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Synthesis of CD-ring modified 1α,25-dihydroxy vitamin D analogues : E-ring analogues

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***Abstract** : Vitamin D analogues, characterized by the absence of both C- and D-rings and by the presence of a five-membered ring formed by connecting C_{12} and C_{21} (E-ring analogues) are described.* Copyright © 1996 Elsevier Science Ltd

Introduction

It is presently well established that the hormonally active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (**1**; 1α,25$(OH)_2D_3$) may generate biological responses via regulation of gene transcription.[1,2] The discovery of the presence of specific vitamin D receptors in more than 30 tissues initiated consideration of possible functions of 1α,25$(OH)_2D_3$ (**1**) outside its classical role in calcium-bone homeostasis. The hormone was found to be capable of regulating cell proliferation and differentiation of a variety of immunological and malignant cells.

In view of this extraordinary flexibility, current research is aimed at the synthesis of analogues with superagonistic potency and especially which can dissociate the cell differentiating effects from the calcemic effects. This has led during the last decade to the synthesis of a large number of analogues with modifications in the side chain, in the A-ring and to a much smaller extent in the central CD-ring skeleton. Especially side chain modifications have yielded interesting analogues with respect to the above differentiation potential, such as 23-yne, 22-oxa, 20-epi derivatives, homo derivatives (i.e., 24-homo, ...) and also combinations thereof.[3]

Several years ago we embarked on an extensive study of the structure-function relationship with focus on the least studied part of the molecule, i.e. the central CD-ring region.[4] To a first approximation the latter may be considered as an isolator between the side chain and the A-ring which are the two moieties that carry the hydroxy groups (i.e., 1α-OH and 25- or 24-OH) that are essential for activity. Moreover, the central part may also enforce a relative orientation of both moieties that is crucial for activity. In this respect we envisioned stripping the molecule to its five-carbon backbone (C-9)-(C-20) and resubstituting it again in various ways.

In the present paper we wish to describe non-steroid "E-ring" analogues that are characterized by the absence of both C- and D-rings and by the presence of a five-membered ring formed by connecting two atoms of the central backbone, i.e., C-13 and C-20 (the numbering is given in relation with the steroid). Next to the parent analogue **2** in this new family (scheme 2) we also describe variations in the side chain (cf. R in scheme 1) which have been shown to confer some differentiation potential, such as the inclusion of a 23-yne bond (scheme 3), of chain elongations (scheme 4), and of 22-oxa and 20-epi modifications (scheme 5).

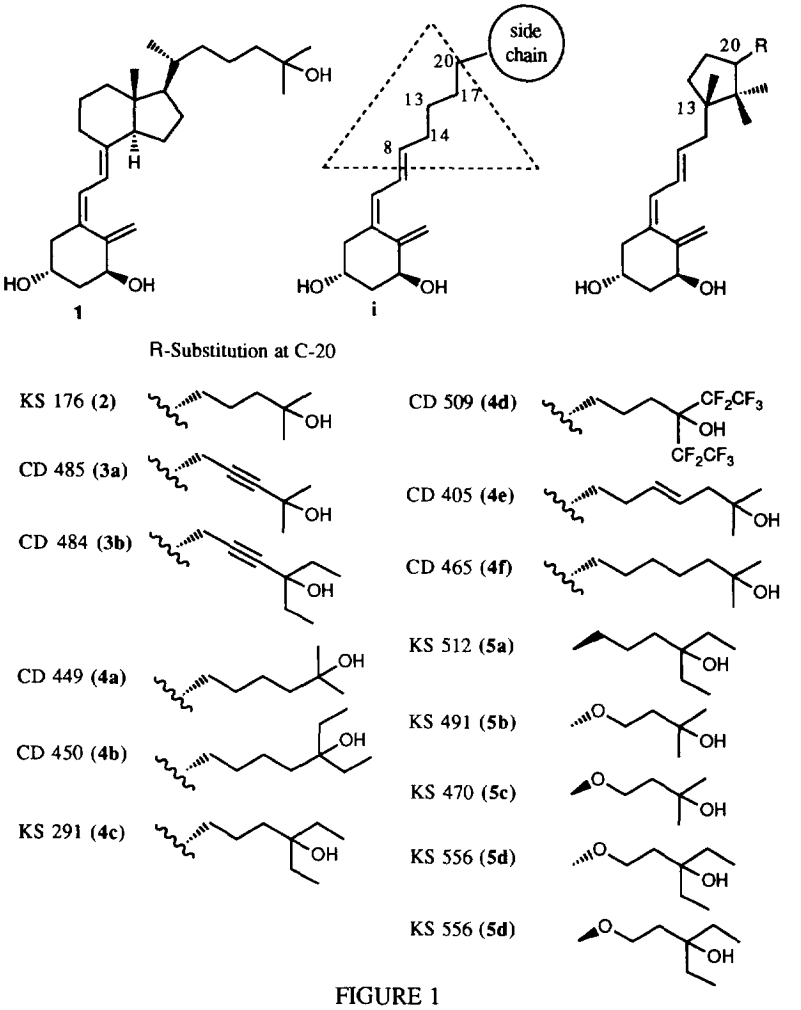
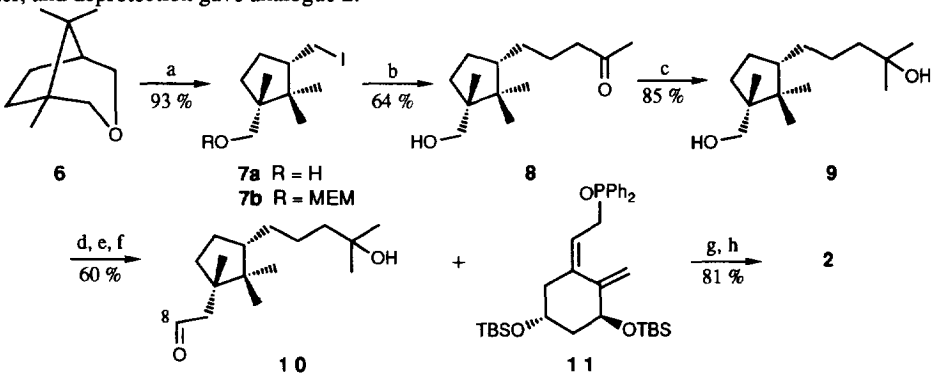


FIGURE 1

An ideal precursor for the central fragment is cyclopentanoid **7a** (scheme 1). It can be obtained from (+)-camphoric acid in 3 steps *via* ether cleavage of known **6**[5], for which we found the method of Olah *et al.*[6] to be most effective. Construction of the analogues will involve (i) formation of side chains with the iodomethyl group as a handle and (ii) introduction of the diene and A-ring *via* the primary alcohol.

For the formation of a variety of side chains several methods were developed. A first method involves conjugate addition to enones and acrylates (schemes 1 and 3). It is noteworthy that no reaction was observed using cuprates derived from **7b**. This is probably due to steric inhibition around C-22. However ultrasonically induced additions[7] led rather efficiently to the desired products; e.g. ketone **8** in this particular case. The synthesis of analogue **2**, with the side chain of the natural metabolite **1**, is shown as a general example in scheme 2. After formation of the side chain, we turned our attention to the introduction of the C-8 formyl function, required for the Lythgoe[8] coupling with the A-ring phosphine oxide presursor **11**.[9] Oxidation of the hydroxy group in **9**, Wittig reaction of the resulting aldehyde with triphenyl methoxymethylene ylide and

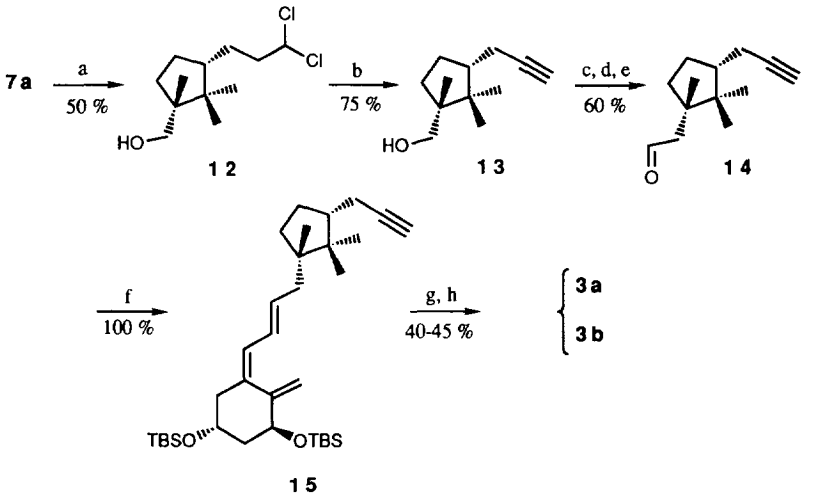
subsequent hydrolysis afforded **10**. Finally, reaction of **10** with the anion of **11**, leading exclusively to the 7,8 E-isomer, and deprotection gave analogue **2**.



(a) NaI, $MeSiCl_3$, CH_3CN, refl. 2 h; (b) CH_2=CH-$COCH_3$, Cu_2I_2:Zn: (1:4), EtOH:H_2O (7:3),))), r.t., 3 h; (c) MeMgBr, THF, r.t., 12 h; (d) $C_5H_5N.SO_3$, Et_3N, DMSO:CH_2Cl_2 (2:1), -5°C, 2 h; (e) $Ph_3PCH_2OCH_3$.Cl, LDA, HMPA:THF, -40°C to r.t., 12 h; (f) HCl, THF, r.t., 45 min; (g) BuLi, THF:HMPA (6:1), -78°C to r.t., 1 h; (h) TBAF, THF, r.t., 24 h

SCHEME 1

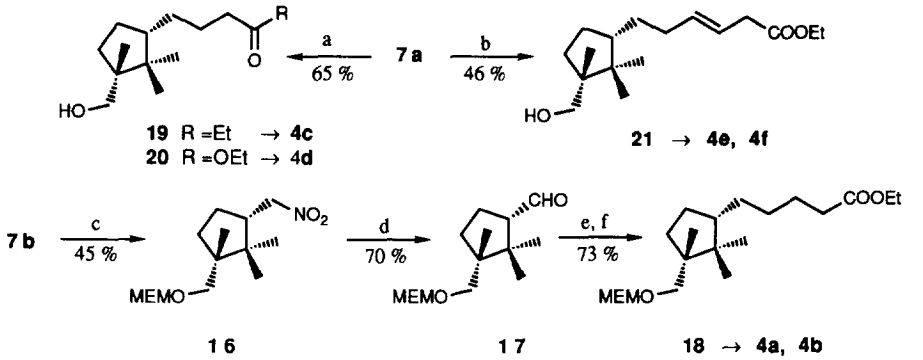
The synthesis of the analogues with a 23-yne side chain (type 3) is based on radical addition[10] of **7a** on 1,1-dichloroethene and subsequent bis-elimination of **12** (scheme 2). The resulting **13** was transformed in aldehyde **14** (as described for **10** from **9**; scheme 2); coupling with **11** gave **15**. Finally the eventual side chain was formed *via* reaction of the anion of **15** with the appropriate ketone.



(a) CH_2=CCl_2, Bu_3SnCl, $NaBH_4$, AIBN, EtOH, -20°C to r.t., 24 h; (b) LDA, THF, -30°C, 1 h; (c) $C_5H_5N.SO_3$, Et_3N, DMSO:CH_2Cl_2 (2:1), -5°C, 3 h; (d) $Ph_3PCH_2OCH_3$.Cl, LDA, HMPA:THF, -40°C to r.t., 24 h; (e) HCl, THF, r.t., 45 min; (f) **11**, BuLi, THF:HMPA (6:1), -78°C to r.t., 1 h; (g) RCOR, LDA, THF:HMPA, -40°C to r.t., 1 h; (h) TBAF, THF, r.t., 48 h

SCHEME 2

Scheme 3 describes the synthesis of analogues (type 4) with elongated side chains. The 26,27-bishomo analogues **4c** and **4d** were respectively constructed from **7a** *via* **19** and **20** as described for the synthesis of **2** (scheme 2). For the synthesis of the decafluoro-26,27-bishomo analogue **4d** ester **20** was treated with pentafluoroethyllithium, prepared *in situ*, according to Gassman and O'Reilly.[11] Ultrasonically induced 1,6-addition of **7a** with ethyl 2,4-pentadienoate led to **21** the precursor for 24-bishomo analogues **4e** and **4f**.



(a) CH_2=CH-COR, Cu_2I_2:Zn (1:4), EtOH:H_2O (7:3),))), -20°C, 2 min; (b) CH_2=CH-CH=CH-COOEt, Cu_2I_2:Zn (1:4), EtOH:H_2O (7:3),))), -20°C, 2 min; (c) $NaNO_2$, urea, DMF, 25°C, 48 h; (d) NaOMe (1 eq); O_3/Me_2S, -78°C, 5 min; (e) $(EtO)_2P(O)CH_2CH$=CH-COOEt, LDA, THF, -78°C, 2 h; (f) H_2/Pd, 4 atm., r.t., 15 h

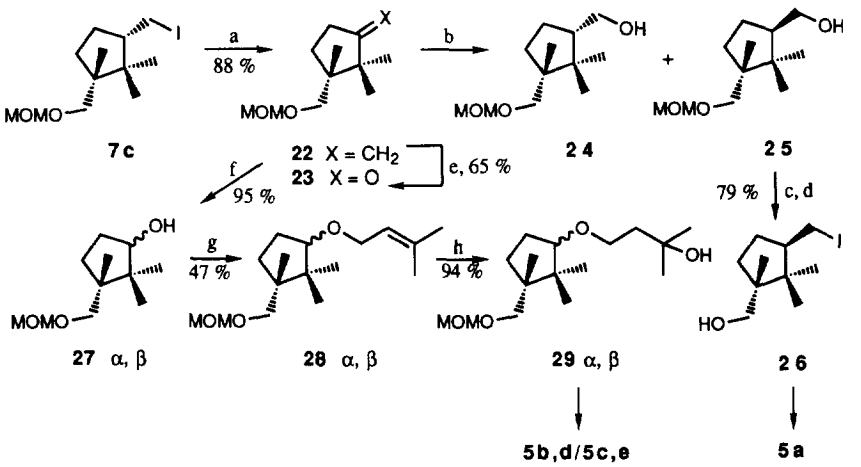
SCHEME 3

The approach, directed towards 24-homo side chains, involves the intermediacy of aldehyde **17**, efficiently obtained *via* alkaline ozonolysis[12] of **16**. Reaction of **17** with the anion of triethyl 4-phosphonocrotonate and subsequent hydrogenation gave **18**. After reaction of the ester function with the appropriate Grignard reagent and primary alcohol deprotection, analogues **4a** and **4b** were obtained as described for **2** from **9** (scheme 2).

It has been demonstrated that some 20-epi vitamin D analogues with selected side chains induce differentiation between calcemic activities and new actions.[13] We therefore decided to evaluate **5a**, the epimer of **4c** (scheme 4).

Inversion of C-20 in **7c** was carried out *via* hydroboration of **22**. Surprisingly the best method for elimination of iodide **7c** was found to be reaction with TBAF.[14] Hydroboration with 9-BBN led with low diastereoselectivity to **24** and **25** in a 2:3 ratio. Both epimers could be separated by HPLC and the structure was proved by hydrolysis of **24** into the known corresponding diol.[4] The desired alcohol **25** was transformed into iodide **26** which was then taken through the same sequence as described for **2** from **7a** (scheme 2).

Also two 22-oxa analogues were synthetized starting from **22**. Double bond cleavage and subsequent reduction of cyclopentanone **23** gave the epimeric alcohols **27**α and **27**β in 2:3 ratio. As both epimers were difficult to separate, ether formation was carried out on the mixture. After formation of the 25-hydroxy group, MOM-ether cleavage and oxidation to the aldehyde both epimers could be separated and the structures proven by NOE experiments. After deprotection of the primary hydroxyl group both intermediates were transformed into respectively **5b,d** and **5c,e** (as described for **2** from **9**).



(a) TBAF, THF, r.t., 24 h; (b) (*i*) 9-BBN, THF, 55°C, 5 h; (*ii*) H_2O_2, NaOH, Δ, 1 h; (c) I_2 PPh_3, imid., Et_2O:MeCN (3:1); (d) Amberlyst-15, MeOH:THF (1:1); (e) OsO_4, $NaIO_4$, THF:H_2O (1:1), r.t., 30 h; (f) $LiAlH_4$, THF, r.t., 15 h; (g) $(CH_3)_2C{=}CHCH_2Cl$, KOH, Crown 6, tol.,))), r.t., 1 h; (h) (*i*) $Hg(OAc)_2$, H_2O, THF, r.t., 1 h; (*ii*) $NaBH_4$

SCHEME 4

The biological evaluation of the E-ring analogues 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-deficient chicks and vitamin D-replete normal NMRI mice. For KS 176 with the natural side chain as in 1α,25(OH)$_2$D$_3$ a mild vitamin D-like biological activity was found. Indeed compound KS 176 displayed about 10 % of the VDR affinity and even higher than 10 % of the biological activity on several cell lines when compared with the antiproliferative or prodifferentiating effect of 1α,25(OH)$_2$D$_3$ while it has only 0.1 % of the calcemic effect. Further modifications of the side chain led to analogues with, compared to 1α,25(OH)$_2$D$_3$, nearly equipotent or even superagonist (CD 509) activity (especially on keratinocytes and MCF-7 cells) whereas all such analogues had poor calcemic effects in vivo (≤ 3 % compared to 1α,25(OH)$_2$D$_3$). It is noteworthy that the parent compound **i** (figure 1) with a "naked" alkyl chain (C-8, C-25) has no receptor binding and is devoid of all activity.

Further details of the biological activity of the non-steroidal E-ring analogues will be published elsewhere.

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References

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) 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.
5. Erickson, G.W.; Fry, J.L. *J. Org. Chem.* **1980**, *45*, 970.
6. Olah, G.A.; Hussain, A.; Singh, B.P.; Meheotra, A.K. *J. Org. Chem.* **1983**, *48*, 3667.

7. Pérez-Sestelo, J.; Mascareñas, J.L.; Castedo, L.; Mouriño, A. *Tetrahedron Lett.* **1994**, 275.
8. 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.
9. Baggiolini, E.G.; Iacobelli, J.A.; Hennessy, B.M.; Batcho, A.D.; Sereno, J.F.; Uskokovic, M.R. *J. Org. Chem.* **1986**, *51*, 3098.
10. Giese, B.; Gonzàlez-Gomez, J.A.; Witzel, T. *Angew. Chem.* **1984**, *96*, 52; Giese, B. *Angew. Chem.* **1985**, *97*, 555.
11. (a) Gassman, P.G.; O'Reilly, N.J. *Tetrahedron Lett.* **1985**, *26*, 5243; (b) Angelastro, M.R.; Burkhart, J.P.; Bey P.; Peet, P. *Tetrahedron Lett.* **1992**, *33*, 3265.
12. Mc Murry, J.E.; Melton, J.; Padgett, H. *J. Org. Chem.* **1974**, *39*, 259.
13. Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M.; Hansen, K. *Biochem. Pharmacol.* **1991**, *42*, 1569.
14. Eliminations using non-nucleophilic voluminous bases failed. Seemingly the steric congestion around C-20 allows only attack of F^-.

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