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Remarkable effect of 2α-modification on the VDR antagonistic activity of 1α-hydroxyvitamin D_3-26,23-lactones

Nozomi Saito,[a] Toshihiro Matsunaga,[a] Toshie Fujishima,[a] Miyuki Anzai,[b] Hiroshi Saito,[b] Kazuya Takenouchi,[b] Daishiro Miura,[b] Seiichi Ishizuka,[b] Hiroaki Takayama[a] and Atsushi Kittaka*[a]

[a] *Faculty of Pharmaceutical Sciences, Teikyo University, Kanagawa 199-0195, Japan. E-mail: akittaka@pharm.teikyo-u.ac.jp; Fax: +81-426-85-3714; Tel: +81-426-85-3715*

[b] *Teijin Institute for Bio-Medical Research, Tokyo 191-8512, Japan; Fax: +81-42-586-8298; Tel: +81-42-586-8220*

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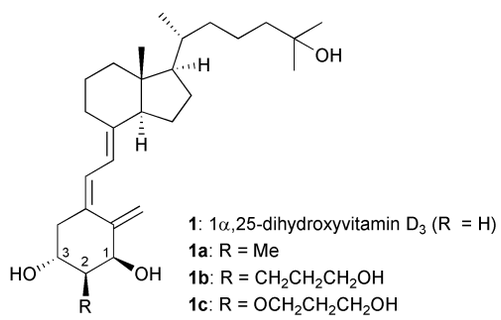
Novel 2α-methyl-, 2α-(3-hydroxypropyl)- and 2α-(3-hydroxypropoxy)-substituted 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone derivatives were efficiently synthesized *via* Reformatsky type allylation and palladium-catalyzed alkenylative cyclization processes, and their biological activities were evaluated. Introducing functional groups into the 2α-position of the vitamin D_3-26,23-lactones resulted in remarkable enhancement of their antagonistic activity on vitamin D receptor (VDR).

Introduction

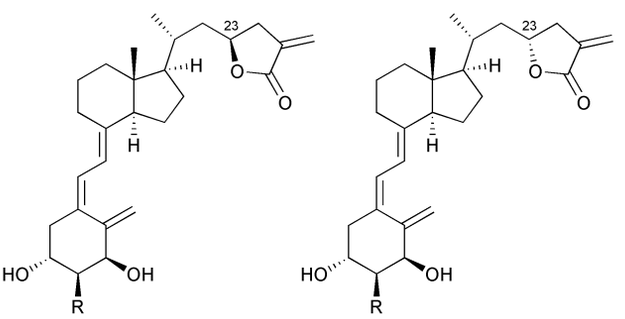
1α,25-Dihydroxyvitamin D_3 (**1**) is the most potent metabolite of vitamin D_3, and regulates various biological events, including calcium and phosphorus homeostasis, cell proliferation and differentiation, and immune reaction.[1,2] Most of the biological responses of **1** are mediated *via* interaction with its specific receptor, vitamin D receptor (VDR), which is one of the nuclear receptor superfamily and acts as a ligand-dependent gene transcription factor with coactivators.[3,4] Recently, we have synthesized several 1α,25-dihydroxyvitamin D_3 analogues, which systematically introduced an alkyl, ω-hydroxyalkyl, and ω-hydroxyalkoxyl group into the C2α position of **1**, to investigate A-ring conformation- and structure–activity relationships.[5–8] Some of these 2α-modified vitamin D_3 analogues exhibited unique biological profiles with potent agonistic activity. In particular, introduction of the 2α-methyl (**1a**),[5] 2α-(3-hydroxypropyl) (**1b**)[6] and 2α-(3-hydroxypropoxy) (**1c**)[7] groups showed 2- to 4-fold higher binding affinity to the bovine thymus VDR relative to the natural hormone **1** (Fig. 1).



1: 1α,25-dihydroxyvitamin D_3 (R = H)
1a: R = Me
1b: R = $CH_2CH_2CH_2OH$
1c: R = $OCH_2CH_2CH_2OH$

Fig. 1 Structures of 1α,25-dihydroxyvitamin D_3 (**1**) and its 2α-substitued analogues **1a**–**c**.

In 1999, studies on the modification of the side-chain structure based on the 1α,25-dihydroxyvitamin D_3-26,23-lactone metabolite[9] derived from active vitamin D_3 (**1**) led to the discovery of TEI-9647 (**2**) and TEI-9648 (**3**) (Fig. 2).[10] Both vitamin D_3 analogues **2** and **3** have an α-methylene-γ-butyrolactone moiety on the side-chain, and are the first specific antagonists of the VDR-mediated genomic action of **1**.[11] That is, vitamin D_3-lactone derivatives **2** and **3** inhibit human leukemia cell (HL-60 cells) differentiation,[10a] as well as 25-hydroxyvitamin D_3-24-hydroxylase gene expression in human osteosarcoma cells[10b] and in HL-60 cells[10d] induced by **1**. Furthermore, it is noteworthy that **2** shows antagonistic action on the genomic-mediated calcium metabolism regulated by **1** *in vivo* in rats.[10e] The unique structures and interesting biological profiles of **2** and **3** prompted us to investigate the structure–activity relationship of the vitamin D_3 lactones **2** and **3** from the standpoint of the anti-D molecules. Particularly, we focused on the introduction of the motifs described above into the C2α position of lactone analogues **2** and **3**. It was expected that such modifications should increase their VDR binding affinity and improve their antagonistic activity to VDR-mediated biological actions. Here, we report the synthesis and biological evaluation of the novel 2α-modified 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone analogues.



2: R = H (TEI-9647)
2a: R = Me
2b: R = $CH_2CH_2CH_2OH$
2c: R = $OCH_2CH_2CH_2OH$

3: R = H (TEI-9648)
3a: R = Me
3b: R = $CH_2CH_2CH_2OH$
3c: R = $OCH_2CH_2CH_2OH$

Fig. 2 Structures of 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones (TEI-9647: **2** and TEI-9648: **3**) and their 2α-modified analogues **2a**–**c** and **3a**–**c**.

Results and discussion

Synthesis of the CD-ring part **6** is shown in Scheme 1. Hydrindan derivative **4** was prepared from vitamin D_2 by the established method.[12] Bromomethylenation of **4** gave **5** in 76% yield. The substitution reaction of tosylate **5** with potassium

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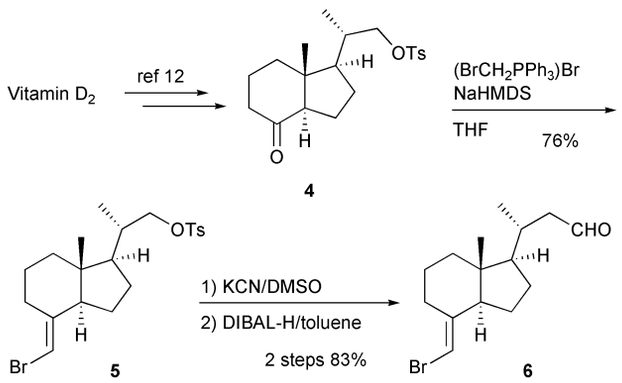
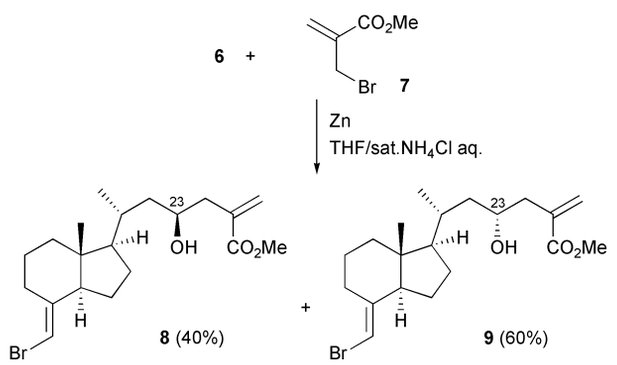


Scheme 1

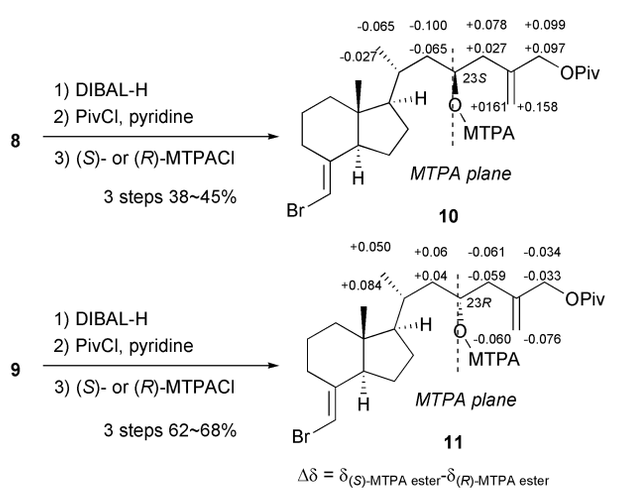
cyanide in DMSO followed by DIBAL-H reduction of the resulting cyano group afforded aldehyde **6** in 83% yield (2 steps from **5**).

The zinc-mediated Reformatsky type allylation of aldehyde **6** with methyl bromomethylacrylate **7** proceeded smoothly to give γ-hydroxyesters **8** and **9** in yields of 40% and 60%, respectively (Scheme 2).



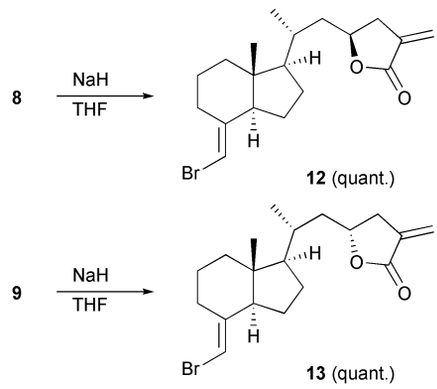
Scheme 2

The absolute configurations at the C23 position (based on steroidal numbering) on the side chain of **8** and **9** were determined using modified Mosher's method[13] (Scheme 3). Thus, γ-hydroxyesters **8** and **9** were transformed into the corresponding (*S*)- and (*R*)-MTPA esters **10** and **11**, respectively. The values of $\Delta\delta = \delta_{(R)\text{-MTPA ester}} - \delta_{(S)\text{-MTPA ester}}$ in the ^{1}H NMR spectra of **10** and **11** were calculated and shown in Scheme 3. These data were considered by applying a modified Mosher's method and the absolute configurations at the C23 position of **10** and **11** were determined to be *S* and *R*, respectively.



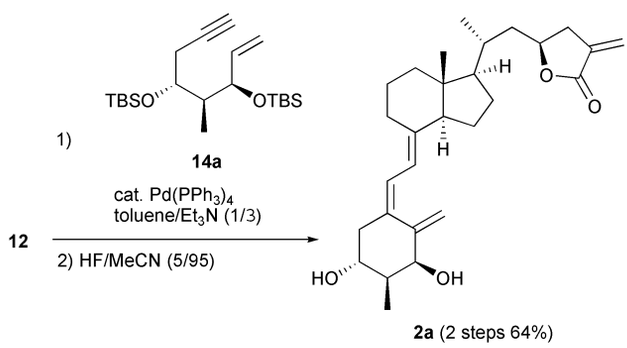
Scheme 3

Treatment of **8** with NaH in THF gave a CD-ring precursor **12** having a lactone moiety in quantitative yield. Similarly, the lactone derivative **13** was also synthesized from γ-hydroxyester **9** in an excellent yield (Scheme 4).



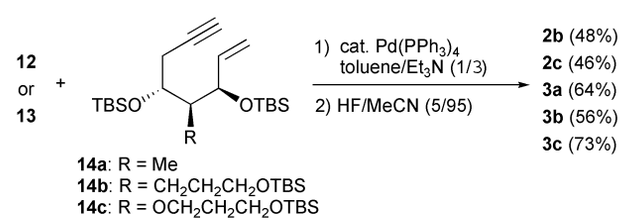
Scheme 4

The palladium-catalyzed coupling reaction[14] of the above bromoolefin **12** and enyne **14a**, which was prepared by our previously reported procedure,[15] followed by deprotection of silyl groups using HF/MeCN provided the desired 2α-methyl-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone **2a** in good yield (Scheme 5).



Scheme 5

Encouraged by this result, we synthesized the other 2α-modified vitamin D_3-26,23-lactone derivatives (**2b**,**c** and **3a**–**c**) as shown in Scheme 6. Thus, the coupling reaction of bromoolefins **12** or **13** and enynes **14a**, **14b**,[6] or **14c**[7] and subsequent deprotection afforded the desired 2α-modified vitamin D_3 analogues having a lactone side chain, *i.e.* 2α-methyl- (**3a**), 2α-(3-hydroxypropyl)- (**2b** or **3b**) and 2α-(3-hydroxypropoxy)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones (**2c** or **3c**), in moderate to good yields.



Scheme 6

Biological activities of vitamin D_3-lactone analogues, thus obtained **2a**–**c** and **3a**–**c**, were evaluated, and the results are summarized in Table 1. Binding affinity to chick intestinal VDR was examined as described previously,[16] and TEI-9647 (**2**) and TEI-9648 (**3**) showed 8 (12%) and 14 (7%) times weaker potency than that of the natural hormone **1**, respectively. Introduction of the A-ring motifs, *i.e.*, 2α-methyl (**2a**), 2α-(3-hydroxypropyl) (**2b**) and 2α-(3-hydroxypropoxy) (**2c**) groups,

Table 1 Biological activities of vitamin D_3-lactone analogues

Compound	VDR binding affinity[a]	Relative antagonistic activity[b]
1	100	—
2 (TEI-9647)	12	100
2a	16	1019
2b	18	2989
2c	16	1160
3 (TEI-9648)	7	6
3a	37	38
3b	33	100
3c	23	66

[a] Chick intestinal VDR. The potency of **1** is normalized to 100. [b] Determined by the NBT-reduction method and relative antagonistic activity was calculated at IC_{50}. The potency of **2** is normalized to 100.

into the vitamin D_3-lactone **2**, little affected the binding affinity to the VDR. On the other hand, the binding affinity of TEI-9648 analogues (**3a**–**c**) to VDR was significantly increased to 3.3–5.3 times that of **3** by the introduction of C2α substituents. Next, the antagonistic activities of **2a**–**c** and **3a**–**c** were assessed in terms of inhibition of HL-60 cell differentiation induced by treatment of the natural hormone **1**, and their activities relative to TEI-9647 (**2**) were calculated based on the IC_{50} values. Interestingly, modification of the C2α position of the original antagonist **2** resulted in marked enhancement of the antagonistic activities. The 2α-methyl analogue **2a** showed *ca.* 10-fold higher activity than **2**. Surprisingly, the antagonistic activity of **2b**, which possesses the ω-hydroxypropyl subsituent on the C2α position, was 30 times stronger than that of **2**. Furthermore, introduction of the 2α-(3-hydroxypropoxy) group (**2c**) improved the activity to *ca.* 12-fold higher than **2**. Although 2α-modified TEI-9648 (**3**) type analogues **3a**–**c** generally showed weaker antagonistic activities than **2**, introduction of the three motifs into **3** increased the activities compared to the original compound **3** (6.3 to 16.7 times more potent than **3**).

Conclusion

We introduced three motifs, 2α-methyl, 2α-(3-hydroxypropyl) and 2α-(3-hydroxypropoxy) substituents, into 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone analogues, and investigated their biological activities. It was found that the modification of the C2α position of the vitamin D_3-lactone skeleton remarkably enhanced their antagonistic activities. The VDR antagonists are expected to be potent therapeutic agents for some diseases caused by hypersensitivity to 1α,25-dihydroxyvitamin D_3, such as Paget's bone disease.[17] We expect that these analogues with potent anti-D activity would contribute to understanding the mechanisms involved in the expression of antagonistic activity on VDR as well as to finding the seeds of new medicines for treating Paget's bone disease.

Experimental

General

All manipulations were performed under an argon atmosphere unless otherwise mentioned. All solvents and reagents were purified when necessary using standard procedures. Column chromatography was performed on silica gel 60 N (Kanto Chemical CO., Inc., 100–210 μm), and flash column chromatography was performed on silica gel 60 (Merck, 0.040–0.063 mm).

(1*R*,4*E*,3a*R*,7a*R*)-4-Bromomethylene-7a-methyl-1-[(1*S*)-methyl-2-*p*-toluenesulfonyloxyethyl]perhydroindene (5)

To a suspension of bromomethyltriphenylphosphonium bromide (5.9 g, 14 mmol) in THF (20 mL) was added a solution of NaHMDS in THF (1.0 M, 14 mL, 14 mmol) at 0 °C and the mixture was stirred at the same temperature for 1 h. To the mixture was added a solution of **4** (1.0 g, 2.7 mmol) in THF (20 mL) at 0 °C, and the resulting mixture was stirred at the same temperature for 2 h. To the mixture was added a saturated NH_4Cl aq. solution at 0 °C, and the aqueous layer was extracted with AcOEt. The organic layer was washed with a saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 20 : 1) to give **5** (920 mg, 76%) as a colorless oil. $[\alpha]_D^{26}$ +72.4 (*c* 1.38, $CHCl_3$); IR (neat) 1647, 1599, 1360, 1176 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.53 (s, 3 H), 1.00 (d, *J* = 6.6 Hz, 3 H), 1.05–1.81 (m, 10 H), 1.88–2.00 (m, 2 H), 2.45 (s, 3 H), 2.91 (m, 1 H), 3.82 (dd, *J* = 9.3, 6.1 Hz, 1 H), 3.96 (dd, *J* = 9.3, 3.2 Hz, 1 H), 5.64 (s, 1 H), 7.34 (d, *J* = 8.1 Hz, 2 H), 7.78 (d, *J* = 8.1 Hz, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.8, 17.0, 21.6, 22.0, 22.4, 36.8, 39.5, 45.4, 51.4, 55.4, 75.3, 97.3, 127.9, 129.8, 133.1, 144.5, 144.6; EI-LRMS *m*/*z* 440 (M^+), 361, 268, 227, 189, 172, 91; EI-HRMS calcd for $C_{21}H_{29}O_3{}^{79}Br$ 440.1021, found 440.1023.

(1*R*,4*E*,3a*R*,7a*R*)-4-Bromomethylene-1-[(1*R*)-2-formyl-1-methylethyl]-7a-methylperhydroindene (6)

To a solution of **5** (1.2 g, 2.8 mmol) in DMSO (3 mL) was added KCN (363 mg, 5.6 mmol), and the mixture was stirred at 70 °C for 1.5 h. The mixture was diluted with Et_2O, and the organic layer was washed with H_2O and saturated NaCl aq. solution, dried over Na_2SO_4 and concentrated. The residue was dissolved in CH_2Cl_2 (5.5 mL). To the solution was added a solution of DIBAL-H in toluene (1.0 M, 3 mL, 3.1 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1.5 h. To the mixture was added, 10% potassium sodium tartrate aq. solution, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/Et_2O = 30 : 1) to give **6** (692 mg, 2.3 mmol in 2 steps) as a colorless oil. $[\alpha]_D^{20}$ +86.1 (*c* 1.08, $CHCl_3$); IR (neat) 2950, 1725, 1381 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.62 (s, 3 H), 1.04 (d, *J* = 6.6 Hz, 3 H), 1.20–1.40 (m, 3 H), 1.45–1.75 (m, 5 H), 1.90 (m, 1 H), 1.95–2.15 (m, 3 H), 2.20 (ddd, *J* = 16.0, 9.3, 3.2 Hz, 1 H), 2.47 (dd, *J* = 16.0, 2.7 Hz, 1 H), 2.89 (m, 1 H), 5.67 (s, 1 H), 9.76 (dd, *J* = 3.2, 1.2 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.8, 20.0, 21.9, 22.4, 27.7, 30.9, 31.7, 39.6, 45.5, 50.7, 55.4, 55.7, 97.7, 144.6, 203.0; EI-LRMS *m*/*z* 298 (M^+) 254, 227, 148; EI-HRMS calcd for $C_{15}H_{23}O^{79}Br$ 298.0932, found 298.0934.

(1*R*,4*E*,3a*R*,7a*R*)-4-Bromomethylene-1-[(1*R*,3*S*)-3-hydroxy-5-methoxycarbonyl-1-methyl-5-hexenyl]-7a-methylperhydroindene (8) and (1*R*,4*E*,3a*R*,7a*R*)-4-Bromomethylene-1-[(1*R*,3*R*)-3-hydroxy-5-methoxycarbonyl-1-methyl-5-hexenyl]-7a-methylperhydroindene (9)

To a solution of **6** (250 mg, 0.84 mmol) in saturated NH_4Cl aq. solution/THF (5 : 1, 4.3 mL) were added **7** (0.21 mL, 1.7 mmol)

and activated zinc dust (219 mg, 3.3 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1.5 h. The mixture was diluted with AcOEt. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 8 : 1) to give **8** (129 mg, 40%) and **9** (198 mg, 60%) as colorless oils, respectively. **8**: $[\alpha]_D^{20}$ +108.3 (*c* 0.23, $CHCl_3$); IR (neat) 1719, 1632, 1439, 1203 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3 H), 0.86 (m, 1 H), 1.02 (d, *J* = 6.3 Hz, 3 H), 1.25–1.34 (m, 4 H), 1.41–1.69 (m, 7 H), 1.90–2.03 (m, 3 H), 2.18 (dd, *J* = 9.0, 13.9 Hz, 1 H), 2.68 (dd, *J* = 1.7, 13.9 Hz, 1 H), 2.87 (m, 1 H), 3.77 (s, 3 H), 3.85 (m, 1 H), 5.64 (s, 1 H), 5.68 (s, 1 H), 6.27 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.3, 19.8, 22.5, 23.0, 28.2, 31.5, 34.5, 40.3, 40.5, 44.2, 45.9, 52.5, 56.2, 56.8, 69.5, 97.7, 128.1, 137.6, 145.1, 168.1; EI-LRMS *m/z* 398 (M^+), 287, 256, 229; EI-HRMS calcd for $C_{20}H_{31}{}^{79}BrO_3$ 398.1457, found 398.1460. **9**: $[\alpha]_D^{20}$ +93.1 (*c* 0.38, $CHCl_3$); IR (neat) 1719, 1630, 1439, 1203 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 0.97 (d, *J* = 6.3 Hz, 3 H,), 1.07 (m, 1 H), 1.21–1.37 (m, 4 H), 1.40–1.69 (m, 7 H), 1.86–2.04 (m, 3 H), 2.86 (dd, *J* = 13.9, 8.2Hz, 1H), 2.51 (dd, *J* = 13.9, 3.7 Hz, 1 H), 2.87 (m, 1 H), 3.77 (s, 3 H), 3.85 (m, 1 H), 5.64 (s, 1 H), 5.66 (s, 1 H), 6.25 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.4, 19.1, 22.5, 23.0, 28.2, 31.5, 33.2, 40.3, 42.0, 44.1, 46.0, 52.5, 56.3, 56.8, 67.9, 97.7, 127.9, 137.6, 145.2, 168.2; EI-LRMS *m/z* 398 (M^+), 366, 287, 256, 229; EI-HRMS calcd for $C_{20}H_{31}{}^{79}BrO_3$ 398.1457, found 398.1452.

Transformation of 8 into the corresponding (*S*)- and (*R*)-MTPA ester 10

To a solution of **8** (58 mg, 0.15 mmol) in toluene (3 mL) was added a solution of DIBAL-H in toluene (1.04 M, 0.63 mL, 0.65 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1 h. To the mixture was added 10% potassium sodium tartrate aq. solution, and the aqueous layer was extracted with Et_2O. The organic layers were combined and washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 2 : 1) to give the corresponding diol (52 mg, 96%) as a colorless oil. $[\alpha]_D^{18}$ −5.6 (*c* 1.54, $CHCl_3$); IR (neat) 3478, 1653 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3 H), 1.00 (d, *J* = 6.3 Hz, 3 H), 1.20–1.35 (m, 4 H), 1.41–1.71 (m, 7 H), 1.90–2.08 (m, 4 H), 2.43 (dd, *J* = 1.7, 4.2 Hz, 1 H), 2.51 (br s, 2 H), 2.88 (m, 1 H), 3.84 (m, 1 H), 4.11 (s, 2 H), 4.98 (s, 1 H), 5.15 (s, 1 H), 5.64 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 19.6, 22.2, 22.6, 27.9, 31.1, 34.2, 39.9, 41.7, 43.8, 45.6, 55.9, 56.5, 66.5, 69.3, 97.4, 114.4, 144.8, 145.8; EI-LRMS *m/z* 370 (M^+), 352, 337, 298, 256, 227; EI-HRMS calcd for $C_{19}H_{31}O_2{}^{79}Br$ 370.1507, found 370.1507. To a solution of the above diol (50 mg, 0.14 mmol) in CH_2Cl_2 (1.3 mL) were added pyridine (43 µL, 0.54 mmol) and pivaloyl chloride (20 µL, 0.16 mmol) at 0 °C, and the mixture was stirred at room temperature for 4 h. To the mixture was added water, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 9 : 1) to give the corresponding pivaloyl ester (30 mg, 48%) as a colorless oil. $[\alpha]_D^{17}$ +51.2 (*c* 1.54, $CHCl_3$); IR (neat) 1730, 1287, 1152 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3 H), 1.01 (d, *J* = 6.3 Hz, 3 H), 1.23 (s, 9 H), 1.25–1.39 (m, 4 H), 1.41–1.47 (m, 2 H), 1.50–1.61 (m, 3 H), 1.63–1.70 (m, 2 H), 1.91–2.05 (m, 5 H), 2.36 (d, *J* = 14.2 Hz, 1 H), 2.88 (m, 1 H), 3.85 (m, 1 H), 4.52 (d, *J* = 13.7 Hz, 1 H), 4.58 (d, *J* = 13.7 Hz, 1 H), 5.05 (s, 1 H), 5.17 (s, 1 H), 5.65 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 19.7, 22.2, 22.7, 27.3, 28.0, 31.1, 34.3, 39.9, 41.8, 43.7, 45.6, 55.9, 56.4, 66.4, 68.1, 97.4, 114.8, 141.2, 144.8, 177.9; EI-LRMS *m/z* 454 (M^+), 375, 357, 299; EI-HRMS calcd for $C_{24}H_{39}O_3{}^{79}Br$ 454.2083, found 454.2086. <Synthesis of (*S*)-MTPA Ester **10**> To a solution of the above pivaloyl ester (17 mg, 37 µmol) in pyridine (0.37 mL) were added (*R*)-MTPACl (14 µL, 74 µmol) and DMAP (4.6 mg, 37 µmol) at 0 °C, and the mixture was stirred at room temperature for 4 h. To the mixture was added water, and the aqueous layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by preparative thin-layer chromatography (hexane/AcOEt = 15 : 1) on silica gel to give the corresponding (*S*)-MTPA ester **10** (20 mg, 80%) as a colorless oil. $[\alpha]_D^{20}$ +21.5 (*c* 1.15, $CHCl_3$); IR (neat) 1736, 1456, 1269, 1157 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.53 (s, 3 H), 0.91 (m, 1 H), 0.97 (d, *J* = 5.8 Hz, 3 H), 1.22 (s, 9 H), 1.26 (m, 1 H), 1.32–1.46 (m, 4 H), 1.48–1.53 (m, 2 H), 1.62–1.67 (m, 3 H), 1.86–1.91 (m, 2 H), 1.95 (br d, *J* = 12.6 Hz, 1 H), 2.34 (dd, *J* = 15.0, 8.7 Hz, 1H), 2.43 (dd, *J* = 15.0, 3.8 Hz, 1 H), 2.87 (m, 1 H), 3.52 (s, 3 H), 4.51 (d, *J* = 13.6 Hz, 1 H), 4.55 (d, *J* = 13.6 Hz, 1 H), 5.00 (s, 1 H), 5.15 (s, 1 H), 5.32 (dddd, *J* = 8.4, 8.4, 4.2, 3.8 Hz, 1 H), 5.63 (s, 1 H), 7.37–7.40 (m, 3 H), 7.52–7.54 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.8, 19.2, 21.9, 22.5, 27.2, 27.7, 30.9, 33.9, 37.7, 38.8, 39.7, 40.2, 45.5, 55.5, 55.7, 56.0, 66.3, 74.2, 84.5 (q, $^2J_{C-F}$ = 27.3 Hz), 97.6, 115.8, 123.3 (q, $^1J_{C-F}$ = 289.1 Hz), 127.4, 128.3, 129.5, 132.1, 139.7, 144.8, 166.1, 177.9; EI-LRMS *m/z* 670 (M^+), 591, 489, 436, 357, 255; EI-HRMS calcd for $C_{34}H_{46}O_5{}^{79}BrF_3$ 670.2481, found 670.2485. <Synthesis of (*R*)-MTPA ester **10**> In a similar manner to that for the synthesis of (*S*)-MTPA ester, a crude product, which was obtained from the above pivaloyl ester (13 mg, 29 µmol), (*S*)-MTPACl (11 µL, 58 µmol) and DMAP (3.5 mg, 29 µmol) in pyridine (0.29 mL), was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 15 : 1) to give the corresponding (*R*)-MTPA ester **10** (18 mg, 94%) as a colorless oil. $[\alpha]_D^{18}$ +55.0 (*c* 1.23, $CHCl_3$); IR (neat) 1742, 1458, 1277, 1167 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 1.04 (d, *J* = 6.0 Hz, 3 H), 1.21 (s, 9 H), 1.25–1.32 (m, 3 H), 1.41–1.49 (m, 2 H), 1.51–1.59 (m, 3 H), 1.64–1.68 (m, 2 H), 1.74 (dd, *J* = 10.2, 9.5 Hz, 1 H), 1.95 (m, 3 H), 2.26 (dd, *J* = 14.8, 8.9 Hz, 1 H), 2.40 (dd, *J* = 14.8, 3.5 Hz, 1 H), 2.88 (m, 1 H), 3.52 (s, 3 H), 4.41 (d, *J* = 14.3 Hz, 1 H), 4.44 (d, *J* = 14.3 Hz, 1 H), 4.84 (s, 1H), 4.97 (s, 1 H), 5.31 (dddd, *J* = 9.5, 8.9, 4.1, 3.5, 1 H), 5.65 (s, 1 H), 7.36–7.69 (m, 3 H), 7.51 (br d, *J* = 6.6 Hz, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.8, 19.1, 22.0, 22.5, 27.2, 27.8, 31.0, 33.9, 37.5, 38.8, 39.8, 40.5, 45.5, 55.4, 55.8, 56.2, 66.2, 74.1, 84.3 (q, $^2J_{C-F}$ = 27.6 Hz), 97.6, 115.9, 123.3 (q, $^1J_{C-F}$ = 288.7 Hz), 127.3, 128.3, 129.5, 132.2, 139.2, 144.8, 166.0, 177.9; EI-LRMS *m/z* 670 (M^+), 591, 489, 436, 357, 255; EI-HRMS calcd for $C_{34}H_{46}O_5{}^{79}BrF_3$ 670.2481, found 670.2483.

Transformation of 9 into the corresponding (*S*)- and (*R*)-MTPA ester 11

To a solution of **9** (65 mg, 0.16 mmol) in toluene (3 mL) was added a solution of DIBAL-H in toluene (1.04 M, 0.63 mL, 0.65 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1.2 h. To the mixture was added 10% potassium sodium tartrate aq. solution, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 2 : 1) to give the corresponding diol (61 mg, quant.) as a colorless oil. $[\alpha]_D^{18}$ +90.9 (*c* 1.46, $CHCl_3$); IR (neat) 3320, 1630, 909 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 0.98 (d, *J* = 6.3 Hz, 3 H), 1.06 (m, 1 H), 1.21–1.36 (m, 3 H), 1.40–1.69 (m, 7 H), 1.87–2.04 (m, 3 H), 2.18 (dd, *J* = 14.2, 8.7 Hz, 1 H), 2.29 (dd, *J* = 14.2, 3.1 Hz, 1 H), 2.41 (br s, 2 H), 2.87 (m, 1 H), 3.85 (m, 1 H), 4.10 (s, 2 H), 4.98 (s, 1 H), 5.14 (s, 1 H), 5.64 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 18.8, 22.2, 22.7, 27.9, 31.1, 32.9, 40.0, 43.3, 43.8, 45.7, 56.0, 56.5, 66.4, 67.6, 97.4, 114.2, 144.8, 145.8; EI-LRMS *m/z* 370 (M^+), 352, 337, 298, 256, 227; EI-HRMS calcd for $C_{19}H_{31}O_2{}^{79}Br$ 370.1507, found 370.1501. To a solution of the above diol (63

mg, 0.17 mmol) in CH_2Cl_2 (0.6 mL) were added pyridine (54 μL, 0.68 mmol) and pivaloyl chloride (36 μL, 0.32 mmol) at 0 °C, and the mixture was stirred at room temperature for 19 h. To the mixture was added water, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 9 : 1) to give the corresponding pivaloyl ester (56 mg, 72%) as a colorless oil. $[\alpha]_D^{17}$ +64.4 (*c* 1.54, $CHCl_3$); IR (neat) 1732, 1285, 1154 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 0.98 (d, J = 6.3 Hz, 3 H), 1.23 (s, 9 H), 1.26–1.40 (m, 4 H), 1.43–1.74 (m, 7 H), 1.87–2.04 (m, 4 H), 2.11–2.22 (m, 2 H), 2.88 (m, 1 H), 3.87 (m, 1 H), 4.54 (s, 2 H), 5.03 (s, 1 H), 5.14 (s, 1 H), 5.64 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 14.3, 18.9, 22.2, 22.7, 27.3, 27.9, 31.1, 32.9, 40.0, 43.2, 43.7, 45.7, 56.0, 56.4, 66.4, 97.4, 114.5, 141.2, 144.8, 178.0; EI-LRMS *m*/*z* 454 (M^+), 375, 357, 299; EI-HRMS calcd for $C_{24}H_{39}O_3{}^{79}Br$ 454.2083, found 454.2086. <Synthesis of (*S*)-MTPA ester **11**> To a solution of the above pivaloyl ester (20 mg, 44 μmol) in pyridine (0.44 mL) were added (*R*)-MTPACl (73 μL, 0.38 mmol) and DMAP (24 mg, 0.20 mmol) at 0 °C, and the mixtre was stirred at room temperature for 2 days. To the mixture was added water, and the aqueous layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by preparative thin-layer chromatography (hexane/AcOEt = 15 : 1) on silica gel to give the corresponding (*S*)-MTPA ester **11** (28 mg, 95%) as a colorless oil. $[\alpha]_D^{19}$ +38.0 (*c* 0.31, $CHCl_3$); IR (neat) 1742, 1458, 1277, 1167 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.48 (s, 3 H), 0.98 (d, J = 6.3 Hz, 3 H), 1.22 (s, 9 H), 1.12–1.30 (m, 3 H), 1.36–1.44 (m, 2 H), 1.50–1.58 (m, 3 H), 1.63–1.68 (m, 2 H), 1.79 (dd, J = 12.6, 11.5 Hz, 1 H), 1.88 (m, 1 H), 1.94–1.99 (m, 2 H), 2.23 (dd, J = 14.4, 5.8 Hz, 1 H), 2.45 (dd, J = 14.4, 6.2 Hz, 1 H), 2.87 (m, 1 H), 3.49 (s, 3 H), 4.49 (d, J = 13.7 Hz, 1 H), 4.53 (d, J = 13.7 Hz, 1 H), 4.94 (s, 1 H), 5.06 (s, 1 H), 5.38 (ddd, J = 11.5, 6.2, 5.8 Hz, 1 H), 5.64 (s, 1 H), 7.36–7.40 (m, 3 H), 7.52 (br d, J = 7.1 Hz, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.7, 18.6, 22.0, 22.5, 27.2, 27.6, 30.9, 32.8, 38.8, 39.1, 39.8, 40.5, 45.5, 55.2, 55.8, 56.0, 66.2, 72.7, 84.6 (q, $^2J_{C-F}$ = 27.6 Hz), 97.6, 115.7, 123.3 (q, $^1J_{C-F}$ = 289.0 Hz), 127.5, 128.4, 129.6, 132.0, 139.3, 144.8, 166.2, 177.9; EI-LRMS *m*/*z* 591 $(M-Br)^+$, 489, 436, 357, 255; EI-HRMS calcd for $C_{34}H_{46}O_5F_3$ (M–Br) 591.3297, found 591.3286. <Synthesis of (*R*)-MTPA ester **11**> In a similar manner to that for the synthesis of (*S*)-MTPA ester, a crude product, which was obtained from the above pivaloyl ester (30 mg, 66 μmol), (*S*)-MTPACl (40 μL, 0.21 mmol) and DMAP (24 mg, 0.20 mmol) in pyridine (0.66 mL), was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 15 : 1) to give the corresponding (*R*)-MTPA ester **11** (38 mg, 86%) as a colorless oil. $[\alpha]_D^{18}$ +63.3 (*c* 0.23, $CHCl_3$); IR (neat) 1740, 1453, 1273, 1165 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.40 (s, 3 H), 0.93 (d, J = 6.0 Hz, 3 H), 1.04 (m, 1 H), 1.13–1.25 (m, 4 H), 1.22 (s, 9 H), 1.34 (m, 1 H), 1.47 (m, 1 H), 1.54 (m, 1 H), 1.61–1.65 (m, 2 H), 1.73 (dd, J = 12.2 11.3 Hz, 1 H), 1.81 (m, 1 H), 1.90–1.95 (m, 2 H), 2.29 (dd, J = 14.3, 6.0 Hz, 1 H), 2.51 (dd, J = 14.3, 6.1 Hz, 1 H), 2.85 (m, 1 H), 3.53 (s, 3 H), 4.52 (d, J = 13.6 Hz, 1 H), 4.57 (d, J = 13.6 Hz, 1 H), 4.99 (s, 1 H), 5.12 (s, 1 H), 5.40 (ddd, J = 11.3, 6.1, 6.0 Hz, 1 H), 5.62 (s, 1 H), 7.37–7.38 (m, 3 H), 7.53 (br d, J = 6.3 Hz, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.7, 18.5, 22.0, 22.5, 27.2, 27.4, 30.9, 32.5, 38.8, 39.3, 39.8, 40.5, 45.5, 55.4, 55.8, 55.9, 66.2, 72.4, 84.4 (q, $^2J_{C-F}$ = 27.6 Hz), 97.5, 115.6, 123.4 (q, $^1J_{C-F}$ = 288.7 Hz), 127.3, 128.3, 129.5, 132.2, 139.5, 144.9, 166.2, 177.9; EI-LRMS *m*/*z* 670 (M^+), 591, 489, 436, 357, 255; EI-HRMS calcd for $C_{34}H_{46}O_5{}^{79}BrF_3$ 670.2481, found 670.2459.

(*S*)-5-{(*R*)-2-[(1*R*,4*E*,3a*R*,7a*R*)-4-Bromomethylene-7a-methylperhydroinden-1-yl]propyl}-3-methylenedihydrofuran-2-one (12)

To a suspension of NaH (60% oil dispersion, 9.6 mg, 0.24 mmol) of THF (1.4 mL) was added a solution of **8** (87 mg, 0.22 mmol) in THF (3.0 mL) at 0 °C, and the mixture was stirred at the same temperature for 30 min. To the mixture was added saturated NH_4Cl aq. solution, and the aqueous layer was extracted with Et_2O. The organic layers were combined and washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 8 : 1) to give **12** (80 mg, quant.) as a colorless oil. $[\alpha]_D^{19}$ +64.5 (*c* 0.19, $CHCl_3$); IR (neat) 1763, 1671, 1437, 1140 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3 H), 1.04 (d, J = 6.3 Hz, 3 H), 1.19–1.70 (m, 11 H), 1.90–2.01 (m, 3 H), 2.54 (dddd, J = 16.8, 6.5, 2.9, 2.9 Hz, 1 H), 2.87 (m, 1 H), 3.05 (m, 1 H), 4.61 (ddt, J = 6.5, 6.9, 6.9 Hz, 1 H), 5.62 (s, 1 H), 5.65 (s, 1 H), 6.22 (br s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 19.4, 22.1, 22.6, 28.0, 31.1, 33.7, 34.2, 39.9, 42.4, 45.6, 55.8, 76.5, 97.6, 121.8, 134.5, 144.6, 170.0; EI-LRMS *m*/*z* 366 (M^+), 287, 229, 201; EI-HRMS calcd for $C_{19}H_{27}O_2{}^{79}Br$ 366.1194, found 366.1204.

(*R*)-5-{(*R*)-2-[(1*R*,4*E*,3a*R*,7a*R*)-4-Bromomethylene-7a-methylperhydroinden-1-yl]propyl}-3-methylenedihydrofuran-2-one (13)

In a similar manner to that for the synthesis of **12** from **8**, a crude product, which was obtained from **9** (112 mg, 0.28 mmol), NaH (60% oil dispersion, 12.3 mg, 0.31 mmol) in THF (5.6 mL), was purified by (hexane/AcOEt = 8 : 1) to give **13** (104 mg, quant.) as a colorless oil. $[\alpha]_D^{19}$ +127.6 (*c* 0.46, $CHCl_3$); IR (neat) 1763, 1667, 1437, 1127 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3 H), 1.02 (d, J = 6.6 Hz, 3 H), 1.20–1.34 (m, 5 H), 1.44–1.69 (m, 4 H), 1.75–1.89 (m, 3 H), 1.95–2.03 (m, 2 H), 2.52 (dddd, J = 16.9, 5.9, 2.9, 2.9 Hz, 1 H), 2.87 (m, 1 H), 3.07 (dddd, J = 16.9, 7.3, 2.3, 2.3 Hz, 1 H), 4.64 (m, 1 H), 5.62 (br s, 1 H), 5.65 (s, 1 H), 6.22 (dd, J = 2.7, 2.3 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.4, 19.1, 22.5, 22.9, 28.0, 31.4, 33.4, 34.9, 40.3, 43.8, 46.0, 56.2, 56.5, 75.4, 97.9, 122.2, 134.9, 144.9, 170.4; EI-LRMS *m*/*z* 366 (M^+), 287, 229, 201; EI-HRMS calcd for $C_{19}H_{27}O_2{}^{79}Br$ 366.1194, found 366.1190.

The general procedure for the synthesis of 2α-substituted 25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones

To a solution of bromoolefin and enyne (1.5 eq. to bromoolefin) in toluene-Et_3N (1 : 3) and $Pd(PPh_3)_4$ (30 mol% to bromoolefin), and the mixture was stirred at 110 °C for 2 h. The mixture was filtered through a silica gel pad, and the filtrate was concentrated. The residue was dissolved in MeCN (1 mL). To the solution was added HF/MeCN (1 : 9, 1 mL) at 0 °C, and the mixture was stirred at room temperature for 2 h. To the mixture was added saturated $NaHCO_3$ aq. solution, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aq. solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel to give the vitamin D_3-lactone analogues.

(23*S*)-25-Dehydro-2α-methyl-1α-hydroxyvitamin D_3-26,23-lactone (2a)

A crude product, which was obtained from **12** (13 mg, 35 μmol), **14a** (20 mg, 53 μmol) and $Pd(PPh_3)_4$ (13 mg, 11 μmol), was treated with HF/MeCN. After usual workup, the crude product was purified by column chromatography on silica gel (hexane/AcOEt = 1 : 1) to give **2a** (10 mg, 64% in 2 steps) as an amorphous solid. $[\alpha]_D^{19}$ +72.2 (*c* 0.15, $CHCl_3$); IR (neat) 3345, 2926, 1752, 1277 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 1.03 (d, J = 6.3 Hz, 3 H), 1.08 (d, J = 6.8 Hz, 3 H), 1.19–2.04 (m, 17 H), 2.23 (dd, J = 13.2, 8.1 Hz, 1 H), 2.54 (dddd, J = 16.8, 6.6, 3.0, 3.0 Hz, 1 H), 2.67 (dd, J = 13.2, 3.9 Hz, 1 H), 2.83 (m, 1 H), 3.05 (dd, J = 16.8, 7.2 Hz, 1 H), 3.84 (m, 1 H), 4.30 (br s, 1 H), 4.59 (ddt, J = 6.6, 7.2, 7.0 Hz, 1 H), 5.00 (s, 1 H), 5.28 (s, 1 H), 5.62 (br s, 1 H), 6.00 (d, J = 11.2 Hz, 1 H), 6.22 (br s, 1 H), 6.39 (d, J = 11.2 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.1, 12.5, 18.6, 22.2, 23.5, 27.6, 29.0, 33.0, 34.5, 40.5, 43.4, 44.2, 46.0,

56.3, 56.9, 71.7, 75.1, 75.4, 113.2, 117.1, 121.9, 124.7, 133.2, 134.8, 142.7, 146.5, 170.4; EI-LRMS *m/z* 440 (M^+) 422, 404, 394, 378, 283; EI-HRMS calcd for $C_{28}H_{40}O_4$ 440.2927, found 440.2920.

(23*S*)-25-Dehydro-2α-(3-hydroxypropyl)-1α-hydroxyvitamin D_3-26,23-lactone (2b)

A crude product, which was obtained from **12** (14 mg, 38 μmol), **14b** (31 mg, 57 μmol) and $Pd(PPh_3)_4$ (13 mg, 11 μmol), was treated with HF/MeCN. After usual workup, the crude product was purified by flash column chromatography on silica gel (AcOEt) to give **2b** (10 mg, 56% in 2 steps) as an amorphous solid. $[\alpha]_D^{21}$ +47.5 (*c* 0.31, $CHCl_3$); IR (neat) 3382, 2944, 1757, 1667, 1437 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 1.03 (d, *J* = 6.3 Hz, 3 H), 1.22–1.73 (m, 19 H), 1.88–2.04 (m, 3 H), 2.25 (dd, *J* = 13.0, 8.8 Hz, 1 H), 2.54 (ddt, *J* = 16.8, 6.3, 3.1 Hz, 1 H), 2.66 (dd, *J* = 13.0, 4.4 Hz, 1 H), 2.82 (m, 1 H), 3.05 (ddt, *J* = 16.8, 7.4, 2.3 Hz, 1 H), 3.70 (t, *J* = 5.0 Hz, 2 H), 3.89 (ddd, *J* = 8.3, 8.3, 4.3 Hz, 1 H), 4.38 (d, *J* = 2.4 Hz, 1 H), 4.59 (dddd, *J* = 7.4, 7.0, 7.0, 6.3 Hz, 1 H), 4.99 (d, *J* = 2.0 Hz, 1 H), 5.28 (m, 1 H), 5.62 (dd, *J* = 2.4, 2.4 Hz, 1 H), 5.99 (d, *J* = 11.2 Hz, 1 H), 6.22 (dd, *J* = 2.8, 2.8 Hz, 1 H), 6.40 (d, *J* = 11.5 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 19.2, 22.2, 22.8, 23.4, 28.0, 29.0, 30.2, 33.7, 34.1, 40.4, 42.3, 45.9, 49.1, 56.2, 56.5, 62.9, 70.5, 73.6, 76.6, 113.7, 117.1, 121.9, 124.7, 132.9, 134.7, 142.9, 146.5, 170.3; EI-LRMS *m/z* 484 (M^+) 466, 448, 438, 389, 338, 309, 253; EI-HRMS calcd for $C_{30}H_{44}O_5$ 484.3189, found 484.3176.

(23*S*)-25-Dehydro-2α-(3-hydroxypropoxy)-1α-hydroxyvitamin D_3-26,23-lactone (2c)

A crude product, which was obtained from **12** (11 mg, 29 μmol), **14c** (25 mg, 45 μmol) and $Pd(PPh_3)_4$ (10 mg, 9 μmol), was treated with HF/MeCN. After usual workup, the crude product was purified by flash column chromatography on silica gel (AcOEt) to give **2c** (11 mg, 73% in 2 steps) as a colorless oil. $[\alpha]_D^{19}$ +41.4 (*c* 0.21, $CHCl_3$); IR (neat) 3379, 2946, 1761, 1279 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 1.02 (d, *J* = 6.4 Hz, 3 H), 1.21–2.01 (m, 16 H), 2.17 (t, *J* = 5.0 Hz, 1 H), 2.24 (dd, *J* = 13.0, 9.3 Hz, 1 H), 2.47 (d, *J* = 3.4 Hz, 1 H), 2.54 (d, *J* = 4.4 Hz, 1 H), 2.56 (m, 1 H), 2.69 (dd, *J* = 13.0, 4.6 Hz, 1 H), 2.82 (m, 1 H), 3.05 (dddd, *J* = 16.9, 7.4, 2.6, 2.3 Hz, 1 H), 3.38 (dd, *J* = 7.5, 3.5 Hz, 1 H), 3.75–3.90 (m, 4 H), 4.06 (m, 1 H), 4.45 (dd, *J* = 3.5, 3.5 Hz, 1 H), 4.59 (dddd, *J* = 7.4, 7.0, 7.0, 7.0 Hz, 1 H), 5.09 (br s, 1 H), 5.39 (br s, 1 H), 5.62 (dd, *J* = 2.3, 2.3 Hz, 1 H), 6.01 (d, *J* = 11.4 Hz, 1 H), 6.22 (dd, *J* = 2.7, 2.6 Hz, 1 H), 6.42 (d, *J* = 11.4 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.1, 19.2, 22.2, 23.4, 28.0, 29.0, 31.9, 33.7, 34.1, 40.4, 40.9, 42.3, 45.9, 56.2, 56.5, 61.3, 68.4, 68.5, 71.9, 76.8, 84.5, 116.2, 117.3, 121.9, 125.4, 131.7, 134.7, 143.1, 144.2, 170.4; EI-LRMS *m/z* 500 (M^+) 482, 464, 406, 390, 352; EI-HRMS calcd for $C_{30}H_{44}O_6$ 500.3138, found 500.3133.

(23*R*)-25-Dehydro-2α-methyl-1α-hydroxyvitamin D_3-26,23-lactone (3a)

A crude product, which was obtained from **13** (16 mg, 44 μmol), **14a** (25 mg, 65 μmol), $Pd(PPh_3)_4$ (15 mg, 13 μmol), was treated with HF/MeCN. After usual workup, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 3 : 2) to give **3a** (9 mg, 47% in 2 steps) as an amorphous solid. $[\alpha]_D^{19}$ +94.1 (*c* 0.48, $CHCl_3$); IR (neat) 3387, 2936, 1748, 1275 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3 H), 1.01 (d, *J* = 6.3 Hz, 3 H), 1.08 (d, *J* = 6.8 Hz, 3 H), 1.23–2.03 (m, 17 H), 2.23 (dd, *J* = 13.3, 7.8 Hz, 1 H), 2.52 (br d, *J* = 16.9, 1 H), 2.67 (dd, *J* = 13.3, 3.5 Hz, 1 H), 2.83 (m, 1 H), 3.06 (br d, *J* = 16.9 Hz, 1 H), 3.85 (m, 1 H), 4.31(br s, 1 H), 4.64 (m, 1 H), 5.00 (s, 1 H), 5.28 (s, 1 H), 5.62 (br s, 1 H), 6.00 (d, *J* = 11.2 Hz, 1 H), 6.22 (br s, 1 H), 6.38 (d, *J* = 11.2 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.2 (2 C), 12.7, 18.8, 22.4, 23.6, 27.7, 29.1, 33.1, 34.6, 40.6, 43.5, 44.3, 46.1, 56.4, 56.9, 71.7, 75.2, 75.4, 113.1, 117.0, 121.8, 124.5, 133.1, 134.7, 142.5, 146.4, 170.1; EI-LRMS *m/z* 440 (M^+) 422, 404, 394, 378, 283; EI-HRMS calcd for $C_{28}H_{40}O_4$ 440.2927, found 440.2932.

(23*R*)-25-Dehydro-2α-(3-hydroxypropyl)-1α-hydroxyvitamin D_3-26,23-lactone (3b)

A crude product, which was obtained from **13** (12 mg, 33 μmol), **14b** (27 mg, 49 μmol), $Pd(PPh_3)_4$ (11 mg, 9.8 μmol), was treated with HF/MeCN. After usual workup, the crude product was purified by flash column chromatography on silica gel (AcOEt) to give **3b** (7 mg, 45% in 2 steps) as an amorphous solid. $[\alpha]_D^{19}$ +93.6 (*c* 0.31, $CHCl_3$); IR (neat) 3382, 2926, 1752, 1645, 1442 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 1.02 (d, *J* = 6.3 Hz, 3 H), 1.26–1.82 (m, 19 H), 1.96–2.04 (m, 3 H), 2.25 (dd, *J* = 13.0, 8.3 Hz, 1 H), 2.52 (m, 1 H), 2.67 (dd, *J* = 13.2, 4.0 Hz, 1 H), 2.84 (m, 1H), 3.04–3.10 (m, 1 H), 3.71 (t, *J* = 5.3 Hz, 2 H), 3.90 (ddd, *J* = 8.3, 8.3, 4.5 Hz, 1 H), 4.38 (d, *J* = 2.0 Hz, 1 H), 4.63–4.65 (m, 1 H), 4.99 (s, 1 H), 5.28 (s, 1 H), 5.62 (br s, 1 H), 6.00 (d, *J* = 11.2 Hz, 1 H), 6.23 (br s, 1 H), 6.39 (d, *J* = 11.2 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 18.4, 22.0, 22.6, 23.2, 27.4, 28.8, 30.0, 32.8, 34.3, 40.3, 43.2, 44.1, 45.8, 48.9, 56.1, 56.7, 62.7, 70.2, 73.4, 74.9, 113.4, 116.9, 121.7, 124.5, 132.7, 134.6, 142.6, 146.4, 170.2; EI-LRMS *m/z* 484 (M^+) 466, 448, 438, 389, 338, 309, 253; EI-HRMS calcd for $C_{30}H_{44}O_5$ 484.3189, found 484.3174.

(23*R*)-25-Dehydro-2α-(3-hydroxypropoxy)-1α-hydroxyvitamin D_3-26,23-lactone (3c)

A crude product, which was obtained from **13** (16 mg, 44 μmol), **14c** (36 mg, 65 μmol) and $Pd(PPh_3)_4$ (15 mg, 13 μmol), was treated with HF/MeCN. After usual workup, the crude product was purified by flash column chromatography on silica gel (AcOEt) to give **3c** (10 mg, 46% in 2 steps) as an amorphous solid. IR (neat) 3393, 2946, 1759, 1278 cm^{-1}; $[\alpha]_D^{18}$ +71.0 (*c* 0.37, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3 H), 1.01 (d, *J* = 6.3 Hz, 3 H), 1.26–2.03 (m, 16 H), 2.23 (dd, *J* = 13.2, 9.0 Hz, 1 H), 2.35 (br s, 1 H), 2.54 (m, 2 H), 2.61 (d, *J* = 3.9 Hz, 1 H), 2.68 (dd, *J* = 4.2 Hz, 1 H), 2.83 (m, 1 H), 3.06 (dd, *J* = 17.3, 7.6 Hz, 1 H), 3.37 (dd, *J* = 7.2, 3.1 Hz, 1 H), 3.76–3.90 (m, 4 H), 4.06 (m, 1 H), 4.44 (br s, 1 H), 4.63 (m, 1 H), 5.01 (br s, 1 H), 5.39 (br s, 1 H), 5.61 (br s, 1 H), 6.01 (d, *J* = 11.0 Hz, 1 H), 6.22 (br s, 1 H), 6.41 (d, *J* = 11.0 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.1, 18.6, 22.2, 23.4, 27.6, 29.0, 31.9, 33.0, 34.5, 40.5, 40.9, 43.4, 46.0, 56.3, 56.9, 61.2, 68.4, 68.5, 71.8, 75.2, 84.4, 116.1, 117.3, 125.4, 131.8, 134.8, 143.1, 144.3, 170.4; EI-LRMS *m/z* 500 (M^+) 482, 464, 406, 390, 352; EI-HRMS calcd for $C_{30}H_{44}O_6$ 500.3138, found 500.3134.

Vitamin D receptor (VDR) binding assay

[26,27-*methyl*-^{3}H]-1α,25-dihydroxyvitamin D_3 (specific activity 6.623TBq $mmol^{-1}$, 15,000 dpm, 15.7 pg) and various amounts of 1α,25-dihydroxyvitamin D_3 and an analogue to be tested were dissolved in 50 μl of absolute ethanol in 12 × 75 mm polypropylene tubes. 0.2 mg of the chick intestinal VDR and 1 mg of gelatin in 1 ml of phosphate buffer solution (25 nM KH_2PO_4, 0.1 M KCl, 1 mM dithiothreitol, pH 7.4) were added to each tube in an ice bath. The assay tubes were incubated in a shaking water bath for 1 h at 25 °C and then chilled in an ice bath. 1 ml of 40% polypropylene glycol 6000 in distilled water was added to each tube, which was then mixed vigorously and centrifuged at 2260 × *g* for 60 min at 4 °C. After the supernatant was decanted, the bottom of the tube containing the pellet was cut off into a scintillation vial containing 10 ml of dioxane-based scintillation fluid and the radioactivity was counted with a Beckman liquid scintillation counter (Model LS6500). The relative potency of the analogues were calculated from their

concentration needed to displace 50% of [26,27-*methyl*-^{3}H]-1α,25-dihydroxyvitamin D_3 from the receptor compared with the activity of 1α,25-dihydroxyvitamin D_3 (assigned a 100% value).

Assay for HL-60 cell differentiation

Nitro blue tetrazolium (NBT)-reducing activity was used as a cell differentiation marker. HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FCS. Exponentially proliferating cells were collected, suspended in fresh medium and seeded in culture plates (Falcon, Becton Dickinson and Company, Franklin Lakes, NJ). Cell concentration at seeding was adjusted to 2×10^4 cells mL^{-1} and the seeding volume was 1 mL/well. An ethanol solution of 1α,25-dihydroxyvitamin D_3 (final concentration: 10^{-8} M) and an analogue (final concentration: 10^{-11} to 10^{-6} M) was added to the culture medium at 0.1% volume and culture was continued for 96 h at 37 °C in a humidified atmosphere of 5% CO_2/air without medium change. The same amount of vehicle was added to the control culture. NBT-reducing assay was performed according to the method of Collins *et al.*[18] Briefly, cells were collected, washed with PBS and suspended in serum-free medium. NBT/TPA solution (dissolved in PBS) was added. Final concentrations of NBT and TPA were 0.1% and 100 ng mL^{-1}, respectively. Then, the cell suspensions were incubated at 37 °C for 25 min. After incubation, cells were collected by centrifugation and resuspended in FCS. Cytospin smears were prepared, and the counter-staining of nuclei was done with Kemechrot solution. At least 500 cells per preparation were observed. The relative potency of the analogues were calculated from the concentration needed to inhibit 50% of cell differentiation by 10^{-8} M 1α,25-dihydroxyvitamin D_3 (IC_{50}) compared with the activity of TEI-9647 (**2**) (assigned a 100% value).

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References

1 (*a*) *Vitamin D*, eds. D. Feldman, F. H. Glorieux and J. W. Pike, Academic Press, New York, 1997; (*b*) R. A. Ettinger and H. F. Deluca, *Adv. Drug Res.*, 1996, **28**, 269.
2 R. Bouillon, W. H. Okamura and A. W. Norman, *Endocr. Rev.*, 1995, **16**, 200.
3 (*a*) R. M. Evans, *Science*, 1988, **240**, 889; (*b*) K. Umezono, K. K. Murakami, C. C. Thompson and R. M. Evans, *Cell*, 1991, **65**, 1255.
4 K. Takeyama, Y. Masuhiro, H. Fuse, H. Endoh, A. Murayama, S. Kitanaka, M. Suzawa, J. Yanagisawa and S. Kato, *Mol. Cell Biol.*, 1999, **19**, 1049.
5 (*a*) K. Konno, S. Maki, T. Fujishima, Z. Liu, D. Miura, M. Chokki and H. Takayama, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 151; (*b*) K. Konno, T. Fujishima, S. Maki, Z. Liu, D. Miura, M. Chokki, S. Ishizuka, K. Yamaguchi, Y. Kan, M. Kurihara, N. Miyata, C. Smith, H. F. DeLuca and H. Takayama, *J. Med. Chem.*, 2000, **43**, 4247.
6 (*a*) Y. Suhara, K.-i. Nihei, H. Tanigawa, T. Fujishima, K. Konno, K. Nakagawa, T. Okano and H. Takayama, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1129; (*b*) Y. Suhara, K.-i. Nihei, M. Kurihara, A. Kittaka, K. Yamaguchi, T. Fujishima, K. Konno, N. Miyata and H. Takayama, *J. Org. Chem.*, 2001, **66**, 8760.
7 A. Kittaka, Y. Suhara, H. Takayanagi, T. Fujishima, M. Kurihara and H. Takayama, *Org. Lett.*, 2000, **2**, 2619.
8 H. Takayama, A. Kittaka, T. Fujishima and Y. Suhara, in *Vitamin D Analogues in Cancer Prevention and Therapy*, eds. J. Reichrath, M. Friedrich and W. Tilgen, Springer-Verlag, Berlin, 2003, pp. 289–317.
9 (*a*) S. Ishizuka, S. Ishimoto and A. W. Norman, *Biochemistry*, 1984, **23**, 1473; (*b*) S. Ishizuka, T. Ohba and A. W. Norman, in *Vitamin D: Molecular, Cellular and Chemical Endocrinology*, eds. A. W. Norman, K. Schaefer, H. G. Grigoleit and D. von Herrath, Walter de Gruyter, Berlin, 1988, pp. 143–144.
10 (*a*) D. Miura, K. Manabe, K. Ozono, M. Saito, Q. Gao, A. W. Norman and S. Ishizuka, *J. Biol. Chem.*, 1999, **274**, 16392; (*b*) K. Ozono, M. Saito, D. Miura, T. Michigami, S. Nakajima and S. Ishizuka, *J. Biol. Chem.*, 1999, **274**, 32376; (*c*) D. Miura, K. Manabe, Q. Gao, A. W. Norman and S. Ishizuka, *FEBS Lett.*, 1999, **460**, 297; (*d*) S. Ishizuka, D. Miura, H. Eguchi, K. Ozono, M. Chokki, T. Kamimura and A. W. Norman, *Arch. Biochem. Biophys.*, 2000, **380**, 92; (*e*) S. Ishizuka, D. Miura, K. Ozono, M. Chokki, H. Mimura and A. W. Norman, *Endocrinol.*, 2001, **142**, 59; (*f*) S. Ishizuka, D. Miura, K. Ozono, M. Saito, H. Eguchi, M. Chokki and A. W. Norman, *Steroids*, 2001, **66**, 227.
11 To date, only two types of VDR antagonists are known. For the other type of VDR antagonist of 25-carboxylic esters ZK159222 and its analogue ZK168281, see: (*a*) M. Herdick, A. Steinmeyer and C. Carlberg, *J. Biol. Chem.*, 2000, **275**, 16506; (*b*) Y. Bury, A. Steinmeyer and C. Carlberg, *Mol. Pharmacol.*, 2000, **58**, 1067; (*c*) M. Herdick, A. Steinmeyer and C. Carlberg, *Chem. Biol.*, 2000, **7**, 885; (*d*) A. Toell, M. M. Gonzalez, D. Ruf, A. Steinmeyer, S. Ishizuka and C. Carlberg, *Mol. Pharmacol.*, 2001, **59**, 1478.
12 I. Hijikuro, T. Doi and T. Takahashi, *J. Am. Chem. Soc.*, 2001, **123**, 3716 and references cited therein.
13 I. Ohtani, T. Kusumi, Y. Kashman and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.
14 B. M. Trost, J. Dumas and M. Villa, *J. Am. Chem. Soc.*, 1992, **114**, 9836.
15 Y. Suhara, A. Kittaka, S. Kishimoto, M. J. Calverly, T. Fujishima, N. Saito, T. Sugiura, K. Waku and H. Takayama, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3255.
16 (*a*) J. A. Eisman, A. J. Hamstra, B. E. Kream and H. F. DeLuca, *Arch. Biochem. Biophys.*, 1976, **176**, 235; (*b*) M. Inaba and H. F. DeLuca, *Biochim. Biophys. Acta*, 1989, **1010**, 20.
17 Recent reports on Paget's bone disease, see: (*a*) C. Menaa, J. Barsony, S. V. Reddy, J. Cornish, T. Cundy and G. D. Roodman, *J. Bone Miner. Res.*, 2000, **15**, 228; (*b*) N. Kurihara, S. V. Reddy, C. Menaa, D. Anderson and G. D. Roodman, *J. Clin. Invest.*, 2000, **105**, 607; (*c*) C. Menaa, S. V. Reddy, N. Kurihara, H. Maeda, D. Anderson, T. Cundy, J. Cornish, F. R. Singer, J. M. Bruder and G. D. Roodman, *J. Clin. Invest.*, 2000, **105**, 1833; (*d*) R. J. Leach, F. R. Singer and G. D. Roodman, *J. Clin. Endo. Metab.*, 2001, **86**, 24; (*e*) S. V. Reddy, N. Kurihara, C. Menaa, G. Landucci, D. Forthal, B. A. Koop, J. J. Windle and G. D. Roodmann, *Endocrinology*, 2001, **142**, 2898; (*f*) W. E. Friedrichs, S. V. Reddy, J. M. Bruder, T. Cundy, J. Cornish, F. R. Singer and G. D. Roodman, *J. Bone Miner. Res.*, 2002, **17**, 145.
18 S. J. Collins, F. W. Ruscetti, R. E. Gallagher and R. C. Gallo, *J. Exp. Med.*, 1979, **149**, 969.