

Pergamon

PII: S0960-894X(96)00301-0

Synthesis of CD-ring modified 1α,25-dihydroxy Vitamin D Analogues : C-ring Analogues

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Abstract : Vitamin D analogues, characterized by the absence of a D-ring (C-ring analogues) are described. Copyright © 1996 Elsevier Science Ltd

The finding that 1α ,25-dihydroxy vitamin D₃ (1; 1α ,25(OH)₂D₃) is active in the regulation of cell proliferation and differentiation, next to its classical role in calcium-bone homeostasis, has led in recent years to the development of analogues which can dissociate the cell differentiating effects from the calcemic effects.^{1,2} Among the different portions of the vitamin D skeleton especially structural modifications of the side-chain and of the A-ring have been studied in the past.³





Some years ago, we have 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 regio.⁴ In the present paper we wish to

describe the synthesis of analogues that lack the ring closed five-membered ring of the CD-ring skeleton, i.e., the "C-ring" analogues 3, 4, 5 and 6.5



(a) DCC, DMAP, CH_2Cl_2 (for 8a : 91%; for 8b : 96%; for 8c : 96%); (b) for 9a : LDA, THF-HMPA; TBDMSCl; reflux 16 h (58\%); for 9b : LICA, THF-HMPA (8/3); TBDMSCl, reflux 18 h (67\%); for 9c : LDA, THF-HMPA; TBDMSCl, reflux 16 h (78\%); (c) LAH, THF (for a : 86%; for b : 80%(after prior conversion to the corresponding methyl ester); for c : 90%); (d) TsCl, pyridine (>95\%); (e) LAH, THF (>95\%); (f) Hg(OAc)₂, NaOH; NaBH₄ (67\%); (g) TESCl, DMAP, imidazole, DMF (92\%); (h) 9-BBN, H₂O₂ (for a : 95%; for b : 92%; for c : 95%); (i) PDC (for a : 80%; for b : 92%; for c : 85%; (j) TBAF, THF, 30°C (~90\%); (k) PPh₃, DEAD, p-NO₂PhCOOH (68\%); (l) K₂CO₃, KOH; (m) TBDMSCl, imidazole, DMAP, DMF (97\%); (n) TBDPSCl, imidazole, DMAP, DMF (95\%); (o) Pd(OH)₂/C, EtOH, cyclohexene, reflux (95\%); (p) NaH, DMSO, HC=C-C(OEE)Me₂ (68\%); (q) 20, n-BuLi; THF, -78°C (for 3a : 88%; for 4b : 88%; for 4c : 89%); (s) PPTS, CH₂Cl₂, r.t. (95\%).

SCHEME 2

The deletion of the five-membered ring in the *trans*-fused perhydrindane system should have important consequences as to the composition of the well known 1,7-sigmatropic shift induced equilibrium between

vitamin and previtamin forms.⁶ It is well established that the reason why the vitamin form predominates in the natural equilibrium (>90 % at room temperature) is due to the constraint imposed by the *trans*-fusion on the 6-membered C-ring.⁶ Hence in the monocyclic case (3 and 5) predominance of the previtamin form is expected. Therefore, next to analogues 3 and 5 which possess the natural 19-methylene group, the corresponding 19-nor derivatives (4 and 6, respectively) have also been considered. As for the side-chain structure, next to the natural 25-OH chain (3a, 4a, 5a, 6a), derivatives characterized by a 24R-OH group (3b, 4b), a 23-yne function (3c, 4c), a formal 16,17-ene bond (5, 6) and a bis-homologation (5b, 6b) are considered.³

The synthesis of analogues 3 and 4 rests on the Ireland-Claisen rearrangement of the homochiral 9. Within the three series **a**, **b** and **c** 9 resulted from the esterification of (R)-3-methyl-2-cyclohexen-1-ol 7 (90 % ee) with the corresponding acid 8. Whereas 8a is (R)-(+)-citronellic acid, 8b (98 % ee) is obtained through a 4-step sequence involving the Bayer-Villiger oxidation of (-)-menthone as first crucial step.⁷ (S)-4-Benzyloxy-3-methylbutanoic acid (8c) (94 % ee) is obtained from (S)-3-methyl- γ -butyrolactone.⁸ The Ireland-Claisen rearrangement is performed on the (Z)-silyl ketone acetal (HMPA is used as cosolvent upon enolate formation) and leads through a boat-like transition state to a diastereomeric mixture strongly in favor of the (R)-17-substituted acid (ratio 10/11 better than 10:1).⁹ In the three series the undesired diastereomer is separated after reduction of the acid mixture to the corresponding alcohols 14 and 15. In the case of the c-series the stereochemical assignment was readily established on the stage of the γ -lactones 12 and 13 (obtained from the mixture of 10c and 11c with iodine in acetonitrile) : in the minor isomer 13 a strong NOE is observed between the angular methyl group and H-17 ($\delta = 2.85$ ppm, ³J = 2.9 Hz), while none is observed in the case of isomer 12 ($\delta = 2.55$ ppm, ³J = 6.8 Hz).

After reduction of the diastereomeric mixture of acids 10 and 11, the obtained alcohols 14 and 15 are separated within each series. The reductive removal of the hydroxy group is further accomplished via tosylation followed by LAH reduction. In each series the necessary 8-ketone function (cf. 17, 18 and 19) is obtained *via* oxidative 9-BBN treatment followed by PDC oxidation of the resulting 8-hydroxyl group. The construction of the desired side-chains varies depending on the series; for the **a**-series : conversion of the trisubstituted double bond to the tertiary alcohol using mercuric acetate, NaOH and sodium borohydride; in the **b**-series : an uneventful sequence including a Mitsunobu inversion; in the **c**-series : the introduction of the 3,3-dimethyl-3-ethoxyethyloxy alkyne fragment *via* tosylate substitution. The eventually obtained cyclohexanone derivatives 17, 18 and 19 are each subjected to the usual Horner-Wittig conditions¹⁰ with phosphine oxides 20¹¹ and 21¹², and lead after removal of the different protective groups to the desired analogues 3a, 3b, 3c and 4a, 4b, 4c.

The synthesis of analogues 5 and 6 with a 17-methylene substituent is based on the conjugate addition of the cuprate derived from enantiopure 23 with 3-methyl-2-cyclohexenone (22). The chiral bromide 23 (99 % ee) was obtained from methyl (R)-3-hydroxy-2-methyl propionate¹³ in a 4 step sequence involving after hydroxy group protection and reduction to the aldehyde, Gilbert's alkynynation¹⁴ followed by bromination. With 23 in hand, we turned our attention to the conjugate addition. We first studied higher order cuprates (R*₂Cu(CN)₂Li₂) which have been successfully applied in the conjugate addition on 3-methyl-2-cyclohexenone in the presence of BF₃-OEt₂ or Me₃SiCl.¹⁵ This method failed, only formation of dimers derived from 23 were found. Also the Noyori-Hooz method, where a CuI/n-Bu₃P complex is formed from the vinyllithium was unsuccessful.¹⁶ After considerable experimentation we found that only the procedure described by Magnus et al. led to a viable route (62 % of 24).¹⁷ The experimental conditions are critical. Reaction of the vinylbromide 23 with 2 eq. t-BuLi in ether/THF at -130°C gave the vinyllithium which was treated immediately with preformed CuI/HMPT complex.¹⁸ The reaction mixture was treated with BF₃.OEt₂, followed by the addition of the enone at -78°C, and gradually warmed to -20°C over 18 hours. After deprotection of the alcohol, careful HPLC (Bio-Sil 90-10 column) separation, the two diastereoisomers 25 and 26 (in a ratio of 1/1) were obtained in >95 % de.



(a) (i) 2 eq t-BuLi, THF, -130°C; (ii) CuI, HMPT, -78°C; (iii) BF3.OEt2, -78°C; (iv) 3-methyl-2-cyclohexenone, -78°C \rightarrow -18°C, 16 h; (b) TBAF, THF, r.t., 2 h; (c) Ph3P, imidazole, I2, THF, 0°C \rightarrow r.t., 6 h; (d) NaBH4, CH₂Cl₂/MeOH (1:1), -78°C \rightarrow -30°C; 5 h; (e) CuI, Zn dust, CH₂=CH-COOCH3, EtOH/H₂O (7:3),))), -10°C; (f) PDC, CH₂Cl₂, 0°C \rightarrow r.t., 24 h; (g) for 28 : n-BuLi, 20, THF, -78°C \rightarrow -20°C, 4 h (85%); for 29 : same procedure with 21 (65%); (h) MeMgBr, THF, r.t., 2 d (98%); (i) TBAF, THF, 0°C \rightarrow r.t., 2 d (90%); (j) EtMgBr, THF, r.t., 2 d (89%).

SCHEME 3

For each, only one C-18 methyl signal was observed in the 500 MHz ¹H NMR in the presence of Eu(hfc)₃ (with control on the racemate). The stereochemistry of the two C-13 epimers was determined by circular dichroism measurement for which the opposite Cotton effects were observed. The desired (S)-epimer 25 shows a negative Cotton effect ($\Delta\epsilon$ -0.02 at 289 nm, CH₃CN). The further elaboration of the side-chain involves conversion of the primary alcohol 25 into the iodide followed by the ultrasonically induced addition with methyl acrylate.¹⁹ This step was performed on the alcohol (obtained after reduction of the cyclohexanone) which was then oxidized back to ketone 27. Finally, the cyclohexanone 27 was coupled with the two A-ring phosphine oxides 20 and 21. The 25-OH substituted analogues were eventually obtained *via* reaction with MeMgBr (a-series) and EtMgBr (b-series) in THF, followed by deprotection.

As expected, upon standing in acetone the "natural" methylene substituted derivatives 3b, 3c and 5a, 5b all lead to equilibria in which the corresponding previtamin forms 30, 31, 32 and 33, respectively, predominates; e.g., starting from 3c at 24° C the equilibrium 3c/31 (77:23) is reached after ca 6 days.



The affinity of the C-ring analogues of 1α ,25-(OH)₂D₃ to the pig intestinal mucosa vitamin D receptor was evaluated as described previously.²⁰ The relative affinity of the analogues was calculated from their concentration needed to displace 50 % of [³H]1 α ,25-(OH)₂D₃ from its receptor compared with the activity of 1α ,25-(OH)₂D₃ (assigned a value of 100 %).

The biological evaluation was determined *in vitro* on different cell lines (HL60, MCF-7, MG-63, keratinocytes).³ The *in vivo* calcemic effect of the C-ring analogs was tested in vitamin D-replete normal NMRI Mice. The C-ring analogue with the natural side-chain as in 1α ,25-(OH)₂D₃ (ZG1368) displayed about 60 % of the VDR affinity and this compound showed an antiproliferative and prodifferentiating activity 10 (HL60) to 60 (MCF-7, keratinocytes) times that of 1α ,25-(OH)₂D₃ and was 2 times less calcemic than 1α ,25-(OH)₂D₃. Other modifications of the side chain (ZG1310V, XM612) also improved the antiproliferative activity of 1α ,25-(OH)₂D₃ and decreased the calcemic activity. The introduction of a double bond between carbon 16 and carbon 17 (CY625) increased the antiproliferative activity of 1α ,25-(OH)₂D₃ (2 times) and showed poor calcemic effects (1 % compared to 1α ,25-(OH)₂D₃). The C-ring analogue with the natural side-chain as in 1α ,25-(OH)₂D₃ but lacking carbon 19 (ZG1423) was less potent than the compound with a normal A-ring but other 19-nor C-ring analogues were equipotent or even superagonist compared to 1α ,25-(OH)₂D₃ with a poor calcemic activity (≤ 20 % compared to 1α ,25-(OH)₂D₃). Further details of the biological activity of the C-ring analogues will be published elsewhere.

Acknowledgements. We thank the "NFWO" and the "Ministerie voor Wetenschapsbeleid" for financial support to the laboratory. We thank Prof. Gawronski (Poznan, Poland) for recording the CD spectra. We thank Prof. Magnus (University of Texas at Austin, USA) for sending the experimental details. We thank THERAMEX S.A. for financial assistance.

References and notes

- 1. Bouillon, R.; Van Baelen, H. Saudi Med. J. 1989, 10, 260.
- 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.
- (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) A similar concept was reported by A. Kutner at

the 9th Vitamin D Workshop, Orlando, USA (28/04 - 02/05 1994). See Kutner, A.; Wilson, S.R.; Zhao, H.; Fitak, H. Chodymski, H.; Halkes, S.J. Polish Patent Appl. No P302010, 1994.

- For the synthesis of "E-ring" analogues, see : Sabbe, K.; D'Hallewyn, C.; De Clercq, P.; Vandewalle, M.; Bouillon, R.; Verstuyf, A. *Bioorg. Med. Chem. Lett.* 1996, 6, 1697.
- For a review, see : Norman, A. W. Vitamin D, the Calcium Homeostatic Steroid Hormone, Academic Press: New York, 1979; pp. 37. Havinga, E. Experientia 1973, 29, 1181.
- 7. Grieco, P.A.; Yokoyama, Y.; Gilman, S.; Ohfune, Y. J. Chem. Soc. 1977, 870.
- Chapleo, C. B.; Hallett, P.; Lythgoe, B.; Waterhouse, I.; Wright, P. W. J. Chem. Soc. Perkin I, 1977, 1211.
- 9. Ireland, R. E.; Wipf, P.; Jia-Ning Xiang J. Org. Chem. 1991, 56, 3572.
- Lythgoe, B.; Moran, T.A.; Nambudiry, M.E.N.; Tideswell, J.; Wright, P.W. J. Chem. Soc., Perkin Trans I 1978, 590; Kocienski, P.J.; Lythgoe, V. J. Chem. Soc., Perkin Trans. I 1978, 1290.
- Baggiolini, E.G.; Iacobelli, J.A.; Hennessy, B.M.; Batcho, A.D.; Sereno, J.F.; Uskokovic, M.R. J. Org. Chem. 1986, 51, 3098.
- (a) Perlman, K.L.; Sicinski, R.R.; Schnoes, H.K.; DeLuca, H.F. Tetrahedron Lett. 1990, 31, 1823;
 (b) Perlman, K.L.; Swenson, R.E.; Paaren, H.E.; Schnoes, H.K.; DeLuca, H.F. Tetrahedron Lett. 1991, 32, 7663.
- Corey, E. J. J. Am. Chem. Soc. 1972, 94, 6190; also see : Roush, W. R.; Palkowitz, A. D.; Palmer, M. A. J. Org. Chem. 1987, 52, 316.
- 14. Gilbert, J. C.; Weerasooriya, U. J. Org. Chem., 1979, 44, 4997.
- Lipshutz, B. H.; Wilhelm, R. S.; Kozlowki, J. Tetrahedron Lett. 1982, 23, 3755; Lipshutz, B. H.;
 Wilhelm, R. S.; Kozlowski, J. J. Org. Chem. 1984, 48, 3938.
- Noyori, R.; Suzuki, M.; Kawagishi, T. Tetrahedron Lett. 1980, 21, 1249; Hooz, J.; Layton, R. B. Can. J. Chem. 1970, 48, 1626.
- 17. Magnus, P.; Quaglioto, D. J. Org. Chem. 1985, 50, 1621; Magnus, P. private communication.
- 18. Semmelhack, M. F.; Yamashita, A.; Tomesch, J. C.; Hirotsu, K. J. Am. Chem. Soc. 1978, 100, 5565.
- 19. Pérez-Sestelo, J.; Mascareñas, J.L.; Mouriño, A. Tetrahedron Lett. 1994, 275.
- 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.; Birkenhäger, J.C. J. Biol. Chem. 1992, 267, 3044.