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Calcitriol derivatives with two different side chains at C-20 24-Hydroxy derivatives as metabolic products and molecular probes for VDR exploration☆

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Abstract

We previously synthesized calcitriol derivatives with two identical side chains emanating at C-20, also known as gemini. In view of the evidence identifying C-24 hydroxylation as the first step in the in the metabolic cascade of calcitriol and gemini, stereochemical differentiation between the possible epimeric 20*R*- and 20*S* side-chain hydroxylated gemini became of interest. We now report the stereoselective synthesis of these compounds. Of these, 1,24(*R*),25-trihydroxy-21-(3-hydroxy-3-methyl-butyl)-20(*R*)-19-nor-cholecalciferol was identified as the main metabolic product of 19-nor-gemini. In general, higher doses of the 24-hydroxylated gemini compounds were required to increase blood calcium levels in mice and to suppress INF-γ release in MLR.

Keywords: Gemini; 24(*R*)-hydroxylase; Metabolism; VDR; 24(*R*)-hydroxy gemini; Side-chain de-symmetrization

1. Introduction

The calcitriol derivatives **1a** and **1b**, featuring two identical side chains emanating at C-20, exert the full spectrum of calcitriol activities, such as binding to the VDR, suppression of increased parathyroid hormone levels in nephrectomized rats and INF-γ release in MLR cells, stimulation of HL-60 leukemia cell-differentiation and inhibition of solid-tumor cell-proliferation [1–3]. The major metabolic conversion of calcitriol ($1\alpha,25(OH)_2D_3$) is initiated by the 24*R*-hydroxylase leading to $1\alpha,24(R),25(OH)_3D_3$, followed by oxidation to the 24-oxo species, 23-hydroxylation and oxidative chain cleavage [4–7]. A similar metabolic fate is expected for **1a** and **1b** and it has indeed been confirmed that **1a** is also subject to 24-hydroxylation but the stereochemical outcome of this process remained unresolved. To clarify the stereochemical implication of this metabolic change by comparison with authentic samples, the two possible epimeric pairs **2a**, **3a** and **2b**, **3b** (Fig. 1) were required whose syntheses are summarized herein.

2. Materials and methods [8]

2.1. Synthetic concepts

A successful de-symmetrization of the two 4-hydroxy-4-methylpentyl groups, also referred to as the gemini side chains, is the key to the synthesis of the pairs **2a**, **3a** and **2b**, **3b**. This task was achieved by the addition of borane to the diastereotopic face of the alkene moiety in **5** [9,10]. Completion of the hydroboration protocol provided the pair of epmeric dihydroxyalkanes **6** and **7** that was easily separated by chromatography (Scheme 1). The absolute configuration of these alkenols is based on a crystallographic analysis [10]. A sequence of steps converted these diols to ketones of the type **9a** and **9b**. For the synthesis of the two epimeric pairs **2a**, **3a** and **2b**, **3b** we chose the convergent and established Wittig–Horner reaction using the Lythgoe phosphine oxide coupling protocol [11,12] (Scheme 1) in which each of the two elaborated ketones **9a** and **9b** was linked to the functionalized (2-cyclohexylethenyl)diphenylphosphine oxides **8a** and **8b**. A single step removed all five silyl protecting groups from each condensation product and led to the target compounds **2a**, **3a** and **2b**, **3b** (Scheme 2).

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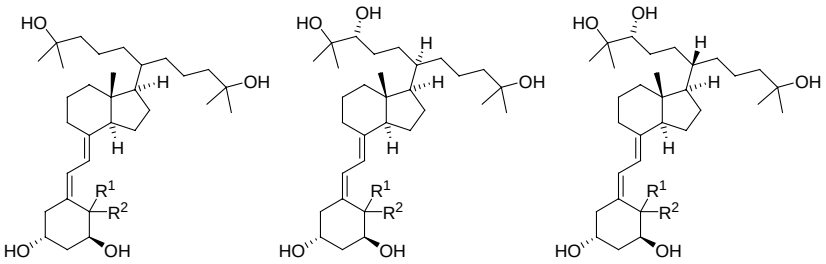
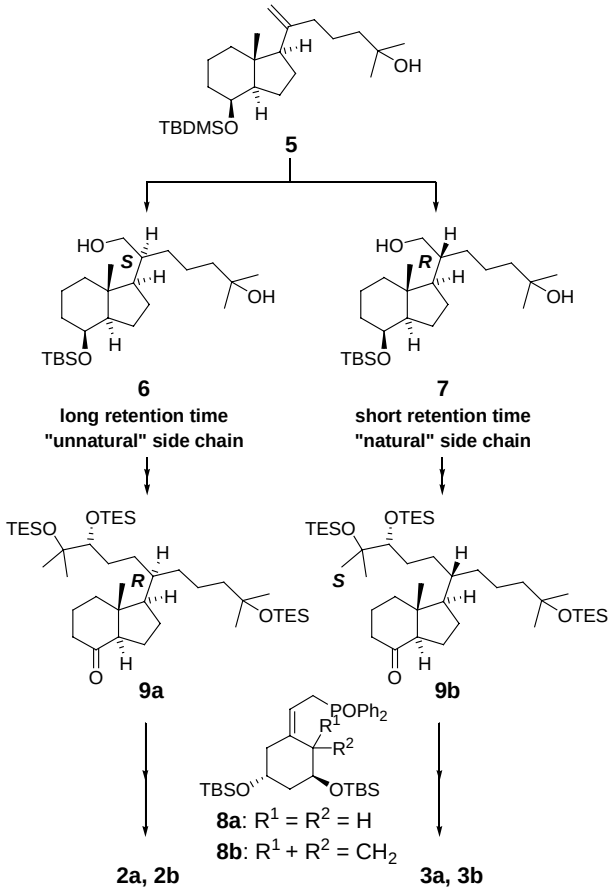


Fig. 1.

2.2. Syntheses of 1,24(R),25-trihydroxy-21-(3-hydroxy-3-methyl-butyl)-20(S)-cholecalciferol (3b)

Compounds **2a**, **3a**, and **2b**, **3b** were prepared by similar synthetic pathways. For illustrative purposes we describe only the synthesis of **3b**.

The iodoalcohol **10**, derived from diol **7**, was treated with sodium benzenesulfinate to give **11a** that was readily converted to the silyl ether **11b**. A following condensation with the 2-oxiranyl-2-propanol, prepared in situ from **12** [13], led to diol **13** as an epimeric mixture which was subjected to reductive de-sulfonylation and de-silylation to furnish the tetraol **14**. The vicinal diol was protected as the oxolane **15**, and then oxidized with pyridinium dichromate to ketone **16**. A mild treatment with acid converted **16** to the ketotriol **17** that was further converted to **9b** with chlorotriethylsilane in *N*,*N*- dimethylformamide and imidazole. Subsequent condensation with **8b** gave **18**. One deprotection step with tetrabutylammonium fluoride liberated all five protected hydroxyl groups and, after chromatographic purification, compound **3b** was obtained.



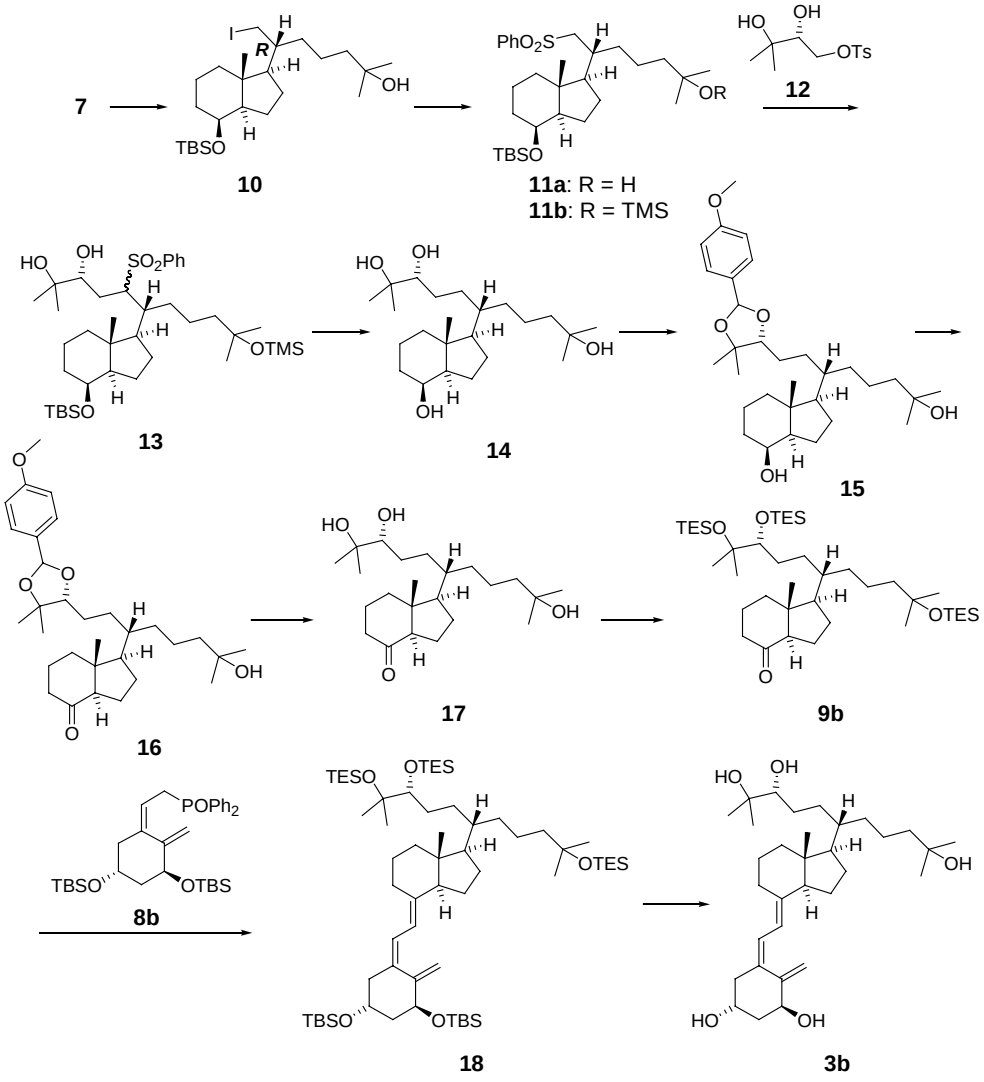
Scheme 1. De-symmetrization of the gemini side chains and synthetic strategy.

3. Results and discussion

It has been shown that metabolism of **1a** in kidney and bone cells produces a 24-hydroxylated species. It stands to reason that this metabolic hydroxylation is also effected by the ubiquitous 24(*R*)-hydroxylase and that it is side-chain specific. Considering the heterotopic environment of the two identical side chains in **1a** and **1b**, mono-24-hydroxylation can potentially occur either on the pro-*R* or pro-*S* side-chains. Hydroxylation of the pro-*R* chain, as shown in **2a** and **2b**, is tantamount to hydroxylation of the chain with the natural configuration. At first glance, one could therefore speculate that the pro-*R* chain should be the preferred substrate thus identifying **2a** and **2b** as the more likely metabolic target. On the other hand, the observation that 20-epi-calcitriol is hydroxylated at a higher rate than its natural 20-*R* counterpart, might suggest preferential hydroxylation at the unnatural side chain thus rendering **3a** and **3b** as the more likely metabolic products. The availability of both epimers by synthesis, as described herein, was instrumental in the identification of the natural pro-*R* side-chain as the preferred metabolic substrate in the case **1a**, and hence of **2a** as its main metabolic product.

As shown in Table 1, neither of the 20*R*,24*R*- nor the 20*S*,24*R*-pentaols **2a**, **2b** and **3b** increased blood calcium levels in mice at doses up to 30 μg/kg. No increase in calcium levels were observed at a ten times higher dose of **3a**. Concomitant with this improved tolerance, a generally higher dose was required to suppress INF-γ release in MLR.

Synthesis of the 20(*S*),24(*R*) epimer **3b**



Scheme 2. Synthesis of the 20(*S*),24(*R*) epimer **3b**.

Table 1
Minimum tolerated dose in mice and IC_{50} for INF-γ release in MLR

Compound	MTD (μg/kg)	MLR IFN-γ IC50 (pM)
1a	3	44
2a	30	781
3a	>300	3856
1b	0.1	4
2b	30	66
3b	30	549

The 20*R*-epimer **2b**, however, was shown to be not only some ten times more active than 20*S*-analog **3b** in the suppression of INF-γ release in MLR, but also to be comparable to **1a**. In view of the ameliorated hypercalcemic effects, chemical 24-hydroxylation of the pro-*R*-side chain in the gemini series may be a useful tool for enhancement of drug performance.

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