Steroids 157 (2020) 108600

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of ursodeoxycholic acid from plant-source (20S)-21-hydroxy-20-methylpregn-4-en-3-one

Jie Wang[a], Xiang-Zhong Gu[b], Li-Ming He[a], Chen-Chen Li[a], Wen-Wei Qiu[a,*]

[a] Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Chemical Engineering, East China Normal University, Shanghai 200241, China

[b] Department of Research and Development, Jiangsu Jiaerke Pharmaceuticals Group Co., Ltd., Zhenglu Town, Changzhou 213111, China

ARTICLE INFO

Keywords:
Ursodeoxycholic acid
Bisnoralcohol
Synthesis

ABSTRACT

A novel synthetic route of producing ursodeoxycholic acid (UDCA) was developed through multiple reactions from cheap and commercially available bisnoralcohol (BA). The key reaction conditions, including solvents, bases and reaction temperatures of the route were investigated and optimized. In the straightforward route for preparation of UDCA, most of the reaction steps have high conversions with average yields of 91%, and overall yield up to 59% (6 steps) from the plant-source BA. Especially in the last step of reduction and hydrolysis, there are five functional groups converted with calcd 97% per conversion in one-pot reaction. This promising route offers economical and efficient strategies for potential large-scale production of UDCA.

1. Introduction

Ursodeoxycholic acid (UDCA) (Fig. 1) is the major bile acid of the bile of black bears which has been used in Chinese traditional medicine as a remedy for liver diseases [1]. It is an active pharmaceutical ingredient (API) widely used in clinics and approved by the US Food and Drug Administration (FDA) for the treatment of primary biliary cholangitis (PBC) since the year 1988 [2,3]. PBC, previously termed primary biliary cirrhosis, is a rare autoimmune inflammatory liver disease, which produces bile duct injury, fibrosis, cholestasis and eventual cirrhosis [4]. UDCA also has a variety of biological activities, such as non-surgical treatment of cholesterol gallstones [5], anti-apoptotic effects [6], anti-inflammatory actions [7], improvement of insulin sensitivity [8], antitumor activity [9] and treatment of Alzheimer's disease [10]. Accordingly, exploitation of economical and efficient synthetic methods of UDCA will not only benefit PBC patients, but also promote its further development and applications.

Cholic acid (CA) can be used as a commercial starting material for preparation of UDCA. For examples, Ferrari et al. provided a low overall yield method by a crucial high-temperatured Wolff-Kishner reduction [11]; Dangate et al. exploited an optimized protection-free route with o-iodoxybenzoic acid as key regioselective oxidant [12]; previously, we also developed a high-yield route (7 steps) by a key elimination reaction of C12-mesylate ester group of CA, in which most of the reaction steps have high conversions with average yields of 94% [13]. Currently, UDCA is usually obtained by epimerization of chenodeoxycholic acid (CDCA) (Fig. 1) by regioselective oxidation of 7α-OH to 7-oxo and then reduction to 7β-OH [14–16]. Hyodeoxycholic acid (HDCA) is a relatively less expensive commercially available bile acid compared with CDCA. Zhou et al. prepared of UDCA from the HDCA (Fig. 1) with a 15% poor total yield [17]. More recently, Dou et al. developed a route based on the HDCA with an improved 26% overall yield via a crucial Shapiro reaction [18]. However, no matter the starting materials of CA, CDCA and HDCA are extracted from the bile acid and belonging to starting materials of animal origin. To our knowledge, there is no synthetic route to UDCA based on a plant-source commercial starting material. The (20S)-21-hydroxy-20-methylpregn-4-en-3-one, also called bisnoralcohol (BA) (Fig. 1), which is obtained from side-chain degradation of the phytosterols by an industrial strain mycobacterium neoaurum [19,20]. Presently, the BA is abundant and commercially available, which price is much cheaper than CDCA. Thus, it is rather valuable to develop an improved synthetic route for large scale production of UDCA using the readily-available starting material BA.

Herein, we report a high-efficiency and economical synthetic route of UDCA from cost-effective and plant-source BA.

2. Experimental

2.1. General procedures

All reagents and chemicals were purchased from commercial

* Corresponding author.
E-mail address: wwqiu@chem.ecnu.edu.cn (W.-W. Qiu).

https://doi.org/10.1016/j.steroids.2020.108600
Received 19 December 2019; Received in revised form 21 January 2020; Accepted 27 January 2020
Available online 14 February 2020
0039-128X/

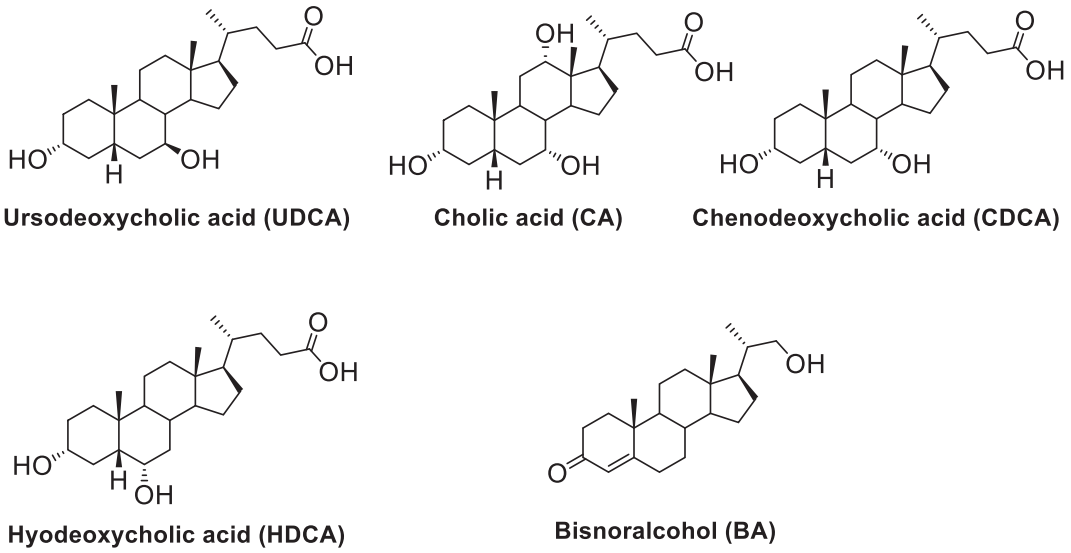


Fig. 1. Structures of bile acids and bisnoralcohol.

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. ^{1}H (400 MHz or 500 MHz) and ^{13}C (100 MHz or 125 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. 3-dioxolane-23,24-bisnorchola-5-en-22-ol (2)

To a solution of bisnoralcohol (10.0 g, 30.26 mmol), ethylene glycol (16.8 mL, 302.60 mmol) and *p*TSA (57 mg, 0.30 mmol) in benzene (300 mL) was refluxed with Dean-Stark column for 24 h. The reaction mixture was treated with saturated $NaHCO_3$ solution (20 mL) and extracted with AcOEt (60 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 (petroleum ether/AcOEt, 3/1, v/v) to give **2** (10.0 g, 88%) as a white solid; mp: 175–177 °C [lit. [21] 173–174 °C]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36–5.32 (m, 1H), 3.97–3.90 (m, 4H), 3.63 (dd, J = 10.5, 3.2 Hz, 1H), 3.35 (dd, J = 10.5, 6.9 Hz, 1H), 2.58–2.53 (m, 1H), 2.11 (dd, J = 14.2, 2.9 Hz,1H), 2.03–1.91 (m, 2H), 1.85–1.72 (m, 3H), 1.69–1.58 (m, 3H), 1.57–1.49 (m, 2H), 1.49–1.39 (m, 2H), 1.36–1.27 (m, 3H), 1.22–1.15 (m, 2H), 1.12–1.07 (m, 1H), 1.04 (d, J = 6.7 Hz, 3H), 1.02 (s, 3H), 1.00–0.97 (m, 1H), 0.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.26, 122.25, 109.60, 68.12, 64.55, 64.34, 56.59, 52.52, 49.79, 42.55, 41.91, 39.74, 38.90, 36.73, 36.45, 32.04, 31.84, 31.19, 27.85, 24.51, 21.16, 19.00, 16.89, 12.07. HRMS (ESI): calcd for $C_{24}H_{38}NaO_3$ $[M + Na]^+$, 397.2713, found 397.2704.

2.2.2. 3-dioxolane-23,24-bisnorchola-5-en-22-al (3)

To a solution of **2** (10.1 g, 26.96 mmol), TEMPO (42 mg, 0.27 mmol) in CH_2Cl_2 (100 mL) was stirred for 20 min under nitrogen atmosphere at 0 °C. Then a solution of $NaHCO_3$ (3.1 g, 36.40 mmol), TBAB (870 mg, 2.70 mmol) in H_2O (40 mL) was added. The reaction mixture was stirred at 0 °C for an additional 20 min, and then NCS (4.1 g, 31.00 mmol) was added. After 5 h, the reaction mixture was allowed to warm to room temperature, treated with sodium thiosulfate pentahydrate (1.3 g in 25 mL H_2O). The reaction mixture was stirred at 0 °C for 20 min. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (50 mL × 2). The organic layer was washed with sodium hydroxide solution, brine, dried with anhydrous Na_2SO_4 and concentrated to give **3** (9.6 g, 95%) as a light yellow solid; mp: 168–171 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.56 (d, J = 3.3 Hz, 1H), 5.36–5.31 (m, 1H), 3.97–3.90 (m, 4H), 2.58–2.53 (m, 1H), 2.39–2.31 (m, 1H), 2.11 (dd, J = 14.2, 2.9 Hz, 1H), 2.00–1.93 (m, 2H), 1.91–1.82 (m, 1H), 1.81–1.73 (m, 2H), 1.68–1.62 (m, 3H), 1.59–1.53 (m, 1H), 1.52–1.44 (m, 3H), 1.40–1.29 (m, 2H), 1.28–1.15 (m, 2H), 1.12 (d, J = 6.8 Hz, 3H), 1.11–1.03 (m, 2H), 1.02 (s, 3H), 0.72 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.20, 140.26, 122.10, 109.54, 64.56, 64.35, 56.11, 51.08, 49.79, 49.61, 43.09, 41.90, 39.58, 36.74, 36.45, 32.01, 31.80, 31.19, 27.16, 24.78, 21.10, 19.00, 13.59, 12.37. HRMS (ESI): calcd for $C_{24}H_{36}NaO_3$ $[M + Na]^+$, 395.2557, found 395.2542.

2.2.3. (20S)-3-oxopregn-4-ene-20-carboxaldehyde (4)

By a similar procedure described for **3**, **4** was obtained as a light yellow solid (yield 95%), mp: 155–157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.55 (s, 1H), 5.71 (s, 1H), 2.45–2.23 (m, 5H), 1.99 (t, J = 13.7 Hz, 2H), 1.91–1.78 (m, 2H), 1.68 (t, J = 10.2 Hz, 2H), 1.43 (m, 5H), 1.30–1.19 (m, 2H), 1.17 (s, 3H), 1.11 (d, J = 5.5 Hz, 3H), 1.06–0.89 (m, 3H), 0.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.00, 199.65, 171.31, 123.99, 55.25, 53.84, 51.04, 49.54, 43.10, 39.39, 38.68, 35.80, 35.68, 34.06, 32.93, 32.05, 27.11, 24.64, 21.06, 17.48, 13.53, 12.44. HRMS(ESI): calcd for $C_{22}H_{32}NaO_2$ $[M + Na]^+$, 351.2295, found 351.2292.

2.2.4. (22E)-3-oxo-4,22-choladien-24-oic acid ethyl ester (5) [22]

To a solution of **4** (9.5 g, 28.92 mmol) and Ph_3P = $CHCOOC_2H_5$ (20.2 g, 57.84 mmol) in toluene (150 mL) was refluxed for 4 h. After cooling, the reaction mixture was concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 3/1, v/v) to give **5** (11.3 g, 98%) as a white solid; mp: 160–162 °C. [lit. [23] 155–157 °C]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (dd, J = 15.3, 9.0 Hz, 1H), 5.71 (d, J = 13.4 Hz, 2H), 4.24–4.09 (m, 2H), 2.45–2.21 (m, 5H), 2.00 (d, J = 12.6 Hz, 2H), 1.80 (m, 1H), 1.76–1.33 (m, 7H), 1.26 (m, 6H), 1.17 (s, 3H), 1.08 (d, J = 6.2 Hz, 3H), 1.05–0.86 (m, 3H), 0.73 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 199.70, 171.51, 167.16, 154.56, 123.94, 119.21, 60.27, 55.78, 54.98, 53.84, 42.82, 39.80, 39.54, 38.69,

35.80, 35.70, 34.08, 32.98, 32.06, 28.19, 24.28, 21.10, 19.31, 17.49, 14.40, 12.32. HRMS(ESI): calcd for $C_{26}H_{38}NaO_3$ [M + Na]$^+$, 421.2713, found 421.2708.

*2.2.5. (E)-3,3-ethylenedioxy-5,22-choladienoic acid ethyl ester (**6**) [22]*

To a solution of **3** (9.6 g, 25.77 mmol) and Ph_3P = $CHCOOC_2H_5$ (18.0 g, 51.54 mmol) in toluene (150 mL) was refluxed for 4 h. After cooling, the reaction mixture was concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 3/1, v/v) to give **6** (11.2 g, 98%) as a white solid; mp: 122–124 °C.

To a solution of **5** (5.0 g, 12.54 mmol), ethylene glycol (7.0 mL, 125.40 mmol) and *p*TSA (25 mg, 0.13 mmol) in toluene (150 mL) was refluxed with Dean-Stark column for 24 h. The reaction mixture was treated with saturated $NaHCO_3$ solution (20 mL) and extracted with AcOEt (50 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 (petroleum ether/AcOEt, 3/1, v/v) to give **6** (4.9 g, 88%) as a white solid; mp: 122–124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (dd, J = 15.6, 8.9 Hz, 1H, H-22), 5.72 (d, J = 15.6 Hz, 1H, H-23), 5.39–5.28 (m, 1H, H-6), 4.16 (q, J = 7.1 Hz, 2H, –OC**H**$_2$CH$_3$), 3.97–3.90 (m, 4H, –OC**H**$_2$C**H**$_2$O–), 2.58–2.53 (m, 1H), 2.26 (d, J = 6.7 Hz, 1H), 2.11 (dd, J = 14.2, 2.9 Hz, 1H), 2.00–1.92 (m, 2H), 1.81–1.73 (m, 2H), 1.72–1.61 (m, 3H), 1.60–1.52 (m, 2H), 1.51–1.41 (m, 2H), 1.37–1.30 (m, 1H), 1.27 (t, J = 7.1 Hz, 3H, –OCH$_2$C**H**$_3$), 1.25–1.18 (m, 3H), 1.08 (d, J = 6.7 Hz, 3H, H-21), 1.04–1.06 (m, 2H), 1.02 (s, 3H, H-19), 1.00–0.97 (m, 1H), 0.71 (s, 3H, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.20 (C-24), 154.84 (C-22), 140.24 (C-5), 122.20 (C-6), 119.07 (C-23), 109.57 (C-3), 64.56 (OCH_2CH_2O), 64.35 (OCH_2CH_2O), 60.22 (O**C**H$_2$CH$_3$), 56.63 (C-14), 55.01 (C-17), 49.78 (C-9), 42.79 (C-13), 41.91 (C-4), 39.85 (C-20), 39.72 (C-12), 36.75 (C-10), 36.45 (C-1), 32.02 (C-8), 31.80 (C-7), 31.20 (C-2), 28.25 (C-16), 24.42 (C-15), 21.14 (C-11), 19.38 (C-21), 19.00 (C-19), 14.42 (OCH_2**C**H$_3$), 12.24 (C-18). HRMS(ESI): calcd for $C_{28}H_{42}NaO_4$ [M + Na]$^+$, 465.2975, found 465.2990.

*2.2.6. (E)-3,3-ethylenedioxy-7-oxo-5,22-choladienoic acid ethyl ester (**7**)*

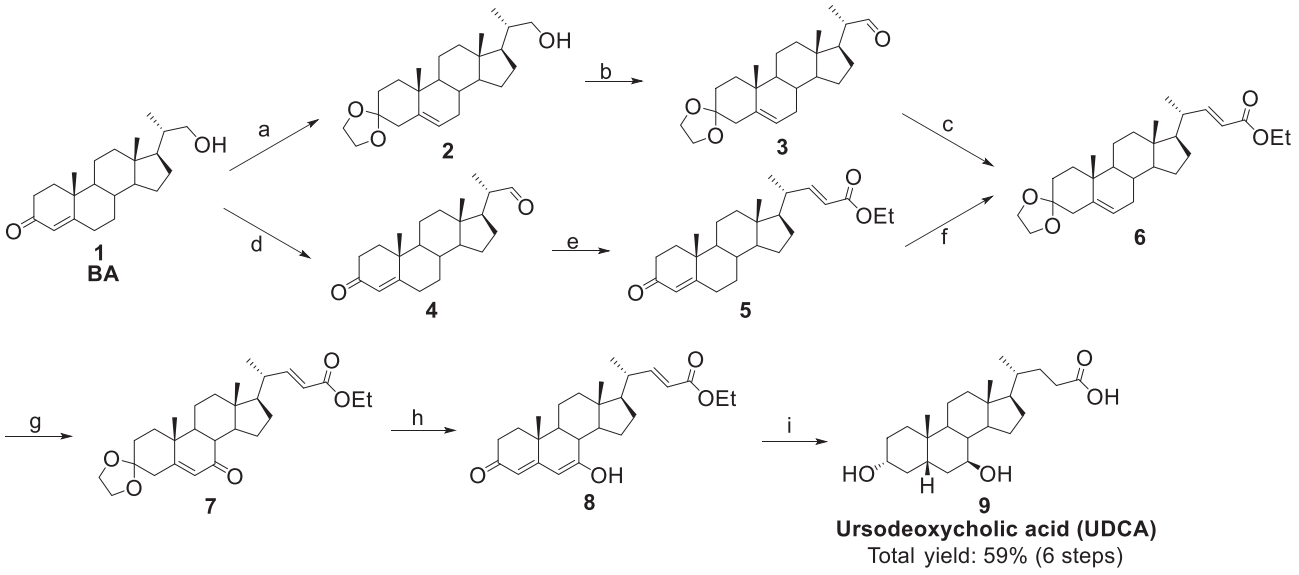
To a solution of **6** (5.0 g, 11.30 mmol) in a mixed solvent (100 mL) of acetone-H_2O (9:1, v/v) was added NHPI (2.0 g, 12.43 mmol), PDC (4.7 g, 12.43 mmol) at room temperature. The reaction mixture was stirred for 20 h and the solid residue was removed by filtration, then the filtrate was evaporated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 3/1, v/v) to give **7** (4.4 g, 85%) as a white solid; mp: 139–141 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (dd, J = 15.6, 9.0 Hz, 1H, H-22), 5.72 (d, J = 15.6 Hz, 1H, H-23), 5.65 (d, J = 1.7 Hz, 1H, H-6), 4.16 (q, J = 7.1 Hz, 2H, –OC**H**$_2$CH$_3$), 3.98–3.90 (m, 4H, –OC**H**$_2$C**H**$_2$O–), 2.66 (dd, J = 14.7, 1.8 Hz, 1H), 2.44–2.36 (m, 1H), 2.34–2.18 (m, 3H), 2.02–1.95 (m, 1H), 1.89–1.83 (m, 2H), 1.78–1.71 (m, 2H), 1.64–1.52 (m, 3H), 1.52–1.43 (m, 1H), 1.27 (m, 8H), 1.19 (s, 3H, H-19), 1.08 (d, J = 6.6 Hz, 3H, H-21), 0.70 (s, 3H, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.52 (C-7), 167.11 (C-24), 164.66 (C-5), 154.53 (C-22), 126.73 (C-6), 119.23 (C-23), 108.98 (C-3), 64.70 (–OCH_2CH_2O–, 64.62 (–OCH_2CH_2O–), 60.24 (–O**C**H$_2$CH$_3$), 53.82, 50.01, 49.65, 45.41, 43.58, 41.84, 39.59, 38.65, 38.35, 35.73, 31.16, 28.34, 26.44, 21.25, 19.58 (C-21), 17.08 (C-19), 14.40 (–OCH_2**C**H$_3$), 12.40 (C-18). HRMS(ESI): calcd for $C_{28}H_{40}NaO_5$ [M + Na]$^+$, 479.2768, found 479.2770.

*2.2.7. (E)-7-hydroxy-3-oxo-4,6,22-choladienoic acid ethyl ester (**8**)*

To a solution of **7** (4.4 g, 9.64 mmol) in a mixed solvent (50 mL) of THF-H_2O (9:1, v/v) was added H_2SO_4 (2 mL) at 0 °C. Then the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was treated with saturated $NaHCO_3$ solution (80 mL) and extracted with AcOEt (30 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 (petroleum ether/AcOEt, 3/1, v/v) to give **8** (3.9 g, 98%) as a white solid; mp: 167–169 °C. ^{1}H NMR (400 MHz, DMSO-***d*$_6$**) δ 10.22 (s, 1H), 6.74 (dd, J = 15.4, 9.0 Hz, 1H), 5.78 (d, J = 15.5 Hz, 1H), 5.29 (s, 1H), 5.27 (s, 1H), 4.09 (dd, J = 13.2, 6.4 Hz, 2H), 2.37–2.13 (m, 5H), 1.94–1.85 (m, 2H), 1.58–1.42 (m, 4H), 1.34–1.25 (m, 3H), 1.20 (m, 6H), 1.05 (d, J = 7.0 Hz, 6H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, DMSO-***d*$_6$**) δ 198.89, 165.98, 165.30, 164.15, 154.50, 118.79, 117.65, 99.83, 59.69, 53.03, 50.42, 48.87, 44.90, 43.47, 38.80, 38.27, 35.45, 32.49, 27.95, 26.30, 25.43, 21.04, 19.19, 16.91, 14.17, 12.09. HRMS(ESI): calcd for $C_{26}H_{36}NaO_4$ [M + Na]$^+$, 435.2506, found 435.2501.

*2.2.8. Ursodeoxycholic acid (**UDCA**)*

To a solution of **8** (2.0 g, 4.85 mmol) in 2-methyl tetrahydrofuran (20 mL) in autoclave was hydrogenated in the presence of Raney-Ni (2.0 g) under H_2 (4.0 MPa) for 24 h at 90 °C. Then *i*-PrOH (20 mL), *t*-



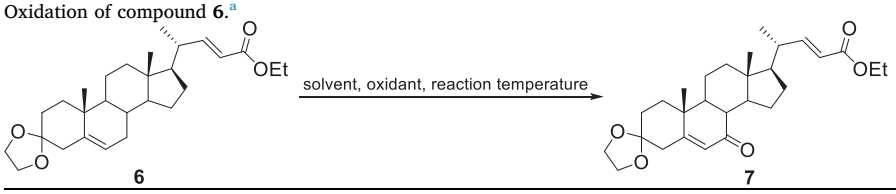
Scheme 1. Synthesis of ursodeoxycholic acid from bisnoralcohol. Reagents and conditions: (a) ethylene glycol, *p*TSA, benzene, reflux, 88%; (b) TEMPO, $NaHCO_3$, TBAB, NCS, CH_2Cl_2, H_2O, 0 °C, 95%; (c) Ph_3 = $CHCOOC_2H_5$, toluene, reflux, 98%; (d) TEMPO, $NaHCO_3$, TBAB, NCS, CH_2Cl_2, H_2O, 0 °C, 95%; (e) Ph_3 = $CHCOOC_2H_5$, toluene, reflux, 98%; (f) ethylene glycol, *p*TSA, benzene, reflux, 88%; (g) PDC, NHPI, acetone, H_2O, rt, 85%; (h) H_2SO_4, THF, rt, 98%; (i) Raney Ni, H_2, *t*-BuONa, 2-methyl tetrahydrofuran, *i*-PrOH, 90 °C, 87%.

BuONa (932 mg, 9.70 mmol) was added and flushed with H_2 (4.0 MPa). The reaction mixture was stirred for 24 h at 90 °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 (100 mL) and extracted with AcOEt (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 **UDCA** (1.65 g, 87%) as a white solid; mp: 200–202 °C [lit. [13] 198–200 °C]. [α]25 D + 60.9 (*c* 1.0, CH_3CH_2OH). [lit. [13] [α]25 D + 59.7 (*c* 1.0, CH_3CH_2OH)].^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (s, 1H, –COO**H**), 4.46 (s, 1H, H-3), 3.88 (d, *J* = 6.7 Hz, 1H, H-7), 3.35–3.24 (m, 2H), 2.26–2.19 (m, 1H), 2.13–2.05 (m, 1H), 1.95–1.81 (m, 2H), 1.78–1.63 (m, 4H), 1.51–1.42 (m, 3H), 1.41–1.28 (m, 7H), 1.23–1.08 (m, 6H), 1.05–0.91 (m, 2H), 0.88 (d, *J* = 6.5 Hz, 6H, H-19, H-21), 0.61 (s, 3H, H-18). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.93 (C-24), 69.75 (C-3), 69.46 (C-7), 55.87 (C-17), 54.70 (C-14), 43.11 (C-13), 43.02 (C-8), 42.20 (C-5), 39.94 (C-12), 39.84 (C-9), 39.73 (C-4), 37.75 (C-6),37.28 (C-20), 34.88 (C-1), 33.78 (C-10), 30.78 (C-22, C-23), 30.26 (C-2), 28.21 (C-16), 26.75 (C-15), 23.34 (C-19), 20.89 (C-11), 18.32 (C-21), 12.06 (C-18). HRMS(ESI): calcd for $C_{24}H_{40}NaO_4$ $[M + Na]^+$, 415.2819, found 415.2834.

3. Results and discussion

The synthesis of UDCA is shown in Scheme 1. Compound **2** was obtained by glycol protection of the C-3 carbonyl group in 88% yield. Oxidation of side chain hydroxyl groups to aldehydes in the presence of TEMPO and NCS gave Compound **3** and **4** in 95% yield respectively. Compound **5** was prepared by witting reaction in 98% yield. The key intermediate compound **6** was afforded by witting reaction from compound **3** in 98% yield or glycol protection of the C-3 carbonyl group from compound **5** in 88% yield. The witting reaction with a resonance-stabilized is known to predominantly deliver trans stereoisomer, and the two isomers (cis- and trans- isomers) show different chemical shifts on ^{1}H NMR and ^{13}C NMR spectra. Herein, the compounds **5** and **6** obtained by witting reaction showed a single trans structure on ^{1}H NMR and ^{13}C NMR spectra respectively. The yield of oxidation at the C-7 position to afford compound 7 was unsatisfactory. Therefore, a model system was employed to find an ideal solvent and optimal reaction conditions (Table 1). After screening various oxidant, we found that the oxidant of PDC/NHPI was the optimal oxidant for this reaction, and the yield was up to 72% (Table 1, entry 1–12). Then we examined several different solvents, and the results showed that acetone-H_2O (9:1, v/v) is the optimal solvent (Table 1, entry 4, 10 and 13–24). We also

Table 1
Oxidation of compound **6**.[a]

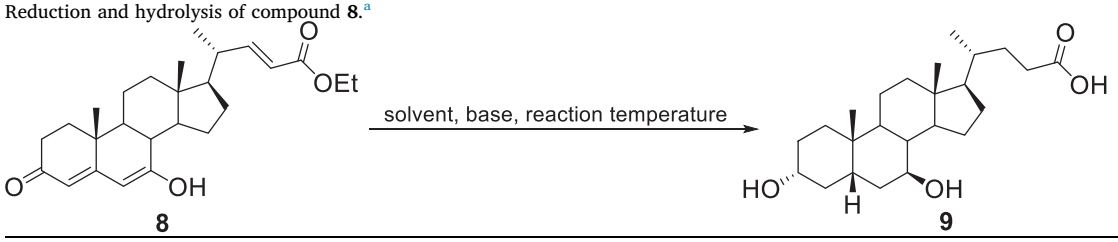


6 7

Entry	Solvent	Oxidant	RT[b](°C)	Yield[c](%)
1	Toluene	$Na_2Cr_2O_7$/NHPI	25	N[d]
2	Toluene	$Na_2Cr_2O_7$/NHPI/Acetic acid	25	54
3	Toluene	$K_2Cr_2O_7$/NHPI/Acetic acid	25	52
4	Toluene	PDC/NHPI	25	66
5	Toluene	PDC/TBHP	25	43
6	Toluene	BPO/NHPI	25	42
7	Acetone	$Na_2Cr_2O_7$/NHPI	25	N[d]
8	Acetone	$Na_2Cr_2O_7$/NHPI/Acetic acid	25	62
9	Acetone	$K_2Cr_2O_7$/NHPI/Acetic acid	25	61
10	Acetone	PDC/NHPI	25	72
11	Acetone	PDC/TBHP	25	59
12	Acetone	BPO/NHPI	25	51
13	DMF	PDC/NHPI	25	38
14	t-BuOH	PDC/NHPI	25	N[d]
15	Ethyl acetate	PDC/NHPI	25	N[d]
16	MeCN	PDC/NHPI	25	70
17	NMP	PDC/NHPI	25	N[d]
18	DCM	PDC/NHPI	25	68
19	MeCN/H_2O (4:1, v/v)	PDC/NHPI	25	77
20	MeCN/H_2O (9:1, v/v)	PDC/NHPI	25	79
21	MeCN/H_2O (14:1, v/v)	PDC/NHPI	25	79
22	Acetone/H_2O (4:1, v/v)	PDC/NHPI	25	80
23	Acetone/H_2O (9:1, v/v)	PDC/NHPI	25	85
24	Acetone/H_2O (14:1, v/v)	PDC/NHPI	25	82
25	Acetone/H_2O (9:1, v/v)	PDC/NHPI	0	65
26	Acetone/H_2O (9:1, v/v)	PDC/NHPI	50	73

[a] All the reactions were performed for 20 h, and the ratio of oxidant/compound **6** was 1.1:1 (mol: mol).
[b] Reaction temperature.
[c] Isolated yield.
[d] No reaction.

Table 2
Reduction and hydrolysis of compound **8**.[a]



Entry	Solvent	Base	RT[b](°C)	Yield[c](%)
1	i-PrOH	t-BuOK	60	38
2	n-BuOH	t-BuOK	60	35
3	t-BuOH	t-BuOK	60	36
4	THF	t-BuOK	60	N[d]
5	i-PrOH	t-BuOK	40	33
6	i-PrOH	t-BuOK	90	61
7	i-PrOH	CH_3ONa	90	44
8	i-PrOH	C_2H_5ONa	90	43
9	i-PrOH	t-BuONa	90	72
10	THF/i-PrOH (1/1, v/v)	t-BuONa	90	81
11	1, 4-dioxane/i-PrOH (1/1, v/v)	t-BuONa	90	70
12	2-methyl tetrahydrofuran/i-PrOH (1/1, v/v)	t-BuONa	90	87
13[e]	i-PrOH	t-BuONa	90	60
14[e]	THF/i-PrOH (1/1, v/v)	t-BuONa	90	65
15[e]	2-methyl tetrahydrofuran/i-PrOH (1/1, v/v)	t-BuONa	90	68

[a] All the reactions were performed for 48 h, and the ratio of Raney-Ni/compound **8** was 1/1 (m/m).
[b] Reaction temperature.
[c] Isolated yield.
[d] No UDCA.
[e] Solvent and base were added together (The rest were added separately).

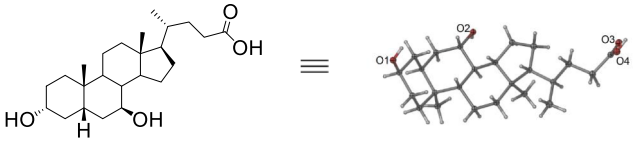


Fig. 2. Single-crystal X-ray structure of ursodeoxycholic acid.

investigated the reaction temperature, and discovered that 25 ˚C was the most suitable temperature, and the yield was up to 85% for the oxidation reaction (entry 23, 25 and 26). Compound **8** was furnished by hydrolysis of the ethylene ketal of compound **7** in the presence of H_2SO_4 and THF in 98% yield. Finally, UDCA was afforded by reduction and hydrolysis of compound **8** under H_2 (4 MPa), Raney-Ni and base in autoclave. After screening various solvents, reaction temperature and bases (Table 2) of the reaction, we found that a mixed solvent of 2-methyl tetrahydrofuran-*i*-PrOH, 90 ˚C and *t*-BuONa were optimum conditions and the yield reach 87%. In this step, five functional groups were converted with calcd 97% per conversion in one-pot reaction. The relative stereo-configuration of UDCA was determined by X-ray analysis [24] (Fig. 2). In this straightforward methodology for preparation of UDCA, most of the conversions are very efficient with an average yield of 91% in 6 steps and overall yield up to 59%.

4. Conclusion

In summary, we have successfully developed an efficient and economical synthetic route of UDCA from cost-effective and commercially available plant-source BA. Simultaneously, the key reaction conditions were investigated and the optimal solvent, base and reaction temperature were determined. Herein, we report a new straightforward methodology for the preparation of UDCA, most of the conversions are efficiently and the overall yield is high. We wish this work may not only suitable for industrialization but also facilitate the research and development of novel UDCA derivatives for PBC disease.

Acknowledgment

This work was supported by Shanghai Science and Technology Council (Grant 18ZR1411200) and Jiangsu Jiaerke Pharmaceuticals Group Co., Ltd.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2020.108600.

References

[1] L.R. Hagey, D.L. Crombie, E. Espinosa, M.C. Carey, H. Igimi, A.F. Hofmann, Ursodeoxycholic acid in the Ursidae: biliary bile acids of bears, pandas, and related carnivores, J. Lipid Res. 34 (11) (1993) 1911–1917.
[2] G. Paumgartner, U. Beuers, Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited, Hepatology 36 (3) (2002) 525–531.
[3] D. Chascsa, E.J. Carey, K.D. Lindor, Old and new treatments for primary biliary cholangitis, Liver Int. 37 (4) (2017) 490–499.
[4] Y.Q. Huang, Recent advances in the diagnosis and treatment of primary biliary cholangitis, World J. Hepatol. 8 (33) (2016) 1419–1441.
[5] G. Salen, A. Colalillo, D. Verga, E. Bagan, G.S. Tint, S. Shefer, Effect of high and low doses of ursodeoxycholic acid on gallstone dissolution in humans, Gastroenterology 78 (6) (1980) 1412–1418.
[6] J.D. Amaral, R.J. Viana, R.M. Ramalho, C.J. Steer, C.M. Rodrigues, Bile acids: regulation of apoptosis by ursodeoxycholic acid, J. Lipid Res. 50 (9) (2009) 1721–1734.
[7] W.K. Ko, S.J. Kim, M.J. Jo, H. Choi, D. Lee, I.K. Kwon, S.H. Lee, I.B. Han, S. Sohn, Ursodeoxycholic acid inhibits inflammatory responses and promotes functional recovery after spinal cord injury in rats, Mol. Neurobiol. 56 (1) (2019) 267–277.
[8] T. Tsuchida, M. Shiraishi, T. Ohta, K. Sakai, S. Ishii, Ursodeoxycholic acid improves insulin sensitivity and hepatic steatosis by inducing the excretion of hepatic lipids in high-fat diet-fed KK-Ay mice, Metabolism 61 (7) (2012) 944–953.
[9] S.C. Lim, H.Q. Duong, K.R. Parajuli, S.I. Han, Pro-apoptotic role of the MEK/ERK pathway in ursodeoxycholic acid-induced apoptosis in SNU601 gastric cancer cells,

Oncol. Rep. 28 (4) (2012) 1429–1434.
[10] S.M. Bell, K. Barnes, H. Clemmens, A.R. Al-Rafiah, E.A. Al-Ofi, V. Leech, O. Bandmann, P.J. Shaw, D.J. Blackburn, L. Ferraiuolo, H. Mortiboys, Ursodeoxycholic acid improves mitochondrial function and redistributes Drp1 in fibroblasts from patients with either sporadic or familial Alzheimer's disease, J. Mol. Biol. 430 (21) (2018) 3942–3953.
[11] M. Ferrari, F. Zinetti, Process for preparing high purity ursodeoxycholic acid, Ferrari M, Zinetti F. Process for preparing high purity ursodeoxycholic acid, US, 2880047 [P], 2014.
[12] P.S. Dangate, C.L. Salunke, K.G. Akamanchi, Regioselective oxidation of cholic acid and its 7β epimer by using o-iodoxybenzoic acid, Steroids 76 (2011) 1397–1399.
[13] X.L. He, L.T. Wang, X.Z. Gu, J.X. Xiao, W.W. Qiu, A facile synthesis of ursodeoxycholic acid and obeticholic acid from cholic acid, Steroids 140 (2018) 173–178.
[14] D.D. Yu, S.S. Andrali, H. Li, M. Lin, W. Huang, B.M. Forman, Novel fxr (farnesoid x receptor) modulators: potential therapies for cholesterol gallstone disease, Bioorg. Med. Chem. 24 (18) (2016) 3986–3993.
[15] X.Z. Hu, A.J. Liu, Method for producing ursodesoxycholic acid by using chenodeoxycholic acid as raw material, China, 102911235 [P], 2013.
[16] J.S. Cao, X.L. Hao, S.H. Wang, Method for preparation of ursodesoxycholic acid from chenodeoxycholic acid, China, 102464692 [P], 2010.
[17] W.S. Zhou, Z.Q. Wang, B. Jiang, Stereocontrolled conversion of hyodeoxycholic acid into chenodeoxycholic acid and ursodeoxycholic acid, J. Chem. Soc. Perkin Trans. 1 (1) (1990) 1–3.
[18] Q. Dou, Z. Jiang, A facile route to ursodeoxycholic acid based on stereocontrolled conversion and aggregation behavior research, Synthesis 48 (04) (2015) 588–594.
[19] X. Li, X. Chen, Y. Wang, P. Yao, R. Zhang, J. Feng, Q. Wu, D. Zhu, Y. Ma, New product identification in the sterol metabolism by an industrial strain Mycobacterium neoaurum NRRL B-3805, Steroids 132 (2018) 40–45.
[20] L.Q. Xu, Y.J. Liu, K. Yao, H.H. Liu, X.Y. Tao, F.Q. Wang, D.Z. Wei, Unraveling and engineering the production of 23,24-bisnorcholenic steroids in sterol metabolism, Sci. Rep. 6 (2016) 21928.
[21] H.S. Kim, B.S. Choi, K.C. Kwon, S.O. Lee, H.J. Kwak, C.H. Lee, Synthesis and antimicrobial activity of squalamine analogue, Bioorg. Med. Chem. 8 (2000) 2059–2065.
[22] G.A. Samaja, O. Castro, L.D. Alvarez, M.V. Dansey, D.S. Escudero, A.S. Veleiro, A. Pecci, G. Burton, 27-Nor-Δ^4-dafachronic acid is a synthetic ligand of Caenorhabditis elegans DAF-12 receptor, Bioorg. Med. Chem. Lett. 23 (2013) 2893–2896.
[23] M. Linker, W. Kreiser, Synthesis of Methyl (20R,22E)- and (20S,22E)-3-Oxochola-1,4,22- trien-24-oate, Helv. Chim. Acta. 85 (2002) 1096–1101.
[24] CCDC 1963315 contains the supplementary crystallographic data of UDCA for this paper. These data can be obtained free of charge viahttps://www.ccdc.cam.ac.uk/ or by emailing data-request@ccdc.cam.ac.uk or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K. (fax, +44 (0)1223 336 033).