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Synthesis and Biological Evaluation of 1α-Hydroxy-25(R and S) -25,26-Epoxy-23-yne Vitamin D3 and of 1α,25(R and S),26-Trihydroxy-23-yne Vitamin D3.

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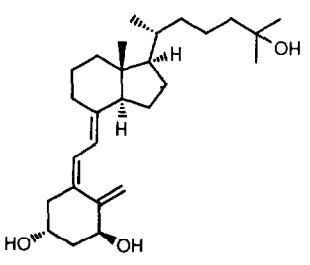
***Abstract** : The synthesis of both 1α-hydroxy-25(R and S)-25,26-epoxy-23-yne vitamin D_3 and of both 1α,25(R and S),26-trihydroxy-23-yne vitamin D_3 is described. Biological evaluation includes the study of calcemic effect, receptor binding and cell differentiation.* © 1997 Published by Elsevier Science Ltd.

The observation that 1α,25-dihydroxy vitamin D_3 (**1** ; calcitriol), the hormonally active metabolite of vitamin D_3, is active in the regulation of cell profileration and differentiation, next to the classical role in calcium-bone homeostasis, has led in recent years to the development of analogues capable of dissociating cell differentiation effects from calcemic effects.[1,2]



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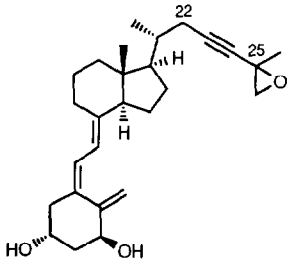
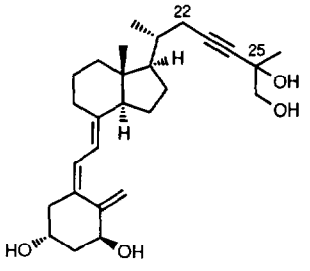
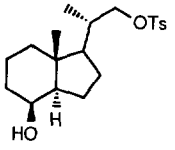


2a (WY322); 25(*R*)
2b (WY319); 25(*S*)
2c (ZXY404); 25-epimers



3a (WY236); 25(*R*)
3b (WY320); 25(*S*)



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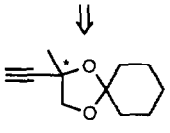

4a (*R*)
4b (*S*)

Scheme 1

A large number of side chain modified analogues have been described during the last decade.[3] In this context we have recently described the biological evaluation of analogues carrying an epoxide function in the

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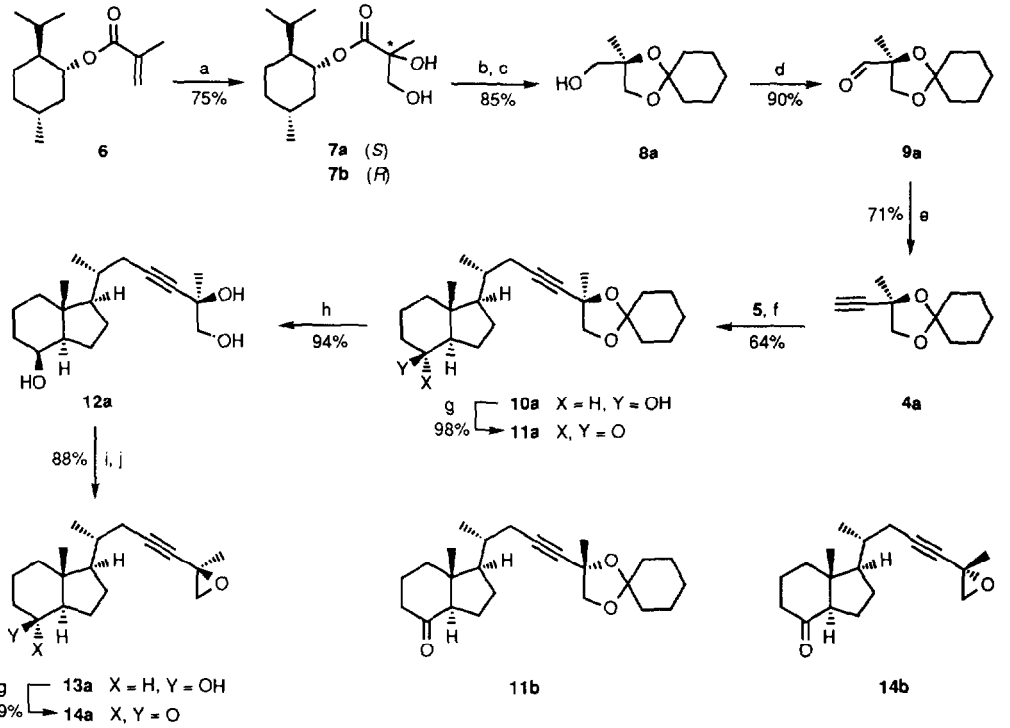
side chain.[4] Several of these analogues were lacking an additional 24-or 25-hydroxy group and surprisingly were among the most active members of the series. Indeed in all potent analogues described in the literature such a hydroxy function is present. Furthermore one of the more potent "epoxy" analogues namely **2c** consisted of an C-25 epimeric mixture. In order to assess the relative biological activity we decided to synthesize both epimers **2a** and **2b** to compare them with the corresponding epimeric 25,26 diols **3a** and **3b** in order to obtain some insight in the mode of action of epoxide **2c**.

Our strategy for the synthesis of the four analogues centers around side chain construction *via* coupling of respectively **4a** and **4b** with the known tosylate **5** of the Inhoffen-Lythgoe diol.[5] For the synthesis of the acetylenic precursors **4a** and **4b** we adapted a method described for the synthesis of 2-*(R)*- and 2-*(S)*-methyl-2-menthylglycerates[6] based on the dihydroxylation of the (-)-menthyl ester of methacrylic acid **6** (scheme 2). The enantiopure **7a** and **7b** were obtained by column chromatographic separation[6,7] of the epimeric mixture.



(a) OsO_4 (cat), NMO, H_2O-Me_2CO, r.t., 6 h; (b) cyclohexanone, PTSA, Na_2SO_4, r.t., 5 h; (c) DIBAL, CH_2Cl_2, -78°C, 1.5 h; (d) $(COCl)_2$, DMSO, Et_3N, -78°C, 2h; (e) $(MeO)_2P(O)CHN_2$, t-BuOK, THF, -78°C → r.t., 20 h; (f) NaH, DMSO, r.t., 5h; (g) PDC, PPTS, CH_2Cl_2, 0°C → r.t., 10 h; (h) $HS(CH_2)_3SH$, $BF_3.OEt_2$, CH_2Cl_2, -50°C → -20°C, 7 h; (i) TsCl, Et_3N, CH_2Cl_2, 0°C, 13 h; (j) DBU, 0°C, 5 h.

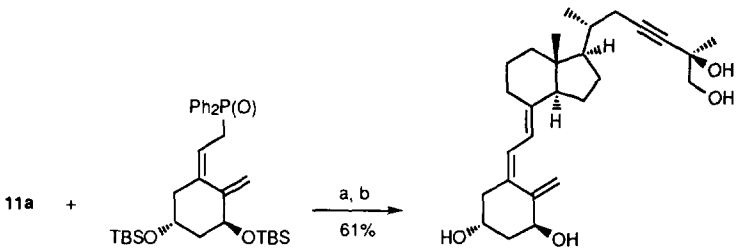
Scheme 2

Both epimers were taken through the same reaction sequence depicted in scheme 2 for the *(R)*-epimer **4a**. After diol protection in **7a**, the chiral auxiliary was removed *via* reduction of the ester function. Swern[8] oxidation of the alcohol **8a**[9] afforded aldehyde **9a**[9] which was transformed into **4a**[9] upon treatment with the anion of dimethyl diazomethylphosphonate.[10]

Coupling of the anion **4a** with tosylate **5** led to **10a** which upon oxidation afforded ketone **11a**[9]. The 25-*(S)*-epimer **11b** was obtained in essentially the same yields starting from **7b**; formation of the analogues **3a** and **3b** is shown in scheme 3.

Compounds **10a** and **10b** were also the intermediates in the synthesis of the respective epoxy-analogues **2a** and **2b**. Cleavage of the cyclohexylidene protective group of the α-diol in **10a** proved troublesome; only ketal exchange using ethanedithiol[11] afforded, in good yield, the triol **12a**.

Selective formation of the tosylate of the primary hydroxy function in **12a** followed by DBU treatment led to epoxide **13a**. Finally, oxidation gave the C-8 ketone **14a**. The epimer **14b**[9] was obtained from 25-*epi*-**10b** as described for **14a** from **10a**.



11b + **15** —a, b→ **3b** (51%)

14a + **15** —a, c→ **2a** (81%)

14b + **15** —a, c→ **2b** (75%)

(a) BuLi, THF, -78°C→ r.t., 4 h; (b) AG-50W-X12, MeOH, r.t., 7 d; (c) TBAF, THF, r.t., 20 h.

Scheme 3

Ketones **11a, b** and **14a, b** were coupled[12] with the lithiated A-ring precursor[13]; deprotection finally led respectively to the title compounds **3a, 3b, 2a** and **2b**.[9] The lower yields in the case of **2a,b** is as for **10a** to **12a**, due to the deprotection of the 25,26-diol.

The affinity of the analogues **2a,b** and **3a,b** to the pig intestinal mucosa vitamin D receptor (VDR) was evaluated as described previously.[14] The relative affinity of the analogues was calculated from their concentration needed to displace 50% of [^{3}H]1α,25(OH)$_2$D$_3$ from its receptor compared with the activity of **1** (assigned a value of 100%).

The biological evaluation (table) was determined in vitro on different cell lines (HL 60, MCF-7, keratinocytes).[3,14] Both epoxy-epimers (**2a,2b**) and the corresponding 25,26 diols (**3a,3b**) demonstrated nearly the same affinity for the VDR (60-80% compared to the natural hormone **1a**) (table). The prodifferentiating (HL 60) and antiproliferative (keratinocytes, MCF-7) activities were exactly the same, being 1.5 (HL 60, MCF-7) and 2.5 (keratinocytes) fold greater than that of 1α,25(OH)$_2$D$_3$. The calcemic effects of the analogues were tested in vitamin D-repleted normal mice by daily administration of the compounds for 7 days and their calcemic potency was more than 100 times decreased compared 1α,25(OH)$_2$D$_3$. The configuration at position 25 (*R* or *S*) in the analogues **3a,b** did not influence the biological activity, in contrast with other 1α,25(OH)$_2$D$_3$ analogues such as 22-ene-26,27-dehydro-1α,24(*S*)-(OH)$_2$D$_3$ (MC 903) or 22-ene-26,27-dehydro-1α,24(*R*)-(OH)$_2$D$_3$[15] Remarkably the 25(*R*) or (*S*) 25,26-epoxy-23-yne-1α,25(OH)$_2$D$_3$ and

their corresponding diols all shared the same biological activity. Whether the mechanisms of action can be explained by the intrinsic activity of the epoxides or by prior metabolism into diols requires further metabolic studies.

Table : Biological activities of 25(*R*) and (*S*)epoxides and corresponding diols.

Compound	VDR	HL-60	MCF-7	Keratinocytes	Calcium serum
2a (WY322)	75	150	150	250	0.2
2b (WY319)	60	150	150	250	0.3
2c (ZXY 404)	70	150	150	250	0.25
3a (WY 236)	80	150	150	250	0.1
3b (WY 320)	70	150	150	250	0.7

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