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Stereospecific microbial reduction of ethyl 1-benzyl-3-oxo-piperidine-4-carboxylate

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Abstract—Microbial reduction of ethyl 1-benzyl-3-oxo-piperidine-4-carboxylate by the majority of evaluated microorganisms gave the ethyl *cis*-(3*R*,4*R*)-1-benzyl-3*R*-hydroxy-piperidine-4*R*-carboxylate as the major product in high diastereo- and enantioselectivities. The 3*R*,4*R*-hydroxy ester was produced in 97.4% diastereomeric excess (de) and 99.8% enantiomeric excess (ee) by *Candida parapsilosis* SC16347, while 99.5% de and 98.2% ee were obtained from reduction by *Pichia methanolica* SC16415. A few of the evaluated microorganisms gave 10–40% of the ethyl *trans*-(3*R*,4*S*)-1-benzyl-3*R*-hydroxy-piperidine-4*S*-carboxylate as the minor product.

1. Introduction

Enantiomerically pure α-substituted-β-hydroxycarboxylates containing two chiral centers are useful building blocks in organic synthesis. Microbial reduction has attracted increasing attention in asymmetric synthesis in recent years.[1–6] It was reported that Baker's yeast reduced the title compound **1** diastereo- and enantioselectively to *cis*-(3*R*,4*R*)-**2a** with de 73% and ee >95% under non-fermenting conditions.[7] The de (73%) of the product was low and separation would be necessary to increase the de and ee to the level required for the synthesis of most pharmaceutical intermediates. The present work describes the microbial reduction of ethyl 1-benzyl-3-oxopiperidine-4-carboxylate **1** to make the ethyl *cis*-(3*R*,4*R*)-1-benzyl-3*R*-hydroxypiperidine-4*R*-carboxylate **2a** in high diastereomeric and enantiomeric excess (Scheme 1).

2. Results and discussion

The racemic *cis* **2a** and **2b** and *trans* **3a** and **3b** ethyl-1-benzyl-3-hydroxypiperidine-4-carboxylate were prepared by reduction of ethyl 1-benzyl-3-oxopiperidine-4-carboxylate **1** with sodium borohydride followed by separation of the two diastereomers by preparative HPLC. The assignment of *cis* and *trans* stereochemistries was based on proton NMR spectroscopy. An achiral HPLC method was developed for determining the extent of reduction and diastereomeric excess (de) of the hydroxy esters and chiral HPLC method was established for determining the enantiomeric excess of the hydroxy esters. In the chiral HPLC method, racemic *cis*-hydroxy ester **2** showed two peaks of equal area at retention times of 10.3 and 12.7 min, whose absolute configurations were established to be *cis*-(3*R*,4*R*)-**2a**, and *cis*-(3*S*,4*S*)-**2b**, respectively (see below). In the same chiral HPLC method, racemic *trans*-hydroxy ester **3** showed two peaks of equal area at 8.5 and 11.4 min, whose absolute configurations were established to be *trans*-(3*R*,4*S*)-**3a**, and *trans*-(3*S*,4*R*)-**3b**, respectively (see below).

Three types of Baker's yeast were evaluated for reduction of **1** under non-fermenting conditions as described in the literature.[7] Sigma type I gave a negligible amount of product. Both Sigma type II and Baker's yeast from Red Star gave one hydroxy ester isomer as the major product with a retention time of 10.3 min in our chiral HPLC method. Since the literature[7] reported the predominant formation of *cis*-(3*R*,4*R*) **2a** with Baker's yeast under the same conditions, the 10.3 min peak in our chiral HPLC was assigned the configuration *cis*-(3*R*,4*R*)-**2a**. The 12.7 min peak in the chiral HPLC was thus assigned to the other *cis*-enantiomer *cis*-(3*S*,4*S*)-**2b**.

Ninety one microorganisms in four multi-well plates were screened at 2 g/L substrate input for the reduction of **1**. Most cultures gave *cis*-(3*R*,4*R*)-**2a** as the major product and many of them showed high ee and high de. Results

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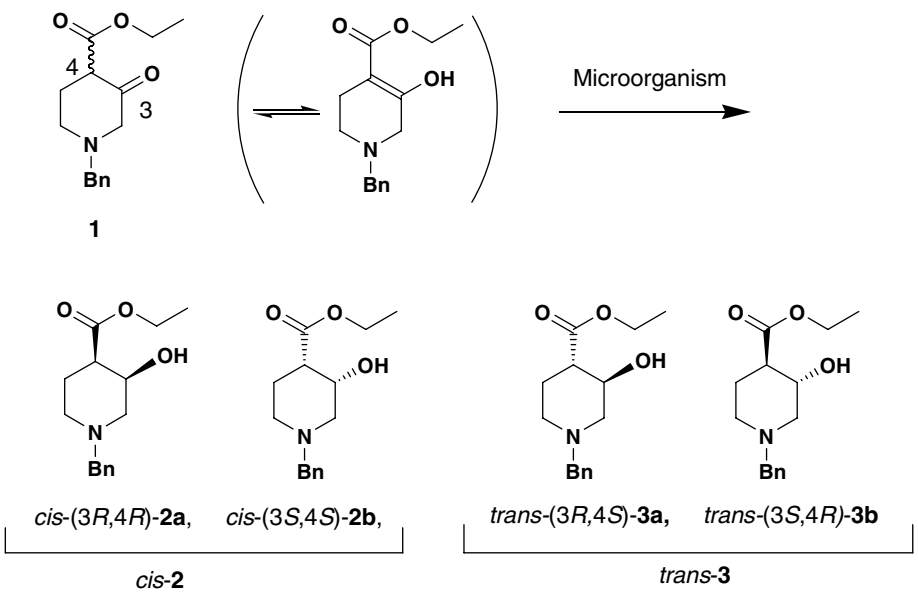


Scheme 1. Microbial reduction of compound **1**.

for the most promising microorganisms and Baker's yeast are shown in Table 1.

Table 1. Selectivity for *cis*-(3*R*,4*R*)-**2a** in the initial screening

Microorganism	SC number	Conversion (%)	ee (%)	de (%)
Pichia methanolica	16415	100	98.2	99.5
Hansenula polymorpha	13865	100	98.7	99.0
Hansenula polymorpha	13895	100	99.8	98.6
Candida parapsilosis	16347	100	99.8	97.4
Baker's yeast Sigma type II		68	79.9	97.0
Baker's yeast Red Star		42	93.9	35.3

The four cultures shown in Table 1 were also grown in flasks, and microbial reductions were carried out at a higher substrate input of 5 g/L. *Pichia methanolica* SC16415 and *Candida parapsilosis* SC16347 gave *cis*-(3*R*,4*R*)-**2a** in high yield with high de and high ee as seen in the initial screening performed at a lower substrate concentration. *Hansennula polymorpha* SC13865 gave *cis*-(3*R*,4*R*)-**2a** as the major product but with lower de (83%), and *H. polymorpha* SC13895 showed lower product yield (17%) to *cis*-(3*R*,4*R*)-**2a** at the higher substrate input (Table 2).

Microbial reductions by growing cultures of *C. parapsilosis* SC16347 with no pH adjustment using 10 g/L substrate input were complete (>99% substrate utilized) in 48 h to give *cis*-(3*R*,4*R*)-**2a** as the major product. The yield was determined to be 68% by HPLC, and the de and ee were 99% and 99.6%, respectively (Table 2). The pH of the growing culture was approximately 5 and was not adjusted. When the pH was adjusted to 7 just after addition of substrate, the substrate utilized was 94% after 48 h to give 44% product yield with 97% de (Table 2). Similar experiments were carried out at 20 g/L substrate input. When the bioreduction was carried out at pH 5.0, 97% substrate was utilized in 48 h and the *cis*-(3*R*,4*R*)-**2a** was obtained as the major product in 59% estimated yield, 98% de and 99.6% ee. With adjustment of the pH to about 7, 80% substrate was utilized in 48 h with 26% estimated yield and 94% de (Table 2). Thus, the native pH of approximately 5 is preferred for the reduction by *C. parapsilosis* SC16347, and both the yield and de were lower at the higher pH of 7.0.

In order to prepare *cis*-(3*R*,4*R*)-**2a** on gram scale, *C. parapsilosis* SC16347 was grown in 1 L of medium. The growing culture was used for reduction of 5 g of the hydrochloride of **1**. Conversion to *cis*-(3*R*,4*R*)-**2a** reached 84% after 56 h.

Table 2. Microbial reduction to *cis*-(3*R*,4*R*)-**2a** in 125-mL flasks

Microorganism	SC number	Substrate input (g/L)	pH	Substrate utilized (%)	Product formed (%)[a]	ee (%)	de (%)
Pichia methanolica	16415	5	NA	99	100	97.8	99
Hansenula polymorpha	13865	5	NA	85	78	ND	83
Hansenula polymorpha	13895	5	NA	18	17	ND	99
Candida parapsilosis	16347	10	~5	99	68	99.6	99
		20	~5	97	59	99.6	98
		10	7	94	44	ND	97
		20	7	80	26	ND	94

Growing culture (20 mL), 28 °C, 200 rpm, 48 h.
NA: pH not adjusted. ND: not determined.
[a] Yield estimated by HPLC.

Both the ee and de were greater than 99%. The crude product was purified by flash chromatography to give 2.2 g of *cis*-(3*R*,4*R*)-**2a** (50% isolated yield) with AP 93, ee 99.3%, de 99.5%, and $[\alpha]_D = +48.4$ (*c* 2.35, $CHCl_3$). The specific rotation of a sample of *cis*-(3*R*,4*R*)-**2a** with de 73% and ee > 95% was reported to be $[\alpha]_D = +27.35$ (*c* 1.81, $CHCl_3$) in the literature.[7] Our sample of *cis*-(3*R*,4*R*)-**2a** had higher de and ee, and thus had a higher specific rotation (+48.4), as expected. The specific rotation of the isolated product further confirmed our assignment of the *cis*-(3*R*,4*R*)-**2a**.

In the initial screening, a few cultures gave *trans*-(3*R*,4*S*)-**3a** as the major product with ee greater than 99% and moderate de. However, the diastereoselectivity changed when the biotransformation was conducted on a gram scale. The reductions gave *cis*-(3*R*,4*R*)-**2a** as the major product and *trans*-(3*R*,4*S*)-**3a** as the minor product with no detectable *trans*-(3*S*,4*R*)-**3b**. Results are shown in Table 3. By repeated flash chromatography of the crude product from several batches of gram scale reactions, 0.9 g of pure *trans*-(3*R*,4*S*)-**3a** was obtained with AP 98, de 96%, ee 99.5%, and $[\alpha]_D = +23.6$ (*c* 1.68, $CHCl_3$).

Table 3. Change in diastereoselectivity when preparing *trans*-(3*R*,4*S*)-**3a**

Microorganism	SC number	Relative % of *trans*-(3*R*,4*S*)-**3a**	
		Initial screening	Gram scale reaction
Hansenula anomala	13830	70	25
Pichia anomala	16139	73	42
Pichia anomala	16143	88	13

In order to establish the absolute configuration of the *trans*-(3*R*,4*S*)-**3a**, two epimerization experiments were carried out (Scheme 2) using *cis*-(3*R*,4*R*)-**2a** and *trans*-(3*R*,4*S*)-**3a** isolated from microbial reduction experiments.

The hydroxy esters were epimerized at C-4 and also partially hydrolyzed to hydroxy acids by treatment with base. The whole mixture was neutralized, and the hydroxy acids generated were re-esterified back to the hydroxy ester. As shown in Table 4, *cis*-(3*R*,4*R*)-**2a** was partially epimerized to 18.6% *trans*-**3a**, with 81.4% remaining *cis*-(3*R*,4*R*)-**2a**. Since the absolute configuration of *cis*-(3*R*,4*R*)-**2a** had been established before, the absolute configuration of *trans*-**3a** isolated from the microbial reduction was, therefore, established as *trans*-(3*R*,4*S*)-**3a**.

Table 4. Epimerization of *cis*-(3*R*,4*R*)-**2a**

Reaction time (h)	Relative % and retention time in chiral HPLC		
	3.8 min Acids mixture	8.5 min *trans*-(3*R*,4*S*)-**3a**	10.3 min *cis*-(3*R*,4*R*)-**2a**
0	Negligible	0.2	99.8
2	Negligible	0.8	99.2
4	Negligible	1.6	98.4
20	18	4.2	77.8
68	33.9	9.3	56.8
92	40.1	10.1	49.8
92[a]	Negligible	18.6	81.4

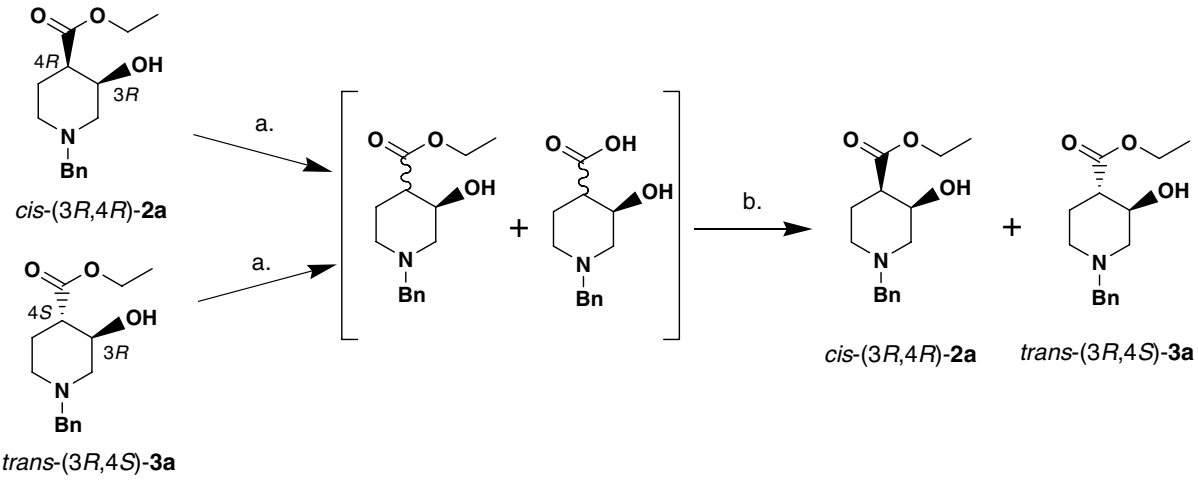
[a] After conversion of hydroxyacids back to ethyl hydroxyesters.

Similarly, *trans*-(3*R*,4*S*)-**3a** was epimerized partially (Table 5). The relative proportion of *cis*-(3*R*,4*R*)-**2a** was increased from 1.8 to 7.7. The *cis*-(3*S*,4*S*)-**2b** and *trans*-(3*S*,4*R*)-**3b** compounds were not evident in the HPLC. Although the

Table 5. Epimerization of *cis*-(3*R*,4*R*)-**2a**

Reaction time (h)	Relative % and retention time in chiral HPLC		
	3.8 min Acids mixture	8.5 min *trans*-(3*R*,4*S*)-**3a**	10.3 min *cis*-(3*R*,4*R*)-**2a**
0	Negligible	98.2	1.8
2	11.1	87.2	1.7
4	20.0	78.3	1.7
20	30.5	67.5	2.0
68	56.6	41.3	2.1
92	67.3	30.3	2.4
92[a]	Negligible	92.3	7.7

[a] After conversion of hydroxyacids back to ethyl hydroxyesters.



Scheme 2. Epimerization of the stereogenic center at C-4. Reagents and conditions: (a) K_2CO_3, EtOH, rt, 92 h; (b) EtOH, DCC, DMAP, rt, 3 h.

epimerization did not reach equilibrium, these experiments conclusively established the absolute configuration of *trans*-(3*R*,4*S*)-**3a**.

3. Experimental

3.1. Chemicals and general methods

Ethyl 1-benzyl-3-oxopiperidine-4-carboxylate hydrochloride was purchased from Aldrich. Other chemicals were purchased from VWR and/or Sigma–Aldrich.

NMR spectra were recorded on a Bruker-300 or 400 NMR spectrophotometer. LC–MS data were recorded on a Shimadzu LC–MS system. Rotation data were recorded on a Perkin–Elmer 241 Polarimeter.

3.2. HPLC methods

Analytical HPLC methods were performed with a gradient of solvent A (0.01 M NH_4OAc in 80:20 of water–methanol) and solvent B (0.01 M NH_4OAc in 5:20:75 of water–methanol–acetonitrile) at ambient temperature and UV detection at 220 nm. Achiral HPLC was performed on a Waters XTerra RP-18 column (3.5 μm, 150 × 4.6 mm) with a flow rate of 1 mL/min and a gradient from 20% to 100% solvent B over 5 min and 100% B for an additional 5 min. The retention times for the substrate **1**, *cis*-**2**, and *trans*-**3** were 7.8, 4.2, and 5.5 min, respectively. Chiral HPLC was performed on a Chiralpak AD-RH column (5 μm, 150 × 4.6 mm) with a flow rate of 0.5 mL/min and a gradient from 60% to 70% solvent B over 15 min. The retention times were 8.5 min for *trans*-(3*R*,4*S*)-**3a**, 10.3 min for *cis*-(3*R*,4*R*)-**2a**, 11.4 min for *trans*-(3*S*,4*R*)-**3b**, and 12.7 min for *cis*-(3*S*,4*S*)-**2b**.

3.3. Microorganisms and medium

Microorganisms were obtained from the Bristol-Myers Squibb culture collection. The SC number denotes the ID number in the culture collection. The multi-well plates were prepared with the cultures and stored at −70 °C before use. One liter of F7 medium contained 10 g malt extract, 10 g yeast extract, 1 g peptone, and 20 g dextrose, and was adjusted to pH 7 and autoclaved for 20 min.

3.4. Ethyl 1-benzyl-3-hydroxypiperidine-4-carboxylate racemic *cis*-2 and *trans*-3

A 250-mL flask equipped with a pH probe was charged with ethyl 1-benzyl-3-oxo-piperidine-4-carboxylate hydrochloride (5.0 g, 16.79 mmol), water (75 mL), and ethyl acetate (40 mL). The pH of the aqueous phase was adjusted to pH 9 with 2 M K_3PO_4 solution (10.5 mL). The contents of the flask were transferred to a separatory funnel to separate the phases. The organic phase was collected. The aqueous phase was extracted with additional ethyl acetate (40 mL). The combined organic phases were dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo to recover 4.46 g of a dark brown oil. The oil was dissolved in ethanol (50 mL). The solution was cooled to 0 °C. $NaBH_4$ (0.54 g, 14.27 mmol) was added portionwise over 30 min. After 30 min, the reaction was concentrated in vacuo to a solid residue. The residue was partitioned between ethyl acetate (80 mL) and water (40 mL). The organic phase was collected, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to recover 4.12 g of crude product as a yellow oil. The oil was subjected to flash chromatography using an ethyl acetate/hexane elution to give 2.2 g of a mixture of racemic *cis*-**2** and *trans*-**3** as a colorless oil. The *cis*-**2** and *trans*-**3** isomers were separated using preparative HPLC. The assignment of *cis*-**2** and *trans*-**3** was based on ^1H NMR shifts.

The racemic *cis*-**2** was isolated as a white solid. ^1H NMR ($CDCl_3$) δ 7.29 (m, 5H), 4.2 (m, 1H), 4.18 (q, 2H), 3.53 (s, 2H), 3.10 (broad, OH), 2.98 (m, 1H), 2.88 (m, 1H), 2.38 (m, 1H), 2.22 (d, 1H), 2.02 (m, 2H), 1.77 (m, 1H), 1.26 (t, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 173.3, 137.8, 129.0, 128.4, 127.3, 66.5, 62.5, 60.6, 59.0, 52.1, 45.6, 22.4, 14.2 ppm; HRMS (ES): exact mass calculated for $C_{15}H_{22}HO_3$ $[M+H]^+$, 264.1600. Found: 264.1607. Elem. Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.41; H, 8.03; N, 5.31. Found: C, 68.37; H, 7.90; N, 5.24.

The racemic *trans*-**3** was isolated as a light yellow oil. ^1H NMR ($CDCl_3$) δ 7.29 (m, 5H), 4.17 (q, 2H), 3.96 (m, 1H), 3.52 (d, 2H), 3.00 (dd, 1H), 2.78 (dd, 1H), 2.28 (m, 1H), 2.0 (m, 3H), 1.75 (m, 1H), 1.26 (t, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 174.3, 137.9, 129.0, 128.3, 127.2, 68.1, 62.7, 60.8, 58.7, 52.3, 48.8, 26.4, 14.2 ppm; HRMS (ES): exact mass calculated for $C_{15}H_{22}NO_3$ $[M+H]^+$, 264.1600. Found: 264.1587.

3.5. Microbial reduction by Baker's yeasts

Three types of Baker's yeasts from *Saccharomyces cerevisiae* were used (Sigma YSC-1, type I; Sigma YSC-2, type II; Red Star, active dry yeast). To 1 g of the Baker's yeast in a 50-mL flask was added 5 mL of tap water. After 40 min on a shaker at 200 rpm and 28 °C, a solution of the hydrochloride of **1** (10 mg) in DMSO (100 μL) was added. The flasks were kept on the same shaker for 20 h. The reaction mixture (0.5 mL) was withdrawn, mixed with 1 mL MeOH, filtered through a 0.2 μm filter, and subjected to HPLC analysis.

3.6. Screening of microorganisms for reduction of 1

Each multi-well plate (Whatman) consists of a total of 24 deep wells (4 × 6). Four multi-well plates containing a total of 91 yeast cultures were used. The plate was taken out of the −70 °C freezer and thawed to room temperature. One milliliter of sterile F7 medium was added to each well. The microorganisms were grown by shaking in a Thermomixer R at 600 rpm at 28 °C for 24 h. A solution of the hydrochloride of **1** (2 mg) in DMSO (20 μL) was added to each well. Biotransformation was carried out by shaking on the same shaker. After 24 h, methanol (1 mL) was added to each well. The mixtures were filtered through a 0.2 μm filter, and the filtrates were analyzed by achiral HPLC to determine the conversion and de by relative area counts and by chiral HPLC to determine the ee.

3.7. Microbial reductions at 5, 10, and 20 g/L substrate input in flasks

The four cultures in Table 1 were grown in flasks for microbial reduction at 5, 10, and 20 g/L substrate input. One thawed frozen vial of microbial culture was inoculated in 100 mL F7 medium (500-mL flask). The flask was placed on a shaker at 28 °C and 200 rpm. After 64 h, the growing culture was transferred (15% transfer) into fresh sterile F7 medium and shaken under the same conditions for 24 h. The growing culture (20 mL) was transferred to a sterile flask (125 mL). A solution or suspension of the substrate (hydrochloride of **1**) in water was added to make the substrate input 5, 10, or 20 g/L. The pH was approximately 5 and was not adjusted. The flasks were placed on a shaker at 28 °C and 200 rpm for 48 h. For analytical samples, 0.5 mL of the reaction mixture was taken out and mixed with 3.5 mL of acetonitrile, filtered through a 0.2 μm filter and subjected to HPLC. The relative conversion was based upon the HPLC area ratio of the product and the remaining substrate. A standard sample of *cis*-(3*R*,4*R*)-**2a** was also analyzed by the same HPLC method. The estimated yield was the HPLC area of the product versus the standard.

Microbial reductions by growing cultures of *C. parapsilosis* SC16347 were carried out under similar conditions using 10 and 20 g/L substrate input. For each substrate input, an additional experiment was also carried out by adjusting the pH to 7 just after substrate addition.

3.8. Microbial reduction on gram scale to prepare *cis*-(3*R*,4*R*)-2a

One thawed frozen vial of *C. parapsilosis* SC16347 was inoculated in 100 mL F7 medium (500-mL flask). The flask was placed on a shaker at 28 °C and 200 rpm. After 64 h, the growing culture was transferred (15% transfer) into fresh sterile F7 medium and shaken under the same conditions for 24 h. The growing culture was again transferred (15% transfer) into 1 L F7 medium (4-L flask) and shaken under the same condition for 24 h. A solution of the hydrochloride of **1** (5 g) in DMSO (50 mL) was added. The flask was shaken under the same conditions for 56 h. The pH was adjusted to 7.5 with 1 M NaOH. The mixture was extracted with EtOAc (3×0.8 L). The aqueous phase was further adjusted to pH 9 and extracted with 1 L of EtOAc. The combined organic extract was concentrated to dryness to give 6.8 g of crude oily product. It was subjected to flash chromatography (160 g silica gel) and eluted with dichloromethane–acetone–NH_4OH (94:5:1) to give 4.3 g of product. This was again subjected to flash chromatography (140 g silica gel) and eluted with dichloromethane–MeOH (97:3). The product fractions were combined, stirred with charcoal overnight at room temperature, filtered through a pad of Celite, and concentrated to dryness to give 2.2 g of *cis*-(3*R*,4*R*)-**2a** (50% isolated yield) with AP 93, ee 99.3%, de 99.5%, and $[\alpha]_D = +48.4$ (*c* 2.35, $CHCl_3$). LC–MS 264 (M+1). ^1H, ^1H–^1H COSY and ^{13}C NMR spectra were recorded in $CDCl_3$. ^1H δ 7.2–7.4 (m, 5H, Ph), 4.19 (m, 1H, 3-CH), 4.15 (q, $J = 7.2$ Hz, 2H, CH_2 in Et), 3.52 (s, 2H, Ph–CH_2), 2.96 (m, 1H, 2-CH_2-A), 2.86 (m, 1H, 6-CH_2-A), 2.35 (m, 1H, 4-CH), 2.20 (m, 1H, 2-CH_2-B), 2.03 (m, 2H, 5-CH_2-A and 6-CH_2-B), 1.73 (m, 1H, 5-CH_2-B), 1.24 (t, $J = 7.2$ Hz, 3H, CH_3 in Et) ppm. ^{13}C δ 172.72, 137.02, 128.68 (2C), 127.92 (2C), 126.89, 65.99, 61.98, 60.08, 58.40, 51.56, 45.10, 21.85, 13.81 ppm.

3.9. Microbial reductions on gram scale to prepare *trans*-(3*R*,4*S*)-3a

One thawed frozen vial of microbial culture of *Pichia anomala* SC16143 was inoculated in 100 mL F7 medium (500-L flask). The flask was placed on a shaker at 28 °C and 200 rpm. After 64 h, the growing culture was transferred (15% transfer) into fresh sterile F7 medium and shaken under the same conditions for 24 h. The growing culture was again transferred (15% transfer) into 1 L F7 medium (4-L flask) and shaken for 24 h at 28 °C and 200 rpm. To the growing culture, a solution of the hydrochloride of **1** (5 g) in DMSO(50 mL) was added. After 24 h, work-up (as described in the previous section) gave 5.4 g of crude product, which contained *cis*-(3*R*,4*R*)-**2a** as the major product with only 13% of *trans*-(3*R*,4*S*)-**3a** and no detectable *trans*-(3*S*,4*R*)-**3b**. A similar result of only 28% *trans*-(3*R*,4*S*)-**3a** and predominantly the *cis*-(3*R*,4*R*)-**2a** was obtained in another experiment at 2 g/L substrate input.

Microbial reductions were carried out at 2 g/L substrate input under similar conditions with the following two microorganisms. *Pichia anomala* SC16139 gave 2.8 g of crude product containing *cis*-(3*R*,4*R*)-**2a** as the major product with 42% of *trans*-(3*R*,4*S*)-**3a** and no detectable *trans*-(3*S*,4*R*)-**3b**. *Hansenula anomala* SC13830 provided 2.7 g of crude product with *cis*-(3*R*,4*R*)-**2a** as the major product and 25% of *trans*-(3*R*,4*S*)-**3a** with no detectable *trans*-(3*S*,4*R*)-**3b**.

Four batches of crude products from the above experiments were combined and subjected to repeated flash chromatography and eluted with dichloromethane–MeOH–NH_4OH (96.5:3:0.5) and heptane–EtOAc (1:1) to give 0.9 g of *trans*-(3*R*,4*S*)-**3a** with AP 98, de 96%, ee 99.5%, and $[\alpha]_D = +23.6$ (*c* 1.68, $CHCl_3$). LC–MS 264 (M+1). ^1H, ^1H–^1H COSY and ^{13}C NMR spectra were recorded in $CDCl_3$. ^1H δ 7.2–7.4 (m, 5H, Ph), 4.16 (q, 2H, $J = 7.2$ Hz, CH_2 in Et), 3.96 (m, 1H, 3-CH), 3.56 (d, $J = 13.2$, 1H, Ph–CH_2-A), 3.52 (d, $J = 13.2$, 1H, Ph–CH_2-B), 3.01 (m, 1H, 2-CH_2-A), 2.80 (m, 1H, 6-CH_2-A), 2.27 (m, 1H, 4-CH), 1.9–2.1 (m, 3H, 2-CH_2-B, 5-CH_2-A and 6-CH_2-B), 1.74 (m, 1H, 5-CH_2-B), 1.25 (t, 3H, $J = 7.2$ Hz, CH_3 in Et) ppm. ^{13}C δ 174.18, 137.30, 129.12 (2C), 128.18 (2C), 127.16, 67.73, 62.39, 60.66, 58.38, 51.96, 48.62, 26.15, 14.07 ppm.

3.10. Epimerization of *cis*-(3*R*,4*R*)-2a

The *cis*-(3*R*,4*R*)-**2a** (10 mg) was dissolved in 2 mL of ethanol. K_2CO_3 (20 mg) was added. The mixture was stirred at room temperature. Analytical samples were taken periodically and subjected to chiral HPLC.

After 92 h, the reaction mixture was filtered. The filtrate was neutralized with 1 M HCl. The whole mixture was

dried under reduced pressure. The residue was dissolved in 5 mL of anhydrous dichloromethane, and 16 mg of DCC, 1 mg of DMAP, and 0.5 mL of EtOH were added. The mixture was stirred at room temperature for 3 h. The mixture was dried.

An analytical sample was taken and subjected to chiral HPLC. The results are listed in Table 3. The *trans*-(3*S*,4*R*)-**3b** (the 11.4 min peak in chiral HPLC) and *cis*-(3*S*,4*S*)-**2b** (12.7 min peak) were negligible in all cases.

3.11. Epimerization of *trans*-(3*R*,4*S*)-3a

The *trans*-(3*R*,4*S*)-**3a** (5 mg) was dissolved in 1 mL of ethanol. K_2CO_3 (10 mg) was added. The mixture was stirred at room temperature. Analytical samples were taken out periodically and subjected to chiral HPLC.

After 92 h, the reaction mixture was filtered. The filtrate was neutralized with 1 M HCl. The whole mixture was dried under reduced pressure. The residue was dissolved in 3 mL of anhydrous dichloromethane, and 8 mg of DCC, 0.5 mg of DMAP, and 0.3 mL of EtOH were added. The mixture was stirred at room temperature for 3 h. The mixture was dried. An analytical sample was taken out and subjected to chiral HPLC. The results are listed in Table 4. The *trans*-(3*S*,4*R*)-**3b** (11.4 min peak) and *cis*-(3*S*,4*S*)-**2b** (12.7 min peak) were negligible in all cases.

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