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Facial synthesis of key intermediate of obeticholic acid via Pd-catalyzed Kumada-Tamao-Corriu cross-coupling reaction

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ABSTRACT

Obeticholic acid (OCA) is used to treatment for Primary Biliary Cholangitis and other Famesoid X Receptor related diseases. Through the palladium catalyzed Kumada-Tamao-Corriu cross-coupling reaction, a novel and efficient method for synthesis of OCA with satisfied yield was successfully developed. The absolute configuration of the key intermediate was confirmed by Single-crystal X-ray Diffraction. It affords good strategy for large-scale synthesis of OCA.

1. Introduction

Obeticholic acid (OCA) is a semi-synthetic bile acid analogue studied and patented by Pellicciari et al which was reported to treat Primary biliary cholangitis (PBC) and Nonalcoholic steatohepatitis (NASH) [1–3]. As a 6-ethyl derivative of the natural human Bile Acid, the OCA is a first-in-class selective FXR (Farnesoid X Receptor) agonist which is ~100-fold more potent than the chenodeoxycholic [4]. Except the PBC and NASH, the OCA is taken as the candidate drug for other liver diseases and other FXR related disorders, such as alcoholic hepatitis, obesity patients with gallstone disease, portal hypertension and bile acid diarrhea [5,6].

Although the OCA is an ideal chemical tool to study the function of FXR, the present synthesis methods suffered from harsh conditions or low yields to obtain the key intermediate 6-alpha-ethyl-7-KLCA (6-alpha-ethyl-7-keto-lithocholic acid), which limited the large-scale preparation of OCA. As described in Scheme 1 route **a**, air sensitive strong base such as *t*-BuLi was needed to generate carbanion at position 6 from 7-KLCA, which *in situ* reacted with ethyl bromide to provide the 6-alpha-ethyl-7-KLCA (**II**) in 12% yield [7]. As shown in Scheme 1 route **b**, an alternative method using Mukaiyama-aldol reaction was developed to synthesize the key intermediate with the yield of 50% [8]. The key intermediate (**IV**) of this route was constructed through reaction of water sensitive silyl enol ether (**III**) with acetaldehyde at −68 °C. It is urgent to develop an easily operated synthetic route of OCA under mild reaction conditions.

Herein, we designed and accomplished a novel and economical synthetic method of intermediate 6-alpha vinyl-7-KLCA (**4**) under mild condition using Kumada cross-coupling from commercially available cholic acid. It could be reduced by hydrogenation, then deprotected and transposed to OCA under alkaline conditions as reported method in the previous literature (Scheme 2) [9]. To our knowledge, this is the first report about synthesizing OCA by using catalyzed cross-coupling reaction in a facial condition.

First, the carboxyl group of 7-KLCA is transformed to methyl ester (compound **1**). Subsequently, the hydroxyl group is protected by acetyl group to obtain compound **2**. Then, the compound **2** is treated with hydrobromic acid and bromine to give the alpha-carbonyl brominated compound **3**, which then coupled with vinyl magnesium bromide to obtain compound **4** under palladium catalyst system. It's noted that inversion of configuration of 6-carbon was observed. The beta configuration of compound **4** was confirmed by X-ray analysis. Compound **5** was obtained from compound **4** by hydrogenation. Under alkaline conditions, acetyl and methyl ester group were removed to obtain compound **6**. At last, the compound **6** was reduced by sodium borohydride to provide desired obeticholic acid.

2. Experimental

2.1. General procedures

All reagents and chemicals were purchased from commercial

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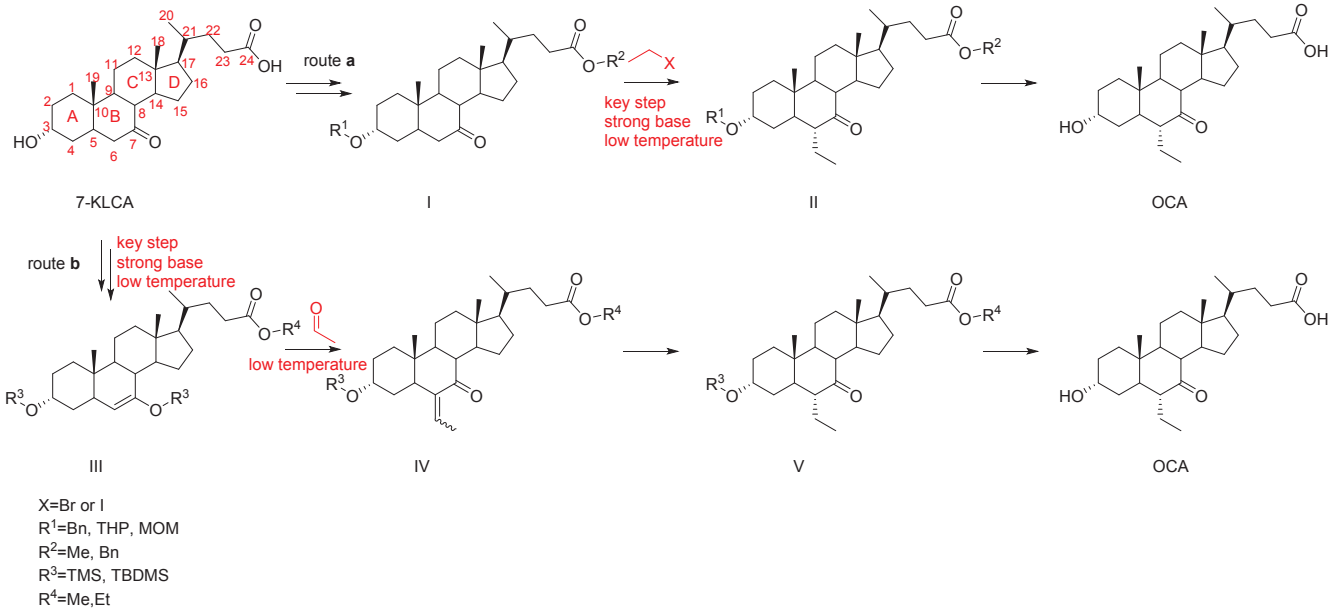
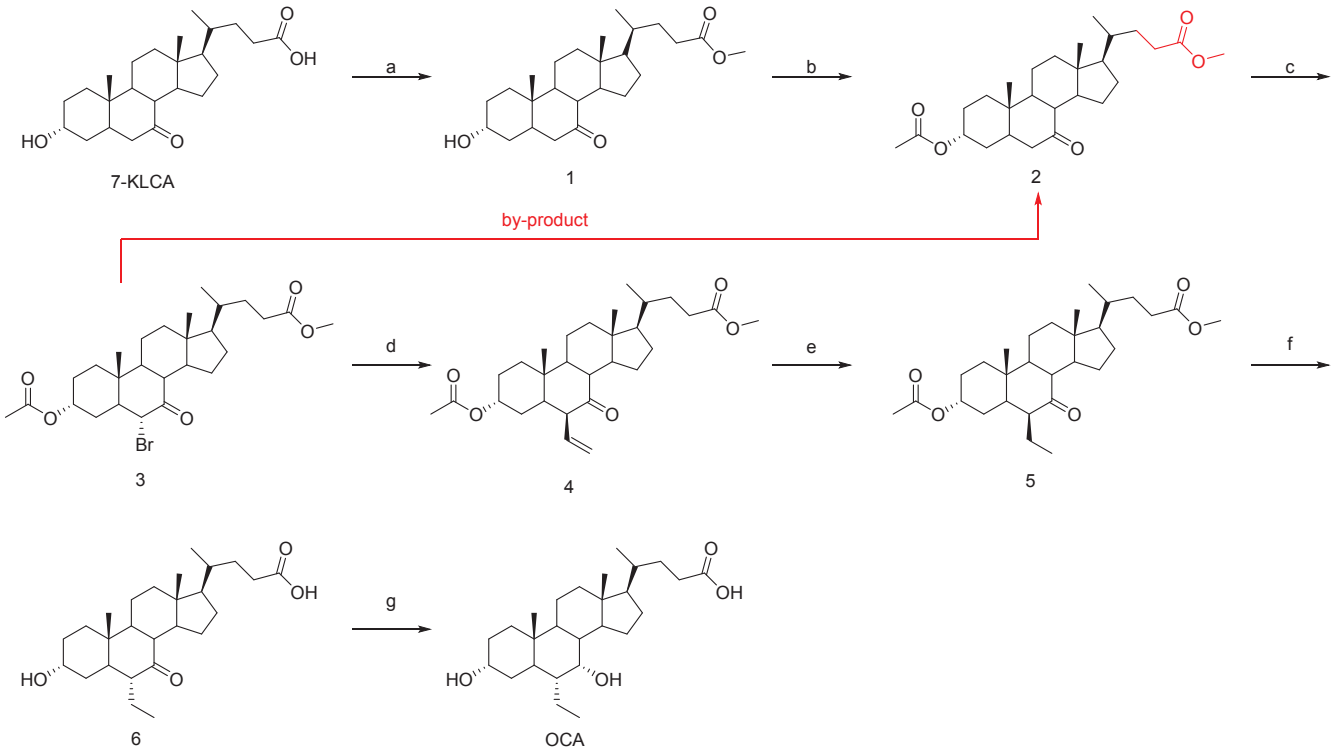


Scheme 1. Previously reported synthesis routes.



Scheme 2. Synthesis of obeticholic acid from 7-KLCA. Reagents and conditions: (a) H_2SO_4, MeOH, rt, 99%; (b) Ac_2O, Pyridine, DMAP, 93%; (c) 48% HBr, Br_2, rt, 76%; (d) $Pd(PPh_3)_4$, (S,S)-Ph-BOX, CH_2 = $CHMgBr$, THF, rt, 31%; (e) Pd/C, H_2, MeOH, 55 °C, 99%; (f) NaOH, H_2O, 95 °C, 2 h, 95%; (g) NaOH, H_2O, $NaBH_4$, 75 °C, 5 h, 72%.

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 or Ar. Column chromatography was performed on silica gel (QingDao Huanghai, 100–200 mesh) using the indicated eluents. Thin-layer chromatography was carried out on silica gel plates (QingDao Huanghai) with a layer thickness of 0.25 mm. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on Bruker 300 MHz spectrometer with $CDCl_3$ or CD_3OD 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 Bruker micro TOF-Q II spectrometer.

2.2. Chemical synthesis

2.2.1. Methyl 3α-dihydroxy-7-keto-5β-cholan-24-UDCA (1)

To a solution of 7-KLCA (10.01 g, 21 mmol) in MeOH (280 mL) was

added H_2SO_4 (6 mL) at room temperature. The reaction mixture was refluxed for 1 h, and then concentrated. The residue was dissolved in CH_2Cl_2 (200 mL). Organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated to give compound **1** (10.29 g, 99%) as a white solid. IR (ATR) cm^{-1}: 3526, 3447, 2937, 2870, 1739, 1718, 1688, 1637. ^1H NMR (300 MHz, $CDCl_3$): 3.60 (1H, s, 24-OAc), 3.53 (1H, tt, 3-H), 2.80(1H, dd, 8-H), 2.37 (1H, s, 3-OH), 1.14(3H, s, 18-H), 0.87 (3H, d, 20-H), 0.59 (3H, s, 19-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 212.18, 174.45, 77.42, 77.00, 76.58, 70.41, 54.58, 51.28, 49.27, 48.75, 45.93, 45.19, 42.55, 42.43, 38.77, 37.11, 35.01, 34.93, 33.99, 30.83, 30.77, 29.59, 28.07, 24.59, 22.85, 21.47, 18.15, 11.83. HRMS (ESI): calcd for $C_{25}H_{40}O_4$ $[M+Na]^+$, 427.2824, found 427.2824.

2.2.2. Methyl 3α-acetoxy-7-keto-5β-cholan-24-UDCA (2)

To a solution of **1** (10.29 g, 25 mmol) in CH_2Cl_2 (90 mL) stirred at 0 °C, Ac_2O (6 mL, 63.4 mmol), TEA (10.5 mL, 75.3 mmol) and DMAP (0.16 g, 1.27 mmol) were added at 0 °C, then return to room temperature. The reaction mixture was stirred overnight. The reaction mixture was washed with H_2O and brine, dried with anhydrous Na_2SO_4 and concentrated to give **2** (10.57 g, 93%) as a yellow solid. IR (ATR) cm^{-1}: 3441, 3389, 3018, 2978, 2952, 2898, 2868, 2852, 1735, 1705. ^1H NMR (300 MHz, $CDCl_3$): 4.65 (1H, tt, 3-H), 3.62 (1H, s, 24-OCH_3), 2.83 (1H, dd, 8-H), 1.96 (3H, s, 3-Ac), 1.17 (3H, s, 18-H), 0.87 (3H, d, 20-H), 0.62 (3H, s, 19-H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.63, 211.49, 174.47, 170.36, 77.42, 77.00, 76.58, 72.83, 54.74, 51.34, 49.39, 48.83, 45.76, 45.15, 42.67, 42.55, 38.86, 35.11, 35.08, 33.75, 33.04, 30.95, 30.91, 29.58, 28.16, 25.97, 24.69, 22.92, 21.64, 21.16, 18.28, 11.96. HRMS (ESI): calcd for $C_{27}H_{42}O_5$ $[M+Na]^+$, 469.2930, found 469.2932.

2.2.3. Methyl 3α-acetoxy-6α-bromo-7-keto-5β-cholan-24-UDCA (3)

Bromine (1.5 mL, 0.5 mmol) was dissolved in acetic acid (21 mL) at 0 °C and added dropwise to compound **2** (10.57 g, 23.7 mmol) at 0 °C. After finished, the solution of 48% HBr in water 1.1 mL was added into mixture. The reaction mixture was stirred at room temperature with TLC monitoring. After the reaction had finished, the mixture was poured into $NaHCO_3$ (aq), extracted with ethyl acetate 20 mL, then washed with $Na_2S_2O_3$(aq), water and brine, dried with anhydrous Na_2SO_4 and concentrated. The residence was recrystallized by 10 mL MeOH at −20 °C, then filtered to give compound **3**(9.433 g, 76%) as a light yellow solid. IR (ATR) cm^{-1}: 3439, 2951, 2875, 2836, 1728. ^1H NMR (300 MHz, $CDCl_3$): 5.17 (1H, d, 6-H), 4.66 (1H, tt, 3-H), 3.62 (1H, s, 24-OCH_3), 2.44 (1H, t, 8-H), 1.99 (3H, s, 3-Ac), 1.26 (3H, s, 18-H), 0.88 (3H, d, 20-H), 0.64 (3H, s, 19-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.28, 174.53, 170.30, 77.42, 77.00, 76.58, 72.37, 59.02, 54.68, 53.89, 51.45, 49.46, 49.25, 42.96, 42.76, 38.60, 38.05, 35.07, 33.90, 30.93, 30.85, 28.46, 28.09, 25.98, 24.40, 23.18, 21.68, 21.18, 18.28, 11.98. HRMS (ESI): calcd for $C_{27}H_{41}BrO_5$ $[M+Na]^+$, 547.2035, found 547.2034.

2.2.4. Methyl 3α-acetoxy-6β-vinyl-7-keto-5β-cholan-24-UDCA (4)

To a solution of **3** (9.43 g, 17.9 mmol) in anhydride THF (94 mL), Pd $[P(Ph)_3]_4$ (0.16 g, 0.9 mmol) and (S,S)-Ph-BOX (0.30 g, 0.9 mmol) was added into a Schlenk flask under nitrogen atmosphere at 0 °C. Then vinylmagnesium bromide (1 M/L in THF, 59 mL) was added dropwise into the mixture and stirred overnight. The reaction was quenched with water (1 mL) then filtered with diatomite. The filtrate was concentrated and extracted with 100 mL ethyl acetate, then evaporated to oil and then purified with silica gel (petroleum ether: ethyl acetate = 20:1) to get compound **4** as a white solid (2.63 g, 31%) and compound **2** (4.82 g, 60%). M.p. 146.2–146.8 °C. $[\alpha]_D^{25}$ + 63.3 (c 1 g/100 mL MeOH). IR (ATR) cm^{-1}: 3449, 3389, 2946, 2874, 1739, 1703, 1653, 1626. ^1H NMR (300 MHz, $CDCl_3$): 6.02 (1H, ddd, 6-vinyl-CH), 5.00 (1H, dd, 6-vinyl-AH) 4.78 (1H, dd, 6-vinyl-BH), 4.64 (1H, tt, 3-H), 3.63 (1H, s, 24-OCH_3), 2.79 (1H, d, 6-H), 2.69 (1H, t, 8-H), 1.99 (3H, s, 3-Ac), 1.26 (3H, s, 18-H), 0.88 (3H, d, 20-H), 0.64 (3H, s, 19-H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.29, 174.50, 170.42, 138.37, 115.41, 77.42, 77.00, 76.58, 72.58, 61.80, 54.72, 51.38, 50.55, 48.61, 46.01, 42.68, 42.43, 38.68, 35.89, 35.14, 34.42, 30.94, 30.90, 28.11, 25.58, 25.05, 24.71, 21.38, 21.20, 18.27, 11.96. HRMS (ESI): calcd for $C_{29}H_{44}O_5$ $[M+Na]^+$, 495.3086, found 495.3184.

2.2.5. Methyl 3α-acetoxy-6β-ethyl-7-keto-5β-cholan-24-UDCA (5)

To a solution of **4** (2.63 g, 5.6 mmol) in MeOH (26 mL), Pd/C (0.26 g, 10% w/w) was added at room temperature. The reaction mixture was heated to reflux and stirred for 4 h. The mixture was filtered with diatomite and the filtrate was evaporated to dryness to get compound **5** (2.64 g, 99%) as a colorless oil. IR (ATR) cm^{-1}: 3448, 2950, 2868, 1742, 1703. ^1H NMR (300 MHz, $CDCl_3$) δ 4.63 (s, 1H), 3.64 (s, 3H), 2.54 (t, J = 11.3 Hz, 1H), 2.33 (dd, J = 12.8, 7.6 Hz, 1H), 2.20 (dd, J = 19.0, 12.5 Hz, 2H), 1.97 (s, 4H), 1.90 (t, J = 11.2 Hz, 3H), 1.86–1.58 (m, 8H), 1.56–1.03 (m, 17H), 0.90 (d, J = 6.1 Hz, 4H), 0.86–0.75 (m, 4H), 0.65 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 215.21, 215.10, 174.60, 170.55, 72.73, 61.83, 54.77, 51.43, 49.19, 48.62, 45.49, 42.90, 42.45, 38.73, 35.56, 35.23, 35.18, 34.91, 30.98, 30.94, 29.64, 28.13, 26.46, 25.87, 25.69, 24.84, 21.43, 21.27, 18.30, 13.01, 12.05. HRMS (ESI): calcd for $C_{26}H_{44}O_4$ $[M+Na]^+$, 443.3135, found 443.3137.

2.2.6. 3α-dihydroxy-6α-ethyl-7-keto-5β-cholan-24-UDCA (6)

A 30% aqueous sodium hydroxide (10 mL) was added into a round bottom flask containing compound **6** (1.00 g, 2.11 mmol, 1equiv). The mixture was stirred at 70 °C for 24 h and then return to room temperature. The mixture was neutralized to pH = 4 by 1 M/L HCl. The organic phase was separated, dried with anhydrous Na_2SO_4 and concentrated to provide compound **8** as colorless oil (0.84 g, 95%). This compound can be crystalized by EtOH to provide get off-white solid. IR (ATR) cm^{-1}: 3395, 2968, 2940, 2871, 2624, 1708. ^1H NMR (300 MHz, $CDCl_3$): δ 5.66 (s, 2H), 3.52 (s, 1H), 2.76–2.61 (m, 1H), 2.46–2.08 (m, 4H), 1.96 (d, J = 12.9 Hz, 2H), 1.86–1.74 (m, 4H), 1.69 (dd, J = 14.0, 6.9 Hz, 3H), 1.55–1.35 (m, 4H), 1.34–1.01 (m, 11*H*), 1.00–0.85 (m, 5H), 0.79 (dd, J = 14.3, 7.0 Hz, 3H), 0.63 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 213.05, 179.54, 77.42, 77.00, 76.58, 71.15, 54.75, 51.97, 50.68, 49.89, 48.93, 43.68, 42.62, 38.94, 35.63, 35.16, 34.20, 31.56, 31.02, 30.73, 29.67, 28.23, 24.55, 23.47, 21.82, 18.77, 18.29, 12.02, 11.93. HRMS (ESI): calcd for $C_{26}H_{44}O_4$ $[M+Na]^+$, 443.3135, found 443.3137.

2.2.7. Obeticholic acid

Compound **8** (1.03 g,2.3 mmol, 1 equiv), NaOH (0.19 g, 4.7 mmol, 2 equiv), and H_2O (7 mL) was added to the reaction flask. The mixture of $NaBH_4$ (0.10 g, 2.80 mmol, 1.2 equiv), NaOH (1.2 mg), and H_2O (1 mL) was added to the stirring mixture at room temperature, and then, the mixture was stirred at 75 °C for 6 h. Then reaction mixture was cooled to room temperature. Citric Acid aqueous solution was added slowly to the reaction mixture until pH = 6 and then the reaction mixture was extracted with CH_2Cl_2 (10 mL) three times. The organic phase was dried by anhydride Na_2SO_4 and evaporated to dryness to get OCA 0.81 g as a white solid, yield: 86%. M.p.104.5–106.5 °C. $[\alpha]_D^{25}$ + 5.1 (c 1 g/100 mL MeOH). IR (ATR) cm^{-1}: 3449, 2964, 2935, 2870, 2611, 1734, 1712. ^1H NMR (300 MHz, $CDCl_3$) δ 4.19 (s, 3H), 3.70 (s, 1H), 3.41 (s, 1H), 2.46–2.31 (m, 1H), 2.30–2.14 (m, 1H), 2.02–1.72 (m, 6H), 1.71–1.54 (m, 3H), 1.53–1.06 (m, 15H), 1.01 (d, J = 11.3 Hz, 1H), 0.90 (dd, J = 16.0, 6.0 Hz, 9H), 0.65 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.13, 77.42, 77.00, 76.58, 72.34, 70.98, 55.78, 50.41, 45.14, 42.72, 41.16, 40.00, 39.57, 35.47, 35.40, 33.76, 33.15, 31.04, 30.81, 30.39, 28.16, 23.64, 23.12, 22.22, 20.72, 18.24, 11.76, 11.64. HRMS (ESI): calcd for $C_{26}H_{44}O_4$ $[M+Na]^+$, 443.3135, found 443.3137.

3. Results and discussion

The synthetic route of OCA is shown in Scheme 2. Compound **1** was obtained by methylation with MeOH and H_2SO_4 in 90% yield, then

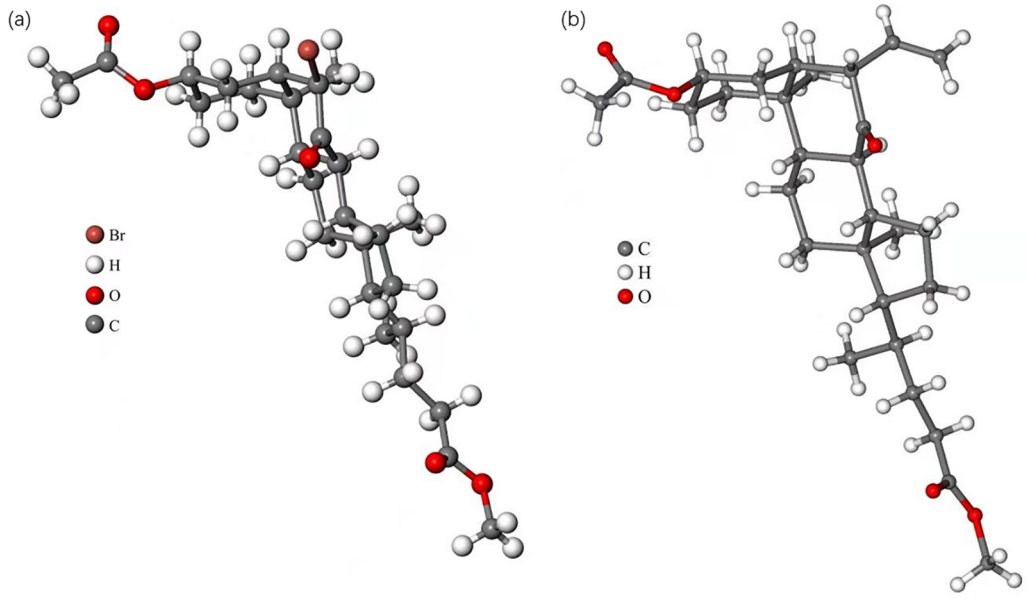


Fig. 1. The single-crystal x-ray diffraction of compound **3**(a) and compound **4**(b).

esterification of 3α-OH with Ac_2O in the presence of pyridine and DMAP provided ester **2** in 90% yield. Compound **3** was prepared by Br_2 with HBr in 94% yield. The key intermediate 4 was obtained by reaction of compound **3** with vinylmagnesium bromide solution in anhydride THF catalyzed by $Pd(PPh_3)_4$ and (S,S)-Ph-BOX at room temperature with 31% yield. In addition, compound **2** was recovered as the byproduct in 60% yield, which could be reutilized to prepare compound **3**. Compound **4**, the key intermediate, was successfully synthesized for the first time. To confirm the absolute configuration of compound **3** and **4**, we successfully obtained their single crystal and characterized the absolute configuration by single-crystal x-ray diffraction. As shown in Fig. 1, configuration inversion of 6-carbon from alpha (compound **3**) to beta (compound **4**) was detected after cross-coupling reaction. Configuration at 6-carbon of compound **5** was retained after reduction of compound **4** under H_2 and Pd/C in MeOH at 75 °C. Demethylation and deacetylation were performed with NaOH in H_2O with configuration conversion to obtained compound **6** in the yield of 95%. In this step, compound **5** was converted into enol formula through keto-enol tautomerism under base catalyzed condition and then transferred into 6-alpha-ethyl ketone. As show in Fig. 1(b), ring A is twist-boat form and 6-ethyl is in axial bond, so compound **4** is a thermodynamically unstable species. Like compound **4**, compound **5** is also a thermodynamically unstable species, so after forming an enol structure, it will release energy and convert into thermodynamically 6-alpha ethyl ketone. Finally, OCA was afforded by stereoselective reduction of 7-ketone group to 7β-OH of compound **6** in 93% yield. The total yield of this route was up to 15%. The higher yield would be obtained when the byproduct of compound **2** generated in the coupling reaction was reutilized.

4. Conclusion

In summary, we have successfully developed a novel efficient synthetic route of Obeticholic acid. Through Kumada-Tamao-Corriu coupling reaction, 6-bromo KLCA (compound **3**) could couple with vinyl magnesium bromide to construct 6-vinyl intermediate (compound **4**), then converted to the target compound acid. The by-product of the cross-coupling reaction is the 6-carbon dehalogenated intermediate (compound **2**), which can be reutilized. This method shows good yield and avoids the use of low temperature and strong alkali conditions. All the compounds have been well verified by HRMS, ^1H NMR, ^{13}C NMR and IR. The absolute configuration of the key intermediates (compounds **3** and **4**) was confirmed by single crystal X-ray diffraction. We hope that this work can provide a new strategy for the synthesis of bile acids and promote the development of new synthetic methods of bile acids analogs. The application of this method to other bile acid analogs is under study.

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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.2020.108657.

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