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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2-METHYL-20-*EPI* ANALOGUES OF 1α,25-DIHYDROXYVITAMIN D_3

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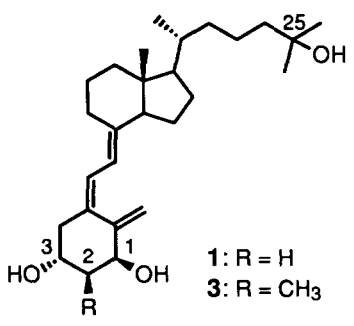
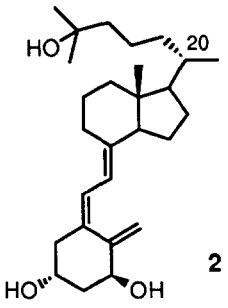
Abstract: Synthesis and biological evaluation of all eight possible A-ring diastereomers of 2-methyl-20-*epi*-1,25-dihydroxyvitamin D_3 are described. Among the analogues synthesized, 2α-methyl-20-*epi*-1α,25-dihydroxyvitamin D_3 exhibited exceptionally high potency. The double modification of 2-methyl substitution and 20-epimerization yielded analogues with unique activity profiles.

The hormonally active form of vitamin D, 1α,25-dihydroxyvitamin D_3 (**1**), has a wide range of activities, including cell-differentiating and antiproliferative activities in addtion to its classical role in calcium homeostasis, which have been utilized to develop therapeutic agents for cancer, psoriasis and osteoporosis.[1,2]

Most of the analogues synthesized so far are modified in the side chain. Among them, 20-*epi*-1α,25-dihydroxyvitamin D_3 (**2**) has a high cell differentiation activity with relatively low calcemic effects.[3,4] This 20-epimerization has led to highly promising analogues such as KH-1060.[3] Modification in the A-ring also produced analogues with a unique biological profile, such as 2β-(hydroxypropoxy)-1α,25-dihydroxyvitamin D_3



1: R = H
3: R = CH_3



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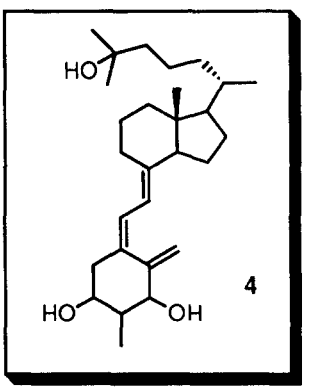

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(ED-71).[5] In view of this A-ring modification as well as the conformation-activity relationships in the A-ring,[6] we have synthesized all eight possible A-ring diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 and found that the potency of the analogues varies with the configuration not only of the C-1 and C-3 hydroxy groups, but also of the 2-methyl group. In particular, 2α-methyl-1α,25-dihydroxyvitamin D_3 (**3**; ααβ-isomer) has higher

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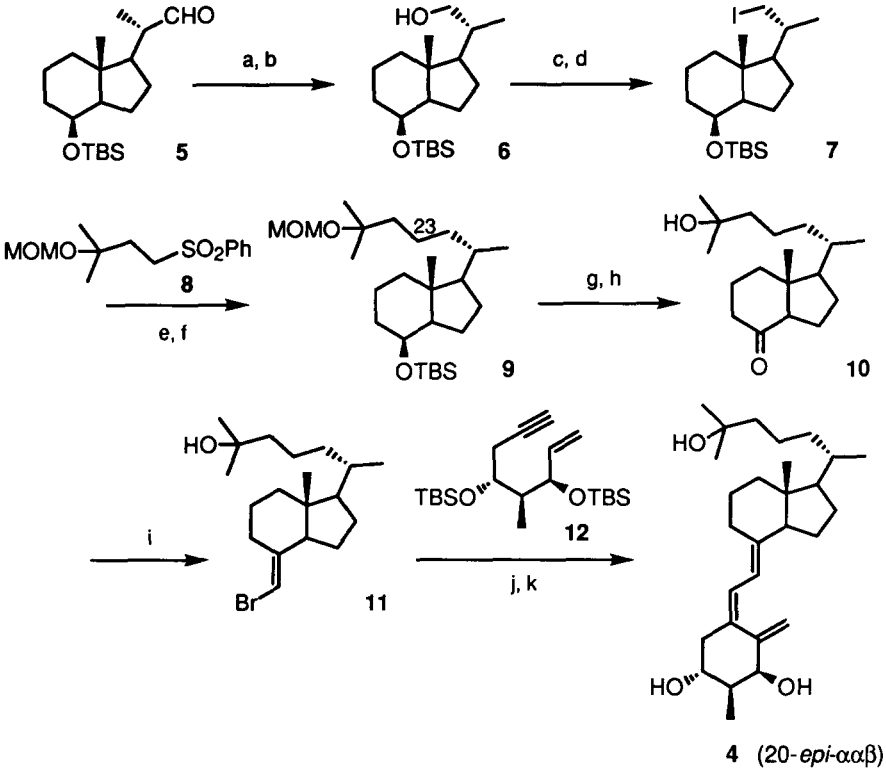
potency than 1α,25-dihydroxyvitamin D_3.[7] These remarkable effects of 2-methyl substitution and 20-epimerization, and the results obtained with hybrid analogues[8] prompted us, in the present work, to design and synthesize all eight possible A-ring diastereomers of 2-methyl-20-*epi*-1,25-dihydroxyvitamin D_3 (**4**) as A-ring analogues with 20-epimerization.

Scheme 1



(a) DBU/ THF, reflux; (b) $NaBH_4$/ MeOH, 0°C, 52% (two steps); (c) TsCl/ pyridine, r.t., 98%; (d) NaI/ DMF, 50°C, 92%; (e) *n*-BuLi, HMPA/ THF, - 78°C, 98%; (f) Na-Hg/ NaH_2PO_4-MeOH-THF, r.t., 98%; (g) TsOH/ MeOH, r.t., 85%; (h) TPAP-NMO-4Å M.S./ CH_2Cl_2, 96%; (i) $Ph_3P^+CH_2Br\cdot Br^-$, NaHMDS/ THF, 57%; (j) $Pd_2(dba)_3$·$CHCl_3$-PPh_3-Et_3N/ toluene, reflux; (k) CSA/ MeOH, r.t., 63% (two steps).

The analogues were synthesized by employing the convergent method of Trost et al.[9] Scheme 1 outlines the synthesis of the 20-*epi*-CD-ring portion **11**, and the subsequent coupling with an A-ring enyne **12**, as exemplified by the ααβ-isomer (the Greek letters denote the configurations at C-1, C-2, and C-3, respectively, in the vitamin D numbering system). The aldehyde **5**[10] was equilibrated under basic conditions to give an approximately 2: 3 mixture of the aldehydes in favor of the 20-epimer. Subsequent reduction of this aldehyde mixture with $NaBH_4$ afforded the corresponding C-20 epimeric alcohols, which were then separated by chromatography to obtain the desired 20-*epi* alcohol **6** in 52% yield. This was converted to the iodide **7** *via* its tosylate. Condensation of the iodide **7** with the side chain moiety **8**[11] using *n*-BuLi as a base in the presence of HMPA furnished a mixture of C-23 epimeric sulfones in 98% yield. Desulfonylation with sodium amalgam in a buffered mixture of methanol and THF[12] produced the desired CD-ring portion **9**. Removal of both protecting groups in **9** with TsOH afforded the corresponding diol in high yield. The resulting secondary alcohol was

oxidized with TPAP-NMO to give the ketone **10** in 96% yield. Finally, bromomethylenation of **10** furnished the requisite 20-*epi*-CD-ring synthon **11**. Each of the eight possible A-ring enynes, prepared separately as we previously reported,[7] was coupled with 20-*epi*-CD-ring **11** using the Pd catalyst followed by deprotection to give the 2-methyl-20-*epi* analogue of 1α,25-dihydroxyvitamin D_3 (**4**).[13] In this way, a set of eight stereoisomers of 2-methyl-20-*epi*-1,25-dihydroxyvitamin D_3 was synthesized.

The biological activities of the synthesized analogues are summarized in Table 1. The potency varies with the stereochemistry of the A-ring substituents. This is similar to the result that we previously reported for the 20-natural counterparts.[7] 2α-Methyl-20-*epi*-1α,25-dihydroxyvitamin D_3 (20-*epi*-ααβ) exhibited exceptionally high potency.

In the vitamin D receptor (VDR) binding assay using bovine thymus VDR,[14] the 2α-methyl-20-*epi* analogue (20-*epi*-ααβ) exhibited 12-fold higher affinity than 1α,25-dihydroxyvitamin D_3, whereas the 2β-methyl-20-*epi* analogue (20-*epi*-αββ) had comparable activity to 1α,25-dihydroxyvitamin D_3. Compared to the corresponding 20-natural isomer, each 20-*epi* analogue had 3- to 10-fold higher affinity for VDR.[7] The VDR binding potency of 20-*epi*-1α,25-dihydroxyvitamin D_3 (**3**) relative to 1α,25-dihydroxyvitamin D_3 (normalized to 100) was reported to be 120 for chick intestinal VDR[3] and 500 for bovine thymus VDR.[4] Thus, the double modification of 2-methyl substitution and 20-epimerization resulted in additive effects on VDR binding. The affinity of vitamin D binding protein (DBP) was tested using fetal calf serum DBP (data not shown).[15] Each 2-methyl-20-*epi* analogue was shown to be a poor ligand of DBP, having approximately 300 times less affinity than 1α,25-dihydroxyvitamin D_3. These results imply that 20-epimerization greatly decreases the DBP binding activity irrespective of the A-ring stereochemistry. Cell differentiation activities towards HL-60 cells[16] were high in the 20-*epi*-ααβ-, 20-*epi*-αββ-, and 20-*epi*-ααα-isomers. In particular, the 20-*epi*-ααβ isomer exhibited 590 times higher potency than 1α,25-dihydroxyvitamin D_3, having comparable activity to KH-1060, the most potent analogue reported to date. Bone calcium mobilization was tested normal SD male rats.[17] The 2α-methyl modification increased the calcemic activity together with VDR binding potency, while the 2β-methyl compound,

Table 1. Biological Activity of 2-Methyl-20-*epi* Analogues of 1α,25-Dihydroxyvitamin D_3[a]

	VDR[c] binding	HL-60 cell differentiation[d]	Ca mobilization[e]
1α,25-$(OH)_2D_3$	100	100	100
20-*epi*-ααβ[b]	1200	59000	655
20-*epi*-αββ	160	2600	115
20-*epi*-ααα	17	730	144
20-*epi*-αβα	<0.1	6	NT[f]
20-*epi*-βαα	<0.1	1	NT
20-*epi*-ββα	7	190	19
20-*epi*-βαβ	<0.1	3	NT
20-*epi*-βββ	<0.1	1	NT

(a) The results for 1α,25-dihydroxyvitamin D_3 are normalized to 100. (b) The Greek letters denote the configurations of C-1, C-2 and C-3, respectively. (c) Bovine thymus. (d) Cell differentiation was assessed in terms of NBT reductivity. (e) Rat serum calcium level. (f) Not tested.

the 20-*epi*-αββ isomer, exhibited similar calcemic activity to 1α,25-dihydroxyvitamin D_3. It is noteworthy that the 20-*epi*-ββα was biologically active in spite of having 1β-hydroxy configuration: compared to 1α,25-dihydroxyvitamin D_3, it showed two-fold greater HL-60 cell differentiation activity, but only one-fifth of the calcium-mobilizing activity. Since the 20-*epi*-βαα isomer, with altered stereochemistry at the 2 position, showed poor activities, these effects of the 20-*epi*-ββα isomer may be due to the combination of 1β configuration and 2β-methyl substitution.

In summary, we have synthesized eight stereoisomers of 2-methyl-20-*epi*-1,25-dihydroxyvitamin D_3, as novel analogues with modifications of both the A-ring and the side chain. These analogues exhibited unique profiles of vitamin D activities depending upon the configuration in the A-ring. Some of them may be useful tools in research on the biology of vitamin D .

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11. The side chain moiety **8**, 2-methyl-4-(phenylsulfonyl)-butan-2-ol MOM-ether, was synthesized *via* three-step conversion (a. MeOH/ H_2SO_4, r.t., quant.; b. MeMgBr/ THF, 0 °C, 96%; c. MOMCl/ $^iPr_2NEt$, r.t., 83%) of commercially available 3-(phenylsulfonyl)propionic acid.
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13. **4** (20-*epi*-ααβ): ^{1}H NMR (400 MHz/ $CDCl_3$/ TMS) δ 0.53(3H, s), 0.85(3H, d, J = 6.7 Hz), 1.08(3H, d, J = 6.8 Hz), 1.21(6H, s), 2.23(1H, dd, J = 7.9, 13.4 Hz), 2.67(1H, dd, J = 4.0, 13.4 Hz), 2.83(1H, dd, J = 4.0, 12.5 Hz), 3.83(1H, ddd, J = 7.9, 4.4, 4.0 Hz), 4.29(1H, d, J = 3.3 Hz), 5.01(1H, m), 5.28(1H, m), 6.01(1H, d, J = 11.3 Hz), 6.39(1H, d, J = 11.3 Hz); UV (EtOH) λ_{max} 266 nm; MS *m/z* 430(M^+), 412(M^+ - H_2O), 394(M^+ - 2 H_2O); HRMS *m/z* 430.3443, calcd. for $C_{28}H_{46}O_3$: 430.3447.
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