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Synthesis of 2α-substituted-14-epi-previtamin D_3 and its genomic activity

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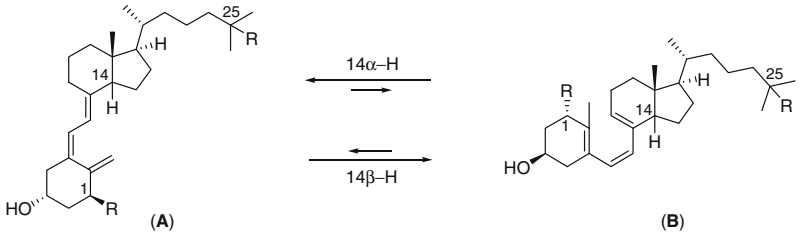
ABSTRACT

We synthesized and isolated 2α-substituted analogs of 14-epi-previtamin D_3 after thermal isomerization at 80 °C for the first time. The VDR binding affinity and transactivation activity of osteocalcin promoter in HOS cells were evaluated, and the 2α-methyl-substituted analog was found to have greater genomic activity than 14-epi-previtamin D_3.

Vitamin D_3 is present in thermal equilibrium with previtamin D_3 via [1,7]-sigmatropic rearrangement. In this equilibrium, the 6-*s*-*trans* isomer, that is, the vitamin D form (**A**), is more stable and major than the 6-*cis* isomer of the previtamin D form (**B**) (Scheme 1). Active vitamin D_3, 1α,25(OH)$_2$$D_3$ (**1**), which is the biologically most active metabolite of vitamin D_3, contains 5–10% of its previtamin D form, 1α,25(OH)$_2$preD_3 (**pre-1**) at 37 °C in similar equilibrium.[1] Most scientists have focused on the analogs of the major vitamin D form for therapeutic evaluation rather than the previtamin D form, because previtamin D_3 is easily transformed to vitamin D_3 through thermal equilibrium and is almost impossible to isolate in the pure form.[1] While **1** is a ligand of the nucleic receptor (vitamin D receptor, VDR), regulates gene transcription, and exhibits various biological responses as a hormone,[2] **pre-1** is believed to be a weak ligand of VDR and a poor activator of the above genomic actions;[3] however, **pre-1** has been studied as a ligand of a putative membrane vitamin D receptor for a long time,[4] and it is well-known that **pre-1** causes various biological rapid



vitamin D_3: R = H (14α–H)
1: R = OH, 1α,25(OH)$_2$$D_3$ (14α–H, 1α,25-dihydroxyvitamin D_3)
14-epi-1: R = OH, 14-epi-1α,25(OH)$_2$$D_3$ (14β–H)

previtamin D_3: R = H (14α–H)
pre-1: R = OH, 1α,25(OH)$_2$preD_3 (14α–H)
14-epi-pre-1: R = OH, 14-epi-1α,25(OH)$_2$preD_3 (14β–H)

Scheme 1. Equilibrium between vitamin D_3 and previtamin D_3.

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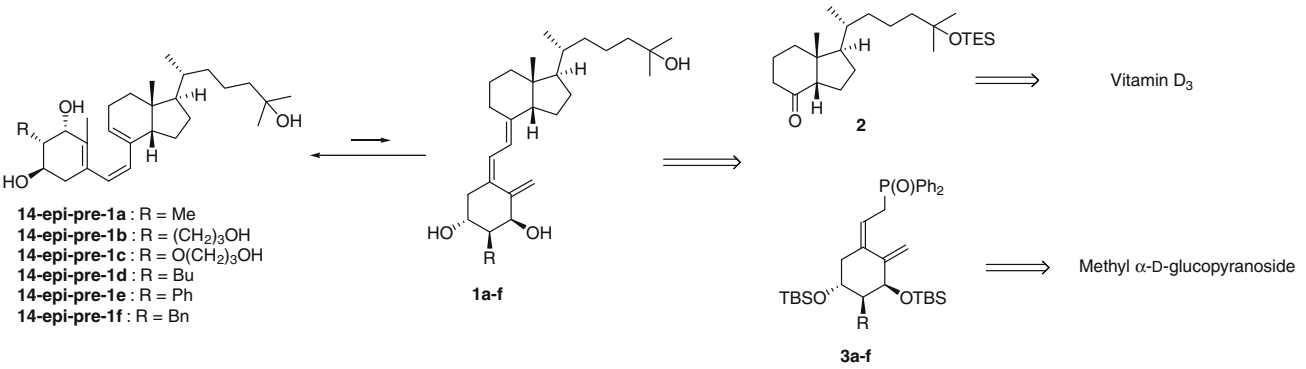
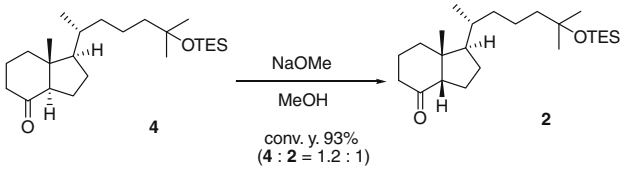


Scheme 2. Retrosynthetic analysis of 2α-substituted 14-epi-1α,25(OH)$_2$preD$_3$.

responses, for example, stimulation of intestinal Ca^{2+} transport (transcaltachia),[5] activation of PKC[6] and MAP[7] kinases, and so on, which are called non-genomic actions.

Okamura et al. reported that the thermal equilibrium ratio between vitamin D form (**A**) and previtamin D form (**B**) at 80 °C was reversed by epimerizing the CD-ring bridgehead hydrogen of C14, that is, 14-epi-1α,25(OH)$_2$preD$_3$ (**14-epi-pre-1**) was major and dominant to 14-epi-1α,25(OH)$_2$D$_3$ (**14-epi-1**).[8] Since it requires a high temperature (80 °C) to reach thermal equilibrium, **14-epi-pre-1** is expected to be isolated stable at room temperature. Using this reverse equilibrium, we focused on the synthesis of the **14-epi-pre-1** analogs with A-ring modification, and aimed to identify the more detailed biological properties and potential as therapeutic agents of the previtamin D$_3$ skeleton.

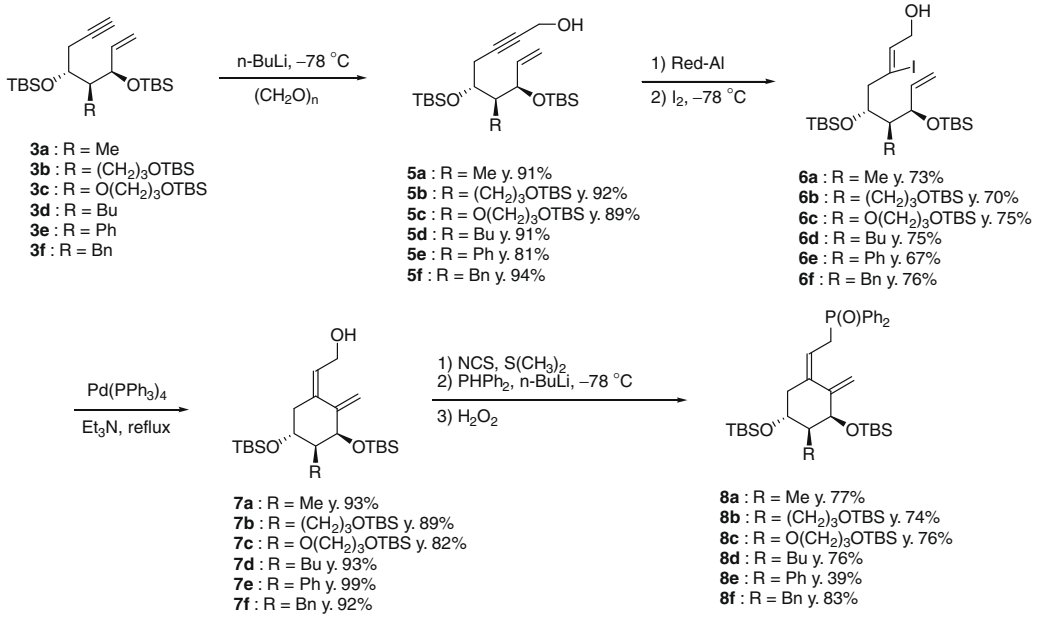
14-epi-pre-1 could be prepared from **14-epi-1** by thermal isomerization, so we planed to synthesize **14-epi-1** analogs as temporary first targets. This strategy would help us to understand the equilibrium between vitamin D$_3$ and previtamin D$_3$. The **14-epi-1** analogs were divided into two fragments, which were CD-ring and A-ring fragments (Scheme 2). The CD-ring fragment could be obtained by epimerization at H14 in Grundmann's ketone derivative, which was derived from vitamin D$_3$.[9] The A-ring fragment could be synthesized from methyl α-D-glucoside, and we could introduce various alkyl groups at the 2α-position as we reported previously.[10] We found that 2α-alkyl and 2α-(ω-hydroxyalkyl) substitution afforded great improvements VDR binding affinity and the subsequent genomic actions.[11] We therefore decided to prepare analogs with 2α-substitutions (**14-epi-pre-1a**∼**1f**) in this Letter.



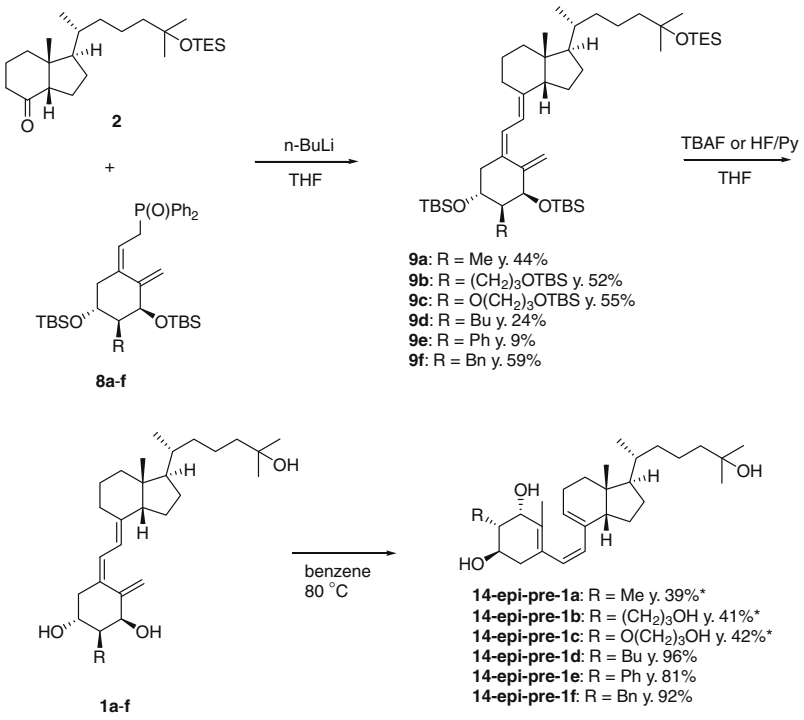
Scheme 3. Synthesis of the CD-ring fragment.

The CD-ring fragment (**2**) was synthesized from the known ketone of TES-protected 25-hydroxy Grundmann's ketone (**4**).[9] According to the literature, epimerization of H14 was successfully conducted by NaOMe with recovery of the starting material (Scheme 3).[8]

A-ring fragments (**8a**–**f**) were prepared from the known enynes **3a**–**f** (Scheme 4),[10a–d] which reacted with *n*-BuLi and then $(CH_2O)_n$ to give alcohols **5a**–**f** in good to excellent yields. Then, hydroalumination and subsequent iodination of the alkyne gave the vinyl iodides **6a**–**f**. Next, cyclization by Heck reaction proceeded smoothly to afford a six-membered A-ring,[12] whose hydroxyls were easily transformed into phosphine oxides in three



Scheme 4. Synthesis of the A-ring fragments.



Scheme 5. Coupling reaction and synthesis of 2α-substituted 14-epi-1α,25(OH)$_2$preD$_3$.

steps to give **8a**–**f**, respectively.[8] As above, we were able to prepare A-ring fragments in good overall yield.

Since both fragments were available, we tried the coupling reaction under basic conditions using *n*-BuLi (Scheme 5).[8] Small excess amounts of the A-ring fragment worked well and we obtained the coupled products **9a**–**f**, although some were obtained in low yield. The silyl protected **14-epi-1** analogs (**9a**–**f**) tend not to lead to isomerization to their previtamin D form, probably because TBS groups at the A-ring should show steric hindrance to reaching the transition state of the [1,7]-sigmatropic hydrogen shift existing between the vitamin D form and previtamin D form. Then, all silyl groups in **9a**–**f** were removed in one step with excess TBAF or HF/pyridine, and most of the deprotected compounds remained in the vitamin D form (**1a**–**f**), and only small amounts of the previtamin D form (**pre-1a**∼**1f**) were produced under these reaction conditions. Isomerization was therefore examined at 80 °C in benzene, and fortunately the portions of the desired **14-epi-pre-1a**∼**1f** increased, and most vitamin D forms were converted into the previtamin D form easily in less than two hours. After thermal equilibrium had been established, the ratio of the compounds was about 5/95 (vitamin D/previtamin D) based on ^{1}H NMR studies. Using HPLC, the mixture of both forms was separated, and pure **14-epi-pre-1a**∼**1f** were used for further biological studies.[13]

The VDR binding affinity and the osteocalcin promoter transactivation activity of the new compounds were evaluated using the chick intestinal VDR and HOS cells, respectively.[14] The results are summarized in Table 1 in comparison with the natural hormone **1** and **14-epi-pre-1**, which was synthesized in a similar manner in our laboratory. The new compounds showed lower activity than the natural hormone **1**; however, some showed higher activity than 14-epi-1α,25(OH)$_2$preD$_3$ (**14-epi-pre-1**). In particular, **14-epi-pre-1a**, the 2α-methyl substituted analog indicated a remarkable increase in VDR binding affinity and transactivation activity. It is worth noting that **14-epi-pre-1** analogs gain genomic activity, and 2α-substitution on the A-ring seems to have great effects on the biological actions of the previtamin D form.

Table 1
Relative binding affinity for chick intestinal VDR and osteocalcin promoter transactivation activity in HOS cells of 2α-substituted 14-epi-1α,25(OH)$_2$preD$_3$[14]

Compound	VDR[a]	Osteocalcin transactivation activity (ED$_{50}$ (nM))
1	100	0.03
14-epi-pre-1	0.5	0.46
14-epi-pre-1a	8.4	0.12
14-epi-pre-1b	1.4	0.69
14-epi-pre-1c	0.17	0.95
14-epi-pre-1d	0.27	5.77
14-epi-pre-1e	<0.03	0.88
14-epi-pre-1f	0.03	30.2

In conclusion, we synthesized the 2α-substituted analogs of **14-epi-pre-1** for the first time and were able to isolate these new analogs (**14-epi-pre1a**∼**1f**) after thermal isomerization at 80 °C. Using them, we evaluated the VDR binding affinity and transactivation activity of osteocalcin promoter in HOS cells, among which, the 2α-methyl-substituted analog (**14-epi-pre-1a**) was found to have greater genomic activity than **14-epi-pre-1**. Further studies of the action mechanisms of **14-epi-pre-1** analogs as well as studies on non-genomic activity are currently in progress in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data (spectroscopic data of **14-epi-pre-1b**∼**1f** and experimental details of testing binding affinity for the chick intestinal VDR and osteocalcin promoter transactivation activity) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.112.

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13. Data for **14-epi-pre-1a**: $[\alpha]_D^{22}$ −11.64 (c 1.61, $CHCl_3$); UV (EtOH) λ_{max} 252.5 nm, λ_{min} 229.5 nm; IR (neat) 3374, 2961, 1456, 1375 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.99–1.98 (m, 34H), 2.50 (dd, J = 16.8, 5.1 Hz, 1H), 3.65 (dt, J = 10.0, 5.5 Hz, 1H), 3.87 (d, J = 2.0 Hz, 1H), 5.58 (d, J = 3.4 Hz, 1H), 5.71 (d, J = 13.1 Hz, 1H), 5.75 (d, J = 13.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 17.6, 19.6, 20.9, 21.8, 22.9, 28.6, 29.1, 29.5, 29.6, 33.9, 34.1, 35.7, 38.4, 41.1, 41.7, 44.3, 51.0, 51.8, 68.2, 71.1, 74.5, 125.6, 127.4, 130.4, 130.8, 132.7, 138.7; EI-LRMS m/z 430 (M^+), 412, 396, 374, 350, 169; EI-HRMS calcd for $C_{28}H_{46}O_3$ (M^+) 430.3446, found 430.3430. For spectroscopic data of **14-epi-pre-1b**∼**1f**, see: Supplementary data.
14. For experimental details of testing binding affinity for the chick intestinal VDR and osteocalcin promoter transactivation activity, see: Supplementary data.