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Preparation of an A-ring building block for the total synthesis of 1α,25-dihydroxy vitamin D_3 and structurally related congeners: lipase-catalyzed stereoselective esterification of a suitable epoxyalcohol

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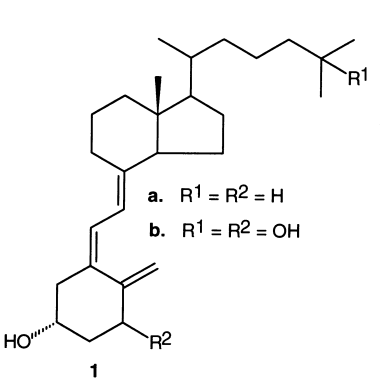
Abstract

An useful A-ring building block for the total synthesis of vitamin D_3 congeners, compound **7**, has been prepared starting from vitamin D_2 by a chemo-enzymatic approach that relies on lipase-catalyzed acylation in an organic solvent for the stereoselective step.

Vitamin D_3 **1a** is a steroid-like vitamin that controls calcium homeostasis and bone mineralization through its hormonally active form 1α,25-dihydroxycholecalciferol, calcitriol **1b**, that also shows many other important biological activities on cell differentiation and proliferation or on immunological competent cells.[1] Therefore, considerable effort has been made towards the synthesis of structurally related congeners that show interesting pharmacological applications, such as, for instance, calcipotriol **2**, that has recently been introduced into clinics for the treatment of psoriasis.[2]



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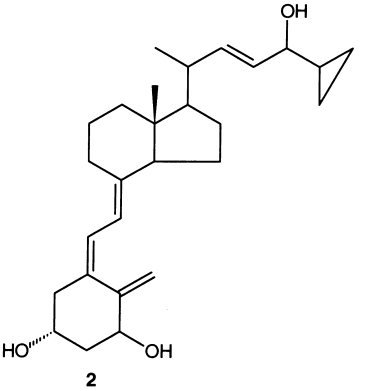

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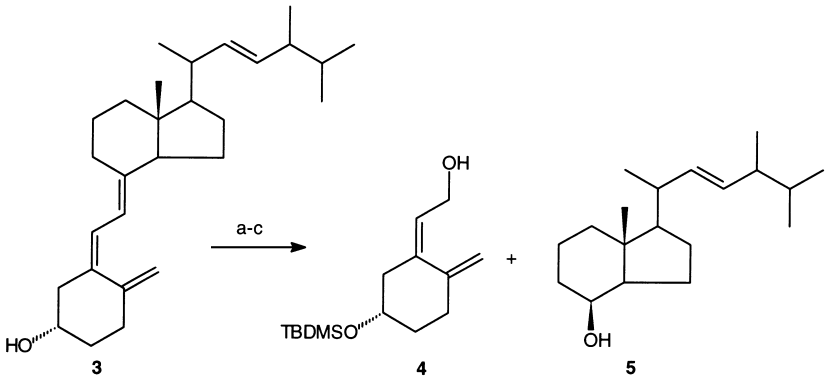
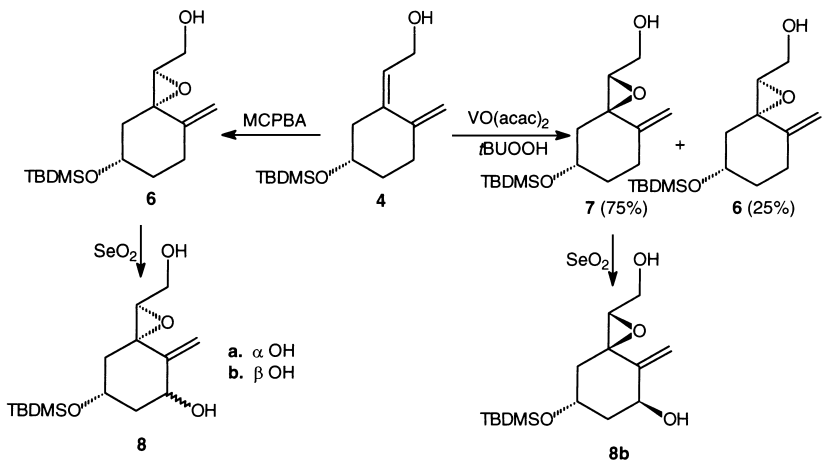
Synthetic approaches to vitamin D_3 congeners include the elaboration of structurally related steroid compounds or total synthesis starting from suitable cyclic precursors.[3] A method for the preparation of the two cyclic fragments corresponding to the A-ring and the de-AB-cholestane derivative by oxidative cleavage, described for vitamin D_3 **1a**,[4] has been applied by us to vitamin D_2 **3** that differs from vitamin D_3 **1a** in the side chain (Scheme 1).[5]



Scheme 1. Reagents: (a) $KMnO_4$, $EtOH/H_2O$; (b) TBDMSCl/imidazole/DMF; (c) $Pb(OAc)_4$, C_6H_6; Red-Al, $C_6H_5CH_3$

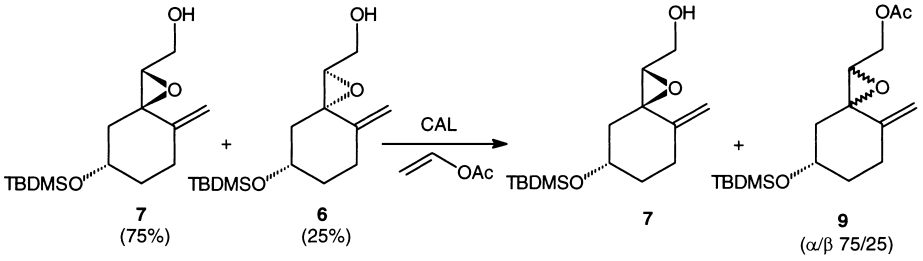
Both cyclic intermediates **4** and **5** are valuable cyclic building blocks for the total synthesis of vitamin D_3 analogues and have been isolated in 44 and 60% yield, respectively.[6] The cleavage of the double bond of compound **5** and further elaboration would permit the construction of modified side chains,[7] whereas for the introduction of the C-1 hydroxy group it has been reported that the epoxidation of the 5,6-double bond in compound **4** is needed in order to protect the 4-position by undesired oxidation (Scheme 2).[8] The stereochemical control of the C-1 hydroxylation depends on the stereochemistry of the 5,6-epoxide, since the oxidation of the stereochemically pure α-epoxide **6** leads mainly to the wrong C-1 α-hydroxylation product **8a**, whereas only from the β-epoxyalcohol **7**, could the epoxytriol **8b** be obtained with the required configuration of the



Scheme 2.

C-1 and C-3 hydroxy groups.[8] However, depending on the epoxidation procedure, the diastereomerically pure α-epoxide **6** (*m*-chloroperbenzoic acid) or prevalent β-epoxyalcohol **7** (vanadyl acetylacetonate/*tert*-butylhydroperoxide, **7**:**6**, 6.3:1 ratio) can be prepared.[8] In our hands, the vanadyl acetylacetonate/*tert*-butylhydroperoxide epoxidation of compound **4** afforded a 3:1 (β:α) diastereomeric ratio of the epoxides[9] and only a careful purification by column chromatography afforded the required epoxyalcohol **7**.[10]

We therefore decided to investigate the stereoselectivity of a lipase-catalyzed acetylation of the above mixture.[11] A few lipases did not work successfully or were not selective for preparative purposes.[12] Only *Candida antarctica* lipase[13] catalyzed the required acetylation and, using vinyl acetate as solvent and reagent, in 0.75 h from the above α/β-epoxides **6** and **7** mixture the pure unreacted β-epoxyalcohol **7** and a 75:25 mixture of α- and β-epoxyacetates **9** was obtained (Scheme 3).[14]



Scheme 3.

We have also verified the enzymatic preference for the α-stereoisomer in the above transesterification, preparing the α-epoxyalcohol **6** that was quantitatively converted to the corresponding acetate in 0.75 h. In conclusion, taking advantage of the stereopreference of the CAL-catalyzed acetylation of a cyclic epoxyalcohol, the synthesis of diastereomerically pure β-epoxyalcohol **7** can be presented as an advantageous method for the preparation of a crucial synthon for the total synthesis of vitamin D_3.[15]

Acknowledgements

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5. The experimental procedure was essentially as described in Ref. 4. One main modification concerns the permanganate oxidation (step a). Filtration on Celite of the final aqueous ethanol solution should be preferred to the described use of silica gel, which made the recovery more tedious and troublesome. Silylation (step b) and

following steps ($Pb(OAc)_4$ and Red-Al) were performed as described and compounds **4** and **5** were isolated in 44 and 60%, respectively, from vitamin D_2 **3**.

6. ^1H NMR (500 MHz, Bruker 500 AM) spectra in $CDCl_3$ are in agreement with the structures of the intermediates in the oxidation procedure. Vitamin D_2 5,6-diol (step a): δ 0.77–0.84 (overlapped d+s, 9H, CH_3-18 and CH_3-26,27), 0.89 (d, 3H, CH_3-21), 0.96 (d, 3H, CH_3-28), 3.72–3.84 (m, 1H, CH-3), 4.84–4.90 (overlapped d+s, 2H, CH-7 and CH-19), 4.98 (s, 1H, CH-19), 5.08–5.24 (m, 2H, CH-22,23), 5.54 (s, 1H, CH-6). 3β-Silyl ether (step b): δ 0.00 (s, 6H, CH_3 Si), 0.78–0.82 (overlapped d+s, 9H, CH_3-18 and CH_3-26,27), 0.88–0.92 (overlapped d+s, 12H, CH_3-21 and $(CH_3)_3C$), 0.96 (d, 3H, CH_3-28), 3.66–3.76 (m, 1H, CH-3), 4.86 (s, 1H, CH-19), 4.90 (d, 1H, CH-7), 4.96 (s, 1H, CH-19), 5.08–5.24 (m, 2H, CH-22,23), 5.50 (d, 1H, CH-6). Compound **4**: δ 0.00 (s, 6H, CH_3Si), 0.90 (s, 9H, $(CH_3)_3C$), 1,45–1.61 (m, 1H, CH-2), 1.61–1.79 (m, 1H, OH), 1.79–1.90 (m, 1H, CH-2), 1.99–2.08 (m, 1H, CH-1), 2.12–2.20 (m, 1H, CH-4), 2.32–2.44 (m, 2H, CH-1,4), 3.78–3.84 (m, 1H, CH-3), 4.11–4.25 (m, 2H, CH_2-7), 4.60 (s, 1H, CH-19), 4.92 (s, 1H, CH-19), 5.41 (t, 1H, CH-6).
7. For instance, ozonolysis/reduction of double bond in compound **5** affords the so-called Inhoffen-Lythgoe diol (75%), that has been used as intermediate for the synthesis of several vitamin D_3 congeners. Among many available examples, see: (a) Sardina, F. J.; Mouriño, A.; Castedo, L. *J. Org. Chem.* **1986**, *51*, 1264. (b) Hatakeyama, S.; Ikeda, T.; Irie, H.; Izumi, C.; Mori, H.; Uenoyama, K.; Yamada, H.; Nishizawa, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1959.
8. Kiegel, J.; Wovkulich, P. M.; Uskokovic, M. R. *Tetrahedron Lett.* **1991**, *32*, 6057.
9. NMR spectra of epoxides **6** and **7** have been reported in detail in Ref. 8; typically, our sample from $VO(acac)_2$/*t*-BuOOH epoxidation showed the most significant resonances at 3.10 (t, 0.24H, CH, 6α-isomer) and 3.16 (dd, 0.76H, CH 6β-isomer) ppm corresponding to a 3:1 ratio of β:α-epimers.
10. Purification of the epoxyalcohol mixture was carried out by column chromatography (neutral aluminum oxide III: crude mixture, 10:1). Elution with hexane:ethyl acetate (8:2) afforded pure β-epoxyalcohol **7** (30% yields).
11. For a recent review on the preparation of chiral synthons by enzymatic acylation and esterification reactions, see: Santaniello, E.; Reza-Elahi, S.; Ferraboschi, P. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; M. Dekker: New York, 2000; pp. 415–460.
12. Negative results were obtained with PCL (*Pseudomonas cepacia* lipase) by transesterification with vinyl acetate in $CHCl_3$ or by alcoholysis of the acetates of the β/α-epoxide mixture with methanol in *tert*-butyldimethylether. *Candida cylindracea* lipase (CCL) transesterification with vinyl acetate in $CHCl_3$ is not selective, since the β/α-acetate mixture **9** is in the same ratio as the starting material.
13. *Candida antarctica* lipase (CAL B, Novozym 435) was a gift from Novo Nordisk (Italy).
14. To a solution of α/β epoxide mixture (0.154 g, 0.54 mmol) in vinylacetate (19 ml) CAL (0.310 g) was added. The reaction mixture was kept at 30°C (0.75h) monitoring the reaction progress by GLC (HP-5 WB oven temperature 200°C). The retention times T_R (min) were as follows: α-epoxide **6**: 7.99; β-epoxide **7**: 7.68; α-epoxide acetate: 9.85; β-epoxide acetate: 10.58. Silica gel column chromatography (10:1) afforded epoxyacetates (α:β, 75:25, 30 mg) by elution with hexane:ethylacetate 9:1 and pure β-epoxide **7** (75 mg) by elution with hexane:ethylacetate 7:3. NMR resonances were as follows: β-epoxide **7**: 3.16 ppm (dd, 1H, CH-6); α/β-epoxide acetate mixture **9**: 3.08 (t, 0.76H, CH, 6α-isomer) and 3.16 (dd, 0.24H, CH, 6β-isomer). For a comparison, the NMR resonances of the acetate mixture prepared (Py/Ac_2O) from the α/β-epoxide **6**:**7** (1:3 ratio): 3.08 (t, 0.25H, CH, 6α-isomer) and 3.16 (dd, 0.75H, CH, 6β-isomer) ppm.
15. For an extensive review on the application of biocatalysis to steroids and, in particular, to the preparation of vitamin D synthons, see: Ferrero, M.; Gotor, V. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; M. Dekker: New York, 2000; pp. 579–631.