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A New Method for the Preparation of *Ent*-Cholesterol from *Ent*-Testosterone

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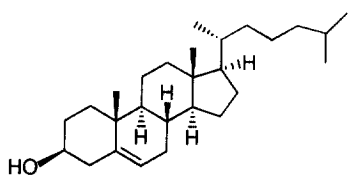
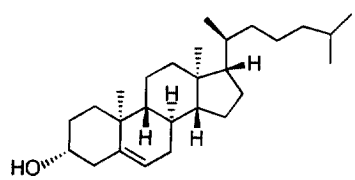
Abstract: An efficient total synthesis of the enantiomer of cholesterol is reported. The enantiomer of testosterone was prepared and converted into a C_{21} 3-silyloxy-$\Delta^{5,17(20)}$-diene according to literature procedures. The additional five carbon atoms of the cholesterol side chain were introduced by an ene reaction. Selective hydrogenation of the resultant Δ^{16} double bond and removal of the 22-hydroxyl group by a mesylation and demesylation sequence gave *ent*-cholesterol in 9.7% overall yield from *ent*-testosterone

Key words: Steroids and Sterols, Ene reactions, Enantiospecificity, Stereocontrol.

Ion channels are membrane bound proteins and the extent to which the membrane modulates ion channel function is an interesting research topic. The effect of the physical properties and composition of the membrane on the function of ion channels has been investigated.[1-3] Biological membranes usually contain both cholesterol and phospholipids. Since these naturally occurring molecules are enantiomerically pure, the interactions between them are enantiospecific. As a long term goal, we want to determine the magnitude to which enantiospecific interactions between cholesterol and phospholipids affect ion channel function. Since substantial quantities of the enantiomer of cholesterol will be required for our future studies, we have developed an improved method for the conversion of *ent*-testosterone[4] to *ent*-cholesterol.[5]



Cholesterol



Ent-Cholesterol

Ent-Testosterone **1** (Scheme 1) was prepared as described by Ohloff et al.[4] Using the procedures reported by Rychnovsky and Mickus,[5] steroid **1** was converted in five steps to steroid **2** [26% overall yield, $[\alpha]_D^{24}$ +42, (c 1.00, $CHCl_3$)]. However, our attempts to continue with the multi-step elaboration of the side chain of *ent*-cholesterol using the methods described by Rychnovsky and Mikus (hydroboration of the exocyclic olefin **2** with 9-BBN

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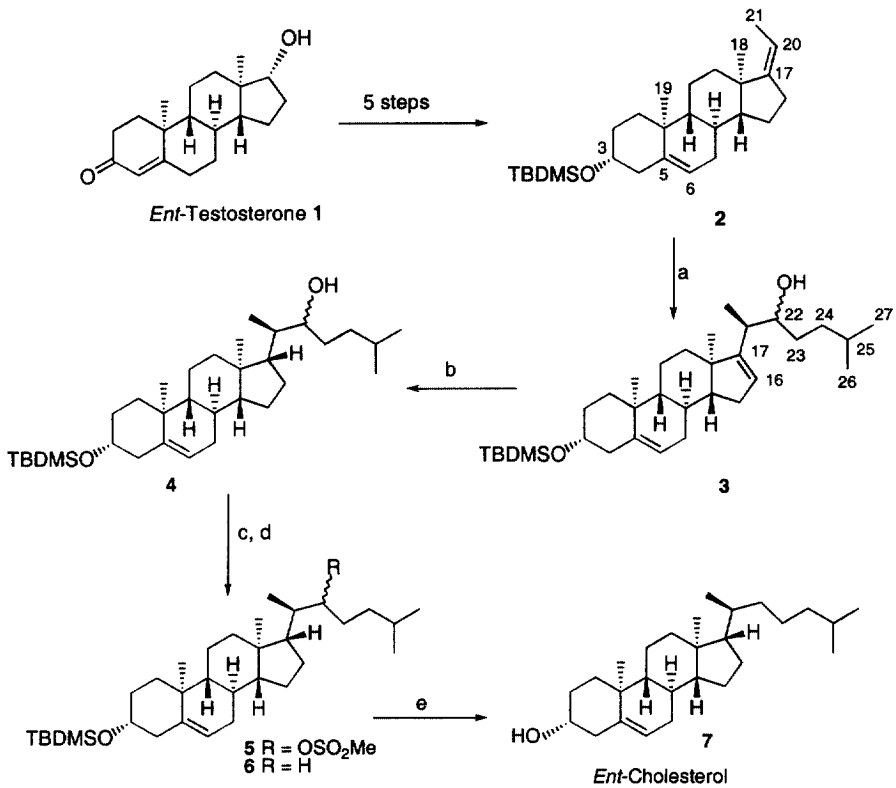
followed by *in situ* coupling with $ClCH_2CN$, nitrile alkylation and reductive decyanation) failed due our inability to successfully carry out the initial hydroboration/coupling reaction. Thus, an alternative method for completing the synthesis of *ent*-cholesterol was considered.



Scheme 1. a) 4-methyl-1-pentanal, Me_2AlCl, CH_2Cl_2; b) Pt/C (5%), H_2 atm., EtOH/Et_2O (1:1); c) $MeSO_2Cl$, Py; d) $LiB(Et)_3H$, THF; e) $(n\text{-}Bu)_4NF$, THF

The ene reaction, which has been applied for the synthesis of other C-17 substituted sterols,[6-8] seemed particularly attractive for construction of the cholesterol side chain from *Z*-olefin **2**. Accordingly, the dimethylaluminium chloride mediated ene reaction of *Z*-olefin **2** with 4-methyl-1-pentanal[9] gave a quantitative yield of the inseparable epimeric 22-alcohols **3** (85:15 ratio as determined by ^1H NMR). As observed previously for steroids of naturally occurring absolute configuration,[7] stereocontrol at C-20 was achieved due to the presence of the methyl group at C-13, which in this case precludes the reaction taking place from the α-face of the steroid.

A typical experimental procedure for the ene reaction involves the addition of steroid **2** to a well stirred mixture of two equivalents of 4-methyl-1-pentanal and dimethylaluminium chloride at –78 °C. After an additional 5 h at –78 °C and 2 h at room

temperature, the reaction mixture was cooled again to –78 °C, 50% aqueous methanol (10 mL) was added, and the product was extracted with ethyl acetate. Excess Lewis acid and longer reaction time gave a different product which was tentatively assigned as the *bis*-steroidal ether formed after *in situ* cleavage of the TBDMS group at O-3 of ene product **3**.

Selective reduction of the Δ^{16} double bond in the presence of the Δ^{5} double bond was achieved by catalytic hydrogenation using 5% platinum on carbon as catalyst in a 1:1 mixture of diethyl ether and ethanol at room temperature for ~1 h. The reaction was ~90% complete at this time. Longer reaction times and higher conversion rates were avoided to prevent possible reduction of the Δ^{5} double bond. The addition of hydrogen to the β-face of the Δ^{16} double bond was expected since approach to the α-face is sterically hindered by the C-18 methyl group. Unreacted steroid **3** was separated from product **4** by silica gel column chromatography and pure samples of the C-22 epimers of steroid **4** were obtained from this purification procedure.

Several methods were tried for removal of the C-22 hydroxyl group of compound **4**. Conversion of the hydroxyl group to an iodo group using methyltriphenoxyphosphonium iodide[10] followed by dehalogenation using $LiB(Et)_3H$ in THF (Super-Hydride®) gave a complex product mixture. A tosylation/detosylation method[11] also was tried, but this approach was unsuccessful because steric hindrance prevented formation of the tosylate. However, the smaller mesylate was readily formed and hydride displacement of the mesylate from steroid **5** by Super-Hydride®[12] gave steroid **6** (62%) along with alcohol **4** (33%). Alcohol **4** can be recycled to give additional amounts of steroid **6**. Mesylate **5** was not purified prior to the hydride displacement step because this compound was found to rearrange to uncharacterized products during silica gel column chromatography. Also notable was the absence of olefin formation during the hydride displacement step. Olefin formation frequently accompanies hydride displacement when mesylates are reacted with $LiAlH_4$.[12]

The synthesis was completed by removal of the TBDMS group using $(n\text{-}Bu)_4NF$ to give *ent*-cholesterol in an overall yield of 36% starting from steroid **2**. Minor impurities (ca 10% as determined by ^1H NMR) were removed by column chromatography followed by HPLC (Beckman Ultrasphere silica column, 5μ, 10 mm x 25 cm eluted with 10% EtOAc in hexanes at 3 mL/min). Purified *ent*-cholesterol had: mp 145 °C (lit mp[5] 146-147 °C); $[\alpha]_D^{24}$ +40.0 (c 1.01, EtOH), lit[5] $[\alpha]_D^{24}$ +40.6 (c 1.01, EtOH). Spectroscopic data[13] are also in complete agreement with literature data.[5] The overall yield for the synthesis of *ent*-cholesterol from *ent*-testosterone by this new method, exclusive of recyclable intermediate, is 9.7%.

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[13] Spectroscopic data:

For **3**: ^{1}H NMR (300 MHz, $CDCl_3$): 5.49 (bs, 1H, 16-H); 5.34 (bd, 1H, J = 4.8 Hz, 6-H); 3.61 (m, 1H, 22-H); 3.50 (m, 1H, 3-H); 1.03 (s, 3H, 19-H); 0.91-0.88 (m, 18H, *t*-BuSi, 21-H, 26-H, 27-H); 0.79(s, 3H, 18-H); 0.05 (s, 6H, $SiMe_2$).

For **4**: ^{1}H NMR (300 MHz, $CDCl_3$) for major isomer: 5.30 (bd, 1H, J = 4.8 Hz, 6-H); 3.60(m, 1H, 22-H); 3.48(m, 1H, 3-H); 1.00 (s, 3H, 19-H); 0.87-0.85 (m, 18H, *t*-BuSi, 21-H, 26-H, 27-H); 0.68 (s, 3H, 18-H); 0.05(s, 6H, $SiMe_2$). ^{1}H NMR (300 MHz, $CDCl_3$) for minor isomer: 5.30 (bd, 1H, J = 4.8 Hz, 6-H); 3.80 (m, 1H, 22-H); 3.48 (m, 1H, 3-H); 1.00 (s, 3H, 19-H); 0.82-0.90 (bs, 15H, *t*-BuSi, 26-H, 27-H), 0.82 (d, 3H, J = 6.6 Hz, 21-H), 0.68 (s, 3H, 18-H), 0.05 (s, 6H, $SiMe_2$). ^{13}C NMR (75 MHz, $CDCl_3$) for major isomer: 141.66, 121.17, 73.92, 72.59, 56.65, 52.50, 50.07, 42.73, 42.17, 40.09, 39.72, 37.27, 36.46, 35.58, 33.12, 31.97, 31.85, 31.79, 28.08, 27.64, 25.84 (3 × C), 24.11, 22.59, 22.45, 20.97, 19.31, 18.14, 11.65, 11.36, −4.75 (2 × C).

For **5**: ^{1}H NMR (300 MHz, $CDCl_3$): 5.30 (bd, 1H, J = 4.8 Hz, 6-H); 4.77 (t, 1H, J = 6.9 Hz, 22-H); 3.50 (m, 1H, 3-H); 3.01 (s, 3H, SO_2Me); 0.99 (s, 3H, C-19); 0.85-0 0.91 (m, 18H, *t*-BuSi, 21-H, 26-H, 27-H); 0.69 (s, 3H, C-18); 0.05 (s, 6H, $SiMe_2$).

For **6**: ^{1}H NMR (300 MHz, $CDCl_3$): 5.32 (bd, 1H, J = 4.9 Hz, 6-H); 3.49 (m, 1H, 3-H); 0.99 (s, 3H, 19-H); 0.88-0.85 (m, 18-H, *t*-BuSi, 21-H, 26-H, 27-H); 0.68 (s, 3H, 18-H); 0.05 (s, 6H, $SiMe_2$).

For **7**: ^{1}H NMR (300 MHz, $CDCl_3$): 5.35 (bd, 1H, J = 5.2 Hz, 6-H); 3.52 (m, 1H, 3-H); 1.00 (s, 3H, 19-H); 0.91 (d, 3H, J = 6.6 Hz, 21-H); 0.867 (d, 3H, J = 6.6 Hz, 26 or 27-H); 0.863 (d, 3H, J = 6.6 Hz, 26 or 27-H); 0.68 (s, 3H, 18-H). ^{13}C NMR (75 MHz, $CDCl_3$): 140.86, 121.79, 71.77, 56.69, 56.07, 50.04, 42.21, 39.68, 39.42, 37.14, 36.40, 36.08, 35.69, 31.79, 31.54, 28.13, 27.90, 24.17, 23.70, 22.71, 22.44, 20.95, 19.27, 18.58, 11.71.