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Article

Ketoreductase/Transaminase, One-Pot, Multikilogram Biocatalytic Cascade Reaction

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ABSTRACT: A biocatalytic cascade to produce tert-butyl ((2*R*,4*R*)-2-methyltetrahydro-2*H*-pyran-4-yl)carbamate **6** has been demonstrated at the multikilogram scale. In this reaction, a racemic ketone is resolved by reducing the undesired ketone using a ketone reductase (KRED). The reduction is stereospecific for the 2-position of substrate (2*S*)-ketone leaving the (2*R*)-ketone unreacted. After the (2*S*)-ketone has been depleted, a transaminase is added to catalyze the enantioselective transamination of the ketone, resulting in formation of the (2*R*, 4*R*)-amine **6**. The product is recovered from the aqueous reaction after Boc protection.

KEYWORDS: *cascade reaction, biocatalysis, transaminase, alcohol dehydrogenase, ketoreductase*

INTRODUCTION

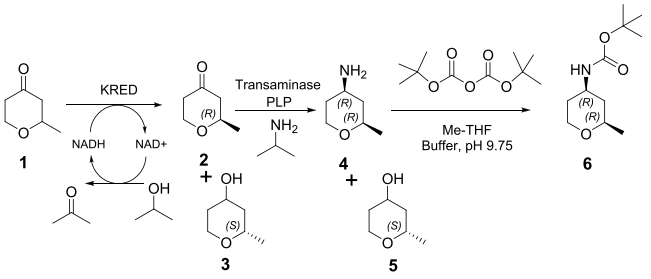
Biocatalysis has been gaining acceptance in the process chemistry community over the past couple of decades. The benefits of using enzymes as catalysts are well known. Foremost is their exquisite chemo-, regio-, and stereoselectivity. Enzymes are also highly regarded as green reagents since these catalysts may operate in aqueous solutions, are readily produced from renewable starting materials, and are biodegradable. Advances in the field of protein engineering have resulted in the availability of stable, process ready enzymes that are commercially available. Biocatalysis has been demonstrated in the commercial scale production of several blockbuster pharmaceuticals, such as Lipitor, Lyrica, Xalkori, and Januvia.[1−4]

One of the more powerful methods used in biocatalysis is the emerging field of multistep enzyme reactions, particularly those run in-situ and commonly referred to as performed in cascade mode. Benefits of cascade reactions include the ability to drive an unfavorable equilibrium and the use of unstable substrates or intermediates. Alleviating the need to isolate intermediates confers the advantage of cost savings associated with the use of solvents and the time required to clean the reaction vessel between steps. Challenges facing cascades include cross reactivity of the enzymes and the compatibility of the steps in terms of pH, temperature, and tolerance of cosolvents.[5]

Cascades may be characterized as being simultaneous or sequential. Simultaneous cascades have the advantage of simplicity and the possibility of driving the thermodynamic equilibrium. Sequential cascades may be preferred when the cascade includes incompatible steps. The steps in a sequential reaction may be compartmentalized using engineering controls such as membrane reactors or flow techniques. Some recent reviews offer an excellent description of the current state of the field of biocatalysis and cascades.[6,7]

This paper describes the synthesis of an intermediate in the synthesis of a compound under development to treat Parkinson's disease. Specifically, tert-butyl ((2*R*,4*R*)-2-methyltetrahydro-2*H*-pyran-4-yl)carbamate **6** was produced using ketoreductase (KRED) and transaminase enzymes (Scheme 1). The compounds in this scheme have previously been described.[8,9] This cascade was conducted in a sequential manner due to the cross reactivity of the enzymes with **1**.

Scheme 1. Production of Tert-Butyl ((2*R*,4*R*)-2-Methyltetrahydro-2*H*-Pyran-4-Yl)Carbamate 6 Using Ketoreductase (KRED) and Transaminase Enzymes



The initial approach to **6** was inspired by a literature report of the synthesis of (*S*)-2-methyltetrahydro-4*H*-pyran-4-one **7** (Scheme 2). This process was attractive because it utilized cheap starting materials and readily available lipase CalB (Novozyme 435). The authors report that the synthetic route in Scheme 2 was used to produce ∼100 kg of the (*S*)-2-pyranone **7**. This resolution could potentially afford the desired (*R*)-2-pyranone as well.

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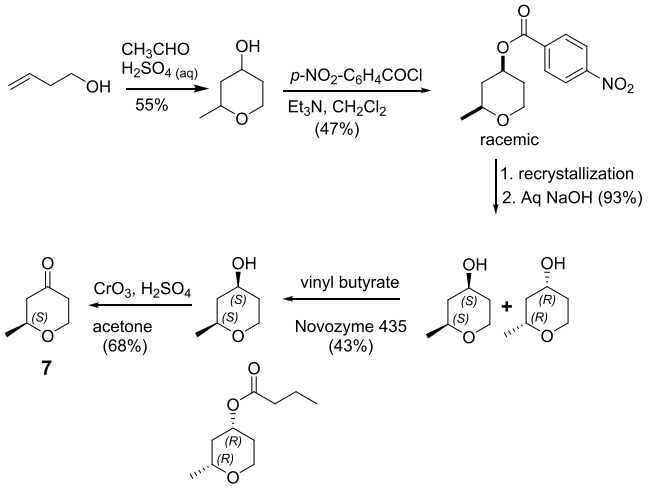
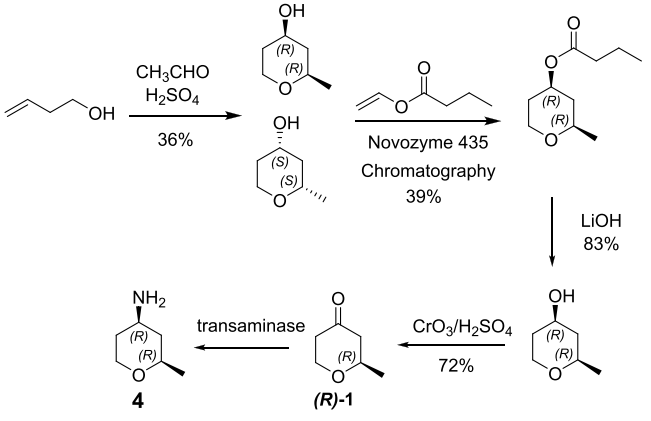
Scheme 2. Literature Report of the Synthesis of 7 Using Stereoselective Enzymatic Resolution



There were some pros and cons for the synthesis using the lipase resolution. The Prins reaction utilized inexpensive starting materials but was more selective toward the cis stereoisomer. The pyranol and butyrate were separated by an aqueous/organic partition followed by chromatography. The Jones oxidation was not favorable on environmental grounds due to the use of chromium. The route developed utilized flow chemistry for the acylation step. Although this route was effective, we often found it to be unreliable when adapted to flow so an alternative route was developed as described below. The newly developed route is based on the literature report and enhanced by the addition of a transaminase enzyme that was used to make (2*R*,4*R*)-2-methyltetrahydro-2*H*-pyran-4-amine **4** (Scheme 3).

Scheme 3. Initial Medicinal Chemistry Route

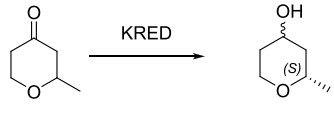


Initially, a dynamic kinetic resolution was envisioned using a transaminase to selectively convert racemic **1** to **4**. This approach was unsuccessful due to the difficulty of epimerizing the C-2 methyl group. Subsequent calculations using an internal Pfizer tool, based on COSMO-RS (Cosmotherm Implementation), predicted that the proton at the 2-position had a pKa of 27. With this result in hand, the route in Scheme 1 was developed. This route relies on an enzyme that can stereospecifically reduce 2*S*-**1**, leaving 2*R*-ketone **1** unreacted. When all the 2*S*-1 is depleted, a transaminase enzyme is added to the reaction vessel to perform a stereoselective transamination of **2** to produce **4**. Amine **4** is then Boc-protected to enable isolation from the aqueous medium, facilitating a two-enzyme, three-step one-pot biocatalytic cascade reaction to afford carbamate **6**.

RESULTS AND DISCUSSION

KRED Screen of Racemic Ketone 1. A screen was conducted to identify an enzyme that could selectively reduce **1**. A total of 338 enzymes from vendors and from an internal Pfizer collection were screened. Most of the enzymes exhibited high selectivity at the 4-position but were not selective at the 2-position, resulting in a mix of diastereomers. Four enzymes did show the desired selectivity at both positions. The most selective of these was ADH-097 from c-LEcta, and this enzyme was chosen for further development (Table 1).

Table 1. KRED Screen for the Conversion of 1 to 3

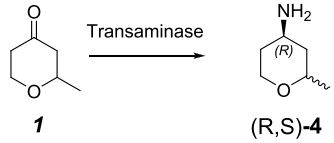


		2S-**1**	2R-**1**	2S,4S-alcohol	2R, 4R-alcohol	2S, 4R-alcohol	2R, 4S-alcohol
Vendor	Enzyme						
c-LEcta	ADH-097	0.0	53.9	0.0	1.0	42.7	2.4
Pfizer	KR124	2.6	49.8	9.8	12.4	8.9	19.1
Almac	AR-115	3.9	72.8	25.7	0.0	0.7	0.9
Almac	N151	4.3	73.2	24.9	0.0	0.8	1.1

Conditions: **1**, 17.5 mM; NADP+, 0.1 mg/mL; NAD+, 0.1 mg/mL; glucose dehydrogenase, 0.1 mg/mL; glucose, 21 mM, enzyme, 2 mg/mL; DMSO, 2%; 30 °C, 1000 rpm, 24 h. Values are expressed as area %.

Transaminase Screen of 1. A screen was conducted to identify enzymes that could enantioselectivity perform a transamination of 2R-**1** to yield **4**. When the screen was performed, chiral **2** was unavailable, so the screen was conducted on the racemate **1** (Table 2). The Pfizer enzymes are wild-type enzymes with the following accession numbers: AT045, XP_381942; AT041, XP_001209325.1; AT043, XP_001818566; and AT042, CAP97304.

Table 2. Transaminase Screen for the Conversion of 1 to Diastereomers 4



		2R, 4R-amine	2R, 4S-amine	2S-amine
Vendor	Enzyme			
Pfizer	AT045	1922546	1036492	1080
Pfizer	AT041	1887991	1620990	1828
Codexis	ATA-051	1111858	845686	5025
Codexis	ATA-P2-B01	1076278	1068425	0
Codexis	ATA-050	1043656	824299	4267
Pfizer	AT043	934092	1113668	1355
Pfizer	AT042	859794	694574	2114
Codexis	ATA-P2-A07	415542	628798	0
Codexis	ATA-P2-A01	379071	728735	0
Almac	Tam-901	340272	458855	0

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Org. Process Res. Dev. 2021, 25, 941−946

Conditions: **1**, 10 mM; enzyme, 2 mg/mL; isopropylamine hydrochloride, 20 mM; pyridoxal phosphate, 3 mM; DMSO, 2%; 100 mM potassium phosphate, pH 7.0, 0.5 mL; 40 °C; 1000 rpm; 18 h. Values are areas under the curve of the HPLC peak.

Process Development. This project was in the early stages of development, and very limited process development was conducted when the project was halted. The KRED enzyme, ADH-097, and transaminase enzyme, ATA-P2-B01, were chosen based upon the screening results and availability. This was a robust process, and when transferred to a third party vendor, proprietary enzymes owned by that vendor were used. Variables in the KRED reaction that were investigated were enzyme loading, the choice of a cofactor recycling system, and the concentration of **1**. The concentration of racemic-**1** was investigated in reactions with 2.5% enzyme loading (weight lyophilized enzyme powder: weight **1**), 5% isopropanol, 100 mM potassium phosphate buffer, pH 7.5, and 30 °C in 1 mL overnight reactions (Figure 1). The results were

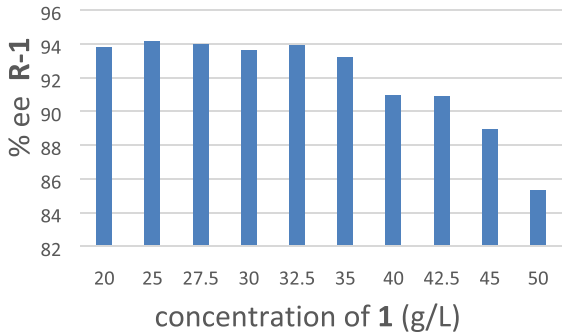

Figure 1. Effect of concentration of **1** in the KRED reaction with ADH-097 when sampled at 24 h.

judged by the %ee of R-**1**. At the highest substrate concentration tested, 50 g/L, the %ee was slightly lower than that at more dilute concentrations, but the result at this concentration was deemed adequate as a starting point for further optimization.

KRED with ADH-097 enzyme loading was investigated in reactions with cofactor recycling with glucose dehydrogenase, 100 mM potassium phosphate buffer, pH 7.5, and 30 °C in 1 mL overnight reactions (Figure 2). The best result was observed with a KRED loading of 2.5%, which was deemed adequate to use for further process development.

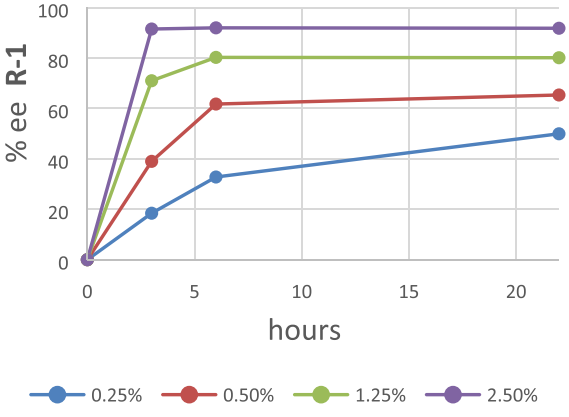

Figure 2. Effect of enzyme loading in the KRED reaction.

The KRED, ADH-097, can perform substrate-coupled recycling of NAD^+. The cosubstrate is isopropanol, which is oxidized to acetone. The concentration of isopropanol and a comparison to enzyme-coupled cofactor recycling with glucose dehydrogenase were investigated (Figure 3). The result with glucose dehydrogenase was superior. The difference in isopropanol concentration was minimal, with concentrations of 2−4% being preferred.

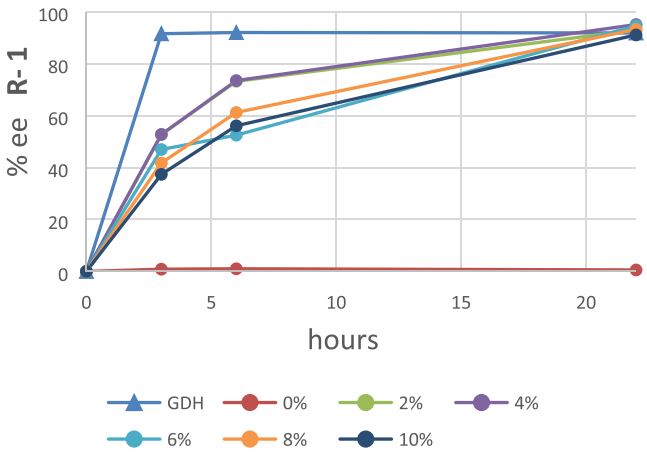

Figure 3. Comparison of cofactor recycling systems.

Standard conditions that have been used for previous transaminase reactions were employed with no process development. These transaminases from Codexis are known to be extremely stable to temperature and organic solvents. It was also known that the preferred amine donor is isopropylamine. In the transaminase reaction, the isopropanol is converted to acetone and an accumulation of acetone can be detrimental. A critical parameter for driving the reaction to completion is in-situ product removal, which may be accomplished by distilling the acetone under vacuum or by means of a nitrogen sweep.

With this minimal amount of development, the process was conducted as a one-pot sequential cascade at the gram scale and then transferred to a third party where it was repeated at the lab scale and then at the multikilogram scale. However, at the multikilogram scale, the third party vendor used a higher loading of their proprietary enzymes, and in order to lessen the chance of emulsions in the final work-up, the decision was made to remove the KRED enzyme following the first step and conduct the reaction as a telescoped process.

CONCLUSIONS

The original route to **4** was long, low yielding, and difficult to carry out on the kilogram scale, required multiple chromatographic separations, and used a chromium oxidation step and an inefficient enzymatic step that used vinyl butyrate as the substrate lowering the atom efficiency. A novel three-step two-enzyme one-pot biocatalytic cascade reaction was developed, which was stereoselective and atom efficient. Chromatography was eliminated, thus greatly decreasing the use of large volumes of organic solvents and silica gel waste. Reactions were run in aqueous buffer, and the heavy metal oxidation step was removed. Although recent advances in biocatalysis using KREDs and transaminases have enabled great many reactions, the disclosure of multikilogram-scale processes using a cascade

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Org. Process Res. Dev. 2021, 25, 941−946

or telescoped reaction that involves a transaminase is uncommon.

EXPERIMENTAL SECTION

Analytical Methods. Chiral GC for **1** and racemic **3**. Column, Supelco BetaDex 225; 30 m × 0.25 mm × 0.25 μm, #24348. Constant flow with helium at 1.2 mL/min; gradient, 60 °C for 0.25 min.

Ramp to 150 °C at 7 °C/min; hold for 2 min; FID detection.

UPLC Method for **1**and **2**. Before analysis, the quenched, clarified samples were derivatized with Marfey's Reagent (CAS 95713−52-3).[10] A 50 μL sample was mixed with 10 μL of aqueous 1 M sodium bicarbonate and 200 μL of Marfey's Reagent (5 g/L in acetonitrile) for 60 min at 40 °C. The reaction was quenched with 10 μL of 1 M HCl and diluted with 230 μL of acetonitrile. Column, Waters BEH C18, 2.1 × 100 mm, 1.7 μm, part # 186002350; column temperature, 45 °C; flow rate, 0.5 mL/min; mobile phase, 0.1% aqueous TFA/ACN; gradient, t = 0 min 95:5, t = 4 min 70:30; UV detection at 340 nm.

Screening of KRED Enzymes. Screening of the enzymes was carried out in a 96-well format. Plates contained 1 mg of enzyme in 20 uL of phosphate buffer. Each well was charged with 100 mM potassium phosphate, 2 mM $MgCl_2$ buffer, pH 7.0 (500 uL); pyranone **1** (1 mg) in DMSO (10 uL); glucose dehydrogenase Codexis CDX901 (0.05 mg); NADP+ (0.05 mg); NAD+ (0.05 mg); and glucose (1.9 mg). The plates were incubated with shaking at 30 °C overnight and quenched with 1.0 mL of ethyl acetate. The organic phase was analyzed using chiral GC.

Screening of Transaminase Enzymes. Screening of the enzymes was carried out in a 96-well format. Plates contained 1 mg of enzyme in 20 μL of phosphate buffer. Each well was charged with 100 mM potassium phosphate buffer, pH 7.5 (500 uL); pyranone **1** (0.57 mg) in DMSO (10 uL); pyridoxal phosphate (0.37 mg); and isopropylamine hydrochloride (0.96 mg). The plates were incubated with shaking at 40 °C overnight and quenched with 0.5 mL of acetonitrile. The mixtures were centrifuged to clarify and analyzed using uPLC after derivatization with Marfey's Reagent (CAS 95713−52-3).

Preparation of 2-Methyl-tetrahydro-pyran-4-ol. Purified water (121.5 kg) was charged into a 500 L glass-lined reactor, and the stirrer was started. Then, sulfuric acid (30.5 kg) was added slowly at <40 °C and stirred for 0.5 h. (3-Buten-1-ol) (31.0 kg, 1.0 equiv) was added and stirred for 0.5 h. Then, the mixture was heated to 50∼60 °C. Paraldehyde (19.8 kg, 0.36 equiv) was added into the above mixture in three portions. Then, the reaction mixture was maintained at 50∼60 °C for 3∼4 h. Meanwhile, the reaction was sampled for GC analysis every 1∼2 h. The reaction was considered complete when the area% of 3-Buten-1-ol was≤1%. The resulting mixture was cooled to 10∼20 °C to give 201.6 kg (assay by NMR with 19.7%) of yellow liquid with GC purity of 73% (GC a/a). This equates to an 82.4% yield from starting 3-buten-1-ol. The solution was directly used in the next step. ^{1}H NMR (400 MHz, DMSO): δ 3.74∼3.78 (m, 1H), 3.51−3.55 (m, 1H), 3.19−3.30 (m, 2H), 1.74−1.78 (m, 2H), 1.66−1.68 (m, 2H), 1.19−1.22 (m, 1H), 1.02−1.05 (m, 3H), 0.93−0.99 (m, 1H).

Preparation of 2-Methyldihydro-2*H*-pyran-4(3*H*)-one (1). 2-Methyltetrahydro-2*H*-pyran-4-ol (200.5 kg, 39.5 kg corrected by the NMR assay with 19.7%, 1.0 equiv) was charged into a 2000 L glass-lined reactor at 10∼20 °C, and the stirrer was started. A solution of sodium hydroxide (29.9 kg) in purified water (116.7 kg) was added to adjust pH to 6∼7. Then, ruthenium (iii) chloride trihydrate (4.5 kg, 0.05 equiv) was added in one portion, and the mixture was cooled to 0∼10 °C. Sodium hypochlorite solution (634.0 kg, 2.5 equiv) was added into the above mixture at 0∼10 °C. Then, the reaction mixture was maintained at 0∼10 °C for 2∼3 h. Meanwhile, the mixture was sampled for GC analysis every 0.5∼2 h. The reaction was considered complete when the area% of 2-methyltetrahydro-2*H*-pyran-4-ol was ≤1%. Dichloromethane (217.1 kg) was added to the reaction mixture. Then, the phases were separated, and the aqueous phase was re-extracted with dichloromethane (106.1 kg). Then, the combined organic phase was filtered with a nutsche filter, which was preloaded with Celite (20.3 kg). The filter cake was soaked with dichloromethane (45.9 kg). The rinsing liquor was combined with the filtrate. A solution of sodium sulfite (11.5 kg) in purified water (116.9 kg) was added to the combined filtrate at 15∼25 °C. Then, the phases were separated, and the aqueous phase was re-extracted with dichloromethane (46.9 kg) at 15∼25 °C. The combined organic phase was concentrated at ≤25 °C under reduced pressure until no more distillate was observed and DCM residual ≤10%. The resulting pale yellow liquid (31.1 kg, assay by NMR with 85.4%) was obtained with GC purity of 96% (GC a/a). This equates to a 68.3% yield from 2-methyl-tetrahydro-pyran-4-ol. The liquid was directly used in the next step. ^{1}H NMR (400 MHz, CDCl3): δ 4.25∼4.30 (m, 1 H), 3.70∼3.76 (m, 1H), 3.64∼3.67 (m, 1H), 2.54∼2.62 (m, 1H), 2.36∼2.42 (m, 1H), 2.25∼2.33 (m, 2H), 1.31 (d, J = 6.4 Hz, 3H).

Preparation of (*R*)-2-Methyltetrahydropyran-4-one (2). Purified water (365.3 kg) was charged into a 1000 L glass-lined reactor, and the stirrer was started. Disodium phosphate dodecahydrate (7.4 kg) and dihydrogen phosphate dihydrate (2.4 kg) were added into the mixture at 20∼25 °C and stirred until solid was dissolved completely. Then, isopropanol (42.0 kg), **1** (31.0 kg, 26.4 kg corrected by the NMR assay with 85.4%), and *β*-dpnh dipotassium salt (0.26 kg) were added into the mixture at 20∼25 °C. Then, the system pH was detected and ensured in the range from 6.5 to 7.5. KRED lysate (6.6 kg) was added into the mixture at 10∼25 °C. Then, the reaction mixture was heated to 23∼27 °C and stirred for 13∼14 h. Meanwhile, the mixture was sampled for GC analysis every 0.5∼1.5 h. The reaction was considered complete when the ee value of **2** was ≥98.0%.

Hydrochloric acid (3.6 kg) was added into the mixture at 10∼25 °C to adjust pH to 2∼3 and stirred for 0.5∼1 h. Sodium hydroxide solution (6.5 kg, 20% w/v), which was pre-prepared, was added dropwise into the mixture at 10∼25 °C to adjust pH to 6∼8. Then, Celite (6.6 kg) was added and stirred for 0.5∼1 h. The mixture was filtered with a stainless steel centrifuge under the protection of nitrogen. The filter cake was rinsed with purified water (26.5 kg). The filtrate was transferred into a 1000 L glass-lined reactor and stirred until it becomes homogeneous for temporary storage. The pale yellow liquid (482.5 kg, assay by Wt % with 2.5%) was obtained with an ee value of 98.0%. This equates to a 46.2% yield from 2-methyldihydro-2*H*-pyran-4(3*H*)-one. The solution was directly used in the next step.

Preparation of (2*R*,4*R*)-2-methyltetrahydro-2*H*-pyran-4-amine (4). Purified water (6.1 kg) and isopropyl amine (25.3 kg) were charged into a 200 L glass-lined reactor

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Org. Process Res. Dev. 2021, 25, 941−946

1, and the stirrer was started. Hydrochloric acid (41.1 kg) was added to adjust the pH of the mixture to 6∼8. The mixture was stirred until it becomes homogeneous. **2** (482.3 kg, 12.2 kg corrected by Wt % with 2.5%) was added into a 1000 L glass-lined reactor 2 at 10∼25 °C. The solution prepared in reactor 1 was added into the mixture at 10∼25 °C. Then, pyridoxal-5-phosphate monohydrate (0.5 kg) was added, and the mixture was stirred until it becomes homogeneous. The pH of the mixture was adjusted to 7.4∼7.6 with the sodium hydroxide solution (25.3 kg, 4.4% w/v) at 10∼25 °C. ATA lysate (192.9 kg) was added into the reaction mixture. Then, the mixture was reacted at 27∼33 °C under the protection of nitrogen for 40∼42 h. The mixture was sampled for pH and GC analysis every 4∼8 h; the reaction was considered complete when pH = 7.4∼7.6 and the content of **2** was ≤2%.

Hydrochloric acid (11.2 kg) was added into the mixture at 27∼33 °C to adjust pH to 2∼3. Celite (18.4 kg) was added into the mixture at 27∼33 °C. Under the protection of nitrogen, the mixture was filtered with a SS1000 hastelloy centrifuge in portions. The filter cake was washed with purified water (71.2 kg). At 10∼25 °C, the filtrate was transferred to reactor 2, and then, the pH was adjusted to 11.5∼12.5 with the sodium hydroxide solution (61.9 kg, 40% w/v). The mixture was concentrated at T ≤ 42 °C under reduced pressure. The mixture was sampled every 3∼5 h for pH change and derivatives of isopropylamine analysis until it was ≤0.2%. A yellow clear liquid (711.1 kg) was obtained with an ee value of 98.1%. The solution was directly used in the next step.

^{1}H NMR (400 MHz, DMSO-d6) ä ppm 1.00−1.24 (m, 5 H), 1.42 (qd, J = 12.19, 4.89 Hz, 2 H), 1.65−1.97 (m, 3 H), 2.18−2.39 (m, 4 H), 3.23 (br. s., 2H), 3.33−3.51 (m, 2 H), 3.74−4.04 (m, 2 H), 6.96−7.26 (m, 3 H), 7.47 (d, J = 8.22 Hz, 2 H), 7.61−8.03 (m, 4 H).

Preparation of tert-butyl (2*R*,4*R*)-2-methyltetrahydro-2*H*-pyran-4-ylcarbamate (6). The solution of **4** was filtered with a stainless steel nutsche filter, and the filter cake was rinsed with purified water (24.5 kg). The filtrate was transferred into the 1000 L reactor, and the stirrer was started. Methyl tert-butyl ether (161.4 kg) was added into the mixture at 10∼25 °C. The di-tert-butyl dicarbonate solution prepared with di-tert-butyl dicarbonate (17.3 kg, 0.9 equiv) and methyl tert-butyl ether (18.3 kg) was added into the mixture at 10∼25 °C. Then, the reaction mixture was heated to 25∼30 °C for 44 h. Meanwhile, the mixture was sampled every 1∼2 h for GC analysis. The reaction was considered complete when the di-tert-butyl dicarbonate content was ≥0.5% and the difference between two consecutive samples was ≤0.5%.

Then, the phases were separated. The aqueous phase was extracted with methyl tert-butyl ether (161.7 kg) twice at 10∼25 °C. The organic phase was combined and filtered with a stainless steel nutsche filter, and the filter cake was rinsed with methyl tert-butyl ether (6.8 kg). The filtrate and the rinsing liquor were combined, which was transferred into the 1000 L reactor and then settled for 0.5∼1 h before separation. The organic phase left in the reactor was concentrated at ≤45 °C under reduced pressure until 90∼135 L left. Purified water (437.4 kg) and sodium chloride (48.6 kg) were added into two 150 L drums. The mixture was stirred until the solid dissolved completely. While maintaining the temperature at 10∼25 °C, the mixture was washed three times with sodium chloride solution (182.1 kg). The organic phase left in the reactor was concentrated at ≤45 °C under reduced pressure until 45∼60 L left. Then, the organic phase was transferred into a 20 L rotary evaporator and continued to concentrate at≤45 °C under reduced pressure until 0.5∼1.0 vol left. *n*-Heptane (13.5 kg) was added into the mixture, and then, the mixture was sampled for methyl tert-butyl ether residual analysis until methyl tert-butyl ether residual ≤0.5%. The mixture was concentrated at ≤45 °C under reduced pressure until 9∼18 L left. *n*-Heptane (13.5 kg) was added into the mixture, and then, the mixture was sampled for methyl tert-butyl ether residual analysis until methyl tert-butyl ether residual ≤0.5%. The mixture was cooled to 10∼25 °C. Then, the mixture was filtered with a 10 L filter flask; the filter cake was rinsed with *n*-heptane (8.2 kg), which was pre-cooled to 0∼10 °C, and then, the filter cake was sampled for purity analysis by HPLC until it was ≥98%. While maintaining the temperature at 15∼30 °C, the filter cake was swept with nitrogen at a tray dryer for 5∼8 h. The mixture was sampled every 5∼10 h for solvent residual analysis until *n*-heptane residual ≤0.5%. A white solid (13.3 kg, assay by Wt % with 98.8%) was obtained with HPLC purity of 99.6%. This equates to a 58.6% yield, which was calculated without correcting by assay from (2*R*,4*R*)-2-methyltetrahydro-2*H*-pyran-4-amine. ^{1}H NMR (400 MHz, DMSO-*d6*) ä ppm 1.00 (d, *J* = 12.13 Hz, 1 H), 1.03−1.15 (m, 3 H), 1.17−1.34 (m, 2 H), 1.34−1.41 (m, 9 H), 1.55−1.79 (m, 2 H), 3.18−3.36 (m, 3 H), 3.38−3.48 (m, 1 H), 3.72−3.90 (m, 1 H), 6.79 (d, *J* = 7.83 Hz, 1 H).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.0c00557.

^{1}HNMR results for isolated compounds described in the Experimental Section (PDF)

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Notes

The authors declare no competing financial interest.

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