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Inhibitors of sterol synthesis: synthesis and spectral properties of derivatives of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one fluorinated at carbon 7 or carbon 9 and their effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured mammalian cells

Abdul U. Siddiqui, Shankar Swaminathan, Frederick D. Pinkerton, Nicolas Gerst, William K. Wilson, Hyunah Choi, George J. Schroepfer, Jr.*

Departments of Biochemistry and Cell Biology and of Chemistry, Rice University, P.O. Box 1892, Houston, TX 77251-1892, USA

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Abstract

As part of a program to prepare $\Delta^{8(14)}$-15-ketosterols that cannot readily be metabolized to cholesterol or side-chain oxygenated species, we have prepared 3β-hydroxy-7α-fluoro-5α-cholest-8(14)-en-15-one (**VII**) and the 9α-hydroxy (**IV**), 9α-fluoro (**VI**) and 7α-fluoro (**VIII**) derivatives of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**II**). Sterol **IV** was prepared by oxidation of the $\Delta^{8,14}$ dienol ethyl ether of the 3β-acetate of **II** with *m*-chloroperbenzoic acid, followed by mild alkaline hydrolysis of the 3β-acetate derivative of **IV**. Treatment of **IV** with hydrogen fluoride-pyridine gave **VI**. The 7α-fluoro-15-ketosterols **VII** and **VIII** were synthesized by treating the 3β,15-bis-trimethylsilyl $\Delta^{7,14}$-dienol ether derivative of the appropriate $\Delta^{8(14)}$-15-ketosterol with *N*-fluoropyridinium triflate, followed by hydrolysis of residual trimethylsilyl ethers and purification by high-performance liquid chromatography. The combined results of ^{1}H and ^{13}C nuclear magnetic resonance (NMR) chemical shifts, ^{1}H-^{1}H coupling constants, ^{1}H-^{19}F long-range coupling constants and molecular modeling indicated that a 7α-fluoro, 9α-fluoro or 9α-hydroxy substituent has negligible effect on the conformation of the 15-ketosterols. ^{1}H and ^{13}C-NMR data are also given for $\Delta^{6,8(14)}$- and $\Delta^{8(14),9(11)}$-15-ketosterols, synthetic byproducts that could not be detected readily in samples of the fluoro-15-ketosterols by chromatographic methods. Mass spectra of **VI** and of previously reported 9α-fluoro and 9α-hydroxy-$\Delta^{8(14)}$-15-ketosterols showed abundant M-62 or M-60 ions that appear to correspond to loss of ketene and HF or H_2O. The 9α-hydroxy-F_7-15-ketosterol **IV**, the 7α-fluoro-15-ketosterol **VII** and the 7α-fluoro-F_7-15-ketosterol **VIII** were of equivalent potency to the parent 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) in lowering the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in CHO-K1 cells. The 9α-fluoro-F_7-15-ketosterol **VI** showed high potency but appeared to be slightly less active than **I**.

Keywords: 15-oxygenated sterols; Fluorination; NMR; Mass spectrometry; Conformational analysis

* Corresponding author.

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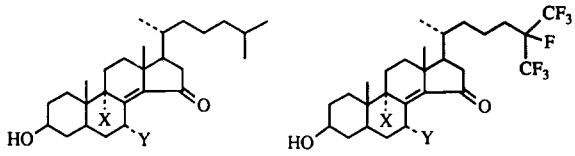
1. Introduction

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) is a potent regulator of cholesterol metabolism. **I** has very high potency not only in the inhibition of sterol synthesis and the lowering of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in cultured mammalian cells [1–5] but also in the inhibition of cholesterol absorption upon its administration to rats [6,7]. In an attempt to construct more potent analogs of **I**, we have prepared 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**II**), in which the oxidation at C-26 (and C-25) is blocked [8]. The F_7-15-ketosterol **II** was shown to have very high potency, equivalent to **I**, in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells and in Hep G2 cells [8]. Moreover, **II** was shown to have favorable properties upon its dietary administration to rats [9]. However, a potentially undesirable feature of **II** is its conversion to 25,26,26,26,27,27,27-heptafluorocholesterol in rats [9]. Taking advantage of our experience with **I** and knowledge of its metabolism in cultured mammalian cells[1] [5], in subcellular preparations of rat liver [10–13] and in intact animals [7,14–19], we have sought analogs of **I** in which the F_7 functionality of **II** was retained but in which enzymatic conversion to F_7-cholesterol did not occur. We have previously demonstrated that the 9α-hydroxy and 9α-fluoro analogs of **I**, i.e. 3β,9α-dihydroxy-5α-cholest-8(14)-en-15-one (**III**) and 3β-hydroxy-9α-fluoro-5α-cholest-8(14)-en-15-one (**V**), are highly active in lowering the levels of HMG-CoA reductase activity in cultured mammalian cells [20], and that dietary administration of **V** lowers serum cholesterol levels in rats [21]. Stimulated by these results, we have prepared the 9α-hydroxy and 9α-fluoro analogs of **II** and the 7α-fluoro analogs of **I** and **II**. Also presented herein are the spectral properties of these 15-ketosterol analogs and their effects on the levels of HMG-CoA reductase activity in CHO-K1 cells. Structures of 15-ketosterols **I** and **II** and their 7α-fluoro, 9α-hydroxy and 9α-fluoro derivatives are shown in Fig. 1.



I	X = H	Y = H	**II**	X = H	Y = H
III	X = OH	Y = H	**IV**	X = OH	Y = H
V	X = F	Y = H	**VI**	X = F	Y = H
VII	X = H	Y = F	**VIII**	X = H	Y = F

Fig. 1. Structures of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**II**) and their 9α-hydroxy and 9α-fluoro derivatives.

2. Experimental procedures and results

2.1. Materials and methods

The following compounds were prepared as described previously: 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) [22], 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**II**) [8], 3β-hydroxy-9α-fluoro-5α-cholest-8(14)-en-15-one (**V**) [23], 3β,9α-dihydroxy-5α-cholest-8(14)-en-15-one (**III**) [23], the 3β-acetate of **III** [23], 3β-hydroxy-5α-cholesta-6,8(14)-dien-15-one (**XIV**) [24], 3β-hydroxy-5α-cholesta-8(14),9(11)-dien-15-one (**XVI**) [25] and its 3β-acetate **XVII** [25]. Bis(trimethylsilyl)trifluoroacetamide (BSTFA), *N*-fluoropyridinium triflate, tetrabutylammonium fluoride, 70% HF-pyridine and *m*-chloroperbenzoic acid (80–85% purity) were purchased from Aldrich Chemical Company (Milwaukee, WI).

Thin-layer chromatography was carried out on plates of silica gel G (Analtech; Newark, DE). Solvent systems used were: SS-1, 30% ethyl acetate in hexane; SS-2, 40% ethyl acetate in hexane; SS-3, 50% ethyl acetate in hexane; SS-4, 50% ether in benzene; SS-5, 10% water in methanol. Medium-pressure liquid chromatography (MPLC) was carried out on columns (500 mm × 10 mm i.d.) of silica gel (20 g; 230–400 mesh; Aldrich Chemical Company). Fractions 20 ml in volume were collected. Analytical reversed-phase high-performance liquid chromatography (HPLC) was performed on a 5-μm Spherisorb ODS-II column (250 mm × 4.6 mm) with UV detection at 259 nm

1. J. St. Pyrek, S. Numazawa, N. Gerst, G.T. Emmons, F.D. Pinkerton and G.J. Schroepfer, Jr., unpublished data.

and a flow rate of 1.0 ml per min. Semi-preparative HPLC was done similarly except that the column was 250 × 9.4 mm and the flow rate was 2 ml/min. Gas chromatography-mass spectrometry (GC-MS) was carried out with falling needle injection and direct introduction of the helium carrier gas into the ion source of the mass spectrometer (Extrel ELQ-400, quadrupole, electron impact at 70 eV). Trimethylsilyl (TMS) ethers were prepared using BSTFA-pyridine (1:1) [13] and injected in hexane solution onto a DB-5 column (15 m × 0.25 mm, 0.1 μm film thickness, bonded phase of 5% phenyl 95% methyl polysiloxane; J & W Scientific; Folsom, CA) that was held at 200°C for 2 min and increased to 290°C at 10°C per min.

Instrumentation for measuring radioactivity, melting points (MP), ultraviolet (UV) spectra (in absolute ethanol), infrared (IR) spectra, low-resolution and high-resolution mass spectra (MS) and ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra have been described previously [8]. Standard Bruker NMR software was used to acquire COSYDEC (ω_1-decoupled ^{1}H-^{1}H correlation spectroscopy, τ_e 0.2 s) [26], HETCOR (^{1}H-^{13}C shift-correlated spectroscopy; ~50 increments, δ 0.6–2.6 in f_1), heteronuclear multiple-quantum coherence (HMQC) [27], heteronuclear multiple-bond correlation (HMBC) [27] and nuclear Overhauser effect (NOE) difference spectra. Overlapped multiplets were isolated by NOE or saturation difference spectroscopy [28,29] using the following conditions: irradiation at low power for 1 s, 90° read pulse, 2.7-s acquisition time, 16 scans per cycle, non-degassed sample, no spinning, 32k points. Modeling of sterols by molecular mechanics was done with PC Model (Macintosh version 4.4; Serena Software; Bloomington, IN), which was also used to predict ^{1}H-NMR vicinal coupling constants. Owing to strong coupling effects at 500 MHz in $CDCl_3$ solution, coupling constants could not be obtained for the C-20, C-22, C-23 and C-24 protons of sterols with a C_8H_{17} side chain or the C-11 protons of 9α-fluoro-15-ketosterols.

^{1}H and ^{13}C-NMR assignments were made from chemical shift comparisons [8,30] and HETCOR data and confirmed by additional spectral results. In the case of dienol ether **XIII** and the dienones, olefinic carbons were distinguished by HMBC spectra. Precise ^{1}H chemical shifts were measured from 1D or COSYDEC spectra as described previously [31], and ^{1}H stereochemical assignments were made by comparisons with other sterol spectra [8,32]. ^{19}F spectra were interpreted as described previously for other F_7 sterols [8]. The orientation of signals in HETCOR spectra [33] of the F_7 sterols showed that $J_{C24-F25}$ and $J_{H24-F25}$ (pro R and pro-S) have the same sign. A similar orientation of signals was noted in COSYDEC spectra. The purity of sterol samples was determined by HPLC, TLC and ^{1}H-NMR (500 MHz, after sufficient magnification of the vertical scale in the methyl and δ 2–6 spectral regions to detect a 1% impurity).

As described previously [8], cell culture experiments were performed by successive incubations of CHO-K1 cells in lipid-rich medium for 48 h and lipid-deficient medium for 18 h, followed by incubation for 4 h with fresh lipid-deficient medium containing sterols, harvesting, and assay for HMG-CoA reductase activity.

2.2. 3β-Acetoxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (IX)

The F_7-15-ketosterol **II** (0.50 g; 0.95 mmol) was dissolved, with heating, in a mixture of acetic anhydride (2 ml) and pyridine (2 ml). After standing overnight at room temperature, the mixture was poured into cold water (100 ml) and extracted with ethyl acetate (100 ml). The separated organic phase was washed successively with 5% HCl (25 ml), 5% $NaHCO_3$ (2 × 50 ml) and water (3 × 100 ml) and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave **IX** as a white solid (532 mg; 98.5% yield); MP 188.5–189.5°C (lit., 187–188°C [8]); purity of 99.4% on HPLC using methanol-water (95:5) as the solvent (t_R 10.5 min); ^{1}H-NMR and MS were essentially identical to spectra of **IX** recorded previously [8].

2.3. 3β-Acetoxy-9α-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (XI)

To a solution of the acetate derivative of the F_7-15-ketosterol **IX** (525 mg; 0.924 mmol) in dry tetrahydrofuran (15 ml) was added triethyl or-

thoformate (0.92 ml; 5.45 mmol; 6 equiv.) and *p*-toluenesulfonic acid (40 mg). After stirring at room temperature for 96 h under nitrogen, water (50 ml) and triethylamine (3 ml) were added slowly, and the resulting mixture was extracted with ether (2 × 100 ml). The organic extract was washed with water (3 × 100 ml), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to give, as indicated by ^{13}C-NMR, the $\Delta^{8,14}$-enol ether **X** as an oil (724 mg) containing triethyl orthoformate (two molar equivalents) and traces of *p*-toluenesulfonic acid.

To a portion (550 mg) of the crude $\Delta^{8,14}$-enol ether in a mixture of dioxane (20 ml) and saturated

Table 1
^{1}H-NMR chemical shifts for $\Delta^{8(14)}$-15-ketosterols functionalized at C-7 or C-9 and synthetic precursors and byproducts thereof[a,b]

	XIX[c]	III	V	VII	XI	IV	VI	XIII[d]	VIII	XVII	XVI	XIV	XV
H-1α	1.78	1.72	1.72	1.26	1.78	1.72	1.72	1.03	1.26	1.47	1.42	1.25	1.25
H-1β	1.47	1.47	1.52	1.72	1.48	1.46	1.52	1.79	1.72	1.91	1.89	1.71	1.71
H-2α	1.89	1.87	1.89	1.86	1.90	1.88	1.89	1.67	1.86	1.96	1.93	1.90	1.90
H-2β	1.45	1.37	1.38	1.38	1.45	1.37	1.38	1.46	1.38	1.57	1.49	1.48	1.49
H-3α	4.72	3.64	3.65	3.67	4.72	3.64	3.65	3.56	3.68	4.70	3.62	3.70	3.70
H-4α	1.75	1.72	1.73	1.66	1.76	1.72	1.73	1.60	1.66	1.76	1.72	1.85	1.85
H-4β	1.31	1.24	1.26	1.26	1.31	1.24	1.26	1.31	1.27	1.37	1.30	1.42	1.43
H-5α	2.18	2.10	2.03	1.84	2.18	2.10	2.04	1.42	1.85	1.50	1.44	2.14†	2.16†
H-6(α)	1.43	1.43	1.45	1.86	1.44	1.44	1.46	1.91	1.86	1.45†	1.46†	5.70	5.72
H-6β	1.28	1.29	1.32	1.53	1.28	1.30	1.32	1.78	1.53	1.45†	1.46†		
H-7(α)	2.01	2.00	1.96		2.01	2.01	1.97	5.85		2.21	2.20	7.50	7.50
H-7β	3.92	3.94	3.93	6.53	3.93	3.94	3.93		6.52	3.98	3.88		
H-9(α)				2.35				1.64†	2.36			2.13	2.14
H-11(α)	1.61	1.61	1.89*†	1.71	1.62	1.62	1.89*†	1.56	1.72	5.71	5.71	1.67	1.69
H-11β	1.96	1.98	1.90*†	1.51	1.97	1.98	1.90*†	1.43	1.51			1.53	1.54
H-12α	1.51	1.50	1.56	1.33	1.52	1.52	1.57	1.28	1.34	2.20	2.19	1.40	1.42
H-12β	2.02	2.02	2.00	2.11	2.02	2.02	2.00	1.99	2.11	2.52	2.52	2.17	2.17
H-16α	2.40	2.40	2.43	2.42	2.38	2.37	2.41	2.19	2.40	2.44	2.43	2.38	2.36
H-16β	2.05	2.05	2.05	2.05	2.05	2.05	2.06	2.10	2.05	2.06	2.07	2.10	2.10
H-17α	1.53	1.53	1.57	1.61	1.54	1.54	1.57	1.52	1.61	1.56	1.56	1.56	1.57
H-18	0.967	0.970	0.953	0.979	0.977	0.981	0.964	0.849	0.990	0.934	0.934	1.016	1.026
H-19	0.818	0.807	0.796	0.697	0.820	0.808	0.798	0.767	0.699	0.949	0.931	0.707	0.710
H-20	1.59	1.59	1.58	1.58†	1.63	1.64†	1.63	1.60	1.62	1.56	1.56	1.58†	1.64†
H-21	1.014	1.015	1.014	1.010	1.046	1.046	1.045	0.940	1.041	0.970	0.968	1.007	1.038
H-22R	1.33	1.33	1.34	1.34†	1.41†	1.42	1.42	1.40	1.42	1.34†	1.34†	1.34†	1.42
H-22S	1.08	1.08	1.09	1.08†	1.17	1.17	1.17	1.11	1.17	1.08	1.08	1.07	1.17
H-23R	1.33†	1.33†	1.34	1.34†	1.48	1.49	1.48	1.47	1.48	1.34†	1.34†	1.34†	1.48
H-23S	1.19	1.19	1.19	1.20	1.64	1.65	1.65	1.66	1.66	1.19	1.19	1.19	1.65
H-24R	1.10*	1.10*	1.10*	1.10*	1.99†	1.98†	1.98†	1.99	1.98	1.11*	1.10*	1.10*	1.99
H-24S	1.15*	1.15*	1.15*	1.15*	2.06	2.06	2.06	2.09	2.06	1.16*	1.15*	1.15*	2.05
H-25	1.52	1.52	1.52	1.52						1.52	1.52	1.52	
H-26	0.864	0.864	0.865	0.867						0.865	0.865	0.864	
H-27	0.867	0.867	0.868	0.870						0.867	0.867	0.867	
Acetate	2.022				2.022					2.032			
	9α-OH		9α-F	7α-F	9α-OH		9α-F	$\Delta^{7,14}$	7α-F	$\Delta^{8(14),9(11)}$		$\Delta^{6,8(14)}$	

[a]Data obtained at 500.1 MHz in 0.01–0.1 M $CDCl_3$ solution at 27°C. Chemical shifts referenced to $Si(CH_3)_4$ signal. Signals marked by asterisks may be interchanged within a column.
[b]Chemical shifts are generally accurate to 0.01 ppm, except for values marked by † (±0.02 ppm).
[c]3β-Acetoxy-9α-hydroxy-5α-cholest-8(14)-en-15-one.
[d]Additional signals for **XIII**: δ 0.12 (s, 3β-OTMS), 0.16 (s, 15-OTMS).

Table 2
^{1}H-^{1}H-NMR coupling constants for $\Delta^{8(14)}$-15-ketosterols functionalized at C-7 or C-9 and synthetic precursors and byproducts thereof[a,b]

	XIX[c]	III	V[d]	VII[e]	XI	IV	VI[d]	XIII	VIII[e]	XVII	XVI	XIV[f]	XV[f]
1α–1β		13.2		13.4		13.0	13.2	13.0	13.2	12.8†	12.6†	12.9	13.6†
1α–2α	3.9†	3.8		3.7			4.0		3.7			4.0†	3.9
1α–2β		13.9		13.8†		13.9	13.6†	14.1†	13.7			13.5†	13.6†
1β–2α	3.3†	3.3		3.5†		3.3†	3.6†	3.6†	3.4	3.4†	3.3†	3.6†	
1β–2β	3.3†	3.8		3.5†		3.9	3.6†	3.6†	3.3	3.4†	3.3†	3.6†	
2α–2β		12.4				12.5			12.6				
2α–3α	4.7†	4.7		4.3†	4.8†		4.3†	4.6†	4.3†	4.7†	4.5†	5.0†	5.1†
2β–3α	11.3†	11.4	11.1†	11.0	11.3†	11.3	11.0	10.9	11.1	11.3†	11.0†	11.1	11.1†
3α–4α	4.9	4.8	4.7†	5.3	4.9	4.9	4.8	4.6†	4.9	4.9	4.8	5.0†	4.9
3α–4β	11.3†	11.1	11.1†	11.0	11.3†		11.1	10.8	11.1	11.3†	11.0†	10.8	11.1†
4α–4β	12.5	12.4	4.7†	12.5	12.5	12.5	12.5	12.4†	12.4	12.3	12.3	12.4	12.4
4α–5α	3.5†	3.4†		2.7	3.6†	3.6†	3.5†		3.0†	2.4†	2.4†	13.5	2.9
4β–5α	12.5	12.7		12.5	12.7	12.7	12.7	12.4†	12.7				
5α–6α	3.7†	3.8		3.2†	3.6†	3.6†	3.6	5.1†	3.6†				
5α–6β	12.7	12.8		13.6	12.7†	12.7†	13.5†		13.7				
6α–6β	13.3	13.3		15.5†		13.3†	13.4	18.6	15.7				
6(α)-7(α)	6.1	5.9	6.0				6.0	5.5				10.0	10.0
6α–7β	2.2	2.2	2.2	3.4	2.2	2.2	2.2		3.2	2.6	3.4†		
6β–7α	13.5	13.4	13.5			13.3†	13.5	2.5†					
6β–7β	4.6	4.6	4.5	2.1	4.6	4.6	4.5		2.2	4.2	3.4†		
7α–7β	14.6	14.6	14.6		14.7	14.7	14.6			19.0	19.1		
9α–11α				7.3					7.2				
9α–11β				10.2					10.3				
11α–11β	14.4	14.0		13.6†					14.1				
11α–12α	3.5†	3.5†		3.5†					3.6†	2.5	2.4†	3.3	
11α–12β	3.2†	3.5†	3.5†	3.5†			3.4†	3.4†	3.5†	6.9	6.9	3.4†	3.4†
11β–12α	14.2	14.0†						13.6†	13.5†			13.7†	
11β–12β	3.6	3.5†	3.5†	3.5†			3.4†	3.4†	3.5†			3.4†	3.4†
12α–12β	12.7†	13.0†	12.7	12.7			12.3†	12.6	12.7	17.3	17.3	12.7	12.6
16α–16β	18.8	18.8	18.9	18.9	18.6	18.6	18.7	15.3	18.8	17.8	17.9	18.6	18.4
16α–17α	7.6	7.7	7.6	7.6	7.9	7.8	7.7	7.9	7.6	6.9	7.0	7.6	7.7
16β–17α	11.9	12.0		11.9	12.2	12.3	12.1	10.4	11.9	12.5	12.6	11.6	11.8
17α–20	9.8	9.8			9.8	9.9	9.8	10.7†					
20–21	6.4	6.4	6.2	6.2	6.6	6.6	6.4	6.4	6.2	6.2	6.3	6.1	6.4
20–22S					8.6	8.7	8.4	8.9	8.4				
22–22					13.3	13.1	13.1	13.2	13.1				
22S–23R					11.0	10.9	10.9	10.7					
22S–23S					4.7	4.9	4.7	4.5					
	9α-OH		9α-F	7α-F	9α-OH		9α-F	$\Delta^{7,14}$	7α-F	$\Delta^{8(14),9(11)}$		$\Delta^{6,8(14)}$	

[a]Data obtained at 500.1 MHz in 0.02–0.1 M $CDCl_3$ solution (27°C). Accuracy is generally ±0.2 Hz, except for couplings marked by † (±0.5 Hz).

[b]Additional couplings: 2α–4α, ~2.1 Hz; 24R–25, 6.6† Hz; 24S–25, 6.6†Hz; 25–26, 6.6 or 6.7 Hz; 25–27, 6.6 or 6.7 Hz (sterols with C_8H_{17} side chain). Unresolved couplings of 1–2 Hz were observed between several pairs of allylic protons.

[c]XIX, 3β-Acetoxy-9α-hydroxy-5α-cholest-8(14)-en-15-one.

[d]^{1}H-^{19}F couplings for 9α-fluoro-15-ketosterols V and VI: H-2β, 1.1 Hz; H-4β, 1.7 Hz; H-5α, 2.0 Hz; H-6β, 2.2 Hz; H-7α, 1.7 Hz; H-11α, ca. 30 Hz; H-11β, ca. 30 Hz.

[e]^{1}H-^{19}F couplings for 7α-fluoro-15-ketosterols VII and VIII: H-4β, 1.5 Hz; H-6α, 12.2 Hz; H-6β, 45.7 Hz; H-7β, 49.0 Hz; H-9α, 2.7 Hz; H-11β, not determined; other couplings <2 Hz.

[f]Multiplicities of vinylic protons signals of $\Delta^{6,8(14)}$-15-ketosterols XIV and XV: H-6, dd, 10.0, 2.3 Hz; H-7, dd, 10.0, 3.4 Hz.

aqueous $NaHCO_3$ (3 ml) was added *m*-chloroperbenzoic acid (159 mg) in a mixture of dioxane (10 ml) and saturated aqueous $NaHCO_3$ (2 ml) over 2 h with stirring. After stirring for an additional 30 min, the reaction mixture was poured into water and the resulting mixture was extracted with ether-CH_2Cl_2 (8:2 ratio, 2 × 100 ml). The combined separated organic phase was washed with 5% $NaHSO_3$ (2 × 100 ml), 5% $NaHCO_3$ (2 × 100 ml) and water (3 × 100 ml) and then dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave an oil (541 mg) which was subjected to MPLC using 15% ethyl acetate in hexane as the eluting solvent. The

Table 3
^{13}C-NMR chemical shifts for $\Delta^{8(14)}$-15-ketosterols functionalized at C-7 or C-9 and synthetic precursors and byproducts thereof[a–d]

	VII	XIII[e]	X[f]	XI	IV	VI	VIII	XVII	XVI	XIV	XV
C-1	36.26	36.98	35.33	29.40	29.63	29.79	36.27	34.84	35.06	35.11	35.13
C-2	30.97	31.84	27.66	26.92	30.85	30.78	30.94	27.47	31.37	30.96	31.02
C-3	70.73	71.50	73.40	72.93	70.52	70.35	70.71	72.88	70.58	71.09	71.11
C-4	37.11	38.34	34.09	33.71	37.88	37.61	37.08	34.02	38.09	35.93	35.99
C-5	36.88	39.75	40.34	35.05	35.25	35.59	36.85	39.74	39.81	45.43	45.47
C-6	34.54	30.47	25.50	28.30	28.40	28.06	34.49	25.72	25.79	138.81	139.10
C-7	82.79	123.60	28.73	22.67	22.77	23.17	82.63	26.45	26.59	124.04	124.01
C-8	143.20	130.84	123.75*	148.73	148.81	144.17	143.69	143.96	144.54	142.68	143.02
C-9	44.92	49.52	137.04	74.13	74.37	95.79	44.90	145.03	145.27	48.90	48.91
C-10	38.30	33.77	36.40	41.31	41.37	41.42	38.34	36.59	36.62	38.06	38.12
C-11	18.98	20.84	21.93	27.76	27.85	25.51	18.95	121.44	121.47	19.24	19.25
C-12	36.21	40.68	37.21	33.52	33.62	33.44	36.20	38.84	38.81	36.37	36.40
C-13	42.59	44.85	43.48	43.11	43.14	43.01	42.58	40.26	40.23	42.65	42.66
C-14	145.90	123.81	121.74*	141.09	141.13	143.14	145.42	137.25	137.13	140.04	139.63
C-15	207.41	144.53	147.80	207.72	207.71	207.65	206.65	205.98	206.35	207.26	206.37
C-16	41.67	38.41	33.86	42.28	42.33	42.19	41.48	44.02	44.09	42.42	42.25
C-17	50.63	54.46	53.26	50.20	50.21	50.07	50.51	50.76	50.68	50.88	50.76
C-18	18.29	17.35	16.02	17.26	17.30	16.82	18.31	15.65	15.65	20.51	20.55
C-19	11.98	12.36	18.11	15.44	15.61	15.13	11.98	20.13	20.27	11.26	11.29
C-20	34.60	33.88	34.00	34.28	34.31	34.34	34.52	34.12	34.09	34.63	34.49
C-21	19.12	18.84	18.78	18.86	18.91	18.89	18.86	18.88	18.86	19.04	18.82
C-22	35.71	35.96	35.85	35.52	35.56	35.56	35.52	35.82	35.79	35.75	35.60
C-23	23.50	18.17	18.15	17.92	17.93	18.03	18.05	23.51	23.48	23.52	18.03
C-24	39.33	29.37	29.32	29.20	29.26	29.27	29.24	39.35	39.34	39.31	29.28
C-25	27.96	g	g	g	g	g	g	27.91	27.94	27.93	g
C-26	22.51	g	g	g	g	121.07	g	22.47	22.50	22.50	g
C-27	22.73	g	g	g	g	121.07	g	22.66	22.71	22.72	g
3β-Ac			21.35	21.34				21.28			
			170.59	170.61				170.43			
	7α-F	$\Delta^{7,14}$	$\Delta^{8,14}$	9α-OH		9α-F	7α-F	$\Delta^{8(14),9(11)}$		$\Delta^{6,8(14)}$	

[a]Chemical shifts referenced to the $CDCl_3$ signal at 77.0 ppm. Data obtained at 75 MHz in $CDCl_3$ solution at a concentration of 0.05–0.2 M. Signals marked by an asterisk may be interchanged.

[b]Multiplicities for side-chain carbons of F_7 sterols: C-23, br d, ~5 Hz; C-24, d, ~20.8 Hz; C-26 and C-27, qd, 287.3, 26.2 Hz.

[c]J_{CF} coupling constants for 7α-fluoro-15-ketosterols **VII** and **VIII**: C-5, ~1 Hz; C-6, 23.3 Hz; C-7, 158.0 Hz; C-8, 14.3 Hz; C-10, ~1 Hz; C-13, 1.5 Hz; C-14, 8.0 Hz; C-15, 2.7 Hz; C-17, 2.0 Hz; C-18, 3.5 Hz; C-19, 1.5 Hz.

[d]J_{CF} coupling constants for 9α-fluoro-15-ketosterol **VI**: C-5, ~4 Hz; C-8, 19.1 Hz; C-9, 172.5 H; C-10, 20.4 Hz; C-11, 25.0 Hz; C-14, 9.1 Hz; C-18, 5.2 Hz; C-19, 3.8 Hz.

[e]Signals of TMS ethers at δ 0.24 and 0.51.

[f]Crude sample of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-15-ethoxy-5α-cholesta-8,14-diene containing triethyl orthoformate (δ 14.9, 59.4, 112.2); ethoxy signals of **X**: δ 15.50, 64.22.

[g]Signal too weak to be measured.

contents of fractions 23–32 were pooled and, after evaporation of the solvent under reduced pressure, gave **XI** (210 mg; 39% yield from **IX**), MP 210.5–211.5°C; single component on TLC in two solvent systems (SS-1, R_f 0.55; SS-4, R_f 0.76); purity of 99.6% by HPLC analysis (solvent, methanol-water (95:5), t_R 5.5 min); IR, ν_{max} 3500, 2980–2920, 1728, 1709, 1628, 1311, 1236, 1221, 1159, 1031 cm^{-1}; high-resolution MS, calcd. for $C_{29}H_{39}O_4F_7$, 584.2737, found 584.2736; ^{1}H-NMR, Table 1; ^{1}H-^{1}H coupling constants, Table 2; ^{13}C-NMR, Table 3; MS, Table 4.

Table 4
Mass spectral data for $\Delta^{8(14)}$-15-ketosterols functionalized at C-7 or C-9[a]

Suggested assignment[b]	Acetate	Free sterols		Trimethylsilyl ethers of			
	XI	**IV**	**VI**	**IV**	**VI**	**VII**	**VIII**
M^+	584* (3)	542* (14)	544 (10)	614 (6)	616 (26)	490 (78)	616 (54)
$M-R_2H$	566* (15)	524 (21)	524* (59)	596 (4)	596 (79)	470 (14)	596 (13)
$M-R_2H-CH_3$	551 (2)	509* (3)	509* (16)	581 (2)	581 (15)	455 (20)	581 (25)
$M-R_1H$	524* (36)	[524]		524 (22)	526 (6)		
$M-R_1H-CH_3$	509 (2)	[509]		509 (1)	511 (5)	385 (4)	
$M-R_1H-R_2H$	506* (13)	506 (6)	506* (7)	506 (8)	506 (23)	380 (56)	506 (94)
$M-R_1H-R_2HCH_3$	491* (19)	491* (10)	491* (42)	491 (6)	491 (57)	365 (58)	491 (69)
$M-R_1H-SC$	285 (4)	285* (5)			287 (5)	287 (11)	287 (10)
$M-R_2H-SC$		[285]	285* (54)		357 (25)		
$M-R_1H-SC-H_2O$	267* (20)	267* (11)	269 (11)	267 (27)	269 (11)	269 (34)	269 (84)
$M-R_1H-SC-R_2H$	[267]	[267]	267* (48)	[267]	267 (100)	267 (51)	267 (100)
$M-R_2H-SC-C_2H_3$	300* (6)	258* (17)	258* (65)	330 (3)	330 (45)		330 (17)
Other ions	467 (4)	467 (3)	482 (33)[c,d]	467 (3)	601 (8)	475 (100)	601 (53)
	441 (4)[c]	441 (2)[c]	477 (8)[c]	416 (15)	554 (14)[d]	472 (11)	598 (14)
	430 (2)[c]	430 (2)[c]	473 (9)	404 (3)	477 (13)	377 (14)	487 (13)
	427 (2)[c]	427 (2)	467 (9)	401 (10)	473 (10)	359 (18)	465 (16)
	416 (35)[c]	416 (15)[c]	449 (5)	374 (2)	465 (9)	351 (7)	377 (14)
	404 (3)[c]	404 (2)[c]	411 (10)	265 (5)	449 (9)	341 (7)	359 (38)
	401 (33)	401 (19)[c]	397 (8)[c]	249 (4)	411 (12)	338 (7)	293 (22)
	389 (3)	389 (3)	335 (5)	212 (16)	329 (18)	331 (6)	212 (52)
	375 (3)	375 (7)[c]	271 (9)	177 (83)	315 (13)	323 (8)	129 (62)
	374 (2)	374 (4)[c]	257 (42)	109 (100)	303 (34)	281 (9)	105 (68)
	273 (4)	177 (100)[c]	145 (100)		293 (8)	267 (34)	
	177 (80)[c]	109 (77)[c]	107 (89)		290 (6)	212 (58)	
	109 (100)[c]	108 (26)	105 (91)		249 (23)	105 (38)	
	108 (65)						
R_1 =	OAc	OH	OH	OTMS	OTMS	OTMS	OTMS
R_2 =	OH	OH	F	OH	F	F	F
	F_7-9α-OH	F_7-9α-OH	F_7-9α-F	F_7-9α-OH	F_7-9α-F	7α-F	F_7-7α-F

[a]Major ions above *m/z* 100 in mass spectra acquired at 70 eV by direct-probe or GC-MS (TMS ethers). Relative intensities as percentage of base peak. R_1 and R_2 are the substituents at C-3 and C-9, respectively. Ions also observed in the high-resolution mass spectrum and compatible (±3.5 millimass units) with the suggested assignments are indicated by an asterisk.
[b]SC, side chain.
[c]High-resolution MS is compatible (±3.5 millimass units) with the following assignments or formulas: *m/z* 482 (**VI**), $C_{25}H_{33}OF_7$ ($M-HF-C_2H_2O$); *m/z* 477 (**VI**), $C_{25}H_{28}OF_7$; *m/z* 441 (**XI** and **IV**), $M-R_1H-C_6H_{11}$; *m/z* 430 (**XI** and **IV**), $M-AcOH-C_7H_{10}$ (error −3.6 mmu); *m/z* 427 (**XI**), $M-AcOH-C_7H_{13}$; *m/z* 416 (**XI** and **IV**), $M-R_1H-C_8H_{12}$; *m/z* 404 (**XI** and **IV**), $M-R_1H-C_9H_{12}$; *m/z* 401 (**IV**), $M-H_2O-C_9H_{15}$; *m/z* 397 (**VI**), $C_{19}H_{20}OF_7$; *m/z* 375 (**IV**), $M-2H_2O-C_{10}H_{11}$; *m/z* 374 (**IV**), $M-2H_2O-C_{10}H_{12}$; *m/z* 177 (**XI** and **IV**), $M-R_1H-SC-C_8H_{12}$; *m/z* 109 (**XI** and **IV**), C_8H_{13}.
[d]Appears to involve loss of ketene.

2.4. 3β,9α-Dihydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (IV)

A solution of **XI** (200 mg; 0.342 mmol) in degassed methanol (4 ml) and degassed tetrahydrofuran (2 ml) was stirred with potassium carbonate (95 mg; 0.68 mmol; 2 equiv.) for 3 h at room temperature under nitrogen. Ethyl acetate (50 ml) and water (50 ml) were added, and the separated organic phase was washed with water (3 × 50 ml), dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The resulting residue (179 mg) was subjected to MPLC on silica gel using 30% ethyl acetate in hexane as the eluting solvent. The contents of fractions 40–70 were pooled and, after evaporation of the solvent under reduced pressure, gave the 9α-hydroxy-F_7-15-ketosterol **IV** (150 mg; 81% yield), MP, 216–217°C; single component on TLC in two solvent systems (SS-2, R_f 0.27; SS-4, R_f 0.19); single component (>99.9%) on HPLC analysis (solvent, methanol-water (9:1), t_R 6.0 min); IR, ν_{max} 3500, 2980–2920, 1686, 1616, 1310, 1236, 1213, 1157 cm^{-1}; high-resolution MS, calcd. for $C_{27}H_{37}O_3F_7$, 542.2631, found 542.2607; ^{19}F-NMR, δ −76.76 and −76.95 (A_3B_3 portion of A_3B_3X system similar to those in spectra of other F_7-sterols [8]: $^3J_{FF}$ ~7 Hz, $^4J_{FF}$ ~9 Hz, F-26 and F-27), −184.30 (*t* of septet, $^3J_{HF}$ ~20 Hz, $^3J_{FF}$ 6.6 Hz, F-25); >99% purity by ^{1}H-NMR; ^{1}H-NMR,

Table 5
Chromatographic properties of 7α-fluoro-, 9α-fluoro- and 9α-hydroxy-15-ketosterols and of $\Delta^{6,8(14)}$- and $\Delta^{8(14),9(11)}$-15-ketosterols[a]

15-Ketosterol		TLC[b]	HPLC[c]	GC[d]	GC-MS[e]
		Free sterols		TMS ethers	
C_8H_{17} side chain					
I	$\Delta^{8(14)}$	0.38	19.8	18.0	9.7
III	9α-hydroxy-$\Delta^{8(14)}$	0.12	7.5	21.8	10.4
V	9α-fluoro-$\Delta^{8(14)}$	0.38	16.3	~17.4[f]	~9.4[f]
VII	7α-fluoro-$\Delta^{8(14)}$	0.38	15.6	~17.2[f]	~9.4[f]
XIV	$\Delta^{6,8(14)}$	0.37	18.5	19.4	9.9[g]
XVI	$\Delta^{8(14),9(11)}$	0.37	17.0	18.0	9.7[g]
$C_8H_{10}F_7$ side chain					
II	$\Delta^{8(14)}$	0.32	10.0	13.0	8.1
IV	9α-hydroxy-$\Delta^{8(14)}$	0.09	5.0	15.3	8.9
VI	9α-fluoro-$\Delta^{8(14)}$	0.32	8.4	~12.7[f]	~7.9[f]
VIII	7α-fluoro-$\Delta^{8(14)}$	0.32	8.1	~12.6[f]	~7.9[f]
XV	$\Delta^{6,8(14)}$	0.32	9.3	13.8	8.4[g]

[a]Chromatographic mobilities for this table were measured at one time when all syntheses were complete, whereas characterizations for purity were done as each analytical sample became available. Differences between retention times and R_f values in this table and those in the Experimental Procedures reflect inadvertent differences in chromatographic conditions.

[b]R_f values from TLC on silica gel G plates developed with 30% ethyl acetate in hexane. Separations were no better at 20%, 40%, or 50% ethyl acetate in hexane.

[c]Retention times (min) for reversed-phase HPLC; elution with 10% water in methanol.

[d]Retention times (min) for GC using a Shimadzu GC-9A chromatograph, splitless injection, nitrogen carrier gas, flame ionization detection and a DB-5 column (30 m × 0.25 mm i.d.; 0.1 μm film thickness) held at 200°C for 3 min and increased to 280°C at 20°C/min.

[e]Retention times (min) for GC-MS using falling needle injection, helium carrier gas and a 15-m DB-5 column held at 200°C for 2 min and increased to 280°C at 10°C/min.

[f]Retention times are approximate owing to extensive decomposition of the fluoro-15-ketosterols in the GC injector. GC-MS of the 9α-fluoro-15-ketosterols showed the $\Delta^{8(14),9(11)}$ dienone TMS ether as the major peak (V) or a significant minor peak (VI). Chromatograms of the 7α-fluoro-15-ketosterols showed, in addition to a minor fluorosterol peak, multiple dienone components, including a major peak corresponding to the $\Delta^{6,8(14)}$ species.

[g]Mass spectra of dienones: TMS ether of **XIV**, *m/z* 470 (9, M^+), 455 (5), 380 (84), 365 (75), 267 (79), 240 (58), 212 (100); TMS ether of **XVI**, *m/z* 470 (100, M^+), 455 (18), 428 (17), 380 (11), 365 (24), 330 (66), 316 (15), 315 (14), 303 (32), 267 (68), 211 (26); TMS ether of **XV**, *m/z* 596 (9, M^+), 581 (5), 506 (97), 491 (80), 465 (14), 267 (83), 240 (64), 212 (100).

Table 1; ^{1}H-^{1}H coupling constants, Table 2; ^{13}C-NMR, Table 3; MS of IV and GC-MS of the 3β-TMS ether of IV (single component at t_R 9.1 min), Table 4; additional chromatographic data, Table 5.

2.5. *3β-Hydroxy-9α,25,26,26,26,27,27,27-octafluoro-5α-cholest-8(14)-en-15-one* (VI)

To a cold solution of the 9α-hydroxy-F_7-15-ketosterol IV (100 mg; 0.185 mmol) in CH_2Cl_2 (20 ml) was added a cooled (−35°C) solution of CH_2Cl_2 (1 ml) and HF-pyridine (1 ml) with stirring. After stirring for 4 h at −35°C, the reaction mixture was poured into water and extracted with ether (3 × 50 ml). The organic phase was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The resulting pale yellow solid (95 mg) was subjected to MPLC using successive elution with 10% ethyl acetate in hexane (400 ml), 15% ethyl acetate in hexane (400 ml) and 20% ethyl acetate in hexane. The contents of fractions 56–67 were pooled and, after evaporation of the solvent, gave VI (86 mg; 86% yield), MP, 134–135°C; single component on TLC in two solvent systems (SS-2, R_f 0.63; SS-4, R_f 0.45); single component (>99.9%) on HPLC analysis (solvent, methanol-water (9:1), t_R 9.5 min); IR, ν_{max} 3400, 2980–2920, 1709, 1633, 1314, 1223, 1159 cm^{-1}; high-resolution MS, calcd. for $C_{27}H_{35}O_2F_7$ (M−HF), 524.2525, found 524.2509; 96% purity by ^{1}H-NMR (containing 4% of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholesta-8(14),9(11)-dien-15-one (XVIII)); ^{19}F-NMR, δ −76.77 and −76.95 (A_3B_3 portion of A_3B_3X system similar to that of IV, F-26 and F-27), −151.34 (br dd, J ~32 and ~30 Hz with several unresolved couplings of 1–2 Hz, F-9α), −184.30 (dd of septet, $^3J_{HF}$ 20.8 and 19.9 Hz, $^3J_{FF}$ 6.6 Hz, F-25); ^{1}H-NMR, Table 1; ^{1}H-^{1}H coupling constants, Table 2; ^{13}C-NMR, Table 3; MS, Table 4; additional chromatographic data, Table 5. Silylation of VI followed by GC-MS analysis resulted in partial decomposition to a 5:1 mixture of the 3β-TMS ether of VI (t_R 8.4 min; MS, Table 4) and a dienone (t_R 8.5 min); MS of the dienone: *m/z* 596 (100, M^+), 554 (13), 491 (25), 330 (47), 315 (25), 303 (34), 267 (72), 211 (68), 143 (97), 129 (100), 105 (88).

2.6. *3β-Hydroxy-7α-fluoro-5α-cholest-8(14)-en-15-one* (VII)

A solution of $\Delta^{8(14)}$-15-ketosterol I (500 mg, 1.25 mmol) in BSTFA-pyridine (1:1 mixture, 3 ml) was heated at 100-110°C in an oil bath for 16 h under nitrogen. The reaction mixture was concentrated in a stream of nitrogen, followed by drying under high vacuum. The residue containing $\Delta^{7,14}$-bis-TMS dienol ether XII was dissolved in dry CH_2Cl_2 (3 ml), *N*-fluoropyridinium triflate (309 mg, 1.25 mmol) was added, and the resulting solution was stirred at room temperature under nitrogen for 36 h. The reaction mixture was poured into ice-water (25 ml), extracted with CH_2Cl_2 (3 × 25 ml) and washed with 5% $NaHCO_3$ solution (25 ml) and water (3 × 50 ml). Evaporation gave a brown solid (672 mg), which ^{1}H-NMR analysis showed to be a mixture of mainly VII, I, XIV and V (8:4:2:1 ratio), present both as their TMS ethers and free sterols. To a solution of a portion (200 mg) of the crude product in dry tetrahydrofuran (4 ml) was added tetrabutylammonium fluoride solution (1.0 M, 1 ml), and the mixture was stirred under nitrogen for 15 min. Monitoring of the reaction by TLC (30% ethyl acetate in hexane) showed disappearance of the TMS ether derivatives (R_f 0.95) and appearance of a single major spot at R_f 0.28. The reaction mixture was diluted with saturated ammonium chloride (1 ml) and extracted with ethyl acetate (3 × 25 ml). The combined organic extracts were washed with water (3 × 50 ml), dried over sodium sulfate and concentrated to a yellow oil. Non-polar materials were removed by filtration through silica gel (5 cm × 1.6 cm column) using elution with 5% ethyl acetate in hexane (200 ml) and 10% ethyl acetate in hexane (100 ml). The desired sterol products were recovered by elution with 15% ethyl acetate in hexane and evaporation of the filtrate to an oil (106 mg). HPLC analysis (SS-5, 1 ml/min) showed the following components: VII (t_R 20.9 min), XIV (t_R 24.9 min), I (t_R 26.3 min) and lesser amounts of V (t_R 21.2 min) and an unidentified compound (t_R 18.3 min). A portion of the crude hydrolyzed product (70 mg) was purified in 10-mg batches by semi-preparative HPLC using 15% water in methanol. Initial purification gave fractions of the desired 7α-fluoro-$\Delta^{8(14)}$-15-ketosterol VII (20 mg of 95% purity and

16 mg of lower purity) and later fractions containing a mixture of $\Delta^{8(14)}$-15-ketosterol **I** and $\Delta^{6,8(14)}$-15-ketosterol **XIV** (19 mg). Material enriched in **VII** was again subjected to HPLC to give **VII** of 97% purity (containing 2% **V** and 1% of an unidentified impurity). This sample (9 mg) was used for cell culture experiments and characterization of **VII**: MP, 76–77°C; single component by TLC in SS-3 (R_f 0.68) and SS-4 (R_f 0.46) and by HPLC in SS-5 (t_R 21.0 min); IR, ν_{max} 3400, 2980–2820, 1711, 1640, 1468, 1383, 1365, 1155, 1123, 1044, 1003, 654 cm^{-1}; UV, λ_{max} (ϵ) 250 nm (11800); high-resolution MS, 418.3220 (35, M^+; calcd. for $C_{27}H_{43}O_2F$, 418.3247), 403.2995 (100, M–CH_3), 400.3153 (11, M–H_2O), 398.3156 (10, M–HF), 383.2938 (25, M–HF–CH_3), 380.3057 (7, M–HF–H_2O), 365.2866 (17, M–HF–H_2O–CH_3), 305.1917 (20, M–SC), 287.1801 (25, M–H_2O–SC), 285.1855 (M–HF–SC), 269.1833 (4), 267.1744 (19, M–HF–H_2O–SC), 258.1613 (5, $C_{17}H_{22}O_2$), 249.1639 (M–HF–$2H_2O$–SC), 105.0703 (22, C_8H_9); ^{19}F-NMR, δ –171.09 (br td, 46.7 and 11.8 Hz, F-7α); ^{1}H-NMR, Table 1; ^{1}H-^{1}H coupling constants, Table 2; ^{13}C-NMR, Table 3; MS, Table 4; additional chromatographic data, Table 5. Silylation of **VII** followed by GC-MS analysis resulted in partial decomposition to a 1:2:2 mixture of the 3β-TMS ether of **VII** (t_R 9.6 min; MS, Table 4), a decomposition product from dehydration (t_R 9.9 min) and a dienone (t_R 10.2 min), in addition to several minor components; MS of the dehydration byproduct: *m/z* 472 (100, M^+), 457 (15), 382 (13), 367 (58), 341 (21), 269 (17), 261 (19), 251 (47), 107 (40); MS of dienone: *m/z* 470 (15, M^+), 380 (100), 365 (58), 338 (9), 267 (26), 240 (20), 212 (57), 129 (16). GC-MS analysis of another sample prepared similarly showed the fluorosterol, two dienone components and no dehydration species.

*2.7. 3β-Hydroxy-7α,25,26,26,26,27,27,27-octafluoro-5α-cholest-8(14)-en-15-one (***VIII***)*

Compound **VIII** was prepared analogously to **VII**. Thus, treatment of **II** (250 mg, 0.48 mmol) with BSTFA-pyridine (1:1, 2 ml) gave bis-TMS ether **XIII** as a pale brown oil, which was analyzed by GC, GC-MS and NMR. ^{1}H and ^{13}C-NMR (Table 3) showed **XIII** contaminated with minor amounts (~10%) of the $\Delta^{8,14}$ dienol ether and other impurities. Treatment of crude **XIII** (~225 mg) in CH_2Cl_2 (2 ml) with *N*-fluoropyridinium triflate (105 mg, 0.42 mmol) at room temperature for 24 h, followed by hydrolysis with tetrabutylammonium fluoride (1 ml), gave a brown solid (223 mg). Filtration through silica gel as described for **VII** gave a yellow solid (101 mg) consisting of **VIII**, **XV** and **II** in an 8:5:1 ratio by ^{1}H-NMR analysis. A portion of the crude hydrolyzed product (60 mg) was purified in 10-mg batches by semi-preparative HPLC using 20% water in methanol. The following components were obtained: 7 mg of **VIII** of 97% purity (containing 3% of **XV** by ^{1}H-NMR), 6 mg of **VIII** of 94% purity (containing 6% of **XV** by ^{1}H-NMR), 19 mg of **VIII** of lower purity, **XV** (6 mg, containing ~14% each of **VIII** and **II** by ^{1}H-NMR) and **II** (6 mg). Characterization of **VIII** of 97% purity (used in cell culture experiments): MP, 151–152°C; single component by TLC in SS-3 (R_f 0.66) and SS-4 (R_f 0.44); HPLC in SS-5, t_R 8.4 min (98% purity, containing **XV**); IR, ν_{max} 3400, 2980–2820, 1709, 1640, 1470, 1449, 1314, 1281, 1221, 1157, 1132, 1045 cm^{-1}; UV, λ_{max} (ϵ) 250 nm (11900); high-resolution MS, 544.2593 (86, M^+; calcd. for $C_{27}H_{36}O_2F_8$, 544.2588), 529.2349 (100, M–CH_3), 526.2579 (12), 524.2496 (26, M–HF), 511.2299 (4), 509.2273 (36, M–HF–CH_3), 506.2402 (23, M–HF–H_2O), 491.2170 (33, M–HF–H_2O–CH_3), 399.1578 (4, $C_{19}H_{22}OF_7$), 305.1903 (11, M–SC), 287.1808 (31, M–H_2O–SC), 285.1830 (8, M–HF–SC), 269.1882 (3, $C_{19}H_{25}O$), 269.1693 (3, M–$2H_2O$–SC), 267.1747 (16, M–HF–H_2O–SC), 258.1605 (6, $C_{17}H_{22}O_2$), 249.1637 (5, M–HF–$2H_2O$–SC), 105.0702 (19, C_8H_9); ^{19}F-NMR, δ –76.76 and –76.93 (A_3B_3 portion of A_3B_3X system similar to that of **IV**, F-26 and F-27), –184.28 (*t* of septet, $^3J_{HF}$ ~20 Hz, $^3J_{FF}$ ~6.6 Hz, F-25), –171.26 (br td, 47.5 and 12.7 Hz, F-7α); ^{1}H-NMR, Table 1; ^{1}H-^{1}H coupling constants, Table 2; ^{13}C-NMR, Table 3; MS, Table 4; additional chromatographic data, Table 5. Silylation of **VIII** of 97% purity followed by GC-MS analysis resulted in partial decomposition to an ~3:2:1 mixture of the 3β-TMS ether of **VIII** (t_R 8.1 min; MS, Table 4) and

two dienones, in addition to minor components; MS of dienones: (t_R 6.6 min) *m/z* 596 (41), 357 (69), 344 (57), 331 (34), 267 (76), 253 (52), 160 (100); (t_R 8.5 min), *m/z* 596 (M^+, 7), 506 (65), 491 (54), 465 (22), 267 (60), 212 (100), 129 (25).

A sample of dienone **XV** containing ~14% each of **VIII** and **II** was characterized as follows: ^{1}H-NMR, Table 1; ^{1}H-^{1}H coupling constants, Table 2; ^{13}C-NMR, Table 3; chromatographic data, Table 5.

2.8. Effects of analogs of 3β-hydroxy-5α-cholest-8(14)-en-15-one on the levels of HMG-CoA reductase activity in CHO-K1 cells

The 9α-hydroxy-F_7-substituted $\Delta^{8(14)}$-15-ketosterol **IV** was equivalent in potency to the parent 15-ketosterol **I** in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells (Table 6). Similar observations were made with the 7α-fluoro-$\Delta^{8(14)}$-15-ketosterol **VII** and the 7α-fluoro-F_7-$\Delta^{8(14)}$-15-ketosterol **VIII**. The 9α-fluoro-F_7-15-ketosterol **VI** showed high potency in suppression of the levels of HMG-CoA reductase activity, but it appeared to be slightly less active than **I**.

3. Discussion

The 9α-fluoro-F_7-15-ketosterol **VI** was synthesized from the F_7-15-ketosterol **II** by a route (Fig. 2) analogous to that used in preparing 9α-fluoro-15-ketosterol **V** from **I** [20,23]. The preparation of the F_7-$\Delta^{8,14}$-dienol ethyl ether from the acetate ester of the F_7-$\Delta^{8(14)}$-15-ketosterol by treatment of the α,β-unsaturated ketone with triethyl orthoformate and an acid catalyst represents an adaptation of procedures described for the preparation of steroidal dienol ethers [34,35] which we have utilized previously for the formation of the $\Delta^{8,14}$-dienol ethyl ether from the 15-ketosterol **I** [20,23] and from the benzoate ester of **I** [23]. In the present study, the $\Delta^{8,14}$-dienol ethyl ether, formed from the acetate ester of **II** by treatment with triethyl orthoformate in the presence of *p*-toluenesulfonic acid, was directly converted to the 9α-hydroxy-15-ketosteryl acetate **XI** by treatment with *m*-chloroperbenzoic acid under controlled conditions, as in our previously described synthesis of 3β-benzoyloxy-9α-hydroxy-5α-cholest-8(14)-en-15-one [23]. Mild alkaline hydrolysis of **XI** gave the 3β,9α-dihydroxy-15-ketosterol **IV**. Treatment of **IV** with HF-pyridine gave the 9α-fluoro-15-ketosterol **VI**; this reaction represents an adaptation of the method of Ambles and Jacquesy [36] which we have utilized previously for the conversion of the 9α-hydroxy-15-ketosterol **III** to the 9α-fluoro-15-ketosterol **V** [20,23] and for the 3β-benzoate ester of **III** to the 3β-benzoate ester of **V** [23].

Allylic fluoro-15-ketosterols readily undergo

Table 6
Effects of 3β,9α-dihydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(4)-en-15-one (**IV**), 3β-hydroxy-9α,25,26,26,26,27,27,27-octafluoro-5α-cholest-8(14)-en-15-one (**VI**), 3β-hydroxy-7α-fluoro-5α-cholest-8(14)-en-15-one (**VII**), 3β-hydroxy-7α,25,26,26,26,27,27,27-octafluoro-5α-cholest-8(14)-en-15-one (**VIII**) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) on the levels of HMG-CoA reductase activity in CHO-K1 cells

Sterol concentration (μM)	HMG-CoA reductase activity (% of control)				
	IV[a]	VI[a]	VII[a]	VIII[a]	I[b]
0.0	100.0	100.0	100.0	100.0	100.0
0.1	61.0 ± 3.8	93.2 ± 4.0	65.5 ± 7.9	63.0 ± 4.4	62.5 ± 2.8
0.25	48.0 ± 4.4	69.3 ± 2.8	45.5 ± 5.9	41.0 ± 1.8	45.8 ± 2.0
0.50	35.4 ± 1.6	49.7 ± 7.2	34.5 ± 2.0	34.2 ± 0.7	36.6 ± 1.6
1.0	27.8 ± 2.2	42.2 ± 6.4	32.7 ± 2.4	38.4 ± 5.2	28.8 ± 1.4
2.5	19.2 ± 0.5	34.2 ± 1.3	28.2 ± 0.7	14.9 ± 1.6	23.6 ± 1.6

[a]Mean ± S.D. for replicate ($n = 3$) assays of HMG-CoA reductase activity.
[b]Mean ± S.E.M. of 40 independent experiments in which triplicate determinations of enzyme activity were made at each concentration.

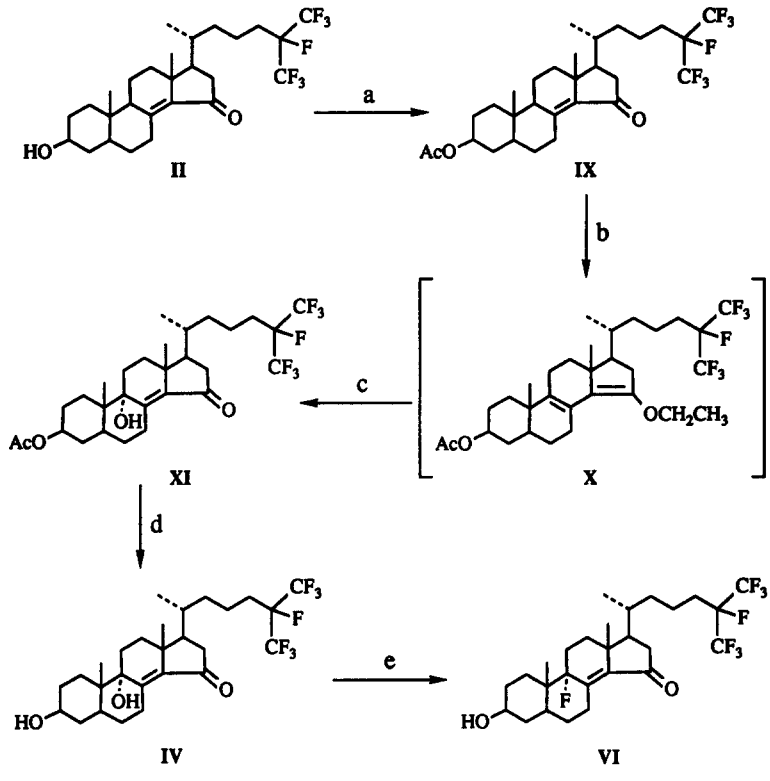


Fig. 2. Chemical synthesis of 3β,9α-dihydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**IV**) and 3β-hydroxy-9α,25,26,26,26,27,27,27-octafluoro-5α-cholest-8(14)-en-15-one (**VI**). Reagents and conditions: (a) Ac_2O, pyridine; (b) $(EtO)_3CH$, pTsOH; (c) *m*-chloroperbenzoic acid, $NaHCO_3$; (d) K_2CO_3; (e) HF-pyridine, −35°C.

elimination[2] to dienones that are virtually inseparable from the fluoro-15-ketosterols or the parent 15-ketosterol on silica gel (Table 5). Therefore, it is desirable to introduce the fluorine in the final synthetic step and from a precursor of markedly different polarity. This strategy has been successfully implemented in the synthesis of 9α-fluoro-15-ketosterols [20,23]. However, attempts to prepare the 7α-fluoro-15-ketosterol **VII** analogously by reaction of 3β,7α-dihydroxy-5α-cholest-8(14)-en-15-one with HF-pyridine gave mainly elimination products. The recent availability of relatively safe and convenient electrophilic fluorinating agents [37], combined with our discovery of conditions for the regioselective synthesis of dienol ethers of $\Delta^{8(14)}$-15-ketosterols [13] suggested an alternative route to **VII**, shown in Fig. 3. Thus, formation of the $\Delta^{7,14}$-dienol TMS ether **XII** of 15-ketosterol **I**, followed by treatment with *N*-fluoropyridinium triflate [37], gave a product containing the desired 7α-fluoro-15-ketosterol **VII** together with **I** and minor amounts of the 9α-fluoro-15-ketosterol **V** and the $\Delta^{6,8(14)}$-15-ketosterol **XIV**. The contaminants, which were similar to **VII** in mobility on silica gel (Table 5), were largely removed by semi-preparative reversed-phase HPLC. The F_7-7α-fluoro-15-ketosterol **VIII** was prepared analogously from the F_7-15-ketosterol **II**. In this case, the 9α-fluoro-15-ketosterol byproduct **VI** was not observed, evidently because

2. The 9α-fluoro-15-ketosterols appeared to eliminate HF to form dienones under basic conditions or upon GC or GC-MS analysis. However, heating of a sample of **V** in ethanol or chloroform showed no decomposition by ^{1}H-NMR.

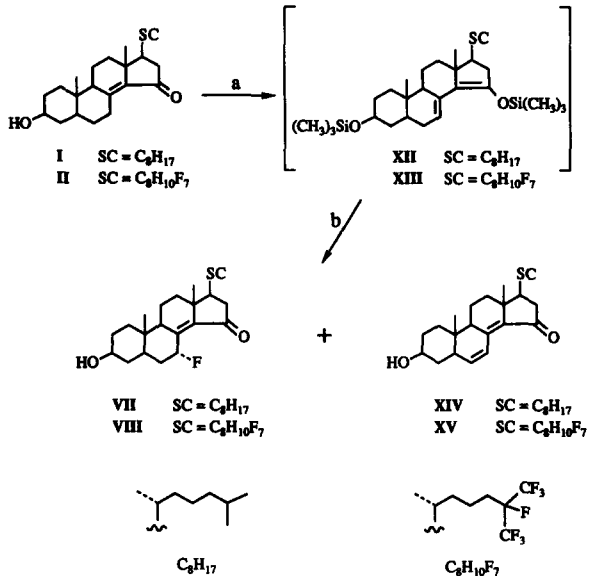


Fig. 3. Chemical synthesis of 3β-hydroxy-7α-fluoro-5α-cholest-8(14)-en-15-one (**VII**) and 3β-hydroxy-7α,25,26,26,26,27,27,27-octafluoro-5α-cholest-8(14)-en-15-one (**VIII**) from the parent 15-ketosterols **I** and **II**. Reagents and conditions: (a) BSTFA-pyridine, 36 h, 23°C; (b) *N*-fluoropyridinium triflate; $(n\text{-Bu})_4NF$.

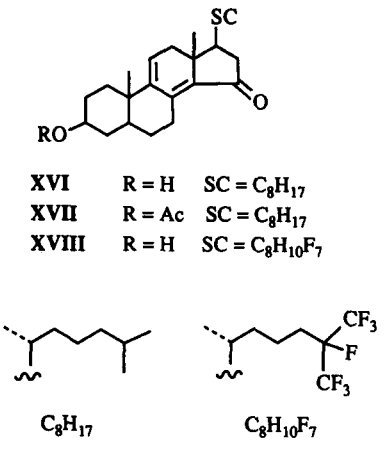


Fig. 4. Structures of 3β-hydroxy-5α-cholesta-8(14),9(11)-dien-15-one (**XVI**), its 3β-acetate **XVII** and its F_7 analog **XVIII**.

of higher purity of the $\Delta^{7,14}$-enol ether intermediate **XIII**.

Prior to biological testing, the 7α-fluoro- and 9α-fluoro-15-ketosterols were analyzed for dienone byproducts. Analysis by chromatographic methods was hampered by lack of resolution (TLC), partial decomposition of the fluoro-15-ketosterols (GC) or difficulty in quantitation (HPLC using UV detection). However, any dienone impurities were visible in the olefinic region of the ^{1}H-NMR spectrum and could be quantified by comparing the heights of resolved methyl singlets. Significant contaminants in the crude 7α-fluoro-15-ketosterols **VII** and **VIII** were the $\Delta^{6,8(14)}$-15-ketosterols **XIV** and **XV**, which were largely or completely removed by semi-preparative HPLC. The 9α-fluoro-15-ketosterol **VI** contained ~4% of the $\Delta^{8(14),9(11)}$-15-ketosterol **XVIII** (Fig. 4), an impurity that was not eliminated by chromatography on either silica gel or reversed-phase media. We have also observed by NMR minor amounts of the $\Delta^{8(14),9(11)}$-15-ketosterol **XVI** as a contaminant in some preparations of 9α-fluoro-15-ketosterol **V**. However, the 500-MHz ^{1}H-NMR spectrum of a sample of **V** used in earlier work [20,23] showed the absence of this dienone byproduct. ^{1}H and ^{13}C-NMR data are given for $\Delta^{6,8(14)}$- and $\Delta^{8(14),9(11)}$-15-ketosterols in Tables 1, 2 and 3, and MS data are in a footnote to Table 5. It should be noted that these dienones may isomerize readily to other dienones[3] or decompose to other products.

Chromatographic mobilities for the dienones and fluoro-15-ketosterols are presented in Table 5. These data illustrate the difficulty of distinguishing the fluoro-15-ketosterols from each other or from dienones by TLC or GC. As observed previously for the parent 15-ketosterols **I** and **II** [8], F_7 sterols have significantly shorter retention times on reversed-phase HPLC and GC than the corresponding sterols with a C_8H_{17} side chain. This effect was more pronounced on a 30-m column than on a 15-m column (operated under somewhat different conditions; see Table 5). The

3. Force-field calculations showed the 14β-$\Delta^{7,9(11)}$-15-one isomer to be lower in heat of formation than the $\Delta^{6,8(14)}$-15-one species **XIV** (by 2.8 kcal/mol) or the $\Delta^{8(14),9(11)}$-15-one isomer **XVI** (by 7.1 kcal/mol). The 14β-$\Delta^{8,11}$ and 14α-$\Delta^{7,9(11)}$ isomers were also lower in heat of formation than **XVI** (by 1.8 and 4.7 kcal/mol).

7α-fluoro- and 9α-fluoro-15-ketosterols decomposed significantly upon GC-MS analysis to mixtures that included dienone components. The availability of GC-MS data for dienone standards (Table 5) permitted tentative identification of some of the dienone species, i.e. $\Delta^{6,8(14)}$ isomers from 7α-fluoro-15-ketosterols **VII** and **VIII** and the $\Delta^{8(14),9(11)}$ isomer in the chromatogram of the 9α-fluoro-15-ketosterol **VI**.

The 7α- and 9α-functionalized $\Delta^{8(14)}$-15-ketosterols described here were also characterized by MS, high-resolution MS, IR and ^1H and ^{13}C-NMR. Mass spectra of the 9α-substituted $\Delta^{8(14)}$-15-ketosterols showed molecular ions of relatively low abundance and fragment ions due to losses of CH_3, side chain, H_2O, CH_3COOH (in the case of **XI**) and HF (in the case of 7α-fluoro- and 9α-fluoro-15-ketosterols). Also noteworthy were ions corresponding to loss of H_2O or HF together with a side-chain fragment ($C_{10}H_{13}F_7$). Unlike mass spectra of the 9α-fluorosterol **VI**, the spectra of 9α-hydroxysterols **IV** and **XI** showed a series of even- and odd-electron ions corresponding to loss of H_2O or CH_3COOH together with a hydrocarbon fragment of 6–10 carbon atoms. The most prominent such ion was *m/z* 416 ($M-CH_3-COOH-C_8H_{12}$ or $M-H_2O-C_8H_{12}$), which in conjunction with loss of side chain gave a very intense ion at *m/z* 177. The *m/z* 100–200 regions of the spectra of 9α-hydroxy-15-ketosterols **IV** and **XI** were dominated by two strong ions (*m/z* 109 and 177), whereas the spectrum of 9α-fluoroketosterol **VI** showed many abundant ions of roughly equal intensity in this region.

An ion of high abundance in the spectra of **VI** and several 9α-fluoro-$\Delta^{8(14)}$-15-ketosterols reported previously [23] is M–62. This ion in the high-resolution mass spectrum of **VI** (*m/z* 482) was compatible only with the formula $C_{25}H_{33}OF_7$, suggesting loss of HF and ketene. Loss of ketene has been reported for many α,β-unsaturated ketones [38], but such fragmentation has not been noted previously for $\Delta^{8(14)}$-15-ketosterols. Among 9α-hydroxy-$\Delta^{8(14)}$-15-ketosterols, the corresponding loss of H_2O and ketene was not observed for the free sterol **IV**; in 3β-acetate **XI** this ion could not be distinguished from loss of CH_3COOH, which gives a fragment of identical elemental composition. However, this ion appears in mass spectra reported for 9α-hydroxy-$\Delta^{8(14)}$-15-ketosterols having a 3β-benzoyloxy or 3-keto group [23]. Among 3β-TMS derivatives, an M–62 ion was observed in 14% abundance for the 9α-fluoro-15-ketosterol **VI**. A $\Delta^{8(14),9(11)}$ dienone appears to be an intermediate species in this fragmentation, judging from the presence of an M–42 ion in the spectrum of the TMS ether of $\Delta^{8(14),9(11)}$-15-ketosterol **XVI** (Table 5) and the absence of M–62 ions in spectra of the 7α-fluoro-15-ketosterols.

GC-MS analysis of the 7α-fluoro-15-ketosterols **VII** and **VIII** showed several peaks whose analysis indicated partial decomposition to dienones in the GC injector. Spectra of **VII** and **VIII** and their TMS ethers were similar to those of the 9α-fluoro-15-ketosterols but showed, among other differences, M^+, $M-CH_3$ and $M-H_2O$ in much greater abundance than the spectra of the TMS ether of 9α-fluoro-15-ketosterol **VI**. Numerous ions of the TMS ether of **VII** were also observed in the mass spectrum of the TMS ether of the $\Delta^{6,8(14)}$-15-ketosterol **XIV** [13]. These ions may be partially the result of thermal elimination in the ion source or contamination from nearby GC peaks of dienones. Abundant ions corresponding to M^+ and $M-CH_3$ demonstrated that a substantial portion of the 7α-fluoro-15-ketosterols does not undergo elimination.

The NMR spectra of the sterols described in this work were fully in accord with the assigned structures. The ^1H, ^{13}C and ^{19}F-NMR spectra of the F_7 sterols showed the expected chemical shifts for the atoms of the $C_8H_{10}F_7$ side chain, and decoupling experiments reaffirmed the stereochemical assignments established previously [8] for the side-chain protons of F_7 sterols. The 7α and 9α-fluoro substituents showed ^{19}F chemical shifts in the general region of those of 9α-fluoro-corticosteroids [39–41] and were nearly insensitive to the F_7 substitution. The ^{13}C chemical shifts of the 9α-hydroxy and 9α-fluoro-$\Delta^{8(14)}$-15-ketosterols were similar to those reported for the corresponding sterols with a C_8H_{17} side chain [30], and the ^{13}C-^{19}F coupling constants were compatible with those reported for other 9α-fluorosteroids [30,33,39,41,42]. Both the 7α-fluoro- and 9α-fluoro-15-ketosterols showed the expected upfield

shifts for γ-*gauche* sp^3 carbons. The magnitude of these shifts, which in the 9α-fluoro-15-ketosterols varied from 3.5 ppm for C-12 to 8.5 ppm for C-5, appeared to be a function of the alignment of the C—F bond with the axial C—H bond of the γ carbon and the distance between these bonds.[4] As in the case of other 9α-fluoro-15-ketosterols [30], a 2.3-ppm downfield shift was observed for C-19 (γ-*anti*) of the F_7 derivative VI, and long-range substituent effects for VI were ≤1.0 ppm except for the unexpected 2.0-ppm upfield shift for C-18. The latter shift, which is much smaller in a 9α-fluorocorticosteroid lacking the $\Delta^{8(14)}$ bond [43], may be attributable to electric field effects [44].

^{1}H-NMR chemical shifts are presented in Table 1 for new sterols described in this work and for 9α-substituted 15-ketosterols with a C_8H_{17} side chain, for which complete ^{1}H data have not been reported. Substituent effects for 7α-fluoro, 9α-fluoro or 9α-hydroxy groups included downfield shifts of 0.3–0.6 ppm for axial protons on γ carbons and smaller upfield shifts (0.1–0.2 ppm) for their equatorial geminal partners.[5] As noted previously for other compounds [45], the magnitude of the downfield shift was generally correlated with the size of the upfield shift of the γ carbon. For example, among the 9α-fluoro-15-ketosterols, large effects were observed for H-1α and C-1 and small effects for H-12α and C-12. These substituent effects, which are similar in magnitude to those observed previously for other sterols bearing axial fluorine or hydroxyl [46], are attributable to polarization of axial C—H bonds of γ carbons by the C—F bond [45].

Comparison of the ^{1}H-^{1}H coupling constants (Table 2) for the substituted 15-ketosterols with those [31] of the parent 15-ketosterol I showed only minor differences. These differences, which were mainly in ring B, did not significantly affect the conformation of that ring. Essentially no differences in coupling constants were observed in the conformationally flexible D ring. The ^{1}H-^{19}F coupling constants of the 7α-fluoro-15-ketosterols ($J_{H6\alpha-F7\alpha}$ 12.2 Hz and $J_{H6\beta-F7\alpha}$ 45.7 Hz) reflected the truly axial orientation of the allylic fluorine. The corresponding coupling constants of the 9α-fluoro-15-ketosterols ($J_{H11\alpha-F9\alpha}$ and $J_{H11\beta-F9\alpha}$, each ~30 Hz) indicated a quasi-axial fluorine whose orientation is determined by the effect of $\Delta^{8(14)}$ double bond on the conformation of ring C (12α-sofa). The corresponding coupling constants in the parent 15-ketosterol I ($J_{H9\alpha-H11\alpha}$ 7.1 Hz and $J_{H9\alpha-H11\beta}$ 10.5 Hz) show that H-9α of I has a similar quasi-axial orientation relative to ring C.

Use of saturation difference spectroscopy at 500 MHz to isolate individual ^{1}H resonances revealed numerous four- and five-bond couplings between F-9α and axial protons of rings A and B. The 9α-fluoro-15-ketosterols showed long-range couplings of >1 Hz to H-2β, H-4β, H-5α, H-6β and H-7α, and the 7α-fluoro-15-ketosterols showed long-range couplings to H-4β and H-9α. Such long-range couplings indicate that the C—F and C—H bonds and the intervening axial bonds are parallel. These geometric conditions are met if rings A and B are in highly symmetrical chair conformations, which are predicted by force-field calculations for the fluoro-15-ketosterols V and VII and for the parent 15-ketosterol I. A similar analysis of long-range ^{1}H-^{19}F couplings has been reported for other fluorinated steroids [39]. In summary, the combined results of the NMR data in Tables 1, 2 and 3, together with long-range ^{1}H-^{19}F couplings and molecular modeling, indicate that a 7α-fluoro, 9α-fluoro or 9α-hydroxy substituent has negligible effects on the conformation of the 15-ketosterols. Apart from electron-withdrawing effects transmitted through bonds and a redistribution of π

4. Molecular modeling indicates that the F9α-C9-C12-H12α pseudo-dihedral angle is −19° and the F9α–H12α distance is 2.78 Å, whereas the F9α-C9-C5-H5α pseudo-dihedral angle is 0° and the F9α–H5α distance is 2.44 Å. The corresponding pseudo-dihedral angles and distances for other γ carbons were: C-7, 3°, 2.58 Å; C-1, −8°, 2.51 Å (9α-fluoro-15-ketosterols) and C-5, −4°, 2.55 Å; C-9, 3°, 2.61 Å (7α-fluoro-15-ketosterols).

5. The following ^{1}H substituent increments (≥0.05 ppm) were observed: 7α-fluoro-15-ketosterols, H-1α, 0.06; H-5α, 0.43; H-6α, 0.38; H-6β, 0.18; H-7β, 2.39; H-9α, 0.51; H-11α, 0.08; H-12α, 0.09; H-16α, 0.06; H-17α, 0.15 ppm; 9α-fluoro-15-ketosterols, H-1α, 0.52; H-1β, −0.20; H-2α, 0.05; H-4α, 0.05; H-5α, 0.62; H-7α, 0.39; H-7β, −0.21; H-11α, 0.25; H-11β, 0.36; H-12α, 0.32; H-12β, −0.10; H-16α, 0.07; H-17α, 0.11; H-19, 0.08 ppm; 9α-hydroxy-15-ketosterols, H-1α, 0.52; H-1β, −0.26; H-5α, 0.68; H-6α, −0.05; H-6β, −0.06; H-7α, 0.43; H-7β, −0.20; H-11β, 0.44; H-12α, 0.26; H-12β, −0.08; H-17α, 0.08; H-19, 0.09 ppm. Shieldings for I were taken from data in Ref. 31.

electron density, the electronic effects of these substituents consisted mainly of moderate polarization of axial C—H bonds of γ carbons.

The new analogs of the 15-ketosterol I were studied with respect to their effects on the level of HMG-CoA reductase activity in cultured cells. We have previously demonstrated that the 9α-hydroxy analog of I is a potent inhibitor of the synthesis of digitonin-precipitable sterols from labeled acetate in both mouse L cells and primary cultures of fetal mouse liver cells, with IC_{50} values of 0.3 and 4.0 μM, respectively, in the two cell types [20]. A major site of the inhibition of sterol synthesis appears to be at the level of HMG-CoA reductase, since the IC_{50} values for reductase lowering in the two cell types were 0.4 and 5.0 μM, respectively. In the present study we have shown that the F_7-9α-hydroxy-$\Delta^{8(14)}$-15-ketosterol **IV** is equivalent in potency to I in lowering the level of reductase activity in CHO-K1 cells.

We have also previously demonstrated that the 9α-fluoro analog of I and its 3-keto derivative are highly active as inhibitors of the synthesis of digitonin-precipitable sterols from labeled acetate in mouse L cells [20]. Both compounds showed IC_{50} values of 0.5 μM. The concentrations of the 9α-fluoro-$\Delta^{8(14)}$-15-ketosterol and its 3-keto derivative required for 50% suppression of the level of HMG-CoA reductase activity were the same (0.2 μM). In the present study we have demonstrated that the 9α-fluoro-F_7-15-ketosterol **VI** lowers HMG-CoA reductase activity in CHO-K1 cells. The potency of **VI** appeared to be slightly less than that of the parent 15-ketosterol I.

The first syntheses of 7α-fluoro analogs of I and II also permitted evaluation of their effects on HMG-CoA reductase in the CHO-K1 cells. Both the 7α-fluoro-$\Delta^{8(14)}$-15-ketosterol **VII** and the 7α-fluoro-F_7-$\Delta^{8(14)}$-15-ketosterol **VIII** were equivalent to I in lowering of reductase activity.

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