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Article

Enantio-Complementary Synthesis of 2-Substituted Pyrrolidines and Piperidines via Transaminase-Triggered Cyclizations

Christian M. Heckmann* and Caroline E. Paul*

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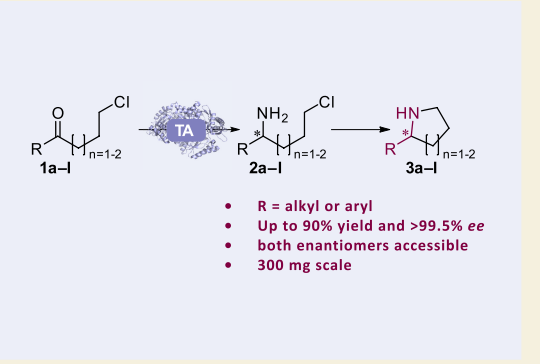
ABSTRACT: Chiral *N*-heterocycles are a common motif in many active pharmaceutical ingredients; however, their synthesis often relies on the use of heavy metals. In recent years, several biocatalytic approaches have emerged to reach enantiopurity. Here, we describe the asymmetric synthesis of 2-substituted pyrrolidines and piperidines, starting from commercially available ω-chloroketones by using transaminases, which has not yet been comprehensively studied. Analytical yields of up to 90% and enantiomeric excesses of up to >99.5% for each enantiomer were achieved, which has not previously been shown for bulky substituents. This biocatalytic approach was applied to synthesize (*R*)-2-(*p*-chlorophenyl)pyrrolidine on a 300 mg scale, affording 84% isolated yield, with >99.5% *ee*.



KEYWORDS: *asymmetric synthesis, biocatalysis, chiral amines, enzyme, N-heterocycles*

INTRODUCTION

Chiral amines are important building blocks of many active pharmaceutical ingredients (APIs) and agrochemicals;[1,2] however, their synthesis by nonenzymatic means usually requires stoichiometric amounts of chiral auxiliaries or the use of chiral rare metal catalysts.[3] On the other hand, an increasingly large portfolio of enzymes has become available for the synthesis of chiral amines, such as transaminases,[4] imine reductases (including reductive aminases),[5] and amine dehydrogenases.[6] These enzymes have found applications in the synthesis of APIs such as antidiabetic drug sitagliptin,[7] LSD1 inhibitor GSK2879552,[8] JAK1 inhibitor abrocitinib,[9] BTK inhibitor nemtabrutinib,[10] and CDK2/4/6 inhibitor PF-06873600,[11] improving the efficiency and sustainability of these processes on up to >100 kg scales.[12] Nitrogen-containing heterocycles represent a privileged structure in many APIs,[13] and thus chiral cyclic amines are especially important building blocks. Several biocatalytic routes toward this moiety have been reported (Figure 1A);[14−23] yet, an attractive strategy, biocatalytic reductive amination of a ketone bearing a leaving group, followed by spontaneous cyclization, remains to be explored (Figure 1B).[7,24,25] Only three examples have been reported in the literature, two of which produce the (*R*)-enantiomer in the 2-position.[7,24] The third example generates a 3-substituted piperidine, but was abandoned during development due to the instability of the aldehyde starting material, and no data on conversions or yield were reported.[25] Transaminases (TAs) are pyridoxal-5′-phosphate (PLP)-dependent enzymes catalyzing the transfer of an amino group from a sacrificial amine donor to a prochiral ketone substrate. Here, we describe their application in the stereoselective synthesis of 2-substituted chiral pyrrolidines and piperidines starting from commercially available ω-chloroketones, employing isopropylamine (IPA) as the amine donor (Figure 1C). Access to both enantiomers is reported for the first time, and the effects of both methyl and electron-rich and -deficient phenyl substituents are explored.

RESULTS AND DISCUSSION

A panel of 10 TAs was selected, composed of five (*S*)- and five (*R*)-selective enzymes, all of which have previously been described. For the (*S*)-selective TAs, *Cv*STA[26] from *Chromobacterium violaceum*, and HEwT (wt[27] and W56G mutant[28]) from *Halomonas elongata*, as well as two TAs that have been engineered to accept bulky−bulky ketones, 3FCR-4M[29] from *Ruegeria* sp. TM1040, and *Pj*STA-R6-8[30] from *Pseudomonas jessenii* were selected. For the (*R*)-selective TAs, *At*RTA[31] from *Aspergillus terreus*, *Ts*RTA[32] from *Thermomyces stellatus*, ATA-117 from *Arthrobacter* sp., as well as its evolved variants for the synthesis of sitagliptin,[7] ATA-117-Rd6, and ATA-117-Rd11 were chosen. All TAs were produced by recombinant expression in *Escherichia coli* BL21(DE3) and used as lyophilized cell-free extracts (CFEs, SI Figure S1).

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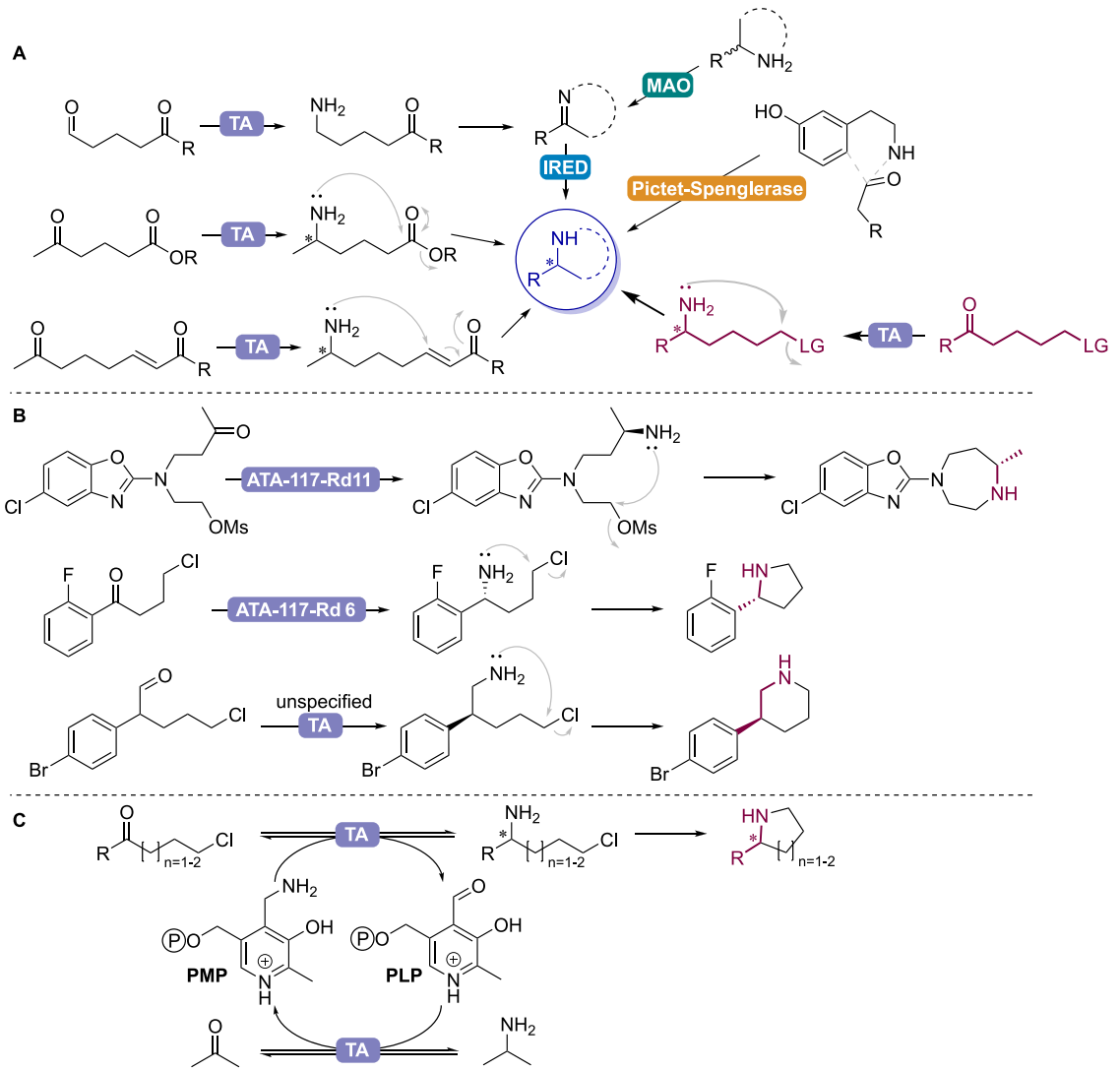

Figure 1. (A) Biocatalytic strategies for the synthesis of chiral cyclic amines. (B) Literature examples of the transamination of carbonyl substrates bearing a terminal leaving group, followed by cyclization. (C) Proposed synthesis of 2-substituted chiral pyrrolidines and piperidines starting from commercially available ω-chloroketones.

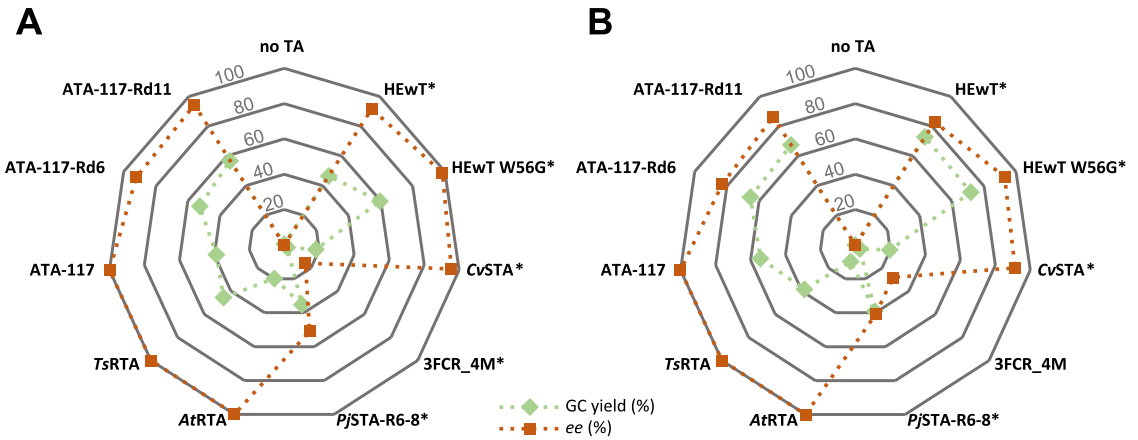

Figure 2. GC yields (determined using a calibration curve of the products) and *ees* for the TA-catalyzed synthesis of (A) 2-methylpyrrolidine **3a** and (B) 2-methylpiperidine **3b**. Enzymes labeled with an asterisk produce the (*S*)-enantiomer, while those without produce the (*R*)-enantiomer. Conditions: TA (10 mg/mL), **1a-b** (50 mM), PLP (1 mM), IPA (0.5), DMSO (5% v/v), KP_i-buffer (100 mM), pH 8, 30 °C, 700 rpm, final volume 0.5 mL. Reaction time: 22 h (**1a**; except *Pj*STA-R6-8: 24 h) or 24 h followed by the addition of NaOH (100 μL, 10 M) and further incubation for 3h (**1b**). Data are the average of duplicates.

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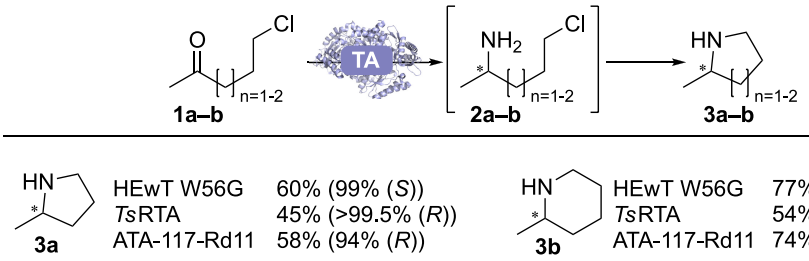
Scheme 1. GC Yields (Determined Using a Calibration Curve of the Products) and *ees* (in Parentheses) for the Best-Performing Variants in the TA-Catalyzed Synthesis of 3a and 3b[a]



[a]Conditions: TA (10 mg/mL), **1a-b** (50 mM), PLP (1 mM), IPA (1 M or 0.5 M (*Ts*RTA and ATA-117)), DMSO (5% v/v), KP_i-buffer (100 mM), pH 8, 30 °C, 700 rpm, final volume 0.5 mL Reaction time: 22 h (**1a**) or 24 h followed by the addition of NaOH (100 μL, 10 M) and further incubation for 3h (**1b**). Data are the average of duplicates.

Initial reactions were set up using 5-chloropentan-2-one **1a** and 6-chlorohexan-2-one **1b** as substrates (Figure 2, SI Figures S2,3), with the best-performing variants summarized in Scheme 1. HEwT W56G showed the highest yields (determined by gas chromatography (GC) with a calibration curve of the product) and enantioselectivity among the (*S*)-selective TAs. Removing the bulky tryptophan side chain alleviates steric clash with the chloro-alkyl chain, allowing for the chloro-alkyl chain to extend into the binding pocket, which one may speculate leads to a more productive positioning (Figure 3), although more detailed

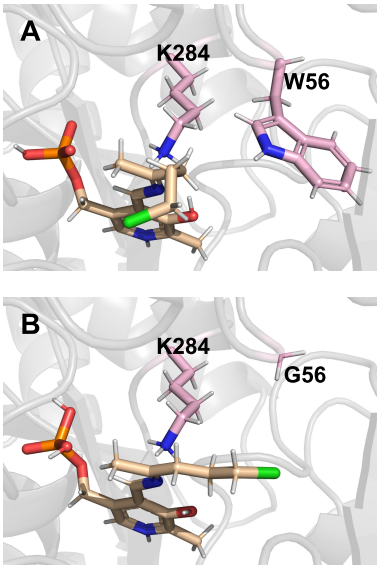


Figure 3. Docked quinonoid intermediate of 5-chloropentan-2-one **1a**. (A) HEwT wt, (B) HEwT W56G. Docking was carried out with the dock_run.mcr macro in YASARA 20.12.24; the figure was generated using Open Source PyMOL 2.5.0.

modeling would be required to confirm this hypothesis. A similar effect on enantioselectivity has previously been reported for HEwT mutants bearing a W56C mutation with *p*-substituted acetophenones.[33] For the (*R*)-selective TAs, a trade-off between GC yield and enantioselectivity was observed. The wild-type TAs showed essentially complete enantioselectivities in almost all cases. On the other hand, the engineered variants of ATA-117 showed higher analytical yields of the product but with reduced *ees*. The improved conversions are likely due to enhanced acceptance of IPA as the amine donor (SI Figures S2,3 see the effect of 1 vs 0.5 M IPA on ATA-117 and the engineered variants), for which the variants were optimized during the evolutionary campaign, as well as their increased stability.[7] The variants having been engineered to accept bulky−bulky ketones, reduced enantioselectivity with the small−bulky ketones used here was expected. While cyclization to the pyrrolidine was spontaneous under the reaction conditions (the chloro-amine was never detected), cyclization to the piperidine required incubation with sodium hydroxide for 3 h, due to the additional degree of freedom conferred by the extra methylene. Significant levels of nonenzymatic hydrolysis of **1a** were observed during the biocatalytic reaction, whereas **1b** was stable and only degraded during the incubation with excess sodium hydroxide (SI Figure S4).

2-Arylpyrrolidines are featured in several bioactive molecules, such as nicotine, larotrectinib,[34] and MSC2530818 (Figure 4).[35]

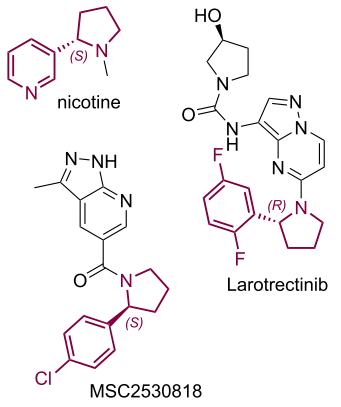


Figure 4. Structures of bioactive molecules containing 2-arylpyrrolidines.

Thus, their synthesis from commercially available 4-chlorobutyrophenones (**1c-k**) was investigated (Scheme 2). Given the bulky−bulky nature of these substrates, the focus was shifted toward the engineered TAs 3FCR-4M, *Pj*STA-R6-8, ATA-117-Rd6, and ATA-117-Rd11. Preliminary tests using ATA-117-Rd6 indicated that higher reaction temperatures of 37 °C and longer reaction times of 48 h were required for these more hindered substrates (Figure 5). Thus, screening of the panel of substrates against the panel of bulky−bulky TAs was carried out under these conditions, the comprehensive results of which can be seen in Supporting Figures S5−15. Hydrolysis and cyclopropane formation were observed as the major nonenzymatic side reactions (identified by liquid chromatography−mass spectrometry (LC-MS), see the SI). The desired products were

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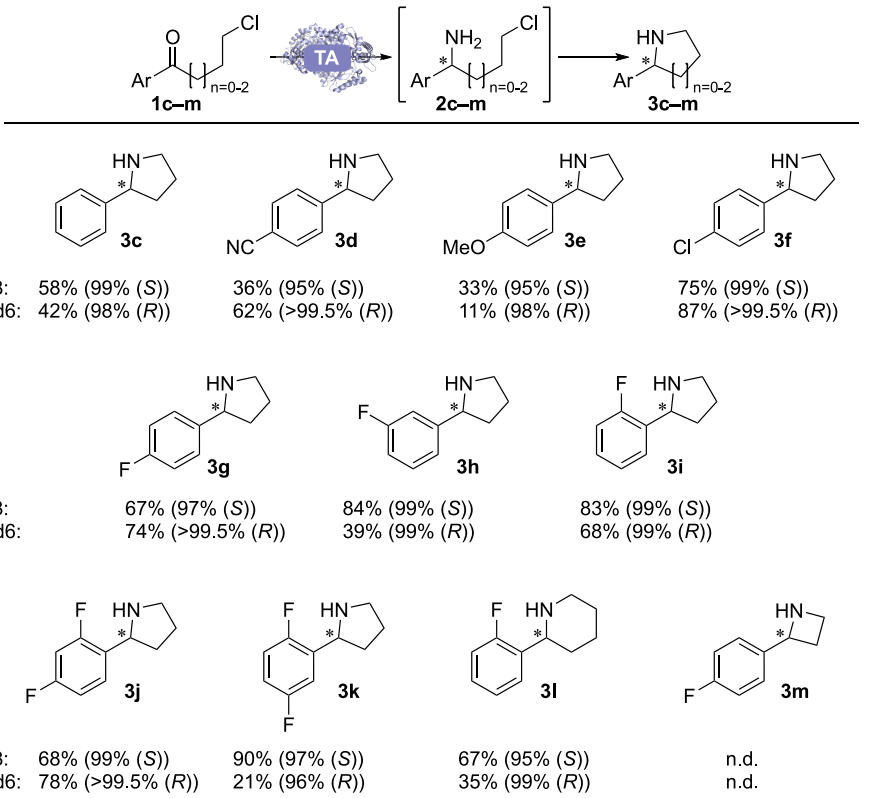
Scheme 2. HPLC Yields (Determined Using a Calibration Curve of the Products) and *ee*s (in Parentheses) for the Best-Performing Variants in the Synthesis of 3c-m[a]



[a]Conditions: TA (10 mg/mL), **1c-g** (50 mM), PLP (1 mM), IPA (1 M), DMSO (20% v/v), KP_i-buffer (100 mM), pH 8, 37 °C, 700 rpm, final volume 0.5 mL Reaction time: 48 h. For **3l**: subsequent addition of NaOH (50 μL, 10 M) and further incubation for 1 h. Data are the average of duplicates. N.d.: not detected.

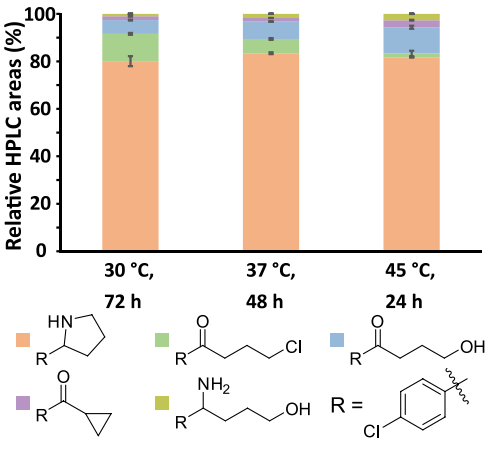


Figure 5. Relative HPLC areas of TA-catalyzed reactions with **1f** as substrate, using varying temperatures (30, 37, and 45 °C). Conditions: ATA-117-Rd6 (10 mg/mL), **1f** (50 mM), PLP (1 mM), IPA (1 M), DMSO (20% v/v), KP_i-buffer (100 mM), pH 8, 700 rpm, final volume 0.5 mL. Data are the average of duplicates; error bars represent standard errors.

identified by comparison to authentic standards (commercial, except **3d** which was synthesized and characterized by NMR, see the SI) as well as LC-MS (see the SI). The absolute configurations were assigned based on the known selectivities of ATA-117-Rd6[7] and *Pj*STA[30] for **1i** and butyrophenone, respectively, as well as consistent elution orders on the chiral GC (see SI), and further confirmed by optical rotation for (*R*)-**3f** (see below). For all substrates, ATA-117-Rd6 was the best (*R*)-selective TA, whereas *Pj*STA-R6-8 was the best (*S*)-selective TA (Scheme 2). ATA-117-Rd6 is less specialized than ATA-117-Rd11 (6 vs 11 rounds of directed evolution toward the synthesis of sitagliptin),[7] and thus may retain more promiscuity. IPA has been reported to be an unsuitable amine donor for 3FCR-4M.[36] The additional mutations in ATA-117-Rd11 are concentrated at the upper back of both the large and small binding pockets, resulting in an enlarged cavity that may influence the dynamics of the substrate entering the active site, as well as the tightness of binding (SI Figure S16).

In the synthesis of 2-arylpyrrolidines **3c-k** (Scheme 2), both enzymes showed excellent (≥95% *ee*) and complementary enantioselectivities, while high-performance liquid chromatography (HPLC) yields ranged from low (10%) to excellent (90%). Of particular interest are products **3f** and **3k**, which are motifs featured in the APIs larotrectinib, and MSC2530818 (Figure 4), respectively. In contrast, recent examples of the asymmetric synthesis of 2-aryl-*N*-heterocycles employing a reductive amination strategy using transition-metal catalysts are mostly limited to <90% *ee*, with only a few exceptions.[37−39] Biocatalytic imine reduction using IREDs generally reaches >99 *ee*;[22] however, the starting imines for this strategy are typically not commercially available. Fanourakis et al.[40] reported the synthesis of (*R*)-**3c** in 33% yield and 89% *ee* starting from readily available 4-phenylbutan-1-ol by employing an asymmetric benzylic C-H activation strategy, followed by Mitsunobu cyclization and deprotection.

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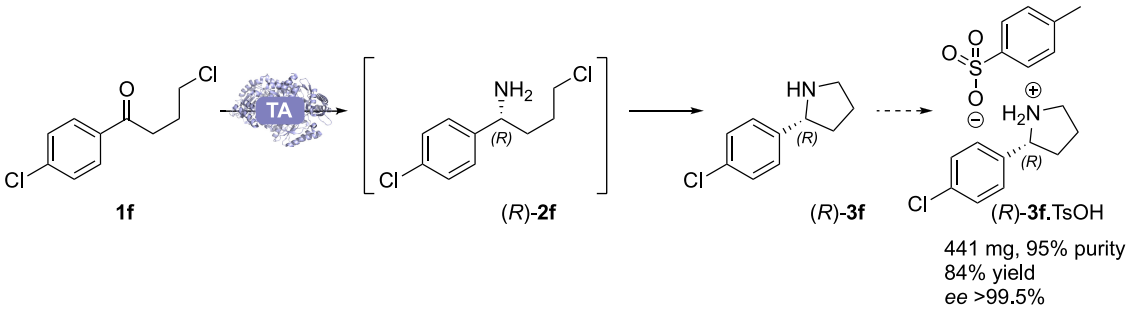
Scheme 3. Preparative-Scale Synthesis of (*R*)-3f from 1f, Catalyzed by ATA-117-Rd6[a]



[a]Conditions: ATA-117-Rd6 (250 mg), **1f** (300 mg), PLP (1 mM), IPA (1 M), DMSO (20% v/v), KP_i-buffer (100 mM), pH 8, 40 °C, vigorous stirring (magnetic stirrer), final volume 25 mL. Reaction time: 72 h.

In the case of ATA-117-Rd6, *para*-substitution with an electron-donating methoxy group resulted in decreased yield, whereas electron-withdrawing chloride, fluoride, and to a lesser extent cyano in that position was beneficial. Fluoride in the *ortho*-position was also beneficial, with di-substitution in both positions showing a synergistic effect. However, a *meta*-fluoro-substituent resulted in decreased yield even in the presence of an additional *ortho*-fluoro group. Docking of the quinonoid intermediates for each substrate (SI Figures S17−26) showed that substitutions on the phenyl ring result in a more twisted configuration around the Cα-N bond. Electron-withdrawing substituents likely increase the yield by increasing the electrophilicity of the carbonyl, increasing its reactivity. The additional increase in yield for the *para*-halogenated compounds may be explained by a π-halogen interaction[41] with Y61 of the neighboring subunit. A structural explanation for the decreased yields with *meta*-fluoride remains elusive.

*Pj*STA-R6-8 largely showed similar trends to ATA-117-Rd6 with some notable exceptions; a *para*-cyano group was detrimental compared to no substitution, whereas *meta*-fluorination was beneficial, with 2′,5′-difluorination being highly beneficial. This effect may be due to the *meta*-fluoro group pointing into the active site entrance, allowing the *ortho*-fluoride to form a hydrogen bond with the PLP hydroxy group and K288. *para*-Fluorination appears to disrupt the possibility for this hydrogen bond, which may explain why 2′,4′-difluorination exhibited similar HPLC yields to para-fluorination and decreased HPLC yields compared to *ortho*-fluorination (SI Figures S27−36).

Curiously, *Pj*STA-R6-8 only showed very slight preference for the electronically activating *para*-nitrile group over the electronically deactivating *para*-methoxy substituent. This preference may be due to steric interactions of the linear nitrile group with M54 and L417, which are reduced with the flexible bent methoxy group, as well as additional beneficial hydrophobic interactions of the methyl group with M54, L57, and M58 (SI Figures S27−29). For most substrates, *Pj*STA-R6-8 showed higher HPLC yields than ATA-117-Rd6. For the synthesis of 2-arylpiperidine **3l**, additional incubation with base was required to complete cyclization, whereas the 3-chloropropiophenone **1m** rapidly degraded and no transaminated product was observed (the major degradation product was 3-isopropylamino-propiophenone, as determined by LC-MS, see SI).

The synthesis of **3f** was then scaled up to 300 mg of starting material using ATA-117-Rd6 (Scheme 3), giving the tosylate salt of (*R*)-**3f** in 84% isolated yield (95% NMR purity, main impurities being water and DMSO), with >99.5% *ee*. As the substrate **1f** is only partially soluble under the reaction conditions, vigorous stirring, higher temperature, and prolonged reaction times were needed to achieve high conversions at this scale due to the lower relative surface area of the aggregated starting material, resulting in reduced mass transfer compared to the analytical scale. The specific rotation (see below) of the free amine of (*R*)-**3f** was consistent with the literature.[42]

CONCLUSIONS

A panel of chiral 2-substituted pyrrolidine and piperidines was synthesized starting from commercially available ω-chloroketones. Both enantiomers could be accessed by choosing the corresponding transaminase, with *ees* >95% in all cases, and analytical yields ranging from 10% to 90%. Chiral azetidines could not be accessed using this strategy as the starting material was not sufficiently stable under the reaction conditions. Isolation of the product on preparative scale was also possible, the product amine being easily precipitated from MTBE using tosic acid. Thus, the strategy described herein represents a powerful and straightforward way to access chiral pyrrolidine and piperidines on a laboratory scale, with potential applications in drug discovery.[43−45] The long reaction times, as well as high enzyme loading (83 wt %) require further improvement of the enzymes identified, for example, by directed evolution. Best results for the generation of (*R*)-2-aryl pyrrolidines and piperidines were obtained with ATA-117-Rd6 rather than the usual choice of ATA-117-Rd11 for bulky-bulky ketones,[46] highlighting that the most engineered enzyme for one specific substrate does not necessarily have the broadest substrate scope.

METHODS

Chemicals were purchased from Merck KGaA (Darmstadt, Germany), TCI Europe N. V. (Zwijndrecht, Belgium), Biosynth s.r.o. (Bratislava, Slovakia), Fluorochem Ltd. (Hadfield, UK), Activate Scientific GmbH (Prien, Germany), abcr GmbH (Karlsruhe, Germany), and Thermo Fisher GmbH (Kandel, Germany). 5-Chloropentan-2-one and 6-chlorohexan-2-one were distilled prior to use, and all other chemicals were used without additional purification. Criterion TGX Stain-Free Precast Gels were used for SDS-PAGE and visualized using a Bio-Rad ChemiDoc Imaging System. Plasmids pET-28a-ATA-117,1 pCH93b-TsRTA,2 pCH93b-AtRTA,3 pMP89a-HEwT,4 and pMP89b-CvSTA5 were received from the Paradisi group, pET-21a-ATA-117-Rd111 was received from the Kroutil group, and pET-20b-PjSTA-R6-86 was received from the Janssen/Fraaije group. pET-28a-ATA-117-Rd61 and pET-28a-3fcr-4M7 were purchased from Synbio Technologies, LLC. (Monmouth Junction, NJ). pMP89a-HEwT W56G8 was prepared using the NEBaseChanger kit (New England Biolabs, Ipswich, MA).

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Transaminase Production

HEwT, HEwT W56G, and *Pj*STA-R6-8 were produced by recombinant expression in *E. coli* BL21(DE3) in ZYP autoinduction medium at 30 °C (24 °C for *Pj*STA-R6-8) overnight.[27] All other transaminases were produced by recombinant expression in *E. coli* BL21(DE3) in TB-lac medium at 25 °C overnight.[32] Lyophilized cell-free extracts were then prepared. Further details can be found in the SI.

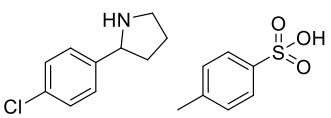
Analytical Scale Reactions

Reactions were set up by combining in the following order: potassium phosphate buffer (100 mM, pH 8), PLP (10 mM stock in buffer) isopropylamine (2 M stock (pH adjusted with HCl) in buffer), substrate (250 or 1 M stock in DMSO), and transaminase (50 mg/mL stock in buffer), to give biotransformations (500 μL) containing the desired concentrations of components as referred to in the manuscript (Table S2). Biotransformations were then incubated at 30 or 37 °C, 700 rpm (Eppendorf ThermoMixer C), for 22−48 h, as indicated in the manuscript. For 6-chlorohexan-2-one (**1b**), sodium hydroxide (100 μL, 10 M) was added, followed by further incubation for 3 h. For *o*-fluoro-5-chlorovalerophenone (**1l**), sodium hydroxide (50 μL, 10 M) was added, followed by further incubation for 1 h.

For 5-chloropentan-2-one (**1a**) and 6-chlorohexan-2-one (**1b**), the reactions were allowed to cool and extracted by adding EtOAc (1 mL), followed by NaOH (10 M, 100 μL; 5-chloropentan-2-one (**1a**) only; adding base after adding solvent suppresses cyclopropanation). The extract was further diluted (400 + 600 μL EtOAc), acetylated (20 μL each of Et_3N + Ac_2O), and analyzed by both achiral and chiral gas chromatography (GC).

For all other substrates, the reactions were allowed to cool and quenched with acetonitrile (1 vol). An aliquot was further diluted (40 μL + 280 μL H_2O + TFA (0.2%) + 280 μL MeCN), centrifuged, and analyzed by high-performance liquid chromatography (HPLC). (Alternatively, 100 μL sample + 450 μL H_2O + HCl (0.2%) + 280 μL MeCN for LC-MS.) The remainder was alkalized with NaOH (10 M, 80 μL; except *o*-fluoro-5-chlorovalerophenone (**1l**)) and extracted into EtOAc (1 mL), acetylated (20 μL each of Et_3N + Ac_2O), and analyzed by chiral GC.

Preparative Scale Reaction—Synthesis of (*R*)-3f·TsOH



In a round-bottom flask equipped with a magnetic stirrer bar, **1f** (300 mg, 1.38 mmol) was dissolved in DMSO (5 mL) after which IPA (12.5 mL of 2 M stock pH adjusted (HCl) stock in KP_i -buffer (100 mM, pH 8)) was added. To the cloudy suspension was then added ATA-117-Rd6 (250 mg), which had been rehydrated in buffer (7.5 mL of PLP (3.33 mM) in KP_i -buffer (100 mM, pH 8)). The reaction was then protected from light (aluminum foil) and stirred vigorously (just below vortex formation) at 40 °C (oil bath) for 72 h. The reaction was allowed to cool, acidified to pH < 3 (320 μL of conc. HCl), and extracted with EtOAc (2 × 10 mL), breaking the emulsion with centrifugation (5000*g* for 1 min). The aqueous phase was then basified to pH >11 (3 mL of 10 M NaOH) and extracted with EtOAc (3 × 10 mL), breaking the emulsion with centrifugation (5000*g* for 1 min). The organic extracts were combined, dried with $MgSO_4$, and concentrated *in vacuo*. The oily residue was then re-dissolved in methyl *tert*-butyl ether (MTBE; 3 mL), and tosic acid monohydrate (270 mg, 1.42 mmol), pre-dissolved in MTBE (5 mL), was slowly added, causing immediate precipitation. After incubation at −20 °C for 30 min, the solid was separated by vacuum filtration, washed with ice-cold MTBE (2 × 2 mL), and dried overnight at 0.34 mbar, giving fine free-flowing white crystals of (*R*)-**3f**.TsOH (441 mg, 95% purity (^{1}H-NMR), 80% purity-adjusted yield). The main impurities detected were DMSO and water (overlapping with the 2-hydrogen of the pyrrolidine ring). $[\alpha]_D^{21}$ = +51° (free amine, *c* 0.883, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$, referenced relative to TMS) δ 1.92−2.18 (3 H, m), 2.19−2.31 (1 H, m), 2.38 (3 H, s), 3.24−3.51 (2 H, m), 4.35−4.52 (m overlapping with water), 7.10−7.17 (4 H, m), 7.26−7.32 (2 H, m), 7.48−7.53 (2 H, m), 8.59 (1 H, br s), 9.35 (1 H, br s); ^{13}C-NMR (101 MHz, $CDCl_3$, absolute referencing relative to ^{1}H-NMR) δ 21.4 (CH_3), 23.5 (CH_2), 31.3 (CH_2), 45.5 (CH_2), 62.7 (CH), 125.8 (CH), 129.0 (CH), 129.1 (CH), 129.4 (CH), 132.6 (C), 135.1 (C), 140.8 (C), 140.9 (C); Retention times on GC and HPLC match those of commercial **3f**.

Analytical methods

Detailed analytical methods are found in the SI.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.3c00103.

Production of enzymes, protocols, analytical measurements, dockings, GC and HPLC chromatograms, and NMR spectra (PDF)

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Author Contributions

CRediT: **Christian M. Heckmann**: conceptualization, data curation, investigation, methodology, writing—original draft, writing—review & editing; **Caroline E. Paul**: conceptualization, funding acquisition, methodology, project administration, supervision, writing—review & editing.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Bommarius, A. S. Biocatalysis: A Status Report. *Annu. Rev. Chem. Biomol. Eng.* **2015**, *6*, 319−345.

(2) Ghislieri, D.; Turner, N. J. Biocatalytic Approaches to the Synthesis of Enantiomerically Pure Chiral Amines. *Top. Catal.* **2014**, *57*, 284−300.

(3) Cabré, A.; Verdaguer, X.; Riera, A. Recent Advances in the Enantioselective Synthesis of Chiral Amines via Transition Metal-Catalyzed Asymmetric Hydrogenation. *Chem. Rev.* **2022**, *122*, 269−339.

(4) Slabu, I.; Galman, J. L.; Lloyd, R. C.; Turner, N. J. Discovery, Engineering, and Synthetic Application of Transaminase Biocatalysts. *ACS Catal.* **2017**, *7*, 8263−8284.
(5) Gilio, A. K.; Thorpe, T.; Turner, N. J.; Grogan, G. Reductive Aminations by Imine Reductases: From Milligrams to Tons. *Chem. Sci.* **2022**, *13*, 4697−4713.
(6) Patil, M. D.; Grogan, G.; Bommarius, A.; Yun, H. Oxidoreductase-Catalyzed Synthesis of Chiral Amines. *ACS Catal.* **2018**, *8*, 10985−11015.
(7) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. Biocatalytic Asymmetric Synthesis of Chiral Amines from Ketones Applied to Sitagliptin Manufacture. *Science* **2010**, *329*, 305−310.
(8) Schober, M.; MacDermaid, C.; Ollis, A. A.; Chang, S.; Khan, D.; Hosford, J.; Latham, J.; Ihnken, L. A. F.; Brown, M. J. B.; Fuerst, D.; Sanganee, M. J.; Roiban, G. D. Chiral Synthesis of LSD1 Inhibitor GSK2879552 Enabled by Directed Evolution of an Imine Reductase. *Nat. Catal.* **2019**, *2*, 909−915.
(9) Kumar, R.; Karmilowicz, M. J.; Burke, D.; Burns, M. P.; Clark, L. A.; Connor, C. G.; Cordi, E.; Do, N. M.; Doyle, K. M.; Hoagland, S.; Lewis, C. A.; Mangan, D.; Martinez, C. A.; Mcinturff, E. L.; Meldrum, K.; Pearson, R.; Steflik, J.; Rane, A.; Weaver, J. Biocatalytic Reductive Amination-Discovery to Commercial Manufacturing Applied to Abrocitinib JAK1 Inhibitor. *Nat. Catal.* **2021**, *4*, 775−782.
(10) Kuhl, N.; Turnbull, B. W. H.; Ji, Y.; Larson, R. T.; Shevlin, M.; Prier, C. K.; Chung, C. K.; Desmond, R.; Guetschow, E.; He, C. Q.; Itoh, T.; Kuethe, J. T.; Newman, J. A.; Reibarkh, M.; Rivera, N. R.; Shang, G.; Wang, Z.; Zewge, D.; Thaisrivongs, D. A. Utilizing Biocatalysis and a Sulfolane-Mediated Reductive Acetal Opening to Access Nemtabrutinib from Cyrene. *Green Chem.* **2023**, *25*, 606−613.
(11) Duan, S.; Widlicka, D. W.; Burns, M. P.; Kumar, R.; Hotham, I.; Desrosiers, J. N.; Bowles, P.; Jones, K. N.; Nicholson, L. D.; Buetti-Weekly, M. T.; Han, L.; Steflik, J.; Hansen, E.; Hayward, C. M.; Strohmeyer, H.; Monfette, S.; Sutton, S. C.; Morris, C. Application of Biocatalytic Reductive Amination for the Synthesis of a Key Intermediate to a CDK 2/4/6 Inhibitor. *Org. Process Res. Dev.* **2022**, *26*, 879−890.
(12) Simić, S.; Zukić, E.; Schmermund, L.; Faber, K.; Winkler, C. K.; Kroutil, W. Shortening Synthetic Routes to Small Molecule Active Pharmaceutical Ingredients Employing Biocatalytic Methods. *Chem. Rev.* **2022**, *122*, 1052−1126.
(13) Vitaku, E.; Smith, D. T.; Njardarson, J. T. Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles among U.S. FDA Approved Pharmaceuticals. *J. Med. Chem.* **2014**, *57*, 10257−10274.
(14) Truppo, M. D.; Turner, N. J. Micro-Scale Process Development of Transaminase Catalysed Reactions. *Org. Biomol. Chem.* **2010**, *8*, 1280.
(15) Simon, R. C.; Zepeck, F.; Kroutil, W. Chemoenzymatic Synthesis of All Four Diastereomers of 2,6-Disubstituted Piperidines through Stereoselective Monoamination of 1,5-Diketones. *Chem. Eur. J.* **2013**, *19*, 2859−2865.
(16) Simon, R. C.; Richter, N.; Busto, E.; Kroutil, W. Recent Developments of Cascade Reactions Involving ω-Transaminases. *ACS Catal.* **2014**, *4*, 129−143.
(17) France, S. P.; Hussain, S.; Hill, A. M.; Hepworth, L. J.; Howard, R. M.; Mulholland, K. R.; Flitsch, S. L.; Turner, N. J. One-Pot Cascade Synthesis of Mono- and Disubstituted Piperidines and Pyrrolidines Using Carboxylic Acid Reductase (CAR), ω-Transaminase (ω-TA), and Imine Reductase (IRED) Biocatalysts. *ACS Catal.* **2016**, *6*, 3753−3759.
(18) Ryan, J.; Šiaučiulis, M.; Gomm, A.; Maciá, B.; O'Reilly, E.; Caprio, V. Transaminase Triggered Aza-Michael Approach for the Enantioselective Synthesis of Piperidine Scaffolds. *J. Am. Chem. Soc.* **2016**, *138*, 15798−15800.
(19) Wang, Y.; Tappertzhofen, N.; Méndez-Sánchez, D.; Bawn, M.; Lyu, B.; Ward, J. M.; Hailes, H. C. Design and Use of de Novo Cascades for the Biosynthesis of New Benzylisoquinoline Alkaloids. *Angew. Chem., Int. Ed.* **2019**, *58*, 10120−10125.
(20) Taday, F.; Ryan, J.; Argent, S. P.; Caprio, V.; Maciá, B.; O'Reilly, E. Asymmetric Construction of Alkaloids by Employing a Key ω-Transaminase Cascade. *Chem. Eur. J.* **2020**, *26*, 3729−3732.
(21) Roddan, R.; Subrizi, F.; Broomfield, J.; Ward, J. M.; Keep, N. H.; Hailes, H. C. Chemoenzymatic Cascades toward Methylated Tetrahydroprotoberberine and Protoberberine Alkaloids. *Org. Lett.* **2021**, *23*, 6342−6347.
(22) Bernhard, L. M.; McLachlan, J.; Gröger, H. Process Development of Enantioselective Imine Reductase-Catalyzed Syntheses of Pharmaceutically Relevant Pyrrolidines. *Org. Process Res. Dev.* **2022**, *26*, 2067−2074.
(23) Pérez-Martín, C.; Rebolledo, F.; Brieva, R. Amine Transaminase Mediated Synthesis of Optically Pure Piperazinones and 1,4-Diazepanones. *Adv. Synth. Catal.* **2022**, *364*, 1326−1336.
(24) Mangion, I. K.; Sherry, B. D.; Yin, J.; Fleitz, F. J. Enantioselective Synthesis of a Dual Orexin Receptor Antagonist. *Org. Lett.* **2012**, *14*, 3458−3461.
(25) Chung, C. K.; Bulger, P. G.; Kosjek, B.; Belyk, K. M.; Rivera, N.; Scott, M. E.; Humphrey, G. R.; Limanto, J.; Bachert, D. C.; Emerson, K. M. Process Development of C-N Cross-Coupling and Enantioselective Biocatalytic Reactions for the Asymmetric Synthesis of Niraparib. *Org. Process Res. Dev.* **2014**, *18*, 215−227.
(26) Kaulmann, U.; Smithies, K.; Smith, M. E. B.; Hailes, H. C.; Ward, J. M. Substrate Spectrum of W-Transaminase from *Chromobacterium Violaceum* DSM30191 and Its Potential for Biocatalysis. *Enzyme Microb. Technol.* **2007**, *41*, 628−637.
(27) Cerioli, L.; Planchestainer, M.; Cassidy, J.; Tessaro, D.; Paradisi, F. Characterization of a Novel Amine Transaminase from *Halomonas Elongata*. *J. Mol. Catal. B: Enzym.* **2015**, *120*, 141−150.
(28) Contente, M. L.; Planchestainer, M.; Molinari, F.; Paradisi, F. Stereoelectronic Effects in the Reaction of Aromatic Substrates Catalysed by *Halomonas Elongata* Transaminase and Its Mutants. *Org. Biomol. Chem.* **2016**, *14*, 9306−9311.
(29) Pavlidis, I. V.; Weiß, M. S.; Genz, M.; Spurr, P.; Hanlon, S. P.; Wirz, B.; Iding, H.; Bornscheuer, U. T. Identification of (S)-Selective Transaminases for the Asymmetric Synthesis of Bulky Chiral Amines. *Nat. Chem.* **2016**, *8*, 1076−1082.
(30) Meng, Q.; Ramírez-Palacios, C.; Capra, N.; Hooghwinkel, M. E.; Thallmair, S.; Rozeboom, H. J.; Thunnissen, A.-M. W. H.; Wijma, H. J.; Marrink, S. J.; Janssen, D. B. Computational Redesign of an ω-Transaminase from *Pseudomonas Jessenii* for Asymmetric Synthesis of Enantiopure Bulky Amines. *ACS Catal.* **2021**, *11*, 10733−10747.
(31) Höhne, M.; Schätzle, S.; Jochens, H.; Robins, K.; Bornscheuer, U. T. Rational Assignment of Key Motifs for Function Guides in Silico Enzyme Identification. *Nat. Chem. Biol.* **2010**, *6*, 807−813.
(32) Heckmann, C. M.; Gourlay, L. J.; Dominguez, B.; Paradisi, F. An (R)-Selective Transaminase From *Thermomyces Stellatus*: Stabilizing the Tetrameric Form. *Front. Bioeng. Biotechnol.* **2020**, *8*, 707.
(33) Planchestainer, M.; Hegarty, E.; Heckmann, C. M.; Gourlay, L. J.; Paradisi, F. Widely Applicable Background Depletion Step Enables Transaminase Evolution through Solid-Phase Screening. *Chem. Sci.* **2019**, *10*, 5952−5958.
(34) Scott, L. J. Larotrectinib: First Global Approval. *Drugs* **2019**, *79*, 201−206.
(35) Czodrowski, P.; Mallinger, A.; Wienke, D.; Esdar, C.; Pöschke, O.; Busch, M.; Rohdich, F.; Eccles, S. A.; Ortiz-Ruiz, M. J.; Schneider, R.; Raynaud, F. I.; Clarke, P. A.; Musil, D.; Schwarz, D.; Dale, T.; Urbahns, K.; Blagg, J.; Schiemann, K. Structure-Based Optimization of Potent, Selective, and Orally Bioavailable CDK8 Inhibitors Discovered by High-Throughput Screening. *J. Med. Chem.* **2016**, *59*, 9337−9349.
(36) Dawood, A. W. H.; Weiß, M. S.; Schulz, C.; Pavlidis, I. V.; Iding, H.; de Souza, R. O. M. A.; Bornscheuer, U. T. Isopropylamine as Amine Donor in Transaminase-Catalyzed Reactions: Better Acceptance through Reaction and Enzyme Engineering. *ChemCatChem* **2018**, *10*, 3943−3949.

https://doi.org/10.1021/jacsau.3c00103
JACS Au XXXX, XXX, XXX−XXX

(37) Zhou, H.; Zhao, W.; Zhang, T.; Guo, H.; Huang, H.; Chang, M. Enantioselective Synthesis of 2-Substituted Pyrrolidines via Intramolecular Reductive Amination. *Synthesis* **2019**, *51*, 2713−2719.
(38) Węglarz, I.; Michalak, K.; Mlynarski, J. Zinc-Catalyzed Asymmetric Hydrosilylation of Cyclic Imines: Synthesis of Chiral 2-Aryl-Substituted Pyrrolidines as Pharmaceutical Building Blocks. *Adv. Synth. Catal.* **2021**, *363*, 1317−1321.
(39) Zhang, Y.; Kong, D.; Wang, R.; Hou, G. Synthesis of Chiral Cyclic Amines via Ir-Catalyzed Enantioselective Hydrogenation of Cyclic Imines. *Org. Biomol. Chem.* **2017**, *15*, 3006−3012.
(40) Fanourakis, A.; Williams, B. D.; Paterson, K. J.; Phipps, R. J. Enantioselective Intermolecular C-H Amination Directed by a Chiral Cation. *J. Am. Chem. Soc.* **2021**, *143*, 10070−10076.
(41) Shah, M. B.; Liu, J.; Zhang, Q.; Stout, C. D.; Halpert, J. R. Halogen-π Interactions in the Cytochrome P450 Active Site: Structural Insights into Human CYP2B6 Substrate Selectivity. *ACS Chem. Biol.* **2017**, *12*, 1204−1210.
(42) Rajender Reddy, L.; Das, S. G.; Liu, Y.; Prashad, M. A Facile Asymmetric Synthesis of Either Enantiomer of 2-Substituted Pyrrolidines. *J. Org. Chem.* **2010**, *75*, 2236−2246.
(43) Li Petri, G.; Raimondi, M. V.; Spanò, V.; Holl, R.; Barraja, P.; Montalbano, A. *Pyrrolidine in Drug Discovery: A Versatile Scaffold for Novel Biologically Active Compounds*; Springer International Publishing, 2021; Vol. *379* DOI: 10.1007/s41061-021-00347-5.
(44) Fryszkowska, A.; Devine, P. N. Biocatalysis in Drug Discovery and Development. *Curr. Opin. Chem. Biol.* **2020**, *55*, 151−160.
(45) Devine, P. N.; Howard, R. M.; Kumar, R.; Thompson, M. P.; Truppo, M. D.; Turner, N. J. Extending the Application of Biocatalysis to Meet the Challenges of Drug Development. *Nat. Rev. Chem.* **2018**, *2*, 409−421.
(46) Sheludko, Y. V.; Slagman, S.; Gittings, S.; Charnock, S. J.; Land, H.; Berglund, P.; Fessner, W. D. Enantioselective Synthesis of Pharmaceutically Relevant Bulky Arylbutylamines Using Engineered Transaminases. *Adv. Synth. Catal.* **2022**, *364*, 2972−2981.

https://doi.org/10.1021/jacsau.3c00103
JACS Au XXXX, XXX, XXX−XXX