A Novel Telescoped Kilogram-Scale Process for Preparation of Obeticholic Acid

Cheng-Wei Li¹,² Cai Wang² Chao Guo² Bin-Hua Lv²* You-Fu Luo¹*

¹State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Medical School, Sichuan University, Chengdu, People’s Republic of China
²Suzhou Zelgen Biopharmaceuticals Co., Ltd., Kunshan, People’s Republic of China

Pharmaceut Fronts 2021;3:e56–e64.

Abstract

A novel scalable four-step process has been developed to improve the synthesis of obeticholic acid (OCA). The key step of this process was the isolation of the amide intermediate, which underwent hydrogenation, basic epimerization, ketone reduction, and amide hydrolysis in a one-pot procedure. The use of efficient single recrystallization for the final purification in this process made the corresponding work-up procedure more concise and environmentally friendly. A kilogram-scale production of OCA following this process could achieve over 70% yield with all impurities controlled below 0.10%.

Introduction

Obeticholic acid (OCA, or 7-ECDCA) is a selective agonist of farnesoid X receptor (FXR) with the 6α-ethyl group substituted from chenodeoxycholic acid (CDCA), and has been under intense investigation by Intercept.¹,² To date, it has been authorized by Food and Drug Administration for the treatment of primary biliary cholangitis, and granted breakthrough therapy designation in nonalcoholic steatohepatitis.³,⁴ Based on the widespread use of OCA as a FXR agonist in therapeutic applications, the synthesis of OCA has attracted extensive attention.⁵⁻⁷ The synthesis strategy of OCA was first focused in Pellicciari et al’s report for the introduction of an alkyl substitution from 7-keto-lithocholic acid (Scheme 1).⁵⁻⁷,⁻¹⁰ However, there are many disadvantage in this process, such as the strict operation with cryogenic temperature, the complicated column purification, the participation of highly toxic reagents including HMPA (hexamethylphosphoramide) and bromoethane, as well as the low overall yield (less than 3.1%). Similar to this strategy, Yu et al improved the process by using pyridinium chlorochromate (a selective oxidant) and iodoethane (a strong nucleophilic reagent), but did not escape the

Keywords
► obeticholic acid
► four-step process
► amide intermediate
► kilogram-scale production

received April 19, 2021
accepted June 3, 2021

DOI https://doi.org/10.1055/s-0041-1731757.
ISSN 2628-5088.

© 2021. The Author(s).
This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution, and reproduction so long as the original work is properly cited. (https://creativecommons.org/licenses/by/4.0/)
Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany
use of HMPA and the column chromatograph.\textsuperscript{13} Later, novel synthesis steps of OCA were explored via using cholic acid and 3-keto-bisnorcholesterol as starting regents.\textsuperscript{12,13} However, it is difficult to scale up the production of OCA with either approach mentioned above. To our acknowledge, the kilogram-scale production of OCA was first reported by Ferrari and Pellicciari in 2006, and this process included eight steps, yet with only 24.6\% total yield achieved (\textbf{Scheme 2}).\textsuperscript{14} It should be noted that 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (\textbf{10}), an intermediate from epimerization claimed in a patent, would be converted to OCA with high chiral selectivity after the NaBH₄ reduction. In 2013, André et al made the process more safe with high quality and yield (30\%) of OCA being achieved.\textsuperscript{15} Although carcinogenic reagents are avoided and the yield is improved, André et al.’s process was not concise enough, for example, a large amount of concentrated caustic soda is involved in the reaction independently in the later stage of the process, leading to tedious work and a high amount of waste.

Considering that in compound \textbf{11}, a selective reduction of 7-ketone group, priority to 6-ethylene, was obtained (\textbf{Scheme 3}),\textsuperscript{16} protection strategies for functional groups, such as 3α-OH and 24-COOH (\textbf{Scheme 4}),\textsuperscript{17–20} have aroused much attention during the synthesis of OCA. However, the use of protection strategies would inevitably lead to yield loss and purification challenges as the number of reaction steps being increased. Thus, exploring an effective, concise, and environmentally friendly process to obtain OCA with robust quality control for commercialization remained significantly urgent.\textsuperscript{21}

Herein, a novel four-step, telescoped, kilogram-scale process to obtain OCA was discovered. The \textit{E}-conformation of \textbf{8} (\textbf{8-E}), a commercially available agent, was used as the key starting material (\textbf{Scheme 5}). Instead of double blocking, only...
the 24-COOH of 8-E was protected as amide 13, which was converted to OCA in a one-pot procedure integrating the previous strong base steps.14,15 Besides, variant process parameters, including the final recrystallization condition, were further investigated, and high-quality OCA with satisfactory yield was successfully obtained.

Results and Discussion

Compound 8-E was Preferred as the Starting Material

Compound 8 is a crude mixture of E/Z isomers with no fixed proportion (Scheme 2). When 8 was coupled with NH₄Cl, crude 13 was obtained as a mixture of E/Z isomers with only 70% yield. Given above, the E-isomer of 8 (8-E), instead of 8, was preferred for the following reasons: (1) 8-E is commercially available at a cheap price (about $1,800/kg); (2) high-grade 8-E from 8 can be easily achieved through recrystallization (►Fig. 1)15 with both the E-isomer ratio and purity being no less than 99.0%, and the specified impurity (7-ketolithocholic acid associated with CDCA) being NMT (no more than) 0.2%; and (3) ultraviolet (UV) absorption of 8-E is strong, making it easier to monitor the target compounds.

Preparation of (E)-3α-Hydroxy-6-ethylidene-7-keto-5β-cholan-24-amide (13, Step 1)

We prepared 13 from 8-E through an amidation reaction, and the process parameter is outlined in ►Table 1. In this...
process, amine NH₄Cl was preferred, as it was identified to be cost-effective, and to have reliable stability and safety. Through using NH₄Cl, compound 13 could separate, as an excellent solid, in nearly quantitative yield, and the subsequent hydrolyses could be completed with least impurities in comparison to other amides. For example, the separated yield of target amides consisted of morpholine (80%, entry 3), CH₂ONH₂-HCl (79%, entry 4), and CH₂NHOC₂H₃-HCl (77%, entry 6), and these amounts of yields were much lower than that of NH₄Cl (96%, Entry 8). It is exciting to notice that the yield of the target product was good when using CH₃NH₂-HCl (90%, entry 1) and pyrrolidine (92%, entry 2). However, the use of pyrrolidine, originated from PyBOP, resulted in the generation of pyrrolidine byproduct 14, the major product in this reaction (Fig. 2). Besides, by using CH₂NH₂-HCl and pyrrolidine, the resulting amides were too stable to be hydrolyzed in the following one-pot procedure, leading to increased impurities of OCA, and thus, neither of them was used in this synthesis step. Furthermore, the use of HONH₂-HCl led to yield loss of the target product (87%, entry 5) and 5% yield of 7-oxime byproduct, yet CH₂NHOH-HCl gave a messier coupling result (entry 7). PyBOP was employed as a coupling reagent according to a reported study. Our data showed that PyBOP resulted in a higher consumption and less impurities (entry 8, 8-E consumed over in 6 hours, 96% yield) when compared with HBTU (entry 10, 8-E consumed in 24 hours, three impurities with 2–5% content), EDCI/1-hydroxybenzotriazole (HOBt) (entry 11, 6% 8-E left after 36 hours), or DIC (entry 12, 25% 8-E left after 24 hours). Hünig's base was beneficial for time-saving and complete consumption in comparison to triethylamine (entry 9, 10% 8-E left after 24 hours). With the optimized conditions in hand, compound 13 was obtained in good yield (96%, entry 8) with high-performance liquid chromatography (HPLC) purity >98%, which was confirmed with ¹H NMR, ¹³C NMR, and HRMS (high-resolution mass spectrometry) spectra. The selected coupling conditions were found to perform well in a kilogram-scale production with a good yield of 96%. Under the reaction conditions provided in Table 1, five major byproducts were confirmed in this step, and they were Z-isomer (13-Z), pyrrolidine amide (14), self-esterified dimer (15), tri(pyrrolidin-1-yl)phosphine oxide (16), as well as HOBt.

**Preparation of OCA through the One-Pot Procedure (Steps 2–4)**

Telescopied synthesis of OCA from compound 13 is shown in Fig. 3. The procedure started with Pd/C catalytic hydrogenation (step 2) and epimerization (step 3), followed by NaBH₄-induced reduction (step 4). Hydrolysis of the amide unit proceeded in both step 3 and step 4. In step 2, the reaction conditions were screened. Based on the fact that the solvent should not only dissolve the substrate effectively but also be mutually soluble with water, several solvents, including methanol, dichloromethane, dioxane, tetrahydrofuran, or a combination of the above solvents, have been selected. In this step, single solvent methanol (methanol:13 = 12:1, v/m) was preferred, despite a better dissolvability of 13 in a binary solvent of methanol and dichloromethane (1:1). Noticeably, blockages tend to occur during filtration when using the two hydrophobic solvents. Furthermore, to favor fast consumption (controlling 13 NMT 0.2%), pressure (3–5 atm) and heat (40–55°C) were also employed. In this step, a mixture of 17α/17β isomers (the ratio is close to 10:90) was obtained with no further filtration, since the isomeric intermediates precipitated quickly after slight cooling of this reaction.

Once NaOH solution was charged to the hydrogenation, epimerization along with hydrolysis (step 3) immediately started under the condition of reflux. In this step, only the residue of 17α was monitored (17α is controlled NMT 1%). Amide hydrolysis should be ignored because the further hydrolysis may continue during the NaBH₄ reduction (Step 4). Thus, the dosage of NaOH and NaBH₄ was screened in the following study. Table 2 notes that NaOH (20 equiv.: the molar ratio to 13 is 20/NaBH₄ (2.0 equiv.) was the best formula (entry 5) with less 7-ketone intermediates left. Low base ability (entries 6 and 7) was associated with inadequate hydrolysis, resulting in retention of amide-blocked impurities (e.g., 18α and 18β). 2–8%, α-conformation is the major). Furthermore, a low amount of NaBH₄ (entries 2 and 3, 1.5 equiv. and 1.0 equiv.) brought byproducts with 7-ketone reserved (10 or Imp-6 is the major with the yield of 6–11%). Although the full consumption of the starting material 10 and the high quality of the target product being obtained, the use of NaOH (entry 4, 30 equiv.) or NaBH₄ (entry 1, 3.0 equiv.) should be given up because of the over-wastage that occurred.

**Crystallization for the Final Purification of Crude OCA**

Once the final in process control (10 is controlled below 1.0%) passed, a routine work-up procedure, including acid quenching, extraction, and distillation, was performed. The purity of the crude product is close to 95% with Imp-1 as the major impurity (~4%). The final purification of OCA is crucial for furnishing qualified active pharmaceutical ingredient.
André et al. highlighted the participation of n-butyl acetate (n-BuOAc) in OCA recrystallization. Feng et al. suggested the potential use of heptane as a wonderful antisolvent in this process. In this study, the crystallization conditions were screened based on the ratio of n-BuOAc/heptane (Table 3, entries 1–5), and we further proposed a two-stage cooling plan: first warm crystallization was used to control the quality, and then a cooler precipitation was performed to get more product (Table 3, entries 5–7). With the optimized recrystallization conditions, the quality and yield of OCA significantly improved (entries 6 and 7; 77 and 75%, respectively). In comparison to second cooling to 10°C, the second cooling to 20°C led to less Imp-1 (the maximum impurity: 0.24% in entry 6; 0.08% in entry 7), and should be chosen as the ideal crystallization conditions. In this article, the final crystallization procedure was confirmed as follows: OCA crude was dissolved in a binary solvent of n-BuOAc: heptane (4.4:0.85), and then refluxed; the clear solution was gradually cooled down to 40°C with the cooling rate being 8 to 15°C/hour for warm crystallization for ~2 hours, followed by a second cooling at a similar rate to 20°C, and the system was held for further precipitation.

After this process of crystallization, a similar impurity profile involving three isomers (Imp-1, 2, and 3), CDCA (Imp-4), and the dimer (Imp-9) was outlined as disclosed (Fig. 4). Two exclusive impurities, Imp-5 (originated from pyrrolidine) and Imp-8 (originated from methanol), were rarely detected. Imp-6 and Imp-7 were generated via the insensitive reduction of the 7-ketone or 6-ethylene group, while Imp-10 was the residue of 8-E. As OCA and most impurities have poor UV absorption for detection, the HPLC-charged aerosol detector (CAD) method was introduced covering all the above impurities in acceptable resolution (Figure S11 [online only]). Our data suggested a >99.5% purity of OCA from a kilogram-scale campaign, and all impurities were controlled NMT 0.10%, which was in

| Entry | Amine | Coupling reagent | Base | Consumption, quality and work-up | Yield | Impact of the resulting amide on one-pot step |
|-------|-------|------------------|------|----------------------------------|-------|---------------------------------------------|
| 1     | CH₃NH₂·HCl | PyBOP          | DIPEA | Consumed over in 6 hours, solid formed | 90%   | Hardly to hydrolyze, much messier than entry 8 |
| 2     | Pyrrolidine | PyBOP          | DIPEA | Consumed over in 6 hours, solid formed | 92%   | Hardly to hydrolyze, much messier than entry 8 |
| 3     | Morpholine | PyBOP          | DIPEA | Consumed over in 6 hours, foam, need extraction | 80%   | Most amide hydrolyze, much messier than entry 8 |
| 4     | CH₃ONH₂·HCl | PyBOP          | DIPEA | Consumed over in 6 hours, foam, need extraction | 79%   | Trace product, messy |
| 5     | HONH₂·HCl | PyBOP          | DIPEA | Consumed over in 6 hours, oxime byproduct (5%), solid formed | 87%   | Similar to entry 8 |
| 6     | CH₃NHOCH₃·HCl | PyBOP        | DIPEA | Consumed over in 6 hours, foam, need extraction | 77%   | Similar to entry 8 |
| 7     | CH₃NHOH·HCl | PyBOP          | DIPEA | Consumed over in 6 hours, a major impurity (11%) | ND    | / |
| 8     | NH₄Cl   | PyBOP          | DIPEA | Consumed over in 6 hours, solid formed | 96%   | Least impurities, quality in control. |
| 9     | NH₄Cl   | PyBOP          | TEA   | 10% 8-E stayed after 24 hours | ND    | / |
| 10    | NH₄Cl   | HBTU           | DIPEA | Consumed over in 24 hours, 3 impurities (2–5%) | ND    | / |
| 11    | NH₄Cl   | EDCI/HOBt      | DIPEA | Consumed over in 36 hours, 6 impurities (2–5%) | ND    | / |
| 12    | NH₄Cl   | DIC            | DIPEA | Consumed over in 24 hours, 7 impurities (2–5%) | ND    | / |
accordance with the ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guideline, and more stricter than that of the documented process (►Table 4), especially for Imp-4 and Imp-9 (both were less than 0.05% in comparison to controlled NMT 0.15% and 3.0% in the documented process, respectively). This could be attributed to the strict quality precontrol in 8-E and the use of complete hydrolysis as an adequate base.

**Conclusion**

In summary, the production of OCA, without the need of separating intermediate 10, was achieved in a four-step process, including three-telescoped steps in one pot integrating previous multiple steps. Key to the process is the selection of amide 13 as a key intermediate obtained by coupling 8-E (a cheap commercially available regent) with NH₄Cl in the presence of PyBOP and DIPEA, which is easily purified and performed well for the one-pot procedure. Process parameters including the solvent, the ratio of reagents (NaOH and NaBH₄), and recrystallization conditions (solvent and the cooling control) were thoroughly studied for optimum yield, safety, and quality considerations. An effective HPLC-CAD method was also introduced to determine the purity of the final crystals. A kilogram-scale production following this process succeeded to furnish the desired product in 72% overall yield and over 99.5% purity, while
total impurities were well below 0.10%. This process is not only concise but also environmentally friendly, scalable, and shows excellent quality control.

Supporting Information

Spectroscopic characterization processes ($^1$H NMR, $^{13}$C NMR, and HRMS) for 13 and OCA, as well as HPLC-CAD results for the possible impurities following OCA synthesis, are included in the Supporting Information (Figs. S1–11 [online only]).

### Table 2

| Entry | NaOH        | NaBH$_4$ | The quality of the crude product                                      |
|-------|-------------|----------|----------------------------------------------------------------------|
| 1     | 20 equiv.   | 3.0 equiv. | 0.15% of 7-ketone intermediate 10 stayed                          |
| 2     | 20 equiv.   | 1.5 equiv. | 6% of 7-ketone intermediate 10 stayed                              |
| 3     | 20 equiv.   | 1.0 equiv. | 11% of 7-ketone intermediate 10 stayed                             |
| 4     | 30 equiv.   | 2.0 equiv. | Little amide intermediate stayed                                   |
| 5     | 20 equiv.   | 2.0 equiv. | 0.2% of amide intermediate (18α is the major), 0.13% of 7-ketone intermediate 10 stayed |
| 6     | 10 equiv.   | 2.0 equiv. | 2% of amide intermediate (18α is the major) and obvious pyrrolidine-coupled amide stayed |
| 7     | 5 equiv.    | 2.0 equiv. | 8% of amide intermediate (18α:18β = 12.5:1) and much pyrrolidine-coupled impurities formed |

### Table 3

| Entry | n-BuOAc (v/m) | Heptane (v/m) | Temp. control and crystallization state | Crystal purity | Imp-1* | Yield |
|-------|---------------|---------------|----------------------------------------|----------------|--------|-------|
| 1     | 3.5           | 0             | Reflux to 25°C naturally, smooth crystallization | 99.8%          | 0.07%  | 56%   |
| 2     | 2.0           | 0             | Reflux to 25°C naturally, too thick to filtration | /              | /      | /     |
| 3     | 2.7           | 0.85          | Reflux to 25°C with fast cooling, thick crystals formed dramatically with separation trouble | 98.6%          | 0.65%  | 84%   |
| 4     | 4.0           | 0.85          | Reflux to 30°C with fast cooling, thick crystals formed dramatically with separation trouble | 99.1%          | 0.37%  | 74%   |
| 5     | 4.4           | 0.85          | Reflux to 40°C naturally, smooth crystallization | 99.7%          | 0.07%  | 62%   |
| 6     | 4.4           | 0.85          | Reflux to 35°C (hold on for 2 hours), second cooling to 10°C, smooth crystallization | 99.2%          | 0.24%  | 77%   |
| 7     | 4.4           | 0.85          | Reflux to 40°C (hold on for 2 hours), second cooling to 20°C, smooth crystallization | 99.6%          | 0.08%  | 75%   |

*Imp-1 was the maximum impurity in this process.

### Experimental Section

#### General

Common reagent-grade chemicals such as PyBOP and NH$_4$Cl were purchased and used without further purification. The key starting material 8-ε was supplied by Xiamen Halosynthec Co., Ltd.. The $^1$H NMR and $^{13}$C NMR spectra data were recorded either on a Bruker 600 MHz or a Bruker 400 MHz NMR spectrometer. Chemical shifts are summarized in parts per million (ppm) using tetramethylsilane as an internal standard and are given in 8 units. Solvents for NMR spectra were DMSO-$d_6$ or CD$_3$OD unless otherwise stated. High-resolution mass spectra were obtained on an Agilent 1100 series HPLC system coupled to an Agilent 6210 ESI-TOF mass spectrometer. The purity of OCA was analyzed on a Thermo Fisher Dionex Ultimate 3000 system with Corona Veo CAD, chromatographic separation was performed on an Agilent InfinityLab Poroshell column at a flow rate of 0.6 mL/minute for a run time of 45 minutes. The mobile phase A was 0.1% formic acid (v/v) and the mobile phase B was acetonitrile.

#### General Procedure for the Synthesis of (E)-3α-Hydroxy-6-ethylidene-7-keto-5β-cholan-24-amide (compound 13)

A 100 L glass tank was charged with 8-ε (4.80 kg), N,N-dimethylformamide (32.10 kg), and PyBOP (7.25 kg) at ice temperature. After that, DIPEA (5.95 kg) was added slowly, the mixture was stirred for 30 minutes, followed by the addition of ammonium chloride (1.00 kg). The content of 8-ε in the process was monitored by HPLC (NMT 0.5%), and then 5% sodium bicarbonate solution was added dropwise. After stirring for another 1 hour, the mixture was centrifuged and washed twice with water. The wet cake (containing /C$_2$450% water) was dissolved in ethyl acetate (25.90 kg) and refluxed for another 1 hour. After cooling down, the solid product was routinely centrifuged, washed twice with water, and then dried in an air-drying oven at 55 to 60°C to yield the title compound as off-white powder (4.60 kg, yield: 96%, Table 2 The ratio screen of NaOH and NaBH$_4$ for this one-pot process

| Entry | NaOH        | NaBH$_4$ | The quality of the crude product                                      |
|-------|-------------|----------|----------------------------------------------------------------------|
| 1     | 20 equiv.   | 3.0 equiv. | 0.15% of 7-ketone intermediate 10 stayed                          |
| 2     | 20 equiv.   | 1.5 equiv. | 6% of 7-ketone intermediate 10 stayed                              |
| 3     | 20 equiv.   | 1.0 equiv. | 11% of 7-ketone intermediate 10 stayed                             |
| 4     | 30 equiv.   | 2.0 equiv. | Little amide intermediate stayed                                   |
| 5     | 20 equiv.   | 2.0 equiv. | 0.2% of amide intermediate (18α is the major), 0.13% of 7-ketone intermediate 10 stayed |
| 6     | 10 equiv.   | 2.0 equiv. | 2% of amide intermediate (18α is the major) and obvious pyrrolidine-coupled amide stayed |
| 7     | 5 equiv.    | 2.0 equiv. | 8% of amide intermediate (18α:18β = 12.5:1) and much pyrrolidine-coupled impurities formed |

#### Crystallization condition screen for the purification of OCA

| Entry | n-BuOAc (v/m) | Heptane (v/m) | Temp. control and crystallization state | Crystal purity | Imp-1* | Yield |
|-------|---------------|---------------|----------------------------------------|----------------|--------|-------|
| 1     | 3.5           | 0             | Reflux to 25°C naturally, smooth crystallization | 99.8%          | 0.07%  | 56%   |
| 2     | 2.0           | 0             | Reflux to 25°C naturally, too thick to filtration | /              | /      | /     |
| 3     | 2.7           | 0.85          | Reflux to 25°C with fast cooling, thick crystals formed dramatically with separation trouble | 98.6%          | 0.65%  | 84%   |
| 4     | 4.0           | 0.85          | Reflux to 30°C with fast cooling, thick crystals formed dramatically with separation trouble | 99.1%          | 0.37%  | 74%   |
| 5     | 4.4           | 0.85          | Reflux to 40°C naturally, smooth crystallization | 99.7%          | 0.07%  | 62%   |
| 6     | 4.4           | 0.85          | Reflux to 35°C (hold on for 2 hours), second cooling to 10°C, smooth crystallization | 99.2%          | 0.24%  | 77%   |
| 7     | 4.4           | 0.85          | Reflux to 40°C (hold on for 2 hours), second cooling to 20°C, smooth crystallization | 99.6%          | 0.08%  | 75%   |

*Imp-1 was the maximum impurity in this process.

A Novel Telescoped Kilogram-Scale Process for Preparation of OCA

Li et al.
HPLC purity: 98.2%). $^1$H NMR (600 MHz, DMSO-$d_6$) δ 7.23 (s, 1H), 6.66 (s, 1H), 5.97 (q, J = 7.2, 13.8 Hz, 1H), 4.55 (d, J = 4.8 Hz, 1H), 3.46–3.42 (m, 1H), 2.60–2.57 (m, 1H), 2.30–2.25 (m, 1H), 2.20 (t, J = 11.4 Hz, 1H), 2.09–2.04 (m, 1H), 1.97–1.79 (m, 5H), 1.68–1.64 (m, 1H), 1.57–1.55 (m, 1H), 1.44–1.31 (m, 5H), 1.25–1.02 (m, 8H), 0.94 (s, 3H), 0.89 (d, J = 6.0 Hz, 3H), 0.60 (s, 3H). $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 203.72, 174.70, 143.69, 128.34, 68.57, 54.09, 50.26, 48.18, 44.88, 43.05, 38.59, 38.50, 37.55, 34.83, 34.20, 34.03, 32.05, 31.44, 29.60, 28.00, 25.65, 22.59, 20.86, 18.45, 12.32, 11.88. HRMS m/z calcd. for C$_{26}$H$_{42}$NO$_3$ [M + H]$^+$ 416.3159, found 416.3157; calcd. for C$_{26}$H$_{41}$NaO$_3$ [M + Na] 438.2979, found 438.2976.

**General Procedure for the Synthesis of 3α,7α-Dihydroxy-6α-ethyl-5β-cholan-24-oic acid (OCA)**

To a solution of 13 (4.19 kg) in methanol (39.81 kg) in a 200 L high-pressure tank, 10% wet palladium on carbon (0.94 kg) was added after flushing with N$_2$. Afterwards, the gas was exchanged with H$_2$, the mixture was stirred at 50°C at a pressure of 4 atm until 13 was NMT 0.2%. To the mixture, sodium hydroxide solution (8.00 kg sodium hydroxide in 50.28 kg water) was added, and then re-fluxed for 3 hours until 17α was NMT 1.0%. The resulting solution was filtrated and washed with water (2 kg/C$_2$), the filtrate was added sodium borohydride (0.76 kg) portion-wise, re-fluxed for another 6 hours, and cooled down. For the resulting mixture,

**Table 4** Comparison of the controlling strategy of related substances between this process and documented process$^{12}$

| Entry                                  | Imp-1 | Imp-2 | Imp-3 | Imp-4 | Imp-5 | Imp-6 | Imp-7 | Imp-8 | Imp-9 | Imp-10 |
|----------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| Documented process limit               | 0.15% | 0.15% | NA$^a$| 3.0%  | NA    | 0.15% | NA    | 0.15% | NA    | 0.15%  |
| This process limit                     | 0.15% | 0.10% | 0.10% | 0.10% | 0.10% | 0.10% | 0.10% | 0.10% | 0.10% | 0.10%  |
| Resolution                             | 1.46  | 6.25  | 2.07  | 5.71  | 26.90 | 8.55  | 1.46  | 40.11 | /     | 8.88   |
| Assay in a kilogram-scale production   | 0.08% | 0.02% | ND$^b$| 0.04% | 0.04% | ND    | 0.03  | 0.02  | 0.03% | ND     |

$^a$NA refers to “not analyzed.”

$^b$ND refers to “not detected.”
the pH was adjusted to pH 4–5 with hydrochloride (21.61 kg), and then it was neutralized with sodium hydroxide, and then distilled. The residue was adjusted to pH 2–3, extracted with ethyl acetate (72.00 kg × 3), washed with water and brine, and then dried. The crude product was crystallized from a binary solvent of n-BuAc and heptane (4:4:0.85). The hot solution was cooled to ~40°C naturally with stirring at first, followed by a second cooling to ~20°C with the subsequent precipitation. The solid product was routinely centrifuged, washed and dried to get an off-white solid (3.14 kg yield; 75%, HPLC purity: 99.6%). 1H NMR (600 MHz, D2O) δ 3.68 (s, 1H), 3.37–3.31 (m, 1H), 2.38–2.33 (m, 1H), 2.25–2.19 (m, 1H), 2.03–2.01 (m, 1H), 1.97–1.74 (m, 7H), 1.63–1.61 (m, 1H), 1.58–1.46 (m, 6H), 1.44–1.30 (m, 7H), 1.24–1.10 (m, 3H), 1.05–1.10 (m, 1H), 0.99 (d, J = 6.6 Hz, 3H), 0.94 (s, 3H), 0.93 (t, J = 7.2 Hz, 3H), 0.72 (s, 3H). 13C NMR (100 MHz, DMSO-d6) δ 174.96, 70.61, 68.41, 55.55, 50.08, 45.33, 42.02, 41.28, 39.94, 35.53, 35.19, 34.94, 33.54, 32.63, 30.83, 30.76, 30.43, 27.83, 23.08, 22.16, 20.41, 18.17, 11.69. HRMS m/z calcld. for C28H44NaO4 [M + Na] 443.3132, found 443.3137.

Conflict of Interest
We declared no conflict of interest.

Acknowledgment
We thank Bingling Qiu (Xiamen Halosyntech Co., Ltd.) for his help in offering the key starting material 8-E.

References
1 Makishima M, Okamoto AY, Repa JJ, et al. Identification of a nuclear receptor for bile acids. Science 1999;284(5418):1362–1365
2 Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999;3(05):543–553
3 FDA News Release. FDA approves Ocaliva for rare, chronic liver disease. Accessed April, 2021 at: https://www.fda.gov/news-events/press-announcements/fda-approves-ocaliva-rare-chronic-liver-disease
4 Our Focus - Intercept Pharmaceuticals. Advanced fibrosis due to NASH puts patients at high risk of progressing to serious outcomes. Accessed April, 2021 at: https://www.interceptpharma.com/our-focus/nash/
5 Pelliccari R, Fiorucci S, Camaioni E, et al. 6-O-ethylchenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. J Med Chem 2002;45(17):3569–3572
6 Pelliccari R. Steroids as agonists for FXR. WO Patent 2002/072598 September, 2002
7 Pelliccari R, Costantino G, Camaioni E, et al. Bile acid derivatives as ligands of the farnesoid X receptor. Synthesis, evaluation, and structure-activity relationship of a series of body and side chain modified analogues of chenodeoxycholic acid. J Med Chem 2004; 47(18):4559–4569
8 Shott LD, Borkovec AB, Knapp WA Jr. Toxicology of hexamethyl-phosphoronic triamide in rats and rabbits. Toxicon Appl Pharmacol 1971;18(03):499–506
9 Steere NV, CXXX. Background information on hexamethylphosphoronic triamide. J Chem Educ 1976;53(01):A12
10 National Toxicology Program. Toxicology and carcinogenesis studies of bromoethane (ethyl bromide) (CAS No. 74-96-4) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicology Program Tech Rep Ser 1989;363:1–186
11 Yu D, Mattern DL, Forman BM. An improved synthesis of 6-ethylchenodeoxycholic acid (6ECDCA), a potent and selective agonist for the Farnesoid X Receptor (FXR). Steroids 2012;77 (13):1335–1338
12 He XL, Wang LT, Gu XZ, Xiao JX, Qiu WW. A facile synthesis of Ursodeoxyxlic acid and obeticholic acid from cholic acid. Steroids 2018;140:173–178
13 Ignacio HS, Yolanda FS, Carlos CL, Alfonso PE, José Angel TH. Process and intermediates for the synthesis of obeticholic acid and derivatives thereof. EP patent 3431486 A1, January, 2019
14 Ferrari M, Pelliccari R. Process for preparing 3α(β)-7α(β)-dihydroxy-6α(β)-alkyl-5β-choloic acid. WO patent 2006122977 A2, November, 2006
15 André S, Heidi W, Emilie J, et al. Preparation, uses and solid forms of obeticholic acid. WO patent 2013192097 A1, December, 2013
16 Sepe V, Unmarino R, D’auria MV, et al. Conioaster E, a small heterodimer partner sparing farnesoid X receptor modulator endowed with a pregnane X receptor agonistic activity, from the marine sponge Theonella swinhoei. J Med Chem 2012;55(01):84–93
17 Zhang J, Yu JH, Ma SM, Xu XN, Xiao P. A method for preparing obeticholic acid and related compound. CN patent 106589039 A, April, 2017
18 Zhang FY, Chen QY, Liu P. Method for preparing obeticholic acid. WO patent 2016045480 A1, March, 2016
19 Zhang SJ, Wang S, Liu I, Tian WW. Method for manufacturing obeticholic acid and intermediate thereof. WO patent 2018010651 A1, January, 2018
20 Mahender RS, Sanjiv T, Nilav P, Sudhir S, Sudharshan R, Dhaval S. An improved process for preparation of obeticholic acid. WO patent 2018220513 A1, December, 2018
21 Pelliccari R, Pruzanski M, Gioiello A. The discovery of obeticholic acid (Ocaliva®): first-class in FXR agonist. In: Fischer J, Klein C, Chalmers WE, Successful Drug Discovery. 3rd ed. Weinheim: Wiley-VCH; 2018:197–244
22 Alinsa J, Barany G, Albericio F, Kates SA. Pyrrolidinone formation as a side reaction during activation of carboxylic acids by phosphonic salt coupling reagents. Lett Pept Sci 1999;6:243–245
23 CosteJ, Le-Nguyen D, Castro B. PyBOP®: a new peptide coupling reagent devoid of toxic by-product. Tetrahedron Lett 1990;31:205–208
24 Feng WD, Zhuo SM, Zhang FL. Process research and impurity control strategy for obeticholic acid, a farnesoid X receptor agonist. Org Process Res Dev 2019;23:1979–1989
25 ICH. ICH Harmonised Tripartite Guideline: Impurities in New Drug Substances. Q3A(R2) 2006. June 18, 2021. Accessed at: https://database.ich.org/sites/default/files/Q3A%28R2%29Guideline.pdf
26 Lv B, Li CW, Guo C. Method for preparing chenodeoxycholic acid derivative. CN patent 111718388 A, September, 2020