Synthetic Studies of Vitamin D Analogues. XI.[1] Synthesis and Differentiation-Inducing Activity of 1α,25-Dihydroxy-22-oxavitamin D_3 Analogues

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Six analogues of 1α,25-dihydroxy-22-oxavitamin D_3 (OCT) (2), 26,27-dimethyl OCT (5), 26,27-diethyl OCT (6), 24-norOCT (7), 24-homoOCT (8), 24-dihomoOCT (9), and 24-trihomoOCT (10) were synthesized from the 20(*S*)-alcohol (11) as the common starting material. In the activity inducing differentiation of human myeloid leukemia cells (HL-60) into macrophages, 26,27-dimethyl OCT (5) and 24-homoOCT (8) showed the highest activities. The binding properties of these analogues to the chick embryonic intestinal 1α,25-dihydroxyvitamin D_3 (1) receptor are also described.

Keywords 1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxy-22-oxavitamin D_3; irradiation; thermal isomerization; differentiation-inducing activity; calcium phosphorous metabolism

Since 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] (**1**), the active form of vitamin D_3, was discovered to induce differentiation of malignant cells in addition to its originally recognized regulatory effect of calcium and phosphorous metabolism, a variety of its analogues have been synthesized to examine structure–activity relationships.[2] We have already synthesized 1α,25-dihydroxy-22-oxavitamin D_3 (OCT) (**2**),[3] 1α,25-dihydroxy-20-oxavitamin D_3 (**3**),[4] and 1α,25-dihydroxy-23-oxa, -thia, and -azavitamin D_3 (**4**)[5] to develop a clinically more useful medicine in which the differentiation-inducing activity of vitamin D is separated from its potential hypercalcemic activity. Among these analogues, OCT (**2**) was chosen as a candidate for antihyperparathyroidism agent and is now under clinical trials.[6]

Recently, it was reported that elongated or truncated side chains of 1α,25-$(OH)_2$-D_3 (**1**) exert a great influence upon the differentiation-inducing activity and the hypercalcemic activity.[7–10] These findings stimulated our interest to investigate the biological actions of 22-oxavitamin D_3 analogues bearing side chains of different sizes. Accordingly, in this paper we wish to describe the synthesis of OCT analogues having various side chains as well as the preliminary evaluation of their differentiation-inducing activities.

Synthesis Our modification of a side chain of OCT (**2**) is based on the following two aspects; 1) introduction of alkyl groups into the C-26/C-27 terminal carbons of OCT (**2**) to provide 26,27-dimethyl OCT (**5**) and 26,27-diethyl OCT (**6**), 2) change the distance from C-22 oxygen to the terminal hydroxy group to give 24-norOCT (**7**), 24-homoOCT (**8**), 24-dihomoOCT (**9**), and 24-trihomoOCT (**10**). The common starting material for the synthesis of each analogue was the 20(*S*)-alcohol (**11**) which was prepared from dehydroepiandrosterone *via* microbiological 1α-hydroxylation as reported previously.[3]

First, we undertook the synthesis of analogues elongated at the C-26/C-27 positions. The 20(*S*)-alcohol (**11**) was alkylated with ethyl acrylate by the phase transfer reaction catalyzed by tetra-*n*-butylammonium hydroxide to afford the ester (**12**) in 56% yield accompanied by the recovery of **11** in 41% yield. Construction of C-26/C-27 parts was

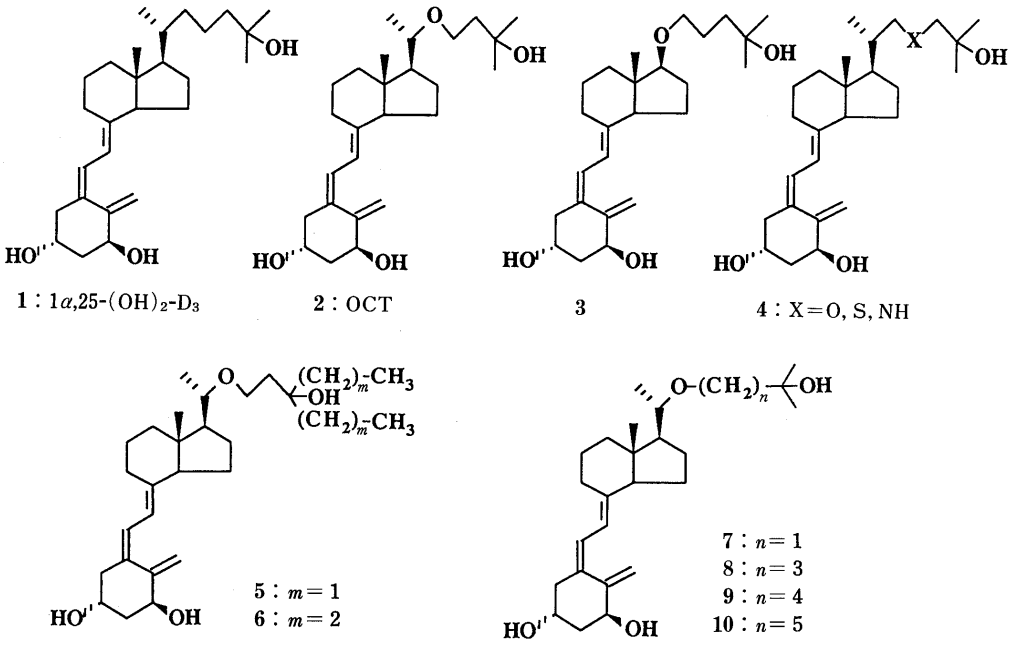
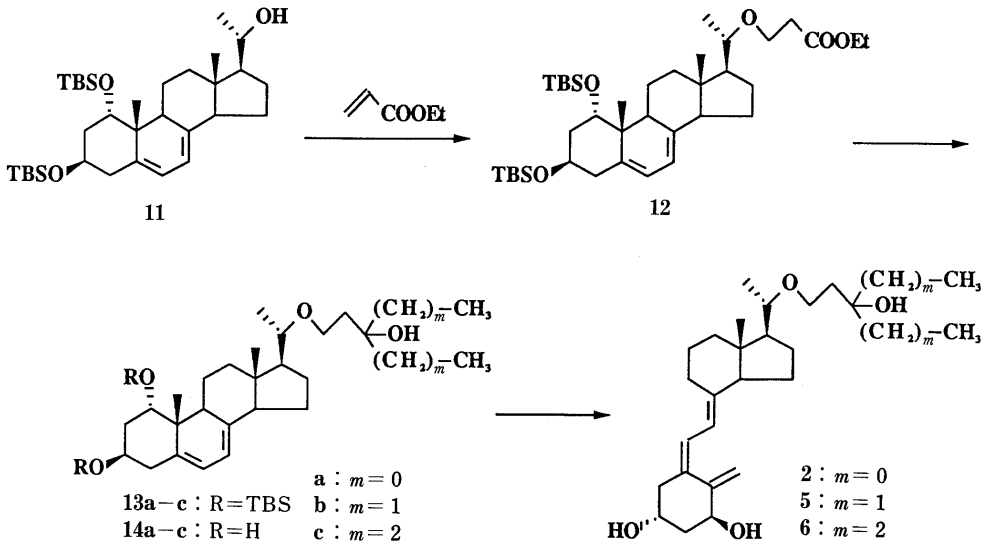


Chart 1



TBS = *tert*-butyldimethylsilyl

Chart 2

performed by alkylation of the carbonyl function of **12** with organometallic reagents. Thus, treatment of **12** with methyllithium in tetrahydrofuran (THF) at −65 °C gave the dimethylated derivative **13a**, in 65% yield, which was then desilylated by tetra-*n*-butylammonium fluoride to give the triol (**14a**) in 85% yield. Subsequent irradiation of **14a** in ethanol at 0 °C under an argon atmosphere using a high pressure mercury lamp through a Vycor filter, followed by thermal isomerization under reflux in ethanol, provided OCT (**2**) in 17% yield. In comparison with the procedure reported previously,[2] the present synthesis of OCT (**2**) is more practical due to facile introduction of the side chain without any formation of by-products.

We similarly synthesized the 26,27-dimethyl analogue (**5**) and the 26,27-diethyl analogue (**6**) from the same intermediate **12**. The ester (**12**) was treated with ethylmagnesium bromide or propylmagnesium bromide at 0 °C to give the alcohols (**13b** and **13c**) in 55% and 33% yields, respectively. Both **13b** and **13c** were desilylated to the triols (**14b** and **14c**), which were irradiated and thermally isomerized to provide 26,27-dimethyl OCT (**5**) and 26,27-diethyl OCT (**6**) in 15% and 5% yields, respectively.

The synthesis of the OCT analogue with a truncated side chain was then carried out. The 20(*S*)-alcohol (**11**) was alkylated with isobutyleneoxide (**15**) in the presence of dibenzo-18-crown-6 and potassium *tert*-butoxide at 100 °C to give the alcohol (**16**), in 47% yield, which was then desilylated to provide the triol (**17**) in 75% yield. The triol (**17**) was irradiated and isomerized to 24-norOCT (**7**) in 13% yield.

The last targets were three OCT analogues elongated at the C-24 position: 24-homoOCT (**8**), 24-dihomoOCT (**9**) and 24-trihomoOCT (**10**). Reaction of **11** with the chloride (**18a**)[11] or the bromide (**18b**)[12] in boiling xylene for 18 h provided the alkylated products **19a** and **19b** in excellent yields. Deketalization of both **19a** and **19b** in methanolic Amberlyst 15 at room temperature for 18 h led to the keto-alcohols (**20a**, **20b**) with concomitant desilylation at the C-3 position. Addition of excess methylmagnesium bromide to **20a** and **20b** completed the side chain formation, giving rise to the diols (**21a**, **21b**), which were desilylated and subsequently converted to 24-homoOCT (**8**) and 24-dihomoOCT (**9**) by irradiation and isomerization. Finally, the coupling reaction of **11** with the bromide (**23**)[13] gave the ether (**24**), which was desilylated, irradiated and isomerized to 24-trihomoOCT (**10**).

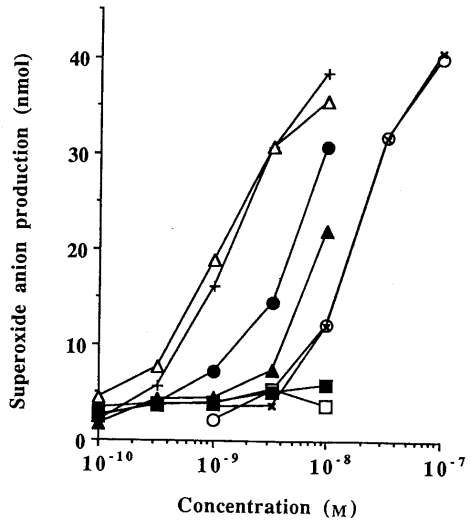


Fig. 1. Comparative Effect of Vitamin D_3 Analogues on the Induction of Superoxide Anion Production of HL-60

○, 1α,25-$(OH)_2$-D_3 (**1**); ●, OCT (**2**); △, 26,27-dimethyl OCT (**5**); ×, 26,27-diethyl OCT (**6**); □, 24-norOCT (**7**); +, 24-homoOCT (**8**); ▲, 24-dihomoOCT (**9**); ■, 24-trihomoOCT (**10**).

Biological Results The preliminary results of the activity inducing differentiation of human myeloid leukemia cells (HL-60) into macrophages *in vitro* estimated by superoxide anion generation[14] are shown in Fig. 1. In the ability of 26,27-dialkylated analogues, 26,27-dimethyl OCT (**5**) ($ED_{50} = 1.48 \times 10^{-9}$ M) showed the highest activity and was approximately threefold as potent as OCT (**2**) ($ED_{50} = 4.90 \times 10^{-9}$ M) at ED_{50}, whereas 26,27-diethyl OCT (**6**) ($ED_{50} = 1.78 \times 10^{-8}$ M) had activity nearly equal to 1α,25-$(OH)_2$-D_3 (**1**) ($ED_{50} = 1.70 \times 10^{-8}$ M) and was about one-fourth of OCT (**2**).

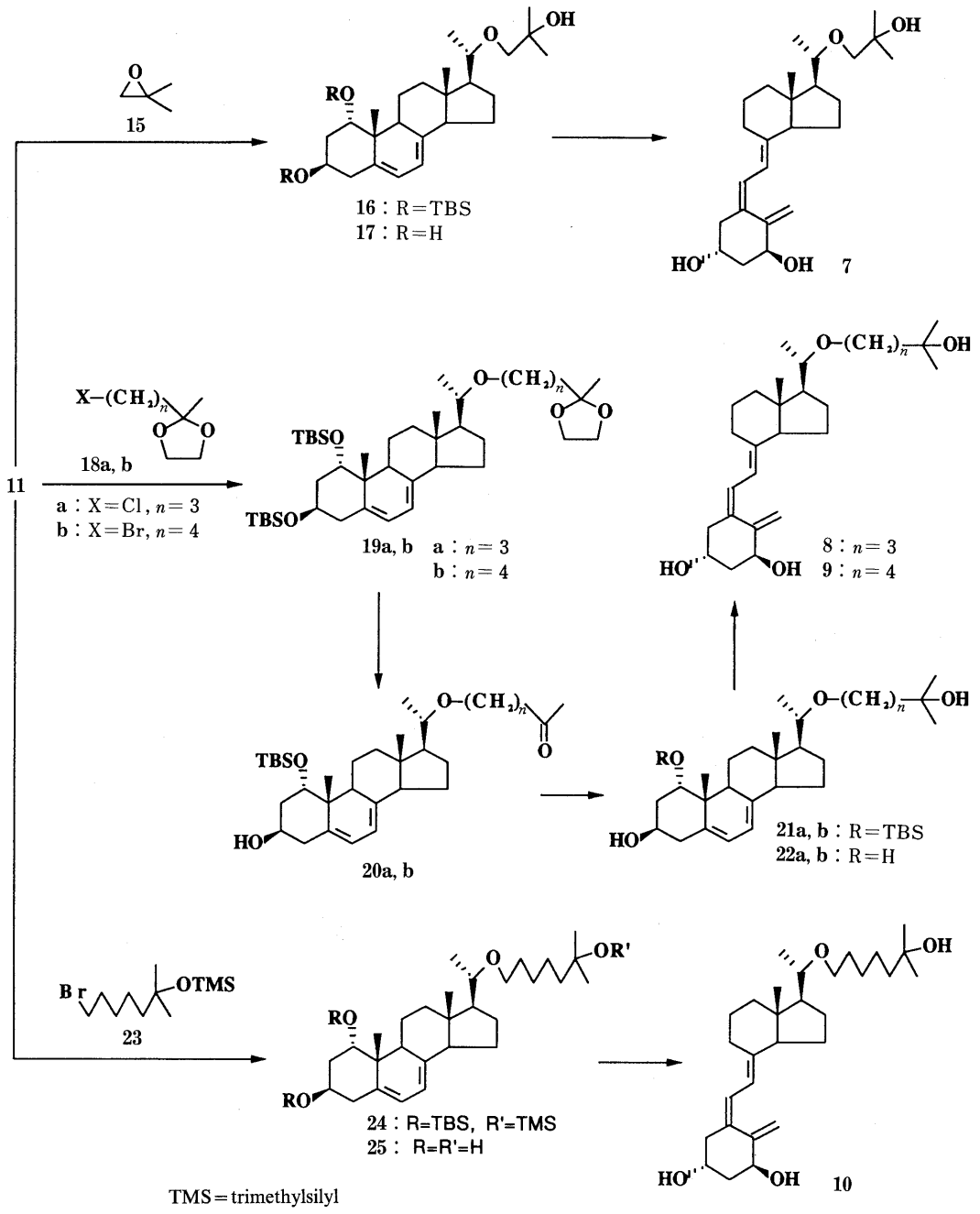


Chart 3

On the other hand, in the case of either an elongated or truncated side chain at the C-24 position, the highest activity to induce differentiation of HL-60 was observed in 24-homoOCT (**8**) ($ED_{50}=1.58\times10^{-9}$ M), which was comparable to 26,27-dimethyl OCT (**5**). 24-DihomoOCT (**9**) ($ED_{50}=8.61\times10^{-9}$ M) was positioned between OCT (**2**) and 1α,25-$(OH)_2$-D_3 (**1**) at ED_{50}. A clear bell-shaped relationship in the truncated or elongated analogues at the C-24 position was formed from 24-norOCT (**7**) to 24-trihomoOCT (**10**).

Figure 2 shows the binding properties of OCT (**2**), 26,27-dimethyl OCT (**5**), 24-homoOCT (**8**), and 1α,25-$(OH)_2$-D_3 (**1**) to the chick embryonic intestinal 1α,25-$(OH)_2$-D_3 receptor.[15] The relative binding potencies to the receptor were 100% for 1α,25-$(OH)_2$-D_3 (**1**), 12.5% for OCT (**2**), 50% for 26,27-dimethyl OCT (**5**), and 9% for 24-homoOCT (**8**), respectively. As the receptor-mediated hypercalcemic action of vitamin D is well known, 26,27-dimethyl OCT (**5**) is expected to exhibit higher calcemic action than OCT (**2**), whereas 24-homoOCT (**8**) comparable to OCT (**2**). Further biological properties, including the regulatory effect of calcium and phosphorous metabolism, are now under investigation.

Experimental

General Methods All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained using a Hitachi 260-30 spectrometer. ^{1}H-Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL FX-200 spectrometer or a JEOL GSX 500 in $CDCl_3$ with tetramethylsilane as an internal standard. Abbreviations used are s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Mass (MS) spectra were carried out on a Shimadzu GCMS-QP 1000 with the ionizing voltage at 20 eV.

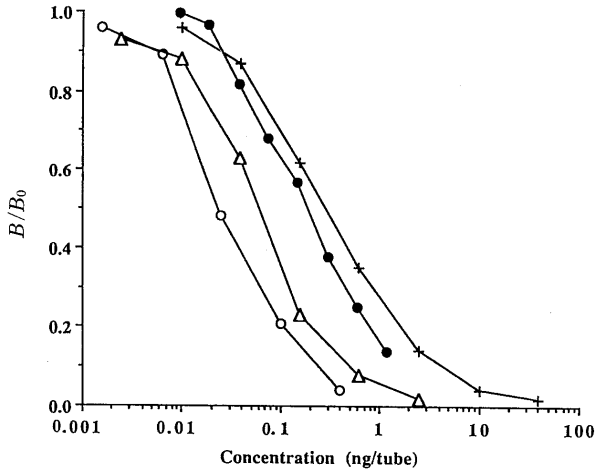


Fig. 2. Competitive Displacement Curves of [^{3}H]-1α,25-$(OH)_2$-D_3 from Chick Embryonic Intestinal 1α,25-$(OH)_2$-D_3 Receptor with Vitamin D_3 Analogues

○, 1α,25-$(OH)_2$-D_3 (**1**); ●, OCT (**2**); △, 26,27-dimethyl OCT (**5**); +, 24-homoOCT (**8**).

Ultraviolet (UV) spectra were recorded with a Shimadzu UV-240 in EtOH. 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 0.5 mm thickness of Merck Kieselgel 60 containing PF_{254} indicator. The phrase "residue upon work-up" refers to the residue when the organic layer was separated, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. All new compounds described in this experimental section were homogeneous on TLC.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(2-ethyloxycarbonylethyloxy)pregna-5,7-diene (12) A mixture of **11** (6.23 g, 11.1 mmol), ethyl acrylate (31 ml), *n*-Bu_4NOH (10% in H_2O, 2 ml), NaOH (50% in H_2O, 125 ml) and toluene (312 ml) was vigorously stirred at room temperature for 15 h. The mixture was then diluted with Et_2O and H_2O. The separated organic layer was washed with saturated NaCl and the residue upon work-up was purified by flash column chromatography with *n*-hexane/AcOEt (5:1) as the eluent to give the recovered alcohol (**11**) (2.55 g, 41% recovery) and **12** (4.09 g, 56%; 94% based on the recovery) as colorless glass, mp 91—93 °C (EtOH). IR (KBr): 1740, 1460, 1380, 1255, 1180, 1095, 1080 cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.59 (3H, s), 0.88 (18H, s), 0.90 (3H, s), 1.17 (3H, d, J=6.1 Hz), 1.26 (3H, t, J=7.2 Hz), 2.52 (2H, br t, J=10.0 Hz), 3.42—3.58 (1H, m), 3.62—3.70 (1H, m), 3.72—3.84 (1H, m), 4.14 (2H, q, J=7.2 Hz), 5.28 (1H, dt, J=5.7, 2.9 Hz), 5.54 (1H, d, J=5.7 Hz). MS *m/z*: 660 (M^+), 471 (100%). UV λ_{max} nm: 293, 281, 270. *Anal.* Calcd for $C_{38}H_{68}O_5Si_2$: C, 69.04; H, 10.37. Found: C, 68.70; H, 10.82.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (13a) A solution of **12** (10.56 g, 16.0 mmol) in THF (100 ml) was added dropwise to a stirred solution of MeLi (1.4 M solution in Et_2O, 100 ml, 140 mmol) in THF (150 ml) at −65 °C. The mixture was then stirred at −65—−70 °C for 15 min, quenched by the addition of saturated NH_4Cl at −65 °C, and extracted with Et_2O. The extract was washed with saturated NaCl and the residue upon work-up was purified by flash column chromatography with *n*-hexane/AcOEt (7:1) as the eluent to give **11** (0.92 g, 10%) and **13a** (6.73 g, 65%) as colorless needles, mp 148—149.5 °C (MeOH). IR (KBr): 3525, 1250, 1100 cm^{-1}. NMR δ: 0.06 (3H, s), 0.07 (6H, s), 0.11 (3H, s), 0.61 (3H, s), 0.88 (18H, s), 0.90 (3H, s), 1.21 (3H, d, J=6.3 Hz), 1.23 (3H, s), 1.24 (3H, s), 3.26 (1H, quintet, J=6.3 Hz), 3.49 (1H, dt, J=9.1, 5.4 Hz), 3.66—3.72 (1H, m), 3.72—3.90 (1H, m), 3.90—4.14 (1H, m), 5.31 (1H, dt, J=5.7, 2.3 Hz, 5.57 (1H, d, J=5.7 Hz). MS *m/z*: 646 (M^+), 457 (100%). UV λ_{max} nm: 293, 281, 270. *Anal.* Calcd for $C_{38}H_{70}O_4Si_2$: C, 70.53; H, 10.90. Found: C, 70.49; H, 10.81.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(3-ethyl-3-hydroxypentyloxy)pregna-5,7-diene (13b) A mixture of EtMgBr (1.01 M solution in Et_2O, 2.3 ml, 2.32 mmol) was added dropwise to a stirred solution of **12** (63 mg, 0.10 mmol) in THF (3 ml) at 0 °C. The mixture was stirred at the same temperature for 40 min, then at room temperature for 3 h. The mixture was quenched by the addition of saturated NH_4Cl at 0 °C and extracted with CH_2Cl_2. The extract was washed with saturated NaCl and the residue upon work-up was purified by preparative TLC developed with *n*-hexane/AcOEt (4.9:1) to give **13b** (36 mg, 55%) as a colorless oil. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.58 (3H, s), 0.85 (6H, t, J=6.3 Hz), 0.88 (18H, s), 0.89 (3H, s), 1.19 (3H, d, J=6.1 Hz), 3.21 (1H, m), 3.36—3.47 (1H, m), 3.52 (1H, br s), 3.65 (1H, br s), 3.72—3.82 (1H, m), 3.98 (1H, m), 4.28 (1H, m), 4.53 (1H, br d), 5.31 (1H, br t), 5.69 (1H, br d). MS *m/z*: 674 (M^+), 485 (100%).

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(3-hydroxy-3-propylhexyloxy)pregna-5,7-diene (13c) A solution of *n*-PrMgBr (2 M solution in THF, 27 ml, 54 mmol) was added dropwise to a stirred solution of **12** (800 mg, 1.21 mmol) in THF (20 ml) at 0 °C. The mixture was then refluxed for 31 h. The mixture was quenched by the addition of saturated NH_4Cl at 0 °C and diluted with *n*-hexane. The insoluble material was filtered off. The filtrate was washed with saturated NaCl, and the residue upon work-up was purified by flash column chromatography with *n*-hexane/AcOEt (13:1) as the eluent to give **13c** (284 mg, 33%) as a colorless oil. IR (neat): 3500, 1440, 1260 cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.56 (3H, s), 0.83 (24H, br s), 0.89 (3H, s), 1.14 (3H, d, J=5.8 Hz), 3.16 (1H, m), 3.36 (1H, m), 3.96 (1H, m), 5.28 (1H, br t), 5.51 (1H, br d). MS *m/z*: 702 (M^+), 409 (100%). UV λ_{max} nm: 293, 281, 270.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(2-hydroxy-2-methylpropyloxy)pregna-5,7-diene (16) A mixture of **11** (561 mg, 1 mmol), *tert*-BuOK (90%, 1.23 g, 11 mmol), dibenzo-18-crown-6 (250 mg) and **15** (2.5 ml) in xylene (30 ml) was stirred at 100 °C for 2 h. The mixture was then diluted with toluene and washed with H_2O and saturated NaCl. The residue upon work-up was purified by flash column chromatography with *n*-hexane/AcOEt (6:1) as the eluent to give **16** (300 mg, 47%) as a colorless powder. IR (neat): 3600, 3475, 1465, 1380, 1250, 1090 cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.59 (3H, s), 0.88 (18H, s), 0.90 (3H, s), 1.17 (6H, s), 3.05 (1H, d, J=8.4 Hz), 3.24—3.38 (1H, br), 3.39 (1H, d, J=8.4 Hz), 3.66—3.72 (1H, br s), 3.92—4.04 (1H, br), 5.28—5.32 (1H, m), 5.59 (1H, d, J=5.7 Hz). MS *m/z*: 632 (M^+), 442 (100%). UV λ_{max} nm: 293, 281, 270.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(4,4-ethylenedioxypentyloxy)pregna-5,7-diene (19a) A mixture of **11** (126 mg, 0.23 mmol), NaH (60%, 120 mg, 3 mmol), and **18a**[11] (561 mg, 3.41 mmol) in xylene (23 ml) was refluxed for 18 h. The mixture was diluted with AcOEt and washed with H_2O and saturated NaCl. The residue upon work-up was purified by flash column chromatography with *n*-hexane/AcOEt (9:1) as the eluent to give crude **19a** (370 mg), which was used without further purification. Purification of crude **19a** by preparative TLC, developed three times with *n*-hexane/AcOEt (5:1), gave analytically pure **19a** as a colorless oil. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.60 (3H, s), 0.88 (18H, s), 0.90 (3H, s), 1.16 (3H, d, J=6.1 Hz), 1.32 (3H, s), 3.22 (2H, m), 3.59 (1H, m), 3.70 (1H, br s), 3.92 (2H, d, J=2.9 Hz), 3.94 (2H, d, J=2.9 Hz), 4.02 (1H, m), 5.27 (1H, br t), 5.58 (1H, br d). MS *m/z*: 688 (M^+), 85 (100%). UV λ_{max} nm: 293, 281, 270.

1α-*tert*-Butyldimethylsilyloxy-3β-hydroxy-20(*S*)-(4-oxopentyloxy)pregna-5,7-diene (20a) A solution of crude **19a** (370 mg) and Amberlyst 15 (135 mg) in MeOH (50 ml) was stirred at room temperature for 18 h. The insoluble material was filtered off. The filtrate was diluted with AcOEt and washed with H_2O, saturated $NaHCO_3$ and saturated NaCl. The residue upon work-up was purified by flash column chromatography with *n*-hexane/AcOEt (4:1) as the eluent to give **20a** (95 mg, 80% from **11**) as a colorless oil. IR (neat): 3425, 1715, 1370, 1250, 1085, 1060 cm^{-1}. NMR δ: 0.08 (3H, s), 0.12 (3H, s), 0.60 (3H, s), 0.88 (9H, s), 0.89 (3H, s), 1.15 (3H, d, J=4.9 Hz), 2.15 (3H, s), 3.21 (2H, m), 3.53 (1H, m), 3.74 (1H, br s), 4.30 (1H, m), 5.35 (1H, m), 5.60 (1H, br d). MS *m/z*: 473 (M^+ − *tert*-Bu), 85 (100%).

1α-*tert*-Butyldimethylsilyloxy-3β-hydroxy-20(*S*)-(4-hydroxy-4-methylpentyloxy)pregna-5,7-diene (21a) A solution of MeMgBr (3 M solution in Et_2O, 0.5 ml, 1.5 mmol) was added dropwise to a stirred solution of **20a** (95 mg, 0.18 mmol) in THF (5 ml) at −10 °C. The mixture was stirred at the same temperature for 20 min, then at room temperature for 3 h. The mixture was treated in the same manner described in the preparation of **13b**. The crude product was purified by flash column chromatography with *n*-hexane/AcOEt (2.6:1) as the eluent to give **21a** (39 mg, 40%) as a colorless oil. IR ($CHCl_3$): 3650, 1080, 1055 cm^{-1}. NMR δ: 0.07 (3H, s), 0.11 (3H, s), 0.61 (3H, s), 0.88 (9H, s), 0.89 (3H, s), 1.23 (3H, d, J=6.1 Hz), 1.21 (6H, s), 3.27 (2H, m), 3.56 (1H, m), 3.74 (1H, br s), 4.04 (1H, m), 5.36 (1H, m), 5.61 (1H, br d). MS *m/z*: 546 (M^+), 101 (100%). UV λ_{max} nm: 293, 281, 270.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(5,5-ethylenedioxyhexyloxy)pregna-5,7-diene (19b) A mixture of **11** (1.20 g, 2.14 mmol), NaH (60%, 295 mg, 7.38 mmol) and **18b**[12] (1.66 g, 7.44 mmol) in xylene (60 ml) was treated in the same manner described in the preparation of **19a**. The crude product was purified by flash column chromatography with *n*-hexane/AcOEt (5.7:1) as the eluent to give **19b** (1.48 g, 99%) as colorless prisms, mp 98.5—100 °C. IR (KBr): 1480, 1470, 1385, 1260, 1095 cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.60 (3H, s), 0.88 (18H, s), 0.90 (3H, s), 1.14 (3H, d, J=6.1 Hz), 1.31 (3H, s), 3.19 (2H, m), 3.53 (1H, m), 3.70 (1H, br s), 3.91 (2H, d, J=3.3 Hz), 3.94 (2H, d, J=3.3 Hz), 4.00 (1H, m), 5.29 (1H, br t), 5.57 (1H, br d). MS *m/z*: 702 (M^+), 99 (100%). UV λ_{max} nm: 293, 281, 270.

1α-*tert*-Butyldimethylsilyloxy-3β-hydroxy-20(*S*)-(5-hydroxy-5-methylhexyloxy)pregna-5,7-diene (21b) A mixture of **19b** (1.48 g, 2.10 mmol) and Amberlyst 15 (540 mg) in MeOH (60 ml) and THF (35 ml) was treated in the same manner described in the preparation of **20a**. The crude product was purified by flash column chromatography with *n*-hexane/AcOEt (2:1) as the eluent to give crude **20b** (970 mg) which was used without further purification. A solution of crude **20b** (300 mg) in THF (11 ml) was added dropwise to a stirred solution of MeMgBr (3 M solution in Et_2O, 1.6 ml, 4.8 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min, then at room temperature for 40 min. The mixture was treated in the same manner described in the preparation of **13b**. The crude product was purified by flash column chromatography with *n*-hexane/AcOEt (4:3) as the eluent to give **21b** (120 mg, 33% from **19b**) as a colorless oil. NMR δ: 0.07 (3H, s), 0.12 (3H, s), 0.61 (3H, s), 0.88 (9H, s), 0.89 (3H, s), 1.15 (3H, d, J=6.1 Hz), 1.21 (6H, s), 3.23 (2H, m), 3.55 (1H, m), 3.73 (1H, br s), 3.99 (1H, m), 5.33 (1H, br t), 5.60 (1H, br d).

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(6-methyl-6-trimethylsilylheptyloxy)pregna-5,7-diene (24) A mixture of **11** (561 mg, 1 mmol), NaH (60%, 268 mg, 7 mmol) and **23**[13] (2.33 g, 8.3 mmol) in xylene (30 ml) was refluxed for 12 h. The mixture was diluted with AcOEt and washed with H_2O and saturated NaCl. The residue upon work-up was chromatographed with *n*-hexane/AcOEt (25:1) as the eluent to give crude **24** (580 mg) which was used without further purification. IR (neat): 1465, 1380, 1365, 1250, 1045 cm^{-1}. MS *m/z*: 628 ($M^+ - tert$-$BuMe_3SiOH$), 131 (100%). UV λ_{max} nm: 293, 282, 270.

1α,3β-Dihydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (14a). General Procedure for Desilylation of 13a—c, 16, 21a, b, and 24 A solution of **13a** (18.4 g, 28.5 mmol) and *n*-Bu_4NF (1 M solution in THF, 290 ml, 0.29 mol) in THF (290 ml) was refluxed mildly for 18 h. The mixture was then diluted with AcOEt, washed with H_2O, 10% HCl, saturated $NaHCO_3$ and saturated NaCl. The residue upon work-up was recrystallized from acetone to give **14a** (10.1 g, 85%) as colorless prisms, mp 186.5—187.5 °C. IR (KBr): 3500, 3420, 1480, 1390, 1170, 1100, 1080, 1065, 1055 cm^{-1}. NMR δ: 0.62 (3H, s), 0.94 (3H, s), 1.22 (3H, d, J=7.2 Hz), 1.24 (6H, s), 3.20—3.32 (1H, m), 3.42—3.56 (1H, m), 3.72—3.91 (2H, m), 4.06 (1H, br), 5.35—5.43 (1H, m), 5.72 (1H, br d, J=5.7 Hz). MS *m/z*: 418 (M^+), 69 (100%). UV λ_{max} nm: 293, 281, 270. *Anal.* Calcd for $C_{26}H_{42}O_4 \cdot 1/3H_2O$: C, 73.55; H, 10.13. Found: C, 73.75; H, 10.53.

1α,3β-Dihydroxy-20(*S*)-(3-ethyl-3-hydroxypentyloxy)pregna-5,7-diene (14b) This (69 mg, 85%) was obtained as a colorless foam from **13b** (123 mg, 0.18 mmol), *n*-Bu_4NF (1 M solution in THF, 2 ml, 2 mmol) and THF (10 ml) after purification by flash column chromatography with CH_2Cl_2/EtOH (12.5:1) as the eluent. IR ($CHCl_3$): 3620, 3555, 1480 cm^{-1}. NMR δ: 0.60 (3H, s), 0.85 (3H, t, J=7.6 Hz), 0.91 (3H, s), 0.97 (3H, t, J=7.2 Hz), 1.20 (3H, d, J=6.1 Hz), 3.24 (1H, m), 3.39—3.50 (1H, m), 3.63—3.85 (2H, m), 4.03 (1H, m), 5.37 (1H, br t), 5.67 (1H, br d). MS *m/z*: 446 (M^+), 56 (100%). UV λ_{max} nm: 293, 282, 271.

1α,3β-Dihydroxy-20(*S*)-(3-hydroxy-3-propylhexyloxy)pregna-5,7-diene (14c) This (60 mg, 31%) was obtained as a colorless oil from **13c** (284 mg, 0.40 mmol), *n*-Bu_4NF (1 M solution in THF, 4 ml, 4 mmol) and THF (10 ml) after purification by flash column chromatography with CH_2Cl_2/EtOH (12.5:1) as the eluent. IR (neat): 3450, 1480, 1400, 1170 cm^{-1}. NMR δ: 0.61 (3H, s), 0.92 (6H, s), 0.92 (3H, t, J=6.3 Hz), 1.20 (3H, d, J=5.8 Hz), 3.23 (1H, m), 3.45 (1H, m), 4.01 (1H, m), 5.34 (1H, m), 5.67 (1H, br d). MS *m/z*: 474 (M^+), 315 (100%). UV λ_{max} nm: 293, 282, 271.

1α,3β-Dihydroxy-20(*S*)-(2-hydroxy-2-methylpropyloxy)pregna-5,7-diene (17) This (142 mg, 75%) was obtained as a colorless powder from **16** (295 mg, 0.47 mmol), *n*-Bu_4NF (1 M solution in THF, 4.7 ml, 4.7 mmol) and THF (4.7 ml) after purification by flash column chromatography with CH_2Cl_2/EtOH (10:1) as the eluent. IR (Nujol): 3350, 1170, 1150, 1090, 1050 cm^{-1}. NMR δ: 0.62 (3H, s), 0.94 (3H, s), 1.19 (6H, s), 3.05 (1H, d, J=8.4 Hz), 3.28—3.48 (1H, m), 3.40 (1H, d, J=8.4 Hz), 3.68—3.82 (1H, m), 3.96—4.16 (1H, br), 5.38—5.44 (1H, br), 5.72 (1H, d, J=5.7 Hz). MS *m/z*: 404 (M^+), 72 (100%). UV λ_{max} nm: 293, 282, 271.

1α,3β-Dihydroxy-20(*S*)-(4-hydroxy-4-methylpentyloxy)pregna-5,7-diene (22a) This (8 mg, 67%) was obtained as a colorless foam from **21a** (15 mg, 0.03 mmol), *n*-Bu_4NF (1 M solution in THF, 2.0 ml, 2 mmol) and THF (2 ml) after purification by preparative TLC developed twice with AcOEt/*n*-hexane (7:1). IR ($CHCl_3$): 3400, 1215 cm^{-1}. NMR δ: 0.61 (3H, s), 0.94 (3H, s), 1.19 (3H, d, J=6.0 Hz), 1.21 (6H, s), 3.24—3.31 (2H, m), 3.58—3.62 (1H, m), 3.77 (1H, br s), 4.03—4.10 (1H, m), 5.39—5.41 (1H, m), 5.73 (1H, dd, J=6.0, 2.6 Hz). MS *m/z*: 432 (M^+), 83 (100%). UV λ_{max} nm: 293, 282, 271.

1α,3β-Dihydroxy-20(*S*)-(5-hydroxy-5-methylhexyloxy)pregna-5,7-diene (22b) This (68 mg, 71%) was obtained as a colorless oil from **21b** (120 mg, 0.21 mmol), *n*-Bu_4NF (1 M solution in THF, 2.2 ml, 2.2 mmol) and THF (10 ml) after purification by flash column chromatography with AcOEt as the eluent. IR (neat): 3480, 1650, 1460, 1375, 1150 cm^{-1}. NMR δ: 0.60 (3H, s), 0.92 (3H, s), 1.17 (3H, d, J=6.1 Hz), 1.21 (6H, s), 3.23 (2H, m), 3.56 (1H, m), 3.74 (1H, br s), 3.92—4.09 (1H, m), 5.39 (1H, br t), 5.68 (1H, br d). MS *m/z*: 446 (M^+), 97 (100%). UV λ_{max} nm: 293, 282, 271.

1α,3β-Dihydroxy-20(*S*)-(6-hydroxy-6-methylheptyloxy)pregna-5,7-diene (25) This (54 mg, 12% from **11**) was obtained as a colorless powder from **24** (580 mg, crude), *n*-Bu_4NF (1 M solution in THF, 40 ml, 40 mmol) and THF (40 ml) after purification by flash column chromatography with CH_2Cl_2/EtOH (10:1) as the eluent. IR (Nujol): 3350, 1195, 1145, 1100, 1060 cm^{-1}. NMR δ: 0.61 (3H, s), 0.95 (3H, s), 1.20 (6H, s), 3.13—3.30 (2H, m), 3.48—3.62 (1H, m), 3.64—3.78 (1H, br), 3.96—4.15 (1H, m), 5.32—5.43 (1H, br), 5.72 (1H, d, J=5.7 Hz). MS *m/z*: 460 (M^+), 68 (100%). UV λ_{max} nm: 293, 282, 271.

1α,3β-Dihydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)-9,10-secopregna-5,7,10(19)-triene (2). General Procedure for Irradiation and Thermal Isomerization of 14a—c, 17, 22a, b, and 25 A solution of **14a** (820 mg, 1.96 mmol) in EtOH (750 ml) was irradiated using a 400 W high pressure mercury lamp with a Vycor filter at 0 °C for 20 min. The mixture was then refluxed mildly for 1.5 h and concentrated *in vacuo* to leave an oil which was submitted to 2-stage flash column chromatography with 1) CH_2Cl_2/EtOH (12.5:1) as the first eluent, 2) AcOEt/*n*-hexane (6:1) as the second eluent to give **2** (136 mg, 17%) as colorless glass, mp 80—84 °C (AcOEt–*n*-hexane). IR (KBr): 3390, 1370, 1145, 1085, 1050 cm^{-1}. NMR δ: 0.54 (3H, s), 1.18 (3H, d, J=6.3 Hz), 1.23 (6H, s), 2.31 (1H, dd, J=13.7, 6.6 Hz), 2.60 (1H, dd, J=13.7, 3.4 Hz), 2.82 (1H, dd, J=12.0, 1.7 Hz), 3.25 (1H, quintet, J=6.3 Hz), 3.47 (1H, dt, J=9.1, 5.4 Hz), 3.75—3.91 (2H, m), 4.16—4.30 (1H, m), 4.36—4.50 (1H, m), 4.98 (1H, t, J=1.4 Hz), 5.32 (1H, t, J=1.4 Hz), 6.02 (1H, d, J=11.4 Hz), 6.36 (1H, d, J=11.4 Hz). MS *m/z*: 418 (M^+), 69 (100%). UV λ_{max} nm: 262, λ_{min} nm: 227.

1α,3β-Dihydroxy-20(*S*)-(3-ethyl-3-hydroxypentyloxy)-9,10-secopregna-5,7,10(19)-triene (5) A solution of **14b** (339 mg, 0.76 mmol) THF (310 ml) was irradiated for 10 min and treated in the same manner described in the general procedure. The crude product was purified by flash column chromatography with CH_2Cl_2/EtOH (12.5:1) as the eluent to give **5** (51.9 mg, 15%) as a colorless foam. NMR δ: 0.53 (3H, s), 0.86 (6H, t, J=8.0 Hz), 1.18 (3H, d, J=7.9 Hz), 3.25 (1H, br t, J=7.4 Hz), 3.38—3.51 (1H, m), 3.72—3.88 (1H, m), 4.14—4.28 (1H, br), 4.36—4.48 (1H, br), 4.98 (1H, s), 5.31 (1H, s), 6.01 (1H, d, J=11.4 Hz), 6.35 (1H, d, J=11.4 Hz). MS *m/z*: 446 (M^+), 97 (100%). UV λ_{max} nm: 263, λ_{min} nm: 227.

1α,3β-Dihydroxy-20(*S*)-(3-hydroxy-3-propylhexyloxy)-9,10-secopregna-5,7,10(19)-triene (6) A solution of **14c** (39 mg, 0.08 mmol) in THF (200 ml) was irradiated for 1.5 min and treated in the same manner described in the general procedure. The crude product was submitted to 3-stage purification; 1) preparative TLC developed with CH_2Cl_2/EtOH (12:1), 2) preparative TLC developed twice with CH_2Cl_2/EtOH (20:1), 3) preparative TLC developed with AcOEt/*n*-hexane (3:1), to give **6** (1.9 mg, 5%) as a colorless foam. NMR δ: 0.53 (3H, s), 0.88 (6H, t, J=6.8 Hz), 1.18 (3H, d, J=6.1 Hz), 3.17—3.28 (1H, m), 3.37—3.48 (1H, m), 4.23 (1H, m), 4.44 (1H, m), 4.99 (1H, br t), 6.02 (1H, d, J=10.9 Hz), 6.37 (1H, d, J=10.9 Hz). MS *m/z*: 456 ($M^+ - H_2O$), 54 (100%). UV λ_{max} nm: 263, λ_{min} nm: 227.

1α,3β-Dihydroxy-20(*S*)-(2-hydroxy-2-methylpropyloxy)-9,10-secopregna-5,7,10(19)-triene (7) A solution of **17** (139 mg, 0.34 mmol) in THF (310 ml) was irradiated for 5 min and treated in the same manner described in the general procedure. The crude product was submitted to

3-stage purification; 1) flash column chromatography with CH_2Cl_2/EtOH (12.5 : 1), 2) flash column chromatography with AcOEt/*n*-hexane (6 : 1) as the eluent, 3) preparative TLC developed four times with CH_2Cl_2/EtOH (12.5 : 1), to give **7** (17.8 mg, 13%) as a colorless foam. NMR δ: 0.53 (3H, s), 1.16 (3H, d, J=6.2 Hz), 1.19 (6H, s), 3.04 (1H, d, J=8.4 Hz), 3.24—3.48 (1H, br), 3.39 (1H, d, J=8.4 Hz), 4.20—4.32 (1H, br), 4.40—4.52 (1H, br), 4.99 (1H, s), 5.33 (1H, s), 6.03 (1H, d, J=11.4 Hz), 6.37 (1H, d, J=11.4 Hz). MS *m/z*: 404 (M^+), 72 (100%). UV λ_{max} nm: 263, λ_{min} nm: 227.

1α,3β-Dihydroxy-20(*S*)-(4-hydroxy-4-methylpentyloxy)-9,10-secopregna-5,7,10(19)-triene (8) A solution of **22a** (33 mg, 0.06 mmol) in EtOH (400 ml) was irradiated for 1.5 min and treated in the same manner described in the general procedure. The crude product was purified by flash column chromatography with AcOEt/*n*-hexane (5 : 1) as the eluent to give **8** (3.4 mg, 13%) as a colorless foam. NMR δ: 0.53 (3H, s), 1.17 (3H, d, J=6.1 Hz), 1.22 (6H, s), 3.26 (2H, m), 3.59 (1H, m), 4.23 (1H, m), 4.43 (1H, m), 5.00 (1H, t, J=1.7 Hz), 5.33 (1H, t, J=1.7 Hz), 6.02 (1H, d, J=11.4 Hz), 6.37 (1H, d, J=11.4 Hz). MS *m/z*: 432 (M^+), 83 (100%). UV λ_{max} nm: 263, λ_{min} nm: 227.

1α,3β-Dihydroxy-20(*S*)-(5-hydroxy-5-methylhexyloxy)-9,10-secopregna-5,7,10(19)-triene (9) A solution of **22b** (45 mg, 0.07 mmol) in EtOH (200 ml) was irradiated for 3 min and treated in the same manner described in the general procedure. The crude product was submitted to 2-stage purification; 1) flash column chromatography with CH_2Cl_2/EtOH (9 : 1) as the eluent, 2) preparative TLC developed twice with AcOEt, to give **9** (6.8 mg, 15%) as a colorless foam. NMR δ: 0.53 (3H, s), 1.16 (3H, d, J=6.1 Hz), 1.21 (6H, s), 3.17—3.25 (2H, m), 3.51—3.58 (1H, m), 4.23 (1H, m), 4.41 (1H, m), 5.00 (1H, br t), 5.33 (1H, br t), 6.03 (1H, d, J=10.9 Hz), 6.38 (1H, d, J=10.9 Hz). MS *m/z*: 446 (M^+), 96 (100%). UV λ_{max} nm: 263, λ_{min} nm: 227.

1α,3β-Dihydroxy-20(*S*)-(6-hydroxy-6-methylheptyloxy)-9,10-secopregna-5,7,10(19)-triene (10) A solution of **25** (54 mg, 0.12 mmol) in THF (310 ml) was irradiated for 2.5 min and treated in the same manner described in the general procedure. The crude product was submitted to 2-stage purification; 1) flash column chromatography with CH_2Cl_2/EtOH (10 : 1) as the eluent, 2) preparative TLC developed three times with CH_2Cl_2/EtOH (8 : 1), to give **10** (6.3 mg, 12%) as a colorless foam. NMR δ: 0.53 (3H, s), 1.15 (3H, d, J=6.2 Hz), 1.21 (6H, s), 3.12—3.28 (2H, m), 3.47—3.60 (1H, m), 4.16—4.28 (1H, br), 4.36—4.46 (1H, br), 5.00 (1H, s), 5.33 (1H, s), 6.02 (1H, d, J=11.4 Hz), 6.38 (1H, d, J=11.4 Hz). MS *m/z*: 460 (M^+), 68 (100%). UV λ_{max} nm: 262, λ_{min} nm: 227.

Differentiation-Inducing Activity HL-60 was cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and 20 μg/ml gentamycin at 37 °C in a humidified atmosphere of 5% CO_2 in air. Induction of differentiation was estimated by the ability of the cell to generate a superoxide anion. Vitamin D-treated cells were obtained by seeding HL-60 at 1×10^5/ml in growth media and by culturing it for 4 d in the presence of various concentrations of vitamin D_3 analogues. The cells were washed free of the compounds and suspended in a 1.5 ml reaction mixture containing 80 μM ferricytochrome c (Sigma Chemical Co., St. Louis, MO.) and 500 ng/ml phorbol myristate acetate (Sigma) in 0.1% gelatin Hanks' balanced salt solution without phenol red. The mixture was incubated at 37 °C for 60 min and centrifuged for 10 min at $400 \times g$ at 4 °C. The reduction of ferricytochrome c was measured by the absorption increase at 550 to 540 nm (molar absorption coefficient, 19.1×10^3/cm) with a Hitachi U-3200 double-beam spectrophotometer.

Binding Affinity with Chick Intéstinal Cytosolic Receptor Chick embryonic intestinal 1α,25-$(OH)_2$-D_3 receptor (Yamasa Shoyu Co., Tokyo, Japan, 0.42 mg/ml) was incubated at 4 °C for 3 h with 10000 dpm of [^{3}H]-1α,25-$(OH)_2$-D_3 (6.67 TBq/mmol) and various concentrations of vitamin D_3 analogues in 0.05 M phosphate buffer, pH 7.4, containing 0.3 M KCl. Bound and free forms of [^{3}H]-1α,25-$(OH)_2$-D_3 were separated by the addition of dextran–charcol and centrifugation. The radioactivity of the receptor bound [^{3}H]-1α,25-$(OH)_2$-D_3 was measured with an Aloka LSC-900.

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