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TETRAHEDRON

Stereoselective Synthesis and Structural Establishment of (25*S*)-24,24-Difluoro-1α,25,26-trihydroxyvitamin D_3, a Major Metabolite of 24,24-Difluoro-1α,25-dihydroxyvitamin D_3

Hiroshi Iwasaki,* Ryuzo Hosotani, Yoichi Miyamoto,§ and Yoshio Nakano

Tsukuba Research Laboratory, NOF Corporation, 5-10 Tokodai, Tsukuba-shi, Ibaraki 300-2635, Japan.

Keiko Yamamoto, and Sachiko Yamada

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101-0062, Japan.

Toshimasa Shinki, and Tatsuo Suda

Department of Biochemistry, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.

Kentaro Yamaguchi

Chemical Analysis Center, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan.

Katsuhiro Konno, and Hiroaki Takayama

Faculty of Pharmaceutical Sciences, Teikyo University, 1091-1 Suarashi, Sagamiko-cho, Kanagawa 199-0195, Japan.

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Abstract: (25*S*)-24,24-Difluoro-1α,25,26-trihydroxyvitamin D_3 (**3a**) and its (25*R*)-epimer (**3b**), either of which is expected to be a major metabolite of 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (**2**), were synthesized. Asymmetric addition to β-ketosulfoxides (**5a**, **5b**) of trimethylaluminum was used as a key process to construct the chiral tertiary alcohol moiety of **3a** and **3b**. The absolute configuration of the tertiary alcohol was determined by X-ray crystallographic analysis of **20** which is a CD-ring analog of the **3a** intermediate. The configuration at the C(25) position of the metabolite was established as *S* by HPLC comparison between the metabolite and chemically synthesized **3a** and **3b**.

INTRODUCTION

The active vitamin D_3 metabolite, 1α,25-dihydroxyvitamin D_3 (**1**), is degraded in the target tissue mainly via the hydroxylation at C(24) catalyzed by 1α,25-dihydroxyvitamin D_3 24-hydroxylase (CYP24).[1] CYP24 is a

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multicatalytic enzyme and catalyzes not only the 24*R*-hydroxylation but also 23*S*-hydroxylation,[2] oxidation of the 24-hydroxy group to a ketone, and cleavage of the C(23)-C(24) bond of (23*S*)-23,25-dihydroxy-24-oxovitamin D_3.[2,3] Perfluorination either at C(24) or C(26) and C(27) inhibits the 24-hydroxylation. Therefore, the high potency of vitamin D analogs perfluorinated at C(24) or C(26) and C(27) is, in part, due to their long life-time. It has been known that 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3 is hydroxylated in the target tissue at the 23-position to give (23*S*)-26,26,26,27,27,27-hexafluoro-1α,23,25-trihydroxyvitamin D_3.[4,5] We reported in a previous paper[6] that incubation of 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (**2**) with rat kidney homogenates yielded 24,24-difuoro-1α,25,26-trihydroxyvitamin D_3 (**3**) as a major metabolite. This metabolite was shown to be a single epimer at C(25) and the enzyme undergoing this transformation was demonstrated to be CYP24. Thus we showed, for the first time, that CYP24 catalyzes hydroxylation at C(26) of vitamin D. Synthesis of (25*S*)- and (25*R*)-24,24-difuoro-1α,25,26-trihydroxyvitamin D_3 (**3a** and **3b**) is important not only to determine the configuration at C(25) of this metabolite but also to know in general the stereochemistry of the 26-hydroxylation by CYP24. In the present paper, we report the stereoselective synthesis of two C(25) epimers of **3** using diastereoselective addition of an organoaluminum reagent to the terminal asymmetric β-ketosulfoxide function as a key step and determination of the stereochemistry of our previously isolated metabolites (Figure 1).

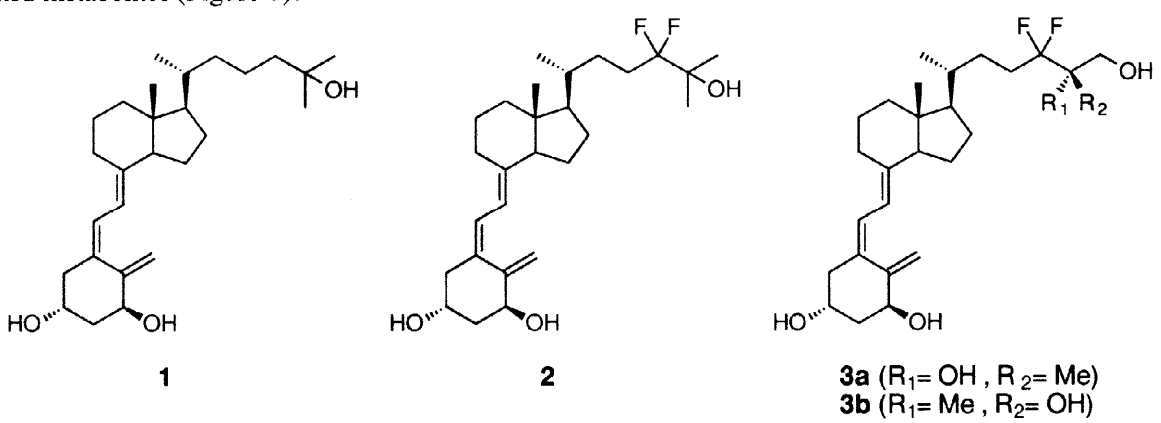
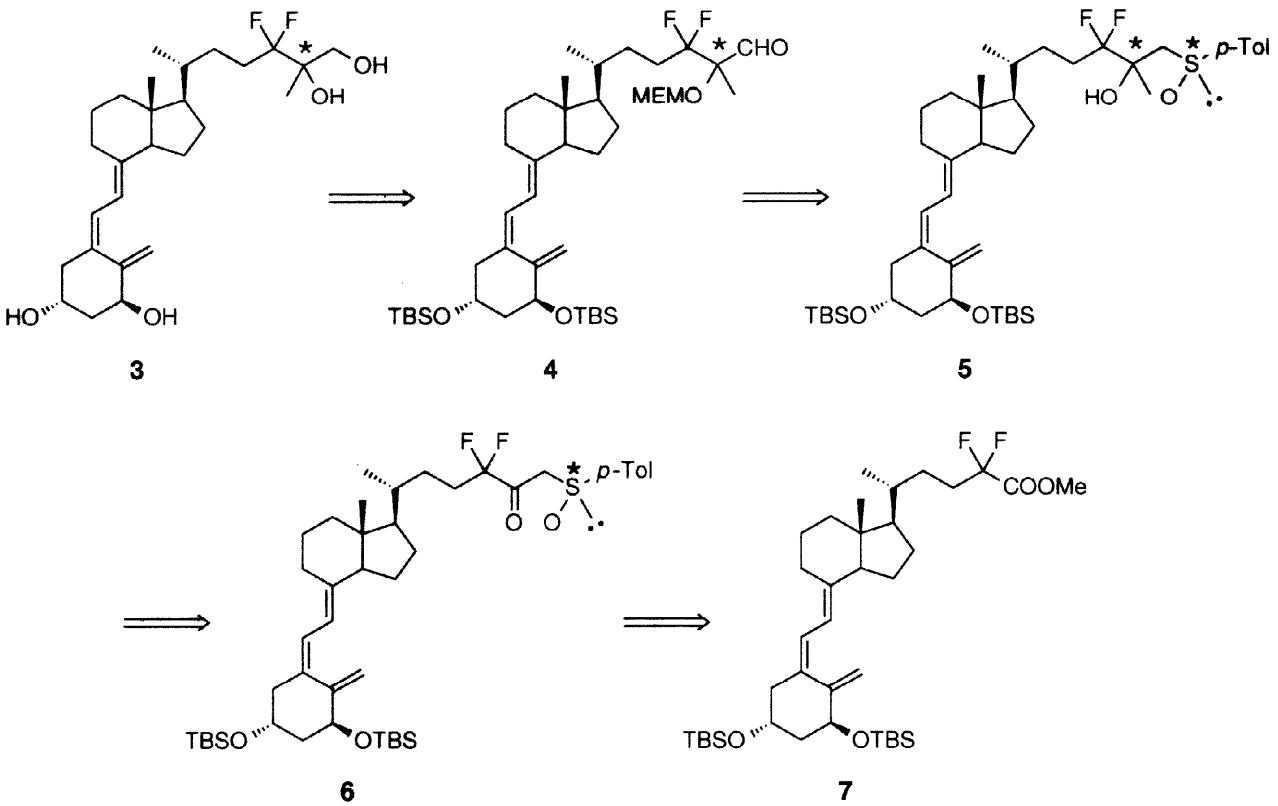


Figure 1. Structures of 1α,25-dihydroxyvitamin D_3 and its C-24 fluorinated analogs.

Our approach to the syntheses of **3a** and **3b** is as follows. We selected diastereomerically pure β-ketosulfoxide **6** as a key intermediate which was assumed to give aldehyde **4** with high diastereoselectivity. The carbonyl group of chiral β-ketosulfoxides is known to be converted into asymmetric secondary or tertiary alcohol with various organoalumimum reagents[7-11] under chelation or non-chelation control conditions and, moreover, the sulfinyl moiety can be converted into an aldehyde by Pummerer rearrangement followed by hydrolysis. Consequently, **6** can be converted into tertiary alcohol **5** with highly stereoselective addition of trimethylaluminum in the presence of a Lewis acid as reported by Carreño *et al.*[9] and then into aldehyde **4**. Furthermore, the β-ketosulfoxide **6** can be readily prepared from α,α-difluorocarboxylic ester **7** by use of Bravo's method.[11] The preparation of **7** from vitamin D_2 has been already reported by Ando *et al.*[12] (Scheme 1)

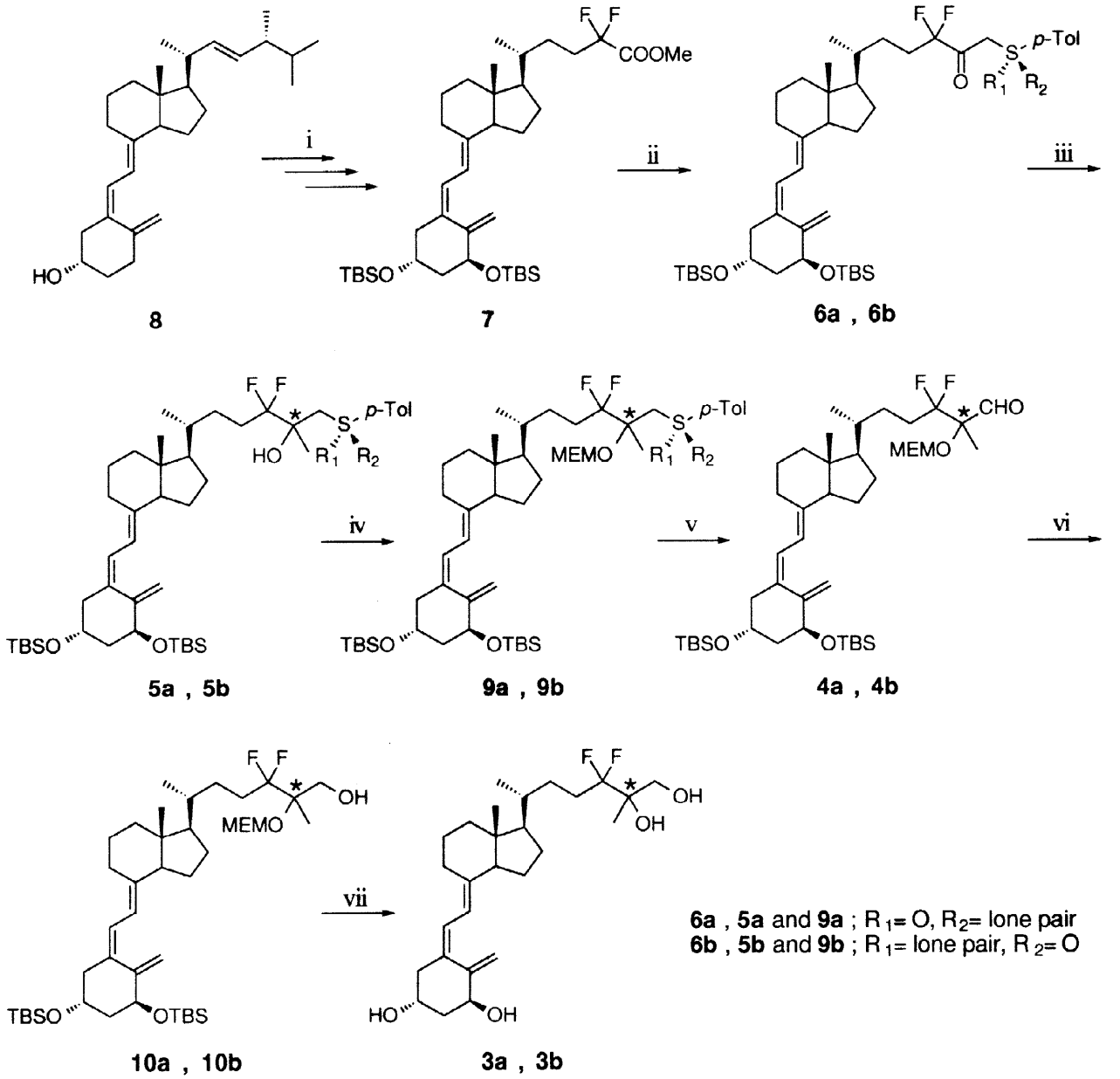


Scheme 1. Retrosynthetic analysis of 24,24-difluoro-1α,25,26-trihydroxyvitamin D_3.

RESULTS AND DISCUSSION

Synthesis of 3a and 3b.

The starting material **7** was converted into β-keto-(*R*)-sulfoxide (**6a**) by treatment with (*R*)-(+)-methyl *p*-tolyl sulfoxide in the presence of lithium diisopropylamide (LDA) in 92% yield. The β-ketosulfoxide **6a** was treated with molecular sieves 4A prior to the next alkylation because the β-ketosulfoxide was actually a mixture of keto and hydrate forms. The stereoselective addition of trimethylalumimun to **6a** was performed under chelation control condition in the presence of $ZnBr_2$ to afford tertiary alcohol (**5a**) in 88% (69% d.e.) yield. The diastereomerically pure **5a** was protected with a methoxyethoxymethyl (MEM) group by treatment with MEM chloride in the presence of NaH to give **9a** in 76% yield. Pummerer rearrangement of **9a** with trifluoroacetic anhydride (TFAA) in the presence of pyridine, followed by hydrolysis with 20% aqueous KOH solution gave an aldehyde (**4a**) in 94% yield. The aldehyde **4a** was reduced with $NaBH_4$ to afford an alcohol (**10a**) in 95% yield. The alcohol **10a** was deprotected by treatment with 10-(+)-camphorsulfonic acid (CSA) in MeOH to give (25*S*)-vitamin D (**3a**) in 72% yield. The (25*R*)-isomer (**3b**) was synthesized similarly from **7** via (*S*)-sulfoxide (**6b**) as a key intermediate (Scheme 2).

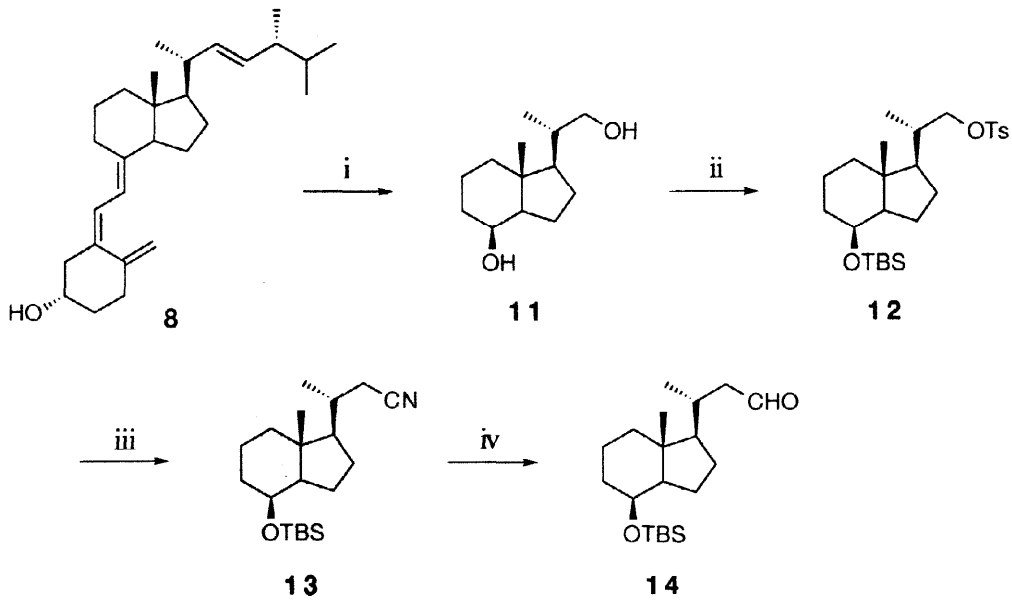


Scheme 2. Reagents: (i) Ref. 12; (ii) (*R*)-(+)- or (*S*)-(-)-methyl *p*-tolyl sulfoxide, LDA, THF (**6a**: 92%, **6b**: 96%); (iii) MS4A, Me_3Al, $ZnBr_2$, CH_2Cl_2 (**5a**: 88%, 69% d.e., **5b**: 78%, 61% d.e.); (iv) MEMCl, NaH, THF (**9a**: 76%, **9b**: 81%); (v) 1) TFAA, pyridine, CH_2Cl_2 2) 20% KOH aq., MeCN (**4a**: 94%, **4b**: 77%); (vi) $NaBH_4$, MeOH (**10a**: 95%, **10b**: 90%); (vii) CSA, MeOH (**3a**: 72%, **3b**: 89%).

The configuration at C(25) of **3a** and **3b** was deduced to be *S* and *R*, respectively, on the basis of the transition state model for the addition proposed by Carreño *et al.*[9] To confirm the stereochemistry, an attempt was made to synthesize a crystalline analog to be subjected to X-ray crystallographic analysis.

Synthesis of CD-ring analog (20).

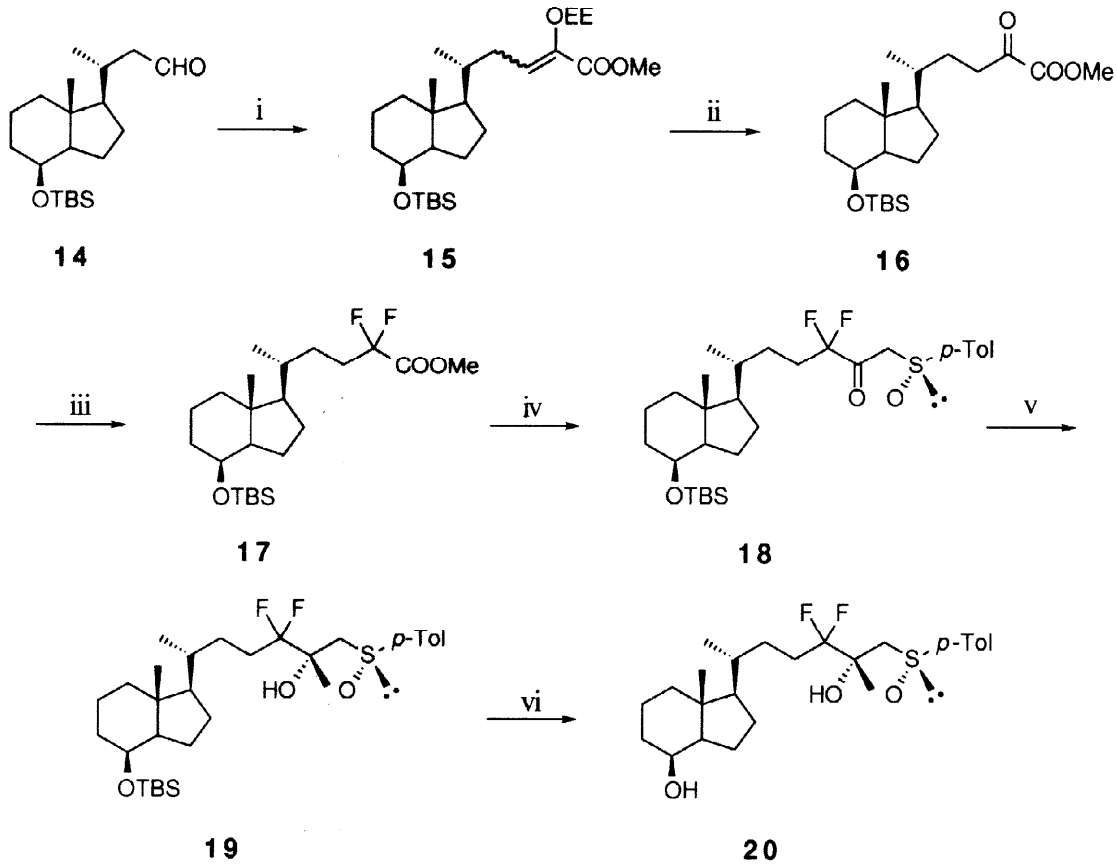
Because of difficulty in obtaining a single crystal of **3a** or **3b** for X-ray crystallographic analysis, we investigated the synthesis of a readily crystallizable CD-ring analog which is structurally closely related to **3a** or **3b**. Inhoffen-Lythgoe diol[13] (**11**) was prepared from vitamin D_2 as previously reported[14] (O_3 then $NaBH_4$) in 74% yield. The primary and secondary alcohols of **11** were sequentially tosylated (*p*-toluenesulfonyl chloride (TsCl), 4-dimethylaminopyridine (DMAP)) and protected (*tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), 2,6-lutidine) to afford **12** in 93% yield. Reaction of **12** with NaCN gave nitrile (**13**) in 89% yield and **13** was further converted into aldehyde **14** by reduction with diisobutylaluminum hydride (DIBAL-H) in 81% yield (Scheme 3).



Scheme 3. Reagents: (i) 1) O_3, CH_2Cl_2 2) $NaBH_4$, MeOH (74%); (ii) 1) TsCl, DMAP, CH_2Cl_2 2) TBSOTf, 2,6-lutidine, CH_2Cl_2 (93%); (iii) NaCN, DMSO (89%); (iv) DIBAL-H, CH_2Cl_2 (81%).

Horner-Wadsworth-Emmons reaction of **14** with trimethyl (ethoxyethyloxy)phosphonoacetate[15] in the presence of LDA afforded an enol ether (**15**) in 90% yield. The enol ether was solvolyzed with pyridinium *p*-toluenesulfonate (PPTS) in MeOH (63% yield) and the α-ketoester (**16**) obtained was converted into an α,α-difluoroester (**17**) by reaction with morphorinosulfur trifluoride (morph-DAST) in 80% yield. In the same way as the synthesis of **6a**, **17** was allowed to react with (*R*)-(+)-methyl *p*-tolyl sulfoxide to afford optically pure β-ketosulfoxide (**18**) in 90% yield, which was further converted into tertiary alcohol (**19**) by selective addition of trimethylaluminum in the presence of $ZnBr_2$ in 82% yield (67% d.e.). Desilylation of **19** with Dowex® 50X4-400 ion exchange resin in MeOH gave a readily crystallizable diol (**20**) in 86% yield (Scheme 4).

Crystalline **20** (m.p. 140~141 °C) was subjected to X-ray crystallographic analysis. The resulting ORTEP drawing shows that the newly generated chiral center of the tertiary alcohol (**20**) has *R* configuration (Figure 2). The stereochemistry is the same as that we had predicted on the basis of the mechanism of the addition reaction.



Scheme 4. Reagents: (i) $(MeO)_2P(O)CH(OEE)COOMe$, LDA, THF (90%); (ii) PPTS, MeOH-CH_2Cl_2 (63%); (iii) morph-DAST, CH_2Cl_2 (80%); (iv) (*R*)-(+)-methyl *p*-tolyl sulfoxide, LDA, THF (90%); (v) MS4A, Me_3Al, $ZnBr_2$, CH_2Cl_2 (82%, 67% d.e.); (vi) Dowex® 50X4-400 ion exchange resin, MeOH (86%).

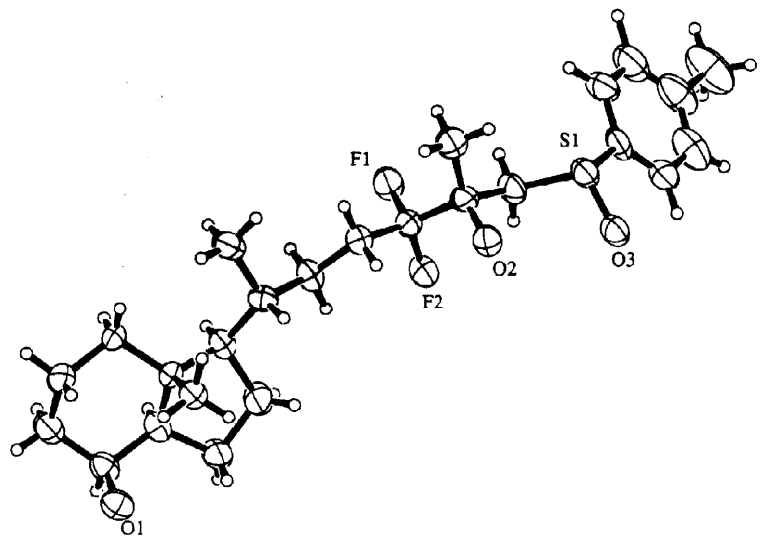
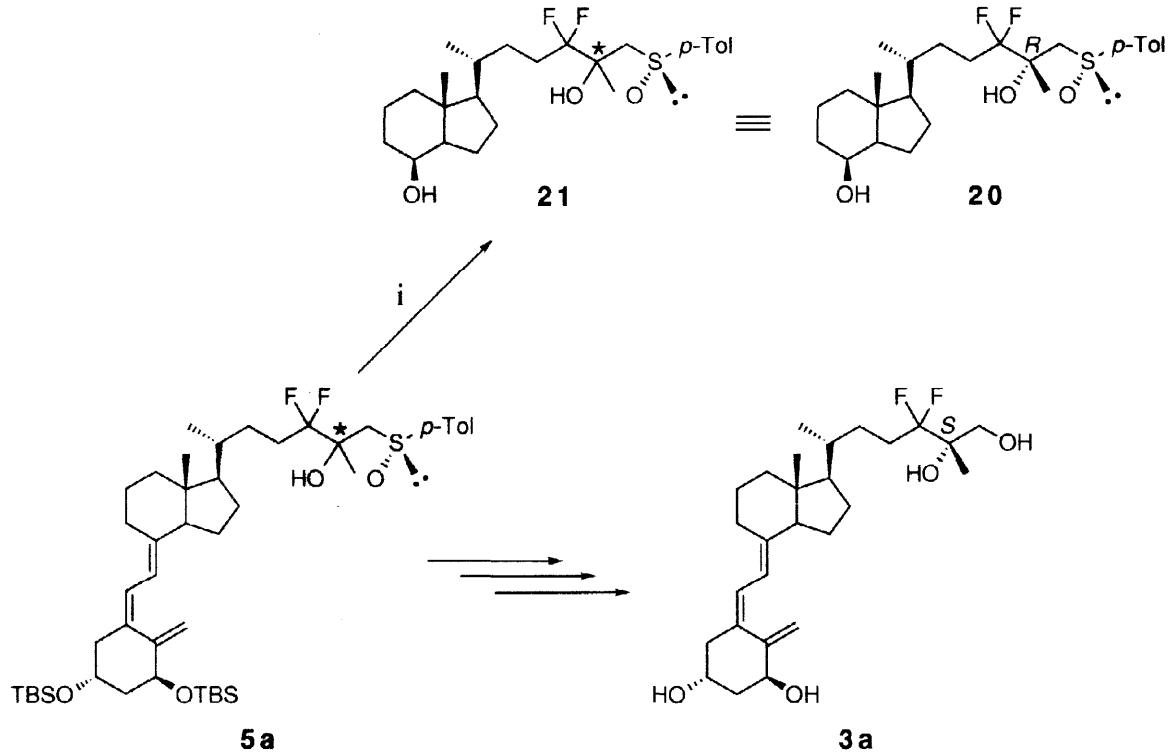


Figure 2. ORTEP drawing of **20**.

In the meantime, the same CD-ring fragment (**21**) was derived from **5a**, which is an intermediate of **3a** and has been derived from (*R*)-(+)-methyl *p*-tolyl sulfoxide, by ozonolysis followed by reduction with $NaBH_4$ in 91% yield. Since all analytical data of **21** were identical with that of **20**, **3a** was determined to be (25*S*)-isomer (Scheme 5).



Scheme 5. Reagents: (i) 1) O_3, CH_2Cl_2 2) $NaBH_4$, MeOH (91%).

HPLC comparison of 3a and 3b with the metabolite of 2.

Next, we compared chemically synthesized **3a** and **3b** with the enzymatically generated metabolite of **2** using a chiral HPLC column (CHIRALCEL® OF[16]). The metabolite was eluted with **3a** (Figure 3). Thus, we determined the structure of the major metabolite of **2** to be (25*S*)-24,24-difluoro-1α,25,26-trihydroxyvitamin D_3. The configuration at C(25) of the metabolite of **2** hydroxylated by CYP24 was now shown opposite[17] compared with that of the C(26) hydroxylated metabolite of **1**, (25*S*)-1α,25,26-trihydroxyvitamin D_3.[18] It has been reported that CYP27 hydroxylates C(25) as well as C(26) of vitamin D_3 and 1α-hydroxyvitamin D_3.[19] These facts support our proposal described previously[6] that there are two C(26) hydroxylation enzymes, CYP24 and CYP27, and indicate that these two enzymes discern between the C(26) and C(27) methyl groups of vitamin D derivatives.

CONCLUSION

The syntheses of (25*S*)- and (25*R*)-24,24-difluoro-1α,25,26-trihydroxyvitamin D_3 (**3a,3b**) were achieved in 7 steps from α,α-difluoroester (**7**) by use of diastereoselective addition of trimethylaluminum to

diastereomerically pure β-ketosulfoxides (**6a** and **6b**) as a key step. The overall yields of **3a** and **3b** were 40% and 37%, respectively. To determine the configuration at C(25) of **3a** and **3b**, we synthesized the CD-ring analog (**20**) from vitamin D_2 in 12 steps and 14% overall yield. By X-ray crystallographic analysis of **20**, the configuration at C(25) was established as *R*. Therefore, **3a** and **3b** have (25*S*)- and (25*R*)- configuration, respectively. By HPLC comparison, we determined the structure of the major metabolite of **2** to be (25*S*)-24,24-difluoro-1α,25,26-trihydroxyvitamin D_3.

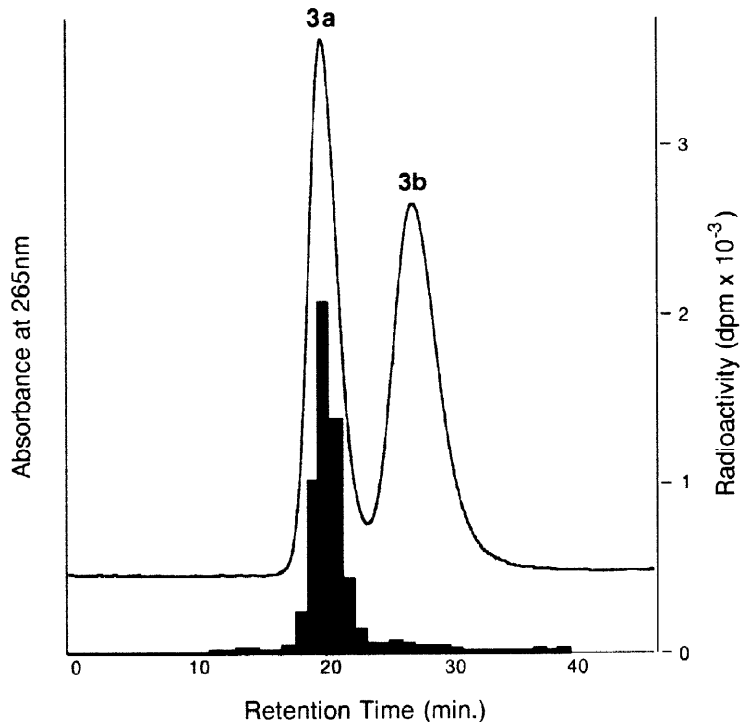


Figure 3. Co-chromatography of the chemically synthesized 24,24-difluoro-1α,25,26-trihydroxy-vitamin D_3 epimers (**3a**, **3b**) (upper chart shown as curves) and 24,24-difluoro-1α,25-dihydroxyvitamin [1β-^{3}H] D_3 metabolite generated in rat kidney homogenates (lower chart shown as a column). Column: CHIRALCEL® OF (4.6 mm i.d. x 250 mm); Mobile phase: hexane:2-propanol= 7:3; Flow rate: 0.5 mL/min.

EXPERIMENTAL

General: ^{1}H-NMR (270 MHz) and ^{13}C-NMR (67.8 MHz) spectra were recorded in $CDCl_3$ on a JEOL JNM-EX270 instrument. Chemical shifts are given in ppm (δ), using tetramethylsilane (TMS) as internal standard. Optical rotations were measured on a HORIBA SEPA-200 polarimeter. Mass spectra were registered on a JEOL JMS-700 instrument. IR spectra were recorded on JASCO FT/IR-7300 instrument. Tetrahydrofuran (THF) was freshly distilled from benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2 and stored over molecular sieves 4A. Column chromatographies were performed with silica gel 60 (70-230 mesh, Merck) and preparative TLC was run on silica gel 60 F_{254} Merck.

(5*Z*,7*E*,20*R*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-oxo-26-[(*R*)-*p*-tolylsulfinyl]-27-nor-9,10-seco-5,7,10(19)-cholestatriene (6a). To a solution of diisopropylamine (230 μL, 1.637 mmol) in THF (10 mL) n-butyllithium (2.34M in hexane solution) (700 μL, 1.638 mmol) was added at 0 °C under an atmosphere of Ar and then stirred for 10 min. After the solution was cooled to -78 °C, a solution of (*R*)-(+)-methyl *p*-tolylsulfoxide (250 mg, 1.624 mmol) of THF (1 mL) was added and stirred for 5 min. Then a solution of **7** (552 mg, 0.812 mmol) in THF (2 mL) was added and stirred for 15 min at -78 °C, then for 1.5 h at room temperature. The reaction mixture was poured into saturated NH_4Cl solution (40 mL) and stirred for 30 min. The mixture was extracted with AcOEt (2x 40 mL), and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel (10 g, hexane:AcOEt= 10:1~5:1) to afford β-ketosulfoxide **6a** (596 mg, 92%) as a white foam. The obtained **6a** was actually a mixture of keto and hydrate forms. **^{1}H-NMR**: δ 0.06 (12H, s), 0.52 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 0.93 (3H, d, J= 5.6 Hz), 1.05~2.05 (16H, m), 2.21 (1H, d, J= 6.9 Hz, 12.9 Hz), 2.43 (3H, s), 2.38~2.50 (1H, m), 2.82 (1H, m), 3.98 (2H, d, J= 14.8 Hz), 4.10~4.25 (1H, m), 4.21 (2H, d, J= 14.8 Hz), 4.34~4.42 (1H, m), 4.86 (1H, d, J= 2.3 Hz), 5.18 (1H, br s), 6.01 (1H, d, J= 11.2 Hz), 6.23 (1H, d, J= 11.2 Hz), 7.35 (2H, d, J= 7.9 Hz), 7.59 (2H, d, J= 7.9 Hz), (3.02 (2H, d, J= 12.9 Hz), 3.11 (2H, d, J= 12.9 Hz), 3.54 (1H, br s), 6.38 (1H, s), 7.38 (2H, d, J= 7.9 Hz)). Chemical shift values (in parentheses) result from the hydrate form. **IR** ($CHCl_3$): 3283 cm^{-1}, 2952 cm^{-1}, 1742 cm^{-1}, 1086 cm^{-1}. **LRMS** (FAB): m/z 821 $(M+H)^+$ (hydrate form), 803 $(M+H)^+$ (keto form), 746 $(M-tBu)^+$, 672 $(M-TBSO)^+$, 139 $(p\text{-TolSO})^+$, 379. **HRMS** (FAB): calcd. for $C_{45}H_{73}O_4F_2Si_2S$ $(M+H)^+$ 803.4736 found 803.4772.

(5*Z*,7*E*,20*R*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-oxo-26-[(*S*)-*p*-tolylsulfinyl]-27-nor-9,10-seco-5,7,10(19)-cholestatriene (6b). β-Ketosulfoxide **6b** (303 mg, 96%) was prepared as a white foam from **7** (269 mg, 0.395 mmol) in the same manner described for the preparation of **6a** by treatment with (*S*)-(-)-methyl *p*-tolyl sulfoxide (130 mg, 0.791 mmol). The obtained **6b** was actually a mixture of keto and hydrate forms. **^{1}H-NMR**: δ 0.06 (12H, s), 0.52 (3H, s), 0.88 (18H, s), 0.93 (3H, d, J= 5.6 Hz), 1.05~2.05 (16H, m), 2.21 (1H, d, J= 7.3 Hz, 13.5 Hz), 2.43 (3H, s), 2.38~2.50 (1H, m), 2.82 (1H, m), 3.98 (2H, d, J= 14.8 Hz), 4.10~4.25 (1H, m), 4.21 (2H, d, J= 14.8 Hz), 4.34~4.42 (1H, m), 4.87 (1H, d, J= 2.3 Hz), 5.18 (1H, br s), 6.01 (1H, d, J= 11.2 Hz), 6.23 (1H, d, J= 11.2 Hz), 7.35 (2H, d, J= 8.3 Hz), 7.59 (2H, d, J= 8.3 Hz), (3.02 (2H, d, J= 12.9 Hz), 3.12 (2H, d, J= 12.9 Hz), 3.57 (1H, br s), 6.38 (1H, s), 7.38 (2H, d, J= 8.3 Hz)). Chemical shift values (in parentheses) result from the hydrate form. **IR** ($CHCl_3$): 3276 cm^{-1}, 2952 cm^{-1}, 1743 cm^{-1}, 1086 cm^{-1}. **LRMS** (FAB): m/z 821 $(M+H)^+$ (hydrate form), 803 $(M+H)^+$ (keto form), 746 $(M-tBu)^+$, 672 $(M-TBSO)^+$, 139 $(p\text{-TolSO})^+$, 379. **HRMS** (FAB): calcd. for $C_{45}H_{73}O_4F_2Si_2S$ $(M+H)^+$ 803.4736 found 803.4742.

(5*Z*,7*E*,20*R*,25*R*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-hydroxy-26-[(*R*)-*p*-tolylsulfinyl]-9,10-seco-5,7,10(19)-cholestatriene (5a). To a suspension of $ZnBr_2$ (90

mg, 0.400 mmol) in CH_2Cl_2 (10 mL) molecular sieves 4A (390 mg) was added and stirred for 2.5 h at room temperature under an atmosphere of Ar. Then a solution of **6a** (320.0 mg, 0.399 mmol) in CH_2Cl_2 (3 mL) which was dried over molecular sieves 4A (400 mg) for 3 h at room temperature was added to the suspension and stirred for 30 min. After the suspension was cooled to -78 °C, Me_3Al (2M in hexane solution) (2.0 mL, 4.00 mmol) was added and stirred for 3 h. The reaction mixture was quenched with saturated NH_4Cl solution (15 mL) and allowed to warm to room temperature with vigorous stirring. 1N-HCl (15 mL) was added to the mixture and extracted with AcOEt (70 mL). The organic phase was washed with brine three times, dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel (10 g, hexane:AcOEt= 10:1~3:1) to afford two diastereomers of tertiary alcohol (less polar (25*R*)-isomer **5a**: 242 mg, 74%; more polar (25*S*)-isomer **22a**: 45 mg, 14%) as a white foam respectively. **5a**: $[\alpha]_D^{20}$= +135.3° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.05 (6H, s), 0.06 (6H, s), 0.52 (3H, s), 0.89 (18H, s), 0.93 (3H, d, J= 4.7 Hz), 1.10~2.25 (19H, m), 1.73 (3H, s), 2.44 (3H, s), 2.35~2.50 (1H, m), 2.80 (1H, d, J= 13.2 Hz), 2.75~2.83 (1H, m), 3.17 (1H, d, J= 13.2 Hz), 4.19 (1H, tt, J= 3.3 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.3 Hz, 5.0 Hz), 4.60 (1H, s), 4.86 (1H, d, J= 1.3 Hz), 5.18 (1H, d, J= 1.3 Hz), 6.01 (1H, d, J= 11.2 Hz), 6.23 (1H, d, J= 11.2 Hz), 7.37 (2H, d, J= 7.9 Hz), 7.57 (2H, d, J= 7.9 Hz). **^{13}C-NMR**: δ -4.76, -4.65, 11.99, 18.17, 18.26, 18.69, 21.47, 21.62, 22.12, 23.49, 25.82, 25,88, 26.42, 26.56, 26.77, 27.57, 28.86, 35.74, 40.50, 40.57, 44.83, 45.78, 46.06, 56.17, 56.30, 60.97, 67.54, 72.09, 75.41, 77.23, 111.25, 117.99, 123.14, 124.06, 124.44, 130.33, 135.07, 139.89, 140.88, 142.46, 148.32. **IR** (KBr): 3314 cm^{-1}, 2951 cm^{-1}, 1254 cm^{-1}, 1087 cm^{-1}. **LRMS** (FAB): m/z 820 $(M+H)^+$, 688 $(M-TBSO)^+$, 556 $(M-2TBSO)^+$, 139 (*p*-TolSO)$^+$, 379, 439. **HRMS** (FAB): calcd. for $C_{46}H_{77}O_4F_2Si_2S$ $(M+H)^+$ 819.5049 found 819.5085. **22a**: $[\alpha]_D^{20}$= +133.9° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.06 (6H, s), 0.07 (6H, s), 0.54 (3H, s), 0.89 (18H, s), 0.96 (3H, d, J= 6.3 Hz), 1.10~2.25 (19H, m), 1.38 (3H, s), 2.43 (3H, s), 2.35~2.50 (1H, m), 2.75~2.90 (1H, m), 2.89 (1H, d, J= 13.5 Hz), 3.05 (1H, d, J= 13.5 Hz), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.6 Hz, 6.3 Hz), 4.74 (1H, s), 4.87 (1H, d, J= 1.7 Hz), 5.18 (1H, d, J= 1.7 Hz), 6.03 (1H, d, J= 11.2 Hz), 6.24 (1H, d, J= 11.2 Hz), 7.35 (2H, d, J= 8.3 Hz), 7.57 (2H, d, J= 8.3 Hz). **^{13}C-NMR**: δ -4.76, -4.65, 12.00, 18.18, 18.53, 18.65, 21.46, 22.14, 23.50, 24.38, 24.44, 25.84, 25,88, 26.67, 27.60, 28.88, 35.78, 40.59, 44.85, 45.80, 46.07, 56.15, 56.30, 61.49, 67.55, 72.11, 75.76, 77.23, 111.28, 117.99, 123.16, 123.97, 124.06, 125.98, 130.20, 130.33, 135.07, 140.90, 141.36, 142.08, 148.32. **IR** (KBr): 3282 cm^{-1}, 2951 cm^{-1}, 1255 cm^{-1}, 1087 cm^{-1}. **LRMS** (FAB): m/z 820 $(M+H)^+$, 688 $(M-TBSO)^+$, 556 $(M-2TBSO)^+$, 139 (*p*-TolSO)$^+$, 379, 439. **HRMS** (FAB): calcd. for $C_{46}H_{77}O_4F_2Si_2S$ $(M+H)^+$ 819.5049 found 819.5082.

(5*Z*,7*E*,20*R*,25*S*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-hydroxy-26-[(*S*)-*p*-tolylsulfinyl]-9,10-seco-5,7,10(19)-cholestatriene (5b). Ketosulfoxide **6b** (260.0 mg, 0.324 mmol) was treated with Me_3Al in the same manner described for the preparation of **5a** to afford two diastereomers of tertiary alcohol **5b** (167 mg, 63%) and its C-25 epimer **22b** (41 mg, 15%) as a white foam respectively. **5b**: $[\alpha]_D^{20}$= -26.9° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.06 (12H, s), 0.53 (3H, s), 0.87 (18H, s),

0.93 (3H, d, J= 6.3 Hz), 1.10~2.25 (19H, m), 1.71 (3H, s), 2.44 (3H, s), 2.35~2.50 (1H, m), 2.81 (1H, d, J= 13.2 Hz), 2.75~2.83 (1H, m), 3.19 (1H, d, J= 13.2 Hz), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.3 Hz, 5.6 Hz), 4.62 (1H, s), 4.86 (1H, d, J= 1.6 Hz), 5.17 (1H, d, J= 1.6 Hz), 6.01 (1H, d, J= 11.2 Hz), 6.23 (1H, d, J= 11.2 Hz), 7.37 (2H, d, J= 8.2 Hz), 7.56 (2H, d, J= 8.2 Hz). **^{13}C-NMR**: δ -4.76, -4.65, 11.99, 18.15, 18.24, 18.67, 21.47, 21.73, 22.12, 23.49, 25.82, 25,88, 26.58, 26.72, 27.58, 28.88, 35.76, 40.57, 44.85, 45.79, 46.07, 56.15, 56.30, 60.86, 67.55, 72.11, 75.43, 77.21, 111.25, 117.99, 123.14, 124.06, 124.47, 130.33, 135.07, 139.91, 140.89, 142.44, 148.34. **IR** (KBr): 3329 cm^{-1}, 2951 cm^{-1}, 1255 cm^{-1}, 1087 cm^{-1}. **LRMS** (FAB): m/z 820 $(M+H)^+$, 687 $(M\text{-}TBSO)^+$, 555 $(M\text{-}2TBSO)^+$, 139 $(p\text{-}TolSO)^+$, 379, 439. **HRMS** (FAB): calcd. for $C_{46}H_{77}O_4F_2Si_2S$ $(M+H)^+$ 819.5049 found 819.5089. **22b**: $[\alpha]_D^{20}$= -21.2° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.06 (12H, s), 0.54 (3H, s), 0.89 (18H, s), 0.95 (3H, d, J= 5.6 Hz), 1.10~2.25 (19H, m), 1.40 (3H, s), 2.43 (3H, s), 2.35~2.50 (1H, m), 2.75~2.90 (1H, m), 2.90 (1H, d, J= 13.9 Hz), 3.05 (1H, d, J= 13.9 Hz), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.35 (1H, dd, J= 3.6 Hz, 6.3 Hz), 4.67 (1H, s), 4.87 (1H, d, J= 1.3 Hz), 5.18 (1H, d, J= 1.3 Hz), 6.02 (1H, d, J= 11.2 Hz), 6.24 (1H, d, J= 11.2 Hz), 7.35 (2H, d, J= 7.9 Hz), 7.57 (2H, d, J= 7.9 Hz). **^{13}C-NMR**: δ -4.76, -4.65, 12.00, 18.15, 18.24, 18.72, 21.44, 22.14, 23.49, 24.28, 25.82, 25,88, 26.65, 27.21, 27.57, 28.88, 35.71, 40.61, 44.85, 45.80, 46.07, 56.21, 56.32, 61.76, 67.55, 72.11, 75.74, 77.23, 111.27, 118.00, 123.14, 123.97, 124.08, 125.89, 130.19, 130.33, 135.11, 140.84, 141.38, 142.05, 148.34. **IR** (KBr): 3290 cm^{-1}, 2951 cm^{-1}, 1255 cm^{-1}, 1087 cm^{-1}. **LRMS** (FAB): m/z 820 $(M+H)^+$, 688 $(M\text{-}TBSO)^+$, 556 $(M\text{-}2TBSO)^+$, 139 $(p\text{-}TolSO)^+$, 379, 439. **HRMS** (FAB): calcd. for $C_{46}H_{77}O_4F_2Si_2S$ $(M+H)^+$ 819.5049 found 819.5082.

(5*Z*,7*E*,20*R*,25*R*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-(methoxyethoxymethyl)oxy-26-[(*R*)-*p*-tolylsulfinyl]-9,10-seco-5,7,10(19)-cholestatriene (9a). To a solution of **5a** (200 mg, 0.244 mmol) in THF (3 mL) NaH (60% oil dispersion) (14 mg, 0.350 mmol) was added and stirred at 0 °C for 5 min under an atmosphere of Ar. Then MEMCl (50 μL, 0.436 mmol) was added to the solution and stirred for 2 h. The reaction mixture was quenched with NH_4Cl solution (30 mL) and extracted with AcOEt (2x 30 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica gel (10 g, hexane:AcOEt= 10:1~3:1) to afford MEM ether **9a** (170.0 mg, 76%) as a colorless oil. $[\alpha]_D^{20}$= +90.2° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.059 (6H, s), 0.064 (6H, s), 0.53 (3H, s), 0.88 (18H, s), 0.94 (3H, d, J= 6.6 Hz), 1.10~2.25 (17H, m), 1.72 (3H, s), 2.42 (3H, s), 2.35~2.50 (1H, m), 2.75~2.83 (1H, m), 2.99 (1H, d, J= 13.9 Hz), 3.21 (1H, d, J= 13.9 Hz), 3.36 (3H, s), 3.55 (2H, t, J= 5.0 Hz), 3.73 (1H, dt, J= 5.0 Hz, 11.5 Hz), 3.89 (1H, dt, J= 5.0 Hz, 11.5 Hz), 4.19 (1H, tt, J= 3.3 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.7 Hz, 6.3 Hz), 4.60 (1H, s), 4.86 (1H, d, J= 2.3 Hz), 4.89 (1H, d, J= 7.6 Hz), 4.99 (1H, d, J= 7.6 Hz), 5.18 (1H, d, J= 2.3 Hz), 6.01 (1H, d, J= 11.2 Hz), 6.24 (1H, d, J= 11.2 Hz), 7.33 (2H, d, J= 7.9 Hz), 7.55 (2H, d, J= 7.9 Hz). **^{13}C-NMR**: δ -4.80, -4.69, 11.99, 17.83, 18.13, 18.22, 18.64, 21.37, 22.10, 23.45, 25.81, 25.84, 27.55, 28.84, 35.69, 40.58, 44.84, 45.75, 46.04, 56.14, 56.26, 58.94, 66.65, 66.81, 67.46, 67.53, 71.70, 71.75, 72.09, 77.22, 91.03,

111.21, 117.99, 123.11, 123.95, 130.06, 135.09, 140.79, 141.53, 142.05, 148.32. **IR** ($CHCl_3$): 2952 cm^{-1}, 1253 cm^{-1}, 1087 cm^{-1}. **LRMS** (FAB): m/z 908 $(M+H)^+$, 139 $(p\text{-TolSO})^+$, 529, 379, 248. **HRMS** (FAB): calcd. for $C_{50}H_{85}O_6F_2Si_2S$ $(M+H)^+$ 907.5574 found 907.5604.

(5*Z*,7*E*,20*R*,25*S*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-(methoxyethoxymethyl)oxy-26-[(*S*)-*p*-tolylsulfinyl]-9,10-seco-5,7,10(19)-cholestatriene (9b). Alcohol **5b** (300 mg, 0.367 mmol) was treated with NaH and MEMCl in the same manner described for the preparation of **9a** to afford MEM ether **9b** (270 mg, 81%) as a colorless oil. $[\alpha]_D^{20}$= -9.9° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.059 (6H, s), 0.062 (6H, s), 0.53 (3H, s), 0.88 (18H, s), 0.92 (3H, d, J= 6.6 Hz), 1.10~2.00 (17H, m), 1.71 (3H, s), 2.21 (1H, dd, J= 7.6 Hz, 13.2 Hz), 2.42 (3H, s), 2.35~2.50 (1H, m), 2.82 (1H, dd, J= 2.3 Hz, 12.9 Hz), 3.01 (1H, d, J= 14.2 Hz), 3.21 (1H, d, J= 14.2 Hz), 3.36 (3H, s), 3.54 (2H, t, J= 4.6 Hz), 3.72 (1H, dt, J= 4.6 Hz, 10.8 Hz), 3.88 (1H, dt, J= 4.6 Hz, 10.8 Hz), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.6 Hz, 6.6 Hz), 4.60 (1H, s), 4.86 (1H, d, J= 2.3 Hz), 4.89 (1H, d, J= 7.6 Hz), 4.98 (1H, d, J= 7.6 Hz), 5.18 (1H, d, J= 2.3 Hz), 6.02 (1H, d, J= 11.2 Hz), 6.24 (1H, d, J= 11.2 Hz), 7.33 (2H, d, J= 7.9 Hz), 7.55 (2H, d, J= 7.9 Hz). **^{13}C-NMR**: δ -4.80, -4.69, 11.99, 17.97, 18.13, 18.22, 18.64, 21.39, 22.10, 23.47, 25.81, 25.86, 26.58, 27.57, 27.96, 28.84, 35.69, 40.58, 44.84, 45.77, 46.06, 56.17, 56.28, 58.96, 66.61, 67.55, 71.68, 72.09, 77.22, 79.19, 91.03, 111.25, 118.00, 123.11, 123.95, 130.06, 135.11, 140.77, 141.51, 142.07, 148.30. **IR** ($CHCl_3$): 2950 cm^{-1}, 1252 cm^{-1}, 1085 cm^{-1}. **LRMS** (FAB): m/z 908 $(M+H)^+$, 832 $(M\text{-}OCH_2CH_2OCH_3)^+$, 139 $(p\text{-TolSO})^+$, 818, 379, 248. **HRMS** (FAB): calcd. for $C_{50}H_{85}O_6F_2Si_2S$ $(M+H)^+$ 907.5574 found 907.5604.

(5*Z*,7*E*,20*R*,25*S*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-(methoxyethoxymethyl)oxy-26-oxo-9,10-seco-5,7,10(19)-cholestatriene (4a). MEM ether **9a** (58 mg, 0.064 mmol) was dissolved in CH_2Cl_2 (1 mL) and cooled to 0 °C under an atmosphere of Ar. Then pyridine (11 μL, 0.136 mmol) and trifluoroacetic anhydride (18 μL, 0.129 mmol) were added to the solution with stirring. After 15 min stirring, MeCN (0.5 mL) and 20% KOH solution (0.5 mL) were added to the reaction mixture. The solution was vigorously stirred at 0 °C for 2 h, then poured into water (20 mL) and extracted with AcOEt (20 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was developed on silica gel preparative TLC (hexane:AcOEt= 2:1) to afford aldehyde **4a** (47 mg, 94%) as a colorless oil. $[\alpha]_D^{20}$= +42.4° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.059 (6H, s), 0.064 (6H, s), 0.53 (3H, s), 0.88 (18H, s), 0.93 (3H, d, J= 6.6 Hz), 1.05~2.05 (18H, m), 1.47 (3H, s), 2.21 (1H, dd, J= 7.3 Hz, 13.2 Hz), 2.45 (1H, dd, J= 3.3 Hz, 13.2 Hz), 2.83 (1H, dd, J= 3.0 Hz, 13.2 Hz), 3.37 (3H, s), 3.52 (2H, t, J= 4.6 Hz), 3.72 (1H, dt, J= 4.6 Hz, 11.2 Hz), 3.84 (1H, dt, J= 4.6 Hz, 11.2 Hz), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.6 Hz, 6.3 Hz), 4.84 (1H, d, J= 7.3 Hz), 4.87 (1H, d, J= 1.7 Hz), 4.94 (1H, d, J= 7.3 Hz), 5.18 (1H, d, J= 1.7 Hz), 6.02 (1H, d, J= 11.2 Hz), 6.23 (1H, d, J= 11.2 Hz), 9.68 (1H, s). **^{13}C-NMR**: δ -4.80, -4.69, 11.97, 12.76, 18.13, 18.23, 18.61, 22.10, 23.45, 25.81, 25.84, 26.32, 27.55,

28.72, 28.84, 35.67, 40.78, 44.84, 45.77, 46.06, 56.17, 56.28, 59.01, 67.53, 67.82, 71.54, 72.08, 77.20, 83.60, 83.99, 91.23, 111.23, 118.02, 123.09, 123.74, 135.13,140.74, 148.32, 198.99. **IR** ($CHCl_3$): 2951 cm^{-1}, 1743 cm^{-1}, 1255 cm^{-1}, 1078 cm^{-1}, 1024 cm^{-1}. **LRMS** (FAB): m/z 784 $(M+H)^+$, 651 $(M\text{-}TBSO)^+$, 379, 248. **HRMS** (FAB): calcd. for $C_{43}H_{77}O_6F_2Si_2S$ $(M+H)^+$ 783.5227 found 783.5195.

(5*Z*,7*E*,20*R*,25*R*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-25-(methoxyethoxymethyl)oxy-26-oxo-9,10-seco-5,7,10(19)-cholestatriene (4b). Aldehyde **4b** (40 mg, 77%) was obtained as a colorless oil from **9b** (60 mg, 0.066 mmol) in the same manner described for the preparation of **4a**. $[\alpha]_D^{20}$= +45.7° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.057 (6H, s), 0.062 (6H, s), 0.53 (3H, s), 0.88 (18H, s), 0.93 (3H, d, J= 6.6 Hz), 1.05~2.05 (18H, m), 1.46 (3H, s), 2.21 (1H, dd, J= 7.3 Hz, 13.2 Hz), 2.45 (1H, dd, J= 3.0 Hz, 13.2 Hz), 2.82 (1H, dd, J= 3.0 Hz, 13.2 Hz), 3.37 (3H, s), 3.52 (2H, t, J= 4.6 Hz), 3.73 (1H, dt, J= 4.6 Hz, 11.2 Hz), 3.84 (1H, dt, J= 4.6 Hz, 11.2 Hz), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.6 Hz, 6.3 Hz), 4.84 (1H, d, J= 7.6 Hz), 4.87 (1H, d, J= 1.7 Hz), 4.94 (1H, d, J= 7.6 Hz), 5.18 (1H, d, J= 1.7 Hz), 6.02 (1H, d, J= 11.2 Hz), 6.24 (1H, d, J= 11.2 Hz), 9.69 (1H, s). **^{13}C-NMR**: δ -4.78, -4.67, 11.99, 12.80, 18.15, 18.24, 18.60, 22.12, 23.47, 25.82, 25.86, 26.35, 27.57, 28.75, 28.86, 35.73, 40.60, 44.85, 45.79, 46.08, 56.19, 56.30, 59.03, 67.53, 67.84, 71.56, 72.11, 77.22, 83.58, 83.97, 91.25, 111.27, 118.04, 123.11, 123.79, 135.15,140.75, 148.34, 199.01. **IR** ($CHCl_3$): 2952 cm^{-1}, 1743 cm^{-1}, 1255 cm^{-1}, 1081 cm^{-1}, 1023 cm^{-1}. **LRMS** (FAB): m/z 784 $(M+H)^+$, 726 $(M\text{-}tBu)^+$, 651 $(M\text{-}TBSO)^+$, 379, 248. **HRMS** (FAB): calcd. for $C_{43}H_{77}O_6F_2Si_2S$ $(M+H)^+$ 783.5227 found 783.5197.

(5*Z*,7*E*,20*R*,25*S*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-26-hydroxy-25-(methoxyethoxymethyl)oxy-9,10-seco-5,7,10(19)-cholestatriene (10a). Aldehyde **4a** (44 mg, 0.056 mmol) was dissolved in MeOH (1 mL) and cooled to 0 °C under an atmosphere of Ar. Then $NaBH_4$ (9 mg, 0.238 mmol) was added to the solution and stirred for 30 min. The reaction mixture was poured into water (20 mL) and extracted with AcOEt (3x 20 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was developed on silica gel preparative TLC (hexane:AcOEt= 2:1) to afford alcohol **10a** (42 mg, 95%) as a colorless oil. $[\alpha]_D^{20}$= +35.8° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.059 (6H, s), 0.064 (6H, s), 0.53 (3H, s), 0.88 (18H, s), 0.93 (3H, d, J= 5.3 Hz), 1.05~2.05 (19H, m), 1.32 (3H, s), 2.21 (1H, dd, J= 7.3 Hz, 13.5 Hz), 2.45 (1H, dd, J= 3.3 Hz, 13.2 Hz), 2.82 (1H, dd, J= 3.0 Hz, 12.9 Hz), 3.39 (3H, s), 3.45~3.65 (3H,m), 3.70~3.90 (3H,m), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.6 Hz, 6.3 Hz), 4.86 (1H, d, J= 1.3 Hz), 4.88 (2H, s), 5.18 (1H, d, J= 1.3 Hz), 6.02 (1H, d, J= 11.2 Hz), 6.24 (1H, d, J= 11.2 Hz). **^{13}C-NMR**: δ -4.76, -4.67, 12.00, 15.08, 18.15, 18.24, 18.67, 22.14, 23.50, 25.82, 25.88, 26.54, 26.59, 27.58, 28.54, 28.88, 35.85, 40.63, 44.85, 45.80, 46.07, 56.32, 56.37, 58.94, 63.43, 67.55, 67.58, 71.50, 72.11, 77.21, 81.74, 90.22, 111.27, 118.00, 123.14, 135.09, 140.88, 148.32. **IR** ($CHCl_3$): 3491 cm^{-1}, 2951 cm^{-1}, 1256 cm^{-1}, 1074 cm^{-1}. **LRMS** (FAB): m/z 786 $(M+H)^+$, 652 $(M\text{-}TBSO)^+$, 379, 248. **HRMS** (FAB): calcd. for $C_{43}H_{79}O_6F_2Si_2S$ $(M+H)^+$ 785.5383 found 785.5386.

(5*Z*,7*E*,20*R*,25*R*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-24,24-difluoro-26-hydroxy-25-(methoxyethoxymethyl)oxy-9,10-seco-5,7,10(19)-cholestatriene (10b). Alcohol **10b** (136 mg, 90%) was afforded as a colorless oil from **4b** (150 mg, 0.192 mmol) in the same manner described for the preparation of **10a**. $[\alpha]_D^{20}$= +55.5° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.060 (6H, s), 0.064 (6H, s), 0.54 (3H, s), 0.88 (18H, s), 0.93 (3H, d, J= 5.0 Hz), 1.05~2.05 (19H, m), 1.32 (3H, s), 2.21 (1H, dd, J= 7.3 Hz, 13.5 Hz), 2.45 (1H, dd, J= 3.6 Hz, 13.2 Hz), 2.83 (1H, dd, J= 2.6 Hz, 13.2 Hz), 3.39 (3H, s), 3.50~3.65 (3H,m), 3.70~3.90 (3H,m), 4.19 (1H, tt, J= 3.6 Hz, 7.3 Hz), 4.37 (1H, dd, J= 3.6 Hz, 6.3 Hz), 4.86 (1H, d, J= 1.7 Hz), 4.88 (2H, s), 5.18 (1H, d, J= 1.6 Hz), 6.02 (1H, d, J= 11.2 Hz), 6.24 (1H, d, J= 11.2 Hz). **^{13}C-NMR**: δ -4.80, -4.69, 11.98, 15.13, 18.13, 18.22, 18.67, 22.12, 23.49, 25.81, 25.84, 26.58, 27.57, 28.46, 28.86, 35.83, 40.59, 44.84, 45.77, 46.06, 56.30, 58.92, 63.33, 67.57, 71.48, 72.09, 77.20, 81.71, 90.19, 111.25, 117.97, 123.13, 125.36, 135.06, 140.86, 148.30. **IR** ($CHCl_3$): 3491 cm^{-1}, 2951 cm^{-1}, 1255 cm^{-1}, 1074 cm^{-1}. **LRMS** (FAB): m/z 786 $(M+H)^+$, 653 $(M-TBSO)^+$, 379, 248. **HRMS** (FAB): calcd. for $C_{43}H_{79}O_6F_2Si_2S$ $(M+H)^+$ 785.5383 found 785.5396.

(25*S*)-24,24-Difluoro-1α,25,26-trihydroxyvitamin D$_3$ (3a). To a solution of **10a** (93 mg, 0.119 mmol) in MeOH (7 mL) 10-(+)-camphorsulfonic acid (45 mg, 0.194 mmol) was added and stirred at room temperature for 2 h under an atmosphere of Ar. The reaction mixture was poured into saturated $NaHCO_3$ solution (40 mL) and extracted with AcOEt (3x 40 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was developed on silica gel preparative TLC (AcOEt) to afford **3a** (40 mg, 72%) as a white solid. $[\alpha]_D^{20}$= +38.6° (c= 0.9, EtOH). **^{1}H-NMR**: δ 0.55 (3H, s), 0.95 (3H, d, J= 6.3 Hz), 1.20~2.10 (21H, m), 1.26 (3H, s), 2.31 (1H, dd, J= 6.6 Hz, 13.2 Hz), 2.60 (1H, dd, J= 2.6 Hz, 13.2 Hz), 2.71 (1H, s), 2.83 (1H, dd, J= 2.6 Hz, 13.2 Hz), 3.46 (1H, dd, J= 9.2 Hz, 11.2 Hz), 3.93 (1H, dd, J= 4.3 Hz, 11.2 Hz), 4.15~4.30 (1H, m), 4.35~4.50 (1H, m), 5.00 (1H, d, J= 1.3 Hz), 5.33 (1H, d, J= 1.3 Hz), 6.02 (1H, d, J= 10.9 Hz), 6.38 (1H, d, J= 10.9 Hz). **^{13}C-NMR**: δ 12.02, 18.65, 22.25, 23.56, 27.48, 27.96, 29.06, 35.67, 40.47, 42.89, 45.30, 45.91, 56.19, 56.32, 65.50, 66.88, 70.85, 74.39, 77.21, 111.80, 117.12, 124.98, 132.97, 143.03, 147.65. **IR** (KBr): 3324 cm^{-1}, 2948 cm^{-1}, 1051 cm^{-1}. **LRMS** (FAB): m/z 469 $(M+H)^+$, 451 $(M+H-H_2O)^+$, 433 $(M+H-2H_2O)^+$, 315, 287, 135, 75. **HRMS** (FAB): calcd. for $C_{27}H_{43}O_4F_2$ $(M+H)^+$ 469.3129 found 469.3130.

(25*R*)-24,24-Difluoro-1α,25,26-trihydroxyvitamin D$_3$ (3b). Vitamin D_3 analog **3b** (32 mg, 89%) was obtained as a white solid from **10b** (60 mg, 0.077 mmol) in the same manner described for the preparation of **4b**. $[\alpha]_D^{20}$= +32.2° (c= 0.9, EtOH). **^{1}H-NMR**: δ 0.55 (3H, s), 0.95 (3H, d, J= 6.6 Hz), 1.20~2.10 (21H, m), 1.26 (3H, s), 2.31 (1H, dd, J= 6.6 Hz, 13.2 Hz), 2.60 (1H, dd, J= 3.6 Hz, 13.2 Hz), 2.78 (1H, s), 2.83 (1H, dd, J= 3.6 Hz, 12.2 Hz), 3.45 (1H, dd, J= 9.2 Hz, 11.9 Hz), 3.94 (1H, dd, J= 4.3 Hz, 11.9 Hz), 4.15~4.30 (1H, m), 4.35~4.50 (1H, m), 5.00 (1H, d, J= 1.7 Hz), 5.33 (1H, d, J= 1.7 Hz), 6.02 (1H, d, J= 11.2 Hz), 6.38 (1H, d, J= 11.2 Hz). **^{13}C-NMR**: δ 12.01, 18.62, 22.23, 23.54, 27.48,

27.91, 29.04, 35.65, 40.45, 42.86, 45.28, 45.90, 56.16, 56.28, 65.48, 66.87, 70.86, 74.34, 77.20, 111.81, 117.11, 124.96, 132.96, 143.02, 147.64. **IR** (KBr): 3346 cm^{-1}, 2948 cm^{-1}, 1051 cm^{-1}. **LRMS** (FAB): m/z 469 $(M+H)^+$, 451 $(M+H-H_2O)^+$, 433 $(M+H-2H_2O)^+$, 315, 287, 135, 75. **HRMS** (FAB): calcd. for $C_{27}H_{43}O_4F_2$ $(M+H)^+$ 469.3129 found 469.3132.

(20*R*)-De-A,B-8β-hydroxy-20-(hydroxymethyl)pregnane (11). Vitamin D_2 (5.00g, 12.63 mmol) was dissolved in CH_2Cl_2 (170 mL), and MeOH (70 mL) which contained $NaHCO_3$ (30 mg) was added. After cooling to -78 °C, a stream of ozone (flow rate: 1 L/min) was passed to the solution until a gray-blue color appeared (100 min). The remaining ozone was purged with a stream of Ar, then $NaBH_4$ (2.00 g, 52.87 mmol) was added and stirred for 30 min. The reaction mixture was allowed to warm to room temperature while stirring for 1 h. The reaction mixture was then acidified by adding 1N-HCl (50 mL) at 0 °C and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel (150 g, hexane:AcOEt= 7:3) to afford Inhoffen-Lythgoe diol **11** (1.98 g, 74%) as a white solid. **^{1}H-NMR**: δ 0.96 (3H, s), 1.03 (3H, d, J= 6.6 Hz), 1.12~1.92 (14H, m), 1.99 (1H, dd, J= 2.6 Hz, 13.2 Hz), 3.39 (1H, dd, J= 6.6 Hz, 10.6 Hz), 3.64 (1H, dd, J= 3.3 Hz, 10.6 Hz), 4.09 (1H, d, J= 2.6 Hz).

(20*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-20-[(*p*-tolylsulfonyl)oxymethyl]pregnane (12). DMAP (2.20 g, 18.02 mmol) and *p*-TsCl (2.23 g, 11.71 mmol) was added to a solution of **11** (1.91 g, 9.01 mmol) in CH_2Cl_2 (40 mL). After stirring overnight at room temperature, the reaction mixture was poured into water (100 mL) and extracted with CH_2Cl_2 (100 mL). The organic solution was washed with 0.5N-HCl, water, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and evaporated. The residue was dissolved in CH_2Cl_2 (40 mL) and then 2,6-lutidine (2.6 mL, 22.53 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (3.1 mL, 13.52 mmol) was added. After stirring at room temperature for 30 min, the solution was poured into water (100 mL) and extracted with CH_2Cl_2 (100 mL). The organic solution was washed with 0.5N-HCl and brine, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica gel (60 g, hexane:AcOEt= 50:1) to afford **12** (4.00 g, 93%) as colorless needles (m.p. 46~47 °C (lit.[20] 50 °C)). **^{1}H-NMR**: δ 0.02 (6H, s), 0.887 (3H, s), 0.892 (9H, s), 0.97 (3H, d, J= 6.6 Hz), 1.07~1.91 (13H, m), 2.47 (3H, s), 3.81 (1H, dd, J= 6.6 Hz, 9.2 Hz), 3.98 (1H, dd, J= 3.0 Hz, 9.2 Hz), 4.00 (1H,br-s), 7.36 (2H, d, J= 8.3 Hz), 7.80 (2H, d, J= 8.3 Hz).

(20*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-20-(cyanomethyl)pregnane (13).[21] Tosylate **12** (1.43 g, 2.98 mmol) was dissolved in DMSO (20 mL) and then NaCN (189 mg, 3.857 mmol) was added to the solution. The reaction mixture was heated at 90 °C for 1 h with stirring, then poured into water (40 mL) and extracted with AcOEt (3x 30 mL). The combined organic phase was washed with water and brine, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica gel (30 g, hexane:AcOEt= 80:1) to

afford nitrile **13** (892 mg, 89%) as a colorless oil. **^{1}H-NMR**: δ 0.00 (3H, s), 0.02 (3H, s), 0.89 (9H, s), 0.93 (3H, s), 1.14 (3H, d, J= 6.6 Hz), 1.10~2.00 (20H, m), 2.23 (1H, dd, J= 6.6 Hz, 16.5 Hz), 2.34 (1H, dd, J= 4.0 Hz, 16.5 Hz), 4.01 (1H, d, J= 2.6 Hz).

(20*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-20-(formylmethyl)pregnane (14). DIBAL-H (1M in hexane) (5.4 mL, 5.40 mmol) was added dropwise to a solution of **13** (892 mg, 2.663 mmol) of CH_2Cl_2 (20 mL) at 0 °C under an atmosphere of Ar. After stirring for 1 h, the reaction mixture was quenched by adding saturated NH_4Cl solution (1.6 mL), diluted with Et_2O (15 mL) and then stirred for 30 min. The mixture was dried over $MgSO_4$ and filtered through Celite. After evaporation, the residue was chromatographed on silica gel (50 g, hexane:AcOEt= 80:1) to afford aldehyde **14** (743 mg, 81%) as a white solid. $[\alpha]_D^{20}$= +29.8° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.002 (3H, s), 0.01 (3H, s), 0.89 (9H, s), 0.96 (3H, s), 1.00 (3H, d, J= 6.3 Hz), 1.03~2.20 (20H, m), 2.46 (1H, dd, J= 2.0 Hz, 13.5 Hz), 4.00 (1H, d, J= 2.6 Hz), 9.75 (1H, dd, J= 1.3 Hz, 3.3 Hz). **^{13}C-NMR**: δ -5.26, -4.90, 13.61, 17.49, 17.93, 18.85, 22.87, 25.71, 27.47, 31.17, 34.24, 40.46, 42.18, 50.68, 52.93, 56.45, 69.23, 77.10, 203.55. **IR** (KBr): 1728 cm^{-1}. **LRMS** (EI): m/z 338 (M^+), 323 $(M\text{-}Me)^+$, 281 $(M\text{-}tBu)^+$. **HRMS** (EI): calcd. for $C_{20}H_{38}O_2Si$ 338.2641 found 338.2648.

(20*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-24-(1'-ethoxyethyl)oxy-24-(methoxy-carbonyl)cholan-23-ene (15). To a solution of diisopropylamine (0.89 mL, 6.59 mmol) in THF (20 mL) n-butyllithium (2.34M in hexane solution) (2.81 mL, 6.59 mmol) was added at 0 °C under an atmosphere of Ar and then stirred for 10 min. After the solution was cooled to -40 °C, a solution of trimethyl (ethoxyethyl-oxy)phosphonoacetate (1.77 g, 6.59 mmol) in THF (5 mL) was added and stirred for 15 min. To this reaction mixture, a solution of **14** (743 mg, 2.198 mmol) in THF (3 mL) was added and stirred at -40 °C for 10 min then at 0 °C for 30 min. The mixture was poured into saturated NH_4Cl solution (100 mL) and extracted with AcOEt (3x 50 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel (30 g, hexane:AcOEt= 50:1) to afford enol ether **15** (957 mg, 90%) as a colorless oil. **^{1}H-NMR**: δ 0.006 (3H, s), 0.01 (3H, s), 0.89 (9H, s), 0.90~2.56 (27H, m), 3.49~3.66 (1H, m), 3.77 and 3.79 (each 3H, s), 3.74~3.90 (1H, m), 4.00 (1H, d, J= 2.3 Hz), 5.01 (0.7H, ddd, J= 1.7 Hz, 5.3 Hz, 10.6 Hz), 5.12 (0.3H, dd, J= 4.9 Hz, 10.2 Hz), 5.72~5.79 (0.7H, m), 6.28~6.36 (0.3H, m). **IR** (neat): 1748 cm^{-1}. **LRMS** (EI): m/z 453 $(M\text{-}Et)^+$, 393 $(M\text{-}OEE)^+$, 367 $(M\text{-}TBS)^+$, 251 $(M\text{-}OTBS)^+$, 215. **HRMS** (EI): calcd. for $C_{21}H_{35}O_4$ $(M\text{-}TBS)^+$ 367.2484 found 367.2502.

(20*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-24-oxo-24-(methoxycarbonyl)cholane (16). Enol ether **15** (957 mg, 1.986 mmol) was dissolved in CH_2Cl_2 (5 mL) and a solution of PPTS (100 mg, 0.397 mmol) in MeOH (2 mL) was added. After stirring at room temperature for 90 min, the reaction mixture was poured into water (50 mL) and extracted with AcOEt (3x 50 mL). The combined organic solution was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica

gel (30 g, hexane:AcOEt= 50:1) to afford α-ketoester **16** (516 mg, 63%) as a colorless oil. $[\alpha]_D^{20}$= +45.2° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ -0.01 (3H, s), 0.01 (3H, s), 0.88 (9H, s), 0.905 (3H, s), 0.906 (3H, d, J= 6.3 Hz), 1.00~2.00 (15H, m), 2.65~2.95 (2H, m), 3.87 (3H, s), 4.00 (1H, d, J= 2.3 Hz). **^{13}C-NMR**: δ -5.26, -4.90, 13.61, 17.53, 17.93, 18.29, 22.92, 25.53, 25.71, 27.07, 28.76, 34.30, 34.64, 36.20, 40.57, 42.08, 52.75, 52.93, 56.23, 69.30, 161.57, 194.71. **IR** (neat): 1732 cm^{-1}. **LRMS** (FAB): m/z 411 $(M+H)^+$, 279 $(M\text{-}TBSO)^+$, 59. **HRMS** (FAB): calcd. for $C_{23}H_{43}O_4Si$ $(M+H)^+$ 411.2931 found 411.2915.

(20*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-24,24-difluoro-24-(methoxycarbonyl)-cholane (17). Morph-DAST (730 μL, 5.925 mmol) was added dropwise to a solution of **16** (486 mg, 1.185 mmol) in CH_2Cl_2 (10 mL) at room temperature under an atmosphere of Ar. After stirring overnight, the reaction mixture was cooled to 0 °C and saturated $NaHCO_3$ solution (50 mL) was added dropwise with vigorous stirring. The mixture was extracted with AcOEt (50 mL) and the organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica gel (10 g, hexane:AcOEt= 100:1) to afford difluoroester **17** (407 mg, 80%) as a colorless oil. $[\alpha]_D^{20}$= +41.2° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ -0.01 (3H, s), 0.01 (3H, s), 0.88 (9H, s), 0.90 (3H, d, J= 6.3 Hz), 0.91 (3H, s), 1.00~2.00 (17H, m), 3.87 (3H, s), 4.00 (1H, d, J= 2.3 Hz). **^{13}C-NMR**: δ -4.76, 13.73, 17.66, 18.06, 18.34, 23.02, 25.84, 26.99, 27.04, 27.10, 31.19, 34.41, 34.57, 40.70, 42.17, 53.06, 53.19, 56.10, 69.42, 116.87, 164.99. **IR** (neat): 1776 cm^{-1}. **LRMS** (EI): m/z 432 (M^+), 417 $(M\text{-}Me)^+$, 375 $(M\text{-}tBu)^+$. **HRMS** (EI): calcd. for $C_{23}H_{42}O_3F_2Si$ 432.2871 found 432.2899.

(20*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-24,24-difluoro-25-oxo-26-[(*R*)-*p*-tolyl-sulfinyl]-27-norcholestane (18). To a solution of diisopropylamine (240 μL, 1.789 mmol) in THF (5 mL) was added n-butyllithium (2.34M in hexane solution) (740 μL, 1.732 mmol) at 0 °C under an atmosphere of Ar and then stirred for 10 min. After cooling to -78 °C, a solution of (*R*)-(+)-methyl *p*-tolylsulfoxide (268 mg, 1.737 mmol) of THF (1 mL) was added and stirred for 5 min. Then a solution of **17** (375 mg, 0.868 mmol) in THF (2 mL) was added and stirred for 15 min at -78 °C and for a further 1.5 h at room temperature. The reaction mixture was poured into saturated NH_4Cl solution (50 mL) and stirred for 30 min. The mixture was extracted with AcOEt (3x 50 mL), and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel (10 g, hexane:AcOEt= 10:1~5:1) to afford β-ketosulfoxide **18** (431 mg, 90%) as a white foam. The obtained **18** was actually a mixture of keto and hydrate forms. **^{1}H-NMR**: δ -0.005 (3H, s), 0.01 (3H, s), 0.87 (3H, d, J= 6.9 Hz), 0.89 (9H, s), 0.90 (3H, s), 0.95~2.04 (17H, m), 2.43 (3H, s), 3.98 (2H, d, J= 14.8 Hz), 3.99 (1H, br s), 4.21 (2H, d, J= 14.8 Hz), 7.35 (2H, d, J= 7.9 Hz), 7.59 (2H, d, J= 7.9 Hz), (3.02 (2H, d, J= 12.9 Hz), 3.11 (2H, d, J= 12.9 Hz), 3.50 (1H, br s), 6.37 (1H, s), 7.37 (2H, d, J= 7.9 Hz)). Chemical shift values (in parentheses) result from the hydrate form. **IR** ($CHCl_3$): 3302 cm^{-1}, 1743 cm^{-1}. **LRMS** (EI): m/z 497 $(M\text{-}tBu)^+$, 139 $(p\text{-}TolSO)^+$. **HRMS** (EI): calcd. for $C_{26}H_{39}O_3F_2SiS$ $(M\text{-}tBu)^+$ 497.2357 found 497.2370.

(20*R*,25*R*)-De-A,B-8β-(*tert*-butyldimethylsilyl)oxy-24,24-difluoro-25-hydroxy-26-[(*R*)-*p*-tolylsulfinyl]cholestane (19). To a suspension of $ZnBr_2$ (195 mg, 0.866 mmol) in CH_2Cl_2 (20 mL) molecular sieves 4A (777 mg) was added and stirred for 3 h at room temperature under an atmosphere of Ar. Then a solution of **18** (345 mg, 0.623 mmol) in CH_2Cl_2 (4 mL) which was dried over molecular sieves 4A (490 mg) for 3 h at room temperature was added to the suspension and stirred for 30 min. After the suspension was cooled to -78 °C, Me_3Al (2M in hexane solution) (3.60 mL, 7.120 mmol) was added and stirred for 3 h. The reaction mixture was quenched with saturated NH_4Cl solution (10 mL) and allowed to warm to room temperature with vigorous stirring. Then the mixture was acidified by adding 1N-HCl (20 mL) and extracted with AcOEt (50 mL). The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel (10 g, hexane:AcOEt= 10:1~3:1) to afford two diastereomers of optically pure tertiary alcohol (less polar (25*R*)-isomer **19**: 282 mg, 68%, more polar (25*S*)-isomer **23**: 55 mg, 13%) as a colorless oil respectively. **19**: $[\alpha]_D^{20}$= +150.1° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.01 (6H, s), 0.89 (9H, s), 0.90 (3H, d, J= 4.6 Hz), 0.92 (3H, s), 1.00~2.10 (17H, m), 1.73 (3H, s), 2.44 (3H, s), 2.81 (1H, d, J= 13.2 Hz), 3.18 (1H, d, J= 13.2 Hz), 4.00 (1H, br s), 4.60 (1H, s), 7.38 (2H, d, J= 7.9 Hz), 7.57 (2H, d, J= 7.9 Hz). **^{13}C-NMR**: δ -4.78, 13.71, 17.66, 18.04, 18.47, 21.47, 21.58, 23.01, 25.82, 26.43, 26.68, 26.99, 27.15, 34.45, 34.86, 40.70, 42.16, 53.05, 56.39, 61.08, 69.45, 75.40, 124.06, 124.45, 130.33, 139.93, 142.44. **IR** ($CHCl_3$): 3358 cm^{-1}, 2932 cm^{-1}, 1253 cm^{-1}, 1025 cm^{-1}. **LRMS** (FAB): m/z 571 $(M+H)^+$, 513 $(M-tBu)^+$, 439 $(M-TBSO)^+$, 139 $(p\text{-TolSO})^+$. **HRMS** (FAB): calcd. for $C_{31}H_{53}O_3F_2SiS$ $(M+H)^+$ 571.3453 found 571.3473. **23**: $[\alpha]_D^{20}$= +126.5° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.01 (6H, s), 0.89 (9H, s), 0.915 (3H, d, J= 6.3 Hz), 0.92 (3H, s), 1.00~2.20 (17H, m), 1.38 (3H, s), 2.43 (3H, s), 2.90 (1H, d, J= 13.5 Hz), 3.05 (1H, d, J= 13.5 Hz), 4.00 (1H, br s), 4.75 (1H, s), 7.35 (2H, d, J= 8.3 Hz), 7.57 (2H, d, J= 8.3 Hz). **^{13}C-NMR**: δ -5.14, -4.78, 13.75, 17.68, 18.04, 18.44, 21.44, 23.04, 24.24, 24.30, 25.82, 26.52, 27.19, 27.46, 34.43, 34.88, 40.70, 42.16, 53.05, 56.35, 61.76, 69.45, 75.67, 123.97, 125.96, 130.19, 141.38, 142.03. **IR** ($CHCl_3$): 3302 cm^{-1}, 2932 cm^{-1}, 1253 cm^{-1}, 1025 cm^{-1}. **LRMS** (FAB): m/z 571 $(M+H)^+$, 513 $(M-tBu)^+$, 439 $(M-TBSO)^+$, 139 $(p\text{-TolSO})^+$. **HRMS** (FAB): calcd. for $C_{31}H_{53}O_3F_2SiS$ $(M+H)^+$ 571.3453 found 571.3473.

(20*R*,25*R*)-De-A,B-24,24-difluoro-8β,25-dihydroxy-26-[(*R*)-*p*-tolylsulfinyl]cholestane (20). Tertiary alcohol **19** (249 mg, 0.437 mmol) was dissolved in MeOH (22 mL) and Dowex® 50X4-400 ion exchange resin (4.65g, prewashed with 1N-HCl then MeOH) was added. After stirring for 4 days, the resin was filtered off and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (10 g, hexane:AcOEt= 2:1) to afford desilylated compound **20** (172 mg, 86%) as colorless needles (m.p. 140~141 °C) along with **19** (30 mg, 12%). $[\alpha]_D^{20}$= +174.0° (c= 1.0, $CHCl_3$). **^{1}H-NMR**: δ 0.91 (3H, d, J= 6.9 Hz), 0.92 (3H, s), 1.00~2.20 (18H, m), 1.72 (3H, s), 2.43 (3H, s), 2.80 (1H, d, J= 13.2 Hz), 3.17 (1H, d, J= 13.2 Hz), 4.07 (1H, br s), 4.61 (1H, s), 7.37 (2H, d, J= 8.3 Hz), 7.56 (2H, d, J= 8.3 Hz). **^{13}C-NMR**: δ 13.53, 17.45, 18.40, 21.47, 21.58, 22.50, 26.40, 26.72, 27.04, 33.60, 34.88, 40.38, 41.89, 52.58, 56.30, 61.26,

69.31, 75.35, 124.04, 124.47, 130.33, 139.93, 142.42. **IR** (KBr): 3360 cm^{-1}, 2934 cm^{-1}, 1375 cm^{-1}, 1205 cm^{-1}, 1029 cm^{-1}. **LRMS** (FAB): m/z 457 $(M+H)^+$, 439 $(M-H_2O)^+$, 139 $(p\text{-TolSO})^+$. **HRMS** (FAB): calcd. for $C_{25}H_{39}O_3F_2S$ $(M+H)^+$ 457.2588 found 457.2609. **Anal.**: calcd. for $C_{25}H_{38}O_3F_2S$, C 65.76, H 8.39; found, C 65.82, H 8.16.

X-ray crystallographic analysis of 20.[22] A crystal with dimensions of 0.25 x 0.15 x 0.30 mm was obtained by recrystallization from AcOEt/hexane. All measurements were made on a Rigaku RAXIS-II imaging plate area detector with graphite monochromated Mo-$K\alpha$ radiation (λ= 0.71069 Å). The data were collected at a temperature of 15±1 °C to a maximum 2θ value of 50.1°. The observed cell parameters are as follows: $C_{25}H_{38}O_3F_2S$, M_r= 456.63, orthorombic, space group $P2_12_12_1$ (#19), lattice constants a = 10.300(8), b = 36.27(3), c = 6.795(2)Å, V= 2538(2)Å^3, Z= 4, D_{calc}= 1.195 g/cm^3, μ= 1.64 cm^{-1}, $F(000)$= 984.00. The structure was solved by direct methods (program SIR92[23]) and expanded using Fourier techniques (program DIRDIF94[24]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1585 observed reflections (I>2.40$\sigma(I)$) and 281 variable parameters and converged (largest parameter shift was 0.03 times its esd) with unweighted and weighted agreement factors of R= 0.068 and R_w= 0.083. All calculations were performed using the teXsan[25] crystallographic software package of Molecular Structure Corporation.

(20*R*,25*R*)-De-A,B-24,24-difluoro-8β,25-dihydroxy-26-[(*R*)-*p*-tolylsulfinyl]cholestane (21) (Ozonolysis of 5a). Compound **5a** (50 mg, 0.061 mmol) was dissolved in CH_2Cl_2 (17 mL), and MeOH (3 mL) which contained $NaHCO_3$ (7 mg) was added. After cooling to -78 °C, a stream of ozone was passed to the solution for 10 min. The remaining ozone was purged with a stream of Ar, then $NaBH_4$ (6 mg, 0.159 mmol) was added and allowed to warm to room temperature while stirring for 1 h. After evaporating the solvent, the residue was suspended in AcOEt (50 mL) and washed with 0.5N-HCl and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was developed on silica gel preparative TLC (hexane:AcOEt= 1:1) to afford CD-ring derivative **21** (25 mg, 91%) as colorless needles. $[\alpha]_D^{20}$= +174.6° (c= 0.5, $CHCl_3$). Other instrumental analysis data corresponded to that of **20**.

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§ Present address: Department of Microbiology, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto-shi, Kumamoto 860-0811, Japan.

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