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Chemical Synthesis of 15-Ketosterols and their Inhibitions of Cholesteryl Ester Transfer Protein

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Abstract—Described herein are the chemical syntheses of 3β-hydroxy-5α-cholest-8(14)-en-15-one and 3β-hydroxy-5α-cholest-8(14),16-dien-15-one from diosgenin and the examinations of their ability to inhibit the cholesteryl ester transfer protein (CETP). Clemmensen reduction of diosgenin gave cholest-5-ene-3β,16β,26-triol. Tosylation of the latter compound gave cholest-5-ene-3β,16β,26-triol 26-tosylate which, upon reduction with $LiAlH_4$, gave cholest-5-ene-3β,16β-diol. Hydrogenation–benzoylation of the latter to 5α-cholest-3β,16β-diol 3β-benzoate followed by mesylation-elimination gave 5α-cholest-16-ene-3β-ol 3β-benzoate. Controlled oxidation of the latter with CrO3–dimethylpyrazole gave 3β-hydroxy-5α,14α-cholest-16-en-15-one 3β-benzoate. Oxidation of Δ^{16}-15-one with SeO_2 gave 3β-hydroxy-5α-cholest-8(14),16-dien-15-one 3β-benzoate along with 3β-hydroxy-5α,14β-cholest-16-en-15-one 3β-benzoate. Selective hydrogenation of the $\Delta^{8(14),16}$-15-ketosteryl ester, followed by base hydrolysis gave 3β-hydroxy-5α-cholest-8(14)-en-15-one. Hydrolysis of 3β-hydroxy-5α-cholest-8(14),16-dien-15-one 3β-benzoate in basic media gave 3β-hydroxy-5α-cholest-8(14),16-dien-15-one. The effects of the 15-ketosterols on the CETP activity were studied *in vitro* by incubating cholesteryl ester donor (HDL), cholesteryl ester acceptor (LDL) and human plasma as a CETP source at 37 °C. 3β-Hydroxy-5α-cholest-8(14)-en-15-one was found to be active in supression of the levels of CETP activity in human plasma.

Introduction

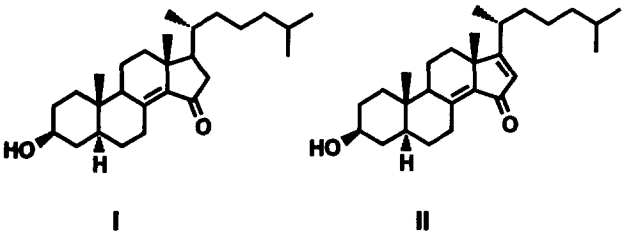
Most of the plasma cholesteryl esters (CE) are generated in the high-density lipoproteins (HDL) fraction by lecithin:cholesterol acyltransferase (LCAT) and are redistributed to very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) by the plasma cholesteryl ester transfer protein (CETP).[1–3] Plasma cholesteryl ester transfer protein is a hydrophobic glycoprotein which plays an important role in reverse cholesterol transport, the process in which cholesterol is transported from peripheral tissues to the liver.[4] Since HDL levels are inversely correlated with atherosclerosis in humans, the activity of the CETP could have an important influence on atherosclerosis. Koizumi *et al.*[5] reported two hyperalphalipoproteinemic patients with large HDL that was clearly separated from LDL. The plasma from these subjects lacked CETP activity. Yokoyama *et al.*[6] reported that homozygous subjects with familial hyperalphalipoproteinemia had impaired plasma cholesteryl ester transfer between HDL and LDL. Animals that express CETP tend to have higher VLDL and LDL cholesterol levels, whereas those with a minimum CETP activity tend to have high HDL cholesterol levels.[7] Because VLDL and LDL are associated with the progression of atherosclerosis, and HDL are considered anti-atherogenic, CETP could be an atherogenic protein. Given other conditions required for atherosclerosis to develop, expression of CETP would accelerate the rate at which the arterial lesions progress.

Recent studies showed that transgenic mice expressing CETP had much worse atherosclerosis than did non-expressing controls, and that the increase in lesion severity was due largely to CETP-induced alterations in the lipoprotein profile.[8] According to the recent concept, CETP appears to be a potent atherogenic agent in the plasma. Consequently, the inhibition of the enzyme could result in the reduction of cholesterol levels in the blood.

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) and a large number of other 15-oxysterols have been known to exhibit a number of biological activities including the inhibition of cholesterol biosynthesis by the competative inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-Co A) reductase activity in cultured mammalian cells.[9–14] Some of these 15-ketosterols also have been found to have significant hypocholesterolemic activity upon oral administration to rodents[15] and non-human primates.[16,17] The reduction of total serum cholesterol levels induced by **I** in rhesus has been shown to be associated with a lowering of the levels of LDL cholesterol, an elevation of the levels of HDL cholesterol, and a shift in the HDL profile to the one in which HDL_2 species predominate.[16a]

Though the elevation of HDL cholesterol level could have resulted from other factors such as the inhibition of HMG-Co A reductase, we suspected that the 15-ketosterols might inhibit the plasma CETP, causing the accumulation of cholesteryl esters in HDL. We also

thought that a simultaneous inhibition of both the HMG-Co A reductase inside cells and the CETP in the plasma by the 15-ketosterols might provide an excellent way to reduce the level of cholesterol in the blood.



I II

Figure 1. Structures of 3β-hydroxy-5α-cholest-8(14)-en-15-one (I) and 3β-hydroxy-5α-cholest-8(14),16-dien-15-one (II).

We now report the chemical synthesis of 3β-hydroxy-5α-cholest-8(14)-en-15-one (I), and 3β-hydroxy-5α-cholest-8(14),16-dien-15-one (II)[18] (Fig. 1) and their effects on the cholesteryl ester transfer protein in human plasma.

Results and Discussion

For the synthesis of 15-ketosterols I and II we chose diosgenin (Fig. 2) as the starting material because it is commercially available in high purity. An appropriate synthetic plan to reach compounds I and II first requires the preparation of a Δ^{16}-sterol like **8** and then the introduction of oxygen at the 15 position. The intermediate 3β-hydroxy-5α-cholest-16-ene 3β-benzoate (**8**) was readily synthesized in six steps from diosgenin in 27% yield. The triol (**3**), which was obtained from diosgenin by Clemmensen reduction[13a,19] was selectively tosylated with *p*-toluenesulfonyl chloride in pyridine to give cholest-5-ene-3β,16β,26-triol 26-tosylate (**4**) in 75% yield, which was then treated with $LiAlH_4$ in ether,[20] yielding the 16-hydroxycholesterol (**5**) in 84% yield.

Hydrogenation of **5** with 5% Pt on C in ethyl acetate, followed by selective protection with benzoyl chloride in pyridine gave 5α-cholest-3β,16β-diol 3β-benzoate (**6**) in 76% yield. The 5α-configuration of **6** was proved by similarity of its ^{13}C NMR spectrum with that of 5α-cholest-8(14)-en-3β-ol 3β-benzoate.[21] Mesylation of **6** with methanesulfonyl chloride in pyridine at 0 °C rising to ambient temperature gave 3β-benzoyloxy-5α-cholest-16β-ol 16β-mesylate (**7**), then the crude product was treated with NaI in DMF[22] at 120 °C for 2 h to afford 5α-cholest-16-en-3β-ol 3β-benzoate (**8**) in 96% yield. With other reaction conditions elimination of mesylate (pyridine, $NaHCO_3$ and triethyl amine) was carried out, but the yield was not high.

In order to introduce oxygen at the 15 position, the intermediate **8** was oxidized with an excess CrO_3–3,5-dimethylpyrazole complex[23] in CH_2Cl_2 at –20 °C for 30 min which gave, after silica gel column chromatography, 3β-hydroxy-5α,14α-cholest-16-en-15-one 3β-benzoate (**9**) in 43% yield along with 3β-hydroxy-5α,14β-cholest-16-en-15-one 3β-benzoate (**10**) and 3β-hydroxy-5α,14α-cholest-16α,17α-epoxy-15-one 3β-benzoate as byproduct in small quantity. Compound **9** showed IR (1709 cm^{-1}) and UV [λ_{max} 233 nm (log ε 4.18)] absorptions characteristic of Δ^{16}-15-one functionality. Owing to the deshielding of the carbonyl group at the 15 position, the ^1H NMR siginal of 7β-H appeared at δ 2.74. Oxidation of **9** with SeO_2 in 2-methyl-2-propanol[24] afforded a mixture of 3β-hydroxy-

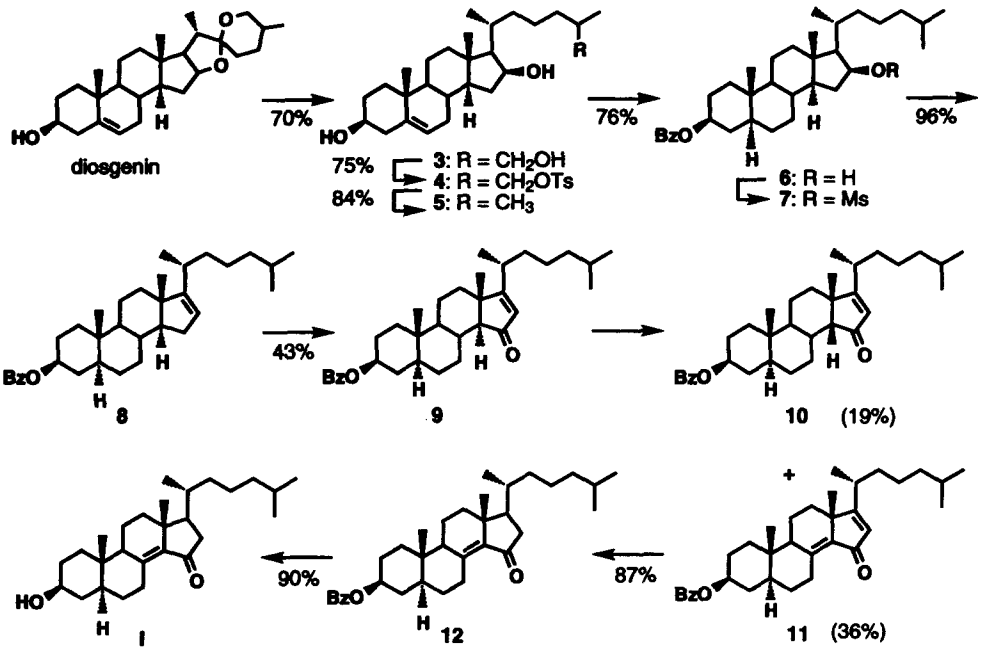


Figure 2. Synthesis of 3β-hydroxy-5α-cholest-8(14)-en-15-one from diosgenin.

5α,14β-cholest-16-en-15-one 3β-benzoate (**10**) (19%) and 3β-hydroxy-5α-cholest-8(14),16-dien-15-one 3β-benzoate (**11**) (36%) which could be separately isolated by silica gel chromatography to purity, while oxidation of compound **9** easily isomerized to **10**.

The 14-H configuration of the compounds **9** and **10** was established by NMR experiments. 2D ^1H–^1H NOESY showed a NOE crosspeak between 18-CH_3 (δ 1.19) and H-14 (1.95) for compound **10**, but the COSY spectrum showed no coupling. This observation revealed that 14-H and 18-CH_3 are oriented on the same plane (i.e. CD ring junction *cis*). The 14β-stereochemistry of the Δ^{16}-15-ketosterol **10** was confirmed by comparing its NMR spectra with those of 14α-Δ^{16}-15-one isomer **9**. The ^1H NMR chemical shifts (14-H, 18-H: δ 1.95, 1.19) of the 14β-Δ^{16} isomer **10** showed similar patterns compared with those of 14α-Δ^{16} isomer **9** (14-H, 18-H : δ 1.95, 1.02), but certain ^{13}C NMR siginals (C-9, C-13, C-14, C-18, C-19: δ 44.02, 48.27, 57.26, 24.37, 10.94) of 14β-Δ^{16} isomer **10** differed somewhat from those of 14α-Δ16 isomer **9** (C-9, C-13, C-14, C-18, C-19: d 54.90, 47.00, 63.79, 23.62, 12.29).[25]

The 14β-H configuration of the 14β-Δ^{16} isomer was apparent from its downfield ^{13}C NMR signal for C-18 (δ 24.37). These results parallel closely those of Schroepfer,[14] who obtained 14β-Δ^8-15-ketosterol by acid catalyzed isomerization of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) and assigned the 14β-configuration to β,γ-unsaturated ketone based on LIS (Lanthanide Induced Shift) experiment.

Finally selective hydrogenation (H_2/5% Pt on C/EtOAc) of 3β-hydroxy-5α-cholest-8(14),16-dien-15-one 3β-benzoate (**11**) yielded 3β-hydroxy-5α-cholest-8(14)-en-15-one 3β-benzoate (**12**) in 87% yield. The 17β-configuration of the latter was proved by similarity of its ^{13}C NMR spectrum with that of the known compound.[21] Hydrolysis of **12** in 1 M ethanolic KOH solution gave 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) in 90% yield. Controlled hydrolysis of 3β-hydroxy-5α-cholest-8(14),16-dien-15-one 3β-benzoate (**11**) in 1 M ethanolic KOH solution provided 3β-hydroxy-5α-cholest-8(14),16-dien-15-one (**II**) in 86% yield. The structure and stereochemistry of each synthetic intermediate were confirmed by ^1H and ^{13}C NMR (Tables 1 and 2) and MS.

CETP inhibition may be an excellent way to treat the hypercholesterolemia because inhibitory reaction takes place in the blood, not in the cell, which could eliminate the severe side effects of HMG-Co A reductase caused by the inhibition of several biosynthetic pathways such as steroid hormone and dolichol syntheses, and also helps to remove cholesterol from the blood by acceleration of the reverse cholesterol transport. Compound **I** has been shown to be a potent inhibitor of sterol synthesis in cultured mammalian cells and of the levels of HMG-Co A reductase activity in these cells.[9] The simultaneous inhibition of HMG-Co A reductase and CETP could be a better way to treat the hypercholesterolemic patients if effective CETP inhibition is developed by derivatization of compound **I**. Initial studies on the CETP inhibition were carried out with compounds **I** and **II**. Among the sterols examined, **I** was active in the reduction of CETP activity *in vitro*. The results presented in Fig. 3 show that compound **I** showed a 50% inhibition of CETP activity at 660 μM.

Although the inhibitory effect of compound **I** required a relatively high concentration, this is one of the first synthetic chemicals that shows inhibition of CETP. Immobilized HDL may hinder the transfer of [^{3}H]CE, because of adsorption of radioactive compound, into the Sepharose beads. The concentration of **I** required for the inhibition of CETP is expected to be significantly reduced when purified CETP is used, instead of whole plasma as used in this study. Because of the simultaneous inhibition of CETP and HMG-Co A reductase it could be a candidate for a new blood cholesterol lowering agent, with improved inhibition activity by derivatization using this compound as a leading material.

Table 1. ^1H NMR chemical shifts[a–c]

Sterol	3α-H	6-H	7-H or 7βH	16-H or 16α-H	18-H_3	19-H_3	21-H_3	26,27-H_3
4[d]	3.51	5.34		4.31	0.880	1.014	0.939	0.878
5[e]	3.43	5.31		4.30	0.890	1.008	0.968	0.867
6	4.99			4.30	0.878	0.878	0.962	0.868
8	4.95			5.27	0.990	0.762	0.990	0.860
9	4.95		2.74	5.62	1.018	0.938	1.103	0.858
10	4.92		2.35	5.92	1.194	0.841	1.101	0.861
11	4.99		4.09	5.92	1.179	0.871	1.099	0.860
12	4.99		4.16		0.987	0.784	1.006	0.868
I	3.64		4.14		0.972	0.714	0.998	0.863
II	3.65		4.07	5.91	1.164	0.799	1.089	0.856

[a]300 MHz Spectra. Chemical shifts (δ) in ppm relative to $(CH_3)_4Si$ in $CDCl_3$ solution.
[b]Multiplicities: 3α-H: *m*. 6H: *d*, $J = 4.5 + 0.5$ Hz. 16H: **5**, **6**, **8**, *m*; **9**, **10**, **11**, **II**, *s*. 18-H_3: *s*. 19-H_3: *s*. 21-H_3: *d*, $J = 6.5 + 0.3$ Hz. 26,27-H_3: *d*, $J = 6.6 + 0.2$ Hz.
[c]Benzoate esters showed at δ 7.30 + 0.10 Hz (*meta*-H), 7.50 + 0.10 Hz (*para*-H), 8.03 + 0.01 Hz (*ortho*-H).
[d]26-H_2 showed at δ 3.89 and 3.80 as AB portion of ABX system: $J_{AB} = 9.3$ Hz, $J_{AX} = 6.4$ Hz, $J_{BX} = 5.8$ Hz. Also for *p*-toluenesulfonate δ 2.45 (Me), 7.78 (*ortho*-H), 7.34 (*meta*-H).
[e]In DMSO-d_6 + $CDCl_3$ solution.

Table 2. ^{13}C NMR assignments[a]

	4 Δ^5 26-OTs 3β,16β-OH	5 Δ^5 16-OH 3β-OH	6 5α 16-OH 3β-OBz	8 5α-Δ^{16} 3β-OBz	9 14α-Δ^{16} 15-Keto 3β-OBz	10 14β-Δ^{16} 15-Keto 3β-OBz	11 5α-$\Delta^{16,8(14)}$ 15-Keto 3β-OBz	12 5α-$\Delta^{8(14)}$ 15-Keto 3β-OBz	I 5α-$\Delta^{8(14)}$ 15-Keto 3β-OH	II 5α-$\Delta^{16,8(14)}$ 15-Keto 3β-OH
C1	37.12	36.43	36.42[b]	36.71	36.24	36.05	36.19	36.37	36.56	36.41
C2	31.50	30.67	27.59	27.62	27.51	27.18	27.33	27.37	31.12	31.16
C3	71.60	70.06	73.90	74.31	74.02	74.20	73.82	73.80	70.80	70.90
C4	42.16	41.51	34.70	34.30	33.99	34.02	33.70	33.78	37.76	37.73
C5	140.86	140.54	44.36	45.04	44.90	44.58	44.41	44.06	44.15	44.55
C6	121.29	120.02	28.19	28.64	28.16	29.70	29.32	29.08	29.18	29.39
C7	31.72	30.95	29.43	31.01[b]	30.33	28.98	27.37	27.52	27.61	27.39
C8	31.41	30.75	33.73	34.20	33.20	33.94	145.17	150.10	150.76	145.52
C9	50.02	49.28	53.81	55.00	54.90	44.02	51.19	50.91	50.89	51.33
C10	36.43	35.22	35.76	35.80	35.88	36.87	38.86	38.74	38.77	38.86
C11	20.63	19.85	20.51	21.17	20.50	19.21	19.71	19.58	19.59	19.72
C12	39.79	38.65	39.78	36.87	36.61	38.20	37.02	36.98	36.98	37.00
C13	42.13	41.30	42.09	47.16	47.00	48.27	45.51	42.59	42.57	45.46
C14	54.42	53.66	53.90	57.31	63.79	57.26	137.92	140.47	140.28	137.90
C15	36.77	36.26	36.34[b]	31.93[b]	207.76	210.46	197.50	207.93	208.19	197.49
C16	72.23	70.58	71.56	120.45	124.36	128.42	127.41	42.42	42.47	127.39
C17	61.30	60.52	61.17	161.03	188.62	191.93	186.88	50.81	50.82	186.86
C18	12.98	12.20	12.89	16.35	23.62	24.37	24.78	18.79	18.79	24.73
C19	19.30	18.54	11.87	12.30	12.29	10.94	12.86	12.88	12.93	12.85
C20	29.67	28.94	31.53	32.34	32.41	32.42	32.76	34.52	34.50	32.72
C21	18.05	17.36	17.74	21.90	21.28	21.08	22.08	19.25	19.24	21.96
C22	35.78	35.67	35.14	35.19	32.54	32.57	30.48	35.85	32.83	30.52
C23	23.33	23.21	23.70	25.33	25.21	25.37	25.44	23.57	23.53	25.37
C24	32.89	39.08	39.11	39.23	38.96	38.94	38.99	39.39	39.37	39.96
C25	32.70	27.12	27.17	27.91	27.84	27.87	27.89	27.96	27.96	27.84
C26	75.12	22.00	22.37	22.64	22.55	22.59	22.61	22.71	22.75	22.53
C27	16.34	21.76	22.15	22.64	22.55	22.53	22.60	22.52	22.55	22.53
C=O			165.57	166.06	166.03	166.02	166.04	166.06		
q	133.11		130.57	131.03	130.89	131.01	130.77	130.83		
o	127.79		129.03	129.51	128.47	129.50	129.52	129.51		
m	129.72		127.82	128.20	128.19	128.21	128.26	128.24		
p	144.54		132.23	132.58	132.63	132.60	132.75	132.70		
Me	21.54									

[a]75.5 MHz: chemical shifts (δ) in ppm referenced to $CDCl_3$ at 77.0 ppm in $CDCl_3$ solution.
[b]Assignments may be interchanged within a column.

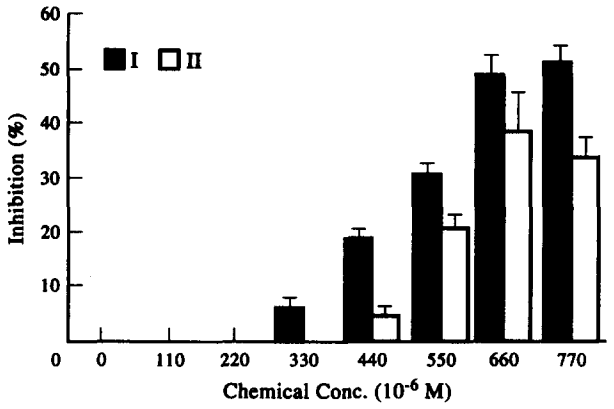


Figure 3. Effects of 3β-hydroxy-5α-cholest-8(14)-en-15-one (I) and 3β-hydroxy-5α-cholest-8(14),16-dien-15-one (II) on levels of CETP in plasma cells. Per cent inhibition relative to control value (no added sterol). Variation is expressed as +S.D. of replicate (n = 2) assays for the experimental values.

Experimental

Materials and methods

Melting points (mp) were determined using a Thomas–Hoover melting point apparatus, and are not corrected. IR spectra were obtained on a Matton GL-6030E spectrophotometer using KBr pellets or thin film on NaCl. UV spectra were measured in methanol on a Shimadzu UV-2100 spectrophotometer. The ^1H and ^{13}C NMR spectra were measured on Bruker AM-300 and/or JEOL GSX-500; unless otherwise stated, all NMR were performed in carefully neutralized $CDCl_3$ solution. The chemical shifts of ^1H NMR spectra are given in ppm downfield from tetramethylsilane and of ^{13}C NMR spectra were referenced to $CDCl_3$ at 77.0 ppm. ^1H and ^{13}C NMR assignments were made from DEPT (distortionless enhancement by polarization transfer), COSY, ^1H–^{13}C shift-correlated (HETCOR), and the two-dimentional NOESY (nuclear Overhauser effect

difference spectroscopy), and comparison with spectra of similar sterols. Low resolution mass spectra (MS) were recorded on a Shimadzu QP-1000 spectrometer with electron energy of 20 or 70 eV and direct sample introduction. High resolution MS were measured on a JEOL JMS-DX 303 spectrometer. TLC analyses were carried out on precoated 0.2 mm HPTLC silica gel 60 plates (E. Merck, Darmstadt); substances were visualized by spraying with 5% ammonium molybdate in 10% H_2SO_4. TLC solvent systems were: (SS-1), EtOAc:hexane 1:1; (SS-2), EtOAc:hexane 1:2; (SS-3), EtOAc:hexane, 1:4; (SS-4), EtOAc:hexane, 1:19; (SS-5), Et_2O:benzene, 1:1; (SS-6), Et_2O:benzene, 1:4. Medium pressure liquid chromatography (MPLC) was performed by using EM Lobar silica gel 60 prepacked columns (40–63 μm; A, B, C type; E. Merck, Darmstadt) equipped with a Fluid Metering Inc. lab pump. For routine column chromatography, E. Merck silica gel (70–230 mesh) was used as adsorbent. Spinning disk thin layer chromatography was performed on a Harrison Research Chromatotron, with a 2 mm thickness silica gel plate. All reactions were performed under argon atmosphere. Solutions were dried over anhydrous Na_2SO_4. Pyridine, Et_2O, EtOAc and CH_2Cl_2 were dried over CaH_2 and distilled following standard procedures.[26] Cholest-5-ene-3β,16β,26-triol,[13a,19] 3β-hydroxy-5α-cholest-8(14)-en-15-one 3β-benzoate (**12**)[18,30] and 3β-hydroxy-5α-cholest-8(14),16-dien-15-one 3β-benzoate (**11**) were prepared previously.[18] Benzoyl chloride, methanesulfonyl chloride, *p*-toluenesulfonyl chloride, $LiAlH_4$, NaI, CrO_3, imidazole, SeO_2, 2-methyl-2-propanol, 3,5-dimethyl- pyrazole were purchased from Aldrich and used as received. Diosgenin was purchased from Steraloids, Inc. (Wilton, NH). 1α,2α-*n*-[^{3}H]Cholesteryl oleate (specific activity 50 Ci mmol^{-1}) was purchased from Amersham Life Science (Amersham, U.K.). Cyanogen bromide-activated Sepharose-4B, Sephadex G-25 were obtained from Sigma Chemical Co. (St. Louis, MO).

Isolation of lipoproteins

Blood (16 h fasting) from normal male volunteers was collected into Na_2-EDTA containing tubes. Plasma was prepared by centrifugation at 5000 *g* for 15 min at 4 °C. The density of the plasma was adjusted to 1.225 by KBr. Lipoproteins were isolated by the method described previously.[27]

Radiolabeling of HDL

[^{3}H]Cholesteryl oleate (50 mCi) was dried under a nitrogen stream and mixed with 0.5 mL HDL (10 mg mL^{-1}). After adding 20 mg of additional HDL, the mixture was gently shaken, and then incubated at 37 °C. The labeled HDL was re-isolated by centrifugation or gel filtration using Sephadex G-25. Labeling efficiencies were usually between 20 and 50%. Labeled HDL was stored at 4 °C in the presence of 1 mM EDTA and 0.02% NaN_3.

Immobilization of [^{3}H]HDL

Radiolabeled HDL was covalently coupled to CNBr-activated Sepharose-4B as described by Pritchard *et al.*[28] After the coupling, any remaining active groups were blocked by incubation in 0.1 M Tris–HCl, pH 8.0, for 1 h at room temp. The [^{3}H]HDL-Sepharose beads were washed several times with 0.1 M Tris–HCl/0.5 M NaCl, pH 8.0, using sintered glass filter. The immobilized [^{3}H]HDL was then stored in 0.1 M Tris–HCl/0.5 M NaCl, pH 8.0, at 4 °C until use. The immobilized [^{3}H]HDL showed no significant difference from fresh prepartion up to 3 months.

Cholesteryl ester transfer assay[29]

Immobilized [^{3}H]HDL donor and unlabeled LDL acceptor were incubated with test sample to be assayed. Donor and acceptor lipoproteins were then separated by a low-speed centrifugation, and then the radioactivity of the supernatant was counted in a Packard TRI-CARB 1600TR liquid scintillation counter. Transfer activity was expressed as the percentage of labeled cholesteryl oleate transferred to the LDL acceptor relative to a control incubation without added CETP source. [^{3}H]HDL-Sepharose beads (50 μl) were mixed with 0.3 mL of human LDL (0.75 mg mL^{-1}) as a CE-acceptor. Human plasma (50 μL) was added to above mixture as a CETP source. Total 0.4 mL of the assay mixture was incubated at 37 °C. Following an incubation, the pellet [^{3}H]HDL-Sepharose beads was separated from the mixture by centrifugation at 3000 *g* for 5 min. Aliquot of supernatant (0.2 mL) was subjected to the scintillation counting to determine the amount of [^{3}H]cholesteryl ester transfered to CE-acceptor.

Chemical syntheses

Cholest-5-ene-3β,16β,26-triol 26-tosylate (4). To a stirred solution of cholest-5-en-3β,16β,26-triol[13a,19] (**3**; 940 mg, 2.25 mmol) in dry pyridine (20 mL) was added a solution of *p*-toluenesulfonyl chloride (645 mg, 3.38 mmol) in pyridine (3 mL) at 0 °C, and the resulting mixture was stirred at room temp. for 6 h. The reaction mixture was diluted with H_2O and extracted with EtOAc, and the combined extracts were washed with 10% HCl, brine and saturated $NaHCO_3$ solution, dried and concentrated to give 1.15 g of crude product. Purification over chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane 1:2) gave **4** (960 mg, 1.68 mmol, 75%), cholest-5-ene-3β,16β,26-triol 3β,26-ditosylate (35 mg, 0.05 mmol, 2.1%), and unreacted cholest-5-ene-3β,16β,26-triol (**3**) (150 mg, 0.36 mmol, 16%). Analytical sample was recrystallized in MeOH:H_2O. Cholest-5-ene-3β,16β,26-triol 26-tosylate (**4**): mp 98–100 °C; single component on TLC in three solvent systems R_f 0.67 (SS-1), 0.34 (SS-3), 0.30 (SS-5); IR (KBr) 3411, 2933, 2856, 1713, 1459, 1378, 1281, 1169, 1115, 1038, 710 cm^{-1}; MS (relative intensity, %) 571 (40, [M–1]), 556 (44, [M–1–Me]), 524 (41, [M–

2Me–H_2O]), 386 (35, [M–Me–OTs]), 350 (17, [M–Me–$2H_2O$-OTs]), 274 (63, [M–SC–Me]), 271 (73, [M–SC–H_2O), 253 (48, [M–SC–$2H_2O$).

Cholest-5-ene-3β,16β-diol (5). To a solution of **4** (520 mg, 0.91 mmol) in dry Et_2O (30 mL) was added $LiAlH_4$ (400 mg), and then stirred at room temp. for 24 h. The resulting mixture was poured into H_2O, extracted twice with Et_2O (50 mL), and the combined extracts were washed with 10% HCl, saturated $NaHCO_3$, brine, dried and concentrated to give 356 mg of yellowish solid which was further purified with chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane, 1:4) to give white solid **5** (310 mg, 0.77 mmol, 84%). Analytical sample was recrystallized in MeOH:H_2O; mp 166–168 °C; single component on TLC in three solvent systems R_f 0.70 (SS-2), 0.10 (SS-3), 0.37 (SS-5); IR (KBr) 3430, 2938, 2851, 1466, 1377, 1049, 1024 cm^{-1}; MS (relative intensity, %) 402 (18, $[M]^+$), 387 (12, [M–Me]), 384 (32, [M–H_2O]), 369 (18, [M–H_2O–Me]), 366 (9, [M–$2H_2O$]), 351 (24, [M–Me–$2H_2O$]), 317 (10), 290 (5), 274 (15, [M–SC–Me]), 271 ([M–SC–H_2O]), 253 (22, [M–SC–$2H_2O$]), 213 (28); high resolution MS on ion at m/z 402, 402.3489 (calcd for $C_{27}H_{46}O_2$ 402.3498).

5α-Cholest-3β,16β-diol 3β-benzoate (6). Compound **5** (500 mg, 1.24 mmol) was hydrogenated in EtOAc (35mL) over 5% Pt/C (190 mg) at room temp. After removal of catalyst by filtration through a short pad of silica gel, the solvent was evaporated to give a solid, which was treated with 0.2 mL of benzoyl chloride in pyridine (2 mL) at 0 °C for 30 min. The resulting mixture was poured into H_2O and extracted with EtOAc (50 mL), the combined extracts were washed with 10% HCl, brine, saturated $NaHCO_3$, brine, dried and concentrated to give 630 mg of solid, which was subjected to MPLC (silica gel, 1.0 × 50 cm, elution with EtOAc:hexane 7:93). Evaporation of fractions gave white solid **6** (470 mg, 0.93 mmol, 76%). Analytical sample was recrystallized in CH_2Cl_2:MeOH; mp 162–164 °C; single component on TLC in three solvent systems R_f 0.60 (SS-3), 0.16 (SS-4), 0.76 (SS-5); IR (KBr) 3570, 2948, 2867, 2850, 1716, 1450, 1277, 1254, 1116, 1027, 714 cm^{-1}; MS (relative intensity, %): 508 (0.3, $[M]^+$), 493 (16, [M–Me]), 490 (10, [M–H_2O]), 379 (23), 215 (20), 105 (100, C_6H_5CO); high resolution MS on ion at m/z 508, 508.3878 (calcd for $C_{34}H_{52}O_3$ 508.3916).

5α-Cholest-16-en-3β-ol 3β-benzoate (8). To a solution of **6a** (260 mg, 0.51 mmol) in dry pyridine (5 mL) was added a soluton of methanesulfonyl chloride (0.06 mL, 0.76 mmol) in dry pyridine (5 mL) at 0 °C and then stirred at room temperature for 30 min. The resulting mixture was diluted with H_2O (30 mL) and extracted with EtOAc (50 mL). The organic layers were washed with 10% HCl, brine, saturated $NaHCO_3$, water, dried and concentrated to give 3β-benzoyloxy-5α-cholest-16-ol 16-mesylate. The resulting 16-mesylate was heated with NaI (350 mg, 2.33 mmol) in DMF (10 mL) at 80 °C for 2 h. The mixture was diluted with H_2O and extracted with EtOAc. The organic layers were washed with brine, saturated $NaHCO_3$, brine, dried and concentrated to give yellow solid (247 mg) which was further purified with chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane, 3:97) to give **8** (240 mg, 0.49 mmol, 96%). Analytical sample was recrystallized in CH_2Cl_2:MeOH; mp 112–114 °C; single component on TLC in three solvent systems R_f 0.46 (SS-4), 0.80 (SS-5), 0.81 (SS-6); IR (KBr) 2933, 1717, 1455, 1273, 1111, 714 cm^{-1}; MS (relative intensity, %): 490 (16, $[M]^+$), 475 (100, [M–Me]), 377 (28, [M–SC]), 353 (33, [M–$C_6H_5CO_2H$–Me]), 215 (20), 105 (100, C_6H_5CO); high resolution MS on ion at m/z 490, 490.3809 (calcd for $C_{34}H_{50}O_2$ 490.3811).

3β-Hydroxy-5α-cholest-16-en-15-one 3β-benzoate (9). 3,5-Dimethylpyrazole (910 mg, 9.47 mmol) was added to a suspension of chromium trioxide (947 mg, 9.47 mmol) in dry CH_2Cl_2 (5 mL) at –20 °C under nitrogen, and the mixture was stirred at –20 °C for 30 min. To the resulting dark red solution was added **8** (230 mg, 0.47 mmol) in one portion, and the resulting mixture was stirred at –20 °C for 1 h. A 5 N NaOH solution (5 mL) was added, and the reaction mixture was stirred for 30 min at 0 °C. The resulting mixture was extracted twice with EtOAc, and the extracts were washed with 10% HCl and H_2O, dried and evaporated to dryness. The yellow residue (170 mg) was further purified with chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane, 5:95) to give white solid **9** (103 mg, 0.2 mmol, 43%). Analytical sample was recrystallized in CH_2Cl_2–MeOH: mp 142–143 °C; single component on TLC in three solvent systems R_f 0.59 (SS-3), 0.12 (SS-4), 0.72 (SS-5); IR (KBr) 2930, 1714, 1701, 1275, 1109, 704 cm^{-1}; MS (relative intensity, %) 504 (24, $[M]^+$), 489 (10, [M–Me]), 391 (10, [M–SC]), 382 (26, [M–$C_6H_5CO_2H$]), 367 (19, [M–$C_6H_5CO_2H$–Me]), 327 (11), 269 (21, [M–SC–$C_6H_5CO_2H$]), 251 (6, [M–SC–$C_6H_5CO_2H$–H_2O]), 208 (100), 105 (99, C_6H_5CO); UV λ_{max} 233 nm (log ε 4.18); high resolution MS on ion at m/z 504, 504.3608 (calcd for $C_{34}H_{48}O_3$ 504.3603).

3β-Hydroxy-5α,14β-cholest-16-en-15-one 3β-benzoate (10) and 3β-hydroxy-5α-cholest-8(14),16-dien-15-one 3β-benzoate (11). A mixture of **9** (97 mg, 0.194 mmol) and selenium dioxide (110 mg, 0.99 mmol) in 2-methyl-2-propanol (10 mL) was refluxed for 24 h under nitrogen. After removal of insoluble material by filtration through a pad of Celite, the filtrate was diluted with H_2O, extracted with EtOAc, and the combined extracts were washed with brine, dried and evaporated to dryness. The crude product was further purified with chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane, 5:95) to give viscous oil **10** (19 mg, 0.038 mmol, 19%) and white solid **11** (35 mg, 0.07 mmol, 36%).

Compound **10**: viscous oil; single component on TLC in three solvent systems R_f 0.55 (SS-3), 0.08 (SS-4), 0.74 (SS-5); IR (neat) 2933, 2867, 1713, 1455, 1277, 1115, 714 cm^{-1}; MS (relative intensity, %) 504 (38, $[M]^+$),

420 (18), 392 (7), 382 (28, [M–$C_6H_5CO_2H$]), 367 (21, [M–$C_6H_5CO_2H$–Me]), 269 (14, [M–SC–$C_6H_5CO_2H$]), 251 (6, [M–SC–$C_6H_5CO_2H$–H_2O]); UV λ_{max} 229 nm (log ε 4.32); high resolution MS on ion at m/z 504, 504.3620 (calcd for $C_{34}H_{48}O_3$ 504.3603).

Compound **11**: mp 164–165 °C (CH_2Cl_2:MeOH); single component on TLC in three solvent systems R_f 0.47 (SS-3), 0.04 (SS-4), 0.72 (SS-5); IR (KBr) 2953, 2928, 1717, 1680, 1636, 1279, 1113, 708 cm^{-1}; MS (relative intensity, %) 502 (100, $[M]^+$), 487 (11, [M–Me]), 417 (14), 389 (5, [M–SC]), 380 (6, [M–$C_6H_5CO_2H$]), 365 (25, [M–$C_6H_5CO_2H$–Me]), 267 (68, [M–SC–$C_6H_5CO_2H$]), 253 (28, [M–SC–$C_6H_5CO_2H$–H_2O]), 239 (26), 105 (100); UV λ_{max} 233 (log ε 4.29), 259 nm (log ε 4.23); high resolution MS on ion at m/z 502, 502.3440 (calcd for $C_{34}H_{46}O_3$ 502.3447).

3β-Hydroxy-5α-cholest-8(14)-en-15-one 3β-benzoate (12). Compound **11** (45 mg, 0.09 mmol) was dissolved in EtOAc (15 mL) and hydrogenated at 1 atm of H_2 at room temp. in the presence of 5% Pt/C (15 mg). After removal of insoluble material through a short pad of silica gel, the solvent was evaporated to give a solid (41 mg) which was further purified with chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane, 5:95) to give white solid **12** (39 mg, 0.078 mmol, 87%). Analytical sample was recrystallized in CH_2Cl_2:MeOH; mp 157–158 °C (lit.[30] 157–158 °C); single component on TLC in three solvent systems R_f 0.66 (SS-3), 0.12 (SS-4), 0.78 (SS-5); IR (KBr) 2951, 2868, 1715, 1619, 1275, 1113, 712 cm^{-1}; MS (relative intensity, %) 505 (100, [M+1]), 504 (3, $[M]^+$), 489 (11, [M–Me]), 486 (8, [M–H_2O]), 391 (14, [M–SC]), 382 (13, [M–$C_6H_5CO_2H$]), 373 (41, [M–SC–H_2O]), 367 (73, [M–$C_6H_5CO_2H$–Me]), 269 (54, [M–SC–$C_6H_5CO_2H$]), 251 (100, [M–SC–$C_6H_5CO_2H$-H_2O]), 105 (100, [C_6H_5CO]); UV λ_{max} 233 nm (log ε 4.27), 260 nm (log ε 4.25); high resolution MS on ion at m/z 504, 504.3605 (calcd for $C_{34}H_{48}O_3$ 504.3603).

3β-Hydroxy-5α-cholest-8(14)-en-15-one (I). Compound **12** (100 mg, 0.198 mmol) in 10 mL of 1 M 95% ethanolic KOH solution was refluxed for 10 min. H_2O and $CHCl_3$ were added and the resulting mixture was extracted with $CHCl_3$. The combined extracts were washed with 2% HCl and H_2O, dried over Na_2SO_4, and evaporated to dryness. The crude product (75 mg) was further purified with chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane, 1:4) to give white solid **I** (71 mg, 0.18 mmol, 90%). Analytical sample was recrystallized in H_2O:MeOH; mp 148–149 °C (lit.[31] 148–149 °C); single component on TLC in three solvent systems R_f 0.57 (SS-1), 0.53 (SS-2), 0.31 (SS-6); IR (KBr) 3452, 2932, 2863, 1701, 1616, 1464, 1045 cm^{-1}; MS (relative intensity, %) 400 (4, $[M]^+$), 367 (2, [M–H_2O–Me]), 305 (27), 262 (100), 183 (12); UV λ_{max} 260 nm (log ε 4.19); high resolution MS on ion at m/z 400, 400.3345 (calcd for $C_{27}H_{44}O_2$ 400.3341).

3β-Hydroxy-5α-cholest-8(14),16-dien-15-one (II). Compound **11** (7 mg, 0.0139 mmol) in 1 M 95% ethanolic KOH solution (5 mL) was stirred at room temp. for 48 h. The reaction mixture was diluted with H_2O, and extracted with $CHCl_3$. The combined extracts were washed with 2% HCl and H_2O, dried over Na_2SO_4, and evaporated to dryness. The crude product was further purified with chromatotron (silica gel, 1 mm thickness disk, elution with EtOAc:hexane, 1:2) to give solid **II** (24 mg, 0.06 mmol, 86%). Analytical sample was recrystallized in H_2O:MeOH; mp 127–129 °C; single component on TLC in three solvent systems R_f 0.56 (SS-1), 0.43 (SS-2), 0.26 (SS-6); IR (KBr) 3469, 3365, 2929, 2859, 1675, 1632, 1462, 1373, 1042 cm^{-1}; MS (relative intensity, %) 398 (100, $[M]^+$), 383 (32, [M–Me]), 313 (39), 285 (19, [M–SC]), 267 (33, [M–SC–H_2O]); high resolution MS on ion at m/z 398.3155 (calcd for C27H40O2 398.3185).

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