Synthesis and metabolic studies of 1α,2α,25-, 1α,4α,25- and 1α,4β,25-trihydroxyvitamin D3

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Review

Synthesis and metabolic studies of 1α,2α,25-, 1α,4α,25- and 1α,4β,25-trihydroxyvitamin D_3

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ABSTRACT

Three different A-ring perhydroxylated trihydroxyvitamin D_3 metabolites were synthesized from their appropriate A-ring precursors and CD-ring for their potential therapeutic applications. We first chemically synthesized 1α,2α,25-trihydroxyvitamin D_3 [1α,2α,25$(OH)_3D_3$] to study its VDR binding affinity because this metabolite is a product of recombinant human CYP3A4 catalysis when 2α-(3-hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 (O2C3), a more potent vitamin D receptor (VDR) binder than 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], is used as the substrate. We found that this metabolite retained 27.3% of the VDR binding affinity compared to 1α,25$(OH)_2D_3$. The k_{cat}/K_m value of CYP24A1 for 1α,2α,25$(OH)_3D_3$ is 60% of that for 1α,25$(OH)_2D_3$. Since the biological activity and the metabolic fate of a naturally occurring C4-hydroxylated vitamin D_2 metabolite found in the serum of rats treated with pharmacological doses of vitamin D_2 have never been described, we next synthesized 1α,4α,25-trihydroxyvitamin D_3 and its diastereoisomer, 1α,4β,25-trihydroxyvitamin D_3, to study their metabolism and biological activities. Both 4-hydroxylated isomers showed weaker VDR binding affinity than 1α,25$(OH)_2D_3$. Although either 4-hydroxylated isomer can be metabolized by CYP24A1 almost at the same level as 1α,25$(OH)_2D_3$, their metabolic patterns catalyzed by uridine 5′-diphosphoglucuronosyltransferase (UGT) are different; only the 4α-hydroxylated analog can be metabolized by UGT to produce a glucuronate conjugate. The results provide important information for the synthesis of new novel chemotherapeutic vitamin D analogs which would be less subjective to degradation and therefore more bioavailable than 1α,25$(OH)_2D_3$.

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Contents

1. Introduction

The physiologically active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], expresses its biological activity through binding to and modulation of the

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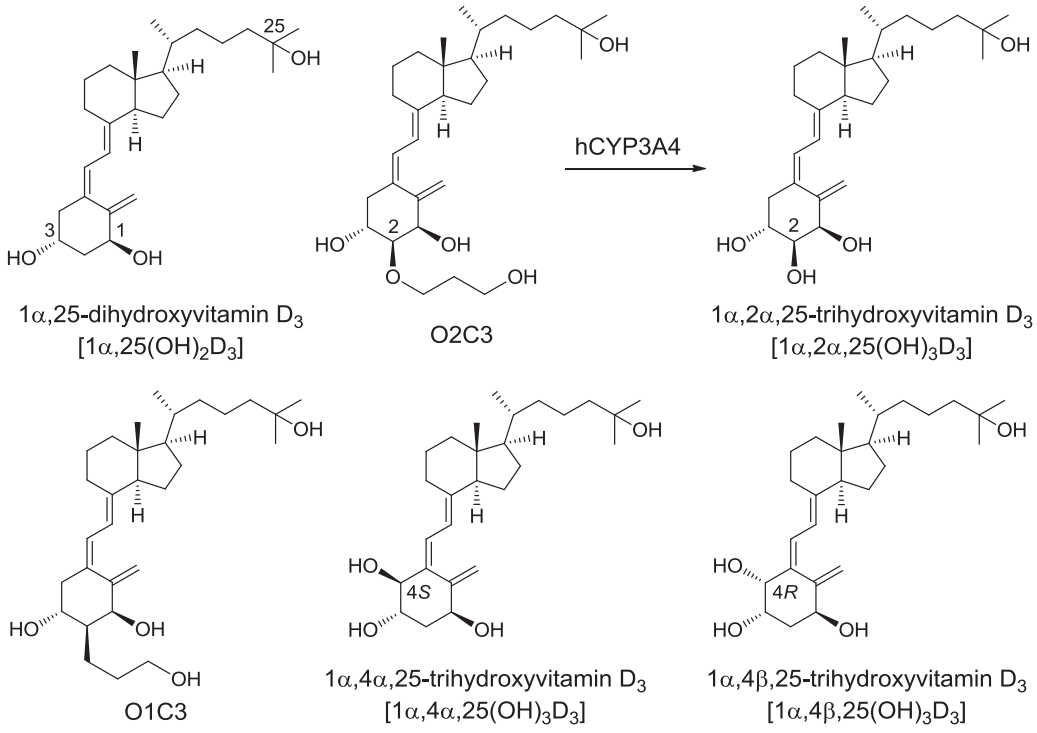
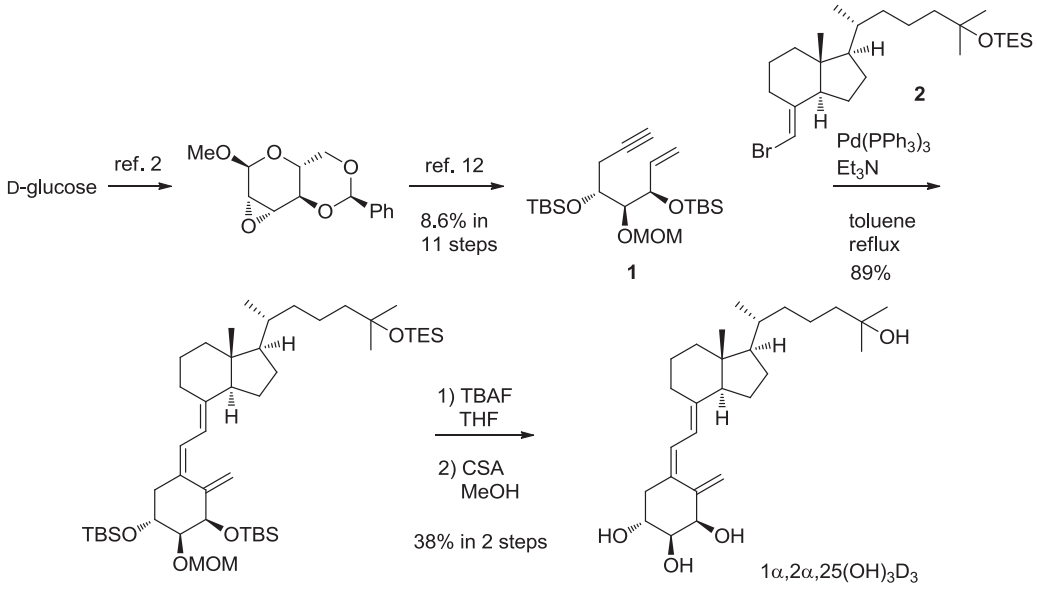
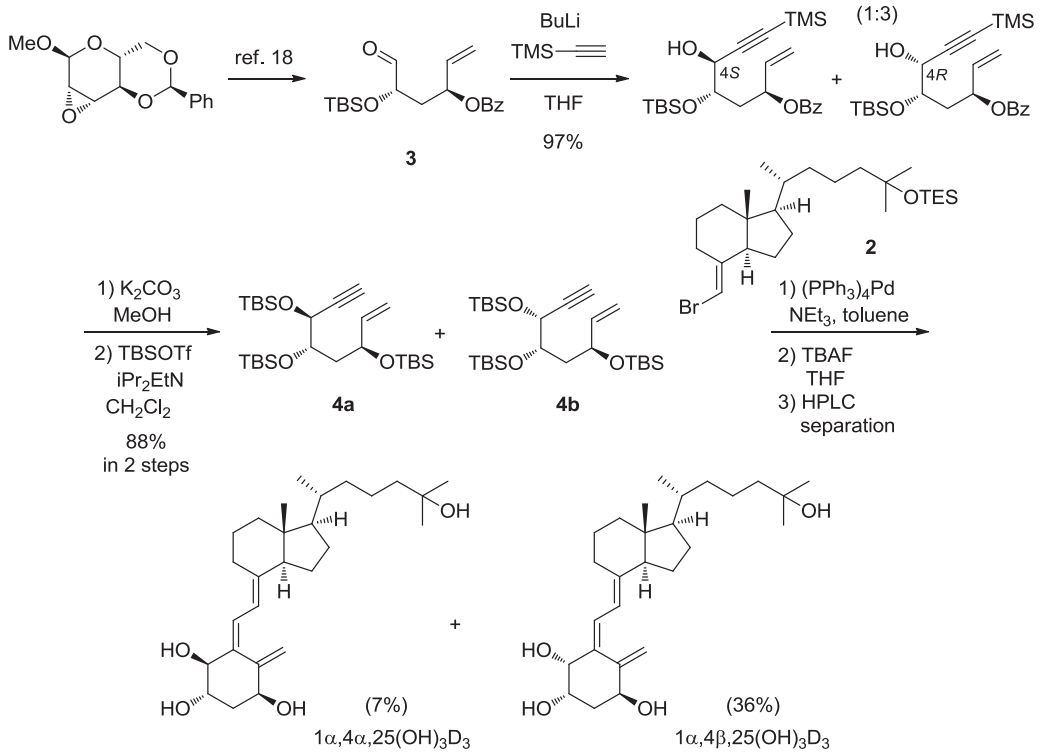


Fig. 1. Structures of $1\alpha,25(OH)_2D_3$ and its hydroxylated analogs O1C3, O2C3, $1\alpha,2\alpha,25(OH)_3D_3$, $1\alpha,4\alpha,25(OH)_3D_3$, and $1\alpha,4\beta,25(OH)_3D_3$.

vitamin D receptor (VDR) [1]. Previously, we synthesized 2α-(3-hydroxypropoxy)-$1\alpha,25(OH)_2D_3$ (O2C3) which possesses 1.8-times greater binding affinity for VDR than the natural hormone $1\alpha,25(OH)_2D_3$ [2–4]. The reason of being the better ligand can be explained by X-ray co-crystallographic analyses of the VDR-O2C3 complex, which shows additional hydrogen bonding between the terminal hydroxy group of the introduced 2α-side chain of the O2C3 and Arg274 in the ligand binding domain (LBD) of the VDR [5]. Although $1\alpha,25(OH)_2D_3$ is inactivated by CYP24A1-dependent catabolism via C-24 and C-23 hydroxylation pathways to calcitroic acid and 26,23-lactone derivatives, respectively [6], it was found that some 2α-substituted active vitamin D analogs like O2C3 were highly resistant to the CYP24A1 catabolism. The k_{cat}/K_m value of O2C3 was only 3% of that for $1\alpha,25(OH)_2D_3$ [7]. Since CYP24A1 is a specific enzyme induced by the VDR-ligand [$1\alpha,25(OH)_2D_3$ or its analogs] complex in the target tissue and inactivates $1\alpha,25(OH)_2D_3$ and its analogs, CYP24A1-resistant ligands would have longer-term biological effects and bioavailability in the target tissues [8]. On the other hand, CYP3A4 is the most important drug-metabolizing cytochrome P450 enzyme with a broad catalytic spectrum [9]. Although $1\alpha,25(OH)_2D_3$ and its analog 2α-(3-hydroxypropyl)-$1\alpha,25$-dihydroxyvitamin D_3 (O1C3) are not the primary substrates for CYP3A4 [8], we demonstrated that O2C3 was metabolized by the human recombinant CYP3A4 to $1\alpha,2\alpha,25$-trihydroxyvitamin D_3 [$1\alpha,2\alpha,25(OH)_3D_3$, Fig. 1] [8]. Its 2β-isomer, a known compound, shows potent $1\alpha,25(OH)_2D_3$-like activities [10], and is one of the eldecalcitol (ED-71) metabolites [11]. To study



Scheme 1. Synthesis of $1\alpha,2\alpha,25(OH)_3D_3$.



Scheme 2. Synthesis of 1α,4α,25(OH)$_3$D$_3$ and 1α,4β,25(OH)$_3$D$_3$.

biological activity of 1α,2α,25(OH)$_3$D$_3$, we chemically synthesized 1α,2α,25(OH)$_3$D$_3$ using the Trost Pd-mediated coupling reaction between an A-ring precursor **1** from D-glucose and a known CD-ring bromoolefin **2** (Scheme 1) [12–14].

On the other hand, a number of hydroxylated vitamin D metabolites were found in the circulation of mammals. For example, 4,25-dihydroxyvitamin D$_2$ was isolated and identified from the serum of rats intoxicated with pharmacological amounts of vitamin D$_2$ [15]. However, the biological significance of the 4-hydroxy group remains unclear. We therefore synthesized 1α,4α,25-trihydroxyvitamin D$_3$ [1α,4α,25(OH)$_3$D$_3$] and 1α,4β,25-trihydroxyvitamin D$_3$ [1α,4β,25(OH)$_3$D$_3$] by the similar manner as 1α,2α,25(OH)$_3$D$_3$, and evaluated their hVDR binding affinity as well as their further metabolism by human CYP24A1 and human liver microsomal fraction to compare with those of the natural hormone 1α,25(OH)$_2$D$_3$.

2. Results and discussion

Synthesis of 1α,2α,25(OH)$_3$D$_3$ was accomplished as shown in Scheme 1. We prepared chiral epoxide from methyl α-D-glucoside [2], which was converted to the enyne A-ring precursor **1** in 11 steps [12]. The CD-ring bromoolefin **2** and enyne **1** were coupled using Pd-catalyst to give the seco-steroidal product [16,17]. Deprotection of *O*-silyl groups gave the target molecule 1α,2α,25(OH)$_3$D$_3$. The isolated product was re-purified by HPLC to test its biological activity.

The other A-ring hydroxylated vitamin D$_3$ analogs of 1α,4α,25(OH)$_3$D$_3$ and 1α,4β,25(OH)$_3$D$_3$ were synthesized as shown in Scheme 2 [18]. The same chiral epoxide from D-glucose was converted to aldehyde **3** [19] followed by ethynylation afforded alcohols as a diastereomeric mixture in a 1:3 ratio. Stereochemistry of each alcohol was determined by the modified Mosher's method [19,20]. After manipulation of the protecting groups, enynes **4a** and **4b** were connected with CD-ring bromoolefin **2** using Pd-catalyst to give the coupling products, and TBAF-deprotection followed by HPLC-separation gave the target molecules 1α,4α,25(OH)$_3$D$_3$ and 1α,4β,25(OH)$_3$D$_3$ [18,19].

Next, hVDR binding affinity was performed for the above three new A-ring perhydroxylated vitamin D$_3$ metabolites, and the results are shown in Table 1. Eldecalcitol metabolite 1α,2β,25(OH)$_3$D$_3$ showed strong VDR binding affinity as compared with O2C3 metabolite 1α,2α,25(OH)$_3$D$_3$ [10]. Although the 2-OH group is not a severe obstacle to VDR binding, the 4-OH group might be steric hindrance when this ligand binds to the ligand binding pocket of the VDR [12,18].

Regarding metabolism, O2C3 is resistant to CYP24A1, but its CYP3A4 metabolite 1α,2α,25(OH)$_3$D$_3$ can be further metabolized by CYP24A1, similar to 1α,25(OH)$_2$D$_3$. The k_{cat}/K_m value of hCYP24A1 for 1α,2α,25(OH)$_3$D$_3$ was found to be 60% of that for 1α,25(OH)$_2$D$_3$ [8]. On the other hand, 1α,4α,25(OH)$_3$D$_3$ and 1α,4β,25(OH)$_3$D$_3$ are metabolized to several metabolites by CYP24A1-dependent multi-step oxidation pathways similar to that for 1α,25(OH)$_2$D$_3$ or its A-ring diastereomers. Interestingly, HPLC profile of the metabolites of 1α,4α,25(OH)$_3$D$_3$ is quite similar to that of 1α,25(OH)$_2$D$_3$, whereas, the profile of 1α,4β,25(OH)$_3$D$_3$ is quite similar to that of 3-epimers of 1α,25(OH)$_2$D$_3$ (Fig. 2) [18,21]. After 60 min incubation, the % conversion of 1α,4α,25(OH)$_3$D$_3$ and

Table 1
Relative hVDR binding affinity of A-ring perhydroxylated analogs.

Compound	VDR Binding affinity[a]
1α,25(OH)$_3$D$_3$	100
O2C3	180[b]
1α,2α,25(OH)$_3$D$_3$	27.3
1α,2β,25(OH)$_3$D$_3$	110[c]
1α,4α,25(OH)$_3$D$_3$	0.9
1α,4β,25(OH)$_3$D$_3$	2.9

[a] The potency of 1α,25(OH)$_3$D$_3$ is normalised to 100.
[b] Ref. [2].
[c] Ref. [10].

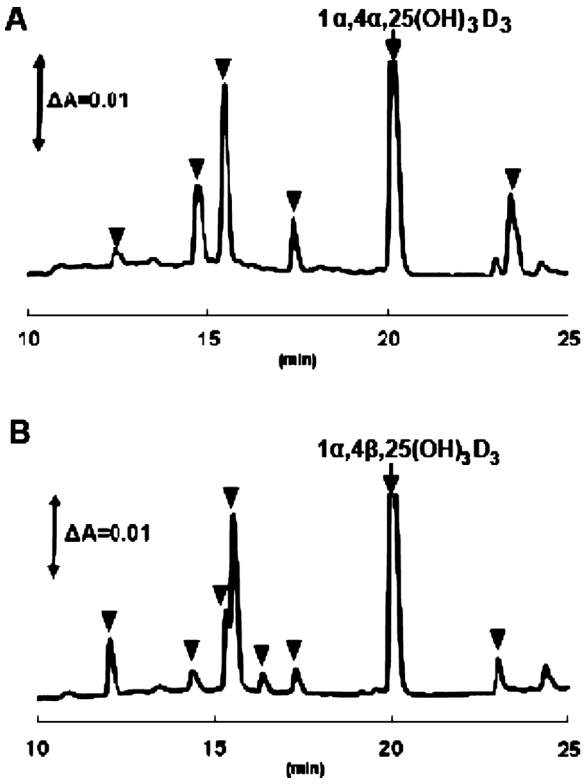


Fig. 2. HPLC profiles of 1α,4α,25- (A) and 1α,4β,25-trihydroxyvitamin D_3 (B) and their metabolites by human CYP24A1. The peaks with arrow heads show putative metabolites by human CYP24A1.

1α,4β,25(OH)$_3$D$_3$ are about 45% and 44%, respectively, which are nearly the same as those for 1α,25(OH)$_2$D$_3$. Both 4-hydroxyvitamin D_3 analogs were treated with human liver microsomal fraction containing drug-metabolizing enzymes, and no metabolite from either analog was detected. The results suggest that both analogs are poor substrates for human hepatic cytochrome P450s. However, addition of UDP-glucuronic acid produced a metabolite (glucuronide) of 1α,4α,25(OH)$_3$D$_3$ whereas no metabol|%1e[Accept]ite of 1α,4β,25(OH)$_3$D$_3$ was observed [18]. Since β-glucuronidase treatment converted the metabolite of 1α,4α,25(OH)$_3$D$_3$ back to 1α,4α,25(OH)$_3$D$_3$ itself, this suggests that the metabolite is a glucuronide of 1α,4α,25(OH)$_3$D$_3$. Neither 1α,4β,25(OH)$_3$D$_3$ nor 1α,25(OH)$_2$D$_3$ was converted to its glucuronide. Therefore, the 4α-OH group of 1α,4α,25(OH)$_3$D$_3$ appeared to be glucuronidated by certain hepatic UGT(s) [18].

3. Conclusion

We synthesized three new A-ring perhydroxylated vitamin D_3 metabolites, 1α,2α,25(OH)$_3$D$_3$, the major O2C3 metabolite catalyzed by CYP3A4, and 1α,4α,25(OH)$_3$D$_3$ and 1α,4β,25(OH)$_3$D$_3$, the tentative 1α,25(OH)$_2$D$_3$ related metabolites. The position and stereochemistry of the synthetically introduced OH group to the A-ring influence hVDR binding affinity and their further metabolism by CYP enzymes. These finding would be useful for creating new novel potential chemotherapeutic ligands for VDR.

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