Synthesis of 1β-hydroxydeoxycholic acid in H-2 and unlabeled forms

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Abstract

1β–hydroxydeoxycholic acid in unlabeled and stable isotope labeled forms was required for use as a biomarker for Cytochrome P450 3A4/5. A lengthy synthesis was undertaken to deliver the unlabeled compound and in the process, to develop a route to the deuterium labeled compound. The synthesis of the unlabeled compound was completed, but in very low yield. Concurrent with the synthetic approach, a biosynthetic route was pursued and this approach proved to be much more rapid and afforded the compound in both unlabeled and deuterium labeled forms in a one-step oxidation from deoxycholic acid and [D_4]deoxycholic acid respectively.

Introduction

Bile acids are steroids which are the major organic components of bile. In humans, cholic acid and chenodeoxycholic acid are primary bile acids (figure 1); they are synthesized in the liver from cholesterol in a multistep process.[1] These compounds are conjugated to taurine or glycine prior to secretion into the intestine and after conjugation are termed bile salts.[2] Intestinal bacteria can then further metabolize these bile salts. One key pathway consists of the hydrolysis of the bile salt to the bile acid and then dehydroxylation of the 7-hydroxyl of cholic acid or chenodeoxycholic acid to give deoxycholic acid (DCA) or lithocholic acid respectively.[3] Bile acids and salts can be reabsorbed into the blood stream by enterohepatic circulation and can undergo further biotransformation in the liver to give a plethora of products among which are hydroxylated versions of DCA.[1,4]

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jlcr.3495

The formation of 1β –hydroxydeoxycholic acid was recently reported to be a biomarker for cytochrome P-450 3A4 activity *in vivo*.[5,6] To further develop this as a potential clinical biomarker, both unlabeled and stable isotope labeled versions of the compound were required.[7,8]

Results

The synthesis of 1β-hydroxydeoxycholic acid (**1)** in unlabeled form was reported previously,[7,8] and we did not anticipate any significant problems in replicating the reported procedures (Scheme 1). Beta-hydroxy ketone **2** was proposed as a key intermediate to serve as the starting material for the incorporation of the deuterium labels *via* a base catalyzed exchange reaction. The protons on the carbon alpha to the ester could also potentially exchange which was not viewed as problematic; however, the beta-ketoalcohol of **2** could potentially eliminate to give enone **3** under the proposed exchange conditions as was observed previously for a similar substrate (Scheme 2).[9] Therefore, this reaction needed to be probed as soon as possible. Assuming the elimination was minimal, once the deuterated alcohol was obtained, it could then be converted to the final compound using the same procedure used for the unlabeled compound.

The synthesis started from the readily available DCA, and the first four steps – esterification of the acid, oxidation of the C-3 hydroxyl to the ketone, acetylation of the C-12 hydroxyl, and dibromination – rapidly delivered dibromoketone **4** for use in the $Cr(OAc)_2$ mediated debromination (see Scheme 3).[10] Attemps to replicate the $Cr(OAc)_2$ debromination failed to give the target compound under a variety of conditions. Therefore, an alternate approach was used in which the C-2 bromide was first eliminated with Li_2CO_3 to give monobromoketone **5** followed by debromination with Zn to afford enone **6**.[11] Epoxidation of **6** under basic conditions provided epoxide **7**,[8,11] but when **7** was reacted with $Cr(OAc)_2$ in EtOH, none of the target compound was observed under a variety of conditions.[12] It has been reported that $Cr(OAc)_2$ is sensitive to oxygen;[13] it is likely that the quality of the reagent used in the debromination of **4** and in the epoxide opening of **6** was not sufficient to catalyze these transformations since these reactions are well precedented.

Therefore, alternate epoxide opening conditions were investigated (Scheme 4). LiBr opening of epoxide **7** followed by Pd-catalyzed hydrogenation gave a mixture of products which were difficult to separate.[14,15] SmI_2 mediated epoxide ring opening gave a poor yield of a low purity compound.[16] Reduction with $Na[PhSeB(OEt)_3]$ in EtOH afforded the target compound in 60% yield and good purity by NMR.[17] This later route appeared promising, but while this development work was proceeding, enough alcohol had been obtained from these probe reactions to investigate the key step for deuterium incorporation.

The reaction was probed with unlabeled materials first to simplify the analysis and under several reaction conditions (LiOH or NaOH, room temp or 100 °C) complete elimination to give the enone and either full or partial acetate hydrolysis (Scheme 5) was observed. This result necessitated a redesign of the proposed route to the labeled compound.

A recent report used a silyl group as a surrogate for a hydroxy group in a similar compound;[14] therefore, enone **6** was reacted with the cuprate formed from $Me_2PhSiCl$ to give **10** after conjugate addition (Scheme 6). The silane was converted to alcohol **8** using a Fleming–Tamao oxidation. Reduction of ketone **8** to alcohol **11** (as a mixture of diastereomeric alcohols) and subsequent hydrolysis of the esters and purification by silica gel chromatography gave an alcohol as the major product in 46% yield for the two steps. The modest yield reflects the difficulty of the separation of the diastereotopic alcohols **1** and **12**. Unfortunately, on carefully analysis by NMR spectroscopy, the isolated alcohol was determined to be the incorrect diastereomer, alcohol **12**. A second preparation was performed in which the diastereomeric alcohols were separated to give **13** in a 20% yield. However, the final purification again proved difficult, and the final compound (**1**) was isolated in only a 14% yield. A detailed NMR analysis was used to determine the structures and is detailed in the supplementary material and summarized in the table.

Insert table and caption here.

The use of enzymes for the functionalization of inert C-H bonds has recently been reviewed.[18] During this work, parallel efforts were ongoing to find a biocatalyst which converted DCA to **1** (Scheme 7).[6] A library of commercially available *Bacillus megaterium* P450 mutants from Codexis was investigated for their ability to convert DCA to **1** in a selective manner and in high conversion. These results were compared with a human liver microsomal incubation of DCA which was thought to produce **1** as the major mono-oxygenated metabolite.[19] The comparison allowed the selection of an engineered P450, MicroCYP0029, as a suitable biocatalyst. A scale up reaction was performed according to the vendors protocol and **1** was formed from DCA as the sole major product and was isolated in 27% chemical yield (10.5 mg from 39 mg) by mass directed preparative HPLC. The use of [D_4]DCA afforded **[D_4]-1** with a high, uniform, isotopic incorporation (>95% D_4 which reflected the isotopic distribution of the starting material) in 33% yield (13 mg from 39 mg). This biosynthetic route was competitively priced to the synthetic route so work was halted on the purely synthetic route. For a small scale preparation (<100mg), it was clear the enzymatic method was superior in price and speed. For larger amounts, a close comparison of the costs of the two routes would be required to discern the less expensive option. Although not investigated, it is likely that a purely synthetic method would provide a much less homogenous isotopomer pattern than that of the enzymatic method.

Experimental:

General: All solvents and reagents were obtained from Aldrich or Alfa or other local sources and were used without further purification except for anhydrous solvents which were pre-treated by passing through a 3A MS column before use.[20] [D_4]Deoxycholic acid was obtained from C/D/N. Chromatographic purifications were conducted using flash chromatography on 100~200 mesh silica gel.

LC/MS were acquired on a Shimadzu LC/MS-2010 (Column: Sepax ODS 50×2.0 mm, 5 um) or Agilent 1200 HPLC-1956 MSD (Column: Shim-pack XR-ODS 30×3.0 mm，2.2 um). ^{1}H NMR spectra were recorded on Bruker Avance 400 MHz for ^{1}H NMR or Bruker Avance 600 MHz instruments for ^{1}H, ^{13}C and 2D-experiments. Samples were dissolved in $CDCl_3$, DMSO-d6 or Methanol-d4. NMR experiments were recorded at 25 degrees C. ^{1}H, ^{13}C chemical shifts were referenced *via* the residual solvent signal, set to 7.27/77.0 ($CDCl_3$), 2.5/39.5 (DMSO-d6) or 3.31/49.0 ppm (Methanol-d4). **1**, **12** and **DCA** were assigned using COSY, ROESY, CH correlation and CH long-range correlation experiments using standard Bruker pulse sequences. Methyl Deoxycholate, Methyl 3-oxo-5β-12α -hydroxy-cholanoate, Methyl 3-oxo-5β -12α -acetoxy-cholanoate, **4**, **6**, **7**, and **8** were characterized by ^{1}H NMR and LC/MS, and the spectra were consistant with previously reported data.[8,21]

(R)-methyl 4-((3R,5R,8R,9S,10S,12S,13R,14S,17R)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (Methyl Deoxycholate)
The procedure of Májer was modified as follows.[22] A mixture of deoxycholic acid (50.0 g, 130 mmol) in 4 M HCl in MeOH (2 mol, 500 mL) was stirred at 20 °C for 12 h at which time TLC indicated the reaction to be complete; therefore, the solvent was evaporated. The resulting crude material was dissolved in EtOAc (300 mL), and the solution was washed with sat. aq. $NaHCO_3$ (3x100 mL) and brine (2x100 mL), and was then dried over $MgSO_4$. After filtering, the solution was concentrated to give methyl deoxycholate (45.0 g, 111 mmol, 85%) as a colorless oil.

(R)-methyl 4-((5R,8R,9S,10S,12S,13R,14S,17R)-12-hydroxy-10,13-dimethyl-3-oxohexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate *(*Methyl 3-oxo-5β-12α -hydroxy-cholanoate)
A modification of the procedure of Aher was used.[23] A flask containing a suspension of methyl deoxycholate (25.0 g, 61.5 mmol) and Ag_2CO_3 (35 g, 170 mmol) in toluene (300 mL) was fitted with a Dean-Stark apparatus, and the solution heated at reflux for 48 hrs at which time analysis by TLC showed the reaction to be complete. The mixture was filtered through a pad of celite, and the celite pad was washed with CH_2Cl_2 (3x500 mL). The combined organic layers were concentrated under reduced pressure to give a residue which was purified by silica gel chromatography (3:1, pet ether:EtOAc) to give methyl 3-oxo-5β -12α -acetoxy-cholanoate (21.0 g, 51.9 mmol, 84% yield) as a yellow oil.

(R)-methyl 4-((5R,8R,9S,10S,12S,13R,14S,17R)-12-acetoxy-10,13-dimethyl-3-oxohexadecahydro-1H-c

yclopenta[a]phenanthren-17-yl)pentanoate (Methyl 3-oxo-5β-12α -acetoxy-cholanoate)

A modification of the procedure of Bonar-Law was used.[24] A mixture of methyl 3-oxo-5β-12α -hydroxy-cholanoate (50.0 g, 124 mmol), Ac_2O (125 mL, 1.33 mol), dimethylaminopyridine (1.5 g, 12 mmol) and pyridine (500 mL) was stirred for 1 hr at 20 °C at which time analysis by TLC showed the reaction to be complete. The solvent was evaporated, and the crude residue was taken up in EtOAc (800 mL). The solution was washed with 1M HCl (2x300 mL), sat. aq. $NaHCO_3$ (2x300 mL), and brine (2x300 mL) and was dried over $MgSO_4$. After filtration, the organic layer was concentrated to dryness to give methyl 3-oxo-5β-12α -acetoxy-cholanoate (42.0 g, 94.0 mmol, 76% yield) as a yellow solid.

(R)-methyl 4-((2R,4S,5S,8R,9S,10R,12S,13R,14S,17R)-12-acetoxy-2,4-dibromo-10,13-dimethyl-3-oxohexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**4**)

A modification of the procedure of Tohma was used.[8] A solution of methyl 3-oxo-5β-12α -acetoxy-cholanoate (44.0 g, 98.5 mmol) in HOAc (160 mL) and $CHCl_3$ (80 mL) was stirred as Br_2 (30.7 g, 192 mmol) was added, and the resulting reaction mixture was stirred for 1 hr at 20 °C at which time assay by LC/MS showed the reaction to be complete. The reaction was diluted with sat aq $NaHCO_3$ (300 mL), and the resulting biphasic mixture was extracted with EtOAc (2x300 mL). The combined organic layers were washed with sat aq $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solution was concentrated to dryness under reduced pressure. The resulting residue was washed three times with MeOH to give compound **4** (38.0 g, 62.8 mmol, 64% yield) as an off-white solid (>90% UV purity at 220 nm by HPLC).

(R)-methyl 4-((4S,5S,8R,9S,10R,12S,13R,14S,17R)-12-acetoxy-4-bromo-10,13-dimethyl-3-oxo-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-17-yl)pentanoate (**5**)

A modification of the procedure of Joly was used.[11] A solution of compound **4** (49.0 g, 81.1 mmol) in DMF (400 mL) was stirred as Li_2CO_3 (8.8 g, 120 mmol) and LiBr (9.72 g, 112 mmol) were added, and the reaction was warmed to 80 °C for 4 hrs. The reaction mixture was diluted with EtOAc (500 mL) and washed with 1 N HCl. The combined organic layers were washed with brine, dried and filtered, and the resulting solution was concentrated to dryness under reduced pressure to give compound **5** (40.0 g, HPLC purity of approximately 80%, 75% yield) as a yellow solid, and **5** was used in the next step without further purification.

(R)-methyl 4-((5R,8R,9S,10R,12S,13R,14S,17R)-12-acetoxy-10,13-dimethyl-3-oxo-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-17-yl)pentanoate (**6**)

A modification of the procedure of Tohma was used.[8] A solution of compound **5** (40.0 g, 80% purity by HPLC, 61.1 mmol) in HOAc (300 mL) was stirred as Zn (16.0 g, 245 mmol) was added, and the reaction was stirred at room temperature for 0.5 h at which time assay by LC/MS showed the reaction to be complete. The mixture was filtered and concentrated under

reduced pressure. The resulting residue was purified by column chromatography on silica gel (80:1, Pet ether:EtOAc) to give compound **6** (22.0 g, 65% yield) as a yellow solid.

(R)-methyl 4-[(1S,2S,5R,8S,9S,10S,12S,13R,14S,17R)-12-acetoxy-1,2-epoxy-10,13-dimethyl-3-oxo-1,2,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]pentanoate (**7**)

A modification of the procedure of Tohma was used.[8] A solution of compound **6** (5.00 g, 11.3 mmol) in dioxane (80 mL) was stirred at 0 °C as a solution of 30% aq. H_2O_2 (20.00 mL, 196 mmol) in 2 N NaOH (10.00 mL) was added dropwise. The solution was stirred for 12 h at 25 °C at which point TLC showed the reaction to be completed. The reaction mixture was diluted with brine (100 mL) and was extracted with ethyl acetate (2x150 mL). The combined organic layers were washed with sat aq $Na_2S_2O_3$ (2x100 mL) and were then dried over Na_2SO_4. After filtering, the solution was concentrated to dryness and then purified by column chromatography (80:1, CH_2Cl_2:MeOH) to afford compound **7** (3 g, 57% yield) as a colorless oil.

(R)-methyl 4-((1R,5R,8S,9S,10S,12S,13R,14S,17R)-12-acetoxy-1-(dimethyl(phenyl)silyl)-10,13-dimethyl-3-oxohexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**10**)

A modification of the procedure of Wolfram was used.[14] A solution of Li (780.0 mg, 112.4 mmol) in 50 mL THF was stirred at 0 °C as dimethylphenylsilyl chloride (9.60 g, 56.3 mmol) was added. After stirring for 6 h at 0 °C, the resulting mixture was added to a suspension of CuCN (1.6 g, 17.9 mmol) in 50 mL THF at -30 °C, and the slurry was stirred at -30 °C for 0.5 hr. A solution of **7** (5.0 g, 11.2 mmol) in 50 mL of THF was then added, and the resulting slurry was stirred at -30 °C for 2 hr. The mixture was diluted with 0.1 N HCl (200 mL) and was extracted with EtOAc (2x300 mL). The combined organic extracts were dried over Na_2SO_4 and filtered, and the organic solution was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (8:1 pet ether:EtOAc) to give silane **10** (4.4 g, 67% yield) as a yellow oil. ^{1}H NMR (400 MHz $CDCl_3$) δ 7.45-7.50 (m 2H), 7.37 - 7.32 (m, 3H), 5.13 (t, *J*=2.9 Hz, 1H), 3.66 (s, 3H), 2.64 (dd, *J*=17.0, 14.0 Hz, 1H), 2.15 – 2.44 (m, 5H), 2.08 (s, 3H), 1.97-2.05 (m, 2H), 1.74-1.96 (m, 5H), 1.55-1.73 (m, 4H), 1.44-1.54 (m, 3H), 1.24-1.43 (m, 4H),0.8-0.95 (m, 1H), 0.98 (s, 3H), 0.81 (d, *J*=6.0 Hz, 3H), 0.72 (s, 3H), 0.38 (s, 3H), 0.33 (s, 3H). The spectrum shows an approximately 1:1.4 molar ratio of **10** to EtOAc.

(R)-methyl 4-((1R,5R,8S,9S,10S,12S,13R,14S,17R)-12-acetoxy-1-hydroxy-10,13-dimethyl-3-oxohexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**8**)

A modification of the procedure of Wolfram was used.[14] A solution of **10** (3.0 g, 5.2 mmol) in AcOH (10 mL) was stirred at room temperature as a solution of $Hg(OAc)_2$ (2.1 g, 6.6

mmol) in 20% aq AcOOH (5 mL, 5.2 mmol) were added, and the resulting mixture was stirred for 3h. The mixture was diluted with sat aq $NaHSO_3$ (100 mL) and was extracted with EtOAc (2x200 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ and were dried over Na_2SO_4. After filtration, the solution was concentrated under reduced pressure, and the resulting residue was purified by column chromatography (4:1 Pet ether; EtOAc) to give **8** (1.6 g, 67% yield) as a white solid.

(R)-methyl 4-((1R,3R/S,5R,8S,9S,10S,12S,13R,14S,17R)-12-acetoxy-1,3-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**11**)

A modification of the procedure of Tohma was used.[8] A solution of compound **8** (1.5 g, 3.2 mmol) in CH_2Cl_2 (5 mL) and THF (10 mL) was stirred as $NaBH_4$ (750. mg, 19.8 mmol) was added and the resulting reaction mixture was stirred at room temperature for 1 hr. TLC showed the reaction was completed; therefore, the reaction mixture was diluted with sat. NH_4Cl (100 mL) and was extracted with EtOAc (300 mL). The organic layer was washed with brine and dried over Na_2SO_4, and after filtration, the organic layer was concentrated under reduced pressure to dryness to give compound **11** (1.3 g) as a colorless oil.

(R)-4-((1R,3R,5R,8S,9S,10S,12S,13R,14S,17R)-1,3,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid (**12**)

A modification of the procedure of Tohma was used.[8] A solution of compound **11** (1.3 g, 2.8 mmol) in MeOH (30 mL) and H_2O (20 mL) was stirred as NaOH (1.1 g, 27.1 mmol) was added. The reaction was warmed to 110 °C and was stirred at that temperature for 12 hr at which time LC-MS showed the reaction to be complete. The mixture was cooled to room temperature, and 0.5 N HCl (10 mL) was added. The reaction mixture was extracted with EtOAc (2x20 mL), and the combined organic layers were dried over Na_2SO_4.
After filtration, the solution was concentrated under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (20:1, CH_2Cl_2:MeOH) to give **12** (0.6 g, 46% yield from **8**) as a white solid (96.5% purity by ^{1}H NMR[25] using fumaric acid as the internal standard). LC/MS [M-H] 407.4. NMR data is compiled in the table.

(R)-methyl 4-((1R,3S,5R,8S,9S,10S,12S,13R,14S,17R)-12-acetoxy-1,3-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**13**)
A modification of the procedure of Tohma was used.[8] A solution of compound **8** (1.5 g, 3.2 mmol) in in CH_2Cl_2 (5 mL) was stirred at 0°C as $NaBH_4$ (600. mg, 15.9 mmol) was added, and the resulting reaction mixture was stirred at at 0 °C for 0.5 hr. TLC showed the reaction to be complete; therefore, it was diluted with sat. NH_4Cl (30 mL) and was extracted with EtOAc (2x30 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, and after filtration, the organic layer was concentrated under reduced pressure to dryness to give compound **11**. Purification by column chromatography on silica gel (3:1 to 1:1, Hexane:EtOAc = 3:1, 1:1) afforded compound **13** (0.30 g, 20%yield) as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 (s, 3H), 0.82 (d, J = 6.4 Hz, 3H), 1.03 – 1.19 (m, 1H),

1.04 (s, 3H), 1.18 – 1.78 (m, 20H), 1.81 – 1.97 (m, 4H), 2.10 (s, 3H), 2.22 (ddd, *J* = 15.6, 9.3, 6.7 Hz, 1H), 2.36 (ddd, *J* = 15.1, 9.9, 5.0 Hz, 1H), 3.69 (s, 3H), 3.85 (s, 1H), 4.14 (dq, *J* = 12.1, 6.0, 5.9, 5.9 Hz, 1H), 5.08 (d, *J* = 2.8 Hz, 1H).

(R)-4-((1R,3S,5R,8S,9S,10S,12S,13R,14S,17R)-1,3,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid (**1**)

A modification of the procedure of Tohma was used.[8] A solution of compound **13** (0.30 g, 0.65 mmol) in MeOH (3 mL) and H_2O (2 mL) was stirred as NaOH (120 mg, 3.0 mmol) was added. The reaction was warmed to 100 °C and was stirred at that temperature for 12 hr at which time TLC showed the reaction to be complete. The mixture was cooled to room temperature, and the pH was adjusted to 4 with 1 N HCl. The reaction mixture was then extracted with EtOAc (2x80 mL), and the combined organic layers were dried over Na_2SO_4. After filtration, the solution was concentrated under reduced pressure, and the resulting residue was purified twice by column chromatography on silica gel (3:1, CH_2Cl_2: MeOH) to give **1** (38 mg, 14% yield) as a white solid (79% purity by NMR[25] using 2,3,5,6-tetrachloronitrobenzene as an internal standard). LC/MS [M-H] 407.4**.** NMR data is compiled in the table.

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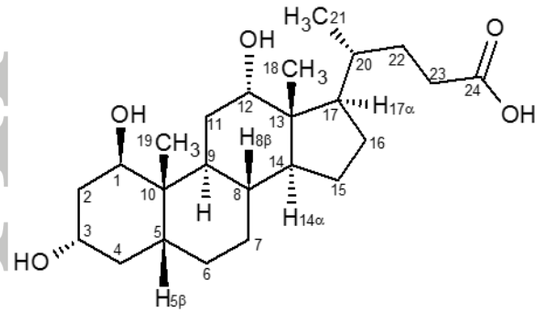
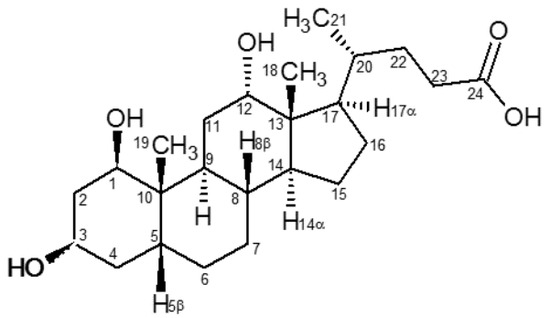
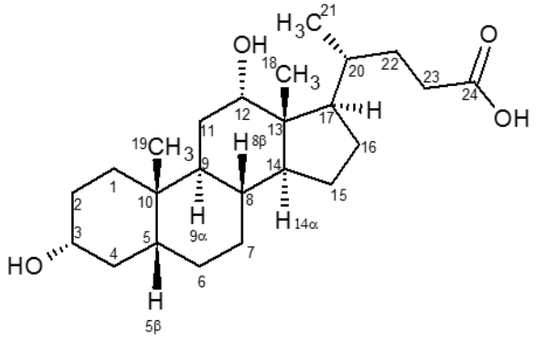
Table. NMR spectra were recorded in D_6-DMSO, and chemical shifts are reported relative to the residual solvent signal set to 2.5 ppm(^{1}H) and 39.5 ppm (^{13}C). Chemical shifts for overlapping ^{1}H signals are taken from relevant 2D experiments with the chemical shift taken at the center of the cross-peak. ^{1}H-Coupling patterns and J values are reported from 1D ^{1}H- spectra for selected, non overlapping, signals (Multipicity, J Hz). For multiplets, the center of the signal is taken as the chemical shift. Abbreviations; s(singlet), d(doublet), t(triplet), dt(doublet of triplet), tt(triplet of triplet), qt(quartet of triplet), m(multiplet), br.(broad), nr. (narrow), n.a.(not applicable), n.d. (not determined).







	(1) 1β-OH deoxycholic acid		(12) 1β-(3β)-OH deoxycholic acid		(Ref) deoxycholic acid	
Position	**^{13}C ppm**	**^{1}H ppm (multiplicity, J)**	**^{13}C ppm**	**^{1}H ppm (multiplicity, J)**	**^{13}C ppm**	**^{1}H ppm (multiplicity, *J*)**
1	71.4	3.59	72.1	3.53 (br.s)	35.2	1.62, 0.88
1-OH	n.a.	4.16 (d, J=4.6)	n.a.	4.77 (br.d,6.0)	n.a.	n.a.
2	37.4	1.64,1.46	31.8	1.68, 1.68	30.2	1.45, 1.27
3	64.6	3.82 (tq, J=9.9,9.9,4.8,4.8,4.8)	66.8	3.99 (br.s)	69.9	3.36 (tt, J10.9,10.9,4.4,4.4)
3-OH	n.a.	4.28 (d, J=4.8)	n.a.	5.14	n.a.	4.45 (br.s)
4	36.0	1.62, 1.29	33.2	1.93, 1.30	36.3	1.63, 1.33
5	35.1	1.69	30.3	1.93	41.6	1.29
6	26.6	1.64, 1.17	25.9	1.75, 1.16	27.0	1.76, 1.17

7	25.7	1.32, 1.08	25.6	1.34, 1.07	26.1	1.33, 1.05
8	35.7	1.35	35.6	1.38	35.6	1.33
9	34.2	1.77	34.1	1.62	32.9	1.80
10	38.0	n.a.	38.8	n.a	33.8	n.a.
11	28.7	1.35,1.30	28.6	1.39, 1.25	28.6	1.36, 1.36
12	70.9	3.76 (br.m)	71.0	3.75	71.0	3.78
12OH	n.a.	4.19 (d, J=4.0)	n.a.	4.14 (br.s)	n.a.	4.2 (br.d, J=3.2)
13	45.7	n.a.	45.8	n.a	46.2	n.a.
14	47.4	1.55	47.4	1.52	47.5	1.57
15	23.5	1.51, 0.98	23.5	1.50, 1.00	23.5	1.50, 0.98,
16	27.0	1.73, 1.18	27.0	1.72, 1.16	27.2	1.75, 1.17
17	46.2	1.74	46.2	1.73	46.2	1.76
18	12.5	0.59 (s)	12.4	0.60 (s)	12.4	0.59 (s)
19	18.2	0.89 (s)	18.5	0.96, (s)	23.1	0.84 (s)
20	35.0	1.28	34.9	1.28	35.0	1.29
21	16.9	0.9 (d, J=6.6Hz)	16.9	0.9 (d, J=6.5)	16.9	0.91 (d, J=6.5Hz)
22	30.8	1.64, 1.18	30.8	1.64, 1.18	30.8	1.64, 1.18
23	31.0	2.09 (ddd, J=15.5,9.1,7.1), 2.21 (ddd, J=15.5,9.6,5.2)	30.8	2.09 (ddd, J=15.6,9.2,6.9), 2.21 (ddd, J=15.6,9.6,5.2)	30.8	2.09 (ddd, J=15.6,9.3,6.9), 2.22 (ddd, J=15.6,9.7,5.2)
24	175.3	n.a.	174.9	n.a.	174.9	n.a.
24OH	n.a	11.91 (br.s)	n.a.	11.92 (br.s)	n.a	11.93 (br.s)

Chemical shifts are reported relative to the residual solvent signal set to 2.5 ppm(^{1}H) and 39.5 ppm (^{13}C).

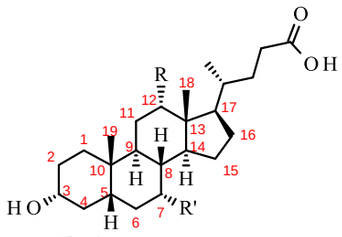
Chemical shifts for overlapping ^{1}H signals are taken from relevant 2D experiments with the chemical shift taken at the center of the cross-peak

^{1}H-Coupling patterns and J values are reported from 1D ^{1}H- spectra for selected, non overlapping, signals (Multipicity, J Hz). For multiplets the center of the signal is taken as the chemical shift

Abbreviations; s(singlet), d(doublet), t(triplet), dt(doublet of triplet), tt(triplet of triplet), qt(quartet of triplet), m(multiplet), br.(broad), nr. narrow, n.a.(not applicable), n.d. (not determined)

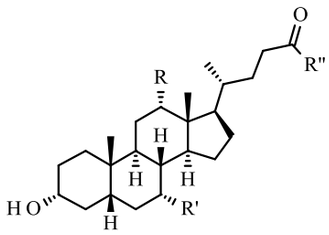
Numbering follows the IUPAC approved atom numbering of steroids.

Bile Acids



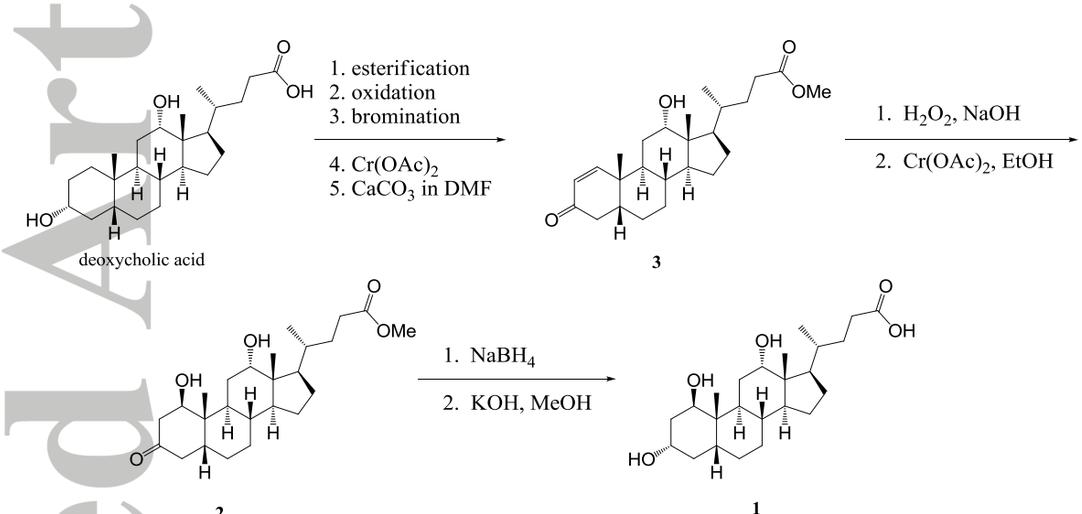
R = OH, R' = OH	cholic acid
R = H, R'= OH	chenodeoxycholoic acid
R = OH, R' = H	deoxycholic acid (DCA)
R = H, R' = H	lithocholic acid

Bile Salts

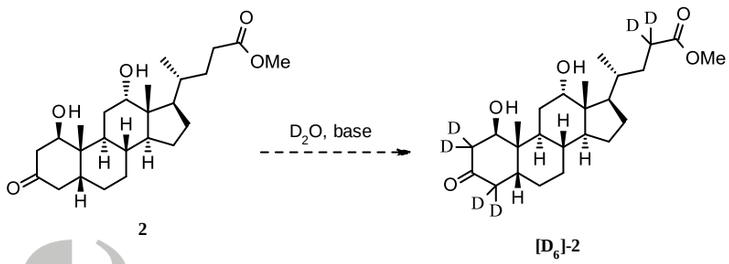


R = OH, R' = OH, R"=$NHCH_2CO_2H$	Glycocholoic acid
R = H, R'= OH, R"=$NHCH_2CH_2SO_3H$	Taurocholoic acid
R = H, R'= OH, R"=$NHCH_2CO_2H$	Glycochenodeoxycholoic acid
R = OH, R' = H, R"=$NHCH_2CH_2SO_3H$	Taurodeoxycholic acid

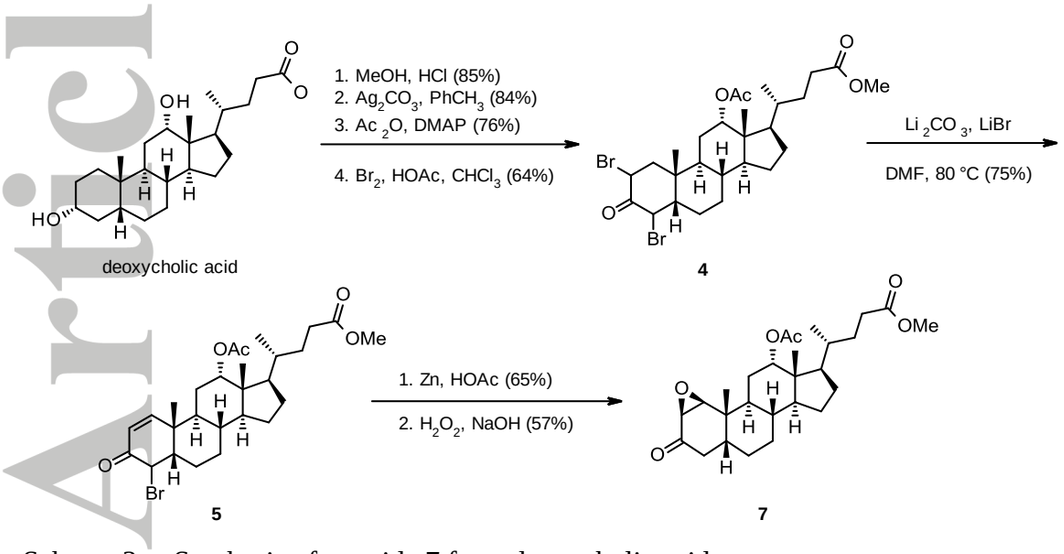
Figure 1. Structures of some common bile acids and bile salts.



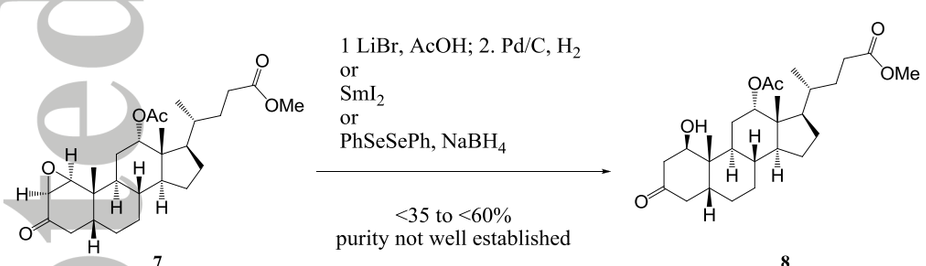
Scheme 1. Previously reported synthesis of 1-beta-hydroxydeoxycholic acid by Tohma.[7,8]



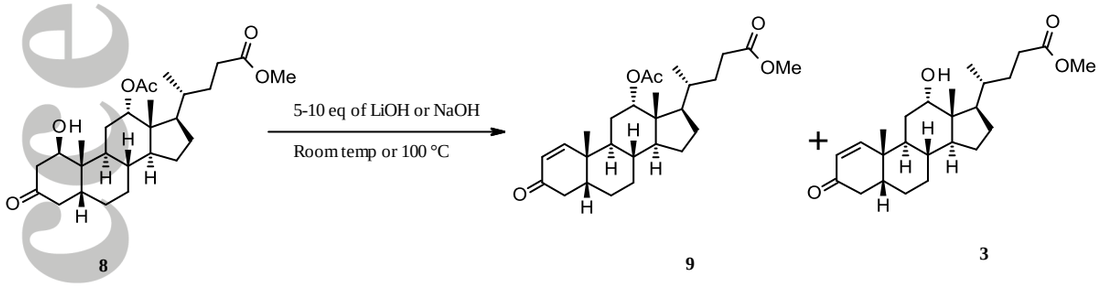
Scheme 2. Proposed key exchange reaction.



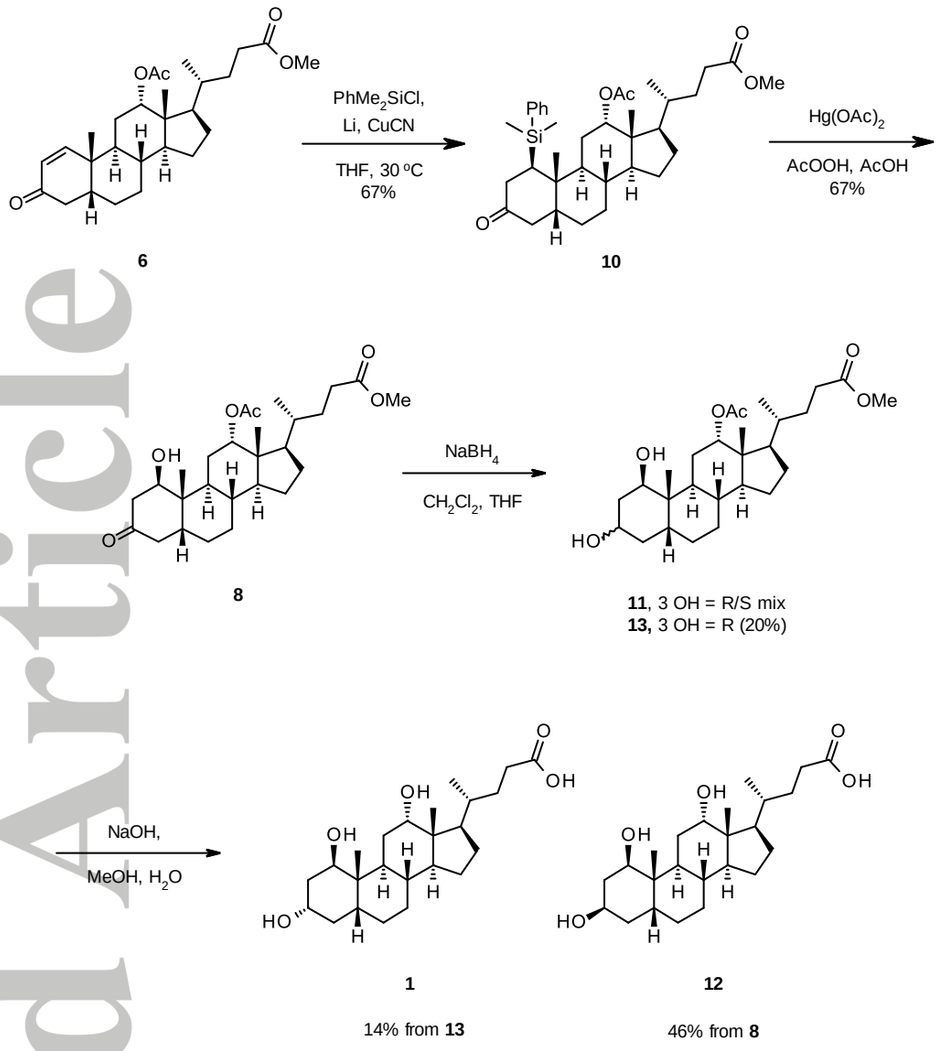
Scheme 3. Synthesis of epoxide **7** from deoxycholic acid.



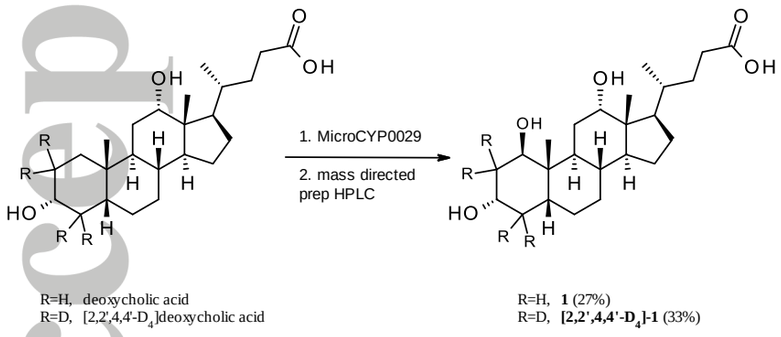
Scheme 4. Attempted ring opening of epoxide **7.**



Scheme 5. Probing the stability of the 1β- hydroxy of **8** to the proposed exchange reaction conditions.



Scheme 6. Conversion of **7** to **1** and **12**.



Scheme 7. Biocatalytic route from DCA to **1**.