

PII: S0960-894X(97)00243-6

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

B. Linclau, 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 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 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 structural modifications of the side-chain and of the A-ring have been especially studied in the past.³





[°] Fax : (32-9) 264 49 98 - E-mail : pierre.declercq.@.rug.ac.be.

B. LINCLAU et al.

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 region⁴ 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 synthetic strategy centers around the advanced intermediate 4 which can be obtained from $(+)-\alpha$ -pinene 5, according to a procedure described by Chapuis and Brauchli.⁷

the highly active analogue 3 (Ro 23-7553)⁶ which has an identical pattern of unsaturated bonds.



a)(*i*) ArSeCN, Bu₃P, THF, r.t.; (*ii*) H₂O₂, NaHCO₃, r.t., 1 h then 55°C, 1 h; b) OsO₄, NMO, acetone, H₂O, 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 \rightarrow r.t., 1.5 h; e) (*i*) LDA, THF, -78°C, 0.5 h; (*ii*) propargylbromide, -78°C \rightarrow 0°C, 2 h; f) LiAlH₄, Et₂O, r.t., 4 h; g) TBSCl, imidazole, DMAP, DMF, r.t., 18 h; h) SOCl₂, pyridine, 0°C, 1 h; i) TBAF, THF, r.t., 2 h; j) TsCl, NEt₃, DMAP, CH₂Cl₂, r.t., 12 h; k) (*i*) BuLi, Et₂O; (*ii*) acetone, Et₂O -78°C, 1 h then r.t., 2 h; l) H₅IO₆, Et₂O, 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 unit¹¹ 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 coupling¹² 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 $[^{3}H]1\alpha,25(OH)_{2}D_{3}$ from its receptor compared with the activity of $1\alpha,25(OH)_{2}D_{3}$ (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 series¹⁴, where the (S)-epimer is mostly the more potent agonist. Further details of the biological activities will be published elsewhere.

Analogue	VDR	HL-60	MG-63	MCF-7	Keratino-	Calcium	Ratio
					cytes	Serum	
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

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

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.

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

References and notes

- 1. Bouillon, R.; Van Baelen, H. Saudi Med. J. 1989, 10, 260.
- 2. DeLuca, H.F.; Burmester, J.; Darwish, H.; Krisinger, J. Comprehensive Medicinal Chemistry, Pergamon Press, New York 1990, vol. 3, 1129.
- 3. Bouillon, R.; Okamura, W.H.; Norman, A.W. Endocrine Reviews 1995, 16, 200.
- 4. (a) Bouillon, R.; De Clercq, P.; Pirson, P.; Vandewalle, M. Novel structural analogues of vitamin D; patent PCT/EP 93.202037.3; priority date 09-07-1993; (b) Sabbe, K.; D'Halleweyn, C.; De Clercq, P.; Vandewalle, M.; Bouillon, R.; Verstuyf, A. Bioorg. Med. Chem. Lett. 1996, 6, 1697; (c) Gui-Dong, Zhu; Yongjun, Chen; Xiaoming, Zhou; Vandewalle, M.; De Clercq, P.; Bouillon, R.; Verstuyf, A. Bioorg. Med. Chem. Lett. 1996, 6, 1703;(d) For an analogous approach see Kutner, A.; Zhao, H.; Fitak, H.; Wilson, S.R. Biorganic Chem. 1995,23, 22.
- 5. Linclau, B.; De Clercq, P.; Vandewalle, M.; Bouillon, R.; Verstuyf, A. Biorg. Med. Chem. Lett. 1997 preceeding paper.
- 6. (a) Uskokovic, M.R.; Baggiolini, E.; Shiuey, S.J.; Iacobelli, J.; Hennessy, B.; Kiegiel, J.; Daniewski, A.R.; Pizzolato, G.; Courtney, L.F.; Horst, R.L.; Vitamin D Gene Regulation, Structure-Function Analysis and Clinical Application. Berlin : Walter de Gruyter, 1991, 139, (b) Chen, T.C.; Persons, K.; Uskokovic, M.R.; Horst, R.L.; Holick, M.F. J. Nutr. Biochem. 1993, 4, 49.
- Chapuis, C.; Brauchli, R. Helv. Chim. Acta 1992, 75, 1527.
 Dauben, W.G.; Brookhart, T. J. Am. Chem. Soc. 1981, 103, 1293; Schmuff, N.R.; Trost, B.M. J. Org. Chem. 1983, 48, 1404; Ibuka, T.; Taga, T.; Nishii, S.; Yamamoto, Y. J. Chem. Soc., Chem. Commun. 1988, 342.
- Danheiser, R.L.; Nowick, J.S. J. Org. Chem. 1991, 56, 1176.
 Black, T.H.; Eisenbeis, S.A.; McDermott, T.S.; Maluleka, S.L. Tetrahedron, 1990, 46, 2307.
- Wu, W.-L.; Wu Y.-L. J. Org. Chem. 1993, 58, 3586.
 Lythgoe, B.; Moran, T.A.; Nambudiry, M.E.N.; Tideswell, J.; Wright, P.W. J. Chem. Soc., Perkin Trans I 1978, 580; Kocienski, P.J.; Lythgoe, B. J. Chem. Soc., Perkin Trans. I 1978, 1290.
- 13. Baggiolini, E.G.; Iacobelli, J.A.; Hennessy, B.M.; Batcho, A.D.; Sereno, J.F.; Uskokovic, M.R. J.Org. Chem. 1986, 51, 3098.
- 14. Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M.; Hansen, K. Biochem. Pharmacol. **1991**, 42, 1569.
- 15. Bouillon, R.; Allewaert, K.; Van Leeuwen, J.P.T.M.; Tan, B.K.; Xiang, D.Z.; De Clercq, P., Vandewalle, M.; Pols, H.A.P.; Bos, M.P.; Van Baelen, H.; Birken häger, J.C. J. Biol. Chem. 1992, 267, 3044.

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