# Synthesis and Biological Activity of 1$\alpha$,2$\alpha$,25-Trihydroxyvitamin $D_3$: Active Metabolite of 2$\alpha$-(3-Hydroxypropoxy)-1$\alpha$,25-dihydroxyvitamin $D_3$ by Human CYP3A4

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Received August 14, 2013; accepted November 1, 2013

**Our previous studies revealed that recombinant human CYP3A4 converted 2$\alpha$-(3-hydroxypropoxy)-1$\alpha$,25-dihydroxyvitamin $D_3$ (O2C3), which was a more potent binder to vitamin D receptor (VDR) than the natural hormone, 1$\alpha$,25-dihydroxyvitamin $D_3$ (1$\alpha$,25(OH)$_2$D$_3$, 1), to 1$\alpha$,2$\alpha$,25-trihydroxyvitamin $D_3$ (2). Here, we synthesized 2 using the Trost Pd-mediated coupling reaction between an A-ring precursor and a CD-ring bromoolefin and evaluated its preliminary biological activity. We found that metabolite 2 from O2C3 was still active as a VDR ligand while maintaining human VDR binding affinity (27.3% of 1$\alpha$,25(OH)$_2$D$_3$) and HL-60 cell differentiation activity (62% of 1$\alpha$,25(OH)$_2$D$_3$).**

**Key words** 1$\alpha$,2$\alpha$,25-trihydroxyvitamin $D_3$; vitamin D receptor; cell differentiation; CYP3A4; CYP24A1

The active metabolite of vitamin $D_3$, 1$\alpha$,25-dihydroxyvitamin $D_3$ [1$\alpha$,25(OH)$_2$D$_3$, **1**], plays important roles in cellular growth, differentiation, apoptosis, and immune responses, in addition to its classical major roles in calcium homeostasis and bone mineralization.[1–4] Actually, **1** and several synthetic analogs of **1** have been used clinically in the treatment of bone diseases, secondary hyperparathyroidism, psoriasis, and osteoporosis.[1,5] Although **1** is inactivated by CYP24A1-dependent catabolism *via* C-24 hydroxylation to calcitroic acid for excretion from the body,[1] it was found that some 2$\alpha$-substituted active vitamin D analogs were highly resistant to CYP24A1, for example, the $k_{cat}/K_m$ value of 2$\alpha$-(3-hydroxypropoxy)-1$\alpha$,25-dihydroxyvitamin $D_3$ (O2C3), which showed 1.8-times greater binding affinity for vitamin D receptor (VDR) than **1**,[6–10] was only 3% of that for **1**.[11] CYP24A1 is the specific enzyme induced by the VDR-ligand (**1** or its analog) complex in the target tissue and inactivates **1** and its analogs; therefore, CYP24A1-resistant ligands would have long-term biological effects on the target tissues.[12] On the other hand, CYP3A4 is a broad-spectrum drug-metabolizing P450 enzyme,[13] but **1** and its analog 2$\alpha$-(3-hydroxypropyl)-1$\alpha$,25-dihydroxyvitamin $D_3$ (O1C3) are not primary substrates for CYP3A4.[12] Recently, however, we demonstrated that O2C3 was metabolized by CYP3A4 and converted to 1$\alpha$,2$\alpha$,25-trihydroxyvitamin $D_3$[12] (**2**, Fig. 1). Its 2$\beta$-isomer (**4**) is a known compound and shows potent 1$\alpha$,25(OH)$_2$D$_3$-like activities,[14] and we report here the synthesis of a new 2$\alpha$-hydroxylated analog **2** using the Trost Pd-mediated coupling reaction between an A-ring precursor **12** and a CD-ring bromoolefin **13** to evaluate its preliminary biological activity.[15–18]

The A-ring precursor **12** for Trost coupling was prepared from the known epoxide **5**[19] in 11 steps (Chart 1). Briefly, treatment of **5** with *p*-methoxybenzyl alkoxide with heating gave methyl 3-*O*-(*p*-methoxybenzyl)altropyranoside **6**, which had the 2$\alpha$-hydorxy group (steroidal numbering) required in target molecule **2**. After 2-*O*-silylation, the *p*-methoxybenzyl (PMB) protecting group was removed by 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) to give **7**, and the methoxymethyl (MOM) group was introduced instead for the next bromination reaction using *N*-bromosuccinimide (NBS). NBS treatment for the resulting *O*-MOM-protected bezylidene acetal gave bromide **8**. Activated Zn-reduction in the presence of $NaBH_3CN$ produced alcohol **9**, which was converted to epoxide **10** *via* tosylation followed by tetrabutylammonium fluoride (TBAF) treatment. Trimethylsilyl (TMS)–ethynylation of **10** afforded enyne **11**, and subsequent solvolysis in $K_2CO_3$–MeOH and *O*-silylation provided enyne **12** (Chart 1).

The CD-ring bromoolefin **13**[20] and enyne **12** obtained above were connected using Pd-catalyst to give the coupling product **14**.[21] Desilylation by TBAF and deacetalization under acidic conditions gave the target molecule **2**[22] (Chart 2). The isolated product was re-purified by HPLC to test biological activity.

The binding affinity of the new analog **2** for the human vitamin D receptor (hVDR)[23] and induction activity of HL-60 cell differentiation[24] are shown in Table 1. The new analog **2** was still active, like its 2$\beta$-diastereoisomer **4**,[14] and these results were different from 4-OH analogs of 1$\alpha$,4$\alpha$,25-trihydroxyvitamin $D_3$ and 1$\alpha$,4$\beta$,25-trihydroxyvitamin $D_3$, which were very weak agonistic ligands for hVDR (for hVDR binding affinity: 0.9% and 2.9% of the natural hormone **1**, respectively).[25] The 2$\alpha$-OH analog **2** showed lower binding affinity for hVDR than that of the natural hormone **1**, and X-ray cocrystallographic analysis of 2$\alpha$-methyl-1$\alpha$,25-dihydroxyvitamin $D_3$, which was a better binder for hVDR than **1**, in the ligand binding domain (LBD) of hVDR explained the 2$\alpha$-methyl group of **2** was fitted in the hydrophobic pocket formed by Phe150, Leu233, and Ser237[10]; therefore, the 2$\alpha$-OH group of **2** at the same position as the 2$\alpha$-methyl group would not be suitable for the pocket to bind the LBD of hVDR. The 2$\beta$-OH group with the different direction in the LBD may not be disturbed in binding.[14]

The authors declare no conflict of interest.

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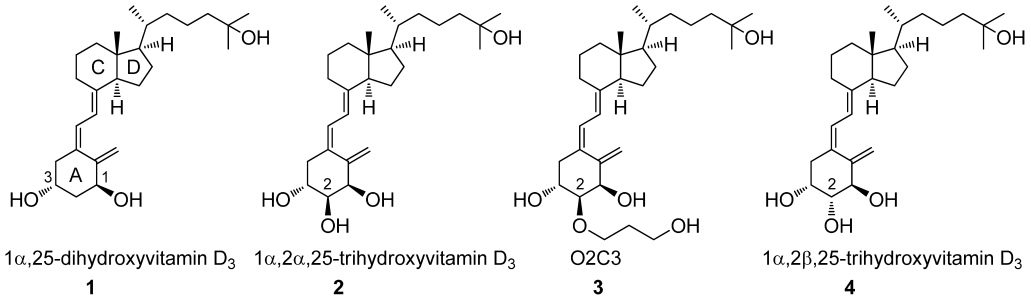


Fig. 1. Structures of 1$\alpha$,25-Dihydroxyvitamin $D_3$ (**1**) and 2-Oxygenated Analogs **2**–**4**

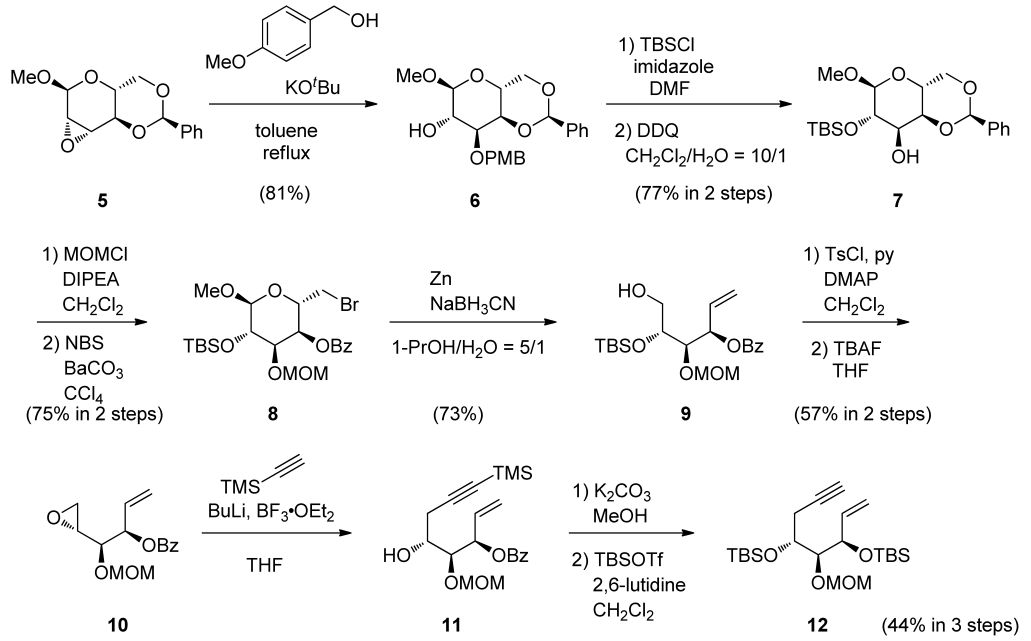


Chart 1. Synthetic Route to A-Ring Precursor **12**

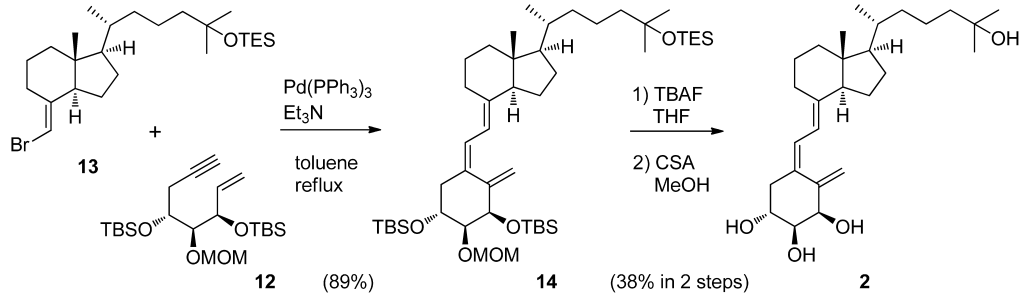


Chart 2. Synthesis of 1$\alpha$,2$\alpha$,25-Trihydroxyvitamin $D_3$ (**2**)

Table 1. Relative Binding Affinity for hVDR and HL-60 Cell Differentiation Activity of **1**–**4**

| Compound | hVDR binding affinity[a)] | HL-60 cell differentiation[a,b)] |
|---|---|---|
| 1$\alpha$,25$(OH)_2D_3$ (**1**) | 100 | 100 |
| 1$\alpha$,2$\alpha$,25$(OH)_3D_3$ (**2**) | 27.3 | 62 |
| O2C3 (**3**) | 180[c)] | 55 |
| 1$\alpha$,2$\beta$,25$(OH)_3D_3$ (**4**) | 110[d)] | 44[d)] |

*a*) The potency of 1$\alpha$,25$(OH)_3D_3$ is normalized to 100. *b*) Relative activity is calculated at $EC_{50}$. *c*) References 6 and 7. *d*) Reference 14.

## Conclusion

We synthesized a new analog of active vitamin $D_3$, 1$\alpha$,2$\alpha$,25-trihydroxyvitamin $D_3$ (**2**), which was the major metabolite of O2C3 by CYP3A4, to study its biological activity. **2** showed moderate hVDR binding affinity and potent HL-60 cell differentiation activity ($EC_{50}$ 68 nM: 62% activity of **1**). The data demonstrate that **2** and **4** with 2$\alpha$- and 2$\beta$-stereochemistries of the 2-OH group on the active vitamin D skeleton maintain VDR binding and HL-60 cell differentiation activity. This is a totally different effect from that of the 4-OH group.[25] Although O2C3 was resistant to CYP24A1, its CYP3A4 metabolite **2** was further metabolized by CYP24A1, similar to **1**. The $k_{cat}/K_m$ value of hCYP24A1 for **2** was 60% of that for **1**.[12] Further biological testing is underway in our laboratories.

**Acknowledgments** This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 25860011 to M.T.) as well as a Grant-in-Aid from the Japan Society for the Promotion of Science (No. 24590021 to A.K.).

## References and Notes

1) Feldman D., Pike J. W., Adams J. S., "Vitamin D," 3rd ed., Elsevier Academic Press, New York, 2011.
2) Brown A. J., Slatopolsky E., *Mol. Aspects Med.*, **29**, 433–452 (2008).
3) Laverny G., Penna G., Uskokovic M., Marczak S., Maehr H., Jankowski P., Ceailles C., Vouros P., Smith B., Robinson M., Reddy G. S., Adorini L., *J. Med. Chem.*, **52**, 2204–2213 (2009).
4) DeLuca H. F., *Nutr. Rev.*, **66** (Suppl. 2), S73–S87 (2008).
5) Kubodera N., *Heterocycles*, **80**, 83–98 (2010).
6) Kittaka A., Suhara Y., Takayanagi H., Fujishima T., Kurihara M., Takayama H., *Org. Lett.*, **2**, 2619–2622 (2000).
7) Saito N., Suhara Y., Kurihara M., Fujishima T., Honzawa S., Takayanagi H., Kozono T., Matsumoto M., Ohmori M., Miyata N., Takayama H., Kittaka A., *J. Org. Chem.*, **69**, 7463–7471 (2004).
8) Kittaka A., *Yakugaku Zasshi*, **128**, 1235–1250 (2008).
9) Takahashi E., Nakagawa K., Suhara Y., Kittaka A., Nihei K., Konno K., Takayama H., Ozono K., Okano T., *Biol. Pharm. Bull.*, **29**, 2246–2250 (2006).
10) Hourai S., Fujishima T., Kittaka A., Suhara Y., Takayama H., Rochel N., Moras D., *J. Med. Chem.*, **49**, 5199–5205 (2006).
11) Abe D., Sakaki T., Kusudo T., Kittaka A., Saito N., Suhara Y., Fujishima T., Takayama H., Hamamoto H., Kamakura M., Ohta M., Inouye K., *Drug Metab. Dispos.*, **33**, 778–784 (2005).
12) Yasuda K., Ikushiro S., Kamakura M., Takano M., Saito N., Kittaka A., Chen T. C., Ohta M., Sakaki T., *J. Steroid Biochem. Mol. Biol.*, **133**, 84–92 (2013).
13) Zhu J., DeLuca H. F., *Arch. Biochem. Biophys.*, **523**, 30–36 (2012).
14) Tsugawa N., Nakagawa K., Kurobe M., Ono Y., Kubodera N., Ozono K., Okano T., *Biol. Pharm. Bull.*, **23**, 66–71 (2000).
15) Synthesis of the A-ring precursor of **2** was reported, see: Posner G. H., Nelson T. D., *J. Org. Chem.*, **56**, 4339–4341 (1991).
16) Previously, synthesis of 1$\alpha$,2$\alpha$,25-trihydroxy-19-norvitamin $D_3$ was reported, see: Sicinski R. R., Perlman K. L., DeLuca H. F., *J. Med. Chem.*, **37**, 3730–3738 (1994). See also refs. 17 and 18.
17) Shimizu M., Iwasaki Y., Shibamoto Y., Sato M., DeLuca H. F., Yamada S., *Bioorg. Med. Chem. Lett.*, **13**, 809–812 (2003).
18) Ono K., Yoshida A., Saito N., Fujishima T., Honzawa S., Suhara Y., Kishimoto S., Sugiura T., Waku K., Takayama H., Kittaka A., *J. Org. Chem.*, **68**, 7407–7415 (2003).
19) Wiggings L. S., *Methods Carbohydr. Chem.*, **2**, 188–191 (1963).
20) Matsuo M., Hasegawa A., Takano M., Nakamura Y., Saito H., Kakuda S., Takagi K., Ochiai E., Horie K., Takimoto-Kamimura M., Takenouchi K., Sawada D., Kittaka A. *ACS Med. Chem. Lett.*, **4**, 671–674 (2013).
21) Trost B. M., Dumas J., Villa M., *J. Am. Chem. Soc.*, **114**, 9836–9845 (1992).
22) Spectroscopic data for **2**: $[\alpha]_D^{22}$ +45.8 ($c$=0.3, $CHCl_3$); UV (EtOH) $\lambda_{max}$ 266.5 nm, $\lambda_{min}$ 227.5 nm; IR (neat) 3379, 2927, 2858, 1724, 1643, 1462, 1377, 1284, 1219, 1142, 1072, 918 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$) $\delta$: 0.53 (s, 3H), 0.93 (d, $J$=6.3 Hz, 3H), 1.21–1.76 (m, 27H), 1.96–2.01 (m, 2H), 2.16–2.26 (m, 2H), 3.70–3.72 (m, 1H), 4.41 (ddd, $J$=7.1, 13.7, 14.1 Hz, 1H), 4.42 (ddd, $J$=7.1, 13.7, 14.2 Hz, 1H), 5.01 (d, $J$=1.7 Hz, 1H), 5.27 (d, $J$=0.98 Hz, 1H), 5.96 (d, $J$=11.2 Hz, 1H), 6.40 (d, $J$=12.0 Hz, 1H). $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$: 12.1, 18.8, 20.8, 22.2, 23.5, 27.6, 29.2, 29.4, 29.7, 36.1, 36.4, 40.5, 41.3, 44.4, 46.0, 56.4, 56.5, 70.5, 71.1, 74.9, 77.3, 117.0, 117.1, 125.9, 128.8, 131.3, 144.1; ESI-HR-MS Calcd for $C_{27}H_{44}O_4$ $[M+Na]^+$ 455.3132, Found 455.3108.
23) Binding affinity for hVDR was evaluated using a 1$\alpha$,25$(OH)_2D_3$ assay kit (Polarscreen Vitamin D Receptor Competitor Assay, Red, Cat. No. PV4569) purchased from Invitrogen.
24) Potency of induction of HL-60 cell differentiation was tested as described in ref. 18.
25) Sawada D., Tsukuda Y., Yasuda K., Sakaki T., Saito H., Takagi K., Takenouchi K., Chen T. C., Reddy G. S., Kittaka A., *Chem. Pharm. Bull.*, **60**, 1343–1346 (2012).