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Concise Synthesis and Biological Activities of 2α-Alkyl- and 2α-(ω-Hydroxyalkyl)-20-*epi*-1α,25-dihydroxyvitamin D_3

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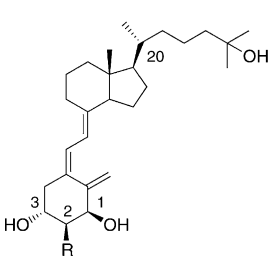
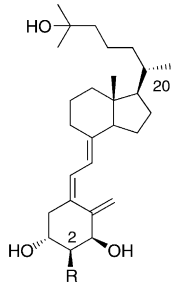
Abstract—We found a concise route to the Trost A-ring precursor enyne for synthesizing 2α-alkylated 1α,25-dihydroxyvitamin D_3 (**1**) from D-glucose. The enynes were coupled with the 20-*epi*-CD ring part to study the effect of the double modification of 2α-substitution and 20-epimerization upon biological activities of **1**. The novel three analogues of 2α-alkyl- and four analogues of 2α-(ω-hydroxyalkyl)-20-*epi*-1α,25-dihydroxyvitamin D_3 (**5b–d** and **6a–d**) showed higher binding affinity for vitamin D receptor (VDR) and more potent activity in induction of HL-60 cell differentiation than those of the natural hormone **1**.

1α,25-Dihydroxyvitamin D_3 (**1**), the hormonally active form of vitamin D_3, mediates intestinal calcium absorption, and bone resorption and mineralization. In addition, **1** has been found to exhibit a variety of biological activities in many tissues and cells since the discovery of the fact that **1** induces cell differentiation and proliferation.[1] This renewed interest has prompted numerous efforts to synthesize a huge number of vitamin D analogues in order to investigate biological roles of this hormone and to develop potential therapeutic agents.[2,3]

Previously, we synthesized A-ring modified analogues **2a–d** and **3a–d**, in which the 2α-alkyl or the 2α-hydroxyalkyl group was introduced to **1**, to study the A-ring conformation- and structure–activity relationships (Fig. 1).[4–6] The resulting analogues exhibited interesting biological activities, in particular 2α-methyl and 2α-(3-hydroxypropyl) analogues (**2a** and **3c**) showed much higher potency than **1** in terms of binding affinity for bovine thymus vitamin D receptor (VDR), elevation of rat serum calcium concentration, and induction of HL-60 cell differentiation.[4,5,6b]





1: 1α,25-dihydroxyvitamin D_3 (R = H)
2a: R = CH_3
2b: R = CH_2CH_3
2c: R = $CH_2CH_2CH_3$
2d: R = $CH_2CH_2CH_2CH_3$
3a: R = CH_2OH
3b: R = CH_2CH_2OH
3c: R = $CH_2CH_2CH_2OH$
3d: R = $CH_2CH_2CH_2CH_2OH$

4: 20-*epi*-1α,25-dihydroxyvitamin D_3 (R = H)
5a: R = CH_3
5b: R = CH_2CH_3
5c: R = $CH_2CH_2CH_3$
5d: R = $CH_2CH_2CH_2CH_3$
6a: R = CH_2OH
6b: R = CH_2CH_2OH
6c: R = $CH_2CH_2CH_2OH$
6d: R = $CH_2CH_2CH_2CH_2OH$

Figure 1. Structures of the natural hormone **1**, its 2α-substituted analogues **2–3**; 20-*epi*-derivative **4**, and its 2α-substituted analogues **5–6**.

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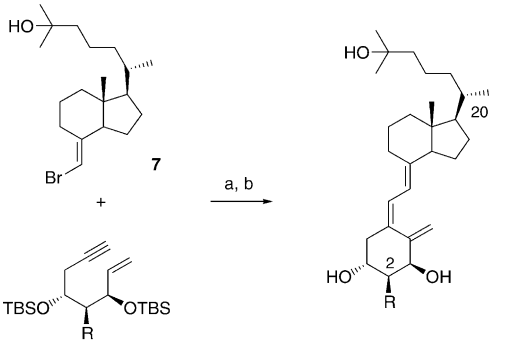
For example, 2α-(3-hydroxypropyl) analogue **3c** exhibited 3-fold higher VDR binding affinity, approximately 500 times higher potency for elevation of rat serum calcium concentration than those of **1**.[4b] These remarkably high activities are unique among vitamin D analogues reported to date. On the other hand, structural modifications of the CD-ring side chain have been intensively studied in the past.[7] Among them, 20-*epi*-1α,25-dihydroxyvitamin D_3 (**4**) is known as one of the typical compounds exhibiting high cell differentiation activity with relatively low calcemic effects.[8–10] It is tempting to speculate that modification at the 2α-position on the A-ring may correlate to the structure of the CD-ring side chain providing potential biological activity, which would be different from its parent hormone. Indeed, we synthesized all possible diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 with 20-epimerization, and found 2α-methyl-20-*epi*-1α,25-dihydroxyvitamin D_3 (**5a**) exhibited exceptionally high potency in VDR binding, transcriptional potency, and HL-60 cell differentiation activity.[11] We expect that combination of this 20-epimerization and 2α-substitution would lead to highly promising analogues.[12] We report here an improved synthesis of the A-ring precursor enynes **8b–d** for 2α-alkylated **4** from D-glucose, utilizing highly active Grignard reagents, and biological evaluation for VDR binding affinity and HL-60 cell differentiation activity of totally seven novel analogues of 2α-substituted 1α,25-dihydroxyvitamin D_3 with 20-epimerization of the CD-ring moiety (**5b–d**, **6a–d**).

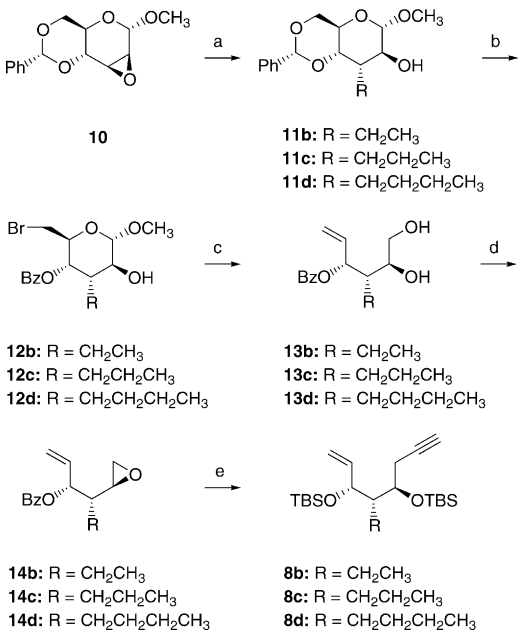
The analogues were synthesized by employing the convergent method of Trost and co-workers using palladium-catalyzed coupling reaction of the A-ring synthon, enyne, with the CD-ring portion.[13] Scheme 1 outlines the synthetic route to the target compounds **5b–d** and **6a–d**. The A-ring precursors **8b–d** were synthesized in eight steps from epoxide **10**, which is readily available from methyl α-D-glucoside (Scheme 2).[14] The reaction of ethyl magnesium chloride with epoxide **10** using toluene as a reaction solvent gave epoxy ring opening product **11b** regioselectively in 74% yield. *n*-Propyl and



8b: R = CH_2CH_3
8c: R = $CH_2CH_2CH_3$
8d: R = $CH_2CH_2CH_2CH_3$
9a: R = CH_2OTBS
9b: R = CH_2CH_2OTBS
9c: R = $CH_2CH_2CH_2OTBS$
9d: R = $CH_2CH_2CH_2CH_2OTBS$

5b: R = CH_2CH_3
5c: R = $CH_2CH_2CH_3$
5d: R = $CH_2CH_2CH_2CH_3$
6a: R = CH_2OH
6b: R = CH_2CH_2OH
6c: R = $CH_2CH_2CH_2OH$
6d: R = $CH_2CH_2CH_2CH_2OH$

Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, TEA/toluene (3:1), reflux; (b) 1 M TBAF in THF; 19–56% for two steps.



Scheme 2. Reagents and conditions: (a) RMgCl, toluene, **11b** (74%), **11c** (75%), and **11d** (83%); (b) NBS, $BaCO_3$, CCl_4, reflux, **12b** (69%), **12c** (85%), and **12d** (68%); (c) Zn powder, $NaBH_3CN$, 1-propanol–H_2O (10:1), 95 °C, **13b** (70%), **13c** (75%), and **13d** (67%); (d) (i) TmCl, DMAP, CH_2Cl_2, (ii) LiHMDS, THF, −78 °C to rt, **14b** (57%), **14c** (76%), and **14d** (72%) for two steps; (e) (i) TMSCCH, BuLi, $BF_3{\cdot}OEt_2$, THF, −78 °C, (ii) K_2CO_3, MeOH, (iii) TBSOTf, 2,6-lutidine, **8b** (72%), **8c** (77%), and **8d** (46%) for three steps.

n-butyl groups could be introduced in the same way. It is important to note that the use of toluene is essential in this step to realize shorter reaction time and higher chemical yield as compared with the use of conventional ethereal solvents such as diethyl ether and THF.[15] Acetals **11b–d** were treated with *N*-bromosuccinimide to give bromo benzoates **12b–d**. Bromides **12b–d** reacted with zinc in the presence of sodium cyanoborohydride to give 1,2-diols **13b–d**, which were converted to epoxides **14b–d** through sulfonylation of the primary hydroxy group, followed by base treatment. A-ring precursors **8b–d** were obtained by the reaction of **14b–d** with lithium acetylide, and protecting group manipulations.

Enynes **9a–d** were prepared by our reported method.[4] Bromoolefin **7** with the 20-*epi*-CD-ring moiety was synthesized from vitamin D_2.[16]

Those synthons thus obtained were coupled using tetrakis(triphenylphosphine)palladium(0) as a catalyst followed by deprotection of the silyl ethers with 1 M TBAF in THF gave the desired compounds **5b–d**[17–19] and **6a–d**.[20–23] These target compounds were purified by recycled reverse-phase HPLC for the biological evaluation.

We determined binding affinity of **5b–d** and **6a–d** to the bovine thymus VDR.[24] As we expected, the VDR affinity of 20-epimers **5b–d** and **6a–d** were increased when compared to that of the corresponding natural side chain derivatives of **2b–d** and **3a–d** (Table 1). Especially, the 2α-hydroxypropyl analogue **6c** showed the highest VDR binding affinity in this series, that is, 3.7-fold

Table 1. Relative binding affinity of 2α-substituted-**1** (**2**–**3**) and 2α-substituted-20-*epi*-**1** (**5**–**6**) for bovine thymus VDR and their potency in induction of HL-60 cell differentiation

Compd	Binding affinity to VDR[a]	HL-60 cell differentiation[a,b]
1	100	100
2a	400[5]	258[5]
2b	40[4]	106[4]
2c	20[4]	44[4]
2d	8[4]	74
3a	20[4]	10[4]
3b	70[4]	86[4]
3c	300[4]	240[4]
3d	120[4]	460
4	400[16]	3571[16]
5a	1200[11]	9688[11]
5b	150	4200
5c	119	1750
5d	33	2300
6a	190	570
6b	320	4030
6c	370	4900
6d	183	3910

[a]The potency of **1** is normalized to 100.
[b]Data are the mean of three separate experiments.

higher than that of **1**. Even 20-epimerization of **3a** provided a better ligand (**6a**) in shape than the natural hormone for the VDR.

Except **5a**, however, simple 20-epimerization of the parent steroid without 2α-(ω-hydroxyalkyl) group (**4**) provided the best results in VDR binding. The modeled structure of **6c** constructed by molecular dynamics calculations (AMBER* force field) using MacroModel ver. 6.5. is shown in Figure 2. Although **6c** (red) could form an additional hydrogen bonding between the terminal hydroxyl of the 2α-hydroxypropyl group and Arg 274 in the ligand binding domain of the VDR, the positions of the two hydroxyls (1α,3β) on the A-ring are slightly different from the original positions in **4** (green).[10] This effect based on the 2α-substituent may decrease the binding affinity to the VDR. Next, we evaluated the HL-60 cell differentiation activity of the analogues **5b**–**d** and **6a**–**d** by NBT reduction method.[25] As shown in Table 1, 20-epimerization raised the induction of differentiation activity of HL-60 cells markedly, it was more than one order. Tendency to magnify the cell differentiation activity was closely related to the VDR binding affinity,[26] and **6c** exhibited the highest potency in this experiment. Although introduction of the 2α-substituent into the 20-*epi* analogue **4** did not cause very dramatic alternation in the activity if compared with **5a**, it would be interesting to examine the other biological activities in vivo. For example, ED-71, which has the 2β-(3-hydroxypropoxyl) group in **1**, shows unique biological activity profiles; high calcemic activity along with a long half-life in plasma, and the clinical trial of ED-71 as a promising candidate for treatment of osteoporosis has been conducted.[27]

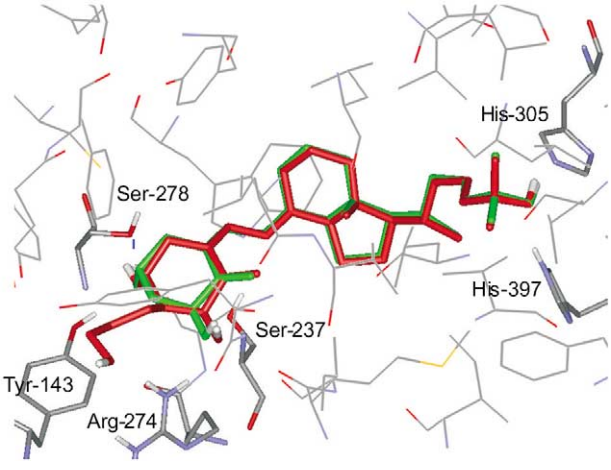


Figure 2. Modeled structure of **6c** (red) with Moras X-ray structure of the VDR-**4** (green) complex.[10]

In summary, we have synthesized seven new analogues of 2α-alkyl- and 2α-(ω-hydroxyalkyl)-20-*epi*-1α,25-dihydroxyvitamin D_3 and evaluated their VDR binding affinity and HL-60 cell differentiation activity. These double modifications on both the A-ring and the side chain increased VDR binding affinity and potency in induction of HL-60 cell differentiation compared to those of the natural hormone **1**. We consider that these results provide important information for vitamin D research with regard to the structure–activity relationships of the A-ring and the side-chain moiety.[28]

Acknowledgements

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References and Notes

1. DeLuca, H. F. *FASEB J.* **1988**, *2*, 224.
2. Recent Advances in Vitamin D Chemistry and Pharmacological Activity. Uskokovic, M. R., Ed. *Bioorg. Med. Chem. Lett. Symposia-in-print* **1993**, *3*, 1775.
3. Jones, G.; Calverley, M. J. *Trends Endocrinol. Metab.* **1993**, *4*, 297.
4. (a) Suhara, Y.; Nihei, K.; Kurihara, M.; Kittaka, A.; Yamaguchi, K.; Fujishima, T.; Konno, K.; Miyata, N.; Takayama, H. *J. Org. Chem.* **2001**, *66*, 8760. (b) Suhara, Y.; Nihei, K.; Tanigawa, H.; Fujishima, T.; Konno, K.; Nakagawa, K.; Okano, T.; Takayama, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1129.
5. (a) Konno, K.; Fujishima, T.; Maki, S.; Liu, Z.-P.; Miura, D.; Chokki, M.; Ishizuka, S.; Yamaguchi, K.; Kan, Y.; Kurihara, M.; Miyata, N.; Smith, C.; DeLuca, F. H.; Takayama, H. *J. Med. Chem.* **2000**, *43*, 4247. (b) Konno, K.; Maki, S.; Fujishima, T.; Liu, Z.-P.; Miura, D.; Chokki, M.; Takayama, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 151.

6. For 2α-hydroxyalkoxyl series, see: (a) Kittaka, A.; Suhara, Y.; Takayanagi, H.; Fujishima, T.; Kurihara, M.; Takayama, H. *Org. Lett.* **2000**, *2*, 2619. For our account, see: (b) Takayama, H.; Kittaka, A.; Fujishima, T.; Suhara, Y. In: *Vitamin D Analogs in Cancer Prevention and Therapy: Recent Results in Cancer Research 164*; Reichrath, J., Friedrich, M., Tilgren, W., Eds.; Springer-Verlag: Berlin, Heidelberg, 2003, p 289.
7. Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200.
8. Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M. J.; Hansen, K. *Biochem. Pharmacol.* **1991**, *42*, 1569.
9. Dilworth, F. J.; Calverley, M. J.; Makin, H. L. J.; Jones, G. *Biochem. Pharmacol.* **1994**, *47*, 987.
10. The crystal structures of the ligand binding domain (LBD) of VDR complexed to **1** and **4** resembled the conformations of LBD, even though the C20 methyl group demonstrated inverted geometry in LBD, see: Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Mitschler, A.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5491.
11. (a) Fujishima, T.; Liu, Z.-P.; Miura, D.; Chokki, M.; Ishizuka, S.; Konno, K.; Takayama, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2145. (b) Nakagawa, K.; Kurobe, M.; Ozono, K.; Konno, K.; Fujishima, T.; Takayama, H.; Okano, T. *Biochem. Pharmacol.* **2000**, *59*, 691.
12. Fujishima, T.; Liu, Z.-P.; Konno, K.; Nakagawa, K.; Okano, T.; Yamaguchi, K.; Takayama, H. *Bioorg. Med. Chem.* **2001**, *9*, 525.
13. Trost, B. M.; Dumas, J.; Villa, M. *J. Am. Chem. Soc.* **1992**, *114*, 9836.
14. Wiggins, L. S. *Methods Carbohydr. Chem.* **1963**, *2*, 188.
15. For example, this reaction proceeded only in 24% when *n*-BuMgCl was used in THF. Honzawa, S.; Yamamoto, Y.; Hirasaka, K.; Takayama, H.; Kittaka, A. *Heterocycles*, in press.
16. Fujishima, T.; Konno, K.; Nakagawa, K.; Kurobe, M.; Okano, T.; Takayama, H. *Bioorg. Med. Chem.* **2000**, *8*, 123.
17. Data for **5b**: $[\alpha]_D^{27}$ −911.9 (*c* 0.007, $CHCl_3$); UV (EtOH) λ_{max} 268 nm, λ_{min} 229 nm; ^1H NMR (400 MHz, $CDCl_3$–D_2O) δ 0.53 (s, 3H), 0.85 (d, 3H, *J* = 6.6 Hz), 1.21 (s, 6H), 2.20 (dd, 1H, *J* = 8.5, 13.2 Hz), 2.66 (dd, 1H, *J* = 4.1, 13.2 Hz), 2.83 (m, 1H), 3.89 (m, 1H), 4.34 (dd, 1H, *J* = 4.5 Hz), 4.99 (d, 1H, *J* = 2.0 Hz), 5.27 (d, 1H, *J* = 2.0 Hz), 6.00 (d, 1H, *J* = 11.3 Hz), 6.40 (d, 1H, *J* = 11.3 Hz); HREIMS calcd for $C_{29}H_{48}O_3$ (M^+) 444.3604, found 444.3594.
18. Data for **5c**: $[\alpha]_D^{27}$ +459.7 (*c* 0.003, $CHCl_3$); UV (EtOH) λ_{max} 268 nm, λ_{min} 228 nm; ^1H NMR (400 MHz, $CDCl_3$–D_2O) δ 0.53 (s, 3H), 0.85 (d, 3H, *J* = 6.6 Hz), 1.21 (s, 6H), 2.26 (dd, 1H, *J* = 8.4, 13.7 Hz), 2.66 (dd, 1H, *J* = 4.2, 13.7 Hz), 2.83 (m, 1H), 3.92 (dt, 1H, *J* = 4.2, 8.4 Hz), 4.34 (d, 1H, *J* = 2.4 Hz), 4.93 (d, 1H, *J* = 2.2 Hz), 5.18 (d, 1H, *J* = 2.2 Hz), 6.05 (d, 1H, *J* = 11.2 Hz), 6.32 (d, 1H, *J* = 11.2 Hz); HREIMS calcd for $C_{30}H_{50}O_3$ (M^+) 458.3760, found 458.3765.
19. Data for **5d**: $[\alpha]_D^{22}$ +1.4 (*c* 0.33, $CHCl_3$); UV (EtOH) λ_{max} 268 nm, λ_{min} 227 nm; ^1H NMR (400 MHz, $CDCl_3$–D_2O) δ 0.53 (s, 3H), 0.85 (d, 3H, *J* = 6.4 Hz), 0.92 (t, 3H, *J* = 7.0 Hz), 1.21 (s, 6H), 2.24 (dd, 1H, *J* = 8.6, 13.0 Hz), 2.66 (dd, 1H, *J* = 4.4, 13.2 Hz), 2.83 (m, 1H), 3.88 (ddd, 1H, *J* = 4.4, 8.4, 8.4 Hz), 4.37 (d, 1H, *J* = 3.2 Hz), 4.99 (d, 1H, *J* = 1.8 Hz), 5.27 (d, 1H, *J* = 1.8 Hz), 6.01 (d, 1H, *J* = 11.2 Hz), 6.40 (d, 1H, *J* = 11.2 Hz); HREIMS calcd for $C_{31}H_{52}O_3$ (M^+) 472.3916, found 472.3909.
20. Data for **6a**: $[\alpha]_D^{27}$ +157.8 (*c* 0.03, $CHCl_3$); UV (EtOH) λ_{max} 269 nm, λ_{min} 226 nm; ^1H NMR (400 MHz, $CDCl_3$–D_2O) δ 0.53 (s, 3H), 0.85 (d, 3H, *J* = 6.6 Hz), 2.31 (m, 2H), 2.66 (dd, 1H, *J* = 4.8, 13.2 Hz), 2.88 (m, 1H), 3.96 (dd, 1H, *J* = 4.4, 11.4 Hz), 4.03 (m, 1H), 4.24 (m, 1H), 4.46 (d, 1H, *J* = 2.9 Hz), 5.01 (d, 1H, *J* = 2.2 Hz), 5.29 (d, 1H, *J* = 2.2 Hz), 5.98 (d, 1H, *J* = 11.5 Hz), 6.45 (d, 1H, *J* = 11.5 Hz); HREIMS calcd for $C_{28}H_{44}O_3$ $(M-H_2O)^+$ 428.3291, found 428.3316.
21. Data for **6b**: $[\alpha]_D^{27}$ +29.5 (*c* 0.007, $CHCl_3$); UV (EtOH) λ_{max} 268 nm, λ_{min} 227 nm; ^1H NMR (400 MHz, $CDCl_3$–D_2O) δ 0.53 (s, 3H), 0.85 (d, 3H, *J* = 6.4 Hz), 1.21 (s, 6H), 2.26 (dd, 1H, *J* = 8.5, 13.4 Hz), 2.66 (dd, 1H, *J* = 4.3, 13.4 Hz), 2.83 (m, 1H), 3.79 (m, 2H), 3.94 (m, 1H), 4.37 (bs, 1H), 5.02 (d, 1H, *J* = 1.8 Hz), 5.30 (bs, 1H), 6.01 (d, 1H, *J* = 11.2 Hz), 6.40 (d, 1H, *J* = 11.2 Hz); HREIMS calcd for $C_{29}H_{48}O_4$ (M^+) 460.3552, found 460.3532.
22. Data for **6c**: $[\alpha]_D^{27}$ +8.3 (*c* 0.01, $CHCl_3$); UV (EtOH) λ_{max} 267 nm, λ_{min} 227 nm; ^1H NMR (400 MHz, $CDCl_3$–D_2O) δ 0.53 (s, 3H), 0.85 (d, 3H, *J* = 6.6 Hz), 1.21 (s, 6H), 2.26 (dd, 1H, *J* = 9.4, 13.5 Hz), 2.66 (dd, 1H, *J* = 4.4, 13.5 Hz), 2.83 (m, 1H), 3.69 (m, 2H), 3.89 (dt, 1H, *J* = 4.2, 8.4 Hz), 4.36 (d, 1H, *J* = 2.9 Hz), 4.99 (d, 1H, *J* = 2.2 Hz), 5.27 (d, 1H, *J* = 2.2 Hz), 6.00 (d, 1H, *J* = 11.4 Hz), 6.40 (d, 1H, *J* = 11.4 Hz); HREIMS calcd for $C_{30}H_{50}O_4$ (M^+) 474.3709, found 474.3726.
23. Data for **6d**: $[\alpha]_D^{27}$ −509.4 (*c* 0.06, $CHCl_3$); UV (EtOH) λ_{max} 268 nm, λ_{min} 227 nm; ^1H NMR (400 MHz, $CDCl_3$–D_2O) δ 0.53 (s, 3H), 0.85 (d, 3H, *J* = 6.6 Hz), 1.21 (s, 6H), 2.26 (dd, 1H, *J* = 9.4, 13.5 Hz), 2.66 (dd, 1H, *J* = 4.4, 13.5 Hz), 2.83 (m, 1H), 3.69 (m, 2H), 3.89 (dt, 1H, *J* = 4.2, 8.4 Hz), 4.36 (d, 1H, *J* = 2.9 Hz), 4.99 (d, 1H, *J* = 2.2 Hz), 5.27 (d, 1H, *J* = 2.2 Hz), 6.00 (d, 1H, *J* = 11.4 Hz), 6.40 (d, 1H, *J* = 11.4 Hz); HREIMS calcd for $C_{31}H_{52}O_4$ $(M-H_2O)^+$ 470.3760, found 470.3763.
24. Imae, Y.; Manaka, A.; Yoshida, N.; Ishimi, Y.; Shinki, T.; Abe, E.; Suda, T.; Konno, K.; Takayama, H.; Yamada, S. *Biochim. Biophys. Acta* **1994**, *1213*, 302.
25. Collins, S. J.; Ruscetti, F. W.; Gallagher, R. E.; Gallo, R. C. *J. Exp. Med.* **1979**, *149*, 969.
26. Discussion on biological activities and VDR binding affinity, see: Masuno, H.; Yamamoto, K.; Wang, X.; Choi, M.; Ooizumi, H.; Shinki, T.; Yamada, S. *J. Med. Chem.* **2002**, *45*, 1825.
27. Kubodera, N.; Sato, K.; Nishii, Y. In *Vitamin D*; Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic: New York, 1997; p 1071.
28. Okano, T.; Nakagawa, K.; Kubodera, N.; Ozono, K.; Isaka, A.; Osawa, A.; Terada, M.; Mikami, K. *Chem. Biol.* **2000**, *7*, 173.