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Synthesis and Biological Evaluation of some 25,26-epoxy-1α,24-dihydroxyvitamin D_3 Analogues

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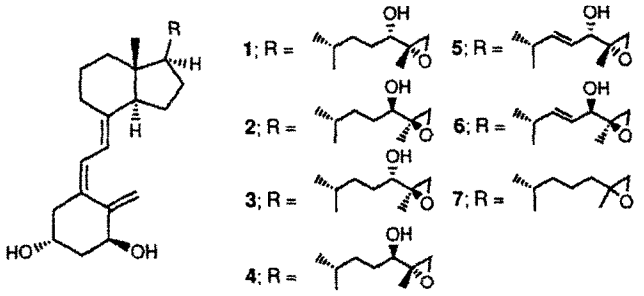
Abstract *: The synthesis of all stereoisomeric 25,26-epoxy-1α,24-dihydroxyvitamin D_3 analogues is described and relies on the Sharpless kinetic resolution of secondary alcohols. It further includes the Julia procedure for side chain construction and the Lythgoe A-ring coupling procedure. Biological evaluation includes the study of calcemic effect, receptor binding and cell differentiation.*

Introduction

The importance of 1α,25-dihydroxyvitamin D_3, the hormonally active metabolite of vitamin D_3 is presently well recognized.[1] Apart from its normal role as calcium regulator[2] other potential properties start to emerge including regulation of cell proliferation and differentiation processes and immune modulation.[2,3]

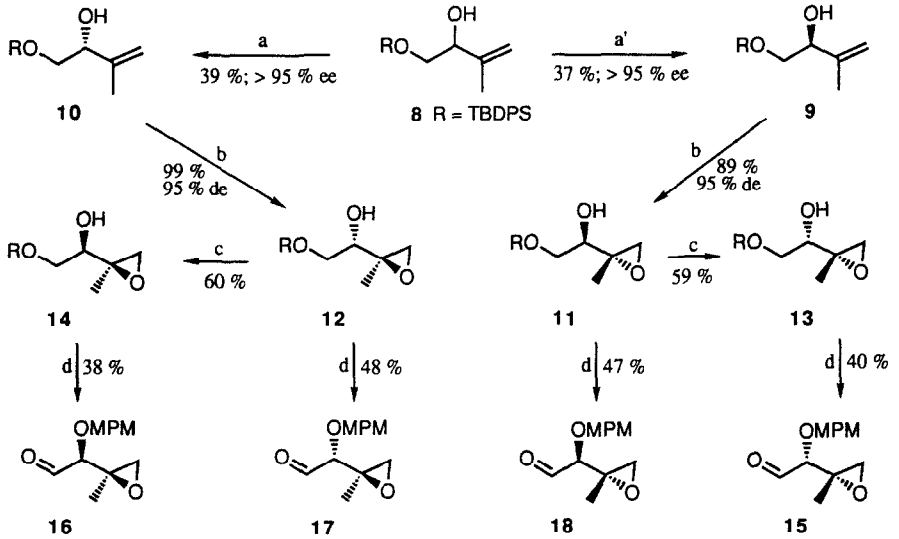
Recently, there has been a growing interest in the development of analogues of 1α,25-$(OH)_2$-D_3 with low calcemic effect but increased cell differentiating ability. A large number of side-chain modified analogues have been described during the last decade;[4] the evaluation of an epoxide function in the side-chain had been neglected until now. We decided therefore to investigate this type of analogues. In this context we present here our first report involving mainly 24-hydroxy-25,26-epoxy substituted side-chains.



Synthesis

The strategy for the side chain construction of the analogues **1**, **2**, **5** and **6** centers around the Julia olefination method[5] involving the sulfone **20**, derived from the Lythgoe-Inhoffen diol[6], and the aldehydes **15** and **16** as the 23,27-carbon fragment.

The homochiral, highly functionalized aldehydes **15** to **18** were obtained via initial Sharpless kinetic resolution[7] of allylic alcohol **8** (scheme 1). In order to minimize the complexation of the catalyst with the homoallylic primary oxy function, a bulky silyl protecting group (TBDPS) was selected. Initially, we directed our efforts towards the formation of optically pure epoxides. When conditions *a* were applied up to 50 % conversion **11** was obtained in 80 % ee; lowering the conversion to 37 % afforded **11** in 93 % ee.

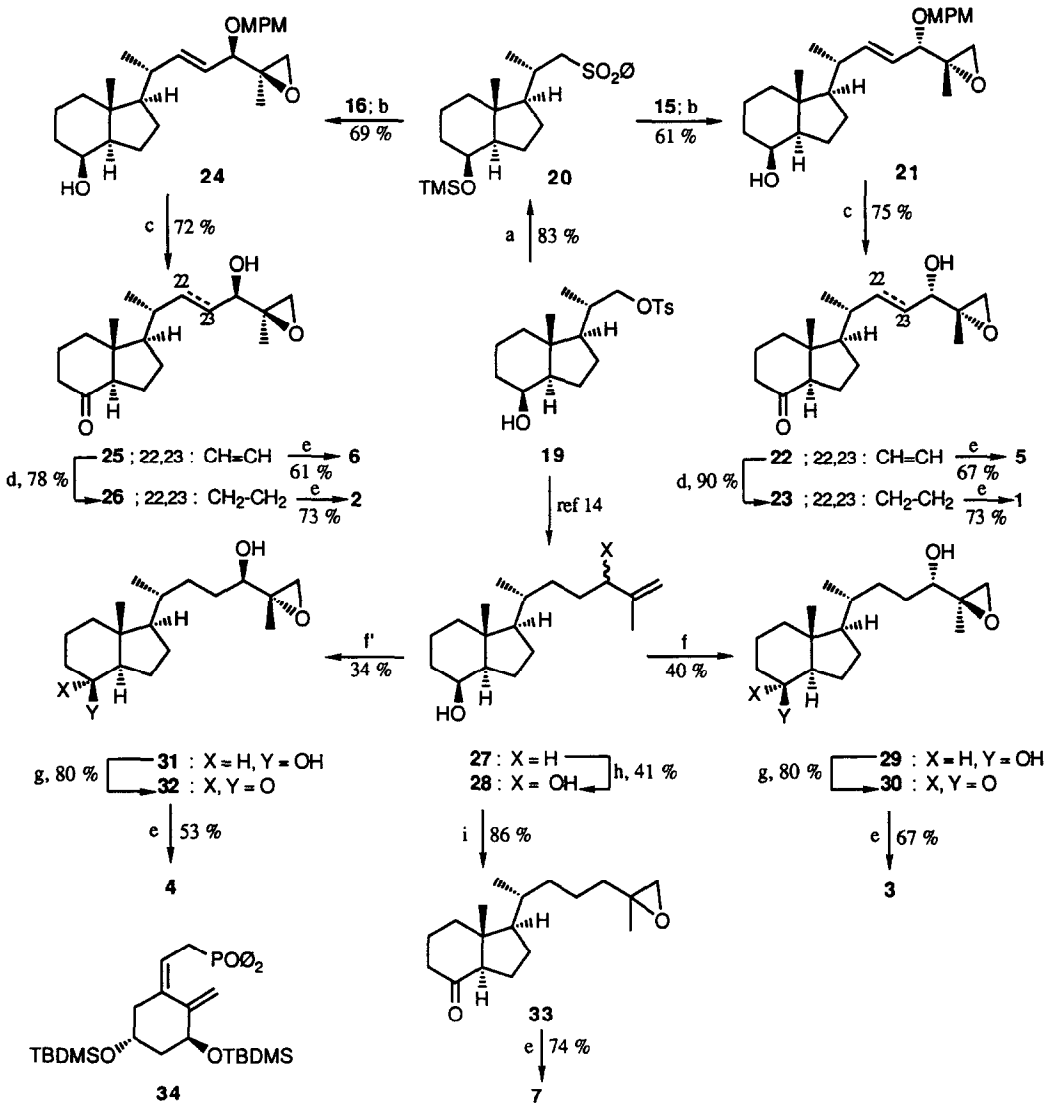


(a) $Ti(OiPr)_4$ (0.50 eq), (-)-DIPT (0.65 eq), TBHP (0.69 eq), 4A sieves (29 w %), CH_2Cl_2, -20°C, 27 h, 60 % conversion; (a') as for a with (+)-DIPT; (b) $VO(acac)_2$, TBHP, toluene, RT, 2 h; (c) *(i)* HCOOH, PPh_3, DEAD, toluene, rt, *(ii)* $NaHCO_3$, MeOH, rt; (d) *(i)* MPMCl, NaH, n-Bu_4NI, THF, rt, *(ii)* TBAF, THF, rt, *(iii)* $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78°C.

SCHEME 1

In order to improve further the optical purity we turned our attention to the enantiomeric allylic alcohols. At 60 % conversion, **9** and **10** were respectively obtained in >95 % ee; only one methyl signal was observed in the 500 MHz ^1H NMR in the presence of $Eu(hfc)_3$ (with control on the racemate). In order to avoid partial silyl migration (5 to 10 %) to the secondary hydroxyl group, the reaction residue had to be chromatographed directly on silica gel, without prior treatment with a NaOH solution.

Vanadium(IV)-catalyzed epoxidation[8] of **9** and **10** led respectively to **11** and **12** in high yield and with excellent diastereoselectivity. The two threo isomers **13** and **14** are available via Mitsunobu inversion[9]; the use of formic acid as the nucleophile led to the corresponding formates (72 %) which upon methanolysis gave the desired alcohols **13** and **14** (ca 83 %) next to ca 10 % of the silyl migrated products. This route allows the formation of the pure homochiral alcohols **11** to **14** with >97 % ee.[10] The different diastereomers can be distinguished on HPLC; the % ee of each alcohol was checked via the NMR of the MTPA esters.[11]



(a) *(i)* PhSH, K_2CO_3, DMSO, 35°C, *(ii)* mCPBA, CH_2Cl_2, rt, *(iii)* N-TMS-imidazole, CH_2Cl_2; (b) *(i)* LDA, THF, -78°C, *(ii)* Ac_2O (freshly distilled from P_2O_5), Et_3N, THF, -78°C → rt, *(iii)* 3.4 % Na (Hg), Na_2HPO_4, MeOH/THF, -20°C, *(iv)* TBAF, THF; (c) *(i)* PDC, CH_2Cl_2, *(ii)* DDQ, CH_2Cl_2-H_2O; (d) 5 % Rh/Al_2O_3, H_2, EtOAc; (e) *(i)* N-TMS-imidazole, CH_2Cl_2, *(ii)* **34**, n-BuLi, THF, -78°C, *(iii)* TBAF, THF, rt; (f) (+)-DIPT, $Ti(O\text{-}i\text{-}Pr)_4$, TBHP, 4A sieves, -20°C, 27 h; (f') (-)-DIPT, 23 h; (g) PDC, CH_2Cl_2, rt, 2.5 h; (h) *(i)* N-TMS-imidazole, CH_2Cl_2, rt, *(ii)* SeO_2, t-BuOOH, CH_2Cl_2, -20°C, 22 h and then CH_3SCH_3, 25-30°C, 5 h, *(iii)* TBAF, THF, rt; (i) *(i)* mCPBA, CH_2Cl_2, rt, 1 h, *(ii)* PDC, CH_2Cl_2, rt.

SCHEME 2

The desired aldehydes **15** and **16** were respectively obtained from **13** and **14** via protective group interchange and subsequent Swern oxidation[12] during which no epimerization was observed (the diastereomeric aldehydes are distinguishable on NMR).

Reaction of aldehyde **15** with lithiated sulfone **20** (scheme 2), followed by quenching with Ac_2O, led to the intermediate β-acetoxysulfone[5,13] which upon reductive elimination and silyl ether cleavage afforded the E-alkene **21** as the sole isomer. Oxidation to the C-8 ketone and deprotection of the 24-hydroxyl function gave **22**; subsequent catalytic hydrogenation led to **23**. Both ketones, after *in situ* formation of the 24-TMS ether, were coupled with lithiated A-ring precursor **34**;[14] deprotection finally led to respective **1** and **5**.[10] Analogues **2** and **6** were obtained in the same way starting from **16** and **20**.

The analogues **3** and **4** were obtained via the known **27**.[15] The best conditions[16] found for allylic oxidation of the TMS-ether of **27** gave epimeric **28** in 41 % yield. Asymmetric epoxidation led to respectively **29** and **31** in good diastereoselectivity. In both cases could the minor isomer be separated by HPLC. Interestingly selective oxidation of the 8-hydroxyl function is possible in these diols. The desired ketones **30** and **32** are thus available from the Lythgoe-Inhoffen diol without the need to protect hydroxyl groups in the sequence. Coupling with lithiated **34** and deprotection gave the analogues **3** and **4**.

In order to determine eventually the influence of the 24-hydroxyl function in **1**, **2**, **3** and **4**, the analogue **7** (epimeric at C-25) was prepared starting from **27**. Epoxidation and subsequent oxidation led to the ketone **33** which was transformed into **7** as described for the other analogues.

Biochemical evaluation

The affinity of the epoxy analogues to the vitamin D receptor was evaluated by their ability to compete with [^{3}H]1α,25-$(OH)_2$-D_3 (specific activity 180 Ci/mmol Amersham, Buckinghamshire, UK) for binding to the high speed supernatant from intestinal mucosa homogenates obtained from normal pigs.[17] The incubation was performed at 4°C for 20 h and phase separation was obtained by addition of dextran-coated charcoal. The affinity for 1α,25-$(OH)_2$-D_3 was $1.06 \pm 0.38 \times 10^{10}$ M^{-1} ($M \pm SD$, $n = 10$). The relative affinity of the epoxy analogues was calculated from their concentration needed to displace 50 % of [^{3}H]1α,25-$(OH)_2$-D_3 from its receptor compared with 1α,25-$(OH)_2$-D_3 (assigned a 100 % volume); all displayed a decreased receptor binding (Table 1).

Biological evaluation

The cell differentiating effect of the epoxy analogues was evaluated by the induction of superoxide production, as measured by 4-nitro-blue tetrazolium (NBT) reduction, in human promyeloid leukemia cells (HL-60 cells) as described previously.[17,18] The concentration needed for induction of black formazan deposits in 50 % of the HL-60 cells by each analogue was calculated and their relative potency compared to that of 1α,25-$(OH)_2$-D_3 (5.2 ± 2.7 nM, $n = 8.1$, assigned as 100 %) (Table 1).

The calcemic effects of the vitamin D analogues was evaluated by measuring serum calcium in vitamin D deficient chicks treated for 10 d by daily i.m. injection of increasing amounts of the analogues.[17,19] Analogues **1** to **6** were only weakly calcemic as their activity was less than 3 % of that of 1α,25-$(OH)_2$-D_3; **7**, which lacks the 24-hydroxyl group has a potency of 14 % compared to the natural hormone.

The cell differentiating effect of several epoxy analogues thus exceeded their calcemic effects more than 30-fold (e.g. **4**, **5** and **6**). Moreover the cell differentiation induced by all but one (i.e. **2**) the analogues markedly exceeded their relative receptor binding properties (Table 1) for unknown reasons.

TABLE 1. Biological activity of side chain epoxy analogues of 1α,25-$(OH)_2$-D_3 as assessed by their relative affinity for the intestinal mucosal vitamin D receptor and their capability to induce differentiation of human promyeloid leukemia cells (HL-60). The activity of all analogues are compared with that of 1α,25-$(OH)_2$-D_3 (assigned a value of 100 %).

Analogue	Receptor binding	HL-60 cell differentiation
1	5	7
2	20	12
3	1	44
4	9	33
5	8	30
6	8	30
7	27	37

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