Synthetic Studies of Vitamin D Analogs. XXII.[1] Synthesis and Antiproliferation Activity of Putative Metabolites of 1α,25-Dihydroxy-22-oxavitamin D_3[2]

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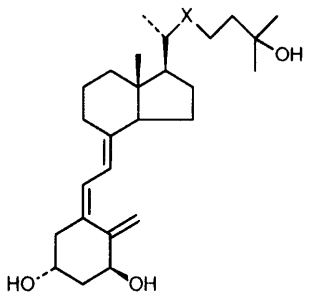
As putative metabolites of 1α,25-dihydroxy-22-oxavitamin D_3 (OCT), 24-hydroxylated OCT in 24(*R*) and 24(*S*) forms, 24-ketoOCT, 26-hydroxylated OCT in 25(*S*) and 25(*R*) forms, pentanorOCT and pentanor-ketoOCT were synthesized from the steroidal 20(*S*)-alcohol. Their antiproliferation activities towards human promyelocytic leukemia cells (HL-60 cells) are also reported. Oxidized derivatives at the C-24 position, 24-ketoOCT, 24(*R*)-hydroxylated OCT and 24(*S*)-hydroxylated OCT, showed activities comparable to or slightly weaker than that of OCT, while 26-hydroxylated OCT was less active than OCT. Truncated OCT, pentanor OCT and pentanor-ketoOCT, were inactive at 10^{-7}—10^{-10} M.

Key words vitamin D_3 analog; 1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxy-22-oxavitamin D_3; OCT; metabolite; antiproliferation activity

1α,25-Dihydroxy-22-oxavitamin D_3 (OCT, **1**), the 22-oxygenated analog of 1α,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$, **2**], has potent *in vitro* differentiation-induction and antiproliferation activities with low *in vivo* calcemic liability.[4–6] Compound **1** is being clinically investigated as a candidate for antihyperparathyroidism injection[7] and antipsoriatic ointment.[8]

During the course of our development of **1**, it was necessary to synthesize possible metabolites of **1** for pharmacokinetic and metabolic studies. It is well known that the active vitamin D_3, **2**, is hydroxylated at C-23, C-24 or C-26 as the first step in its metabolism. The hydroxylated metabolites of **2** are further oxidized to keto-alcohol, lactone (calcitriol lactone) or carboxylic acid (calcitroic acid).[9–13] On the assumption that oxidation pathway of **1** to **2** would be similar, we undertook the synthesis of OCT derivatives oxidized at C-23, C-24 or C-26 as putative metabolites of **1**. In this paper we wish to describe the synthesis of 24-hydroxylated OCT (**3** and **4**) in 24(*R*) and 24(*S*) forms,[14] 24-ketoOCT (**5**),[15,16] 26-hydroxylated OCT (**6** and **7**) in 25(*S*) and 25(*R*) forms and truncated OCT, pentanorOCT (**8**) and pentanor-ketoOCT (**9**), which might be derived from the 23-hydroxylated hemiacetal (**10**). Their antiproliferation activities towards human promyelocytic leukemia cells (HL-60 cells), measured in a preliminary biological evaluation of these compound, are also reported.



1 X = O: OCT
2 X = CH_2: 1,25(OH)$_2$D$_3$

Chart 1

The common starting material for the synthesis of each oxidized OCT was the 20(*S*)-alcohol (**11**), which was prepared from dehydroepiandrosterone *via* microbiological 1α-hydroxylation as described previously.[4] First, we synthesized **3** and **4**. The 20(*S*)-alcohol (**11**) was alkylated with the (*R*)-epoxide (**12a**) and the (*S*)-epoxide (**12b**) prepared from D-mannitol and L-serine,[17] in the presence

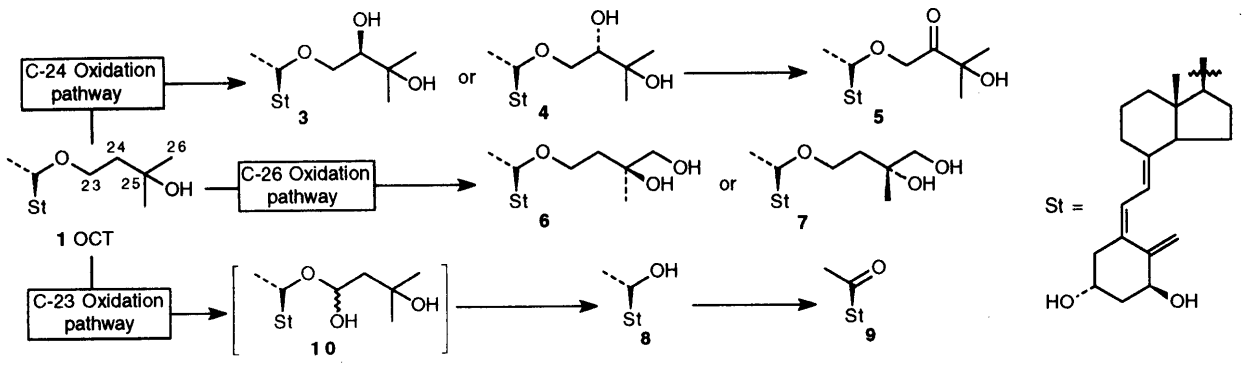


Chart 2

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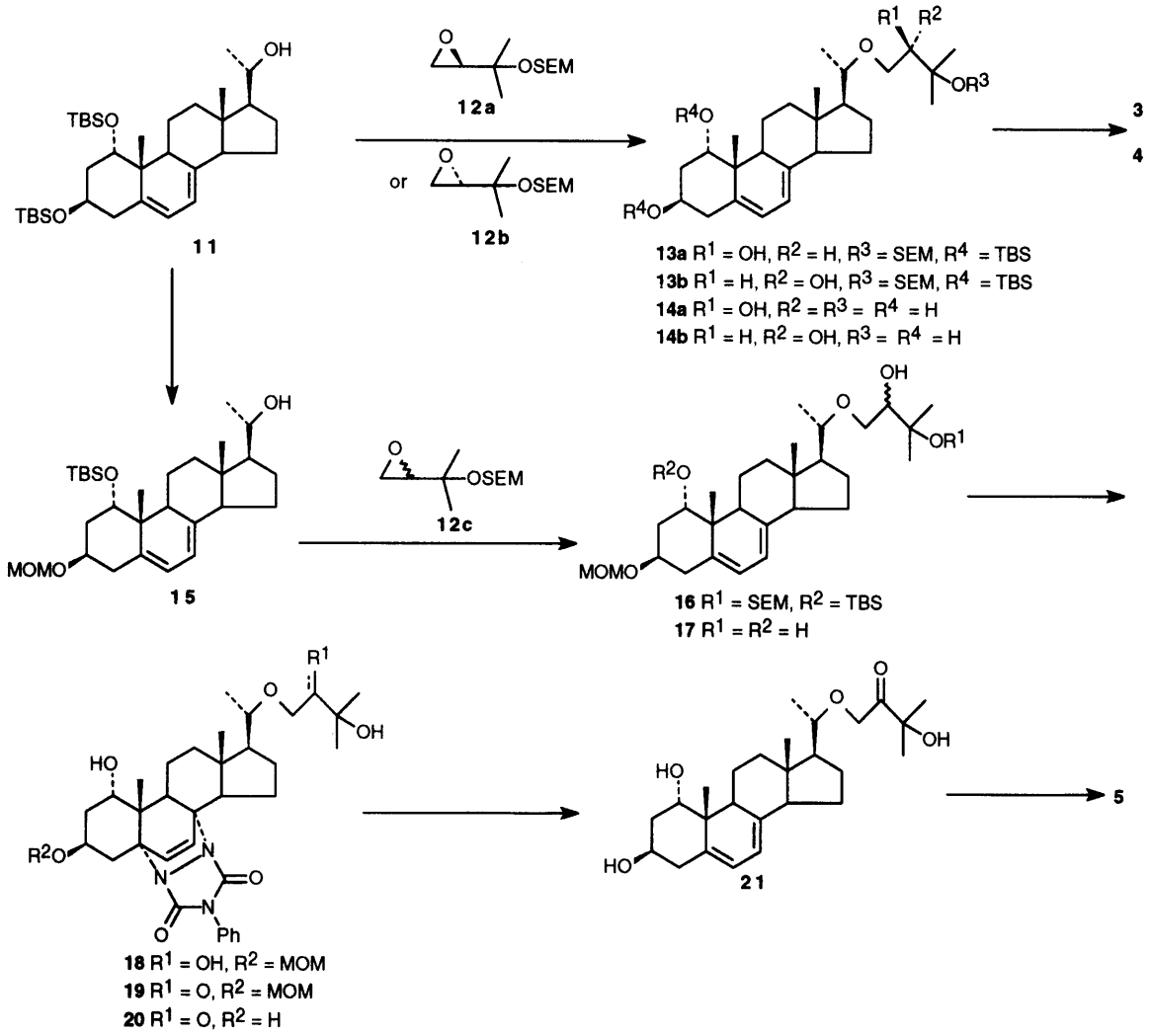
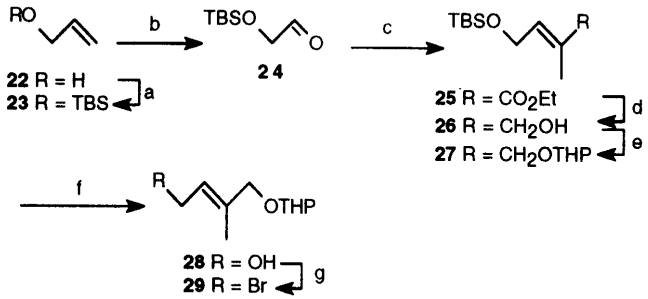


Chart 3

of dibenzo-18-crown-6 and potassium *tert*-butoxide to give the alcohol (**13a** or **13b**) in 24% and 10% yields, respectively. The alcohols (**13a** and **13b**) were then separately deprotected with tetrabutylammonium fluoride (TBAF) in *N*,*N*′-dimethylpropyleneurea (DMPU) at 80 °C to give the tetraols (**14a** and **14b**) in 77% and 70% yields, respectively. Subsequent irradiation of **14a** and **14b** in ethanol at 0 °C using a high-pressure mercury lamp through a Vycor filter followed by thermal isomerization under reflux in ethanol gave rise to 24(*R*)-hydroxylated OCT (**3**) and 24(*S*)-hydroxylated OCT (**4**) in 17% and 13% yields, respectively. For the synthesis of **5**, the *tert*-butyldimethylsilyl (TBS) group used for protection of the 3-hydroxy part in **11** was changed to a methoxymethyl (MOM) group in **15** after extensive examination of deprotection conditions. Thus, the alcohol (**15**) was alkylated with the racemic epoxide (**12c**)[18] to give the ether (**16**) in 73% yield. The silyl ether moiety in **16** was cleaved by TBAF to afford the triol (**17**). To protect allylic positions of the 5,7-diene in **17** from the next oxidation step, **17** was converted to the 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) adduct (**18**). The Swern oxidation of **18** gave the 24-ketone (**19**) in 44% yield, and this was converted to the 5,7-diene (**21**) by retro Diels–Alder reaction[19] (65%) of the triol (**20**) obtained by the deprotection (68%) of **19**.



Reagents: a, TBSCl, Et_3N; b, i) O_3, ii) Ph_3P; c, $Ph_3PC(Me)CO_2Et$; d, DIBAH; e, dihydropyran; f. TBAF; g. Ph_3P, CBr_4

Chart 4

The 5,7-diene (**21**) was then transformed to **5** by irradiation and thermal isomerization.

Next, the synthesis of **6** and **7** was examined. After many fruitless attempts, we focused our attention on a route using the Katsuki–Sharpless epoxidation.[20] Thus, the bromide (**29**) was prepared from the allyl alcohol (**22**) as follows: a) silylation giving **23**, b) ozonolysis giving **24**, c) Horner–Emmons reaction giving **25**, d) reduction giving **26**, e) tetrahydropyranyl (THP) ether formation

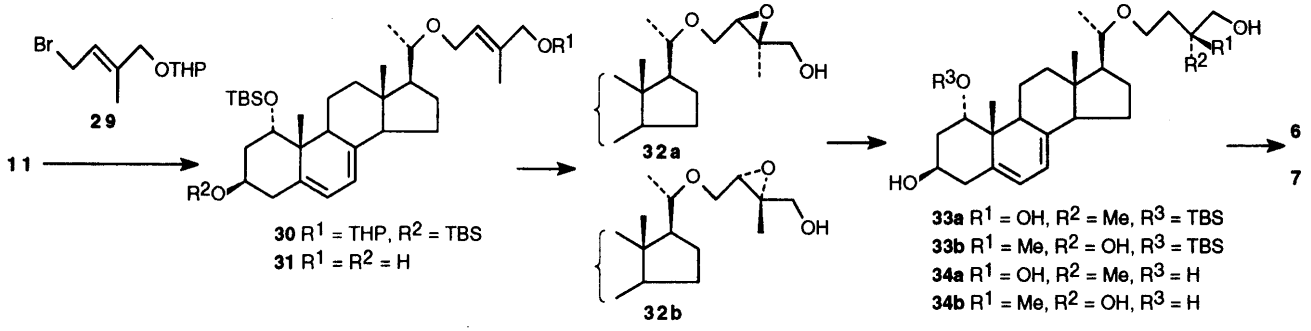


Chart 5

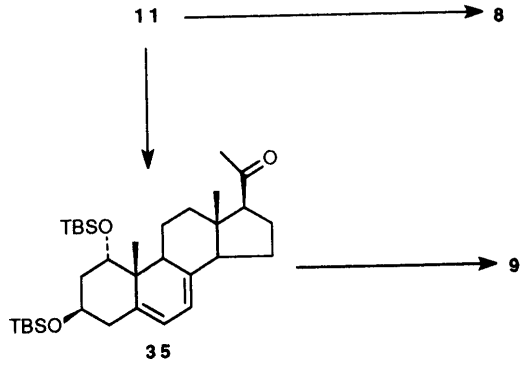


Chart 6

Table 1. Biological Properties of OCT and its Putative Metabolites

Compd.	VDR binding affinity		Anti proliferation activity	
	B/B_{50} (pg/tube)	Relative to OCT	ED_{50} ($\times 10^{-10}$ mol dm^{-3})	Relative to OCT
1 (OCT)	21	1	4.6	1
3	72	1/3	7.0	1/2
4	470	1/22	14	1/3
5	42	1/2	5.0	1
6	640	1/30	30	1/7
7	530	1/25	39	1/9
8	19500	1/929	>1000	<1/217
9	7650	1/364	>1000	<1/217

giving **27**, f) desilylation giving **28** and g) bromination giving **29**.

The reaction between **11** and **29** in the presence of potassium hydride resulted in the formation of the ether (**30**) in 94% yield based upon the recovery of **11**. The THP moiety in **30** was cleaved using pyridinium *p*-toluenesulfonate (PPTS) in methanol to afford the allyl alcohol (**31**) with concomitant desilylation at the C-3 position in 82% yield. The subsequent Katsuki–Sharpless epoxidation[20] of **31** with *tert*-butylhydroperoxide in the presence of (−) or (+)-diisopropyl tartrate and titanium (IV) tetraisopropoxide provided the epoxide (**32a** or **32b**) in 89% and 86% yields, respectively.[21] Regioselective cleavage of the epoxy-rings in **32a** and **32b** with diisobutylaluminum hydride (DIBAH) was achieved at the less congested C-24 position to give the triols (**33a** and **33b**). Both **33a** and **33b** were then desilylated to the tetraols (**34a** and **34b**) in 86% and 77% yields from the epoxides (**32a** and **32b**), which were irradiated and thermally isomerized to **6** and **7** with 25(*S*) and 25(*R*)-configurations in 18% and 19% yields, respectively.

The last targets were truncated OCT, pentanorOCT (**8**) and pentanor-ketoOCT (**9**), which might be formed metabolically through C-23 oxidation *via* the hemiacetal (**10**). Thus, **11** was irradiated, thermally isomerized and desilylated to give **8** in 25% yield. Finally, the ketone (**35**), obtained from **11** by the Swern oxidation in 86% yield,[22] was converted to **9** by irradiation, thermal isomerization and desilylation as described previously.[23]

In a preliminary biological evaluation of the putative metabolites of **1**, the antiproliferation activity towards HL-60 cells[24] was examined (Table 1). The OCT derivatives oxidized at the C-24 position, **5**, **3** and **4**, showed activities comparable to or slightly weaker than that of **1**, while **6** and **7** were less active than **1**. On the other hand, truncated OCT derivatives, **8** and **9**, were inactive at 10^{-7}—10^{-10} M. Relative antiproliferation activity of each derivative is well correlated with the affinity for bovine thymus vitamin D receptor (VDR), as also shown in Table 1. In metabolic experiments, **8** has been identified as the main metabolite of **1**,[25] together with minor oxidized metabolites such as **3**, **4**, **5**, **6** and **7**, whose structures were confirmed by direct comparison with the authentic samples synthesized in these experiments. The results imply that metabolism of **1** might be a deactivation pathway as regards its biological response. The metabolism of **1** will be discussed in detail elsewhere.[26]

Experimental

General Methods Optical rotations were measured with JASCO DIP-370 and Horiba SEPA-200 polarimeters. Ultraviolet (UV) spectra were recorded with a Shimadzu UV-240 in EtOH. Infrared (IR) spectra were obtained using Hitachi 270-30 and JASCO IR-700 spectrometers. ^{1}H-Nuclear magnetic resonance (NMR) spectra were recorded on JEOL JNM-FX-90A, JEOL FX-200, JEOL JNM-GX-500 and Hitachi R-3000 spectrometers in $CDCl_3$ with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. Mass spectra (MS) were obtained on Shimadzu GCMS-QP 1000 and JEOL JMS-DX303. High-resolution mass spectra (HRMS) were obtained using VG Auto Spec Q and JEOL JMX-AX500 instruments. All air-sensitive reactions were carried out under an atmosphere of dry argon or nitrogen. Preparative TLC was performed on 20 × 20 cm plates coated with 0.5 mm thickness of Merck Kieselgel 60 containing F_{254} indicator. The phrase "residue upon work-up" refers to the residue obtained when the organic layer was separated and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure.

(20*S*,24*R*)-1α,3β-Bis(*tert*-butyldimethylsilyloxy)-24-hydroxy-25-[2-(trimethylsilyl)ethoxymethyloxy]-22-oxacholesta-5,7-diene (13a) A mixture of the 20(*S*)-alcohol (**11**) (874 mg, 1.56 mmol), *tert*-BuOK (90%; 2.13 g, 17.1 mmol), dibenzo-18-crown-6 (405 mg, 1.12 mmol) and **12a**

(809 mg, 3.48 mmol) in toluene (51 ml) was stirred at 80 °C for 3.5 h, then poured into H_2O and extracted with AcOEt. The extract was washed with saturated NaCl. The residue upon work-up was submitted to 2-stage purification, 1) flash column chromatography with hexane–AcOEt (10 : 1) as the eluent, and 2) preparative TLC developed three times with hexane–AcOEt (10 : 1), to give **13a** (297 mg, 24%) as a pale yellow oil. UV λ_{max} nm: 270, 281, 293. IR (neat): 3520, 2960, 1100 cm^{-1}. NMR δ: 0.02 (9H, s, 3 × $SiCH_3$), 0.05 (3H, s, $SiCH_3$), 0.06 (6H, s, 2 × $SiCH_3$), 0.11 (3H, s, $SiCH_3$), 0.61 (3H, s, 18-CH_3), 0.88 (21H, s, 2 × $SiC(CH_3)_3$, 19-CH_3), 0.90 (2H, t, J=8.5, $SiCH_2$), 1.20 (3H, d, J=6.1, 21-CH_3), 1.25 (6H, s, 26-CH_3, 27-CH_3), 2.97—3.00 (1H, m), 3.24—3.30 (1H, m), 3.37—3.68 (4H, m), 3.63 (2H, t, J=8.5, $SiCH_2C\underline{H}_2O$), 3.94—4.08 (1H, m, 3-CH), 4.78 (2H, s, OCH_2O), 5.28—5.33 (1H, m, 7-CH), 5.53—5.57 (1H, m, 6-CH). MS m/z: 792 (M^+), 603 (100%).

(20*S*,24*S*)-1α,3β-Bis(*tert*-butyldimethylsilyloxy)-24-hydroxy-25-[2-(trimethylsilyl)ethoxymethyloxy]-22-oxacholesta-5,7-diene (13b) A mixture of **11** (874 mg, 1.56 mmol), *tert*-BuOK (90%, 2.13 g, 17.1 mmol), dibenzo-18-crown-6 (405 mg, 1.12 mmol) and **12b** (1.00 g, 4.30 mmol) in toluene (51 ml) was treated in the same manner as described for the preparation of **13a**. The crude product was submitted to 3-stage purification, 1) flash column chromatography with hexane–AcOEt (15 : 1) as the eluent, 2) flash column chromatography with hexane–AcOEt (20 : 1) as the eluent, and 3) preparative TLC developed with hexane–AcOEt (10 : 1), to give **13b** (118 mg, 10%) as a pale yellow oil. UV λ_{max} nm: 270, 281, 293. IR (neat): 3620, 2980, 1100 cm^{-1}. NMR δ: 0.02 (9H, s, 3 × $SiCH_3$), 0.05 (3H, s, $SiCH_3$), 0.06 (6H, s, 2 × $SiCH_3$), 0.11 (3H, s, $SiCH_3$), 0.61 (3H, s, 18-CH_3), 0.89 (21H, s, 19-CH_3, 2 × $SiC(CH_3)_3$), 0.90 (2H, t, J=8.5, $SiCH_2$), 1.20 (3H, d, J=5.8, 21-CH_3), 1.25 (6H, s, 26-CH_3, 27-CH_3), 2.97—3.01 (1H, m), 3.27—3.35 (1H, m), 3.35—3.77 (3H, m), 3.64 (2H, t, J=8.5, $SiCH_2C\underline{H}_2O$), 3.94—4.11 (1H, m, 3-CH), 4.79 (2H, s, OCH_2O), 5.28—5.32 (1H, m, 7-CH), 5.54—5.57 (1H, m, 6-CH). MS m/z: 792 (M^+), 603 (100%).

(20*S*,24*R*)-1α,3β,24,25-Tetrahydroxy-22-oxacholesta-5,7-diene (14a) A mixture of **13a** (297 mg, 0.37mmol), TBAF (solution in THF; 5.6 ml, 5.6 mmol) and molecular sieves 4 A (1.00 g) in DMPU (5 ml) was stirred at 80 °C for 6 h and at room temperature for 15.5 h. The insoluble material was removed by filtration. The filtrate was poured into H_2O and extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ and NaCl. The residue upon work-up was purified by flash column chromatography with CH_2Cl_2–EtOH (12 : 1) as the eluent to give **14a** (125 mg, 77%) as a white powder. UV λ_{max} nm: 271, 282, 293. IR (KBr): 3430, 3300, 2945, 1070 cm^{-1}. NMR δ: 0.62 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.21 (3H, d, J=6.1, 21-CH_3), 1.22 (3H, s, 26-CH_3), 1.24 (3H, s, 27-CH_3), 3.28—3.35 (1H, m), 3.39—3.47 (2H, m), 3.73—3.83 (2H, m), 3.96—4.13 (1H, m, 3-CH), 5.35—5.42 (1H, m, 7-CH), 5.69—5.74 (1H, m, 6-CH). MS m/z: 434 (M^+), 129 (100%).

(20*S*,24*S*)-1α,3β,24,25-Tetrahydroxy-22-oxacholesta-5,7-diene (14b) A mixture of **13b** (118 mg, 0.15 mmol), TBAF (solution in THF; 2.3 ml, 2.3 mmol) and molecular sieves 4 A (400 mg) in DMPU (2 ml) was treated in the same manner as described for the preparation of **14a**. The crude product was purified by flash column chromatography with CH_2Cl_2–EtOH (10 : 1) as the eluent to give **14b** (45 mg, 70%) as a colorless powder. UV λ_{max} nm: 270, 281, 292. IR (KBr): 3450, 2970, 1095 cm^{-1}. NMR δ: 0.61 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 1.21 (3H, d, J=5.8, 21-CH_3), 1.22 (3H, s, 26-CH_3), 1.25 (3H, s, 27-CH_3), 3.24—3.31 (1H, m), 3.37—3.46 (2H, m), 3.71—3.77 (2H, m), 3.96—4.13 (1H, m, 3-CH), 5.25—5.28 (1H, m, 7-CH), 5.47—5.52 (1H, m, 6-CH). MS m/z: 434 (M^+), 59 (100%).

(20*S*)-1α-*tert*-Butyldimethylsilyloxy-20-hydroxy-3β-methoxymethyloxypregna-5,7-diene (15) A mixture of **11** (1.48 g, 2.64 mmol), Ac_2O (1.5 ml, 15.9 mmol) and pyridine (3.0 ml, 37.1 mmol) was stirred at room temperature for 14 h. The mixture was diluted with AcOEt and washed with 10% HCl and saturated $NaHCO_3$. The residue upon work-up was purified by flash column chromatography with AcOEt–hexane (1 : 14) as the eluent to give (20*S*)-20-acetyloxy-1α,3β-bis(*tert*-butyldimethylsilyloxy)pregna-5,7-diene (1.13 g, 71%) as a pale yellow oil. UV λ_{max} nm: 270, 281, 293. IR (neat): 2955, 2935, 2855, 1740, 1250, 1100, 835 cm^{-1}. NMR δ: 0.05 (3H, s, $SiCH_3$), 0.06 (6H, s, 2 × $SiCH_3$), 0.11 (3H, s, $SiCH_3$), 0.63 (3H, s, 18-CH_3), 0.88 (9H, s, $SiC(CH_3)_3$), 0.89 (12H, s, 19-CH_3, $SiC(CH_3)_3$), 1.24 (3H, d, J=6.1, 21-CH_3), 2.00 (3H, s, CH_3CO), 3.70 (1H, br s, 1-CH), 3.95—4.14 (1H, m, 3-CH), 4.86—5.03 (1H, m, 20-CH), 5.29—5.38 (1H, m, 7-CH), 5.58 (1H, br d, J=5.6, 6-CH). MS m/z: 602 (M^+), 73 (100%).

A mixture of the above-mentioned acetate (860 mg, 1.43 mmol), Amberlyst 15 (550 mg), MeOH (50 ml) and THF (20 ml) was stirred at room temperature for 14.5 h. The insoluble material was removed by filtration. The filtrate was concentrated *in vacuo*. The residue upon work-up was purified by flash column chromatography with AcOEt–hexane (1 : 3) as the eluent to give (20*S*)-20-acetyloxy-1α-*tert*-butyldimethylsilyloxy-3β-hydroxypregna-5,7-diene (688 mg, 99%) as a colorless oil. UV λ_{max} nm: 270, 281, 293. IR (neat): 3425 br, 2945, 1735, 1245, 1060, 830 cm^{-1}. NMR δ: 0.04 (3H, s, $SiCH_3$), 0.09 (3H, s, $SiCH_3$), 0.59 (3H, s, 18-CH_3), 0.89 (12H, s, 19-CH_3, $SiC(CH_3)_3$), 1.21 (3H, d, J=6.3, 21-CH_3), 1.97 (3H, s, CH_3CO), 3.71 (1H, br s, 1-CH), 3.93—4.12 (1H, m, 3-CH), 4.84—5.00 (1H, m, 20-CH), 5.26—5.34 (1H, m, 7-CH), 5.58 (1H, br d, J=5.6, 6-CH). MS m/z: 488 (M^+), 43 (100%).

Chloromethyl methyl ether (3.9 ml, 51.3 mmol) was added dropwise to a stirred solution of the above-mentioned alcohol (688 mg, 1.41 mmol) and diisopropylethylamine (2 ml, 68.9 mmol) in THF (20 ml) at 0 °C. The mixture was stirred at room temperature for 20 h, then poured into 10% HCl, extracted with AcOEt and washed with saturated $NaHCO_3$ and saturated NaCl. The residue upon work-up was purified by flash column chromatography with AcOEt–hexane (1 : 9) as the eluent to give (20*S*)-20-acetyloxy-1α-*tert*-butyldimethylsilyloxy-3β-methoxymethyloxypregna-5,7-diene (632 mg, 84%) as a colorless oil. UV λ_{max} nm: 270, 281, 293. IR (neat): 2950, 2875, 1730, 1240, 1040, 830 cm^{-1}. NMR δ: 0.04 (3H, s, $SiCH_3$), 0.09 (3H, s, $SiCH_3$), 0.59 (3H, s, 18-CH_3), 0.85 (12H, s, 19-CH_3, $SiC(CH_3)_3$), 1.20 (3H, d, J=6.3, 21-CH_3), 1.96 (3H, s, CH_3CO), 3.32 (3H, s, OCH_3), 3.71 (1H, br s, 1-CH), 3.80—4.00 (1H, m, 3-CH), 4.60 and 4.66 (each 1H, d, J=6.3, OCH_2O), 4.81—4.97 (1H, m, 20-CH), 5.23—5.33 (1H, m, 7-CH), 5.58 (1H, br d, J=5.4, 6-CH). MS m/z: 532 (M^+), 45 (100%).

$LiAlH_4$ (90 mg, 2.37 mmol) was added portionwise to a stirred solution of the above-mentioned ether (632 mg, 1.19 mmol) in THF (20 ml) at 0 °C. The mixture was stirred at the same temperature for 30 min, then NaOH (1 mol solution, 0.2 ml) and Rochelle salt solution were added and the whole was extracted with CH_2Cl_2. The residue upon work-up was purified by flash column chromatography with AcOEt–hexane (1 : 4) as the eluent to give **15** (582 mg, 100%) as a colorless oil. UV λ_{max} nm: 270, 281, 293. IR (neat): 3270 br, 2950, 1145, 1100, 1085, 1040, 835 cm^{-1}. NMR δ: 0.04 (3H, s, $SiCH_3$), 0.09 (3H, s, $SiCH_3$), 0.59 (3H, s, 18-CH_3), 0.85 (12H, s, 19-CH_3, $SiC(CH_3)_3$), 1.20 (3H, d, J=6.3, 21-CH_3), 3.32 (3H, s, OCH_3), 3.71 (1H, br s, 1-CH), 3.82—4.01 (1H, m, 3-CH), 4.60 and 4.67 (each 1H, d, J=6.8, OCH_2O), 5.24—5.33 (1H, m, 7-CH), 5.58 (1H, dd, J=2.1, 5.5, 6-CH). MS m/z: 490 (M^+), 45 (100%).

(20*S*)-1α-*tert*-Butyldimethylsilyloxy-24-hydroxy-25-[2-(trimethylsilyl)ethoxymethyloxy-22-oxacholesta-5,7-diene (16) A mixture of **15** (582 mg, 1.19 mmol), **12c** (1.26 g, 5.43 mmol), *tert*-BuOK (892 mg, 7.15 mmol) and dibenzo-18-crown-6 (118 mg, 0.33 mmol) in toluene (30 ml) was stirred at 100 °C for 8 h, then poured into saturated NaCl and extracted with AcOEt. The residue upon work-up was purified by flash column chromatography with AcOEt–hexane (1 : 7) as the eluent to give crude **16** (625 mg), which was used without further purification. MS m/z: 722 (M^+), 73 (100%).

(20*S*)-1α,24,25-Trihydroxy-3β-methoxymethyloxy-22-oxacholesta-5,7-diene (17) A mixture of crude **16** (423 mg), TBAF (solution in THF; 6.0 ml, 6.00 mmol), molecular sieves 4 A (765 mg) and 1,3-dimethyl-2-imidazolidinone (DMI) (3.0 ml) was stirred at 100 °C for 3.5 h. The insoluble material was removed by filtration. The filtrate was diluted with AcOEt and washed with saturated NaCl. The residue upon work-up was purified by flash column chromatography with AcOEt–hexane (1 : 3) as the eluent to give **17** (108 mg, 29% from **15**) as a colorless oil. UV λ_{max} nm: 271, 281, 293. IR (neat): 3460 br, 3270 br, 2925, 2870, 1370, 1145, 1100, 1030 cm^{-1}. NMR δ: 0.61 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.08—1.20 (9H, m, 21-CH_3, 26-CH_3, 27-CH_3), 3.08—3.40 (4H, m), 3.38 (3H, s, OCH_3), 3.75 (1H, br s, 1-CH), 3.77—3.91 (1H, m, 3-CH), 4.70 (2H, s, OCH_2O), 5.27—5.33 (1H, m, 7-CH), 5.72 (1H, br d, J=3.6, 6-CH). MS m/z: 478 (M^+), 59 (100%).

PTAD Adduct of (20*S*)-1α,24,25-Trihydroxy-3β-methoxymethyloxy-22-oxacholesta-5,7-diene (18) PTAD (60 mg, 0.34 mmol) in CH_2Cl_2 (10 ml) was added to a stirred solution of **17** (40 mg, 0.08 mmol) in CH_2Cl_2 (5 ml) at room temperature. The mixture was stirred at room temperature for 13.5 h, and concentrated *in vacuo*. The residue was purified by flash column chromatography with AcOEt–hexane (2 : 3) as the eluent to give **18** (37 mg, 68%) as a colorless foam. UV λ_{max} nm: 205. IR (neat): 3455br, 2960, 1410, 1035 cm^{-1}. NMR δ: 0.81 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 1.15—1.27 (9H, m, 21-CH_3, 26-CH_3, 27-CH_3), 3.21—3.49 (3H, m), 3.38 (3H, s, OCH_3), 3.66—3.79 (1H, m), 3.88 (1H,

br s, 1-CH), 4.62—4.87 (1H, m, 3-CH), 4.70 and 4.80 (each 1H, d, J= 6.5, OCH_2O), 6.26 and 6.40 (each 1H, d, J=8.1, 6-CH, 7-CH), 7.23—7.46 (5H, m, PhH). MS m/z: 653 (M^+), 45 (100%).

PTAD Adduct of (20*S*)-1α,25-Dihydroxy-3β-methoxymethyloxy-24-oxo-22-oxacholesta-5,7-diene (19) DMSO (0.11 ml, 1.50 mmol) was added to a stirred solution of triphosgene (79 mg, 0.27 mmol) in CH_2Cl_2 (0.3 ml) at −65 °C. The mixture was stirred at the same temperature for 10 min, then a solution of **18** (95 mg, 0.15 mmol) in CH_2Cl_2 (0.5 ml) was added dropwise. Stirring was continued at the same temperature for 15 min. Then triethylamine (0.26 ml, 1.90 mmol) was added and the mixture was stirred at the same temperature for 10 min and at room temperature for 20 min. The mixture was diluted with CH_2Cl_2, and washed with H_2O and saturated NaCl. The residue upon work-up was purified by preparative TLC developed with AcOEt to give **19** (42 mg, 44%) as a colorless powder. UV λ_{max} nm: 207. IR (KBr): 3460 br, 2925, 1725, 1680, 1410, 1040 cm^{-1}. NMR δ: 0.83 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 1.22 (3H, d, J=6.1, 21-CH_3), 1.38 (6H, s, 26-CH_3, 27-CH_3), 3.38 (3H, s, OCH_3), 3.90 (1H, br s, 1-CH), 4.22 and 4.43 (each 1H, d, J=16.7, 23-CH_2), 4.65—4.83 (1H, m, 3-CH), 4.71 and 4.82 (each 1H, d, J=6.7, OCH_2O), 6.26 (1H, d, J=8.3, 7-CH), 6.42 (1H, d, J=8.3, 6-CH), 7.27—7.41 (5H, m, PhH). MS m/z: 476 (M^+ −PTAD), 45 (100%).

PTAD Adduct of (20*S*)-1α,3β,25-Trihydroxy-24-oxo-22-oxacholesta-5,7-diene (20) A mixture of **19** (38 mg, 0.06 mmol) and HCl (6 mol solution, 0.5 ml) in MeOH (14 ml) was stirred at room temperature for 17.5 h. The mixture was poured into saturated $NaHCO_3$, and extracted with CH_2Cl_2. The residue upon work-up was purified by preparative TLC developed with AcOEt to give **20** (24 mg, 68%) as a colorless powder. UV λ_{max} nm: 206. IR (KBr): 3430 br, 2960, 2925, 1735, 1675, 1405 cm^{-1}. NMR δ: 0.83 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.22 (3H, d, J=6.1, 21-CH_3), 1.39 (6H, s, 26-CH_3, 27-CH_3), 3.89 (1H, br s, 1-CH), 4.22 and 4.44 (each 1H, d, J=16.7, 23-CH_2), 4.79—4.95 (1H, m, 3-CH), 6.27 (1H, d, J=8.4, 7-CH), 6.41 (1H, d, J=8.4, 6-CH), 7.27—7.45 (5H, m, PhH). MS m/z: 432 (M^+ −PTAD), 59 (100%).

(20*S*)-1α,3β,25-Trihydroxy-24-oxo-22-oxacholesta-5,7-diene (21) A solution of **20** (24 mg, 0.04 mmol) in DMI (2.5 ml) was stirred at 140 °C for 2.5 h. The mixture was diluted with AcOEt and washed with saturated NaCl. The residue upon work-up was purified by preparative TLC developed with AcOEt to give **21** (11 mg, 65%) as a colorless powder. UV λ_{max} nm: 271, 282, 293. IR (KBr): 3410 br, 2920, 1725, 1455, 1375, 1045 cm^{-1}. NMR δ: 0.62 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.23 (3H, d, J=6.0, 21-CH_3), 1.39 (6H, s, 26-CH_3, 27-CH_3), 3.76 (1H, br s, 1-CH), 4.02—4.16 (1H, m, 3-CH), 4.26 and 4.45 (each 1H, d, J=16.8, 23-CH_2), 5.37—5.43 (1H, m, 7-CH), 5.72 (1H, br d, J=4.0, 6-CH). MS m/z: 432 (M^+), 59 (100%).

3-*tert*-Butyldimethylsilyloxy-1-propene (23) A mixture of **22** (8.16 ml, 120 mmol), TBSCl (15.0 g, 100 mmol), triethylamine (50.2 ml, 360 mmol) and 4-dimethylaminopyridine (1.47 g, 12.0 mmol) in CH_2Cl_2 (254 ml) was stirred at room temperature for 20 h, then diluted with Et_2O, and washed with H_2O, cold 10% HCl, saturated $NaHCO_3$ and saturated NaCl. The residue upon work-up was distilled under reduced pressure to give **23** (13.8 g, 80%) as a colorless oil, bp 49—50 °C (16 mmHg). IR (neat): 1254, 1007, 918 cm^{-1}. NMR δ: 0.07 (6H, s, 2 × $SiCH_3$), 0.91 (9H, s, $SiC(CH_3)_3$), 4.17 (2H, dt, J=1.7, 4.4, OCH_2), 5.01—5.38 (2H, m, $C\underline{H}_2$=CH), 5.73—6.09 (1H, m, CH_2=C$\underline{H}$). HRMS m/z: 172.1258 (Calcd for $C_9H_{20}OSi$: 172.1283).

Ethyl 4-*tert*-Butyldimethylsilyloxy-2-methyl-2-butenoate (25) Ozone was bubbled into a stirred mixture of **23** (1.00 g, 5.81 mmol) and $NaHCO_3$ (1.15 g, 13.7 mmol) in CH_2Cl_2 (60 ml) at −82 °C for 45 min. Excess ozone was removed by bubbling nitrogen for 30 min. Ph_3P (2.30 g, 8.72 mmol) was added and stirring was continued at room temperature for 1 h. The obtained aldehyde **24** was used without isolation. (Carbethoxyethylidene)triphenylphosphorane (4.88 g, 12.8 mmol) was added to the above mixture and stirring was continued at room temperature for 14 h. The mixture was concentrated *in vacuo*, and the residue was extracted with hexane. The insoluble material was removed by filtration. The filtrate was concentrated *in vacuo*. The crude product was purified by chromatography with Et_2O–hexane (1 : 20) as the eluent to give **25** (1.27 g, 84% from **23**) as a colorless oil. IR (neat): 1715, 1243 cm^{-1}. NMR δ: 0.08 (6H, s, 2 × $SiCH_3$), 0.90 (9H, s, $SiC(CH_3)_3$), 1.28 (3H, t, J=7.1, $CH_2C\underline{H}_3$), 1.79 (3H, br s, 2-CH_3), 4.19 (2H, q, J=7.1, $C\underline{H}_2CH_3$), 4.33 (2H, br d, J=5.6, 4-CH_2), 6.76 (1H, br t, J=5.6, 3-CH). HRMS m/z: 258.1661 (Calcd for $C_{13}H_{26}O_3Si$: 258.1651).

4-(Tetrahydropyran-2-yloxy)-3-methyl-2-butenol (28) DIBAH (2.53 ml, 14.2 mmol) was added to a stirred solution of **25** (1.22 g, 4.73 mmol) in CH_2Cl_2 (27 ml) at −74 °C. The mixture was stirred at the same temperature for 45 min, then the reaction mixture was quenched by adding 10% NaOH (0.72 ml) at the same temperature. Stirring was continued at room temperature for 1.3 h. The mixture was diluted with CH_2Cl_2 and THF. The insoluble material was removed by filtration. The filtrate was dried with $MgSO_4$ and concentrated *in vacuo* to give the crude alcohol (**26**) (1.12 g), which was used without further purification. A solution of the crude alcohol (**26**) (1.12 g), dihydropyran (1.75 ml, 18.9 mmol) and PPTS (119 mg, 0.47 mmol) in CH_2Cl_2 (11 ml) was stirred at room temperature for 3.5 h. The mixture was concentrated under reduced pressure at room temperature, then diluted with Et_2O and washed with H_2O. The residue upon work-up gave the crude ether (**27**) (1.59 g), which was used without further purification. A solution of the crude ether (**27**) (1.59 g) and TBAF (1 mol solution in THF; 8.52 ml, 8.52 mmol) in THF (47 ml) was stirred at room temperature for 50 min, then diluted with Et_2O and washed with H_2O and saturated NaCl. The residue upon work-up was purified by chromatography with Et_2O–hexane (2 : 1) as the eluent to give **28** (735 mg, 84% from **25**) as a colorless oil. *Anal.* Calcd for $C_{10}H_{18}O_3$: C, 64.47; H, 9.75. Found: C, 64.16; H, 9.83. IR (neat): 3402, 868, 812 cm^{-1}. NMR δ: 1.66 (3H, s, 3-CH_3), 1.20—1.96 (6H, m, THP), 2.12 (1H, br t, OH), 3.27—4.32 (6H, m, 1-CH_2, 4-CH_2, $CH_2C\underline{H}_2O$), 4.58 (1H, br t, OCHO), 5.64 (1H, dt, J=1.2, 6.8, 2-CH). MS m/z: 155 (M^+ −CH_2OH).

4-Bromo-1-(tetrahydropyran-2-yloxy)-2-methyl-2-butene (29) A mixture of **28** (650 mg, 3.50 mmol), Ph_3P (1.01 g, 4.19 mmol), CBr_4 (1.74 g, 5.24 mmol) and $NaHCO_3$ (881 mg, 10.5 mmol) in CH_2Cl_2 (40 ml) was stirred at room temperature for 4 h, then diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and saturated NaCl. The residue upon work-up was purified by chromatography with Et_2O–hexane (1 : 5) as the eluent to give **29** (515 mg, 59%) as a colorless oil. IR (neat): 1121, 663 cm^{-1}. NMR δ: 1.74 (3H, s, 3-CH_3), 1.14—2.09 (6H, m, THP), 3.34—4.33 (6H, m, 1-CH_2, 4-CH_2, $CH_2C\underline{H}_2O$), 4.60 (1H, br t, OCHO), 5.83 (1H, br t, J=7.9, 2-H). MS m/z: 247 (M^+ −H).

(20*S*)-1α,3β-Bis(*tert*-butyldimethylsilyloxy)-26-(tetrahydropyran-2-yloxy)-22-oxacholesta-5,7,24-triene (30) Potassium hydride (64 mg, 1.61 mmol) was added to a stirred solution of **11** (300 mg, 0.54 mmol) in THF (10 ml) at 0 °C. The mixture was stirred at room temperature for 1.5 h. The bromide **29** (267 mg, 1.07 mmol) in THF (11 ml) was added, and the resulting mixture was refluxed for 1.3 h. The reaction was quenched by adding H_2O at room temperature. The whole was then diluted with Et_2O and washed with saturated NaCl. The residue upon work-up was purified by chromatography with Et_2O–hexane (1 : 10) as the eluent to give **30** (197 mg, 94% based upon the recovery of **11**) as a colorless oil. IR (neat): 2934, 1253, 1077, 1022, 835 cm^{-1}. NMR δ: 0.06 (6H, s, 2 × $SiCH_3$), 0.11—2.81 (24H, m), 0.11 (6H, s, 2 × $SiCH_3$), 0.60 (3H, s, 18-CH_3), 0.87 (21H, s, 19-CH_3, 2 × $SiC(CH_3)_3$), 1.19 (3H, d, J=7.0, 21-CH_3), 3.21—4.27 (9H, m), 4.75 (1H, br t), 5.25—5.74 (3H, m, 6-CH, 7-CH, 24-CH). HRMS m/z: 728.5230 (Calcd for $C_{43}H_{76}O_5Si_2$: 728.5232).

(20*S*)-1α-*tert*-Butyldimethylsilyloxy-3β,26-dihydroxy-22-oxacholesta-5,7,24-triene (31) A solution of **30** (669 mg, 0.92 mmol) and PPTS (79 mg, 0.31 mmol) in MeOH (8.4 ml) was stirred at room temperature for 26 h. The mixture was extracted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and saturated NaCl. The residue upon work-up was purified by chromatography with Et_2O–hexane (2 : 1) as the eluent to give **31** (400 mg, 82%) as a colorless foam. $[\alpha]_D^{30}$ −7.67° (c=1.62 in $CHCl_3$). *Anal.* Calcd for $C_{32}H_{54}O_4Si$: C, 72.40; H, 10.26. Found: C, 72.50; H, 10.21. IR (neat): 3366, 1255, 1147, 1086, 836 cm^{-1}. NMR δ: 0.06 (3H, s, $SiCH_3$), 0.11—2.81 (20H, m), 0.11 (3H, s, $SiCH_3$), 0.60 (3H, s, 18-CH_3), 0.87 (12H, s, 19-CH_3, $SiC(CH_3)_3$), 1.19 (3H, d, J=6.1, 21-CH_3), 3.21—4.27 (7H, m), 5.25—5.74 (3H, m, 6-CH, 7-CH, 24-CH). HRMS m/z: 530.3820 (Calcd for $C_{32}H_{54}O_4Si$: 530.3792).

(20*S*,24*R*,25*S*)-1α-*tert*-Butyldimethylsilyloxy-24,25-epoxy-3β,26-dihydroxy-22-oxacholesta-5,7-diene (32a) A mixture of (−)-diisopropyl-D-tartrate (123 mg, 0.53 mmol), molecular sieves 4A (55 mg) and $Ti(OPr^i)_4$ (0.14 ml, 0.47 mmol) in CH_2Cl_2 (6.9 ml) was stirred at −25 °C for 10 min, then *tert*-butylhydroperoxide (1.04 mol solution in CH_2Cl_2; 0.69 ml, 0.72 mmol) and **31** (170 mg, 0.32 mmol) in CH_2Cl_2 (6.9 ml) were added at −25 °C. The resulting mixture was stirred at −25 °C for 2 h, 10% tartaric acid was added, and stirring was continued at the same temperature for 30 min. The mixture was then diluted with CH_2Cl_2. The insoluble material was removed by filtration. The filtrate was washed with saturated $NaHCO_3$, and the residue upon work-up was purified by

chromatography with Et_2O–hexane (5:1) as the eluent to give **32a** (156 mg, 89%) as a colorless oil. $[\alpha]_D^{31}$ −1.76° (c=1.45 in $CHCl_3$). IR (neat): 3404, 1255, 1147, 1087, 1066, 868, 812, 770 cm^{-1}. NMR δ: 0.07 (3H, s, $SiCH_3$), 0.11 (3H, s, $SiCH_3$), 0.61 (3H, s, 18-CH_3), 0.63–1.77 (9H, m), 0.88 (9H, s, $SiC(CH_3)_3$), 1.21 (3H, d, J=6.0, 21-CH_3), 1.31 (3H, s, 19-CH_3), 1.59 (3H, s, 25-CH_3), 1.86—2.09 (4H, m), 2.29 (1H, br t, J=13.6, 23-CH), 2.49 (1H, br d, J=13.6, 23-CH), 2.80 (1H, br s, 24-CH), 3.19—3.81 (8H, m), 4.07 (1H, br s, 1-CH), 5.33 (1H, br d, J=6.8, 7-CH), 5.61 (1H, br d, J=6.8, 6-CH). HRMS m/z: 546.3734 (Calcd for $C_{32}H_{54}O_5Si$: 546.3741).

(20*S*,24*S*,25*R*)-1α-*tert*-Butyldimethylsilyloxy-24,25-epoxy-3β,26-dihydroxy-22-oxacholesta-5,7-diene (32b) This (151mg, 86%) was obtained as a colorless oil from **31** (170 mg, 0.32 mmol) in the same manner as described for the preparation of **32a**. $[\alpha]_D^{30}$ −16.72° (c=1.92 in $CHCl_3$). IR (neat): 3400, 1254, 1147, 1086, 1064, 867, 833 cm^{-1}. NMR δ: 0.07 (3H, s, $SiCH_3$), 0.12 (3H, s, $SiCH_3$), 0.63 (3H, s, 18-CH_3), 0.88 (9H, s, $SiC(CH_3)_3$), 0.96—1.79 (9H, m), 1.19 (3H, d, J=5.9, 21-CH_3), 1.31 (3H, s, 19-CH_3), 1.60 (3H, s, 25-CH_3), 1.87—2.09 (4H, m), 2.31 (1H, br t, J=13.6, 23-CH), 2.50 (1H, br d, J=13.6, 23-CH), 2.80 (1H, br s, 24-CH), 3.22—3.77 (8H, m), 4.06 (1H, br s, 1-CH), 5.33 (1H, br d, J=6.8, 7-CH), 5.63 (1H, br d, J=6.8, 6-CH). HRMS m/z: 546.3720 (Calcd for $C_{32}H_{54}O_5Si$: 546.3741).

(20*S*,25*S*)-1α,3β,25,26-Tetrahydroxy-22-oxacholesta-5,7-diene (34a) A mixture of **32a** (150 mg, 0.28 mmol) and DIBAH (solution in toluene; 2.82 ml, 2.82 mmol) in toluene (3.4 ml) was stirred at 0 °C for 4 h, then 10% NaOH (0.68 ml) and THF (5 ml) were added and stirring was continued at 60 °C for 30 min. The mixture was diluted with CH_2Cl_2 and THF. The insoluble material was removed by filtration. The filtrate was dried over $MgSO_4$ and concentrated *in vacuo* to give the crude triol **33a** (124 mg), which was used without further purification. A mixture of the crude triol **33a** (122 mg) and TBAF (solution in THF; 0.67 ml, 0.67 mmol) in THF (9 ml) was refluxed for 14 h, then diluted with AcOEt and washed with H_2O, 10% HCl, saturated $NaHCO_3$ and saturated NaCl. The residue upon work-up was purified by chromatography with $CHCl_3$–MeOH (20:1) as the eluent to give **34a** (84 mg, 86%) as a colorless powder. $[\alpha]_D^{29}$ −14.73° (c=1.12 in MeOH). IR (KBr): 3380, 2936, 1649, 1052 cm^{-1}. NMR δ: 0.61 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.18 (3H, s, 25-CH_3), 1.22 (3H, d, J=6.1, 21-CH_3), 1.24—1.79 (12H, m), 1.85—2.04 (4H, m), 2.14 (1H, dq, J=2.4, 13.1), 2.34 (1H, br t, J=7.0), 2.53 (1H, ddd, J=1.8, 2.4, 15.9), 2.69—2.78 (1H, m), 2.96 (1H, br s), 3.26—3.34 (1H, m, 20-CH), 3.34—3.43 (2H, m, 23-CH_2), 3.46 (1H, br d, J=11.0), 3.77 (1H, br s), 3.88 (1H, dt, J=3.1, 9.3, 1-H), 4.06 (1H, sextet, J=6.1, 3-CH), 5.40 (1H, dd, J=2.4, 5.5, 7-CH), 5.73 (1H, dd, J=2.4, 5.5, 6-CH). HRMS m/z: 434.3007 (Calcd for $C_{26}H_{42}O_5$: 434.3032).

(20*S*,25*R*)-1α,3β,25,26-Tetrahydroxy-22-oxacholesta-5,7-diene (34b) This (83 mg, 77%) was obtained as a colorless powder from **32b** (145 mg, 0.27 mmol) in the same manner as described for the preparation of **34a**, without isolation of the crude triol **33b**. $[\alpha]_D^{29}$ −14.65° (c=1.29 in MeOH). IR (KBr): 3372, 2934, 2874, 1647, 1054 cm^{-1}. NMR δ: 0.62 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.20 (3H, s, 25-CH_3), 1.21 (3H, d, J=6.1, 21-CH_3), 1.39—1.79 (12H, m), 1.86—2.06 (4H, m), 2.14 (1H, dq, J=2.4, 7.0), 2.34 (1H, br t, J=7.0), 2.54 (1H, ddd, J=1.8, 2.4, 7.0), 2.64 (1H, br s), 2.68—2.76 (1H, m), 3.29 (1H, q, J=7.0, 20-CH), 3.37—3.46 (2H, m, 23-CH_2), 3.55 (1H, dt, J=3.1, 9.3), 3.74—3.79 (2H, m), 4.02—4.10 (1H, m, 3-CH), 5.40 (1H, dd, J=2.4, 5.5, 7-CH), 5.73 (1H, dd, J=2.4, 5.5, 6-CH). HRMS m/z: 434.3021 (Calcd for $C_{26}H_{42}O_5$: 434.3032).

(24*R*)-24-Hydroxy-22-oxacalcitriol (3). General Procedure for Irradiation and Thermal Isomerization of 14a, 14b, 21, 34a and 34b A solution of **14a** (33.5 mg, 0.08 mmol) in EtOH (200 ml) was irradiated using a 400 W high-pressure mercury lamp with a Vycor filter at 0 °C for 2.5 min. The mixture was then refluxed mildly for 2.5 h and concentrated *in vacuo*. The crude product was purified by preparative TLC developed twice with AcOEt to give **3** (5.7 mg, 17%) as a colorless foam. $[\alpha]_D^{20}$ +44.00° (c=0.29 in EtOH). UV λ_{max} nm: 262, λ_{min} nm: 227. NMR δ: 0.53 (3H, s, 18-CH_3), 1.19 (3H, d, J=6.1, 21-CH_3), 1.22 (3H, s, 26-CH_3), 1.24 (3H, s, 27-CH_3), 3.26—3.32 (1H, m, 20-CH), 3.37—3.47 (2H, m, 23-CH, 24-CH), 3.73—3.82 (1H, m, 23-CH), 4.16—4.27 (1H, m, 3-CH), 4.38—4.45 (1H, m, 1-CH), 4.99 (1H, s, 19-CH), 5.33 (1H, s, 19-CH), 6.02 (1H, d, J=11.5, 7-CH), 6.37 (1H, d, J=11.5, 6-CH). MS m/z: 434 (M^+), 134 (100%). HRMS m/z: 434.3023 (Calcd for $C_{26}H_{42}O_5$: 434.3032).

(24*S*)-24-Hydroxy-22-oxacalcitriol (4) A solution of **14b** (30.0 mg, 0.07 mmol) in EtOH (200 ml) was irradiated for 3.0 min. The mixture was treated according to the general procedure. The crude product was submitted to 2-stage purification, 1) preparative TLC developed twice with AcOEt, and 2) preparative TLC developed twice with CH_2Cl_2–EtOH (10:1), to give **4** (3.9 mg, 13%) as a colorless foam. $[\alpha]_D^{20}$ +29.00° (c=0.19 in EtOH). UV λ_{max} nm: 263, λ_{min} nm: 227. NMR δ: 0.54 (3H, s, 18-CH_3), 1.19 (3H, d, J=5.8, 21-CH_3), 1.22 (3H, s, 26-CH_3), 1.25 (3H, s, 27-CH_3), 3.22—3.29 (1H, m, 20-CH), 3.34—3.46 (2H, m, 23-CH, 24-CH), 3.76 (1H, br d, J=6.3, 23-CH), 4.14—4.25 (1H, m, 3-CH), 4.37—4.44 (1H, m, 1-CH), 4.99 (1H, s, 19-CH), 5.33 (1H, s, 19-CH), 6.02 (1H, d, J=11.0, 7-H), 6.37 (1H, d, J=11.0, 6-H). MS m/z: 434 (M^+), 134 (100%). HRMS m/z: 434.3039 (Calcd for $C_{26}H_{42}O_5$: 434.3032).

24-Oxo-22-oxacalcitriol (5) A solution of **21** (11.0 mg, 0.02 mmol) in EtOH (200 ml) was irradiated for 1.8 min. The mixture was treated according to the general procedure. The crude product was purified by preparative TLC developed with AcOEt to give **5** (1.10 mg, 10%) as a colorless oil. UV λ_{max} nm: 263, λ_{min} nm: 227. NMR δ: 0.53 (3H, s, 18-CH_3), 1.21 (3H, d, J=6.3, 21-CH_3), 1.40 (6H, s, 26-CH_3, 27-CH_3), 4.18—4.27 (1H, m, 3-CH), 4.22 and 4.43 (each 1H, d, J=16.3, 23-CH_2), 4.39—4.48 (1H, m, 1-CH), 5.00 (1H, s, 19-CH), 5.33 (1H, s, 19-CH), 6.03 (1H, d, J=12.0, 7-CH), 6.37 (1H, d, J=12.0, 6-CH). MS m/z: 432 (M^+), 59 (100%).

(25*S*)-26-Hydroxy-22-oxacalcitriol (6) A solution of **34a** (22.7 mg, 0.05 mmol) in EtOH (200 ml) was irradiated for 2.5 min. The mixture was treated according to the general procedure. The crude product was submitted to 2-stage purification, 1) preparative TLC developed twice with AcOEt–EtOH (25:1), and 2) preparative TLC developed with CH_2Cl_2–EtOH (20:3), to give **6** (4.1 mg, 18%) as a colorless foam. $[\alpha]_D^{20}$ +65.85° (c=0.09 in EtOH). UV λ_{max} nm: 264, λ_{min} nm: 227. IR (neat): 3385, 2920, 2865, 1050, 730 cm^{-1}. NMR δ: 0.54 (3H, s, 18-CH_3), 1.19 (3H, s, 25-CH_3), 1.19 (3H, d, J=5.9, 21-CH_3), 3.21—3.34 (1H, m, 20-CH), 3.42 (2H, s, 25-CH_2OH), 3.44—3.61 (1H, m, 23-CH), 3.69—3.81 (1H, m, 23-CH), 4.22 (1H, br s, 3-CH), 4.43 (1H, br s, 1-CH), 5.00 (1H, s, 19-CH), 5.33 (1H, s, 19-CH), 6.02 (1H, d, J=11.2, 7-CH), 6.37 (1H, d, J=11.2, 6-H). MS m/z: 434 (M^+), 85 (100%). HRMS m/z: 434.3048 (Calcd for $C_{26}H_{42}O_5$: 434.3032).

(25*R*)-26-Hydroxy-22-oxacalcitriol (7) This (3.9 mg, 19%) was obtained as a colorless foam from **34b** (20.1 mg, 0.05 mmol) in the same manner as described for the preparation of **6**. $[\alpha]_D^{20}$ +49.35° (c=0.08 in EtOH). UV λ_{max} nm: 263, λ_{min} nm: 227. IR (neat): 3375, 2920, 2865, 1050 cm^{-1}. NMR δ: 0.53 (3H, s, 18-CH_3), 1.18 (3H, s, 25-CH_3), 1.20 (3H, d, J=7.3, 21-CH_3), 3.25—3.57 (4H, m, 20-CH, 23-CH, 25-CH_2OH), 3.81—3.93 (1H, m, 23-CH), 4.23 (1H, br s, 3-CH), 4.42 (1H, br s, 1-CH), 4.99 (1H, s, 19-CH), 5.33 (1H, s, 19-CH), 6.02 (1H, d, J=11.6, 7-CH), 6.37 (1H, d, J=11.6, 6-CH). MS m/z: 434 (M^+). 85 (100%). HRMS m/z: 434.3048 (Calcd for $C_{26}H_{42}O_5$: 434.3032).

(1*S*,3*R*,20*S*)-1,3,20-Trihydroxy-9,10-secopregna-5,7,10(19)-triene (8) The 20(*S*)-alcohol **11** (1.25 g, 2.24 mmol) in THF (750 ml) was irradiated using a 400 W high-pressure mercury lamp with a Vycor filter at 0 °C for 23 min. The mixture was then refluxed for 3 h and concentrated *in vacuo*. The residue in THF (45 ml) was added to TBAF (solution in THF; 22.4 ml, 22.4 mmol). The mixture was stirred at room temperature for 15 h, and concentrated *in vacuo*. The residue was extracted with AcOEt, and washed with 5% HCl, 5% NaOH and saturated NaCl. The residue upon work-up was submitted to 3-stage purification, 1) flash column chromatography with CH_2Cl_2–EtOH (100:9) as the eluent, 2) flash column chromatography with AcOEt–hexane (3:1) as the eluent, and 3) flash column chromatography with CH_2Cl_2–EtOH (10:1) as the eluent to give **8** (187 mg, 25%) as a colorless foam. $[\alpha]_D^{20}$ +55.10° (c=0.01 in EtOH). UV λ_{max} nm: 263, λ_{min} nm: 227. NMR δ: 0.55 (3H, s, 18-CH_3), 1.23 (3H, d, J=6.6, 21-CH_3), 2.33 (1H, dd, J=6.0, 13.1), 2.61 (1H, dd, J=2.9, 13.1), 2.85 (1H, dd, J=2.9, 10.8), 3.62—3.76 (1H, m, 20-CH), 4.17—4.31 (1H, m, 3-CH), 4.37—4.51 (1H, m, 1-CH), 5.00 (1H, s, 19-CH), 5.33 (1H, s, 19-CH), 6.04 (1H, d, J=11.7, 7-CH), 6.37 (1H, d, J=11.7, 6-CH). MS m/z: 332 (M^+), 134 (100%). HRMS m/z: 332.2369 (Calcd for $C_{21}H_{32}O_3$: 332.2351).

VDR Binding Assay The binding affinity of OCT (**1**) and its putative metabolites **3**—**9** with the calf thymus vitamin D receptor was tested using a $1,25(OH)_2D_3$ assay kit purchased from Incstar (Stillwater, MN). Calf thymus $1,25(OH)_2D_3$ receptor was incubated at 20 °C for 1 h with various concentrations of $1,25(OH)_2D_3$ (**2**) (1.25—80 pg/tube), OCT (**1**) (1.25—80 pg/tube) or its putative metabolites **3**—**9** (2.5—204, 800 pg/tube). After the incubation period, 15000 dpm 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)$_2$D$_3$ were separated by addition of dextran–charcoal suspension and centrifugation. The radioactivity was measured with an Aloka LSC-700.

Assessment of HL-60 Cell Growth HL-60 cells were kindly provided by Dr. Inaba, Osaka City University, Medical School. Cells were cultured at 37 °C in RPMI 1640 medium (Nissui Pharmaceutical, Japan) supplemented with 10% heat-inactivated fetal calf serum and 60 μg/ml of kanamycin in a humidified atmosphere of 5% CO_2 in air. Under these conditions, the doubling time of HL-60 cells was 24 h. Vitamin D-induced cells were obtained by seeding HL-60 at 1×10^5/ml in growth medium and culturing for 72 h in the presence of $10^{-10}-10^{-7}$ M OCT **1** or its putative metabolites **3**—**9** dissolved in EtOH. Control cultures contained the EtOH vehicle at 0.1% (v/v). After the incubation period, cells were harvested and the cell number was determined using a hemacytometer. Cell viability was determined in terms of trypan blue exclusion. The number of cells counted in triplicate experiments was expressed as a percentage of the control. Data are expressed as the mean of triplicate counts ± standard error.

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