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A facile synthesis of ursodeoxycholic acid and obeticholic acid from cholic acid

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ABSTRACT

A novel synthetic route of producing ursodeoxycholic acid (UDCA) and obeticholic acid (OCA) was developed through multiple reactions from cheap and readily-available cholic acid. The reaction conditions of the key elimination reaction of mesylate ester group were also investigated and optimized, including solvent, base and reaction temperature. In the straightforward synthetic route for preparation of UDCA and OCA, most of the reaction steps have high conversions with average yields of 94% and 92%, and overall yield up to 65% (7 steps) and 36% (11 steps) from cholic acid, respectively. This promising route offers economical and efficient strategies for potential large-scale production of UDCA and OCA.

1. Introduction

Primary biliary cholangitis (PBC), previously termed primary biliary cirrhosis, is a rare autoimmune inflammatory liver disease, which produces bile duct injury, fibrosis, cholestasis and eventual cirrhosis. PBC mainly affects middle-aged women, with a sex ratio of at least 9:1 female to male [1,2]. Ursodeoxycholic acid (UDCA) (Fig. 1), one of the secondary bile acids, is the only drug approved by the US Food and Drug Administration (FDA) for the treatment of PBC since the year 1988 [3,4]. UDCA in patients has been shown to improve serum biochemistries, liver transplantation-free, and ameliorate the overall morbidity and mortality associated with PBC [5–7]. In addition, the clinical properties of UDCA include non-surgical treatment of cholesterol gallstones, anti-apoptotic effects, decreasing serum TNF-α concentrations and improving muscle and hepatic insulin sensitivity [8–11]. UDCA was the recommended first-line drug for treatment of PBC until 2016 when obeticholic acid (OCA, also known as 6-ECDCA or INT-747) (Fig. 1) was approved by US FDA and European Medicines Agency [12]. OCA is a farnesoid X receptor (FXR) agonist, which has been evaluated as a second-line therapy in PBC [13]. It is used in patients who show an inadequate response to UDCA or who are unable to tolerate UDCA [14].

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in developed countries, which is defined as the presence of hepatic steatosis in patients due to causes other than excessive alcohol use [15,16]. NAFLD is a metabolic syndrome and associated with insulin resistance, obesity, dyslipidemia and hypertension [17,18]. Nonalcoholic steatohepatitis (NASH) is the most extreme form of NAFLD and the leading cause of hepatic morbidity and mortality [19]. It is also regarded as a major cause of cirrhosis, which ultimately lead to hepatocellular carcinoma and has become the second leading indication for liver transplantation in United States [20]. NASH affects 2–5% of adults in United States and other Western countries [21]. Despite the significant burden to the public health system, there are no current FDA-approved therapies for this disease [22]. It is gratifying that there are several drugs in the phase III trial in NASH patients. For instance, elafibranor (GFT505), a dual peroxisome proliferator-activated receptor alpha/delta (PPARα/δ) agonist, is being studied by Genfit; Liraglutide, a glucagon-like peptide-1 (GLP-1) agonist, is being studied by Novo Nordisk; OCA, a bile acid derivative that acts as an agonist of the FXR, is being studied by Intercept Pharmaceuticals [23]. Among these novel agents, the OCA is a very promising medication and gets FDA breakthrough therapy designation for NASH with liver fibrosis [22]. Accordingly, exploitation of economical and efficient synthetic methods of UDCA and OCA will not only benefit PBC patients, but also promote the further development of NASH medications.

UDCA is usually synthesized from its relatively expensive epimer chenodeoxycholic acid (CDCA) (Fig. 1) by regioselective oxidation of 7α-OH to 7-oxo and then reduction to 7β-OH [24–26]. Recently other chemical methods for the preparation of UDCA have also been developed. Zhou et al. synthesized of UDCA from low cost hyodeoxycholic acid (HDCA) (Fig. 1) with a 15% poor total yield [27]. Subsequently

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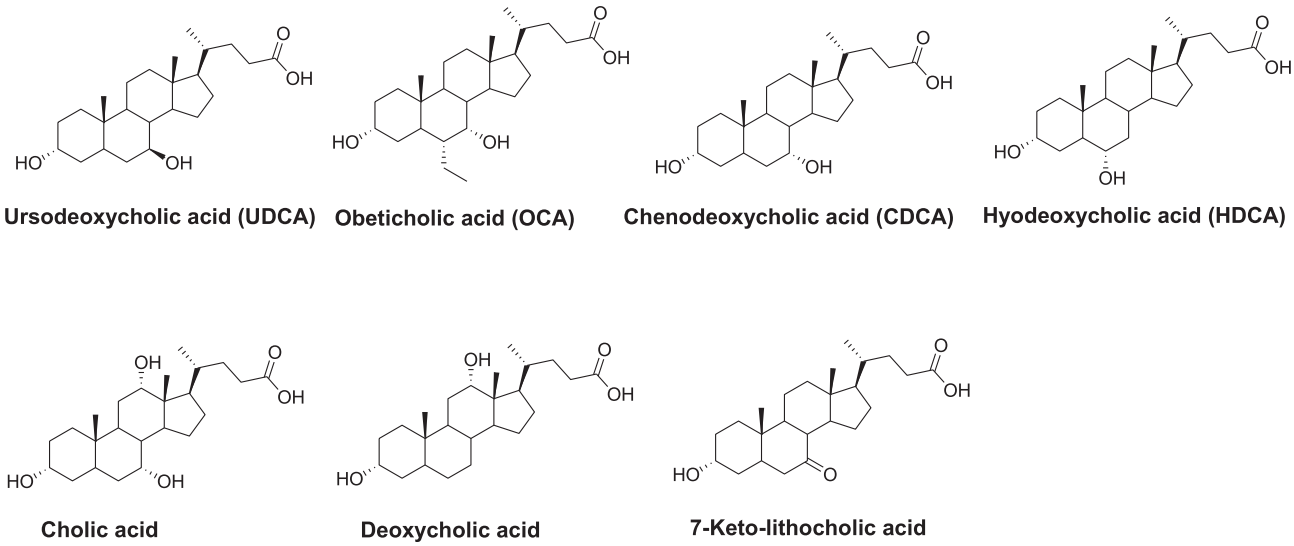


Fig. 1. Structures of obeticholic acid, 7-keto-lithocholic acid and bile acids.

Dou et al. developed a route from HDCA with an improved 26% overall yield via a key Shapiro reaction [28]. UDCA can also be synthesized from cholic acid (Fig. 1). For instance, Ferrari et al. provided a 10% low overall yield method by a crucial high-temperatured Wolff–Kishner reduction [29]; Dangate et al. also exploited an optimized protection-free route (53% overall yield) with o-iodoxybenzoic acid (IBX) as key regioselective oxygenant and hydrazine hydrate as key Wolff–Kishner reducing agent from cholic acid [30]. Cholic acid is a bile acid and relatively much cheap than CDCA, which can be commercially and abundantly available. Thus, it is rather valuable to develop an improved synthetic route for large scale production of UDCA using the cost-effective and readily-available starting material cholic acid.

OCA is generally synthesized from CDCA. For examples, Yu et al. reported a 5 steps approach (20% overall yield), and the key reactions are regioselective oxidation of the 7α-OH with PCC, enolate formation with LDA/HMPA and alkylation with iodoethane [31]; Valentina et al. also used the CDCA as the starting material for preparation of OCA with an improved synthetic strategy (32% overall yield), and the crucial steps are aldol addition with acetaldehyde, selective reduction of the C7-ketone with NaBH4 and $CeCl_3$, and stereoselective hydrogenation of exocyclic double bond [32]. Alexander et al. exploited a synthetic route to OCA with a 3% poor overall yield from the cheap deoxycholic acid [33] (Fig. 1). In addition, a procedure provided by Pellicciari et al. starts with the expensive 7-keto-lithocholic acid (Fig. 1) and gives a very low overall yield (2–3%) [34]. However, there is no efficient procedure available for preparation of OCA that begins with cholic acid.

Herein, we report an efficient and economical synthetic route of UDCA and OCA from cost-effective cholic acid.

2. Experimental

2.1. General procedures

All reagents and chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. When needed, the reactions were carried out in oven-dried glassware under a positive pressure of dry N_2. Column chromatography was performed on silica gel (QinDao, 200–300 mesh) using the indicated eluents. Thin-layer chromatography was carried out on silica gel plates (QinDao) with a layer thickness of 0.25 mm. Melting points were determined using the MEL-TEMP 3.0 apparatus and uncorrected. ^1H (400 MHz or 500 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker 400 MHz or 500 MHz spectrometer with $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane (TMS) as the internal standard. All chemical shift values were reported in units of δ (ppm). The following abbreviations were used to indicate the peak multiplicity: s = singlet; d = doublet; t = triplet; m = multiplet; br = broad. High-resolution mass data were obtained on a BrukermicroOTOF-Q II spectrometer.

2.2. Chemical synthesis

2.2.1. 3α,12α-Dihydroxy-7-keto-5β-cholan-24-oic acid (1)

To a solution of cholic acid (18 g, 44.1 mmol) in a mixed solvent (400 mL) of acetone-H_2O (3:1, v/v) was added NBS (11.4 g, 64.0 mmol) at room temperature. The flask was covered with aluminum foil to keep the reaction in the dark. The reaction mixture was stirred at room temperature for 2 h and then added a saturated solution of $NaHSO_3$ (50 mL). The mixture was evaporated under reduced pressure to remove acetone, and then poured into H_2O (100 mL) and extracted with CH_2Cl_2 (100 mL × 3). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and then concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 20/1, v/v) to give **1** [35] (16.5 g, 92%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 4.00 (s, 1H), 3.64–3.58 (m, 1H), 1.17 (s, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.68 (s, 3H).

2.2.2. Methyl 3α,12α-dihydroxy-7-keto-5β-cholan-24-oate (2)

To a solution of **1** (8.5 g, 20.9 mmol) in MeOH (100 mL) was added concentrated H_2SO_4 (2 mL) at room temperature. The reaction mixture was refluxed for 2 h, and then concentrated. The residue was dissolved in DCM (100 mL). Organic layer was washed with 5% aqueous NaOH and brine, dried over anhydrous Na_2SO_4 and concentrated to give compound **2** [36] (8.7 g, 99%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 4.00 (s, 1H), 3.66 (s, 3H), 3.58 (s, 1H), 1.18 (s, 3H), 0.97 (d, J = 5.2 Hz, 3H), 0.68 (s, 3H).

2.2.3. Methyl 3α-acetoxy-12α-hydroxy-7-keto-5β-cholan-24-oate (3)

To a solution of **2** (8.7 g, 20.7 mmol) in dry CH_2Cl_2 (80 mL), Ac_2O (2.5 mL, 26.8 mmol), pyridine (3.3 mL, 41.2 mmol) and DMAP (122 mg, 1 mmol) were added at room temperature. The reaction mixture was stirred for 2 h under nitrogen atmosphere at room temperature and concentrated. The residue was poured into H_2O (30 mL) and extracted with AcOEt (30 mL × 3). The organic layer was washed with aqueous 2 M HCl and brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 3/1, v/v) to give **3** [37] (8.6 g, 90%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 4.71–4.63 (m, 1H), 4.00 (s, 1H), 3.66 (s, 3H), 1.99

(s, 3H), 1.18 (s, 3H), 0.97 (d, J = 6.0 Hz, 3H), 0.68 (s, 3H).

2.2.4. Methyl 3α-acetoxy-12α-methanesulfonate-7-keto-5β-cholan-24-oate (4)

To a solution of **3** (8.6 g, 18.6 mmol) in dry pyridine (80 mL), MsCl (2.9 mL, 37.2 mmol) was added at room temperature. The reaction mixture was stirred for 8 h under nitrogen atmosphere at room temperature and then concentrated. The residue was poured into aqueous 2 M HCl (50 mL) and extracted with AcOEt (50 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 5/1, v/v) to give **4** (9.9 g, 99%) as a white solid; mp: 75–77 °C. IR (ATR) cm^{-1}: 1730, 1710, 1244, 1171, 905. ^1H NMR (400 MHz, $CDCl_3$) δ 5.12 (s, 1H), 4.70–4.62 (m, 1H), 3.66 (s, 3H), 3.06 (s, 3H), 1.99 (s, 3H), 1.20 (s, 3H), 0.98 (d, J = 6.4 Hz, 3H), 0.76 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.7, 174.4, 170.6, 82.9, 72.8, 51.5, 49.0, 46.4, 45.9 (2C), 45.1, 41.4, 39.4, 36.4, 35.0, 34.8, 33.7, 33.3, 30.9 (2C), 27.8, 27.6, 25.7, 24.0, 22.8, 21.3, 17.8, 12.5. HRMS (ESI): calcd for $C_{28}H_{44}NaO_8S$ $[M+Na]^+$: 563.2675, found 563.2649.

2.2.5. Methyl 3α-acetoxy-7-keto-5β-chol-11-enoate (5)

To a solution of **4** (9.5 g, 17.6 mmol) in NMP (80 mL), CH_3COOK (17.2 g, 176 mmol) was added at room temperature. The reaction mixture was stirred for 12 h under nitrogen atmosphere at 135 °C. After cooling, the residue was poured into H_2O (150 mL) and extracted with AcOEt (60 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 10/1, v/v) to give **5** (7.2 g, 92%) as a white solid; mp: 138–140 °C. IR (ATR) cm^{-1}: 1731, 1712, 1244, 1038. ^1H NMR (500 MHz, $CDCl_3$) δ 6.21 (dd, J = 10.5 Hz, 3.0 Hz, 1H), 5.33 (dd, J = 10.5 Hz, 2.0 Hz, 1H), 4.74–4.67 (m, 1H), 3.66 (s, 3H), 1.99 (s, 3H), 1.14 (s, 3H), 1.01 (d, J = 6.5 Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.9, 174.4, 170.4, 139.7, 123.8, 72.7, 51.4, 50.6, 47.4, 47.2, 45.4, 44.9, 44.7, 44.5, 35.8, 35.4, 33.7, 33.6, 31.0, 30.8, 28.6, 25.9, 23.5, 23.0, 21.2, 18.3, 17.0. HRMS (ESI): calcd for $C_{27}H_{40}NaO_5$ $[M+Na]^+$: 467.2776, found 467.2768.

2.2.6. 3α-Hydroxy-7-keto-5β-chol-11-en-24-oic acid (6)

To a solution of **5** (7.0 g, 15.7 mmol) in a mixed solvent (60 mL) of MeOH-THF (4:1, v/v), NaOH (6.3 g, 157 mmol) and H_2O (5 mL) were added at room temperature. The reaction mixture was refluxed for 4 h under nitrogen atmosphere. After cooling, the reaction mixture was acidified to pH 5 with aqueous 1 M HCl and evaporated under reduced pressure to remove MeOH and THF. The residue was poured into H_2O (80 mL) and extracted with CH_2Cl_2 (50 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 10/1, v/v) to give **6** (5.7 g, 93%) as a white solid; mp: 214–216 °C. IR (ATR) cm^{-1}: 3275, 2920, 2863, 1728, 1698, 1278, 1052. ^1H NMR (400 MHz, $CDCl_3$) δ 6.20 (d, J = 10.4 Hz, 1H), 5.35 (d, J = 10.0 Hz, 1H), 3.62 (s, 1H), 1.13 (s, 3H), 1.02 (d, J = 6.0 Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 210.7, 174.7, 138.7, 124.5, 69.0, 50.1, 47.4, 46.4, 45.3, 44.5, 44.4, 44.0, 38.1, 35.3, 35.0, 33.6, 30.7, 30.6, 29.8 28.2, 23.1, 22.8, 18.2, 16.8. HRMS (ESI): calcd for $C_{24}H_{36}NaO_4$ $[M+Na]^+$: 411.2493, found 411.2506.

2.2.7. Ursodeoxycholic acid (UDCA)

To a solution of **6** (4.0 g, 10.3 mmol) in *i*-PrOH (100 mL) in autoclave, was added Raney-Ni (4.0 g), *t*-BuOK (1.2 g, 10.8 mmol) and KBH_4 (1.6 g, 29.4 mmol), then flushed with H_2 (4.0 MPa). The reaction mixture was stirred for 24 h at 40 °C. After cooling, the reaction mixture was acidified to pH 5 with AcOH and the catalyst was removed by filtration through celite, then the filtrate was evaporated under reduced pressure. The residue was poured into H_2O (200 mL) and extracted with CH_2Cl_2 (50 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 20/1, v/v) to give **UDCA** (3.8 g, 93%) as a white solid; mp: 198–200 °C [lit. [17] 195–197 °C]. $[\alpha]_D^{25}$ +59.7 (c 1.0, CH_3CH_2OH). ^1H NMR (400 MHz, $CDCl_3$) δ 3.63–3.57 (m, 2H), 0.94 (d, J = 6.0 Hz, 6H), 0.68 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.8, 69.7, 69.4, 55.8, 54.7, 43.1, 43.0, 42.2, 39.9, 39.8, 39.7, 38.7, 37.7, 37.2, 34.8, 33.7, 30.7, 30.2, 28.2, 26.7, 23.3, 20.8, 18.3, 12.0. HRMS (ESI): calcd for $C_{24}H_{40}O_4$ $[M+H]^+$, 393.2984, found 393.3005.

2.2.8. Methyl 3α-hydroxy-7-keto-5β-chol-11-enoate (7)

To a solution of **5** (7.0 g, 15.7 mmol) in MeOH (50 mL), NaOH (690 mg, 17.3 mmol) was added at room temperature. The reaction mixture was stirred for 1.5 h under nitrogen atmosphere at room temperature. The reaction mixture was acidified to pH 5 with aqueous 1 M HCl and evaporated under reduced pressure to remove MeOH. The residue was poured into H_2O (80 mL) and extracted with AcOEt (50 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 2/1, v/v) to give **7** (6.3 g, 99%) as a white solid; mp: 94–96 °C. IR (ATR) cm^{-1}: 3362, 3240, 1732, 1710, 1265, 1064. ^1H NMR (400 MHz, $CDCl_3$) δ 6.20 (d, J = 10.0 Hz, 1H), 5.35 (d, J = 10.0 Hz, 1H), 3.66 (s, 3H), 3.62 (s, 1H), 1.13 (s, 3H), 1.01 (d, J = 6.0 Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.8, 174.5, 139.5, 124.0, 70.6, 51.5, 50.6, 47.5, 47.3, 45.7, 45.2, 44.9, 44.5, 38.0, 35.9, 35.4, 34.1, 31.1, 30.9, 29.8, 28.7, 23.5, 23.1, 18.3, 17.0. HRMS (ESI): calcd for $C_{25}H_{38}NaO_4$ $[M+Na]^+$: 425.2663, found 425.2662.

2.2.9. Methyl 3α,7-trimethylsilyloxy-5β-chol-11-enoate (8)

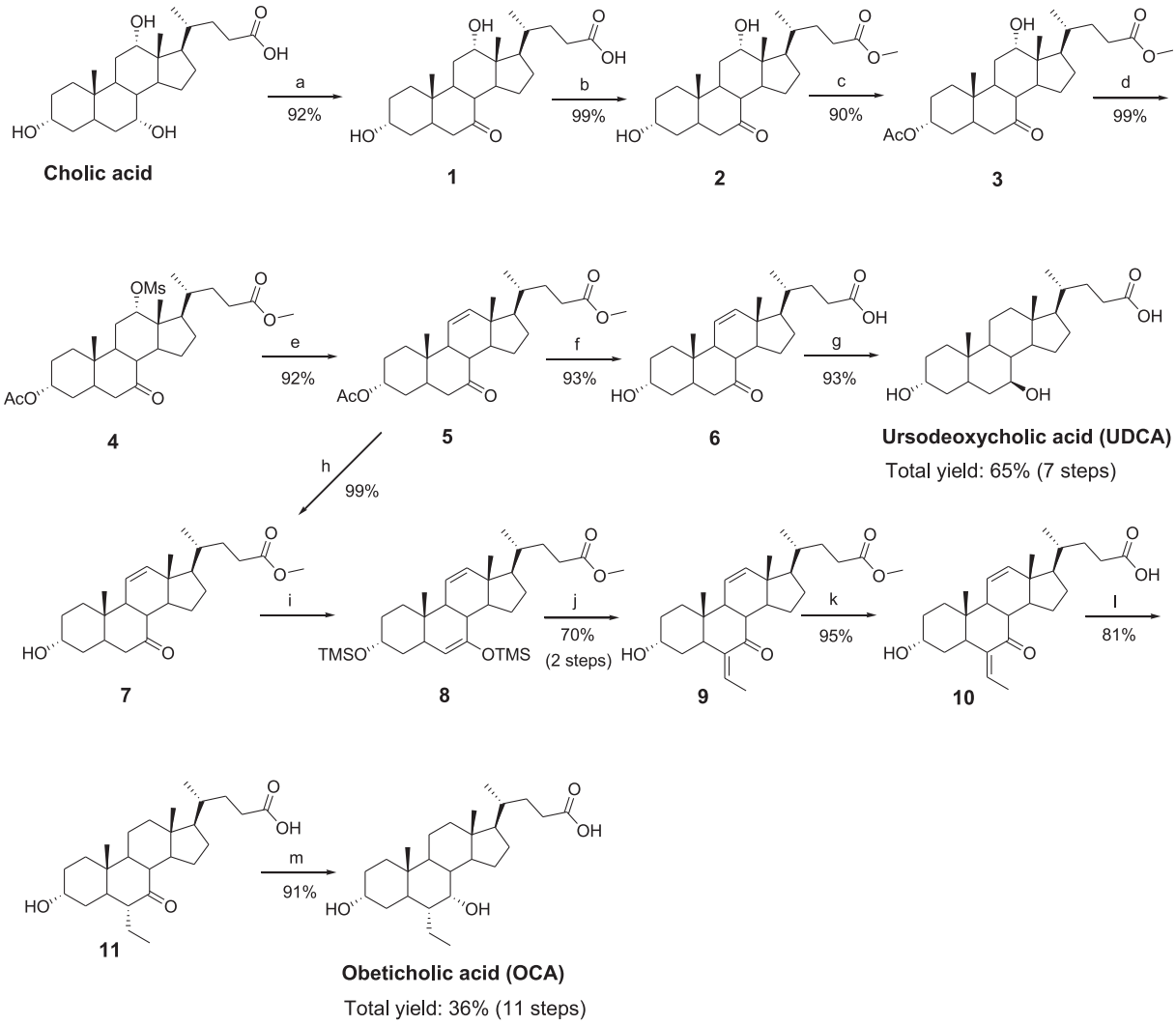
To a solution of LDA (100 mL, 100 mmol) in dry THF (100 mL), TMSCl (12.7 mL, 100 mmol) was added under nitrogen atmosphere at −78 °C. The reaction mixture was stirred for 20 min, then a solution of **7** (4.0 g, 10.0 mmol) in dry THF (50 mL) was added dropwise in 10 min. The reaction mixture was stirred at −78 °C for an additional 1 h, and then triethylamine (16.6 mL, 120 mmol) was added. After 1 h, the reaction mixture was allowed to warm to room temperature, treated with aqueous saturated solution of $NaHCO_3$ (30 mL). The organic phase was separated, and the aqueous phase was extracted with AcOEt (50 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated to give **8** as a yellow residue, which was subjected to next step without any purification.

2.2.10. Methyl 3α-hydroxy-6-ethyliden-7-keto-5β-chol-11-enoate (9).

To a solution of **8** in CH_2Cl_2 (100 mL), was added acetaldehyde (1.1 mL, 20.0 mmol) under nitrogen atmosphere at −60 °C. The reaction mixture was added dropwise $BF_3{\cdot}OEt_2$ (12.6 mL, 100 mmol). The reaction mixture was stirred for 2 h at −60 °C and allowed to warm to 35 °C and stirred for 2 h at this temperature. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ and extracted with AcOEt (50 mL × 3). The combined organic phases were washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 40/1, v/v) to give **9** (3.0 g, 70%, over two steps) as a white solid; mp: 74–76 °C. IR (ATR) cm^{-1}: 3418, 1734, 1688, 1264, 1168, 1061, 1020. ^1H NMR (400 MHz, $CDCl_3$) δ 6.23 (d, J = 10.0 Hz, 1H), 6.16 (q, J = 6.0 Hz, 1H), 5.41 (d, J = 10.4 Hz, 1H), 3.66 (br, 4H), 1.69 (d, J = 6.4 Hz, 3H), 1.01 (d, J = 6.0 Hz, 3H), 0.98 (s, 3H), 0.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.5, 174.5, 143.7, 140.0, 129.5, 123.7, 70.4, 51.5, 50.4, 48.7 46.6, 45.7, 45.5, 41.4, 38.3, 36.0, 35.0, 34.5, 31.1, 30.9, 29.7, 29.0, 24.5, 23.7, 18.4, 17.0, 12.6. HRMS (ESI): calcd for $C_{27}H_{40}NaO_4$ $[M+Na]^+$: 451.2834, found 451.2819.

2.2.11. 3α-Hydroxy-6-ethyliden-7-keto-5β-chol-11-en-24-oic acid (10)

To a solution of **9** (2.0 g, 4.7 mmol) in MeOH (100 mL), NaOH (560 mg, 14.0 mmol) and H_2O (6 mL) were added at room temperature. The reaction mixture was stirred for 3 h under nitrogen atmosphere at 50 °C. After cooling, the reaction mixture was acidified to pH 5 with aqueous 1 M HCl and evaporated under reduced pressure to remove MeOH. The residue was



Scheme 1. Synthesis of ursodeoxycholic acid and obeticholic acid from cholic acid. Reagents and conditions: (a) NBS, acetone, H_2O, rt, 92%; (b) MeOH, H_2SO_4, reflux, 99%; (c) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, 90%; (d) MsCl, pyridine, 99%; (e) CH_3COOK, NMP, 135 °C, 92%; (f) NaOH, H_2O, MeOH, THF, reflux, 93%; (g) Raney-Ni, KBH_4, H_2 (4 MPa), *t*-BuOK, *i*-PrOH, 40 °C, 93%; (h) NaOH, MeOH, rt, 99%; (i) TMSCl, LDA, Et_3N, THF, −78 °C; (j) MeCHO, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −60 °C, 70% (over two steps); (k) NaOH, H_2O, MeOH, 50 °C, 95%; (l) (1)H_2 (4 MPa), Pd/C, MeOH, 70 °C; (2) NaOH (30%), reflux, 81%; (m) NaOH (50%), $NaBH_4$, reflux, 91%.

poured into H_2O (60 mL) and extracted with CH_2Cl_2 (30 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 20/1, v/v) to give **10** (1.8 g, 95%) as a white solid; mp: 103–104 °C. IR (ATR) cm^{-1}: 3405, 2918, 2882, 1264, 1060, 1021. ^1H NMR (400 MHz, $CDCl_3$) δ 6.24 (dd, J = 10.4, 2.4 Hz, 1H), 6.16 (q, J = 6.8 Hz, 1H), 5.42 (d, J = 10.4 Hz, 1H), 3.71–3.63 (m, 1H), 1.70 (d, J = 7.2 Hz, 3H), 1.03 (d, J = 6.4 Hz, 3H), 0.99 (s, 3H), 0.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.6, 179.2, 143.6, 140.1, 129.7, 123.7, 70.5, 50.4, 48.7, 46.6, 45.7, 45.5, 41.4, 38.2, 36.0, 35.0, 34.5, 31.1, 30.7, 29.6, 29.0, 24.5, 23.7, 18.4, 17.0, 12.6. HRMS (ESI): calcd for $C_{26}H_{38}NaO_4$ $[M+Na]^+$: 437.2661, found437.2662.

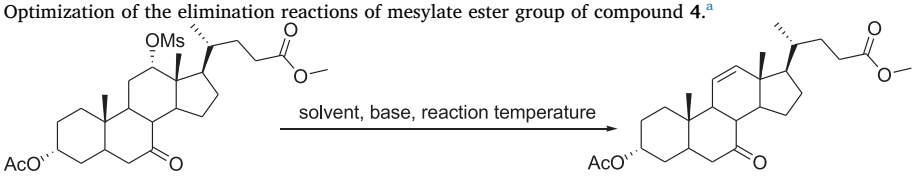
2.2.12. 3α-Hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (11)

To a solution of **10** (1.7 g, 4.1 mmol) in MeOH (120 mL), 10% palladium on carbon (170 mg) was added under nitrogen atmosphere at room temperature in autoclave. Then the autoclave was flushed with H_2 (4.0 MPa) and stirred for 24 h at 70 °C. After cooling, the catalyst was removed by filtration through Celite, and the filtrate was evaporated under reduced. The residue was poured into 30% aqueous solution of NaOH (140 mL) and refluxed for 5 h under nitrogen atmosphere. Then the reaction mixture was acidified to pH 5 with H_3PO_4 (85%) and extracted with CH_2Cl_2 (30 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 20/1, v/v) to give **11** (1.4 g, 81%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 3.56–3.51 (m, 1H), 1.22 (s, 3H), 0.93 (d, J = 6.4 Hz, 3H), 0.80 (t, J = 7.2 Hz, 3H), 0.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.0, 179.5, 71.2, 54.8, 52.0, 50.7, 50.0, 49.0, 43.7, 42.7, 39.0, 35.7, 35.2, 34.3, 31.6, 31.1, 30.8, 29.7, 28.3, 24.6, 23.5, 21.9, 18.8, 18.3, 12.1, 12.0.

2.2.13. Obeticholic acid (OCA)

To a solution of **11** (1.3 g, 3.1 mmol) in H_2O (100 mL), was added NaOH (1.0 g, 32.5 mmol) and the reaction mixture was heated to reflux. Then a solution of $NaBH_4$ (130 mg, 3.4 mmol) in H_2O (30 mL) was added in 10 min. The reaction mixture was reflux for 4 h. After cooling, the reaction mixture was acidified to pH 5 with aqueous 1 M HCl and extracted with CH_2Cl_2 (30 mL × 3). The organic layer was washed with brine, dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 10/1, v/v) to give **OCA** (1.2 g, 91%) as a white solid; mp: 120–122 °C [lit. [38] 120–124 °C]. $[\alpha]_D^{25}$ +5.20 (*c* 1.8, CH_3OH). ^1H NMR (400 MHz, $CDCl_3$) δ 3.70 (s, 1H), 3.45–3.37 (m, 1H), 0.93 (d, J = 6.4 Hz, 3H), 0.92–0.88 (m, 6H), 0.66 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.9, 70.6,

Table 1
Optimization of the elimination reactions of mesylate ester group of compound **4**.[a]



4 **5**

Entry	Solvent	Base	RT[b] (°C)	Yield[c] (%)
1	DMPU	CH_3COOK	125	90
2	NMP	CH_3COOK	125	90
3	DMF	CH_3COOK	125	56
4	DMSO	CH_3COOK	125	82
5	Toluene	CH_3COOK	reflux	N[d]
6	NMP	MeONa	125	C[e]
7	NMP	NaOH	125	C
8	NMP	K_2CO_3	125	84
9	NMP	*t*-BuOK	125	C
10	NMP	Na_2CO_3	125	75
11	NMP	Cs_2CO_3	125	70
12	NMP	CH_3COOK	115	80
13	NMP	CH_3COOK	135	92
14	NMP	CH_3COOK	145	81

[a] All the reactions were performed for 12 h, and the ratio of base/compound **4** was 10:1 (mol: mol).
[b] Reaction temperature.
[c] Isolated yield.
[d] No reaction.
[e] Complex.

68.4, 55.5, 50.1, 45.3, 42.0, 41.2, 39.9, 39.3, 35.5, 35.2, 34.9, 33.5, 32.6, 30.7 (2C), 30.4, 27.8, 23.1 (2C), 22.1, 20.4, 18.1, 11.7 (2C). HRMS (ESI): calcd for $C_{26}H_{44}O_4$ $[M+Na]^+$, 443.3135, found 443.3137.

3. Results and discussion

The synthesis of UDCA is shown in Scheme 1. Compound **1** was obtained by regioselective oxidation of the 7α-OH to 7-ketone group with NBS in 92% yield. Protection of the C-24 carboxyl group in the presence of H_2SO_4 and MeOH gave ester **2** in 99% yield, then selective esterification of the 3α-OH with Ac_2O in the presence of pyridine and DMAP provided ester **3** in 90% yield. Compound **4** was prepared by protection of 12α-OH with MsCl under pyridine in 99% yield. The key intermediate **5** was afforded by reaction of **4** under CH_3COOK in *N*-methyl-2-pyrrolidone (NMP) with 92% yield. For this elimination reaction of mesylate ester group of **4**, the efficient solvent is 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) according to our previously reported procedure [39]. However, the DMPU is expensive and searching for an inexpensive solvent is important for industrialization. Therefore, a model system was employed to find an ideal solvent and optimal reaction conditions (Table 1). After examining several different solvents, we found the economical NMP was as efficient as DMPU, and the yield was up to 90% (Table 1, entry 1–5). Then we screened various bases, and the results showed that the CH_3COOK was the optimal base for this reaction (entry 2 and 6–11). Finally, we also investigated the reaction temperature, and discovered that 135 °C was the most suitable temperature, and the yield reached 92% for the elimination reaction (entry 2 and 12–14). Compound **6** was produced by hydrolysis of **5** with NaOH and H_2O in a mixed solvent of MeOH-THF in 93% yield. Finally, UDCA was afforded by reduction of C11-C12 double bond and stereoselective reduction of 7-ketone group to 7β-OH (no 7α-OH isomer was detected based on ^1H NMR spectrum) of **6** under H_2 (4 MPa), KBH_4, Raney-Ni, *t*-BuOK and isopropanol in autoclave with 93% yield. In this step, we combined the reduction of double bond and carbonyl group in a one-pot synthesis and made the process high yield and efficient. Why the reduction of 7-ketone group offered a dominant 7β-OH stereoisomer? We think maybe both the chiral inducement of compound 6 itself and the stereoselectivity of potassium *tert*-butoxide contribute to the stereoselective reduction [40]. In this straightforward methodology for preparation of UDCA, most of the conversions are very efficient with an average yield of 94% in 7 steps and overall yield up to 65%.

OCA was also synthesized according to the pathway described in Scheme 1. Compound **7** was furnished by regioselective hydrolysis of the 3-acetate group of the key intermediate **5** under NaOH in 99% yield. The silyl enol ether **8** was obtained by reaction of **7** with TMSCl, and subsequent aldol addition with acetaldehyde in the presence of boron trifluoride etherate gave the desired compound **9** (70% yield, over two steps). Hydrolysis of the C-24 methyl ester of **9** under NaOH in MeOH and H_2O provided compound **10** in 95% yield. Reduction of two double bonds of **10** under H_2 (4 MPa) and palladium in carbon in autoclave (the ratio of 6α-ethyl/6β-ethyl is 1/3 based on ^1H NMR spectrum), and then transformation of the 6β-ethyl to 6α-ethyl in NaOH aqueous solution gave compound **11** in 81% yield. The product OCA was afforded by stereoselective reduction of 7-ketone group to 7α-OH with $NaBH_4$ in NaOH aqueous solution in 91% yield. In this novel methodology for preparation of OCA, most of the conversions are very efficient with an average yield of 91% in 11 steps (from cholic acid) and overall yield up to 36%.

4. Conclusion

In summary, we have successfully developed an efficient and economical synthetic route of UDCA and OCA from cost-effective and readily-available cholic acid. Simultaneously, the reaction conditions of the key elimination reaction of mesylate ester group of compound **4** were also investigated and the optimal solvent, base and reaction temperature were determined. In our novel methodologies for preparation of UDCA and OCA, most of the conversions are efficiently and overall yields are very high. We wish this work may not only suitable for industrialization but also facilitate the research and development of novel UDCA and OCA derivatives for PBC or NASH disease.

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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.2018.10.009.

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