Electronic Supplementary Information

Impact of Aqueous Micellar Media on Biocatalytic Transformations Involving Transaminase (ATA); Applications to Chemoenzymatic Catalysis

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1. General experimental information
   
a. TLC

   Thin layer chromatography (TLC) was performed using Silica Gel 60 F254 plates (Merck, 0.25 mm thick), and visualized with a UV lamp and ninhydrin stain. Flash chromatography was done in glass columns using Silica Gel 60 (EMD, 40-63 μm).

b. NMR

   $^1$H and $^{13}$C NMR spectra were recorded on either a Varian Unity Inova 400 MHz (400 MHz for $^1$H, 100 MHz for $^{13}$C), a Varian Unity Inova 500 MHz (500 MHz for $^1$H, 125 MHz for $^{13}$C), a Varian Unity Inova 600 MHz (600 MHz for $^1$H), Bruker (400 MHz for $^1$H, 100 MHz for $^{13}$C, 376 MHz for $^{19}$F), or Bruker (500 MHz for $^1$H, 125 MHz for $^{13}$C, 471 MHz for $^{19}$F).

   Deuterated NMR solvents were purchased from Cambridge Isotopes Laboratories. DMSO-$d_6$, CD$_3$OD, and CDCl$_3$ were used as solvents. Residual peaks for CHCl$_3$ in CDCl$_3$ ($^1$H = 7.26 ppm, $^{13}$C = 77.00 ppm), (CH$_3$)$_2$SO in (CD$_3$)$_2$SO ($^1$H = 2.50 ppm, $^{13}$C = 39.52 ppm) or MeOH in MeOD ($^1$H = 3.31 ppm, $^{13}$C = 49.00 ppm) have been assigned as internal standards. The chemical shifts are reported in ppm. The coupling constants $J$ value are given in Hz. Data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, bd = broad doublet, t = triplet, q = quartet, quin = quintet, m = multiplet), coupling constant (if applicable) and integration.

c. HRMS

   Mass spectra were obtained from the UC Santa Barbara and UC Irvine Mass Spectrometry Facility.

   LCT-Premier (ESI)

   Mass spectra were acquired via ESI-MS using a Waters LCT Premier mass spectrometer equipped with an Alliance 2695 Separations module. Samples dissolved in methanol were directly infused into the mass spectrometer with no chromatography performed. Accurate mass data was calibrated using sodiated polyethylene glycol or sodiated polyethylene glycol monomethyl ether as an internal standard for positive ions and clusters of sodium formate as an internal standard for negative ions.

   GCT-Premier (GC-EI and GC-CI)

   Mass spectra were acquired via GC-MS using a Waters GCT Premier mass spectrometer equipped with an Agilent 7890A GC oven and J&W Scientific DB-5ms+DG narrow bore column using
helium carrier gas. Samples dissolved in DCM were injected into the GC injector port which was maintained at 260 °C. The GC oven temperature was maintained at 50 °C for one minute then raised to 290 °C at a rate of 10 °C per minute to elute the sample. Accurate mass data were calibrated using perfluorotributylamine or 2,4,6-tris(trifluoromethyl)-1,3,5-triazine as a co-injected standard. Where applicable, methane reagent gas was used to perform chemical ionization (CI) experiments.

d. Aminotransferase (ATA) and PLP cofactor

Aminotransaminase (ATA) Screening Kit containing 24 amine transaminase enzymes and PLP were purchased from Codex® and were used as received. The enzyme powder was stored at -18 °C until use. Purchasing website: https://www.codexis-estore.com/product-page/codex-amine-transaminase-ata-screening-kit.

e. Reagents

Triethanolamine was purchased from Sigma-Aldrich. Ketone reagents were purchased from Sigma-Aldrich, Combi-Blocks, Alfa Aesar, TCI, or Acros Organics and used without further purification. TPGS-750-M was synthesized according to the published procedure1 or was obtained from PHT international. Brij 30, Tween 60, Triton X-100 were purchased from Sigma-Aldrich. PTS 600 was purchased from Cambridge Major Laboratories Inc. Solutol was purchased from Gattefosse.

2. Experimental procedures

a. TPGS-750-M preparation

**DL-α-Tocopherol Succinate.** To a solution of DL-α-Tocopherol (4.30 g, 10.00 mmol) and succinic anhydride (1.50 g, 15.00 mmol) in toluene (20 mL) was added Et3N (0.35 mL, 2.50 mmol) at 22 °C with stirring, and the stirring was continued at 60 °C for 5 h. Water was added to the reaction mixture, which was then extracted with CH2Cl2. The combined organic layers were washed with 1 N HCl (3 x 50 mL) and water (2 x 30 mL), dried over anhydrous Na2SO4, and concentrated in vacuo affording a yellow liquid, which was purified by flash column chromatography on silica gel eluting with a 10% EtOAc/ hexane to 35% EtOAc/hexanes gradient.
to afford DL-R-tocopherol succinate (5.25 g, 99%) as a white solid, mp 64-67°C. IR (neat) 2926, 1757, 1714, 1576, 1455, 1415, 1377, 1251, 1224, 1151, 1078, 926 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 2.94 (t, J = 6.8 Hz, 2H), 2.84 (t, J = 6.8 Hz, 2H), 2.59 (t, J = 6.8 Hz, 2H), 2.09 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.85-1.71 (m, 2H), 1.56-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.88-0.84 (m, 12H).¹³C NMR (100 MHz, CDCl₃) δ 178.6, 171.0, 149.7, 140.7, 126.9, 125.1, 123.2, 117.6, 75.2, 39.6, 37.8, 37.7, 37.6, 37.5, 33.0, 32.9, 31.3, 29.2, 28.8, 28.2, 25.0, 24.6, 24.0, 22.9, 22.8, 21.2, 20.8, 19.95, 19.88, 13.0, 12.2, 12.0; MS ESI+ m/z 554 [M + Na]⁺. HRMS ESI+ m/z calcd for C₃₃H₅₄O₅Na [M + Na]⁺: 553.3869; found: 553.3876.

**TPGS-750-M.** A mixture containing DL-R-tocopherol succinate (2.97 g, 5.60 mmol), poly(ethylene glycol) monomethylether-750 (4.00 g, 5.33 mmol) and p-TsOH (0.15 g, 0.79 mmol) in toluene (20 mL) was refluxed for 5 h using a Dean-Stark trap. After cooling to rt, the mixture was poured into saturated aqueous NaHCO₃ solution and extracted with CH₂Cl₂. The combined organic layers were washed with saturated NaHCO₃ (3 x 50 mL), brine (2 x 30 mL), dried over anhydrous Na₂SO₄, and concentrated in vacuo to afford TPGS-750-M (6.60 g, 98%) as a waxy solid. IR (neat) 2888, 1755, 1739, 1465, 1414, 1346, 1281, 1245, 1202, 1109, 947, 845 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 4.28-4.26 (m, 2H), 3.71-3.54 (m, PEG), 3.38 (s, 3H), 2.93 (t, J = 7.2 Hz, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.58 (t, J = 6.8 Hz, 2H), 2.08 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.84-1.70 (m, 2H), 1.55-1.04 (m, 24H), 0.87-0.83 (m, 12H).¹³C NMR (100 MHz, CDCl₃) δ 172.2, 170.9, 149.5, 140.6, 126.7, 125.0, 123.0, 117.4, 94.5, 75.1, 72.0, 70.64, 70.56, 69.1, 64.0, 59.0, 39.4, 37.6, 37.5, 37.4, 37.3, 32.8, 32.7, 31.1, 29.2, 28.9, 28.0, 24.8, 24.5, 22.8, 22.7, 21.1, 20.6, 19.8, 19.7, 13.0, 12.1, 11.8; MS (ESI) m/z 1272 [M + Na]⁺

b. **Surfactant solution preparation**

TPGS-750-M is also commercially available from Sigma-Aldrich (catalog number 733857). The 2 wt% TPGS-750-M aqueous solution was prepared by mixing TPGS-750-M wax (10.0 g) with HPLC grade water (490.0 g), and stir until dissolved.

c. **Triethanolamine buffer solution preparation**

To a 500 mL beaker equipped with a magnetic stirrer, HPLC grade water (200.0 mL), triethanolamine (5.0 g), and isopropylamine (18.0 g) were added. Under gentle stirring at rt, concentrated HCl was added dropwise. After pH reached 8.5 (indicated by a pH meter), the total
volume was then adjusted to 300.0 mL by adding HPLC grade water. The buffer solution was stored at 4 °C until use.

d. 2 wt % TPGS-750-M in triethanolamine buffer solution preparation

To a 500 mL beaker equipped with a magnetic stirrer, triethanolamine (5.0 g), 2 wt % TPGS-750-M aqueous solution (200.0 mL), and isopropylamine (18.0 g) were added. Under gentle stirring at room temperature, concentrated HCl was added dropwise, monitoring the pH by a pH meter. After the pH reached 8.5, the total volume was then adjusted to 300.0 mL by adding 2 wt % TPGS-750-M aqueous solution in a graduate cylinder. The buffer solution was stored under 4 °C until use.

Alternative Method: Desired amount (2 wt %, 4 wt %, or 6 wt %) of non-ionic surfactant (TPGS-750-M, solutol, PTS-600, or Brij 30, Tween 60 or Triton X-100) was weighed into a beaker, then stirred in triethanolamine buffer solution (pH 8.5) until surfactant dissolved.

e. Conversion monitoring in buffer and TPGS-750-M/buffer

To evaluate the impact of TGPS-750-M on the conversion of different ketone substrates by transaminase, comparative monitoring has been performed. There is one time monitoring per vial.
(t = 30 min, 1 h, 3 h, 5 h, and 24 h). To each of the five 5 mL vials equipped with a magnetic stir bar was added the ketone (0.01 mmol). Triethanolamine buffer at pH = 8.5 (1.0 mL) (with or without 2 wt % TPGS-750-M) was then added. Each reaction vial was capped by a screw cap and stirred at 50 °C for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP) (0.266 mg/mL or 1 mM), and transaminase (1.0 mg) were then added sequentially and stirred vigorously (1000 rpm) at 50 °C.

After the desired time is reached, the reaction mixture was basified with 5 N NaOH (~0.30 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (1.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (3.0 mL x 1) and dried over anhydrous MgSO₄. The conversion was determined by ¹H NMR.

Example using the NMR spectrum to determine the extent of conversion of the nonracemic amine 8 after 24 h (7.42 ppm (t) → 7.36 ppm (t)).
f. Screening of the aqueous reaction medium involving various surfactants.

Ketone substrate (0.01 mmol) was added to a 5 mL vial equipped with a magnetic stir bar. Triethanolamine buffer at pH = 8.5 (1.0 mL) (with or without 2, 4, 6 wt % surfactant) was then added and stirred at 50 °C for 2 min to afford an emulsified solution. Pyridoxal 5'-phosphate (PLP) (0.266 mg/mL or 1 mM), and transaminase (1.0 mg) was then added sequentially and stirred vigorously (1000 rpm) at 50 °C. The reaction vial was capped with a screw cap, and after 24 h, the reaction mixture was basified with 5 N NaOH (~0.30 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (1.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (3.0 mL x 1) and dried over anhydrous MgSO₄. The conversion was determined by ¹H NMR.

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\[ \text{Table 1. Screening of the aqueous reaction medium involving various surfactants. Conversions were determined by } \text{¹H NMR. Reaction conditions: ketone substrate (0.01 mmol), isopropyl amine (1.3 M), PLP (1.0 mM), ATA (1.0 mg), aqueous buffer pH = 8.5 (1.0 mL), 50 °C, 24 h. } \text{³ TPGS-750-M (6 wt %) / triethanolamine (TEA) buffer was used.} \]
```

<table>
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<th>Substrate</th>
<th>Enzyme</th>
<th>Buffer only</th>
<th>2 wt % TPGS-750-M/buffer</th>
<th>2 wt % Solutol/buffer</th>
<th>4 wt % Solutol/buffer</th>
<th>6 wt % PTS600/buffer</th>
<th>2 wt % Brij30/buffer</th>
<th>2 wt % Triton X-100/buffer</th>
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</tbody>
</table>

g. Synthesis of nonracemic amines using transaminases

The ketone substrate (0.10 mmol) was added to a 20 mL vial equipped with a magnetic stir bar. A solution of 2 wt % TPGS-750-M in a triethanolamine buffer (10 mL, pH = 8.5) was then added and the mixture stirred at 50 °C for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP) (0.266 mg/mL or 1 mM), and transaminase (10 mg) were then added sequentially. The reaction vial was capped with a screw cap and stirred vigorously (1000 rpm) at 50 °C.
<table>
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<th>Chemical species</th>
<th>Concentration</th>
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<td>ketone substrate</td>
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<tr>
<td>triethanolamine</td>
<td>142.5 mM</td>
</tr>
<tr>
<td>isopropylamine</td>
<td>1.3 M</td>
</tr>
<tr>
<td>PLP</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>ATA</td>
<td>10 mg per 0.1 mmol ketone</td>
</tr>
</tbody>
</table>

Workup: The reaction mixture was basified with 5 N NaOH (~1.5 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (5.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (30 mL x 1) and dried over anhydrous MgSO₄. Volatiles were evaporated under reduced pressure. The residue was analyzed by NMR, HRMS, and HPLC.

Some amine products were readily isolated and directly subjected to chiral HPLC analysis. Others, however, due to their lack of stability (e.g., air oxidation) or solubility issues (e.g., in water) required derivatization to facilitate their isolation and characterization.

Cbz protection: To a stirred solution of the crude product in DCM (1.0 mL) was added sodium carbonate (12 mg, 0.30 mmol, 3 equiv), and benzyl chloroformate (85.3 mg, 70.5 µL, 0.50 mmol, 5.0 equiv). Upon completion of the reaction, the solvents were evaporated. The Cbz-protected amines were purified via column chromatography with 0-10% EtOAc/hexane.

Acetyl derivatization: To a stirred solution of the extracted crude product in DCM (1.0 mL) were added triethylamine (30.3 mg, 41.8 µL, 0.30 mmol, 3 equiv) and Ac₂O (30.6 mg, 20.4 µL, 0.30 mmol, 3.0 equiv). Upon completion of the reaction, the solvents were evaporated. Purification of the crude material was done via flash chromatography.
h. General procedures for the synthesis of racemic amines

General procedure A was employed for preparation of the corresponding racemic amines $14_{\text{rac}}$ and $36_{\text{rac}}$. General procedure B was used for the corresponding racemic amines $8_{\text{rac}}, 10_{\text{rac}}, 12_{\text{rac}}, 13_{\text{rac}}, 16_{\text{rac}}, 17_{\text{rac}}, 18_{\text{rac}}, 20_{\text{rac}}, 21_{\text{rac}}, 22_{\text{rac}}, 23_{\text{rac}}, 24_{\text{rac}}, 25_{\text{rac}}, 26_{\text{rac}}$, and $31_{\text{rac}}$ ($\text{rac} = \text{racemic}$). The protecting group installation procedures are the same for both the racemic and nonracemic amines.

- Procedure A

Ammonium formate (5 equiv) and ketone (1.0 mmol, 1.0 equiv) were added to a 4 mL vial equipped with a magnetic stir bar. $[\text{Ir(COD)Cl}_2]^2$ (1.0 mg, 0.50 mol %) was added in a glove box. Methanol was added via syringe under argon. The vial was sealed and refluxed overnight. Upon completion, the resulting mixture was extracted with EtOAc (1 v x 5). The organic layers were separated and washed with 1 N HCl (1 v x 3). The aqueous layers were combined, basified with 5 N NaOH to pH 12–13 and extracted with EtOAc (1 v x 4). The separated organic layer was dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. Volatiles were evaporated under reduced pressure and analyzed by NMR.

- Procedure B

To a solution of ketone (1.0 mmol, 1.0 equiv) in methanol was added ammonium acetate (0.15 g, 2.0 mmol, 2.0 equiv) and sodium cyanoborohydride (0.31 g, 5.0 mmol, 5.0 equiv). The resulting mixture was stirred at 60 °C. (Caution: gas evolution) After 12 h, the reaction mixture was basified with 5 N NaOH to pH 12–13 (indicated by pH paper). The resulting mixture was extracted with EtOAc (1 v x 5). The organic layers were separated and washed with 1 N HCl (1 v x 3). The aqueous layers were combined, basified with 5 N NaOH to pH 12–13 and extracted with EtOAc (1 v x 4). The separated organic layer was dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. The racemic amine was analyzed by NMR.
i. Experimental procedure for a 3-step sequence (DMP/ATA/CbzCl)

To a 4 mL vial equipped with a magnetic stirrer, 1-(4-bromophenyl)ethan-1-ol (20 mg, 0.10 mmol, 1 equiv), Dess-Martin periodinane (63.6 mg, 0.15 mmol, 1.5 equiv) and 2 wt % TPGS-750-M (0.2 mL) were added. The reaction vial was capped with a screw cap. The mixture was stirred vigorously at 45 °C until complete consumption of the starting material monitored by TLC. The reaction solution was then transferred to a 20 mL vial equipped with a magnetic stir bar, 2 wt % TPGS-750-M/triethanolamine buffer (9.8 ml, pH = 8.5), PLP (2.6 mg), and ATA-260 (5.0 mg) was added sequentially and stirred (1000 rpm) at 50 °C for 21 h. The reaction mixture was basified with 5 N NaOH (~1.5 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (5 mL x 5). The organic layer was separated with the aid of a centrifuge (low speed for 5 min). The combined organic phases were collected and dried over anhydrous MgSO₄. Volatiles were evaporated under reduced pressure. To a stirred solution of the crude product in 2 wt % TPGS-750-M (0.2 mL), sodium hydroxide (12.0 mg, 0.30 mmol, 3.0 equiv), and benzyl chloroformate (85.3 mg, 70.5 µL, 0.50 mmol, 5.0 equiv) were added and stirred at rt. The reaction mixture was then loaded on silica gel and purified by flash column chromatography to yield (S)-benzyl (1-(4-bromophenyl)ethyl)carbamate (28.7 mg, 86% yield, >99%ee, Rₜ = 0.43, 10% EtOAc/hexanes) as a white solid.
j. Experimental procedure for 1-pot sequence (ERED/ATA/S\textsubscript{N}Ar)

To a 20 mL vial equipped with a magnetic stirrer, (E)-3-methyl-4-phenylbut-3-en-2-one (20 mg, 0.127 mmol, 1.0 equiv), ERED-103 (40 mg), GDH-105 (8 mg), glucose (46 mg, 0.26 mmol, 2.0 equiv), and NADP\textsuperscript{+} (2 mg) were suspended in 2 wt% of TPGS-750-M in phosphate buffer (pH = 7, 4 mL). The reaction was stirred at 35 °C for 12 h. The reaction progress was monitored by \(^1\text{H} \) NMR. After the reaction reached completion, the substrate concentration was adjusted to 10 mM by adding 8.72 mL of triethanolamine buffer solution (triethanolamine, [118 mM], pH = 8.5). ATA-256 (50 mg) was then added and stirred (1000 rpm) at 50 °C for 21 h. The reaction progress was monitored by \(^1\text{H} \) NMR. After completion, potassium phosphate tribasic monohydrate (29 mg, 0.127 mmol, 1 equiv) and 2,4,5-trichloropyrimidine (23 µL, 0.254 mmol, 2.0 equiv,) were added and the mixture was stirred at 45 °C for 21 h. The resulting mixture was extracted with EtOAc (10 mL x 5), and the combined organic extracts were dried over anhydrous MgSO\textsubscript{4}. The volatiles were evaporated under reduced pressure and the crude residue was purified by flash column chromatography (0 to 25% EtOAc/hexane) to yield 2,5-dichloro-N-((2R,3S)-3-methyl-4-phenylbutan-2-yl)pyrimidin-4-amine. (60.5 mg, 99% yield, >99% ee, R\textsubscript{f} = 0.36, 10% EtOAc/hexane).
k. Experimental procedure for (S)-rivastigmine

To a 5 mL vial equipped with a magnetic stir bar was added 3'-hydroxyacetophenone (0.1 mmol, 1 equiv), triethylamine (0.2 mmol, 2 equiv), and N-ethyl-N-methylcarbamoyl chloride (0.2 mmol, 2 equiv) was added sequentially. The reaction was stirred at 55 °C for 4 h. After completion of the reaction (monitored by $^1$H NMR), 2 wt % TPGS-750-M/triethanolamine buffer (pH 8.5) was added in (3.3 mL x 3) and transferred to a 20 mL vial. To the stirred resulting reaction mixture, PLP (2.6 mg) and ATA-256 (5 mg) were added sequentially and heated to 50 °C and stirred for 21 h. After completion of the reaction, the solution was basified to pH 12–13 and extracted with EtOAc (3 x 4 mL). The combined organic layers were evaporated to provide crude amine intermediate. To the stirred solution of crude amine intermediate in 2 wt % TPGS-750-M (0.5 mL) was added formaldehyde (37% in water; 0.8 mmol, 8 equiv, 65.0 uL) and NaBH(OAc)$_3$ (0.6 mmol, 0.6 equiv). The reaction was allowed to stirred at rt for 10 h. After completion, the mixture was loaded on silica gel and purified by flash column chromatography (2 v/v % MeOH in DCM + 1 v/v% Et$_3$N) to provide (S)-rivastigmine (23.7 mg, 95% yield, >99% ee) as a colorless oil.
3. Supplementary Tables
   a. E Factor calculation details presented in Table 2

TABLE 2. COMPARISON OF LITERATURE ROUTES TO (S)-RIVASTIGMINE.

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<th>no. of pots</th>
<th>total time [h]a</th>
<th>yield [%]b</th>
<th>ee [%]c</th>
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*Sum of reaction time of each step. Overall yield was considered. Represents the % ee of (S)-rivastigmine. Mass of organic solvents used in the reaction and workup divided by the mass of product. The unusually high E Factor calculated up to the point of final purification (as this is highly variable) is attributed to the EtOAc needed in this particular case to extract amine from the ATA reaction mixture; recovery and recycling of this solvent was not attempted.

**Entry 1:**
Overall Yield: 0.99*0.94*0.97*0.91 = 82%  
>96% ee  
Reaction Time: 4+.3+20+17+8 = 49.3 h  
Acetone: 130 ml / 0.784 g/ml = 165.816 g  
CH₂Cl₂: 10 ml / 1.33 g/ml = 7.519 g  
MeOH: 8 ml / 0.792 g/ml = 10.101 g  
Total waste: 183.436 g  
Product: 66 mg  
E Factor: 2779

**Entry 2:**
Overall Yield: = 0.85*0.82*.92 = 64%  
99% ee  
Reaction Time: 0.25+1+0.25+0.5+3+16+3+0.5+1+4 = 29.5 h  
EtOH: 157 L / 0.789 g/ml = 198.986 kg  
EtOAc: 736 L / 0.902 g/ml = 815.964 kg  
THF: 135 L / 0.889 g/ml = 151.856 kg  
Heptane: 50 L / 0.684 g/ml = 73.099 kg  
Total waste: 1239.905 kg  
Product: 4.6 kg  
E Factor: 269.544
Entry 3:
Overall Yield: $0.98 \times 0.92 \times 0.93 \times 0.95 \times 0.98 = 82\%$
$>96\%$ ee
Reaction Time: $3 \text{ d} + 2 \text{ d} + 40 \text{ h} + 12 \text{ h} + 5 \text{ h} = 177\text{ h}$
EtOH: $20 \text{ ml} / 0.789 \text{ g/ml} = 25.348 \text{ g}$
mesitylene: $7 \text{ ml} / 0.864 \text{ g/ml} = 8.102 \text{ g}$
Et$_2$O: $10 \text{ ml} / 0.713 \text{ g/ml} = 12.399 \text{ g}$
EtOAc: $90 \text{ ml} / 0.902 \text{ g/ml} = 99.778 \text{ g}$
THF: $10 \text{ ml} / 0.889 \text{ g/ml} = 11.249 \text{ g}$
Total waste: $156.876 \text{ g}$
Product: $48 \text{ mg}$
E Factor: 3268

Entry 5:
Overall Yield: $0.86 \times 0.80 \times 0.99 \times 0.92 \times 0.97 = 61\%$
$>99\%$ ee
EtOAc: $147 \text{ ml} / 0.902 \text{ g/ml} = 162.971 \text{ g}$
THF: $6 \text{ ml} / 0.889 \text{ g/ml} = 6.748 \text{ g}$
CH$_2$Cl$_2$: $2.4 \text{ ml} / 1.33 \text{ g/ml} = 1.804 \text{ g}$
Total waste: $171.523 \text{ g}$
Product: $28 \text{ mg}$
E-factor: 6125

Entry 6:
Overall Yield: $0.89 \times 0.76 \times 1 = 66\%$
$99\%$ ee
Reaction Time: $5 + 24 + 16.5 = 45.5 \text{ h}$
THF: $100 \text{ ml} / 0.889 \text{ g/ml} = 112.486 \text{ g}$
EtOAc: $130 \text{ ml} / 0.902 \text{ g/ml} = 144.124 \text{ g}$
CH$_2$Cl$_2$: $6 \text{ ml} / 1.33 \text{ g/ml} = 4.511 \text{ g}$
Total waste: $261.121 \text{ g}$
Product: $72 \text{ mg}$
E-factor: 3626

Entry 7:
Overall Yield: $0.99 \times 0.94 \times 0.97 \times 0.91 = 82\%$
$>96\%$ ee
Reaction Time: $4 + 21 + 10 = 35 \text{ h}$
EtOAc: $12 \text{ ml} / 0.902 \text{ g/ml} = 13.3 \text{ g}$
Total waste: $13.3 \text{ g}$
Product: $23.7 \text{ mg}$
E-factor: 561
b. Screening of transaminases in aqueous buffer

Table S1. Screening of ATAs in aqueous buffer

The ketone substrate (0.01 mmol) was added to a 4 mL vial equipped with a magnetic stir bar. Triethanolamine buffer at pH = 7.5 (1 mL) was then added and stirred at 30 °C for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP) (0.266 mg/mL or 1 mM), and transaminase (1.0 mg) was then added sequentially and stirred vigorously (1000 rpm) under 30 °C. The reaction vial was capped with a screw cap, and after 24 h, the reaction mixture was basified with 5 N NaOH (~0.30 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (1.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (3.0 mL x 1) and dried over anhydrous MgSO₄. The conversion was determined by ¹H NMR.
c. Optimization of temperature and pH for transamination in aqueous buffer

![Chemical structure](image)

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*Table S2. Optimization of temperature and pH effect for transamination in aqueous buffer*

The ketone substrate (0.01 mmol) was added to a 4 mL vial equipped with a magnetic stir bar. Triethanolamine buffer at pH = 7.5 or 8.5 (1 mL) was then added and stirred at 30 °C, 40 °C or 50 °C for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP) (0.266 mg/mL or 1 mM), and transaminase (1.0 mg) was then added sequentially and stirred vigorously (1000 rpm) under 30 °C, 40 °C, or 50 °C. The reaction vial was capped with a screw cap, and after 24 h, the reaction mixture was basified with 5 N NaOH (~0.30 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (1.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (3.0 mL x 1) and dried over anhydrous MgSO₄. The conversion was determined by ¹H NMR.
d. Concentration effect of transamination in aqueous buffer vs. in surfactant for 24 h

\[
\text{Concentration effect of transamination in aqueous buffer vs surfactant for 24 h}
\]

\[
\text{Table S3. (a) Concentration effect of transamination in aqueous buffer vs surfactant for 24 h; (b) time screening of transamination in 2 wt % TPGS-750-M/buffer (0.01 M)}
\]

To evaluate the concentration effect of transamination in aqueous buffer vs. that in surfactant, a triethanolamine buffer solution was prepared for each concentration (0.01 M, 0.02 M, and 0.04 M).

- For a concentration of 0.01 M
To a 50 mL beaker equipped with a magnetic stirrer, triethanolamine (0.5 g), 2 wt % TPGS-750-M aqueous solution or aqueous solution (20.0 mL), and isopropylamine (1.80 g) were added. Concentrated HCl was added dropwise under gentle stirring at rt, monitoring the pH with a pH meter. After the pH reached 8.5, the total volume was then adjusted to 30.0 mL by adding 2 wt % TPGS-750-M aqueous solution or water in a graduated cylinder. The buffer solution was stored at 4 °C until use.
The ketone substrate (0.04 mmol) was added to a 5 mL vial equipped with a magnetic stir bar. Triethanolamine buffer at pH = 8.5 (4 mL), with or without 2 wt % surfactant, was then added and stirred at 50 ºC for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP) (0.266 mg/mL), and transaminase (4.0 mg) were then added sequentially and the reaction mixture was stirred vigorously (1000 rpm) under 50 ºC. The reaction vial was capped with a screw cap, and after 24 h, the reaction mixture was basified with 5 N NaOH (~0.60 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (2.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (5.0 mL x 1) and dried over anhydrous MgSO₄. The conversion was determined by ¹H NMR.

For a concentration of 0.02 M

To a 50 mL beaker equipped with a magnetic stirrer, triethanolamine (0.25 g), 2 wt % TPGS-750-M aqueous solution or aqueous solution (10.0 mL), and isopropylamine (1.80 g) were added. Concentrated HCl was added dropwise under gentle stirring at rt, monitoring the pH with a pH meter. After the pH reached 8.5, the total volume was then adjusted to 15.0 mL by adding 2 wt % TPGS-750-M aqueous solution or water in a graduated cylinder. The buffer solution was stored at 4 ºC until use.

Ketone substrate (0.04 mmol) was added to a 5 mL vial equipped with a magnetic stir bar. Triethanolamine buffer at pH = 8.5 (2 mL; with or without 2 wt % surfactant) was then added and the reaction mixture stirred at 50 ºC for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP; 0.53 mg/mL) and transaminase (4.0 mg) were then added sequentially and stirred vigorously (1000 rpm) under 50 ºC. The reaction vial was capped with a screw cap, and after 24 h, the reaction mixture was basified with 5 N NaOH (~0.60 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (2.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (5.0 mL x 1) and dried over anhydrous MgSO₄. The conversion was determined by ¹H NMR.

For a concentration of 0.04 M

To a 25 mL beaker equipped with a magnetic stirrer, triethanolamine (0.125 g), 2 wt % TPGS-750-M aqueous solution or aqueous solution (5.0 mL), and isopropylamine (1.80 g) were added.
Concentrated HCl was added dropwise under gentle stirring at rt, monitoring the pH using a pH meter. After the pH reached 8.5, the total volume was then adjusted to 7.5 mL by adding 2 wt % TPGS-750-M aqueous solution or water in a graduated cylinder. The buffer solution was stored at 4 °C until use.

The ketone substrate (0.04 mmol) was added to a 5 mL vial equipped with a magnetic stirrer. Triethanolamine buffer at pH = 8.5 (1 mL), with or without 2 wt % surfactant, was then added and stirred at 50 °C for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP) (1.07 mg/mL), and transaminase (4.0 mg) was then added sequentially and stirred vigorously (1000 rpm) under 50 °C. The reaction vial was capped with a screw cap, and after 24 h, the reaction mixture was basified with 5 N NaOH (~0.60 mL) to pH 12–13 (indicated by a pH indicator paper). The resulting mixture was extracted with EtOAc (2.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 minutes. The combined organic phases were collected and washed with distilled water (5.0 mL x 1) and dried over anhydrous MgSO4. The conversion was determined by 1H NMR.

e. **Time screening of transamination in 2 wt % TPGS-750-M/buffer**

The ketone substrate (0.04 mmol) was added to a 5 mL vial equipped with a magnetic stir bar. A solution of 2 wt % TPGS-750-M in a triethanolamine buffer at pH = 8.5 (4 mL) was then added and stirred at 50 °C for 2 min to afford an emulsified solution. Pyridoxal 5’-phosphate (PLP; 0.266 mg/mL or 1 mM), and transaminase (4.0 mg) were then added sequentially and stirred vigorously (1000 rpm) under 50 °C. The reaction vial was capped with a screw cap, and after 24 h, 48 h, or 72 h, the reaction mixture was basified with 5 N NaOH (~0.60 mL) to pH 12–13 (indicated by pH indicator paper). The resulting mixture was extracted with EtOAc (2.0 mL x 5). The organic layer was separated with the aid of a low-speed centrifuge for 5 min. The combined organic phases were collected and washed with distilled water (5.0 mL x 1) and dried over anhydrous MgSO4. The conversion was determined by 1H NMR.
4. Product characterization (NMR, HRMS, and chirality assessment)

\begin{center}
\includegraphics[width=1.0\textwidth]{product_structure}
\end{center}

Benzyl (S)-(1-(6-(2-fluoro-3-(trifluoromethyl)phenyl)pyridin-2-yl)ethyl)carbamate 8

Following the general procedure with enzyme ATA-256, the product 16 was obtained as a white solid, 35.6 mg from a 0.1 mmol batch, 85% yield, 97% ee. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.19 (t, $J = 7.5$ Hz, 1H), 7.76 (t, $J = 7.7$ Hz, 1H), 7.72 (ddd, $J = 7.9$, 2.5, 1.2 Hz, 1H), 7.66 (t, $J = 7.2$ Hz, 1H), 7.40 – 7.25 (m, 7H), 5.99 (d, $J = 7.8$ Hz, 1H), 5.18 – 5.08 (m, 2H), 5.01 (t, $J = 7.1$ Hz, 1H), 1.54 (d, $J = 6.8$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 161.4, 158.7, 156.7, 155.7, 151.1, 137.4, 136.5, 135.1, 128.6, 128.5, 128.2, 128.1, 127.5, 127.0, 124.2, 123.3, 123.2, 121.6, 120.5, 66.7, 51.6, 22.9. $^{19}$F NMR (471 MHz, CDCl$_3$) $\delta$ -61.26, -119.48. HRMS TOF MS EI+ $m/z$ calcd C$_{22}$H$_{18}$F$_4$N$_2$O$_2$ [M]$^+$: 418.1304; found 418.1314. The enantioselectivity was determined by HPLC analysis Chiralcel® 5 µm OD-H column 150 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) $t_1 = 6.697$ min (minor) $t_2 = 7.296$ min (major).

(R)-Benzyl (1-(4-(trifluoromethyl)phenyl)ethyl)carbamate 10

Following the general procedure with enzyme ATA-025, the product was obtained as a yellow oil, 29.1 mg from a 0.1 mmol batch, 88% yield, 79% ee. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.50 (d, $J = 8.0$ Hz, 2H), 7.33 (d, $J = 8.1$ Hz, 7H), 5.07 – 4.89 (m, 3H), 4.86 – 4.75 (m, 1H), 1.40 (d, $J = 7.0$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 155.5, 136.2, 129.7, 128.5, 128.2, 126.2, 125.7, 125.6, 125.6, 125.4, 66.9, 50.5, 22.5. $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.49. HRMS TOF MS EI+ $m/z$ calcd C$_{17}$H$_{16}$F$_3$NO$_2$ [M+Na]$^+$: 346.1031; found 346.1031. The enantioselectivity was determined by HPLC analysis (Phenomenex® 3 µm Lux Cellulose-2 column 150 x 2 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) $t_1 = 0.416$ min (minor) $t_2 = 0.873$ min (major).
(S)-E-4-Phenylbut-3-en-2-amine 12

Following the general procedure with enzyme ATA-260, the product was obtained as a yellow oil, 90 mg from a 120 mg batch, 75% yield, >99% ee. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.01 (s, 2H), 7.44 – 7.27 (m, 5H), 6.69 (d, $J$ = 16.0, 1H), 6.28 – 6.22 (m, 1H), 3.98 (s, 1H), 1.34 (d, $J$ = 6.8 Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 135.5, 134.7, 128.6, 126.9, 125.4, 50.1, 19.2. The enantioselectivity was determined by HPLC analysis (Chiralcel OD-H 250 mm x 4.6 mm, 5 $\mu$m column, $n$-hexane : 0.1% TFA in methanol = 95:5, flow rate 1.0 mL/min) $t_1$ = 18.520 min (major). Spectral data matched those previously reported.3

(S), E-Benzyl-(3-methyl-4-phenylbut-3-en-2-yl)carbamate 13

Following the general procedure with enzyme ATA-256, the product was obtained as a white solid, 19.2 mg from a 0.1 mmol batch, 65% yield, >99% ee. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.47 – 7.28 (m, 7H), 7.21 (q, $J$ = 7.2 Hz, 3H), 6.46 (s, 1H), 5.25 – 5.04 (m, 2H), 4.84 (s, 1H), 4.35 (s, 1H), 1.85 (s, 3H), 1.34 (d, $J$ = 6.9 Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 155.5, 138.9, 137.6, 136.6, 129.0, 128.5, 128.1, 128.0, 126.4, 124.9, 66.7, 53.7, 20.1, 14.8. HRMS TOF MS EI+ m/z calcd C$_{19}$H$_{21}$NO$_2$ [M]$^+$: 295.1572; found 295.1564. The enantioselectivity was determined by HPLC analysis (Chiralcel® 5 $\mu$m OD-H column 150 x 4.6 mm, isopropanol : $n$-hexane = 10:90, flow rate 1.0 mL/min) $t_1$ = 10.611 min (major).

(S)-2-Ethylhexyl (E)-3-(4-(1-(((benzyloxy)carbonyl)amino)ethyl)phenyl)acrylate 14
Following the general procedure with enzyme ATA-256, the product was obtained as a colorless oil, 15.5 mg from a 0.1 mmol batch, 45% yield, >99% ee. **$^1$H NMR** (500 MHz, CDCl₃) δ 7.65 (d, $J$ = 16.0 Hz, 1H), 7.50 (dd, $J$ = 8.2, 2.4 Hz, 2H), 7.33 (d, $J$ = 8.1 Hz, 2H), 6.42 (d, $J$ = 16.0 Hz, 1H), 5.63 (d, $J$ = 7.9 Hz, 1H), 5.15 – 5.10 (m, 1H), 4.19 – 4.07 (m, 2H), 2.00 (s, 3H), 1.49 (d, $J$ = 7.0 Hz, 3H), 1.41 (m, 3H), 0.95 – 0.82 (m, 12H).

**$^{13}$C NMR** (101 MHz, CDCl₃) δ 168.7, 158.7, 145.4, 143.9, 133.7, 128.4, 126.7, 118.4, 67.4, 48.6, 38.9, 30.5, 29.7, 29.0, 23.8, 23.5, 23.0, 21.7, 14.0, 11.0. **TOF MS** CI+ $m/z$ calcd C₂₁H₃₂NO₃ [M⁺]: 345.2304; found 345.2313. The enantioselectivity of the unprotected primary amine was determined by HPLC analysis (Phenomenex® 5 µm Lux Cellulose-1 column 250 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) $t_1$ = 6.333 min (major).

![Chemical Structure](image)

**(S)-Benzyl (1-(4-iodophenyl)ethyl)carbamate 16**

Following the general procedure with enzyme ATA-260, the product was obtained as a white solid, 28.6 mg from a 0.1 mmol batch, 75% yield, >99% ee. **$^1$H NMR** (400 MHz, CDCl₃) δ 7.65 (d, $J$ = 8.0 Hz, 2H), 7.34 (s, 5H), 7.06 (d, $J$ = 7.9 Hz, 2H), 5.15 – 4.99 (m, 3H), 4.86 – 4.71 (m, 1H), 1.44 (d, $J$ = 7.0 Hz, 3H). **$^{13}$C NMR** (101 MHz, CDCl₃) δ 155.4, 143.3, 137.7, 136.3, 128.5, 128.2, 127.9, 92.6, 66.8, 50.3, 22.4. **HRMS TOF MS** EI+ $m/z$ calcd C₁₆H₁₆INO₂ Na [M+Na]⁺: 404.0124; found 404.0128. The enantioselectivity was determined by HPLC analysis (Phenomenex® 5 µm Lux Cellulose-1 column 250 x 4.6 mm, isopropanol: n-hexane = 10:90, flow rate 1.0 mL/min) $t_1$ = 13.488 min (major).

![Chemical Structure](image)

**(S)-Benzyl(1-(4-chlorophenyl)ethyl)carbamate 17**

Following the general procedure with enzyme ATA-260, the product was obtained as a white solid 24.0 mg from a 0.1 mmol batch, 81% yield, >99% ee. **$^1$H NMR** (400 MHz, CDCl₃) δ 7.38 – 7.19 (m, 9H), 5.15 – 5.02 (m, 2H), 4.98 (s, 1H), 4.88 – 4.75 (m, 1H), 1.45 (d, $J$ = 6.9 Hz, 3H). **$^{13}$C NMR** (101 MHz, CDCl₃) δ 156.1, 141.1, 136.3, 133.0, 128.8, 128.5, 128.2, 127.3, 66.8, 50.8, 22.4. **HRMS TOF**
MS EI+ m/z calcd C_{18}H_{16}ClNO_{2} Na [M+Na]^+: 312.0767; found 312.0767. The enantioselectivity was determined by HPLC analysis (Chiralcel® 5 µm OD-H column 150 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) t\textsubscript{1} = 9.987 min (major).

\[(S)-\text{Benzyl (1-}(4-\text{hydroxyhex-1-yn-1-yl)}\text{phenyl)ethyl)carbamate 19}\]

Following the general procedure with enzyme ATA-256, the product was obtained as a white solid, 26.3 mg, 75% yield, >99% ee. \textit{\textsuperscript{1}H NMR} (400 MHz, CDCl\textsubscript{3}) δ 7.35 (d, \(J = 8.0\) Hz, 7H), 7.21 (d, \(J = 7.8\) Hz, 2H), 5.18 – 4.74 (m, 5H), 3.71 (t, \(J = 6.1\) Hz, 2H), 2.45 (t, \(J = 6.5\) Hz, 2H), 1.72 (dq, \(J = 14.8, 7.8\) Hz, 5H), 1.45 (d, \(J = 6.9\) Hz, 3H). \textit{\textsuperscript{13}C NMR} (101 MHz, CDCl\textsubscript{3}) δ 155.5, 142.9, 136.4, 131.8, 128.5, 128.1, 125.8, 122.9, 89.9, 80.6, 66.8, 62.5, 50.5, 31.9, 30.9, 25.0, 19.2, 14.1. \textbf{HRMS} TOF MS EI+ m/z calcd C_{22}H_{25}NO_{3} Na [M+Na]^+: 374.1732; found 374.1732. The enantioselectivity of the unprotected primary amine was determined by HPLC analysis (Agilent® Poroshell 120 2.7 µm chiral-V column 50 x 4.6 mm, NH\textsubscript{4}COOH pH = 3.5 (15 mM) : n-hexane = 10:90, flow rate 1.0 mL/min) t\textsubscript{1} = 3.780 min (major).

\[(S)-1-\text{(2-Iodophenyl)ethan-1-amine 20}\]

Following the general procedure with enzyme ATA-260, the product was obtained as a clear oil, 100.0 mg from a 190.0 mg batch, 52% yield, >99% ee. \textit{\textsuperscript{1}H NMR} (400 MHz, CDCl\textsubscript{3}) δ 8.33 (s, 1H), 7.87 (q, \(J = 0.8\) Hz, 1H), 7.52 (d, \(J = 8.0\) Hz, 1H), 7.39 (t, \(J = 8.0\) Hz, 1H), 7.27 (s, 1H), 7.07-7.03 (m, 1H), 4.73 (t, \(J = 8\) Hz, 1H), 1.59 (d, \(J = 8.0\) Hz, 3H). \textit{\textsuperscript{13}C NMR} (100 MHz, CDCl\textsubscript{3}) δ 140.2, 140.0, 130.6, 129.4, 126.1, 98.7, 55.7, 20.0. \textbf{HRMS} TOF MS EI+ m/z calcd C_{9}H_{11}IN [M+H]^+: 247.993626; found 247.9946. The enantioselectivity was determined by HPLC analysis (Chiralpak® AY-H 5 µ
column 250 mm x 4.6 mm, n-hexane: 0.1% diethylamine in ethanol = 95:5, flow rate 1.0 mL/min) t₁ = 12.579 min (major).

(S)-r-Butyl -4-(((benzyloxy)carbonyl)amino)azepane-1-carboxylate 21

Following the general procedure with enzyme ATA-260, the product was obtained as a colorless oil, 21.6 mg from a 0.1 mmol batch, 62% yield, 83% ee. ¹H NMR (500 MHz, CDCl₃) δ 7.33 (d, J = 3.5 Hz, 5H), 5.06 (s, 2H), 4.77 – 4.65 (m, 1H), 3.67 (s, 1H), 3.52 (d, J = 16.9 Hz, 2H), 3.33 (s, 1H), 3.14 – 3.05 (m, 1H), 2.05 – 1.97 (m, 1H), 1.91 – 1.83 (m, 1H), 1.81 (s, 1H), 1.51 (d, J = 39.7 Hz, 3H), 1.43 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 155.5, 155.4, 136.5, 128.5, 128.1, 128.1, 79.4, 66.6, 51.5, 51.3, 46.5, 45.4, 42.6, 42.3, 35.3, 35.0, 33.6, 33.3, 28.5, 24.2, 24.2. HRMS TOF MS EI⁺ m/z calcd C₁₉H₂₈N₂O₄ Na [M+Na]⁺: 371.1947; found 371.1946. The enantioselectivity was determined by HPLC analysis (Phenomenex® 5 µm Lux Cellulose-1 column 250 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 0.5 mL/min) t₁ = 24.767 min (major) t₂ = 28.886 min (minor).

(S)-r-Butyl-3-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate 22

Following the general procedure with enzyme ATA-260, the product was obtained as a white solid, 24.6 mg from a 0.1 mmol batch, 75% yield, >99% ee. ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 7.32 (m, 5H), 5.18 (d, J = 13.8 Hz, 3H), 3.56 (dd, J = 12.5, 4.6 Hz, 2H), 3.52 – 3.35 (m, 2H), 2.13 – 2.02 (m, 2H), 1.45 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 154.6, 154.5, 154.4, 154.2, 140.9, 134.9, 128.7, 128.7, 128.6, 128.5, 128.4, 127.7, 127.0, 79.6, 77.7, 69.9, 65.4, 60.4, 51.8, 51.5, 43.9, 43.5, 31.5, 30.8, 29.7, 28.5, 21.2, 14.2. (rotamer was observed). The enantioselectivity was determined by HPLC analysis (Chiralcel® 5 µm OD-H column 150 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) t₁ = 1.295 min (major). Spectral data matched those previously reported.⁴
(S)-Benzy1 3-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate 23

Following the general procedure with enzyme ATA-260, the product was obtained as a black oil, 156.0 mg from a 196.0 mg batch, 80% yield, 98.4% ee. \(^1\)H NMR (400 MHz, d\(_6\)-DMSO) 7.96 (s, 3H), 7.37-7.31 (m, 4 H), 5.07 (d, J = 1.2 Hz, 2H), 3.81- 3.48 (m, 6H), 3.42-3.34 (m, 2H), 2.16 (d, J = 4 Hz, 1H), δ 1.94 (d, J = 80 Hz, 1H). \(^1\)C NMR (100 MHz, DMSO-d\(_6\)) 136.9, 128.0, 66.0, 49.3, 43.5, 40.1, 38.9, 29.0. The enantioselectivity was determined by HPLC analysis (Chiralpak® AD-H 250 mm x 4.6 mm, 5 µ column, n-hexane: 0.1% DEA in ethanol = 75:25, flow rate 1.0 mL/min) t\(_1\) = 12.829 min (major) t\(_2\) = 15.665 min (minor). Spectral data matched those previously reported.\(^5\)

(S)-Benzyl-(1-phenylpropyl)carbamate 24

Following the general procedure with enzyme ATA-260, the product was obtained as a white solid, 42.6 mg from a 0.2 mmol scale batch, 79% yield, >99% ee. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 7.19 (dd, J = 9.0, 5.8 Hz, 6H), 7.12 (d, J = 6.4 Hz, 4H), 5.00 – 4.82 (m, 3H), 4.46 (q, J = 7.9 Hz, 1H), 1.65 (hept, J = 7.0 Hz, 2H), 0.75 (t, J = 7.4 Hz, 3H). \(^1\)C NMR (101 MHz, CDCl\(_3\)) δ 155.7, 142.3, 136.5, 128.7, 128.6, 128.5, 128.1, 127.3, 126.4, 66.7, 56.9, 29.6, 10.6. The enantioselectivity was determined by HPLC analysis (Chiralcel® 5 µm OD-H column 150 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) t\(_1\) = 9.777 min (major). Spectral data matched those previously reported.\(^6\)

(S)-1-(Furan-2-yl)ethan-1-amine 25
Following the general procedure with enzyme ATA-260, the product was obtained as a yellow oil, 61 mg from an 88 mg batch, 70% yield, 98.9% ee. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.37 (d, $J = 1.2$ Hz, 1H), 6.37 – 6.33 (m, 2H), 4.42 (s, $J = 8.0$ Hz, 1H), 1.62 (d, $J = 8.0$ Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 150.3, 143.5, 110.7, 108.5, 44.9, 17.1. The enantioselectivity was determined by HPLC analysis (Chiralpak® IG 250 mm x 4.6 mm, 5 μ column, n-hexane: 0.1% diethylamine in ethanol = 90:10, flow rate 1.0 mL/min) $t_1 = 8.250$ min (major) $t_2 = 9.016$ min (minor). Spectral data matched those previously reported.7

![Structure](image1)

**(S)-1-(6-Bromopyridin-2-yl)ethan-1-amine 26**

Following the general procedure with enzyme ATA-260, the product was obtained as a brown oil, 110 mg from a 160 mg batch, 69% yield, 95.7% ee. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 8.33 (s, 2H), 7.85 (t, $J = 7.6$ Hz, 1H), 7.689 (d, $J = 8.0$ Hz, 1H), 7.56 (d, 7.6 Hz, 1H), 4.52 (d, $J = 7.2$Hz, 1H), 1.46 (d, $J = 6.8$ Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-d$_6$) δ 159.3, 158.3, 140.7, 127.8, 121.2, 39.5, 19.6. The enantioselectivity was determined by HPLC analysis (Chiralpak® AY-H 250 mm x 4.6 mm, 5 μ column, n-hexane: 0.1% diethylamine in isopropanol = 90:10, flow rate 0.8 mL/min) $t_1 = 11.630$ min (major) $t_2 = 12.872$ min (minor). Spectral data matched those previously reported.8

![Structure](image2)

**(S)-Benzyl (1-(4-bromophenyl)ethyl)carbamate 29**

Following the general procedure with enzyme ATA-260, the product was obtained as a white solid, 29.4 mg from a 0.1 mmol batch, 88% yield, >99% ee. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.45 (d, $J = 8.1$ Hz, 2H), 7.34 (s, 5H), 7.18 (d, $J = 8.1$ Hz, 2H), 5.16 – 4.93 (m, 3H), 4.86 – 4.72 (m, 1H), 1.45 (d, $J = 6.9$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.4, 142.6, 136.3, 131.7, 131.5, 128.5, 128.2, 127.7, 121.1, 66.8, 50.2, 22.4. HRMS TOF MS EI+ m/z calcd C$_{16}$H$_{16}$BrNO$_2$ Na [M+Na]$^+$: 356.0262; found
356.0273. The enantioselectivity was determined by HPLC analysis (Chiralcel® 5 µm OD-H column 150 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) t₁ = 10.709 min (major).

2,5-Dichloro-N-((2R,3S)-3-methyl-4-phenylbutan-2-yl)pyrimidin-4-amine 32

Following the experimental procedure used for the 1-pot sequence (ERED/ATA/S_NAr), the product was obtained as a colorless oil, 60.5 mg from a 0.127 mmol batch, 99% yield, >99% ee, 82:18 dr. ^1H NMR (500 MHz, CDCl₃) δ 7.99 (s, 1H), 7.33 – 7.25 (m, 3H), 7.24 – 7.20 (m, 1H), 7.20 – 7.11 (m, 2H), 5.31 (d, J = 9.3 Hz, 1H), 4.37 (dqt, J = 8.1, 6.5, 4.2 Hz, 1H), 2.76 (dt, J = 13.5, 6.5 Hz, 1H), 2.44 (td, J = 14.1, 13.6, 8.6 Hz, 1H), 2.16 – 2.02 (m, 1H), 1.31 – 1.21 (m, 4H (mixed with a diastereomer)), 0.93 (dd, J = 29.4, 6.9 Hz, 3H). ^13C NMR (126 MHz, CDCl₃) δ 158.5, 158.2, 158.1, 158.0, 153.4, 153.3, 140.3, 140.1, 129.0, 128.9, 128.5, 128.4, 128.3, 126.2, 126.1, 126.0, 113.0, 113.0, 52.2, 50.6, 50.2, 43.2, 39.9, 39.8, 39.5, 38.9, 36.0, 32.2, 31.9, 29.7, 27.7, 22,, 22.5, 17.6, 16.3, 15.3, 14.6, 10.0. HRMS TOF MS EI⁺ m/z calcd C_{15}H_{17}Cl_{2}N_{3}H [M+H]^+: 310.0878; found 310.0893. The dr was determined by ^1H NMR (4.37 ppm int. = 1.00; 4.26 ppm int. = 0.22); the enantioselectivity was determined by HPLC analysis (Phenomenex® 5 µm Lux Cellulose-1 column 250 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) t₁ = 17.219 min (minor) t₂ = 20.928 (major).

(S)-Rivastigmine

Following the experimental procedure for (S)-rivastigmine, the product was obtained as a colorless oil, 23.7 mg from a 0.1 mmol batch, 95% yield, >99% ee. ^1H NMR (500 MHz, CDCl₃) δ 7.28 (t, J = 7.8 Hz, 1H), 7.11 (dd, J = 7.7, 1.4 Hz, 1H), 7.08 – 7.03 (m, 1H), 7.00 (d, J = 8.0 Hz, 1H), 3.44 (dq, J
= 31.3, 7.2 Hz, 2H), 3.24 (q, J = 6.7 Hz, 1H), 3.02 (d, J = 37.1 Hz, 3H), 2.20 (d, J = 0.9 Hz, 6H), 1.39 – 1.33 (m, 3H), 1.26 – 1.17 (m, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 154.6, 154.4, 151.5, 145.8, 145.7, 128.9, 124.2, 124.2, 120.7, 120.7, 120.2, 65.6, 44.0, 43.2, 34.2, 33.8, 29.7, 27.1, 20.1, 13.2, 12.5. The enantioselectivity was determined by HPLC analysis Chiralcel® 5 µm OD-H column 150 x 4.6 mm, isopropanol : n-hexane = 10:90, flow rate 1.0 mL/min) $t_1$ = 5.565 min (major). Spectral data matched those previously reported. 9
5. NMR spectra

$^1$H NMR spectrum of TPGS-750-M

$^{13}$C NMR spectrum of TPGS-750-M
$^1$H NMR spectrum of benzyl (S)-(1-(6-(2-fluoro-3-(trifluoromethyl)phenyl)pyridin-2-yl)ethyl)carbamate 8

$^{13}$C NMR spectrum of benzyl (S)-(1-(6-(2-fluoro-3-(trifluoromethyl)phenyl)pyridin-2-yl)ethyl)carbamate 8
19F NMR spectrum of benzyl (S)-(1-(6-fluoro-3-(trifluoromethyl)phenyl)pyridin-2-yl)ethyl)carbamate 8

1H NMR spectrum of (R)-benzyl (1-(4-(trifluoromethyl)phenyl)ethyl)carbamate 10
$^{13}$C NMR spectrum of (R)-benzyl (1-(4-(trifluoromethyl)phenyl)ethyl)carbamate 10

$^{19}$F NMR spectrum of (R)-benzyl (1-(4-(trifluoromethyl)phenyl)ethyl)carbamate 10
$^1$H NMR spectrum of (S,E)-4-phenylbut-3-en-2-amine 12

$^{13}$C NMR spectrum of (S,E)-4-phenylbut-3-en-2-amine 12
$^1$H NMR spectrum of $(S,E)$-benzyl-(3-methyl-4-phenylbut-3-en-2-yl)carbamate 13

$^{13}$C NMR spectrum of $(S,E)$-benzyl-(3-methyl-4-phenylbut-3-en-2-yl)carbamate 13
$^1$H NMR spectrum of 2-ethylhexyl (E)-3-(4-(((benzoxycarbonyl)amino)ethyl)phenyl)acrylate 14

$^{13}$C NMR spectrum of 2-ethylhexyl (E)-3-(4-(((benzoxycarbonyl)amino)ethyl)phenyl)acrylate 14
H NMR spectrum of (S)-benzyl(1-(4-iodophenyl)ethyl)carbamate 16

\[ \text{H NMR spectrum of (S)-benzyl(1-(4-iodophenyl)ethyl)carbamate 16} \]

\[ \text{C NMR spectrum of (S)-benzyl(1-(4-iodophenyl)ethyl)carbamate 16} \]

\[ \text{C NMR spectrum of (S)-benzyl(1-(4-iodophenyl)ethyl)carbamate 16} \]
$^1$H NMR spectrum of (S)-benzyl(1-(4-chlorophenyl)ethyl)carbamate 17

$^{13}$C NMR spectrum of (S)-benzyl(1-(4-chlorophenyl)ethyl)carbamate 17
$^1$H NMR spectrum of (S)-benzyl(1-(4-bromophenyl)ethyl)carbamate 18

$^{13}$C NMR spectrum of (S)-benzyl(1-(4-bromophenyl)ethyl)carbamate 18
$^1$H NMR spectrum of (S)-benzyl (1-(4-(6-hydroxyhex-1-yn-1-yl)phenyl)ethyl)carbamate 19

$^{13}$C NMR spectrum of (S)-benzyl (1-(4-(6-hydroxyhex-1-yn-1-yl)phenyl)ethyl)carbamate 19
$^1$H NMR spectrum of (S)-1-(2-iodophenyl)ethan-1-amine 20

$^{13}$C NMR spectrum of (S)-1-(2-iodophenyl)ethan-1-amine 20
$^1$H NMR spectrum of (S)-tert-butyl-4-(((benzyloxy)carbonyl)amino)azepane-1-carboxylate 21

$^{13}$C NMR spectrum of (S)-tert-butyl-4-(((benzyloxy)carbonyl)amino)azepane-1-carboxylate 21
$^1$H NMR spectrum of (S)-benzyl 3-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate 23

$^{13}$C NMR spectrum of (S)-benzyl 3-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate 23
$^1$H NMR spectrum of benzyl (S)-(1-phenylpropyl)carbamate 24

$^{13}$C NMR spectrum of benzyl (S)-(1-phenylpropyl)carbamate 24
$^1$H NMR spectrum of (S)-1-(furan-2-yl)ethan-1-amine 25

$^{13}$C NMR spectrum of (S)-1-(furan-2-yl)ethan-1-amine 25
1H NMR spectrum of (S)-1-(6-bromopyridin-2-yl)ethan-1-amine 26

13C NMR spectrum of (S)-1-(6-bromopyridin-2-yl)ethan-1-amine 26
$^1$H NMR spectrum of 2,5-dichloro-$N$-((2R,3S)-3-methyl-4-phenylbutan-2-yl)pyrimidin-4-amine 32

$^{13}$C NMR spectrum of 2,5-dichloro-$N$-((2R,3S)-3-methyl-4-phenylbutan-2-yl)pyrimidin-4-amine 32
\( ^1 \text{H NMR spectrum of (S)-rivastigmine} \)

\( ^{13} \text{C NMR spectrum of (S)-rivastigmine} \)
6. HPLC traces

HPLC analysis of benzyl (S)-(1-(6-(2-fluoro-3-(trifluoromethyl) phenyl)pyridin-2-yl)ethyl)carbamate 8
HPLC analysis of \((R)-\text{benzyl} \ (1-(4-(\text{trifluoromethyl})\text{phenyl})\text{ethyl})\text{carbamate} \ 10\)
HPLC analysis of (S,E)-4-phenylbut-3-en-2-amine 12
HPLC analysis of (S,E)-benzyl-(3-methyl-4-phenylbut-3-en-2-yl)carbamate 13
HPLC analysis of (S)-2-ethylhexyl (E)-3-(4-(((benzyloxy)carbonyl)amino)ethyl)phenyl)acrylate 14
HPLC analysis of (S)-benzyl(1-(4-iodophenyl)ethyl)carbamate 16
HPLC analysis of (S)-benzyl(1-(4-chlorophenyl)ethyl)carbamate 17
HPLC analysis of (S)-benzyl(1-(4-bromophenyl)ethyl)carbamate 18
HPLC analysis of (S)-benzyl (1-(4-(6-hydroxyhex-1-yn-1-yl)phenyl)ethyl)carbamate 19

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HPLC analysis of (S)-1-(2-iodophenyl)ethan-1-amine 20
HPLC analysis of (S)-\textit{tert}-butyl -4-(((benzyloxy)carbonyl)amino)azepane-1-carboxylate 21
HPLC analysis of (S)-tert-butyl-3-(((benzyloxy)carbonyl)amino)pyrrolidine-1-carboxylate 22
HPLC analysis of $(S)$-benzyl 3-(((benzoyloxy)carbonyl)amino)pyrrolidine-1-carboxylate 23
HPLC analysis of (S)-benzyl-(1-phenylpropyl)carbamate 24
HPLC analysis of (S)-1-(furan-2-yl)ethan-1-amine 25
HPLC analysis of (S)-1-(6-bromopyridin-2-yl)ethan-1-amine 26
HPLC analysis of 2,5-dichloro-N-((2R,3S)-3-methyl-4-phenylbutan-2-yl)pyrimidin-4-amine 32
HPLC analysis of (S)-rivastigmine

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7. References


