

Synthesis and Biological Evaluations of C-23-Modified 26,26,26,27,27,27-F₆-Vitamin D₃ Analogues

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Abstract—A convenient synthetic method which could allow flexible modification at C-23 of 26,26,26,27,27,27-hexafluoro-1 α ,25-dihydroxyvitamin D₃ (**3**) has been developed. An effective construction of hexafluoroacetone (HFA) aldol part on the side chain of **10** was achieved by aldol reaction with HFA gas. This route is also attractive as an approach to diverse 26,27-modified vitamin D₃ analogues. The preliminary biological activities of 23-modified 26,27-F₆ vitamin D₃ analogues are evaluated. The potency of VDR affinities of the C-23-substituted analogues (keto group (**4**); OH group (**5a,5b**); fluorine atom (**6a,6b**); and oxetane ring (**7a,7b**)) was found to vary depending upon both the nature and stereochemistry of the substituents. In contrast, the HL-60 cell differentiation property was less varied than VDR affinity, and depended upon the nature rather than the stereochemistry of the substituents. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

1 α ,25-Dihydroxyvitamin D₃ (**1**; 1 α ,25(OH)₂D₃), the hormonally active form of vitamin D₃, plays an important role in regulating cell proliferation and differentiation in a variety of cell types.¹ A large number of side chain modified analogues have been reported during the last decade.² Among them, 26,26,26,27,27,27-hexafluoro-1 α ,25-dihydroxyvitamin D₃ (**3**; 26,27-F₆-1 α ,25(OH)₂D₃) exhibits a characteristic potency with some biological activities to 1.^{3–5} Our laboratory has already reported that one analogue of **3**, 26,27-F₆-1 α ,23(S),25(OH)₃D₃ (**5a**) showed a binding affinity similar to **3**, to the vitamin D₃ receptor (VDR),⁶ whereas 1 α ,23(S),25(OH)₃D₃ (**2**) had a lower binding affinity than **1** (**2**: 12% compared to **1**).² Data suggested that modification at C-23 of **3** would produce an unexpected biological effect, compared to **1**. Therefore we planned to prepare C-23-modified analogues of **3** and evaluate their biological activities. At present, no convenient synthetic methods to introduce the substituents at C-23 of **3** have been reported, so we worked to develop a novel synthetic method. Our intended modifications at C-23 can be categorized by introducing the following groups: (i) ketone (**4**); (ii)

OH group (**5a,5b**); (iii) fluorine atom (**6a,6b**); and (iv) oxetane ring (**7a,7b**). In particular, modifications including (iii) and (iv) at C-23 in vitamin D₃ have not been attempted previously. This report concerns a novel synthetic method which enables flexible modifications at C-23 of **3** via the key-intermediate **10** and the biological activities of the resulting analogues.

Results and Discussion

Synthesis

As shown in Scheme 1, the synthetic plan relied upon construction of a side chain of the key-intermediate **10** using an aldol reaction of **13** with hexafluoroacetone (HFA). Tosylate **14**, which was prepared from readily available vitamin D₂ in six steps,^{7,8} could be transformed into methyl ketone **13**. In practice, methyl ketone **13** was prepared as described in Scheme 2. Displacement of the tosyl group in **14** with KCN and subsequent reduction by diisobutylaluminium hydride (DIBALH) afforded aldehyde **16**. The reaction of **16** with methylmagnesium bromide followed by oxidation gave the desired methyl ketone **17**. Following the 1 α -hydroxylation procedure developed by Andrews et al.,^{8,9} **18** was obtained in 41% yield. Silylation of **18** and successive photoisomerization gave methyl ketone **13**. As previously reported in the literature, HFA aldols can be prepared from enol silyl

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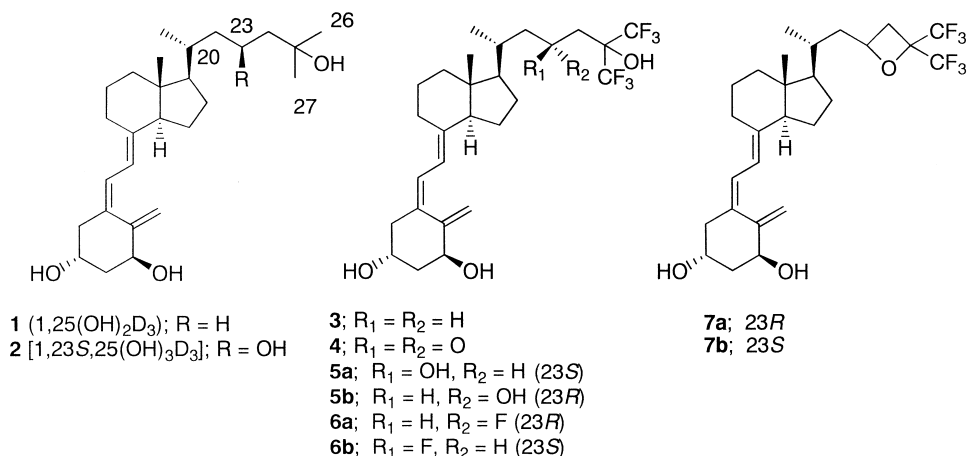
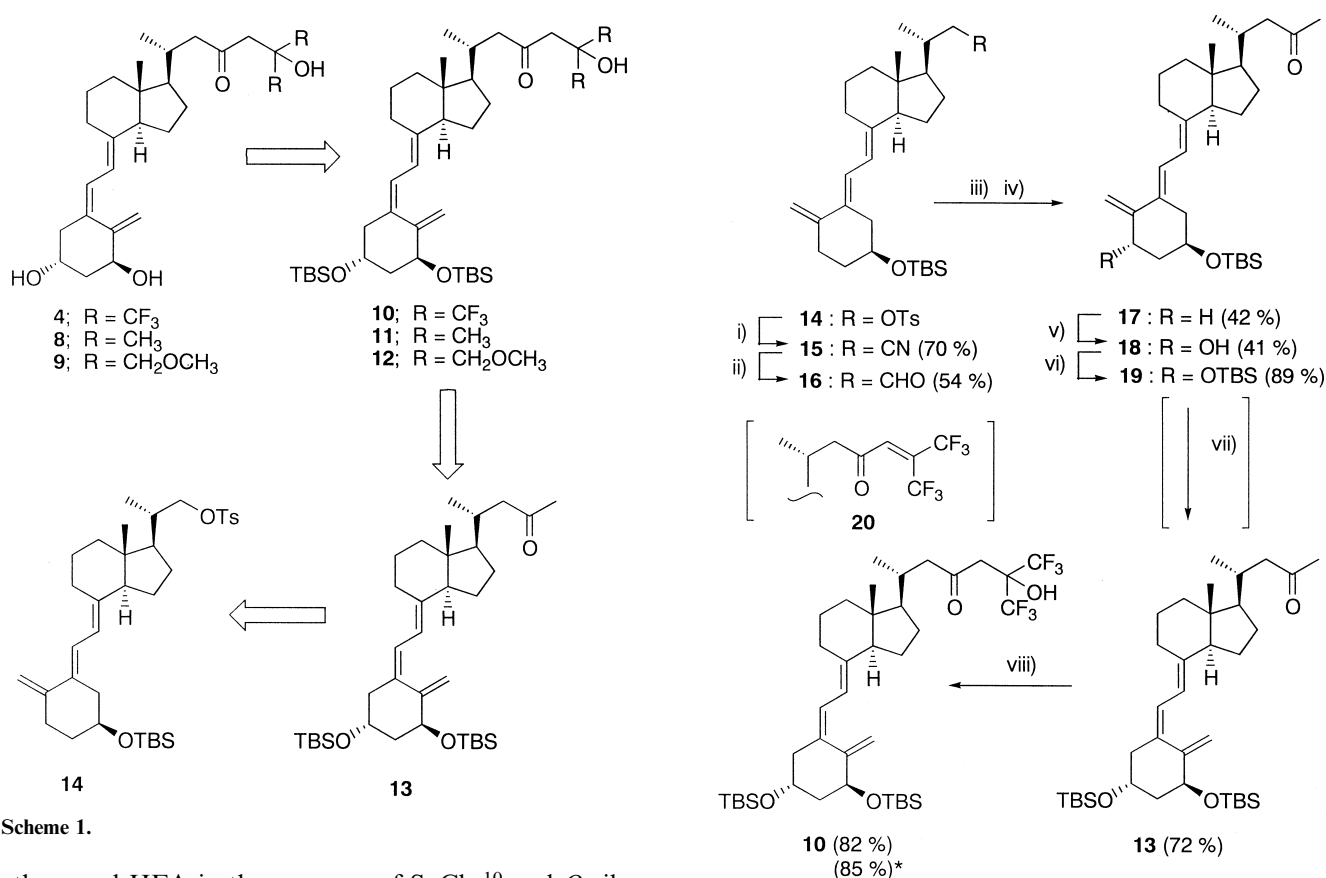


Figure 1.



Scheme 1.

ethers and HFA in the presence of SnCl₄,¹⁰ and *O*-silylated HFA aldols can be obtained in the absence of SnCl₄.¹¹ To prevent the risk of degrading the labile vitamin D triene unit by Lewis acids, and to simplify the reaction procedure from the methyl ketone to the HFA aldol, we examined basic conditions using lithium bases; Lithiation of **13** with LDA (1.1–3.0 equiv) in THF at –70 °C followed by the addition of an excess amount of HFA gas gave the undesired 24-dehydrate compound **20** (15%), along with recovery of starting material **13** (80%).¹² Switching the base to lithium bis(trimethylsilyl) amide [LiN(TMS)₂] (1.4 equiv)¹³ gave the desired HFA aldol adduct **10** (82%) as a major product with a small amount of recovered **13** (4%). In this case, **20** was not obtained.

Scheme 2. (i) KCN, DMSO, 90 °C, 30 min; (ii) DIBALH, THF, –70 °C → rt; (iii) MeMgBr, Et₂O, rt, 10 min; (iv) TPAP, NMO, MS4A, CH₂Cl₂, rt, 15 min; (v) SeO₂, NMO, MeOH–CH₂Cl₂, reflux, 2 h; (vi) TBSCl, imidazole, DMF, rt, 3 h; (vii) Hg lamp, Anthracene, NEt₃, rt, 1 h; (viii) CF₃COCF₃, LiN(TMS)₂, THF, –70 °C, 30 min *Considering the recovery of **13**.

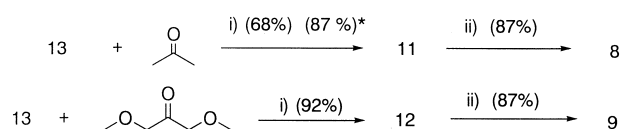
One of the desired analogues, 26,27-F₆-1α,25(OH)₂-23-oxo-D₃ (**4**) was obtained from **10** by removing protecting groups. Reduction of **10** with NaBH₄ and separation of the resulting two isomers by column chromatography readily afforded the more polar isomer **21a** and the less polar isomer **21b**. Removal of the protecting groups of **21a** and **21b** gave 26,27-F₆-1α,23(S),25(OH)₃D₃ (**5a**) and 26,27-F₆-1α,23(R),25(OH)₃D₃ (**5b**) respectively. The

stereochemical assignment at C-23 of **5a** was confirmed by X-ray crystallographic analysis of monohydrate form of **5a**. The assignment at C-23 of **5b** could therefore be informed from this data. Introduction of fluorine at C-23 of **21b** was performed by treatment with diethylaminosulfur trifluoride (DAST)¹⁴ via S_N2 displacement. In this step, the formation of significant amount of undesired elimination product **22** was observed by ¹H NMR analysis of crude product (the molar ratio of 23S-F derivative and **22** (2:1)).¹⁵ The following deprotection and removal of the elimination product gave **6b**. The epimer **6a** was derived from **21a** by the same procedure. For the synthesis of the respective oxetane analogues, mesylation of **21a** and treatment with KOH in MeOH followed by deprotection using tetrabutylammonium fluoride (TBAF) gave the corresponding oxetane **7a**. The epimer **7b** was also taken through the same procedure, from **21b**.

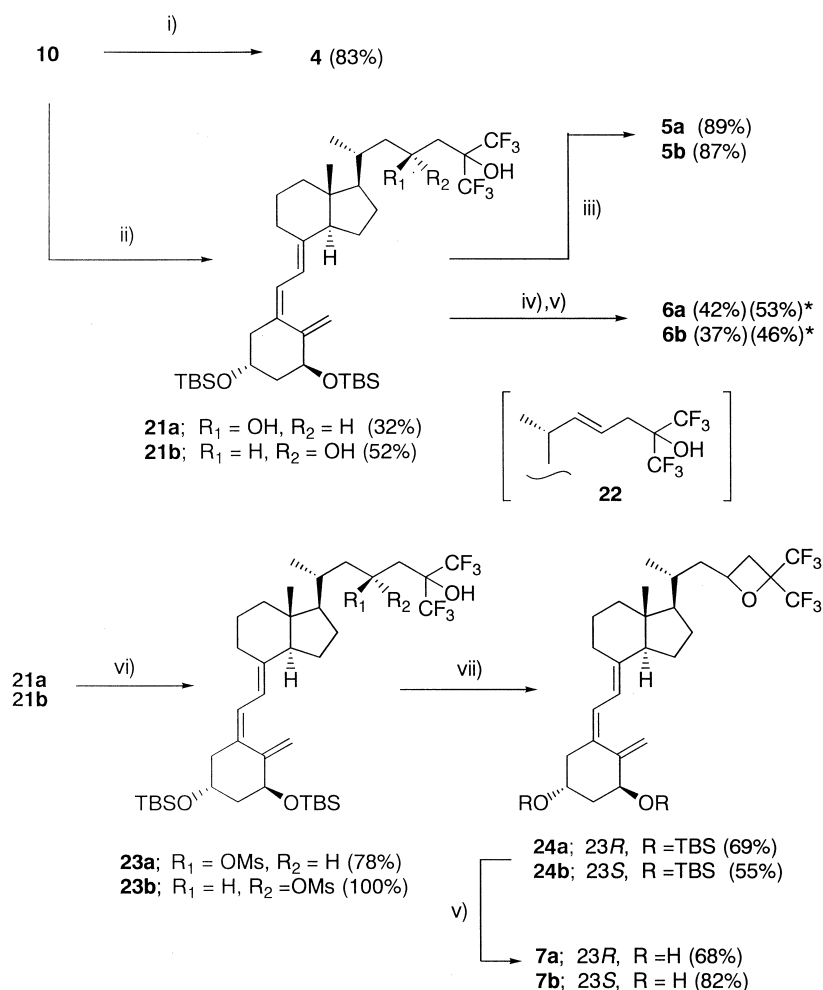
This method via an aldol reaction is also attractive as an approach to new types of 26,27-modified analogues for biological screening purposes. In a preliminary study, we have already synthesized two compounds (**8**¹⁶ and **9**) using acetone and dimethoxyacetone¹⁷ with satisfactory yields (Schemes 1 and 4). Introduction of two extra methoxy groups at C-26 and C-27 has not been previously reported.

Biological evaluations

Biological evaluations of the 26,27-F₆ analogues described above, together with **3** (for comparison) are summarized in Table 1. The binding affinity to VDR was determined by a competitive binding assay using the cytosolic receptor from chick intestine.¹⁸ The activity in differentiating HL-60 cells was examined by nitro blue tetrazolium (NBT) reduction.¹⁹ The potency of VDR affinities of the C-23-substituted analogues (**5a**, **5b**, **6a**, **6b**) was found to vary depending upon both the nature and stereochemistry of the substituents (30–1040% of **3**). Remarkably, *S*-isomers (**5a**, **6b**) were more potent than *R*-isomers (**5b**, **6a**), especially when fluorine was substituted. In contrast, the HL-60 cell differentiation property was less varied than VDR affinity (47–200% of **3**), and this depended upon the nature rather than the



Scheme 4. (i) LiN(TMS)₂, THF, −78 °C, (ii) MsOH, MeOH, rt, 1 h
*Considering the recovery of **13**.



Scheme 3. (i) TsOH, MeOH, rt, 2 h; (ii) NaBH₄, THF-MeOH, −10 °C, 10 min; (iii) MsOH, MeOH, rt, 1 h; (iv) DAST, CH₂Cl₂, −50 °C→rt, 1 h; (v) TBAF-THF, rt, overnight; (vi) MsCl, pyridine, 0 °C, 1 h; (vii) 5% KOH-MeOH; rt, 10 min. *Considering the recovery of starting material.

Table 1. Comparison of biological activities of 23-modified 26,27-F₆ analogues and **3**^a

Compound	VDR	HL-60
3	(100)	(100)
4 [23-keto]	130	— ^c
5a [23(S)-OH]	100 ^b	47
5b [23(R)-OH]	30	54
6a [23(R)-F]	160	200
6b [23(S)-F]	1040	140
7a [23(R)-oxetane]	30	54
7b [23(S)-oxetane]	7	64

^aThe results for **3** are normalized to 100.^bThe data was already reported in ref 6.^c**4** was 40-fold more active than **1**. Comparison of HL-60 cell differentiating activity between **1** and **3** was described in ref 4 (**3**: 10-fold compared to **1**). The data suggested **4** is more active than **3**.

stereochemistry of the substituents. For the oxetane compounds, the *S*-isomer (**7b**) leads to a drop in VDR affinity (7% of **3**) without significantly decreased HL-60 cell differentiating property, whereas the *R*-isomer (**7a**) has decreased binding to both VDR and HL-60 cell differentiation. As described in the literature, introduction of *S*/*R*-OH, keto group at C-23 of **1** gave significant decreased binding affinity to VDR compared to **1**.^{2,16} On the other hand, among 26,27-F₆ analogues, *S*-OH and keto-modifications exhibited a similar and more potent binding affinity than **3** respectively. And *S*-OH modification showed decreased binding affinity, which was more moderate than that of **1**.

Conformational analysis

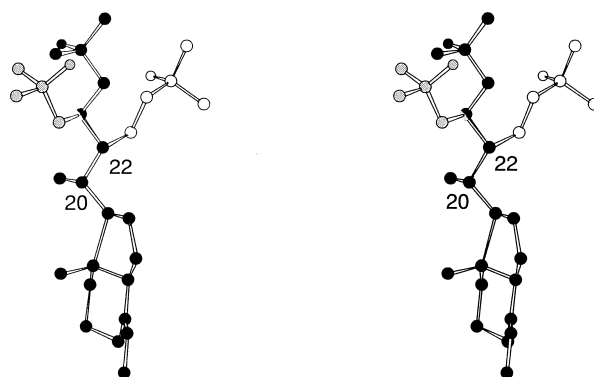
To comprehend the modificational effects of the substituents at C-23 of **3** on VDR affinity, we focused on the side chain conformers possessing the biologically important functional groups (25-OH group). Conformational searches of side chains of **3** and C-23-modified analogues were subjected to a Monte Carlo simulation (2000 iterations) using the MM3 force field²⁰ and the GB/SA Solvation Model (in a water matrix)²¹ including MacroModel (v4.5).²² It was carried out on model compounds in which the A ring and diene system up to C-5 were replaced by a H atom. Rotations were applied to the rotatable C–C bonds of the side chain and the 23-OH bond (in the case of **5a** and **5b**). All conformers within 50 kJ/mol of the global minimum were saved and ranked by relative energy. The data suggested that the side chains of all C-23-modified compounds clearly have fewer degrees of freedom than that of **3** (Table 2). Compounds (**4**, **5a**, **5b**, **6a**, **6b**) possess three families of conformers at low energy levels (0–6 kJ/mol) (Figure 2 and Table 3)²³ (The torsion angle at C₁₇–C₂₀–C₂₂–C₂₃, and C₂₀–C₂₂–C₂₃–C₂₄ *anti-anti* (conformer A; black), *gauche* (+)-*anti* (conformer B; white), *anti-gauche* (+) (conformer C; gray)). The stereochemistry of the substituents at C-23 of **3** influenced the conformational behavior of the side chains significantly. First, with the respect to hydroxy compounds, the *R*-isomer (**5b**) and *S*-isomer (**5a**) adopt the characteristic conformers A and C respectively in high population at a low energy level (0–6 kJ/mol). Next, with the respect to the fluorinated analogues (**6a,6b**), *R*- and *S*-isomers have higher con-

Table 2. Conformational distribution for C-23-modified vitamin D₃ analogues (2000 iterations)

Compound	Number of conformers	
	≤50 kJ/mol ^a	≤10 kJ/mol ^a
3	815	64
4 [23-keto]	262	12
5a [23(S)-OH]	480	4
5b [23(R)-OH]	534	13
6a [23(R)-F]	700	27
6b [23(S)-F]	436	9
7a [23(R)-oxetane]	44	12
7b [23(S)-oxetane]	29	5

^aRelated energy above global minimum energy conformer.**Table 3.** Conformers of 23-modified analogues at low energy level (0–6 kJ/mol)^a

Compound	Conformation type	Δ <i>E</i> (kJ/mol) ^b	Mol%
4 [23-keto]	A	0	57
	B	3.1	9
	C	3.8	7
	B	4.2	6
	C	4.5	5
5a [23(S)-OH]	C	0	86
5b [23(R)-OH]	A	0	67
6a [23(R)-F]	A	0	60
	B	2.8	20
6b [23(S)-F]	C	0	32
	B	0.5	27
	A	1.1	21

^aHigh proportioned conformers (> 5 mol% (Boltzmann distribution at 298 K)).^bRelative energy above global minimum energy conformer.**Figure 2.** Superposition of three families of conformers of **5a**, **5b**, **6a** and **6b** at 0–6 kJ/mol (stereoview) as their deprotonated and defluorinated forms. The substituents at C-23 were omitted to see the orientation of side chains clearly.

formational flexibility than hydroxy compounds, because of the reduced steric repulsion between the 23-substituents and the 18-methyl group. At an energy level (0–6 kJ/mol), conformer C was adopted by the *S*-isomer (**6b**), not by the *R*-isomer (**6a**). In the case of 23-keto compound (**4**) the side chain adopted more expanded mobility than those of the other compounds (**5a,5b,6a,6b**), because of reduced steric repulsion between carbonyl group and the 18-methyl group. As the result, in the case of hydroxy and fluorinated analogues, one explanation was provided by conformational analysis and biological data. Conformer

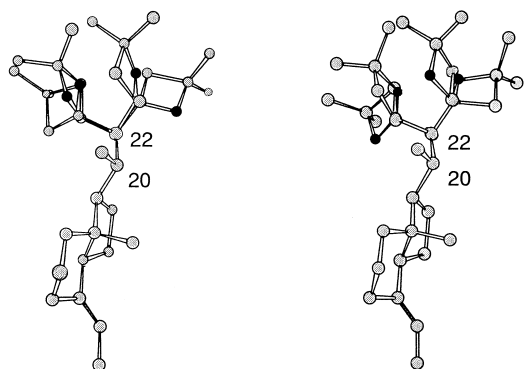


Figure 3. Superposition of four families of conformers of two oxetane compound (**7a**; left, **7b**; right) at 0–8 kJ/mol as their deprotonated and defluorinated forms.

C adopted by *S*-isomers (**5a,6b**) might be more appropriate to keep the size and lipophilicity of the molecule favorable to fit into the VDR cavity than *R*-isomers (**5b,6a**), especially when fluorine is substituted. On the other hand, oxetane analogues (**7a,7b**) adopt four families of conformers within 8 kJ/mol from the lowest energy conformer.²³ As shown in Figure 3, the four conformers of **7a** were nearly superimposable on those of **7b**, except for the positions of the oxygen atom in the oxetane ring. The positional change of the oxygen atom in the oxetane ring may explain the difference of binding to the VDR.

Conclusion

We developed a convenient synthetic method which enables flexible modification at C-23 of **3**. An effective construction of the side chain of **10** was achieved by aldol reaction with HFA. As the result of preliminary biological evaluations of 26,27- F_6 analogues, with respect to VDR affinities, the modification at C-23 of **3** influenced biological activities significantly. It depends on both the nature and stereochemistry of the substituents. In contrast, the HL-60 cell differentiation property was less varied than VDR affinity. It was also found that *R/S*-OH and keto-modificational effects of **3** to VDR affinity provided a different biological profile compared to **1**.

Experimental

All reactions involving oxygen- or moisture-sensitive compounds were carried out under a dry nitrogen atmosphere. Reaction temperatures refer to external bath temperatures. Reactions were monitored by thin layer chromatography (TLC) using Merck TLC plates (silica gel 60 F_{254} , 0.25 mm thickness). After ultraviolet illumination at 254 nm, the plates were visualized by immersion in a solution of phosphomolybdic acid in MeOH followed by heating. Column chromatography purification was performed with Merck silica gel (silica gel 60, 70–230 mesh), and PLC purification was performed with Merck PLC plates (silica gel 60 F_{254} , 1 mm or 0.5 mm thickness). ^1H NMR data were measured in CDCl_3 . Chemical shifts are reported as δ units (ppm) downfield from tetramethylsilane (δ 0.00) using residual solvent

signal as an internal standard: δ 7.26. Dichloromethane, tetrahydrofuran (THF), and diethyl ether (Et_2O) was dried over molecular sieves 4Å (MS-4Å). The purity of products (**4**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**) was judged to be at least 98% by HPLC analysis (UV, 265 nm). (*) The yields were considered with the recovery of starting materials.

3 β -[(*t*-Butyldimethylsilyl)oxy]-20(*S*)-(cyanomethyl)-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (15). To a suspension of **14** (24.5 g, 40.9 mmol) in DMSO (250 mL) was added potassium cyanide (13.0 g, 265 mmol) and heated for 30 min at 90 °C. The reaction mixture was cooled in an ice bath, and then diluted with water, and extracted with ethyl acetate. The organic layer was washed with water, brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc :hexane, 1:10) to give **15** (13.0 g, 70%) as an amorphous solid. IR (neat): 2932, 2858, 2247, 1467, 1254, 1098, 836 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06–0.07 (6H, 2s), 0.58 (3H, s), 0.88 (9H, s), 1.18 (1H, d, J = 6.5 Hz), 3.85 (1H, m), 4.64 (1H, br s), 4.92 (1H, br s), 5.85 (1H, d, J = 11.5 Hz), 6.46 (1H, d, J = 11.5 Hz).

3 β -[(*t*-Butyldimethylsilyl)oxy]-20(*S*)-(formylmethyl)-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (16). Diisobutylaluminum hydride (DIBALH, 0.93 M in hexane, 2.27 mL, 2.11 mmol) was added to a solution of **15** (640 mg, 1.41 mmol) in THF (12 mL) at –70 °C. After warming to room temperature, the reaction mixture was stirred for 30 min. When the starting material was detected by TLC, DIBALH (0.93 M in hexane solution, 2.27 mL, 2.11 mmol) was added to the reaction mixture at –70 °C. After the starting material was consumed completely, 5% aqueous HCl was added. The mixture was extracted with ethyl acetate. The organic layer was washed with 5% aqueous HCl, saturated NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc :hexane, 1:10) to give **16** (350 mg, 54%) as an amorphous solid. IR (neat): 2929, 2856, 1728 1471, 1251, 1098, 836 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06–0.07 (6H, 2s), 0.61 (3H, s), 0.88 (9H, s), 1.03 (1H, d, J = 6.5 Hz), 3.86 (1H, m), 4.64 (1H, br s), 4.93 (1H, br s), 5.86 (1H, d, J = 11.5 Hz), 6.49 (1H, d, J = 11.5 Hz), 9.77 (1H, d, J = 2.5 Hz).

3 β -[(*t*-Butyldimethylsilyl)oxy]-20(*S*)-(propyl-23-oxo)-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (17). Methylmagnesium bromide (0.99 M in Et_2O , 1.04 mL, 1.03 mmol) was added to a solution of **16** (350 mg, 0.766 mmol) in Et_2O (7 mL) at room temperature. After stirring for 10 min, 10% aqueous NH_4Cl was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc :hexane, 1:5) to give the corresponding alcohol as an amorphous solid (195 mg, 54%). IR (neat): 3370, 2930, 2856, 1471, 1253, 1098, 836 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06–0.07 (6H, 2s), 0.57 and 0.59 (total 3H, s), 0.88 (9H, s), 1.60 (3H, s),

3.86 (2H, m), 4.64 (1H, br s), 4.92 (1H, br s), 5.85 (1H, d, $J=11.5$ Hz), 6.47 (1H, d, $J=11.5$ Hz). Tetrapropylammonium perruthenate (TPAP, 26 mg, 0.074 mmol) was added in one portion to a stirred mixture of the alcohol (175 mg, 0.37 mmol), 4-methylmorpholine *N*-oxide (NMO, 65 mg, 0.55 mmol) and powdered 4 Å molecular sieves (125 mg) in dichloromethane (4 mL) at room temperature. After 15 min, the reaction mixture was passed through silica gel chromatography (EtOAc:hexane, 1:10) to give **17** (136 mg, 78%) as an amorphous solid. IR (neat): 2949, 2856, 1717 1470, 1252, 1098, 836 cm^{-1} . ^1H NMR (300 MHz) δ : 0.05–0.07 (6H, 2s), 0.59 (3H, s), 0.88 (9H, s), 0.94 (3H, d, $J=6.5$ Hz), 2.12 (3H, s), 3.85 (1H, m), 4.64 (1H, br s), 4.92 (1H, br s), 5.85 (1H, d, $J=11.5$ Hz), 6.46 (1H, d, $J=11.5$ Hz).

1 α -Hydroxy-3 β -[(*t*-butyldimethylsilyl)oxy]-20(*S*)-(n-propyl-23-oxo)-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (18). NMO (151 mg, 1.29 mmol) was added to a solution of **17** (117 mg, 0.249 mmol) in dichloromethane (1.5 mL), and then a solution of selenium dioxide (SeO_2 , 28 mg, 0.252 mmol) in methanol (1.5 mL) was added, and the mixture was heated under reflux for 2 h. After cooling to room temperature, water was added and extracted with dichloromethane. The organic layer was washed with saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 1:10) and the further HPLC purification (Zorbax BP SIL, 10 mm $\times$ 25 cm, EtOAc:hexane, 1:10) to give 1 α -hydroxy compound **18** (50 mg, 41%) as an amorphous solid. IR (neat): 3436, 2950, 2856, 1713, 1472, 1253, 1078, 836 cm^{-1} . ^1H NMR (270 MHz) δ : 0.07 (6H, s), 0.59 (3H, s), 0.88 (9H, s), 0.94 (3H, d, $J=6.5$ Hz), 2.13 (3H, s), 4.20 (1H, m), 4.50 (1H, br s), 4.94 (1H, br s), 5.06 (1H, br s), 5.85 (1H, d, $J=11.5$ Hz), 6.50 (1H, d, $J=11.5$ Hz).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-20(*S*)-(n-propyl-23-oxo)-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (19). A solution of **18** (50 mg, 0.103 mmol) in DMF (0.5 mL) containing imidazole (20 mg, 0.294 mmol) and *t*-butyldimethylsilyl chloride (TBSCl, 25 mg, 0.166 mmol) was stirred at room temperature for 3 h. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with 1N HCl, saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 1:10) to give **19** (55 mg, 89%) as an amorphous solid. IR (neat): 2952, 2857, 1718, 1470, 1253, 1083, 835 cm^{-1} . ^1H NMR (270 MHz) δ : 0.05–0.06 (12H, 2s), 0.58 (3H, s), 2.13 (3H, s), 4.22 (1H, m), 4.53 (1H, m), 4.94 (1H, br s), 4.98 (1H, br s), 5.82 (1H, d, $J=11.5$ Hz), 6.45 (1H, d, $J=11.5$ Hz). MS(EI) m/z : 600 (M^+), 585 (M^+-CH_3), 543 (M^+-tBu), 486 (M^+-2tBu), 468 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}$), 453 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}-\text{CH}_3$). HRMS(EI) m/z : 600.4409 (M^+) (calcd for $\text{C}_{36}\text{H}_{64}\text{O}_3\text{Si}_2$ 600.4394).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-20(*S*)-(n-propyl-23-oxo)-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (13). A solution of **19** (430 mg, 0.715 mmol) in toluene (200 mL) containing triethylamine (3 drop), anthracene (400 mg, 2.24 mmol) was irradiated for 1 h to give, after PLC

purification (1 mm, EtOAc:hexane, 1:5), **13** (308 mg, 72%) as an amorphous solid. IR (neat): 2927, 2857, 1718, 1472, 1256, 1075, 829 cm^{-1} . ^1H NMR (270 MHz) δ : 0.05–0.06 (12H, 2s), 0.57 (3H, s), 0.88 (18H, s), 0.93 (3H, d, $J=6.5$ Hz), 2.12 (3H, s), 4.18 (1H, m), 4.36 (1H, m), 4.86 (1H, d, $J=2.5$ Hz), 5.17 (1H, br s), 6.01 (1H, d, $J=11.0$ Hz), 6.23 (1H, d, $J=11.0$ Hz). MS(EI) m/z : 600 (M^+), 585 (M^+-CH_3), 543 (M^+-tBu), 486 (M^+-2tBu), 468 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}$), 453 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}-\text{CH}_3$). HRMS(EI) m/z : 600.4421 (M^+) (calcd for $\text{C}_{36}\text{H}_{64}\text{O}_3\text{Si}_2$ 600.4394).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-23-oxo-9,10-seccholesta-5(*Z*),7(*E*),10(19)-trien-25-ol (10). *n*-Butyllithium (1.64 M solution hexane, 9.1 mL, 14.9 mmol) was added dropwise to a solution of 1,1,1,3,3,3-hexamethyldisilazane (3.33 mL, 15.8 mmol) in THF (30 mL) at -70°C . The resulting mixture was stirred for 10 min. A solution of **13** (6.30 g, 10.5 mmol) in THF (35 mL) was added to the reaction mixture. After stirring for 10 min, hexafluoroacetone gas was blown through the mixture for 30 min. The reaction mixture was quenched with 10% aqueous NH_4Cl and extracted with ethyl acetate. The organic layer was washed with 1N HCl, saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 1:30) to give **10** (6.56 g 82%, (85%)* as an amorphous solid with the recovery of **13** (0.25 g, 4%). IR (neat): 3306, 2929, 1709, 1462, 1258, 1089, 834 cm^{-1} . ^1H NMR (270 MHz) δ : 0.04–0.06 (12H, 2s), 0.57 (3H, s), 0.88 (18H, s), 0.96 (3H, d, $J=6.2$ Hz), 2.88 (2H, br s), 4.20 (1H, m), 4.37 (1H, m), 4.85 (1H, d, $J=2.3$ Hz), 5.18 (1H, br s), 6.01 (1H, d, $J=11.0$ Hz), 6.23 (1H, d, $J=11.0$ Hz), 6.88 (1H, br s). MS(EI) m/z : 766 (M^+), 751 (M^+-CH_3), 709 (M^+-tBu), 697 (M^+-CF_3), 652 (M^+-2tBu), 634 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}$), 619 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}-\text{CH}_3$). HRMS(EI) m/z : 766.4299 (M^+) (calcd for $\text{C}_{39}\text{H}_{64}\text{O}_4\text{F}_6\text{Si}_2$ 766.4247).

26,26,26,27,27,27-Hexafluoro-23-oxo-9,10-seccholesta-5(*Z*),7(*E*),10(19)-trien-1 α ,3 β ,25-triol (4). *p*-Toluene-sulfonic acid monohydrate ($\text{TsOH}\cdot\text{H}_2\text{O}$, 35 mg) was added to a solution of **10** (185 mg, 0.241 mmol) in methanol (2.5 mL). After stirring for 2 h at room temperature, the reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaHCO_3 , brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 1:1) to give **4** (108 mg, 83%) as an amorphous powder. UV (EtOH): λ_{max} 264 nm. ^1H NMR (270 MHz) δ : 0.59 (3H, s), 0.97 (3H, d, $J=6.0$ Hz), 2.88 (2H, br s), 4.24 (1H, m), 4.43 (1H, m), 4.99 (1H, br s), 5.33 (1H, br s), 6.01 (1H, d, $J=11.5$ Hz), 6.37 (1H, d, $J=11.5$ Hz), 6.86 (1H, s). MS(EI) m/z : 538 (M^+), 520 ($\text{M}^+-\text{H}_2\text{O}$), 502 ($\text{M}^+-2\text{H}_2\text{O}$). HRMS(EI) m/z : 538.2555 (M^+) (calcd for $\text{C}_{27}\text{H}_{36}\text{O}_4\text{F}_6$ 538.2518).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-9,10-seccholesta-5(*Z*),7(*E*),10(19)-trien-23-(*S*),25-diol (21a) and 1 α ,3 β -bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-9,10-seccholesta-5(*Z*),7(*E*),10(19)-trien-23(*R*),25-diol (21b). Sodium borohydride (514 mg, 13.6 mmol) was added to a solution

of **10** (7.0 g, 9.13 mmol) in THF (60 mL) and methanol (60 mL) at -10°C . After stirring for 10 min, the reaction mixture was quenched with 1N HCl and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was separated by silica gel chromatography (EtOAc:hexane, 1:20) to give **21a** [2.27 g, 32%; $R_f=0.25$ (EtOAc:hexane, 1:10)] as a white powder and **21b** [3.65 g, 52%, $R_f=0.5$ (EtOAc:hexane, 1:10)] as a white powder.

21a: IR (neat): 3308, 2953, 2857, 1472, 1207, 1075, 835 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06 (12H, s), 0.56 (3H, s), 0.88 (18H, s), 0.98 (3H, d, $J=5.9\text{ Hz}$), 4.20 (1H, m), 4.38 (2H, m), 4.86 (1H, d, $J=2.3\text{ Hz}$), 5.18 (1H, br s), 6.02 (1H, d, $J=11.0\text{ Hz}$), 6.23 (1H, d, $J=11.0\text{ Hz}$), 6.31 (1H, br s). MS(EI) m/z : 768 (M^+), 753 (M^+-CH_3), 711 (M^+-tBu), 636 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}$), 621 ($\text{M}^+-2t\text{Bu}-\text{CH}_3-\text{H}_2\text{O}$). HRMS(EI) m/z : 768.4432 (M^+) (calcd for $\text{C}_{39}\text{H}_{66}\text{O}_4\text{F}_6\text{Si}_2$ 768.4404).

21b: IR (neat): 3326, 2953, 2857, 1472, 1211, 1075, 835 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06 (12H, s), 0.56 (3H, s), 0.88 (18H, s), 1.00 (3H, d, $J=6.3\text{ Hz}$), 4.20 (1H, m), 4.37 (2H, m), 4.86 (1H, d, $J=2.3\text{ Hz}$), 5.18 (1H, br s), 6.02 (1H, d, $J=11.2\text{ Hz}$), 6.24 (1H, d, $J=11.2\text{ Hz}$), 6.28 (1H, br s). MS(EI) m/z : 768 (M^+), 753 (M^+-CH_3), 711 (M^+-tBu), 636 ($\text{M}^+-2t\text{Bu}-\text{H}_2\text{O}$), 621 ($\text{M}^+-2t\text{Bu}-\text{CH}_3-\text{H}_2\text{O}$). HRMS(EI) m/z : 768.4402 (M^+) (calcd for $\text{C}_{39}\text{H}_{66}\text{O}_4\text{F}_6\text{Si}_2$ 768.4404).

26,26,26,27,27,27-Hexafluoro-9,10-secocholesta-5(Z),7(E),10(19)-trien-1 α ,3 β ,23(S),25-tetraol (5a). Methanesulfonic acid (MsOH, 0.06 mL) was added to a solution of **21a** (1.0 g, 1.30 mmol) in methanol (15 mL). After stirring for 1 h at room temperature, the reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 3:2) to give **5a** (624 mg, 89%) as an amorphous powder. UV (EtOH): λ_{max} 264 nm. ^1H NMR (270 MHz) δ : 0.57 (3H, s), 0.98 (3H, d, $J=5.6\text{ Hz}$), 4.23 (1H, br s), 4.38 (1H, m), 4.42 (1H, m), 4.99 (1H, br s), 5.33 (1H, br s), 6.02 (1H, d, $J=11.5\text{ Hz}$), 6.29 (1H, br s), 6.37 (1H, d, $J=11.5\text{ Hz}$). MS(EI) m/z : 540 (M^+), 522 ($\text{M}^+-\text{H}_2\text{O}$), 507 ($\text{M}^+-\text{H}_2\text{O}-\text{CH}_3$), 504 ($\text{M}^+-2\text{H}_2\text{O}$), 489 ($\text{M}^+-2\text{H}_2\text{O}-\text{CH}_3$). HRMS(EI) m/z : 540.2631 (M^+) (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_4\text{F}_6$ 540.2674).

26,26,26,27,27,27-Hexafluoro-9,10-secocholesta-5(Z),7(E),10(19)-trien-1 α ,3 β ,23(R),25-tetraol (5b). MsOH (0.06 mL) was added to a solution of **21b** (1.64 g, 2.13 mmol) in methanol (15 mL). After stirring for 1 h at room temperature, the mixture was treated in the same manner described in the preparation of **4**. The residue was purified by silica gel chromatography (EtOAc:hexane, 3:2) to give **5b** (1.00 g, 87%) as an amorphous powder. UV (EtOH): λ_{max} 264 nm. ^1H NMR (270 MHz) δ : 0.58 (3H, s), 1.00 (3H, d, $J=6.3\text{ Hz}$), 4.23 (1H, m), 4.33 (1H, m), 4.42 (1H, m), 5.00 (1H, br s), 5.33 (1H, br s), 6.01 (1H, d, $J=10.5\text{ Hz}$), 6.37 (1H, d, $J=10.5\text{ Hz}$), 6.41 (1H, br s). MS(EI) m/z : 540 (M^+), 522 ($\text{M}^+-\text{H}_2\text{O}$), 507 ($\text{M}^+-\text{H}_2\text{O}$).

CH_3), 504 ($\text{M}^+-2\text{H}_2\text{O}$), 489 ($\text{M}^+-2\text{H}_2\text{O}-\text{CH}_3$). HRMS(EI) m/z : 540.2618 (M^+) (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_4\text{F}_6$ 540.2674).

23(R),26,26,26,27,27,27-Heptafluoro-9,10-secocholesta-5(Z),7(E),10(19)-trien-1 α ,3 β ,25-triol (6a). DAST (35 μL , 0.26 mmol) was added to a solution of **21a** (50 mg, 0.065 mmol) in dichloromethane (1.0 mL) at -50°C . The mixture was stirred for 10 min at the same temperature, warmed to room temperature and stirred for 1 h. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 1:10) to give crude mixture (30 mg) with the recovery of **21a** (10 mg, 20%). To a solution of the crude mixture (30 mg) in THF (1.0 mL) was added tetrabutylammonium fluoride (TBAF, 1M in THF, 0.5 mL) at room temperature and stirred overnight. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (ethanol:hexane, 1:5) and the further HPLC purification (Zorbax BP SIL, 10 mm $\times$ 25 cm, ethanol:hexane, 1:10) to give **6a** (15 mg, 42% (53%)*) as an amorphous solid. UV (EtOH): λ_{max} 265 nm. ^1H NMR (270 MHz) δ : 0.58 (3H, s), 1.00 (3H, d, $J=6.6\text{ Hz}$), 4.23 (1H, br s), 4.43 (1H, m), 5.00 (1H, br s), 5.0–5.3 (1H, m), 5.32 (1H, br s), 6.02 (1H, d, $J=11.5\text{ Hz}$), 6.38 (1H, d, $J=11.5\text{ Hz}$). MS(FAB+) m/z : 542 (M^+), 525 ($\text{M}^+-\text{H}_2\text{O}+\text{H}$), 523 (M^+-F), 505 ($\text{M}^+-\text{F}-\text{H}_2\text{O}$). HRMS(FAB+) m/z : 542.2597 (M^+) (calcd for $\text{C}_{27}\text{H}_{37}\text{O}_3\text{F}_7$ 542.2619).

23(S),26,26,26,27,27,27-Heptafluoro-9,10-secocholesta-5(Z),7(E),10(19)-trien-1 α ,3 β ,25-triol (6b). DAST (35 μL , 0.26 mmol) was added to a solution of **21a** (50 mg, 0.065 mmol) in dichloromethane (1.0 mL) at -50°C . The mixture was stirred for 10 min at the same temperature, warmed to room temperature and stirred for 1 h. The reaction mixture was treated in the same manner described in the preparation of **6a** to give **6b** (13 mg, 37%, (46%)*) as an amorphous solid with the recovery of **21b** (10 mg, 20%). UV (EtOH): λ_{max} 265 nm. ^1H NMR (270 MHz) δ : 0.57 (3H, s), 1.01 (3H, d, $J=6.3\text{ Hz}$), 4.23 (1H, br s), 4.43 (1H, m), 5.00 (1H, br s), 5.0–5.3 (1H, m), 5.33 (1H, br s), 6.02 (1H, d, $J=11.0\text{ Hz}$), 6.38 (1H, d, $J=11.0\text{ Hz}$). MS (FAB+) m/z : 542 (M^+), 525 ($\text{M}^+-\text{H}_2\text{O}+\text{H}$), 523 (M^+-F), 505 ($\text{M}^+-\text{F}-\text{H}_2\text{O}$). HRMS(FAB+) m/z : 542.2595 (M^+) (calcd for $\text{C}_{27}\text{H}_{37}\text{O}_3\text{F}_7$ 542.2619).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-23(S)-[(methanesulfonyl)oxy]-26,26,26,27,27,27-hexafluoro-9,10-secocholesta-5(Z),7(E),10(19)-trien-25-ol (23a). Methanesulfonyl chloride (MsCl, 0.25 mL, 3.23 mmol) was added to a solution of **21a** (100 mg, 0.130 mmol) in pyridine (1.0 mL). The reaction mixture was stirred at 0°C for 1 h. Water was added to the mixture and extracted with ethyl acetate. The organic layer was washed with 1N HCl, saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 1:10) to give **23a** (86 mg, 78%) as an amorphous solid.

^1H NMR (300 MHz) δ : 0.06 (12H, s), 0.55 (3H, s), 0.88 (18H, s), 1.03 (3H, d, $J=5.9$ Hz), 2.83 (1H, m), 3.07 (3H, s), 4.19 (1H, m), 4.38 (1H, m), 4.59 (1H, br s), 4.85 (1H, br s), 5.19 (2H, m), 6.02 (1H, d, $J=11.3$ Hz), 6.23 (1H, d, $J=11.3$ Hz).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-23(*R*),25-epoxy-26,26,27,27,27-hexafluoro-9,10-secocholesta-5(*Z*),7(*E*),10(19)-triene (24a). To a solution of **23a** (65 mg, 0.077 mmol) in methanol (0.5 mL) was added 5% KOH-MeOH (1.0 mL) at room temperature. After stirring for 10 min, the reaction mixture was concentrated and purified by PLC (0.5 mm, EtOAc:hexane, 3:2) to give **24a** (40 mg, 69%). ^1H NMR (300 MHz) δ : 0.06 (12H, s), 0.54 (3H, s), 0.88 (18H, s), 0.93 (3H, d, $J=6.6$ Hz), 2.82 (1H, m), 4.19 (1H, m), 4.37 (1H, m), 4.86 (1H, br s), 5.01 (1H, m), 5.18 (1H, br s), 6.02 (1H, d, $J=11.3$ Hz), 6.23 (1H, d, $J=11.3$ Hz).

23(*R*),25-Epoxy-26,26,26,27,27,27-hexafluoro-9,10-secocholesta-5(*Z*),7(*E*),10(19)-triene (7a). To a solution of **24a** (40 mg, 0.053 mmol) in THF (1.0 mL) was added TBAF (1M in THF, 0.5 mL) at room temperature and stirred overnight. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 3:2) to give **7a** (19 mg, 68%) as an amorphous solid. UV (EtOH): λ_{max} 265 nm. ^1H NMR (300 MHz) δ : 0.55 (3H, s), 0.93 (3H, d, $J=6.6$ Hz), 2.60 (2H, m), 2.83 (1H, m), 4.24 (1H, m), 4.43 (1H, m), 5.00 (2H, m), 5.32 (1H, br s), 6.02 (1H, d, $J=11.5$ Hz), 6.37 (1H, d, $J=11.5$ Hz). MS(FAB+) m/z : 522 (M^+), 505 ($\text{M}^+ - \text{H}_2\text{O} + \text{H}^+$), 487 ($\text{M}^+ - 2\text{H}_2\text{O} + \text{H}^+$). HRMS(FAB+) m/z : 522.2574 (M^+) (calcd for $\text{C}_{27}\text{H}_{36}\text{O}_3\text{F}_6$ 522.2568).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-23(*R*)-[(methanesulfonyl)oxy]-26,26,26,27,27,27-hexafluoro-9,10-secocholesta-5(*Z*),7(*E*),10(19)-trien-25-ol (23b). MsCl (0.5 mL, 6.46 mmol) was added to a solution of **21a** (200 mg, 0.260 mmol) in pyridine (2.0 mL). The same treatment as described in the preparation of **23a** gave **23b** (220 mg, 100%) as an amorphous solid. ^1H NMR (300 MHz) δ : 0.06 (12H, s), 0.55 (3H, s), 0.88 (18H, s), 1.01 (3H, d, $J=6.3$ Hz), 3.08 (3H, s), 4.19 (1H, m), 4.38 (1H, m), 4.86 (1H, br s), 4.90 (1H, br s), 5.19 (2H, m), 6.02 (1H, d, $J=11.2$ Hz), 6.25 (1H, d, $J=11.2$ Hz).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-23(*R*),25-epoxy-26,26,27,27,27-hexafluoro-9,10-secocholesta-5(*Z*),7(*E*),10(19)-triene (24b). To a solution of **23b** (580 mg, 0.685 mmol) in MeOH (3.0 mL) was added 5% KOH-MeOH (5.0 mL). The same treatment as described in the preparation of **24a** gave **24b** (283 mg, 55%) as an amorphous solid. ^1H NMR (300 MHz) δ : 0.06 (12H, s), 0.52 (3H, s), 0.88 (18H, s), 0.96 (3H, d, $J=6.2$ Hz), 2.82 (1H, m), 4.19 (1H, m), 4.38 (1H, m), 4.86 (1H, br s), 5.00 (1H, quint., $J=7.2$ Hz), 5.18 (1H, br s), 6.01 (1H, d, $J=11.2$ Hz), 6.23 (1H, d, $J=11.2$ Hz).

23(*S*),25-Epoxy-26,26,26,27,27,27-hexafluoro-9,10-secocholesta-5(*Z*),7(*E*),10(19)-triene (7b). To a solution of

24b (118 mg, 0.157 mmol) in THF (1.0 mL) was added TBAF (1M in THF, 0.5 mL) at room temperature and stirred overnight. The same treatment as described in the preparation of **7a** gave **7b** (67 mg, 82%) as an amorphous solid. ^1H NMR (300 MHz) δ : 0.53 (3H, s), 0.96 (3H, d, $J=6.2$ Hz), 2.83 (1H, m), 4.23 (1H, m), 4.44 (1H, m), 5.00 (2H, m), 5.33 (1H, br s), 6.01 (1H, d, $J=11.1$ Hz), 6.37 (1H, d, $J=11.1$ Hz). MS(FAB+) m/z : 522 (M^+), 505 ($\text{M}^+ - \text{H}_2\text{O} + \text{H}^+$), 487 ($\text{M}^+ - 2\text{H}_2\text{O} + \text{H}^+$). HRMS(FAB+) m/z : 522.2549 (M^+) (calcd for $\text{C}_{27}\text{H}_{36}\text{O}_3\text{F}_6$ 522.2568).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-23-oxo-9,10-secocholesta-5(*Z*),7(*E*),10(19)-trien-25-ol (11). To a solution of $\text{LiN}(\text{TMS})_2$ (1.04 M in hexane, 0.63 mL, 0.66 mmol) in THF (1.0 mL) was added dropwise a solution of **13** (300 mg, 0.50 mmol) in THF (1.0 mL) at -78°C . After stirring for 10 min, acetone (60 μL , 0.82 mmol) was added. After stirring for 10 min, the reaction mixture was quenched by 1N HCl and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 . The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc:hexane, 1:20 $\rightarrow$ 1:10) to give **11** (224 mg, 68% (87%)) as an amorphous solid with recovery of **13** (66 mg, 22%). ^1H NMR (300 MHz) δ : 0.05 (12H, s), 0.56 (3H, s), 0.86 (18H, s), 0.93 (3H, d, $J=6.4$ Hz), 2.55–2.58 (2H, m), 3.98 (1H, s), 4.18 (1H, m), 4.36 (1H, m), 4.85 (1H, br s), 5.16 (1H, br s), 6.00 (1H, d, $J=11.2$ Hz), 6.22 (1H, d, $J=11.2$ Hz).

23-Oxo-9,10-secocholesta-5(*Z*),7(*E*),10(19)-trien-1 α ,3 β ,25-triol (8). MsOH (20 μL) was added to a solution of **11** (86 mg) in methanol (5.0 mL). The same treatment as described in the preparation of **5a** and purification by silica gel chromatography (EtOAc:hexane, 2:1) gave **8** (49 mg, 87%) as an amorphous solid. ^1H NMR (300 MHz) δ : 0.58 (3H, s), 0.94 (3H, d, $J=6.4$ Hz), 1.24 (6H, s), 2.56–2.58 (2H, m), 3.97 (1H, s), 4.22 (1H, m), 4.42 (1H, m), 5.00 (1H, br s), 5.33 (1H, br s), 6.01 (1H, d, $J=11.4$ Hz), 6.37 (1H, d, $J=11.4$ Hz). MS(FAB+) m/z : 431 ($\text{M}^+ + 1$), 430 (M^+), 413 ($\text{M}^+ - \text{H}_2\text{O} + \text{H}^+$). HRMS(FAB+) m/z : 430.3042 (M^+) (calcd for $\text{C}_{27}\text{H}_{42}\text{O}_4$ 430.3083).

1 α ,3 β -Bis[(*t*-butyldimethylsilyl)oxy]-26,27-dimethoxy-23-oxo-9,10-secocholesta-5(*Z*),7(*E*),10(19)-trien-25-ol (12). To a solution of $\text{LiN}(\text{TMS})_2$ (1.04 M in hexane, 1.26 mL, 1.31 mmol) in THF (5.0 mL) was added dropwise a solution of **13** (600 mg, 1.0 mmol) in THF (3.0 mL) at -78°C . After stirring for 10 min, dimethoxyacetone (0.35 g, 3.0 mmol) was added. The same treatment as described in the preparation of **11** and purification by silica gel chromatography (EtOAc:hexane, 1:3) gave **12** (660 mg, 92%) as an amorphous solid. ^1H NMR (300 MHz) δ : 0.04 (12H, s), 0.55 (3H, s), 0.85 (18H, s), 0.91 (3H, d, $J=6.4$ Hz), 2.65 (2H, s), 3.32 (10H, s), 4.04 (1H, s), 4.17 (1H, m), 4.34 (1H, m), 4.83 (1H, br s), 5.15 (1H, br s), 5.99 (1H, d, $J=11.2$ Hz), 6.20 (1H, d, $J=11.2$ Hz).

26,27-Dimethoxy-23-oxo-9,10-secocholesta-5(*Z*),7(*E*),10(19)-trien-1 α ,3 β ,25-triol (9). MsOH (16 μL) was added to a solution of **11** (69 mg, 0.096 mmol) in methanol (4.0 mL). The same treatment as described in the preparation of **5a** silica gel chromatography (EtOAc:

hexane, 3:1) gave **9** (41 mg, 87%) as an amorphous solid. ^1H NMR (300 MHz) δ : 0.56 (3H, s), 0.92 (3H, d, $J=6.4$ Hz), 2.65 (2H, s), 3.33 (10H, s), 4.04 (1H, s), 4.20 (1H, m), 4.41 (1H, m), 4.97 (1H, br s), 5.31 (1H, br s), 6.00 (1H, d, $J=11.4$ Hz), 6.34 (1H, d, $J=11.4$ Hz). MS(FAB+) m/z : 491 ($\text{M}^+ + 1$), 490 (M^+), 473 ($\text{M}^+ - \text{H}_2\text{O} + \text{H}^+$). HRMS(FAB+) m/z : 490.3333 (M^+) (calcd for $\text{C}_{29}\text{H}_{46}\text{O}_6$ 490.3297).

Measurement of vitamin D₃ receptor binding affinity.

Chick intestinal $1\alpha,25$ -dihydroxyvitamin D₃ receptor was obtained from Yamasa Biochemical (Chiba, Japan) and dissolved in 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose, 25 mM KCl, 5 mM MgCl₂, 1 mM EDTA and 12 mM thioglycerol just before use. The receptor solution (0.2 mL, 0.08 mg protein) was incubated with 1.8 nM [^3H]- $1\alpha,25$ -dihydroxyvitamin D₃ (180 Ci/nmol) and $1\alpha,25$ -dihydroxyvitamin D₃ or analogue at various concentration for 24 h at 4 °C. The bound and free [^3H]- $1\alpha,25$ -dihydroxyvitamin D₃ were separated by treatment with dextran-coated charcoal and centrifuged at 3000 rpm for 15 min at 4 °C. The radioactivity of supernatant with ACS-II (Amersham, UK) was counted.

Measurement of cellular differentiation. Human leukemia HL-60 cells were incubated as described previously.¹⁹ The incubation was carried out for 4 days and at the end of the fourth day, superoxide production was measured by nitro blue tetrazolium (NBT) reduction. The number of cells containing intracellular black-blue formazan deposits was determined by light microscopy. At least 200 cells were counted in duplicate per determination.

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References and Notes

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