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SYNTHESIS OF DEUTERIUM- AND TRITIUM-LABELED 25,26,26,26,27,27,27-HEPTAFLUOROCHOLESTEROL

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Summary

25,26,26,26,27,27,27-Heptafluorocholesterol (F_7-cholesterol) is a novel analog of cholesterol with potential value in biological investigations. We have combined an isotope-labeling strategy described for 15-ketosterols with modifications in the original synthesis of F_7-cholesterol to prepare analogs containing deuterium or tritium at the C-23 position. ^{1}H, ^{2}H, and ^{13}C nuclear magnetic resonance spectra indicated high chemical purity of the deuterium-labeled analog and confirmed the presence of deuterium only at C-23. The tritium-labeled analog was synthesized in high radiochemical purity with a specific activity of 472 mCi/mmol.

Key words: side-chain fluorinated cholesterol, tritium, deuterium, NMR

Introduction

26-Hydroxycholesterol, a potent regulator of sterol synthesis and of the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in mammalian cells (1), is a major oxysterol present in blood plasma (2) and in atherosclerotic lesions (3). The enzymatic conversion of cholesterol to 26-hydroxycholesterol and to the corresponding carboxylic acid and the efflux of the 26-oxygenated products from cells has been proposed as a defense mechanism to reduce intracellular levels of cholesterol (4). To block enzymatic side-chain oxidation, we have synthesized 25,26,26,26,27,27,27-heptafluorocholesterol (F_7-cholesterol) (5). To explore the metabolism of the F_7-cholesterol, we now describe the synthesis of its [23-^{2}H] and [23-^{3}H] analogs.

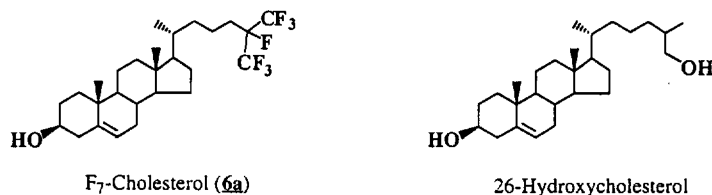

Figure 1. Structures of F_7-cholesterol and 26-hydroxycholesterol.

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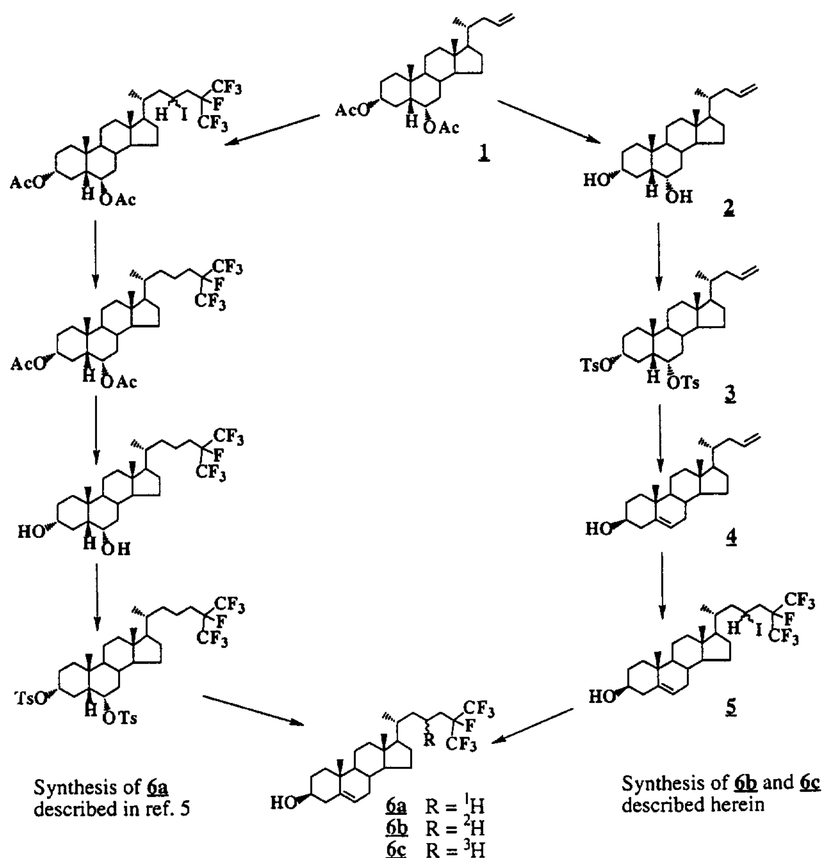
Results and Discussion

We sought to introduce deuterium and tritium labels into F_7-cholesterol at C-23 by reducing a 23-iodide intermediate with tributyltin deuteride or tritide. This strategy was used to prepare deuterium- and tritium-labeled F_7-$\Delta^{8(14)}$-15-ketosterols (6), for which the iodide reduction represented the final or penultimate step of the synthesis (5, 7, 8).

Scheme 1 depicts two syntheses of F_7-cholesterol (6). On the left is shown the original synthesis reported (5, 9) for the unlabeled sterol (6a). This synthesis is unsuitable for preparing tritium-labeled material because the 23-iodide reduction occurs at an early step and because one of the subsequent steps requires a careful chromatographic separation. For the purpose of making isotopically labeled F_7-cholesterol, we have modified the original synthesis in order to introduce the Δ^5 bond prior to the 23-iodide reduction. This modified synthesis is shown on the right side of Scheme 1.



Scheme 1. Two syntheses of F_7-cholesterol (6) from 5β-chol-23-ene-3α,6α-diol diacetate (1).

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As shown in Scheme 1, our synthesis began with 5β-chol-23-ene-3α,6α-diol diacetate (1), an intermediate described in the preparation of unlabeled F_7-cholesterol (5). The 3α,6α-diacetoxy moiety was unmasked to the $\Delta^{5,23}$-3β-hydroxy-C_{24}-steroid 4 by the same series of reactions described in the original synthesis of F_7-cholesterol (5). The differences in side-chain functionality did not adversely affect the reaction yields or the chromatographic purification of 4 from a complex mixture of byproducts. Likewise, the Δ^5 double bond did not interfere with construction of the fluorine-substituted side chain by addition of heptafluoroisopropyl iodide (i-C_3F_7I) to 4 in the presence of the free-radical initiator triethylborane (5). Iodide 5 was obtained in high purity as an 88:12 mixture of 23*R*- and 23*S*-iodo isomers accompanied by only traces (0.3%) of the deiodinated byproduct 6a. Byproducts analogous to 6a, which are often produced in much higher amounts in the addition of i-C_3F_7I to Δ^{23} steroids, have been attributed to abstraction of hydrogen from the reaction medium by a free-radical intermediate (6). Reduction of iodide 5 with tributyltin deuteride of 97% isotopic purity afforded the 23-deuterio-F_7-cholesterol 6b showing a high level of deuterium incorporation (95% d_1, 5% d_0). Variable and generally lower levels of deuterium incorporation had been observed in similar preparations of 23-deuterio-15-ketosterols owing to contamination of the 23-iodides with deiodinated material analogous to 6a (6). The ^2H nuclear magnetic resonance (NMR) spectrum of 6b showed a 3:2 ratio of 23*R*- and 23*S*-deuterio isomers, a result consistent with findings reported for similar reductions (6). NMR analysis confirmed the absence of tributyltin species (10) and indicated a purity of >99%.

The tritium-labeled F_7-cholesterol 6c was prepared analogously by reduction of iodide 5 with [^3H]tributyltin hydride. The synthesis of 6c was designed to introduce tritium at the final step in order to minimize manipulations of radiolabeled material. The reaction gave predominantly a single sterol product that was readily purified by medium-pressure liquid chromatography (MPLC) on silica gel. Thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) of 6c, shown in Figure 2, indicated high radiochemical purity and chromatographic mobility identical to that of the unlabeled F_7-cholesterol 6a. The specific activity of 6c was 472 mCi/mmol.

^1H, ^2H, and ^{13}C NMR data for 6b and its synthetic precursors (Table 1) were compatible with the structures presented. The NMR data were measured with high precision (11), which permitted determination of very small deuterium isotope shifts. Comparison of ^1H and ^{13}C NMR data for 6a and 6b indicated the following upfield shifts for introduction of deuterium at C-23: ~0.008 ppm for the C-22 and C-24 protons, ~0.016 ppm for the C-23 protons, ~0.10 ppm for C-22 and C-24 (12). Isotope shifts at other positions were negligible (≤0.002 ppm for ^1H).

Low- and high-resolution mass spectra of deuterium-labeled F_7-cholesterol 6b and its synthetic precursors were compatible with the assigned chemical structures and with mass spectra reported for similar compounds. In particular, the Δ^5-3β-hydroxysteroids 4, 5, 6, and the 23-hydroxy derivatives of 6a showed the major fragment ions described previously (13) for cholesterol, including abundant ions for M – 15, M – 18, M – 33, M – 85, M – 111, *m/z* 255, 231, and 213 and minor ions at M – 139 and *m/z* 273.

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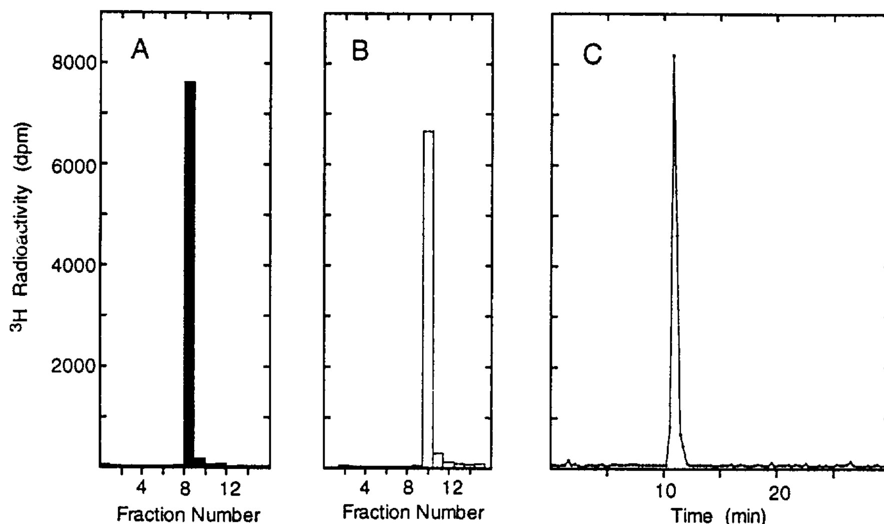

Figure 2. Radiochemical purity of tritium-labeled F_7-cholesterol (6c) by radio-TLC (A and B) and radio-HPLC (C). Conditions are described in the Experimental section.

Experimental

5β-Chol-23-ene-3α,6α-diol diacetate (1) was prepared as described previously (5, 9). The following reagents were obtained commercially: tributyltin deuteride (97% isotopic purity) and tri-*n*-butyltin chloride (96% purity) from Aldrich Chemical Co. (Milwaukee, WI); 2,2'-azobisisobutyronitrile (AIBN) from Janssen Chimica (San Diego, CA); 2-iodoheptafluoropropane from PCR (Gainesville, FL); and sodium borotritide (14) from Amersham (Arlington Heights, IL).

Melting points (mp) were measured with a Thomas-Hoover apparatus in sealed, evacuated capillary tubes. Analytical TLC was performed using precoated silica gel G plates (0.25-mm thickness; Analtech, Newark, DE). TLC plates were charred by spraying with 5% ammonium molybdate in 10% sulfuric acid followed by heating. MPLC was done on columns dry packed with silica gel (230-400 mesh; EM Science, Gibbstown, NJ). HPLC was performed on a 5-µm Spherisorb ODS-II column (250 × 4.6 mm i.d.; Custom LC, Houston, TX) at 1.0 ml/min with a Waters model 510 pump, Rheodyne 7125 injector, and UV detection at 210 nm. Radioactivity was measured on a Packard model 1500 liquid scintillation analyzer using ScintiVerse (Fisher Scientific; Fair Lawn, NJ) as scintillation fluid. Low-resolution and high-resolution mass spectra (MS) were recorded on a VG ZAB-HF double sector instrument by electron impact at 70 eV and are reported as *m/z* (relative intensity, suggested assignment). Ions attributable to losses of CH_3, side chain, H_2O, or combinations thereof are marked by an asterisk. Ions showing exact masses within ±3.0 millimass units of the indicated or implied assignments are marked by †. NMR spectra were recorded on a Bruker AMX500 or AC250 spectrometer as described previously (5, 11). ^1H NMR

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spectra (500 MHz) were measured at 25°C on $CDCl_3$ solutions (5 to 15 mM) and referenced to internal tetramethylsilane; ^{13}C NMR spectra (126 MHz) were measured at 22°C on $CDCl_3$ solutions (20 to 100 mM) and referenced to $CDCl_3$ at 77.0 ppm; ^{19}F NMR spectra (235 MHz) were measured at ~22°C on $CDCl_3$ solutions (≤50 mM) and referenced to internal $CFCl_3$ (0 ppm for the tallest line); ^2H NMR spectra (77 MHz) were acquired unlocked with inverse-gated proton decoupling at 25°C in $CHCl_3$ solution (~20 mM) and referenced to $CDCl_3$ at δ 7.26. Accuracy of ^1H NMR shieldings is ±0.001 ppm (15) except for values marked by † (±0.003 ppm). Assignments of the C-24 protons of Δ^{23} steroids were established from their vicinal couplings (~10 Hz, pro-*E* or ~15 Hz, pro-*Z*). The purity of steroid samples was estimated by ^1H NMR (500 MHz spectrum; methyl region and δ 2.5 – 7.0 region) and confirmed by TLC and HPLC; steroids 2, 3, 4, and 6b showed ≥99% purity by NMR.

5β-Chol-23-ene-3α,6α-diol (2) To a solution of 5β-chol-23-ene-3α,6α-diol diacetate (1, 1.0 g, 2.25 mmol) in tetrahydrofuran (10 ml) and methanol (10 ml) was added potassium carbonate (1.0 g). The mixture was stirred under nitrogen at room temperature for 24 h, poured into water, and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated to a yellow solid that was purified by MPLC (500 × 25 mm i.d. column; elution with ethyl acetate-hexane 7:3). Evaporation of fractions 90-100 gave 2 as a white solid (700 mg, 86% yield): mp, 155-156°C; single component by TLC (R_f 0.36; ethyl acetate-hexane 1:1); MS, *m/z* 360 (4, M^+), 342*† (53), 327* (32), 324*† (26), 319† (8, M – C_3H_5), 309*† (18), 301† (100, M – C_3H_7O), 283† (65, M – H_2O – C_3H_7O), 271† (14, M – $C_5H_{13}O$), 255* (10), 231† (36, $C_{16}H_{23}O$), 215 (42, $C_{16}H_{23}$), 213 (38, $C_{16}H_{21}$); high-resolution MS, calcd. for $C_{24}H_{40}O_2$, 360.3028; obsd., 360.3027; ^1H and ^{13}C NMR, Table 1.

5β-Chol-23-ene-3α,6α-diol bis(*p*-toluenesulfonate) (3) To a mixture of diol 2 (320 mg, 0.89 mmol) and 4-dimethylaminopyridine (5 mg) in pyridine (5 ml) was added *p*-toluenesulfonyl chloride (600 mg, 3.15 mmol). The mixture was stirred at room temperature under nitrogen for 30 h, poured over cold 5% sulfuric acid, and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to a white foam. Purification by MPLC (500 × 10 mm i.d. column; elution with ethyl acetate-hexane 8:92) afforded 3 as a white foamy product (490 mg, 82% yield): mp, 75-76°C; single component by TLC (R_f 0.52; ethyl acetate-hexane 1:3); ^1H NMR, δ 7.786, 7.723, 7.353, 7.333 (two AA'XX' systems, J_{AX} ~8.4 Hz); 2.468 and 2.462 (s, tosylate CH_3); 5.744 (strongly coupled dddd, ~15, ~10, ~8, ~6 Hz, H-23), 4.979† (m, H-24*E*), 4.976† (m, H-24*Z*), 4.790 (dt, 12.3, 4.9 Hz, H-6β), 4.299 (tt, 11.4, 4.8 Hz, H-3β), 2.149 (ddddd, 13.8, 6.2, 3.2, 1.7, 1.4 Hz, H-22*S*), 1.93 (m, H-12β), 1.845 (dtd, 13.6, 9.6, 6.3 Hz, H-16α), 1.634 (dtd, 12.5, 4.3, ~0.9 Hz, H-7β), 1.611 (td, 12.8, 11.3 Hz, H-4α), 1.227 (q, 12.3 Hz, H-7α), 0.886 (d, 6.6 Hz, H-21), 0.801 (s, H-19), 0.598 (s, H-18).

Chola-5,23-dien-3β-ol (4) A mixture of the ditosylate 3 (0.45 g, 0.67 mmol) and potassium acetate (1.0 g, 10.2 mmol) were dissolved in a heated solution (110°C) of *N,N*-dimethylformamide (10 ml) and water (1.5 ml). The reaction was stirred at this temperature for 5 h, poured into water (50 ml), and (after cooling) extracted with ethyl acetate. The organic phase was washed with water,

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dried over Na_2SO_4, and evaporated to a light yellow solid (270 mg). The crude product was dissolved in methanol-tetrahydrofuran (1:1, 8 ml), followed by addition of KOH (250 mg) and water (0.2 ml). The mixture was stirred at 25°C for 3 h, poured into water (100 ml), and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated to a yellow solid that was purified by MPLC (750 × 10 mm i.d. column, elution with ethyl acetate-hexane 5:195 (1000 ml) and ethyl acetate-hexane 9:191). Fractions 2-5 gave an oil (40 mg) identified by ^1H and ^{13}C NMR as a 11:6:1:1:1 mixture of chola-3,5,23-triene, chola-2,5,23-triene, chola-2,4,23-triene, 3β-chlorochola-5,23-diene, and an unidentified steroid. Fractions 7-59 gave a yellow residue (28 mg), which was identified by ^1H and ^{13}C NMR as chiefly a 20:10:5:2:1 mixture of chola-4,23-dien-3β-ol, chola-4,23-dien-3α-ol, chola-6,23-dien-3β-ol, chola-5,23-dien-3α-ol, and chola-3,23-dien-5α-ol (tentative). Fractions 62-90 gave 4 as a white solid (140 mg, 61% yield): mp, 129-130°C; single component by TLC (R_f 0.26; ethyl acetate-hexane 15:85); MS, *m/z* 342† (100, M^+), 327*† (18), 324*† (43), 309*† (24), 301† (15, M – C_3H_5), 285 (9), 283† (26, M – C_3H_5 – H_2O), 273*† (8), 257† (34, M – C_5H_9O), 255*† (11), 231† (48, $C_{16}H_{23}O$), 213† (28, $C_{16}H_{21}$), 203† (16, $C_{15}H_{23}$), 107† (63, C_8H_{11}); high-resolution MS, calcd. for $C_{24}H_{38}O$, 342.2923; obsd., 342.2918; ^1H and ^{13}C NMR, Table 1.

23-Iodo-25,26,26,26,27,27,27-heptafluorocholest-5-en-3β-ol (5) To a slurry of chola-5,23-dien-3β-ol (4, 100 mg, 0.29 mmol) in hexane (2 ml) were added 2-iodoheptafluoropropane (0.1 ml) and triethylborane (1.0 M solution in hexane, 0.1 ml) in a nitrogen atmosphere. The mixture was stirred at room temperature for 5 h in a foil-wrapped flask. Evaporation of the solvent at < 40°C yielded a yellow residue which was purified by MPLC (500 × 10 mm i.d. column; elution with ethyl acetate-hexane 4:96). Fractions 42-58 gave 5 as a white solid (138 mg, 74% yield); single component by TLC (R_f 0.38; ethyl acetate 1:3); 87:13 mixture of 23*R*-iodo and 23*S*-iodo isomers by HPLC (t_R 10.8 min and 11.9 min; water-methanol 2:98; UV detection at 210 nm); 88:12 mixture of 23*R*-iodo and 23*S*-iodo isomers by ^1H NMR (containing 0.3% of 6a); MS, *m/z* 638† (100, M^+), 623*† (23), 620*† (65), 605*† (36), 578† (7, M – C_3H_8O), 553† (37, M – C_5H_9O), 527† (60, M – $C_7H_{11}O$), 499 (15), 273*† (9), 255*† (16), 231 (10), 213 (28), 107† (53, C_8H_{11}); high-resolution MS, calcd. for $C_{27}H_{38}OF_7I$, 638.1856; obsd., 638.1858; ^1H and ^{13}C NMR, Table 1.

[23-^{2}H]25,26,26,26,27,27,27-Heptafluorocholesterol (6b) To a solution of iodide 5 (50 mg, 0.078 mmol) in tetrahydrofuran was added AIBN (2 mg) and tributyltin deuteride (40 μl). The mixture was stirred at room temperature under nitrogen for 6 h, followed by evaporation under nitrogen to a white residue that was dissolved in ethyl acetate-hexane 3:7 and filtered through silica gel. Evaporation of the filtrate gave a white solid (40 mg) that was purified on MPLC (500 × 10 mm i.d. column; elution with ethyl acetate-hexane 1:9). Evaporation of fractions 30-38 gave a white solid (27 mg), which was again filtered through silica gel to remove long-chain nonsteroidal impurities and provide an analytical sample of 6b (25 mg): mp, 149.5-150°C (lit. mp for 6a, 149-150.5°C (5)); single component by TLC (R_f 0.43; ethyl acetate-hexane 3:7 and R_f 0.51; acetone-toluene 1:4); MS (95% d_1, 5% d_0), *m/z* 513† (100, M^+), 498*† (26), 495*† (68), 480*† (50), 453† (10, M – C_3H_8O), 428† (47, M – C_5H_9O), 402† (78, M – $C_7H_{11}O$), 374† (22, M – $C_9H_{15}O$), 332

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(8), 320† (8, $C_{14}H_{17}{}^2HF_7$), 273* (12), 255*† (22), 231† (15, $C_{16}H_{23}O$), 213† (30, $C_{16}H_{21}$), 107† (53, C_8H_{11}); high-resolution MS, calcd. for $C_{27}H_{38}{}^2HOF_7$, 513.2952; obsd., 513.2959; ^{1}H and ^{13}C NMR, Table 1; ^{2}H NMR, 3:2 ratio of singlets at δ 1.437† (^{2}H-23*R*) and 1.630† (^{2}H-23*S*); ^{19}F NMR, –76.74 and –76.95 (A_3B_3 portion of A_3B_3X system; J_{AB} ~9 Hz, J_{AX} ~7 Hz, F-26 and F-27), –184.14 (dd of septet, 21, 20, 6.7 Hz).

In addition to <u>**6b**</u>, two polar components were isolated by MPLC. Fractions 111-115 gave a white solid (6 mg; ~97% purity by ^{1}H NMR): single component by TLC (R_f 0.34; ethyl acetate-hexane 3:7 and R_f 0.46; acetone-toluene 1:4); MS, *m/z* 528† (100, M^+), 513*† (23), 510*† (66), 495*† (36), 468 (8, M – C_3H_8O), 443† (40, M – C_5H_9O), 417† (54, M – $C_7H_{11}O$), 389 (10, M – $C_9H_{15}O$), 293† (22, $C_{11}H_{12}OF_7$), 273*† (16), 255*† (25), 231† (15, $C_{16}H_{23}O$), 213† (34, $C_{16}H_{21}$), 107† (66, C_8H_{11}); high-resolution MS calcd. for $C_{27}H_{39}O_2F_7$, 528.2838; obsd., 528.2854; identified by NMR as (23*R*)-25,26,26,26,27,27,27-heptafluorocholest-5-ene-3β,23-diol (16). Fractions 120-126 gave a white solid (5 mg; ~97% purity by ^{1}H NMR): single component by TLC (R_f 0.29; ethyl acetate-hexane 3:7 and R_f 0.44; acetone-toluene 1:4); MS, *m/z* 528† (100, M^+), 513*† (23), 510*† (66), 495*† (36), 468† (8, M – C_3H_8O), 443† (40, M – C_5H_9O), 417 (52, M – $C_7H_{11}O$), 389† (8, M –$C_9H_{15}O$), 293† (22, $C_{11}H_{12}OF_7$), 273*† (15), 255*† (25), 231† (15, $C_{16}H_{23}O$), 213† (34, $C_{16}H_{21}$), 107† (66, C_8H_{11}); high-resolution MS, calcd. for $C_{27}H_{39}O_2F_7$, 528.2838; obsd., 528.2811; identified by NMR as (23*S*)-25,26,26,26,27,27,27-heptafluorocholest-5-ene-3β,23-diol (16).

[23-^{3}H]25,26,26,26,27,27,27-Heptafluorocholesterol (<u>6c</u>) Tributyltin tritide was prepared as described previously (6b) by adding a solution of tri-*n*-butyltin chloride (10.4 μl, 0.038 mmol) in absolute ethanol (0.5 ml) to a suspension of sodium borotritide (250 mCi, 6.5 Ci/mmol, 0.038 mmol) in absolute ethanol (1.0 ml), followed by stirring under nitrogen for 1 h at room temperature and evaporation under nitrogen to a cloudy oil. To this oil were added iodide <u>**5**</u> (19.0 mg, 0.0298 mmol; 88:12 mixture of 23*R* and 23*S* epimers), AIBN (2.0 mg), and anhydrous tetrahydrofuran (2.0 ml). The reaction mixture was stirred at room temperature under nitrogen for 6 h, followed by evaporation to dryness under nitrogen, dissolution in ethyl acetate (120 ml), washing with saturated sodium chloride (2 × 50 ml), and drying over Na_2SO_4. Radio-TLC of the crude material showed the major radioactive product comigrating with <u>**6a**</u> (R_f 0.53 in ethyl acetate-hexane 1:1). The crude oily product was adsorbed onto silica gel (300 mg) and subjected to MPLC (700 × 10 mm i.d. column; elution with ethyl acetate-hexane 1:99 (600 ml) and ethyl acetate-hexane 5:95). Apart from fractions 1-2, nearly all the radioactivity was eluted in fractions 76-94, which were pooled and evaporated to give <u>**6c**</u> as a white solid (13 mg; 472 mCi/mmol). This product was stored at –20°C in benzene (25 ml) and analyzed by radio-TLC and radio-HPLC as follows. TLC plates spotted with <u>**6c**</u> were developed with ethyl acetate-hexane 1:1 or acetone-toluene 3:7, followed by scraping of 1-cm sections into 7-ml vials for scintillation counting. The resulting chromatograms (Figures 2A and 2B) showed a single radioactive component comigrating with <u>**6a**</u> (R_f 0.53 for ethyl acetate-hexane 1:1 and R_f 0.67 for acetone-toluene 3:7). For radio-HPLC, a solution of <u>**6c**</u> (1 μl, 16400 dpm) in methanol-benzene 99:1 containing <u>**6a**</u> was injected onto a Spherisorb ODS-II column (250

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Table 1. ^{1}H and ^{13}C NMR data for ^{2}H-F$_7$-cholesterol (6b) and synthetic precursors.

^{1}H NMR data^{a}					^{13}C NMR data^{b}				
Atom	2	4	5^{c}	6b	Atom	2	4	5^{c}	6b
H-1α	1.794	1.078	1.081	1.079	C-1	35.53	37.20	37.21	37.23
H-1β	1.058	1.847†	1.848†	1.846†	C-2	30.19	31.61	31.61	31.64
H-2α	1.346	1.838†	1.841†	1.840†	C-3	71.58	71.77	71.77	71.78
H-2β	1.693	1.500	1.505	1.502	C-4	29.19	42.24	42.24	42.28
H-3	3.626	3.525	3.524	3.527	C-5	48.39	140.72	140.74	140.76
H-4α	1.416	2.294	2.297	2.296	C-6	68.07	121.67	121.59	121.63
H-4β	1.892	2.235	2.238	2.237	C-7	34.96	31.86	31.83	31.88
H-5	1.623				C-8	34.81	31.85	31.82	31.86
H-6	4.062	5.352	5.353	5.353	C-9	39.84	50.05	50.01	50.07
H-7α	1.139	1.529†	1.532†	1.532†	C-10	35.93	36.46	36.46	36.49
H-7β	1.662	1.974	1.979	1.974	C-11	20.71	21.03	21.04	21.05
H-8β	1.455	1.460†	1.473†	1.460†	C-12	39.80	39.62	39.73	39.73
H-9α	1.376†	0.929	0.936	0.934	C-13	42.78	42.30	42.52	42.36
H-11α	1.401†	1.508†	1.524†	1.516†	C-14	56.10	56.66	56.72	56.69
H-11β	1.196†	1.457†	1.469†	1.461†	C-15	24.21	24.27	24.17	24.24
H-12α	1.140†	1.158	1.172	1.170	C-16	28.17	28.15	27.92	28.21
H-12β	1.974	2.000	2.023	2.007	C-17	55.74	55.61	55.85	55.89
H-14α	1.135†	0.999†	0.998	1.003†	C-18	12.02	11.87	11.95	11.84
H-15α	1.592	1.590	1.600	1.595	C-19	23.48	19.38	19.40	19.38
H-15β	1.083†	1.090†	1.117	1.089†	C-20	35.76	35.76	37.51	35.50^{d}
H-16α	1.877	1.862	1.821	1.816	C-21	18.53	18.60	17.33	18.48
H-16β	1.283	1.292	1.341	1.256	C-22	40.54	40.60	47.21	35.91
H-17α	1.130	1.109	1.214	1.096	C-23	137.31	137.38	22.91	*e*
H-18	0.651	0.690	0.735	0.686	C-24	115.72	115.66	40.81	29.32
H-19	0.910	1.009	1.015	1.010	C-25			*e*	*e*
H-20	1.473	1.486	1.641	1.419†	C-26			*e*	*e*
H-21	0.917	0.931	0.901	0.939	C-27			*e*	*e*
H-22R	1.798	1.802	1.185	1.407†					
H-22S	2.173	2.175	1.952	1.088					
H-23R	5.769	5.775		1.421†					
H-23S			4.343	1.614					
H-24R	4.985†	4.983†	2.820	1.963†					
H-24S	4.988†	4.985†	3.026	2.036†					

^{a} ^{1}H NMR data obtained at 500 MHz in 5-15 mM CDCl$_3$ solution at 25°C. ^{1}H chemical shifts are generally accurate to ±0.001 ppm except for values marked by † (ca. ±0.003 ppm). R and S denote pro-*R* and pro-*S* (or pro-*E* and pro-*Z*) hydrogens. ^{1}H-^{1}H and ^{1}H-^{19}F coupling constants were similar to those of Δ^{5} sterols (11), Δ^{23} steroids (5, 7, 8), and F$_7$-sterols (5, 7, 8). ^{b} ^{13}C NMR data (±0.03 ppm) obtained at 126 MHz at 22°C in 20-80 mM CDCl$_3$ solution and referenced to the CDCl$_3$ signal at 77.0 ppm (data for 6b acquired at 25°C). ^{c} NMR signals for the minor C-23 epimer of 5: δ_C 50.7 (C-22), 28.3 (C-16), 19.0 (C-21), 11.8 (C-18); δ_H, 4.35 (m, H-23), 2.76 (m, H-24), 2.098 (dddt, 14.8, 9.2, 3.2, 0.6 Hz, H-22*S*), 1.89 (m, H-22*R*), 1.88 (m, H-16α), 1.009 (s, H-19), 0.993 (d, 6.5 Hz, H-21), 0.696 (s, H-18). ^{d} Two signals, δ 35.51, 35.49. ^{e} Weak signals not observed.

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× 4.6 mm i.d.), eluted with methanol-water 98:2 at 1 ml/min, and collected as 0.3-min fractions. The fractions were diluted with ScintiVerse and subjected to scintillation counting. The resulting chromatogram (Figure 2C) showed a single peak comigrating with 6a (t_R 11.1 min; UV detection at 210 nm).

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References and Notes

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10. Tributyltin species were monitored in reductions of 15-ketosterols by examining various ^1H and ^{13}C NMR spectral regions (7), the most important being a ^1H triplet at δ 0.93. This signal would be obscured in **6b** by the H-21 doublet (δ 0.94), but traces of tributyltin would be observable as multiplets at δ_H 1.30 and 1.36 (in open spectral regions of **6b** at 500 MHz) and in the COSYDEC spectrum (notably at δ 0.93). After MPLC purification of **6b**, no trace of tributyltin signals were observed in its NMR spectra. The relative ease of removal of tributyltin materials compared with similar reduction of a 15-ketosterol (7) is attributable to the very low loading on the MPLC column described herein.

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12. (a) These calculations are based on NMR data of higher precision than those given in previously (5, 12b) for **6a**. The observed isotope shifts represent weighted averages of isotope shifts for the 23*R*- and 23*S*-deuterio species. (b) Gerst N., Pinkerton F.D., Kisic A., Wilson W.K., Swaminathan S., and Schroepfer G.J., Jr. – J. Lipid Res. 35: 1040-1056 (1994).

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14. The specific activity and level of ^3H of the sodium borotritide described herein were those provided by the commercial supplier.

15. Up to 100 mM, only small changes (usually ≤0.002 ppm) in ^1H NMR chemical shift were observed for most steroids. However, substantial differences (0.004-0.012 ppm) in chemical shift between spectra acquired at 2 mM and 25 mM concentrations in $CDCl_3$ were observed for most signals in rings A and B of diol **2**.

16. The structure elucidation of the 3β,23-diols and their characterization by NMR will be presented elsewhere.

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