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

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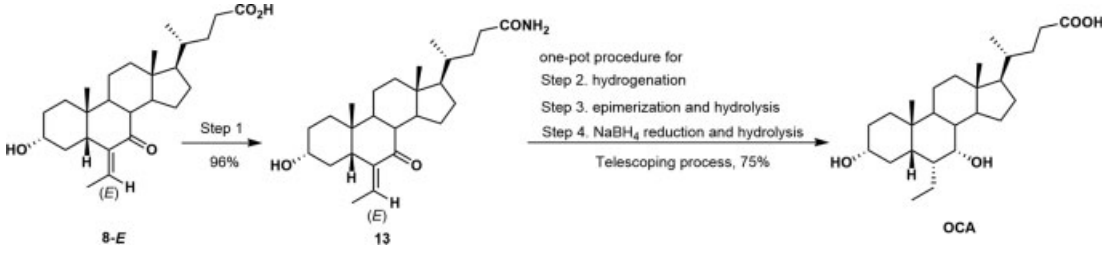

Abstract

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%.

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

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**).[5,6,8–10] 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

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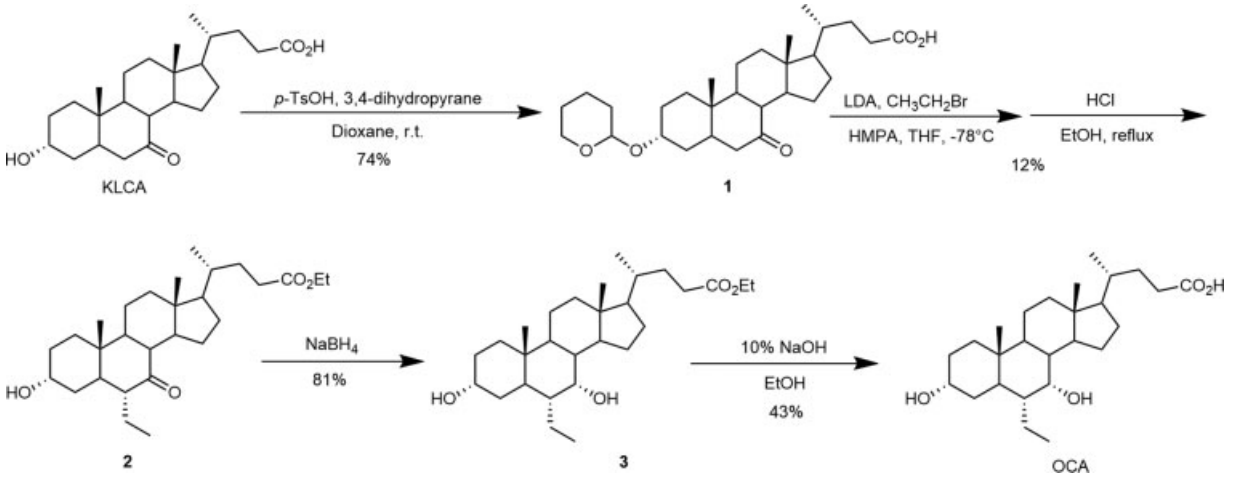
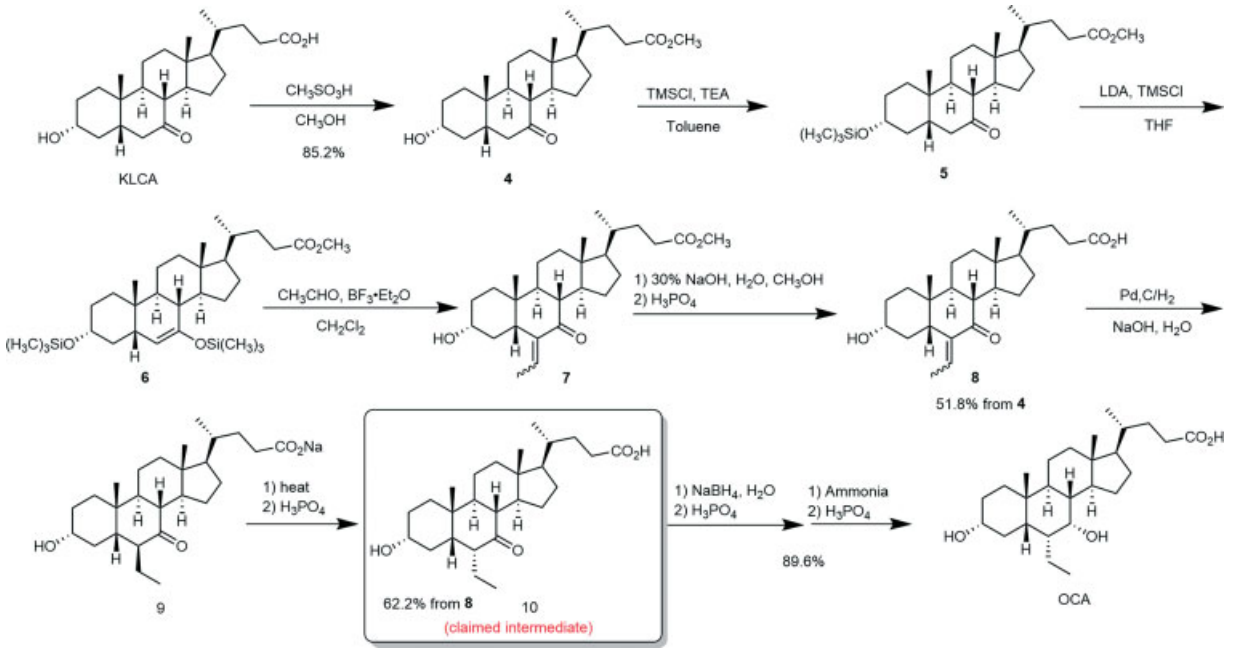


Scheme 1 Alkyl substitution approach to prepare OCA by Pellicciari et al (total yield: 3.1%). OCA, obeticholic acid.

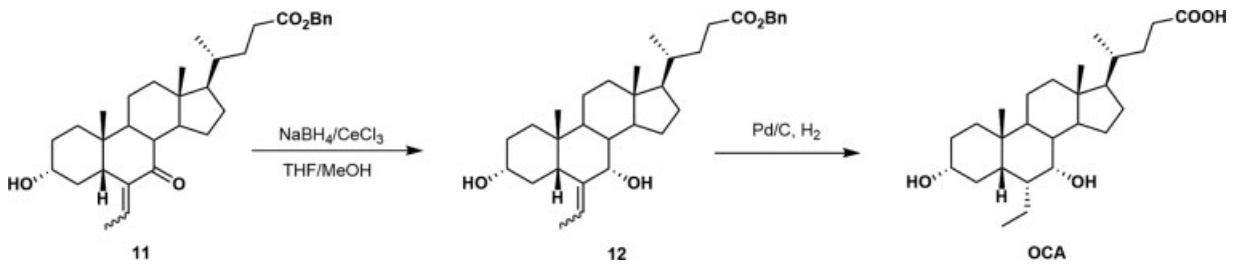


Scheme 2 The first kilogram-scale process illustrated in 8 steps by Ferrari and Pellicciari (total yield: 24.6%).

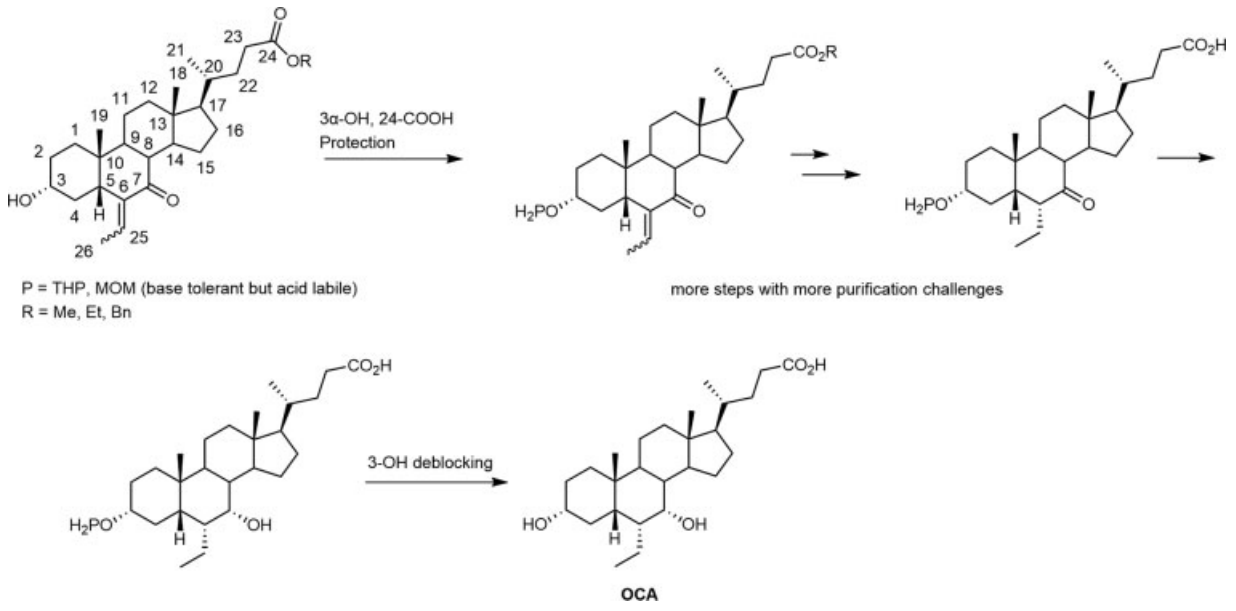
use of HMPA and the column chromatograph.[11] Later, novel synthesis steps of OCA were explored via using cholic acid and 3-keto-bisnorcholenol as starting regents.[12,13] However, it is difficult to scale up the production of OCA with either approach mentioned above. To our acknowledge, the kilogram-scale 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 (**Scheme 2**).[14] It should be noted that 3α-hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (**10**), an intermediate from epimerization claimed in a patent, would be converted to OCA with high chiral selectivity after the $NaBH_4$ reduction. In 2013, André et al made the process more safe with high quality and yield (30%) of OCA being achieved.[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 **11**, a selective reduction of 7-ketone group, priority to 6-ethylene, was obtained (**Scheme 3**),[16] protection strategies for functional groups, such as 3α-OH and 24-COOH (**Scheme 4**),[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.[21]

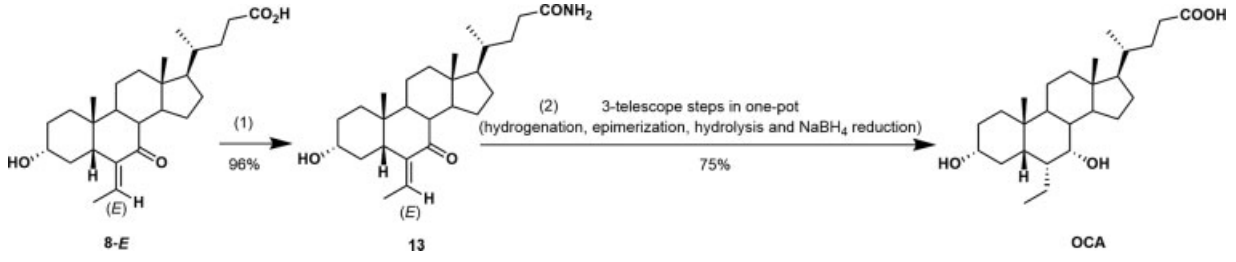
Herein, a novel four-step, telescoped, kilogram-scale process to obtain OCA was discovered. The *E*-conformation of **8** (**8-*E***), a commercially available agent, was used as the key staring material (**Scheme 5**). Instead of double blocking, only



Scheme 3 Alternative strategy by reducing 7-ketone priority to hydrogenation by Sepe et al.



Scheme 4 Double blocking strategy for 3α-OH and 24-COOH.



Scheme 5 Telescoped process of OCA in this study. (1) NH_4Cl, PyBOP, DMF, DIPEA, 0°C–r.t.. (2) H_2, 10% Pd/C, MeOH, 50°C, 4 atm; then NaOH, H_2O, reflux; followed with $NaBH_4$, reflux. OCA, obeticholic acid.

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 NH_4Cl, 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

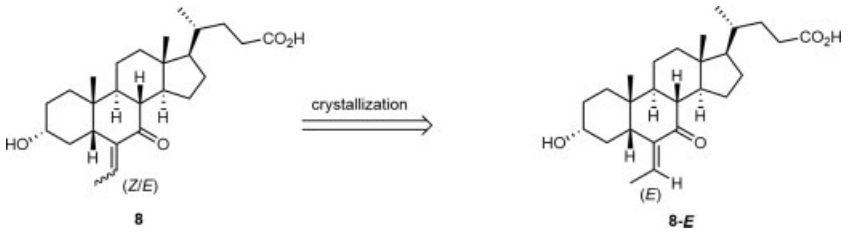


Fig. 1 8-***E*** could crystallize from configuration mixed **8**.

process, amine NH_4Cl was preferred, as it was identified to be cost-effective, and to have reliable stability and safety. Through using NH_4Cl, 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), CH_3ONH_2·HCl (79%, entry 4), and CH_3NHOCH_3·HCl (77%, entry 6), and these amounts of yields were much lower than that of NH_4Cl (96%, Entry 8). It is exciting to notice that the yield of the target product was good when using CH_3NH_2·HCl (90%, entry 1) and pyrrolidine (92%, entry 2). However, the use of pyrrolidine, originated from PyBOP, resulted in the generation of pyrrolidide byproduct **14**, the major product in this reaction (►**Fig. 2**).[22] Besides, by using CH_3NH_2·HCl and pyrrolidine, the resulting amides were too stable to hydrolyze 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 $HONH_2$·HCl led to yield loss of the target product (87%, entry 5) and 5% yield of 7-oxime byproduct, yet CH_3NHOH·HCl gave a messier coupling result (entry 7).

PyBOP was employed as a coupling reagent according to a reported study.[23] 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 over 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 ^{1}HNMR, ^{13}C 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)

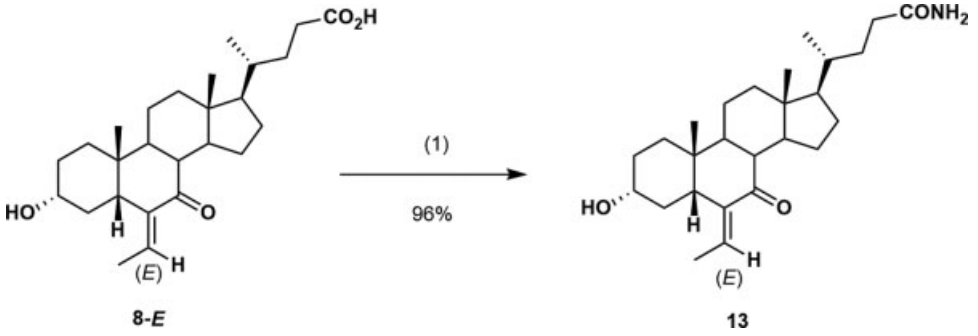
Telescoped synthesis of OCA from compound **13** is shown in ►**Fig. 3**. The procedure started with Pd/C catalytic hydrogenation (*step 2*) and epimerization (*step 3*), followed by $NaBH_4$-induced reduction (*step 4*). Hydrolysis of the amide unit proceeded in both *step 3* and *step 4*. In *step 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 tan, 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 $NaBH_4$ reduction (**Step 4**). Thus, the dosage of NaOH and $NaBH_4$ was screened in the following study. ►**Table 2** notes that NaOH (20 equiv.: the molar ratio to **13** is 20)/$NaBH_4$ (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 retainment of amide-blocked impurities (e.g., **18α** and **18β**, 2–8%, α-conformation is the major). Furthermore, a low amount of $NaBH_4$ (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 $NaBH_4$ (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.

Table 1 Coupling condition screened for intermediate 13 (solvent: DMF)



8-*E* 13

Entry	Amine	Coupling reagent	Base	Consumption, quality and work-up	Yield	Impact of the resulting amide on one-pot step
1	CH_3NH_2·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	CH_3ONH_2·HCl	PyBOP	DIPEA	Consumed over in 6 hours, foam, need extraction	79%	Trace product, messy
5	$HONH_2$·HCl	PyBOP	DIPEA	Consumed over in 6 hours, oxime byproduct (5%), solid formed	87%	Similar to entry 8
6	CH_3NHOCH_3·HCl	PyBOP	DIPEA	Consumed over in 6 hours, foam, need extraction	77%	Similar to entry 8
7	CH_3NHOH·HCl	PyBOP	DIPEA	Consumed over in 6 hours, a major impurity (11%)	ND	/
8	NH_4Cl	PyBOP	DIPEA	Consumed over in 6 hours, solid formed	96%	Least impurities, quality in control.
9	NH_4Cl	PyBOP	TEA	10% **8-*E*** stayed after 24 hours	ND	/
10	NH_4Cl	HBTU	DIPEA	Consumed over in 24 hours, 3 impurities (2–5%)	ND	/
11	NH_4Cl	EDCI/HOBt	DIPEA	6% **8-*E*** stayed after 36 hours	ND	/
12	NH_4Cl	DIC	DIPEA	25% **8-*E*** stayed after 24 hours	ND	/

André et al highlighted the participation of *n*-butyl acetate (*n*-BuOAc) in OCA recrystallization.[15] Feng et al suggested the potential use of heptane as a wonderful antisolvent in this process.[24] 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 for OCA recrystallization: at first warm crystallization was used to control the quality, 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**).[15,24] 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 insufficient reduction for 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

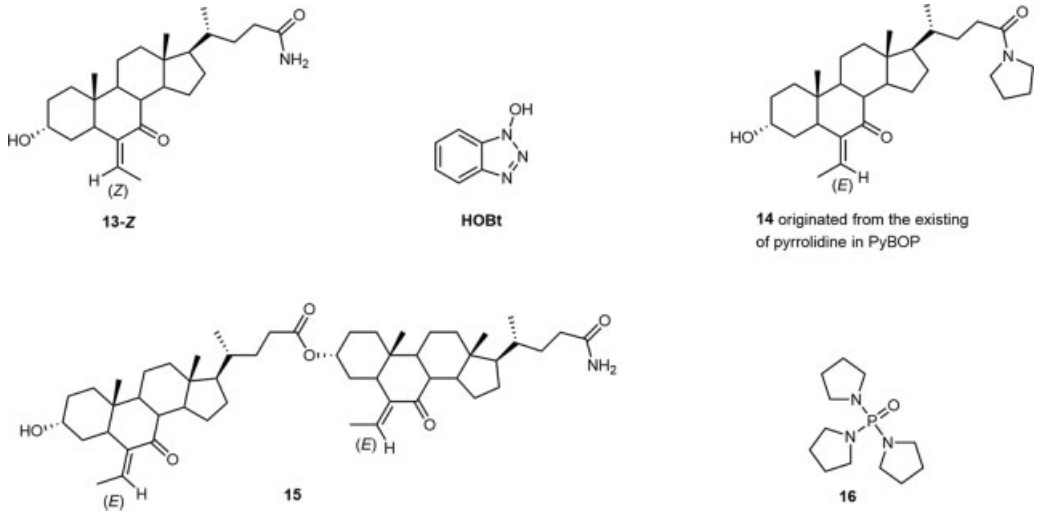
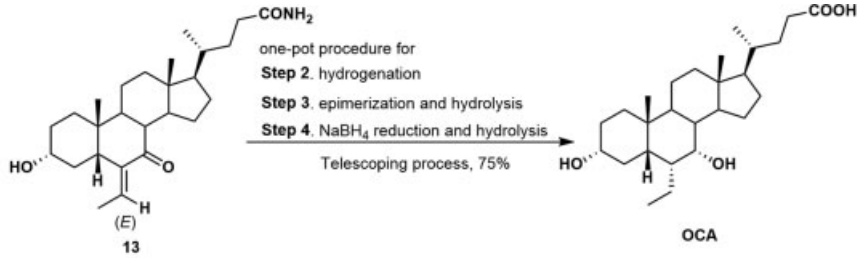


Fig. 2 Possible byproducts in amide-coupling reaction.



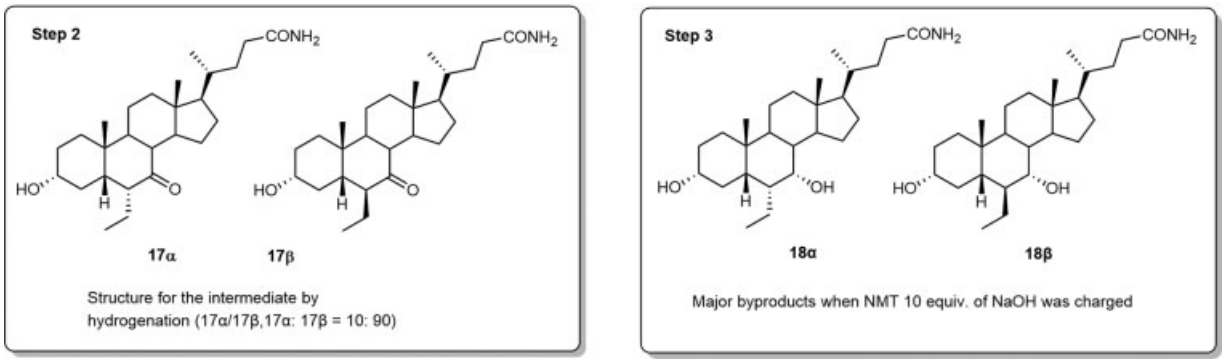


Fig. 3 Telescoped production of OCA. OCA, obeticholic acid.

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**),[25] 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).[15] 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.[26] 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 NH_4Cl 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 $NaBH_4$), 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

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

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])**.

Experimental Section

General

Common reagent-grade chemicals such as PyBOP and NH_4Cl were purchased and used without further purification. The key starting material **8-*E*** was supplied by Xiamen Halosyntech 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 δ units. Solvents for NMR spectra were DMSO-d_6 or CD_3OD 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-*E*** (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-*E*** 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 ~50% 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 3 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[a]	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%

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

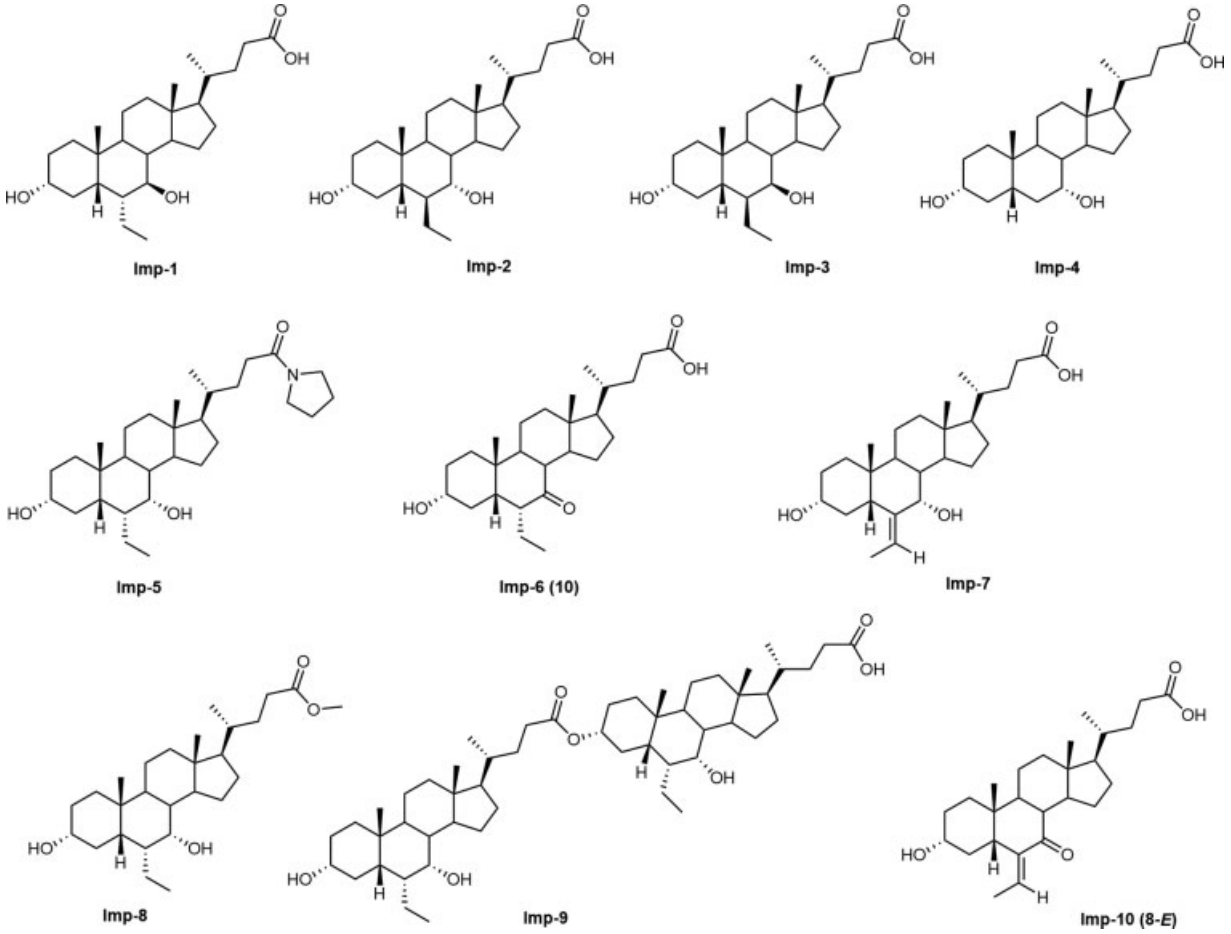


Fig. 4 Related substances after the crystallization.

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, 4H), 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 $C_{26}H_{41}NNaO_3$ [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 refluxed for 3 hours until **17α** was NMT 1.0%. The resulting solution was filtrated and washed with water (2 kg × 2), the filtrate was added sodium borohydride (0.76 kg) portion-wise, refluxed 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%	0.15%	NA	0.15%	NA
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 distillated. 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*-BuOAc 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%). ^{1}H NMR (600 MHz, CD_3OD) 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). ^{13}C NMR (100 MHz, DMSO-d_6) 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 calcd. for $C_{26}H_{44}NaO_4$ [M + Na] 443.3132, found 443.3137.

Conflict of Interest

We declared no conflict of interest.

Acknowledgment

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