Synthetic Studies of Vitamin D Analogues. XVII.[1] Synthesis and Differentiation-Inducing Activity of 1α,24-Dihydroxy-22-oxavitamin D_3 Analogues and Their 20(*R*)-Epimers[2]

Noboru KUBODERA,* Hiroyoshi WATANABE, Katsuhito MIYAMOTO, Masahiko MATSUMOTO, Setsu MATSUOKA, and Takehiko KAWANISHI

Exploratory Research Laboratories, Chugai Pharmaceutical Co., Ltd., 1–135 Komakado, Gotemba, Shizuoka 412, Japan. Received February 9, 1993

Four vitamin D_3 analogues, 1α,24(*S*)- and 1α,24(*R*)-dihydroxy-22-oxavitamin D_3 (5 and 6) and their 20(*R*)-epimers (7 and 8) were synthesized from the 20(*S*)-alcohol (10). In tests of activity to induce differentiation of human myeloid leukemia cells (HL-60) to macrophages, 5 showed comparable activity to 1α,25-dihydroxy-22-oxavitamin D_3 (OCT) (2), and the other three analogues (6, 7 and 8) were less active than OCT (2). The binding properties of these analogues to the chick embryonic intestinal 1α,25-dihydroxyvitamin D_3 (1) receptor were evaluated. Furthermore, 20(*R*)-OCT (9) was synthesized and its biological properties were compared with those of OCT (2) and the 20(*R*)-epimers (7 and 8).

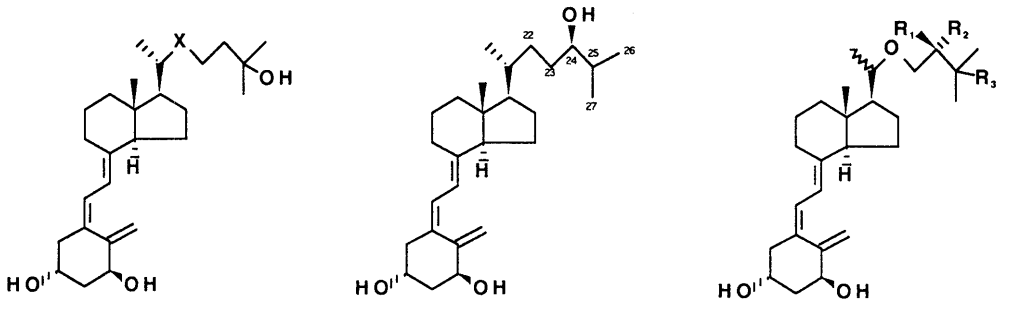
Keywords vitamin D_3 analogue; 1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxy-22-oxavitamin D_3; 1α,24(*S*)-dihydroxy-22-oxavitamin D_3; differentiation-inducing activity; 20(*R*)-epimer

In recent years, considerable attention has been focused on the synthesis of analogues of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] (**1**), aiming to separate the differentiation-inducing activity of human myeloid leukemia cells (HL-60) from the regulatory effect on calcium and phosphorus metabolism.[3,4] During our study of side chain modification of **1**, we have initially obtained 1α,25-dihydroxy-22-oxavitamin D_3 (OCT) (**2**),[5,6] which shows high activity in inhibition of cellular proliferation and stimulation of cell differentiation with remarkably low calcemic activity.[7] OCT (**2**) is being clinically investigated for suppression of secondary hyperparathyroidism.[8]

The recent discovery that 1α,24(*R*)-dihydroxyvitamin D_3 (**3**)[9,10] and MC-903 (**4**),[11] both hydroxylated at the C-24 position, exhibit essentially the same cell differentiation activity but reduced hypercalcemic action as compared with 1α,25-$(OH)_2$-D_3 (**1**), stimulated our interest in 22-oxygenated analogues possessing a 24-hydroxy group instead of the 25-hydroxy moiety. In this paper we wish to describe the synthesis of 1α,24(*S*)- and 1α,24(*R*)-dihydroxy-22-oxavitamin D_3, (**5**) and (**6**), and their 20(*R*)-epimers (**7** and **8**) as well as a preliminary evaluation of their differentiation-inducing activities. Furthermore, the present report deals with the synthesis of 20(*R*)-OCT (**9**), which was recently prepared by an alternative route,[12] and a comparison of its biological properties with those of OCT (**2**) and the 20(*R*)-epimers (**7** and **8**).

Synthesis First, the 20(*S*)-alcohol (**10**) was alkylated with the (*S*)-epoxide (**11**)[13] (prepared from D-valine by means of a 3-step sequence) or the (*R*)-epoxide (**12**)[11] (prepared from L-valine), in the presence of dibenzo-18-crown-6 and potassium *tert*-butoxide at 100 °C for 3 h to give the 24(*S*)-alcohol (**13**) or the 24(*R*)-alcohol (**14**) in 44% or 48% yields, respectively, based upon the recovery of **10**. Desilylation of **13** and **14** with tetrabutylammonium fluoride provided the triols (**15** and **16**) in 76% and 57% yields, respectively, which were then converted to 1α,24(*S*)- and 1α,24(*R*)-dihydroxyvitamin D_3 (**5** and **6**) by irradiation in ethanol at 0 °C using a high-pressure mercury lamp through a Vycor filter, followed by thermal isomerization under reflux in ethanol, in 13% and 14% yields, respectively.

Next, we turned our attention to the synthesis of 20(*R*)-vitamin D_3 analogues (**7**, **8** and **9**), since biological comparison of vitamin D_3 analogues and their 20(*R*)-epimers is at an early stage.[14,15] Thus, the 20(*S*)-alcohol (**10**) was converted to the 20(*R*)-alcohol (**18**) by following



1 : X = CH_2; 1α,25-$(OH)_2$-D_3
2 : X = O; OCT
3 : 1α,24(*R*)-$(OH)_2$-D_3
4 : 22*E*-ene-26,27-cyclo; MC-903
5 : 20(*S*), R_1 = OH, R_2 = H, R_3 = H
6 : 20(*S*), R_1 = H, R_2 = OH, R_3 = H
7 : 20(*R*), R_1 = OH, R_2 = H, R_3 = H
8 : 20(*R*), R_1 = H, R_2 = OH, R_3 = H
9 : 20(*R*), R_1 = H, R_2 = H, R_3 = OH

Chart 1

© 1993 Pharmaceutical Society of Japan

NII-Electronic Library Service

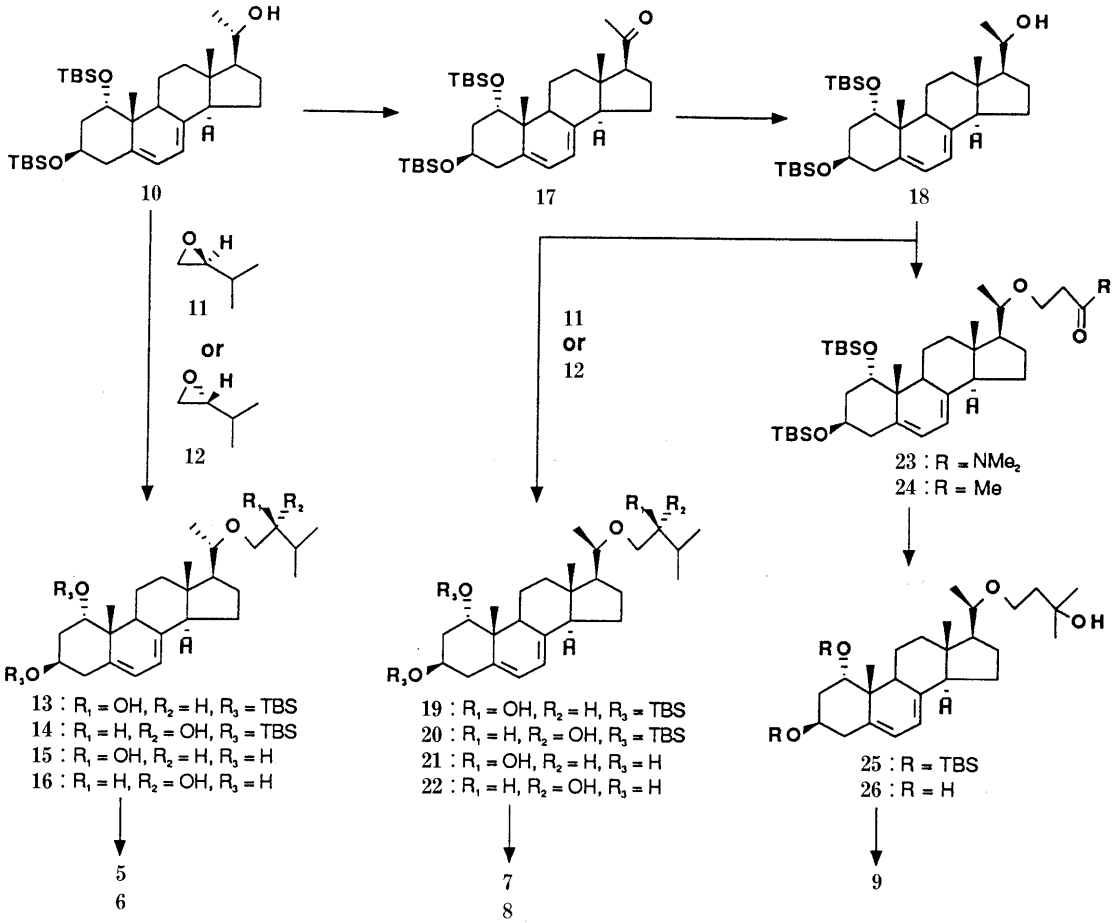
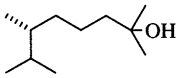
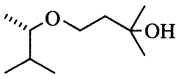
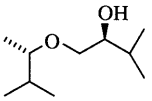
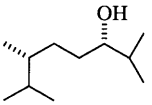
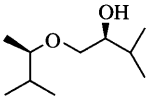
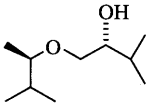
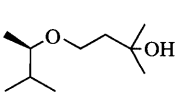


Chart 2

a 2-step sequence, because the direct inversion of **10** to **18** using the Mitsunobu reaction was unsuccessful due to the preferential occurrence of an elimination reaction. Oxidation of **10** with dimethyl sulfoxide (DMSO) and trichloromethyl chloroformate under alternative Swern conditions,[16] gave the ketone (**17**) in 85% yield. Hydride reduction of **17** by lithium aluminum hydride occurred predominantly from the less congested α-face to afford the 20(*R*)-alcohol (**18**) in 80% yield accompanied with the 20(*S*)-alcohol (**10**) (14% yield), which was readily separated by flash column chromatography. The 20(*R*)-alcohol (**18**) was alkylated with the (*S*)-epoxide (**11**) or the (*R*)-epoxide (**12**) to give the alcohol (**19** or **20**), which was then transformed into 20(*R*)-vitamin D_3 analogue (**7** or **8**) by desilylation, irradiation and thermal isomerization.

Finally, 20(*R*)-OCT (**9**) was synthesized as follows. Although the alkylation of the 20(*R*)-alcohol (**18**) was not accomplished with ethyl acrylate, in contrast to the 20(*S*)-alcohol (**10**),[6] *N*,*N*-dimethylacrylamide worked well as a Michael acceptor in the presence of sodium hydride to give the amide (**23**) in 61% yield. Organocerium reagent, prepared from methylmagnesium bromide and cerium(III) chloride at −15 °C,[17,18] reacted with the amide (**23**) to furnish the 24-ketone (**24**) in 19% yield accompanied with the recovered amide (**23**) in 35% yield, the dimethylated product (**25**) in 2.5% yield, and the retro-Michael product (**18**) in 2.9% yield, though the reaction conditions were not optimized. The 24-ketone (**24**) was further methylated with methylmagnesium bromide at 0 °C to afford **25**, in 91% yield, and this was desilylated (68%), irradiated and

Table I. Biological Properties of Various Vitamin D_3 Analogues

	Compound	Differentiation-inducing activity		Binding affinity to receptor	
		ED_{50} (M)	Relative potency	B/B_0(pg)	Relative potency
1		3.76×10^{-9}	1	33.1	1
2		1.16×10^{-9}	3.24	189.7	0.17
5		1.37×10^{-9}	2.75	98.1	0.34
6		9.17×10^{-9}	0.41	2047.1	0.016
7		4.68×10^{-8}	0.08	9842.2	0.003
8		2.40×10^{-8}	0.16	1606.9	0.021
9		1.46×10^{-9}	2.58	408.2	0.08

NII-Electronic Library Service

thermally isomerized to 20(*R*)-OCT (**9**) in 15% yield.

Biological Results The preliminary results of testing of the activity to induce differentiation of HL-60 to macrophages *in vitro* estimated from superoxide anion production,[6] are shown in Table I. 1α,24(*S*)-Dihydroxy-22-oxavitamin D_3 (**5**) showed comparable activity with OCT (**2**), whereas its 24(*R*)-epimer (**6**) was less active than **2**. In the case of MC-903 (**4**) and its 24(*S*)-epimer, similar relationships between the configuration at the C-24 center and the differentiation-inducing activity were also reported.[19] Among the three 20(*R*)-vitamin D_3 analogues (**7**, **8** and **9**), only 20(*R*)-OCT (**9**) exhibited a similar differentiation-inducing activity to OCT (**2**), while **7** and **8** were moderately active. It is suggestive that in a series of 22-oxygenated analogues of vitamin D_3, the differentiation-inducing activity of HL-60 corresponds well to the binding potency to chick embryonic intestinal 1α,25-$(OH)_2$-D_3 receptor[6] (Table I). Further pharmacological properties including calcemic activity are under investigation.

Experimental[20]

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-[2(*S*)-hydroxy-3-methylbutyloxy]pregna-5,7-diene (13). General Procedure for the Synthesis of 13, 14, 19 and 20 A mixture of **10** (561 mg, 1 mmol), *tert*-BuOK (90%, 1.50 g, 12 mmol), dibenzo-18-crown-6 (250 mg) and **11** (860 mg, 10 mmol) in toluene (30 ml) was stirred at 100 °C for 3 h. The mixture was then diluted with toluene, washed with H_2O and saturated NaCl, and dried over $MgSO_4$. The residue after removal of the solvent *in vacuo* was purified by flash column chromatography with *n*-hexane–AcOEt (5 : 1) as the eluant to give the recovered **10** (130 mg, 23% recovery) and crude **13**. Further purification of crude **13** by preparative TLC, developed three times with *n*-hexane–AcOEt (10 : 1), gave analytically pure **13** (220 mg, 44% yield based upon recovery) as a colorless foam. Other alkylated products (**14**, **19** and **20**) were similarly obtained in 48%, 27% and 28% yields, respectively. Spectroscopic data are given in Table II.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20-oxopregna-5,7-diene (17) DMSO (2.2 ml, 31.1 mmol) was added to a stirred solution of trichloromethyl chloroformate (1.5 ml, 8.1 mmol) in CH_2Cl_2 (13 ml) at −65 °C. The reaction mixture was stirred for 10 min and **10** (4.15 g, 7.4 mmol) in CH_2Cl_2 (20 ml) was added dropwise. Stirring was continued for 20 min at the same temperature, then triethylamine (5.2 ml, 37.0 mmol) was added and the mixture was stirred at the same temperature for 10 min and at room temperature for 1 h. The mixture was diluted with CH_2Cl_2, washed with H_2O and saturated NaCl, and dried over $MgSO_4$. The residue after removal of the solvent *in vacuo* was purified by flash column chromatography with *n*-hexane–AcOEt (9 : 1) as the eluant to give **17** (3.54 g, 86%) as colorless needles, mp 137—139 °C (MeOH). IR (KBr): 1700, 1460, 1355, 1250, 1085, 1075 cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (3H, s), 0.08 (3H, s), 0.12 (3H, s), 0.58 (3H, s), 0.88 (9H, s), 0.90 (9H, s), 2.14 (3H, s), 3.71 (1H, br s), 3.92—4.12 (1H, m), 5.28—5.36 (1H, m), 5.59 (1H, d, J = 5.6 Hz). MS *m/z*: 558 (M^+), 370 (100%). UV λ_{max} nm: 293, 282, 271. *Anal.* Calcd for $C_{33}H_{58}O_3Si_2$: C, 70.91; H, 10.38. Found: C, 70.68; H, 10.40.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*R*)-hydroxypregna-5,7-diene (18) A solution of **17** (774 mg, 1.38 mmol) in tetrahydrofuran (THF) (10 ml) was added dropwise to a stirred mixture of $LiAlH_4$ (105 mg, 2.76 mmol) in THF (10 ml) at 0 °C. The mixture was stirred at room temperature for 1 h, then the reaction was quenched with aqueous Et_2O, and the whole was extracted with Et_2O. The extract was washed with H_2O and saturated NaCl, and dried over $MgSO_4$. The residue after removal of the solvent *in vacuo* was purified by flash column chromatography with *n*-hexane–AcOEt (15 : 1) as the eluant to give **10** (111 mg, 14%) and **18** (620 mg, 80%). **18**: Colorless foam softening at 65—73 °C. IR (Nujol): 3320, 1250 (br) cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.70 (3H, s), 0.88 (18H, s), 0.91 (3H, s), 1.16 (3H, d, J = 6.2 Hz), 3.68—3.82 (2H, br), 3.96—4.15 (1H, br), 5.28—5.33 (1H, m), 5.58 (1H, d, J = 6.2 Hz). MS *m/z*: 560 (M^+), 73 (100%). UV λ_{max} nm: 293, 282, 271. *Anal.* Calcd for $C_{33}H_{60}O_3Si_2$: C, 70.65; H, 10.78. Found; C, 70.65; H, 10.72.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*R*)-(2-dimethylaminocarbonylethyloxy)pregna-5,7-diene (23) A mixture of **18** (627 mg, 1.12 mmol), *N,N*-dimethylacrylamide (277 mg, 2.80 mmol) and NaH (60%, 56 mg, 1.40 mmol) in THF (3.5 ml) was stirred at room temperature for 15 h. The reaction was then quenched with H_2O, and the mixture was extracted with Et_2O, washed with H_2O and saturated NaCl, and dried over $MgSO_4$. The residue after removal of the solvent *in vacuo* was purified by flash column chromatography with *n*-hexane–AcOEt (3 : 2) as the eluant to give **23** (499 mg, 61%) as a colorless oil. IR (neat): 1660, 1465, 1250, 1100 cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.62 (3H, s), 0.88 (18H, s), 0.90 (3H, s), 1.09 (3H, d, J = 6.2 Hz), 2.94 (3H, s), 3.03 (3H, s), 3.23—3.40 (1H, m), 3.52—3.72 (2H, m), 3.75—3.90 (1H, m), 3.92—4.14 (1H, m), 5.24—5.31 (1H, m), 5.56 (1H, d, J = 6.2 Hz). UV λ_{max} nm: 293, 282, 271. *Anal.* Calcd for $C_{38}H_{69}NO_4Si_2$: C, 69.14; H, 10.54; N, 2.12. Found: C, 68.84; H, 10.42; N, 2.02.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*R*)-(3-oxobutyloxy)pregna-5,7-diene (24) $CeCl_3 \cdot 7H_2O$ (767 mg, 2.06 mmol) was heated at 250 °C in a dry oven for 1 h, then at 140 °C *in vacuo* (0.5 mmHg) with stirring for 1 h,

TABLE II. Spectral Data for Alkylated Products (**13**, **14**, **19** and **20**)

	IR (neat, cm^{-1})	^{1}H-NMR ($CDCl_3$) δ ppm	MS *m/z*	UV λ_{max} nm	Formula	Analysis (%) Calcd(Found) C	H
13	3600, 3480, 1465, 1370, 1255, 1090	0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.61 (3H, s), 0.86 (3H, s), 0.88 (18H, s), 0.92 (3H, d, J = 6.6 Hz), 0.97 (3H, d, J = 6.6 Hz), 1.19 (3H, d, J = 6.0 Hz), 3.20—3.36 (1H, m), 3.36—3.49 (2H, m), 3.70 (1H, br s), 3.96—4.16 (1H, m), 5.28—5.36 (1H, m), 5.58 (1H, d, J = 6.2 Hz)	646 (M^+) 457 (100%)	293, 282, 271	$C_{38}H_{70}O_4Si_2 \cdot 1/2H_2O$	69.56 (69.81	10.91 11.25)
14	3600, 3480, 1460, 1370, 1250	0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.61 (3H, s), 0.86 (3H, s), 0.88 (18H, s), 0.93 (3H, d, J = 7.0 Hz), 0.98 (3H, d, J = 6.6 Hz), 1.20 (3H, d, J = 6.0 Hz), 3.09 (1H, t, J = 8.8 Hz), 3.22—3.33 (1H, m), 3.36—3.48 (1H, m), 3.67 (1H, dd, J = 3.0, 9.1 Hz), 3.70 (1H, br s), 3.94—4.12 (1H, m), 5.28—5.35 (1H, m), 5.58 (1H, d, J = 6.2 Hz)	646 (M^+) 87 (100%)	293, 282, 271	$C_{38}H_{70}O_4Si_2 \cdot 1/4H_2O$	70.04 (69.97	10.91 11.20)
19	3600, 3500, 1470, 1460, 1370, 1250, 1090	0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.64 (3H, s), 0.87 (18H, s), 0.88 (3H, s), 0.90 (3H, d, J = 6.8 Hz), 0.95 (3H, d, J = 6.8 Hz), 1.10 (3H, d, J = 6.2 Hz), 3.09 (1H, t, J = 8.6 Hz), 3.28—3.39 (1H, m), 3.39—3.52 (1H, m), 3.60—3.72 (2H, m), 3.92—4.12 (1H, m), 5.23—5.34 (1H, m), 5.54 (1H, d, J = 5.6 Hz)	514 (M^+ − ^{t}BuMe$_2$SiOH) 457 (100%)	293, 281, 270	$C_{38}H_{70}O_4Si_2 \cdot 1/2H_2O$	69.56 (69.60	10.91 11.19)
20	3600, 3470, 1460, 1370, 1250, 1090	0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.64 (3H, s), 0.87 (3H, s), 0.88 (18H, s), 0.92 (3H, d, J = 6.8 Hz), 0.98 (3H, d, J = 6.8 Hz), 1.10 (3H, d, J = 6.2 Hz), 3.26—3.40 (2H, m), 3.30—3.56 (2H, m), 3.70 (1H, br s), 3.96—4.14 (1H, m), 5.24—5.33 (1H, m), 5.56 (1H, d, J = 5.6 Hz)	646 (M^+) 301 (100%)	293, 282, 271	$C_{38}H_{70}O_4Si_2 \cdot 1/2H_2O$	69.56 (69.80	10.91 10.85)

NII-Electronic Library Service

and cooled to room temperature. THF (3 ml) was added and stirring was continued at room temperature for 1 h. Methylmagnesium bromide (1 mol in THF, 1.87 ml, 187 mmol) was added at −15 °C and stirring was continued at the same temperature for 30 min. To the resultant suspension, a solution of **23** (412 mg, 0.62 mmol) in THF (4 ml) was added dropwise at −15 °C. The mixture was stirred at −15 °C for 1 h, and at 0 °C for 1 h, then the reaction was quenched by adding saturated NH_4Cl at 0 °C and the whole was extracted with Et_2O. The extract was washed with H_2O and saturated NaCl, and dried over $MgSO_4$. The residue after removal of the solvent *in vacuo* was purified by flash column chromatography with *n*-hexane–AcOEt (3 : 2) as the eluant to give recovered **23** (137 mg, 35%) and a mixture of **24**, **25** and **18**. The mixture was re-purified by preparative

TABLE III. Spectral Data for the Triols (**15**, **16**, **21**, **22** and **26**)

	IR (neat, cm^{-1})	^{1}H-NMR ($CDCl_3$) δ ppm	MS *m/z*	UV λ_{max} nm	$[\alpha]_D$	Formula	Analysis (%) Calcd (Found) C	H
15	3370, 1095, 1055	0.61 (3H, s), 0.90 (3H, d, J=7.0 Hz), 0.94 (3H, s), 0.97 (3H, d, J=6.8 Hz), 1.19 (3H, d, J=6.2 Hz), 3.24—3.36 (2H, m), 3.36—3.48 (2H, m), 3.75 (1H, br s), 3.96—4.13 (1H, m), 5.34—5.42 (1H, m), 5.72 (1H, br d, J=3.8 Hz)	418 (M^+) 87 (100%)	293, 282, 271	−35.60 (c=0.5, EtOH, 24 °C)	$C_{26}H_{42}O_4 \cdot 1/4H_2O$	73.81 (73.65	10.13 10.13)
16	3400, 1460, 1370, 1090, 1050	0.62 (3H, s), 0.91 (3H, d, J=7.0 Hz), 0.93 (3H, s), 0.97 (3H, d, J=6.6 Hz), 1.20 (3H, d, J=6.0 Hz), 3.10 (1H, t, J=8.8 Hz), 3.24—3.34 (1H, m), 3.36—3.48 (1H, m), 3.68 (1H, dd, J=3.0, 9.1 Hz), 3.75 (1H, br s), 3.96—4.13 (1H, m), 5.34—5.42 (1H, m), 5.72 (1H, br d, J=4.0 Hz)	418 (M^+) 87 (100%)	293, 282, 271	−21.19 (c=0.5, EtOH, 24 °C)	$C_{26}H_{42}O_4 \cdot 5/4H_2O$	70.79 (70.82	10.17 9.89)
21	3400, 1460, 1370, 1050	0.66 (3H, s), 0.91 (3H, d, J=6.8 Hz), 0.93 (3H, s), 0.98 (3H, d, J=6.8 Hz), 1.11 (3H, d, J=6.2 Hz), 3.14 (1H, t, J=8.6 Hz), 3.28—3.38 (1H, m), 3.38—3.50 (1H, m), 3.63 (1H, dd, J=3.0, 9.0 Hz), 3.7 (1H, br s), 3.96—4.15 (1H, m), 5.28—5.38 (1H, m), 5.70 (1H, br d, J=4.0 Hz)	418 (M^+) 87 (100%)	293, 282, 271	−65.77 (c=0.675, EtOH, 23 °C)	$C_{26}H_{42}O_4 \cdot 2H_2O$	68.69 (68.92	10.20 10.03)
22	3400, 1465, 1370, 1100, 1050	0.65 (3H, s), 0.90 (3H, d, J=6.8 Hz), 0.95 (3H, s), 0.98 (3H, d, J=6.8 Hz), 1.09 (3H, d, J=6.2 Hz), 3.24—3.40 (2H, m), 3.40—3.50 (2H, m), 3.75 (1H, br s), 3.96—4.13 (1H, m), 5.29—5.38 (1H, m), 5.70 (1H, br d, J=5.7 Hz)	418 (M^+) 73 (100%)	293, 282, 271	−72.14 (c=2.19, EtOH, 23 °C)	$C_{26}H_{42}O_4 \cdot 7/4H_2O$	69.38 (69.17	10.19 10.33)
26	3400, 1460, 1370, 1265, 1150	0.64 (3H, s), 0.93 (3H, s), 1.14 (3H, d, J=5.8 Hz), 1.24 (6H, s), 3.20—3.36 (1H, m), 3.40—3.52 (1H, m), 3.73 (1H, br s), 3.78—3.92 (1H, m), 3.96—4.12 (1H, m), 5.28—5.38 (1H, m), 5.67 (1H, d, J=6.2 Hz)	418 (M^+) 69 (100%)	293, 281, 270		$C_{26}H_{42}O_4 \cdot 1/2H_2O$	73.03 (73.35	10.14 10.39)

TABLE IV. Spectral Data for Vitamin D_3 Analogues (**5**, **6**, **7**, **8** and **9**)

	IR (neat, cm^{-1})	^{1}H-NMR ($CDCl_3$) δ ppm	MS *m/z*	UV (nm) λ_{max}	λ_{min}	$[\alpha]_D$	Formula	HRMS Calcd (Found)
5	3390, 1470, 1450, 1370, 1050	0.53 (3H, s), 0.90 (3H, d, J=6.8 Hz), 0.97 (3H, d, J=6.6 Hz), 1.17 (3H, d, J=6.2 Hz), 3.22—3.36 (2H, m), 3.36—3.49 (2H, m), 4.16—4.28 (1H, br), 4.37—4.48 (1H, br), 5.00 (1H, s), 5.34 (1H, s), 6.02 (1H, d, J=11.4 Hz), 6.37 (1H, d, J=11.4 Hz)	418 (M^+) 87 (100%)	263	227	56.00 (c=0.1, EtOH, 24 °C)	$C_{26}H_{42}O_4$	418.3083 (418.3078)
6	3390, 1470, 1450, 1375, 1055	0.53 (3H, s), 0.90 (3H, d, J=6.8 Hz), 0.97 (3H, d, J=6.8 Hz), 1.18 (3H, d, J=6.0 Hz), 3.08 (1H, t, J=8.8 Hz), 3.20—3.32 (1H, m), 3.36—3.48 (1H, m), 3.67 (1H, dd, J=3.0, 9.1 Hz), 4.17—4.28 (1H, br), 4.37—4.48 (1H, br), 4.99 (1H, s), 5.33 (1H, s), 6.02 (1H, d, J=11.4 Hz), 6.37 (1H, d, J=11.4 Hz)	418 (M^+) 87 (100%)	263	227	46.00 (c=0.1, EtOH, 24 °C)	$C_{26}H_{42}O_4$	418.3083 (418.3080)
7	3390, 1450, 1370, 1055	0.57 (3H, s), 0.90 (3H, d, J=6.8 Hz), 0.97 (3H, d, J=6.8 Hz), 1.10 (3H, d, J=6.2 Hz), 3.10 (1H, t, J=8.6 Hz), 3.24—3.35 (1H, m), 3.35—3.48 (1H, m), 3.61 (1H, dd, J=3.0, 9.1 Hz), 4.15—4.27(1H, br), 4.26—4.34 (1H, br), 4.97 (1H, s), 5.30 (1H, s), 5.97 (1H, d, J=11.4 Hz), 6.36 (1H, d, J=11.4 Hz)	418 (M^+), 87 (100%)	263	227	−24.24 (c=0.165, EtOH, 23 °C)	$C_{26}H_{42}O_4$	418.3083 (418.3124)
8	3400, 1450, 1370, 1110, 1060	0.57 (3H, s), 0.91 (3H, d, J=6.8 Hz), 0.98 (3H, d, J=6.8 Hz), 1.10 (3H, d, J=6.2 Hz), 3.23—3.40 (2H, m), 3.40—3.53 (2H, m), 4.16—4.28 (1H,br), 4.37—4.48 (1H, br), 4.99 (1H, s), 5.31 (1H, s), 5.99 (1H, d, J=11.4 Hz), 6.38 (1H, d, J=11.4 Hz)	418 (M^+) 87 (100%)	263	227	−30.68 (c=0.365, EtOH, 23 °C)	$C_{26}H_{42}O_4$	418.3083 (418.3080)
9	3390, 1455, 1370, 1060	0.55 (3H, s), 1.13 (3H, d, J=6.0 Hz), 1.23 (6H, s), 3.16—3.32 (1H, m), 3.36—3.58 (1H, m), 3.70—3.90 (1H, m), 4.13—4.27 (1H, br), 4.34—4.48 (1H, br), 4.99 (1H, s), 5.30 (1H, s), 5.99 (1H, d, J=11.6 Hz), 6.38 (1H, d, J=11.6 Hz)	418 (M^+) 69 (100%)	263	227	−66.00 (c=0.1, EtOH, 24 °C)	$C_{26}H_{42}O_4$	418.3083 (418.3058)

NII-Electronic Library Service

TLC, developed twice with *n*-hexane–AcOEt (5 : 1), to give **25** (10 mg, 2.5%), **18** (10 mg, 2.9%) and **24** (74 mg, 19%). **24**: Colorless foam. IR (neat): 1710, 1465, 1370, 1360, 1250, 1100 (br) cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.62 (3H, s), 0.88 (18H, s), 0.91 (3H, s), 1.09 (3H, d, J=6.0 Hz), 2.18 (3H, s), 2.68 (2H, t, J=6.3 Hz), 3.20—3.37 (1H, m), 3.44—3.58 (1H, m), 3.69 (1H, br s), 3.72—3.88 (1H, m), 3.93—4.16 (1H, m), 5.24—5.32 (1H, m), 5.57 (1H, d, J=5.7 Hz). MS *m/z*: 630 (M^+), 73 (100%). UV λ_{max} nm: 293, 282, 271. *Anal.* Calcd for $C_{37}H_{60}O_4Si_2 \cdot 1/4H_2O$: C, 69.92; H, 10.55. Found: C, 69.74; H, 10.95.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*R*)-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (25) Methylmagnesium bromide (1 mol/l in THF, 0.55 ml, 0.55 mmol) was added dropwise to a stirred solution of **24** (70 mg, 0.11 mmol) in THF (6 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h, then the reaction was quenched by adding saturated NH_4Cl at 0 °C and the whole was extracted with Et_2O. The extract was washed with H_2O and saturated NaCl, and dried over $MgSO_4$. The residue after removal of the solvent *in vacuo* was purified by flash column chromatography with *n*-hexane–AcOEt (7 : 1) as the eluant to give **25** (66 mg, 91%) as a colorless foam. IR (neat): 3500, 1460, 1370, 1250, 1090 cm^{-1}. NMR δ: 0.05 (3H, s), 0.06 (6H, s), 0.10 (3H, s), 0.63 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 0.91 (3H, s), 1.14 (3H, d, J=5.8 Hz), 1.24 (3H, s), 1.25 (3H, s), 3.18—3.32 (1H, m), 3.36—3.52 (1H, m), 3.66 (1H, br s), 3.73—3.90 (1H, m), 3.95—4.13 (1H, m), 5.24—5.32 (1H, m), 5.57 (1H, d, J=6.2 Hz). MS *m/z*: 646 (M^+), 113 (100%). UV λ_{max} nm: 293, 281, 270. *Anal.* Calcd for $C_{38}H_{70}O_4Si_2 \cdot 1/4H_2O$: C, 70.04; H, 10.91. Found: C, 69.92; H, 11.29.

1α,3β-Dihydroxy-20(*R*)-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (26). General Procedure for Desilylation of 13, 14, 19 and 20 A solution of **25** (71.8 mg, 0.11 mmol) and *n*-Bu_4NF (1 mol/l in THF, 1.1 ml, 1.1 mmol) in THF (1.1 ml) was refluxed gently for 14 h. The mixture was then diluted with AcOEt, washed with H_2O, 10% HCl, saturated $NaHCO_3$ and saturated NaCl, and dried over $MgSO_4$. The residue after removal of the solvent *in vacuo* was purified by flash column chromatography with CH_2Cl_2–EtOH (10 : 1) as the eluant to give **26** (31.6 mg, 68%) as a colorless foam. Other triols (**15**, **16**, **21**, **22**) were similarly obtained in 76%, 57%, 63% and 93% yields, respectively. Spectroscopic data are given in Table III.

1α,3β-Dihydroxy-20(*R*)-(3-hydroxy-3-methylbutyloxy)-9,10-secopregna-5,7,10(19)-triene (9). General Procedure for Irradiation and Thermal Isomerization of 15, 16, 21 and 22 A solution of **26** (27.4 mg, 0.07 mmol) in EtOH (200 ml) was irradiated using a 400 W high-pressure mercury lamp with a Vycor filter at 0 °C for 3 min. The solution was then refluxed gently for 2 h and concentrated *in vacuo* to leave an oil. The crude product was purified by preparative TLC developed with CH_2Cl_2–EtOH (10 : 1) to give **9** (4.6 mg, 15%) as a colorless foam. Other vitamin D_3 analogues (**5**, **6**, **7**, **8**) were similarly obtained in 13%, 14%, 17% and 15% yields, respectively. Spectroscopic data are given in Table IV.

Acknowledgment We are grateful to Dr. Masatomo Hamana, Professor Emeritus of Kyushu University, for his encouragement and useful discussions.

References and Notes

1) Part XVI: N. Kubodera, H. Watanabe, K. Miyamoto, M. Matsumoto, *BioMed. Chem. Lett.*, in press.
2) A part of this work has appeared as a preliminary communication (ref. 1).
3) N. Ikekawa, Y. Fujimoto, *Yuki Gosei Kagaku Kyokai Shi*, **46**, 455 (1988).
4) V. K. Osterm, H. F. DeLuca, *Steroids*, **49**, 73 (1987).
5) E. Murayama, K. Miyamoto, N. Kubodera, T. Mori, I. Matsunaga, *Chem. Pharm. Bull.*, **34**, 4410 (1986).
6) N. Kubodera, H. Watanabe, T. Kawanishi, M. Matsumoto, *Chem. Pharm. Bull.*, **40**, 1494 (1992).
7) J. Abe, M. Morikawa, K. Miyamoto, S. Kaiho, M. Fukushima, C. Miyaura, E. Abe, T. Suda, Y. Nishii, *FEBS Lett.*, **226**, 58 (1987).
8) A. J. Brown, C. R. Ritter, J. L. Finch, J. Morrissey, K. J. Martin, E. Murayama, Y. Nishii, E. Slatopolsky, *J. Clin. Invest.*, **84**, 728 (1989).
9) H. Tanaka, E. Abe, C. Miyaura, R. Kuribayashi, K. Konno, Y. Nishii, T. Suda, *Biochem. J.*, **204**, 713 (1982).
10) M. Yoshida, S. Ishizuka, A. Hoshi, *J. Pharmacobio-Dyn.*, **7**, 962 (1984).
11) L. Binderup, E. Bramm, *Biochem. Pharmacol.*, **37**, 889 (1988).
12) M. J. Calverley, K. Hansen, L. Binderup, PCT Int. Appl., WO 90/09991 (1990) [*Chem. Abstr.*, **114**, 164629*k* (1991)].
13) B. Koppenhoefer, V. Schurig, *Org. Syn.*, **66**, 160 (1987).
14) L. Binderup, S. Latini, E. Binderup, C. Bretting, M. Calverley, K. Hansen, *Biochem. Pharmacol.*, **42**, 1569 (1991).
15) L. Binderup, S. Latini, A. M. Kissmeyer, Proceedings of the 8th Workshop on Vitamin D, Paris, July 1991, p. 478.
16) S. Takano, K. Inomata, S. Tomita, M. Yanase, K. Samizu, K. Ogasawara, *Tetrahedron Lett.*, **29**, 6619 (1988).
17) T. Imamoto, Y. Sugiura, N. Takiyama, *Tetrahedron Lett.*, **25**, 4233 (1984).
18) T. Mikami, M. Kato, N. Kubodera, K. Ochi, Abstract of the 112th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, March 1992, pp. 2—62.
19) M. J. Calverley, *Tetrahedron*, **43**, 4609 (1987).
20) General methods are described in the experimental section of ref. 6.

NII-Electronic Library Service