Preparation and Biological Activity of 24-Epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_2

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A new fluorinated analog of vitamin D_2, 24-epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_2, was efficiently synthesized starting from (*R*)-4-isopropyl-3-propionyl-2-oxazolidinone with high stereochemical control. In all four physiological test systems, the fluorinated vitamin D_2 analog was found to be slightly less active than 1α,25-dihydroxyvitamin D_3.

Key words vitamin D; fluorinated vitamin D analog; calcium regulation; 1α,25-dihydroxyvitamin D_3

1α,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$, **1**), the hormonally active form of vitamin D_3, is essential to the regulation of calcium and phosphorus metabolism in animals. Among its functions are intestinal calcium transport, bone calcium mobilization, calcification of epiphyseal plate cartilage and elevation of plasma calcium and phosphorus concentration. These physiological properties and the application of **1** as a medicine to treat bone diseases such as osteoporosis have prompted the synthesis of vitamin D_3 analogs for enhancing and modifying biological activity.[1] Kobayashi *et al.* reported the synthesis of a fluorinated analog, 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3 (**2**), which displays enhanced and prolonged activity in various bioassays, compared to **1**,[2] thus showing the ability of fluorine atoms to inhibit metabolic hydroxylation at the C-26 and C-27 positions, a step in the deactivation of **1**.[3] DeLuca *et al.* found that 24-epi-1α,25-dihydroxyvitamin D_2 (**3**) regulates intestinal calcium transport and calcification of epiphyseal plate cartilage but without promoting bone calcium mobilization or elevation of plasma calcium concentration.[4] These findings prompted the authors to search for an efficient synthesis of the new fluorinated analog, 24-epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_2 (24-epi-26,27-F_6-1,25-$(OH)_2D_2$, **4**). This paper describes in detail the stereocontrolled synthesis of **4** along with preliminary results on its biological activities.

The synthesis was initiated by the enantioselective construction of the side chain. Diastereoselective reaction of the boron enolate derived from the chiral *N*-acyloxazolidinone **5** with hexafluoroacetone gave the carboximide **6** in 80% yield.[5] The reduction of **6** with lithium borohydride ($LiBH_4$) in tetrahydrofuran (THF) at 0 °C afforded the diol **7** in 64% yield. Treatment of **7** with (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) in pyridine gave the corresponding (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetate **8** (MTPA ester **8**), whose ^1H- and ^{19}F-NMR spectra showed **7** to be enantiomerically pure. The selective silylation of **7** with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in dichloromethane (CH_2Cl_2) gave the *tert*-butyldimethylsilyl (TBDMS) ether **9** in 85% yield. The methoxymethylation of **9** with chloromethyl methyl ether (MOMCl) and diisopropylethylamine (iso-Pr_2NEt) in CH_2Cl_2 at room temperature provided the corresponding methoxymethyl (MOM) ether **10** in 81% yield. Desilylation of **10** with tetrabutylammonium fluoride (Bu_4NF) in THF afforded the alcohol **11** in quantitative yield. This alcohol **11** was treated with *p*-toluenesulfonyl chloride (TsCl) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in pyridine at room temperature for 16 h to give the tosylate **12** in 97% yield.

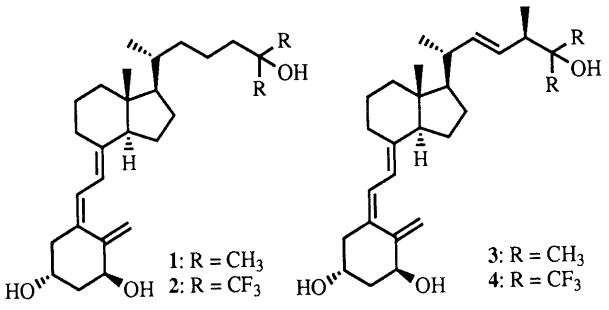
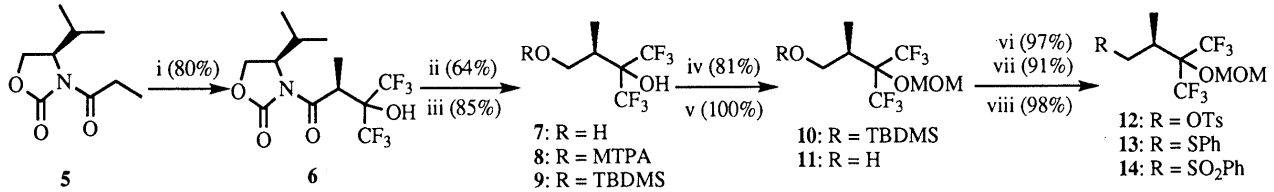


Chart 1



(i) (a) Bu_2BOTf, Et_3N (b) hexafluoroacetone; (ii) $LiBH_4$; (iii) TBDMSCl, imidazole; iv) MOMCl, iso-Pr_2NEt; (v) Bu_4NF; (vi) TsCl in pyridine; (vii) NaH, PhSH; (viii) MCPBA, Na_2HPO_4

Chart 2

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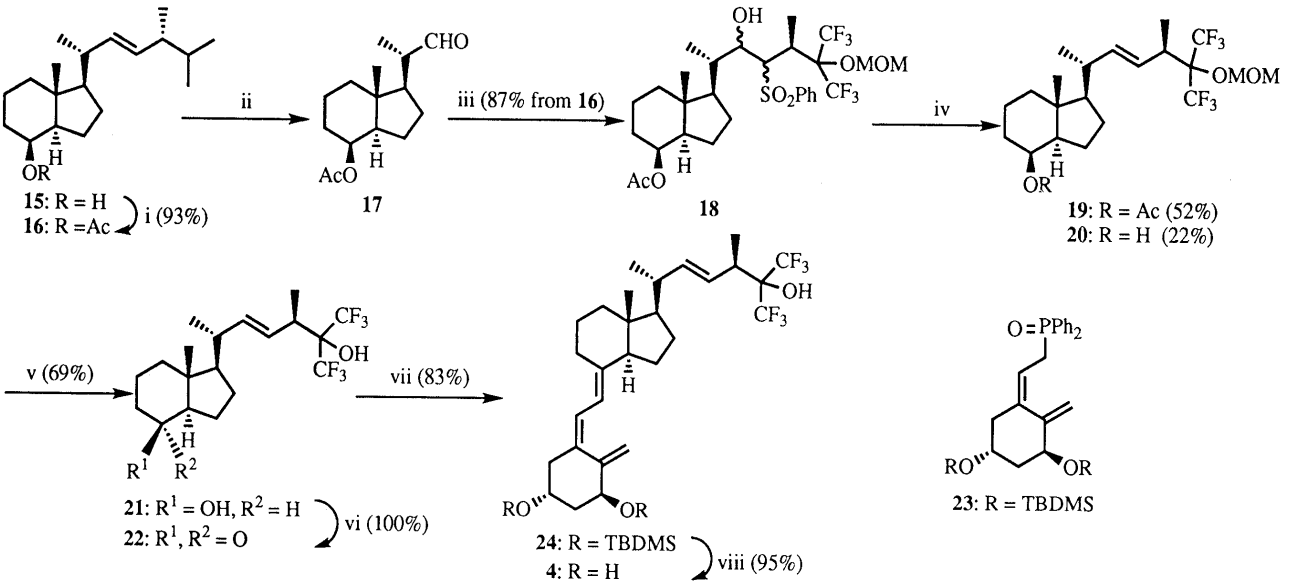

(i) Ac_2O, Et_3N, DMAP in CH_2Cl_2; (ii) (a) O_3, pyridine in CH_2Cl_2 (b) Zn, AcOH; (iii) **14**, BuLi, $MgBr_2{\cdot}OEt_2$; (iv) Na-Hg, Na_2HPO_4; (v) (a) MeLi (b) conc. HCl-dioxane, 60°C; (vi) PCC oxidation; (vii) **23**, BuLi; (viii) AG 50W-X4 in MeOH

Chart 3

Displacement of the tosyl group of **12** with sodium thiophenoxide (NaSPh) provided the sulfide **13** in 91% yield. The oxidation of **13** with *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 at 6 °C gave the sulfone **14** in 98% yield.

To introduce a double bond at C-22 (vitamin D numbering) in a stereocontrolled manner, the condensation of the aldehyde **17** with the sulfone **14** was examined. The alcohol **15**, prepared from vitamin D_2 according to the oxidation procedure of Toh and Okamura,[6] was converted to the acetate **16** in 93% yield by acetylation with acetic anhydride (Ac_2O) in the presence of triethylamine (Et_3N) and DMAP in CH_2Cl_2. The reaction of **16** with ozone was carried out in the presence of pyridine in CH_2Cl_2 at −78 °C. Reduction with zinc powder and acetic acid (AcOH) afforded the crude aldehyde **17**, which was directly treated with the carbanion derived from the sulfone **14** and butyllithium (BuLi), in the presence of magnesium bromide diethyl etherate ($MgBr_2{\cdot}OEt_2$) in THF at −78 to 0 °C to give the alcohol **18** as a mixture of diastereomers in 87% yield in two steps. Treatment of **18** with 5% sodium amalgam (5% Na–Hg) in the presence of sodium hydrogen phosphate (Na_2HPO_4) in THF-methanol (MeOH) at 0 °C afforded the acetate **19** in 52% yield along with the alcohol **20** (22%). Deacetylation of **19** with methyllithium (MeLi) in THF and demethoxymethylation of the resulting alcohol **20** in 1,4-dioxane containing concentrated hydrochloric acid (HCl) at 60 °C gave the corresponding diol **21** in 69% yield in two steps. The diol **21** was oxidized with pyridinium chlorochromate (PCC) in CH_2Cl_2 to give the ketone **22** in quantitative yield.

According to the general approach of Lythgoe *et al.*,[7] the Horner–Wittig reaction of ketone **22** with the phosphinoyl carbanion, prepared from the phosphine oxide 23[8] and BuLi, in THF at −78 to −40 °C afforded the bis(TBDMS) ether **24** in 83% yield. After the desilylation of **24** with cation exchange resin (50W-X4) in MeOH at room temperature,[9] 24-epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_2 (**4**) was obtained as a colorless amorphous powder in 95% yield.

Table 1. Binding Affinities of 1,25-$(OH)_2D_3$ (**1**) and 24-Epi-26,27-F_6-1,25-$(OH)_2D_2$ (**4**) to Chick Intestinal Cytosol Receptor (VDR) and Vitamin D-Deficient Rat Serum DBP

Compound	VDR (IC_{50})[a] (M)	DBP (IC_{50})[a] (M)
1,25-$(OH)_2D_3$ (**1**)	4.0×10^{-10}	5.4×10^{-7}
24-Epi-26,27-F_6-1,25-$(OH)_2D_2$ (**4**)	2.9×10^{-9}	6.8×10^{-6}

a) IC_{50} (M), the 50% inhibitory concentration, was calculated from the displacement curves of [^{3}H]-1,25-$(OH)_2D_3$ and [^{3}H]-25-(OH)D_3 with **1** and **4**, respectively.

Table 1 shows the results of *in vitro* binding assays. The fluorinated vitamin D_2 analog **4** was 8-fold less effective than 1,25-$(OH)_2D_3$ (**1**) for binding to the chick embryonic intestinal 1,25-$(OH)_2D_3$ receptor (VDR). As regards binding affinity to vitamin D binding protein (DBP) from vitamin D-deficient rats, **4** was 13-fold less active than 1,25-$(OH)_2D_3$ (**1**). In *in vivo* experiments, each compound was given to 8-week-old Wistar male rats orally once a day for 3 weeks (0.04—0.625 μg/kg/d). The femurs were excised to measure specific gravity of bone, and blood samples were collected to measure the serum calcium level. As shown in Fig. 1, **4** did not increase the specific gravity of bone, thus indicating that **4** is less active than **1** in increasing bone content of calcium and phosphorus. Figure 2 shows that **4** was less effective than **1** for raising serum calcium in rats.

Experimental

Melting points were determined on a Yanako MP-500D hot stage microscope without correction. Optical rotations were measured in a 1.0 dm cell with a JASCO DIP-370 polarimeter. IR spectra were obtained on a Perkin Elmer 1600 FT-IR. ^{1}H-NMR and ^{19}F-NMR spectra were recorded at 200 MHz and 188 MHz, respectively, on a Varian Gemini-200 instrument. ^{1}H-NMR data are given in parts per million (ppm) downfield

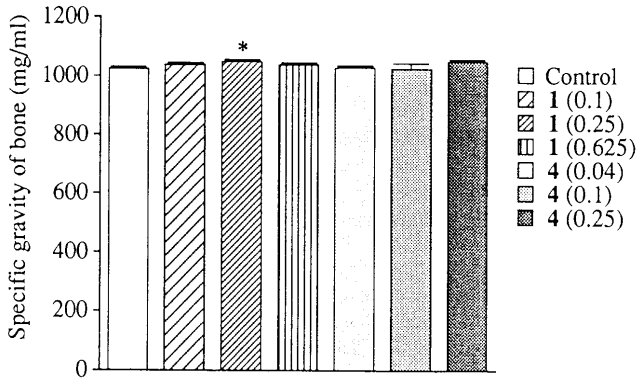

Fig. 1. Increasing Effects of 1,25-$(OH)_2D_3$ (**1**) and 24-Epi-26,27-F_6-1,25-$(OH)_2D_2$ (**4**) on Specific Gravity of Bone in Rats

Each compound was given orally once a day for 3 weeks. Doses are given in μg/kg/d. The rats were killed and the femurs were excised. The weight and volume of the femurs dissected free of soft tissues were measured. Specific gravity was calculated as weight (mg)/volume (ml). $*p<0.05$ *vs.* control.

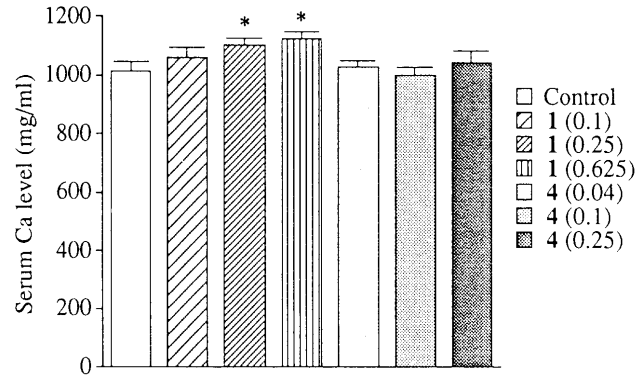

Fig. 2. Increasing Effects of 1,25-$(OH)_2D_3$ (**1**) and 24-Epi-26,27-F_6-1,25-$(OH)_2D_2$ (**4**) on Serum Calcium in Rats

Each compound was given orally once a day for 3 weeks (0.04—0.625 μg/kg/d). Blood samples were collected from the main artery to measure the serum Ca level. $*p<0.01$ *vs.* control.

from tetramethylsilane (TMS) as the internal standard. ^{19}F-NMR data are given in ppm upfield from CCl_3F as the internal standard. The abbreviations used are as follows: s = singlet, d = doublet, t = triplet, q = quartet, sep = septet, m = multiplet, br = broad. Coupling constants (*J* values) are given in hertz (Hz). Low- and high-resolution MS analyses were performed using a Kratos Concept-1H double-focusing magnetic sector spectrometer. Elemental analysis was conducted at Toray Research Center, Inc., Tokyo. Kieselgel 60 (Merck, 230—400 mesh) was used for column chromatography. Thin-layer chromatography (TLC) was carried out with pre-coated Kieselgel $60F_{254}$ plates (Merck). All reactions were carried out under an argon atmosphere with magnetic stirring in oven-dried glassware.

(2′*S*,4*R*)-4-Isopropyl-3-(4′,4′,4′-trifluoro-3′-hydroxy-2′-methyl-3′-trifluoromethylbutyryl)-2-oxazolidinone (6) To a solution of (*R*)-4-isopropyl-3-propionyl-2-oxazolidinone (**5**) (776 mg, 4.19 mmol) in dry CH_2Cl_2 (5 ml) was added dibutylboron triflate (Bu_2BOTf) (1.32 g, 4.81 mmol) at −78 °C over 2 min. After 10 min at this temperature, Et_3N (760 ml, 5.45 mmol) was added over 10 min and the reaction mixture was warmed to 0 °C. After 1 h at 0 °C, the solution was cooled to −78 °C and gaseous hexafluoroacetone (1.5 ml at −78 °C, 11.9 mmol) was added with a cannula. After 0.5 h at −78 °C, the reaction mixture was brought to and held at −30 °C for 2 h, then the reaction was quenched with pH 7.0 phosphate buffer (0.1 M, 5 ml) and MeOH (10 ml), followed by the addition of 30% H_2O_2 MeOH (5 ml 25 ml). After 1 h at 0 °C, the mixture was concentrated *in vacuo*. The residue was diluted with 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (3 : 1, v/v) gave **6** (1.18 g, 80%). **6**: colorless needles (hexane–Et_2O), mp 96.1—97.9 °C. $[\alpha]_D^{24}$ −47.9° (*c* = 0.98, $CHCl_3$). IR (KBr): 3295, 1769, 1682 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, d, *J* = 6.9 Hz), 0.94 (3H, d, *J* = 7.0 Hz), 1.43 (3H, dq, *J* = 7.1, 2.7 Hz), 2.37 (1H, dsep, *J* = 7.1, 2.8 Hz), 4.28—4.49 (3H, m), 4.75 (1H, q, *J* = 7.0 Hz), 6.53 (1H, s). ^{19}F-NMR ($CDCl_3$) δ: 73.32 (3F, dq, *J* = 11.5, 1.5 Hz), 76.17 (3F, q, *J* = 11.5 Hz). MS *m/z*: 351 (M^+), 282, 223, 175, 86, 69. *Anal.* Calcd for $C_{12}H_{15}F_6NO_4$: C, 41.0; H, 4.3; N, 4.1. Found: C, 41.1; H, 4.5; N, 4.1.

(*R*)-4,4,4-Trifluoro-2-methyl-3-trifluoromethylbutane-1,3-diol (7) A solution of **6** (1.12 g, 3.19 mmol) in dry THF (10 ml) was treated with $LiBH_4$ (350 mg, 16.0 mmol) in five portions at 0 °C. The mixture was stirred at 0 °C for 3.5 h, and the reaction was quenched with pH 7.0 phosphate buffer. The whole was extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–Et_2O (1 : 1, v/v) gave **7** (464 mg, 64%). **7**: colorless oil. $[\alpha]_D^{25}$ −7.75° (*c* = 0.76, $CHCl_3$). IR (neat): 3300 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.12 (3H, d, *J* = 7.3 Hz), 2.27 (1H, s), 2.48—2.70 (1H, m), 3.87 (1H, dd, *J* = 11.0, 4.1 Hz), 4.10 (1H, t, *J* = 11.0 Hz), 6.21 (1H, s). ^{19}F-NMR ($CDCl_3$) δ: 72.34 (3F, q, *J* = 10.0 Hz), 76.59 (3F, q, J = 10.0 Hz). MS *m/z*: 208 (M − 18), 139. High-resolution MS (HRMS) Calcd for $C_6H_6OF_6$ (M − H_2O): 208.032. Found: 208.031.

(*R*)-MTPA Ester of 7 (8) A solution of **7** (20 mg, 88 mmol) in dry pyridine (0.5 ml) was treated with (*S*)-MTPACl (23 mg, 91 mmol) at 0 °C. The reaction mixture was brought to and left at room temperature for 16 h, diluted with H_2O and extracted with Et_2O. The combined extracts were washed with 2 N aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. Evaporation of the solvent gave crude **8** (35 mg). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, d, *J* = 7.2 Hz), 2.50—2.67 (1H, m), 3.52 (3H, s), 4.32 (1H, dd, *J* = 11.0, 4.0 Hz), 4.68 (1H, dd, *J* = 11.0, 10.0 Hz), 5.70 (1H, s), 7.43—7.51 (5H, m). ^{19}F-NMR ($CDCl_3$) δ: 71.59 (3F, s), 73.43 (3F, q, *J* = 10.9 Hz), 76.36 (3F, q, *J* = 10.9 Hz).

(*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]-1,1,1-trifluoro-3-methyl-2-trifluoromethylbutan-2-ol (9) Imidazole (441 mg, 6.48 mmol) and TBDMSCl (370 mg, 2.45 mmol) was added to a solution of **7** (444 mg, 1.96 mmol) in dry CH_2Cl_2 (5 ml) at room temperature. The reaction mixture was stirred for 1 h, diluted with CH_2Cl_2, washed with saturated aqueous NH_4Cl and brine, dried over $MgSO_4$ and filtered. After removal of the solvent by evaporation, chromatography of the residue with hexane–EtOAc (30 : 1, v/v) gave **9** (564 mg, 85%). **9**: colorless oil. $[\alpha]_D^{25}$ −12.9° (*c* = 1.07, $CHCl_3$). IR (neat): 3318 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.12 (6H, s), 0.91 (9H, s), 1.08 (3H, d, *J* = 7.1 Hz), 2.43—2.62 (m, 1H), 3.75 (1H, dd, *J* = 10.4, 4.4 Hz), 3.97 (1H, dd, *J* = 10.7, 10.4 Hz), 6.74 (1H, s). ^{19}F-NMR ($CDCl_3$) δ: 72.24 (3F, q, *J* = 10.0 Hz), 76.62 (3F, q, *J* = 10.0 Hz). MS *m/z*: 283 (M − 57). HRMS Calcd for $C_8H_{13}F_6O_2Si$ (M-*tert*-Bu): 283.059. Found: 283.058.

(*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-4,4,4-trifluoro-3-[(methoxymethyl)oxy]-2-methyl-3-trifluoromethylbutane (10) MOMCl (2.0 ml, 26.3 mmol) and iso-Pr_2NEt (10 ml, 57.4 mmol) was added to a solution of **9** (997 mg, 2.93 mmol) in dry CH_2Cl_2 (15 ml) at 0 °C, and the reaction mixture was stirred at room temperature for 5 d. The reaction was quenched with saturated aqueous NH_4Cl and the whole was extracted with Et_2O. The combined extracts were washed with brine, dried over $MgSO_4$ and filtered. The solvent was evaporated and chromatography of the residue was carried out with hexane–EtOAc (40 : 1, v/v) to afford the starting material **9** (77 mg, 7.7%) and **10** (911 mg, 81%). **10**: colorless oil. $[\alpha]_D^{26}$ +13.2° (*c* = 1.27, $CHCl_3$). IR (neat): 1164, 1135, 1099, 1046 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s), 0.89 (9H, s), 1.23 (3H, d, *J* = 7.0 Hz), 2.36—2.54 (1H, m), 3.40—3.51 (1H, m), 3.48 (3H, s), 3.93 (1H, dd, *J* = 10.0, 4.5 Hz), 4.93 (1H, d, *J* = 11.0 Hz), 4.96 (1H, d, *J* = 11.0 Hz). ^{19}F-NMR ($CDCl_3$) δ: 69.40 (s). MS *m/z*: 353 (M − 31), 339. HRMS Calcd for $C_{13}H_{23}O_2F_6Si$ (M − OMe): 353.137. Found: 353.136.

(*R*)-4,4,4-Trifluoro-3-[(methoxymethyl)oxy]-2-methyl-3-trifluoromethylbutan-1-ol (11) A solution of **10** (340 mg, 0.88 mmol) in dry THF (2.5 ml) was treated with Bu_4NF (1.0 M in THF, 1.5 ml, 1.5 mmol) at room temperature. The reaction mixture was stirred at this temperature for 1 h, diluted with CH_2Cl_2, washed with brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (10 : 1, v/v) gave **11** (238 mg, quantitative). **11**: colorless oil. $[\alpha]_D^{26}$ −7.79° (*c* = 0.95, $CHCl_3$). IR (neat): 3384 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, d, *J* = 7.1 Hz), 1.50—1.80 (1H, m), 2.32—2.60 (1H, m), 3.50 (3H, s), 3.48—3.59 (1H, m), 3.95—4.07 (1H, m), 4.93 (1H, d, *J* = 6.6 Hz), 5.03 (1H, d, *J* = 6.6 Hz). ^{19}F-NMR ($CDCl_3$) δ: 69.44 (s). MS *m/z*: 239 (M − 31), 225, 207. HRMS Calcd for $C_7H_9F_6O_2$

(M−OMe): 239.051. Found: 239.050.

(*R*)-1,1,1-Trifluoro-2-[(methoxymethyl)oxy]-3-methyl-4-[(*p*-toluenesulfonyl)oxy]-2-trifluoromethylbutane (12) TsCl (680 mg, 3.57 mmol) and DMAP (20 mg, 0.16 mmol) were added to a solution of **11** (476 mg, 1.76 mmol) in dry pyridine (1.0 ml). The reaction mixture was stirred at room temperature for 10 h and diluted with Et_2O. The organic layer was washed with 0.5 N aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (10 : 1, v/v) gave **12** (728 mg, 97%). **12**: colorless oil. $[\alpha]_D^{26}$ +16.4° (c=1.16, $CHCl_3$). IR (neat): 1598, 1367 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, d, J=6.5 Hz), 2.46 (3H, s), 2.55—2.78 (1H, m), 3.39 (3H, s), 3.83—3.93 (1H, m), 4.38 (1H, dd, J=10.0, 2.8 Hz), 4.86 (1H, d, J=6.4 Hz), 4.94 (1H, d, J=6.4 Hz), 7.30—7.40 (2H, m), 7.75—7.83 (2H, m). ^{19}F-NMR ($CDCl_3$) δ: 69.24 (3F, q, J=9.8 Hz), 69.51 (3F, q, J=9.8 Hz). MS m/z: 424 (M^+). HRMS Calcd for $C_{15}H_{18}O_5F_6S$ (M^+): 424.078. Found: 424.076.

(*S*)-1,1,1-Trifluoro-2-[(methoxymethyl)oxy]-3-methyl-4-phenylthio-2-trifluoromethylbutane (13) A solution of PhSH (300 μl, 2.92 mmol) in DMF (2 ml) was added dropwise to a suspension of NaH (60%, 110 mg, 2.75 mmol) in dry THF (2 ml) at 0 °C. The mixture was stirred at room temperature for 10 min, then a solution of **12** (723 mg, 1.70 mmol) in dry DMF–THF (2 ml–4 ml) was added to the NaSPh solution at 0 °C. The mixture was stirred at room temperature for 14 h, the reaction was quenched with saturated aqueous NH_4Cl and the whole was extracted with Et_2O. The combined extracts were washed with brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (60 : 1, v/v) gave **13** (563 mg, 91%). **13**: colorless oil. $[\alpha]_D^{25}$ +51.3° (c=1.02, $CHCl_3$). IR (neat): 1584 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, d, J=6.6 Hz), 2.35—2.55 (1H, m), 2.67 (1H, t, J=12.2 Hz), 3.42 (3H, s), 3.48 (1H, d, J=12.2 Hz), 4.79 (2H, s), 7.27—7.39 (5H, m). ^{19}F-NMR ($CDCl_3$) δ: 68.27 (3F, q, J=9.8 Hz), 69.00 (3F, q, J=9.8 Hz). MS m/z: 362 (M^+), 317. HRMS Calcd for $C_{14}H_{16}F_6O_2S$ (M^+): 362.077. Found: 362.078.

(*S*)-4-Benzenesulfonyl-1,1,1-trifluoro-2-[(methoxymethyl)oxy]-3-methyl-2-trifluoromethylbutane (14) A solution of **13** (543 mg, 1.50 mmol) and Na_2HPO_4 (1.06 g, 7.47 mmol) in dry CH_2Cl_2 (10 ml) was treated with MCPBA (70%, 920 mg, 3.73 mmol) at 0 °C. After 40 min at 6 °C, the reaction mixture was diluted with Et_2O, washed with saturated aqueous $NaHSO_3$, 5% aqueous NaOH and brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (10 : 1–5 : 1, v/v) gave **14** (579 mg, 98%). **14**: colorless oil. $[\alpha]_D^{22}$ +21.0° (c=0.83, $CHCl_3$). IR (neat): 1586, 1153 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (3H, d, J=6.0 Hz), 2.96—3.20 (2H, m), 3.42 (3H, s), 3.62 (1H, d, J=11.0 Hz), 4.88 (1H, d, J=6.2 Hz), 4.95 (1H, d, J=6.2 Hz), 7.55—7.74 (3H, m), 7.91—7.98 (2H, m). ^{19}F-NMR ($CDCl_3$) δ: 68.92 (s). MS m/z: 394 (M^+), 363. HRMS Calcd for $C_{14}H_{16}F_6O_4S$ (M^+): 394.068. Found: 394.067.

[1*R*-(1α,3a*β*,4α,7aα)]-4-Acetoxyoctahydro-1-[(1*R*,2*E*,4*R*)-1,4,5-trimethyl-2-hexenyl]-7a-methyl-1*H*-indene (16) [1*R*-(1α,3a*β*,4α,7aα)]-Octahydro-7a-methyl-1-[(1*R*,2*E*,4*R*)-1,4,5-trimethyl-2-hexenyl]-1*H*-inden-4-ol[5] (**15**, 5.45 g, 19.6 mmol), Et_3N (8.2 ml, 58.8 mmol) and DMAP (20 mg, 0.16 mmol) were dissolved in dry CH_2Cl_2 (45 ml), then Ac_2O (2.8 ml, 29.7 mmol) was added at 0 °C. After 30 h at room temperature, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The combined extracts were washed with 5% aqueous HCl and saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (20 : 1, v/v) gave **16** (5.85 g, 93%). **16**: colorless oil. IR (neat): 1738 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, d, J=6.7 Hz), 0.83 (3H, d, J=6.7 Hz), 0.89 (3H, s), 0.91 (3H, d, J=6.8 Hz), 1.00 (3H, d, J=6.6 Hz), 1.10—2.09 (15H, m), 2.04 (3H, s), 5.07—5.31 (3H, m). MS m/z: 320 (M^+), 260. HRMS Calcd for $C_{21}H_{36}O_2$ (M^+): 320.272. Found: 320.272.

[1*R*-[1α(*S),3a*β*,4α,7aα]]-4-Acetoxyoctahydro-α,7a-dimethyl-1*H*-indene-1-acetaldehyde (17)** A mixture of O_3 and O_2 was passed into a stirred solution of **16** (592 mg, 1.85 mmol) and pyridine (0.4 ml) in dry CH_2Cl_2 (25 ml) at −78 °C for 50 min until the orange color persisted. Zn powder (600 mg) and AcOH (3 ml) were then added. The mixture was stirred at −78 °C for 30 min and 0 °C for 30 min, diluted with Et_2O and passed through a Celite column. The residue was washed with Et_2O. The combined filtrates were washed with 2 N aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. Concentration of the ethereal solution *in vacuo* gave the crude aldehyde **17** (467 mg). **17**: light yellow oil. IR (neat) : 1737, 1725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s), 1.12 (3H, d, J=6.9 Hz), 1.20—1.21 (12H, m), 2.05 (3H, s), 2.28—2.45 (1H, m), 5.18 (1H, s), 9.58 (1H, d, J=3.2 Hz). MS m/z: 252 (M^+), 192. HRMS Calcd for $C_{15}H_{24}O_3$ (M^+): 252.173. Found: 252.172.

[1*R*-[1α,3a*β*,4α,7aα]]-4-Acetoxyoctahydro-*β*(*S*),7a-dimethyl-α-[(2*S*)-1-benzenesulfonyl-4,4,4-trifluoro-3-[(methoxymethyl)oxy]-3-trifluoromethyl-2-methylbutyl]-1*H*-indene-1-ethanol (18) A solution of the sulfone **14** (730 mg, 1.85 mmol) in dry THF (11 ml) was treated dropwise with BuLi (2.39 M in hexanes, 1.15 ml, 3.34 mmol) at −78 °C. The mixture was stirred at 0 °C for 10 min and at −78 °C for 5 min, then a solution of $MgBr_2 \cdot OEt_2$, prepared from Mg (turnings, 110 mg, 4.52 mmol) and 1,2-dibromoethane (380 μl, 4.41 mmol) in dry Et_2O (4.6 ml) at room temperature, was added at −78 °C. The whole was stirred for 10 min, and a solution of the crude aldehyde **17** (467 mg) in dry THF (4.6 ml) was added dropwise at −78 °C. Stirring was continued at this temperature for 10 min and at 0 °C for 10 min, then the reaction was quenched with saturated aqueous NH_4Cl. The aqcueous layer was separated and extracted with Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent by evaporation, chromatography of the residue with hexane–EtOAc (6 : 1, v/v) gave **18** (1.04 g, 87% from **16**). **18**: colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.50—0.62 (3H, m), 0.75—0.84 (3H, m), 0.89—2.00 (17H, m), 2.03—2.06 (3H, m), 2.41—2.53 (0.7H, m), 3.10—3.15 (1H, m), 3.41—3.50 (3.3H, m), 3.70—4.20 (2H, m), 5.15 (2H, s), 7.48—7.70 (3H, m), 7.83—7.95 (2H, m). ^{19}F-NMR ($CDCl_3$) δ: 65.52—65.94 (3F, m), 68.31—70.08 (3F, m).

[1*R*-(1α,3a*β*,4α,7aα)]-4-Acetoxyoctahydro-7a-methyl-1-[(1*R*,2*E*,4*R*)-6,6,6-trifluoro-5-[(methoxymethyl)oxy]-1,4-dimethyl-5-trifluoromethyl-2-hexenyl]-1*H*-indene (19) Anhydrous Na_2HPO_4 (3 g, 21.1 mmol) and 5% Na–Hg (1.0 g) was added to a solution of **18** (415 mg, 0.64 mmol) in dry THF–MeOH (30 ml–10 ml) at 0 °C. After 3 h at the same temperature, the reaction mixture was diluted with Et_2O and passed through a Celite column. The residue was washed with Et_2O. The combined filtrates were washed with brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (30 : 1—10 : 1, v/v) gave **19** (161 mg, 52%) and [1*R*-(1α,3a*β*,4α,7aα)]-octahydro-7a-methyl-1-[(1*R*,2*E*,4*R*)-6,6,6-trifluoro-5-[(methoxymethyl)oxy]-1,4-dimethyl-5-trifluoromethyl-2-hexenyl]-1*H*-inden-4-ol (**20**, 62 mg, 22%). **19**: colorless oil. $[\alpha]_D^{25}$ +44.7° (c=1.11, $CDCl_3$). IR (neat): 1736 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, s), 0.99 (3H, d, J=6.7 Hz), 1.11—2.00 (16H, m), 2.03 (3H, s), 2.92 (1H, m), 3.48 (3H, s), 4.91 (1H, d, J=6.4 Hz), 5.00 (1H, d, J=6.4 Hz), 5.14 (1H, d, J=2.3 Hz), 5.36—5.43 (2H, m). ^{19}F-NMR ($CDCl_3$) δ: 72.18 (3F, q, J=9.4 Hz), 74.83 (3F, q, J=9.4 Hz). MS m/z: 488 (M^+), 457, 443. HRMS Calcd for $C_{23}H_{34}F_6O_4$ (M^+): 488.236. Found: 488.237. **20**: colorless oil. $[\alpha]_D^{24}$ +39.3° (c=0.95, $CDCl_3$). IR (neat): 3250 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, s), 0.98 (3H, d, J=6.6 Hz), 1.23 (3H, d, J=7.0 Hz), 1.10—2.13 (14H, m), 2.90—3.02 (1H, m), 3.49 (3H, s), 4.09 (1H, s), 4.92 (1H, d, J=6.4 Hz), 5.03 (1H, d, J=6.4 Hz), 5.37—5.43 (2H, m). ^{19}F-NMR ($CDCl_3$) δ: 68.81 (s). MS m/z: 446 (M^+), 428, 415. HRMS Calcd for $C_{21}H_{32}F_6O_3$ (M^+): 446.226. Found: 446.225.

[1*R*-(1α,3a*β*,4α,7aα)]-Octahydro-7a-methyl-1-[(1*R*,2*E*,4*R*)-6,6,6-trifluoro-5-hydroxy-1,4-dimethyl-5-trifluoromethyl-2-hexenyl]-1*H*-inden-4-ol (21) A solution of **19** (156 mg, 0.32 mmol) in dry THF (1.2 ml) was treated with MeLi (1.40 M in ether, 340 μl, 0.48 mmol) at −78 °C. After 5 min at the same temperature, the reaction mixture was allowed to warm to room temperature, then the reaction was quenched with saturated aqueous NH_4Cl and the whole was extracted with Et_2O. The combined extracts were washed with brine, dried over $MgSO_4$ and filtered. Concentration *in vacuo* gave the crude alcohol **20** (153 mg), which was dissolved in 1,4-dioxane–concentrated HCl (3 ml–0.3 ml). The reaction mixture was heated at 60 °C for 15 min and cooled to room temperature, followed by quenching with saturated aqueous $NaHCO_3$ and extraction with Et_2O. The combined extracts were washed with brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (10 : 1—6 : 1, v/v) gave **21** (88 mg, 69% from **19**). colorless oil. $[\alpha]_D^{24}$ +48.2° (c=1.02, $CDCl_3$). IR (neat): 3484, 3281 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, s), 1.03 (3H, d, J=6.6 Hz), 1.18 (3H, d, J=7.1 Hz), 1.10—2.20 (14H, m), 2.70—2.83 (1H, m), 3.06 (1 H, s), 4.08 (1H, s), 5.30 (1H, dd, J=15.2, 10.4 Hz), 5.55 (1H, dd, J=15.2, 9.0 Hz). ^{19}F-NMR ($CDCl_3$) δ: 72.15 (3F, q, J=9.5 Hz), 74.85 (3F, q, J=9.5 Hz). MS m/z: 402 (M^+), 384. HRMS Calcd for $C_{19}H_{28}F_6O_2$ (M^+): 402.199 Found: 402.198.

[1*R*-(1α,3a*β*,4α,7aα)]-Octahydro-7a-methyl-1-[(1*R*,2*E*,4*R*)-6,6,6-trifluoro-5-hydroxy-1,4-dimethyl-5-trifluoromethyl-2-hexenyl]-4*H*-inden-

4-one (22) To a suspension of PCC (276 mg, 1.28 mmol) in dry CH_2Cl_2 (3 ml) was added in one portion a solution of **21** (130 mg, 0.32 mmol) in dry CH_2Cl_2 (1.5 ml) at 0 °C. After 3 h at room temperature, the reaction mixture was diluted with Et_2O and passed through a Celite pad. The residue was washed with ether. The combined filtrates were washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue on a Florisil column with hexane–Et_2O (1 : 1, v/v) gave **22** (129 mg, quantitative). **22**: colorless needles (hexane–Et_2O), mp 155.0—156.0 °C. $[\alpha]_D^{24}$ +32.5° (c=0.93, $CDCl_3$). IR (KBr) : 3319, 1694 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.67 (3H, s), 1.08 (3H, d, J=6.7 Hz), 1.28 (3H, d, J=7.2 Hz), 1.46—2.38 (12H, m), 2.48 (1H, dd, J=11.2, 7.2 Hz), 2.70—2.88 (1 H, m), 2.98 (1H, s), 5.35 (1H, dd, J=15.3, 10.6 Hz), 5.58 (1H, dd, J=15.3, 8.9 Hz). ^{19}F-NMR ($CDCl_3$) δ: 72.22 (3F, q, J=9.8 Hz), 74.52 (3F, q, J=9.8 Hz). MS m/z: 400 (M^+). *Anal.* Calcd for $C_{19}H_{26}F_6O_2$: C, 56.9; H, 6.5. Found: C, 57.1 H, 6.5.

24-Epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_2 Bis-(*tert*-butyldimethylsilyl)ether (24) A solution of phosphine oxide **23** (1.20 g, 2.06 mmol) in dry THF (14 ml) was treated with BuLi (2.39 M in hexanes, 800 μl, 1.91 mmol) at −78 °C. After 10 min at −78 °C, a solution of **22** (93 mg, 0.23 mmol) in dry THF (1 ml) was added. The reaction mixture was brought to and held at −40 °C for 1 h, then the reaction was quenched with saturated aqueous NH_4Cl and the whole was extracted with Et_2O. The combined extracts were washed with brine, dried over $MgSO_4$ and filtered. After evaporation of the solvent, chromatography of the residue with hexane–EtOAc (30 : 1—10 : 1, v/v) gave **24** (148 mg, 83%). **24**: colorless oil. $[\alpha]_D^{25}$ +59.2° (c=0.96, $CDCl_3$). IR (neat): 3490, 1654, 1637 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (12H, s), 0.56 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 1.06 (3H, d, J=6.7 Hz), 1.28 (3H, d, J=7.1 Hz), 1.18—2.27 (15H, m), 2.44 (1H, dd, J=13.0, 3.8 Hz), 2.70—2.86 (2H, m), 3.04 (1H, s), 4.14—4.25 (1H, m), 4.36—4.41 (1H, m), 4.85 (1H, d, J=2.4 Hz), 5.18 (1H, dd, J=2.4, 1.0 Hz), 5.31 (1H, dd, J=15.2, 10.3 Hz), 5.59 (1H, dd, J=15.2, 9.0 Hz), 6.01 (1H, d, J=11.2 Hz), 6.22 (1H, d, J=11.2 Hz). ^{19}F-NMR ($CDCl_3$) δ: 72.00 (3F, q, J=9.5 Hz), 74.78 (3F, q, J=9.5 Hz). FAB-MS m/z: 765 (M+1). HRMS (FAB-MS) Calcd for $C_{40}H_{67}F_6O_3Si_2$ (M+H): 765.454. Found: 765.452.

24-Epi-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_2 (4) A solution of **24** (136 mg, 0.18 mmol) in MeOH (2 ml) was added to a suspension of a cation exchange resin (AG 50W-X4, 100—200 mesh from Dow Chemical Co., prewashed with MeOH) in MeOH (20 ml). The reaction mixture was stirred at room temperature for 5 h. After filtration, the MeOH solution was concentrated *in vacuo* and chromatography of the residue with hexane–EtOAc (1 : 2, v/v) gave **4** (90 mg, 95%). **4**: a colorless powder (MeOH–H_2O), mp 111.0—112.7 °C. $[\alpha]_D^{24}$ +71.2° (c=0.47, MeOH). UV λ_{max}^{MeOH} nm (ε): 264 (17634). IR (KBr): 3422, 1654, 1637 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.58 (3H, s), 1.02 (3H, d, J=6.6 Hz), 1.18 (3H, d, J=7.0 Hz), 1.20—1.82 (12H, m), 1.89 (2H, t, J=5.6 Hz), 1.96—2.10 (3H, m), 2.26 (1H, dd, J=13.4, 6.8 Hz), 2.52 (1H, dd, J=13.4, 3.5 Hz), 2.70—2.90 (2H, m), 4.10—4.20 (1H, m), 4.33—4.39 (1H, m), 4.90 (1H, s), 5.29 (1H, s), 5.40—5.45 (2H, m), 6.08 (1H, d, J=11.0 Hz), 6.32 (1H, d, J=11.0 Hz). ^{19}F-NMR ($CDCl_3$) δ: 71.15 (3F, q, J=9.0 Hz), 72.60 (3F, q, J=9.0 Hz). MS m/z: 536 (M^+), 519. HRMS Calcd for $C_{28}H_{38}F_6O_3$ (M^+): 536.273. Found: 536.270. *Anal.* Calcd for $C_{28}H_{38}F_6O_3$: C, 62.7; H, 7.1. Found: C, 62.6; H, 7.1.

Biological Studies. Binding Affinity with Chick Intestinal Cytosol Receptor[10] Chick embryonic intestinal cytosol receptor (Yamasa Shoyu Co., Tokyo, Japan, 0.5 mg/ml) was incubated at 0 °C for 24 h with [^{3}H]-1,25-$(OH)_2D_3$ and various concentrations of 1,25-$(OH)_2D_3$ (**1**) and 24-epi-26,27-F_6-1,25-$(OH)_2D_2$ (**4**) in TED Mo buffer A (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10 mM $NaMoO_4$, pH=7.4). Bound and free [^{3}H]-1,25-$(OH)_2D_3$ were separated by the addition of dextran–charcol and centrifugation. The radioactivity of the receptor-bound [^{3}H]-1,25-$(OH)_2D_3$ was measured.

Binding Affinity with Vitamin D-Deficient Rat Serum DBP[11] DBP, prepared from a 2000-fold-diluted serum from a vitamin D-deficient rat using 0.05 M phosphate buffer (pH 7.4), was incubated at 25 °C for 1 h with [^{3}H]-25-$(OH)D_3$ and various concentrations of 1,25-$(OH)_2D_3$ (**1**) and 24-epi-26,27-F_6-1,25-$(OH)_2D_2$ (**4**). At the end of incubation, dextran–charcol was added to the assay mixture and this preparation was mixed for 15 s. The mixture was left for 30 min and then centrifuged at 4 °C. The supernatant was transferred into a scintillation vial to measure the radioactivity.

***In Vivo* Testing**[12] Compounds **1** and **4** were each given to 8-week-old Wistar male rats orally once a day for 3 weeks (0.04—0.625 μg/kg/d). Blood samples were collected from the main artery and the serum Ca level was measured by the method of Connerty and Briggs.[13] The rats were killed, and the right and left femurs were excised. The weight and volume of the femurs dissected free of soft tissues were measured. Specific gravity was calculated as weight (mg)/volume (ml).

Acknowledgments The authors are grateful to Drs. M. Ohzeki and S. Higuchi, Taisho Pharmaceutical Co., Ltd., for conducting tests to assess biological activity. The authors also thank Prof. N. Ikekawa, Iwaki Meisei University and Dr. Y. Tanaka, Albany Medical College for valuable comments.

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