# LABELED BILE ACIDS IV: BASE-CATALYZED ONE-POT SYNTHESIS OF DEUTERIUM LABELED BILE ACIDS (1)

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## SUMMARY

Deuterium labeled bile acids are synthesized from their keto acid analogs by a one-pot base-catalyzed enolization exchange with deuterated hydroxylic solvents (OD) followed by in situ reduction of the ketone. The stereochemistry of the alcohol, especially that at C-7, can be controlled either by steric approach reduction with sodium borodeuteride or thermodynamically by reduction with sodium in n-butanol.

Key Words: Bile acids, chenodeoxycholic, ursodeoxycholic, deuterium-labeled, enolization, exchange.

## INTRODUCTION

The carbonyl group is certainly the most widely used and versatile functional group for the introduction of deuterium into steroids and

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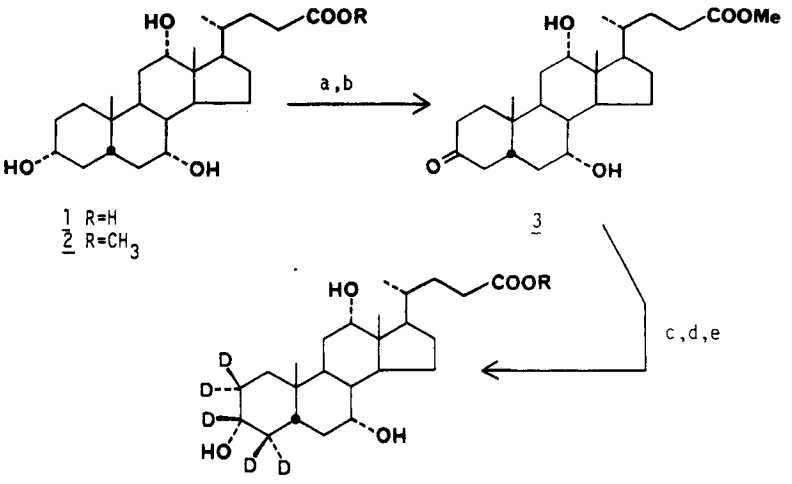
the most common deuterium labeling technique is the exchange of active hydrogens *via* enolization. This is due to the simplicity of the reactions as well as the relatively low price and the accessibility of the required reagents. All of the above are pertinent in our effort to synthesize deuterium-labeled bile acids necessary for the elucidation of the qualitative and quantitative aspects of the biosynthetic pathways involved in the catabolism of cholesterol to bile acids. For such studies to be feasible, the metabolites must be labeled with a deuterium enrichment of at least M+3 (2).

## DISCUSSION

Ketones labeled with deuterium on their adjacent carbons have limited use in biological systems, especially *in vivo* experiments, due to the exchange of the incorporated isotopes with protons in the medium. However, the exchange capability of deuterium ceases as soon as the enolizable ketone is reduced.

Cholic 1 and chenodeoxycholic acids 5 are convenient starting materials. Scheme I depicts the strategy of introducing deuterium at ring A *via* the ketone at C-3. The ketone 3 was synthesized by an Oppenauer oxidation (3) of the methyl cholate 2. However, the same Oppenauer oxidation procedure proceeded rather sluggishly for methyl chenodeoxycholate 5b. This problem was overcome by a quick and simple sequence of acetylation (4), selective hydrolysis of the 3-acetoxy group (5) followed by Jones oxidation (Scheme II) which gave ketone 8 in good overall yield. The same sequence can also be applied to ursodeoxycholic acid 10a in order to synthesize ketone 13. However, selectivity in terms of the hydrolysis of the 3-acetoxy group had to be monitored more carefully. 3-Keto bile acids can also be synthesized in good yield by the selective oxidation of bile acid methyl esters with the more expensive reagent, silver carbonate-celite, in refluxing toluene (21).

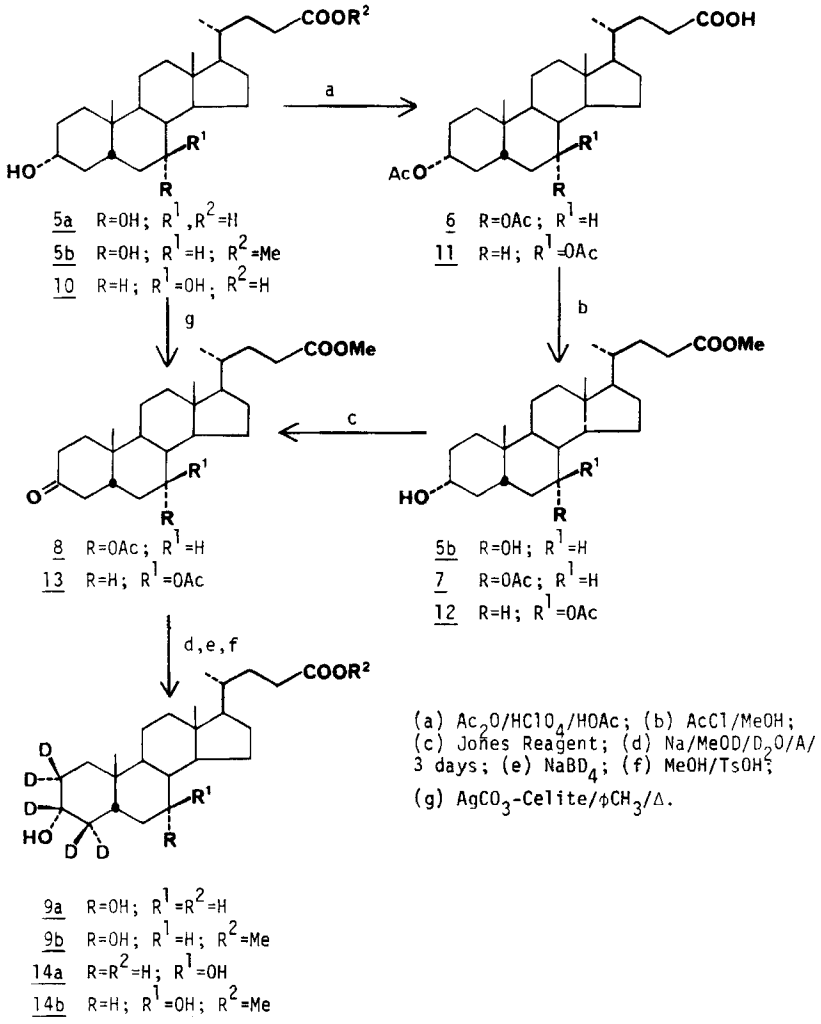
Scheme I




(a) MeOH/TsOH; (b) $Al(O\text{-}iPr)_3/\phi CH_3$/acetone/Δ; (c) $Na/MeOD/D_2O$/Δ/3 days; (d) $NaBD_4$; (e) MeOH/TsOH.

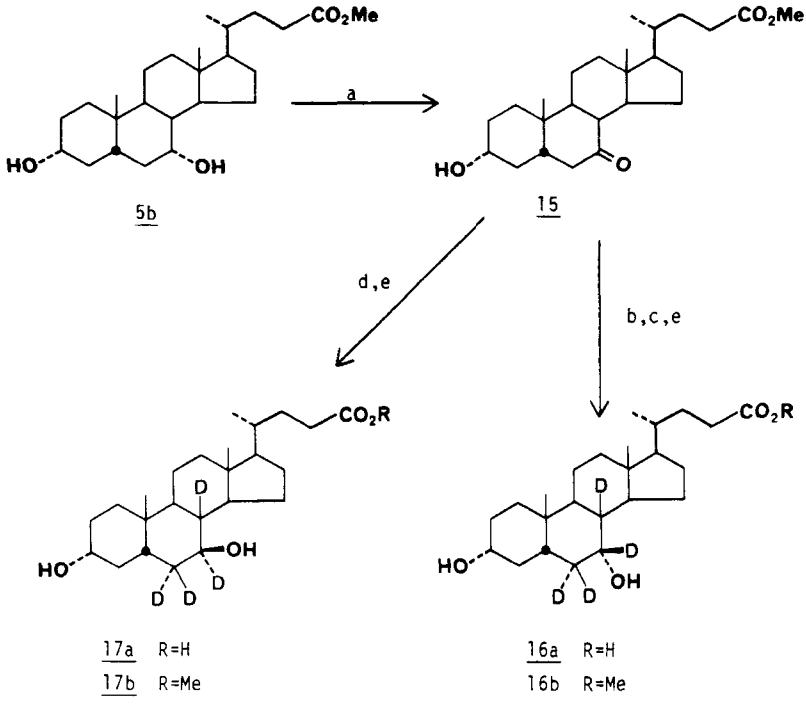
Deuterium was introduced by the base-catalyzed exchange of the 3-keto bile acids 3, 8 and 13 in a mixture of 5% sodium deuterioxide in methanol-OD. Reactions with sodium borohydride in aqueous methanol are known to proceed smoothly, especially when stabilized with sodium methoxide (6). Hence sodium borodeuteride was added directly into the crude reaction mixture when the deuterium exchange was completed. The 3-keto group, in all cases (3, 8 and 13), was reduced predominantly to the 3α-hydroxy moiety, 4a, 9a and 14a respectively, due largely to steric approach control (7), the effective reagent being the larger and more reactive methoxyborodeuteride $[Na(OCH_3)_3BD]$ (8).

Scheme II




The introduction of deuterium into ring B via the C-7 carbonyl group is shown in Scheme III. Ketone 15 was synthesized by the selective oxidation procedure of Haslewood (9). The configuration at C-7 of the reduced product could be controlled either by a one-pot synthesis via the enolization exchange, followed by reduction with

Scheme III




(a) $K_2CrO_4$/NaOAc/HOAc; (b) Na/MeOD/$D_2O$/Δ/3 days; (c) $NaBD_4$; (d) Na/n-butanol (OD)/120°C; (e) MeOH/TsOH.

sodium borodeuteride to give the steric control product (the axial 7α-alcohol 16a) or by the facile sodium reduction in hot n-butanol (OD) to give the equatorial 7β-alcohol 17a (20). These methods were described in our previous publication (10). In the case where a ketone is adjacent to a hydrogen-bearing asymmetric center, epimerization may occur during the enolization exchange. However, no epimerization at C-8 was detected during the exchange of 7-ketones (11, 12). The TLC, NMR, IR and melting points, when compared to an authentic sample, were identical within experimental errors.

For a stable isotope labeled analog, it is assumed that the substance is identical in all aspects, except mass, to the parent compound. Unfortunately, the molecular ion of bile acids, in general, is often not registered on the spectrogram if analysis is conducted by electron impact mass spectrometry. This is due to premature loss of their hydroxyl (as $H_2O$) or derivatized moiety (as TMSOH). However, the mass spectral fragmentations of a number of bile acids, as their hydroxy or acetoxy methyl esters, especially that of cholic 1, chenodeoxycholic 5a and ursodeoxycholic 10a acids, have been extensively studied (13,14) and these results were used to calculate the percentages of deuterium content in our final products by comparisons of the relative peak intensities. Where deuterium is incorporated at C-6, C-7 and C-8, loss of one deuterium is inevitable during fragmentation due to loss of HDO and TMSOD (if derivatized) since the C-7 (and C-12) alcohol dehydrates faster than the C-3 alcohol (13,14).

## EXPERIMENTAL

Melting points were determined on a Kofler melting point apparatus and are uncorrected. The IR spectra of crystals were determined as KBr pellets and of oils as a film on sodium chloride windows. The NMR spectra were obtained in deuteriochloroform solution, or otherwise stated, by using tetramethylsilane as an internal reference and were recorded on a 90 MHz Varian EM-390 spectrometer.

GC-MS spectra of labeled bile acids were recorded as the trimethylsilyl derivatives of the respective methyl esters using a Hewlett Packard 5992-A GC/MS system. The GC column used was a borosilicate glass capillary column (6 ft x 2 mm) containing 3% SP2100 on 80/100 Supelcoport. Column temperatures were 240-290°C.

TMS-Derivatization. About 0.5 mg of a sample was dissolved in 1 mL of TRI-SIL/TBT (Pierce Chemical Co.) in 2 mL tapered vials

equipped with Tuf-Bond teflon-silicone discs and open-top screw caps. The mixture was incubated at 60°C for 2 h before use.

Methyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate (2) was prepared from cholic acid 1 with methanol and p-toluenesulfonic acid according to known procedures (17), m.p. 154-156°C (ethyl acetate) [Lit. (4) 156-157°C].

Methyl 7α,12α-dihydroxy-3-oxo-5β-cholan-24-oate (3) was prepared by the Oppenauer oxidation (3) of the methyl cholate 2, m.p. (ether) 171-172°C [Lit. (3) 171-172°C].

[2α,2β,3β,4α,4β-$^{2}H_{5}$]-Methyl-3α,7α,12α-trihydroxy-5β-cholan-24-oate (4b). The ketone 3 (700 mg, 1.6 mmol) was suspended in a freshly prepared 5% deuterated methanolic sodium hydroxide solution (OD) (190 mg sodium, 13 mL methanol (OD) 99 atom % D, 6.8 mL deuterium oxide 99.8 atom % D). The mixture was refluxed for 3 days in a nitrogen atmosphere. It was then cooled and sodium borodeuteride (100 mg) was added. This was stirred at room temperature overnight, then quenched with cold 10% hydrochloric acid and filtered to give the crude deuterated cholic acid 4a (612 mg, 87%) as a solid. The crude acid was refluxed with a 5% methanolic potassium hydroxide solution (20 mL) for 2 h to remove any exchangeable deuterium from the molecule. The product was acidified, methylated (as for compound 2) and chromatographed on preparative TLC (3 x 20% acetone/hexane) yielding the deuterated methyl cholate 4b; m.p. 153-155°C (ethyl acetate) [Lit. (4) 156-157°C]; IR $\nu_{max}$ 3400 (OH), 2180, 2100 (C-D), 1720 (C=O), 1190, 1080, 1020 (C-O) $cm^{-1}$; NMR δ 0.67 (3H, s, 18-Me), 0.91 (3H, s, 19-Me), 0.97 (3H, d, J = 7 Hz, 21-Me), 3.67 (3H, s, 24-COOMe), 3.86 (1H, m, w/2 ∿ 8 Hz, 7-H), 3.98 (1H, m, w/2 ∿ 8 Hz, 12-H). MS m/e 628 $[(M+5)-CH_3]^+$, 463 $[(M+5)-2\ TMSOH]^+$, 348 $[(M+5)-2\ TMSOH\text{-side chain}]^+$. Isotopic purity: 75% (M+5), 27% (M+4).

Methyl 7α-acetoxy-3α-hydroxy-5β-cholan-24-oate (7). Chenodeoxycholic acid 5 was acetylated according to the procedure of Fieser and Rajagopalan (4). The diacetate 6 (1.78 g, 3.7 mmol) was selectively hydrolyzed (5) with methanol (19 mL) and acetyl chloride (0.94 mL) and the product was worked up according to published procedure (5) to yield a crude oil 7 (1.60 g, 96%) which was sufficiently pure (TLC, NMR) to be used in the next reaction step. An analytically pure sample was prepared by chromatography on TLC (60% ethyl acetate/hexane) and crystallization from ether/hexane; m.p. 112-114°C [Lit. (18) 115-117°C]; IR $\nu_{max}$ 3350 (OH), 1720 (C=O), 1250 (C-O) $cm^{-1}$; NMR δ 0.67 (3H, s, 18-Me), 0.93 (3H, s, 19-Me), 0.93 (3H, d, J = 6 Hz, 21-Me), 2.07 (3H, s, 7-OCOMe), 3.43 (1H, br.m., w/2 ∿ 17 Hz, 3-H), 3.63 (3H, s, 24-COOMe), 4.80 (1H, m, w/2 ∿ 8 Hz, 7-H).

Methyl 7α-acetoxy-3-oxo-5β-cholan-24-oate (8). The alcohol 7 (1.72 g, 3.84 mmol) was oxidized with Jones reagent to give ketone 8 (1.66 g, 96%). An analytical sample was crystallized from hexane, m.p. 116-118°C [Lit. (18) 119-120°C]; IR $\nu_{max}$ 1725 (C=O), 1230 (C-O) $cm^{-1}$; NMR δ 0.68 (3H, s, 18-Me), 0.92 (3H, d, J = 6 Hz, 21-Me), 1.03 (3H, s, 19-Me), 2.03 (3H, s, 3-OAc), 3.60 (3H, s, 24-COOMe), 4.90 (1H, m, w/2 ∿ 8 Hz, 7-H).

[2α,2β,3β,4α,4β-$^2H_5$]-Methyl 3α,7α-dihydroxy-5β-cholan-24-oate (9b). The ketone 8 (1.41, 3.1 mmol) was deuterated according to the procedure as described for compound 4b. This yielded the crude pentadeuterated chenodeoxycholic acid 9a (1.16 g, 81%) as a white solid which was methylated in the usual manner with p-toluenesulfonic acid and methanol and chromatographed (5 x 20% acetone/hexane giving 9b as an oil, IR $\nu_{max}$ 3400 (OH), 2100 (CD), 1730 (C=O), 1250 (C-O) $cm^{-1}$; NMR δ 0.67 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 0.93 (3H, d, J = 6 Hz, 21-Me), 3.60 (3H, s, 24-COOMe), 3.77 (1H, m, w/2 = 8 Hz, 7-H); MS m/e 465 $[(M+5)\text{-TMSOD}]^+$, 374 $[(M+5)\text{-(TMSOH + TMSOD)}]^+$; Isotopic purity: 63% (M+5), 37% (M+4).

Methyl 7β-acetoxy-3α-hydroxy-5β-cholan-24-oate (12). Ursodeoxycholic acid 10 (350 mg, 0.89 mmol) was acetylated according to the procedure for compound 6 to give the diacetoxy acid 11 (340 mg, 80%) as a white solid, m.p. 90-92°C; IR $\nu_{max}$ 3200 (COOH), 1720 (C=O), 1240, 1020 (C-O) $cm^{-1}$; NMR δ 0.70 (3H, s, 18-Me), 0.97 (3H, d, J = 6 Hz, 21-Me), 0.98 (3H, s, 19-Me), 1.98 (3H, s, 7-acetoxy Me), 2.03 (3H, s, 3-acetoxy Me), 4.67 (2H, br.m., w/2 ∿ 22 Hz, 3 and 7-H).

The crude diacetoxy acid 11 was selectively hydrolyzed as described for compound 6 yielding the 3α-alcohol 12 (284 mg, 71% overall) as an oil, IR $\nu_{max}$ 3400 (OH), 1720 (C=O), 1230, 1020 (C-O) $cm^{-1}$; NMR δ 0.70 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 0.98 (3H, d, J = 6 Hz, 21-Me), 1.98 (3H, s, 7-acetoxy Me), 3.50 (1H, br.m., w/2 ∿ 18 Hz, 3-H), 2.63 (3H, s, 24-COOMe), 4.73 (1H, br.m., w/2 ∿ 20 Hz, 7-H).

Methyl 7β-acetoxy-3-oxo-5β-cholan-24-oate (13). Alcohol 12 (297 mg, 0.66 mmol) was oxidized with Jones reagent to give the ketone 13 (227 mg, 77%) as crystals. An analytical sample was recrystallized from ethyl acetate/hexane, m.p. 88-90°C; IR $\nu_{max}$ 1720 (C=O), 1250, 1020 (C-O) $cm^{-1}$; NMR δ 0.75 (3H, s, 18-Me), 0.93 (3H, d, J = 6 Hz, 21-Me), 1.08 (3H, s, 19-Me), 3.62 (3H, s, 24-COOMe), 4.73 (1H, br.m., w/2 ∿ 18 Hz, 7-H).

[2α,2β,3β,4α,4β-$^2H_5$]-Methyl 3α,7β-dihydroxy-5β-cholan-24-oate (14b). The ketone 13 (478 mg, 1.06 mmol) was deuterated according to procedures for compound 4b to give the labeled product 14a (375, 89%) as crude crystals which was then esterified as described for compound 2 and purified by preparative TLC (5 x 30% ethyl acetate/hexane) to give the methyl ester 14b (320 mg, 76% overall) as colorless needles. An analytical sample was recrystallized from aqueous methanol, m.p. 157-159°C [Lit. (19) 159-161°C]; IR $\nu_{max}$ 3350 (OH), 2100 (C-D), 1720 (C=O), 1250 (C-O) $cm^{-1}$; NMR δ 0.68 (3H, s, 18-Me), 0.94 (3H, d, J = 6 Hz, 21-Me), 0.93 (3H, s, 19-Me), 3.55 (1H, br.m., w/2 ∿ 20 Hz, 7-H),

3.65 (3H, s, 24-COOMe); MS m/e 542 $[(M+5)-CH_3]^+$, 465 $[(M+5)-TMSOH]^+$, 374 $[(M+5)-(TMSOH + TMSOD)]^+$. Isotopic purity: 64% (M+5), 36% (M+4).

Methyl 3α-hydroxy-7-oxo-5β-cholan-24-oate (15). The diol 5b was selectively oxidized at C-7 with potassium chromate according to the Haslewood procedure (9) to give the hydroxy ketone 13 as a crystalline solid. An analytical sample was prepared by recrystallization from methanol, m.p. 104-106°C [Lit. (18) 107-109°C], IR $\nu_{max}$ 3400 (OH), 1720 (C=O), 1180 (C-O) $cm^{-1}$; NMR δ 0.65 (3H, s, 18-Me), 0.95 (3H, d, J = 6 Hz, 21-Me), 1.20 (3H, s, 19-Me), 3.5 (1H, br.m., w/2 ∿ 17 Hz, 3-H), 3.62 (3H, s, 24-COOMe).

[6α,6β,7β,8β-$^2H_4$]-Methyl 3α,7α-dihydroxy-5β-cholan-24-oate (16b). The hydroxy ketone 15 (300 mg, 0.7 mmol) was deuterated as described for compound 4b to yield the deuterated methyl chenodeoxycholate 16b (184 mg, 60%) as an oil, IR $\nu_{max}$ 3350 (OH), 2100 (C-D), 1720 (C=O), 1250 (C-O) $cm^{-1}$; NMR δ 0.67 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 0.93 (3H, d, J = 6 Hz, 21-Me), 3.43 (1H, br.m., w/2 ∿ 18 Hz, 3-H), 3.62 (3H, s, 24-COOMe); MS m/e 373 $[(M+4)-(TMSOH + TMSOD)]^+$, 358 $[(M+4)-(THSOH + TMSOD + CH_3)]^+$. Isotopic purity: 56% (M+3), 39% (M+2), 5% (M).

[6α,6β,7α,8β-$^2H_4$]-Methyl 3α,7β-dihydroxy-5β-cholan-24-oate (17b). The hydroxy ketone 15 (450 mg, 1.1 mmol) in deuterated n-butanol (OD) (98 + atom % D, 10 mL) was heated to 120°C in an $N_2$ atmosphere to which sodium pieces (∿ 600 mg ∿ 26 mmol) were added and the reaction was maintained at 120°C for 1/2 h. Worked up according to the procedure for compound 4b yielded the deuterated methyl ursodeoxycholate 17b (300 mg, 67%) as a white solid. An analytical sample was recrystallized from ether/hexane, m.p. 148-150°C [Lit. (19) 152°C]; IR $\nu_{max}$ 3350 (OH), 2100 (C-D), 1720 (C=O), 1260 (ester) $cm^{-1}$; NMR δ 0.67 (3H, s, 18-Me), 0.92 (3H, d, J = 6 Hz, 21-Me), 0.93 (3H, s, 18-Me), 3.63 (1H, m. w/2 ∿ 18 Hz, 3-H), 3.67 (3H, s, 24-COOMe);

MS m/e 539 $[(M+4)-CH_3]^+$, 463 $[(M+4)-TMSOD]^+$, 374 $[(M+4)-(TMSOH + TMSOD)]^+$, 358 $[(M+4)-(TMSOH + TMSOD + CH_3)]^+$. Isotopic purity 60% (M+4), 40% (M+3).

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