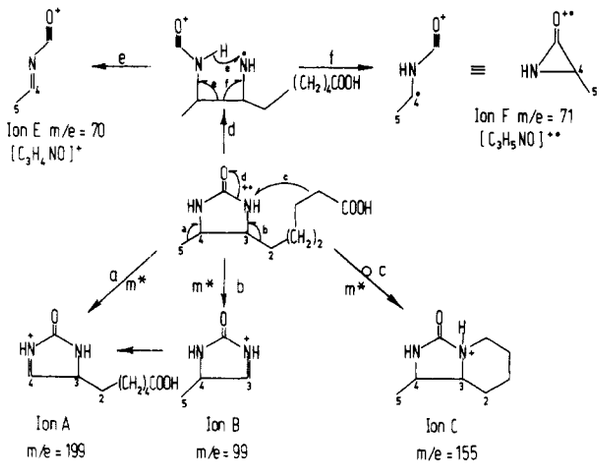
Scheme II



E, and F. When labeling occurs at C-2, C-3, C-4, and C-5 positions (runs 5–8), deuterium content at C-2 and C-5 is deduced from the relative heights of the peak due to ions A, B, C, E, and F.

Experimental Section

NMR spectra were recorded on a Varian HA 100 spectrometer in D_2O and chemical shifts are reported in parts per million (δ) from external HMDS. The gas chromatograph used was a GIRDEL 3000 unit. The mass spectra were obtained using a ATLAS CH_5 mass spectrometer (source at 210 °C). Scintillation counting was carried out with a Intertechnique SL_{30} spectrometer in Bray's liquor. All the results were corrected for quenching by external standard method.

Material. LiB^3H_4 (1 Ci/mmol) was obtained from C.E.A. Saclay France. All the deuterated silanes and germanes used in this study were prepared by $LiAlD_4$ reduction of the corresponding chloro compounds according to known methods.[13] No ≡SiH or ≡GeH could be detected by NMR.

The 4-methyl-5-(ω-carboethoxyamyl)-2-imidazolone (**1a**) and *N,N'*-diacetyl-4-methyl-5-(ω-carboethoxyamyl)-2-imidazolone (**1b**) were prepared in good yield according to Duschinky and Dolan.[2]

Preparation of Labeled Dethiobiotins. A typical reduction experiment was performed as follows.

Reduction of 4-Methyl-5-(ω-carboethoxyamyl)-2-imidazolone. To a mixture of 360 mg (1.5×10^{-3} mol) of **1a** in 1 mL of CF_3COOH, 180 mg (1.5×10^{-3} mol) of Et_3SiH is added gradually. The mixture is kept at 50 °C under shaking. The reduction progress is followed by NMR (disappearance of the $C(5)H_3$—C(4)= signal, singlet at δ 1.95/Me_4Si). The reduction is completed after 20 h. The excess of CF_3COOH and $Et_3SiOOCCF_3$ which has been produced is removed in vacuo. The crude material is then acetylated by two short refluxings with 5 mL of acetic anhydride, the excess of which is distilled off.

The cis/trans ratio **2b/3b** is determined on the crude acetylated mixture by GLC (SE 30 10% on Chromosorb G, WHMDS), **2b/3b** = 1/1. The preparative separation of **2b** and **3b** is performed by silica gel TLC (eluent: ethyl acetate–chloroform, 2/8).

After separation of **2b** and **3b**, saponification of each isomer with 1 N sodium hydroxide (20 °C, 2 h) afforded the corresponding dethiobiotins **2c** and **3c**.

Purification of **2c** and **3c** is carried out on a Dowex AG 50 WX_2 formate column. Dethiobiotin is eluted with 0.05 M formic acid. Total yield (**2c** + **3c**) = 70%: **2c**, mp 159 °C (lit. mp 159 °C);[4] **3c**, mp 156 °C.

The structures of **2c** and **3c** are determined by NMR and mass spectrometry. **2c:** NMR δ 1.44 (3 H, d, J = 6 Hz, CH_3CH), 4.11 (2 H, m, H_3, H_4), 2.45 (2 H, t, J = 7 Hz, $-CH_2COOH$); mass spectrum *m/e* 214 (M^+), 199, 155, 99. **3c:** NMR δ 1.54 (3 H, d, J = 5, 7 Hz, CH_3CH), 3.76 (2 H, m, H_3, H_4), 2.45 (3 H, t, J = 7 Hz, $-CH_2COOH$); mass spectrum *m/e* 214 (M^+), 199, 155, 99.

When the reduction is carried out with Et_3SiD in CF_3COOH, the deuterium distribution at C-4, and consequently at C-3, can be deduced from the integration of the signals: δ 1.43 (3 H, s, CH_3CD) and 1.44 (3 H, d, J = 6 Hz, CH_3CH). However, a better accuracy is obtained by mass spectroscopy.

[^{3}H]Triethylsilane. LiB^3H_4 (88 mg, 4×10^{-3} mol, 1 Ci/mmol) was allowed to react with Et_3SiCl (0.6 g, 4×10^{-3} mol) in 4 mL of dry triglyme under nitrogen at 50 °C. Et_3Si^3H was distilled and trapped at −70 °C in vacuo. Its chemical purity was controlled by GLC (SE 30 20% on Chromosorb Z): yield, 98%, 470 mg; 0.2 Ci/mmol.

Tritiated Dethiobiotin. Et_3Si^3H, prepared as described, was transferred into a flask containing 242 mg (0.75×10^{-3} mol) of *N,N'*-diacetyl-4-methyl-5-(ω-carboethoxyamyl)-2-imidazolone in 2 mL of CF_3COOH (freshly distilled on H_2SO_4). After 60 h at room temperature, excess Et_3Si^3H, CF_3COOH, and $Et_3SiOOCCF_3$ produced were removed in vacuo. Saponification and deacetylation of the crude residue with 1 N sodium hydroxide at 20 °C during 2 h afforded dethiobiotin. The pH of the solution was adjusted to 8 and then the solution was taken on to a Dowex AG 50 WX_2 formate column for purification. Dethiobiotin eluted with 0.05 M formic acid was obtained in 75% yield: 120 mg; mp 159 °C (198 mCi/mmol). The structure and purity were controlled by mass spectrometry and radiochromatography.

Acknowledgments. We are indebted to Dr. Pichat for helpful advice and for allowing one of us (G.G) to prepare (±)-[^{3}H]dethiobiotin in his laboratory (Service des Molecules Marquees, C.E.A. Saclay).

Registry No.—**2a**, 63526-69-2; **2a** C-3 deuterated derivative, 63466-48-8; **2a** C-4 deuterated derivative, 63466-49-9; **2a** deuterated derivative, 63466-50-2; **2b**, 63466-51-3; **2b** C-3 deuterated derivative, 63466-52-4; **2b** C-4 deuterated derivative, 63466-53-5; **2b** deuterated derivative, 63466-54-6; **2b** C-3 tritiated derivative, 63466-55-7; **2b** C-4 tritiated derivative, 63466-56-8; **2c**, 636-20-4; **3a**, 63526-70-5; **3a** C-3 deuterated derivative, 63466-57-9; **3a** C-4 deuterated derivative, 63466-58-0; **3a** deuterated derivative, 63466-59-1; **3b**, 63466-60-4; **3b** C-3 deuterated derivative, 63466-61-5; **3b** C-4 deuterated derivative, 63466-62-6; **3b** deuterated derivative, 63466-63-7; **3b** C-3 tritiated derivative, 63466-64-8; **3b** C-4 tritiated derivative, 63466-65-9; **3c**, 34879-36-2; Et_3SiT, 63466-66-0; LiB^3H_4, 23683-78-5; Et_3SiCl, 994-30-9; Et_3SiD, 1631-33-0; Ph_3SiD, 18536-60-2; Ph_2SiD_2, 17950-94-6; Ph_3GeD, 2816-42-4; Et_3SiH, 617-86-7; *cis*-3,4-dimethyl-2-imidazolinone, 63466-67-1; *trans*-3,4-dimethyl-2-imidazolinone, 63466-68-2; *cis*-3,4-dimethyl-*N,N'*-diacetylimidazolin-2-one, 63466-69-3; *trans*-3,4-dimethyl-*N,N'*-diacetylimidzolin-2-one, 63466-70-6.

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(10) We also checked that this competing side reaction still occurs in a more simple olefin, namely 1-methylcyclohexene, reduction of which by Et_3SiD/CF_3COOD affords a polydeuterated methyl cyclohexane containing one to five deuterium atoms per mole.
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Nucleophilic Substitution with Inversion of Alcohol Configuration with the Reagent Complex Triphenylphosphine–Diethyl Azodicarboxylate–Carboxylic Acid. A Convenient Preparation of Epicholesterol

Lorenzo P. L. Piacenza

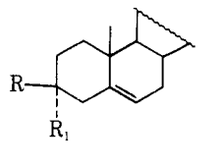
Chemistry Department, University of Durban-Westville, Durban 4000, South Africa

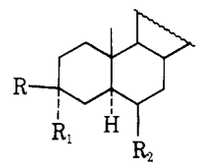
Received April 25, 1977

Several methods have been reported recently for preparing epicholesterol (**1**) from cholesterol (**2**) in fair to good yield.[1,2]

A procedure is described here which is amenable to large-scale synthesis of pure epicholesterol without contamination by cholesterol and it is of good yield.

The reagent complex triphenylphosphine–diethyl azodicarboxylate–carboxylic acid (Bose reagent)[3] is an excellent system for inverting the alcohol configuration in unhindered molecules.[3-5] However, although the initial report by Bose et al.[3] stated that cholesterol (**2**) was readily converted to its epimeric benzoate (**3**), product mixtures were obtained on





	R	R_1		R	R_1	R_2
(1)	H	OH	(4)	OH	H	OH
(2)	OH	H	(5)	H	$PhCO_2$	OH
(3)	H	$PhCO_2$	(6)	H	$PhCO_2$	CH_3SO_3
(7)	$PhCO_2$	H				

repeating the reaction and these contained only a small amount of the required epimer **3**.[6] Since homoallylic participation of the double bond of the reacting substrate **2** interfered with the required reaction pathway, a different preparative route for **3** was successfully attempted. The starting material was 5α-cholestane-3β,6β-diol (**4**), available in 72% yield from cholesteryl acetate via a facile three-step synthesis involving hydroboration, Jones oxidation of the resulting mixture of 6α- and 6β-alcohols to the 3,6-dione and its $NaBH_4$ reduction.[7] The Bose reagent using benzoic acid as the carboxylic acid reacted selectively with the equatorial 3β-hydroxyl group of **4** resulting in an 88% conversion to 3α-benzoyloxy-5α-cholestan-6β-ol (**5**). Mesylation of the 6β-hydroxyl group of **5** gave **6** accompanied by some of the required elimination product **3**. The pure 6β-mesylate **6** on treatment with Li_2CO_3 in DMF gave epicholesteryl benzoate (**3**) in 84% yield. Finally, alkaline hydrolysis of **3** generated a 90% yield of epicholesterol (**1**). The overall yield from the diol **4** was 67% when all intermediates were isolated and purified. Since impure fractions and mother liquors were not worked up for further material, the yield could be made substantially higher if this was pursued, especially since all the steps were fairly clean, and crude material could be taken right through the sequence, thus requiring only a final purification.

The melting points and optical rotations of the two cholesterol epimers **1** and **2** are very similar (mp 142–143 and 148–149 °C, respectively; $[\alpha]_D$ −42° and −39°, respectively) so that these two criteria of identity and purity cannot be used in this instance. However, the corresponding benzoates **3** and **7** are readily distinguished (mp 99–100 and 143–150 °C, respectively; $[\alpha]_D$ −29° and −15°, respectively). Furthermore, differentiation by the NMR spectrum of the benzoates is the method of choice, since the H-3α signal of **7** follows the pattern of axial proton signals by appearing upfield of the H-3β signal of **3** by 0.42 ppm, and the olefinic proton at C-6 becomes shielded by 0.15 ppm when the benzoate group is in the 3α orientation (see Experimental Section for values) so that any impurity of one isomer in the other can be detected readily on a semiquantitative basis. The free alcohols **1** and **2** can also be distinguished by their NMR spectra as the H-3 resonances are separated by 0.49 ppm, although the olefinic proton at C-6 absorbs in practically the same region for both compounds.

The above inversion reaction was carried out successfully on cholesteryl α-epoxide, but failed with 5α,6β-dibromo-5α-cholestane, since the triphenylphosphine reacted with the halogen substituents giving complex mixtures of products.

Experimental Section

General. All melting points were determined on a Kofler hot stage and are uncorrected. NMR spectra were recorded in $CDCl_3$ solution with Me_4Si as an internal standard on a Varian T60 and Varian HA100 spectrometer. Silica gel for column chromatography was Merck grade 7734. All crystalline compounds gave satisfactory analytical data.

3α-Benzoyloxy-5α-cholestan-6β-ol (5). To a stirred solution of the diol **4**[7] (2.00 g, 4.95 mmol), PPh_3 (2.59 g, 9.90 mmol), and benzoic acid (1.21 g, 9.90 mmol) in dry THF (30 mL) under argon at room temperature (rt) was added dropwise a solution of diethyl azodicarboxylate in dry THF (1.72 g, 9.90 mmol in 10 mL) over a 5-min period. TLC (15% ethyl acetate in benzene) indicated almost complete reaction of the diol **4** after 10 min. After 15 h, the solvent was removed under vacuum, and the residue was partially dissolved in a mixture of ethyl acetate and benzene (15:85, respectively), when the insoluble, crystalline material $C_2H_5O_2CNHNHCO_2C_2H_5$, mp 132–134 °C (lit.[8] 135 °C), was filtered off and the filtrate chromatographed on silica gel using the same solvent mixture as above. Compound **5** was eluted (pure by TLC) as an oil, 2.23 g, which could not be induced to crystallize.

6β-Mesylate (6). To a solution of the above product **5** (2.22 g) in dry pyridine (15 mL) was added CH_3SO_2Cl (4 g) at −5 °C. The mixture was left at 5 °C overnight before it was worked up with water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed once with dilute HCl followed by twice with water, then dried, and evaporated leaving a gum consisting of a mixture of two compounds. Chromatography on silica gel with benzene followed by 5% ethyl acetate in benzene gave pure epicholesteryl benzoate (**3**), mp 100–102 °C (yield 0.862 g), followed by a combined fraction containing both **3** and **6** as 1.255 g of oil.

Epicholesteryl Benzoate (3). The oil containing both **3** and **6** (1.255 g) was dissolved in dry DMF (15 mL) and heated under argon with Li_2CO_3 (2.0 g) at 110 °C for 1.5 h. The cooled reaction mixture was poured into a slurry of ice in dilute HCl and extracted twice with ethyl acetate. The combined organic phases were washed once with water, once with saturated $NaHCO_3$ solution, and finally four times with water before drying. The oil obtained on evaporation was essentially **3** but contained small quantities of polar material (TLC). This oil was chromatographed with hexane–benzene (1:1 mixture) giving first a compound (0.008 g) which was tentatively identified as 5α-cholest-6-enyl 3α-benzoate (M^+ 490), followed by epicholesteryl benzoate (**3**) (0.933 g) which was crystallized from THF–methanol: mp 101–103 °C (lit.[9] 99.5 °C); NMR δ 5.26 (2 H, m, $W_{1/2}$ = 16 Hz, H-3β and H-6), *compare* cholesteryl benzoate (**7**) δ 4.84 (1 H, m, $W_{1/2}$ = 20 Hz, H-3α), 5.41 (1 H, m, $W_{1/2}$ = 9 Hz, H-6).

Epicholesterol (1). A solution of the benzoate (**3**) (0.955 g) and KOH (1.0 g) in methanol (50 mL) and THF (2 mL) was refluxed for 3 h when TLC indicated complete hydrolysis of the ester. The reaction mixture was cooled, poured into dilute HCl and twice extracted with ethyl acetate, and the extract was washed with water. Drying and evaporation of the organic phase left a solid which was crystallized from acetone as leaflets (0.672 g): mp 140–142 °C (lit.[10] 142–143 °C); NMR δ 4.02 (1 H, m, $W_{1/2}$ = 8 Hz, H-3β), 5.43 (1 H, m, $W_{1/2}$ = 9 Hz, H-6).

Registry No.—**1**, 474-77-1; **3**, 42921-42-6; **4**, 570-85-4; **5**, 63528-70-1; **6**, 63528-71-2; **7**, 604-32-0; triphenylphosphine, 603-35-0; diethyl azodicarboxylate, 4114-28-7; benzoic acid, 65-85-0; 5α-cholest-6-enyl 3α-benzoate, 63528-72-3.

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