Pure & Appl. Chem., Vol. 70, No. 2, pp. 373–376, 1998.
Printed in Great Britain.

Synthesis of novel vitamin D analogs

R. M. Moriarty,[a] R. Penmasta,[b] M. S. Rao,[b] L.Guo, F. Werner,[a] R. G. Mehta[c]

[a]*Department of Chemistry, University of Illinois at Chicago.* [b]*Steroids Ltd., Chicago, IL.*
[c]*Department of Surgical Oncology, College of Medicine, University of Illinois, Chicgo.*

Abstract: Structural modifications of the side-chain of vitamin D lead potentially to analogs in which a separation is effected between calcemic activity and cellular differentiation. Vitamin D analogs are used clinically for diseases involving cell proliferation. We have discovered an important lead compound, namely, 24- (*R*)-ethyl-1α-hydroxyvitamin D_3 (**5d**) also called 1α-hydroxyvitamin D_5, which is active as an anti-cancer agent. The renal metabolism of **5d** results in hydroxylation at C_{26} (C_{27}), to yield **9** or **10**. Synthesis of this metabolite is presented.

BACKGROUND

1α,25-Dihydroxyvitamin D_3 (**1**) is the hormonally active form of vitamin D_3 and it is essential for the control of calcium homeostasis in the animals and humans. Recently, a striking development has occurred in the area of vitamin D. The main focus of many vitamin D studies has shifted from the more or less classical calcium homeostasis perspective to the regulation of a multitude of other cellular processes. This shift may be traced basically to the diversity of receptors for 1α,25-dihydroxyvitamin D_3 (**1**)[1] which have been discovered throughout the human body. In addition to the more typical sites of action, i.e., intestinal mucosa, bone and kidney, other calcium binding proteins have been discovered in rat brain, spinal cord and hematopoietic cells.

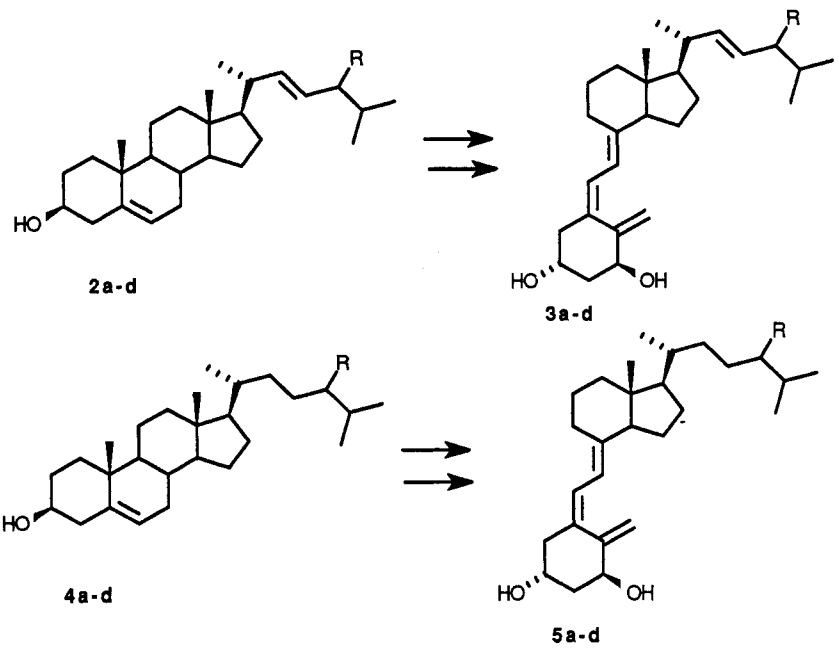
1α,25-Dihydroxyvitamin D_3 (**1**) plays an important role in the area of cancer therapy because of its effect upon three phenomena: cellular induction of differentiation, suppression of cellular proliferation, and both balanced against reduced or low calcemic activity.

The central synthetic problem in vitamin D chemistry is obtention of an analog in which a separation exists between calcemic activity and cellular differentiation activity and cancer agents. At the levels needed for *in vivo* antileukemic agents, 1α,25-dihydroxyvitamin D_3 (**1**) induces toxic blood levels of calcium. Thus, a 1α,25-dihydroxyvitamin D analog in which the side chain is elongated by one carbon atom shows a differentiation activity for leukemia cells about ten times greater than **1** itself but this homolog is approximately equipotent to **1** in calcemic activity.[2] The differentiation:calcemic activity ratio is improved but this homolog could not be called selective. Addition of two extra carbon atoms, to form a *bis*–homo analog, namely, 24-bishomo-1α,25-dihydroxyvitamin D_3 (**6**) leads to a superior compound[2] which is devoid of calcemic activity at concentrations where malignant cell antiproliferative and cell differentiation activity are potent.

These side-chain modified analogs are synthesized by a number of different routes, all of which basically involve cleavage of the existing side-cahin from a precursor vitamin D and the reattachment of the appropriate synthon via Wittig, Julia or Grignard coupling. In our work we followed a different approach. Nature provides a rich array of side-chain alkylated cholesteryl derivatives. These encompass either a methyl or ethyl group at C_{24} and an E-double bond at C_{22-23}, i.e. **2a-d, 4a-d**. Each of these naturally occurring steroids could be converted into a 1α-hydroxyvitamin, i.e., **2a-d** to **3a-d**; **4a-d** to **5a-d**.

Vitamin D_5 (24 R-ethyl vitamin D_3) (**8**) was first reported over fifty years ago and was formed from irradiation of 7-dehydrositosterol (**7**) and showed no effect on blood serum calcium in the chicken.[3,4] In a modern context this is a very important factor since lack of calcemic activity suggests the examination of the 1α-hydroxy analog i.e., **5d**, as an anticancer agent. In fact it proved to be extremely active in blocking breast cancer. 1α-OH-D_5 (**5d**) was shown to inhibit growth and induce differentiation in UISO-BCA-1 and UISO-BCA-4 human breast carcinoma cell lines.[5] Furthermore 1α-OH-D_5 (**5d**) is non-calcemic.

The synthesis of 1α-OH-D_5 (**5d**) which proceeds from stigmasterol (**2c**) is as follows:



1 = **5** = R = H, C_{25}-OH

2a = R = 24 (*R*) CH_3 = Ergosterol → **3a** = 1α-OH-D_2

2b = R = 24 (*S*) CH_3 = 24-*epi*-Ergosterol → **3b** = 1α-OH-*epi*-D_2

2c = R = 24 (*S*) CH_3CH_2 = Stigmasterol → **3c** = 1α-OH-22,23-dehydro-24 (*S*)-ethyl-D_3

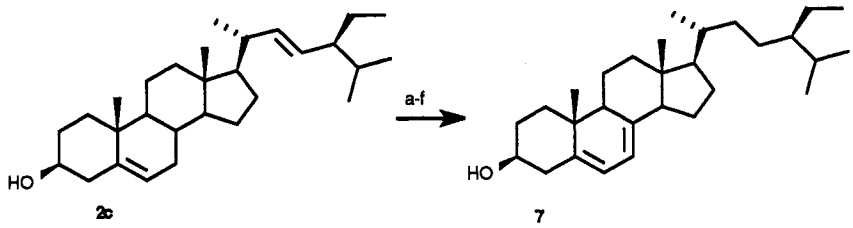
2d = R = 24 (*R*) CH_3CH_2 = 24-*epi*-Stigmasterol → **3d** = 1α-OH-22,23-dehydro-24 (*R*)-ethyl-D_3

4a = R = 24 (*R*) CH_3 = Campesterol → **5a** = 1α-OH-D_7

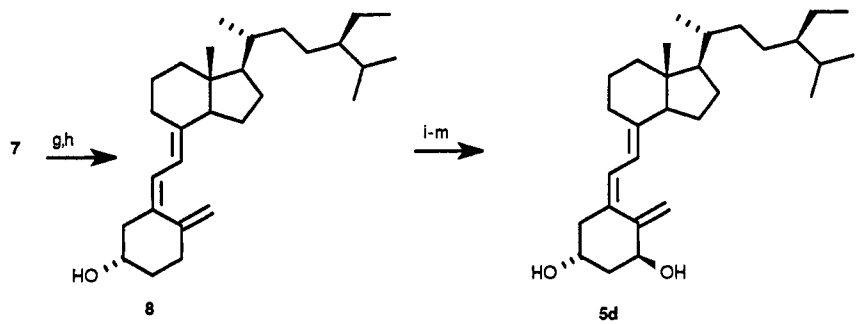
4b = R = 24 (*S*) CH_3 = Brassicasterol → **5b** = 1α-OH-D_4

4c = R = 24 (*S*) CH_3CH_2 = Clionosterol → **5c** = 1α-OH-24*epi*-D_5

4d = R = 24 (*R*) CH_3CH_2 = β- Sitosterol → **5d** = 1α-OH-D_5

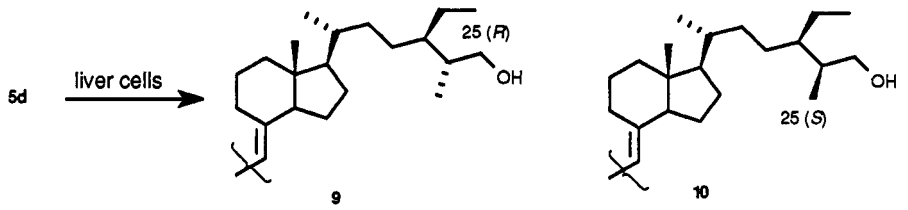


a) TsCl/pyridine, b) CH_3CO_2K, CH_3OH, c) H_2-Pt/C/EtOAc, d) $(CH_3CO)_2O$/AcOH
e) dibromatin/hexane; Bu_4NBr/THF, collidine, f) $LiAlH_4/Et_2O$

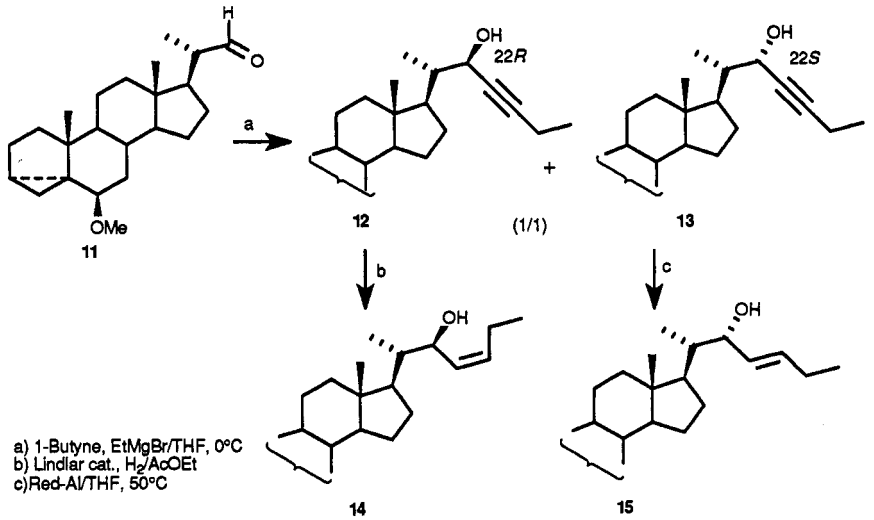


g) hv, Et_2O, C_6H_6, h) Reflux, EtOH, i) TsCl/pyridine, j) $CH_3OH/NaHCO_3$ (gives **6**), Heat,
k) SeO_2, t-BuOOH, l) DMSO/AcOH, m) maleic anhydride/EtOAc, chromatographic separation of *cis* from *trans* isomer

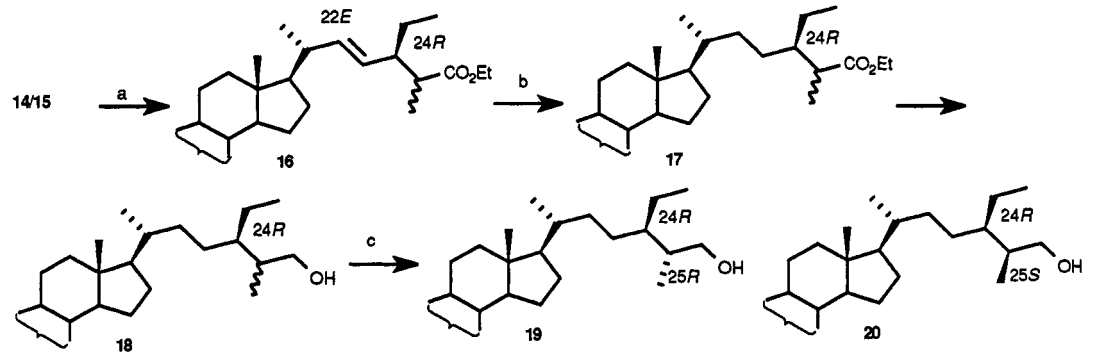
The oxidative metabolism of **5d** in normal human liver cell HHOI proved interesting.[6] This system is capable of C_{25} and C_{27} hydroxylation. The stereochemistry of enzymatic hydroxylation at C_{24} of the side-chain of vitamin D analogs, as in the case of 1α-OH-D_2, affords 1α,24 *S* $(OH)_2D_2$.[7] This involves retention of configuration at C_{24}. It would appear that the hydroxylation at C_{24} is stereoselesctive[8] and occurs with retentiøn of configuration in the case of D_2 and *epi* D_4. In the case of D_4 and D5 the substituent at C_{24} is reversed relative to D_2 and *epi*-D_4 and hydroxylation occurs at C_{25} (C_{26}) (**5a** to **9** or **10**). Since a new chiral center is created at C_{25} it becomes a synthetic problem to prepare the two C_{26} hydroxylated compounds related to vitamin D_5 and determine which is the natural metabolite i.e., **9** or **10**



The syntheses of **9** and **10** start from stigmasterol (**2c**) which is converted to the *p*-toluenesulfonate and solvolysis in methanol/ KOAc to yield the i-steroid 22*E*,24*S*-6β-methoxy-24-ethyl-3α,5α-cyclocholest-22-ene (**6**) which upon ozonolysis yielded aldehyde **11**.



Claisen rearrangements **14** to **16** and **15** to **16** provide the correct side-chain for **19** to **20**.



The C_{25} epimeric C_{26} (C_{27}) hydroxylated stigmasterols are converted to the 1α-hydroxyvitamin D_5 **9** and **10** using the methods described earlier.

REFERENCES

1. DeLuca, H. F.; Schnoes, H. K. *Annu. Rev. Biochem.*, **52**, 411 (1983).
2. Osteem, V. K.; Tanaka,Y.; Prahl J.; DeLuca, H. F.; Ikekawa, N. *Proc. Nat. Acad. Sci. USA*, **84**, 2610 (1987).
3. Wunderlich, Hoppe-Seylers *Z. Physiol. Chem.*, **241**, 116, 119 (1936).
4. Napoli, J. L.; Fivizzani, M. A.; Schnocs, H. K.; DeLuca, H. F. *Arch. Biochem. Biophys.*, **197**, 119 (1979).
5. Mehta, R.; Moriarty, R. M.; Mehta, R. R.; Penmasta, R.; Lazzaro, G.; Liang, G. *J. Nat. Cancer. Inst.*, **89**, 212 (1997).
6. Unpublished results with G. Jones, Queen University, 1995.
7. Strugnell, S.; Byford, V.; Makin, H. L.; Moriarty, R. M.; Gilardi, R.; LeVan, L.; Knutson, J. C.; Bishop, C. W.; Jones, G. *Biochem. J.*, **310**, 233 (1995).
8. DeLuca, H. F.; Schnoes, H. K. *Annu. Rev. Biochem.*, **52**, 411 (1983).