Synthesis and Biological Evaluation of 4-Substituted Vitamin D and 14-Epi-Previtamin D Analogs

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We synthesized the 4-hydroxy and 4-methoxy analogs of active vitamin D_3 (1α,25(OH)$_2$D$_3$, 1) and its C14-epimer with the previtamin D_3 form of 14-epi-1α,25(OH)$_2$preD$_3$ (14-epi-pre1). Their vitamin D receptor (VDR) binding affinity and osteocalcin promoter transactivation activity in HOS cells were evaluated, and had lower activity than the natural hormone (1) and 14-epi-pre1, respectively.

Key words vitamin D_3; previtamin D_3; C4-modified seco-steroid; vitamin D receptor; osteocalcin

It is well-known that 1α,25-dihydroxyvitamin D_3 (1α,25(OH)$_2$D$_3$: **1**), which is the biologically most active metabolite of vitamin D_3, plays an important role in the body as a hormone, such as in calcium and phosphorus homeostasis, cellular differentiation, and immune responses, and inevitably, is involved in several diseases.[1—8] Therefore, its analogs could be good candidates for therapeutic agents, and so far, a large number of derivatives have been synthesized. Vitamin D analogs, such as alphacalcidol, doxercalciferol, paricalcitol, calcipotriol, tacalcitol, and maxacalcitol, are clinically used for osteoporosis, renal failure, secondary hyperparathyroidism, and psoriasis patients.[1,9]

One of our previous research projects investigated previtamin D_3 analogs[10] and we became interested in modification at the 4-position of 14-epi-previtamin D_3. Among the huge library of vitamin D_3 derivatives, few compounds possess substituents at the 4-position, and their biological effects remain unclear, except 4-fluorovitamin D analogs.[11,12] We expected that 4-oxy-substitution of the previtamin D form, especially, could construct a new hydrogen bond in vitamin D receptor (VDR), and also 4-substitution would help us to understand the genomic activity of the pre-form compounds.[10] At the beginning of the study of 4-substitution in vitamin D_3, we report the synthesis and biological evaluation of 4-hydroxy and 4-methoxy analogs of 14-epi-1α,25-dihydroxyprevitamin D_3 (14-epi-1α,25(OH)$_2$preD$_3$, **14-epi-pre1**) and also of 1α,25(OH)$_2$D$_3$ (**1**), that is, compounds **3** and **2**, respectively (Fig. 1).

The target compounds were divided into two fragments, A-ring and CD-ring parts, which were individually synthesized and coupled in a later step. The synthesis of the A-ring fragment is shown in Chart 1. The hydroxy group of the known compound **4** was protected as a *tert*-butyldimethylsilyl (TBS) ether, and transformed into a bromide using *N*-bromosuccinimide (NBS) and $BaCO_3$.[13] Then, it reacted with activated zinc powder in 1-PrOH/H_2O to give aldehyde **5**. The addition reaction of lithium (trimethylsilyl)acetylide to **5** proceeded smoothly to afford alcohol **6a**, a mixture of diastereomers (major/minor 3/1). After the addition reaction, subsequent *in situ* reaction of the resultant lithium alkoxide with MeI afforded methoxy compound **6b** as a mixture of diastereomers (major/minor 3/1). These hydroxy or methoxy groups of **6a** and **6b** corresponded to the 4-position in the A-ring (steroid numbering), that is, we were able to construct the 4-substitution. Next, deprotection of both trimethylsilyl (TMS) and Bz groups in **6a** and **6b** was conducted by K_2CO_3 in MeOH, and the following protection of the resultant hydroxyls by TBS groups gave the A-ring fragments **7a** and **7b**, respectively.

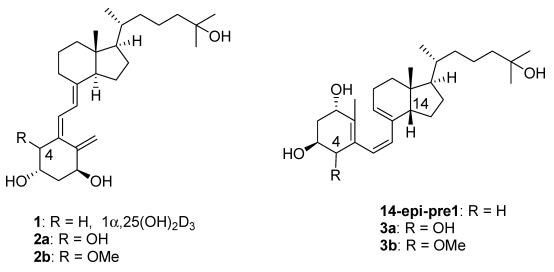

Fig. 1. Structures of Vitamin D_3 and Previtamin D_3 Analogs

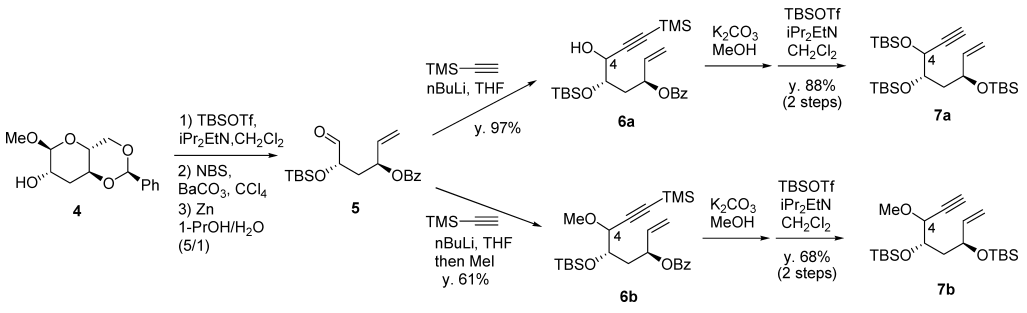

Chart 1

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Stereochemistry at the 4-position of **6a** and **6b** was determined by using Mosher esters as follows[14]: Diastereomers of compound **6a** were separated by HPLC, and both reacted with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA chloride) to give the corresponding (*S*)- and (*R*)-MTPA esters (**6a-MTPAs**), respectively (Fig. 2). Using the four esters, the values of $\Delta\delta$ ($=\delta_{(S)\text{-MTPA ester}}-\delta_{(R)\text{-MTPA ester}}$) in the 400 MHz ^{1}H-NMR spectra were calculated, and the configurations at C4 were determined by applying the modified Mosher's method[14]; therefore, we decided the absolute configurations at C4 of the major isomer of **6a** and **6b** were *R* configuration.

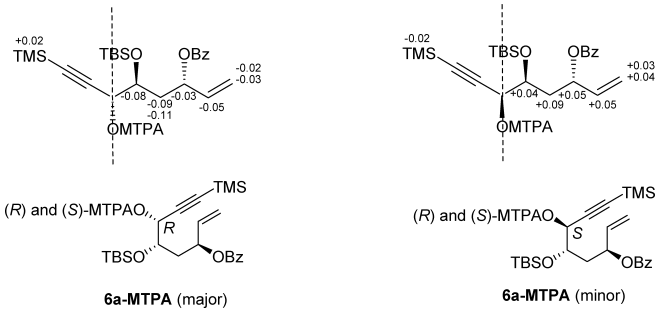


Fig. 2. Determination of the Absolute Configuration of **6a-MTPAs** (Major and Minor)

As in our preceding paper,[10] we transformed **7a**[15] to **9a** and **7b**[15] to **9b**, to try to connect with the CD-ring fragment (**8**), which was prepared from vitamin D_3, to obtain the 14-epi-1α,25(OH)$_2$preD$_3$ analogs (**10a**, **b**) under basic conditions (Chart 2); however, we could not obtain any coupled products **10a**, **b** due to steric hindrance of the substituent at the 4-position of **9**. Therefore, we used Trost's procedure for the coupling reaction.[16]

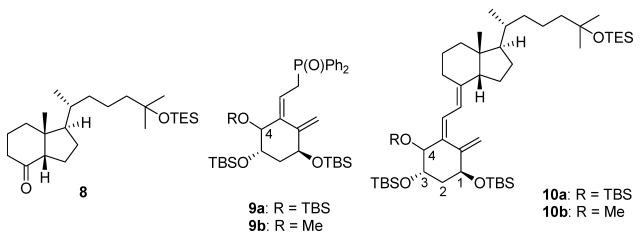


Chart 2

The desired CD-ring fragment **11** was synthesized using **8**[10] by the Wittig reaction (Chart 3)[16,17] and the coupling reaction with **7a** or **7b** was conducted under Trost's coupling conditions (Chart 4). Fortunately, the reaction was straightforward, and the desired coupled compounds **12a** and **12b** were obtained, respectively. Subsequent treatment with tetrabutylammonium fluoride (TBAF) afforded the deprotected compounds, which were then heated at 80 °C in benzene to promote [1,7]-sigmatropic rearrangement. Finally, we could obtain the 4-hydroxy (**3a**) and 4-methoxy (**3b**) analogs of 14-epi-1α,25(OH)$_2$preD$_3$.[18] The major diastereomers at C4 of compounds **3a** and **3b** were purified by HPLC to give **3a*R***[19] and **3b*R***,[20] respectively, and were used for biological evaluation.

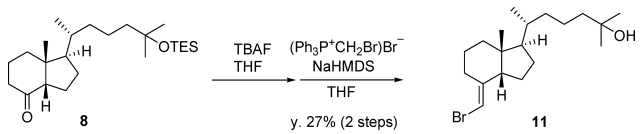


Chart 3

The similar coupling reaction between **7a** and the known CD-ring fragment **14** was applied to synthesize compound **2a** (Chart 5).[16] The reaction gave the coupled product, whose silyl groups were removed by TBAF to afford the desired compound **2a**. The major diastereomer at C4 of this compound was purified by HPLC to give 1α,4β,25-trihydroxyvitamin D_3 (**2a*R***[21]), which was used for biological testing.

VDR binding affinity and osteocalcin promoter transactivation activity of the new compounds were evaluated using the chick intestinal or human VDR, and human osteoblast cell line (HOS) cells, respectively. The results are summarized in Table 1 in comparison with the natural hormone **1** and **14-epi-pre1**, which was prepared in our laboratory.[10] The new compounds had lower activity than **1**, and in terms of the binding affinity for VDR, compounds **3a*R*** and **3b*R*** showed almost the same activity as **14-epi-pre1**. As above, the 4-substitution of vitamin D_3 and 14-epi-previtamin D_3 was thought to be ineffective for VDR binding affinity and osteocalcin promoter transactivation activity.

In conclusion, we synthesized the 4-hydroxy and 4-methoxy analogs of 1α,25(OH)$_2$D$_3$ (**1**) and 14-epi-

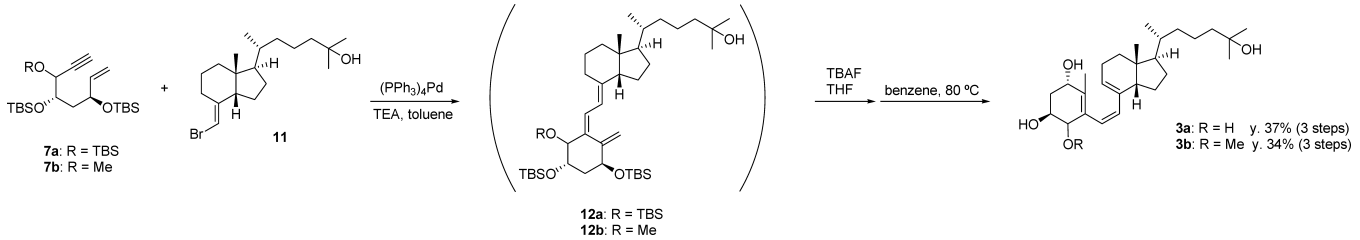


Chart 4

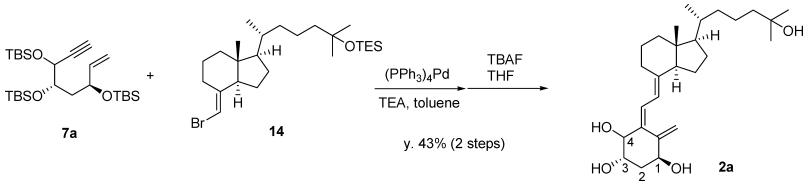
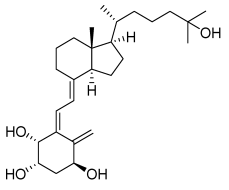


Chart 5

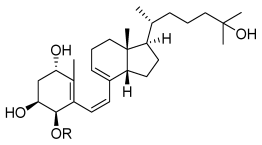
Table 1. Relative Binding Affinity for VDR and Osteocalcin Promoter Transactivation Activity in HOS Cells of **1**, **14-epi-pre1**, and New Compounds

Compound	VDR[a,b,c]	Osteocalcin transactivation activity (ED_{50} (nM))
1	100[b,c]	0.03
2a*R*	2.9[c]	0.22
14-epi-pre1	0.5[b]	0.46
3a*R*	0.1[b]	20.5
3b*R*	0.95[b]	40.5

a) The potency of **1** is normalized to 100. *b*) Chick intesinal VDR was used. *c*) Human VDR was used.



2a*R*



3a*R*: R = H
3b*R*: R = Me

1α,25(OH)$_2$preD$_3$ (**14-epi-pre1**) for the first time. We evaluated VDR binding affinity and osteocalcin promoter transactivation activity in HOS cells. Unfortunately, the biological activities were not improved by 4-substitution; however, the information on new compounds will be useful to create novel potential ligands for VDR.

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15) Enynes **7a** and **7b** were used as diastereomixtures, since it was difficult to separate each diastereomer at this stage without HPLC, respectively.
16) Trost B. M., Dumas J., Villa M., *J. Am. Chem. Soc.*, **114**, 9836—9845 (1992).
17) Deprotection of the TES group was essential for easy separation of the coupled compound from the starting materials in the next reaction (Chart 5).
18) Spectroscopic data of 14-epi-1α,25(OH)$_2$preD$_3$, see: Maynard D. F., Trankle W. G., Norman A. W., Okamura W. H., *J. Med. Chem.*, **37**, 2387—2393 (1994).
19) In ^{1}H-NMR analyses, there were no 6-*s*-*trans* signals. Therefore, we estimated the ratio of the two isomers 6-*cis*/6-*s*-*trans* as 95/<5. Spectroscopic data for **3a*R***: $[\alpha]_D^{19}$ −7.8 (c=0.023, $CHCl_3$); UV (EtOH) λ_{max} 250.0 nm, λ_{min} 228.0 nm; IR (neat) 3379, 1466, 1377, 1215 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.86—1.56 (m, 33H), 3.30—3.34 (m, 3H), 3.62—3.66 (m, 1H), 4.03—4.10 (m, 1H), 4.22 (d, J=5.6 Hz, 2H), 4.49 (d, J=2.4 Hz, 1H), 5.73 (t, J=3.7 Hz, 1H), 5.92 (d, J=5.9 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.9, 19.5, 19.8, 20.5, 21.7, 29.0, 29.3, 29.4, 29.4, 29.8, 34.1, 44.3, 44.4, 45.6, 51.6, 53.4, 68.3, 69.5, 71.1, 71.6, 77.1, 77.2, 77.5, 100.5, 119.5, 126.0, 132.3; EI-HR-MS Calcd for $C_{27}H_{44}O_4$ [M++Na]$^+$ 455.3132, Found 455.3139.
20) In ^{1}H-NMR analyses, there were no 6-*s*-*trans* signals. Therefore, we estimated the ratio of the two isomers 6-*cis*/6-*s*-*trans* as 95/<5. Spectroscopic data for **3b*R***: $[\alpha]_D^{19}$ −142.5 (c=0.07, $CHCl_3$); UV (EtOH) λ_{max} 250.5 nm, λ_{min} 228.0 nm; IR (neat) 3387, 1466, 1377, 1240, 1213 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.89 (s, 3H), 0.93 (d, J=6.4 Hz, 3H), 1.04—1.08 (m, 1H), 1.18—2.08 (m, 28H), 2.16 (s, 2H), 3.47 (s, 3H), 3.67 (d, J=6.4 Hz, 1H), 4.03—4.12 (m, 2H), 5.69 (s, 1H), 5.86 (d, J=12.5 Hz, 1H), 5.98 (d, J=12.5 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.2, 19.6, 19.8, 21.6, 21.9, 23.1, 29.1, 29.6, 33.9, 34.1, 35.8, 40.9, 44.4, 45.8, 52.0, 54.8, 56.4, 59.9, 65.6, 66.5, 69.1, 69.9, 71.0, 126.1, 127.4, 133.2, 137.1, 138.0; EI-HR-MS Calcd for $C_{27}H_{44}O_4$ [M+Na]$^+$ 469.3288, Found 469.3274.
21) Spectroscopic data for **2a*R***: $[\alpha]_D^{21}$ −87.3 (c=0.01, $CHCl_3$); UV (EtOH) λ_{max} 266.0 nm, λ_{min} 212.5 nm; IR (neat) 3383, 1215 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.93 (d, J=6.4 Hz, 3H), 1.19—2.17 (m, 31H), 2.87 (d, J=12.0 Hz, 1H), 3.72 (ddd, J=7.1, 7.1, 14.2 Hz, 2H), 4.19 (s, 1H), 4.27 (s, 1H), 4.42 (d, J=6.1 Hz, 1H), 5.0 (d, J=2.0 Hz, 1H), 5.4 (s, 1H), 6.02 (d, J=11.7 Hz, 1H), 6.66 (d, J=11.7 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.5, 19.8, 20.0, 20.1, 20.8, 22.0, 23.3, 29.1, 29.3, 29.6, 29.7, 30.1, 34.3, 36.1, 38.2, 41.5, 44.7, 45.9, 51.8, 51.9, 52.6, 69.9, 71.5, 77.5, 126.0, 126.3, 134.7, 138.7; EI-HR-MS Calcd for $C_{27}H_{44}O_4$ [M+Na]$^+$ 455.3132, Found 455.3113.