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Synthesis and Biological Activity of the 1α,25-Dihydroxyvitamin D_3 Diastereomer with Unnatural Configuration at the Rings C/D Side-Chain Moiety

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Abstract—1α,25-Dihydroxyvitamin D_3 diastereomer, differing from the parent compound in configuration at four asymmetric carbon atoms in the rings C/D and side chain (C13, C14, C17 and C20), was synthesized and shown to have a significant affinity for the vitamin D receptor.

1α,25-Dihydroxyvitamin D_3 (**1**, Scheme 1), the major active metabolite of vitamin D_3, exerts hormonal control over important physiological processes, including calcium and phosphorus metabolism, cellular differentiation and immune reactions.[1] A great deal of attention has been devoted to synthesis of analogues of this natural compound that would discriminate certain types of its activity.[2] One of the interesting lines of the structure–activity studies is concerned with stereoisomers of compound **1**. Epimers of **1** differing in configuration at one of the stereogenic centres, Cl, C3, C14 or C20, have been studied.[3] These studies have shown, among other important results, that the 1α,25-dihydroxyvitamin D_3 epimer **2** differing in configuration at C20 and, in consequence, in the side-chain orientation, exhibits high affinity to the vitamin D receptor and enhanced cell-differentiation activity.[3d] The activity of the 20-*iso*-epimer posed a question of the importance of the natural configuration at all remaining stereogenic centres in the northern, 'terpenoid' portion of 1α,25-dihydroxy vitamin D_3 for its recognition by the receptor.

In order to evaluate the effect of configurational changes on activity of vitamin D analogues we conducted synthesis and biological evaluation of compound **3** which combines 'natural' ring A and C/D rings side-chain portion with inverted configuration at all asymmetric carbon atoms.[4] Our synthesis of the C/D rings side-chain building block **14** of the target compound **3** is illustrated in Scheme 2. Asymmetric Robinson annulation[5] of 2-methylcyclopentan-1,3-dione **5** with (phenylsulfanyl)methyl vinyl ketone **4** using (*R*)(+)-phenylalanine as the catalyst was carried out according to the procedure of Hagiwara and Uda,[6] involving a gradual increase of the reaction temperature, to afford dione **6** with 89% ee in 71% yield. Dione **6** was reduced[7] into *trans*-hydrindane derivative **7** and the latter was transformed in the usual way into ester **8**. Alkylation[8] of **8** by means of LDA (2 equiv) and bromide **9** (prepared from α-acetyl-γ-butyrlactone via 1-bromopent-4-one[9] in 30% overall yield) gave compound **10** contaminated with a byproduct which could not be separated by chromatography. Under optimized conditions with the use of 1.5 equivalent of LDA the alkylation products were obtained with 76% yield in an estimated ratio of 95:5 (by ^{1}H NMR). Reduction of the alkylation product with DIBAL afforded a mixture of alcohols which were separated by chromatography. The major product, isolated in 85% yield, was identified as **12**. To the side-product structure, **13** was assigned on the grounds of its spectral and analytical properties. Formation of **13** indicated that under conditions of alkylation of **8** along with ester enolate, some α-sulfonyl anion was generated which resulted in the side product **11**. Further reduction of alcohol **12** provided the required building block **14**.

A common ring A precursor **15** was oxidized with Dess–Martin reagent[10] to give a mixture of aldehyde **16** and its tautomer with pyrane ring[11] **17** in a ratio of ca. 2:1,

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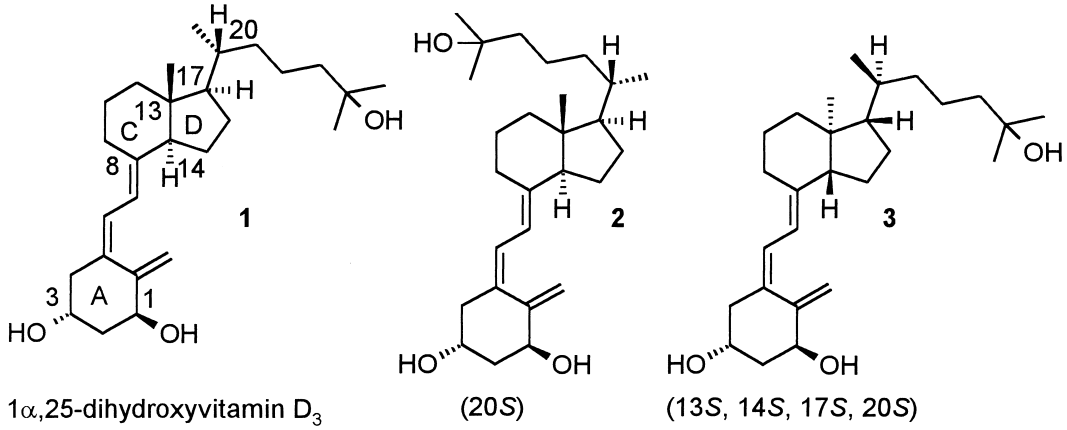
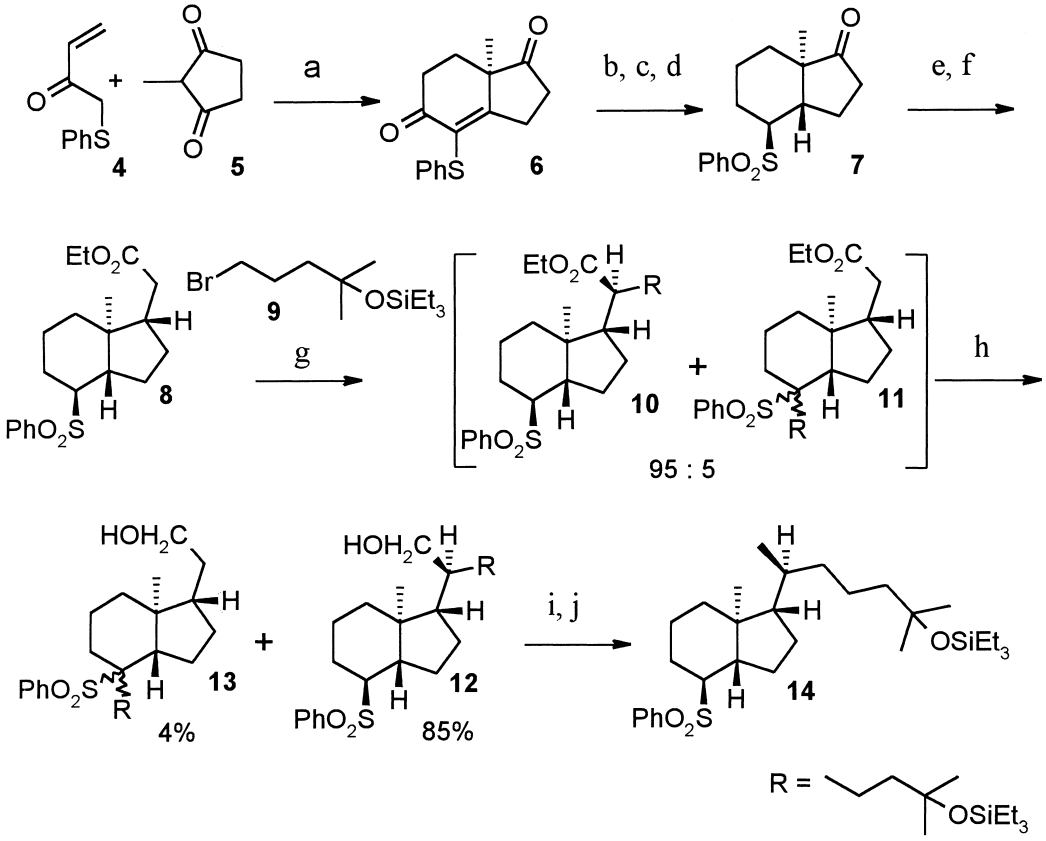
by ^{1}H NMR. The mixture was purified by filtration through a silica gel column, dried and used immediately for the next reaction (Scheme 3).

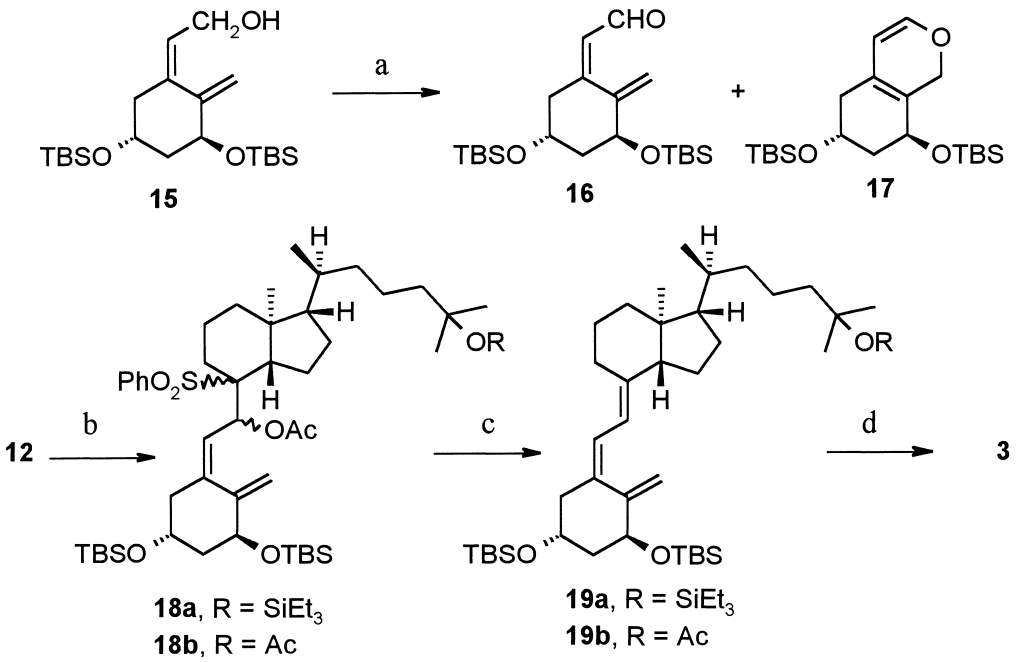
For coupling of the building blocks a modified Kocienski procedure[12] for executing the Julia olefination reaction[13] in vitamin D synthesis was used. It should be noted that the Julia–Kocienski method has never been used for the preparation of 1α,25-dihydroxyvitamin D_3 derivatives. Sulfone **14** (1 equiv) was treated first with butyllithium to generate α-sulfonyl anion and then, at −78 °C, with a mixture of aldehyde and its tautomer **16**–**17** (0.5 equiv). After 1 h, an excess of freshly distilled acetyl chloride was added and the mixture was stirred at room temperature for 15 h. A mixture of acetoxy sulfones **18a** and **18b** was obtained. The mixture was submitted to reduction with 6% sodium amalgam without purification. The resulting product was filtered through a silica gel column to give a mixture of silyloxy and acetoxy trienes **19a** and **19b** in 45% yield



Scheme 1. Structure of 1α,25-dihydroxyvitamin D_3 (**1**) and its analogues differing in configuration of stereogenic centres in C/D rings side-chain portion.



Scheme 2. Synthesis of the C/D rings side-chain building block: (a) (*R*)-(+)-phenylalanine, DMF/Et_3N/D-CSA, 25→55 °C, 71%, 89% ee; (b) *m*-CPBA, CH_2Cl_2; (c) $LiAlH_4$, THF, reflux; (d) Jones reagent, acetone, 77% overall from **6**; (e) NaH, $(EtO)_2P(O)CH_2CO_2Et$, DMF/HMPA, −20→25 °C, 80%; (f) H_2/Pd, EtOH, 97%; (g) LDA, THF/HMPA, then **9** −78→25 °C, 76%; (h) DIBAL-H, CH_2Cl_2, −20→25 °C, 85% of **12** and 4% of **13**; (i) MsCl, CH_2Cl_2, −20 °C; (j) $LiEt_3BH$, THF, 84% overall from **12**.



Scheme 3. Preparation of the ring A building block and the coupling reaction. (a) Dess–Martin reagent, CH_2Cl_2, 90%; (b) *n*-BuLi, THF, then **16** and **17** and after 1 h AcCl, −78→25 °C; (c) Na–Hg, MeOH/Na_2HPO_4, −20 °C, 45% overall from **12**; (d) TBAF/4 Å MS, THF, 80%.

from **15** (ratio 60:40, respectively, by NMR). These experiments show that under the conditions of olefination simultaneously with acetylation of the very sterically hindered hydroxy group at C7, partial exchange of the protective triethylsilyl group for the acyl group at the C25 has also occurred. The mixture **19** was next treated with TBAF·H_2O and 4 Å molecular sieves in THF and the products were separated by flash chromatography to give triol **3** (27% yield from **16**–**17**) and its C25-acetate (8.4% yield). HPLC analysis of thus prepared vitamin D analogue **3** (Nucleosil, EtOAc–CH_2Cl_2, 1:1) indicated its contamination with small amounts of double bond isomers.[14] Finally, a sample of **3** was purified by HPLC and its structure was confirmed by spectroscopic measurements (MS, UV, ^{1}H NMR, CD).

In the biological screening[15] the vitamin D analogue **3** displayed significant binding affinity to the vitamin D receptor (11% compared to 1α,25-dihydroxyvitamin D_3). In cellular systems derivative **3** exerted weak agonistic activity. Thus, the differentiation of HL 60 cells to monocytes is induced at dosages above 100 nM and the inhibition of the proliferation of human peripheral blood mononuclear cells (PBMC) occurs in the same dose range. Upon administration to mice, the compound is much better tolerated than 1α,25-dihydroxyvitamin D_3. Whereas 1α,25-dihydroxyvitamin D_3 induces hypercalcemia at dosages as low as 0.1 μg/kg, analogue **3** does not affect serum calcium levels at 10 μg/kg.

In conclusion, (1*S*,14*S*,17*S*,20*S*)-1α,25-dihydroxyvitamin D_3 (**3**) differing from the parent natural product in configuration at four asymmetric carbon atoms was synthesized and shown to have significant binding affinity for the vitamin D receptor.

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