Supporting Information

Dramatic Enhancement of Antagonistic Activity on Vitamin D Receptor: A Double Functionalization of 1α-Hydroxyvitamin D_3-26,23-Lactones

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General

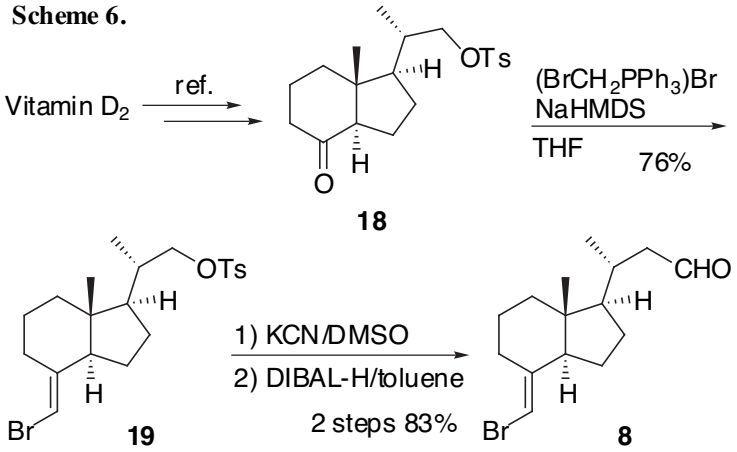
All manipulations were performed under an argon atmosphere unless otherwise mentioned. All solvents and reagents were purified when necessary using standard procedures. Column chromatography was performed on silica gel 60 N (Kanto Chemical CO., Inc., 100-210 μm), and flash column chromatography was performed on silica gel 60 (Merck, 40-63 μm).

Experimental Section

Synthesis of Aldehyde 8

The aldehyde **8** was synthesized from known compound **18** (Scheme 6).

Scheme 6.



Compound 19: 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 **18** (Hijikuro, I.; Doi, T.; Takahashi, T. *J. Am. Chem. Soc.* **2001**, *123*, 3716) (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 **19** (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.

Compound 8: To a solution of **19** (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

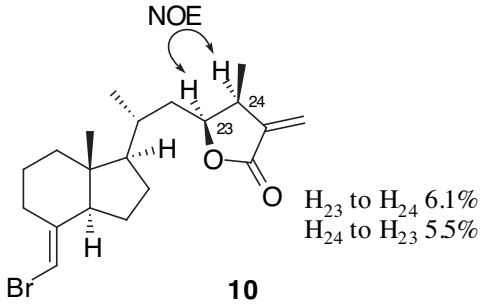
solution of DIBAL-H in toluene (1.0 M, 3 mL, 3.1 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1.5 h. To the mixture was added, 10% potassium sodium tartrate aq. solution, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aq. solution, 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 **8** (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.

Synthesis of *syn*-lactones 10 and 11.

***syn*-Lactones 10 and 11**: To a suspension of $CrCl_3$ (811 mg, 5.1 mmol) in THF (26 mL) was added $LiAlH_4$ (97 mg, 2.6 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. To the mixture were added a solution of **9** (494 mg, 2.6 mmol) in THF (8 mL) and a solution of **8** (385 mg, 1.3 mmol) at room temperature, and the resulting mixture was stirred at the same temperature for 1 h. To the mixture was added H_2O at 0 °C, 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 = 10/1) to give a mixture of **10** and **11** (467 mg, 95 %, ratio of 1 to 1.2). Further separation was performed by recycle-HPLC (column: SHIMADZU Shim-pack PREP-SIL(H)KIT, eluent: hexane/AcOEt = 3/1, flow rate: 10 mL/min, detector:UV (235 nm)). **Spectral data of 10**: $[\alpha]_D^{25}$ +41.3 (*c* 1.09, $CHCl_3$); IR (neat) 1751, 1662, 1631, 1265, 1163 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.58 (s, 3 H), 1.06 (d, *J* = 6.9 Hz, 3 H), 1.14 (d, *J* = 7.0 Hz, 3 H), 1.22-1.51 (m, 5 H), 1.52-1.72 (m, 6 H), 1.96 (m, 1 H), 1.98-2.05 (m, 2 H), 2.88 (m, 1 H), 3.11 (dddq, *J* = 2.0, 2.0, 6.8, 7.0 Hz, 1 H), 4.60 (ddd, *J* = 8.3, 6.8, 5.2 Hz, 1 H), 5.84 (d, *J* = 2.1 Hz, 1 H), 5.65 (s, 1 H), 6.19 (d, *J* = 2.1 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 14.6, 19.8, 22.1, 22.6, 27.8, 31.1, 34.4, 36.4, 38.1, 39.8, 45.6, 55.7, 56.1, 80.1, 97.5, 120.4, 141.2, 144.7, 170.1; EI-LRMS *m/z* 380 (M^+), 301, 227, 147, 105; EI-HRMS calcd for $C_{20}H_{29}O_2{}^{79}Br$ 380.1351, found 380.1347. **Spectral data of 11**: $[\alpha]_D^{25}$ +179.4 (*c* 1.28, $CHCl_3$); IR (neat) 1765, 1664, 1631, 1267, 1124 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 1.01 (d, *J* = 6.6 Hz, 3 H), 1.10 (ddd, *J* = 13.3, 10.8, 1.9 Hz, 1 H), 1.13 (d, *J* = 7.1 Hz, 3 H), 1.20-1.35 (m, 3 H), 1.40-1.71 (m, 6 H), 1.75 (m, 1 H), 1.86 (m, 1 H), 1.97 (ddd, *J* = 12.4, 6.7, 1.1 Hz, 1 H), 2.03 (br d, *J* = 12.4 Hz, 1 H), 5.76 (m, 1 H), 3.17 (ddq, *J* = 2.5, 7.7, 7.1 Hz, 1 H), 4.68 (ddd, *J* = 11.8, 7.7, 1.9 Hz, 1 H), 5.53 (d, *J* = 2.8 Hz, 1 H), 5.65 (s, 1 H), 6.22 (d, *J* = 2.8 H ,1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0,13.9, 18.5, 22.1, 22.6, 27.6, 31.1, 32.5, 36.9, 37.7, 39.9, 45.6, 55.9, 56.3, 78.3, 97.5, 120.5, 140.6, 144.6, 170.1; EI-LRMS *m/z* 380 (M^+), 301, 227, 147, 105; EI-HRMS calcd for $C_{20}H_{29}O_2{}^{79}Br$ 380.1350, found 380.1353.

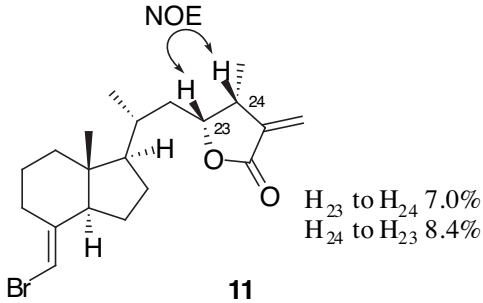
The relative stereochemistries of **10** and **11** were determined by NOE experiments to be *syn*-orientation, respectively (Figure 4).

Figure 4.



H_{23} to H_{24} 6.1%
H_{24} to H_{23} 5.5%

10



H_{23} to H_{24} 7.0%
H_{24} to H_{23} 8.4%

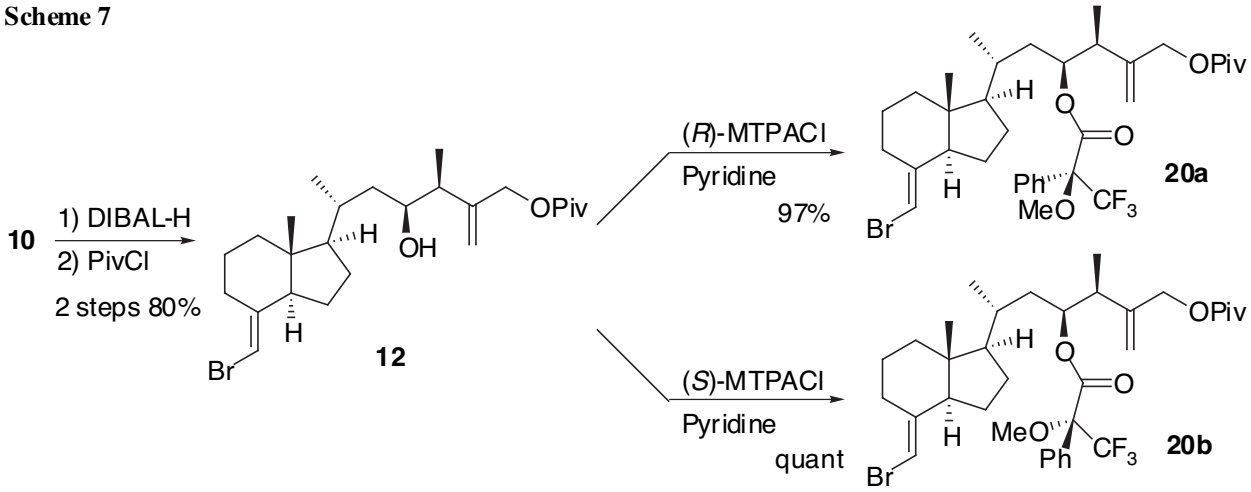
11

Determination of absolute stereochemistries of C23 and C24 positions on lactone ring.

Transformation of 10 into MTPA ester 20

The lactone derivative **10** was transformed into MTPA esters **20a** and **20b** (Scheme 7).

Scheme 7



Compound 12: To a solution of **10** (17.5 mg, 46 μmol) in toluene (1 mL) was added a solution of DIBAL-H (1.04 M solution in toluene, 0.18 mL, 0.19 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. To the mixture was added 10% potassium sodium tartrate aq. solution at 0 °C, 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 = 3/1) to give diol (17 mg, 95%) as a colorless oil. $[\alpha]_D^{27}$ +74.1 (*c* 2.28, $CHCl_3$); IR (neat) 3239, 1631, 1024 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3 H), 1.00 (d, *J* = 6.6 Hz, 3 H), 1.02 (d, *J* = 7.1 Hz, 3 H), 1.15-1.72 (m, 11 H), 1.86-2.06 (m, 3 H), 2.01 (dq, *J* = 2.1, 7.1 Hz, 1 H), 2.70-3.05 (m, 3 H), 3.56 (ddd, *J* = 7.4, 6.0, 2.4 Hz, 1 H), 4.04 (dd, *J* = 12.9, 0.49 Hz, 1 H), 4.13 (dd, *J* = 12.9, 0.73 Hz, 1 H), 4.96 (s, 1 H), 6.26 (br d, *J* = 0.98 Hz, 1 H), 5.63 (br s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.4, 12.0, 19.5, 22.2, 22.6, 28.0, 31.1, 34.6, 39.9, 40.4, 41.7, 45.6, 55.8, 56.6, 65.1, 72.1, 97.4, 113.1, 144.8, 151.31; EI-LRMS *m/z* 384 (M^+), 298, 254, 227, 175, 147; EI-HRMS calcd for $C_{20}H_{33}O_2{}^{79}Br$ 384.1664, found 384.1664.

To a solution of the diol (220 mg, 0.57 mmol) in CH_2Cl_2 (2.9 mL) were added pyridine (0.19 mL, 2.4 mmol) and PivCl (0.09 mL, 0.73 mmol) at 0 °C, and the mixture was stirred at the same temperature for 7 h. To the mixture was added H_2O, 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 = 10/1) to give **12** (226 mg, 84%) as a colorless oil. $[\alpha]_D^{26}$ +59.5 (*c* 1.25, $CHCl_3$); IR (neat) 3503, 1730, 1649, 1284, 1153 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3 H), 1.05 (d, *J* = 6.6 Hz, 3 H), 1.04 (d, *J* = 6.9 Hz, 3 H), 1.15-1.80 (m, 12 H), 1.23 (s, 9 H), 1.90-2.10 (m, 3 H), 2.26 (dq, *J* = 2.8, 6.9 Hz, 1 H), 2.87 (m, 1 H), 3.79 (m, 1 H), 4.52 (d, *J* = 13.7 Hz, 1 H), 4.59 (d, *J* = 13.7 Hz, 1 H), 5.02 (s, 1 H), 5.17 (d, *J* = 1.2 Hz, 1 H), 5.63 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 12.0, 19.6, 22.2, 22.6, 27.3 (3 C), 28.0, 31.1, 34.6, 38.9, 39.9, 40.4, 40.7, 45.6, 55.9, 56.9, 66.1, 70.8, 97.4, 112.6, 144.8, 146.9, 177.9; EI-LRMS *m/z* 468 (M^+), 389, 299, 269, 227, 170, 147; EI-HRMS calcd for $C_{25}H_{41}O_3{}^{79}Br$ 468.2239, found 468.2240.

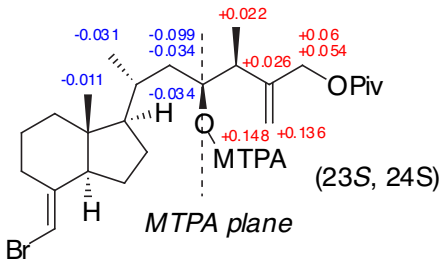
(*S*)-MTPA ester <u>20a</u>: To a solution of **12** (16 mg, 33 μmol) in pyridine (1.5 mL) was added (*R*)-(-)-methoxytrifluoromethylphenylacetyl chloride (MTPACl) (10 μL, 53 μmol) at 0 °C, and the mixture was stirred at room temperature for 16 h. To the mixture was added H_2O, the aqueous layer was 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 = 10/1) to give **20a** (22 mg, 97%) as a colorless oil. $[\alpha]_D^{22}$ +22.2 (*c* 1.69, $CHCl_3$); IR (neat) 1738, 1730, 1651, 1277, 1155 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 0.10 (d, *J* = 6.1 Hz, 3 H), 1.09 (d, *J* = 6.9 Hz, 3 H), 1.22 (s, 9 H), 1.20-1.40 (m, 4 H), 1.44 (m, 1 H), 1.50-1.61 (m, 3 H), 1.61-1.71 (m, 2 H), 1.76 (ddd, *J* = 8.9, 8.3, 7.2 Hz, 1 H), 1.88-2.01 (m, 3 H), 2.47 (dq, *J* = 3.0, 6.9 Hz, 1 H), 2.88 (m, 1 H), 3.49 (s, 3 H), 4.55 (d, *J* = 13.5 Hz, 1 H), 4.62 (d, *J* = 13.5 Hz, 1 H), 4.96 (s, 1 H), 5.16 (s, 1 H), 5.35 (ddd, *J* = 8.9, 4.6, 3.0 Hz, 1 H), 5.65 (dd, *J* = 1.7, 1.7 Hz, 1 H), 7.35-7.42 (m, 3 H), 7.48-7.52 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.8, 12.1, 19.1, 22.0, 22.5, 27.2 (3 C), 27.8, 31.0, 33.9, 37.5, 37.8, 38.8, 39.8, 45.5, 55.3, 55.7, 56.3, 66.3, 76.5, 84.7 (q, $^2J_{C\text{-}F}$ = 27.6 Hz), 97.6, 114.5, 122.4 (q, $^1J_{C\text{-}F}$ = 288 Hz), 127.7 (2 C), 128.3 (2 C), 129.5, 131,4, 144.8, 145.0, 166.1, 178.0; EI-LRMS *m/z* 605 (M^+-Br), 452, 435, 371, 227, 189; EI-HRMS calcd for $C_{35}H_{48}O_5F_3$ (M^+-Br) 605.3454, found 605.3449.

(*R*)-MTPA ester <u>20b</u>: In a similar manner to that for the synthesis of **20a** from **12**, a crude product, which was obtained from **12** (16 mg, 35 μmol), (*S*)-(+)-MTPACl (10 μL, 53 μmol) in pyridine at 50 °C for 24 h, was purified by flash column chromatography on silica gel (hexane/AcOEt = 10/1) to give **20b** (24 mg, quant) as a colorless oil. $[\alpha]_D^{27}$ +51.4 (*c* 1.72, $CHCl_3$); IR (neat) 1740, 1655, 1381, 1273, 1020 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 1.03 (d, *J* = 5.8 Hz, 3 H), 1.07 (d, *J* = 6.9 Hz, 3 H), 1.21 (s, 9 H), 1.25-1.41 (m, 4 H), 1.46 (m, 1 H), 1.52-1.73 (m, 5 H), 1.85 (ddd, *J* = 10.2, 8.9, 8.9 Hz, 1 H), 1.90-2.30 (m, 3 H), 2.46 (dq, *J* = 6.9, 2.8 Hz, 1 H), 2.88 (m, 1 H), 3.52 (s, 3 H), 4.50 (d, *J* = 13.5 Hz, 1 H), 4.55 (d, *J* = 13.6 Hz, 1 H), 5.01 (s, 1 H), 5.34 (ddd, *J* = 8.9, 4.3, 2.8 Hz, 1 H), 5.66 (s, 1 H), 7.35-7.42 (m, 3 H), 7.50-7.54 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.8, 12.0, 19.1, 22.0, 22.5, 27.2 (3 C), 27.9, 31.0, 33.9, 37.3, 38.1, 38.8, 39.8, 45.5, 55.4, 55.7, 56.3, 66.2, 76.2, 84.3 (q, $^2J_{C\text{-}F}$ = 27.6 Hz), 97.7, 114.4, 123.4 (q, $^1J_{C\text{-}F}$ = 287.9 Hz), 127.4, 128.2 (2 C), 129.5 (2 C), 132.3, 144.5, 144.8, 165.9, 177.3; EI-LRMS *m/z* 605 (M^+-Br), 452, 435, 371, 227, 189; EI-HRMS calcd for $C_{35}H_{48}O_5F_3$ 605.3453 (M^+-Br), found 605.3448.

The values of $\Delta\delta = \delta_{(S)\text{-MTPA ester}} - \delta_{(R)\text{-MTPA ester}}$ in the ^1H NMR spectra of **20** were calculated and shown in Figure 5. These data were considered by applying a modified Mosher's method reported by Kusumi *et al*. (Ohtani, I.; Kusumi, T.; Koshman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092), and the absolute configurations at the C23 position of **20** was determined to be 23*S*. From this result and NOE experiment shown in Figure 4, the absolute stereochemistry at the C24 position

of **20** was determined to be 24*S*.

Figure 5.

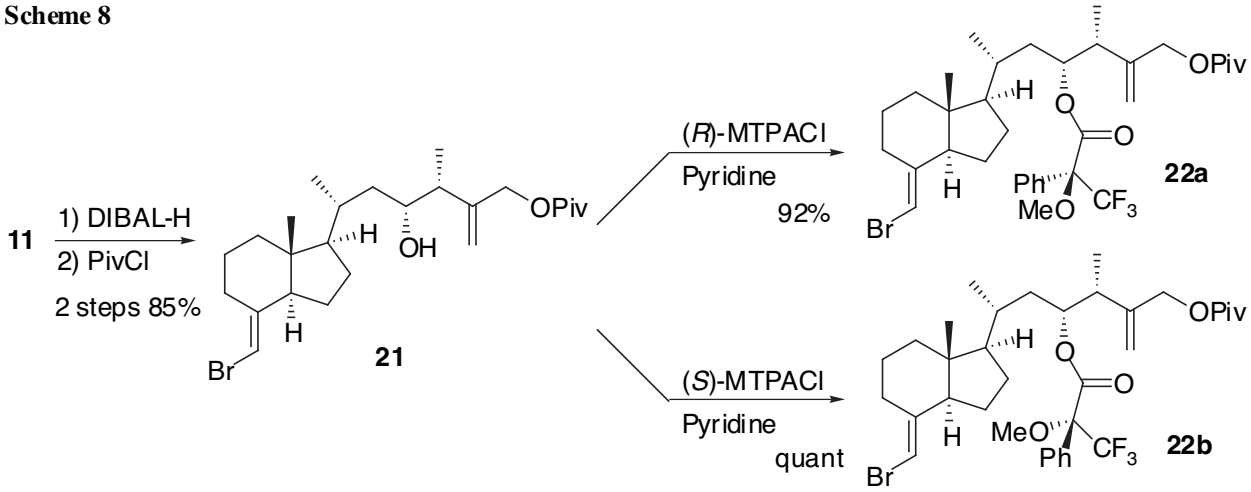


$\Delta\delta = \delta_{(S)\text{-ester}} - \delta_{(R)\text{-ester}}$

Transformation of 11 into MTPA ester 22

The lactone derivative **11** was transformed into MTPA esters **22a** and **22b** (Scheme 8).

Scheme 8



Compound 21: To a solution of **11** (15 mg, 40 μmol) in toluene (1 mL) was added a solution of DIBAL-H (1.04 M solution in toluene, 0.15 mL, 0.16 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. To the mixture was added 10% potassium sodium tartrate aq. solution at 0 °C, 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 = 3/1) to give diol (14 mg, 93%) as an amorphous solid. $[\alpha]_D^{25}$ +124.1 (*c* 1.12, $CHCl_3$); IR (nujol) 3250, 1714, 1635 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 0.96 (d, *J* = 6.6 Hz, 3 H), 1.03 (m, 1 H), 1.07 (d, *J* = 7.0 Hz, 3 H), 1.20-1.38 (m, 3 H), 1.40-1.73 (m, 7 H), 1.85-2.06 (m, 3 H), 2.30 (dq, *J* = 3.9, 7.0 Hz, 1 H), 2.53 (br s, 2 H), 2.88 (m, 1 H), 3.71 (ddd, *J* = 10.6, 3.9, 1.8 Hz, 1 H), 4.06 (br d, *J* = 12.9, 1 H), 4.13 (br d, *J* = 12.9 Hz, 1 H), 4.93 (s, 1 H), 5.17 (br s, 1 H), 5.64 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 14.5, 18.8, 22.1, 22.6, 27.8, 31.1, 33.0, 39.9, 40.1, 44.3, 45.6, 56.0, 56.4, 65.6, 71.2, 97.4, 112.5, 144.8, 151.0; EI-LRMS *m/z* 384 (M^+), 254, 227, 175, 147, 106, 86; EI-HRMS calcd for $C_{20}H_{33}O_2{}^{79}Br$ 384.1664, found 384.1667.

To a solution of the diol (187 mg, 0.49 mmol) in CH_2Cl_2 (3 mL) were added pyridine (0.12 mL, 1.48 mmol) and PivCl (90 μL, 0.73 mmol) at 0 °C, and the mixture was stirred at the same temperature for 16 h. To the mixture was added H_2O, 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 = 10/1) to give **21** (207 mg, 91%) as a colorless oil. $[\alpha]_D^{25}$ +90.3 (*c* 1.52, $CHCl_3$);

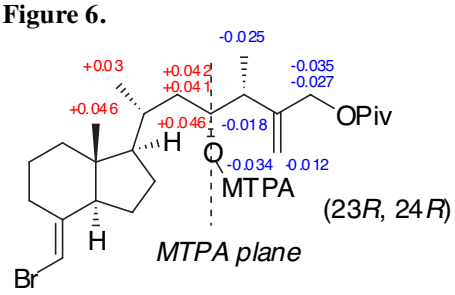
IR (neat) 3510, 1730, 1649, 1284, 1153, 1032 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3 H), 0.95 (d, *J* = 6.6 Hz, 3 H), 1.02 (m, 1 H), 1.09 (d, *J* = 6.9 Hz, 3 H), 1.14-1.73 (m, 11 H), 1.22 (s, 9 H), 1.85-2.06 (m, 3 H), 2.15 (dq, *J* = 5.3, 6.9 Hz, 1 H), 2.86 (m, 1 H), 3.71 (m, 1 H), 4.54 (s, 2 H), 4.97 (s, 1 H), 5.12 (s, 1 H), 5.63 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 14.1, 18.9, 22.2, 22.7, 27.3 (3 C), 27.9, 31.1, 33.1, 38.9, 40.0. 41.1, 43.7, 45.7, 56.0, 56.4, 66.3, 70.3, 97.4, 112.3, 144.8, 146.9, 177.9; EI-LRMS *m/z* 468 (M^+), 389, 299, 269, 227, 170, 147; EI-HRMS calcd for $C_{25}H_{41}O_3{}^{79}Br$ 468.2239, found 468.2234.

(*S*)-MTPA ester <u>22a</u>: To a solution of **21** (21 mg, 33 μmol) in pyridine (1.5 mL) were added (*R*)-(-)-MTPACl (10 μL, 53 μmol) and DMAP (2.8 mg, 23 μmol) at 0 °C, and the mixture was stirred at room temperature for 24 h. To the mixture was added H_2O, 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 = 10/1) to give **22a** (29 mg, 92%) as a colorless oil. $[\alpha]_D{}^{23}$ +46.6 (*c* 1.02, $CHCl_3$); IR (neat) 1738, 1651, 1630, 1381 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.47 (s, 3 H), 0.98 (d, *J* = 6.1 Hz, 3 H), 1.05 (d *J* = 6.9 Hz, 3 H), 1.10 (m, 1 H), 1.20 (m, 1 H), 1.22 (s, 9 H), 1.25 (m,1 H), 1.27 (m, 1 H), 1.40 (m, 1 H), 1.48-1.80 (m, 5 H), 1,78 (ddd, *J* = 13.7, 11.2, 1.1 Hz, 1 H), 1.87 (m, 1 H), 1.92-2.01 (m, 2 H), 2.35 (dq, *J* = 6.9, 6.9 Hz, 1 H), 2.87 (m, 1 H), 3.51 (s, 3 H), 4.51 (d, *J* = 13.5 Hz, 1 H), 4.53 (d, *J* = 13.5 Hz, 1 H), 4.94 (s, 1 H), 5.10 (s. 1 H), 5.34 (ddd, *J* = 11.2, 6.9, 1.4 Hz, 1 H), 5.64 (br s, 1 H), 7.35-7.42 (m, 2 H), 7.54-7.58 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.7, 15.2, 18.5, 22.0, 22.5, 27.2 (3C), 27.6, 30.9, 33.0, 38.8, 39.3, 39.8, 41.4, 45.5, 55.3, 55.8, 56.0, 66.1, 76.3, 84.5 (q, $^2J_{C\text{-}F}$ = 27.6 Hz), 97.6, 113.5, 123.3 (q, $^1J_{C\text{-}F}$ = 289.1 Hz), 127.6, 128.4 (2 C), 129.6 (2 C), 131.9, 144.9, 145.2, 166.3, 177.9; EI-LRMS *m/z* 684 (M^+), 605, 452, 435, 371, 227, 189; EI-HRMS calcd for $C_{35}H_{48}O_5{}^{79}BrF_3$ 684.2637, found 684.2645.

(*R*)-MTPA ester <u>22b</u>: In a similar manner to that for the synthesis of **22a** from **21**, a crude product, which was obtained from **21** (20 mg, 35 μmol), (*S*)-(+)-MTPACl (10 μL, 53 μmol) and DMAP (2.6 mg, 21 μmol) in pyridine at room temperature for 24 h, was purified by flash column chromatography on silica gel (hexane/AcOEt = 10/1) to give **22b** (29 mg, quant) as a colorless oil. $[\alpha]_D{}^{23}$ +79.6 (*c* 2.14, $CHCl_3$); IR (neat) 1738, 1649, 1630, 1381 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.40 (s, 3 H), 0.96 (d, *J* = 6.1 Hz, 3 H), 1.07 (d *J* = 7.2 Hz, 3 H), 1.16 (m, 1 H), 1.20 (m, 1 H), 1.23 (s, 9 H), 1.26 (m,1 H), 1.36 (m, 1 H), 1.45-1.70 (m, 5 H), 1,74 (dd, *J* = 12.9, 10.7 Hz, 1 H), 1.82 (m, 1 H), 1.90-2.00 (m, 2 H), 2.37 (dq, *J* = 6.9, 6.3 Hz, 1 H), 2.86 (m, 1 H), 3.50 (s, 3 H), 4.52 (d, *J* = 14.0 Hz, 1 H), 4.56 (d, *J* = 14.0 Hz, 1 H), 4.96 (s, 1 H), 5.13 (s. 1 H), 5.38 (ddd, *J* = 10.7, 6.3, 1.4 Hz, 1 H), 5.62 (br s, 1 H), 7.35-7.42 (m, 2 H), 7.54-7.58 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 11.7, ;15.3, 18.5, 22.0, 22.5, 27.2 (3 C), 27.5, 30.9, 32.7, 38.8, 39.3, 39.8, 41.5, 45.5, 55.2, 55.8, 56.0, 66.1, 76.1, 84.6 (q, $^2J_{C\text{-}F}$ = 27.6 Hz), 97.5, 113.6, 123.4 (q, $^1J_{C\text{-}F}$ = 289.1 Hz), 127.6, 128.4 (2 C), 129.6 (2 C), 131.6, 144.9, 145.3, 166.3, 177.9; EI-LRMS *m/z* 684 (M^+), 605, 452, 435, 371, 227, 189; EI-HRMS calcd for $C_{35}H_{48}O_5{}^{79}BrF_3$ 684.2637, found 684.2629.

The values of $\Delta\delta = \delta_{(S)\text{-MTPA ester}} - \delta_{(R)\text{-MTPA ester}}$ in the ^1H NMR spectra of **22** were calculated and shown in Figure 6. These data were considered by applying a modified Mosher's method, and the absolute configurations at the C23 positions of **22** were determined to be 23*R*. From this result and NOE experiment shown in Figure 4, the absolute stereochemistry at the C24 position of **22** was determined to be 24*R*.

Figure 6.



$\Delta\delta = \delta_{(S)\text{-ester}} - \delta_{(R)\text{-ester}}$

Synthesis of *anti*-lactones 14 and 16.

Compound 13: To a solution of **12** (210 mg, 0.45 mmol) in CH_2Cl_2 (2.2 mL) were added tetrapropylammonium perruthenate (TPAP, 16 mg, 46 μmol) and *N*-methylmorpholine *N*-oxide (NMO, 79 mg, 0.67 mmol) at room temperature, and the mixture was stirred at the same temperature for 4 h. After the mixture was filtered through silica gel short column (Et_2O), the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 20/1) to give ketone (195.6 mg, 94%) as colorless oil. $[\alpha]_D^{24}$ +124.3 (*c* 2.93, $CHCl_3$); IR (neat) 1732, 1649, 1631, 1280, 1147 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 0.92 (d, *J* = 6.4 Hz, 3 H), 1.20 (d, *J* = 7.1 Hz, 3 H), 1.22 (s, 9 H), 1.29 (m, 1 H), 1.35-1.75 (m, 7 H), 1.83 (m, 1 H), 1.93-2.10 (m, 3 H), 2.26 (dd, *J* = 16.4, 9.9 Hz, 1 H), 2.52 (dd, *J* = 16.4, 2.8 Hz, 1 H), 2.88 (m, 1 H), 3.18 (q, *J* = 7.1 Hz, 1 H), 4.53 (s, 2 H), 5.06 (s, 1 H), 5.21 (s, 1 H), 5.64 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 15.3, 20.1, 22.1, 22.6, 27.3 (3 C), 27.7, 31.0, 32.8, 38.8, 39.8, 45.6, 47.5, 51.5, 55.6, 58.9, 65.8, 97.5, 114.9, 143.0, 144.6, 177.6, 209.7; EI-LRMS *m/z* 466 (M^+), 387, 366, 279, 237, 175; EI-HRMS calcd for $C_{25}H_{39}{}^{79}BrO_3$ 466.2083, found 466.2083.

To a solution of the above ketone (34 mg, 71.7 μmol) in THF (1 mL) was added $LiAlH(O^tBu)_3$ (1.0 M solution in THF, 0.72 mL, 0.72 mmol) at 0 °C, and the mixture was stirred at the same temperature for 9 h. To the mixture was added saturated NH_4Cl 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 (hexane/AcOEt = 10/1) to give **13** (31 mg, 91%) as a colorless oil. $[\alpha]_D^{23}$ +71.1 (*c* 2.06, $CHCl_3$); IR (neat) 3514, 1728, 1649, 1286, 1153 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 0.96 (d, *J* = 6.6 Hz, 3 H), 1.03 (d, *J* = 7.1 Hz, 3 H), 1.16 (m, 1 H), 1.22 (s, 9 H), 1.20-1.80 (m, 10 H), 1.91 (m, 1 H), 1.98 (ddd, *J* = 12.4, 6.8, 1.5 Hz, 1 H), 2.03 (m, 1 H), 2.16 (m, 1 H), 2.21 (br s, 1 H), 2.87 (m, 1 H), 3.59 (m, 1 H), 4.50 (d, *J* = 13.9 Hz, 1 H), 4.58 (d, *J* = 13.9 Hz, 1 H), 5.04 (s, 1 H), 5.11 (d, *J* = 1.2 Hz, 1 H), 5.63 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 16.3, 18.8, 22.2, 22.7, 27.3 (3 C), 27.8, 31.1, 32.8, 38.9, 40.0, 41.0, 45.7, 46.1, 56.0, 56.3, 65.3, 71.1, 97.4, 113.2, 144.9, 146.3, 178.1; EI-LRMS *m/z* 468 (M^+), 390, 229, 178, 68, 57; EI-HRMS calcd for $C_{25}H_{41}{}^{79}BrO_3$ 468.2239, found 468.2243.

***tanti*-Lactone 14**: To a solution of **13** (31 mg, 65 μmol) in toluene (1 mL) was added DIBAL-H (1.04 M solution in toluene, 0.31 mL, 0.32 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. To the mixture was added 10% potassium sodium tartrate aq. solution at 0 °C, 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 dissolved in CH_2Cl_2 (2 mL). To the solution was added MnO_2 (113 mg, 1.3 mmol), and the mixture was stirred at room temperature for 3 days. After the mixture was filtered through silica gel short column (Et_2O), the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 10/1) to give **14** (10 mg, 81% in 2 steps) as a colorless oil. $[\alpha]_D^{19}$ +205.8 (*c* 1.16, $CHCl_3$); IR (neat) 1759, 1663, 1630, 1314 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 3 H), 1.02 (d, *J* = 6.6 Hz, 3 H), 1.23 (d, *J* = 6.6 Hz, 3 H), 1.20-1.95 (m, 12 H), 1.98 (ddd, *J* = 12.2, 5.4, 1.7 Hz, 1 H), 2.03 (br d, *J* = 13.2 Hz, 1 H), 2.61 (m, 1 H), 2.89 (m, 1 H), 4.07 (ddd, *J* = 10.7, 7.3, 2.2 Hz, 1 H), 5.53 (d, *J* = 3.1 Hz, 1 H), 5.65 (d, *J* = 1.7 Hz, 1 H), 6.22 (d, *J* = 3.1 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 16.3, 18.8, 22.2, 22.6, 27.7, 31.1, 33.0, 40.0, 40.9, 41.6, 45.7, 55.9, 56.1, 82.4, 97.6, 120.5, 140.7, 144,6, 170.1; EI-LRMS *m/z* 380 (M^+), 301, 227, 147; EI-HRMS calcd for $C_{20}H_{29}{}^{79}BrO_2$ 380.1351, found 380.1345.

Compound <u>15</u>: To a solution of **21** (Scheme 8, 273 mg, 0.58 mmol) in CH_2Cl_2 (2.9 mL) were added TPAP (20 mg, 58 μmol) and NMO (102 mg, 87 μmol) at room temperature, and the mixture was stirred at the same temperature for 4 h. After the mixture was filtered through silica gel short column (Et_2O), the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 30/1) to give **15** (252 mg, 93%) as a colorless oil. $[\alpha]_D^{21}$ +25.2 (*c* 1.35, $CHCl_3$); IR (neat) 1732, 1716, 1651, 1631, 1371, 1147 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 0.85 (d, *J* = 6.6 Hz, 3 H), 1.17 (d, *J* =7.1 Hz, 3 H), 1.18 (s, 9 H), 1.19-1.30 (m, 3 H), 1.35-1.70 (m, 5 H), 1.79 (m, 1 H), 1.88-2.05 (m, 3 H), 2.22 (dd, *J* = 16.7, 9.9 Hz, 1 H), 2.45 (dd, *J* = 16.7, 2.4 Hz, 1 H), 2.82 (m,1 H), 3.18 (q, *J* = 7.1 Hz, 1 H), 4.46 (d, *J* = 14.7 Hz, 1 H), 4.50 (d, *J* = 14.7 Hz, 1 H), 4.99 (s, 1 H), 5.16 (s, 1 H), 5.59 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 15.5, 19.7, 22.0, 22.5, 27.2 (3 C), 27.7, 30.9, 32.2, 38.8, 39.7, 45.5, 47.6, 50.1, 55.5, 55.8, 65.7, 97.4, 114.6, 143.0, 144.5, 177.4, 209.0; EI-LRMS *m/z* 466 (M^+), 387, 364, 279, 237, 175, 137; EI-HRMS calcd for $C_{25}H_{39}{}^{79}BrO_3$ 466.2082, found 466.2086.

***anti*-Lactone <u>16</u>**: To a solution of **15** (185 mg, 0.40 mmol) in toluene (4 mL) was added DIBAL-H (1.04 M solution in toluene, 1.9 mL, 2.0 mmol) at -78 °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 at 0 °C, 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 dissolved in CH_2Cl_2 (2 mL). To the solution was added MnO_2 (668 mg, 7.7 mmol), and the mixture was stirred at room temperature for 38 h. After the mixture was filtered through silica gel short column (Et_2O), the filtrate was concentrated. The residue was purified by preparative thin-layer chromatography on silica gel (hexane/AcOEt = 10/1) to give **16** (60 mg, 40 % in 2 steps) as a colorless oil. $[\alpha]_D^{21}$ +84.2 (*c* 0.92, $CHCl_3$); IR (neat) 1755, 1664, 1628, 1313, 1153; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3 H), 1.07 (d, *J* = 6.1 Hz, 3 H), 1.25 (d, *J* =6.8 Hz, 3 H), 1.20-1.75 (m, 11 H), 1.90-2.10 (m, 3 H), 2.64 (m, 1 H), 2.88 (m, 1 H), 4.07 (dt, *J* = 6.3, 5.6 Hz, 1 H), 5.53 (d, *J* = 3.1 Hz, 1 H), 5.65 (s, 1 H), 6.22 (d, *J* = 3.1 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 17.3, 19.6, 22.2, 22.6, 28.0, 31.1, 34.5, 39.8, 40.8, 41.5, 45.6, 55.8 (2 C), 84.0, 97.5, 120.7, 140.5, 144.7, 170.1; EI-LRMS *m/z* 380 (M^+), 301, 227, 147; EI-HRMS calcd for $C_{20}H_{29}O_2{}^{79}Br$ 380.1351, found 380.1354.

General Procedure for the synthesis of vitamin D_3-lactones: Method A. To a solution of an A-ring precursor (1.3 or 1.5 equiv. to a CD-ring precursor) and the CD-ring precursor in toluene were added Et_3N and $Pd(PPh_3)_4$ (30 mol % to the CD-ring precursor), and the mixture was stirred at 110 °C. After the mixture was filtered through silica gel pad, the filtrate was concentrated. The crude product was dissolved in MeOH. To the solution was added (+)-10-camphorsulfonic acid (CSA) at 0 °C, and the mixture was stirred at room temperature. 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 or preparative thin-layer chromatography on silica gel to give the vitamin D_3-lactone derivative. Further purification for biological assays was conducted by reversed-phase recycle HPLC (YMC-Pack ODS column, 20 X 150 mm, 9.9 mL/min, CH_3CN:H_2O = 85:15).

General Procedure for the synthesis of vitamin D_3-lactones: Method B. To a solution of an A-ring precursor (1.5 equiv. to a CD-ring precursor) and the CD-ring precursor in toluene were added Et_3N and $Pd(PPh_3)_4$ (30 mol % to the CD-ring precursor), and the mixture was stirred at 110 °C. After the mixture was filtered through silica gel pad, the filtrate was concentrated. The crude product was dissolved in MeCN (1 mL). To the solution was added 10% solution of conc. HF in MeCN (1 mL) at 0 °C, and the mixture was stirred at room temperature. 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 or preparative thin-layer chromatography on silica gel to give the vitamin D_3-lactone derivative. Further purification for biological assays was conducted by reversed-phase recycle HPLC (YMC-Pack ODS column, 20 X 150 mm, 9.9 mL/min, CH_3CN:H_2O = 85:15).

(23*S*,24*S*)-25-Dehydro-1α-hydroxy-24-mehtylvitamin D_3-26,23-lactone (4): According to the General Procedure (Method A), a crude product, which was obtained from **10** (35 mg, 92 μmol), **17** (44 mg, 0.12 mmol), Et_3N (1.5 mL) and $Pd(PPh_3)_4$ (32 mg, 28 μmol) in toluene (3 mL) at 110 °C for 1.5 h, was treated with CSA (47 mg, 0.20 mmol) in MeOH (3 mL) for 1.5 h. After usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/3) to give **4** (20 mg, 48% in 2 steps) as a colorless oil. UV (EtOH) λ_{max} = 265 nm; $[\alpha]_D^{18}$ –17.0 (*c* 0.52, $CHCl_3$); IR (neat) 3395, 1755, 1638, 1269, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3 H), 1.05 (d, *J* = 6.6 Hz, 3 H), 1.13 (d, *J* = 7.1 Hz, 3 H), 1.20-1.75 (m, 13 H), 1.87-1.95 (m, 2 H), 1.96-2.08 (m, 3 H), 2.31 (dd, *J* = 13.4, 6.6 Hz, 1 H), 2.59 (dd, *J* = 13.4, 3.4 Hz, 1 H), 2.82 (dd, *J* = 12.5, 4.4 Hz, 1 H), 3.11 (dddq, *J* = 2.2, 2.2, 6.8, 7.1 Hz, 1 H), 4.22 (m, 1 H), 4.43 (m, 1 H), 4.59 (ddd, *J* = 8.2, 6.8, 5.3 Hz, 1 H), 4.99 (dd, *J* = 1.5, 1.5 Hz, 1 H), 5.32 (dd, *J* = 1.5, 1.5 Hz, 1 H), 5.53 (d, *J* = 2.2 Hz, 1 H), 6.01 (d, *J* = 11.2 Hz, 1 H), 6.18 (d, *J* = 2.2 Hz, 1 H), 6.37 (d, *J* = 11.2 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 14.7, 19.9, 22.4, 23.6, 27.8, 29.1, 34.5, 36.4, 38.2, 40.8, 42.9, 45.3, 46.0, 56.2, 56.9, 66.8, 70.8, 80.3, 111.6, 117.1, 120.4, 124.3, 132.9, 141.3, 142.6, 147.5, 170.2; EI-LRMS *m/z* 440 (M^+), 422, 404, 251, 105; EI-HRMS calcd for $C_{28}H_{40}O_4$ 440.2987, found 440.2932.

(23*S*,24*R*)-25-Dehydro-1α-hydroxy-24-mehtylvitamin D_3-26,23-lactone (5): According to the General Procedure (Method A), a crude product, which was obtained from **16** (14 mg, 37 μmol), **17** (18 mg, 48 μmol), Et_3N (1 mL) and $Pd(PPh_3)_4$ (13 mg, 11 μmol) in toluene (2 mL) at 110 °C for 1.5 h, was treated with CSA (20 mg, 86 μmol) in MeOH (1.5 mL) for 1.5 h. After usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/2) to give **5** (7.8 mg, 48% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max} = 265 nm; $[\alpha]_D^{23}$ +19.7 (*c* 0.30, $CHCl_3$); IR (neat) 3400, 1759, 1630 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3 H), 1.06 (d, *J* = 5.9 Hz, 3 H), 1.28 (d, *J* = 6.8 Hz, 3 H), 1.25-1.80 (m, 13 H), 1.85-2.10 (m, 5 H), 2.32 (dd, *J* = 13.6, 6.4 Hz, 1 H), 2.55-2.70 (m, 2 H), 2.83 (m, 1 H), 4.07 (dt, *J* = 5.9, 6.4 Hz, 1 H), 4.23 (m, 1 H), 4.43 (m, 1 H), 5.00 (s, 1 H), 5.33 (s, 1 H), 5.53 (d, *J* = 2.9 Hz, 1 H), 6.01 (d, *J* = 11.1 Hz, 1 H), 6.22 (d, *J* = 2.9 Hz, 1 H), 6.37 (d, *J* = 11.1 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 17.2, 19.5, 22.3, 23.5, 27.9, 29.0, 34.5, 40.4, 40.7, 41.5, 42.9, 15.2, 45.9, 56.2, 56.5, 66.9, 70.8, 84.2, 111.8, 117.2, 120.8, 124.9, 133.0, 140.8, 142.8, 147.6, 170.4; EI-LRMS *m/z* 440 (M^+), 422, 404, 251, 105; EI-HRMS calcd for $C_{28}H_{40}O_4$ 440.2927 found 440.2929.

(23*R*,24*S*)-25-Dehydro-1α-hydroxy-24-mehtylvitamin D_3-26,23-lactone (6): According to the General Procedure (Method A), a crude product, which was obtained from **14** (19 mg, 49 μmol), **17** (27 mg, 74 μmol), Et_3N (1 mL) and $Pd(PPh_3)_4$ (17 mg, 15 μmol) in toluene (2 mL) at 110 °C for 1.5 h, was treated with CSA (27 mg, 0.12 mmol) in MeOH (1.5 mL) for 1.5 h. After usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/2) to give **6** (7.8 mg, 52% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max} = 265 nm; $[\alpha]_D^{18}$ +81.3 (*c* 0.27, $CHCl_3$); IR (neat) 3383, 1765, 1643, 1247, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3 H), 1.01 (d, *J* = 6.4 Hz, 3 H), 1.22 (d, *J* = 6.8 Hz, 3 H), 1.20-1.38 (m, 4 H), 1.40-2.10 (m, 14 H), 2.31 (dd, *J* = 13.4, 6.4 Hz, 1 H), 2.55-2.65 (m, 2 H), 2.82 (dd, *J* = 12.2, 3.9 Hz, 1 H), 4.07 (ddd, *J* = 10.5, 7.3, 2.0 Hz, 1 H), 4.22 (m, 1 H), 4.42 (dd, *J* = 7.6, 4.4 Hz, 1 H), 4.99 (s, 1 H), 5.32 (dd, *J* = 1.7, 1.4 Hz, 1 H), 5.52 (d, *J* = 2.9 Hz, 1 H), 6.01 (d, *J* = 11.2 Hz, 1 H), 6.21 (d, *J* = 2.9 Hz, 1 H), 6.36 (d, *J* = 11.2 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 16.3, 18.8, 22.4, 23.6, 27.7, 29.1, 33.1, 40.6, 40.9, 41.6, 43.0, 45.3, 46.1, 56.4, 56.9, 66.9, 70.8, 82.5, 111.7, 117.1, 120.4, 124.7, 133.0, 140.8, 142.5, 147.4, 170.2; EI-LRMS *m/z* 440 (M^+), 422, 404, 251, 105; EI-HRMS calcd for $C_{28}H_{44}O_4$ 440.2927, found 440.2920

(23*R*,24*R*)-25-Dehydro-1α-hydroxy--24-mehtylvitamin D_3-26,23-lactone (7): According to the General Procedure (Method A), a crude product, which was obtained from **11** (37 mg, 96 μmol), **17** (46 mg, 0.12 mmol), Et_3N (1.5 mL) and $Pd(PPh_3)_4$ (33 mg, 29 μmol) in toluene (3 mL) at 110 °C for 1.5 h, was treated with CSA (47 mg, 0.20 mmol) in MeOH (1.5 mL) for 45 min. After usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/2) to give **7** (24 mg, 57% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max} = 265 nm; $[\alpha]_D^{23}$ +113.9 (*c* 0.38, $CHCl_3$); IR (neat) 3420, 1757, 1658 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.57 (s, 3 H), 1.02 (d, *J* = 6.4 Hz, 3 H), 1.08 (m, 1 H), 1.13 (d, *J* = 7.3 Hz, 3 H), 1.15-1.35 (m, 3 H), 1.40-2.10 (m, 14 H), 2.31 (dd, *J* = 13.4, 6.6 Hz, 1 H), 2.59 (dd, *J* = 13.4, 3.3 Hz, 1 H), 2.83 (dd, *J* = 12.1, 3.8 Hz, 1 H), 3.16 (dq, *J* = 7.8, 7.3 Hz, 1 H), 4.23 (m, 1 H), 4.43 (m, 1 H), 4.67 (ddd, *J* = 11.8, 7.8, 2.0 Hz, 1 H), 4.99 (s, 1 H), 5.33 (s, 1 H), 5.52 (d, *J* = 2.7 Hz, 1 H), 6.01 (d, *J* = 11.3 Hz, 1 H), 6.21 (d, *J* = 2.7 Hz, 1 H), 6.36 (d, *J* = 11.3 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 13.9, 18.6, 22.4,

23.6, 27.7, 29.1, 32.6, 37.0, 37.8, 40.6, 42.9, 45.3, 46.1, 56.4, 57.1, 66.8, 70.7, 78.4, 111.7, 117.1, 120.5, 124.7, 133.0, 140.7, 142.5, 147.4, 170.2; EI-LRMS *m/z* 440 (M^+), 422, 404, 378, 289, 209,105; EI-HRMS calcd for $C_{28}H_{40}O_4$ 440.2927, found 440.2935.

(23*S*,24*S*)-25-Dehydro-2α,24-dimethyl-1α-hydroxyvitamin D_3-26,23-lactone (4a): According to the General Procedure (Method B), a crude product, which was obtained from **10** (19 mg, 49 μmol), **17a** (28 mg, 74 μmol), Et_3N (1.5 mL) and $Pd(PPh_3)_4$ (17 mg, 15 μmol) in toluene (3 mL) at 110 °C for 1.5 h, was treated with conc. HF in MeCN for 1.5 h. After usual work up, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 1/1) to give **4a** (15 mg, 68% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max} = 267 nm; $[\alpha]_D^{19}$ +4.39 (*c* 1.13, $CHCl_3$); IR (neat) 2281, 1774, 1651, 1603, 1263, 1136 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3 H), 1.05 (d, *J* = 6.6 Hz, 3 H), 1.08 (d, *J* = 6.8 Hz, 3 H), 1.13 (d, *J* = 7.1 Hz, 3 H), 1.20-1.75 (m, 13 H), 1.85-2.10 (m, 4 H), 2.23 (dd, *J* = 13.6, 7.9 Hz, 1 H), 2.66 (dd, *J* = 13.6, 4.0 Hz, 1 H), 2.82 (m, 1 H), 3.11 (m, 1 H), 3.84 (m, 1 H), 4.31 (m, 1 H), 4.59 (m, 1 H), 5.00 (d, *J* = 1.7 Hz, 1 H), 5.27 (br s, 1 H), 5.53 (d, *J* = 2.3 Hz, 1 H), 6.01 (d, *J* = 11.4 Hz, 1 H), 6.18 (d, *J* = 2.3 Hz, 1 H), 6.38 (d, *J* = 11.4 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 12.7, 14.7, 19.8, 22.4, 23.6, 27.9, 29.1, 34.5, 36.4, 38.2, 40.5, 43.5, 44.3, 46.0, 56.2, 56.9, 71.7, 75.4, 80.3, 113.1, 117.0, 120.4, 124.6, 133.0, 141.2, 143.1, 146.4, 170.2; EI-LRMS *m/z* 454 (M^+), 436, 418, 265, 166, 148; EI-HRMS calcd for $C_{29}H_{42}O_4$ 454.3083, found 454.3095.

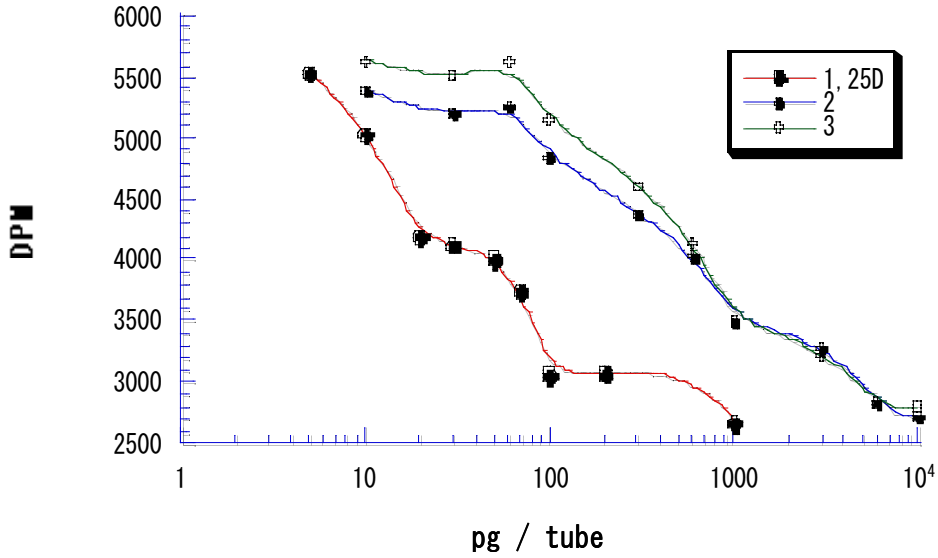
(23*S*,24*R*)-25-Dehydro-2α,24-dimethyl-1α-hydroxyvitamin D_3-26,23-lactone (5a): According to the General Procedure (Method B), a crude product, which was obtained from **16** (12 mg, 31 μmol), **17a** (19 mg, 74 μmol), Et_3N (2 mL) and $Pd(PPh_3)_4$ (11 mg, 9.3 μmol) in toluene (2 mL) at 110 °C for 1.5 h, was treated with conc. HF in MeCN for 2.5 h. After usual work up, the crude product was purified by column chromatography on silica gel (hexane/AcOEt = 1/1) to give **5a** (10 mg, 71% in 2 steps) as an amorphous solid. UV (EtOH) λ_{max} = 267 nm; $[\alpha]_D^{21}$ +45.0 (*c* 0.64, $CHCl_3$); IR (neat) 350, 1761, 1667, 1644, 1248, 1066 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (s, 3 H), 1.06 (d, *J* = 6.8 Hz, 3 H), 1.08 (d, *J* = 7.3 Hz, 3 H), 1.25 (d, *J* = 6.8 Hz, 3 H), 1.30-1.75 (m, 14 H), 1.85-2.10 (m, 3 H), 2.23 (dd, *J* = 13.4, 8.1 Hz, 1 H), 2.55-2.75 (m, 2 H), 2.82 (m, 1 H), 3.83 (m, 1 H), 4.07 (m, 1 H), 4.31 (m, 1 H), 5.00 (s, 1 H), 5.27 (s, 1 H), 5.53 (d, *J* = 2.9 Hz, 1 H), 6.00 (d, *J* = 11.2 Hz, 1 H), 6.22 (d, *J* = 2.9 Hz ,1 H), 6.38 (d, *J* = 11.2 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 12.7, 17.4, 19.7, 22.4, 23.6, 28.1, 29.1, 34.6, 40.5, 40.8, 41.6, 43.5, 44.3, 46.0, 56.2, 56.6, 71.7, 75.4, 84.1, 113.1, 117.0, 120.6, 124.6, 133.0, 140.6, 142.7, 146.4, 170.1; EI-LRMS *m/z* 454 (M^+), 436, 418, 265, 166, 148; EI-HRMS calcd for $C_{29}H_{42}O_4$ 454.3083, found 454.3083.

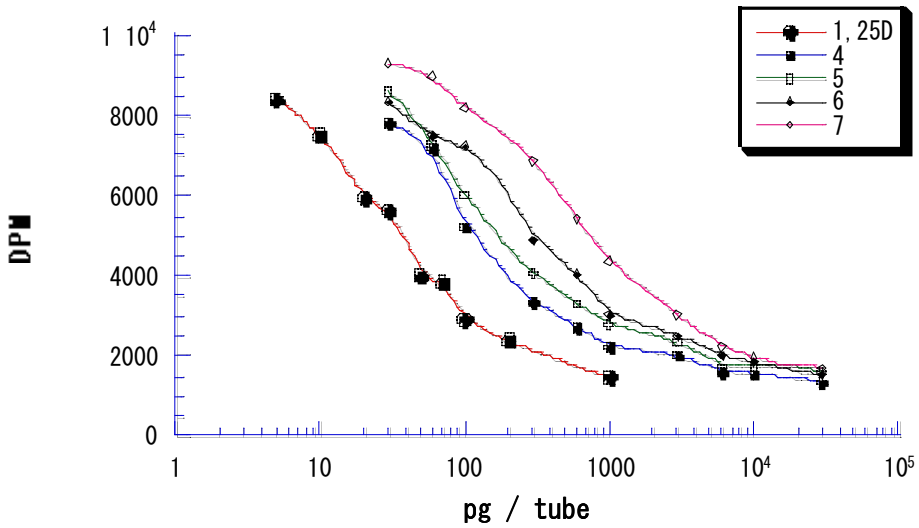
Vitamin D receptor (VDR) binding assay: [26,27-*methyl*-^{3}H]-1α,25-dihydroxyvitamin D_3 (specific activity 6.623 TBq/mmol, 15,000 dpm, 15.7 pg) and various amounts of 1α,25-dihydroxyvitamin D_3 and an analogue to be tested were dissolved in 50 mL of absolute ethanol in 12 x 75-mm polypropylene tubes. 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 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 the mixed vigorously and centrifuged at 2,260 x *g* for 60 min at 4 °C.

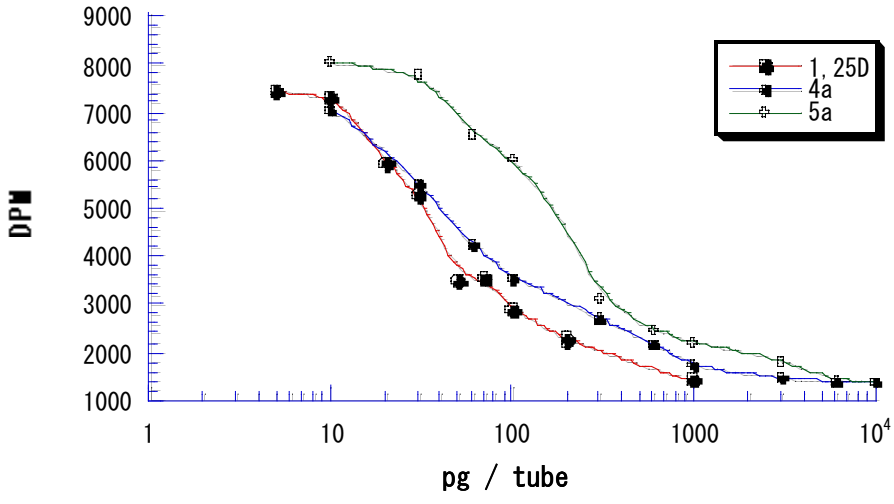
After the supernatant was decanted, the bottom of the tube containing the pellet was cut off into a scintillation vial containing 10 ml of dioxane-based scintillation fluid and the radioactivity was counted with a Beckman liquid scintillation counter (Model LS6500). The relative potency of the analogues 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 x 10^4 cells/mL 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.[19] 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, 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.

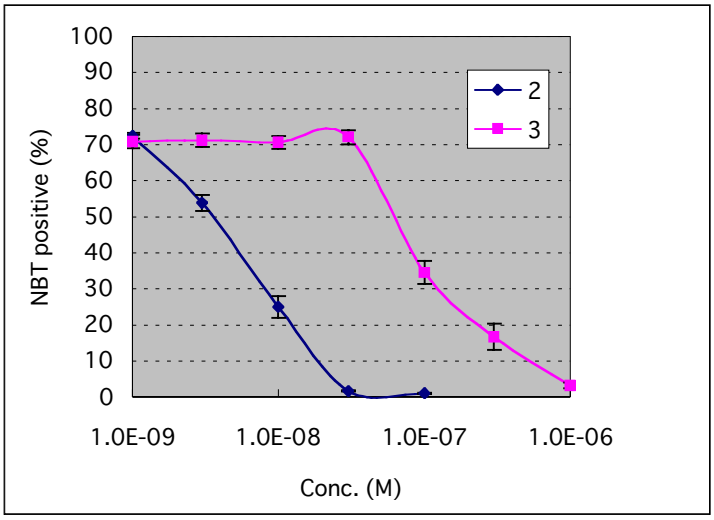
Charts of VDR binding affinity

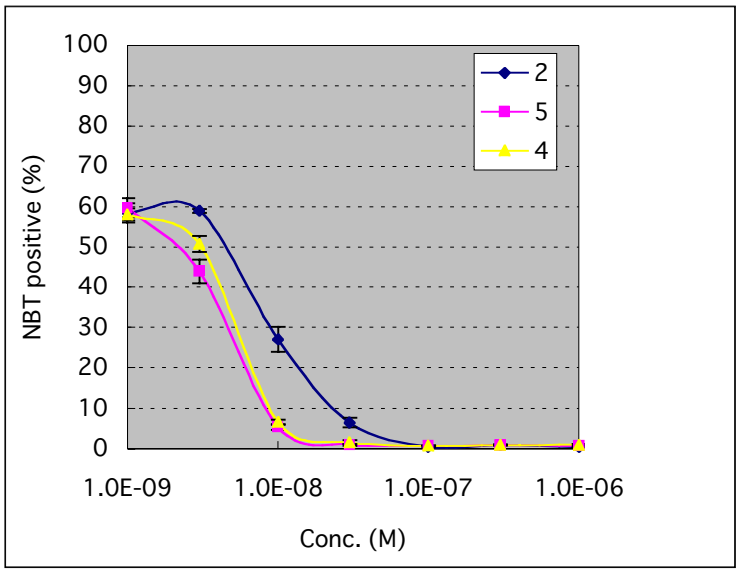


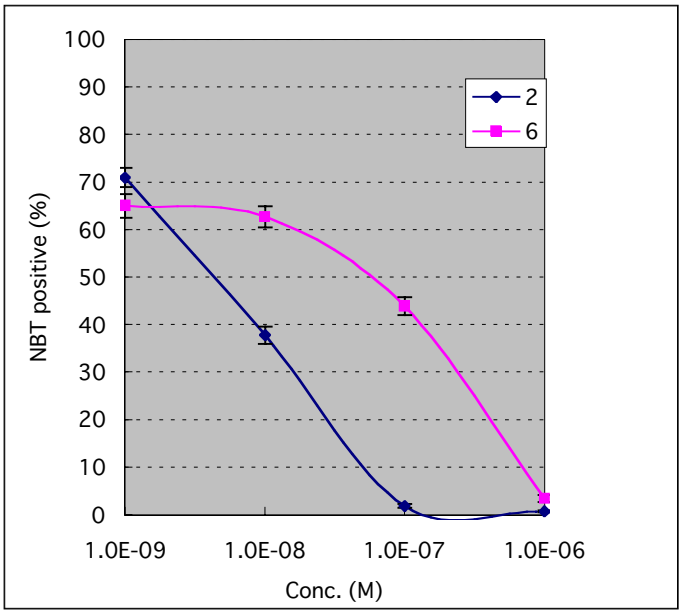


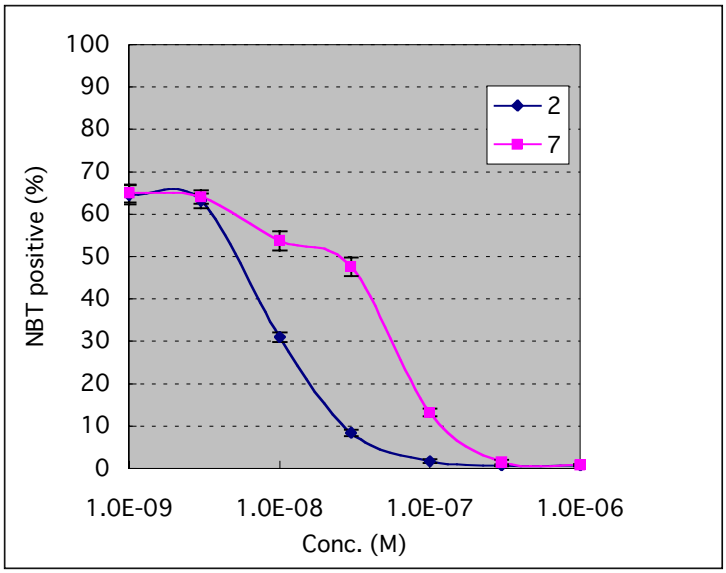


Charts of HL-60 cell differentiation





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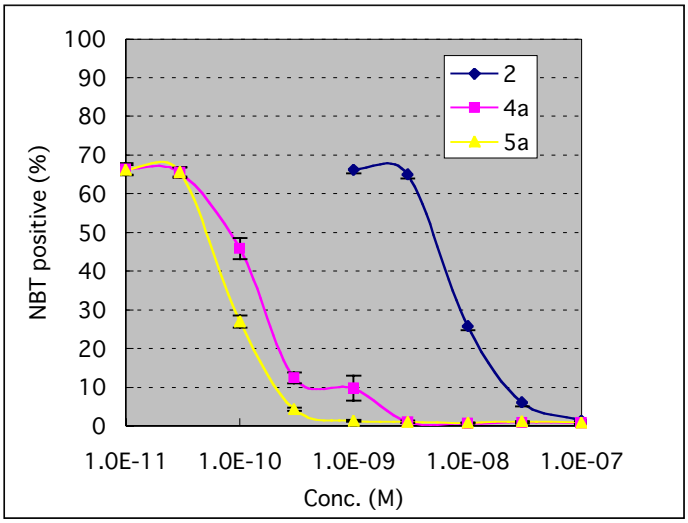
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NBT positive (%)
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1.0E-08
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Conc. (M)