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Synthetic Studies of Vitamin D Analogs. XXIV.[1] Synthesis of Active Vitamin D_3 Analogs Substituted at the 2β-Position and Their Preventive Effects on Bone Mineral Loss in Ovariectomized Rats[2]

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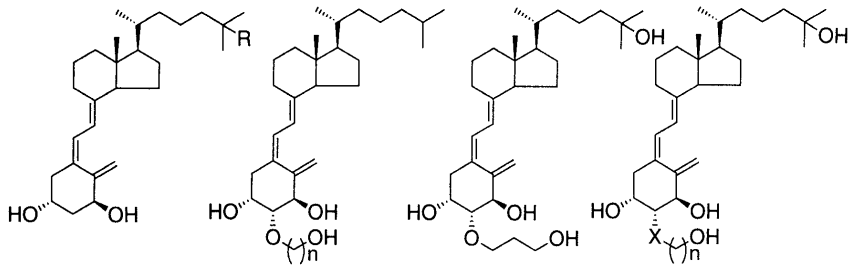
Analogs related to 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) (4), oxa-type and carba-type analogs of vitamin D_3 bearing substituents at the 2β-position of 1α,25-dihydroxyvitamin D_3 (1), were synthesized from the α-epoxides (6 and 13). Three analogs, ED-71 (4) and two carba-type analogs (16 and 26), showed potent preventive effects on bone mineral loss in pre-osteoporosis model rats. ED-71 (4) was concluded to be an optimized analog and a promising candidate for the treatment of osteoporosis.

Key words vitamin D_3 analog; 1α,25-dihydroxyvitamin D_3; 1α-hydroxyvitamin D_3; 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3; ED-71; ovariectomized rat

Various analogs of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$] (**1**), a hormonally active form of vitamin D_3, have been synthesized in attempts to separate differentiation-induction and antiproliferation activities from calcemic activity, with the aim of obtaining useful analogs for the medical treatment of psoriasis, secondary hyperparathyroidism, cancer, immunological disorders, *etc.*[3] There is also intense interest in obtaining compounds more active than 1α,25$(OH)_2D_3$ (**1**) in terms of regulatory effects in calcium and phosphorus metabolism, with the aim of treating bone diseases such as osteoporosis.[4]

A well-known synthetic pro-drug of 1α,25$(OH)_2D_3$ (**1**) is 1α-hydroxyvitamin D_3 (1αOHD_3) (**2**), which has been used clinically for the treatment of hypovitaminosis, chronic renal failure, osteoporosis, *etc.*[5,6] We reported previously that in a modification study of 1αOHD_3 (**2**), a number of vitamin D_3 analogs (**3**) bearing a hydroxyalkoxy group at the 2β-position were synthesized and the analog possessing a hydroxypropoxy group ($n=3$ in **3**) showed the strongest activity in elevating plasma calcium levels in rats fed a low calcium, vitamin D-deficient diet (low Ca/D-deficient diet).[4] We then confirmed that a hydroxypropoxy analog possessing a 25-hydroxy substituent, 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) (**4**), increased plasma calcium levels in rats on a low Ca/D-deficient diet more significantly than did 1α,25$(OH)_2D_3$ (**1**).[4] Although ED-71 (**4**) was also shown to have preventive and therapeutic effects in osteoporosis model rats in recent reports,[7-9] other analogs bearing various substituents at the 2β-position with a 25-hydroxy moiety still remain to be synthesized and evaluated biologically. Here we wish to report the synthesis of several analogs substituted at the 2β-position of 1α,25$(OH)_2D_3$ and also the preventive effects of these analogs on bone mineral loss in pre-osteoporosis model rats, that is, in ovariectomized (OVX) rats,[10] in comparison with ED-71 (**4**) (Chart 1).

Our 2β-modified derivatives of 1α,25$(OH)_2D_3$ (**1**) were of the following two types: oxa-type analogs (**5**, X=O), based on our previous studies,[4] and carba-type analogs (**5**, $X=CH_2$), arising from a new interest in the bioisosters of oxa-type analogs.[11] In both oxa-type and carba-type analogs, substituents at the 2β-position were varied in length, as shown in **5**, bearing $-X(CH_2)_{2-5}OH$. First, we undertook the synthesis of oxa-type analogs in a similar manner to that employed for the synthesis of ED-71 (**4**).[4] Treatment of the α-epoxide (**6**)[4] with various glycols in the presence of potassium *tert*-butoxide (^{t}BuOK) resulted in stereo- and regioselective opening of the epoxy ring in **6**, and introduction of hydroxyalkoxy groups into the 2β-position to give pro-vitamin D_3 analogs (**7**—**9**) in 30%, 84% and 82% yields, respectively. The three pro-vitamin



1:R=OH;1α,25$(OH)_2D_3$
2:R=H;1αOH D_3
3:n=2-5
4:ED-71
5:X=O or CH_2
n=2-5

Chart 1

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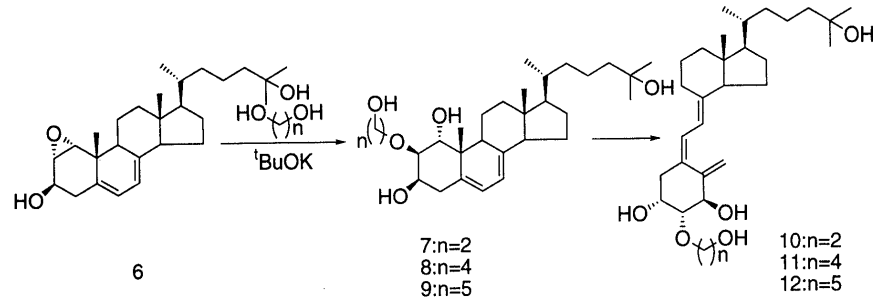
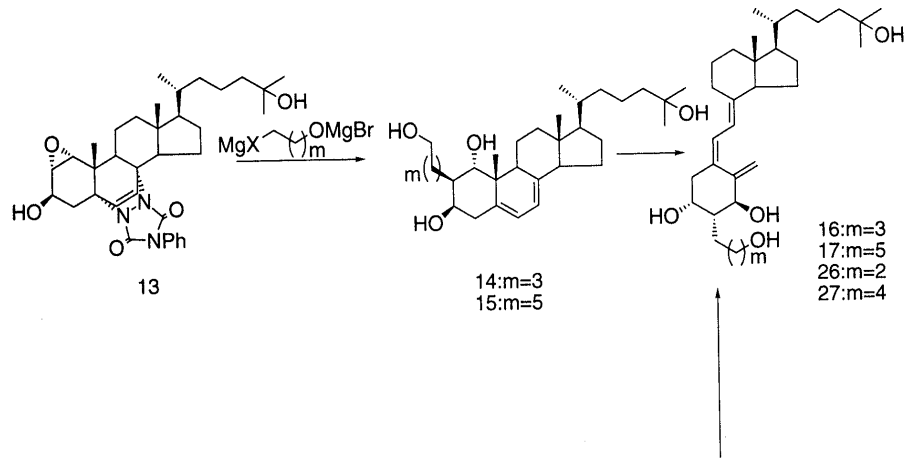


Chart 2



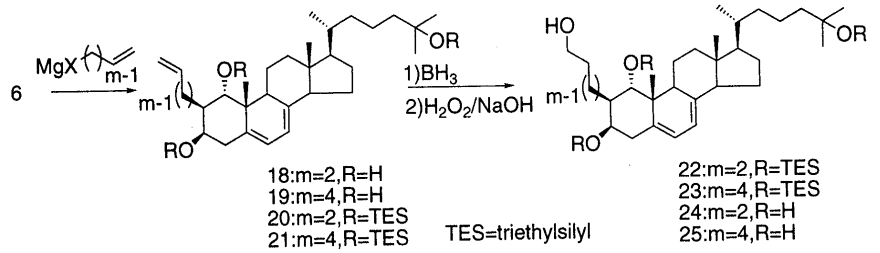


Chart 3

D_3 (**7**—**9**) were then converted to vitamin D_3 analogs (**10**—**12**) by irradiation at 0 °C using a high-pressure mercury lamp (400 W, Vycor filter), followed by thermal isomerization in boiling ethanol (Chart 2).

The synthesis of carba-type analogs was then carried out. Direct introduction of the hydroxyalkyl substituent at the 2β-position was successfully achieved by using Grignard reagents, with the hydroxy moieties protected as their magnesium alkoxide salts (Chart 3). Thus, the α-epoxide 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) adduct (**13**)[4] was treated with the Grignard reagent derived from 4-hydroxybutyl bromide or 6-hydroxyhexyl bromide and magnesium (Mg), in the presence of ethylmagnesium bromide (EtMgBr). EtMgBr was used to generate the magnesium alkoxide functionality from the corresponding hydroxyalkyl bromide. The pro-vitamin D_3 analogs (**14** and **15**) were obtained directly in 33% and 47% yields, respectively, with concomitant removal of the PTAD moiety to regenerate the 5,7-diene system.[12] The pro-vitamin D_3 analogs (**14** and **15**) were converted to carba-type analogs (**16** and **17**) by irradiation and subsequent thermal isomerization in 24% and 21% yields, respectively. Because the direct introduction of hydroxyalkyl substituent into the 2β-position in the cases of 3-hydroxypropyl bromide, or 5-hydroxypentyl bromide and Mg, in the presence of EtMgBr, gave a complicated mixture, we examined another route to **26** and **27**. The hydroboration-oxidation reaction of the alkylated products (**20** and **21**) prepared from the α-epoxide (**6**) and 3-bromo-1-propene or 5-bromo-1-pentene in the presence of Mg, after silylation of the hydroxy groups in **18** and **19**, provided the hydroxyalkylated products (**22** and **23**). Desilylation of **22** and **23** gave the pro-vitamin D_3 analogs (**24** and **25**), which were irradiated and thermally isomerized to the carba-type analogs (**26** and **27**) in 24% and 22% yields, respectively (Chart 3).

Finally, to evaluate the importance of the 2β-terminal hydroxy group for the activity, oxa-type (**30**) and carba-type (**31**) derivatives which lack the terminal hydroxy functionality were synthesized in the same manner as described for the oxa-type (**10**—**12**) and carba-type analogs (**16** and **17**), respectively, as shown in Chart 4.

The preventive effects of the synthesized analogs on bone mineral loss in OVX rats were evaluated. Wistar–Imamichi female rats (8-week-old) were ovariectomized and fed normal diet *ad libitum* for 2 weeks. The rats were

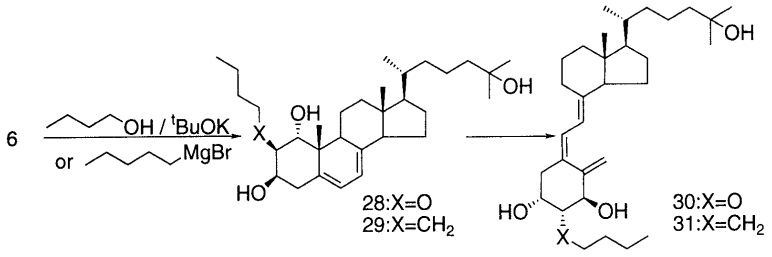


Chart 4

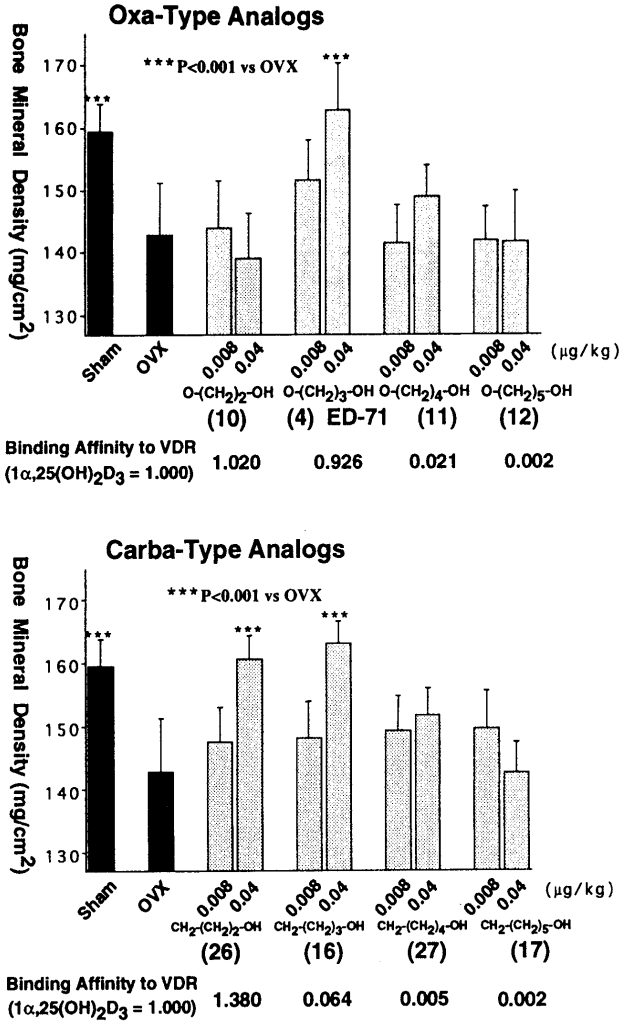


Fig. 1. Spinal BMD in Pre-Osteoporosis Model Rats and Binding Affinity to VDR of Oxa-Type and Carba-Type Analogs

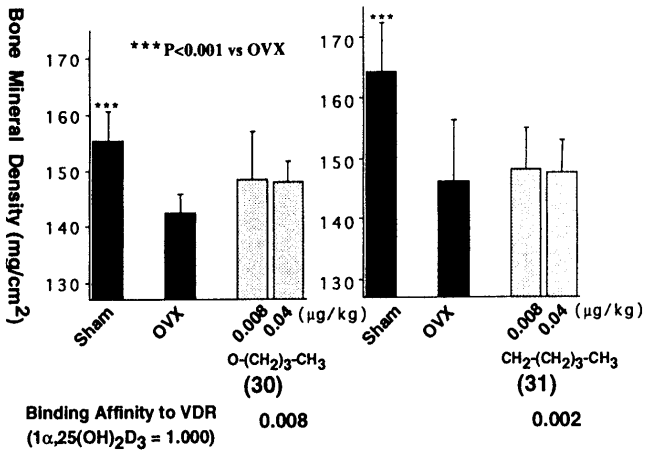


Fig. 2. Spinal BMD in Pre-Osteoporosis Model Rats and Binding Affinity to VDR of Vitamin D_3 Analogs

then orally administered various vitamin D_3 analogs at doses of 0.008 and 0.04 μg/kg, 5 times a week for 6 weeks. The sham and OVX groups were administered medium-chain triglyceride (MCT) alone. The bone mineral density (BMD) of spine (L2-L5) bone mass was measured using a computerized bone measuring apparatus. The rats used in the experiments are considered to be a pre-osteoporosis model, and these analogs showed clear preventive effects against bone mineral loss.[10]

As shown in Fig. 1, three analogs, $-CH_2(CH_2)_2OH$ (**26**), $-CH_2(CH_2)_3OH$ (**16**), and $-O(CH_2)_3OH$ (ED-71) (**4**), significantly increased the BMD. Although significant increases in BMD were not induced by the other five analogs, oxa-type $-O(CH_2)_4OH$ (**11**) and carba-type $-CH_2(CH_2)_4$-OH (**27**) showed moderate, dose-dependent activity. The serum calcium values of all groups were within the normal ranges (data not shown). The relative binding potencies of the analogs to calf thymus vitamin D receptor (VDR) are also shown in Fig. 1 ($1\alpha,25(OH)_2D_3 = 1.000$). From these results, it appears that some degree of binding affinity to VDR is necessary in analogs to realize preventive effects in OVX rats, but high binding affinity to VDR alone is not sufficient to show preventive activity in OVX rats [*e.g.* $-O(CH_2)_2OH$ (**10**)].

Comparisons of $-O(CH_2)_3OH$ (ED-71) (**4**) and $-CH_2(CH_2)_3OH$ (**16**) with $-O(CH_2)_3CH_3$ (**30**) and $-CH_2(CH_2)_3CH_3$ (**31**), in terms of preventive effects on BMD in OVX rats, show several interesting phenomena. Compounds **30** and **31** were chosen as analogs lacking the terminal hydroxy moiety but possessing substituents of similar sizes to **4** and **16**. As shown in Fig. 2, $-O(CH_2)_3CH_3$ (**30**) and $-CH_2(CH_2)_3CH_3$ (**31**) showed no efficacy in OVX rats at doses of 0.008 or 0.04 μg/kg. However, $-O(CH_2)_3$-OH (ED-71) (**4**) and $-CH_2(CH_2)_3OH$ (**16**) reproducibly showed preventive activities (data not shown). This suggests that the terminal hydroxyl group of the 2β-substituents in this series plays an important role in the effect on BMD in OVX rats. Although the structure-activity relationship of vitamin D_3 analogs substituted at the 2β-position in OVX rats remains to be fully established, ED-71 (**4**) appears to be an optimized analog possessing preventive activity in the pre-osteoporosis model rats. Phase I clinical studies of ED-71 (**4**) as a promising candidate for the treatment of osteoporosis are under way.

Experimental

General Methods Infrared (IR) spectra were obtained using a Hitachi 270-30 or Horiba FT-730 spectrometer. ^{1}H-Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL FX-200 or EX-270 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. Ultraviolet (UV) spectra were recorded with a Shimadzu UV-240 in EtOH. Mass spectra (MS) were obtained on a Shimadzu GCMS-QP 1000. High-resolution mass spectra (HRMS) were obtained using a VG Auto Spec Q instrument. All air-sensitive reactions were carried out under an atmosphere of dry argon or nitrogen. Flash column chromatography was carried out with Merck Kieselgel 60, 230—400 mesh. Preparative TLC was performed on 20 × 20 cm plates coated with 0.5 mm thickness of Merck Kieselgel 60 containing F_{254} indicator.

(2*R*)-2-(5-Hydroxypentoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (9). General Procedure for the Synthesis of 7, 8, 9 and 28 A mixture of the α-epoxide (**6**) (20.0 mg, 0.05 mmol), ^{t}BuOK (90%, 16.5 mg, 0.15 mmol), dibenzo-18-crown-6 (405 mg, 0.01 mmol) and 1,5-pentanediol (2 ml) was stirred at 80 °C for 14 h, then poured into H_2O and extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left an oily residue, which was purified by preparative TLC developed with CH_2Cl_2–EtOH (10 : 1) to give **9** (20.6 mg, 82%) as a yellow oil. IR (neat): 3400 (br), 2950, 2870, 1380, 1070, 760 cm^{-1}. NMR δ: 0.63 (3H, s), 0.96 (3H, d, J=6.6), 1.04 (3H, s), 1.22 (6H, s), 3.43—4.20 (7H, m), 5.32—5.41 (1H, m), 5.63—5.75 (1H, m). UV λ_{max} nm: 271, 281, 294. MS m/z: 518 (M^+), 59 (100%). Other compounds, **7**, **8** and **26**, were similarly obtained. (2*R*)-2-(2-Hydroxyethoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (**7**): IR (neat): 3400 (br), 2950, 2890, 1380, 1050 cm^{-1}. NMR δ: 0.63 (3H, s), 0.96 (3H, d, J=6.6), 1.08 (3H, s), 1.22 (6H, s), 3.50—4.11 (7H, m), 5.35—5.42 (1H, m), 5.68—5.74 (1H, m). UV λ_{max} nm: 271, 281, 294. MS m/z: 476 (M^+), 59 (100%). (2*R*)-2-(4-Hydroxybutoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (**8**): IR (neat): 3400 (br), 2950, 2900, 1480, 1390, 770 cm^{-1}. NMR δ: 0.63 (3H, s), 0.96 (3H, d, J=6.3), 1.06 (3H, s), 1.22 (6H, s), 3.41—4.00 (7H, m), 5.35—5.40 (1H, m), 5.68—5.73 (1H, m). UV λ_{max} nm: 270, 281, 293. MS m/z: 504 (M^+), 55 (100%). (2*R*)-2-Butoxy-1α,3β,25-trihydroxycholesta-5,7-diene (**28**): IR (neat): 3430 (br), 2950, 2900, 1480, 1390, 1100 cm^{-1}. NMR δ: 0.63 (3H, s), 0.93 (3H, t, J=7.3), 0.96 (3H, d, J=6.3), 1.07 (3H, s), 1.21 (6H, s), 3.40—3.52 (1H, m), 3.66—3.77 (2H, m), 3.84—4.00 (2H, m), 5.35—5.41 (1H, m), 5.67—5.74 (1H, m). UV λ_{max} nm: 271, 282, 293. MS m/z: 488 (M^+), 59 (100%).

(2*R*)-2-(4-Hydroxybutyl)-1α,3β,25-trihydroxycholesta-5,7-diene (14). General Procedure for the Synthesis of 14 and 15 A mixture of 3 M EtMgBr solution in Et_2O (4.30 ml, 12.9 mmol) and tetrahydrofuran (THF) (5 ml) was stirred, and a solution of 4-chloro-1-butanol (1.20 g, 11.0 mmol) in THF (5 ml) was added dropwise to it at −35 °C to −45 °C during 0.3 h. The stirring was continued at −20 °C for 0.25 h and at room temperature for 0.5 h. Mg (271 mg, 11.2 mmol) was then added and the resulting mixture was refluxed for 13.5 h. Next, a solution of the α-epoxide PTAD adduct (**13**) (100 mg, 0.17 mmol) in THF (5 ml) was added dropwise at room temperature. The reaction mixture was then refluxed for 2 h, quenched by the addition of saturated NH_4Cl, and filtered using Hyflo Super-Cell. The filtrate was extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left an oily residue, which was purified by flash column chromatography with AcOEt–EtOH (50 : 1) as the eluent to give **14** (27.0 mg, 33%) as a white powder. IR (neat): 3390 (br), 2945, 2875 cm^{-1}. NMR δ: 0.62 (3H, s), 0.95 (3H, d, J=6.3), 0.99 (3H, s), 1.20 (6H, s), 3.46 (2H, t, J=6.1), 3.72 (1H, br s), 4.11—4.26 (1H, m), 5.28—5.35 (1H, m), 5.57—5.64 (1H, m). UV λ_{max} nm: 272, 282, 294. MS m/z: 488 (M^+), 59 (100%). The other compound, **15**, was similarly obtained. (2*R*)-2-(6-Hydroxyhexyl)-1α,3β,25-trihydroxycholesta-5,7-diene (**15**): IR (neat): 3355 (br), 2935, 2860 cm^{-1}. NMR δ: 0.62 (3H, s), 0.96 (3H, d, J=6.6), 0.99 (3H, s), 1.21 (6H, s), 3.61 (2H, t, J=6.3), 3.75 (1H, br s), 4.09—4.23 (1H, m), 5.29—5.37 (1H, m), 5.65 (1H, br d, J=5.1). UV λ_{max} nm: 272, 282, 294. MS m/z: 516 (M^+), 43 (100%).

(2*R*)-2-(4-Pentenyl)-1α,3β,25-trihydroxycholesta-5,7-diene (19). General Procedure for the Synthesis of 18, 19 and 29 A solution of 5-bromo-1-pentene (1.01 ml, 8.50 mmol) in THF (3 ml) was stirred and Mg (207 mg, 8.50 mmol) was added at room temperature. The resulting mixture was stirred at the same temperature for 2 h. A solution of the α-epoxide (**6**) (101 mg, 0.24 mmol) in THF (3 ml) was added dropwise. The resulting mixture was refluxed for 3.5 h, poured into saturated NH_4Cl and extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left an oily residue, which was purified by preparative TLC developed with CH_2Cl_2–EtOH (20 : 1) to give **19** (50.0 mg, 42%) as a colorless oil. IR (neat): 3375 (br), 2950, 2900, 2870, 1380, 1060, 760 cm^{-1}. NMR δ: 0.62 (3H, s), 0.96 (3H, d, J=6.3), 1.01 (3H, s), 1.22 (6H, s), 3.77 (1H, br s), 4.16—4.26 (1H, m), 4.92—5.08 (2H, m), 5.33—5.39 (1H, m), 5.64—5.70 (1H, m). UV λ_{max} nm: 271, 281, 294. MS m/z: 485 (M^+), 55 (100%). Other compounds, **18** and **29**, were similarly obtained. (2*R*)-2-(2-Propenyl)-1α,3β,25-trihydroxycholesta-5,7-diene (**18**): IR (neat): 3375 (br), 2940, 2875, 1380, 1040, 905 cm^{-1}. NMR δ: 0.62 (3H, s), 0.95 (3H, d, J=6.3), 1.02 (3H, s), 1.21 (6H, s), 3.74 (1H, br s), 4.13—4.26 (1H, m), 4.95—5.15 (2H, m), 5.30—5.39 (1H, m), 5.67 (1H, br d, J=6.3), 5.70—5.99 (1H, m). UV λ_{max} nm: 272, 282, 293. MS m/z: 456 (M^+), 59 (100%). (2*R*)-2-Pentyl-1α,3β,25-trihydroxycholesta-5,7-diene (**29**): IR (neat): 3400 (br), 2950, 2860, 1470, 1380, 1060, 1040, 910 cm^{-1}. NMR δ: 0.62 (3H, s), 1.01 (3H, s), 1.21 (6H, s), 3.78 (1H, br s), 4.08—4.21 (1H, m), 5.34—5.40 (1H, m), 5.65—5.70 (1H, m). UV λ_{max} nm: 271, 282, 293. MS m/z: 486 (M^+), 59 (100%).

(2*R*)-2-(4-Pentenyl)-1α,3β,25-tris(triethylsililoxy)cholesta-5,7-diene (21). General Procedure for the Synthesis of 20 and 21 Triethylsilyl chloride (260 ml, 1.55 mmol) was added dropwise to a stirred solution of **19** (50.0 mg, 0.10 mmol) and imidazole (1.10 g, 15.5 mmol) in 1,3-dimethyl-2-imidazolidinone (3 ml) at room temperature. The resulting mixture was stirred at room temperature for 48 h, poured into saturated NaCl and extracted with AcOEt and *n*-hexane. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left an oily residue which was purified by preparative TLC developed with *n*-hexane–AcOEt (40 : 1) to give **21** (49.0 mg, 58%) as a colorless oil. IR (neat): 2950, 2920, 2870, 1460, 1380, 1240, 1090, 1010, 820, 740, 730 cm^{-1}. NMR δ: 0.50—0.70 (21H, m), 0.88—1.00 (33H, m), 1.19 (6H, s), 3.87 (1H, br s), 4.13—4.22 (1H, m), 4.90—5.05 (2H, m), 5.29—5.33 (1H, m), 5.55—5.62 (1H, m), 5.73—5.90 (1H, m). UV λ_{max} nm: 272, 282, 294. MS m/z: 826 (M^+), 369 (100%). The other compound, **20**, was similarly obtained. (2*R*)-2-(2-Propenyl)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene(**20**): IR (neat): 2950, 2870, 1455, 1375, 1230, 1085, 1000, 735, 715 cm^{-1}. NMR δ: 0.48—0.70 (21H, m), 0.87—1.05 (33H, m), 1.19 (6H, s), 3.80 (1H, br s), 4.12—4.26 (1H, m), 4.97 (1H, br s), 5.04 (1H, dd, J=1.2, 7.3), 5.26—5.35 (1H, m), 5.60 (1H, br d, J=4.1), 5.67—5.97 (1H, m). UV λ_{max} nm: 272, 282, 294. MS m/z: 798 (M^+), 103 (100%).

(2*R*)-2-(5-Hydroxypentyl)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene (23). General Procedure for the Synthesis of 22 and 23 A stirred solution of **21** (49.0 mg, 0.06 mmol) in THF (3 ml), was treated with BH_3 (1 M solution in THF, 340 ml, 0.34 mmol) at room temperature. The resulting mixture was stirred at room temperature for 24 h, then NaOH (3 M solution in H_2O, 0.5 ml) and H_2O_2 (30% solution in H_2O, 0.4 ml) were added. The mixture was stirred at room temperature for 0.5 h and extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vauo* left an oily residue which was purified by preparative TLC developed with *n*-hexane–AcOEt (10 : 1) to give **23** (14.0 mg, 34%) as a colorless oil. IR (neat): 3375 (br), 2950, 2870, 1460, 1380, 1080, 1050, 1010, 820, 740, 730 cm^{-1}. NMR δ: 0.52—0.68 (21H, m), 0.91—1.00 (33H, m), 1.19 (6H, s), 3.60—3.69 (2H, m), 3.78 (1H, br s), 4.14—4.22 (1H, m), 5.28—5.34 (1H, m), 5.50—5.60 (1H, m). UV λ_{max} nm: 272, 282, 294. The other compound, **22**, was similarly obtained. (2*R*)-2-(3-Hydroxypropyl)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene (**22**): IR (neat): 3375 (br), 2955, 2880, 1460, 1240, 1090, 1050, 1010, 740, 725 cm^{-1}. NMR δ: 0.49—0.71 (21H, m), 0.85—1.04 (33H, m), 1.19 (6H, s), 3.65 (2H, t, J=5.1), 3.75 (1H, br s), 4.14—4.28 (1H, m), 5.26—5.35 (1H, m), 5.59 (1H, br d, J=5.1). UV λ_{max} nm: 272, 282, 294. MS m/z: 816 (M^+), 103 (100%).

(2*R*)-2-(5-Hydroxypentyl)-1α,3β,25-trihydroxycholesta-5,7-diene (25). General Procedure for the Synthesis of 24 and 25 A mixture of **23** (17.0 mg, 0.02 mmol) and tetra-*n*-butylammonium fluoride (1 M solution in THF, 302 μl, 0.30 mmol) in THF (3 ml) was refluxed for 24 h, poured into H_2O and extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left an oily residue which was purified by preparative TLC developed with CH_2Cl_2–EtOH (5 : 1) to give **25** (6.2 mg, 62%) as a colorless powder. IR (neat): 3400 (br), 2960, 2900, 1480, 1070 cm^{-1}. NMR δ: 0.63 (3H, s), 0.96 (3H, d, J=6.3), 1.02 (3H, s), 1.22 (6H, s), 3.65 (2H, t, J=6.4), 3.76 (1H, br s), 4.17—4.27 (1H, m), 5.32—5.40 (1H, m), 5.65—5.72 (1H, m). UV λ_{max} nm: 271, 282, 293. MS m/z: 502 (M^+), 59 (100%). The other compound, **24**, was similarly obtained. (2*R*)-2-(3-Hydroxypropyl)-1α,3β,25-trihydroxycholesta-5,7-diene (**24**): IR (neat): 3390 (br), 2950,

2880, 1470, 1380, 1065, 760 cm^{-1}. NMR δ: 0.62 (3H, s), 0.96 (3H, d, $J=6.1$), 1.02 (3H, s), 1.22 (6H, s), 3.69 (2H, br), 3.75 (1H, br s), 4.15—4.28 (1H, m), 5.31—5.41 (1H, m), 5.63—5.72 (1H, m). UV λ_{max} nm: 272, 282, 293. MS *m/z*: 474 (M^+), 59 (100%).

(2*R*)-2-(5-Hydroxypentoxy)calcitriol (12). General Procedure for Irradiation and Thermal Isomerization of 7, 8, 9, 14, 15, 24, 25, 28 and 29 A solution of **9** (16.1 mg, 0.03 mmol) in EtOH (200 ml) was irradiated using a 400 W high-pressure mercury lamp with a Vycor filter at 0 °C for 2 min. The mixture was then refluxed mildly for 2 h and concentrated *in vacuo*. The crude product was purified by preparative TLC developed with CH_2Cl_2–EtOH (10 : 1) to give **12** (3.13 mg, 19%) as a colorless foam. IR (neat): 3400 (br), 2930, 2860, 1090, 910, 740 cm^{-1}. NMR δ: 0.55 (3H, s), 0.94 (3H, d, $J=6.6$), 1.22 (6H, s), 3.45—4.40 (7H, m), 5.08 (1H, s), 5.50 (1H, s), 6.04 (1H, d, $J=10.5$), 6.36 (1H, d, $J=10.5$). UV λ_{max} nm: 264; λ_{min} nm: 228. MS *m/z*: 518 (M^+), 69 (100%). HRMS *m/z*: 518.4014 (Calcd for $C_{32}H_{54}O_5$: 518.3971). Other compounds, **10** (0.85 mg), **11** (3.7 mg), **16** (4.0 mg), **17** (7.9 mg), **26** (1.1 mg), **27** (1.4 mg), **30** (0.89 mg) and **31** (12.0 mg), were similarly obtained. (2*R*)-2-(2-Hydroxyethoxy)calcitriol (**10**): IR (neat): 3400 (br), 2940, 2880, 1380, 1090, 760 cm^{-1}. NMR δ: 0.55 (3H, s), 0.94 (3H, d, $J=6.3$), 1.22 (6H, s), 3.48—4.22 (7H, m), 5.09 (1H, s), 5.46 (1H, s), 6.03 (1H, d, $J=11.2$), 6.37 (1H, d, $J=11.2$). UV λ_{max} nm: 264; λ_{min} nm: 226. MS *m/z*: 476 (M^+), 59 (100%). HRMS *m/z*: 433.3318 ($[M-C_2H_3O]^+$ Calcd for $C_{27}H_{45}O_4$: 433.3318). (2*R*)-2-(4-Hydroxybutoxy)calcitriol (**11**): NMR δ: 0.55 (3H, s), 0.94 (3H, d, $J=5.9$), 1.21 (6H, s), 3.15—3.20 (1H, m), 3.45—4.38 (6H, m), 5.08 (1H, s), 5.48 (1H, s), 6.05 (1H, d, $J=11.6$), 6.36 (1H, d, $J=11.6$). UV λ_{max} nm: 264; λ_{min} nm: 226. MS *m/z*: 504 (M^+), 59 (100%). HRMS *m/z*: 504.3780 (Calcd for $C_{31}H_{52}O_5$: 504.3815). (2*R*)-2-(4-Hydroxybutyl)calcitriol (**16**): IR (neat): 3850 (br), 2945, 2875 cm^{-1}. NMR δ: 0.55 (3H, s), 0.94 (3H, d, $J=5.9$), 1.22 (6H, s), 3.68 (2H, t, $J=6.1$), 4.01—4.12 (1H, m), 4.16 (1H, br s), 5.02 (1H, s), 5.37 (1H, s), 6.03 (1H, d, $J=11.3$), 6.34 (1H, d, $J=11.3$). UV λ_{max} nm: 264; λ_{min} nm: 229. MS *m/z*: 488 (M^+), 133 (100%). HRMS *m/z*: 488.3896 (Calcd for $C_{31}H_{52}O_4$: 488.3865). (2*R*)-2-(6-Hydroxyhexyl)calcitriol (**17**): IR (neat): 3410 (br), 2970, 2865 cm^{-1}. NMR δ: 0.55 (3H, s), 0.94 (3H, d, $J=6.1$), 1.22 (6H, s), 3.64 (2H, t, $J=6.6$), 3.99—4.09 (1H, m), 4.15 (1H, br s), 5.02 (1H, s), 5.37 (1H, s), 6.03 (1H, d, $J=10.7$), 6.34 (1H, d, $J=10.7$). UV λ_{max} nm: 262; λ_{min} nm: 228. MS *m/z*: 516 (M^+), 59 (100%). HRMS *m/z*: 516.4152 (Calcd for $C_{33}H_{56}O_4$: 516.4178). (2*R*)-2-(3-Hydroxypropyl)calcitriol (**26**): IR (neat): 3367 (br), 2942, 2871, 1376, 1214, 1056, 755 cm^{-1}. NMR δ: 0.55 (3H, s), 0.94 (3H, d, $J=6.1$), 1.22 (6H, s), 3.70 (2H, t, $J=6.3$), 4.02—4.10 (1H, m), 4.17 (1H, br s), 5.03 (1H, s), 5.38 (1H, s), 6.03 (1H, d, $J=11.7$), 6.35 (1H, d, $J=11.7$). UV λ_{max} nm: 264; λ_{min} nm: 228. MS *m/z*: 474 (M^+), 59 (100%). HRMS *m/z*: 474.3702 (Calcd for $C_{30}H_{50}O_4$: 474.3709). (2*R*)-2-(5-Hydroxypentyl)calcitriol (**27**): IR (neat): 3349 (br), 2962, 2929, 2873, 1261, 1079, 1052, 802 cm^{-1}. NMR δ: 0.55 (3H, s), 0.94 (3H, d, $J=5.9$), 1.22 (6H, s), 3.63 (2H, t, $J=6.4$), 4.00—4.09 (1H, m), 4.10—4.18 (1H, m), 5.02 (1H, s), 5.37 (1H, s), 6.03 (1H, d, $J=11.6$), 6.35 (1H, d, $J=11.6$). UV λ_{max} nm: 264; λ_{min} nm: 228. MS *m/z*: 502 (M^+), 59 (100%). HRMS *m/z*: 502.4070 (Calcd for $C_{32}H_{54}O_4$: 502.4022). (2*R*)-2-Butoxycalcitriol (**30**): IR (neat): 3390 (br), 2962, 2929, 2871, 1683, 1376, 1261, 1097, 1031, 802 cm^{-1}. NMR δ: 0.55 (3H, s), 0.91—0.99 (6H, m), 1.22 (6H, s), 3.22 (1H, dd, $J=9.1, 3.7$), 3.47—3.58 (1H, m), 3.65—3.77 (1H, m), 4.23 (1H, br s), 4.29 (1H, d, $J=6.6$), 5.08 (1H, s), 5.50 (1H, s), 6.08 (1H, d, $J=12.2$), 6.34 (1H, d, $J=12.2$). UV λ_{max} nm: 264; λ_{min} nm: 228. MS *m/z*: 488 (M^+), 59 (100%). HRMS *m/z*: 488.3869 (Calcd for $C_{31}H_{54}O_4$: 488.3866). (2*R*)-2-Pentylcalcitriol (**31**): IR (neat): 3400 (br), 2945, 2871, 1468, 1377, 1068, 910, 758 cm^{-1}. NMR δ: 0.55 (3H, s), 0.89 (3H, t, $J=6.8$), 0.94 (3H, d, $J=6.3$), 1.22 (6H, s), 5.02 (1H, s), 5.38 (1H, s), 6.04 (1H, d, $J=11.2$), 6.34 (1H, d, $J=11.2$). UV λ_{max} nm: 264; λ_{min} nm: 228. MS *m/z*: 486 (M^+), 133 (100%). HRMS *m/z*: 486.4071 (Calcd for $C_{32}H_{54}O_3$: 486.4071).

Evaluation of the Preventive Effects on Bone Mineral Loss Wistar–Imamichi female rats (8-week-old) were ovariectomized and fed *ad libitum* normal diet containing 1.2% Ca for 2 weeks. The rats were then orally administered vitamin D_3 analogs, **4**, **10**, **11**, **12**, **16**, **17**, **26**, **27**, **30** and **31**, at various doses in MCT as the vehicle 5 times a week for 6 weeks. The sham and OVX groups were administered MCT alone. BMD of spine (L2-L5) bone mass was measured by a dual X-ray absorptiometer (DEXA, Aloka DCS-600, Tokyo, Japan). The results are expressed as the mean ± standard error of the mean. The statistical significance of differences between the OVX and experimental groups was analyzed by the use of Student's *t*-test.

VDR Binding Assay The binding affinity of analogs, **4**, **10**, **11**, **12**, **16**, **17**, **26**, **27**, **30** and **31**, with the calf thymus VDR was tested using a $1\alpha,25(OH)_2D_3$ assay kit purchased from INCSTAR (Stillwater, MN). Calf thymus VDR was incubated at 20 °C for 1 h with various concentrations of $1\alpha,25(OH)_2D_3$ (**1**) and analogs. After the incubation period, 15000 dpm of [^{3}H]-$1\alpha,25(OH)_2D_3$ was added and the mixture was incubated at 20 °C for 1 h. Bound and free forms of [^{3}H]-$1\alpha,25(OH)_2D_3$ were separated by addition of dextran–charcoal suspension and centrifugation. The radioactivity was measured with an Aloka LSC-700 instrument.

References and Notes

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