Synthetic Studies of Vitamin D Analogues. XVII.¹⁾ Synthesis and Differentiation-Inducing Activity of 1 α ,24-Dihydroxy-22-oxavitamin D₃ Analogues and Their 20(R)-Epimers²⁾

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Four vitamin D₃ analogues, 1 α ,24(S)- and 1 α ,24(R)-dihydroxy-22-oxavitamin D₃ (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 1 α ,25-dihydroxy-22-oxavitamin D₃ (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 1 α ,25-dihydroxyvitamin D₃ (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 D₃ analogue; 1 α ,25-dihydroxyvitamin D₃; 1 α ,25-dihydroxy-22-oxavitamin D₃; 1 α ,24(S)-dihydroxy-22-oxavitamin D₃; differentiation-inducing activity; 20(R)-epimer

In recent years, considerable attention has been focused on the synthesis of analogues of 1 α ,25-dihydroxyvitamin D₃ [1 α ,25-(OH)₂-D₃] (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 1 α ,25-dihydroxy-22-oxavitamin D₃ (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 1 α ,24(R)-dihydroxyvitamin D₃ (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 1 α ,25-(OH)₂-D₃ (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 1 α ,24(S)- and 1 α ,24(R)-dihydroxy-22-oxavitamin D₃ (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 1 α ,24(S)- and 1 α ,24(R)-dihydroxyvitamin D₃ (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 D₃ analogues (7, 8 and 9), since biological comparison of vitamin D₃ 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

Chart 1

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 D₃ 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 D₃ 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

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. 1 α ,24(*S*)-Dihydroxy-22-oxa-vitamin D₃ (**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 D₃ 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 D₃, the differentiation-inducing activity of HL-60 corresponds well to the binding potency to chick embryonic intestinal 1 α ,25-(OH)₂-D₃ receptor⁶⁾ (Table I). Further pharmacological properties including calcemic activity are under investigation.

Experimental²⁰⁾

1 α ,3 β -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 H₂O and saturated NaCl, and dried over MgSO₄. 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.

1 α ,3 β -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 CH₂Cl₂ (13 ml) at -65 °C. The reaction mixture was stirred for 10 min and **10** (4.15 g, 7.4 mmol) in CH₂Cl₂ (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 CH₂Cl₂, washed with H₂O and saturated NaCl, and dried over MgSO₄. 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 $\lambda_{\max}$ nm: 293, 282, 271. Anal. Calcd for C₃₃H₅₈O₃Si₂: C, 70.91; H, 10.38. Found: C, 70.68; H, 10.40.

1 α ,3 β -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 LiAlH₄ (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 Et₂O, and the whole was extracted with Et₂O. The extract was washed with H₂O and saturated NaCl, and dried over MgSO₄. 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 $\lambda_{\max}$ nm: 293, 282, 271. Anal. Calcd for C₃₃H₆₀O₃Si₂: C, 70.65; H, 10.78. Found: C, 70.65; H, 10.72.

1 α ,3 β -Bis(*tert*-butyldimethylsilyloxy)-20(*R*)-(2-dimethylaminocarbonyl-ethyloxy)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 H₂O, and the mixture was extracted with Et₂O, washed with H₂O and saturated NaCl, and dried over MgSO₄. 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 $\lambda_{\max}$ nm: 293, 282, 271. Anal. Calcd for C₃₈H₆₉NO₄Si₂: C, 69.14; H, 10.54; N, 2.12. Found: C, 68.84; H, 10.42; N, 2.02.

1 α ,3 β -Bis(*tert*-butyldimethylsilyloxy)-20(*R*)-(3-oxobutyloxy)pregna-5,7-diene (24**)** CeCl₃·7H₂O (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 <i>m/z</i>	UV $\lambda_{\max}$ 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, <i>J</i> = 6.6 Hz), 0.97 (3H, d, <i>J</i> = 6.6 Hz), 1.19 (3H, d, <i>J</i> = 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, <i>J</i> = 6.2 Hz)	646 (M ⁺) 457 (100%)	293, 282, 271	C ₃₈ H ₇₀ O ₄ Si ₂ ·1/2H ₂ O	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, <i>J</i> = 7.0 Hz), 0.98 (3H, d, <i>J</i> = 6.6 Hz), 1.20 (3H, d, <i>J</i> = 6.0 Hz), 3.09 (1H, t, <i>J</i> = 8.8 Hz), 3.22–3.33 (1H, m), 3.36–3.48 (1H, m), 3.67 (1H, dd, <i>J</i> = 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, <i>J</i> = 6.2 Hz)	646 (M ⁺) 87 (100%)	293, 282, 271	C ₃₈ H ₇₀ O ₄ Si ₂ ·1/4H ₂ O	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, <i>J</i> = 6.8 Hz), 0.95 (3H, d, <i>J</i> = 6.8 Hz), 1.10 (3H, d, <i>J</i> = 6.2 Hz), 3.09 (1H, t, <i>J</i> = 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, <i>J</i> = 5.6 Hz)	514 (M ⁺ – tBuMe ₂ SiOH) 457 (100%)	293, 281, 270	C ₃₈ H ₇₀ O ₄ Si ₂ ·1/2H ₂ O	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, <i>J</i> = 6.8 Hz), 0.98 (3H, d, <i>J</i> = 6.8 Hz), 1.10 (3H, d, <i>J</i> = 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, <i>J</i> = 5.6 Hz)	646 (M ⁺) 301 (100%)	293, 282, 271	C ₃₈ H ₇₀ O ₄ Si ₂ ·1/2H ₂ O	69.56 (69.80)	10.91 (10.85)

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})	$^1\text{H-NMR}$ (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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$ $\cdot 1/4\text{H}_2\text{O}$	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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$ $\cdot 5/4\text{H}_2\text{O}$	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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$ $\cdot 2\text{H}_2\text{O}$	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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$ $\cdot 7/4\text{H}_2\text{O}$	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		$\text{C}_{26}\text{H}_{42}\text{O}_4$ $\cdot 1/2\text{H}_2\text{O}$	73.03 (73.35)	10.14 (10.39)

TABLE IV. Spectral Data for Vitamin D₃ Analogues (**5**, **6**, **7**, **8** and **9**)

	IR (neat, cm^{-1})	$^1\text{H-NMR}$ (CDCl_3) δ ppm	MS m/z	UV (nm)		$[\alpha]_D$	Formula	HRMS
				λ_{max}	λ_{min}			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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$	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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$	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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$	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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$	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)	$\text{C}_{26}\text{H}_{42}\text{O}_4$	418.3083 (418.3058)

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 $\text{C}_{37}\text{H}_{60}\text{O}_4\text{Si}_2 \cdot 1/4\text{H}_2\text{O}$: C, 69.92; H, 10.55. Found: C, 69.74; H, 10.95.

1 α ,3 β -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 $\text{C}_{38}\text{H}_{70}\text{O}_4\text{Si}_2 \cdot 1/4\text{H}_2\text{O}$: C, 70.04; H, 10.91. Found: C, 69.92; H, 11.29.

1 α ,3 β -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*-Bu₄NF (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.

1 α ,3 β -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 D₃ analogues (**5**, **6**, **7**, **8**) were similarly obtained in 13%, 14%, 17% and 15% yields, respectively. Spectroscopic data are given in Table IV.

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References and Notes

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