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

Wu Yong, Shi Ling, C. D'Halleweyn, D. Van Haver, P. De Clercq, M. Vandewalle*

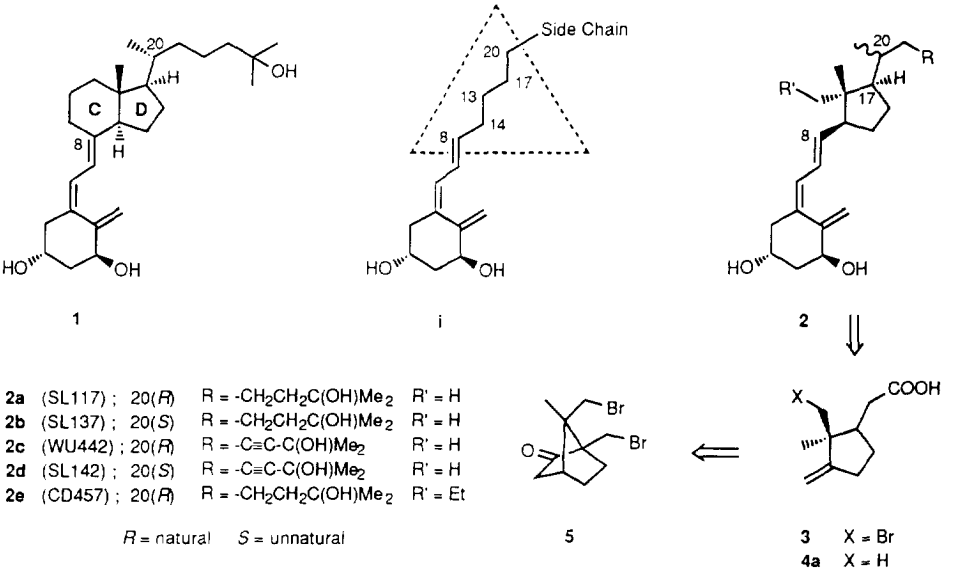
University of Gent, Department of Organic Chemistry, Laboratory for Organic Synthesis, Krijgslaan, 281 (S.4), B-9000 GENT (Belgium)°

R. Bouillon and A. Verstuyf

Laboratorium voor Experimentele Geneeskunde en Endocrinologie, K.U. Leuven, Onderwijs en Navorsing Gasthuisberg, Herestraat, 49, B-3030 LEUVEN (Belgium)

***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 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 especially structural modifications of the side-chain and of the A-ring have been studied in the past.[3]

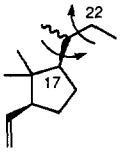


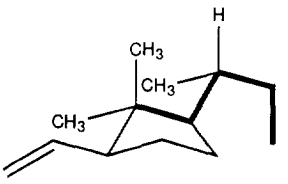
Scheme 1

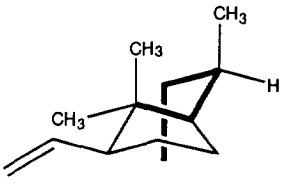
Some years ago, we embarked on an extensive study of the structure-function relationship with the focus on the least studied part of the molecule, i.e. the central CD-ring region.[4] In this respect we decided stripping

° Fax : (32-9) 264.49.98 - E-mail : pierre.declercq.@.rug.ac.be

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 1α- 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]





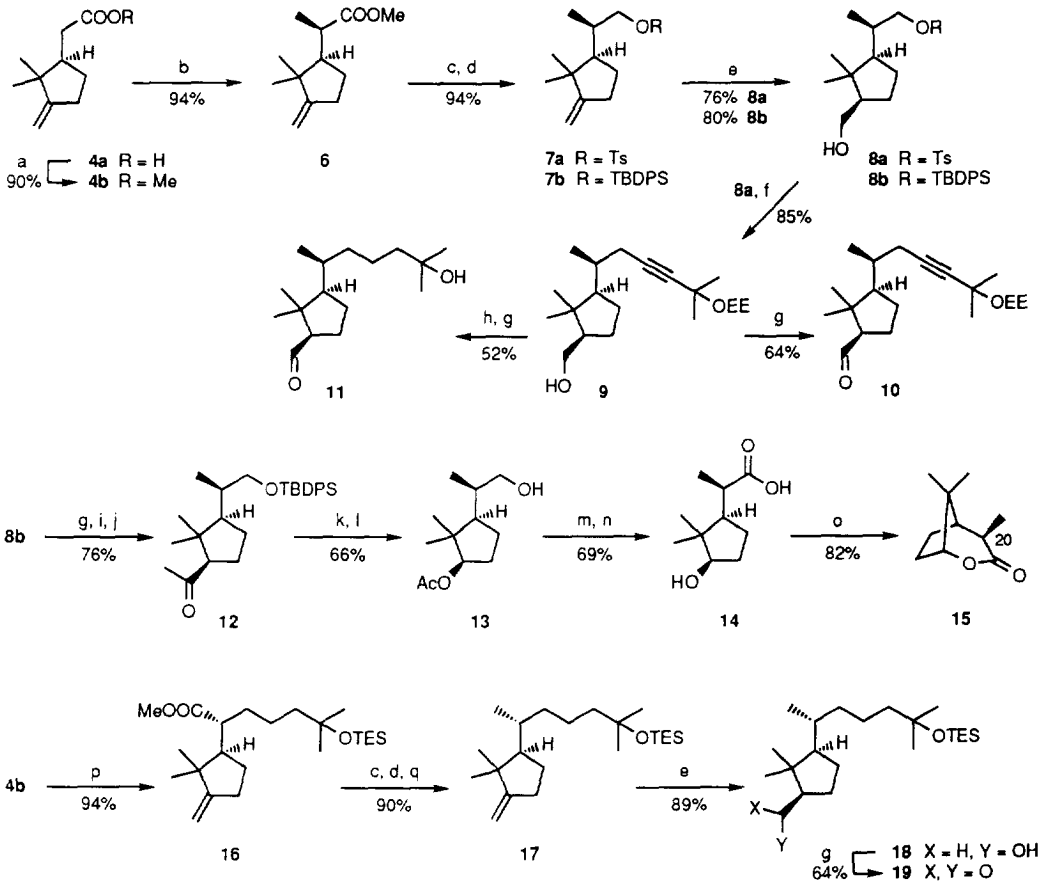


Torsional angle	**2a** 20-(*R*)		**2b** 20-(*S*)	
	τ (°)	mole%	τ (°)	mole%
C_{13}-C_{17}-C_{20}-C_{22}	180	89	-58	87
	87	11	-145	13
C_{17}-C_{20}-C_{22}-C_{23}	61	50	-61	56
	-168	50	-175	35
			74	9

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 base-induced fragmentation of 9,10-dibromocamphor **5** (to **3**) and reductive removal of the bromine atom in **3**.[6] Construction 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-H_2O, 30% H_2O_2, 0°C→ r.t. 12h; (f) NaH, DMSO, HC≡C-C(OEE)Me_2, r.t. 2h; (g) SO_3.py, CH_2Cl_2; DMOS (1:2), Et_3N, 0°C; (h) H_2/Pd, 3 atm., EtOAC, 5% Et_3N, 12h; (i) MeMgBr, THF, -25°C, 1h; (j) PDC, CH_2Cl_2, r.t. 40h; (k) MCPBA, $CHCl_3$, r.t. 4d; (l) TABF,THF, r.t. 48h; (m) PDC, DMF, r.t. 22h; (n) LiOH, CH_3OH, r.t. 7h; (o) PTSA, CH_2Cl_2, r.t. 24h; (p) LDA, HMPA, Br$(CH_2)_3$C(OTES)Me_2, -78°C, 2h; (q) $LiAlH_4$, THF, Δ, 12h.

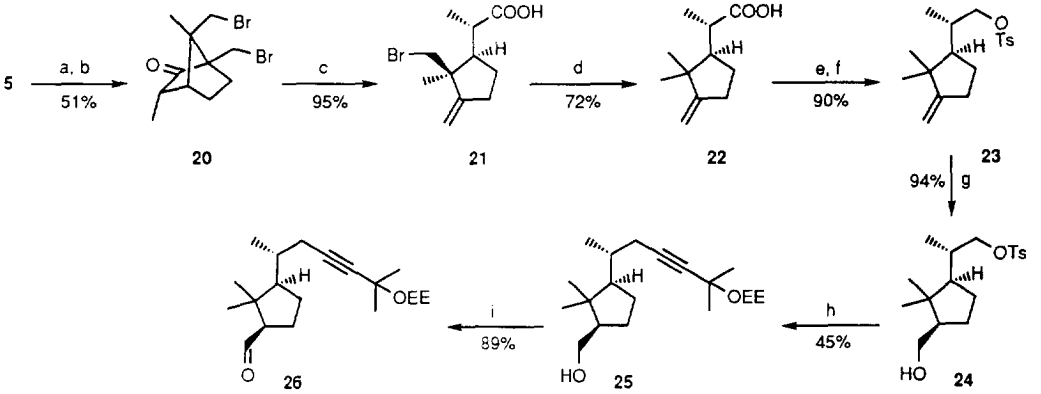
Scheme 2

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 Wicha[8] on steroids and of Money[9] 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**.

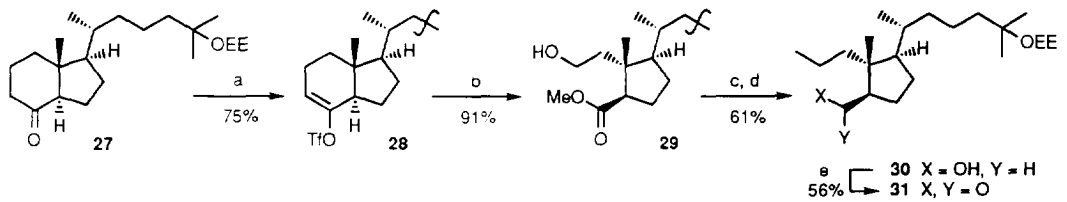
The synthesis of analogue **2a** with the natural 20-*(R)* configuration also starts from the enolate anion derived from intermediate **4b**; alkylation (>95% d.e.) with the appropriate bromide established the full side chain in **16**. The relative C-20 configuration is deduced in analogy with the transformation 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, H_2O (5:1), r.t. 1h; (d) $(n\text{-}Bu)_3SnH$, r.t. 9h; (e) $LiAlH_4$, THF, r.t. 16h; (f) TsCl,py, r.t. 18h; (g) (i) 9-BBN, THF, r.t. 6h; (ii) 2N NaOH, 35% H_2O_2, 0°C→ r.t. 12h; (h) NaH, DMSO, HC≡C(OEE)Me_2; (i) SO_3.py, CH_2Cl_2-DMSO (1:2) Et_3N, 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).



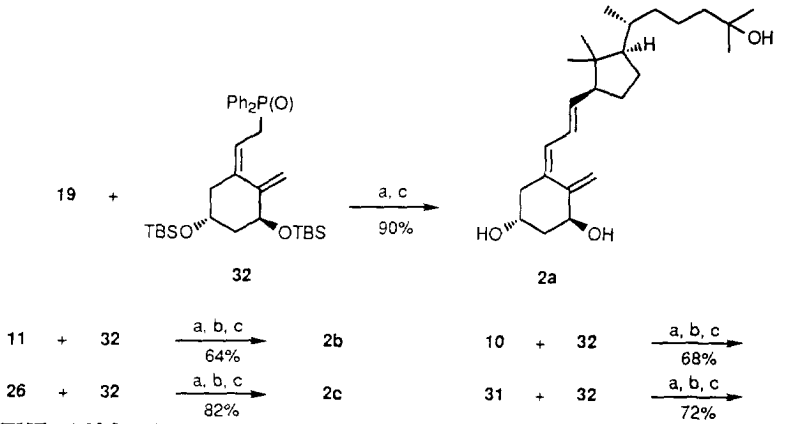
(a) LDA,THF,-78°C, 2h, then PhNTf, 0°C, 18h; (b) O_3, $NaHCO_3$, MeOH, -78°C, then $NaBH_4$, MeOH, -78°C, 18h; (c) TsCl, py, -4°C, 18h; (d) $LiAlH_4$, THF, Δ, 36h; (e) PDC, CH_2Cl_2, r.t. 6h.

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 coupling[7] 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→-20°C, 2h; (b) PPTS, CH_2Cl_2, 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 [^{3}H] 1H] 1α,25(OH)$_2$D$_3$ 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 1α,25(OH)$_2$D$_3$. All analogues were 10 to more than 100-fold less calcemic than 1α,25(OH)$_2$D$_3$ (**1**) Further details of the biological activity of this D-ring analogues will be published elsewhere.

Table

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

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