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# The first synthesis and biological testing of the enantiomer of 1α,25-dihydroxyvitamin $D_3$†

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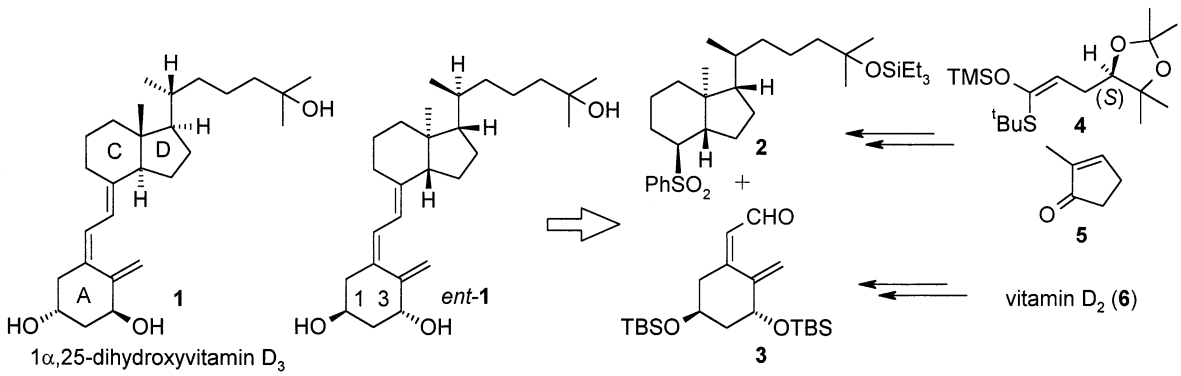
**Abstract**—The 1α,25-dihydroxyvitamin $D_3$ enantiomer was synthesized and examined in biological tests. The ring A precursor was prepared from vitamin $D_2$ employing the Mitsunobu reaction for inversion of the configuration at C-3 and $SeO_2$ hydroxylation at C-1. The CD rings-side chain portion was synthesized from an optically active hexanoic acid derivative using diastereoselective tandem Mukaiyama–Michael addition and vinylsulfone reduction as the key steps. The ring A and CD rings building blocks were combined using the Julia alkenylation reaction. 1α,25-Dihydroxyvitamin $D_3$ enantiomer shows no significant affinity to the vitamin D receptor. 

A great deal of attention has been given to the synthesis of 1α,25-dihydroxyvitamin $D_3$, **1** (Scheme 1), which acts as a hormone controlling calcium homeostasis and shows a broad spectrum of biological activity.[1] There is a continuous search for analogues of **1** devoid of calcemic activity but retaining cell differentiating and anti-proliferation activities, suitable for treatment of cancer and certain skin disorders in humans.[2] Equally needed are analogues which would selectively induce intestine or bone calcium absorption thus providing a means to mediate calcium transport disorders[3] and analogues suppressing immunological responses.[4] In pursuit of new types of biologically active compounds it appeared of interest to synthesize and examine the enantiomer of 1α,25-dihydroxyvitamin $D_3$ (*ent*-**1**) and some related stereo-isomers with profound changes in molecular geometry. Unnatural enantiomers of various natural products have been synthesized.[5] However, reports on enantiomers of hormonally active compounds are scarce. *ent*-Equilenin was synthesized and showed to have rather low estrogenic activity.[6] The prostaglandin $E_1$ enantiomer exhibited small activity in certain tests.[7] However,




**Scheme 1.** The target compound *ent*-**1** and the general synthetic plan.

*Keywords*: enantioselective synthesis; Mukaiyama–Michael reaction; Mitsunobu reaction; vitamin D.

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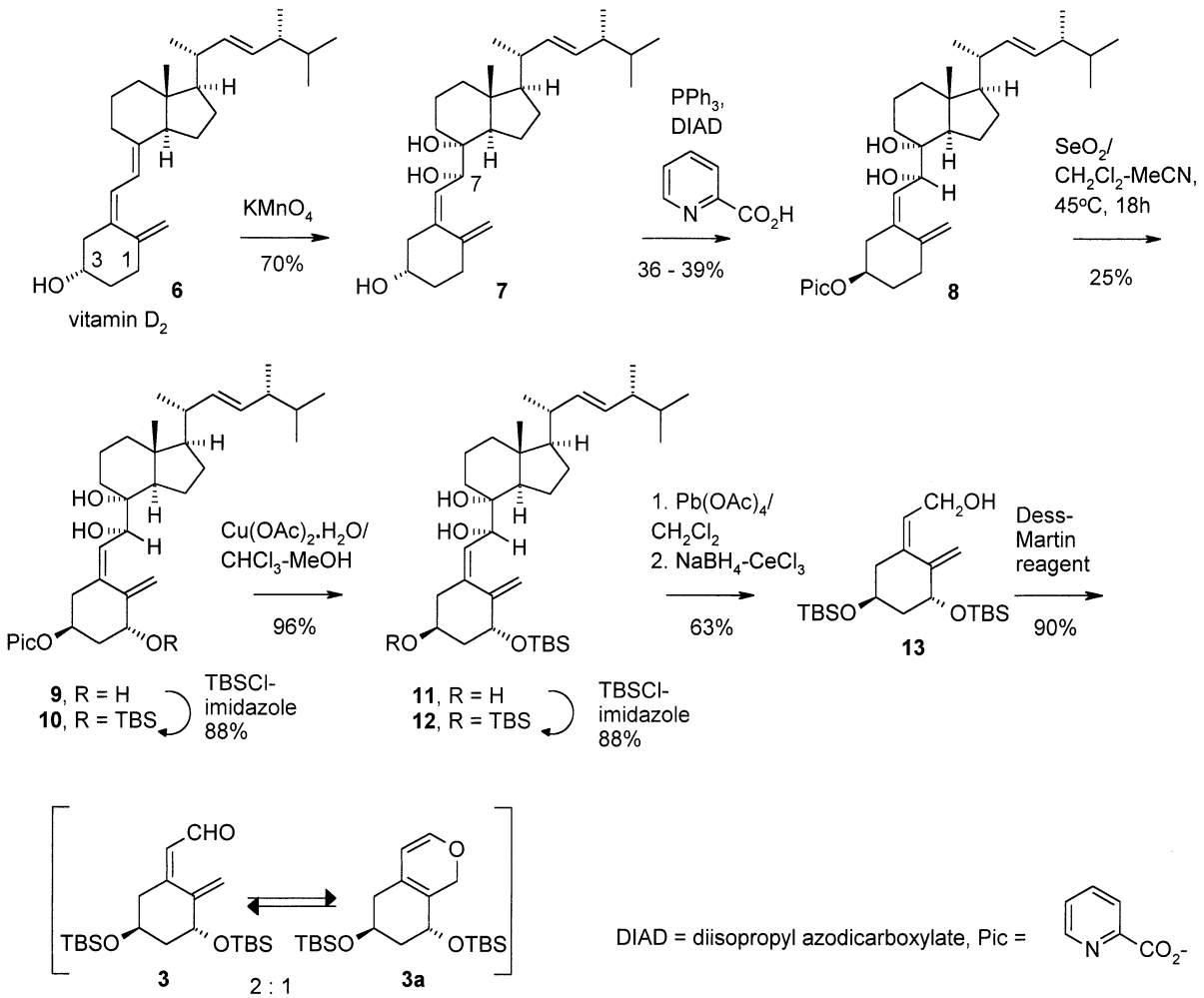
prostaglandin analogues with inverted configuration at all but one of the stereogenic centers show high and specific activity.[8] In principle, enantiomers may also display activities which are not inherent in the parent compounds.[9]

In this communication we report the synthesis and the results of some biological tests of *ent*-1α,25-dihydroxyvitamin $D_3$ (*ent*-**1**, Scheme 1). The general plan of the synthesis of compound *ent*-**1** includes: (1) coupling of the building blocks **2** and **3** using the Julia alkenylation reaction,[10] (2) preparation of the ring A building block **3** from inexpensive vitamin $D_2$, **6**, (Scheme 2) using the Mitsunobu reaction for the inversion of configuration at C-3 and allylic oxidation at C-1 as the key reactions, and (3) a new enantioselective synthesis of the CD rings-side chain building block **2** based upon 1,3-asymmetric induction[11] in the conjugate addition reaction of optically active ketene acetal **4** and unsaturated ketone **5**.

Triol **7** (Scheme 2), which is readily accessible by $KMnO_4$ dihydroxylation of vitamin $D_2$ **6**, has served as a convenient starting material for synthesis of numerous vitamin D congeners.[12,13] Since in the silylation reaction of **7**, the hydroxyl group at C-3 proved more reactive than the remaining hydroxyl groups,[12] it was challenging to submit this acid labile triol to a regioselective configuration inversion reaction. Our initial experiments with the Mitsunobu reaction[14] using *p*-nitrobenzoic acid as the nucleophile provided dehydration products only. However, treatment of **7** with picolinic acid, $Ph_3P$ and DIAD according to the procedure of Sammakia and Jacobs[15] afforded picolate **8** in 36–39% yield, which was readily purified by column chromatography.

Compound **8** was subjected to $SeO_2$ oxidation[16] to give the 1-hydroxy derivative **9** in a 20% yield along with unreacted starting material. Although yields of the above described transformations were rather low, the intermediate **9** was prepared form vitamin $D_2$ in just three steps. After protection of the 1-hydroxy group with the TBS group, the picolate ester group in **10** was hydrolyzed in a mixture of chloroform and methanol using $CuOAc_2 \cdot H_2O$ as the catalyst.[15] Alcohol **11** was transformed into the derivative **12** in the usual way. Efficient transformation of **9** into **11** suggests potential applications of the picolate ester moiety as a protective group.

Oxidative cleavage of the vicinal diol **12** followed by reduction of the crude product[17] and chromatography

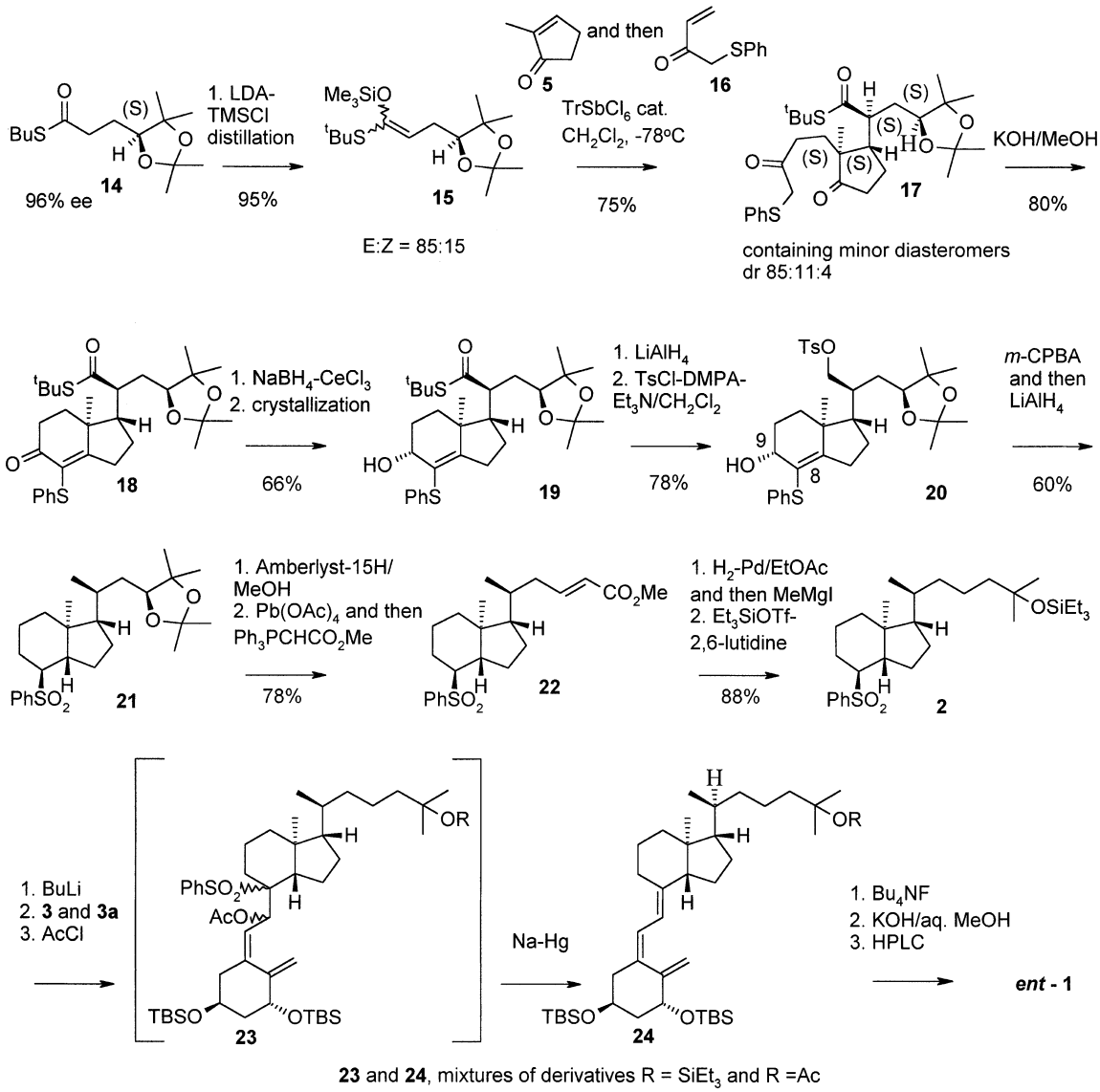



**Scheme 2.** Synthesis of the ring A building block, **3**.

afforded alcohol **13**. Dess–Martin oxidation[18] of **13** gave aldehyde **3** along with its dihydropyrone tautomer **3a** in a ratio of 2:1, respectively (by $^1$H NMR). This mixture was used for coupling with the CD rings-side chain building block.

A new enantioselective synthesis of the building block **2** was developed based upon the asymmetric Mukaiyama–Michael reaction.[19] Optically active thioester‡ **14** (96% ee, Scheme 3), was treated with LDA and then with TMSCl to give ketene acetals **15**. The latter were allowed to react with enone **5** in the presence of 5 mol% of $TrSbCl_6$. When the majority of the reagents were consumed, the second Michael acceptor **16** was added.[20] The reaction product consisted of three diasteromers in a ratio of 85:11:4 (by HPLC). All our attempts to separate the major component, to which structure **17** was later assigned, failed. The mixture was subjected to cyclization to give **18** along with minor diastereomers. After $NaBH_4$–$CeCl_3$ reduction[21] of these α,β-unsaturated ketones a crystalline material was obtained, which was recrystallized to afford allylic alcohol **19** in a 53% yield from **17**. The structure of **19** was determined by X-ray analysis.[11]

Compound **19** was transformed into the key building block **2** using routine operations indicated in Scheme 3. However, one or two brief comments are in order. DIBAL-H which is the reagent of choice for reduction of the thioester group could not be applied to **19** due to susceptibility of the dioxolane ring to reductive opening. Accordingly, **19** was reduced to the corresponding diol using $LiAlH_4$ in THF at reflux. Vinylsulfide **20** was oxidized with *m*-CPBA to the corresponding vinylsulfone and this derivative was then treated with $LiAlH_4$ in THF without purification. Reduction of the vinylsulfone moiety, the adjacent C–O bond[22] and the tosyloxy group at C-21 occurred simultaneously to afford the




**Scheme 3.** Synthesis of CD rings—side chain building block **2** and coupling of building blocks **2** and **3** to afford *ent*-**1**.

‡ This compound was prepared[11] from methyl 5-methyl-hept-4-enoate using the Sharpless asymmetric dihydroxylation[27] and the Weinreb ester group interconversion.[28]

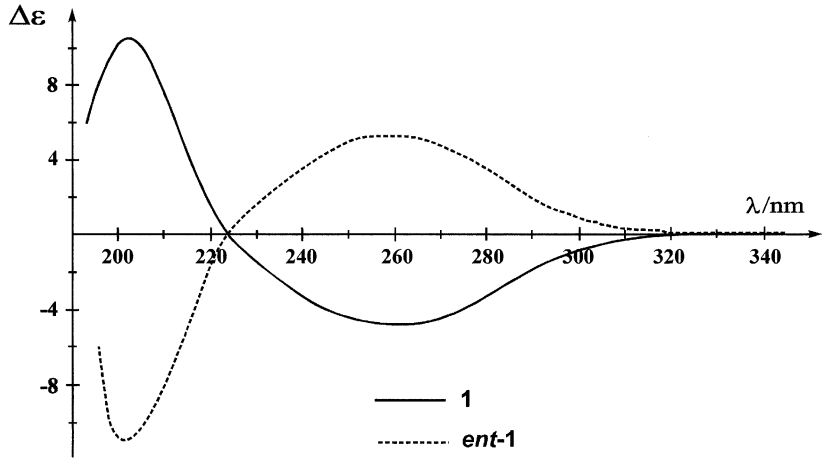



**Figure 1.** CD spectra of 1α,25-dihydroxyvitamin $D_3$ (**1**) and its enentiomer (*ent*-**1**).

*trans*-hydrindane derivative **21**. Ultimately, the key building block **2** was obtained from thioester **14** in eleven steps in a 12% overall yield.

In contrast to Kocienski's[23] procedure for executing the Julia alkylation, the coupling of sulfone **2** and aldehyde **3** was accomplished using an excess of the sulfone. Treatment of **2**, in THF, with BuLi (1.2 mol equivalent), at −20°C, followed with a mixture of **3** and **3a** (0.66 mol equivalents), at−78°C, afforded the adduct which was quenched with AcCl. The crude product **23** was then reduced with 6% sodium amalgam in methanol in the presence[24] of $Na_2HPO_4$. A mixture of silyloxy and acetoxy trienes **24** was obtained in a 25% overall yield from **3**. This mixture was allowed to react with TBAF·$3H_2O$ in THF and then with methanolic KOH to give the respective trihydroxy trienes as a mixture of geometric isomers[25] in a ratio of 94:5:1. The major isomer, *ent*-**1**, was separated by preparative HPLC. Its retention time on an analytical HPLC column was the same as that of natural 1α,25-dihydroxyvitamin $D_3$ and the $^1H$ NMR spectrum was identical to that of the natural compound.

A confirmation of the enantiomeric relation of the synthetic material with **1** was obtained from the CD spectra of both compounds. As shown in Fig. 1, 1α,25-dihydroxyvitamin $D_3$ **1** and *ent*-**1** show a Cotton effect of the same magnitude but of different sign.

Compound *ent*-**1** shows no significant affinity to the vitamin D receptor. It was inactive in tests for differentiation of promyelocytic leukemia HL-60 cells.[26]

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