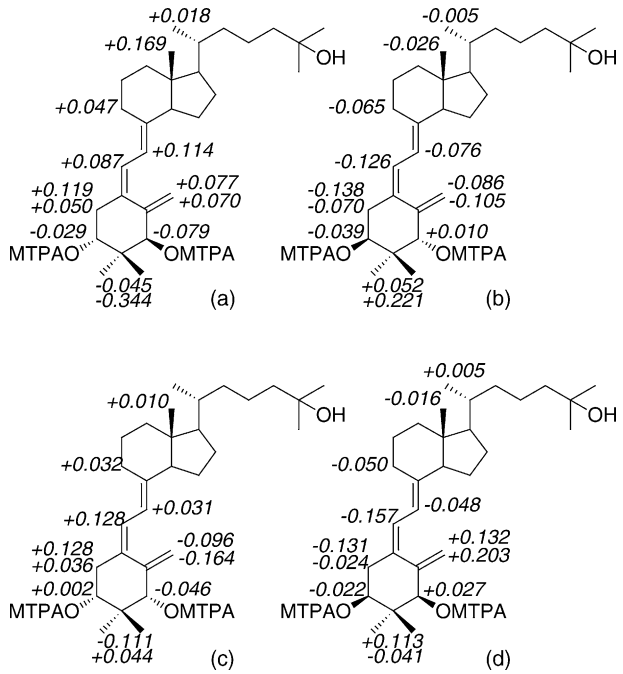


Fig. 2. Determination of absolute configuration at the C1 and C3 positions of **4a–d** by ^1H NMR analyses of their bis-MTPA esters.

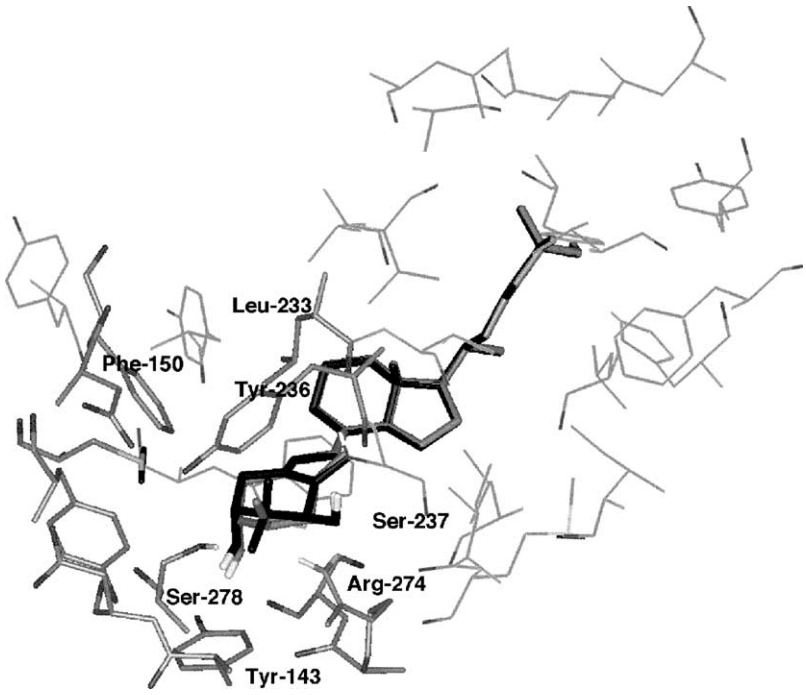


Fig. 3. Overlay of the modeled VDR/2α-methyl-1α,25-dihydroxyvitamin D_3 (**2**: in grey) complex with that of 2,2-dimethyl-1α,25-dihydroxyvitamin D_3 (**4a**: in black) in the LBD. The 34 amino acid residues in the LBD of mutant human VDR [1] complexed with **1**, were used for the calculation.

followed by deprotection with TBAF, proceeded smoothly to give the vitamin analogues **4a,b** and **4c,d**, respectively. The absolute stereochemistry of the diols in the A-ring was determined by ^{1}H NMR analyses of their bis-MTPA esters (Fig. 2) [13]. In this way, syntheses of two sets of four stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 and 2,2-ethano-1,25-dihydroxyvitamin D_3 were accomplished.

Table 1 summarizes the relative VDR binding affinity and HL-60 cell differentiation-inducing activity of the synthesized compounds in comparison with the natural hormone **1**, together with the 2-methyl analogues (**2**,**3**). The 2,2-dimethyl analogue **4a** showed 3% of the affinity of **1**, which suggests that introduction of the second methyl group into **2** results in an approximately 100-fold reduction of the affinity to VDR. The other diastereomers of the 2,2-dimethy-1,25-dihydroxyvitamin D_3 (**4b–d**) showed weaker affinity compared with their mono-methyl parents. On the other hand, the 2,2-ethano analogue (**5a**), having cyclopropane at C2, instead of the *gem*-methyl substituents, showed 70% of the affinity of **1**. Cell differentiation-inducing activity of the 2,2-ethano analogue **5a** was comparable to that of the natural hormone **1**, whereas the 2,2-dimethyl analogue **4a** exhibited one-third of the activity of **1**, with the reduced VDR affinity. The potency of **4a** to induce cell differentiation appears to have been inherited from the 2α-methyl derivative **2**.

Table 1
Relative biological activity of the A-ring analogues[a]

Compounds	VDR[b] affinity	HL-60 cell differentiation[c]
1	100	100
2	400[d]	200[d]
3	13[d]	10[d]
4a	3	30
4b	0.005	NT[e]
4c	<0.001	NT
4d	0.06	NT
5a	70	100

[a] The potency of 1α,25-dihydroxyvitamin D_3 (**1**) is normalized to 100.
[b] Bovine thymus.
[c] Cell differentiation was assessed in terms of NBT reducing activity.
[d] References [4,5].
[e] Not tested.

Docking studies of the A-ring analogues were carried out based on the X-ray crystal structure of deletion mutant VDR [1]. Fig. 3 depicts a superposition of the modeled 2α-methyl analogue (**2**) and the 2,2-dimethyl analogue (**4a**) in the LBD of VDR. In the case of **2**, the axial 2α-methyl group reaches closer to the 'upper' lipophilic pocket formed by Leu-233, Tyr-236 and Phe-150, leading to enhanced interaction [6]. The docking model of the 2,2-dimethyl analogue **4a** shows that the equatorial 2β-methyl group approaches the side-chain of Tyr-143, which causes a significantly less favorable binding mode. This steric clash caused by the *gem*-methyl substituents in **4a** may be avoided by conversion to cyclopropane at C2, as in **5a**.

In summary, we have efficiently synthesized all possible A-ring stereoisomers of 2,2-dimethyl-1,25-dihydroxyvitamin D_3 (**4**) and 2,2-ethano-1,25-dihydroxyvitamin D_3 (**5**) by employing the convergent method using palladium catalyst.

The 2,2-dimethyl analogues exhibited the reduced VDR binding affinity in comparison with the 2-methyl analogues. The 2,2-ethano analogue **5a** showed a comparable activity to the natural hormone in terms of VDR affinity, as well as cell differentiation-inducing activity. Despite the reduced VDR affinity, the dimethyl analogue **4a** exhibited up to one-third of the activity of **1** in HL-60 cell differentiation, suggesting that **4a** would retain the unique activity afforded by 2α-methyl substitution. These features can be explained, in part, by the modeling studies based on the X-ray crystal structure of VDR complexed with **1**.

Acknowledgements

This work was supported in part by a Grant from the Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences.

References

[1] N. Rochel, J.M. Wurtz, A. Mitschler, B. Klaholz, D. Moras, The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand, Mol. Cell 5 (2000) 173–179.

[2] A.W. Norman, R. Bouillon, M.C. Farach-Carson, J.E. Bishop, L.-X. Zhou, L. Nemere, J. Zhao, K.R. Muralidoharan, W.H. Okamura, Demonstration that 1β,25-dihydroxyvitamin D_3 is the nongenomic but not genomic biologic responses and biologic profiles of the three A-ring diastereomers of 1α25-dihydroxyvitamin D_3, J. Biol. Chem. 268 (1993) 20022–20030.

[3] M.G. Bischof, M.-L. Siu-Caldera, A. Weiskopf, P. Vouros, H.S. Cross, M. Peterlik, G.S. Reddy, Differentiation related pathways of 1α,25-dihydroxycholecalciferol metabolism in human colon adenocarcinoma-derived Caco-2 cells: production of 1α,25-dihydroxy-3-epi-cholecalciferol, Exp. Cell. Res. 241 (1998) 194–201.

[4] K. Konno, S. Maki, T. Fujishima, Z.-P. Liu, D. Miura, M. Chokki, H. Takayama, A novel and practical route to A-ring enyne synthon for 1α,25-dihydroxyvitamin D_3 and 2-methyl-1,25-dihydroxyvitamin D_3, Bioorg. Med. Chem. Lett. 8 (1998) 151–156.

[5] K. Konno, T. Fujishima, S. Maki, Z.-P. Liu, D. Miura, M. Chokki, S. Ishizuka, K. Yamaguchi, Y. Kan, M. Kurihara, N. Miyata, C. Smith, H.F. DeLuca, H. Takayama, Synthesis, biological evaluation, and conformational analysis of A-ring diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 and their 20-epimers: Unique activity profiles depending on the stereochemistry of the A-ring and at C-20, J. Med. Chem. 43 (2000) 4247–4265.

[6] T. Fujishima, K. Konno, K. Nakagawa, M. Tanaka, T. Okano, M. Kurihara, N. Miyata, H. Takayama, Synthesis and biological evaluation of all A-ring stereoisomers of 5,6-*trans*-1,25-dihydroxyvitamin D_3 and their 20-epimers: Possible binding modes of potent A-ring analogues to vitamin D receptor, Chem. Biol. 8 (2001) 1011–1024.

[7] K. Nakagawa, M. Kurobe, K. Konno, T. Fujishima, H. Takayama, T. Okano, Structure specific control of differentiation and apoptosis of human promyelocytic leukemia (HL-60) cells by A-ring diastereomers of 2-methyl-1α,25-dihydroxyvitamin D_3 and its 20-epimer, Biochem. Pharmacol. 59 (2000) 1937–1947.

[8] A. Kittaka, Y. Suhara, H. Takayanagi, T. Fujishima, M. Kurihara, H. Takayama, A concise and efficient route to 2α-(ω-hydroxyalkoxy)-1α25-dihydroxyvitamin D_3: Remarkably high affinity to vitamin D receptor, Org. Lett. 2 (2000) 2619–2622.

[9] Y. Suhara, H. Nihei, H. Tanigawa, T. Fujishima, K. Konno, T. Nakagawa, T. Okano, H. Takayama, Synthesis and biological evaluation of novel 2α-substituted 1α,25-dihydroxyvitamin D_3 analogues, Bioorg. Med. Chem. Lett. 10 (2000) 1129–1132.

[10] Y. Suhara, K. Nihei, M. Kurihara, A. Kittaka, K. Yamaguchi, T. Fujishima, K. Konno, N. Miyata, H. Takayama, Efficient and versatile synthesis of novel 2α-substituted 1α,25-dihydroxyvitamin D_3 analogues and their docking to vitamin D receptor, J. Org. Chem. 66 (2001) 8711–8760.

[11] B.M. Trost, J. Dumas, M. Villa, New strategies for the synthesis of vitamin D metabolites via Pd-catalyzed reactions, J. Am. Chem. Soc. 114 (1992) 9836–9845.

[12] T. Fujishima, A. Kittaka, K. Yamaoka, K. Takeyama, S. Kato, H. Takayama, Synthesis of 2,2-dimethyl-1,25-dihydroxyvitamin D_3: A-ring structural motif that modulates interactions of vitamin D receptor with transcriptional coactivators, Org. Biomol. Chem. 1 (2003) 1863–1869.

[13] K. Konno, T. Fujishima, Z.-P. Liu, H. Takayama, Determination of absolute configuration of 1,3-diols by the modified Mosher's methods using their di-MTPA esters, Chirality 14 (2002) 72–80.