

Synthesis and Biological Evaluations of A-Ring Isomers of 26,26,26,27,27,27-Hexafluoro-1,25-dihydroxyvitamin D₃

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Abstract—The activated vitamin D₃ derivative 26,27-F₆-1 α ,25(OH)₂D₃ (**2a**), its three A-ring diastereomers (**2b**, **2c**, **2d**), and 5,6-*trans* isomer (**2e**) were prepared. Two analogues (**2b**, **2c**) of these isomers were synthesized by a palladium catalyzed coupling reaction using vinyl bromide **5** and enynes (**6a**, **6b**), which were derived from readily commercially available 2*S*-(+)-glycidyl *p*-toluenesulfonate **7**, as a common starting material. Competitive vitamin D receptor (VDR) binding affinities of these diastereomers of **2a** were evaluated. Interestingly, the stereochemical effects at C-1,3 of **2a** were considerably more moderate than those of 1 α ,25(OH)₂D₃ (**1**). In particular, isomerization at the 5,6-double bond of **2a** only slightly reduced VDR affinity, whereas 5,6-*trans*-1 α ,25(OH)₂D₃ had a significantly lower binding affinity than **1**. © 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 important roles in regulating cell proliferation and differentiation of a variety of cell types, keratinocytes, immunological and malignant cells.¹ These actions are believed to be mediated via binding to the vitamin D receptor (VDR), which belongs to the superfamily of nuclear receptors for glucocorticoids, estrogens and retinoic acid. Efforts are currently aimed at the development of more therapeutically useful analogues of 1 α ,25(OH)₂D₃, the effects of which on cell proliferation and differentiation may be useful for treatment of disorders ranging from psoriasis to cancer.² A large number of side-chain-modified analogues have been described during the last decade.³ In the A-ring and the triene system, several isomers possessing unnatural configurations and geometries of 1 α ,25(OH)₂D₃ (**1**)^{4–8} were synthesized and their biological activities including VDR affinities were evaluated. On the other hand, previous studies have demonstrated that 26,26,26,27,27,27-hexafluoro-1 α ,25-dihydroxyvitamin D₃ (**2a**; 26,27-F₆-1 α ,25(OH)₂D₃) showed potentiation of various aspects of some biological activities of vitamin D₃.^{9,10} Therefore, we decided to investigate the extent to which A-ring stereochemical modification of **2a** influences VDR affinities. In addition, we also confirmed the

geometric effect of the 5,6-double bond of **2a**. This report concerns a synthetic method to A-ring diastereomers and 5,6-*trans* isomer of **2a** and their VDR binding affinities.

Results and Discussion

Synthesis

Our synthetic plan for the synthesis of these desired analogues is described in Scheme 1. For the preparation of **2c** and **2d**, the convergent method developed by Trost et al.¹¹ was thought to be useful. The enynes **6a**, **6b** could possibly be derived from **7**. As for the preparation of **2a** and bromolefin **5**, we decided to apply the method, which was recently developed by our laboratory,¹² to prepare 26,27-F₆ vitamin D₃ analogues via aldol reaction using hexafluoroacetone (HFA). In practice, **2a** was prepared in the following method, as shown in Scheme 2. Treatment of aldehyde **4**, which was prepared from readily available vitamin D₂,¹³ with methylmagnesium bromide followed by TPAP oxidation¹⁴ gave methylketone **8**. HFA aldol reaction and subsequent reduction of the 23-keto group by NaBH₄ afforded 23*R*-alcohol **10** as the major product.¹² Conversion to the acetate **11**, using Ac₂O/pyridine, was followed by the introduction of the MOM group at the 25-OH group and subsequent removal of the acetyl group gave secondary alcohol **13**. Formation of thionocarbonate **14** and reduction by tributylstannane¹⁵ afforded **3**. Deprotection of **3** with

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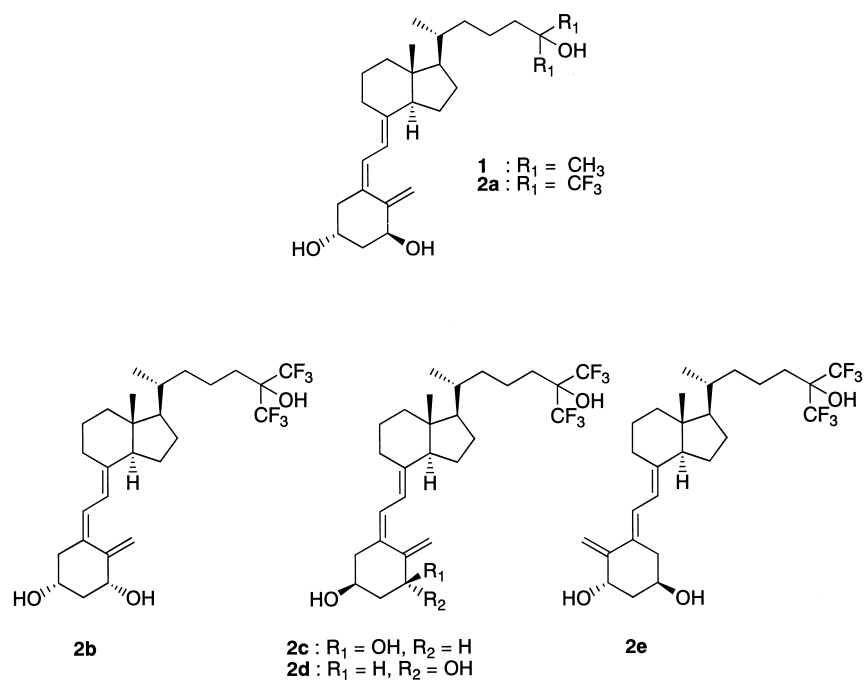
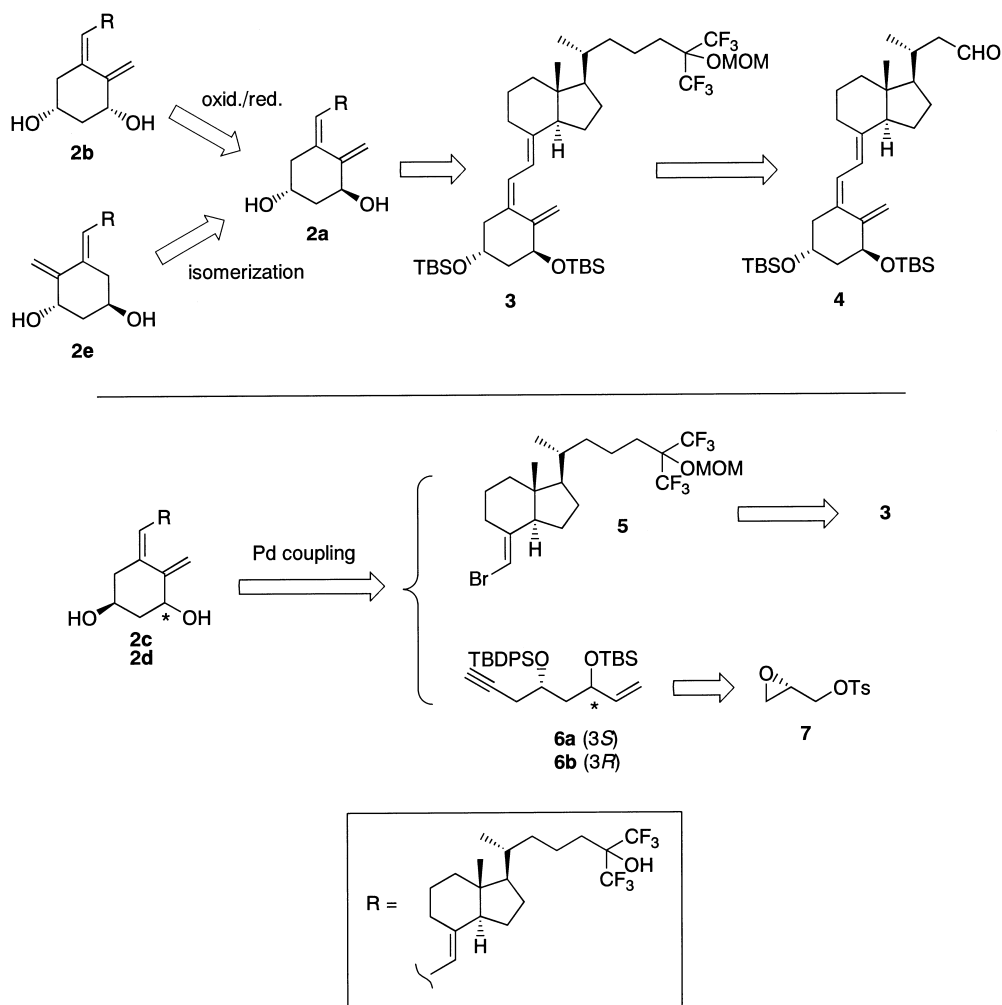
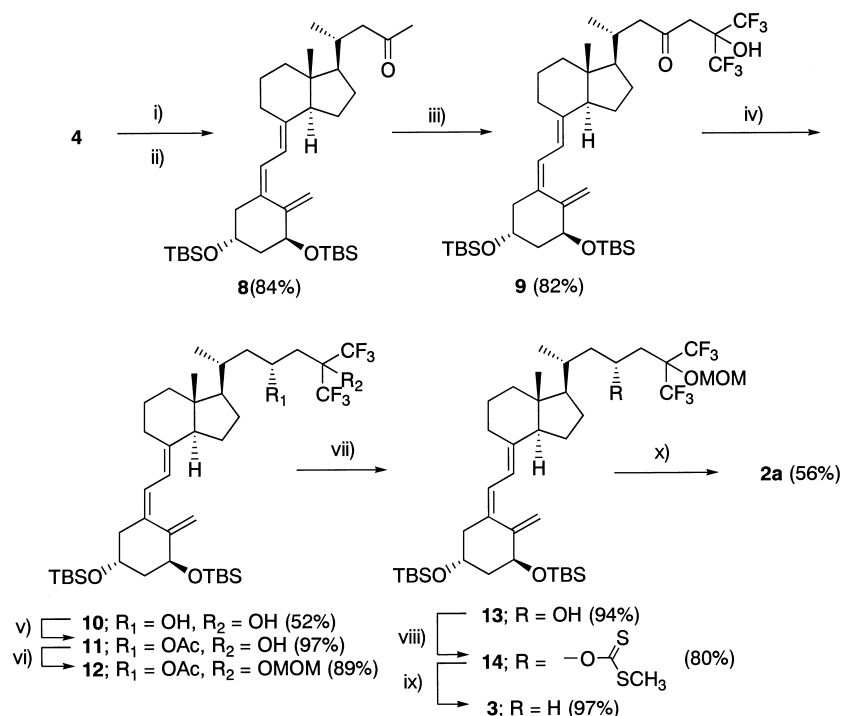
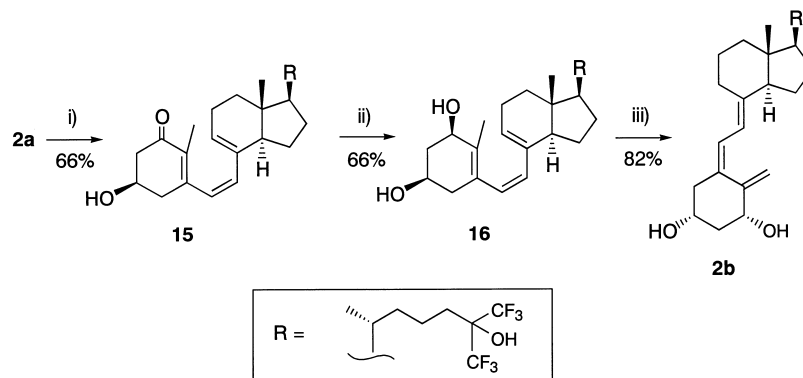


Figure 1.

Scheme 1. Synthetic plan for the synthesis of A-ring isomers of **2a**.



Scheme 2. The preparation of **2a** from **4**. (i) MeMgBr, Et₂O, 0 °C, 30 min; (ii) TPAP, NMO, MS4A, CH₂Cl₂, rt, 15 min; (iii) CF₃COCF₃, LiN(TMS)₂, THF, –70 °C, 30 min; (iv) NaBH₄, THF–MeOH, –10 °C, 10 min; (v) Ac₂O, pyridine, rt, 12 h; (vi) MOMCl, *i*Pr₂NEt, CHCl₃, reflux, 1 h; (vii) KOH–MeOH, 0 °C, 1 h; (viii) NaH, CS₂, rt, overnight then CH₃I; (ix) nBu₃SnH, toluene, reflux, 1 h; (x) MsOH, MeOH, rt, 2 h.

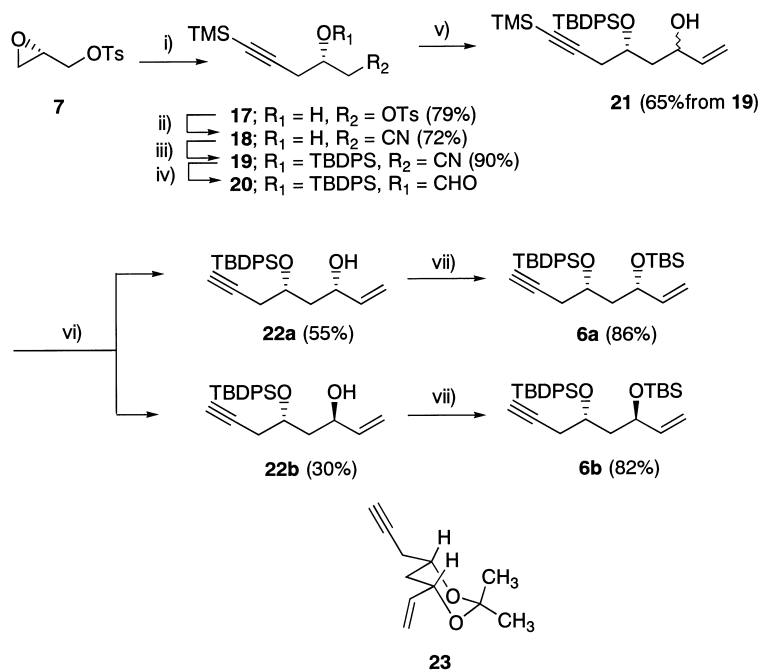


Scheme 3. The preparation of 1-epimer **2b** from **2a**. (i) Dess–Martin periodinane, CH₃CN, rt, 4 h; (ii) NaBH₄, MeOH, 0 °C, 1.5 h; (iii) acetone, 80 °C, 6 h.

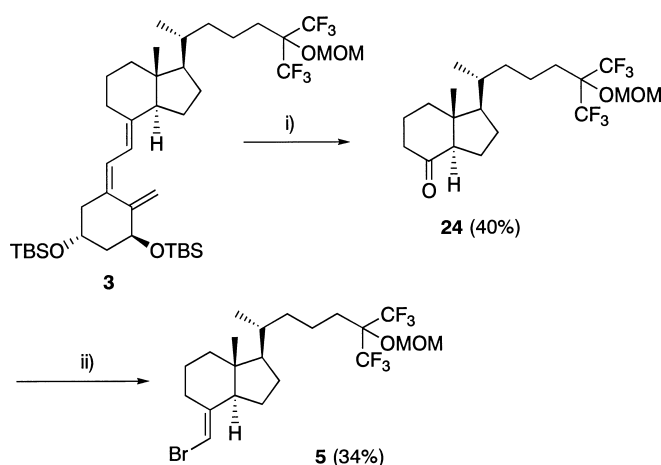
methanesulfonic acid in MeOH gave 26,27-F₆-1 α ,25(OH)₂D₃ (**2a**). The 1-*epi* isomer **2b** was prepared from **2a** in the same manner as described in the preparation of the 1-*epi* isomer of **1**.⁴ Dess–Martin oxidation^{16,17} of **2a** afforded ketone **15**, which upon reduction with NaBH₄ followed by thermal isomerization gave **2b** (Scheme 3).

The other two diastereomers (**2c**, **2d**) were synthesized by Trost's convergent method using a palladium-catalyzed coupling reaction.¹¹ This method has already been applied to the synthesis of A-ring diastereomers of **1** and its 20-*epi* analogues by Takayama et al.^{5,6,18} In this report, we also employed Trost's method for the preparation of two A-ring diastereomers of **2a**. However, as for the preparation of the A-ring precursor enyne compounds (**6a** and **6b**), we chose a readily commercially available chiral building block moiety, 2*S*-(+)-glycidyl *p*-

toluenesulfonate (**7**), as the common starting material; the addition of TMS-acetylene to the epoxide **7** gave **17**,¹⁹ which was treated with KCN in DMSO to afford **18**. The protection of secondary alcohol with the *t*-butyldiphenylsilyl (TBDPS) group followed by reduction with DIBALH gave aldehyde **20**. Grignard addition with vinylmagnesium bromide and subsequent removal of the TMS group of **21** under basic conditions gave a 1.8:1 ratio of the two diastereomer mixture (**22a** and **22b**). These isomers were separated by HPLC. To assign the stereochemistry, **22a** was converted to acetonide **23** whose ¹³C NMR spectrum showed signals for the geminal dimethyl group indicative of a *syn*-1,3-diol chemistry.^{11,20,21} Protection of the alcohol with the *t*-butyldimethylsilyl (TBS) group gave the desired 1,7-enynes **6a** and **6b**, respectively. The corresponding CD-ring fragment was derived from **3** as shown in Scheme 5. Ozonolysis of **3** gave ketone **24**. The highly *E*-selective bromoolefination



Scheme 4. The preparation of enyne compounds (**6a**, **6b**) from **7**. (i) Trimethylsilylacetylene, $n\text{BuLi}$, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, THF, -70°C ; (ii) KCN, DMSO, 40°C , 3 h; (iii) TBDPSCl, imidazole, DMF, 40°C , 2 h; (iv) DIBALH, CH_2Cl_2 , -10°C , 30 min; (v) vinylmagnesium bromide, THF, $-70^\circ\text{C} \rightarrow \text{rt}$, 30 min; (vi) K_2CO_3 , MeOH, rt, 1 h; (vii) TBSCl, imidazole, DMF, 40°C , 2 h.



Scheme 5. Preparation of the CD ring fragment **5** from **3**. (i) O_3 , PPh_3 , -78°C , 30 min; (ii) $\text{LiN}(\text{TMS})_2$, bromotriphenylphosphonium bromide, toluene, -78°C , 30 min.

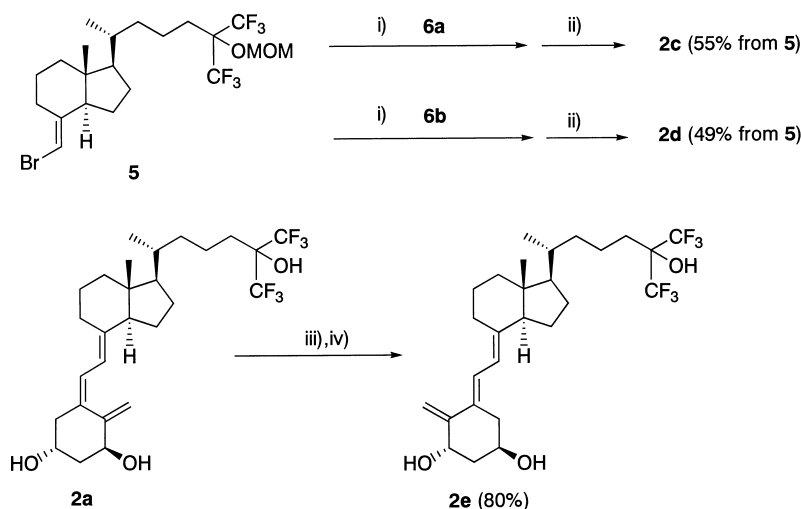
of CD-ring ketone derivatives by bromomethyl-triphenylphosphorane, generated using $\text{NaN}(\text{TMS})_2/\text{THF}$ was also reported by Trost et al.¹¹ However, in the case of **24**, bromoolefin **5** was obtained in only 30% yield under the identical reaction conditions. After investigations, we found that the use of $\text{LiN}(\text{TMS})_2/\text{toluene}$ provided **5** in a slightly increased yield (34%), without decreasing the geometrical selectivity ($E:Z = 100:1$), along with recovered **24** (45%). The generation of the lithium enolate of **24** in situ would account for the recovered ketone. As a result, due to the recovery of **24**, vinyl bromide **5** was obtained in 62% yield. Palladium-catalyzed coupling reaction of vinyl bromide **5** and 1,7-enyne **6a** by $\text{Pd}_2(\text{dba})_3 \cdot \text{CHCl}_3$ followed by deprotection with methanesulfonic acid in MeOH gave 3-*epi* isomer **2c**. The 1 β ,3 α -isomer, **2d**, was synthesized from **5** and 1,7-enyne **6b** by the same method as for **2c**. As for the pre-

paration of 5,6-*trans*-26,27- F_6 -1 α ,25(OH) $_2\text{D}_3$ (**2e**), the same procedure used for the preparation of 5,6-*trans*-1,25(OH) $_2\text{D}_3$ was applied via a cheletropic reaction.⁸

Biological evaluation

Biological evaluations of A-ring isomers of **3** are summarized in Table 1. The binding affinity to VDR was determined by a competitive binding assay using the cytosolic receptor from chick intestine.²²

While the analogous epimerization of the 1-OH group (**2a–2b**) provided a 4-fold reducing effect on binding affinity, that of the 3-OH group (**2a–2c**) had a modest effect (1.5-fold reduction). Double epimerization of the 1,3-OH groups (**2a–2d**) led to a substantial decrease in binding affinity (7-fold reduction). These results show that



Scheme 6. Synthesis of **2c**, **2d**, **2e**. (i) $(\text{dba})_3\text{Pd}_2\cdot\text{CHCl}_3$, NEt_3 , toluene, reflux, 10 min; (ii) MsOH , MeOH , rt, 1 h; (iii) liquified SO_2 , CH_2Cl_2 , reflux, 30 min; (iv) NaHCO_3 , EtOH , reflux, 90 min.

Table 1. Binding activities of four A-ring isomers of 26,27- F_6 - $1\alpha,25(\text{OH})_2\text{D}_3$ (**2a**)^a for the $1\alpha,25(\text{OH})_2\text{D}_3$ receptor in the chick small intestine

Modification type	26,27- F_6 - $1,25(\text{OH})_2\text{D}_3$	$1,25(\text{OH})_2\text{D}_3$
Natural type	45	(100) ^b
1-Epimer	11	(23) ^b
3-Epimer	32	(71) ^b
1,3-Epimer	6.6	(15) ^b
5,6- <i>trans</i> -Isomer	41	(91) ^b

^aThe results for $1\alpha,25(\text{OH})_2\text{D}_3$ are normalized to 100.

^bThe results for 26,27- F_6 - $1\alpha,25(\text{OH})_2\text{D}_3$ are normalized to 100.

^cref 4, chick intestinal.

^dref 7, chick intestinal.

the inversion of stereochemistry at C_1 to an unnatural type (**1 β**) caused a more pronounced reduction on binding affinity than that of the 3-OH group. Inversion of the 1α -OH group of **2a** resulted in a modest reduction, whereas it has been reported that inversion of the 1α -OH group of **1**, which has a higher binding affinity than **2a**, lowered binding affinity by roughly two orders of magnitude.⁴ 5,6-*trans*-26,27- F_6 - $1\alpha,25(\text{OH})_3\text{D}_3$ (**2e**) showed a slightly decreased binding affinity compared to **2a**, whereas 5,6-*trans*- $1\alpha,25(\text{OH})_3\text{D}_3$ had a significantly lower binding affinity than **1**.⁷

Conclusion

26,27- F_6 - $1\alpha,25(\text{OH})_2\text{D}_3$ (**2a**), its three A-ring diastereomers (**2b**, **2c**, **2d**), and 5,6-*trans* isomer **2e** were synthesized from aldehyde **4**. Two analogues (**2b**, **2c**) of these isomers were synthesized by Trost's convergent method. Comparative VDR affinities of all of the isomers of **2a** were evaluated. We found that the stereochemistry of C-1,3 position or the C-5,6 olefinic geometry affects the VDR affinity less in the case of **2a**, compared to the series of **1**. We considered it is because the binding affinity of the A-ring moiety of **2a** to VDR is lower than that of **1**. To our knowledge, among vitamin D_3 analogues, this is the first case which shows such a biological profile.

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). ^1H NMR data were measured in CDCl_3 . Chemical shifts are reported as δ units (ppm) downfield from tetramethylsilane (δ 0.0) using the residual solvent signal as an internal standard: δ 7.26.

(5Z,7E)-(1S,3R,20S)-1,3-Bis[*t*-butyldimethylsilyloxy]-20-(propyl-23-one)-9,10-*seco*-5,7,10(19)-pregnatriene (8). Methylmagnesium bromide (0.99 M in THF, 45.5 mL) was added to the solution of **4** (19.7 g, 33.6 mmol) in Et_2O (415 mL) at 0°C . After stirring for 30 min at the same temperature, saturated NH_4Cl was added, and the mixture 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 (ethyl acetate: hexane, 1:5) to give an alcohol as a colorless oil (18.4 g, 91%). IR (neat): 3353 (br), 2952, 2856, 1471, 1256, 1075, 833 cm^{-1} . ^1H NMR (270 MHz) δ : 0.05–0.06 (12H, m), 0.54 and 0.56 (total 3H, 2s), 3.91 (1H, m), 4.20 (1H, m), 4.36 (1H, m), 4.86 (1H, d, $J=2.0$ Hz), 5.17 (1H, br s), 6.01 (1H, d, $J=11.0$ Hz), 6.24 (1H, d, $J=11.0$ Hz). Tetrapropylammonium perruthenate (600 mg, 1.71 mmol) was added in one portion to a stirred mixture of the alcohol (10.0 g, 16.6 mmol) 4-methylmorpholine *N*-oxide (2.93 g, 25.0 mmol) and powdered 4 Å molecular sieves (5.0 g) in dichloromethane (133 mL) at room temperature. After 15 min, the reaction mixture was passed through silica gel chromatography (dichloromethane only) to give **8** (9.12 g, 92%) as a colorless oil. 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 ($\text{M}^+-2\text{tBu}-\text{H}_2\text{O}$), 453 ($\text{M}^+-2\text{tBu}-\text{H}_2\text{O}-\text{CH}_3$). HR-MS(EI) m/z : 600.4421 (M^+) (calcd for $\text{C}_{36}\text{H}_{64}\text{O}_3\text{Si}_2$ 600.4394).

(5Z,7E)-(1S,3R)-1,3-Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-23-oxo-9,10-*seco*-5,7,10(19)-cholestatrien-25-ol (9). This compound was prepared as previously reported.¹² IR (neat): 3306, 2929, 1709, 1462, 1258, 1089, 834 cm^{-1} . ^1H NMR (270 MHz) δ : 0.04–0.06 (total 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 ($\text{M}^+-2\text{tBu}-\text{H}_2\text{O}$), 619 ($\text{M}^+-2\text{tBu}-\text{H}_2\text{O}-\text{CH}_3$). HR-MS(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).

(5Z,7E)-(1S,3R,23R)-1,3-Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-9,10-*seco*-5,7,10(19)-cholestatriene-23,25-diol (10). This compound was prepared as previously reported.¹² 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, $J=6.3$ Hz), 4.20 (1H, m), 4.37 (2H, m), 4.86 (1H, d, $J=2.3$ Hz), 5.18 (1H, br s), 6.02 (1H, d, $J=11.2$ Hz), 6.24 (1H, d, $J=11.2$ Hz), 6.28 (1H, br s). MS(EI) m/z : 768 (M^+), 753 (M^+-CH_3), 711 (M^+-tBu), 636 ($\text{M}^+-2\text{tBu}-\text{H}_2\text{O}$), 621 ($\text{M}^+-2\text{tBu}-\text{CH}_3-\text{H}_2\text{O}$). HR-MS(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).

(5Z,7E)-(1S,3R,23R)-23-Acetoxy-1,3-bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-9,10-*seco*-5,7,10(19)-cholestatrien-25-ol (11). **10** (2.50 g, 3.25 mmol) was dissolved in pyridine: Ac_2O (2:1, 30 mL) and stirred at room temperature for 12 h. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with 5% HCl, saturated aqueous NaHCO_3 , and brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:20) to give **11** (2.56 g, 97%) as a colorless oil. IR (neat): 3301 (br), 2953, 2875, 1709, 1250, 1076, 835 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06, 0.07 (each 6H, s), 0.55 (3H, s), 0.87, 0.88 (each 9H, s), 0.92 (3H, d, $J=6.6$ Hz), 2.13 (3H, s), 2.44 (1H, dd, $J=13.2, 3.6$ Hz), 2.83 (1H, d, $J=10.9$ Hz), 4.19 (1H, m), 4.38 (1H, m), 4.87 (1H, br s), 4.97 (1H, br d, $J=10.2$ Hz), 5.19 (1H, br s), 6.02 (1H, d, $J=11.2$ Hz), 6.23 (1H, d, $J=11.2$ Hz), 6.50 (1H, s). MS(EI) m/z : 810 (M^+), 506 ($\text{M}^+-\text{H}_2\text{O}$), 488 ($\text{M}^+-2\text{H}_2\text{O}$). HR-MS(EI) m/z : 810.4561 (M^+) (calcd for $\text{C}_{41}\text{H}_{68}\text{O}_5\text{F}_6\text{Si}_2$ 810.4510).

(5Z,7E)-(1S,3R,23R)-23-Acetoxy-1,3-Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-25-methoxymethoxy-9,10-*seco*-5,7,10(19)-cholestatriene (12). A solution of **11** (2.74 g, 3.38 mmol), chloromethylmethyl ether (5.13 mL, 67.5 mmol), $i\text{Pr}_2\text{NEt}$ (17.7 mL, 0.101 mol) in CHCl_3 (20 mL) was heated under reflux for 1 h. After

cooling, the reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with 5% HCl, saturated aqueous NaHCO_3 , and brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:20) to give **12** (2.58 g, 89%) as a colorless oil. IR (neat): 2931, 2857, 1747, 1461, 1216, 1077 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06 (12H, s), 0.52 (3H, s), 0.87 (18H, s), 0.99 (3H, d, $J=5.9$ Hz), 2.02 (3H, s), 2.82 (1H, dd, $J=13.5, 3.3$ Hz), 3.47 (3H, s), 4.19 (1H, m), 4.38 (1H, m), 4.86 (1H, br s), 4.90 (1H, d, $J=6.6$ Hz), 4.99 (1H, d, $J=6.6$ Hz), 5.18 (1H, br s), 5.41 (br s), 5.99 (1H, d, $J=11.4$ Hz), 6.22 (1H, d, $J=11.4$ Hz).

(5Z,7E)-(1S,3R,23R)-Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-25-methoxymethoxy-9,10-*seco*-5,7,10(19)-cholestatrien-23-ol (13). **12** (2.58 g, 3.02 mmol) was dissolved in 5% KOH-MeOH (30 mL) and stirred at 0 °C for 1 h. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:20) to give alcohol **13** (2.31 g, 94%) as a colorless oil. IR (neat): 3505 (br), 2952, 2857, 1470, 1214, 835 cm^{-1} . ^1H NMR (270 MHz) δ : 0.04, 0.05 (each 6H, s), 0.56 (3H, s), 0.87 (18H, s), 0.97 (1H, d, $J=6.6$ Hz), 2.48 (1H, m), 2.58 (1H, d, $J=2.3$ Hz), 2.82 (1H, m), 3.49 (3H, s), 4.19 (2H, m), 4.36 (1H, m), 4.86 (1H, br s), 5.02 (2H, s), 5.16 (1H, br s), 6.02 (1H, d, $J=11.2$ Hz), 6.24 (1H, d, $J=11.2$ Hz).

(5Z,7E)-(1S,3R,23R)-1,3-Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-25-methoxymethoxy-23-(methylthiothiocarbonyloxy)-9,10-*seco*-5,7,10(19)-cholestatriene (14). A mixture of **13** (2.31 g, 2.84 mmol), CS_2 (0.22 mL, 3.66 mmol), 60% NaH (114 mg, 2.85 mmol), imidazole (19 mg, 0.28 mmol) and THF (30 mL) was stirred overnight at room temperature. Then CH_3I (0.27 mL, 4.33 mmol) was added and stirred at room temperature for 2 h. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:20) to give **14** (2.05 g, 80%) as a colorless oil. IR (neat): 2951, 2856, 1214, 1075 cm^{-1} . ^1H NMR (270 MHz) δ : 0.06 (12H, s), 0.52 (3H, s), 0.87, 0.88 (each 9H, s), 0.99 (3H, d, $J=5.6$ Hz), 2.55 (3H, s), 3.47 (2H, s), 4.19 (1H, m), 4.38 (1H, m), 4.86 (1H, br s), 4.94 (1H, d, $J=6.6$ Hz), 5.00 (1H, d, $J=6.6$ Hz), 5.19 (1H, br s), 6.02 (1H, d, $J=11.2$ Hz), 6.21 (2H, m).

(5Z,7E)-(1S,3R)-1,3-Bis[(*t*-butyldimethylsilyl)oxy]-26,26,26,27,27,27-hexafluoro-25-methoxymethoxy-9,10-*seco*-5,7,10(19)-cholestatriene (3). A solution of **14** (2.05 g, 2.27 mmol) in toluene (4.0 mL) was added dropwise to a solution of $n\text{Bu}_3\text{SnH}$ (1.83 mL, 6.80 mmol) in toluene (30 mL) at 100 °C and the solution was heated under reflux for 1 h. After cooling, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel chromatography (hexane only $\rightarrow$ ethyl acetate:hexane, 1:10) to give **3** (1.75 g, 97%) as a colorless oil. IR (neat): 2953, 2857, 1212, 1082 cm^{-1} . ^1H NMR (270 MHz) δ :

0.06 (12H, s), 0.53 (3H, s), 0.87 (18H, s), 0.94 (3H, d, $J=6.3$ Hz), 2.21 (1H, m), 2.83 (1H, d, $J=9.6$ Hz), 3.46 (3H, s), 4.19 (1H, m), 4.38 (1H, m), 4.86 (1H, br s), 4.92 (2H, s), 5.18 (1H, br s), 6.02 (1H, d, $J=10.9$ Hz), 6.24 (1H, d, $J=10.9$ Hz).

(5Z,7E)-(1S,3R)-26,26,26,27,27,27-Hexafluoro-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2a). Methanesulfonic acid (1.0 mL) was added to a solution of **3** (200 mg, 0.251 mmol) in methanol (10 mL) at room temperature and stirred for 2 h. The reaction mixture was quenched with ice water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaHCO_3 , brine, and dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 3:2) to give **2a** (74 mg, 56%) as an amorphous powder. UV (EtOH): λ_{max} 265 nm. ^1H NMR (270 MHz) δ : 0.55 (3H, s), 0.94 (3H, d, $J=6.3$ Hz), 2.93 (1H, br s), 4.24 (1H, m), 4.37 (1H, m), 5.00 (1H, br s), 5.33 (1H, br s), 6.01 (1H, d, $J=11.3$ Hz), 6.38 (1H, d, $J=11.3$ Hz). MS(EI) m/z : 524 (M^+), 506 ($\text{M}^+-\text{H}_2\text{O}$), 491 ($\text{M}^+-\text{CH}_3-\text{H}_2\text{O}$), 488 ($\text{M}^+-2\text{H}_2\text{O}$), 473 ($\text{M}^+-2\text{H}_2\text{O}-\text{CH}_3$).

(6Z)-(3R)-26,26,26,27,27,27-Hexafluoro-9,10-*seco*-5(10),6,8-cholestatriene-3,25-diol-1-one (15). Dess–Martin periodinane reagent (10 mg, 23.5 μmol) was added to a solution of **2a** (5.0 mg, 9.5 μmol) in CH_3CN (2.5 mL) at room temperature and stirred for 4 h. The reaction mixture was quenched with a 1:1 mixture of saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ and NaHCO_3 solution. The organic layer was dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:2) to give **15** (3.3 mg, 66%) as an amorphous solid. ^1H NMR (270 MHz) δ : 0.72 (3H, s), 0.97 (3H, d, $J=6.6$ Hz), 1.79 (3H, s), 2.40–2.60 (2H, m), 2.72–2.86 (2H, m), 2.96 (1H, m), 3.17–3.29 (2H, m), 4.23 (1H, m), 5.49 (1H, m), 6.03 (1H, d, $J=11.9$ Hz), 6.14 (1H, d, $J=11.9$ Hz).

(6Z)-(1R,3R)-26,26,26,27,27,27-Hexafluoro-9,10-*seco*-5(10),6,8-cholestatriene-1 β ,3 β ,25-triol (16). NaBH_4 (11.2 mg, 89.3 μmol) was added to a solution of **15** (3.3 mg, 6.31 μmol) in methanol (0.5 mL) at 0 °C and stirred for 1.5 h. The reaction mixture was quenched with 1N HCl solution, and extracted with ethyl acetate. The organic layer was washed with 1N HCl, saturated aqueous NaHCO_3 , brine and dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:1) to give **16** (2.2 mg, 66%) as an amorphous solid. ^1H NMR (270 MHz) δ : 0.71 (3H, s), 0.97 (3H, d, $J=6.6$ Hz), 1.81 (3H, s), 2.64 (1H, m), 2.92 (1H, m), 4.02 (1H, m), 4.24 (1H, m), 5.56 (1H, m), 5.79 (1H, d, $J=12.2$ Hz), 5.96 (1H, d, $J=12.2$ Hz).

(5Z,7E)-(1R,3R)-26,26,26,27,27,27-Hexafluoro-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2b). A solution of **16** (2.2 mg, 4.19 μmol) in acetone (1 mL) was placed in a sealed tube and heated at 80 °C for 6 h. After cooling, the reaction mixture was concentrated in vacuo. The crude product was purified by silica gel chromatography (ethyl acetate:hexane, 1:1) and further HPLC purification

(Zorbax BP SIL, $\phi 4.6$ mm $\times$ 25 cm, methanol:dichloromethane:hexane = 3:50:50) to give **2b** (1.8 mg, 82%) as an amorphous solid. UV (EtOH): λ_{max} 264 nm. ^1H NMR (500 MHz) δ : 0.55 (3H, s), 0.95 (3H, d, $J=6.7$ Hz), 2.48 (1H, m), 2.56 (1H, m), 2.85 (1H, m), 4.10 (1H, m), 4.35 (1H, m), 5.01 (1H, br s), 5.29 (1H, br s), 6.02 (1H, d, $J=11.3$ Hz), 6.44 (1H, d, $J=11.3$ Hz). MS(EI) m/z : 524 (M^+), 506 ($\text{M}^+-\text{H}_2\text{O}$), 491 ($\text{M}^+-\text{CH}_3-\text{H}_2\text{O}$), 488 ($\text{M}^+-2\text{H}_2\text{O}$), 473 ($\text{M}^+-2\text{H}_2\text{O}-\text{CH}_3$). HR-MS(EI) m/z : 524.2698 (M^+) (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_3\text{F}_6$ 524.2725).

(S)-2-Hydroxy-5-trimethylsilyl-1-*p*-toluenesulfonyloxy-4-pentyne (17). To a solution of trimethylsilylacetylene (642 mg, 6.54 mmol) in THF (6.0 mL) was added *n*-BuLi (1.47 M in hexane, 4.7 mL) dropwise at –70 °C. After stirring for 30 min, $\text{BF}_3\cdot\text{Et}_2\text{O}$ (1.10 mL, 8.50 mmol) and then **7** (1.34 g, 5.90 mmol) was added. The reaction mixture was stirred for 30 min at the same temperature, then the reaction was quenched with saturated aqueous NH_4Cl solution and the whole was extracted with ethyl acetate. The organic layer was washed with water, brine, dried over MgSO_4 concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:3) to give **17** (1.52 g, 79%) as a colorless oil. ^1H NMR (270 MHz) δ : 0.12 (9H, s), 2.46 (3H, s), 2.45–2.48 (2H, m), 3.94–4.20 (3H, m), 7.36 (2H, d, $J=8.4$ Hz), 7.81 (2H, d, $J=8.4$ Hz).

(S)-1-Cyano-2-hydroxy-5-trimethylsilyl-4-pentyne (18). To the suspension of **17** (400 mg, 1.23 mmol) in DMSO (8 mL) was added potassium cyanide (96 mg, 1.48 mmol) and the mixture was heated at 40 °C for 3 h. The reaction mixture was cooled in an ice bath, diluted with water, and extracted with ethyl acetate. The organic layer was washed with water, brine, dried over MgSO_4 concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:3) to give **18** (160 mg, 72%) as a colorless oil. IR (neat): 3458(br), 2961, 2901, 2256, 2178, 1250, 1029 cm^{-1} . ^1H NMR (270 MHz) δ : 0.17 (9H, s), 2.40 (1H, d, $J=5.7$ Hz), 2.59 (2H, d, $J=5.9$ Hz), 2.68 (2H, d, $J=5.5$ Hz), 4.08 (1H, m). MS(EI) m/z : 166 (M^+-CH_3), 148 ($\text{M}^+-\text{CH}_3-\text{H}_2\text{O}$). HR-MS(EI) m/z : 166.0672 (M^+-CH_3) (calcd for $\text{C}_8\text{H}_{12}\text{NOSi}$ 166.0688).

(S)-2-[(*t*-Butyldiphenylsilyl)oxy]-1-cyano-5-trimethylsilyl-4-pentyne (19). To a solution of **18** (239 mg, 1.32 mmol) in DMF (2.0 mL) was added *t*-butyldiphenylsilyl chloride (TBDPSCl, 797 mg, 2.90 mmol), imidazole (272 mg, 4.0 mmol) and stirred at 40 °C for 2 h. The reaction mixture was poured into water and extracted with water. The organic layer was washed with water and brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:10) to give **19** (497 mg, 90%) as a colorless oil. IR (neat): 3072, 2959, 2859, 2253, 2179, 1428 cm^{-1} . ^1H NMR (270 MHz) δ : 0.10 (9H, s), 1.06 (9H, s), 2.44–2.65 (4H, m), 4.05 (1H, m), 7.35–7.70 (10H, m). MS(EI) m/z : 419 (M^+), 404 (M^+-CH_3), 362 (M^+-tBu). HR-MS(EI) m/z : 419.2126 (M^+) (calcd for $\text{C}_{25}\text{H}_{33}\text{NOSi}_2$ 419.2101).

(3S,5S)/(3R,5S)-5-[(*t*-Butyldiphenylsilyl)oxy]-3-hydroxy-8-trimethylsilyl-1-octen-7-yne (21). Diisobutyl aluminium

hydride (DIBALH, 1.0 M, 1.5 mL) was added to a solution of **19** (323 mg, 0.770 mmol) in dichloromethane (1.5 mL) at -10°C and stirred for 30 min. The reaction mixture was quenched with 1N HCl and extracted with ethyl acetate. The organic layer was washed with 1N HCl, saturated aqueous NaHCO_3 , brine, dried over MgSO_4 , and concentrated in vacuo to give **20** (412 mg) as a crude product. ^1H NMR (270 MHz) δ : 0.13 (9H, s), 1.06 (9H, s), 2.34 (2H, m), 2.68 (2H, m), 5.34 (1H, q, $J=5.9$ Hz), 7.41 (6H, m), 7.70 (4H, m), 9.74 (1H, t, $J=2.2$ Hz). Vinylmagnesium bromide (1.0 M in THF, 7.0 mL) was added dropwise to a solution of **20** (412 mg) in THF (4 mL) at -70°C , then warmed up to room temperature and stirred for 30 min. The reaction mixture was quenched with 1 N HCl and extracted with ethyl acetate. The organic layer was washed with 1N HCl, saturated aqueous NaHCO_3 , brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:10) to give **21** (226 mg, 65% from **19**) as a colorless oil. ^1H NMR (300 MHz) δ : 0.11, 0.12 (total 9H, 2s), 1.07 (9H, s), 1.72–1.94 (2H, m), 2.27–2.52 (2H, m), 4.06, 4.13 (total 1H, 2m), 4.31, 4.41 (1H, 2m), 4.99–5.24 (2H, m), 5.70–5.85 (1H, m), 7.40 (6H, m), 7.70 (4H, m). MS(FAB+) m/z : 451 ($\text{M}^+ + \text{H}$), 433 ($\text{M}^+ - \text{H}_2\text{O} + \text{H}$). HR-MS(FAB+) m/z : 451.2457 ($\text{M}^+ + \text{H}$) (calcd for $\text{C}_{27}\text{H}_{39}\text{O}_2\text{Si}_2$ 451.2489).

(3*S*,5*S*)-5-[(*t*-Butyldiphenylsilyl)oxy]-3-hydroxy-1-octen-7-yne (22a**) and (3*R*,5*S*)-5-[(*t*-Butyldiphenylsilyl)oxy]-3-hydroxy-1-octen-7-yne (**22b**).** To a solution of **21** (226 mg, 0.501 mmol) in methanol (4 mL) was added K_2CO_3 (100 mg) and stirred for 1 h at room temperature. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:10) to give a crude product. The crude diastereomer mixture was separated by HPLC (DAISO, IR-120-10/20-S, $\phi 50\text{ mm} \times 25\text{ cm}$, ethyl acetate:hexane, 1:30) to give **22a** (104 mg, 55%, retention time 61.3 min) and **22b** (57 mg, 30%, retention time 50.3 min).

(22a) IR (neat): 3452, 3308, 2932, 1472, 1427 cm^{-1} . ^1H NMR (270 MHz) δ : 1.07 (9H, s), 1.85–1.90 (2H, m), 1.94 (1H, t, $J=2.7$ Hz), 2.23–2.33 (2H, m), 4.06 (1H, m), 4.32 (1H, m), 5.03 (1H, d, $J=10.6$ Hz), 5.15 (1H, d, $J=17.2$ Hz), 5.77 (1H, ddd, $J=5.6, 10.6, 17.2$ Hz), 7.43 (6H, m), 7.69 (4H, m). MS(FAB+) m/z : 379 ($\text{M}^+ + \text{H}$), 361 ($\text{M}^+ - \text{H}_2\text{O} + \text{H}$). HR-MS(FAB+) m/z : 379.2084 ($\text{M}^+ + \text{H}$) (calcd for $\text{C}_{24}\text{H}_{31}\text{O}_2\text{Si}$ 379.2093).

(22b) IR (neat): 3452, 3308, 2932, 1472, 1427 cm^{-1} . ^1H NMR (270 MHz) δ : 1.08 (9H, s), 1.74–1.90 (2H, m), 1.93 (1H, t, $J=2.6$ Hz), 2.23–2.45 (2H, m), 4.12 (1H, m), 4.38 (1H, m), 5.05 (1H, d, $J=10.2$ Hz), 5.21 (1H, d, $J=17.2$ Hz), 5.81 (1H, ddd, $J=5.6, 10.2, 17.2$ Hz), 7.43 (6H, m), 7.69 (4H, m). MS(FAB+) m/z : 379 ($\text{M}^+ + \text{H}$), 361 ($\text{M}^+ - \text{H}_2\text{O} + \text{H}$). HR-MS(FAB+) m/z : 379.2105 ($\text{M}^+ + \text{H}$) (calcd for $\text{C}_{24}\text{H}_{31}\text{O}_2\text{Si}$ 379.2093).

(3*S*,5*R*)-3-[(*t*-Butyldimethylsilyl)oxy]-5-[(*t*-butyldiphenylsilyl)oxy]-1-octen-7-yne (6a**).** To a solution of **22a**

(112 mg, 0.296 mmol) in DMF (1.5 mL) was added TBSCl (89 mg, 0.591 mmol), imidazole (60 mg, 0.881 mmol), and stirred at 40°C for 2 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:10) to give **6a** (125 mg, 86%) as a colorless oil. IR (neat): 3313, 3072, 2857, 1472, 1253 cm^{-1} . ^1H NMR (270 MHz) δ : -0.03 (6H, s), 0.81 (9H, s), 1.05 (9H, s), 1.84 (2H, m), 1.91 (1H, m), 2.29 (2H, m), 3.90 (1H, m), 4.21 (1H, m), 4.86–5.00 (2H, m), 5.58 (1H, m), 7.40 (6H, m), 7.71 (4H, m). MS(EI) m/z : 435 ($\text{M}^+ - \text{Bu}$). HR-MS(EI) m/z : 435.2129 ($\text{M}^+ - \text{Bu}$) (calcd for $\text{C}_{26}\text{H}_{35}\text{O}_2\text{Si}_2$ 435.2176).

(3*R*,5*S*)-[(*t*-Butyldimethylsilyl)oxy]-5-[(*t*-butyldiphenylsilyl)oxy]-1-octen-7-yne (6b**).** To a solution of **22a** (70 mg, 0.185 mmol) in DMF (1 mL) was added TBSCl (56 mg, 0.372 mmol), imidazole (38 mg, 0.558 mmol), and stirred at 40°C for 2 h. The reaction mixture was treated in the same manner described in the preparation of **7a**. The residue was purified by silica gel chromatography (ethyl acetate:hexane, 1:10) to give **6b** (75 mg, 82%) as a colorless oil. IR (neat): 3313, 3072, 2857, 1472, 1253 cm^{-1} . ^1H NMR (270 MHz) δ : -0.07 (3H, s), -0.04 (3H, s), 0.82 (9H, s), 1.06 (9H, s), 1.74 (1H, m), 1.93–2.04 (3H, m), 2.29 (2H, m), 3.93 (1H, m), 4.08 (1H, m), 4.90–5.06 (2H, m), 5.63 (1H, ddd, $J=6.9, 10.7, 17.2$ Hz), 7.40 (6H, m), 7.69 (4H, m). MS(EI) m/z : 435 ($\text{M}^+ - \text{Bu}$). HR-MS(EI) m/z : 435.2183 ($\text{M}^+ - \text{Bu}$) (calcd for $\text{C}_{26}\text{H}_{35}\text{O}_2\text{Si}_2$ 435.2176).

Ketone (24**).** A mixture of O_3 and O_2 was passed into a stirred solution of **3** (1.45 g, 1.81 mmol) in dichloromethane (150 mL) and MeOH (30 mL) at -78°C for 30 min. Then PPh_3 (2.0 g, 7.63 mmol) was added and stirred at -78°C for 30 min, then warmed up to 0°C and stirred for 30 min. The resulting reaction mixture was concentrated in vacuo. The crude mixture was purified by silica gel chromatography (ethyl acetate:hexane, 1:10–1:5) to give **24** (315 mg, 40%) as an amorphous solid. IR (neat): 2960, 2881, 1714, 1212, 1049, 933 cm^{-1} . ^1H NMR (270 MHz) δ : 0.63 (3H, s), 0.96 (3H, d, $J=5.9$ Hz), 2.45 (1H, dd, $J=11.5, 7.6$ Hz), 3.45 (3H, s), 4.91 (2H, s). MS(EI) m/z : 432 (M^+), 417 ($\text{M}^+ - \text{CH}_3$), 389 ($\text{M}^+ - \text{CH}_3 - \text{CO}$), 387 ($\text{M}^+ - \text{CH}_2\text{OCH}_3$). HR-MS(EI) m/z : 432.2120 (M^+) (calcd for $\text{C}_{20}\text{H}_{30}\text{O}_3\text{F}_6$ 432.2099).

Vinyl bromide (5**).** To a solution of hexamethyldisilazane (0.16 mL, 0.757 mmol) in toluene (1.2 mL) was added *n*-BuLi (1.6 M in hexane, 0.39 mL, 0.624 mmol) at -70°C . After stirring for 10 min, bromomethyltriphenylphosphonium bromide (338 mg, 0.775 mmol) was added to the solution and the mixture was warmed up to 0°C and stirred for 10 min. After cooling to -70°C , a solution of **24** (67 mg, 0.155 mmol) in toluene (0.5 mL) was added dropwise to the mixture at the same temperature. After stirring for 30 min, NH_4Cl solution was added and warmed up to room temperature. The resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate: hexane, 1:10)

to give vinyl bromide **5** (27 mg, 34%) as a colorless oil and starting material **24** (30 mg, 45% recovery). IR (neat): 2951, 1467, 1377, 1213 cm^{-1} . ^1H NMR (270 MHz) δ : 0.57 (3H, s), 0.94 (1H, d, $J=6.3$ Hz), 2.86 (1H, m), 3.46 (3H, s), 4.92 (2H, s), 5.65 (1H, s). MS(EI) m/z : 508 (M^+), 463 ($\text{M}^+-\text{CH}_3\text{OCH}_2$). HR-MS(EI) m/z : 508.1406 (M^+) (calcd for $\text{C}_{21}\text{H}_{31}\text{O}_2\text{F}_6\text{Br}$ 508.1412).

(5Z,7E)-(1S,3S)-26,26,26,27,27,27-Hexafluoro-9,10-secosteroid-5,7,10(19)-cholestatriene-1,3,25-triol (2c). A solution of $(\text{dba})_3\text{Pd}_2\text{-CHCl}_3$ (4 mg, 3.86 μmol), triphenylphosphine (11 mg, 41.9 μmol) in triethylamine (0.4 mL) toluene (0.4 mL) was stirred at room temperature for 10 min. A solution of **5** (16 mg, 31.4 μmol) and **6a** (20 mg, 40.6 μmol) in toluene (0.4 mL) was added dropwise and heated under reflux for 1 h. The reaction mixture was concentrated in vacuo, and purified by silica gel chromatography (ethyl acetate: hexane, 1:20) to give silylated 3-epimer (24 mg). Methansulfonic acid (0.1 mL) was added to a solution of the product (24 mg) in methanol (5 mL) at room temperature and stirred for 1 h. The reaction mixture was poured into ice 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 (ethyl acetate:hexane, 3:2) to give **2c** (9.0 mg, 55% from **5**) as an amorphous solid. UV (EtOH): λ_{max} 264 nm. ^1H NMR (500 MHz) δ : 0.55 (3H, s), 0.95 (3H, d, $J=6.4$ Hz), 2.56 (1H, m), 2.85 (1H, m), 4.05 (1H, m), 4.30 (1H, m), 5.00 (1H, br s), 5.30 (1H, br s), 6.02 (1H, d, $J=11.3$ Hz), 6.44 (1H, d, $J=11.3$ Hz). MS(EI) m/z : 524 (M^+), 506 ($\text{M}^+-\text{H}_2\text{O}$), 491 ($\text{M}^+-\text{CH}_3\text{-H}_2\text{O}$), 488 ($\text{M}^+-2\text{H}_2\text{O}$), 473 ($\text{M}^+-2\text{H}_2\text{O-CH}_3$). HR-MS(EI) m/z : 524.2687 (M^+) (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_3\text{F}_6$ 524.2725).

(5Z,7E)-(1R,3S)-26,26,26,27,27,27-Hexafluoro-9,10-secosteroid-5,7,10(19)-cholestatriene-1,3,25-triol (2d). A solution of $(\text{dba})_3\text{Pd}_2\text{-CHCl}_3$ (4 mg, 3.86 μmol), triphenylphosphine (11 mg, 41.9 μmol) in triethylamine (0.4 mL) toluene (0.4 mL) was stirred at room temperature for 10 min. A solution of **5** (20.1 mg, 39.3 μmol) and **6b** (22 mg, 44.6 μmol) in toluene (0.4 mL) was added dropwise and heated under reflux for 1 h. The reaction mixture was concentrated in vacuo, and purified by silica gel chromatography (ethyl acetate: hexane, 1:20) to give silylated 1,3-epimer (24 mg). Methansulfonic acid (0.1 mL) was added to a solution of the product (24 mg) in methanol (3.0 mL) at room temperature, and stirred for 1 h. The reaction mixture was poured into ice 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 (ethyl acetate:hexane, 3:2) to give **2d** (10.1 mg, 49% from **5**) as an amorphous solid. ^1H NMR (500 MHz) δ : 0.55 (3H, s), 0.94 (3H, d, $J=6.7$ Hz), 2.30 (1H, dd, $J=13.3, 7.5$ Hz), 2.62 (1H, dd, $J=13.3, 4.0$ Hz), 2.83 (1H, dd, $J=11.8, 4.0$ Hz), 4.20 (1H, m), 4.42 (1H, m), 5.01 (1H, br s), 5.32 (1H, m), 6.01 (1H, d, $J=11.3$ Hz), 6.39 (1H, d, $J=11.3$ Hz). UV (EtOH): λ_{max} 264 nm. MS(EI) m/z : 524 (M^+), 506 ($\text{M}^+-\text{H}_2\text{O}$), 491 (M^+-CH_3), 488 ($\text{M}^+-2\text{H}_2\text{O}$), 473 ($\text{M}^+-\text{CH}_3\text{-H}_2\text{O}$). HR-MS(EI) m/z : 524.2691 (M^+) (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_3\text{F}_6$ 524.2725).

(5E,7E)-(1S,3R)-26,26,26,27,27,27-Hexafluoro-9,10-secosteroid-5,7,10(19)-cholestatriene-1,3,25-triol (2e). A solution of **2a** (50 mg, 9.53 μmol) in dichloromethane (3 mL) was cooled to -15°C , and then liquified SO_2 (10 mL) was added. The solution was stirred under reflux for 30 min. The SO_2 was distilled off, and the residue was dried in vacuo. The following purification by silica gel chromatography (ethyl acetate only) afford a mixture of (6S) and (6R) SO_2 -adducts as an amorphous powder. The SO_2 adduct was dissolved in EtOH (5 mL), and NaHCO_3 (100 mg) was added to the solution. The reaction mixture was heated under reflux for 90 min. The solvent was removed, and the crude product was purified by silica gel chromatography (ethyl acetate:hexane, 2:1) to give **2e** (40 mg, 80% from **2a**) as an amorphous solid. UV (EtOH): λ_{max} 274 nm. ^1H NMR (270 MHz) δ : 0.57 (3H, s), 0.95 (3H, d, $J=6.7$ Hz), 2.28 (1H, m), 2.87 (2H, m), 4.23 (1H, m), 4.50 (br s), 4.98 (1H, br s), 5.13 (1H, br s), 5.88 (1H, d, $J=11.6$ Hz), 6.58 (1H, d, $J=11.6$ Hz). MS(EI) m/z : 524 (M^+), 506 ($\text{M}^+-\text{H}_2\text{O}$), 488 ($\text{M}^+-2\text{H}_2\text{O}$). HR-MS(EI) m/z : 524.2700 (M^+) (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_3\text{F}_6$ 524.2725).

Measurement of vitamin D₃ receptor binding affinity.²² 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 MgCl_2 , 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.

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