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A Concise and Efficient Route to 2α-(ω-Hydroxyalkoxy)-1α,25-dihydroxyvitamin D_3: Remarkably High Affinity to Vitamin D Receptor[1]

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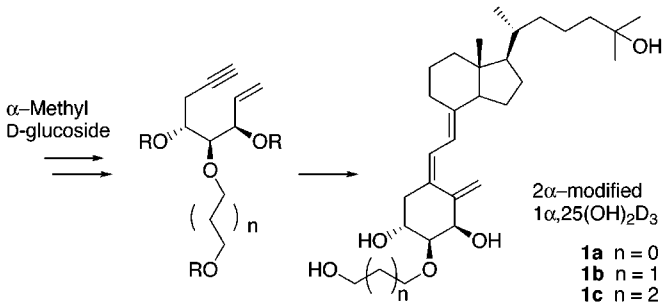
ABSTRACT



A convenient and potentially valuable synthetic approach to the novel 2α-functionalized 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2$$D_3$] derivatives (1a–c), which are the C2-epimer of ED-71 and its analogues, has been developed. The C2α-modified ring A precursors (1,7-enynes 16, *n* = 0, 1, and 2) were constructed stereoselectively starting from D-glucose in high yield. In the synthesized 2α-(ω-hydroxyalkoxy)-1α,25(OH)$_2$$D_3$ derivatives, 1a and 1b showed a greater binding affinity to vitamin D receptor (VDR), up to 1.8 times that of the native hormone.

The seco-steroid hormone 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2$$D_3$] is the most potent metabolite of vitamin D_3 and regulates primarily calcium homeostasis, as well as cell proliferation and differentiation and immunology.[2] Some of these biological responses to this class of compounds may have high potential in the treatment of rickets, renal osteodystrophy, osteoporosis, psoriasis, certain cancers, AIDS, and Alzheimer's disease.[2,3] One of the major problems in the clinical use of 1α,25(OH)$_2$$D_3$ is the effective doses, which likely induce fatal hypercalcemia.[2] The search for a noncalcemic therapeutic agent, and for convenient synthetic methods of finely modified compounds, has been greatly stimulated by medical needs.

2β-(3-Hydroxypropoxy)-1α,25(OH)$_2$$D_3$ (ED-71) was developed by Chugai Pharmaceutical Co. as a promising candidate for the treatment of osteoporosis.[2,4] Recently, we reported synthesis of the A-ring modified analogues, 2-methyl-1α,25(OH)$_2$$D_3$, and found that the 2α-isomer **2** showed higher potency than the native hormone in terms of binding affinity to vitamin D receptor (VDR), elevation of serum Ca concentration, and induction of HL 60 cell differentia-

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(1) This paper is dedicated (by A.K.) to Prof. Dr. A. Eschenmoser on the occasion of his 75th birthday.

(2) (a) *Vitamin D*; Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic Press: New York, 1997. (b) Ettinger, R. A.; DeLuca, H. F. *Adv. Drug Res.* **1996**, *28*, 269−312.

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tion.[5] Further modification of the 2α-methyl group, in particular, introduction of the 2α-(3-hydroxypropyl) group into the A-ring of 1α,25(OH)$_2$D$_3$ (**3**), increased by 500 times the potency of calcium-mobilizing activity.[6] Most of the biological actions of 1α,25(OH)$_2$D$_3$ are considered to be mediated by the vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily acting as a ligand-dependent transcription factor with coactivators.[7] Accordingly, we planned to synthesize new analogues (**1**) containing the hydroxyalkoxyl group at the C2 position, like ED-71 but with the α-orientation, to understand the detail structure−activity relationships, particularly on the ring A (Figure 1).

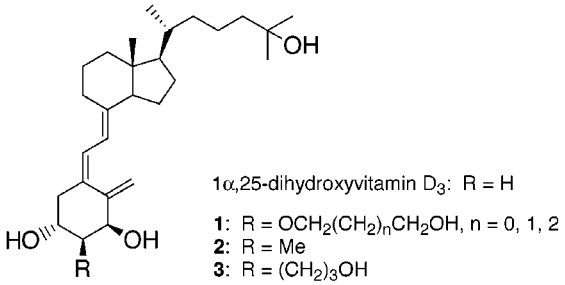
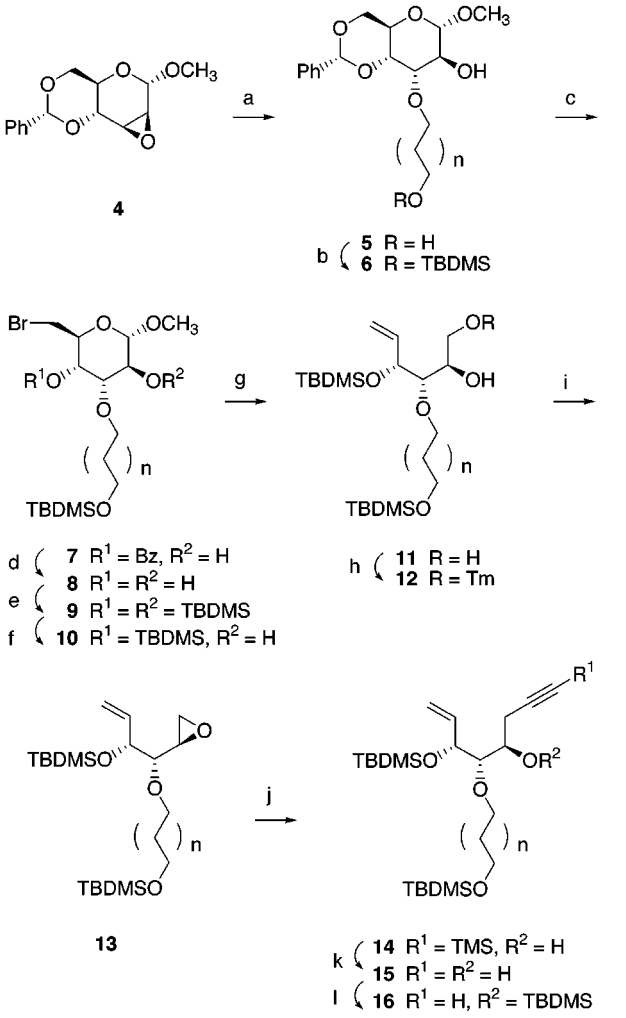


Figure 1. Structures of 1α,25-dihydroxyvitamin D$_3$ [1α,25-(OH)$_2$D$_3$] and its 2α-substituted analogues **1**−**3**.

The original approach to ED-71 from lithocholic acid allows only 2β-substitution of 1α,25(OH)$_2$D$_3$.[4a] On the basis of convergent synthesis, A-ring synthons were elegantly constructed using a C2 chiral epoxide[4b] or the adaptation of a polyol chiron.[4c,d] We wish to report here a new concise and efficient synthetic route to 2α-(ω-hydroxyalkoxy)-1α,25(OH)$_2$D$_3$ derivatives (**1a**−**c**) from D-glucose and their considerable binding affinity to VDR.

To create 1α,2α,3β stereochemistry on the ring A of the target vitamin D analogues (**1**), the known crystalline epoxide **4**, which is readily available from methyl α-D-glucoside,[8] was chosen as the chiral template. The regiospecific ring opening[9] by a suitable alkanediol[4a] at the C3 position affords the altrose configuration, in which C2, C3, and C4 asymmetric centers satisfy the corresponding desired 3β, 2α, and 1α stereochemistries of **1**, respectively. As illustrated in Scheme 1, for example, n = 1, **4** was heated with 1,3-propanediol under basic conditions to yield methyl 3-*O*-(3-hydroxypropyl)altropyranoside **5** (n = 1). After selective

Scheme 1[a]



[a] Conditions and yields: (a) HOCH$_2$(CH$_2$)$_n$CH$_2$OH, KO^{t}Bu, 110 °C, 14 h, n = 0 (88%), n = 1 (94%), and n = 2 (93%); (b) TBDMSCl, imidazole, DMF, n = 0 (93%), n = 1 (92%), and n = 2 (99%); (c) NBS, BaCO$_3$, CCl$_4$, reflux, 35 min, n = 0 (71%), n = 1 (74%), and n = 2 (76%); (d) catalytic NaOMe, MeOH, n = 0 (95%), n = 1 (96%), and n = 2 (99%); (e) TBDMSCl, imidazole, DMF, n = 0 (93%), n = 1 (94%), and n = 2 (85%); (f) catalytic Bu$_4$NF, THF, n = 0 (43%), n = 1 (71%), and n = 2 (68%); (g) Zn, NaBH$_3$CN, 1-propanol−H$_2$O (10:1), 95 °C, 45 min, and then, NaBH$_4$, n = 0 (71%), n = 1 (72%), and n = 2 (75%); (h) TmCl, DMAP, CH$_2$Cl$_2$, n = 0 (82%), n = 1 (84%), and n = 2 (92%); (i) LiHMDS, THF, −78 °C to rt, n = 0 (92%), n = 1 (99%), and n = 2 (90%); (j) TMSCCH, BuLi, BF$_3$OEt$_2$, THF, −78 °C, n = 0 (91%), n = 1 (92%), and n = 2 (94%); (k) K$_2$CO$_3$, MeOH, n = 0 (93%), n = 1 (96%), and n = 2 (63%); (l) TBDMSOTf, 2,6-lutidine, CH$_2$Cl$_2$, 0 °C, n = 0 (92%), n = 1 (90%), and n = 2 (80%).

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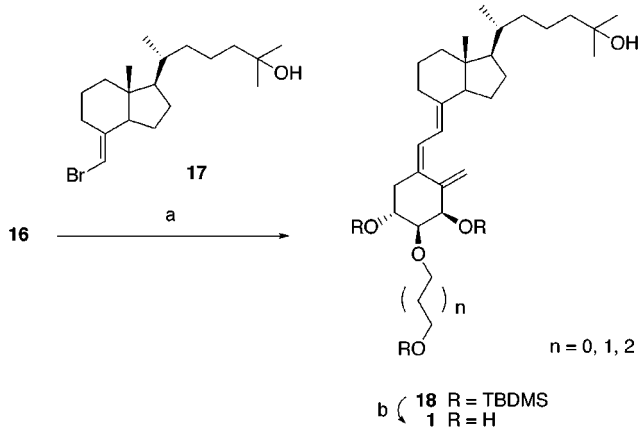
protection of the primary alcohol, treatment of benzylidene acetal **6** with NBS[10] gave bromide **7** in 74% yield. Exchange of the protecting group on the C4 hydroxyl group was accomplished through mild solvolysis of the benzoate, persilylation, and selective deprotection of the C2 hydroxyl group to obtain bis-*O*-silylated **10** in good yield.[11] Reaction of bromide **10** with activated zinc powder generated an aldehyde, which was directly reduced to alcohol **11**.[9,12] Sulfonylation of the primary alcohol and LiHMDS treatment afforded epoxide **13**, into which was subsequently introduced the acetylene unit with ring opening. Removal of the terminal TMS group under basic conditions and the protection of the secondary alcohol with TBDMS provided 1,7-enyne **16** ($n = 1$) in high yield (Scheme 1), which is suitable for the following Trost−Dumas palladium-catalyzed coupling reaction.[13] The other A-ring precursors (**16**) were also prepared by a similar procedure using ethylene glycol ($n = 0$) and 1,4-butanediol ($n = 2$) instead of 1,3-propanediol.

Palladium-mediated alkylative cyclization with vinyl bromide of the CD fragment **17**[13] from Grundmann's ketone and subsequent deprotection furnished the target 2α-substituted 1α,25-dihydroxyvitamin D_3 derivatives **1** in considerable yields (Scheme 2).

Scheme 2[a]



[a] Conditions and yields: (a) catalytic $(Ph_3P)_4Pd$, Et_3N−toluene (1:1), reflux, $n = 0$ (75%), $n = 1$ (52%), and $n = 2$ (69%); (b) Bu_4NF, THF, $n = 0$ (78%), $n = 1$ (61%), and $n = 2$ (70%).

The receptor binding potency of **1a**−**c** was evaluated using bovine thymus VDR,[14] and the results are summarized in Table 1. Interestingly, the highest binding affinity of this series occurred with **1b** ($n = 1$), which is 1.8 times higher than that of 1α,25$(OH)_2D_3$.

Recently, studies on the three-dimensional structural elucidation of the 1α,25$(OH)_2D_3$ docking VDR ligand binding domain have been carried out.[15] It is noteworthy that there is a rather hydrophobic cavity around the C2 position surrounded by Phe-150, Tyr-143, Tyr-147, and Tyr-236. Interestingly, Asp-144 is found at the end of this long cavity. The 1α-hydroxyl group forms hydrogen bonds with Ser-237

Table 1. Relative Binding Affinity of the Series of **1** for Bovine Thymus 1α,25-Dihydroxyvitamin D_3 Receptor (VDR)

compounds	VDR[a]
1α,25$(OH)_2D_3$	100
1a ($n = 0$)	120
1b ($n = 1$)	180
1c ($n = 2$)	40

[a] The potency of 1α,25$(OH)_2D_3$ is normalized to 100.

and Arg-274. The potent VDR affinity of **1a** and **1b** could be explained by molecular mechanics calculations based on the crystal structure established by Moras et al.[15a] In a preliminary calculation,[16] the C2α terminal hydroxyl group of **1b** is likely to reach toward not only Asp-144 but also Tyr-236 to create additional hydrogen bond networks. On the other hand, the C2α terminal hydroxyl group of **1a** probably forms a hydrogen bond with Arg-274. Formation of the new hydrogen bonds could be one of the reasons for the high affinity. Furthermore, based on the calculation, the 2α-substituted A-ring, except for the 2α-methyl case, fits into the cavity in the α-conformation to minimize steric repulsion. In this conformation, the 2α-substituent adopts the equatorial position and the 1α-hydroxyl group retains hydrogen bonding with Ser-237 (Figure 2). Very recently,

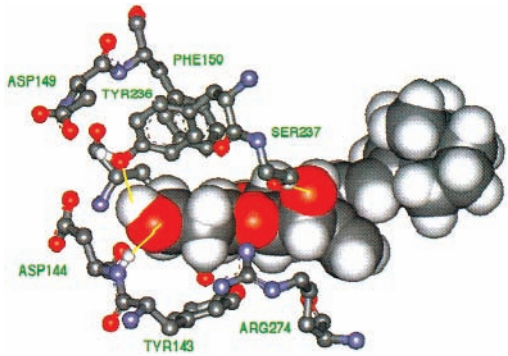


Figure 2. Computer calculation of **1b** in the VDR ligand binding domain.

we reported that 2α-(3-hydroxypropyl)-1α,25$(OH)_2D_3$ (**3**) showed a 3-fold increase in binding activity to VDR.[6] The

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(11) In the case of $n = 1$, recovered **9** (23%) and a mixture of diols (15%), which could be quantitatively recycled to the tris-*O*-TBDMS derivative **9**, were obtained. Then, the recovered **9** and the recycled **9** were combined and desilylated again. Overall yield of **10** for these three steps was 72%. For $n = 0$ and 2, this recycled step was also applied and overall yields were noted in Scheme 1.
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distance between the C2-atom and the terminal hydroxyl group of **3** is almost the same as that for compound **1a**. It is conceivable that different binding activities of these analogues could be due to matching of the hydrophobicity just around the C2 position.

In conclusion, we have developed an efficient synthetic route to the new biologically active 2α-(ω-hydroxyalkoxy)-1α,25(OH)$_2$D$_3$ (**1a**−**c**) through the new A-ring synthon, enyne **16**, from α-D-glucose. It was found that VDR binding affinity of **1b** is 1.8 times higher than that of the native hormone. This increased affinity was elucidated by molecular mechanics calculations based on the crystal structure of VDR bound to its natural ligand. We believe this synthetic strategy could be applicable to a wide range of novel 2α-substituted 1α,25(OH)$_2$D$_3$ derivatives by simply using various nucleophiles toward epoxide **4**. Further synthetic and biological studies in this area are currently in progress in our laboratory.

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Supporting Information Available: Experimental detail and characterization data for compounds **1a**−**c** and **5**−**10** (represented by $n = 1$) and charts of VDR binding assays for **1a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Calculated by MacroModel ver. 6.5 (Schrodinger, Inc.) on a SGI O2 Workstation. The C17 steroidal side chain was omitted for the calculation.