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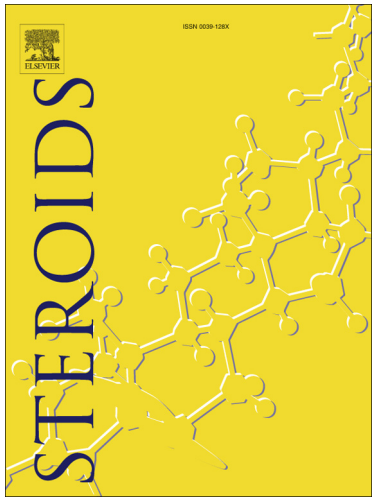

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Efficient synthesis of cholic acid derivates through stereoselective C-H functionalization from hyodeoxycholic acid

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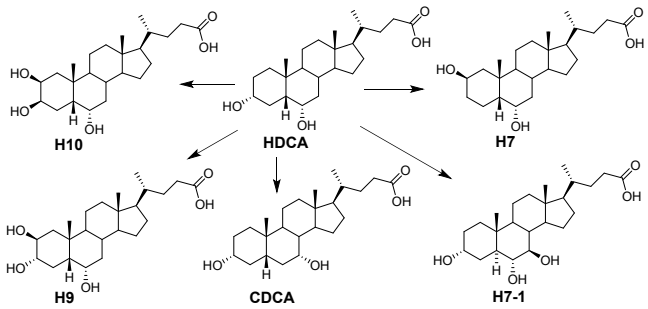
ABSTRACT

Five cholic acid derivatives (including *allo*-ω-muricholic acid and CDCA) were synthesized from hyodeoxycholic acid *via* selective oxidation of C3- or C6-hydroxyl groups by IBX and NBS oxidants and stereocontrolled conversion. The hydroxyl group could be introduced through hydrolyzing α-Br keto with K_2CO_3 aqueous solution or through oxidizing the double bond by monoperoxyphthalic acid. The reduction of C6-O6 carbonyl to methylene could undergo with PTSH, $NaBH_3CN$ and $ZnCl_2$ only at 5β configuration. A feasible synthetic route of CDCA from HDCA has been established to avoid the epimerization with the yield of 45 % (8 steps). These strategies provided good yields, stereoselectivity and reproducibility for the preparation of cholic acid derivates and CDCA.

1. Introduction

Bile acids are well-known for their stereochemically structural variability and biological activities [1], and therefore there have been many efforts toward the syntheses of these natural products and their analogues over the past decades [2-4]. Ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA), as two of the major bile acids, have an important medical application due to its ability to resolve cholesterol gallstones and other hepatobiliary diseases [5-9]. Meanwhile, CDCA has also been an important raw material applied in the syntheses of obeticholic acid and UDCA, respectively [10-11]. CDCA is mainly derived from the bile of geese and ducks, and the output has been limited to some extent compared with cholic acid (CA) and hyodeoxycholic acid (HDCA) derived from the bile of pigs. Two routes have been developed for the synthesis of CDCA from CA as the raw material [12-13], and two routes for the synthesis of UDCA from HDCA [14-15]. However, these routes from HDCA or CA are difficult to achieve industrialization due to harsh reaction conditions and low overall yield.

HDCA, CDCA and UDCA are isomers featuring the structural difference in OH position and stereostructure, and have the significant differences in biological activity [16-17], which suggest that the positions and stereostructures of the hydroxyl groups in bile acids play the crucial role in the pharmacologic actions. So, the transformation from HDCA to CDCA and/or UDCA is an important route to promote high economic and social value of HDCA. Common organic synthesis can hardly achieve this goal through direct hydroxylation of aliphatic C-H bond due to the chemical stability and rigidity although the hydroxylation of aliphatic C-H bond is a very common biological transformation in the living things. Therefore, the detail study on hydroxyl transformation can provide valuable strategies for the synthesis of cholic acid derivates (Scheme 1). In this context, the syntheses of five cholic acid derivates (including allo-ω-muricholic acid and CDCA) have been systematically investigated, and the important clues are found in selective oxidation and elimination reaction.



Scheme 1. The chemical structures of cholic acid derivates

2. Experimental

2.1. General

Hyodeoxycholic acid was a gift from Zhongshan Beiling Bio Technology Co. LTD (Purity: 99.3%). The reagents, chemicals,

solv -0.4 were used as received and solvents were dried and freshly distilled according to standard procedures.

Melting points were measured using a SGWX-4A apparatus (Shanghai Precision Instruments, China). ^{1}H- and ^{13}C-NMR spectra were obtained using a Bruker AVANCE III HD 600 spectrometer (Bruker, Germany) operating at 600 MHz for ^{1}H and 151 MHz for ^{13}C. Chemical shift values were given in δ (ppm) relative to the residual solvent peaks: δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 1.58 and δ_C 39.5 for DMSO-d_6, δ_H 3.31 and δ_C 49.0 for methanol-d_4, and coupling constants were reported in Hz. ROESY experiments were carried out with the use of the standard Bruker program package. High-resolution mass spectra (HRMS) were acquired with a maXis impact spectrometer (Bruker, Germany). Crystallographic data were collected on a Gemini E X-ray single crystal diffractometer (Agilent Technologies, USA) with an Eos CCD detector operating at 40 kV and 30 mA using Cu Kα radiation (λ = 1.54184 Å). FTIR spectra were recorded using KBr discs on VERTEX 70 spectrometer (Bruker, Germany). Optional rotations were recorded on AUTOPOL IV automatic polarimeter. TLC was performed on precoated glass backed TLC sheets (silica gel GF254) and visualized by spraying with phosphomolybdic acid followed by heating. Column chromatography was conducted with silica gel 3: 100–200 mesh (Qingdao Haiyang Chemical Co, China).

2.2. Chemical synthesis

*2.2.1. Methyl 3α, 6α-dihydroxyl-5β-cholanoate (**H1**)*

To a solution of HDCA (1.0002 g, 2.5 mmol) in anhyd MeOH (15 mL) was added concd HCl (100 μL, 3.2 mmol) under stirring, and then the mixture was heated to refluxing for 4 h. The mixture was quenched with saturated $NaHCO_3$ aq (10 mL) and evaporated under reduced pressure. After extracting with EtOAc (20 mL) by three portions, the organic layers were combined and washed with saturated $NaHCO_3$ aq (10 mL×2), and brine (10 mL×3). After the organic phase was dried by anhyd $MgSO_4$, the solvent was evaporated under reduced pressure to afford compound **H1** (1.0251 g, 99% yield) as a white solid; mp 55-57 °C; $[\alpha]_D^{20}$ = +2 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3395 (O-H), 1741 (C=O), 1175 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.04 (dt, J = 12.0, 4.7 Hz, 1H, 6β-H), 3.65 (s, 3H, OCH_3), 3.59 (dt, J = 10.6, 4.6 Hz, 1H, 3β-H), 0.90 (d, J = 6.7 Hz, 3H, 21-H), 0.89 (s, 3H, 19-H), 0.63 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.7 (C-24), 71.5 (C-3), 68.0 (C-6), 56.1, 55.9, 51.4, 48.4, 42.8, 39.9, 39.8, 35.9, 35.5, 35.3, 35.0, 34.8, 31.1, 30.1, 30.2, 29.2, 28.1, 24.2, 23.4, 20.7, 18.2, 12.0; HRMS (ESI, m/z) Calcd for $C_{25}H_{42}O_4$ 406.3083; found: 429.2976 $[M + Na]^+$ (Calcd 429.2981).

*2.2.2. Methyl 6α-hydroxyl-3-oxo-5β-cholanoate (**H2**)*

NBS (0.1602 g, 0.9 mmol) was added to a solution of **H1** (0.2001 g, 0.5 mmol) containing acetone (12 mL), AcOH (33 μL, 0.6 mmol) and H_2O (4 mL) by ten portions at 0 °C. The mixture was allowed to warm to rt after stirring for about 30 min, and then quenched after 1 h with saturated Na_2SO_3 aq (10 mL). After extracting with EtOAc (20 mL) by three portions, the organic layers were combined and washed with saturated Na_2SO_3 aq (10 mL×2), saturated $NaHCO_3$ aq (10 mL×2), and brine (10 mL×3). After dried by anhyd $MgSO_4$ and filtered, the solution was concentrated under reduced pressure to give the crude product, which was purified by column chromatography through silica gel (eluted with 4:1 hexane: ethyl acetate) to afford compound **H2** (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3462 (O-H), 1742 and 1724 (C=O), 1170 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.09 (dt, J = 11.6, 4.6 Hz, 1H, 6β-H), 3.67 (s, 3H, -OCH_3), 1.02 (s, 3H, 19-H), 0.92 (d, J = 6.4 Hz, 3H, 21-H), 0.67 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 212.7 (C-3), 174.7 (C-24), 67.6 (C-6), 56.1, 55.9, 51.5, 50.1, 42.8, 40.2, 39.8, 37.1, 37.1, 36.2, 36.0, 35.3, 34.6, 34.4, 31.0, 30.9, 28.1, 24.2, 22.8, 21.1, 18.3, 12.1; HRMS (ESI, m/z) Calcd for $C_{25}H_{40}O_4$ 404.2927; found: 427.2817 $[M + Na]^+$ (Calcd 427.2824).

*2.2.3. Methyl 6α-acetoxyl-3-oxo-5β-cholanoate (**H3**)*

H2 (0.2001 g, 0.5 mmol) in EtOAc (10 mL) was reacted with Ac_2O (151 μL, 1.6 mmol) in the cat equivalent of DMAP and Et_3N (170 μL, 1.2 mmol) for 3.5 h at r.t. The solution was then washed with 0.5 M HCl aq (15 mL×2), saturated $NaHCO_3$ aq (25 mL×3), and brine (25 mL×2). The resulting organic solution was dried by anhyd $MgSO_4$. After filtered, the filtrate was evaporated under reduced pressure to afford compound **H3** (0.2112 g, 99% yield) as a white solid; mp 108-110 °C; $[\alpha]_D^{20}$ = +5.4 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 1733 and 1699 (C=O), 1159 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.17 (dt, J = 18.0, 7.2Hz, 1H, 6β-H), 3.67 (s, 3H, -OCH_3), 2.04 (s, 3H, CH_3CO), 1.07 (s, 3H, 19-H), 0.93 (d, J = 6.4 Hz, 3H, 21-H), 0.68 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.9 (C-3), 174.6 (C-24), 170.3, 70.5 (C-6), 56.1, 55.9, 51.4, 47.1, 42.8, 40.3, 39.7, 36.9, 36.8, 36.7, 36.3, 35.3, 34.4, 31.0, 30.9, 30.8, 28.0, 24.0, 22.6, 21.2, 21.0, 18.2, 12.0; HRMS (ESI, m/z) Calcd for $C_{27}H_{42}O_5$ 446.3032; found: 469.2927 $[M + Na]^+$ (Calcd 469.2930).

*2.2.4. Methyl 6α-acetoxyl-2α-bromo-3-oxo-5β-cholanoate (**H4**)*

To a stirred solution of compound **H3** (0.2002 g, 0.5 mmol) in EtOAc (20 mL) at rt was added $CuBr_2$ (0.2001 g, 0.9 mmol), and the mixture was heated at reflux. The reaction process was monitored by TLC. After compound **H3** was depleted for about 3 h, the reaction was quenched with H_2O (20 mL). The organic layer was separated and washed with H_2O (20 mL×2), and brine (20 mL×3). After the organic layer was dried by anhyd $MgSO_4$ and filtered, the filtrate was evaporated under reduced pressure to afford compound **H4** (0.2231 g, 95% yield) as a yellow solid; mp 58-61 °C; $[\alpha]_D^{20}$ = -29.8 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 1738 (C=O), 1237 and 1031 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.10 (dt, J = 12.0, 4.7 Hz, 1H, 6β-H), 4.70 (dd, J = 14.1, 5.4 Hz, 1H, 2β-H), 3.63 (s, 3H, -OCH_3), 1.99 (s, 3H, CH_3CO), 1.06 (s, 3H, 19-H), 0.90 (d, J = 6.4 Hz, 3H, 21-H), 0.65 (s, 3H, 18-H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 201.4 (C-3), 174.6 (C-24), 170.1, 70.0 (C-6), 56.0, 55.9, 52.3, 51.5, 49.0, 47.9, 42.8, 40.8, 40.1, 39.6, 36.6, 35.2, 34.4, 31.1, 30.9, 30.8, 28.0, 24.0, 22.2, 21.3, 21.2, 18.3, 12.0; HRMS (ESI, m/z) Calcd for $C_{27}H_{41}BrO_5$ 524.2137; found: 547.2022 $[M + Na]^+$ (Calcd 547.2035).

*2.2.5. Methyl 6α-acetoxyl-2β-hydroxyl-3-oxo-5β-cholanoate (**H5**)*

To a stirred solution of compound **H4** (0.2001 g, 0.4 mmol) in a mixture of acetone (15 mL) and H_2O (5 mL) was added K_2CO_3 (0.1602 g, 1.2 mmol) at 45 °C. After 3 h, the solution was evaporated under reduced pressure. The resulting crude production was dissolved in EtOAc, and the organic layer was washed with 1 M HCl aq (15 mL×2), H_2O (30 mL×2), and brine (30 mL×3). The organic layer was then dried by anhyd $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting product was purified by column chromatography through silica gel (eluted with 10: 1 hexane: ethyl acetate) to afford compound **H5** (0.1593 g, 90% yield) as a white solid; mp 126-128 °C; $[\alpha]_D^{20}$ = -10.6 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3493 (O-H), 1734

and m/z) (dt, J = 18.6, 6.6Hz, 1H, 6β-H), 4.22 (dd, J = 13.0, 6.3 Hz, 1H, 2α-H), 3.66 (s, 3H, -OCH_3), 3.48 (s, 1H, 2-OH), 2.04 (s, 3H, CH_3CO), 1.08 (s, 3H, 19-H), 0.92 (d, J = 6.5 Hz, 3H, 21-H), 0.68 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.3 (C-3), 174.6 (C-24), 170.1, 71.1 (C-6), 70.2 (C-2), 56.1, 55.9, 51.5, 48.7, 46.3, 42.9, 41.5, 39.7, 38.5, 35.3, 35.1, 34.5, 31.1, 31.0, 30.9, 28.1, 24.0, 22.5, 21.2, 21.1, 18.3, 12.0; HRMS (ESI, m/z) Calcd for $C_{27}H_{42}O_6$ 462.2981; found: 485.2878 $[M + Na]^+$ (Calcd 485.2879).

*2.2.6. Methyl 6α-acetoxyl-2β-hydroxyl-5β-cholanoate (**H6**)*

PTSH (0.0411g, 0.2 mmol) was added by ten portions to a stirred solution of compound **H5** (0.1011 g, 0.2 mmol) in MeOH (15 mL) over 15 min. The mixture was stirred at rt for 15 min, and heated to reflux temp to maintain for 45 min, and then allowed to stir at rt for 15 min. The reaction process was monitored by TLC. After compound **H5** was depleted, a mixture of $NaBH_3CN$ (0.0213 g, 0.3 mmol) and $ZnCl_2$ (0.0212 g, 0.2 mmol) in MeOH (2 mL) was added to the reaction solution. After the mixture was allowed to stand for 3 h at reflux, the reaction was quenched with 0.1 M NaOH aq (10 mL) and evaporated under reduced pressure. After extracting with Et_2O (20 mL) by three portions, the combined organic layers were washed with H_2O (10 mL×2), and brine (10 mL×3), and dried by anhyd $MgSO_4$. After filtered, the filtrate was concentrated under reduced pressure to give the crude product, which was purified by column chromatography through silica gel (eluted with 5: 1 hexane: ethyl acetate) to afford compound **H6** (0.0663 g, 68% yield) as colorless oily liquid; $[\alpha]_D^{20}$ = -12.9 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3462 (O-H), 1740 (C=O), 1170 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.17 (dt, J = 18.0, 6.0 Hz, 1H, 6β-H), 3.72-3.66 (m, 1H, 2α-H), 3.65 (s, 3H, -OCH_3), 2.04 (s, 3H, CH_3CO), 1.1 (s, 3H, 19-H), 0.90 (d, J = 6.6 Hz, 3H, 21-H), 0.70 (s, 3H, 18-H); ^{13}C NMR (151 MHz, CDCl3) δ 174.7 (C-24), 170.6, 71.8 (C-6), 66.1 (C-2), 56.1, 55.9, 51.5, 46.3, 46.2, 42.9, 41.4, 39.9, 38.9, 35.4, 35.3, 34.7, 31.3, 31.0, 30.9, 28.1, 24.1, 23.8, 21.4, 20.9, 19.8, 18.3, 12.0; HRMS (ESI, m/z) Calcd for $C_{27}H_{44}O_5$ 448.3189; found: 471.3065 $[M + Na]^+$ (Calcd 471.3086).

*2.2.7. 2β, 6α-Dihydroxyl-5β-cholanoate (**H7**)*

To a stirred solution of compound **H6** (0.0399 g, 0.1 mmol) in MeOH (3 mL) and H_2O (1 mL) was added NaOH (0.0115 g, 0.3 mmol) at rt. The mixture was heated at reflux for 1 h, and then adjusted to pH 1-2 with 1 M HCl aq to afford compound **H7** as white solid (0.0301 g, 86% yield). mp 169-172 °C; $[\alpha]_D^{20}$ = -12 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}): 3512 and 3405 (O-H), 1714 (C=O), 1025 (C-O); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.96 (s, 1H), 4.28 (s, 1H), 4.25 (s, 1H), 3.91-3.83 (m, 1H, 6β-H), 3.50-3.39(m, 1H, 2α-H), 0.88 (t, J = 3.1 Hz, 6H, 19, 21-H), 0.61 (s, 3H, 18-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 175.3 (C-24), 66.6 (C-6), 64.8 (C-2), 56.1, 55.9, 49.8, 47.3, 42.8, 41.3, 39.9, 38.4, 36.1, 35.3, 35.3, 34.8, 31.2, 31.1, 28.1, 24.6, 24.3, 21.1, 19.1, 18.6, 12.3; HRMS (ESI, m/z) Calcd for $C_{24}H_{40}O_4$ 392.2927; found: 415.2815 $[M + Na]^+$ (Calcd 415.2824).

2.2.8. *Methyl 6α-acetoxyl-2β, 3-dihydroxyl-5β-cholanoate* (**H8**)

$NaBH_4$ (0.0412 g, 1.1 mmol) was added to a solution of **H5** (0.1021 g, 0.2 mmol) containing MeOH (10 mL) at 0 °C. Then the mixture was allowed to warm to rt and quenched after 1 h with H_2O (30 mL). After extracting with EtOAc (20 mL) by three portions, the organic layer was washed with saturated $NaHCO_3$ aq (15 mL×3), and brine (15 mL×3), and dried by anhyd $MgSO_4$. After filtered, the solvent was evaporated under reduced pressure to afford compound **H8** (0.0953 g, 95% yield) as a white oily liquid; $[\alpha]_D^{20}$ = -9.2 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3452 Calcd for $C_{27}H_{44}O_6$ 464.3138; found: 487.3024 $[M + Na]^+$ (Calcd 487.3036). Compound **H8** was then purified by column chromatography through silica gel (eluted with 8: 1 acetone: hexane) to afford compound **H8** (3α, 50% yield) as a white solid and compound **H8** (3β, 50% yield) as a white solid; **H8** (3α) ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.19 (dt, J = 12.1, 4.8 Hz, 1H, 6β-H), 4.07-3.99 (m, 1H, 2α-H), 3.72-3.67 (m, 1H, 3β-H), 3.65 (s, 3H, -OCH_3), 2.00 (s, 3H, CH_3CO), 1.01 (s, 3H, 19-H), 0.90 (d, J = 6.5 Hz, 3H, 21-H), 0.63 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.8 (C-24), 170.5, 70.9 (C-3), 68.9 (C-6), 67.4 (C-2), 56.2, 55.9, 51.5, 42.9, 40.6, 39.9, 39.4, 38.5, 38.3, 35.3, 34.6, 31.2, 31.1, 30.9, 28.1, 26.3, 24.0, 23.6, 21.4, 21.1, 18.3, 12.0. **H8** (3β) ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.12 (dt, J = 12.3, 4.8 Hz, 1H, 6β-H), 3.65 (s, 3H, -OCH_3), 3.53 (ddd, J = 12.6, 8.9, 4.1 Hz, 1H, 2α-H), 3.39 (ddd, J = 11.4, 8.9, 5.0 Hz, 1H, 3α-H), 2.02 (s, 3H, CH_3CO), 1.00 (s, 3H, 19-H), 0.90 (d, J = 6.5 Hz, 3H, 21-H), 0.63 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.8 (C-24), 170.5, 76.3 (C-3), 71.2 (C-6), 70.9 (C-2), 56.0, 55.9, 51.5, 45.4, 43.2, 42.8, 41.4, 39.8, 38.8, 35.3, 34.7, 31.3, 31.1, 30.9, 28.1, 27.9, 24.1, 23.2, 21.4, 20.9, 18.3, 12.0.

*2.2.9. 2β, 3, 6α-Trihydroxyl-5β-cholanoate (**H9/H10**)*

To a stirred solution of compound **H8** (3α)(0.1011 g, 0.2 mmol) in MeOH (4 mL) and H_2O (1 mL) was added NaOH (0.0441 g, 1.1 mmol) at room temperature. The mixture was heated at reflux for 1 h, and then adjusted to pH 1-2 with 1 M HCl aq to afford compound 2β, 3α, 6α - trihydroxyl - 5β - cholanoate **H9** (0.0794 g, 90% yield) as white solid; mp 52-55 °C; $[\alpha]_D^{20}$ = -9.2 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3443 (O-H), 1704 (C=O), 1025 and 1023 (C-O); HRMS (ESI, m/z) Calcd for $C_{24}H_{40}O_5$ 408.2876; found: 431.2751 $[M + Na]^+$ (Calcd 431.2773). **H9** ^{1}H NMR (600 MHz, CD_3OD) δ 4.08 (dt, J = 11.1, 4.2 Hz, 1H, 6β-H), 4.02-3.96 (m, 1H, 2α-H), 3.68-3.61 (m, 1H, 3β-H), 1.00 (s, 3H, 19-H), 0.98 (d, J = 6.5 Hz, 3H, 21-H), 0.72 (s, 3H, 18-H); ^{13}C NMR (151 MHz, CD_3OD) δ 176.7 (C-24), 68.9 (C-3), 67.1 (C-6), 67.0 (C-2), 56.1, 55.9, 42.6, 42.1, 40.4, 39.9, 37.8, 37.7, 35.3, 34.8, 33.9, 30.9, 30.6, 27.8, 25.5, 23.8, 22.9, 20.9, 17.4, 11.0.

2β, 3β, 6α-Trihydroxyl-5β-cholanoate H10 was prepared as a white solid according to the similar synthetic method of compound **H9**. ^{1}H NMR (600 MHz, CD_3OD) δ 4.02 (dt, J = 11.4, 4.4 Hz, 1H, 6-H), 3.55-3.42 (m, 1H, 2-H), 3.33-3.27 (m, 1H, 3-H), 0.98 (d, J = 7.1 Hz, 6H,19,21-H), 0.72 (s, 3H, 18-H); ^{13}C NMR (151 MHz, CD_3OD) δ 176.8 (C-24), 75.9 (C-3), 70.4 (C-6), 67.0 (C-2), 56.1, 55.9, 48.4, 43.7, 42.6, 41.3, 39.9, 37.9, 35.3, 34.8, 34.1, 30.9, 30.6, 27.8, 27.4, 23.8, 22.5, 20.7, 17.3, 11.0.

*2.2.10. Methyl 3α-hydroxyl-6-oxo-5β-cholanoate (**H2-1**)*

IBX (0.5201 g, 1.9 mmol) was added to a stirred solution of compound **H1** (0.5003 g, 1.2 mmol) in tert-BuOH (25 mL) at rt. The mixture was heated at reflux for 1 h, and then quenched with 10% Na_2SO_3 aq (25 mL), and the mixture was evaporated under reduced pressure. EtOAc (40 mL) was added to the residue and filtered. The filtrate was washed with 10% Na_2SO_3 aq (25 mL×2), saturated $NaHCO_3$ aq (25 mL×2), and brine (20 mL×3). The organic layer was dried by anhyd $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by column chromatography through silica gel (eluted with 4: 1 hexane: ethyl acetate) to afford compound **H2-1** (0.3582 g, 72% yield) as a white solid; mp 134-137 °C; $[\alpha]_D^{20}$ = -47.2 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3507 (O-H), 1730 and 1679 (C=O), 1100 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.68 (s, 3H, -OCH_3), 3.66-3.59 (m, 1H, 3β-H), 0.94 (d, J = 5.9 Hz, 3H, 21-H), 0.85 (s, 3H, 19-H), 0.66 (s, 3H, 18-H). ^{13}C NMR (151

55.8, 51.5, 43.1, 42.9, 40.0, 39.6, 37.9, 37.1, 35.3, 34.9, 34.4, 31.1, 30.9, 29.9, 27.9, 23.9, 23.2, 20.8, 18.2, 11.9; HRMS (ESI, m/z) Calcd for $C_{25}H_{40}O_4$ 404.2927; found: 427.2814 $[M + Na]^+$ (Calcd 427.2824).

*2.2.11. Methyl 3α-acetoxy-6-oxo-5β-cholanoate (**H3-1**)*

Compound **H2-1** (0.2001 g, 0.5 mmol) dissolved in EtOAc (10 mL) was reacted with Ac_2O (151 μL, 1.6 mmol) in the cat equivalent of DMAP and Et_3N (170 μL, 1.2 mmol). The mixture was allowed to stand for 3.5 h at rt. The organic layer was washed with 0.5 M HCl aq (15 mL×2), saturated $NaHCO_3$ aq (25 mL×3), and brine (25 mL×2). The organic layer was dried by anhyd $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to offer compound **H3-1** (0.2164 g, 98%) as a white solid; mp 146-149 °C; $[\alpha]_D^{20}$ = -21.6 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 1733 and 1703(C=O), 1162 (C-O); ^1H NMR (600 MHz, $CDCl_3$) δ 4.76-4.63 (m, 1H, 3β-H), 3.68 (s, 3H, -OCH_3), 2.04 (s, 3H, CH_3CO), 0.94 (d, J = 6.4 Hz, 3H, 21-H), 0.86 (s, 3H, 19-H), 0.68 (s, 18-H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 212.8 (C-6), 174.6 (C-24), 170.3, 72.4 (C-3), 59.1, 56.9, 55.9, 51.5, 43.1, 42.8, 39.9, 39.6, 37.9, 37.1, 35.3, 34.1, 31.1, 31.0, 30.9, 27.9, 26.2, 23.9, 23.1, 21.2, 20.9, 18.3, 11.9; HRMS (ESI, m/z) Calcd for $C_{27}H_{42}O_5$ 446.3032; found: 469.2930 $[M + Na]^+$ (Calcd 469.2930).

*2.2.12. Methyl 3α-acetoxy-7α-bromo-6-oxo-5β-cholanoate (**H4-1**)*

The synthesis of **H4-1** was similar to that of **H4** as a yellow solid. (0.9981 g, 85% yield); mp 53-56 °C; $[\alpha]_D^{20}$ = +44.2 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 1739 and 1711 (C=O), 1166 (C-O); ^1H NMR (600 MHz, $CDCl_3$) δ 5.15-5.13 (m, 1H, 3β-H), 4.21 (d, J = 2.4 Hz, 1H, 7β-H), 3.68 (s, 3H, -OCH_3), 3.59 (dd, J = 11.2, 4.4 Hz, 1H, 5β-H), 2.07 (s, 3H, CH_3CO), 0.95 (d, J = 8.0 Hz, 3H, 21-H), 0.76 (s, 3H,19-H), 0.71 (s, 3H,18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 205.3 (C-6), 174.6 (C-24), 170.4, 68.5 (C-3), 58.7 (C-7), 55.5, 52.4, 51.5, 46.2, 45.9, 42.7, 41.6, 40.2, 38.7, 35.2, 32.2, 30.9, 30.9, 27.7, 25.2, 24.8, 22.7, 21.4, 20.5, 18.2, 12.4, 12.0; HRMS (ESI, m/z) Calcd for $C_{27}H_{41}BrO_5$ 524.2137; found: 547.2018 $[M + Na]^+$ (Calcd 547.2035).

*2.2.13. Methyl 3α-acetoxyl-7β-hydroxyl-6-oxo-5α-cholanoate (**H5-1**)*

To a stirred solution of compound **H4-1** (0.1803 g, 0.3 mmol) in acetone (5 mL) and H_2O (5 mL) was added K_2CO_3 (0.0951 g, 0.7 mmol) at 50 °C. The reaction process was monitored by TLC. After compound **H4-1** was depleted, the solution was evaporated under reduced pressure. The crude production was dissolved in EtOAc (20 mL), and the organic phase was washed with 1 M HCl aq (15 mL×2), H_2O (30 mL×2), and brine (30 mL×3). The organic layer was separated and dried by anhyd $MgSO_4$. After filtering, the solution was concentrated under reduced pressure to give the crude product, which was purified by column chromatography through silica gel (eluted with 10: 1 hexane: ethyl acetate) to afford compound **H5-1** (0.0811 g, 51% yield) as a white solid; mp 116-119 °C; $[\alpha]_D^{20}$ = +16.8 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3461 (O-H), 1735 and 1703 (C=O), 1164 (C-O); ^1H NMR (600 MHz, $CDCl_3$) δ 5.18-5.10 (m, 1H, 3β-H), 3.83-3.80 (m, 1H, 7α-H), 3.69 (d, J = 3.4 Hz, 5α-H), 3.67 (s, 3H, -OCH_3), 2.04 (s, 3H, CH_3CO), 0.94 (d, J = 6.4 Hz, 3H, 21-H), 0.71 (s, J = 5.8 Hz, 3H, 19-H), 0.68 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.5 (C-6), 174.6 (C-24), 170.2, 78.9 (C-7), 68.4 (C-3), 56.9, 55.3, 51.6, 51.5, 50.1, 46.9, 43.6, 41.5, 39.5, 35.3, 32.5, 31.1, 31.0, 28.3, 26.1, 25.2, 24.8, 21.4, 21.1, 18.4, 12.5, 12.1; HRMS (ESI, m/z) Calcd for $C_{27}H_{42}O_6$ 462.2981; found: 485.2876 $[M + Na]^+$ (Calcd 485.2879).

*(**H6-1**)*

$NaBH_4$ (0.0143 g, 0.4 mmol) was added to a solution of **H5-1** (0.0341 g, 0.1 mmol) containing MeOH (5 mL) at 0 °C. Then the mixture was allowed to warm to rt and quenched after 1 h with H_2O (30 mL). After extracting with EtOAc (15 mL) by three portions, the organic layers were washed with saturated $NaHCO_3$ aq (15 mL×3), and brine (15 mL×3), and dried by anhyd $MgSO_4$. After filtered, the solvent was evaporated under reduced pressure to afford compound **H6-1** (0.0312 g, 90% yield) as a white solid; mp 151-153 °C; $[\alpha]_D^{20}$ = +30.4 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3533 and 3453 (O-H), 1737 and 1715 (C=O), 1159 (C-O); ^1H NMR (600 MHz, $CDCl_3$) δ 5.16-5.10 (m, 1H, 3β-H), 3.67 (s, 3H, -OCH_3), 3.65-3.61 (m, 1H, 6α-H), 3.34 (dd, J = 9.8, 3.7 Hz, 1H, 7α-H), 2.04 (s, 3H, CH_3CO), 1.00 (s, 3H, 19-H), 0.94 (d, J = 6.3 Hz, 3H, 21-H), 0.71 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.7 (C-24), 170.6, 74.4 (C-7), 70.1 (C-3), 55.4 (C-6), 54.9, 52.3, 51.5, 43.6, 41.2, 39.8, 38.3, 35.3, 35.1, 34.8, 31.1, 31.0, 30.1, 29.7, 28.6, 27.2, 26.1, 21.5, 20.5, 18.4, 15.1, 12.1; HRMS (ESI, m/z) Calcd for $C_{27}H_{44}O_6$ 464.3138; found: 487.3031 $[M + Na]^+$ (Calcd 487.3036).

*2.2.15. 3α, 6β, 7β-Trihydroxyl-5α-cholanoate (**H7-1**)*

Compound **H7-1** (0.0161 g, 91% yield) was synthesized as a white solid according to the similar synthetic method of compound **H9**; mp 238-241 °C; $[\alpha]_D^{20}$ = +28.8 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3462 and 3382 (O-H), 1679 (C=O), 1123 (C-O); ^1H NMR (600 MHz, CD_3OD) δ 4.08-4.04 (m, 1H, 3β-H), 3.56-3.46 (m, 1H, 6α-H), 3.21 (dd, J = 10.0, 3.7 Hz, 1H, 7α-H), 0.98 (s, 3H, 19-H), 0.96 (d, J = 6.5 Hz, 3H, 21-H), 0.73 (s, 3H, 18-H); ^{13}C NMR (151 MHz, CD_3OD) δ 176.7 (C-24), 76.5 (C-7), 75.1 (C-6), 65.9 (C-3), 55.8, 55.2, 52.9, 43.3, 40.3, 39.9, 37.8, 35.2, 35.1, 34.0, 32.6, 30.9, 30.6, 28.3, 28.2, 26.8, 20.3, 17.5, 14.2, 11.3; HRMS (ESI, m/z) Calcd for $C_{24}H_{40}O_5$ 408.2876; found: 431.2768 $[M + Na]^+$ (Calcd 431.2773).

*2.2.16. Methyl 3α-acetoxyl-6-hydroxy-5β-cholanoate (**H3-2**)*

$NaBH_4$ (1.1 g, 28.0 mmol) was added to a solution of **H3-1** (2.5 g, 5.6 mmol) containing MeOH (50 mL), the mixture was allowed to warm to rt after stirring for about 30 min, and then quenched after 2 h with AcOH (30 mL). MeOH was evaporated, the residue was dissolved in EtOAc (50 mL), saturated $NaHCO_3$ aq was added to adjust the pH to neutral. The organic layer was washed with brine (20 mL×3), and then dried by anhyd $MgSO_4$. After filtered, the solvent was evaporated under reduced pressure to afford **H3-2** (2.3 g, 92% yield) as a white solid; mp 97-99 °C; **H3-2** (6α): $[\alpha]_D^{20}$ = +17.0 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3453 (O-H), 1735 (C=O), 1172 (C-O); ^1H NMR (600 MHz, $CDCl_3$) δ 4.71-4.64 (m, 1H, 3β-H), 3.74-3.72 (m, 1H, 6β-H), 3.65 (s, 3H, -OCH_3), 2.01 (s, 3H, CH_3CO), 1.10 (s, 3H, 19-H), 0.90 (d, J = 6.5 Hz, 3H, 21-H), 0.66 (s, 3H, 18-H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.8 (C-24), 170.6, 73.8 (C-6), 72.9 (C-3), 56.3, 55.9, 51.5, 48.4, 42.8, 407, 40.0, 35.5, 35.4, 34.5, 34.4, 32.3, 31.1, 31.0, 30.7, 28.2, 26.2, 25.5, 24.2, 21.4, 20.6, 18.3, 12.1; HRMS (ESI, m/z) Calcd for $C_{27}H_{44}O_5$ 448.3189; found: 471.3082 $[M + Na]^+$ (Calcd 471.3086).

*2.2.17. Methyl 3α-acetoxyl-6-toluenesulfonyl-5β-cholanoate (**H3-3**)*

After para-toluenesulfonyl chloride (1.0677 g, 5.6 mmol) was added to a solution of **H3-2** (0.5000 g, 1.1 mmol) containing py (10 mL), the mixture was allowed to warm to rt after stirring for about 4 h, and then the reaction was continued for 24 h. Pyr indene was evaporated, the residue was dissolved in EtOAc (15 mL).The organic layer was washed with saturated $NaHCO_3$ aq

(5 m … d by adding Celite, ethanol was evaporated, the residue was dissolved in EtOAc (15 mL), and the organic layer was washed with brine (5 mL×3). And then dried by anhyd $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by column chromatography through silica gel (eluted with 10: 1 hexane: ethyl acetate) to afford compound **H3-6** (0.2713 g, 90% yield) as a colourless oil; mp 58-60 °C; $[\alpha]_D^{20}$ = +9.1 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3462 (O-H), 1735 (C=O), 1164 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.60-4.54 (m, 1H, 3β-H), 3.81 – 3.76 (m, 1H, 7β-H), 3.60 (s, 3H, -OCH_3), 1.94 (s, 3H, CH_3CO), 0.86 (d, J = 6.5 Hz, 3H, 21-H), 0.84 (s, 3H, 19-H), 0.59 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.7 (C-24), 170.8, 74.3 (C-3), 68.4 (C-7), 55.8, 51.5, 50.4, 42.7, 41.2, 39.5, 39.4, 35.3, 35.3, 35.0, 34.9, 34.4, 32.8, 31.0, 30.9, 28.1 , 26.7, 23.7, 22.7, 21.4, 20.5, 18.2, 11.7; HRMS (ESI, m/z) Calcd for $C_{27}H_{44}O_5$ 448.3189; found: 471.3082 $[M + Na]^+$ (Calcd 471.3086).

After filtered, the solvent was evaporated under reduced pressure to afford **H3-3** (0.6180 g, 92% yield) as colorless oil; mp 93-95 °C; FTIR (KBr, cm^{-1}) 1738 (C=O), 1168 (C-O), 1025, 663 and 554 (S-O); **H3-3** (6α): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 4.64-4.52 (m, 1H, 3β-H), 4.43-4.39 (m, 1H, 6β-H), 3.65 (s, 3H, -OCH_3), 1.99 (s, 3H, CH_3CO), 1.01 (s, 3H, 19-H), 0.89 (d, J = 6.3 Hz, 3H, 21-H), 0.62 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.7 (24-C), 170.4, 144.5, 134.5, 130.2, 129.8, 127.6, 127.1, 83.6 (C-6), 72.9 (C-3), 55.9, 55.9, 51.5, 45.7, 42.8, 40.1, 39.8, 35.3, 34.9, 34.3, 32.0, 31.6, 31.1, 30.9, 30.8, 28.1, 26.1, 25.0, 23.9, 21.6, 21.3, 20.4, 18.2, 12.0; HRMS (ESI, m/z) Calcd for $C_{34}H_{50}O_7S$ 602.3277; found: 625.3163 $[M + Na]^+$ (Calcd 625.3175).

*2.2.18. Methyl 3α-acetoxyl-6-ene-5β-cholanoate (**H3-4**)*

Compound **H3-3** (0.6191 g, 1.0 mmol) was dissolved in DMF (15 mL) and water (2 mL), and KOAc (1.008 g, 10.2 mmol) was added and refluxed for 4 h. DMF was evaporated, the residue was dissolved in EtOAc (20 mL). The organic layer was washed with saturated $NaHCO_3$ aq (5 mL×3), and brine (5 mL×3), and then dried by anhyd $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by column chromatography through silica gel (eluted with 10:1 hexane: ethyl acetate) to afford compound **H3-4** (0.3972 g, 90% yield) as a white solid; mp 123-125°C; $[\alpha]_D^{20}$ = +19.3 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 3444 (C=C), 1734 (C=O), 1165 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.49 (ddd, J = 9.9, 4.8, 2.5 Hz, 1H), 5.44 (d, J = 10.1 Hz, 1H), 4.70-4.63 (m, 1H, 3β-H), 3.66 (s, 3H, -OCH_3), 2.02 (s, 3H, CH_3CO), 0.92 (d, J = 6.5 Hz, 3H, 21-H), 0.85 (s, 3H, 19-H), 0.68 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.6 (24-C), 170.5, 130.3, 128.2, 73.6 (C-3), 55.8, 54.7, 51.4, 43.5, 43.3, 40.1, 39.8, 37.6, 35.9, 35.3, 34.1, 33.2, 31.1, 31.0, 28.2, 26.6, 23.8, 22.6, 21.3, 20.5, 18.2, 12.0; HRMS (ESI, m/z) Calcd for $C_{27}H_{42}O_4$ 430.3083; found: 453.2976 $[M + Na]^+$ (Calcd 453.2981).

*2.2.19. Methyl 3α-acetoxyl-6α, 7α-epoxyl-5β-cholanoate (**H3-5**)*

30% H_2O_2 (5 mL, 0.2 mol) was added dropwise to a stirred solution of phthalic anhydride (2.5 g, 16.9 mmol) in Et_2O (15 mL), after the mixture was stirred at rt for 24 h, a solution of **H3-4** (1.0 g, 2.3 mmol) in toluene (15 mL) was added, then Et_2O was evaporated in vacuo, stirring was continuing for 12 h. The mixture was washed with saturated Na_2CO_3 aq (5 mL×3), and brine (5 mL×3), and then dried by anhyd $MgSO_4$. After filtered, the solvent was evaporated under reduced pressure to afford compound **H3-5** (1.0 g, 96% yield) as a white solid; mp 121-123 °C; $[\alpha]_D^{20}$ = +21.7 (c = 0.5, CH_2Cl_2); FTIR (KBr, cm^{-1}) 1742 (C=O), 1192 (C-O-C), 1171 (C-O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.73-4.65 (m, 1H, 3β-H), 3.66 (s, 3H, -OCH_3), 3.12-3.06 (m, 2H), 2.02 (s, 3H, CH_3CO), 0.91 (d, J = 6.5 Hz, 3H, 21-H), 0.83 (s, 3H, 19-H), 0.69 (s, 3H, 18-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.6 (C-24), 170.7, 72.9 (C-3), 55.6, 55.1, 54.0, 51.8, 51.4, 43.2, 40.2, 39.6, 35.8, 35.3, 34.7, 33.8, 32.5, 31.1, 31.0, 29.5, 28.3, 26.2, 23.9, 23.4, 21.3, 20.2, 18.2, 11.9; HRMS (ESI, m/z) Calcd for $C_{27}H_{42}O_5$ 446.3032; found: 469.2928 $[M + Na]^+$ (Calcd 469.2930).

*2.2.20. Methyl 3α-acetoxyl-7α-hydroxyl-5β-cholanoate (**H3-6**)*

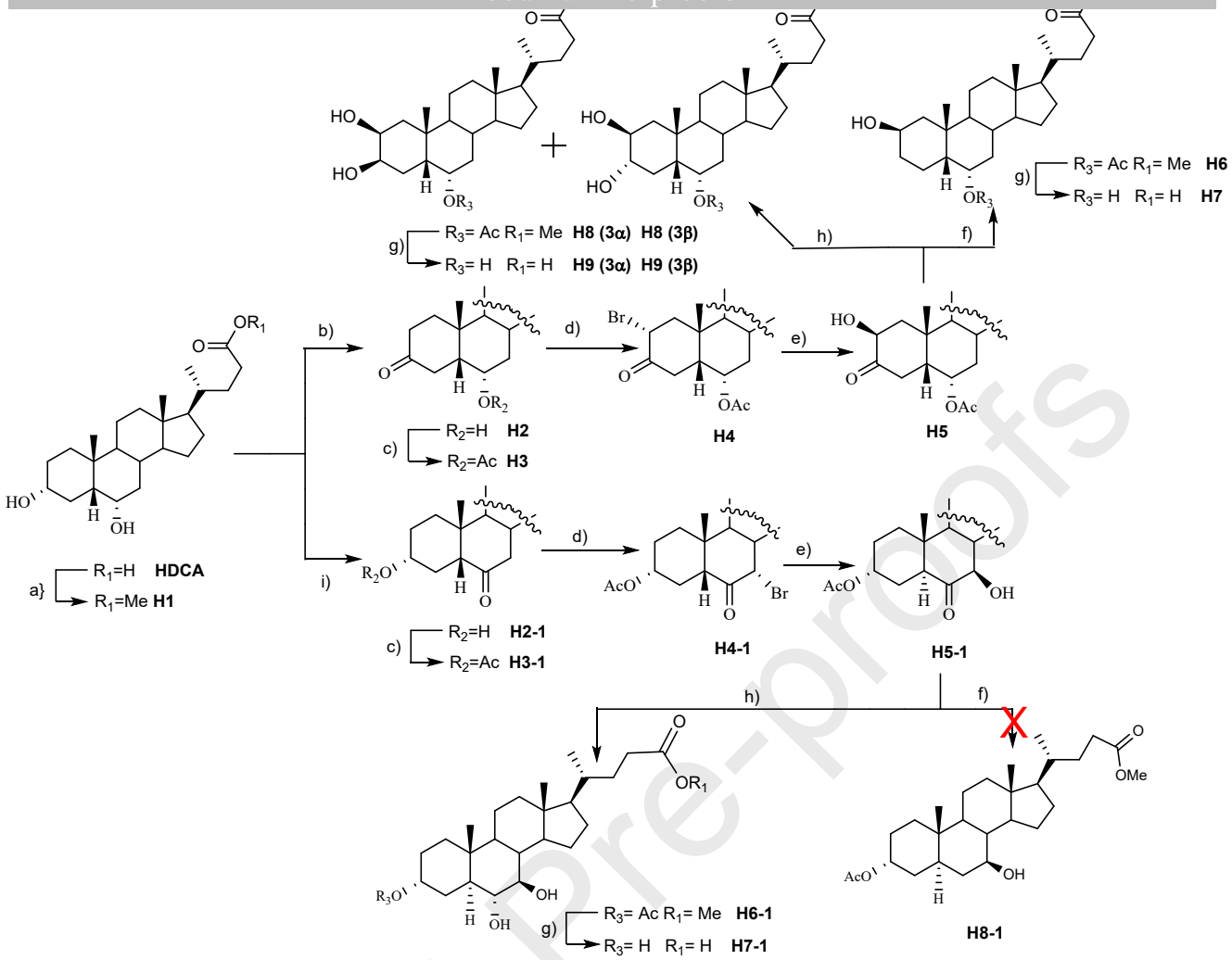
In a medium pressure hydrogenator, compound **H3-5** (0.3000 g , 0.1 mmol) was dissolved in ethanol (15 mL), 0.2500 g of 10% Pd/C (10% by mass of palladium in the palladium carbon catalyst) was added. The hydrogen pressure was set to 0.45 MPa, the reaction temperature was 90 °C and the reaction was carried out

2.2.21. Chenodeoxycholic acid

CDCA (0.0427 g, 97% yield) was synthesized as a white solid according to the similar synthetic method of compound **H9**; mp 119-121 °C; $[\alpha]_D^{20}$ = +12.5 (c = 0.5, MeOH); FTIR (KBr, cm^{-1}) 3450 and 3304 (O-H), 1703 (C=O); ^{1}H NMR (600 MHz, CD_3OD) δ 3.81-3.78 (m, 1H, 7β-H), 3.40 – 3.35 (m, 1H, 3β-H), 0.96 (d, J = 6.5 Hz, 3H, 21-H), 0.93 (s, 3H, 19-H), 0.70 (s, 3H, 18-H); ^{13}C NMR (151 MHz, CD_3OD) δ 178.4 (C-24), 72.1 (C-3), 68.6 (C-7), 55.7, 50.4, 42.7, 41.4, 39.8, 39.6, 39.4, 35.3, 35.3, 35.0, 34.5, 32.8, 30.8, 30.6, 29.7, 28.1, 23.7, 22.7, 20.5, 18.2, 11.7; HRMS (ESI, m/z) Calcd for $C_{24}H_{40}O_4$ 392.2927; found: 391.2864 $[M-H]^-$ (Calcd 391.2848). The results were in good agreement with the CDCA standard sample.

3. Results and discussion

In our efforts to optimize the synthetic process of CDCA from HDCA according to the literature [14-15] and to develop new cholic acid derivates [18-19], we were very surprised to find that the oxidants had an weird effect on the selective oxidation [20] of C3-OH or C6-OH in HDCA (**Scheme 2**). NMR spectra provided the most direct evidence to identify the site of selective oxidation. The ^{1}H NMR spectra of all compounds indicated two tertiary methyls at δ_H 0.9-1.0 and 0.6-0.7 (each 3H, s, CH3-18/CH3-19) and one secondary methyl at δ_H 1.30 (3H, d, J = ~6.5 Hz, CH3-21), which showed characteristics of a typical structures of cholic acid derivates. The chemical shifts of H atoms at C3 and C6 sites (δ_{H3} and δ_{H6}) in **H1** were assigned to be 3.64(m) and 4.07(m) ppm, respectively. δ_{H3} disappeared in **H2** when NBS acted as oxidant and a δ_c of 212.72 ppm was found, which suggested that NBS could selectively oxidize C3-OH in **H1** (**Scheme 2**). Rather, δ_{H6} disappeared and a δ_c of 213.86 ppm was found when IBX as oxidant was applied. These results indicated that the selective oxidation of C6-OH could be achieved by IBX as oxidant, rather than NBS, which was obviously different from the literature reported [15]. In order to further demonstrate our results, the chemical structures of **H2-1** and **H3-1** were determined by X-ray single crystal diffraction (**Figure 1**). In the crystal structures, the C6-O6 bond lengths were 1.204(3) Å in **H2-1** and 1.211(5) Å in **H3-1**, apparently shorter than the C3-O3 bond lengths of 1.417(3) Å in **H2-1** and of 1.464(4) Å in **H3-1**, suggesting that IBX could selectively oxidized C6-OH to the carbonyl compound (The oxygen atoms were defined in the same order as the carbon atoms for clarity).



Scheme 2. Synthetic Route I.

Reaction conditions: a) concd HCl, MeOH, 99%; b) NBS, AcOH, 83%; c) Ac_2O, DMAP, Et_3N, 99%; d) $CuBr_2$, 95%; e) K_2CO_3, 90%; f) PTSH, $NaBH_3CN$, $ZnCl_2$, 68%; g) NaOH aq, then HCl aq, 90%; h) $NaBH_4$, 95%; i) IBX, 72%.

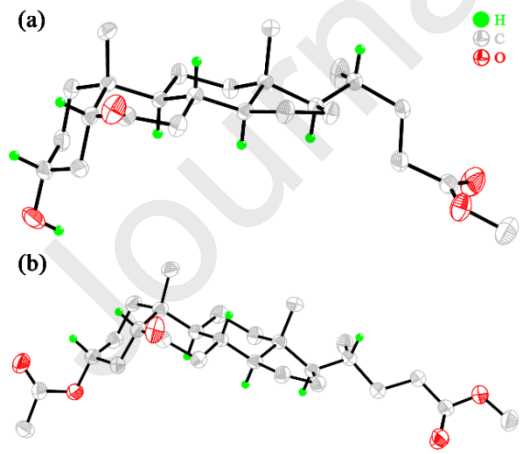


Figure 1. X-ray crystal structures of **H2-1** (a) and **H3-1** (b).

Based on the above results, two routes had been applied to introduce additional hydroxyl group or to transfer hydroxyl group in HDCA. In **Scheme 2**, C3-OH in **H1** was selectively oxidized to afford **H2** by NBS. Next, Ac_2O and Et_3N were used for the protection of C6-OH to produce **H3**. The α-H halogenation of C3-O3 keto in **H3** was carried out with $CuBr_2$ as halogen reagent and ethyl acetate as solvent to afford **H4** [21-22]. The hydrolysis of α-bromo keto **H4** with K_2CO_3 as base and acetone/H_2O as solvent was developed to provide the α-hydroxyl keto intermediate **H5** with a good yield of 90%. The reduction of C3-O3 carbonyl in **H5** to methylene was carried out by Shapiro reaction with PTSH, $NaBH_3CN$ and $ZnCl_2$ to afford **H6** according to the literatures [23-24], and the deprotection of **H6** produced a new isomer of HDCA, **H7**, which stereostructure was also confirmed by single crystal X-ray diffraction shown in **Figure 2**. C2-OH adopted a β-configuration, and C2-O2 bond length in ring A was 1.449(4) /1.454(3) Å, nearly equal to that of C6-O6 in ring B (1.454(3) / 1.437(3) Å). When C3-O3 carbonyl in ring A of **H5** was reduced by $NaBH_4$ and separated by column chromatography through silica gel to produce two pure isomers **H8** (3α) and **H8** (3β), which could be deprotected to afford the corresponding products (2β, 3α, 6α-**H9** and 2β, 3β, 6α-**H10**).

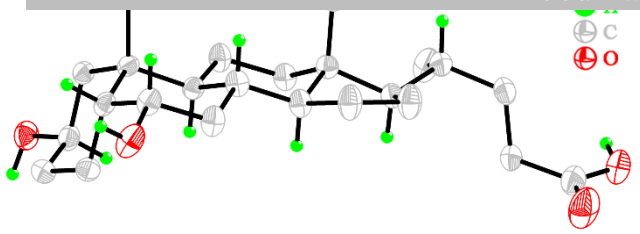


Figure 2. X-ray crystal structure of **H7**

On the other hand, C6-OH in intermediate **H1** could be selectively oxidized by IBX in *t*-BuOH to afford **H2-1** as the major product. After protected by Ac_2O and Et_3N, the α-H halogenation of C6-O6 keto in **H3-1** and hydrolysis of **H4-1** were carried out as followed the above steps to produce methyl 3α-acetoxyl-7β-hydroxyl-6-oxo-5α-cholanoate **H5-1** in a yield of 51%. The C6-O6 bond length of 1.206(3) Å was obviously shorter than C7-O7 (1.430(3) Å) and C3-O3 (1.468(6) Å) in **H5-1** (**Figure 3**), and equal to the C6-O6 bond lengths of 1.204(3) Å in **H2-1** and of 1.211(5) Å in **H3-1** within error, which implied that the introduction of the hydroxyl group at C7 was successful with 7β configuration. However, compared single crystal structure of **H5-1** with **H2-1** and **H3-1**, what's surprising to us was that H5 atom at C5 had a chiral inversion to form an epimerization from 5β to 5α configuration during the hydrolysis process. Furthermore, the attempt to reduce C6-O6 keto of **H5-1** to methylene was unsuccessful by Shapiro reaction and Huang-Minglong reduction, which might be due to the different stereostructure caused by the C5-H epimerization. But it was successful to be reduced by $NaBH_4$ to produce methyl 3α-acetoxyl-6α, 7β-dihydroxyl-5α-cholanoate **H6-1**, which could be hydrolyzed to afford a new epimer of ω-muricholic acid (*allo*-ω-muricholic acid), **H7-1.**

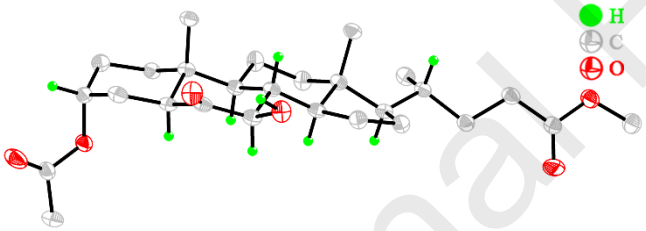
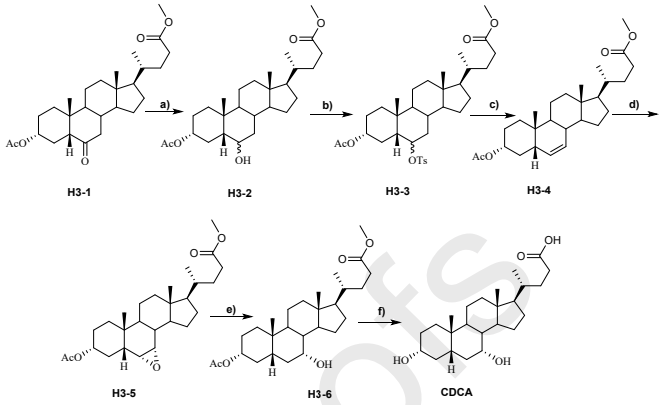


Figure 3. X-ray crystal structure of **H5-1**

In order to further identify the epimerization from 5β-H to 5α-H at C5, the 2D NOESY spectra of **H2-1**, **H3-1, H4-1** and **H5-1** were investigated carefully (ESI, Table S1-4, Fig. S1-4). H-5 (δ = 2.13 ppm for **H2-1**, 2.18 ppm for **H3-1** and 3.59 ppm for **H4-1**) and H-7 (δ = 4.20 ppm for **H4-1**) is related to H-3 (δ = 3.64 ppm for **H2-1**, 4.69 ppm for **H3-1** and 5.13 ppm for **H4-1**), which suggested that H-3, H-5 and H-7 adopted the β configuration in **H2-1**, **H3-1** and **H4-1**. However, no correlation is observed between H-5 (δ = 2.61 ppm), H-7 (δ = 3.81 ppm) and H-3(δ = 5.13 ppm) in **H5-1**, which suggested that H-5 and H-7 happened to be configuration inversions during the hydrolysis process from **H4-1** to **H5-1**. The results were in good agreement with the single crystal x-ray diffraction, but the mechanism was ill-defined yet.

In order to synthesize CDCA from HDCA, a new strategy had been proposed as shown in **Scheme 3**. $NaBH_4$ was used for reduction of C6=O6 carbonyl in **H3-1** to afford a 6-hydroxyl compound, which was treated with *p*-toluenesulfonxyl chloride, and then followed with KOAc to produce methyl an encouraging result that the elimination reaction happened at C6-C7 bond, not in C5-C6, attested by a single crystal X-ray diffraction study. The C6-C7 bond length was 1.328(6)/1.321(7) Å, obviously short than the C-C single bond lengths (about 1.50 Å) in the crystal structure of **H3-4** (**Figure 4**).



Scheme 3. The transformation route of HDCA to CDCA

Reaction Conditions: a) $NaBH_4$, 92%; b) *p*-toluenesulfonxyl chloride, 92%; c) KOAc, 90%; d) monoperoxyphthalic acid, 96%; e) 10% Pd/C, H_2 (4 atm); 90%; f) NaOH aq, then HCl aq; 97%

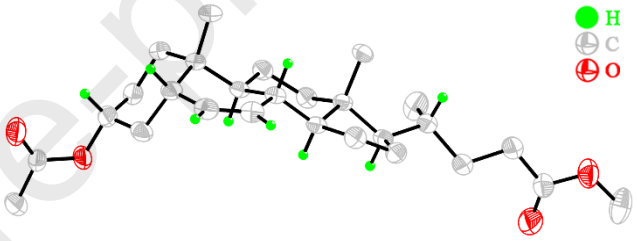


Figure 4. X-ray crystal structure of **H3-4**

In order to estimate how thermodynamically favorable for the potential elimination products (methyl 3α-acetoxyl-6-ene-24-choloate, **H3-4**, and methyl 3α-acetoxyl-5-ene-24-choloate), quantum chemical calculations were carried out at the B3LYP level of density functional theory using 6-31G* basis sets for all atoms. Thus, we found that the hypothetical formation of **H3-4** was energetically favorable with ΔH energy of –1.85 kJ/mol over that of methyl 3α-acetoxyl-5-ene-24-choloate (ESI, Table S5-7). **H3-4** was oxidized by monoperoxyphthalic acid to form an oxirane **H3-5**, which crystal structure featured that the oxirane adopted the α configuration (**Figure 5**). **H3-5** could undergo hydrogenation catalyzed by Pd/C and deprotection to produce CDCA by a regioselective conversion. The route was verified to be mild and efficient with overall 45% yield from HDCA (8 steps).

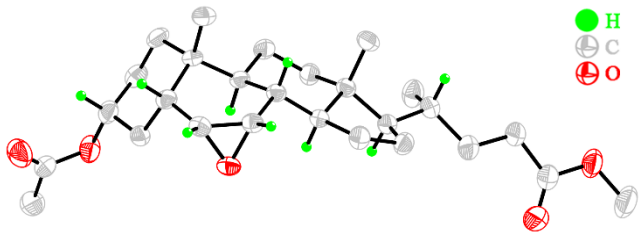


Figure 5. X-ray crystal structure of **H3-5**

In conclusion, five cholic acid derivatives including CDCA were synthesized from hyodeoxycholic acid by selective oxidation and stereocontrolled conversion. Two environmentally friendly oxidants, IBX and NBS, were found to be capable of selectively oxidizing C6-OH or C3-OH groups of methyl hyodeoxycholic ester, respectively. C6=O keto could be reduced by $NaBH_4$ to afford a 6-hydroxyl mixture with a dominant 6α configuration, and undergo the elimination reaction through *p*-toluenesulphonate ester and KOAc to specifically produce a C6=C7 double compound. The carbonyl in ring A could be easily reduced by Shapiro reaction with PTSH, $NaBH_3CN$ and $ZnCl_2$, but the reduction reaction can't happen to C6-O6 keto in 5α-configuration. These findings can provide theoretical basis and reference for the further study of cholic acids.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https:// doi. Org/10. 1016/j.steroids.*****

Abbreviations

CA	Cholic acid
CDCA	Chenodeoxycholic acid
DMAP	4-Dimethylaminopyridine
DMF	*N, N*-Dimethylformamide
HDCA	Hyodeoxycholic acid
IBX	2-Iodoxybenzoic acid
NBS	N-Bromosuccinimide
Py	Pyridine
PTSH	para-Toluenesulfonhydrazide
UDCA	Ursodeoxycholic acid

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Highlights

- The hydroxyl groups in hyodeoxycholic acid are selectively oxidized by 2-iodoxybenzoic acid and *N*-bromosuccinimide as oxidants.
- Hydrolyzing 7α-Br in 6-keto hyodeoxycholic acid can cause the steric inversion from 5β to 5α configuration.
- The reduction of 6-keto to methylene could undergo only at 5β configuration with *para*-toluenesulfonhydrazide, $NaBH_3CN$ and $ZnCl_2$.
- Quantum chemical calculations were carried out to evaluate the potential elimination products.
- A feasible synthetic route of chenodeoxycholic acid from hyodeoxycholic acid has been established with the yield of 45 % (8 steps).

Graphical Abstract

Efficient synthesis of cholic acid derivates through stereoselective C-H functionalization from hyodeoxycholic acid

Yu-yan Liang, Huan Huang, Yang Li, Rong-kai Du, Jing Li, Yong-hong Liu, Shan Li, and Lei Zhang