Stereocontrolled Synthesis of the 22*E*,24*β*(*S*)-Trifluoromethyl Steroidal Side Chain and Its Application to the Synthesis of Fluorinated Analogues of Naturally Occurring Sterols

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Introduction

Organofluorine compounds have recently attracted considerable attention in the fields of agrochemistry, pharmaceuticals, and materials science.[1] Several antiviral, antitumor, and antifungal agents have been developed in which fluorinated substituents play a key role in their biological activities.[2] Specifically, fluorinated analogues have been recognized as useful tools for pharmacological and physiological studies of natural products since the introduction of a fluorine atom into a molecule often leads to a significant change in its physical and biological properties.[3] In view of their unique biological properties, fluorinated steroids have been widely studied.[4] Selective fluorination of the side chain of Vitamin D_3 is a good example in which fluorine has been used to block hydroxylation or to modify the reactivity of the neighboring hydroxyl group.[5]

Trifluoromethyl substituents may also have applications in biologically active drugs and agrochemicals.[6] Substantial effort has been made to develop methods for introducing a trifluoromethyl group into a specific position in biologically active compounds to elucidate structure−activity relationships and to find new analogues with higher activity.[7] Several naturally occurring biologically active steroids have a 24-methyl group in their side chain, such as the well-known plant growth regulator brassinosteroid, brassinolide, from the pollen of *Brassica napus*,[8] typhasterol from *Typha latifolia*,[9] and marine sterols, such as occelasterol from the annelid *Pseudopotamilla occelata*[10] and crinosterol from the sponge *Jaspis stellifera*.[11] A biological evaluation of brassinolide[12] has confirmed a 24*β*(*S*)-methyl group in the side chain of these compounds confers more potent biological activity than the C24α(*R*)-configuration. Despite the fact that several methods have been developed to stereoselectively insert a 24-methyl side chain into steroids,[13] there are no reports regarding the stereocontrolled synthesis of a C24-trifluoromethyl-bearing side chain. In view of the importance of the 24*β*(*S*)-methyl group in the biological activity of steroids, we became interested in the stereocontrolled synthesis of the 22*E*,24*β*(*S*)-trifluoromethyl side chain of steroids and its application to the synthesis of 24*β*(*S*)-trifluoromethyl-substituted crinosterol (**1**) and typhasterol (**2**) (Figure 1).

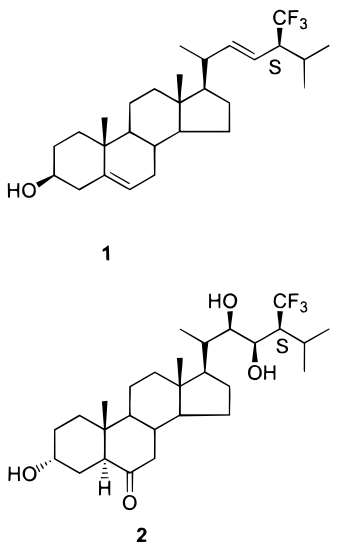

Figure 1.

Results and Discussion

1,3-Dipolar cycloaddition between nitrile oxides and alkenes is one of the most convenient methods for

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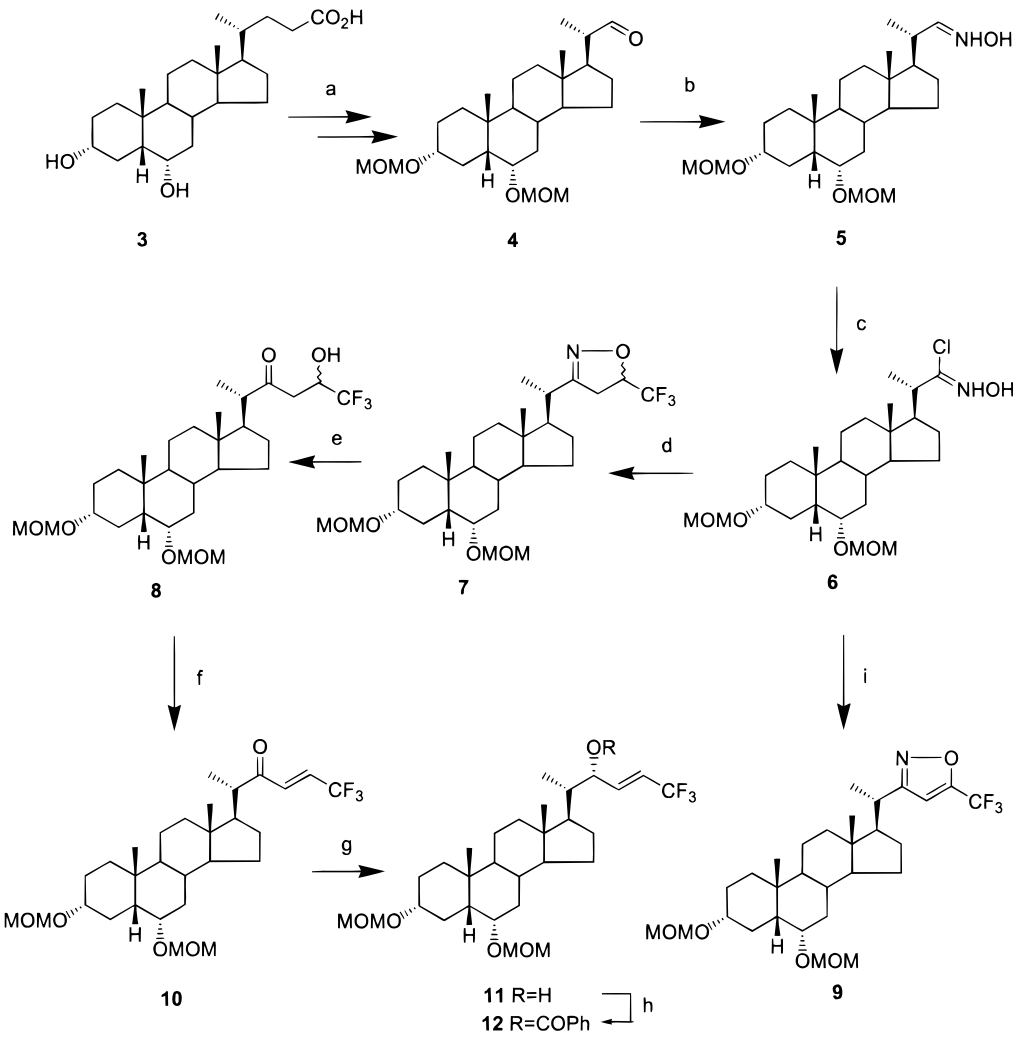
Scheme 1[a]



[a] Reagents and conditions: (a) ref 17; (b) $NH_2OH{\cdot}HCl$/py, rt; (c) NCS/$CHCl_3$; (d) $CF_3CH{=}CH_2/NEt_3/CHCl_3$, rt; (e) H_2/Ra-Ni/H_3BO_3/MeOH, rt; (f) MsCl/NEt_3/CH_2Cl_2, rt; (g) DIBAL-H/THF, −78 °C; (h) PhCOCl/Py, rt; (i) $CF_3(Br)CH{=}CH_2/NEt_3/HCCl_3$.

preparing 1,3-keto-alcohols or α,β-unsaturated ketones via cleavage of the N−O bond of the cycloadduct isoxazoline intermediate.[14] Various applications of 1,3-dipolar cycloadditions in the synthesis of heterocyclic side chains of steroids have been reported.[15]

Optically active isoxazolines can be generated by introducing chirality into either the dipole or dipolarophile.[16] We postulated that chiral steroidal nitrile oxide might undergo stereoselective 1,3-dipolar cycloaddition with the dipolarophile trifluoropropene. Thus, C22-steroidal aldehyde **4**, derived from hydroxycholic acid by established procedures,[17] was condensed with hydroxylamine to give C22-steroidal aldehyde oxime **5** in 75% yield (Scheme 1). Upon treatment with *N*-chlorosuccinimide in chloroform, **5** was converted to C22-steroidal chlorooxime **6**, a precursor to nitrile oxide, which was reacted with trifluoropropene overnight in the presence of triethylamine at room temperature to give steroidal trifluoromethyl isoxazoline **7** in 80% yield. The 1,3-dipolar cycloaddition reaction between steroidal nitrile oxide and trifluoropropene proceeded with a high degree of regioselectivity. However, the reaction was not diastereoselective. The ^{19}F NMR spectrum of **7** has two peaks at −2 ppm and −4 ppm, both with a coupling constant of 7 Hz, in a ratio of 1:1. The 1,3-dipolar cycloaddition steroidal nitrile oxide with trifluoropropene provided a convenient method for the synthesis of trifluoromethyl-containing heterocyclic steroid. The steroidal trifluoromethyl isoxazole **9** could also be formed regioselectively in 85% yield by reacting **6** with 2-bromo-trifluoropropene and triethylamine at room temperature. With steroidal isoxazoline **7** in hand, we next sought to convert the steroidal isoxazoline ring into an α,β-unsaturated ketone side chain. Compound **7** was smoothly converted to 22-one-24-OH **8** in excellent yield by hydrogenation over W-2 Raney nickel in a mixed solvent of methanol and water containing 3 equiv of boric acid. Elimination of the 24-hydroxyl group with methanesulfonyl chloride and triethylamine in methylene chloride gave the 23*E*-24-CF_3-unsaturated ketone **10** quantitatively. It is well-known that the hydride reduction of 22-keto-steroids with $NaBH_4$ or DIBAL-H in THF at −78 °C gives the anti-

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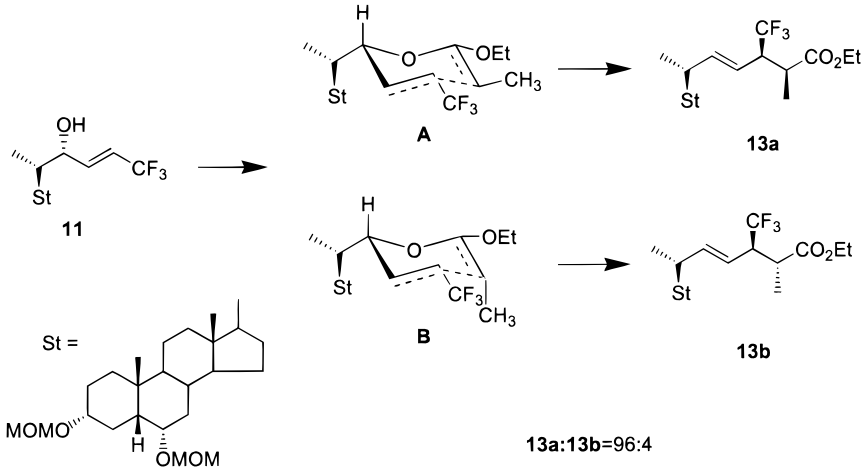
Scheme 2



Cram product (22β(R)-OH) as the main product.[18] Interestingly, reduction of 22-keto-23-ene **10** with $NaBH_4$ at 0 °C or DIBAL-H at −78 °C proceeded with high Cram selectivity to predominantly give the Cram isomer (22α-(*S*)-OH) **11** in ratios of 8:2 (75% yield) and 9:1 (85% yield), respectively. The absolute configuration of the 22-carbon atom of the allylic alcohol was determined by the "exciton chirality method" developed by Harada and Nakanishi.[19] The C22-hydroxyl group in **11** was converted into the corresponding benzoate **12** with benzoyl chloride and pyridine in CH_2Cl_2. The exciton-split CD curves of the benzoate showed a strong positive first Cotton effect in the region of the $\pi-\pi^*$ transition around 251 nm (CD: λ_{max} 251 nm, $\Delta\epsilon = +21.57$). The stereochemistry of C22 was thus assigned to be *S*.

To exploit the above results, we decided to construct 24β(*S*)-CF_3 via Johnson−Claisen rearrangement, a powerful synthetic transformation for the stereoselective construction of carbon−carbon bonds.[20] Considering the bulkiness of the steroidal moiety and the trifluoromethyl group, the six-membered chairlike transition state **A** inherent in Johnson−Claisen rearrangement, in which the CF_3 group and the methyl group occupy the equatorial positions, is more favorable than transition state **B** (Scheme 2). Therefore, Johnson−Claisen rearrangement would result in exclusive *E*-selectivity at the newly created C22-olefinic bond. In addition, a complete transfer of chirality could be achieved to obtain the highly functionalized trifluoromethyl group in a *syn* relationship. When 22S,23*E*-allylic alcohol **11** was heated with triethyl orthopropionate in xylene at 130 °C for 5 h, two sets of peaks (two doublets) in the ^{19}F NMR spectrum of the rearrangement product were observed in a ratio of 96:4. Due to the effect of the electron-withdrawing carboxylate group, the main *syn*-isomer **13a** has an upfield signal in the ^{19}F NMR spectrum at −8 ppm, while the ^{19}F NMR spectrum of the *anti*-isomer **13b** shows a downfield shift at −10 ppm.[21]

Finally, the C26-carbonyl ester in **13** was transformed into the corresponding C26-methyl group in **15** in 91% yield by reduction with $LiAlH_4$ in THF followed by tosylation of the resulting C26-alcohol **14** with methanesulfonyl chloride in pyridine and removal of the toluenesulfonyl group with $LiAlH_4$.[22] The two methoxymethyl protecting groups in **15** were cleaved with pyridinium *p*-toluenesulfonate in *tert*-butyl alcohol to give (22*E*,24S)-3α,6β-dihydroxy-24-trifluoromethyl-5β-cholestan-22-en (**16**), which contains the fully elaborated (22*E*,24S)-24-CF_3 side chain of sterol. Compound **16** is a critical intermediate for preparing steroids bearing the C24β(*S*)-CF_3 side chain. We intended to apply this key intermediate to the synthesis of fluorinated analogues of naturally occurring steroids, such as crinosterol and typhasterol. Crinosterol is a good starting material for the synthesis of brassinolide.[23] Thus, 24β(*S*)-trifluoromethyl crinosterol **1** was prepared in 72% yield by treating **16** with toluenesulfonyl chloride in pyridine, followed by heating the resultant tosylate in DMF with potassium acetate for 4 h and hydrolysis with potassium hydroxide (Scheme 3). To synthesize fluorinated analogues of typhasterol, the 6β-hydroxyl group in **16** was selectively oxidized with pyridinium dichromate (PDC) at room temperature for 6 h while simultaneously epimerizing C5-βH to C5-αH with 2.5% HCl in methanol to give the 3α-hydroxyl-5α-ketone **16** in 60% yield. Osmium tetraoxide-catalyzed asymmetric dihydroxylation (ADH) has been studied for the construction of a (22*R*,23*R*)-22,23-dihydroxyl group as a side chain of brassinolide using different cinchona alkaloids as chiral ligands and potassium ferrocyanide as a co-oxidant.[24] It was found that the 24β(*S*)-methyl 22*E*-double bond of the side chain of sterol was hydroxylated with $(DHQD)_2$-PHAL as a ligand to yield predominantly the desired natural (22*R*,23*R*)-isomer.[25] This method was also successfully applied to the asymmetric

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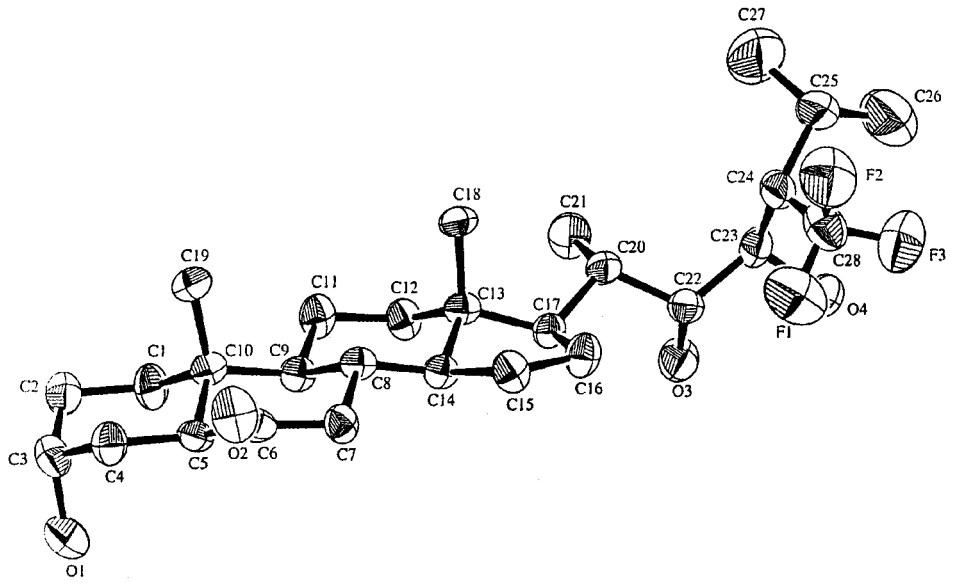
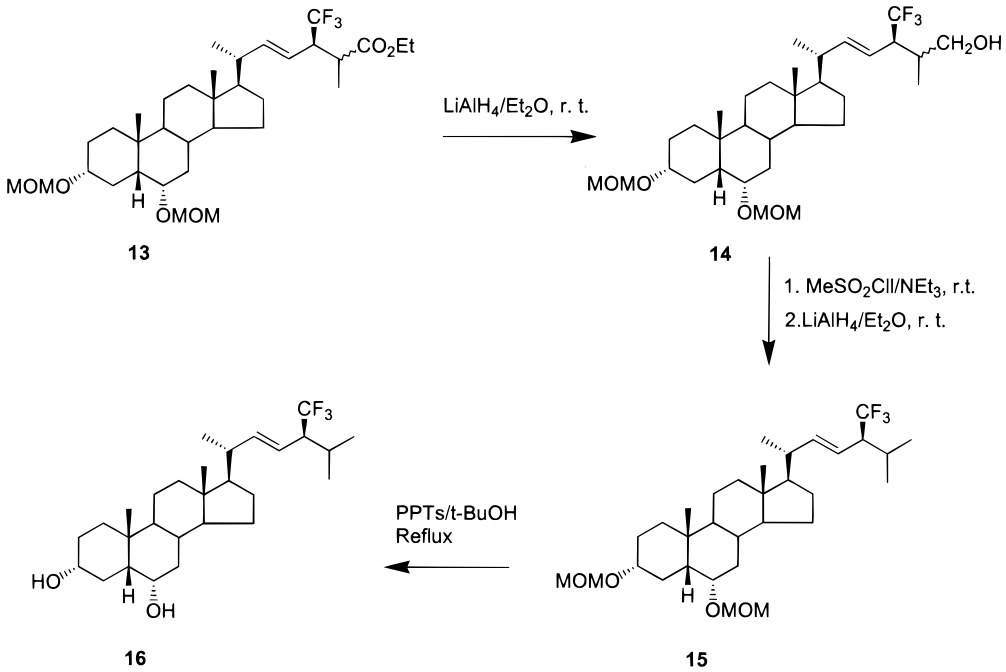


Figure 2.

Scheme 3



oxidation of the 24β(*S*)-trifluoromethyl-22*E*-double bond in **17**. Treatment of **17** with a mixture of $(DHQD)_2$-PHAL (3.0 equiv), $K_3Fe(CN)_6$ (3.0 equiv), and OsO_4 (0.0125 equiv) in a *tert*-butyl alcohol−water mixture gave (22*R*,-23*R*,24*S*)-3α,22,23-trihydroxyl-24-trifluoromethyl-5β-cholestan-6-one (**2**) as the sole product in 88% yield. The final structure was confirmed by X-ray crystallography (Figure 2), which demonstrated that the product, including the stereocenters resulting from Johnson−Claisen rearrangement, was as predicted.

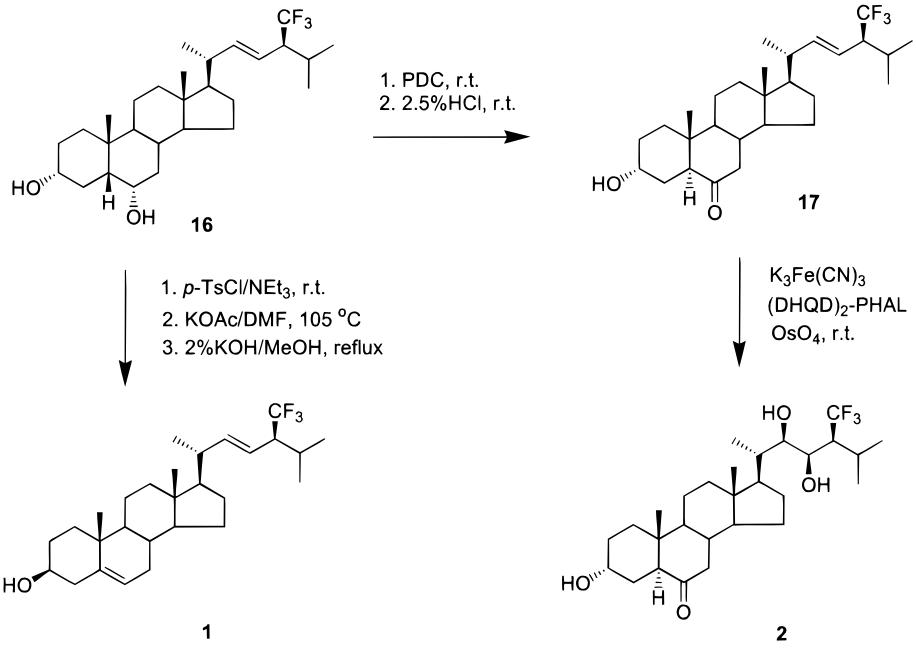
Conclusion

In conclusion, a practical stereocontrolled pathway to the 22*E*,24β(*S*)-CF_3 side chain of sterol was established by means of 1,3-dipolar cycloaddition and stereocontrolled Johnson−Claisen rearrangement of the (22S,23*E*)-24-CF_3 allylic alcohol of sterol with triethyl orthopropionate. Two fluorinated analogues **1** and **2** of naturally occurring crinosterol and typhasterol were successfully synthesized. The 22R,23R-vicinal hydroxyl groups in the side chain of 24β(*S*)-trifluoromethyl sterol were stereoselectively introduced by Sharpless's asymmetric dihyroxylation with $(DHQD)_2$-PHAL as a ligand. The key intermediate **16** may be useful for the preparation of biologically active sterols containing a C24β(*S*)-trifluoromethyl group.

Experimental Section

3α, 6α-Bis(methoxymethoxy)-23,24,25,26,27-pentanor-5β-cholestan-22-aldehyde Oxime (5). To a solution of steroidal aldehyde **4**[17] (2.5 g, 5.7 mmol) in pyridine (20 mL) was added hydroxylamine hydrochloride (0.6 g, 8.7 mmol). The reaction was stirred at room-temperature overnight and then poured into 10% HCl solution. After the usual workup, the crude product was purified by flash chromatography on silica gel and eluted with petroleum/ethyl acetate in a ratio of 10:1 to give steroidal oxime

Scheme 4



5 (1.94 g, 75%) as an amorphous solid: ^{1}H NMR(CD_3COCD_3) δ 0.78 (s, 3H), 0.98 (s, 3H), 2.69 (m, 1H), 3.75 (s, 6H), 3.53 (m, 1H), 3.93 (m, 1H), 4.7 (s, 2H), 4.82 (s, 2H), 7.1 (d, J = 8 Hz, 1H); IR (KBr) 3350(OH), 1454 cm^{-1}; MS *m/e* (relative intensity) 434 (M^+-OH), 387 (5), 327 (40), 310 (100). Anal. Calcd for $C_{26}H_{45}NO_5$: C, 69.14; H, 10.04, N, 3.10. Found: C, 69.20; H, 10.14; N, 3.15.

2-[3α,6α-Bis(methoxymethoxy)-23,24,25,26,27-pentanor-5*β*-cholestan-22-yl]-4-(trifluoromethyl)isoxazoline (7). Compound **5** (424 mg, 0.94 mmol) in chloroform (25 mL) was treated with *N*-chlorosuccinimide (130 mg, 0.97 mmol) at room temperature for 30 min. The mixture was transferred to an autoclave containing excess trifluoropropene and triethylamine (0.33 mL), and the reaction was allowed to proceed at room-temperature overnight. The resulting mixture was filtered, and the filtrate was subjected to the usual workup. The crude product was purified by flash chromatography on silica gel (eluted with petroleum/ethyl acetate = 10:1) to give **7** (442 mg, 82%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.60 (s, 3H), 0.91 (s, 3H), 1.16 (d, J = 6.7 Hz, 3H), 2.79 (m, 1H), 3.15 (m, 2H), 3.45 (s, 3H), 3.48 (s, 3H), 3.54 (m, 1H), 3,91 (m, 1H), 4.58 (s, 2H), 4.71 (q, J = 6.2 Hz, 2H), 4.79 (m, 1H); ^{19}F NMR (CD_3COCD_3) δ −2 (d, J = 7 Hz, CF_3); IR (neat) 1725, 1625, 1452 cm^{-1}; MS *m/e* (relative intensity) 545 (M^+, 8), 481 (10), 421 (50), 45 (100); HRMS calcd for $C_{29}H_{46}F_3NO_5$: 545.3328; found: 545.3363.

2-[3α,6α-Bis(methoxymethoxy)-23,24,25,26,27-pentanor-5*β*-cholestan-22-yl]-4-(trifluoromethyl)isoxazole (9). Compound **5** (100 mg, 0.22 mmol) in chloroform (5 mL) was treated with *N*-chlorosuccinimide (30 mg, 0.23 mmol) at room temperature for 30 min. To the mixture was added 2-bromo-trifluoropropene (195 mg, 1.1 mmol) and triethylamine (0.06 mL), and the reaction was allowed to proceed at room-temperature overnight. The mixture was filtered, and the filtrate was subjected to the usual workup. The crude product was purified by flash chromatography on silica gel (eluted with petroleum/ethyl acetate = 10: 1) to give **9** (101 mg, 85%) as a colorless oil: $[\alpha]^{19}_D$ = −6.52 (*c*, 4.92, $CHCl_3$). ^{1}H NMR (CD_3COCD_3) δ 0.71 (s, 3H), 0.92 (s, 3H), 3.05 (m, 1H), 3.31 (s, 6H), 3.49 (m, 1H), 3.95 (m, 1H), 4.61 (s, 2H), 4.66 (s, 2H), 7.22 (s, 1H); ^{19}F NMR (CD_3COCD_3) δ −12.5 (s); IR (neat) 2941, 1728, 1624 cm^{-1}; MS *m/e* (relative intensity) 482 (M^+, 4), 419 (20), 45 (100). Anal. Calcd for $C_{29}H_{44}F_3NO_5$; C, 64.07; H, 8.16; N, 2.58; F, 10.48. Found: C, 64.20; H, 8.26; N, 2.47; F, 10.39.

3α,6α-Bis(methoxymethoxy)-26,27-bisnor-24-(trifluoromethyl)-5*β*-cholestan-22-one-23-ol (8). A mixture of **7** (50 mg, 0.092 mmol) and boric acid (17 mg, 0.27 mmol) in a mixed solvent of methanol and water (10 mL, 5:1) was hydrogenated over W-2 Raney nickel (10 mg) at room temperature for 2 h. The catalyst was filtered off. After methanol was removed, the residue was dissolved in methylene chloride and subjected to the usual workup to give **8** (50 mg, 99%) as a colorless oil: ^{1}H NMR (CD_3COCD_3) δ 0.74 (s, 3H), 0.96 (s, 3H), 1.12 (d, J = 6.5 Hz, 3H), 2.62 (m, 1H), 2.82 (m, 2H), 3.27 (s, 3H), 3.31 (s, 3H), 3,48 (m, 1H), 3.93 (m, 1H), 4.55 (m, 1H), 4.62 (s, 2H), 4.65 (s, 2H); ^{19}F NMR (CD_3COCD_3) δ +2.6 (d, J = 9.1 Hz); IR (neat) 3393 (OH), 1718 (C=O) cm^{-1}; MS *m/e* (relative intensity) 531 (M^+ − OH, 5), 484 (10), 424 (30), 45 (100). Anal. Calcd for $C_{29}H_{47}F_3O_5$; C, 63.48; H, 8.63; F, 10.39. Found: C, 63.47, H, 9.13, F, 10.50.

(23*E*)-3α,6α-Bis(methoxymethoxy)-25,26,27-trinor-24-(trifluoromethyl)-5*β*-cholestan-22-one-23-ene (10). Compound **8** (35 mg, 0.064 mmol) in dry methylene chloride (3 mL) was treated with methanesulfonyl chloride (6 μL, 0.077 mmol) and triethylamine (22 μL, 0.16 mmol) at 0 °C for 3 h, and the mixture was subjected to the usual workup. The crude product was purified by chromatography on silica gel (eluted with petroleum/ethyl acetate = 10:1) to give **10** (31 mg, 91%) as a colorless oil: $[\alpha]^{19}_D$ = −13.3 (*c* = 2.55, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.74 (s, 3H), 0.96 (s, 3H), 1.12 (d, J = 6.5 Hz, 3H), 2.72 (m, 1H), 3.31 (s, 3H), 3.42 (s, 3H), 3.51 (m, 1H), 3.92 (m, 1H), 4.59 (s, 2H), 4.61 (q, J = 6.5 Hz, 2H), 6.68 (m, 1H), 6.82 (d, $J_{23,24}$ = 16.2 Hz, 1H). ^{19}F NMR ($CDCl_3$) δ −12.3 (d, J = 4 Hz); IR (neat) 1708 (C=O), 1687 (C=C) cm^{-1}; MS *m/e* (relative intensity) 532 (M^+ + 2, 8), 498 (10), 466 (50), 45 (100); HRMS calcd for C_{29} $H_{45}F_3O_4$: 530.3219; found: 530.3215.

(22S,23*E*)-3α,6α-Bis(methoxymethoxy)-25,26,27-trinor-24-(trifluoromethyl)-5*β*-cholestan-22-ol-23-ene (11). DIBAL-H (5 mL, 5 mmol, 1 M in THF) was added to a solution of **10** (2 g, 3.8 mmol) in THF (20 mL) under argon at −78 °C for 30 min. Ethyl acetate (10 mL) was then added to quench the reaction. The temperature was allowed to warm to room temperature. After the usual workup, the crude product was purified by chromatography on silica gel (eluted with petroleum/ethyl acetate = 15:1) to give **11** (1.4 g, 69%) as a colorless oil: $[\alpha]^{19}_D$ = −32.5 (*c* = 0.63, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H), 0.90 (s, 3H), 3.34 (s, 3H), 3.35 (s, 3H), 3.52 (m, 1H), 3.92 (m, 1H), 4.39 (d, J = 6.6 Hz, 1H), 4.64 (s, 2H), 4.70 (q, J = 6.6 Hz, 2H), 5.93 (m, 1H), 6.41 (d, J = 16.1 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ −14.0 (d, J = 4 Hz); IR(neat) 3460 (OH), 1682 (C=C) cm^{-1}; MS *m/e* (relative intensity) 532 (M^+, 11), 463 (30), 408 (50), 283 (90), 45 (100); HRMS calcd for $C_{29}H_{47}F_3O_4$: 532.3375; found: 532.3370.

Ethyl (24R,22*E*)-3α,6α-Bis(methoxymethoxy)-24-(trifluoromethyl)-5*β*-cholestan-22-en-26-oate (13). A solution of **11** (60 mg, 0.12 mmol), triethyl orthopropionate (0.2 mL), and propionic acid (0.01 mL) in xylene (2 mL) was heated at 130 °C for 2 h under argon. The solvent was evaporated under vacuum,

and the residue was purified by chromatography on silica gel (eluted with petroleum/ethyl acetate = 10:1) to yield **13** (66 mg, 95%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H), 0.91 (s, 3H), 2.14 (m, 1H), 2.21 (t, J = 6 Hz, 3H), 2.81 (m, 1H), 3.40 (s, 3H), 3.42 (s, 3H), 3.54 (m, 1H), 3.93 (m, 1H), 4.15 (q, J = 6 Hz, 2H), 4.61 (s, 2H), 4.81 (q, J = 6.6 Hz, 4H), 5.35 (dd, $J_{22,23}$ = 15.2 Hz, $J_{21,22}$ = 9.8 Hz, 1H), 5.59 (dd, $J_{22,23}$ = 15.2 Hz, $J_{23,24}$ = 9 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ −8 (d, J = 10 Hz, *syn*-isomer), −10 (d, J = 8 Hz, *anti*-isomer); IR (neat) 1740 (CO_2) cm^{-1}; MS *m/e* (relative intensity) 618 (M^+, 3), 554 (5), 522 (11), 45 (100). Anal. Calcd for $C_{34}H_{55}F_3O_6$: C, 66.21; H, 8.99; F, 9.24. Found: C, 66.24; H, 9.54; F, 9.35.

(24S,22*E*)-3α,6α-Bis(methoxymethoxy)-24-(trifluoromethyl)-5*β*-cholestan-22-en-26-ol (14). To a suspension of lithium aluminum hydride (20 mg, 0.5 mmol) in dry ether (2 mL) was added **13** (62 mg, 0.01 mmol) at room temperature, and the mixture was allowed to stand for 1 h. The reaction was quenched with ethyl acetate (1 mL) and acidified with 10% HCl to pH 3. After the usual workup, the crude product was subjected to flash chromatography (eluted with petroleum/ethyl acetate in ratio of 5:1) to yield **14** (59 mg, 96%) as an oil: ^{1}H NMR (CD_3COCD_3) δ 0.70 (s, 3H), 0.91 (s, 3H), 1.93 (m, 1H), 2.11 (m, 1H), 3.27 (s, 3H), 3.32 (s, 3H), 3.42 (m, 2H), 3.65 (m, 1H), 3.92 (m, 1H), 4.61 (s, 2H), 4.65 (s, 2H), 5.28 (dd, $J_{22,23}$ = 15.2 Hz, $J_{21,22}$ = 9.8 Hz, 1H), 5.56 (dd, $J_{22,23}$ = 15.2 Hz, $J_{23,24}$ = 9 Hz, 1H). ^{19}F NMR (CD_3COCD_3) δ −8.5 (d, J = 10 Hz, syn-isomer), −10.5 (d, J = 8 Hz, anti-isomer); IR (neat) 3472 (OH), 1671 (C=C)cm^{-1}; MS *m/e* (relative intensity) 531 (M^+, 4), 510 (10), 255 (48), 45 (100). Anal. Calcd for $C_{32}H_{53}F_3O_5$: C 66.87; H, 9.29; F, 9.92. Found: C, 66.80; H, 9.17; F, 9.72.

(24S,22*E*)-3α,6α-Bis(methoxymethoxy)-24-(trifluoromethyl)-5*β*-cholestan-22-ene (15). A solution of **14** (59 mg, 0.11 mmol) in dry methylene chloride (3 mL) was treated with methanesulfonyl chloride (25 μL, 0.32 mmol) and triethylamine (50 μL, 0.35 mmol) at room-temperature overnight. After removal of the solvent, the residue was dissolved in dry ether (2 mL) and treated with lithium aluminum hydride (20 mg, 0.5 mmol). The mixture was stirred at room temperature for 1 h. The reaction was quenched with ethyl acetate (0.5 mL), and 10% HCl (1 mL) was then added. After the usual workup, the crude product was subjected to flash chromatography (eluted with petroleum/ethyl acetate = 15:1) to give **15** (55 mg, 95%) as a colorless oil: $[\alpha]^{19}_D$ = +2.38 (c = 4.30, $CHCl_3$). ^{1}H NMR (CD_3COCD_3) δ 0.65 (s, 3H), 0.92 (s, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.99 (s, 3H), 1.01 (d, J = 5.3 Hz, 3H), 2.45 (m, 1H), 3.25 (s, 3H), 3.30 (s, 3H), 3.45 (m, 1H), 3.79 (m, 1H), 4.44 (s, 2H), 4.54 (q, J = 7 Hz, 2H), 5.2 (dd, $J_{22,23}$ = 15.2 Hz, $J_{21,22}$ = 9.6 Hz, 1H), 5.41 (dd, $J_{22,23}$ = 15.2 Hz, $J_{23,24}$ = 8.9 Hz, 1H); ^{19}F NMR (CD_3COCD_3) δ −10 (d, J = 8 Hz); IR (neat) 2937, 2885, 1560 cm^{-1}; MS *m/e* (relative intensity) 497 ($M^+ - CH_3OCH_2O$, 10), 467 (10), 451 (12), 435 (58), 255 (84), 45 (100). Anal. Calcd for $C_{22}H_{53}F_3O_4$: C, 68.79; H, 9.56; F, 10.20. Found: C, 68.85; H, 9.81; F, 10.27.

(24S,22*E*)-3α,6α-Dihydroxy-24-(trifluoromethyl)-5*β*-cholestan-22-ene (16). Compound **15** (240 mg, 0.5 mmol) was refluxed with pyridinium *p*-toluenesulfonate (600 mg) in *tert*-butyl alcohol (10 mL) for 4 h. After *tert*-butyl alcohol was evaporated under reduced pressure, the residue was dissolved in ethyl acetate and washed with 10% $NaHCO_3$ and brine. After the usual workup, the crude product was recrystallized from ethanol to give **16** (158 mg, 78%): mp 163.5−164.5 °C; $[\alpha]^{19}_D$ = +6.97 (c = 0.559, acetone); ^{1}H NMR (CD_3COCD_3) δ 0.74 (s, 3H), 0.92 (s, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.99 (s, 3H), 1.12 (d, J = 5.3 Hz, 3H), 2.55 (m, 1H), 3.50 (m, 1H), 4.0 (m, 1H), 5.29 (dd, $J_{21,22}$ = 9.6 Hz, $J_{22,23}$ = 15.2 Hz, 1H), 5.57 (dd, $J_{23,24}$ = 8.97 Hz, $J_{22,23}$ = 15.2 Hz, 1H); ^{19}F NMR (CD_3COCD_3) δ −10 (d, J = 8 Hz); IR (KBr) 3377, 1456, 1373 cm^{-1}; MS *m/e* (relative intensity) 452 ($M^+ - H_2O$, 7), 436 (11), 419 (11), 255 (100). Anal. Calcd for $C_{28}H_{45}F_3O_2$: C, 71.45; H, 9.64; F, 12.11. Found: C, 71.23; H, 9.74; F, 12.27.

(24S,22*E*)-3α-Hydroxyl-24-(trifluoromethyl)-5α-cholestan-6-one-22-ene (17). To a solution of **16** (130 mg, 0.28 mmol) in methylene chloride (5 mL) was added pyridinium dichromate (112 mg, 0.3 mmol) at room temperature, and the mixture was stirred for 2 h. The mixture was diluted with ether (10 mL), and the resulting precipitate was filtered off. After removal of the solvent, the residue was dissolved in 2.5% HCl methanol solution (5 mL) and allowed to stand at room temperature for 2 days. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography to give **17** (77 mg, 60%): mp 184.9−185.3 °C; $[\alpha]^{19}_D$ = −14.4 (c = 1.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.67 (s, 3H), 0.75 (s, 3H), 0.95 (d, J = 6.7 Hz,3H), 0.99 (s, 3H), 1.02 (d, J = 6.8 Hz, 3H), 2.45 (m, 1H), 2.75 (t, J = 7.8 Hz, 1H), 4.19 (m, 1H), 5.22 (dd, $J_{21,22}$ = 9.6 Hz, $J_{22,23}$ = 15.2 Hz, 1H), 5.57 (dd, $J_{23,24}$ = 8.97 Hz, $J_{22,23}$ = 15.2 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ −10 (d, J = 9.3 Hz); IR (KBr) 3372 (OH), 1710 (C=O) cm^{-1}; MS *m/e* (relative intensity) 451 (M^+ − OH, 12), 436 (10), 419 (100). Anal. Calcd for $C_{28}H_{45}F_3O_2$: C, 71.76; H, 9.25; F, 12.16. Found: C, 71.40; H, 9.44; F, 12.31.

(24S,22*E*)-3*β*-Hydroxy-24-(trifluoromethyl)-cholestan-5,22-diene (1). A solution of **16** (40 mg) in pyridine (2 mL) was treated overnight with toluenesulfonyl chloride (80 mg). Pyridine was removed under reduced pressure. Ethyl acetate (10 mL) was added, and the solution was washed successively with 10% HCl, saturated $NaHCO_3$ and brine and then dried over Na_2SO_4. After removal of the solvent, the residue was heated at 105 °C with potassium acetate (0.8 mg) in DMF (2 mL) containing H_2O (0.5 mL) for 2 h. DMF was distilled under reduced pressure, 2% KOH/MeOH (2 mL) was added, and the mixture was stirred at room temperature for 4 h. After the usual workup, the crude product was recrystallized from methanol to give **1** (32 mg, 72%): mp 157.4−158.1 °C; $[\alpha]^{19}_D$ = −9.84 (c = 0.758, acetone); ^{1}H NMR ($CDCl_3$) δ 0.71 (s, 3H), 0.81 (d, J = 7 Hz, 3H), 0.83 (d, J = 7H, 3H), 0.98 (s, 3H), 2.63 (m, 1H), 3.42 (m, 1H), 5.19 (m, 1H), 5.30 (d, J = 9 Hz, 1H), 5.55 (d, J = 9 Hz, 1H). ^{19}F NMR ($CDCl_3$) δ −9 (d, J = 8 Hz); IR (KBr) 3349 (OH), 2936,1600,-1467 cm^{-1}; MS *m/e* (relative intensity) 452 (M^+, 29), 434 (30), 419 (37), 255 (100); HRMS calcd for $C_{28}H_{45}F_3O$: 452.3226; found: C, 452.3293.

(22*R*,23*R*,24*S*)-3α,22,23-Trihydroxy-24-(trifluoromethyl)-5α-cholestan-6-one (2). To a well-stirred mixture of $(DHQD)_2$-PHAL (39 mg, 0.5 mmol), potassium ferrocyanide (98.7 mg, 0.3 mmol), potassium carbonate (41.4 mg, 0.3 mmol), methane sulfonamide (29.5 mg, 0.1 mmol), and osmium tetraoxide (0.0125 mmol) in *tert*-butyl alcohol/water (2 mL, 1:1) was added a solution of **17** (40 mg, 0.1 mmol) in *tert*-butyl alcohol/water (5 mL, 1:1). The reaction mixture was kept at room temperature for 4 days. Sodium sulfide (200 mg) was then added. The mixture was stirred for another 2 h and then diluted with ethyl acetate (20 mL). After the usual workup, the crude product was purified by chromatography (eluent:petroleum/ethyl acetate = 1:9) to give **2** (35 mg, 88%). Single crystals of **2** were obtained in methanol solution and subjected to X-ray analysis: mp 242.5−243.1 °C; ^{1}H NMR (CD_3OD) δ 0.79 (s, 3H), 0.81 (d, J = 8 Hz, 6H), 0.98 (s, 3H), 2.97 (m, 1H), 3.77 (s, 1H), 3.90 (s, 1H), 4.02 (m, 1H); ^{19}F NMR (CD_3OD) δ −17 (d, J = 9.1 Hz); IR (KBr) 3460 (OH), 1690 (C=O) cm^{-1}; MS *m/e* (relative intensity) 520 (M^+, 25), 485 (OH,-100), 467 (20), 347 (15); HRMS calcd for $C_{28}H_{45}F_3O_4$: 502.3270; found: 502.3261.

Supporting Information Available: X-ray information for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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