

Remarkable effect of 2 α -modification on the VDR antagonistic activity of 1 α -hydroxyvitamin D₃-26,23-lactones

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Novel 2 α -methyl-, 2 α -(3-hydroxypropyl)- and 2 α -(3-hydroxypropoxy)-substituted 25-dehydro-1 α -hydroxyvitamin D₃-26,23-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 2 α -position of the vitamin D₃-26,23-lactones resulted in remarkable enhancement of their antagonistic activity on vitamin D receptor (VDR).

Introduction

1 α ,25-Dihydroxyvitamin D₃ (**1**) is the most potent metabolite of vitamin D₃, 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 1 α ,25-dihydroxyvitamin D₃ analogues, which systematically introduced an alkyl, ω -hydroxyalkyl, and ω -hydroxyalkoxyl group into the C2 α position of **1**, to investigate A-ring conformation- and structure-activity relationships.⁵⁻⁸ Some of these 2 α -modified vitamin D₃ analogues exhibited unique biological profiles with potent agonistic activity. In particular, introduction of the 2 α -methyl (**1a**),⁵ 2 α -(3-hydroxypropyl) (**1b**)⁶ and 2 α -(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).

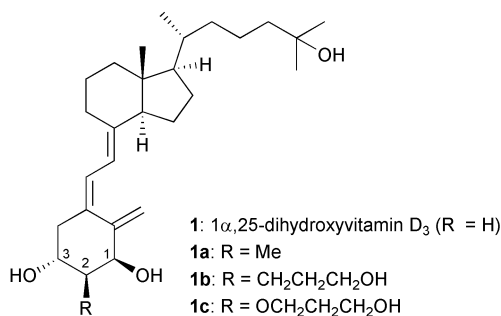


Fig. 1 Structures of 1 α ,25-dihydroxyvitamin D₃ (**1**) and its 2 α -substituted analogues **1a–c**.

In 1999, studies on the modification of the side-chain structure based on the 1 α ,25-dihydroxyvitamin D₃-26,23-lactone metabolite⁹ derived from active vitamin D₃ (**1**) led to the discovery of TEI-9647 (**2**) and TEI-9648 (**3**) (Fig. 2).¹⁰ Both vitamin D₃ 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 D₃-lactone derivatives **2** and **3** inhibit human

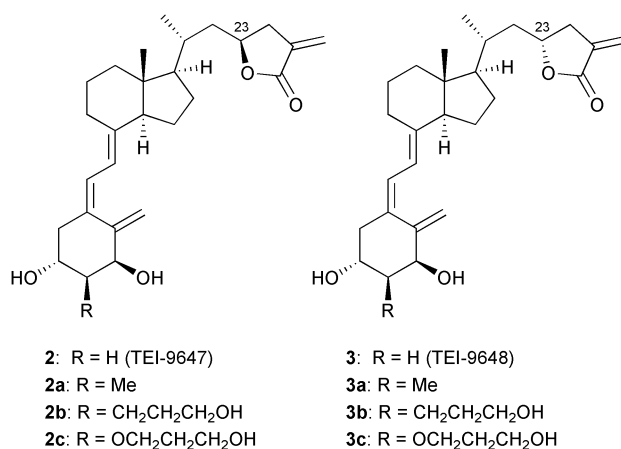
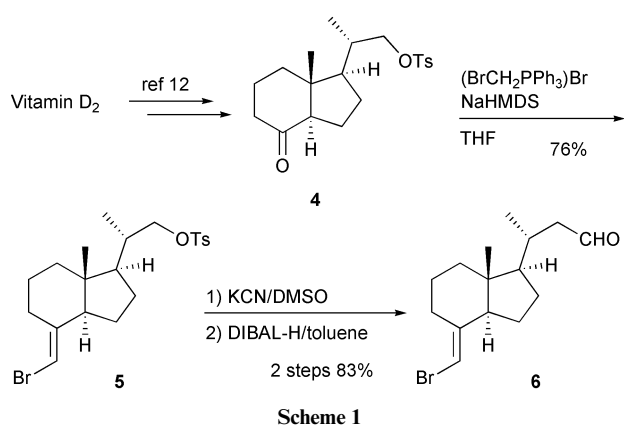


Fig. 2 Structures of 25-dehydro-1 α -hydroxyvitamin D₃-26,23-lactones (TEI-9647: **2** and TEI-9648: **3**) and their 2 α -modified analogues **2a–c** and **3a–c**.

leukemia cell (HL-60 cells) differentiation,^{10a} as well as 25-hydroxyvitamin D₃-24-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 D₃ 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 C2 α 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 2 α -modified 25-dehydro-1 α -hydroxyvitamin D₃-26,23-lactone analogues.

Results and discussion

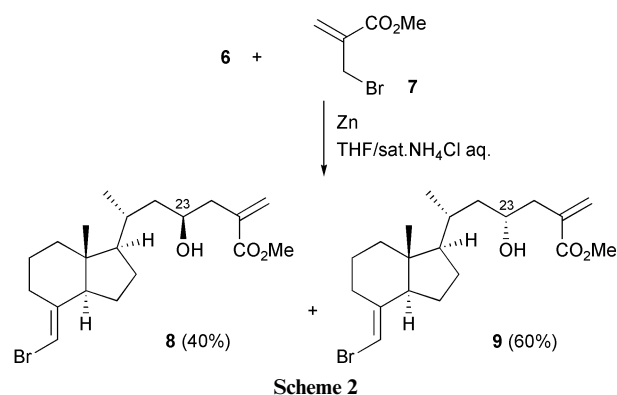
Synthesis of the CD-ring part **6** is shown in Scheme 1. Hydrindan derivative **4** was prepared from vitamin D₂ by the established method.¹² Bromomethylenation of **4** gave **5** in 76% yield. The substitution reaction of tosylate **5** with potassium



Scheme 1

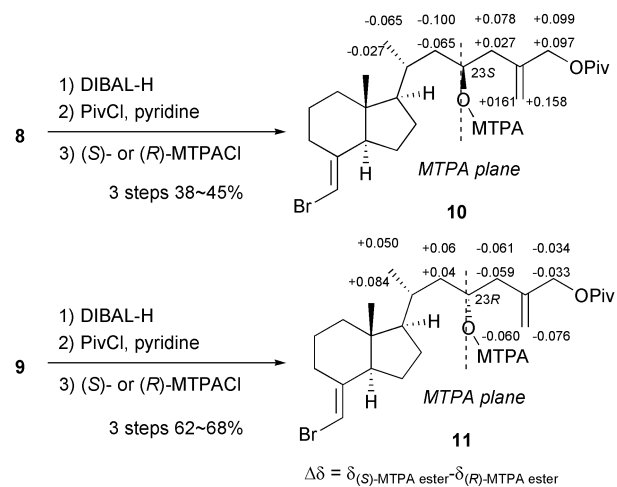
cyanide in DMSO followed by DIBAL-H reduction of the resulting cyano group afforded aldehyde **6** in 83% yield (2 steps from **5**).

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).



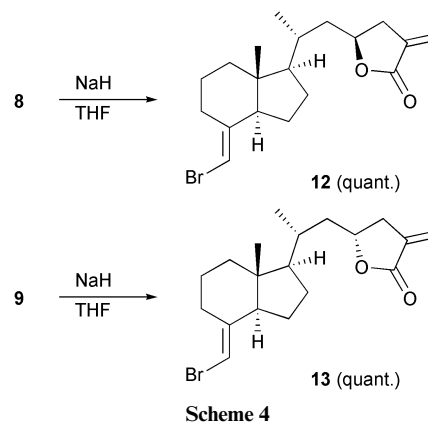
Scheme 2

The absolute configurations at the C23 position (based on steroidal numbering) on the side chain of **8** and **9** were determined using Mosher's method¹³ (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 ¹H 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.



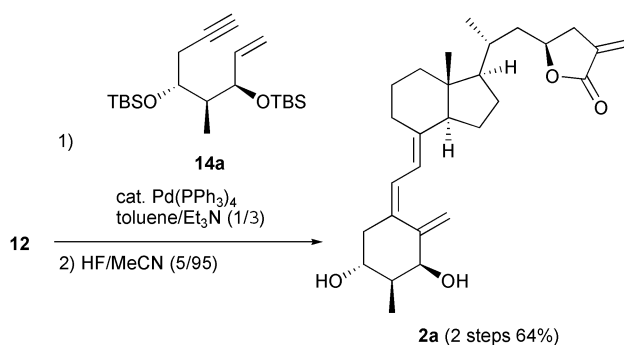
Scheme 3

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).



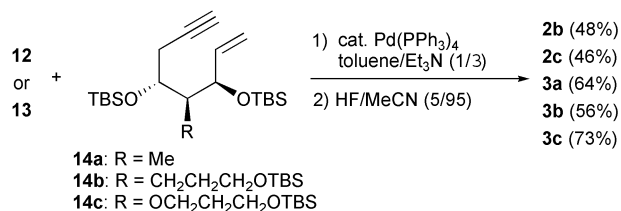
Scheme 4

The palladium-catalyzed coupling reaction¹⁴ 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 2 α -methyl-25-dehydro-1 α -hydroxyvitamin D₃-26,23-lactone **2a** in good yield (Scheme 5).



Scheme 5

Encouraged by this result, we synthesized the other 2 α -modified vitamin D₃-26,23-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 2 α -modified vitamin D₃ analogues having a lactone side chain, *i.e.* 2 α -methyl- (**3a**), 2 α -(3-hydroxypropyl)- (**2b** or **3b**) and 2 α -(3-hydroxypropoxy)-25-dehydro-1 α -hydroxyvitamin D₃-26,23-lactones (**2c** or **3c**), in moderate to good yields.



Scheme 6

Biological activities of vitamin D₃-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 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.* 2 α -methyl (**2a**), 2 α -(3-hydroxypropyl) (**2b**) and 2 α -(3-hydroxypropoxy) (**2c**) groups,

Table 1 Biological activities of vitamin D₃-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 IC₅₀. The potency of **2** is normalized to 100.

into the vitamin D₃-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 C2 α 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 IC₅₀ values. Interestingly, modification of the C2 α position of the original antagonist **2** resulted in marked enhancement of the antagonistic activities. The 2 α -methyl analogue **2a** showed *ca.* 10-fold higher activity than **2**. Surprisingly, the antagonistic activity of **2b**, which possesses the ω -hydroxypropyl substituent on the C2 α position, was 30 times stronger than that of **2**. Furthermore, introduction of the 2 α -(3-hydroxypropoxy) group (**2c**) improved the activity to *ca.* 12-fold higher than **2**. Although 2 α -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, 2 α -methyl, 2 α -(3-hydroxypropyl) and 2 α -(3-hydroxypropoxy) substituents, into 25-dehydro-1 α -hydroxyvitamin D₃-26,23-lactone analogues, and investigated their biological activities. It was found that the modification of the C2 α position of the vitamin D₃-lactone skeleton remarkably enhanced their antagonistic activities. The VDR antagonists are expected to be potent therapeutic agents for some diseases caused by hypersensitivity to 1 α ,25-dihydroxyvitamin D₃, 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 μ m), and flash column chromatography was performed on silica gel 60 (Merck, 0.040–0.063 mm).

(1R,4E,3aR,7aR)-4-Bromomethylene-7a-methyl-1-[(1S)-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 NH₄Cl 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 Na₂SO₄, 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. [α]_D²⁶ +72.4 (*c* 1.38, CHCl₃); IR (neat) 1647, 1599, 1360, 1176 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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 C₂₁H₂₉O₃⁷⁹Br 440.1021, found 440.1023.

(1R,4E,3aR,7aR)-4-Bromomethylene-1-[(1R)-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 Et₂O, and the organic layer was washed with H₂O and saturated NaCl aq. solution, dried over Na₂SO₄ and concentrated. The residue was dissolved in CH₂Cl₂ (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 Et₂O. The organic layer was washed with saturated NaCl aq. solution, dried over Na₂SO₄ and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/Et₂O = 30 : 1) to give **6** (692 mg, 2.3 mmol in 2 steps) as a colorless oil. [α]_D²⁰ +86.1 (*c* 1.08, CHCl₃); IR (neat) 2950, 1725, 1381 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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 C₁₅H₂₃O⁷⁹Br 298.0932, found 298.0934.

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

To a solution of **6** (250 mg, 0.84 mmol) in saturated NH₄Cl 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 Na₂SO₄, 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, CHCl₃); IR (neat) 1719, 1632, 1439, 1203 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₂₀H₃₁⁷⁹BrO₃ 398.1457, found 398.1460. **9**: $[\alpha]_D^{20} + 93.1$ (*c* 0.38, CHCl₃); IR (neat) 1719, 1630, 1439, 1203 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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, 8.2 Hz, 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₂₀H₃₁⁷⁹BrO₃ 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 Et₂O. The organic layers were combined and washed with saturated NaCl aq. solution, dried over Na₂SO₄, 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, CHCl₃); IR (neat) 3478, 1653 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₁₉H₃₁O₂⁷⁹Br 370.1507, found 370.1507. To a solution of the above diol (50 mg, 0.14 mmol) in CH₂Cl₂ (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 Et₂O. The organic layer was washed with saturated NaCl aq. solution, dried over Na₂SO₄, 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, CHCl₃); IR (neat) 1730, 1287, 1152 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₂₄H₃₉O₃⁷⁹Br 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 Na₂SO₄, 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, CHCl₃); IR (neat) 1736, 1456, 1269, 1157 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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, ²*J*_{C-F} = 27.3 Hz), 97.6, 115.8, 123.3 (q, ¹*J*_{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 C₃₄H₄₆O₅⁷⁹BrF₃ 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, CHCl₃); IR (neat) 1742, 1458, 1277, 1167 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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, ²*J*_{C-F} = 27.6 Hz), 97.6, 115.9, 123.3 (q, ¹*J*_{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 C₃₄H₄₆O₅⁷⁹BrF₃ 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 Et₂O. The organic layer was washed with saturated NaCl aq. solution, dried over Na₂SO₄, 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, CHCl₃); IR (neat) 3320, 1630, 909 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₁₉H₃₁O₂⁷⁹Br 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. $[\alpha]_{\text{D}}^{17} + 64.4$ (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 $\text{C}_{24}\text{H}_{39}\text{O}_3$ 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 mixture 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. $[\alpha]_{\text{D}}^{19} + 38.0$ (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, $^2J_{\text{C-F}}$ = 27.6 Hz), 97.6, 115.7, 123.3 (q, $^1J_{\text{C-F}}$ = 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 $\text{C}_{34}\text{H}_{46}\text{O}_5\text{F}_3$ (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. $[\alpha]_{\text{D}}^{18} + 63.3$ (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, $^2J_{\text{C-F}}$ = 27.6 Hz), 97.5, 115.6, 123.4 (q, $^1J_{\text{C-F}}$ = 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 $\text{C}_{34}\text{H}_{46}\text{O}_5$ 670.2481, found 670.2459.

(*S*)-5-{(*R*)-2-[(1*R*,4*E*,3*aR*,7*aR*)-4-Bromomethylene-7*a*-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. $[\alpha]_{\text{D}}^{19} + 64.5$ (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 $\text{C}_{19}\text{H}_{27}\text{O}_2$ 366.1194, found 366.1204.

(*R*)-5-{(*R*)-2-[(1*R*,4*E*,3*aR*,7*aR*)-4-Bromomethylene-7*a*-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. $[\alpha]_{\text{D}}^{19} + 127.6$ (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 $\text{C}_{19}\text{H}_{27}\text{O}_2$ 366.1194, found 366.1190.

The general procedure for the synthesis of 2 α -substituted 25-dehydro-1 α -hydroxyvitamin D₃-26,23-lactones

To a solution of bromoolefin and enyne (1.5 eq. to bromoolefin) in toluene- Et_3N (1 : 3) and $\text{Pd}(\text{PPh}_3)_4$ (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 D₃-lactone analogues.

(23*S*)-25-Dehydro-2 α -methyl-1 α -hydroxyvitamin D₃-26,23-lactone (2a**)**

A crude product, which was obtained from **12** (13 mg, 35 μmol), **14a** (20 mg, 53 μmol) and $\text{Pd}(\text{PPh}_3)_4$ (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. $[\alpha]_{\text{D}}^{19} + 72.2$ (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.

(23S)-25-Dehydro-2 α -(3-hydroxypropyl)-1 α -hydroxyvitamin D₃-26,23-lactone (2b)

A crude product, which was obtained from **12** (14 mg, 38 μ mol), **14b** (31 mg, 57 μ mol) and Pd(PPh₃)₄ (13 mg, 11 μ mol), 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. $[\alpha]_D^{25} +47.5$ (c 0.31, CHCl₃); IR (neat) 3382, 2944, 1757, 1667, 1437 cm^{-1} ; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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.

(23S)-25-Dehydro-2 α -(3-hydroxypropoxy)-1 α -hydroxyvitamin D₃-26,23-lactone (2c)

A crude product, which was obtained from **12** (11 mg, 29 μ mol), **14c** (25 mg, 45 μ mol) and Pd(PPh₃)₄ (10 mg, 9 μ mol), 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. $[\alpha]_D^{25} +41.4$ (c 0.21, CHCl₃); IR (neat) 3379, 2946, 1761, 1279 cm^{-1} ; ¹H NMR (400 MHz, CDCl₃) 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 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); ¹³C NMR (150 MHz, CDCl₃) 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.

(23R)-25-Dehydro-2 α -methyl-1 α -hydroxyvitamin D₃-26,23-lactone (3a)

A crude product, which was obtained from **13** (16 mg, 44 μ mol), **14a** (25 mg, 65 μ mol), Pd(PPh₃)₄ (15 mg, 13 μ mol), 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. $[\alpha]_D^{25} +94.1$ (c 0.48, CHCl₃); IR (neat) 3387, 2936, 1748, 1275 cm^{-1} ; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (150

MHz, CDCl₃) 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.

(23R)-25-Dehydro-2 α -(3-hydroxypropyl)-1 α -hydroxyvitamin D₃-26,23-lactone (3b)

A crude product, which was obtained from **13** (12 mg, 33 μ mol), **14b** (27 mg, 49 μ mol), Pd(PPh₃)₄ (11 mg, 9.8 μ mol), 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. $[\alpha]_D^{25} +93.6$ (c 0.31, CHCl₃); IR (neat) 3382, 2926, 1752, 1645, 1442 cm^{-1} ; ¹H NMR (400 MHz, CDCl₃) 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, 1 H), 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); ¹³C NMR (100 MHz, CDCl₃) 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.

(23R)-25-Dehydro-2 α -(3-hydroxypropoxy)-1 α -hydroxyvitamin D₃-26,23-lactone (3c)

A crude product, which was obtained from **13** (16 mg, 44 μ mol), **14c** (36 mg, 65 μ mol) and Pd(PPh₃)₄ (15 mg, 13 μ mol), 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} ; $[\alpha]_D^{25} +71.0$ (c 0.37, CHCl₃); ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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-³H]-1 α ,25-dihydroxyvitamin D₃ (specific activity 6.623TBq $mmol^{-1}$, 15,000 dpm, 15.7 pg) and various amounts of 1 α ,25-dihydroxyvitamin D₃ and an analogue to be tested were dissolved in 50 μ l of absolute ethanol in 12 \times 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 KH₂PO₄, 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 \times 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-³H]-1 α ,25-dihydroxyvitamin D₃ from the receptor compared with the activity of 1 α ,25-dihydroxyvitamin D₃ (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 1 α ,25-dihydroxyvitamin D₃ (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% CO₂/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 1 α ,25-dihydroxyvitamin D₃ (IC₅₀) compared with the activity of TEI-9647 (2) (assigned a 100% value).

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