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A Chemoenzymatic Route to Chiral Intermediates Used in the Multikilogram Synthesis of a Gamma Secretase Inhibitor

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ABSTRACT: A chemoenzymatic route for the production of an intermediate to a gamma secretase inhibitor is described. The route is robust and was run at multikilogram scale. The process employs both a transaminase catalyzed reductive amination of a substituted tetralone and an alcohol dehydrogenase catalyzed reduction of an α-ketoester to generate the two chiral centers in the molecule, with nearly perfect stereoselectivity. The process also features simple isolation schemes, including a direct drop isolation of the aminotetralin phosphate salt.

INTRODUCTION

Over the past two decades, biocatalysis has become an important technology for the pharmaceutical, fine chemical, and agro industries. Many examples of robust and scalable enzymatic processes have enabled process chemists to gain a level of familiarity with, and trust in biocatalytic reactions that was previously absent. Biocatalysis has been demonstrated in the commercial scale production of several blockbuster pharmaceuticals such as Lipitor, Lyrica, Xalkori, and Januvia.[1−4]

Biocatalysis is a useful tool for chemical synthesis due to the many attractive properties of enzymes, including the ability to operate under mild reaction conditions, and chemo- and regio- and stereoselectivity. Enzymes are also highly regarded as green reagents and are readily produced from renewable starting materials and are biodegradable.

Many shortcomings of biocatalysts have recently been alleviated. These include the availability of enzymes in commercial quantities, the increased number of enzyme classes available with a broad substrate specificity, and the ability to engineer enzymes that are highly stable under desirable process conditions.

Advances in related fields have dramatically improved the utility of enzymes in process chemistry. Efficient and low-cost rapid DNA sequencing and DNA synthesis, as well as novel bioinformatics and protein engineering techniques, have enabled access to a wide selection of robust enzymes that can readily be used in process chemistry.

Historically, because of their availability, hydrolase enzymes produced for the textile, paper, and food industries were the most commonly used biocatalysts. Over the past two decades new classes of enzymes such as nitrilases, aldolases, alcohol dehydrogenases, and transaminases have become reliable tools. Thanks to the continuing advances in the field, we can look forward to industrially useful enzymes such as imine reductases, amine dehydrogenases, and oxidases. Some recent reviews offer an excellent description of the current state of the field of biocatalysis.[5−7]

This paper is focused on the synthesis of compound **6**, an intermediate for the gamma secretase inhibitor **7**. From a retrosynthetic analysis (Scheme 1) the molecule can be made using various disconnections. Two approaches considered were the reductive amination of tetralone **1** with norvaline or the reductive amination of ketoester **3** with tetralin **2**. Both approaches were attempted but were low yielding, especially using hydrogenation methods. A limited panel of commercial imine reductase enzymes were screened for the ability to catalyze the reductive amination, but no reaction was observed.[8] Alternately, the coupling of an activated alcohol, such as **5**, with aminotetralin **2** was investigated.

The option of using biocatalysis to produce **2** and hydroxyester **4** (Scheme 2) was considered attractive due to the possibility of achieving high stereoselectivity. The enzymatic amination of tetralones has previously been reported using a variety of transaminases. Both (*S*)-selective and (*R*)-selective activity has been demonstrated using transaminases from *Vibrio fluvialis, Chromobacterium violaceum*, and *Paracoccus fluorescens*, as well as a variant from *Arthrobacter citreus*.[9] Additionally, the asymmetric enzymatic reduction of α-ketoesters is well-known.[10]

We favored using biocatalysis because it delivered **2** as a stable intermediate while the tetralone was prone to oxidation. The reduction of **3** worked well with alcohol dehydrogenases and asymmetric chemical reductions (hydrogenation) to afford **4**. With **4** in hand we were able to demonstrate alkylation with the triflate which was successful.

Here we describe the use of a transaminase to convert **1** to **2** and an alcohol dehydrogenase to convert **3** to **4**. After activating **4** as a triflate, these chiral intermediates are coupled to form **6** which is the key building block in a route for the synthesis of **7**, a selective gamma secretase inhibitor with potential antitumor activity (Scheme 2).

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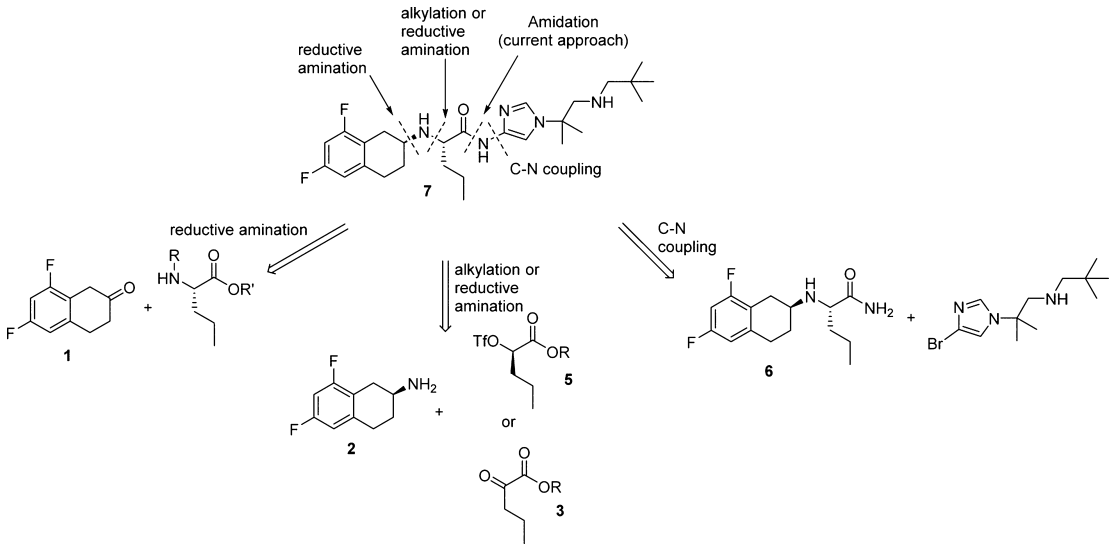
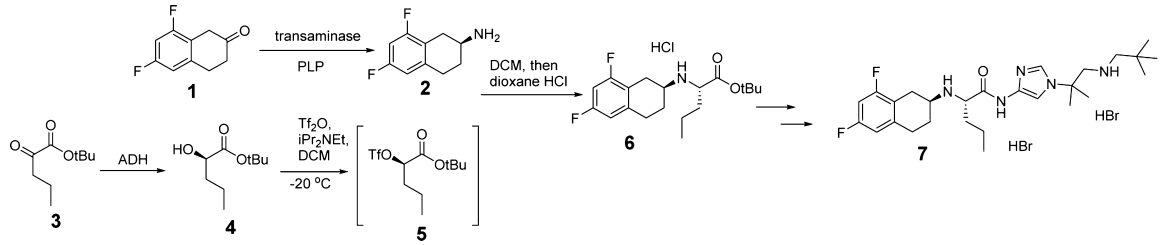
Scheme 1. Retrosynthetic Analysis

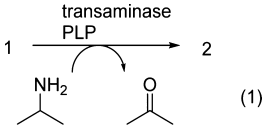


Scheme 2. Route to 6 Using Transaminase and Alcohol Dehydrogenase Enzymes



RESULTS AND DISCUSSION

Based on our experience with using transaminases to produce chiral amines with excellent stereoselectivity, we decided to screen our enzyme collection for the conversion of **1** to **2**.



The ATA-47 enzyme from c-LEcta showed the most promise in the initial screen (Table 1). A rescreen with five additional transaminases from c-LEcta that were not included in the initial screen, but were known to have selectivity similar to ATA-47, were tested with a higher substrate concentration. In this screen, the substrate was dissolved in methanol to facilitate addition to the reactions. Alternatively, a more enzyme friendly cosolvent such as DMSO could have been used. The results are shown in Table 2.

ATA-47 showed the best enantioselectivity, whereas ATA-69 showed the highest activity. Further tests with these two enzymes were carried out to confirm their activities and selectivities at different enzyme−substrate ratios and with varying amounts of *t*-amyl alcohol added as described in Table 3.

The reactivity in the presence of cosolvents was evaluated due to the poor solubility of **1** in aqueous reaction buffer. Addition of toluene, cyclohexane, isopropyl acetate, *t*-amyl alcohol, dimethylformamide (DMF), or methanol (at 3%) was tested. Another solvent, DMSO, which is commonly used in

Table 1. Results from a Screen of 224 Transaminase Enzymes for the Conversion of 1 to 2[a]

vendor	enzyme name	% ee (*S*)-**2**	% conversion
c-LEcta	ATA-47	>99%	32.9
Johnson Matthey	JM-TA-120	>99%	21.1
evocatal	1.2.104	>99%	18.4
Asymchem	116890	>99%	14.2
Codexis	ATA-256	>99%	14.0
Almac	TAm-105	>99%	11.9
Asymchem	116889	>99%	6.9
Codexis	ATA-260	>99%	6.0
Pfizer	*A. denitrificans* (AAP92672)	>99%	4.7
vendor	enzyme name	% ee (*R*)-**2**	% conversion
Pfizer	*A. terreus* (XP_001209325.1)	>99%	29.4
c-LEcta	ATA-72	>99%	16.4
c-LEcta	ATA-71	>99%	15.4
Codexis	ATA-P2-A01	>99%	12.7
Almac	TAm-901	>99%	9.0
Codexis	ATA-117	>99%	8.9
c-LEcta	ATA-73	>99%	8.8
Pfizer	*A. fumigatus* (XP_748821)	>99%	7.7
Codexis	ATA-P2-A01	>99%	7.1
Pfizer	*A. oryzae* (XP_001818566)	>99%	5.6

[a]Identities of the transaminases that are not from vendors are indicated by their accession numbers. Conditions: ketone, 2 mg/mL; enzyme, 2 mg/mL, DMSO, 2%; isopropylamine hydrochloride, 2 equiv; 100 mM potassium phosphate with 3 mM pyridoxal phosphate, pH 7.5, 40 °C, 18 h.

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Table 2. Screen of Additional Transaminase Enzymes[a]

enzyme	enzyme mass (mg)	conversion at 16 h (HPLC a/a %)	ee (%)	comments
ATA-40	30	68	97	on running a further 24 h went to 98% conversion
ATA-47	30	58	>99%	on running a further 24 h went to 98% conversion
ATA-50	20	12	43	not suitable
ATA-64	20	12	98	minimal further conversion with increased reaction time.
ATA-69	20	99	86	good activity
ATA-81	20	5	74	not suitable

[a]Conditions: 10 mg/mL substrate concentration in phosphate buffer, pH 7.8, 30 °C with 3.3% methanol cosolvent.

Table 3. Screen of ATA-69 and ATA-47 at a 10 g/L Substrate Concentration, Varying Enzyme Load, and Varying *t*-Amyl Alcohol Concentrations

enzyme	enzyme mass (mg)	ketone mass (mg)	*t*-amyl alcohol mass (mg)	conversion (HPLC a/a %)	ee (%)	comments
ATA-69	1.6	29	100	82 (66 h)	89	28% conv 16 h
ATA-69	3.2	29	100	93 (66 h)	89	65% conv 16 h
ATA-69	3.2	60	160	low		<1% conv 16 h
ATA-69	3.0	60	80	40 (42 h)	86	
ATA-47	6.9	60	80	58 (42 h)	100	
ATA-47	6.9	61	160	7 (42 h)		

transaminase reactions is an option that may be investigated in future development efforts. At this concentration, the addition of *t*-amyl alcohol and DMF resulted in a marginal increased activity of ATA-47 over the use of methanol, whereas toluene and isopropyl acetate hindered the reaction. In all cases the very high enantioselectivity of the enzyme was unchanged by the addition of these solvents. Interestingly, however, further tests with and without added solvent showed that the 2-phase (solid/liquid) reaction proceeded efficiently to completion without the need for the addition of any solvents to help solubilize the tetralone. Conveniently, it was observed that the product aminotetralin **2** does precipitate as the phosphate salt as it was formed, resulting in a slurry to slurry transamination process step.

Despite a higher activity, the suboptimal enantioselectivity of ATA-69 was confirmed, and this enzyme was not evaluated in further studies. Further work was continued with ATA-47 and ATA-40.

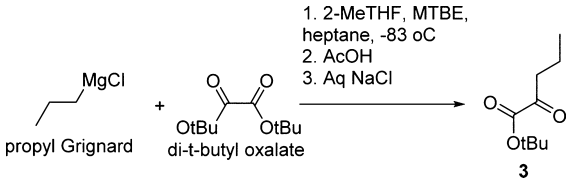
Further studies with ATA-47 and ATA-40 included an examination of enzyme loading, reaction temperature, pH, and isopropylamine concentration (up to 1.8 M). The performance of the two enzymes were comparable in except that ATA-47 consistently gave product with >99% ee, whereas ATA-40 produced a lower quality product having an ee of 97%. Based on the desire to maintain a high % ee, ATA-47 was chosen for scale-up.

The transamination reaction was run using ATA-47 to produce the desired (*S*)-**2** as a phosphate salt. The reaction was conducted in an aqueous isopropylamine phosphate buffer supplemented with the required pyridoxal phosphate cofactor without any added cosolvent. A key finding during process development was the need for a stream of nitrogen overlaying the reaction. This prevented oxidation of the tetralone and also removed the acetone coproduct, thus ensuring the reaction proceeded to completion. As described above, the aminotetralin phosphate salt precipitated as it was formed; therefore, product isolation was achieved by a simple filtration followed by water washes to remove the enzyme, pyridoxal phosphate, and isopropylamine buffer. Acetone washes were then carried out to remove soluble organic impurities and traces of unreacted **1**. After drying, the aminotetralin phosphate salt was isolated with 95% yield and >99% purity and purity (HPLC, 210 nm a/a). The undesired (*R*)-**2** was not detected.

To assess the optical purity of (*S*)-**2**, the (*R*)-**2** was prepared as an analytical standard. The transaminase from *Aspergillus terreus* was produced through fermentation, and a clarified lysate was used as the biocatalyst. This transaminase was used to convert 14 g of **1** to 19 g of (*R*)-**2** under similar conditions to those used to make the (*S*)-**2**. The product was isolated as the phosphate salt with an isolated yield of 89%. No (*S*)-**2** was detected.

With the aminotetralin in hand, the next step was to prepare **3** that is the substrate for the chiral reduction using an alcohol dehydrogenase. **3** was prepared from *n*-propyl Grignard added to a solution of di-*t*-butyl oxalate in MTBE and *n*-heptane at −80 °C (Scheme 3). Analysis of the reaction showed that 2.8%

Scheme 3. Preparation of 3



of the racemic **4** was formed as a byproduct. This racemic **4** is believed to be formed by reduction of the enol form of **3** to the racemate **4** during quench. The origin of the hydride could be from a beta-hydride elimination of excess propylmagnesium chloride from which the magnesium delivers the hydride to the ketone or in a Michael type fashion to the enol form of the ketone. The level of the racemic **4** formed was greatly reduced by switching the quench from a slow addition of the aqueous solution to a fast addition of the reaction mixture into aqueous citric acid to immediately neutralize anionic species and avoid transfer of a hydride to the ketone. This impurity was impossible to avoid and its separation from the **3** by fractional distillation also proved to be very difficult. However, since it had been observed that low concentrations of the undesired diastereomer could be readily removed during the crystallization of **6**, the ketoester oil was used directly in the next step without further purification.

A total of 190 alcohol dehydrogenases were screened for the reduction of **3** to **4**. These were all obtained from commercial sources. Enzymes that produced (*S*)-**4** or (*R*)-**4** with high selectivity were identified. The best hits are listed in Table 4. In this screen, the NAD(P)H cofactor was recycled using glucose and glucose dehydrogenase.

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Table 4. Results of the Screen of 190 Alcohol Dehydrogenases for the Conversion of 3 to 4[a]

vendor	enzyme	% ee (*R*)-**4**	% conversion
Codexis	KRED-P1-B10	>99	100
c-LEcta	ADH-107L	>99	100
c-LEcta	ADH-108L	>99	100
Almac	A161	>99	100
Almac	A411	>99	100
Almac	A631	>99	100
Almac	A671	>99	100
Almac	AR-108	>99	100
Codexis	KRED-NADH-110	95.3	100
Codexis	KRED−P1-B12	95.2	100
vendor	enzyme	% ee (*S*)-**4**	% conversion
Almac	A641	>99	100
Codexis	KRED-P3-G09	91.9	100
Johnson Matthey	JM-ADH-104	88.8	100
Codexis	KRED−P1-B04	88.4	100
Codexis	KRED−P3-C09	88.1	100
Asymchem	117297	86.3	100

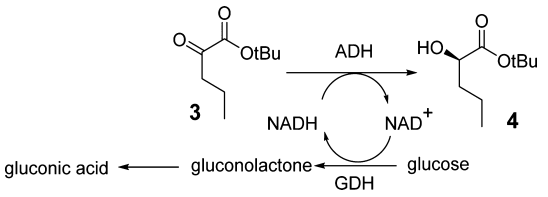
[a]Conditions: ketone, 2 mg/mL; enzyme, 2 mg/mL, DMSO, 2%, glucose, 1.2 equiv, NAD+, 0.1 mg/mL, NADP+, 0.1 mg/mL, glucose dehydrogenase, 0.1 mg/mL; 100 mM potassium phosphate buffer with 2 mM $MgCl_2$, pH 7.0, 30 °C, 18 h.

On the basis of cost, availability, and performance, the c-LEcta enzymes, ADH-107L and ADH-108L, were selected for further development. In initial tests, isopropanol was used for recycling the cofactor, which has advantages over the use of glucose dehydrogenase, since there is no need for a second enzyme and there is no requirement for pH control. With isopropanol, ADH-107L proved the most active; however, at increased concentrations of **3** and isopropanol it was found that increasing amounts of an undesired late eluting (on GC) impurity were formed and the selectivity of the enzyme was marginally reduced. The use of ADH-108L with isopropanol was unsuitable as it was unstable to higher concentrations of this solvent. Since the decision to switch to a glucose/glucose dehydrogenase cofactor recycle system was chosen, the identity of the late eluting peak was not pursued. An option that was not explored was the rescreening of enzymes using substrate coupled cofactor recycle with isopropanol serving as the reductant. This may have identified enzymes that were more tolerant of increased concentrations of isopropanol.

Further evaluation of ADH-108L with a glucose/glucose dehydrogenase cofactor recycle system demonstrated higher activity and stability when compared to ADH-107L with the isopropanol system. Under these conditions, it was found to be 7 fold more active per unit mass than ADH-107L. ADH-108L with the use of glucose/glucose dehydrogenase for cofactor recycle was selected for scale-up development. The conditions of temperature, cofactor concentration, ketoester/glucose concentration, pH range, and enzyme loading were investigated with the aim of developing a reaction that was robust and reproducible with respect to both conversion and selectivity with minimal enzyme loading.

Following optimization, **3** was reduced with ADH-108L to produce the desired (*R*)-**4**. The reaction was conducted at 24 °C and at an initial pH of 7.0, in phosphate buffer. The NADH cofactor was recycled using glucose dehydrogenase which converted glucose to gluconic acid, and the pH was maintained by periodic titration with concentrated sodium hydroxide (Scheme 4). The reaction was nearly complete (>95%) within 6 h but was left to stir overnight at which point the residual **3**

Scheme 4. Reduction of 3 to 4



was <0.1%. After extraction, washing, and solvent stripping, the **4** recovered as an oil (29.2 kg, 88% yield, 96.7% ee). Since the starting ketoester had 2.8% racemic-**4**, this represents an enantioselectivity over the enzymatic reaction of 99.5%.

To assess the optical purity of (*R*)-**4**, (*S*)-**4** was also produced as an analytical standard. One gram of **3** was reduced using Almac CRED A641 to produce (*S*)-**4** with an isolated yield of 77% and a stereopurity of 92.1% ee. The NADPH cofactor was recycled using glucose dehydrogenase. Control reactions showed that background KRED activity in the glucose dehydrogenase preparation was responsible for the production of (*R/S*)-**4**, and this accounted for the lower than expected stereopurity.

The free amine from **2** phosphate salt was prepared by suspension in aqueous sodium hydroxide and extraction with MTBE. The MTBE solvent was displaced with DCM, and the solution of **2** was stored cold under nitrogen until required. The triflate **5** was formed by slowly adding trifluoromethanesulfonic anhydride to a chilled solution of **4** in DCM and diisopropylethylamine. The free **2** was charged to the solution of **5** and the mixture was allowed to warm to 30 °C. This reaction was quenched with potassium bicarbonate and the phases separated. The solvent was stripped under vacuum and displaced with 1,4-dioxane to precipitate the diisopropylethylamine triflate salt which was removed by filtration. The HCl salt of the coupled product **6** in 1,4-dioxane was formed by the addition of water and HCl gas. Prior to HCl addition, water was added to the 1,4-dioxane to a concentration of 2.5% as it had been shown that this assisted in solubilizing impurities and enabled a purer product to be formed during crystallization.

The stereochemical integrity of **2** and **4** was verified upon intercepting **6** which is an intermediate common to both the current route as well as the original early development/Medchem route.[11]

CONCLUSION

The work described in this paper uses transaminases and alcohol dehydrogenases to deliver two target chiral compounds with a high stereopurity. The availability of a large selection of commercially available enzymes for both of these classes of enzymes made the identification of potentially useful enzymes facile. The use of bioinformatics enabled the establishment of an in-house collection of transmainases, including the identification of the (*R*)-selective transaminase that was used in the production of the undesired (*R*)-**2** which was used for synthesis of analytical standards.[12] The stability of the commercially available enzymes enabled their use under process conditions at the multikilogram scale.

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EXPERIMENTAL SECTION

Analytical Methods. Methods were as follows: chiral GC for **3** and **4**; column, Agilent CP-cyclodextrin-β-2,3,6-M-19 (25 m × 0.25 mm × 0.25 μm); constant flow with helium at 1.2 mL/min; gradient, 60−150 °C at 10 °C/min; FID detection.

UPLC Method for **1** and **2** proceeded as follows. Before analysis, the quenched, clarified samples were derivatized with Marfey's Reagent (CAS 95713-52-3).[13] 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 × 50 mm, 1.7 μm, part no. 186002350; column temperature, 45 °C; flow rate, 0.4 mL/min; mobile phase, 0.1% aqueous TFA/ACN; gradient, t = 0 min 95:5, t = 5 min 5:95; UV detection at 340 nm.

Enzymes and Reagents. Aminotransaminase (ATA-47), alcohol dehydrogenase (ADH-108L), and glucose dehydrogenase (GDH-03L) were from c-LEcta GmbH, Leipzig, Germany. Pyridoxal phosphate monohydrate (CAS 41468-25-1) was from Boaray Ltd., Shanghai China. NAD hydrate (CAS 53-84-9, product code OR301) was supplied by Europa Bioproducts, Ely, Cambridge, UK. 6,8-Difluoro-2-tetralone (CAS 843644-23-5) was prepared to order by Usun Fine Chemical Products Ltd., Nanjing, China and by Ash Ingredients Inc., GlenRock, NY, USA. Di-*t*-butyl oxalate (CAS 691-64-5) was from Aceto France SAS, Paris, France. 1-Chloropropane (CAS 540-54-5, product code C0266) was purchased from TCI Deutschland GmbH, Eschborn, Germany. Trifluoromethanesulfonic anhydride (CAS 358-23-6) was from Central Glass, Halle/Westfallen, Germany. Glycerin (CAS 56-81-5, Gycamed 99.7% grade) was obtained through Univar GmbH, Essen, Germany. Glucose monohydrate (CAS 50-99-7) was produced by Cargill (grade C*Dex 02001) and obtained through Chemische Fabrik GmbH, Moenchengladbach, Germany. Isopropylamine (CAS 75-31-0, produced by Oxea) was obtained from IMCD Deutschland GmbH, Koeln, Germany. *N*,*N*-Diisopropylethylamine (CAS 7087-68-5, 98% technical grade) was obtained from BASF, Ludwigshafen, Germany. All other materials were standard plant quality.

Screening of Alcohol Dehydrogenase Enzymes. The screening of the enzymes was carried out in 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); ketoester **3** (1 mg) in DMSO (10 uL); glucose dehydrogenase Codexis CDX901 (0.05 mg); $NADP^+$ (0.05 mg); NAD^+ (0.05 mg); and glucose (1.3 mg). The plates were incubated 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. The screening of the enzymes was carried out in 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 μL); **1** (1 mg) in DMSO (10 μL); pyridoxal phosphate (0.37 mg); and isopropylamine hydrochloride (1.05 mg). The plates were incubated 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 *tert*-Butyl 2-oxopentanoate 3. The Grignard solution was prepared and used directly in the reaction for preparation of ketoester **3**. A stainless steel Grignard reaction vessel was charged with magnesium turnings (13.4 kg, 1.08 equiv), a solution of *n*-propylmagnesium chloride in THF prepared in the laboratory to help with the initiation of the reaction (0.7 kg) and methyl THF (155 kg). The mixture was brought to reflux, and 1-chloropropane was charged (2.4 kg). A sample after 2.5 h showed that the reaction had not yet started, and an extra 0.6 kg of a solution of *n*-propylmagnesium chloride in THF prepared in the laboratory was added. After 6 h, the reaction had started, and the remainder of the 1-chloropropane (37.6 kg) was slowly added over 90 min. The reaction was held at reflux for a further 2.5 h and then cooled to 20−25 °C. A sample the next morning showed nondetectable levels of 1-chloropropane, and the solution was transferred via a tube with a filter to a low temperature charging vessel.

The low temperature vessel was charged with di-*t*-butyl oxalate (37.5 kg, 1 equiv), MTBE (167 kg), and *n*-heptane (154 kg). The solution was cooled to −83 °C, and the freshly prepared Grignard solution (1.05 equiv) was added over 105 min, maintaining the temperature between −79 and −83 °C. A sample taken 1 h after complete addition showed 96% conversion to the desired **3**. The reaction was quenched by the direct addition of a 33% solution of acetic acid (2 equiv) in MTBE maintaining the reaction mixture at ≤ −80 °C. The reaction mixture was then warmed to −20 °C and transferred to another (glass-lined) reactor. The low temperature vessel and transfer lines were washed through with 30 kg MTBE. When the mixture content had warmed to 5 °C, water was added during which the temperature rose to 16 °C. The phases were separated at 19 °C. The aqueous phase (pH 4.5) was discarded, and the organic phase was further washed with 10% aqueous sodium chloride which was separated and discarded. The organic phase was given a final wash with 7.5 L of water, which was also separated and discarded. The organic phase was stripped under vacuum to give 33.8 kg of oil with a combined purity of 85.7% (GC a/a), based on **3** (including the enol ester) plus racemic-**4**. This equates to a 90.7% yield from starting di-*t*-butyl oxalate. ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.7, 160.8, 83.8, 40.9, 27.7, 16.6, 13.5. ^1H NMR ($CDCl_3$, 400 MHz): δ 2.67 (t, J = 8.0 Hz, 2H), 1.61−1.54 (m, 2H), 1.48 (s, 9H), 0.90 (t, J = 7.1 Hz, 3H).

Preparation of (*S*)-6,8-Difluoro-1,2,3,4-tetrahydronaphthalen-2-amine 2. Aqueous isopropylamine/potassium phosphate buffer, pH 7.3, was prepared by adding water (718 kg), 85% phosphoric acid, (107.2 kg), isopropylamine (93 kg, 11.6 equiv), and 45% potassium hydroxide (4.5 kg) to the tank. 8 L of this buffer was removed, and 6,8-difluorotetralone (24.8 kg, 1 equiv) was added. The transaminase enzyme, c-LEcta, ATA-47 (110 g), and pyridoxal phosphate monohydrate (861 g) were each prepared as two separate solutions/suspensions in 4 L of buffer and added to the reactor at room temperature after the 6,8-difluorotetralone addition. The biotransformation was run at 37−39 °C with a constant stream of nitrogen sparged through the reaction suspension to remove the acetone. After 3 days the reaction showed <0.5% starting **1** and was cooled to 20−25 °C, filtered, and washed four times with water (1000 kg in total), followed by three washes with acetone (480 kg in total). The amine salt was then dried on the filter by passing a stream of nitrogen through the bed at room temperature.

A total of 30.9 kg of salt was recovered. The isolated yield over this step was 94%. An analysis showed nondetectable levels of (*R*)-**2** (after derivatization with Marfey's reagent). The

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HPLC purity (a/a 210 nm) was 99.5%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.99−6.75 (m, 2H), 3.10−2.95 (m, 2H), 2.90−2.65 (m, 4H), 2.30−2.15 (m, 1H), 1.90−1.75 (m, 1H), 1.50−1.35 (m, 1H).

Fermentation To Produce (*R*)-Selective Transaminase. The gene for a transaminase from *Aspergillus terreus* was synthesized, cloned into the pJExpress411 vector, and transformed into *E. coli* BL21(DE3) cells. These cells were cultured in Terrific Broth and induced with IPTG in order to express soluble transaminase enzyme. The cells were harvested by centrifugation, suspended in buffer, and lysed using homogenization. The homogenate was clarified by centrifugation, and the resulting crude lysate was used as the biocatalyst to prepare (*R*)-**2**.

Preparation of *tert*-Butyl-(*R*)-2-hydroxypentanoate 4. The reaction was carried out in an aqueous sodium phosphate buffered solution without the addition of any cosolvent. The formed gluconic acid was neutralized by the periodic addition of sodium hydroxide as required. To the vessel was added 0.3 M phosphate buffer, pH 7.0 (300 kg), followed by glucose monohydrate (61 kg, 1.8 equiv) and glycerol (135 kg). The alcohol dehydrogenase, c-LEcta ADH-108L (56 g), glucose dehydrogenase, c-LEcta GDH-03L (66 g), and NAD^+ hydrate (130 g) were then added as a preprepared solution in buffer. The starting **1** (35 kg, 1 equiv) was added, and the reaction was performed at 22−25 °C. Samples were taken every 30−60 min, the pH measured, and 33% sodium hydroxide added as required. The reactions were nearly complete after 5−6 h, after which the reactions were stirred overnight for complete conversion. The reaction was worked up by extraction into MTBE (three extractions with a total of 550 kg MTBE) followed by two water back-washes with 40 L of water each wash. The MTBE solution was then stripped under vacuum to give an oil. The final distillation conditions were 10−20 mbar vacuum, with a sump temperature 36−40 °C. The oil was purified using wiped-film distillation. The wall temperature was 90−93 °C; the vacuum was 6−8 mbar, and the cooling bath temperature was 15−20 °C. A total of 29.1 kg distilled oil was obtained with a corrected yield of 88%. The % ee of the *t*-butyl (*R*)-**2**-hydroxypentanoate was 96.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.20−4.00 (m, 1H), 2.80 (s, 1H), 1.85−1.65 (m, 2H), 1.46 (s, 9H), 1.45−1.31 (m, 2H), 0.85 (s, 3H).

Coupling of (*S*)-6,8-Difluoro-1,2,3,4-tetrahydronaphthalen-2-amine 2 and *tert*-Butyl-(*R*)-2-hydroxypentanoate 4. The free amine (24.7 kg) was released from the phosphate salt by suspending it in water (237 kg) plus MTBE (690 kg) and then adding 33% sodium hydroxide (14.9 kg) to pH 10.8−11.2. The phases were separated and the aqueous phase re-extracted two more times with MTBE. The combined MTBE phases were back-washed with a little water, and the phases were separated and then dried by the addition of sodium sulfate (38 kg). The sodium sulfate was filtered off and washed with a little MTBE. The combined MTBE phases were stripped under vacuum and at a temperature <30 °C to an oil, and then dichloromethane (DCM) was added. The aminotetralin **2**/DCM solution was stored at 0−5 °C, under nitrogen until required. The amine solution, had an HPLC purity of 99.5% a/a (210 nm), and the DCM:MTBE ratio was 95:5.

Hydroxyester **4** (21.6 kg, 1.11 equiv corrected), DCM (570 kg), and diisopropylethylamine (29.1 kg, 2.18 equiv) were charged to a low temperature vessel and the contents cooled to −20 to −25 °C. Trifluoromethanesulfonic anhydride (31.1 kg, 1.07 equiv) was then slowly added, maintaining the temperature below −20 °C. After complete addition, the reaction was stirred for 1 h after which a sample showed complete conversion of the added trifluoromethanesulfonic anhydride. Then free aminotetralin **2**/DCM solution (145 kg, 1.0 equiv) was added; the reaction was warmed to 30 °C, stirred overnight, and continued until a sample showed <2% residual free **2** remaining. At this point, HPLC showed 94.4% product **6** and 1.9% incorrect diastereomer (ratio 98.0:2.0). The reaction was quenched by the addition of a 12% potassium bicarbonate aqueous solution (210 kg) after which the phases were separated. The DCM/product containing phase was stripped under vacuum and the solvent exchanged to 1,4-dioxane at a sump temperature ≤30 °C. The diisopropylethylamine triflate salt precipitated and was filtered at 20−25 °C. The product **6** in 1,4-dioxane was returned to the vessel and the water content adjusted to 2.5%. HCl gas (3.6 kg) was then added, maintaining the temperature between 24 and 29 °C, until a sample showed a pH of approximately 3. The temperature was then adjusted to 25 °C and the product filtered. The filter cake was washed 4 times with 1,4-dioxane (196 kg) and partially dried under a stream of nitrogen to give 41.8 kg damp product. HPLC analysis showed 99.3% and 99.4% purity by HPLC (a/a; 210 nm) with 0.1% of the incorrect diastereomer. The coupled product **6** was dried in a tray drier at 40−45 °C, high vacuum, and under a stream of nitrogen to yield a total of 32.1 kg of **6** (80% overall yield on tetralone 1 and 61% overall yield on di-*t*-butyl oxalate). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.95 (s, 1H), 9.36 (s, 1H), 7.07−7.00 (m, 1H), 6.90−6.85 (m, 1H), 4.13 (s, 1H), 3.48−3.35 (m, 1H), 3.27−3.18, (m, 1H), 2.97−2.75 (m, 3H), 2.30−2.18 (m, 1H), 2.00−1.78 (m,3H), 1.45 (s, 9H), 1.44−1.25 (m, 2H), 0.91 (t, 3H).

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Notes

The authors declare no competing financial interest.

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