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Syntheses and Preventive Effects of Analogues Related to 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) on Bone Mineral Loss in Ovariectomized Rats[1]

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Abstract—Analogues related to 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) (**2**), 26,27-dimethyl ED-71 (**3**) and 26,27-diethyl ED-71 (**4**), were synthesized from lithocholic acid (**5**). In the study of the preventive effects of these analogues and ED-71 (**2**) on bone mineral loss in ovariectomized rats, 26,27-dimethyl ED-71 (**3**) showed the most potent activity.

Introduction

Various analogues of 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (**1**), a hormonally active form of vitamin D_3, have been investigated in attempts to separate differentiation-induction and antiproliferation activities from calcemic activity, with the aim of obtaining useful analogues for the medical treatment of psoriasis, secondary hyperparathyroidism, cancer, etc.[2] There is also an intense interest in obtaining analogues more potent than $1,25(OH)_2D_3$ (**1**) in terms of regulatory effects in calcium and phosphorous metabolism, with the aim of treating bone diseases such as osteoporosis.[3] 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) (**2**) is an analogue of $1,25(OH)_2D_3$ (**1**), bearing a hydroxypropoxy substituent at the 2β-position.[3] ED-71 (**2**) is characterized by highly calcemic activity and long half-life in plasma arising from the strong affinity to vitamin D binding protein (DBP).[4] The clinical trial of ED-71 (**2**) as a promising candidate for the treatment of osteoporosis has been conducted in Japan, based on the preventive and therapeutic effects on bone mineral loss in osteoporosis model rats.[5] We recently reported that in the modification studies of ED-71 (**2**) at the 2β-position, **2** was proved to be an optimized analogue possessing preventive activity in the pre-osteoporosis model rats.[6] On the other hand, the modification at the side chain of active vitamin D analogues generally exerts great influence on the biological functions of those analogues.[7] In the case of ED-71 (**2**), however, the synthesis and biological character of analogues modified at the side chain yet remain to be evaluated. In this paper, we describe the synthesis of 26,27-dimethyl ED-71 (**3**) and 26,27-diethyl ED-71 (**4**) and their preventive effects on bone mineral loss in pre-osteoporosis model rats, that is ovariectomized (OVX) rats (Fig. 1).

Results and Discussion

Synthesis

To synthesize 26,27-dimethyl ED-71 (**3**) and 26,27-diethyl ED-71 (**4**), the ester (**23**) was needed as the key intermediate for the introduction of the elongated side chains. The ester (**23**) was prepared from lithocholic acid (**5**) as follows:

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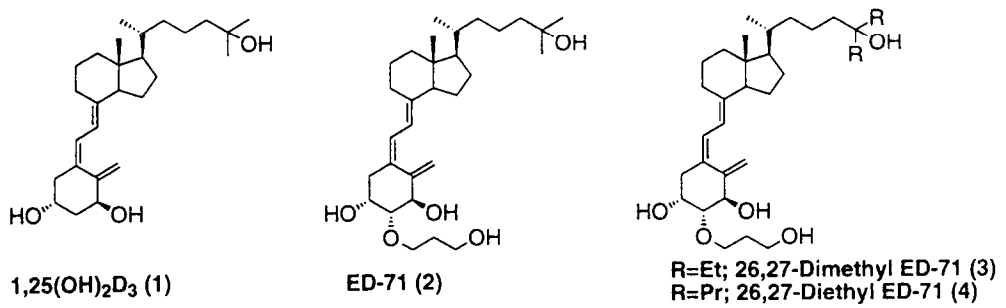


Figure 1. Structures of active vitamin D_3 and ED-71 analogues.

(a) Methylation of **5** with acetyl chloride/methanol giving **6**, quantitatively.
(b) Silylation of **6** with *tert*-butyldimethylsilyl chloride (TBSCl) giving **7** in 83% yield.
(c) Reduction of **7** with sodium borohydride ($NaBH_4$) giving **8** in 83% yield.
(d) Iodination of **8** with iodine/imidazole/triphenylphosphine (PPh_3) giving **9** in 96% yield.
(e) Cyanation of **9** with sodium cyanide (NaCN) giving **10** in 97% yield.
(f) Ethanolysis of **10** with hydrochloric ethanol giving **11**, quantitatively.
(g) Dienone formation with N-bromosuccinimide (NBS) then dichlorodicyanoquinone (DDQ)[8] giving **12** in 46% yield.
(h) Deconjugation of **12** with potassium *tert*-butoxide (*tert*-BuOK)[9] then methylation with trimethylsilyl diazomethane ($TMSCHN_2$) giving **13** in 44% yield.
(i) Reduction of **13** with $NaBH_4$ giving **14** in 96% yield.[9]
(j) Acetylation of **14** with acetic anhydride and pyridine giving **15** in 96% yield.
(k) Bromination of **15** with NBS[9] in the presence of 2,2′-azobisisobutyronitrile (AIBN) then dehydrobromination with γ-collidine giving **16**.
(l) Adduct formation of **16** and 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) giving **17** in 42% yield from **15**.
(m) Hydrolysis of **17** with aqueous potassium hydroxide/methanol giving **18** in 82% yield.
(n) Silylation of **18** giving **19** in 95% yield.
(o) Epoxidation of **19** with *m*-chloroperbenzoic acid (MCPBA) giving **20** in 78% yield.[10]
(p) Retrocycloaddition of **20** giving **21** in 75% yield.[11]
(q) Desilylation of **21** with tetrabutylammonium fluoride (TBAF) giving **22** in 95% yield.
(r) Introduction of hydroxypropoxy substituent giving **23** in 45% yield.

Having the key intermediate ester (**23**) in our hands, treatment of **23** with ethylmagnesium bromide (EtMgBr) or propylmagnesium bromide (PrMgBr) gave **24** (94% yield) and **25** (95% yield) which were converted to 26,27-dimethyl ED-71 (**3**) and 26,27-diethyl ED-71 (**4**) by irradiation at 0 °C using a high pressure mercury lamp (400 W, Vycor filter), followed by thermal isomerization in boiling ethanol, in 14% and 18% yields, respectively (Fig. 2).

Biological effects

The preventive effects of the synthesized analogues on bone mineral loss in OVX rats were evaluated. Wister-Imamichi female rats (8-week-old) were ovariectomized and fed normal diet ad libitum for 2 weeks. The rats were then orally administered vitamin D_3 analogues 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 trigliceride (MCT) as the vehicle. The bone mineral density (BMD) of spine (L2-L5) was measured using a computerized bone measuring apparatus. As shown in Figure 3, ED-71 (**2**) and 26,27-dimethyl ED-71 (**3**) showed dose-dependent and significant increase of spine BMD, whereas 26,27-diethyl ED-71 (**4**) showed no efficacy in OVX rats at doses of 0.008 and 0.04 μg/kg. The most potent effect toward BMD was obtained with 26,27-dimethyl ED-71 (**3**) in this evaluation. The serum calcium values of all groups were within the normal ranges (data are not shown). The relative binding affinity of analogues to calf thymus vitamin D receptor (VDR)[6] and rat DBP[4] are also shown in Figure 3 ($1,25(OH)_2D_3 = 1$). It is suggestive that in this series of ED-71 analogues modified at 26,27-position, increase of BMD corresponds well to the binding potency to VDR. The short half-life in plasma of 26,27-dimethyl ED-71 (**3**) might be expected due to weaker binding affinity to DBP compared to ED-71 (**2**) (Fig. 3). Detailed and further studies of 26,27-dimethyl ED-71 (**3**) on toxicology, pharmacokinetics, metabolism, stability, etc. are necessary to be a clinical candidate for the treatment of osteoporosis.

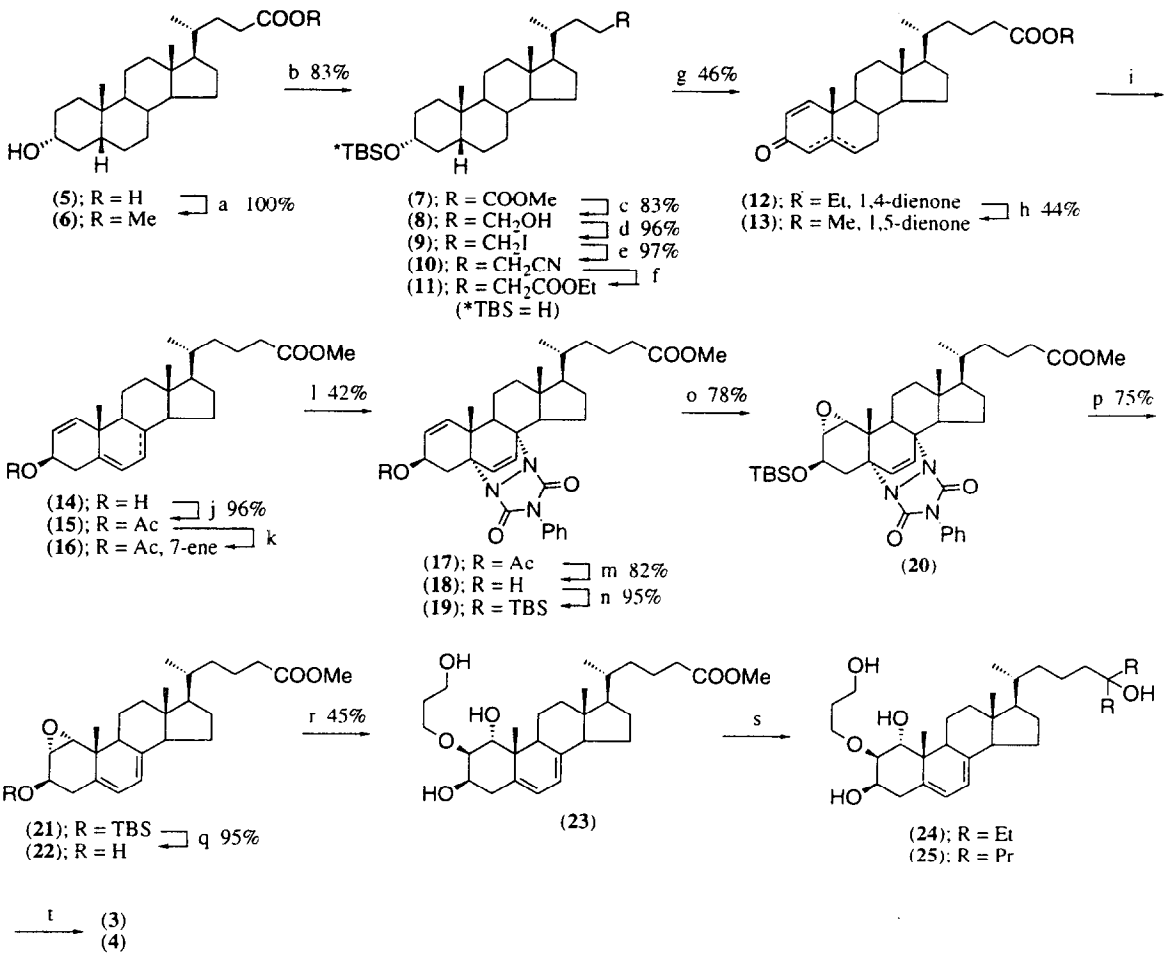


Figure 2. a) AcCl/MeOH; b) TBSCl/imidazole; c) $NaBH_4$; d) I_2/imidazole/Ph_3P; e) NaCN; f) HCl/EtOH; g) 1) NBS, 2) DDQ; h) 1) *tert*-BuOK, 2) $TMSCHN_2$; i) $NaBH_4$; j) Ac_2O/pyridine; k) 1) NBS/AIBN, 2) γ-collidine; l) PTAD; m) KOH/MeOH; n) TBSCl/imidazole; o) MCPBA; p) DMI heat; q) TBAF; r) 1) $HO(CH_2)_3OH$/*tert*-BuOK, 2) $TMSCHN_2$; s) EtMgBr or PrMgBr; t) 1) Hv, 2) heat.

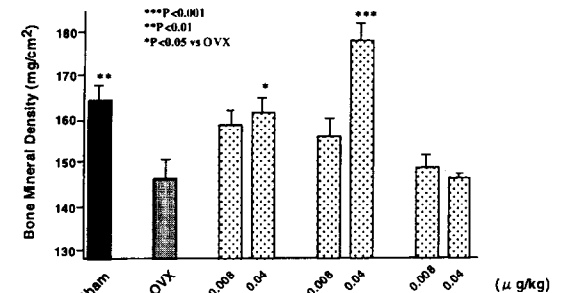
Conclusion

As the analogues related to ED-71 (**2**), 26,27-dimethyl ED-71 (**3**) and 26,27-diethyl ED-71 (**4**) were synthesized from lithocholic acid (**5**). Among these analogues, 26,27-dimethyl ED-71 (**3**) showed the most potent efficacy on spine BMD in OVX rats.

Experimental

General. Melting points were taken on a Yanagimoto micro melting point apparatus and are uncollected. Infrared (IR) spectra were recorded with a Hitachi 270-30 spectrometer or JEOL JIR-6000 and proton nuclear magnetic resonance (NMR) spectra with a JEOL FX-200 or JEOL JNM-270EX in $CDCl_3$ with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. Mass spectra (MS) were obtained with a Shimadzu GCMS QP-1000 or a Hitachi M1200H and ultra violet (UV) spectra with a Shimadzu UV-240. High-resolution mass spectra (HRMS) were obtained using a VG Auto Spec Q instrument. All 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, and preparative thin layer chromatography (TLC) was performed on 20×20 cm plates coated with 0.25 mm thickness of Merck Kieselgel 60 coating F_{254} indicator.

Methyl 3α-hydroxycholanate (6). To a stirred suspension of lithocholic acid **5** (2.53 kg, 6.72 mol) in MeOH (25 L), was added AcCl (240 mL, 3.38 mol) dropwise at room temperature. The resulting mixture was stirred at room temperature for 3.3 h. H_2O (25 L) was added to the mixture. The resulting precipitate was collected by



Binding Affinity to VDR	0.36	0.71	0.0061
Binding Affinity to DBP	3.3	0.33	N.B.
$(1,25(OH)_2D_3$ (1) =1)			

Figure 3. Spinal BMD in OVX rats and binding affinity to DBP and VDR of ED-71 analogues.

filtration and dried at 50 °C to give crude **6** (2.68 kg, quantitatively) as a colorless powder, which was used without further purification. Analytically pure **6** was obtained by recrystallization from MeCN. Mp 129 °C. IR (KBr) 3519, 2933, 2861, 1722, 1714, 1302 cm^{-1}. NMR δ 0.64 (3H, s), 0.90 (3H, d, J = 5.3 Hz), 0.91 (3H, s), 2.15–2.41 (1H, m), 3.58–3.66 (1H, m), 3.66 (3H, m). MS (m/z) 390 (M^+), 373 (100%).

Methyl 3α-(*tert*-butyldimethylsilyloxy)cholanate (7). A mixture of crude **6** (2.68 kg, 6.86 mol), TBSCl (1.65 kg, 9.25 mol) and imidazole (1.49 kg, 21.9 mol) in DMF (13 L) was stirred at room temperature for 30 min. MeOH (26 L) was added to the mixture. The resulting mixture was cooled below 0 °C. The precipitate was collected by filtration, washed with MeOH (1.3 L) and dried at 50 °C to give crude **7** (2.85 kg, 83%) as a colorless powder, which was used without further purification. Analytically pure **7** was obtained by recrystallization from MeCN. Mp 94–95 °C. IR (KBr) 2935, 2862, 1734, 1097, 873, 835 cm^{-1}. NMR δ 0.06 (6H, s), 0.63 (3H, s), 0.89 (9H, s), 0.90 (3H, d, J = 5.3 Hz), 0.91 (3H, s), 2.20–2.35 (1H, m), 3.50–3.70 (1H, m), 3.66 (3H, s). MS (m/z) 504 (M^+), 373 (100%).

3α-(*tert*-Butyldimethylsilyloxy)-20(*R*)-(3-hydroxypropyl)-pregnane (8). To a refluxed mixture of crude **7** (2.84 kg, 5.63 mol) and $NaBH_4$ (1.42 kg, 37.5 mol) in THF (28 L), was added a mixture of MeOH (6.15 L) and THF (10 L) dropwise. The resulting mixture was taken up with H_2O (14 L) and *n*-hexane (14 L) at room temperature. The organic layer was separated, washed with H_2O (7 L), dried over $MgSO_4$ and evaporated. The residue was taken up with MeOH (14 L) and MeCN (28 L) and cooled to −18 °C. The resulting precipitate was collected by filtration and dried at 50 °C to give crude **8** (2.23 kg, 83%) as a colorless powder, which was used without further purification. Analytically pure **8** was obtained by recrystallization from MeCN. mp. 75 °C. IR (KBr) 3325, 2926, 2866, 2862, 1468, 1448, 1377, 1053, 1051, 1016 cm^{-1}. NMR δ 0.06 (6H, s), 0.63 (3H, s), 0.89 (9H, s), 0.89 (3H, d, J = 5.3 Hz), 0.90 (3H, s), 3.50–3.70 (3H, m). MS (m/z) 476 (M^+), 345 (100%).

3α-(*tert*-Butyldimethylsilyloxy)-20(*R*)-(3-iodopropyl)-pregnane (9). To a stirred solution of crude **8** (2.23 kg, 4.68 mol), PPh_3 (1.53 kg, 4.68 mol) and imidazole (400 g, 5.86 mol) in CH_2Cl_2 (22.3 L), was added I_2 (1.31 kg, 10.3 mol) at room temperature. Insoluble material was filtered out and the filtrate was concentrated in vacuo. The residue was taken up with MeOH (22 L). The resulting precipitate was collected by filtration and dried at 50 °C to give crude **9** (2.63 kg, 96%) as a colorless powder, which was used without further purification. Analytically pure **9** was obtained by recrystallization from MeCN. Mp 82–85 °C. IR (KBr) 2951, 2924, 2862, 2854, 1470, 1464, 1462, 1373, 1248, 1097, 1078, 872, 833, 775 cm^{-1}. NMR δ 0.06 (6H, s), 0.63 (3H, s), 0.89 (9H, s), 0.90 (3H, d, J = 7.3 Hz), 0.92 (3H, m), 3.10–3.22 (2H, m), 3.53–3.60 (1H, m). MS (m/z) 586 (M^+), 354 (100%).

3α-(*tert*-Butyldimethylsilyloxy)-20(*R*)-(3-cyanopropyl)-pregnane (10). A mixture of crude **9** (2.00 kg, 3.41 mol) and NaCN (183 g, 3.55 mol) in DMSO (15 L) and THF (5 L) was stirred at 60 °C for 2 h. To the stirred mixture, *n*-hexane (10 L) and H_2O (10 L) were added. The organic layer was separated, washed with H_2O (10 L×2), dried over $MgSO_4$, and evaporated. The residue was taken up with MeOH (10 L). The resulting precipitate was collected by filtration and dried at 50 °C to give crude **10** (1.53 kg, 95%) as a colorless powder, which was used without further purification. Analytically pure **10** was obtained by recrystallization from MeCN. Mp 148–149 °C. IR (KBr) 2929, 2864, 2243, 1471, 1462, 1387, 1383, 1252, 1176, 1106, 1101, 1057, 1007, 953, 931, 901, 874, 837, 775, 668 cm^{-1}. NMR δ 0.06 (6H, s), 0.63 (3H, s), 0.88–0.93 (15H, s, s, d), 2.30 (2H, t, J = 6.9 Hz), 3.53–3.59 (1H, m). MS (m/z) 485 (M^+), 486 (100%).

3α-Hydroxy-20(*R*)-(3-ethoxycarbonylpropyl)pregnane (11). To a stirred solution of HCl gas (2.3 kg) in EtOH (8 L), was added crude **10** (765 g, 1.34 mol). The resulting mixture was refluxed for 1 h and concentrated in vacuo. The residue was dissolved in AcOEt (5 L) and THF (2.5 L), washed with H_2O and saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated to give crude **11** (1.52 kg, quantitatively) as a colorless powder, which was used without further purification. Analytically pure **11** was obtained by recrystallization from MeCN. Mp 105–108 °C. IR (KBr) 3500, 2980, 2939, 2864, 2862, 1707, 1466, 1464, 1462, 1377, 1288, 1257, 1255 cm^{-1}. NMR δ 0.62 (3H, s), 0.90 (3H, s), 0.90 (3H, d,

J=6.2 Hz), 2.17–2.28 (2H, m), 3.54–3.65 (1H, m), 4.11 (2H, q, J=6.9 Hz).

20(*R*)-(3-Ethoxycarbonylpropyl)pregna-1,4-dien-3-one (12). To a stirred mixture of crude **11** (102 g, 242 mmol) and NBS (130 g, 730 mmol) in *tert*-BuOH (981 mL) and H_2O (39.2 mL), was added HBr (19.6 mL) at room temperature. The resulting mixture was stirred at room temperature for 30 min. $Na_2S_2O_4$ (30 g) in H_2O (500 mL) was added to the mixture. The resulting mixture was stirred at room temperature for 30 min, extracted with AcOEt, washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated. To the residue in AcOEt (981 mL), was added DDQ (121 g, 533 mmol). The resulting mixture was refluxed for 3.5 h. The insoluble material was filtered out. The filtrate was washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (1:3) as an eluent to give **12** (46.1 g, 46%) as a pale yellow powder. IR (neat) 2950, 2880, 1735, 1670, 1185, 890 cm^{-1}. NMR δ 0.74 (3H, s), 0.93 (3H, d, J=6.6 Hz), 1.23 (3H, s), 1.25 (3H, t, J=7.3 Hz), 4.12 (2H, q, J=7.3 Hz), 6.06 (1H, s), 6.22 (1H, d, d, J=2.0, 10.2 Hz), 7.06 (1H, d, J=10.2 Hz). MS (m/z) 412 (M^+), 122 (100%).

20(*R*)-(3-Methoxycarbonylpropyl)pregna-1,5-dien-3-one (13). To a stirred solution of *tert*-BuOK (676 mg, 5.42 mmol) in 1,3-dimethyl-2-imidazolidinone (DMI, 4.8 ml), was added **11** (500 mg, 1.21 mmol) in THF (2.4 ml) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. AcOH (2.5 mL) was added to the mixture. The resulting mixture was diluted with AcOEt, washed with H_2O, dried over $MgSO_4$ and evaporated. To the residue in MeOH (4.7 mL) and THF (4.7 mL), was added $TMSCHN_2$ (2.0 M solution in *n*-hexane, 4.7 ml, 9.4 mmol). The resulting mixture was stirred at room temperature for 4.5 h and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (3:17) as an eluent to give **13** (240 mg, 44%) as a colorless powder. IR (neat) 2950, 2875, 1740, 1690, 1190, 1165, 780 cm^{-1}. NMR δ 0.73 (3H, s), 0.95 (3H, d, J=6.3 Hz), 1.22 (3H, s), 3.67 (3H, s), 5.37–5.48 (1H, m), 5.88 (1H, d, J=10.2Hz), 6.98 (1H, d, J=10.2 Hz). MS (m/z) 398 (M^+, 100%).

3β-Hydroxy-20(*R*)-(3-methoxycarbonylpropyl)pregna-1,5-diene (14). To a stirred mixture of **13** (55 mg, 137 μmol) in MeOH (1.5 mL) and THF (1 mL), was added $NaBH_4$ (10.4 mg, 275 μmol) at 0 °C. The resulting mixture was stirred at 0 ° for 1.5 h, quenched with acetone and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (3:7) as an eluent to give **14** (50 mg, 90%) as a colorless oil. IR (neat) 3390 (br), 2940, 2800, 1735, 1440, 1170, 1025, 765 cm^{-1}. NMR δ 0.69 (3H, s), 0.94 (3H, d, J=6.6 Hz), 1.09 (3H, s), 4.11–4.26 (1H, m), 5.41 (1H, brd, J=4.4 Hz), 5.54 (1H, d, J=10.2 Hz), 5.78 (1H, d, d, J=1.7, 10.0 Hz). MS (m/z) 400 (M^+), 371 (100%).

3β-Acetyloxy-20(*R*)-(3-methoxycarbonylpropyl)pregna-1,5-diene (15). A mixture of **14** (337 mg, 842 μmol), Ac_2O (5 mL, 53.0 mmol), *N*,*N*-dimethylaminopyridine (DMAP; 1.15 g, 9.41 mmol), and pyridine (5 mL, 61.8 mmol) was stirred at room temperature for 3 h, poured into diluted HCl solution, extracted with AcOEt, washed with saturated $NaHCO_3$ and saturated $NaCl_g$ dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (1:9) as an eluent to give **15** (358 mg, 96%) as a colorless oil. IR (neat) 2935, 2860, 1735, 1430, 1370, 1235, 1020 cm^{-1}. NMR δ 0.69 (3H, s), 0.94 (3H, d, J=6.3 Hz), 1.10 (3H, s), 2.06 (3H, s), 3.67 (3H, s), 5.15–5.29 (1H, m), 5.40–5.52 (2H, m), 5.86 (1H, d, d, J=1.9, 10,0 Hz). MS (m/z) 442 (M^+), 118 (100%).

PTAD Adduct of 3β-Acetoxy-20(*R*)-(3-methoxycarbonylpropyl)pregna-1,5,7-triene (17). A mixture of **15** (15.5 g, 35.1 mmol), NBS (7.79 g, 43.8 mmol), and AIBN (1.72 g, 10.5 mmol) in *n*-hexane (213 mL) was refluxed for 30 min. The insoluble material was filtered out. The filtrate was concentrated in vacuo. The resulting residue and γ-collidine (17.3 mL, 131 mmol) in toluene (142 mL) was refluxed for 1 h. The insoluble material was filtered out. The filtrate was diluted with AcOEt, washed with 2 N HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and evaporated to give crude **16**, which was used without purification. To a stirred solution of crude **16** in CH_2Cl_2 (170 mL), was added PTAD (3.00 g, 17.1 mmol) at room temperature. The resulting solution was stirred at room temperature for 15 h and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (2:3) as an eluent to give **17** (9.06 g, 42%) as a pale brown oil. IR (neat) 2955, 1735, 1700, 1400, 1245, 730 cm^{-1}. NMR δ 0.82 (3H, s), 0.95 (3H, d, J=6.1 Hz), 1.10 (3H, s), 2.05 (3H, s), 3.49 (1H, d, d, J=8.5, 14.9 Hz), 3.66 (3H, s), 5.79 (1H, d, d, J=4.0, 10.0 Hz), 5.90 (1H, d, J=10.0 Hz), 5.92–6.05 (1H, m), 6.27 (1H, d, J=8.2 Hz), 6.49 (1H, d, J=8.2 Hz), 7.23–7.53 (5H, m). MS (m/z) 615 (M^+), 381 (100%). UV λ_{max} nm 205.

PTAD Adduct of 3β-Hydroxy-20(*R*)-(3-methoxycarbonylpropyl)pregna-1,5,7-triene (18). A mixture of **17** (9.06 g, 14.7 mmol) and KOH (3.05 g, 54.4 mmol) in MeOH (539 mL) was stirred at room temperature for 1 h. 50% AcOH solution (200 mL) was added. The resulting mixture was extracted with AcOEt, washed with saturated NaCl, dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (2:1) as an eluent to give **18** (9.94 g, 82%) as a pale yellow foam. IR (neat) 3240 (br), 2950,

1735, 1700, 1400, 1025 cm^{-1}. NMR δ 0.81 (3H, s), 0.95 (3H, d, J=6.1 Hz), 1.08 (3H, s), 3.37 (1H, d, d, J=8.2, 14.7 Hz), 3.66 (3H, s), 4.97–5.08 (1H, m), 5.75 (2H, brs), 6.28 (1H, d, J=8.2 Hz), 6.45 (1H, d, J=8.2 Hz), 7.23–7.44 (5H, m). MS (*m/z*) 573 (M^+), 141 (100%). UV λ_{max} nm 207.

PTAD Adduct of 3β-(*tert*-Butyldimethylsilyloxy)-20(*R*)-(3-methoxycarbonylpropyl)pregna-1,5,7-triene (19). To a stirred solution of **18** (60.0 mg, 105 μmol) and imidazole (71.9 mg, 1.09 mmol) in DMI (1ml), was added TBSCl (78.8 mg, 523 μmol) at room temperature. The resulting mixture was stirred at room temperature for 2 h, poured into H_2O, extracted with AcOEt-*n*-hexane (1:1), washed with saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC developed with AcOEt-*n*-hexane (1:4) to give **19** (68 mg, 95%) as a colorless oil. IR (neat) 2955, 2855, 1740, 1700, 1400, 1255, 1050, 835, 730 cm^{-1}. NMR δ 0.08 (3H, s), 0.10 (3H, s), 0.81 (3H, s), 0.89 (9H, s), 0.95 (3H, d, J=6.1 Hz), 1.10 (3H, s), 3.32 (1H, d, d, J=7.9, 14.5 Hz), 3.66 (3H, s), 4.90–5.01 (1H, m), 5.57–5.72 (2H, m), 6.26 (1H, d, J=8.3 Hz), 6.44 (1H, d, J=8.3 Hz), 7.23–7.46 (5H, m). MS (*m/z*) 512 (M^+-PTAD), 75 (100%). UV λ_{max} nm 203.

PTAD Adduct of 1α,2α-Epoxy-3β-(*tert*-butyldimethylsilyloxy)-20(*R*)-(3-methoxycarbonylpropyl)pregna-5,7-diene (20). A mixture of **19** (5.08 g, 7.39 mmol) and MCPBA (4.16 g, 16.9 mmol) in CH_2Cl_2 (149 mL) was stirred at room temperature for 4.5 h. 5% $Na_2S_2O_4$ solution was added. The resulting mixture was extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (1:3) as an eluent to give **20** (4.03 g, 78%) as a pale yellow foam. IR (neat) 2960, 2870, 1745, 1700, 1400, 1090, 840 cm^{-1}. NMR δ 0.12 (6H, s), 0.83 (3H, s), 0.91 (9H, s), 0.95 (3H, d, J=6.3 Hz), 1.09 (3H, s), 3.14 (1H, d, J=3.8 Hz), 3.15–3.26 (1H, m), 3.22 (1H, d, J=3.8 Hz), 3.66 (3H, s), 4.83–4.98 (1H, m), 6.19 (1H, d, J=8.3 Hz), 6.41 (1H, d, J=8.3 Hz), 7.20–7.48 (5H, m). MS (*m/z*) 528 (M^+-PTAD), 119 (100%). UV λ_{max} nm 207.

1α,2α-Epoxy-3β-(*tert*-butyldimethylsilyloxy)-20(*R*)-(3-methoxycarbonylpropyl)pregna-5,7-diene (21). A mixture of **20** (950 mg, 1.35 mmol) in DMI (50 mL) was stirred at 140 °C for 5.5 h. The resulting mixture was poured into H_2O, extracted with *n*-hexane, washed with H_2O, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (1:9) as an eluent to give **21** (533 mg, 75%) as a colorless powder. IR (neat) 2945, 2850, 1735, 1435, 1245, 1075, 840, 775 cm^{-1}. NMR δ 0.11 (3H, s), 0.13 (3H, s), 0.63 (3H, s), 0.93 (9H, s), 0.97 (3H, d, J=6.3 Hz), 1.04 (3H, s), 3.01 (1H, d, J=3.7 Hz), 3.27 (1H, d, J=3.7 Hz), 3.67 (3H, s), 3.76–3.90 (1H, m), 5.34–5.43 (1H, m), 5.69 (1H, brd, J=5.6 Hz). MS (*m/z*) 528 (M^+), 73 (100%). UV λ_{max} nm 290, 278, 268.

1α,2α-Epoxy-3β-hydroxy-20(*R*)-(3-methoxycarbonylpropyl)pregna-5,7-diene (22). A mixture of **21** (533 mg, 1.01 mmol) and TBAF (1.0 M solution in THF, 5.03 ml, 5.03 mmol) in THF (10 mL) was stirred at 70 °C for 1 h. The resulting mixture was diluted with AcOEt, washed with saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with AcOEt-*n*-hexane (7:13) as an eluent to give **22** (398 mg, 95%) as a colorless foam. IR (neat) 3455 (br), 2930, 2860, 1730, 1435, 1045, 835, 730 cm^{-1}. NMR δ 0.63 (3H, s), 0.97 (3H, d, J=6.3 Hz), 1.04 (3H, s), 3.04 (1H, d, J=3.4 Hz), 3.33 (1H, d, J=3.4 Hz), 3.67 (3H, s), 3.88 (1H, d, d, J=6.3, 10.5 Hz), 5.34–5.44 (1H, m), 5.70 (1H, brd, J=4.4 Hz). MS (*m/z*) 414 (M^+), 56 (100%). UV λ_{max} nm 290, 278, 268.

1α,3β-Dihydroxy-2β-(3-hydroxypropoxy)-20(*R*)-(3-methoxycarbonylpropyl)pregna-5,7-diene (23). A mixture of **22** (673 mg, 1.63 mmol) and *tert*-BuOK (545 mg, 4.86 mmol) in 1,3-propanediol (9.38 ml, 129 mmol) was stirred at 110 °C for 14 h. H_2O (500 ml) was added and the stirring was continued at 110 °C for 30 min. AcOH (600 mL) was added at 0 °C and the stirring was continued at 0 °C for 10 min. MeOH (1.3 mL), THF (4 mL), and $TMSCHN_2$ (2.0 M solution in *n*-hexane, 6.00 mL, 12.0 mmol) were added and the resulting mixture was stirred at room temperature for 1 h. The mixture was poured into saturated NaCl, extracted with AcOEt, washed with saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with EtOH-CH_2Cl_2 (3:25) as an eluent to give **23** (348 mg, 44%) as a pale yellow powder. IR (neat) 3385 (br), 2940, 2870, 1735, 1440, 1385, 1165, 1095, 1050, 1030 cm^{-1}. NMR δ 0.62 (3H, s), 0.96 (3H, d, J=6.1 Hz), 1.06 (3H, s), 3.49–4.03 (7H, m), 3.67 (3H, s), 5.30–5.41 (1H, m), 5.70 (1H, brd, J=5.4 Hz). MS (*m/z*) 490 (M^+), 131 (100%). UV λ_{max} nm 293, 281, 271.

26,27-Dimethyl-2β-(3-hydroxypropoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (24) General Procedure for the Synthesis of 24 and 25. To a stirred solution of **23** (12.0 mg, 24.5 μmol) in THF (2 mL), was added EtMgBr (1.04 M solution in THF, 490 μL, 510 μmol) at room temperature. The resulting solution was stirred at room temperature for 2 h, quenched by H_2O, extracted with AcOEt, washed with H_2O, dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography with EtOH-CH_2Cl_2 (1:10) as an eluent to give **24** (12.0 mg, 95%) as a colorless powder. IR (KBr) 3400 (br), 2960, 2950, 2880, 1460, 1380, 1140, 1100, 1060 cm^{-1}. NMR δ 0.62 (3H, s), 0.86 (6H, t, J=7.5 Hz), 0.95 (3H, d, J=6.6 Hz), 1.07 (3H, s), 3.58–4.01

(7H, m), 5.32–5.40 (1H, m), 5.67–5.73 (1H, m). MS (m/z) 518 (M^+), 87 (100%). UV λ_{max} nm 293, 282, 271. The other compound, **25**, was similarly obtained using PrMgBr (2.0 M solution in THF) and $CeCl_3$.[12]

26,27-Diethyl-2β-(3-hydroxypropoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (25). IR (neat) 3400 (br), 2950, 2870, 1470, 1380, 1100, 1060, 760 cm^{-1}. NMR δ 0.63 (3H, s), 0.91–0.99 (9H, m), 1.07 (3H, s), 3.67–4.00 (7H, m), 5.34–5.40 (1H, m), 5.67–5.72 (1H, m). MS (m/z) 546 (M^+), 56 (100%). UV λ_{max} nm 293, 281, 271.

26,27-Dimethyl-2β-(3-hydroxypropoxy)-1α,3β,25-trihydroxy-9,10-secocholesta-5,7,10(19)-triene (3). General Procedure for the Synthesis of 3 and 4. A solution of **24** (9.30 mg, 18.0 μmol) in EtOH (200 ml) was irradiated using a 400 W high-pressure mercury lamp with a Vycor filter at 0 °C for 1.5 min. The mixture was then refluxed mildly for 3.5 h and concentrated in vacuo. The residue was purified by preparative TLC developed with EtOH-CH_2Cl_2 (1:10) to give **3** (1.30 mg, 14%) as a colorless oil. IR (neat) 3400 (br), 2950, 2890, 1460, 1380, 1120, 1080, 760 cm^{-1}. NMR δ 0.55 (3H, s), 0.86 (6H, t, J = 7.6 Hz), 0.93 (3H, d, J = 6.3 Hz), 3.26 (1H, d, d, J = 2.8, 9.0 Hz), 3.68–3.97 (4H, m), 4.21–4.37 (2H, m), 5.08 (1H, s), 5.49 (1H, s), 6.04 (1H, d, J = 11.2 Hz), 6.36 (1H, d, J = 11.2 Hz). MS (m/z) 518 (M^+), 87 (100%). UV λ_{max} nm 264, λ_{min} nm 228. HRMS calcd for $C_{32}H_{54}O_5$ 518.3972, Found 518.3965. The other compound, **4**, was similarly obtained.

26,27-Diethyl-2β-(3-hydroxypropoxy)-1α,3β,25-trihydroxy-9,10-secocholesta-5,7,10(19)-triene (4). IR (neat) 3400 (br), 2960, 2940, 2880, 1110, 1080 cm^{-1}. NMR δ 0.55 (3H, s), 0.87–0.94 (9H, m), 3.27 (1H, d, d, J = 3.0, 8.9 Hz), 3.70–3.97 (4H, m), 4.23–4.35 (2H, m), 5.08 (1H, s), 5.49 (1H, s), 6.04 (1H, d, J = 11.2 Hz), 6.36 (1H, d, J = 11.2 Hz). MS (m/z) 546 (M^+), 56 (100%). UV λ_{max} nm 264, λ_{min} nm 228. HRMS calcd for $C_{34}H_{56}O_4$ (M-H_2O) 528.4179, Found 528.4163.

Evaluation of the preventive effects on bone mineral loss. Wister-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 various vitamin D_3 analogues, **2**, **3** and **4**, in MCT as vehicle at several doses 5 times a week for 6 weeks. The sham and OVX groups were administered MCT. 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 the difference between OVX and experimental groups was analyzed by the use of Student's t-test.

VDR binding assay. The binding affinity of analogues, **1**, **2**, **3** and **4**, with the calf thymus VDR was tested using a $1,25(OH)_2D_3$ assay kit purchased from INCSTAR (Stillwater, MN). Calf thymus VDR was incubated at 20 °C for 1 h with various concentrations of **1**, **2**, **3**, and **4** and. After the incubation period, 15,000dpm of [^{3}H]-$1,25(OH)_2D_3$ was added and the mixture was incubated at 20 °C for 1 h. Bound and free forms of [^{3}H]-$1,25(OH)_2D_3$ were separated by addition of dextran-charcoal suspension and centrifugation. The radioactivity was measured with an Aloka LSC-700.

DBP binding assay. The binding affinity of analogues, **1**, **2**, **3** and **4**, with rat DBP was performed according to Revelle et al.[13,14]

References and Notes

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