Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 11, pp. 1461-1464, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00242-4

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

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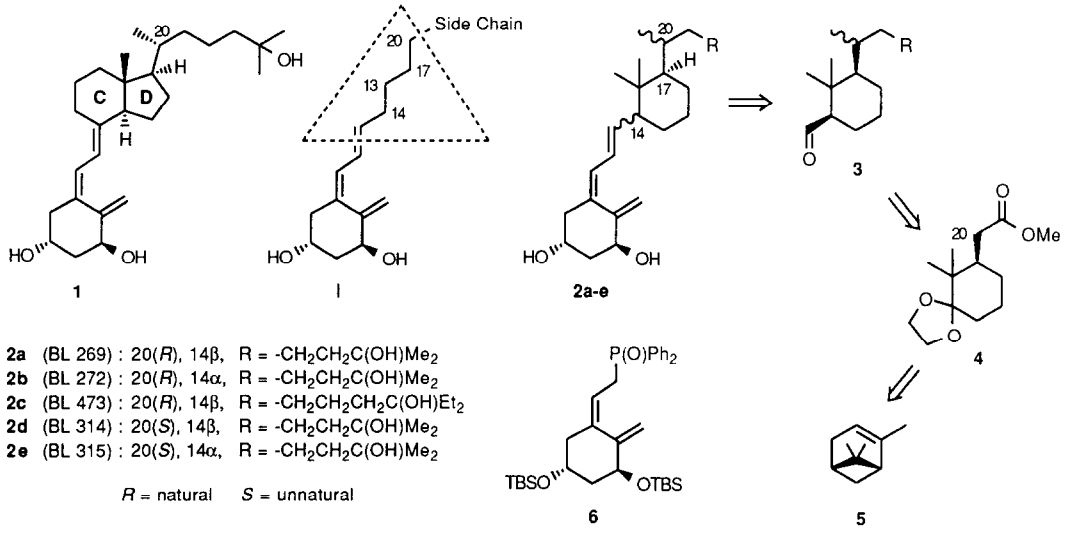
***Abstract** : Vitamin D analogues, characterized by a cyclohexane D-ring and by the absence of a C-ring are described.* © 1997 Elsevier Science Ltd.

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

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] In this respect we decided stripping the

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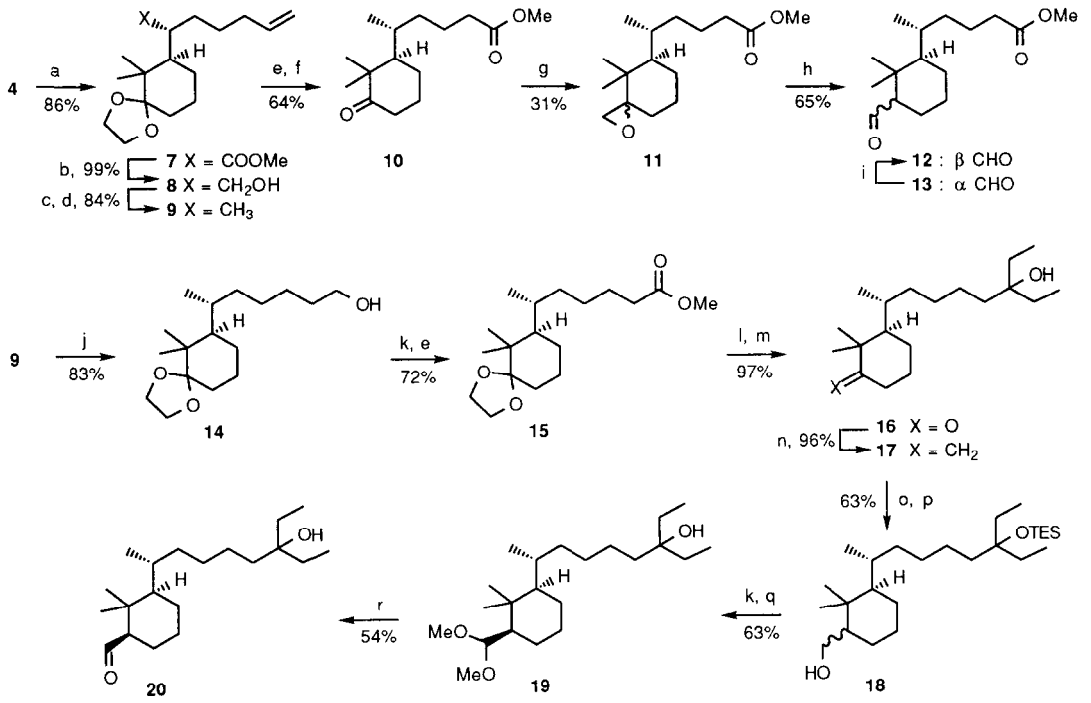
molecule to its five-carbon backbone (C-8 to C-20 : **i**) and resubstituting it again in various ways. In the present paper we describe the synthesis of analogues **2** where the central part is replaced by only a cyclohexane D-ring (scheme 1). We decided to select a "D-ring" carrying a *gem*-dimethyl 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** and which are known to have an influence on restricting the side chain orientations.[3] Also the C-20 configuration influences the side chain orientation. Indeed it has been shown[5] that analogues of **1** with the unnatural 20-*(S)*-configuration can induce interesting differentiations between calcemic effects and new actions. We therefore decided to also introduce both C-20 configurations together with some other side chain modifications.

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.[6] The methoxycarbonyl substituent allows for the construction of the side chain while the keto function is a handle for the introduction of the C-8 formyl group in **3** needed for the Lythgoe coupling[7] with the A-ring precursor **6**.[8]



a) LDA, THF, 5-Br-1-pentene, HMPA, -78°C → r.t., 5 h; b) $LiAlH_4$, Et_2O, r.t., 4 h; c) TsCl, Et_3N, DMAP, CH_2Cl_2, r.t., 20 h; d) $LiAlH_4$, Et_2O, Δ, 5 h; e) O_3, CH_2Cl_2 : 2.5 M NaOH, MeOH (4 : 1), -78°C; f) PPTS, Me_2CO, H_2O, Δ, 3 h; g) $Me_2S{=}CH_2$, THF, r.t., 2 h; h) $BF_3.OEt_2$, THF, r.t., 10 h; i) NaOMe, MeOH, r.t., 12 h; j) (*i*) 9-BBN, THF, r.t., 3.5 h; (*ii*) EtOH, NaOH, H_2O_2, 50°C, 1 h; k) SO_3.py, DMSO, Et_3N, CH_2Cl_2, -8°C, 6 h; l) EtMgBr, THF, r.t., 3 h; m) PTSA, Me_2CO, H_2O, r.t., 30 h; n) $Ph_3P{=}CH_2$, THF, Δ, 3 h; o) TESCl, imid., DMF, r.t., 35 h. p) (*i*) $BH_3.Me_2S$, hexane, r.t., 3 h; (*ii*) H_2O_2, EtOH, NaOH, 65°C, 1 h; q) Amberlyst-15, MeOH, THF, r.t., 12 h; r) $LiBF_4$, 2% H_2O in MeCN, r.t., 3 h.

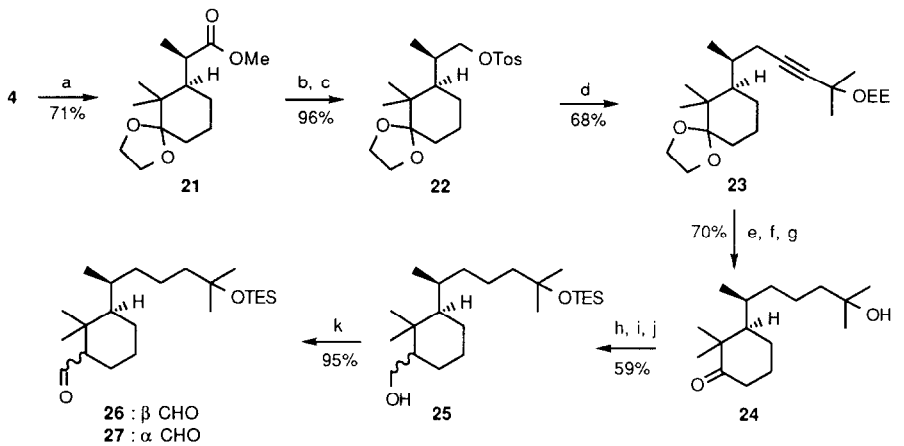
Scheme 2

For the synthesis of analogues **2a,b,c** with 20-*R* configuration (scheme 2) we decided to alkylate **4** with a 4-pentenyl group which would provide us with some flexibility for the construction of different side chains. Epimer **7** was obtained in 95% d.e. and separation was possible by preparative HPLC. This result is in accord

with Wicha's observation on steroids.[9] The relative configuration[10] was proven at the stage of **8** and 20-*epi*-**8** (minor epimer) on the basis of MM2 conformational analysis[11] and n.O.e. experiments. In 20-*epi*-**8** a 3.7% n.O.e. enhancement was observed between one of the 21-hydroxymethyl protons and the axial methyl group while no enhancement was found for **8**; this is consistent with their calculated side chain orientations.

Reductive removal of the hydroxy group, *via* the tosylate, gave **9** which *via* ozonolysis[12] and acetal hydrolysis led to **10**. Reaction with dimethylsulfonium methylide[13], afforded an epimeric mixture of epoxides **11** (6:4). Lewis acid induced isomerization of **11** led to a separable mixture of aldehydes **12** and **13** (6:4 ratio). Base catalyzed equilibration gave **12** and **13** (ratio 6.5:1).[14]

The synthesis of analogue **2c**, with a 24, 26, 27-trishomo side chain, now involved initial hydroboration of **9**, followed by formation of methyl ester **15** which then led to **16**. For the synthesis of the aldehyde **20** we decided to adopt a different route than described for **12**, involving Wittig olefination and hydroboration. However again C-14 epimers **18** (circa 1:1) were obtained; separation was impossible as was the case for the corresponding aldehydes **20** and *epi*-**20**. We then found that HPLC separation was possible at the stage of acetals **19** and *epi*-**19**. Deprotection[15] gave, without epimerization, the desired precursor **20**.[14]



a) (*i*) LDA, THF, -30°C,; (*ii*) MeI, HMPA, -78°C, 3 h then r.t., 2 h; b) $LiAlH_4$, Et_2O, r.t., 4 h; c) TsCl, NEt_3, DMAP, CH_2Cl_2, r.t., 20 h; d) (*i*) NaH, DMSO,65°C, 1.5 h; (*ii*) HC ≡ C(OEE)Me_2 then **23**, DMSO, r.t., 2 h; e) PPTS, n-PrOH, r.t., 1 h; f) H_2 (1 atm), 5% Rh/Al_2O_3, EtOAc, r.t., 2 h; g) PPTS, acetone, H_2O, r.t., 30 h; h) TESCl, imidazole, DMF, r.t., 35 h; i) $Ph_3P(Me)Br$, t-BuOK, THF, Δ, 3 h; j) (*i*) $BH_3.Me_2Sl$, hexane, r.t., 3 h; (*ii*) H_2O_2, EtOH, NaOH, 65°C, 1 h; k) SO_3.py, DMSO, NEt_3, CH_2Cl_2, -8°C, 6 h.

Scheme 3

For the synthesis of the 20-*epi* analogues **2d,e** ester **4** was now methylated to **21** (scheme 3), as the sole isomer (compare **4** to **7**). The side chain was introduced *via* substitution of tosylate **22** with lithiated 3-(ethoxy-ethoxy)-3-methyl-but-1-yn. After hydrogenation and acetal hydrolysis, **24** was transformed into epimeric alcohols **25** (circa 1:1) as described for **16**. In contrast to **18**, epimers **25** could by separated by preparative HPLC. Finally oxidation afforded respectively the precursors **26** and **27**.[14]

Construction of the title compounds **2** involves the Lythgoe coupling[7] of aldehydes **12**, **13**, **20**, **26** and **27**, with the A-ring phosphine oxide **6**[8] and subsequent deprotection (TBAF). For the synthesis of **2a** and **2b**, the coupled products were reacted with MeMgBr prior to deprotection.

The affinity of the D-ring analogues **2** to the pig intestinal mucosa vitamin D receptor (VDR) was evaluated as described previously.[16] 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 (see table) was determined *in vitro* on different cell lines (HL-60, MCF-7, MG-63, keratinocytes)[3]. The *in vivo* effect was tested in vitamin D-deplete normal NMRI mice by measuring calcium levels in serum. The values in the table are given in relation for those of the natural hormone **1** (value 100). BL 269 (**2a**) has a stronger affinity for the VDR compared to **1**. This analogue shows an antiproliferative (MCF-7, keratinocytes) and prodifferentiating (MG-63) activity 3 times that of **1** and is 20 times less calcemic. It is noteworthy that the 20(*S*)-epimer **2d** with unnatural configuration is devoid of all activity. This stands in sharp contrast to analogues with the natural ring where the 20(*S*) configuration mostly induces higher biological activities.[5] Further details of the biological activities will be published elsewhere.

Table

Analogue	VDR	HL-60	MG-63	MCF-7	Keratino-cytes	Calcium Serum
2a (BL 269)	125	90	300	300	300	4
2b (BL 272)	1	2	3	1	30	<0.1
2c (BL 473)	60	600	800	3000	1500	20
2d (BL 314)	0.9	1	1	0	8	<0.1
2e (BL 315)	0.5	1	1	0	8	0.1

Acknowledgements. We thank the "NFWO", the 'Ministerie voor Wetenschapsbeleid" and THERAMEX S.A. for the financial support to the laboratory.

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(*Received in Belgium* 10 *January* 1997; *accepted* 6 *May* 1997)