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Synthesis of [25, 26, 26, 26, 27, 27, 27-d_7]-Cholesterol

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SUMMARY

An efficient synthesis of [25, 26, 26, 26, 27, 27, 27-d_7]-cholesterol **1**, a useful tool for the measurement of cholesterol absorption, is described. Commercially available stigmasterol is converted into the known aldehyde **2** which was then transformed to the d_7-cholesterol **1** through a reaction sequence of 8 steps in an overall yield of 22.5%.

Key words: Cholesterol, deuterium, stigmasterol.

INTRODUCTION

Measurement of cholesterol absorption provides important information on the relationship between diet and blood cholesterol levels. Several methods have been developed to measure the extend of absorption in humans (1-6). Most of the methods used ^{14}C- or ^{3}H-labeled cholesterol as the radioactive tracers for the studies (1-5). However, the radiotracer method cannot be used for routine and repeated measurements for cholesterol absorption. Recently, deuterated cholesterol was reported to be used for measurement of cholesterol absorption in humans (7). Deuterated cholesterol is a reliable marker for measuring cholesterol in humans under various experimental conditions. The added advantage of the tracer is that it can be used for studies in children, in women of child-bearing age, and can be repeated as often as necessary. One of the most commonly used deuterated cholesterol is [25, 26, 26, 26, 27, 27, 27-d_7]-cholesterol **1** (Fig. 1). The synthesis of several deuterium-labeled forms of cholesterol

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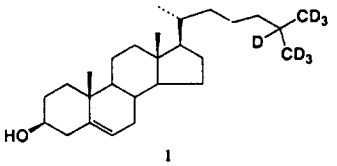
have been reported in the literature (8-12). However, the synthesis of d_7-cholesterol **1** has not been published. Here we report an efficient synthesis of **1** starting from the commercially available stigmasterol.

Figure 1. Structure of Deuterium Labeled Cholesterol



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RESULTS AND DISCUSSION

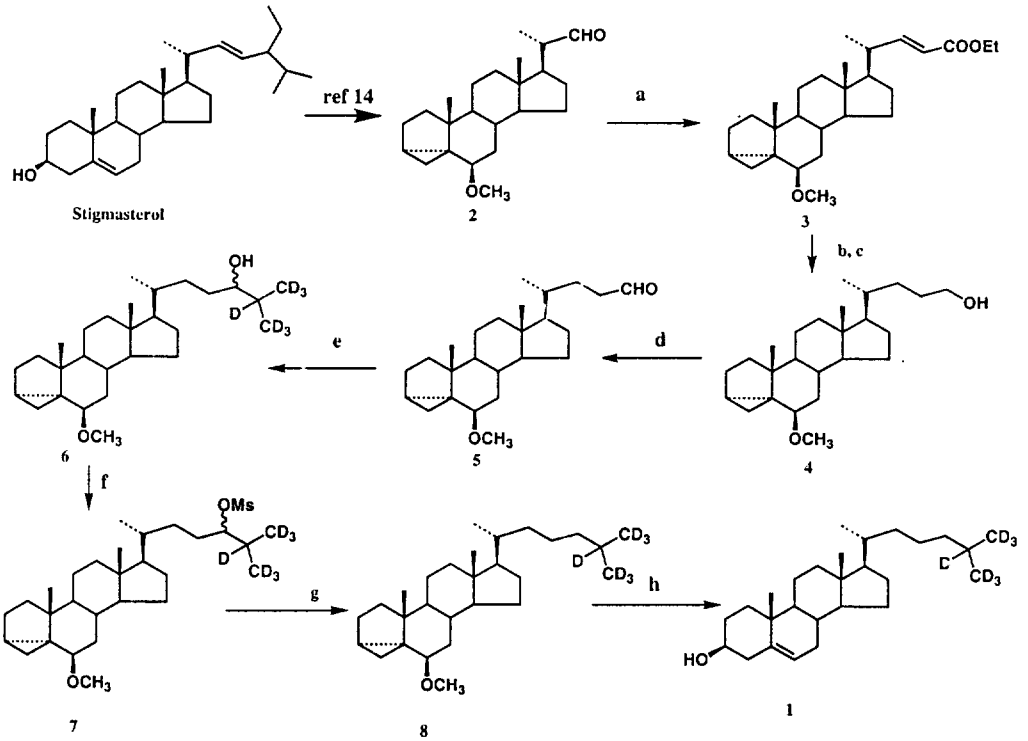
The critical strategy for the chemical synthesis of the d_7-cholesterol is the protection of the 3β-hydroxy-Δ^5-ene functional group. The protecting group should be able to withstand conditions such as palladium catalyzed hydrogenation, LAH reduction and Grignard reactions. One of the most ideal protecting groups can be formed by converting the 3β-hydroxy-Δ^5-ene to 3,5-cyclo-6-methyl ether moiety (13).

The synthesis of [25, 26, 26, 26, 27, 27, 27-d_7]-cholesterol is summarised in figure 2. Commercially available stigmasterol was used as the starting material, which was converted into the aldehyde **2** by known procedure (14). The crude aldehyde **2** was subjected to Horner-Wadsworth-Emmons (HWE) reaction under mild Masamune-Roush's condition (15), yielding α, β-unsaturated ester **3**. After purification by chromatography, compound **3** was obtained as a single spot on TLC, but ^{1}H NMR spectra indicated that it was contaminated with some other byproducts, which had the same R_f value as compound **3**. The 19-CH_3 singlet and 21-CH_3 doublet showed a pair of peaks. In the ^{1}H NMR spectra, two olefinic protons had a coupling constant of 15.6 Hz, indicating the configuration of the double bond to be *trans*. The mixture was used for the next step without further purification. Hydrogenation of α,β-unsaturated ester **3**, followed by reduction of the ester functionality with $LiAlH_4$ gave the pure alcohol **4** (43 % overall yield from **2**). Oxidation of **4** with PCC afforded aldehyde **5**. Reacting **5** with deuterated Grignard reagent: d_7-isopropylmagnesium bromide gave alcohol **6**. Mesylation of **6** yielded mesylate **7** which on treatment with $NaBH_4$ in DMSO gave the product **8** (53% for 4 steps). Finally, triflic acid catalyzed solvolysis of the three- membered

ring (14) of compound **8** in dioxane/water (9 : 1) led to the target molecule: [25, 26, 26, 26, 27, 27, 27-d_7]-cholesterol **1** in 99% yield. The purity of d_7-cholesterol was found to be checked over 98% by GC-MS.

In conclusion, an efficient synthesis of [25, 26, 26, 26, 27, 27, 27-d_7]-cholesterol **1**, a useful tool for measuring cholesterol in humans has been developed. From the crude aldehyde **2**, d_7-cholesterol (**1**) was obtained through a reaction sequence of 8 steps in an overall yield of 22.5 % with high purity (>98%).

Figure 2. Synthesis of Deuterium Labeled Cholesterol



Reagents and Conditions: a. $(EtO)_2P(O)CH_2COOEt$, iPr_2NEt, LiCl, CH_3CN, r.t, 3 days; b. H_2, 5% Pd/C, EtOAc, r.t, 6 h; c. $LiAlH_4$, THF, rt, 2 h, (43% from crude aldehyde **2**); d. PCC, KOAc, Celite, CH_2Cl_2, r.t, 1 h; e. $(CD_3)_2CDMgBr$, THF, -78° C, 0.5 h; -78°C to r.t, 1 h; f. MsCl, Et_3N, CH_2Cl_2, 0°C, 2 h, 81% from **4**; g. $NaBH_4$, DMSO, 90°C, 3 h, 66%; h. Triflic acid, dioxane-H_2O (9:1), reflux 1.5 h, 99%.

EXPERIMENTAL

General. Chemicals (including d_7-isopropyl bromide) and silica gel were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). The chemicals were checked for purity by thin layer chromatography and NMR spectroscopy. Stigmasterol was obtained from Sigma Chemical Company (St. Louis, MO, USA). Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were obtained with a Bruker WH-300 (300 MHz) spectrophotometer. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA, USA). High and low resolution mass spectra were recorded on a Varian MATCH-311A mass spectrometer under EI conditions.

6β–Methoxy-3α,5-cyclo-5α-cholan-24-ol (4)

To the stirred suspension of LiCl (20 g, 471 mmol) in dry acetonitrile (1.5 L) under nitrogen at r.t was added triethyl phosphonoacetate (74 ml, 373 mmol), diisopropylethylamine (53 mL, 305 mmol) and finally the crude aldehyde **2** (25.84 g, 75.1 mmol) in acetonitrile (300 mL). The reaction mixture was stirred at r.t for 3 days and then concentrated *in vacuo*. To the residue was added CH_2Cl_2 (400 mL) and water (300 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 200 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was purified by silica gel chromatography using pet ether/EtOAc (6:1) as eluent, to give compound **3** as a single spot on TLC. But its ^1H NMR spectra indicated it was contaminated with some by-product. Without further efforts on the purification, the mixture was used directly for the next step. The crude compound **3** was dissolved in ethyl acetate (500 mL) and hydrogenated at r.t in the presence of 5 % Pd/C for 6 h. It was filtered and the filtrate was concentrated *in vacuo*, giving crude saturated ester which was used directly for the reduction. Thus the residue was dissolved in anhydrous THF (150 mL) and added dropwise to the suspension of $LiAlH_4$ (3.6 g, 97.74 mmol) in anhydrous THF (350 ml) at 0°C under nitrogen atmosphere, and the reaction mixture was stirred for 2 h at r.t. The reaction was quenched with sequential addition of water (3.6 mL), 15 % NaOH (3.6 mL) and water (10.8 mL). The resulting mixture was stirred at r.t for 1 h and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by chromatography using pet ether/ EtOAc (5:2) as eluent, yielding pure alcohol **4** (12 g, 43% overall yield). m.p: 98 - 99°C [lit. 95 - 97°C (16)]; ^1H NMR (300 MHz, $CDCl_3$) δ 0.70 (s, 3 H, 19-CH_3), 0.90 (d, 3 H,

J = 6.6 Hz, 21-CH_3), 1.0 (s, 3 H, 18-CH_3), 2.75 (m, 1 H, H-6), 3.31 (s, 3 H, OCH_3), 3.60 (m, 2 H, CH_2OH) ppm; m/z 374 (M^+); Observed (M^+): 374.3185; $C_{25}H_{42}O_2$ require: 374.3185.

6β–Methoxy-24ξ-mesyl-3α,5-cyclo-5α–25,26,26,26,27,27,27-d_7-cholestane (7)

To a solution of alcohol **4** (5.8 g, 15.51 mmol), in methylene chloride (CH_2Cl_2 , 300 mL) was added KOAc (4.9 g, 50 mmol), celite (6 g) and PCC (10.5 g, 48.72 mmol). The reaction mixture was stirred at r.t for 1 h and then diluted with ether (300 mL) and filtered through a Florisil column. The filtrate was concentrated *in vacuo*. TLC showed the product was pure enough for the next step. The analytical sample of aldehyde 6β–Methoxy-3α,5-cyclo-5α-cholan-24-ol **5** was obtained by silica gel chromatography using pet ether/EtOAc (4:1) as eluent. m.p: 46.5 - 48.5°C [lit. 46 - 47°C (17)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.69 (s, 3 H, 19-CH_3), 0.90 (d, 3 H, J = 6.6 Hz, 21-CH_3), 1.0 (s, 3 H, 18-CH_3), 2.75 (m, 1 H, H-6), 3.30 (s, 3 H, OCH_3), 9.74 (s, 1 H, CHO) ppm; m/z 372 (M^+); Observed (M^+): 372.3028; $C_{25}H_{40}O_2$ require: 372.2989.

The crude aldehyde **5** was used directly for the next step. d_7-Isopropyl bromide (3.23 g, 24.8 mmol) was added to a suspension of magnesium turnings (596 mg, 24.83 mmol) in anhydrous THF (60 mL). Initiation of the reaction was achieved by mild heating. The reaction mixture was stirred for 1 h with occasional heating with a heat gun. The Grignard reagent that was formed was cooled to -78°C and was added the solution of the crude aldehyde **5** in anhydrous THF (40 mL) under nitrogen atmosphere. The reaction mixture was stirred at -78°C for 30 min and slowly warmed to r.t over 1 h and then quenched with saturated NH_4Cl (80 mL). EtOAc (100 mL) was added to the mixture and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 x 60 mL). The combined organic layers was dried (Na_2SO_4) and concentrated *in vacuo* to give the crude Grignard adduct alcohol 6β–Methoxy-3α,5-cyclo-5α–25,26,26,26,27,27,27-d_7-cholestan-24ξ-ol **6**. An analytical sample of **6** was obtained as a gum by chromatography using petroleum ether/EtOAc (5:1) as eluent. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.69 (s, 3 H, 19-CH_3), 0.89,0.90 (2d, 3H, J = 6 Hz, ratio: 1:1, 21-CH_3), 0.99 (s, 3 H, 18-CH_3), 2.74 (m, 1 H, H-6), 3.29 (s+m, 4 H, OCH_3 and CHOH), ppm; m/z 423 (M^+); Observed (M^+): 423.4094; $C_{28}H_{41}D_7O_2$ require: 423.4109.

The crude product was dissolved in methylene chloride (100 mL) and cooled to 0°C. To this solution was added triethylamine (4.32 mL, 31 mmol) followed by dropwise addition of

methanesulfonyl chloride (1.86 mL, 24 mmol). The reaction mixture was stirred at 0°C for 2 h, washed with saturated $NaHCO_3$ (2 x 50 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by chromatography using pet ether/EtOAc (5:1) as eluent, yielding pure mesylate **7** (6.3 g, 81.1 % from **4**) as a gum. ^1H NMR (300 MHz, $CDCl_3$) δ 0.69 (s, 3 H, 19-CH_3), 0.91 (d, 3 H, J = 6.6 Hz, 21-CH_3), 0.99 (s, 3 H, 18-CH_3), 2.75 (m, 1 H, H-6), 2.98 (s, 3 H, CH_3SO_2), 3.30 (s, 3 H, OCH_3), 4.49 (m, 1 H, CHOMs) ppm. m/z 501 (M^+); Observed (M^+): 501.3869; $C_{29}H_{43}D_7O_4S$ require: 501.3847.

6β-Methoxy-3α,5-cyclo-5α-25,26,26,26,27,27,27-d_7-cholestane (8)

To a solution of mesylate **7** (6 g, 12 mmol) in anhydrous DMSO (80 mL) was added sodium borohydride (2.74 g, 72 mmol). The reaction mixture was stirred under nitrogen atmosphere at 90°C for 3 h and then cooled to r.t. Water (160 mL) was added and the mixture was extracted with ether (3 x 150 mL). The combined organic layers were washed with water (2 x 100 ml), brine (100 ml) and dried (Na_2SO_4). After removal of the solvent *in vacuo*, the residue was purified by chromatography using petroleum ether/CH_2Cl_2 (1:1) as eluent, giving pure deoxygenated product **8** (3.21g, 65.9%). m.p: 79.5-80.5°C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.69 (s, 3 H, 19-CH_3), 0.91 (d, 3 H, J = 6.6 Hz, 21-CH_3), 1.0 (s, 3 H, 18-CH_3), 2.75 (m, 1 H, H-6), 3.30 (s, 3 H, OCH_3). m/z 407 (M^+); Observed (M^+): 407.4144; $C_{28}H_{41}D_7O$ require: 407.4142.

[25, 26, 26, 26, 27, 27, 27-d_7]-Cholesterol 1

To a solution of compound **8** (110 mg, 0.27 mmol) in a mixture of dioxane (9 mL) and water (1 mL) was added one drop of triflic acid. The reaction mixture was refluxed for 1.5 h and cooled to r.t. Saturated $NaHCO_3$ (10 mL) and EtOAc (10 mL) was added to the cooled mixture. The organic layer was separated and the aqueous layer was extracted with EtOAc (15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by chromatography using EtOAc/CH_2Cl_2/pet ether (3:2:2) as eluent, affording d_7-cholesterol **1** (105 mg, 99%). An analytical sample was obtained from crystallization with methanol. m.p: 147.5 - 148.5°C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.65 (s, 3 H, 19-CH_3), 0.89

(d, 3 H, J = 6.6 Hz, 21-CH_3), 1.01 (s, 3 H, 18-CH_3), 3.50 (m, 1 H, H-3), 5.33 (m, 1 H, H-6) ppm; GC-MS: 393 (M^+), one peak; m/z 393 (M^+); Observed (M^+): 393.3988; $C_{27}H_{39}D_7O$ require: 393.3969.

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