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The Synthesis of CD - ring modified 1α,25-dihydroxy vitamin D analogues : Six-membered D-ring analogues II.

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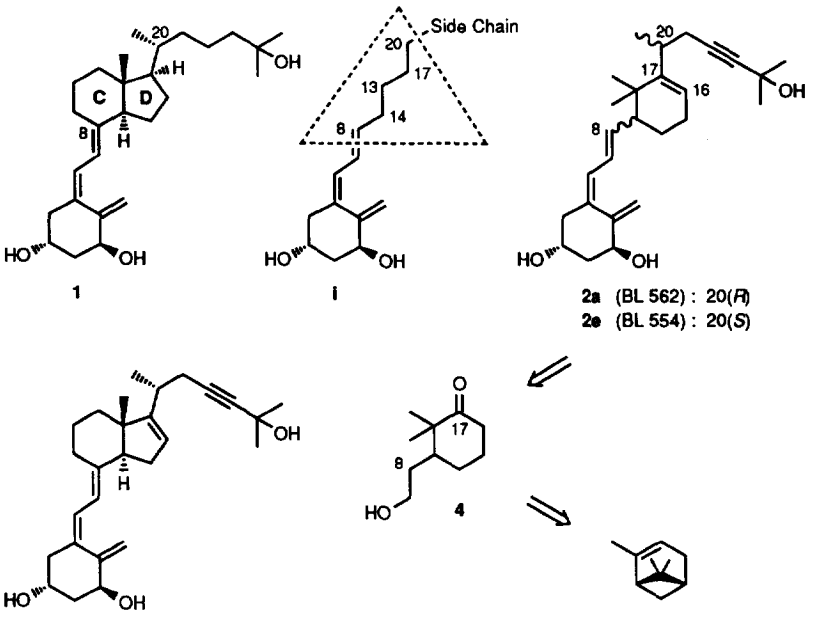
***Abstract** : Vitamin D analogues, characterized by a six-membered D-ring and by the absence of a C-ring are described.* © 1997 Elsevier Science Ltd.

The observation that 1α,25-dihydroxy-vitamin D_3 (**1**; calcitriol) is active in the regulation of cell proliferation 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 differentiating effects from calcemic effects.[1,2] Among the three fragments of the vitamin D skeleton structural modifications of the side-chain and of the A-ring have been especially studied in the past.[3]



Scheme 1

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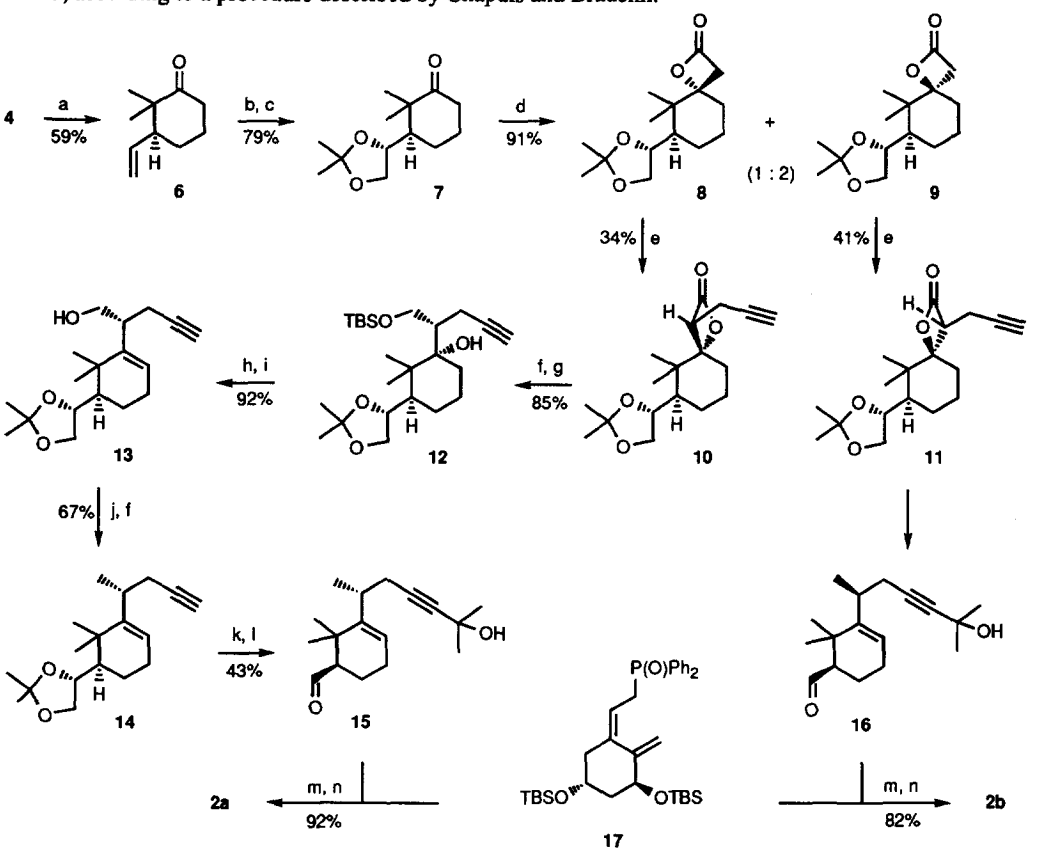
Some years ago, we embarked on an extensive study of the structure-function relationship focussing on the least studied part of the molecule, i.e. the central CD-ring region[4] by stripping the molecule to its five-carbon backbone (C-8 to C-20 : **i**) and resubstituting it again in various ways. In this context we undertook *inter alea* a study of analogues where the central part is replaced by only a six-membered D-ring. In the preceeding paper, analogues based on a cyclohexane D-ring have been described. Presently we want to report on analogues **2a,b** with a cyclohexene D-ring (16, 17-ene; vitamin D numbering).[5] In analogy with the cyclohexane D-ring analogues a *gem*-dimethyl group at C-13 was incorporated in order to restrict the side chain conformations. We also decided to select the 23-yne side chain with both C-20 configurations. This choice was made in relation to the highly active analogue **3** (Ro 23-7553)[6] which has an identical pattern of unsaturated bonds.

The synthetic strategy centers around the advanced intermediate **4** which can be obtained from (+)-α-pinene **5**, according to a procedure described by Chapuis and Brauchli.[7]



a)(*i*) ArSeCN, Bu_3P, THF, r.t.; (*ii*) H_2O_2, $NaHCO_3$, r.t., 1 h then 55°C, 1 h; b) OsO_4, NMO, acetone, H_2O, r.t., 12 h; c) acetone, PTSA, r.t., 12 h; d) (*i*) MeC(O)SPh, LDA, THF, -78°C,0.5 h; (*ii*) **7**, -78°C, 0.5 h→r.t., 1.5 h; e) (*i*) LDA, THF, -78°C, 0.5 h; (*ii*) propargylbromide, -78°C→0°C, 2 h; f) $LiAlH_4$,Et_2O, r.t., 4 h; g) TBSCl, imidazole, DMAP, DMF, r.t., 18 h; h) $SOCl_2$, pyridine, 0°C, 1 h; i) TBAF, THF, r.t., 2 h; j) TsCl, NEt_3, DMAP, CH_2Cl_2, r.t., 12 h; k) (*i*) BuLi, Et_2O; (*ii*) acetone, Et_2O -78°C, 1 h then r.t., 2 h; l) H_5IO_6, Et_2O, r.t., 12 h; m) BuLi, THF, -78°C, r.t., 4 h; n) TBAF,THF, r.t., 14 h.

Scheme 2

Prior to the formation of the side chain, the two-carbon chain in **4** was transformed into a latent formyl function as present in **7**. Although unimportant for the present purpose, it is noteworthy that hydroxylation of **6** led in 93% d.e. to the 8-(*S*)-diol resulting from the rotamer preferred by the 1,3- allylic strain.

Several methods have been described in the vitamin D literature where a 17-keto function is the handle for introducing the side chain in conjunction with the formation of a 16-17 double bond.[8] However, starting from **7**, these methods proved to be unsuccessful because of formation of unseparable diastereoisomeric mixtures. We therefore developed an approach *via* the spiro-δ–lactones **8** and **9**, which were obtained *via* the method of Danheiser *et al*[9] (in a 1:2 ratio). Although also here low diastereoselectivity was observed, this approach has the advantage that the epimers **8** and **9** are easily separated by preparative HPLC. The relative configuration was proven by n.O.e experiments; for **8** with an equatorial lactone methylene group, enhancements (6.7 and 3.3%) were observed between one of the lactone α-H and both methyl groups, while for **9** one enhancement (11.8%) was found between this proton and the equatorial methyl group. Reaction of their enolate anions with 3-bromo-1-propyne led respectively to **10** and **11** as the sole products. Again structure proof was provided by n.O.e. (as for **8** and **9**).

Eliminative-spirolactone opening *via* the procedure described by Black *et al*[10] led to a complex reaction mixture. Therefore a step-wise transformation was adopted. Subsequent to reduction of the lactone and temporary protection of the primary hydroxy function, the tertiary alcohol in **12** was selectively eliminated to the endocyclic double bond. After removal of the 21-hydroxy group in **13**, reaction of lithiated **14** with acetone, followed by acetal hydrolysis and cleavage of the α-diol unit[11] afforded aldehyde **15** required for coupling with the A-ring fragment **17**. As described for **15** from **10**, lactone **11** was transformed into the 20-*epimer* **16**.

Construction of the title compounds **2** involves the Lythgoe coupling[12] of aldehydes **15** and **16** with the A-ring phosphine oxide **17**[13] and subsequent deprotection.

The affinity of the D-ring analogues **2** to the pig intestinal mucosa vitamin D receptor (VDR) was evaluated as described previously.[15] 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 1α,25(OH)$_2$D$_3$ (**1** assigned a value of 100%).

The biological evaluation was determined *in vitro* on different cell lines (HL-60, MCF-7, MG-63, keratinocytes)[3]. The *in vivo* effect of the D-ring analogues was tested in vitamin D-replete normal NMRI mice by measuring calcium levels in serum. Analogue **2a** shows 60% of the VDR affinity. This analogue is as potent as **1** to inhibit cell proliferation and is thousand times less calcemic. An the other hand analogues **2b** (BL554) with the unnaturel 20-(*S*)-configuration has nearly no affinity of cell proliferation or stimulation of cell differentiation. This stands in sharp contrast to biological activities of C-20 epimers in the natural series[14], where the (*S*)-epimer is mostly the more potent agonist. Further details of the biological activities will be published elsewhere.

Table. Biological activities of **2a, 2b** and **3**.

Analogue	VDR	HL-60	MG-63	MCF-7	Keratino-cytes	Calcium Serum	Ratio
2a (BL 562)	60	85		100	200	<0.1	2000
2b (BL 554)	0.7	10	20	4	7	0.25	28
3 (Ro 23-7553)	70	1000	1000	4250	8000	10	1300

It is interesting to note that analogue **2a** has a much lower intrinsic (specific) activity compared to **3** (Ro 23-7553). However **2a** has a ratio (e.g. keratinocytes/Ca^{++}serum $\geq$ 2000) than is the case for **3** (ratio *circa* 1300). This ratio is an indication of the relative toxicity.

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