LABELED BILE ACIDS V:

Deuterium and Carbon-13 Labeled Cholic, Chenodeoxycholic and Ursodeoxycholic Acids (1)

Chee Kong Lai, Chang Yon Byon, Marcel Gut*,
Danuta Mostowicz[Δ] and Warren G. Anderson**
Worcester Foundation for Experimental Biology
Shrewsbury, MA 01545

SUMMARY

This paper describes a general, simple and short procedure for the introduction of stable isotopes (deuterium and carbon-13) to the side chain of bile acids. The bisnorcholyl aldehydes of cholic and chenodeoxycholic acids are key intermediates, while the isotope is introduced by the Wittig condensation of [1,2-$^{13}C_2$]-(carbethoxymethylene)triphenylphosphorane.

Key Words: Bile acids, Cholic, Chenodeoxycholic, Ursodeoxycholic, Deuterium and carbon-13 labeled, [1,2-$^{13}C_2$]-(Carbethoxymethylene)triphenylphosphorane.

INTRODUCTION

In a previous publication (2) we reported an efficient method for labeling the side chain of 3β-hydroxychol-5-en-24-oic acid by the condensation of bisnorcholyl aldehyde with [1,2-$^{13}C_2$]-(carbethoxymethylene)triphenylphosphorane (3). We now describe its general application towards the syntheses of labeled cholic, chenodeoxycholic

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* Author to whom correspondence is to be addressed.

Δ Present address: Polish Academy of Science, 01-224 Warszawa, Poland

**Present address: Biomeasure, Hopkinton, MA 01748

0362-4803/84/070627-11$01.10

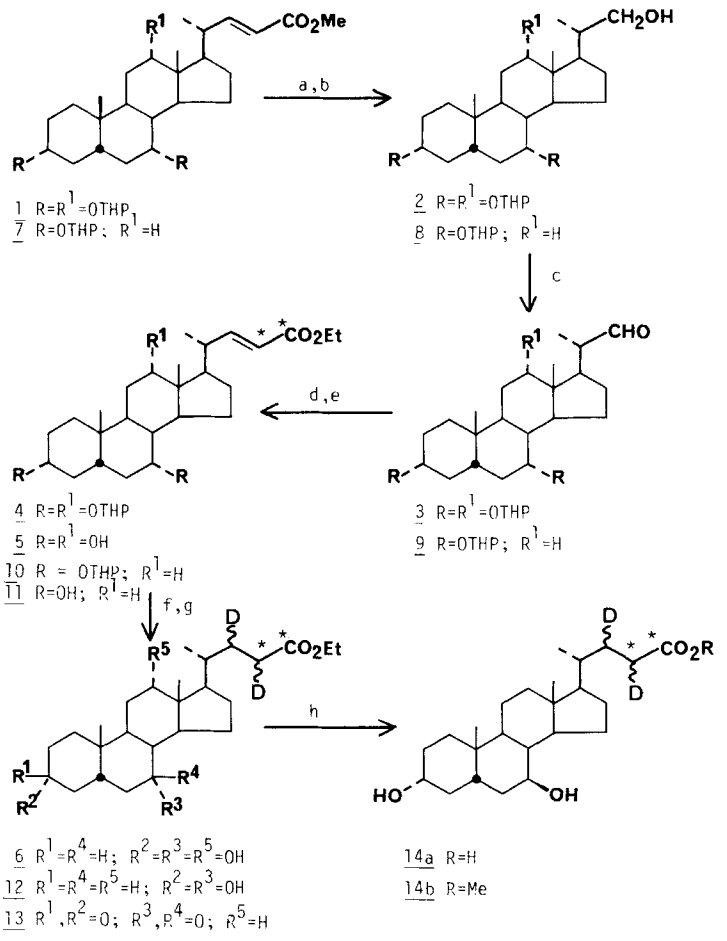
Received February 15, 1984
Revised March 26, 1984

and ursodeoxycholic acids, all of which are useful tracers in the study of the catabolism of cholesterol in the liver.

DISCUSSION

The known conjugated esters of cholic 1 and chenodeoxycholic 7 acids (4) were convenient starting materials. The side chain of the respective conjugated esters 1 and 7 were degraded via ozonolysis

SCHEME



(a) O_3/py/CH_2Cl_2; (b) Vitride; (c) PCC/CH_2Cl_2; (d) $\phi_3P{=}^{*}CH^{*}COOEt$ (17); (e) TsOH/MeOH; (f) Pd-C/D_2/EtOD; (g) Jones reagent; (h) Na/n-BuOH/120°C

followed by reductive workup with Vitride (5) to give the bisnorcholyl alcohols 2 and 8 which were then oxidized with pyridium chlorochromate to form the aldehydes 3 and 9, respectively. Attempts to generate the aldehydes directly by ozonolysis (6) gave the products in very poor yields. The aldehydes 3 and 9 are relatively unstable, and were therefore used directly without further purification for the next step of the reaction sequence.

Condensation of the aldehyde 15 with (carbethoxymethylene)triphenylphosphorane yielded the (E)-ethyl 3β-tetrahydropyranyloxychola-5,22-dien-24-oate (16) exclusively. (See references (2) and (3) for data and detail of preparation of 16.) The (E)-alkene stereochemistry was rationalized by the coupling constant value (16 Hz) which was comparable to that acceptable for the (E) configuration (4). Similarly, Wittig condensation of the aldehydes 3 and 9 with [1,2-$^{13}C_2$]-(carbethoxymethylene)triphenylphosphorane (17) formed the respective conjugated esters 4 and 10. The coupling pattern of the C-23 proton of the ^{13}C-labeled conjugated esters 4 and 10 is more complex than its original ABX system. The two arms of H_B is now coupled to ^{13}C-23 ($\sim$ 160 Hz) and then further long range coupled to ^{13}C-24 ($\sim$ 3 Hz), creating altogether four pairs of doublets (Fig. 1).

The conjugated esters were saturated with deuterium to yield the (M+4) labeled compound. The labeled methyl ursodeoxycholate 14 was synthesized from labeled ethyl chenodeoxycholate 12 in a high yield two step reaction: Oxidation of 12 with Jones reagent gave the diketone 13 which was then reduced with sodium/n-butanol resulting predominantly in the isomer 14a.

EXPERIMENTAL

Melting points were determined on a Kofler melting point apparatus and are uncorrected. The UV spectra were determined in acetonitrile solutions on a Perkin-Elmer 202 spectrophotometer. The IR spectra of

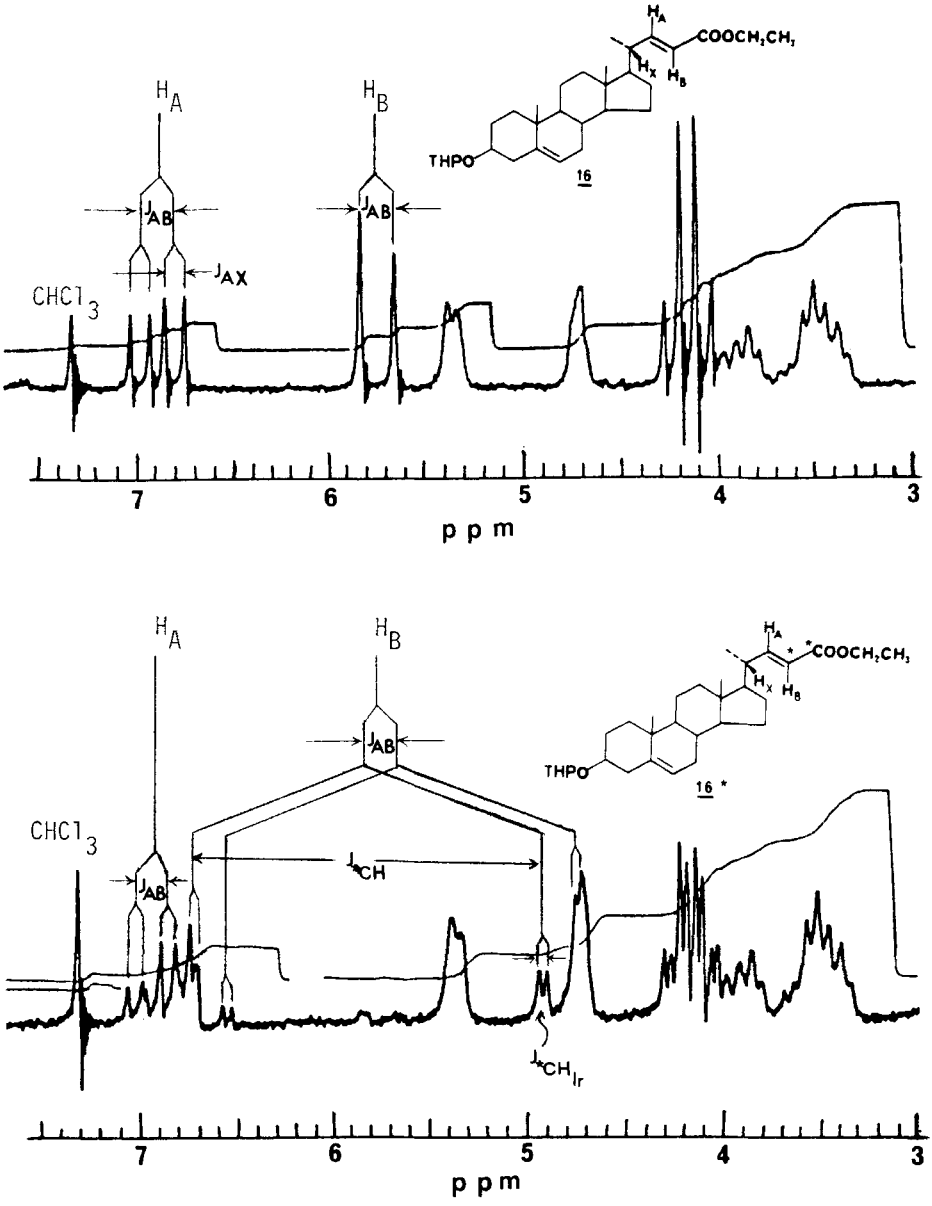


Figure 1. 90 MHz ^{1}H NMR of ^{13}C-labeled and unlabeled ethyl 3β-tetrahydropyranyloxychola-3,22-dien-24-oate (2). "*" indicates ^{13}C-labeling.

crystals were determined as KBr pellets, and of oils as a film on sodium chloride windows. The NMR spectra were obtained in deuteriochloroform solution using tetramethylsilane as an internal reference and were recorded on a 90 MHz Varian EM-390 spectrometer. Mass spectra were recorded on a Nuclide 1290 G spectrometer using a direct insertion probe and a Hewlett Packard 5992A GC/MS system where applicable. Elemental analyses were performed by the Microanalysis Laboratory of the University of Massachusetts at Amherst.

3α,7α12α-Tri[(tetrahydro-2H-pyran-2-yl)oxy]-22,23-bisnor-5β-cholan-24-ol (2). A solution of the conjugated ester 1 (208 mg, 0.3 mmol) in methylene chloride (40 mL) and pyridine (1 mL) was cooled to -78°C and treated with ozone until a blue coloration was formed. The vessel was flushed with nitrogen and sodium dihydrido-bis(2-methoxyethoxy) aluminate (180 mg, 0.9 mmol) was added. The mixture was stirred at -78°C for 1 hr then allowed to warm to 0°C over a 1 hr period and 2N sulfuric acid was added to decompose any excess hydride. The mixture was poured into water and the product was extracted with methylene chloride, the organic layers washed successively with 10% sulfuric acid and saturated aqueous sodium bicarbonate. Evaporation of solvents yielded the crude alcohol 2 which was purified by preparative TLC (50% acetone/hexane) to give the alcohol 2 (150 mg, 78%) as an oil. IR ν_{max} 3400 (OH), 1080, 1020, 980 (C-O) cm^{-1}; ^1H NMR δ 0.68 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.03 (3H, d, J = 6 Hz, 21-Me), 3.67 (11H, br.m., w/2 ∿ 60 Hz, -OCH- and $-OCH_2-$), 4.67 (3H, m, w/2 ∿ 11 Hz, -O-CH-O-).

Anal. Calcd. for $C_{37}H_{62}O_7$: C, 71.80; H, 10.10. Found: C, 71.91; H, 10.00.

3α,7α,12α,Tri[(tetrahydro-2H-pyran-2-yl)oxy]-22,23-bisnor-5β-cholan-24-al (3). The alcohol 2 was oxidized with pyridinium chlorochromate in methylene chloride (2) to the aldehyde 3 which was purified by filtration through a column of florisil. Evaporation of solvent gave the

aldehyde 3 (71%) as an oil. The crude aldehyde was used immediately for the next step. IR ν_{max} 2700 (C-H of aldehyde), 1720 (C=O), 1020, 980 (THP ether) cm^{-1}; ^1H NMR δ 0.72 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.08 (3H, d, J = 6 Hz, 21-Me), 3.63 (9H, br.m., w/2 ~ 35 Hz, -O-CH- and -O-CH_2-), 4.63 (3H, m, w/2 ~ 10 Hz -O-CH-O-), 9.43 (1H, m, w/2 ~ 6 Hz, -CHO).

[23,24-$^{13}C_2$]-(E)-Ethyl 3α,7α,12α-tri[(tetrahydro-2H-pyran-2-yl)oxy]-5β-chol-22-en-24-oate (4). The ester 4 was synthesized from the aldehyde 3 according to a procedure already described (2). The crude material was purified by preparative TLC (30% ethyl acetate/hexane) to give the ester 4 (29%) as an oil. IR ν_{max} 1720 (^{12}COOEt, weak), 1670 (^{13}COOEt, strong), 1610 (^{13}C=C), 1030, 980 (C-O) cm^{-1}; ^1H NMR δ 0.70 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.07 (3H, d, J = 6 Hz, 21-Me), 1.29 (3H, t, J = 7 Hz, 24-*COOCH$_2$CH$_3$), 3.67 (9H, br.m., w/2 ~ 31 Hz, -O-CH- and -O-CH_2-), 4.19 (2H, dq, J = 7 Hz, $J_{*CH_{1r}}$ = 3 Hz, 24-*COOCH$_2$CH$_3$), 4.77 (3H, m, w/2 ~ 16 Hz, -O-CH-O-), ABX system H_A 6.94 (1H, dd, J_{AB} = 16 Hz, J_{AX} = 7 Hz, 22-H, H_B 5.67 (1H, ddd, J_{AB} = 16 Hz, J_{*CH} = 160 Hz, $J_{*CH_{1r}}$ = 3 Hz, 23-H).

[23,24-$^{13}C_2$]-(E)-Ethyl 3α,7α,12α-trihydroxy-5β-chol-22-en-24-oate (5). The THP ether 4 (200 mg) was hydrolyzed in the usual manner with p-toluenesulfonic acid in methanol. Purification of the crude product by preparative TLC (50% acetone/hexane) gave the triol 5 (124 mg, 98%). An analytical sample was prepared by crystallization from ether; m.p. 173-175°C; IR ν_{max} 3400 (OH), 1660 (*C=O), 1600 (*C=C) cm^{-1}; UV (acetonitrile) λ_{max} 215 nm (ε 12,900); ^1H NMR δ 0.72 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.17 (3H, d, J = 6 Hz, 21-Me), 1.29 (3H, t, J = 7 Hz, 24-*COOCH$_2$CH$_3$). 3.50 (1H, br.m., w/2 ~ 20 Hz, 3-H), 3.86 (1H, m, w/2 ~ 9 Hz, 7-H), 3.97 (1H, m, w/2 ~ 9 Hz, 12-H), 4.20 (2H, dq, J = 7 Hz, $J_{*CH_{1r}}$ = 3 Hz,

24-*COOC$\underline{H}_2$CH$_3$), ABX system H_A 6.94 (1H, dd, $\underline{J}_{AB}$ = 16 Hz, $\underline{J}_{AX}$ = 7 Hz, 22-H), H_B 5.67 (1H, ddd, $\underline{J}_{AB}$ = 16 Hz, $\underline{J}_{*CH}$ = 160 Hz, $\underline{J}_{*CH_{1r}}$ = 3 Hz, 23-H); ^{13}C NMR δ 167.2 (d, $\underline{J}_{*C*C}$ = 74.5 Hz, ^{13}C-23); MS m/e 472 [(M+2) -3TMSOH]$^+$, [M - (side chain + 3TMSOH)]$^+$; Isotopic purity: 87.6% (M+2), 12.4% (M).

[23,24-$^{13}C_2$-22ξ,23ξ-$^{2}H_2$]-Ethyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate (6). To the olefin 5 (45 mg, 0.10 mmol), in deuterated ethanol (OD) (2 mL, 99.5 + atom % D) was added 5% platinum-on-charcoal (20 mg) and placed in a hydrogenator under a deuterium atmosphere (99.5 + atom % D). The mixture was stirred vigorously overnight and then filtered through a pad of celite. The filtrate was worked up in the usual manner and purified on preparative TLC (50% acetone/hexane) to give the ester 6 (35 mg, 76%) as colorless prisms. An analytical sample was prepared by recrystallization from ethyl acetate; m.p. 157-159°C [Lit. 162°C (7)]; IR ν_{max} 3350 (OH), 2150 (CD), 1670 (*C=O), 1180, 1070, 1040 (C-O) cm^{-1}; ^{1}H NMR δ 0.68 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 1.02 (3H, d, $\underline{J}$ = 6 Hz, 21-Me), 1.25 (3H, t, $\underline{J}$ = 7 Hz, 24-*COOC$\underline{H}_2$C$\underline{H}_3$), 3.45 (1H, br.m., w/2 ∿ 20 Hz, 3-H), 3.86 (1H, m, w/2 ∿ 9 Hz, 7-H), 3.98 (1H, m, w/2 ∿ 9 Hz, 12-H), 4.12 (2H, dq, $\underline{J}$ = 7 Hz, $\underline{J}_{*CH_{1r}}$ = 3 Hz, 24-*COOC$\underline{H}_2$CH$_3$); MS m/e 476 [(M+4) -2TMSOH]$^+$, 386 [(M+4) -3THSOH]$^+$, 253 [M - (side chain) + 3TMSOH)]$^+$; Isotopic purity: 69% (M+4), 31% (M+3).

3α,7α-Di[tetrahydro-2H-pyran-2-yl)oxy]-22,23-bisnor-5β-cholan-24-ol (8). A solution of the conjugated ester 7 (4) (1.24 mg, 2.16 mmol) was ozonized according to the procedure for compound 2. Reductive workup of the solution, followed by chromatography yielded the alcohol 8 (840 mg, 75%) as an oil; IR ν_{max} 3400 (OH), 1080, 1020, 980 (C-O) cm^{-1}; ^{1}H NMR δ 0.67 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.05 (3H, d, $\underline{J}$ = 6 Hz, 21-Me), 3.50 (4H, br.m., w/2 ∿ 27 Hz, -OCH), 3.83 (4H, br.m., w/2 ∿ 33 Hz, -OCH- and -OC$\underline{H}_2$-), 4.63 (2H, m, w/2 ∿ 17 Hz, -O-CH-O-).

Anal. Calcd. for $C_{32}H_{54}O_5$: C, 74.09; H, 10.49. Found: C, 74.01; H, 10.53.

3α,7α-Di[tetrahydro-2H-pyran-2-yl)oxy]-22,23-bisnor-5β,cholan-24-al (9). The alcohol 8 (855 mg, 1.45 mmol), oxidized according to the procedure for compound 3 with pyridinium chlorochromate yielded the aldehyde 9 (630 mg, 74% as an oil which was sufficiently pure for immediate use in the next reaction sequence; IR ν_{max} 2700 (C-H aldehyde), 1720 (C=O), 1020, 980 (THP ether) cm^{-1}; ^1H NMR δ 0.70 (3H, s, 18-Me), 0.92 (3H, s, 19-Me), 1.15 (3H, d, J = 7 Hz, 21-Me), 3.64 (6H, br.m., w/2 ∿ 35 Hz, -O-CH- and -O-CH_2), 4.64 (2H, m, w/2 ∿ 10 Hz, -O-CH-O-), 9.45 (1H, m, w/2 ∿ 6 Hz, -CHO).

[23,24-$^{13}C_2$]-(E)-Ethyl 3α,7α-di[(tetrahydro-2H-pyran-2-yl)oxy]-5β-chol-22-en-24-oate (10). The ester 10 was synthesized from the aldehyde 9 according to the procedure described previously (2). The crude material was purified by preparative TLC (30% ethyl acetate/hexane) to give the ester 10 (30%) as an oil; IR ν_{max} 1670 (*C=O), 1610 (*C=C), 1070, 980 (C-O) cm^{-1}; ^1H NMR δ 0.67 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.09 3H, d, J = 7 Hz, 21-Me), 1.28 (3H, t, J = 7 Hz, 24-*COOCH$_2$CH$_3$), 3.50 (4H, m, w/2 ∿ 19 Hz, -O-CH- and -O-CH_2-), 3.87 (2H, m, w/2 ∿ 25 Hz, -O-CH-), 4.20 (2H, dq, J = 7 Hz, $J_{^*CH_{1r}}$ = 3 Hz, 24-*COOCH$_2$CH$_3$), 4.69 (2H, m, w/2 ∿ 16 Hz, -O-CH-O-), ABX system H_A 6.94 (1H, dd, J_{AB} = 16 Hz, J_{AX} = 7 Hz, 22-H), H_B 5.67 (1H, ddd, J_{AB} = 16 Hz, $J_{^*CH}$ = 160 Hz, $J_{^*CH_{1r}}$ = 3 Hz, 22-H).

[23,24-$^{13}C_2$]-(E)-Ethyl 3α,7α-dihydroxy-5β-chol-22-en-24-oate (11). The THP ether 10 (50 mg, 0.12 mmol) was hydrolyzed in the usual manner (4) with p-toluenesulfonic acid in methanol. Purification of the crude product by preparative TLC (50% acetone/hexane) gave the diol 11 (42 mg, 83%) as an oil; IR ν_{max} 3400 (OH), 1670 (*C=O), 1610 (*C=C) cm^{-1};

UV (acetonitrile) λ_{max} 216 nm (ε 10,700); ^{1}H NMR δ 0.71 (3H, s, 18-Me), 0.92 (3H, s, 19-Me), 1.10 (3H, d, J = 6 Hz, 21-Me), 1.29 (3H, t, J = 7 Hz, 24-*COOCH$_2$CH$_3$), 3.49 (1H, br.m., w/2 ∿ 20 Hz, 3-H), 3.87 (1H, m, w/2 ∿ 9 Hz, 7-H), 4.20 (2H, dq, J = 7 Hz, $J_{*CH_{1r}}$ = 3 Hz, 24-*COOCH$_2$CH$_3$), ABX system H_A 6.94 (1H, dd, J_{AB} = 16 Hz, J_{AX} = 7 Hz, 22-H), H_B 5.67 (1H, ddd, J_{AB} = 16 Hz, J_{*CH} = 160 Hz, $J_{*CH_{1r}}$ = 3 Hz, 22-H); ^{13}C NMR δ 167.2 (d, J_{*C*C} = 74.5 Hz, ^{13}C-24), 118.7 (d, J_{*C*C} = 74.5 Hz, ^{13}C-23); MS m/e 374 [(M+2) -TMSOH]$^+$, 384 [(M+2) -2TMSOH]$^+$, 255 [M - (side chain + 2TMSOH)]$^+$; Isotopic purity: 81.1% (M+2), 18.9% (M).

[23,24-$^{13}C_2$-22ξ,23ξ-$^2H_2$]-Ethyl 3α,7α-dihydroxy-5β-cholan-24-oate (12). The olefin 11 (50 mg, 0.12 mmol) was saturated with deuterium following the same procedure used for compound 6. Purification by preparative TLC (50% acetone/hexane) yielded the labeled ethyl chenodeoxycholate 12 (42 mg, 83%) as an oil; IR ν_{max} 3300 (OH), 2150 (CD), 1670 (*C=O), 1160, 1070 (C-O) cm^{-1}; ^{1}H NMR δ 0.66 (3H, s, 18-Me), 0.91 (3H, s, 19-Me), 0.93 (3H, d, J = 6 Hz, 21-Me), 1.25 (3H, t, J = 7 Hz, 24-*COOCH$_2$CH$_3$), 3.41 (1H, br.m., w/2 ∿ 20 Hz, 3-H), 3.82 (1H, m, w/2 ∿ 9 Hz, 7-H), 4.09 (2H, dq, J = 7 Hz, $J_{*CH_{1r}}$ = 3 Hz, 24-*COOCH$_2$CH$_3$); MS m/e 388 [(M+4) -2TMSOH]$^+$, 255 [M - side chain + 2TMSOH)]$^+$; Isotopic purity: 71% (M+4), 29% (M+3).

[23,24-$^{13}C_2$-22ξ,23ξ-$^2H_2$]-Ethyl 3,7-dioxo-5β-cholan-24-oate (13). The labeled ethyl chenodeoxycholate 12 (60 mg, 0.14 mmol) was oxidized with Jones reagent as previously described (4). Worked up in the usual manner yielded the dioxo compound 13 (53 mg, 87%). An analytical sample was recrystallized from acetone/hexane to give colorless needles, m.p. 125-127°C; IR ν_{max} 2180 (C-D), 1720 (C=O), 1690 (*C=O), 1175 (C-O) cm^{-1}; ^{1}H NMR δ 0.67 (3H, s, 18-Me), 0.93 (3H, d, J = 6 Hz, 21-Me), 1.20

(3H, s, 19-Me), 1.27 (3H, t, $\underline{J}$ = 7 Hz, 24-$^{*}COOCH_2\underline{CH_3}$), 4.1 (2H, dq, $\underline{J}$ = 7 Hz, $\underline{J}_{*CH_{1r}}$ = 3 Hz, 24-$^{*}COO\underline{CH_2}CH_3$).

[23,24-$^{13}C_2$-22ξ,23ξ-$^{2}H_2$]-Methyl 3α,7α-dihydroxy-5β-cholan-24-oate (14b). The labeled dioxo compound 13 (40 mg, 0.09 mmol) in hot anhydrous n-butanol (120°C) was treated with sodium according to the procedure described previously (4). Worked up, methylated and chromatographed according to that procedure (3), yielded the labeled methyl ursodeoxycholate 14b (28 mg, 70%) as a white solid. An analytical sample was recrystallized from ether/hexane, m.p. 149-151°C [Lit. unlabeled compound (8) 152°C]. IR ν_{max} 3330 (OH), 2180 (C-D), 1720 (C=O), 1690 (*C=O), 1150 (C-O) cm^{-1}; ^{1}H NMR δ 0.68 (3H, s, 18-Me), 0.93 (3H, d, $\underline{J}$ = 6 Hz, 21-Me), 0.94 (3H, s, 19-Me), 3.55 (2H, br.m., w/2 ∿ 20 Hz, 3 and 7-H), 3.65 (3H, d, $\underline{J}_{*CH_{1r}}$ = 3 Hz, 24-*COOMe); MS m/e 410 $[M+4]^+$, 392 $[(M+4)-H_2O]^+$, 374 $[(M+4)-2H_2O]^+$, 359 $[(M+4)-(2H_2O+CH_3)]^+$. 273 [M-side chain]$^+$, 225 $[M-(2H_2O$ + side chain)$]^+$; Isotopic purity: 39% (M+4), 45% (M+3), 13% (M+2), 3% (M+1).

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3. However, we did not report in detail the complex NMR pattern of these condensation products (Fig. 1): NMR of 16*, δ 0.70 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.07 (3H, d, $\underline{J}$ = 7 Hz, 21-Me), 1.26 (3H, t, $\underline{J}$ = 7 Hz, 24-$^{*}COOCH_2\underline{CH_3}$), 3.50 (2H, m, w/2 ∿ 20 Hz, -O-$\underline{CH_2}$), 3.89 (1H, m, w/2 ∿ 14 Hz, -O-CH-), 4.15 (2H, dq, $\underline{J}_{*CH_{1r}}$ = 3 Hz,

$\underline{J}$ = 7 Hz, 24-*COO$\underline{CH}_2$CH$_3$), 4.73 (1H, m, w/2 ∿ 9 Hz, -O-CH-O-) ABX system H_A 6.91 (1H, dd, $\underline{J}_{AB}$ = 16 Hz, $\underline{J}_{AX}$ = 6 Hz, 22-H), H_B 5.63 (1H, ddd, $\underline{J}_{AB}$ = 16 Hz, $\underline{J}_{*CH}$ = 160 Hz, $\underline{J}_{*CH_{1r}}$ = 3 Hz, 23-H). For *C-H coupling, see Stothers, J. B. - In Carbon-13 NMR Spectroscopy, Academic Press, New York, 1972, Chpt. 10.

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