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

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Abstract : Vitamin D analogues characterized by the absence of a C-ring (D-ring analogues) are described. © 1997 Published by Elsevier Science Ltd.

The observation that 1α ,25-dihydroxy vitamin D₃ (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 especially structural modifications of the side-chain and of the A-ring have been studied in the past.³



Scheme 1

Some years ago, we embarked on an extensive study of the structure-function relationship with the focus on the least studied part of the molecule, i.e. the central CD-ring region.⁴ In this respect we decided stripping

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the molecule to its five-carbon backbone (C-8 to C-20, i) and resubstituting it again in various ways. In the present paper we wish to describe the synthesis of analogues lacking the six-membered ring of the CD-skeleton, i.e., the "D-ring analogues" with general formula 2. We decided to select a "D-ring" carrying a *gem*-dimethyl group at C-13 (steroid numbering) as these substituents mimic respectively the angular C-18 methyl group and C-12 in the parent steroid 1, which are known to have an influence on restricting the side-chain orientations.³ It is generally assumed that the relative position in space of the 1 α - and 25-hydroxy groups is important for the biological activity and that the side chain occupies a very restricted topology at the binding site of the vitamin D receptor (VDR).³



Torsional angle —	2a 20-(<i>R</i>)		2b 20-(<i>S</i>)		
	τ (°)	mole%	τ (°)	mole%	
C ₁₃ -C ₁₇ -C ₂₀ -C ₂₂	180	89	-58	87	
	87	11	-145	13	
C ₁₇ -C ₂₀ -C ₂₂ -C ₂₃	61	50	-61	56	
	-168	50	-175	35	
			74	9	

Fig 1

As shown in figure 1 the relative configurations of the methyl group at C-20 has a profound influence on the rotameric distribution of the side-chain in particular at bonds 17-20 and 20-22. In the preferred configuration of the two diastereomers the further chain bearing the 25-hydroxyl group is pointing downwards but in different regions in space. So in analogy with analogues of 1 with the unnatural 20(S)-configuration that were found to induce interesting differentiations between calcemic effects and new actions,⁵ we decided to introduce both C-20 configurations in our "D-ring analogues".

An ideal chiral template for the central fragment is cyclopentanoid 4a, readily available from baseinduced fragmentation of 9,10-dibromocamphor 5 (to 3) and reductive removal of the bromine atom in 3.6Construction of the analogues will involve (i) formation of side chains with 20-*R* and 20-*S* configuration and (ii) producing a C-8 aldehyde function (vitamin D numbering) needed for coupling with the A-ring.⁷



(a) MeOH, PTSA, r.t. 36h: (b) LDA, HMPA, MeI,-78°C,1,5h; (c) DIBAL-H,toluene,-78°C, 1h; (d)TsCl, py, 0°C, 12h; (e)(i) 9-BBN, THF, r.t. 2 h; (ii) 2N NaOH-H₂O, 30% H₂O₂, 0°C \rightarrow r.t. 12h; (f) NaH, DMSO, HC=C-C(OEE)Me₂, r.t. 2h: (g) SO₃.py, CH₂Cl₂: DMOS (1:2), Et₃N, 0°C; (h) H₂/Pd, 3 atm., EtOAC, 5% Et₃N, 12h; (i) MeMgBr. THF, -25°C, 1h; (j) PDC, CH₂Cl₂, r.t. 40h; (k) MCPBA, CHCl₃, r.t. 4d; (l) TABF,THF, r.t. 48h: (m) PDC, DMF, r.t. 22h; (n) LiOH, CH₃OH, r.t. 7h; (o) PTSA, CH₂Cl₂, r.t. 24h; (p) LDA, HMPA, Br(CH₂)₃C(OTES)Me₂, -78°C, 2h; (q) LiAlH₄, THF, Δ , 12h.

Scheme 2

For the synthesis of analogues with the unnatural 20-(S) configuration the enolate anion of ester 4b was methylated yielding 6 as the single stereoisomer (scheme 2). This result is in accord with the observations of Wicha⁸ on steroids and of Money⁹ on an closely related compound and is fully proven at the stage of 15 (vide infra). Reduction of 6 and tosylate formation, in order to introduce the remaining C-atoms of the side chain led to 7a.

We now addressed the stereoselective hydroboration of the methylene function in 7a, in order to obtain alcohol 8a, as the latent functionality of the C-8 formyl group (vide infra). The epimer 8a was obtained in 85% d.e.. The complete side chain was formed via substitution of tosylate 8a with lithiated 3-(1-ethoxy)ethoxy-3-methyl-butyn-1. Oxidation afforded precursor aldehyde 10, while hydrogenation followed by oxidation gave aldehyde 11. The stereochemical outcome of the hydroboration step was proven via 8b (from 7b in 91% d.e.). Oxidation of the hydroxy group in 8b, followed by reaction with methyllithium and oxidation afforded ketone 12. Baeyer-Villiger oxidation and silyl ether cleavage led to 13 which was transformed into 14. Lactone formation ascertained the *cis* relation of the two substituents in 14 and hence in 8a,b. Furthermore, n.O.e experiments on lactone 15 further confirm the relative configuration at C-20, arising from methylation of 4b.

The synthesis of analogue 2a with the natural 20-(R) configuration also starts from the enolate anion derived from intermediate 4b; alkylation (>95% d.e.) with the appropriate bromide established the full side chain in 16. The relative C-20 configuration is deduced in analogy with the transformation of 6 to 7a and is ascertained by the eventual formation of 19, the C-20 epimer of 11. The classical 3-step transformation of the carbomethoxy group in 16 into the C-21 methyl group then led to 17. In analogy with the hydroboration of 7a and b, olefin 17 is transformed in 18 obtained as the single isomer (>95% d.e.). Finally oxidation afforded the C-8 aldehyde precursor 19.



(a) LDA, HMPA, THF, MeI, 0°C, 1.5h; (b) AcOH, HCl (10:1), 80°C, 16h; (c) KOH, DMSO, H₂O (5:1), r.t. 1h; (d) (n-Bu)₃SnH, r.t. 9h; (e) LiAlH₄, THF, r.t. 16h; (f) TsCl,py, r.t. 18h; (g) (i) 9-BBN, THF, r.t. 6h; (ii) 2N NaOH, 35% H₂O₂, 0°C \rightarrow r.t. 12h; (h) NaH, DMSO, HC=C(OEE)Me₂; (i) SO₃.py, CH₂Cl₂-DMSO (1:2) Et₃N, 0°C.

Scheme 3

An alternative approach for the synthesis of analogues with the 20-(R) configuration takes advantage of the reported stereochemical outcome of the methylation of 9,10-dibromocampor 5 (scheme 3). Money *et al* ¹⁰ have shown that the *endo*-epimer 20 is the thermodynamically more stable isomer (ratio *endo:exo* 9:1). Base induced fragmentation of 20 led to 21 which was debrominated to give 22^{11} . The required precursor aldehyde 26 was then constructed starting from 22 in analogy with the transformation of 6 to 10 (scheme 2).



(a) LDA, THF, -78°C, 2h, then PhNTf, 0°C, 18h; (b) O₃, NaHCO₃, MeOH, -78°C, then NaBH₄, MeOH, -78°C, 18h; (c) TsCl, py, -4°C, 18h; (d) LiAlH₄, THF, Δ , 36h; (e) PDC, CH₂Cl₂, r.t. 6h.

Scheme 4

Analogue 2e was synthesized starting from the known ketone 27 (scheme 4)¹². Ozonolysis of enol triflate 28, formed via the kinetic enolate of 27, followed by reductive work-up gave hydroxy-ester 29. Removal of the hydroxy function, via reduction of the corresponding tosylate and concomitant reduction of the ester group afforded alcohol 30. The C-8 precursor aldehyde 31 was then obtained upon oxidation.

It is worth mentioning that for the above described intermediates chemical shift values for the C-21 methyl protons are between δ 0.82-0.85 for the 20-(S) and between δ 0.93-0.96 for the 20-(R) configuration. This is in analogy with the values for both 20-(S) and 20-(R) epimers of vitamin D where respectively 0.81 and 0.91 is observed.

Construction of the title compounds 2 involves the Lythgoe coupling⁷ of aldehyde precursors 10, 11, 19, 26 and 31 with the A-ring phosphine oxide 32^{13} followed by deprotection of the hydroxy functions (scheme 5).



(a) n-BuLi, THF, -78°C \rightarrow -20°C, 2h; (b) PPTS, CH₂Cl₂, silica gel, r.t., (c) TBAF, THF, r.t. 12h.

Scheme 5

The affinity of the D-ring analogues 2 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 [³H] 1H] 1α ,25(OH)₂D₃ from its receptor compared with the activity of 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* calcemic effect was tested in vitamin D-replete normal NMRI mice by measuring calcium levels in serum and urine. The affinity of the D-ring analogues for VDR varied between 40 and 80% compared to 1 (table). The antiproliferative activity was comparable to 1, except for CD 457 (2e) that was 10 times less potent than $1\alpha.25(OH)_2D_3$. All analogues were 10 to more than 100-fold less calcemic than $1\alpha.25(OH)_2D_3$. (1) Further details of the biological activity of this D-ring analogues will be published elsewhere.

Analogue	VDR	HL-60	MG-63	MCF-F	Keratino- cytes	Calcium Serum
2a (SL 117)	80	85	30	85	90	0.3
2b (SL 137)	70	100	10	85	100	3
2c (WU 442)	40	100		200		0.25
2d (SL 142)	40	100	10	150	200	0.5
2e (CD 457)	80	10	8	1	85	1

Table

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