Supporting Information

Enantioselective Synthesis of Pharmaceutically Relevant Bulky Arylbutylamines Using Engineered Transaminases

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TABLE OF CONTENTS

Supplement

Supplement

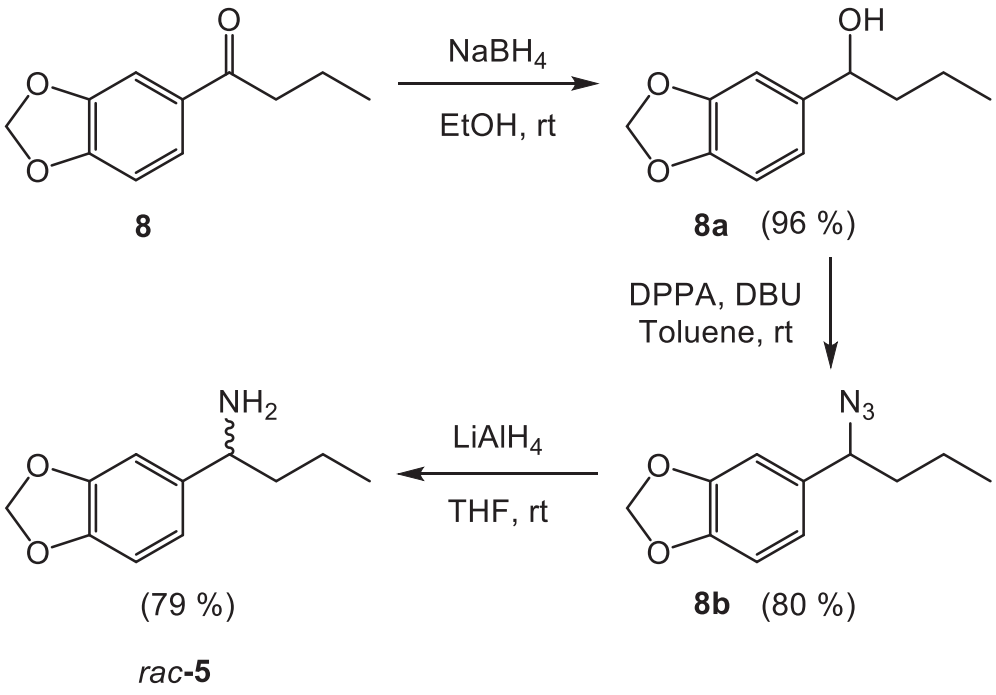
S1. Experimental Section

S1.1 Chemicals and Equipment

3′,4′,-(Methylenedioxy)butyrophenone (**8**) and (*S*)-1-phenylethanamine (**2**) were purchased from Alfa Aesar (Kandel, Germany). Isopropylamine (**11**), (*R*)-1-phenylethanamine (**2**), *p*-nitrobenzylamine (**10**) and *rac*-1-phenylbutylamine hydrochloride (**6**) were purchaised from Sigma-Aldrich (Steinheim, Germany). Pyruvic acid (**4**) sodium salt and L-alanine (**3**) were obtained from TCI (Eschborn, Germany). Acetophenone (**1**) was purchased from Honeywell Riedel-de Haën AG (Seelze, Germany) and butyrophenone (**9**) was obtained from Merck (Darmstadt, Germany). Commercial laboratory chemicals, reagents and components of nutrition medium were purchased from the aforementioned companies. Solvents for liquid chromatography were obtained from Honeywell Riedel-de Haën AG (Seelze, Germany) and Fischer Scientific GmbH (Schwerte, Germany).

NMR spectra were recorded on a Bruker ARX300 and DRX500 instrument. Incubation of 96-well microtiter plates were performed in an Heidolph Titramax/Inkubator 1000 (Heidolph, Germany) and values of optical density were measured using a microtiter plate reader SPECTRAmax 190 (Molecular Devices LLC., San Jose, USA).

S1.2 Synthesis of Racemic Benzo[1,3]dioxol-5-yl-butylamine (5)[1]



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Under an inert atmosphere, 3′,4′-(methylenedioxy)butyrophenone (**8**) (3.0 g, 15.61 mmol, 1 eqv.) was dissolved in anhydrous ethanol (24 mL) and the solution was cooled to 0°C. Sodium borohydride (1.8 g, 46.82 mmol, 3 eqv.) was added portion-wise over the course of 15 min and the mixture was stirred at 0 °C for 3 h. Upon complete consumption of starting material, ethanol was evaporated and the residue was dissolved in 1 M HCl (20 mL). Extraction with ethyl acetate (3× 20 mL) and removal of volatiles in vacuo yielded α-propylpiperonylalcohol (**8a**) (96%, 2.9 g, 14,93 mmol) as yellowish oil.

Under an inert atmosphere, α-propylpiperonylalcohol (**8a**) (2.9 g, 14,93 mmol, 1 eqv.) was dissolved in anhydrous toluene (40 mL), the solution was cooled to 0 °C and neat diphenylphosphoryl azide (5.94 mL, 31.2 mmol, 2.1 eqv.) was added drop-wise over the course of 10 min. Neat 1,8-diazabicyclo[5.4.0]undec-7-ene (4.67 mL, 31.2 mmol, 2.1 eqv.) was added drop-wise over the course of 30 min and the mixture was stirred at room temperature for 5 h. Water (20 mL) was added to quench the reaction and the phases were separated. The aqueous phase was extracted with ethyl acetate (20 mL) and the combined organic phases were subsequently washed with cold 1 M HCl (20 mL) and brine (20 mL). Removal of volatiles in vacuo and silica gel column chromatography (hexane:ethyl acetate 15:1) yielded α-propylpiperonylazide (**8b**) (80%, 2.7 g, 12.5 mmol) as yellowish oil.

Under an inert atmosphere, a solution of 1 M lithium aluminum hydride in tetrahydrofuran (18.8 mL, 18.75 mmol, 1.5 eqv.) was carefully added to tetrahydrofuran (10 mL) at 10 °C. Upon addition of α-propylpiperonylazide (**8b**) (2.7 g, 12.5 mmol, 1 eqv.) over the course of 1 h, the solution was stirred at room temperature until full consumption of starting material according to TLC. The reaction was terminated by addition of a 1 M aqueous solution of sodium potassium tartrate (80 mL) and this mixture was stirred at room temperature overnight before separating the two resulting phases. The upper phase was concentrated to 5 mL in vacuo and the resulting residue was dissolved in toluene (30 mL). The organic phase was extracted with 1 M HCl (3× 30 mL) and the pH of the combined aqueous phases was adjusted to 14. Extraction with toluene (3× 30 mL), washing with brine (20 mL) and removal of volatiles in vacuo yielded benzo[1,3]dioxol-5-yl-butylamine (**5**) (79%, 1.91 g, 9.90 mmol) as brown oil.

8b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 – 6.73 (m, 3H), 5.97 (s, 2H), 4.34 (t, J = 7.3 Hz, 1H), 1.88 – 1.60 (m, 2H), 1.47 – 1.23 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75

MHz, $CDCl_3$) δ 148.05, 147.43, 133.71, 120.65, 108.18, 107.03, 101.20, 66.03, 38.19, 19.49, 13.69.

S1.3 Derivatization with 9-Fluorenylmethyl Chloroformate (Fmoc-Cl)[2]

Reaction sample or purified amine **5** or **6** solution (25 μL, 10-50 mM amine concentration) was mixed with 75 μL of sodium borate buffer solution (7 mM, pH 8.0) and 75 μL of Fmoc-Cl (40 mM in acetonitrile), and then incubated at 30°C and 450 rpm for 15 min. After this time, 80 μL of L-alanine (**3**) (100 mM in water:acetonitrile 2:1) were added to neutralize the Fmoc excess and incubated at 30°C and 450 rpm for 5 min. The sample was centrifuged (13000 rpm, 5 min) before HPLC analysis.

S1.4 High-Throughput Screening of a Commercial TA Library

Acetophenone UV assay:[3] The reactions were performed in 96-well UV transparent flat-bottom microtiter plates (Thermo Fisher Scientific, Weaverville, USA) in a total volume of 200 μl. Each well contained 1 mM (*R*)- or (*S*)-**2**, 1 mM **4** and 0.1 mM PLP in 0.1 M KPi buffer (pH 8.0). Reactions were started by adding TA CFE (40 μL, 5 mg mL^{-1} solution in KPi buffer (pH 8.0)). The plate was incubated at 30°C and the absorbance of the reaction product, acetophenone (**1**), was detected in the dynamic mode at 260 nm and 1 min interval between measurements. Specific activity was quantified at the linear stage of the enzyme reaction (2-12 min) using a calibration curve built for the standard solutions of **1** (Figure S29).

4-Nitrophenethylamine (***10***) *assay*:[4] The reactions were performed in 96-well flat-bottom microtiter plates (Sarstedt, Nümbrecht, Germany) in a total volume of 200 μl. Each well contained 5 mM **10**, 2 mM **8**, 0.1 mM PLP, 4% DMSO in 0.1 M KPi buffer, pH 8.0. Reactions were started by adding TA CFE (40 μL, 5 mg mL^{-1} solution in KPi buffer (pH 8.0)). The plate was incubated at 30°C and 450 rpm for 14 h, and the formation of the colour was visually detected. The control plates contained all components of the reaction mixture except for the amino acceptor **8**. The same mixture containing 1 mg mL^{-1} *Cv*-ATA WT CFE and 2 mM pyruvate (**4**) as an amino acceptor was used as the reaction positive control.

S1.5 Protein concentrations

Protein concentrations were determined with the Pierce™ bicinchoninic acid assay kit (Thermo Scientific, Rockford, USA) according to the manufacturer protocol.

S2 Figures

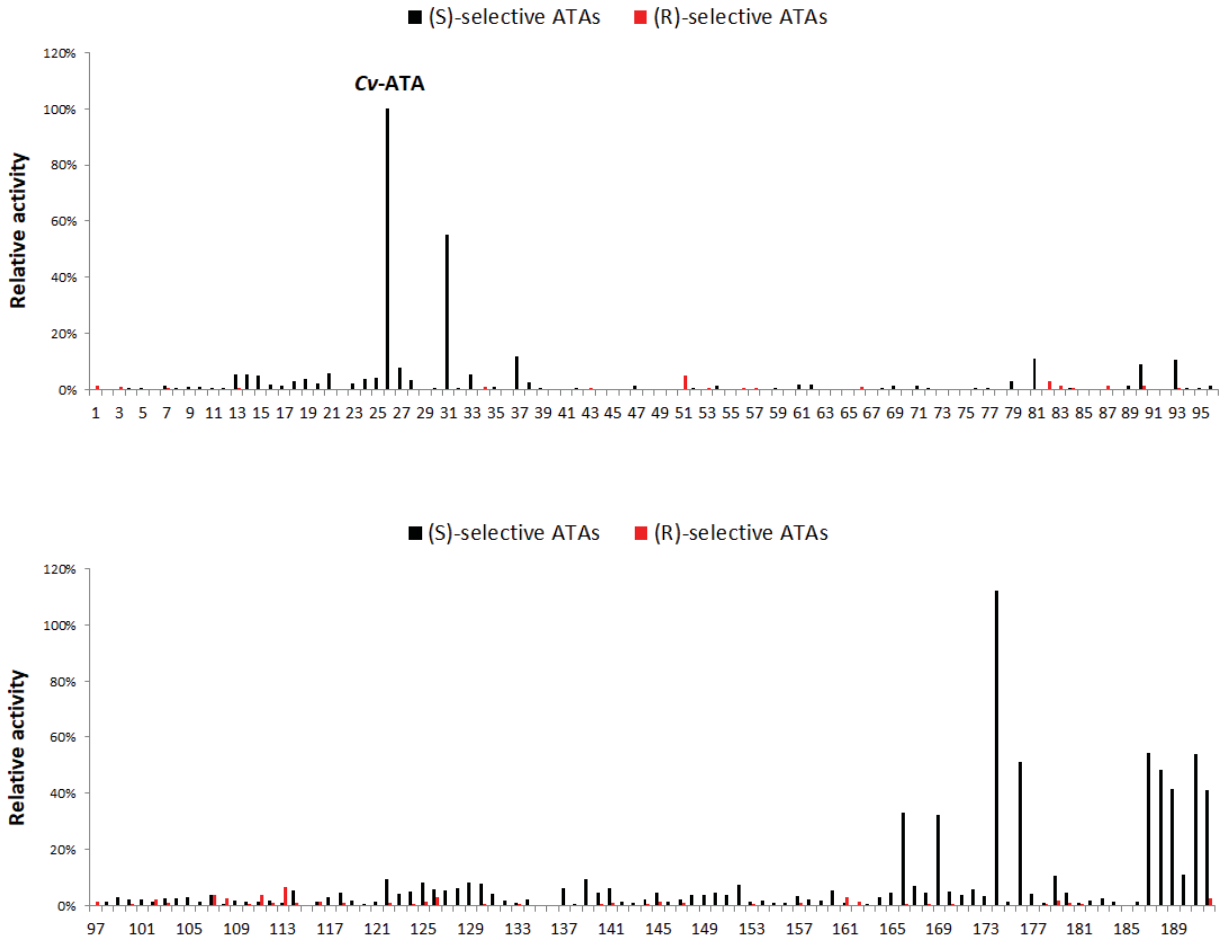

Figure S1. Screening of commercial panel of 192 TAs against (*R*)/(*S*)-MBA (**2**) and pyruvate (**4**). The activity was determined by the amount of produced acetophenone (**1**), and the activity of *Cv*-ATA WT was set as 100% (0.62 µmol min^{-1} mg^{-1} of total soluble protein in CFE).

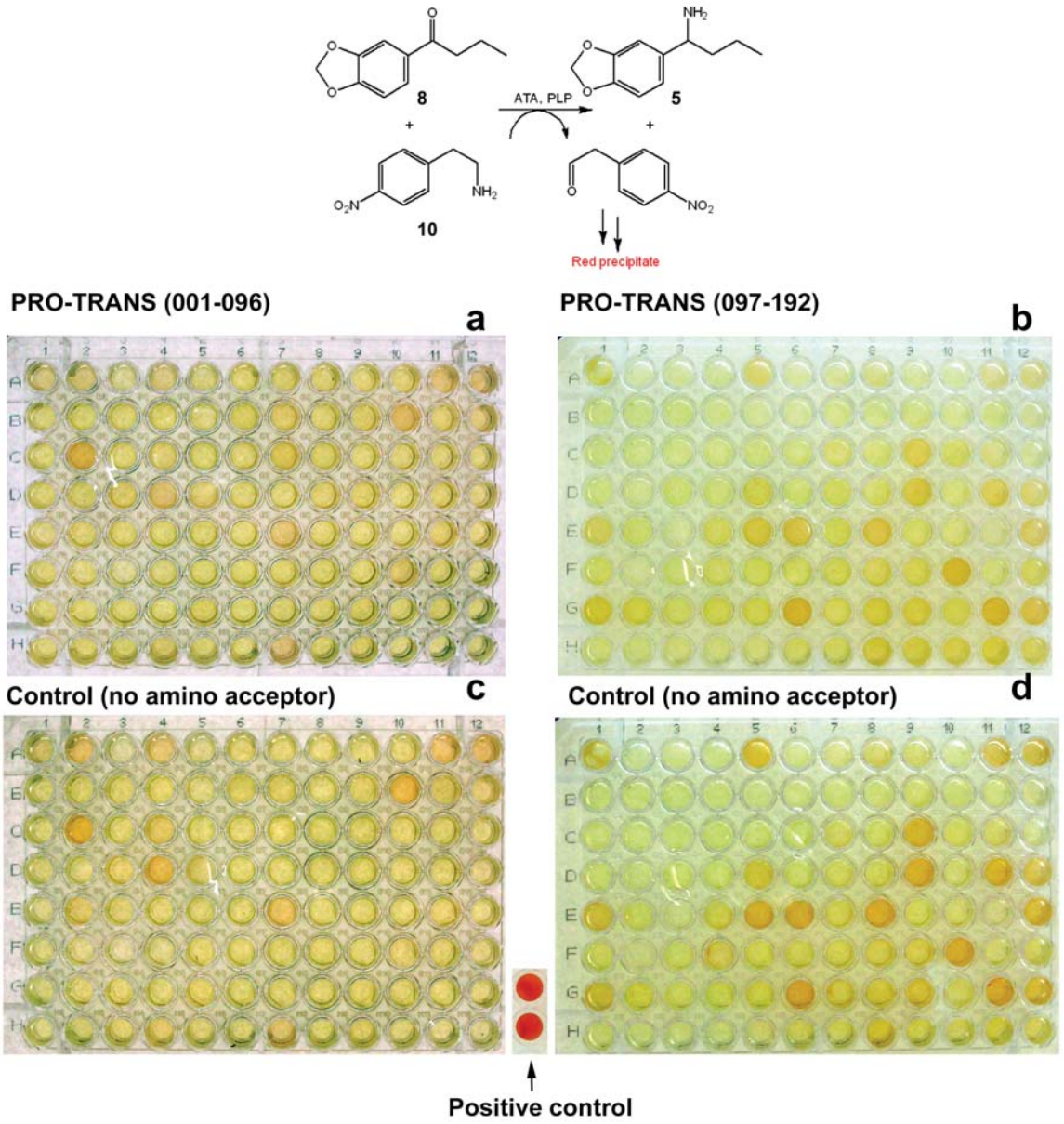

Figure S2. Screening of commercial genomic and metagenomic TA library (**a**, **b**) against **10** as amino donor and **8** as amino acceptor. The pictures of PRO-TRANSP(001-096) (left) and PRO-TRANSP(097-192) (right) were prepared at different light conditions.

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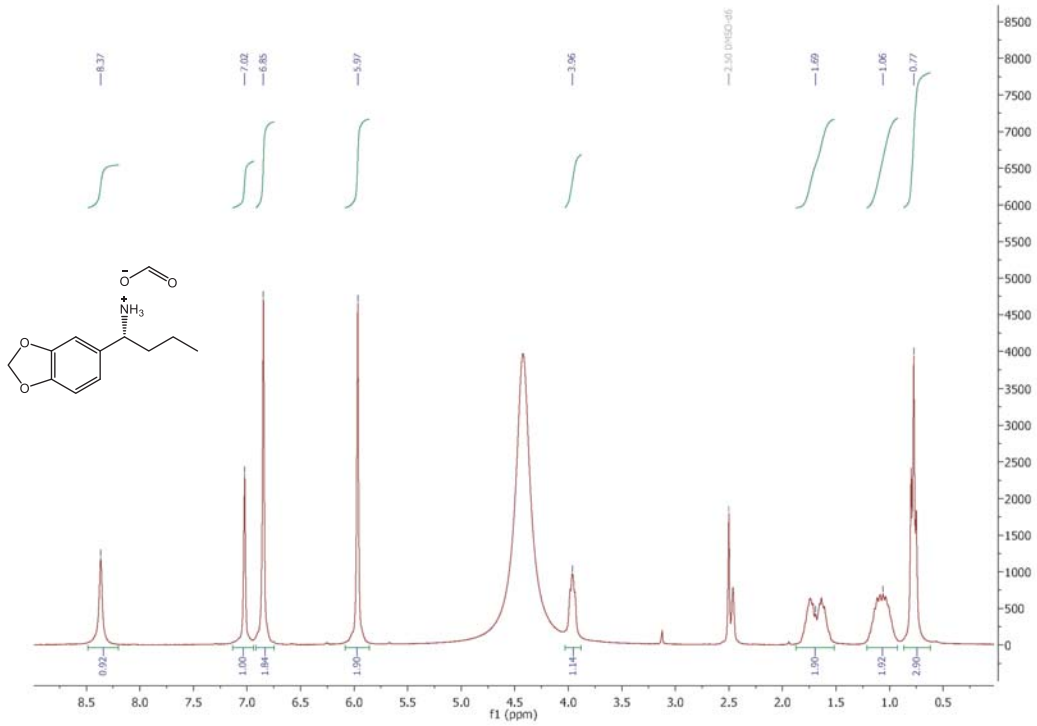

Figure S3. ^{1}H NMR spectrum of (*R*)-**5** formate salt in DMSO-d6. The product was purified after asymmetric stereoselective synthesis by ATA-117-Rd11.

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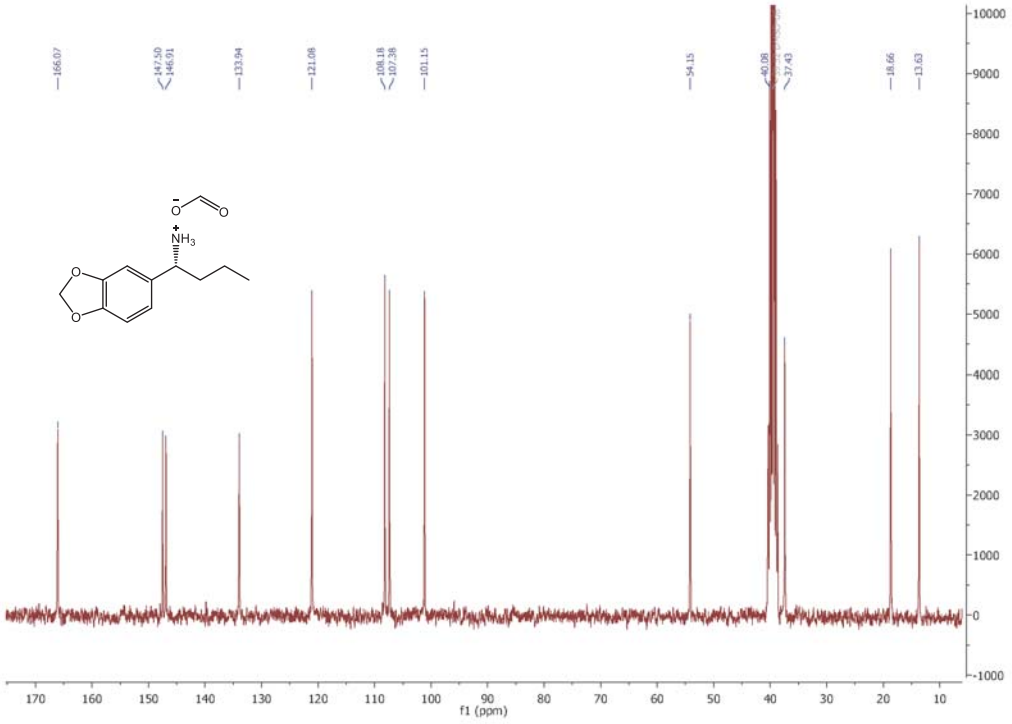

Figure S4. ^{13}C NMR spectrum of (*R*)-**5** formate salt in DMSO-d6. The product was purified after asymmetric stereoselective synthesis by ATA-117-Rd11.

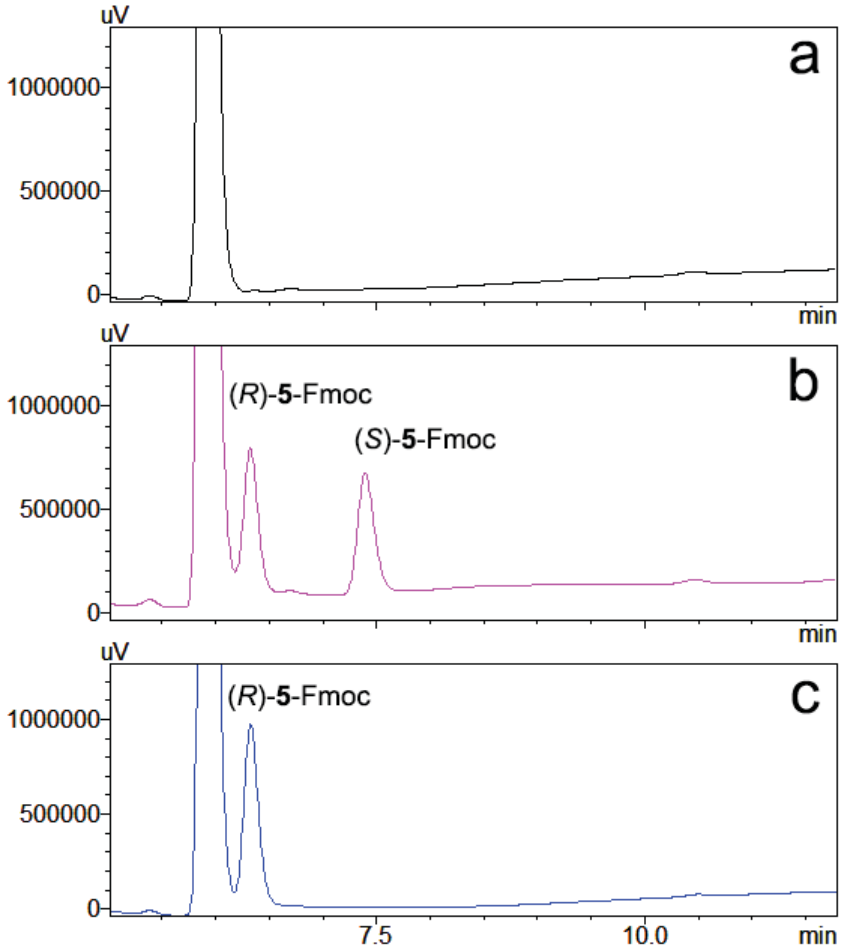

Figure S5. Chiral HPLC of (*R*)- and (*S*)-**5** stereoisomers after Fmoc derivatization: **a**) control sample lacking the amine product; **b**) reference sample of *rac*-**5**-Fmoc; **c**) purified product after asymmetric stereoselective synthesis by ATA-117-Rd11. HPLC was performed with a mobile phase consisting of 5% (v/v) isopropyl alcohol and 0.1% (v/v) diethylamine in acetonitrile.

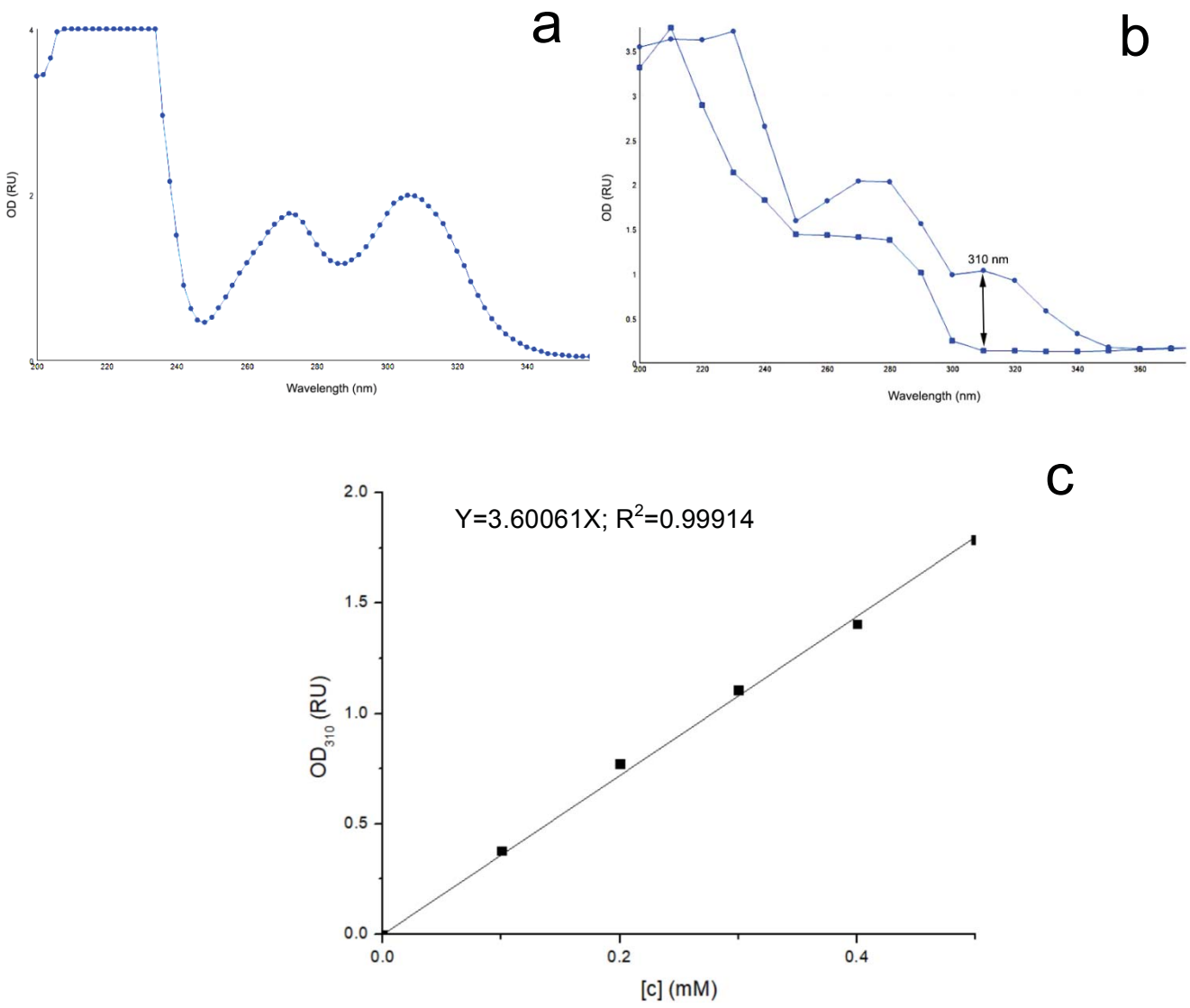

Figure S6. Parameters of the UV-assay for determination of ketone **8** concentrations in the reaction mixture. **a**) UV-spectrum of **8** (0.5 mM in MeOH); **b**) UV-profiles of the reaction mixture containing 0.5 mM *rac*-**5**, 0.5 mg mL^{-1} *Escherichia coli* CFE, 0.05 mM PLP, 50% DMSO (squares), and the same with 0.25 mM **8** (circles); the difference between absorbance values in experimental and background samples at 310 nm is indicated with the arrow; **c**) Calibration curve built for the standard solutions of **8** (0.0-0.5 mM) in the reaction mixture (total volume 200 µl) containing 0.0-0.5 mM *rac*-**5**, 0.05 mM PLP and 0.25 mg mL^{-1} CFE of *E. coli* harbouring the vector without ATA gene.

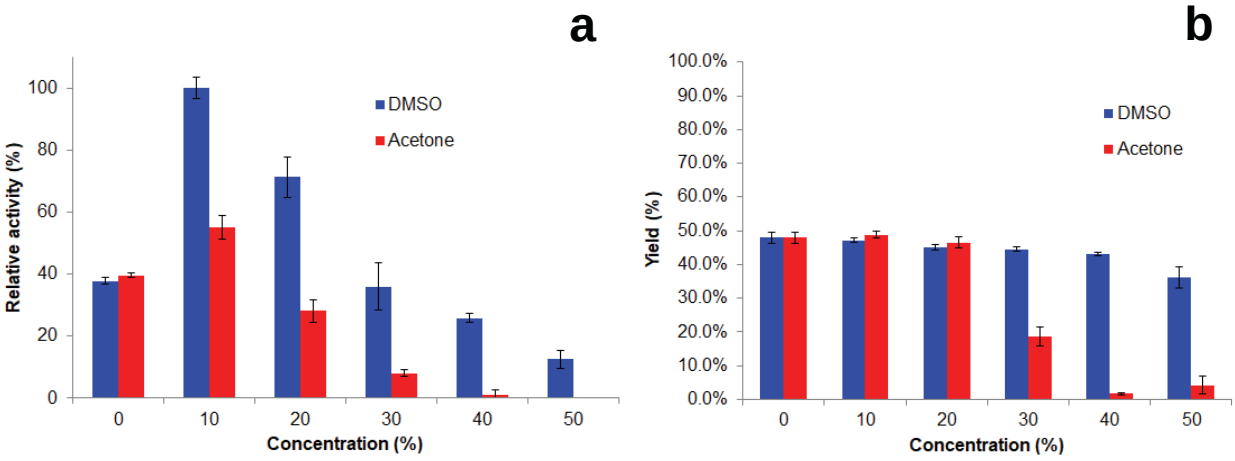

Figure S7. Biochemical characterization of *Cv*-ATA L59A with regards to the influence of acetone and DMSO concentrations: **a**) relative activity; **b**) yield. Specific activity was quantified by the amount of **8** produced in 10 min (pH, DMSO) or 20 min (acetone); the activity at 10% DMSO (1.11±0.04 µmol min-1 mg-1 of TSP) was set as 100%. The yield was determined after 2 h. Bars represent standard deviation of a minimum of three replicates.

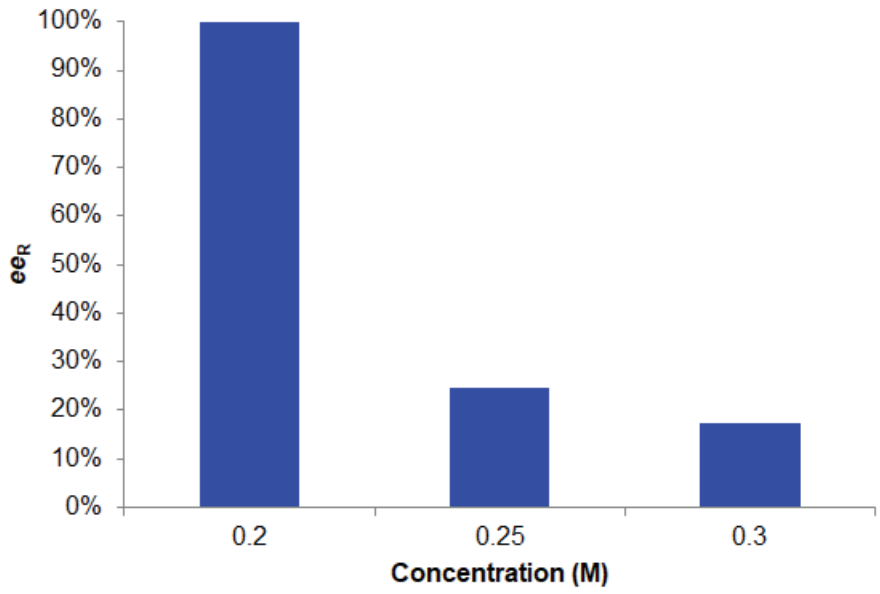

Figure S8. Enantiomeric excess of (*R*)-**5** after kinetic resolution of *rac*-**5** by *Cv*-ATA L59A. Reaction conditions (total volume 0.1 mL): 0.2/0.25/0.3 M *rac*-**5**, 0.2/0.25/0.3 M **4**, 0.5 mM PLP and 2.75 mg mL^{-1} purified *Cv*-ATA L59A in 100 mM KPi buffer (pH 7.5, 30°C). Enantiomeric excess (ee_R) was estimated by quantifying the areas of peaks of (*R*)-**5** and (*S*)-**5** from chiral HPLC.

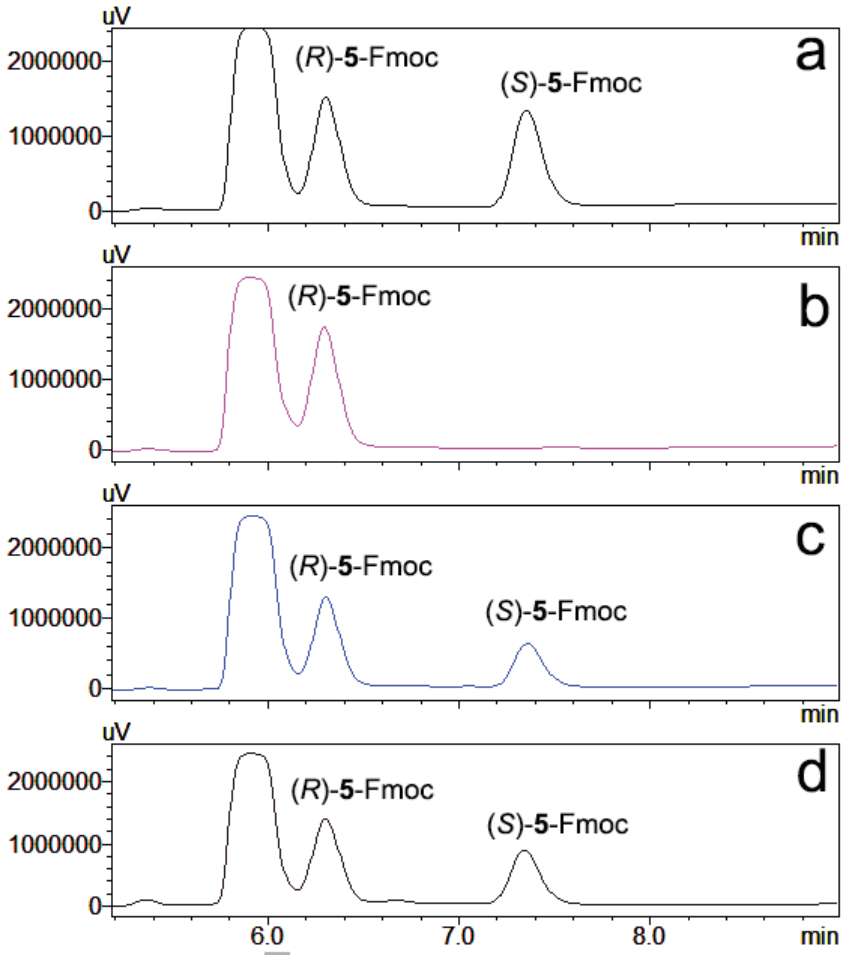

Figure S9. Chiral HPLC of (*R*)- and (*S*)-**5** enantiomers after Fmoc derivatization: **a**) reference sample of *rac*-**5**-Fmoc; **b**) residual amine substrate **5**-Fmoc after kinetic resolution of 0.2 M *rac*-**5** by *Cv*-ATA L59A; **c**) residual amine substrate **5**-Fmoc after kinetic resolution of 0.25 M *rac*-**5** by *Cv*-ATA L59A; **d**) residual amine substrate **5**-Fmoc after kinetic resolution of 0.3 M *rac*-**5** by *Cv*-ATA L59A. Reaction conditions (total volume 0.1 mL): 0.2/0.25/0.3 M *rac*-**5**, 0.2/0.25/0.3 M **4**, 0.5 mM PLP and 2.75 mg mL^{-1} purified *Cv*-ATA L59A in 100 mM KPi buffer (pH 7.5, 30°C). HPLC was performed with a mobile phase consisting of 5% (v/v) isopropyl alcohol and 0.1% (v/v) diethylamine in acetonitrile.

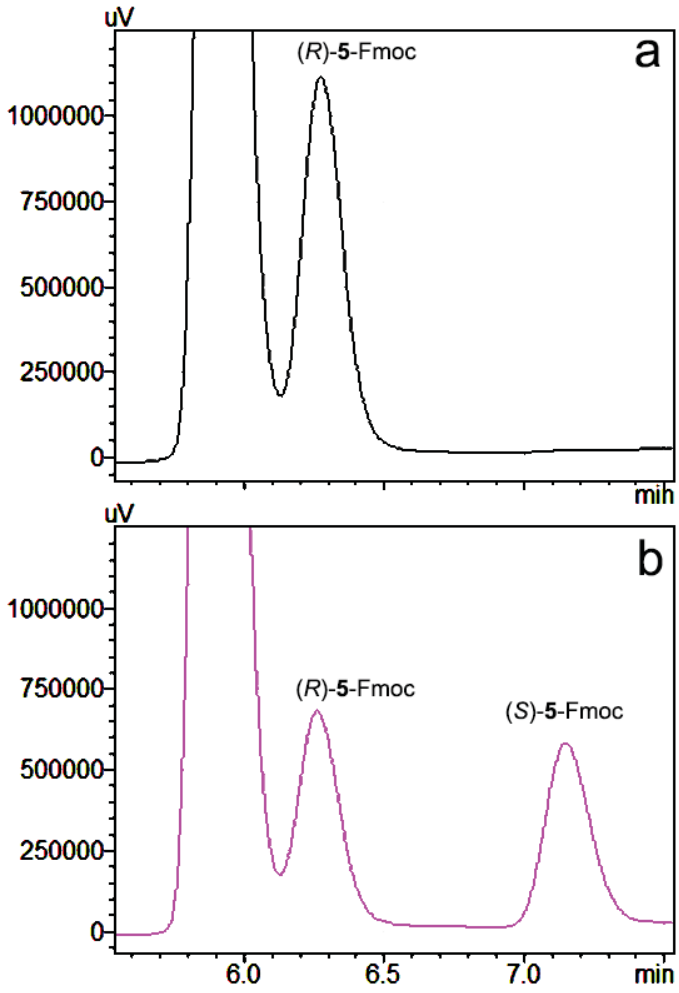

Figure S10. Chiral HPLC of (*R*)-**5** and (*S*)-**5** after Fmoc derivatization: **a**) purified amino donor substrate **5**-Fmoc after kinetic resolution catalyzed by *Cv*-ATA L59A; **b**) reference sample of *rac*-**5**-Fmoc. HPLC was performed with a mobile phase consisting of 5% (v/v) isopropyl alcohol and 0.1% (v/v) diethylamine in acetonitrile.

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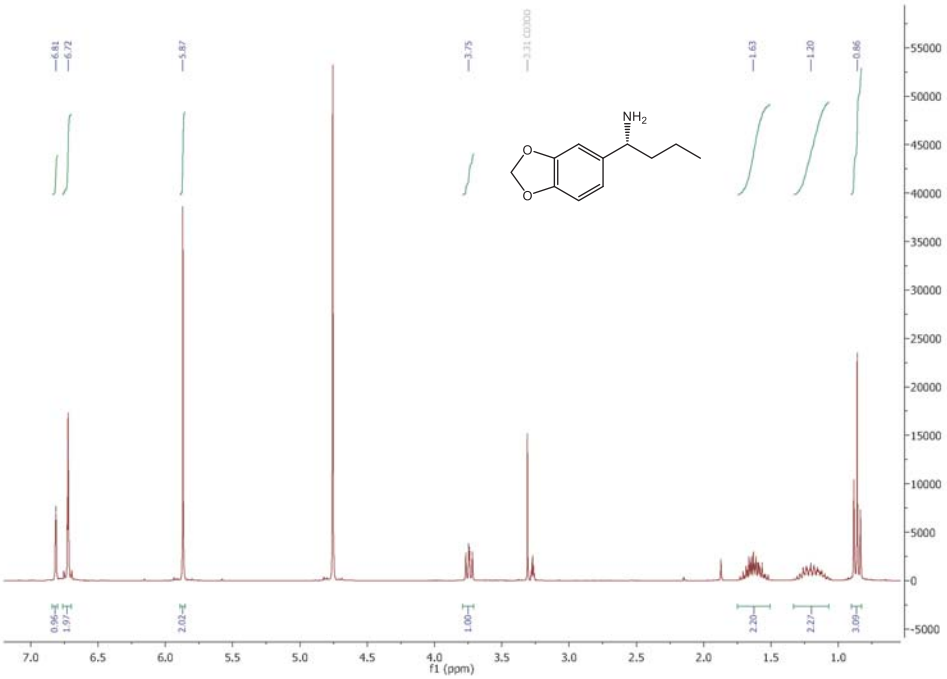

Figure S11. ^{1}H NMR spectrum of the residual substrate (*R*)-**5** after kinetic resolution catalyzed by *Cv*-ATA L59A (in methanol-*d*4).

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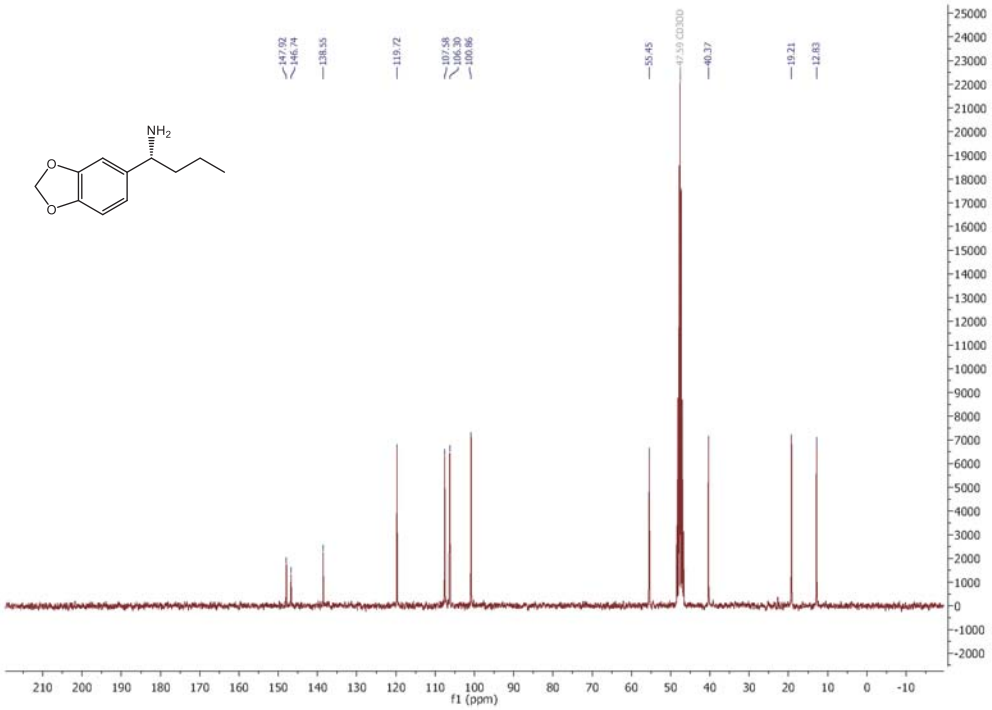

Figure S12. ^{13}C NMR spectrum of the residual substrate (*R*)-**5** after kinetic resolution catalyzed by *Cv*-ATA L59A (in methanol-*d*4).

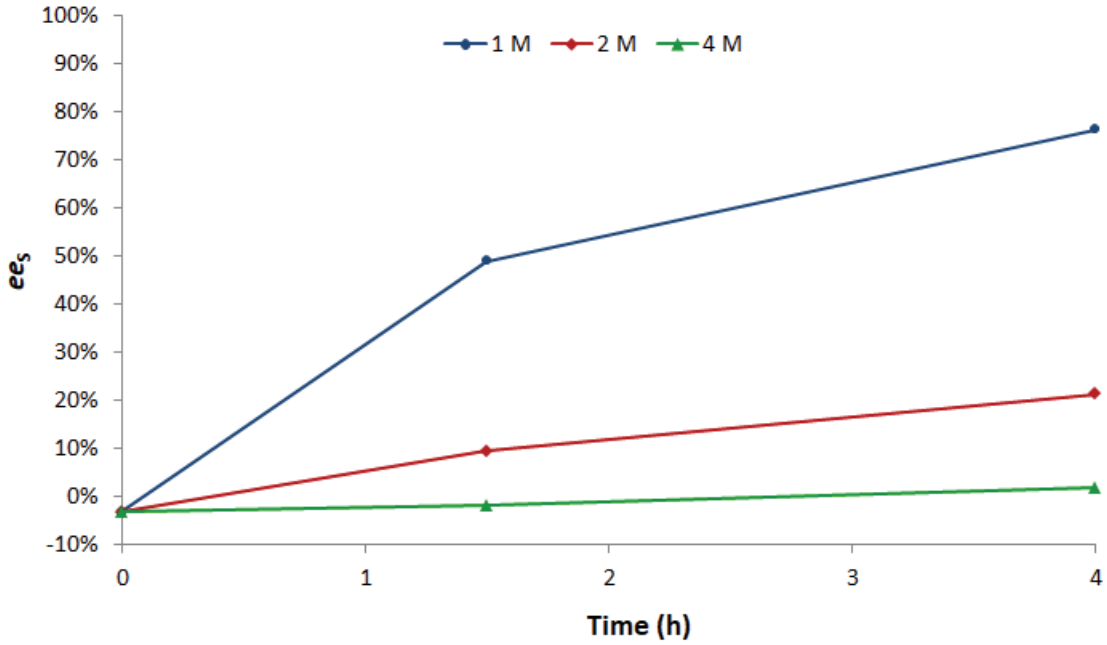

Figure S13. ATA-117-Rd11 catalyzed kinetic resolution of *rac*-**5**. Reaction conditions (reaction volume 1 mL): 0.1 M *rac*-**5** (dropwise during 12 min), 1 M, 2 M or 4 M **12**, 0.5 mM PLP and 30 mg mL^{-1} ATA-117-Rd11 CFE in 0.1 M KPi buffer; pH 8.5; 45°C; 450 rpm. Enantiomeric excess (ee_S) was estimated by quantifying the areas of peaks of (*R*)-**5** and (*S*)-**5** from chiral HPLC.

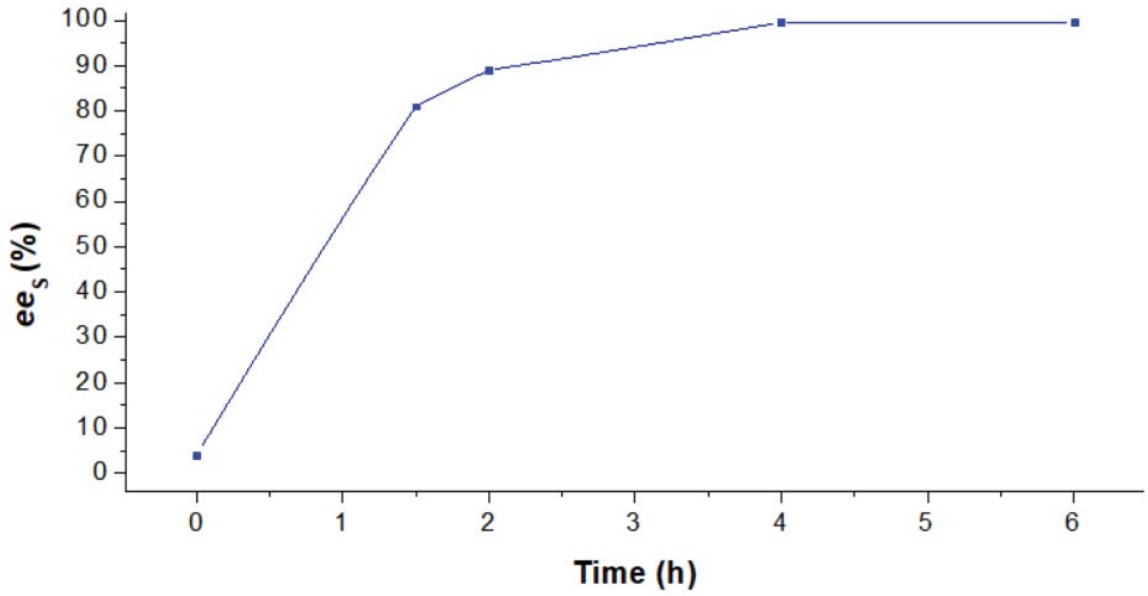

Figure S14. ATA-117-Rd11 catalyzed kinetic resolution of *rac*-**5**. Reaction conditions (total volume 10 mL in 50 mL round-bottom flask): 0.1 M *rac*-**5**, 1.2 M **12**, 0.5 mM PLP and 30 mg mL^{-1} ATA-117-Rd11 CFE in 0.1 M KPi buffer; pH 8.5; 45^{o}C; magnetic stirring. Enantiomeric excess (*ee*$_S$) was estimated by quantifying the areas of peaks of (*R*)-**5** and (*S*)-**5** from chiral HPLC.

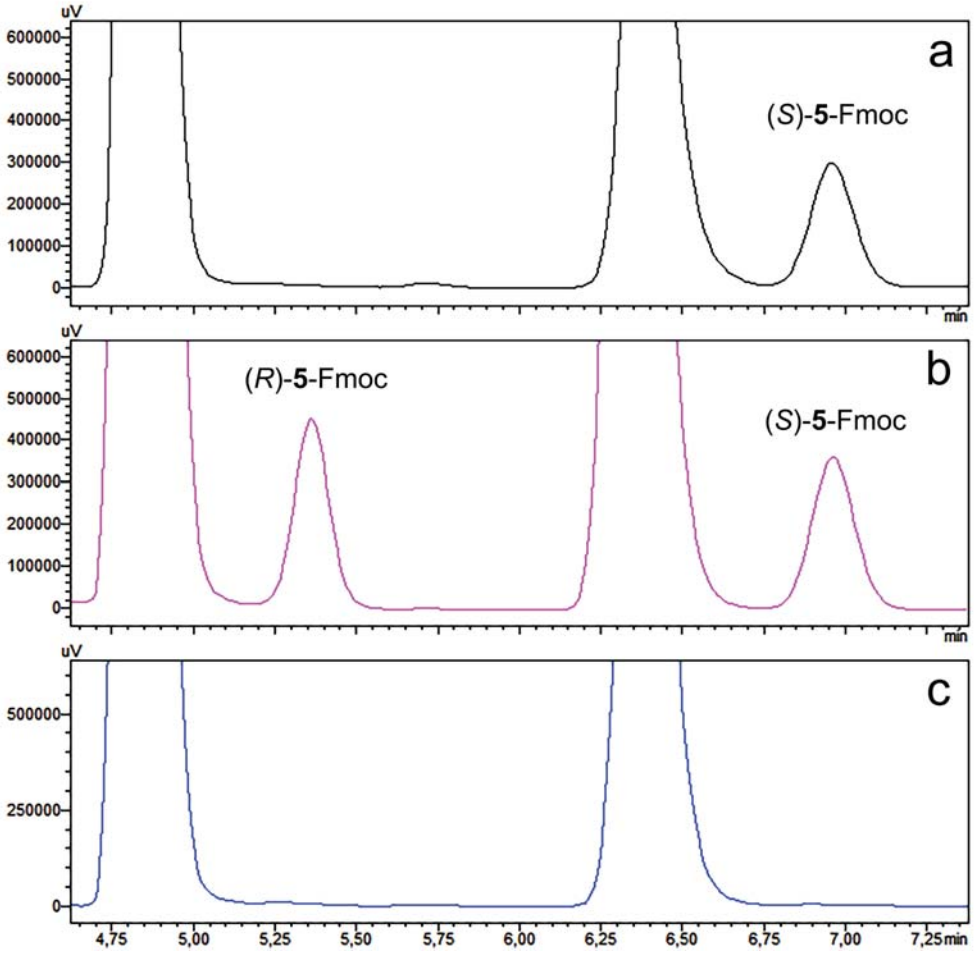

Figure S15. Chiral HPLC of (*R*)-**5** and (*S*)-**5** after Fmoc derivatization: **a**) purified residual amine substrate **5**-Fmoc after kinetic resolution catalyzed by ATA-117-Rd11; **b**) reference sample of *rac*-**5**-Fmoc; **c**) control sample lacking the amine **5** after Fmoc derivatization. HPLC was performed with a mobile phase consisting of 99% (v/v) acetonitrile and 0.1% (v/v) formic acid.

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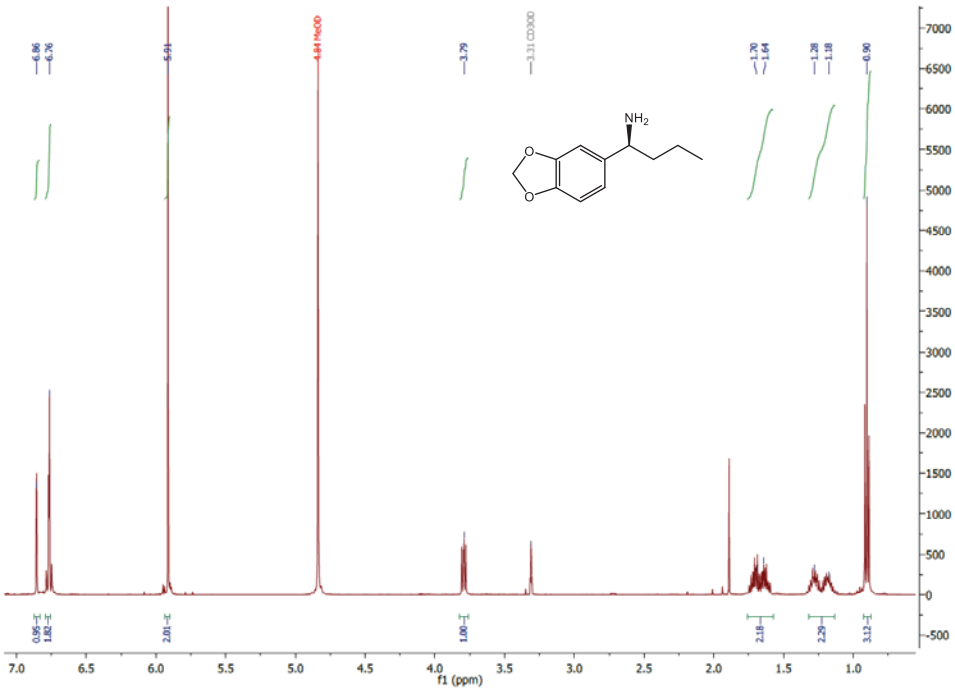

Figure S16. ^{1}H NMR spectrum of the residual substrate (*S*)-**5** after kinetic resolution catalyzed by ATA-117-Rd11 (in methanol-*d*4).

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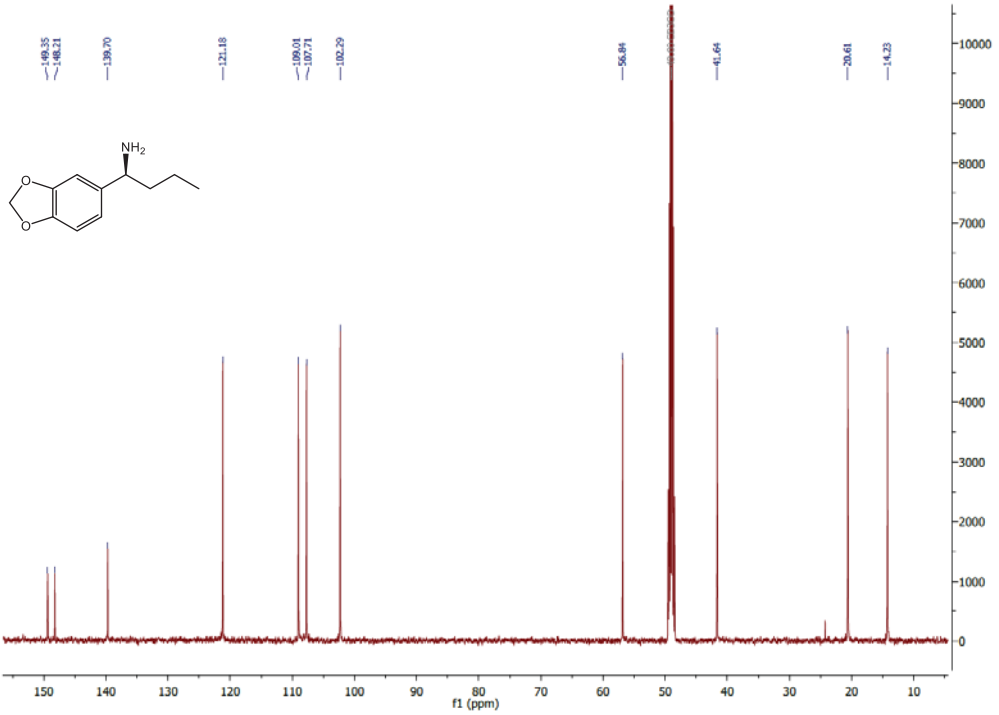

Figure S17. ^{13}C NMR spectrum of the residual substrate (*S*)-**5** after kinetic resolution catalyzed by ATA-117-Rd11 (in methanol-*d*4).

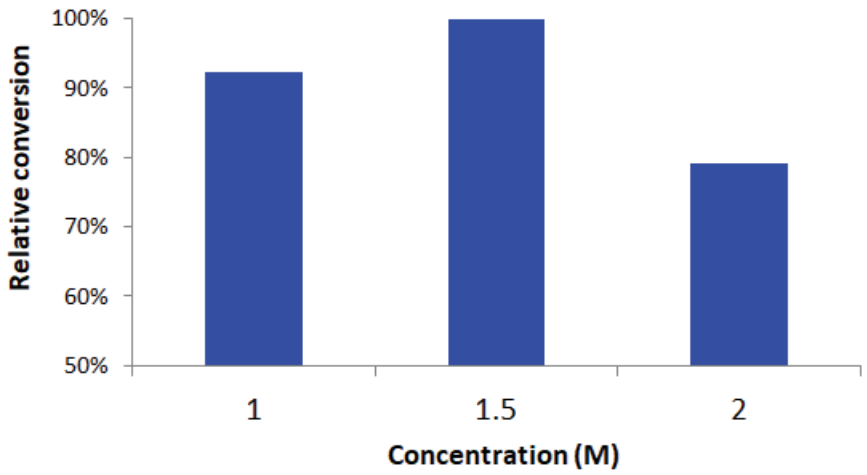

Figure S18. Tolerance of *Cv*-ATA L59A to different concentrations of **11** as an amino donor; yield was determined after 22 h incubation, and the yield at 1.5 M **11** (27%) was set as 100%. Reaction conditions (total volume 0.4 mL): 1 M, 1.5 M or 2 M **11**, 50 mM **9**, 0.5 mM PLP, 2.2 mg mL^{-1} of purified *Cv*-ATA L59A in 100 mM KPi buffer pH 8.0.

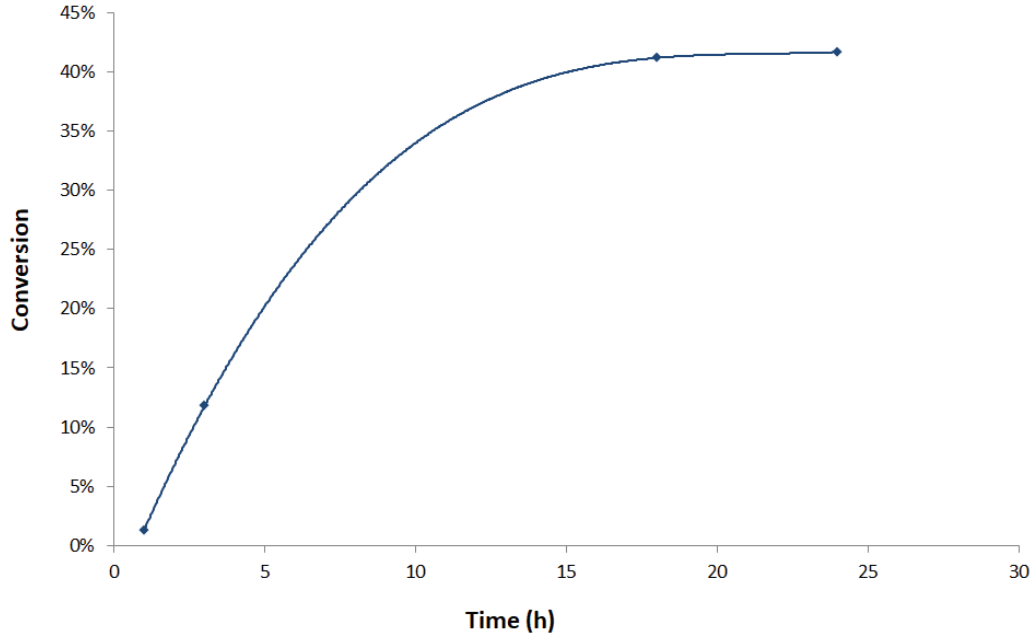

Figure S19. *Cv*-ATA L59A catalyzed conversion of **9**. The conversion was estimated by quantifying the concentration of **9** from HPLC analysis. Reaction conditions: total volume 9 mL; 1.5 M **11**, 50 mM **9**, 0.5 mM PLP, 20% DMSO and 4.8 mg mL^{-1} purified *Cv*-ATA L59A in 0.1 M KPi buffer (pH 8.0), 30^{o}C under magnetic stirring for 24 h**.**

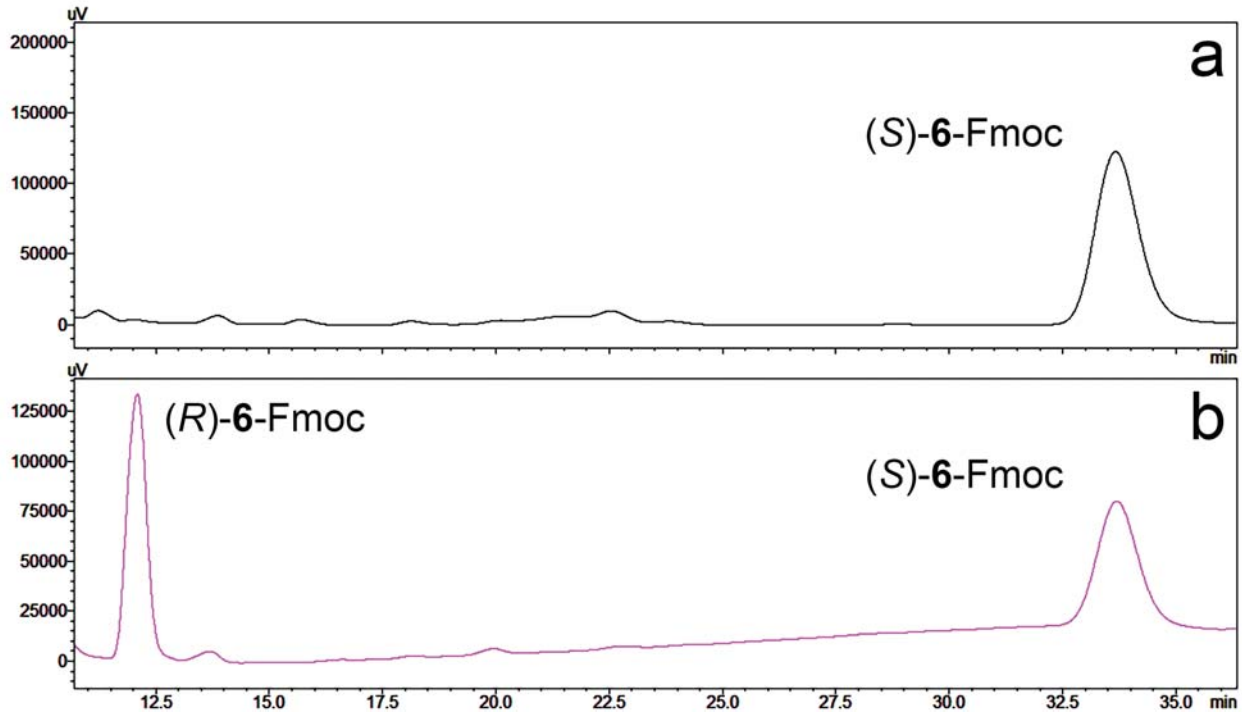

Figure S20. Chiral HPLC of (*S*)-**6** and (*R*)-**6** after Fmoc derivatization: **a**) purified product (*S*)-**6**-Fmoc after stereoselective synthesis catalyzed by *Cv*-ATA L59A; **b**) reference sample of *rac*-**6**-Fmoc. HPLC was performed with a mobile phase consisting of 25% (v/v) isopropyl alcohol and 0.1% (v/v) diethylamine in hexane.

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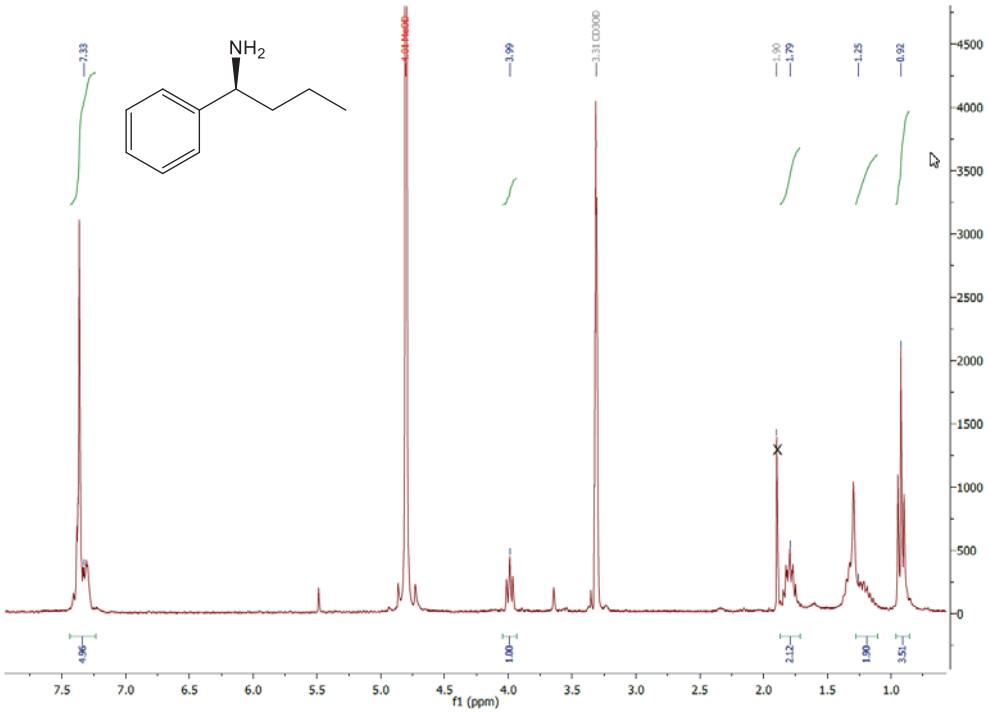

Figure S21. ^{1}H NMR spectrum of (*S*)-**6** in methanol-*d*4. The product was purified after asymmetric stereoselective synthesis by *Cv-ATA* L59A.

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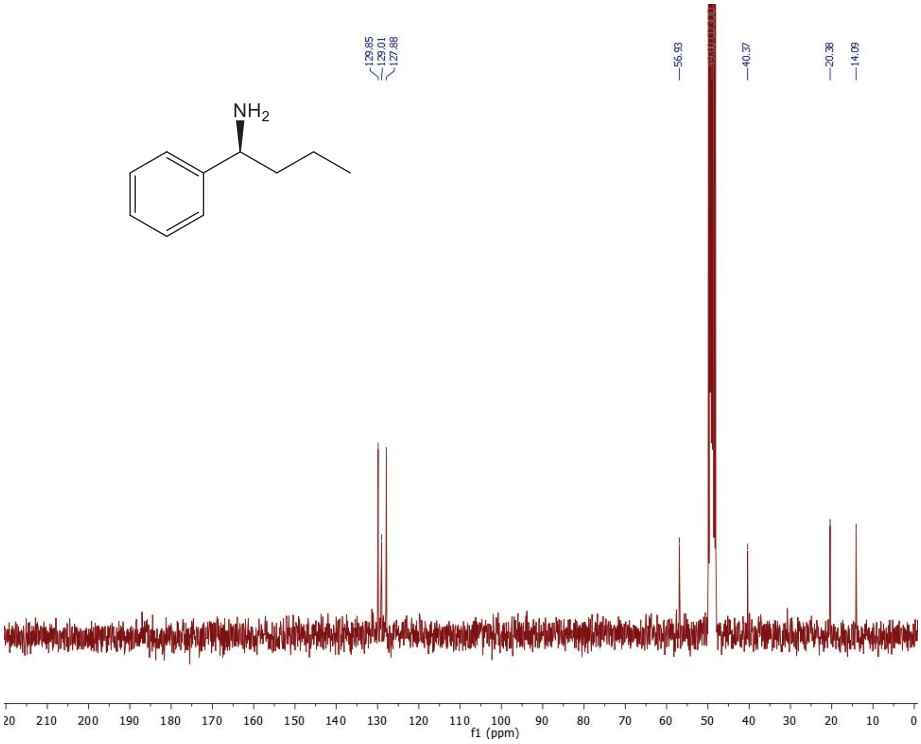

Figure S22. ^{13}C NMR spectrum of (*S*)-**6** in methanol-*d*4. The product was purified after asymmetric stereoselective synthesis by *Cv-ATA* L59A.

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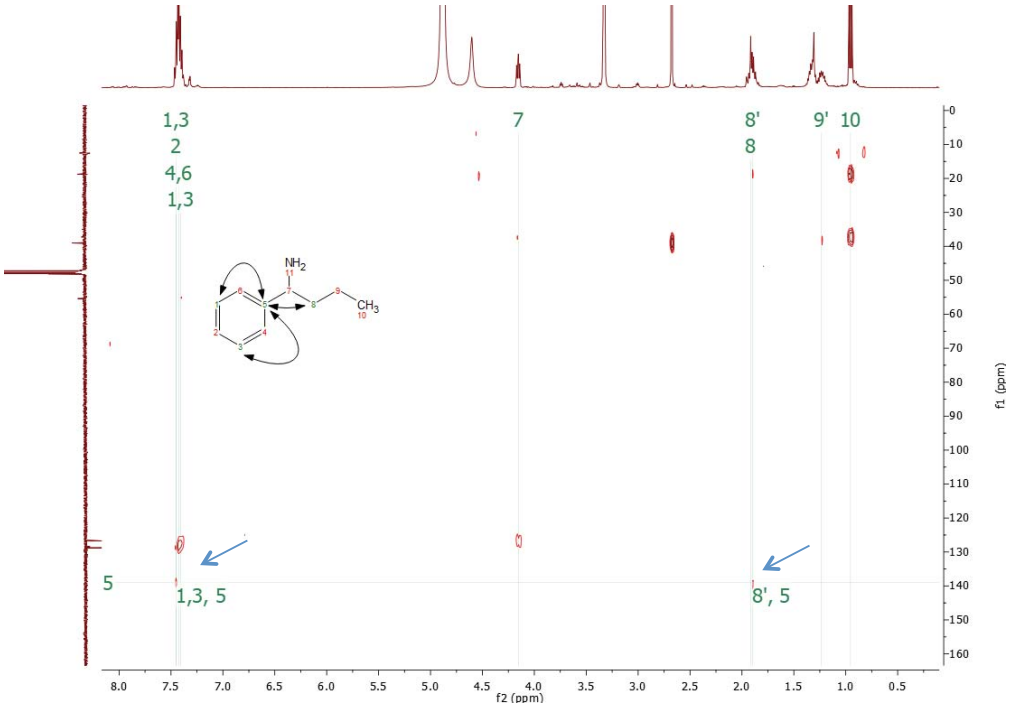

Figure S23. ^{1}H-^{13}C HMBC spectrum of (*S*)-**6** in methanol-*d*4. The product was purified after asymmetric stereoselective synthesis by *Cv-ATA* L59A. The *ipso*-carbon:proton interactions are indicated with arrows.

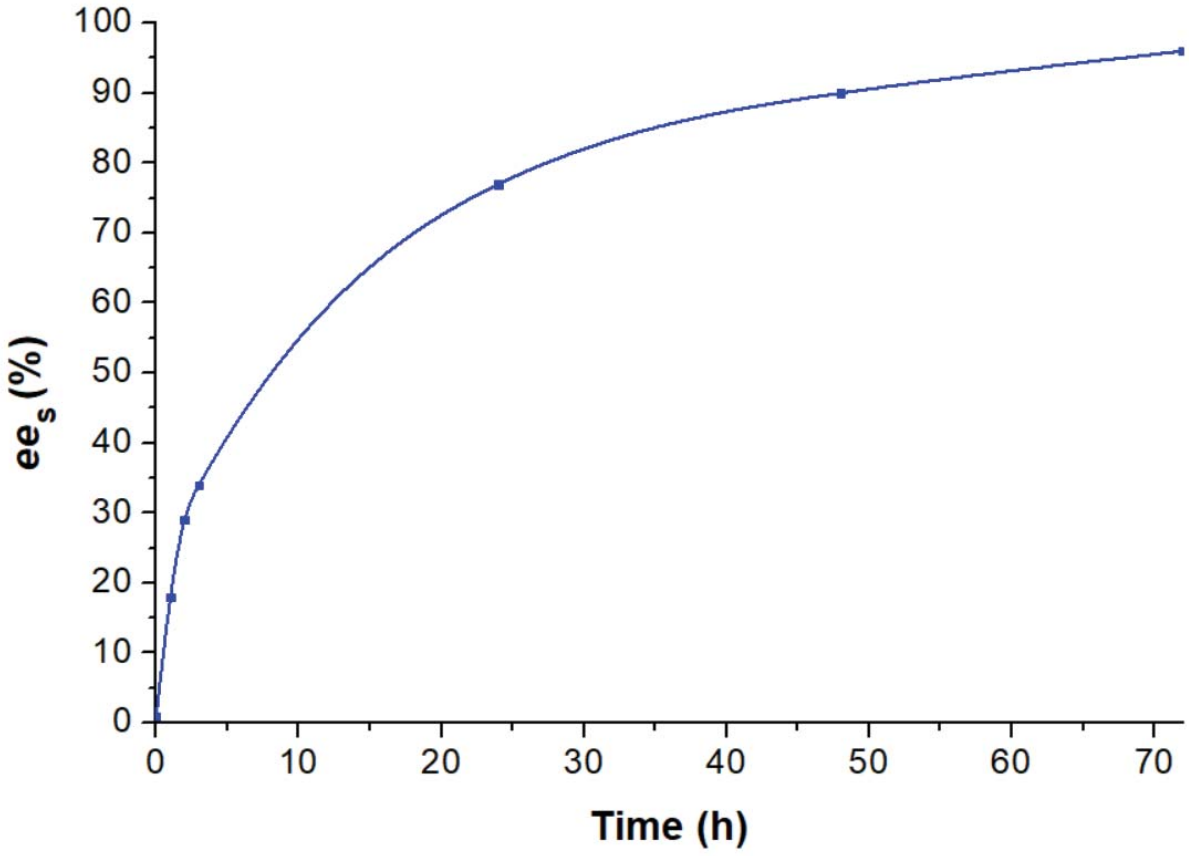

Figure S24. ATA-117-Rd11 catalyzed kinetic resolution of *rac*-**6**. Reaction conditions (total volume 3.3 mL in 10 mL round-bottom flask): 0.1 M *rac*-**6**, 1.2 M **12**, 0.5 mM PLP and 30 mg mL^{-1} ATA-117-Rd11 CFE in 0.1 M KPi buffer; pH 8.5; 45^{o}C; magnetic stirring. Enantiomeric excess (*ee*$_S$) was estimated by quantifying the areas of peaks of (*R*)-**5** and (*S*)-**5** from chiral HPLC.

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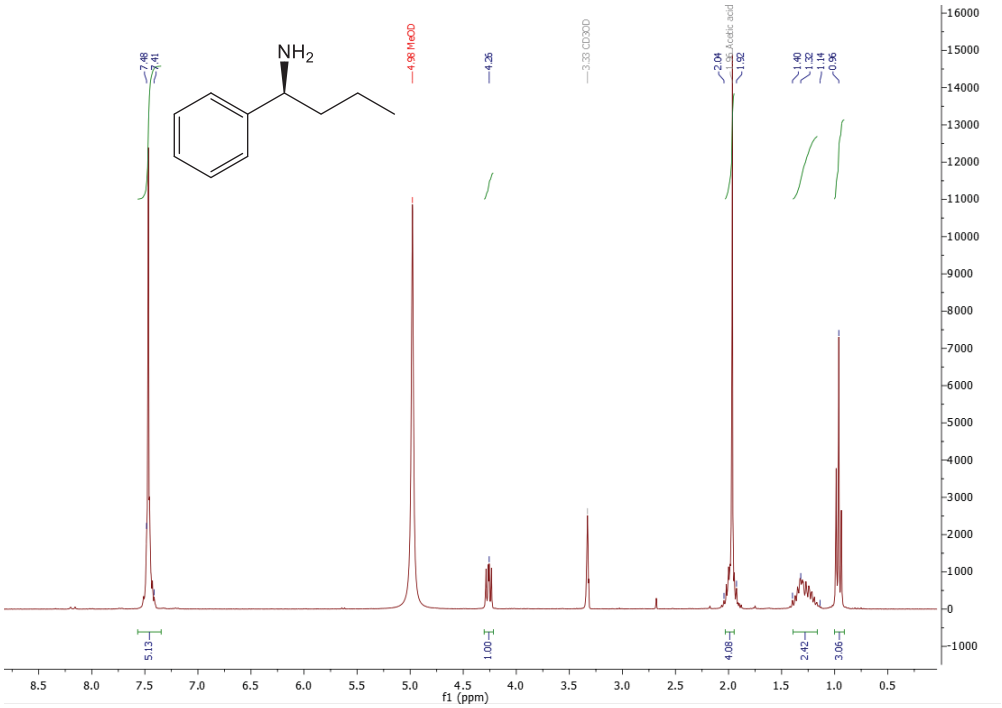

Figure S25. ^{1}H NMR spectrum of (*S*)-**6** in methanol-*d*4. Product was purified after ATA-117-Rd11 catalyzed kinetic resolution of *rac*-**6**.

Supplement

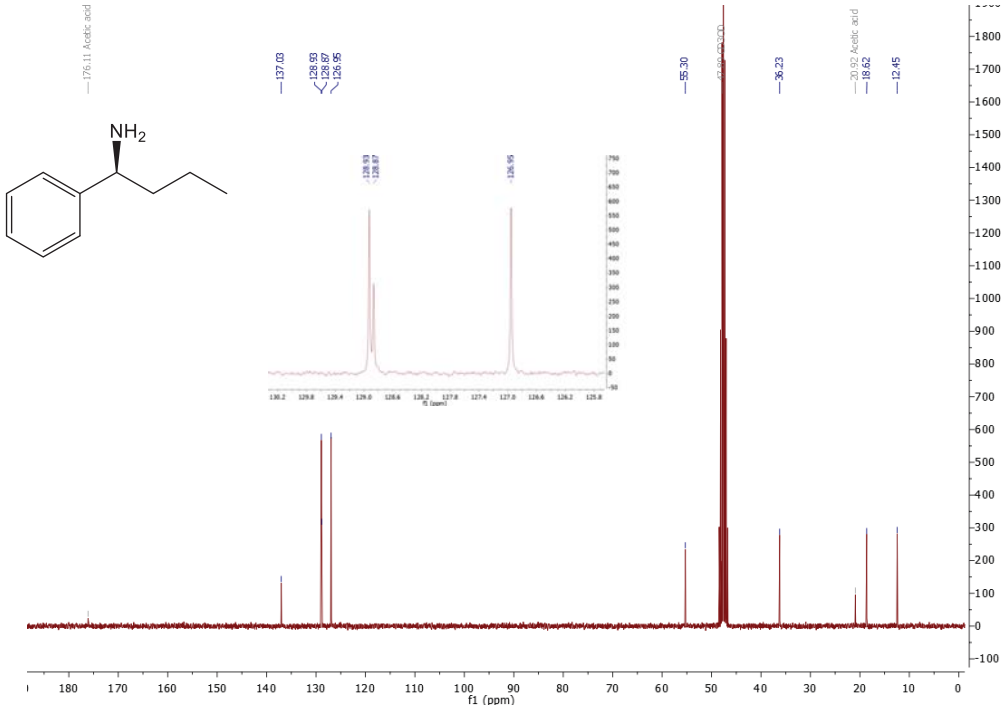

Figure S26. ^{13}C NMR spectrum of (*S*)-**6** in methanol-*d*4. Product was purified after ATA-117-Rd11 catalyzed kinetic resolution of *rac*-**6**.

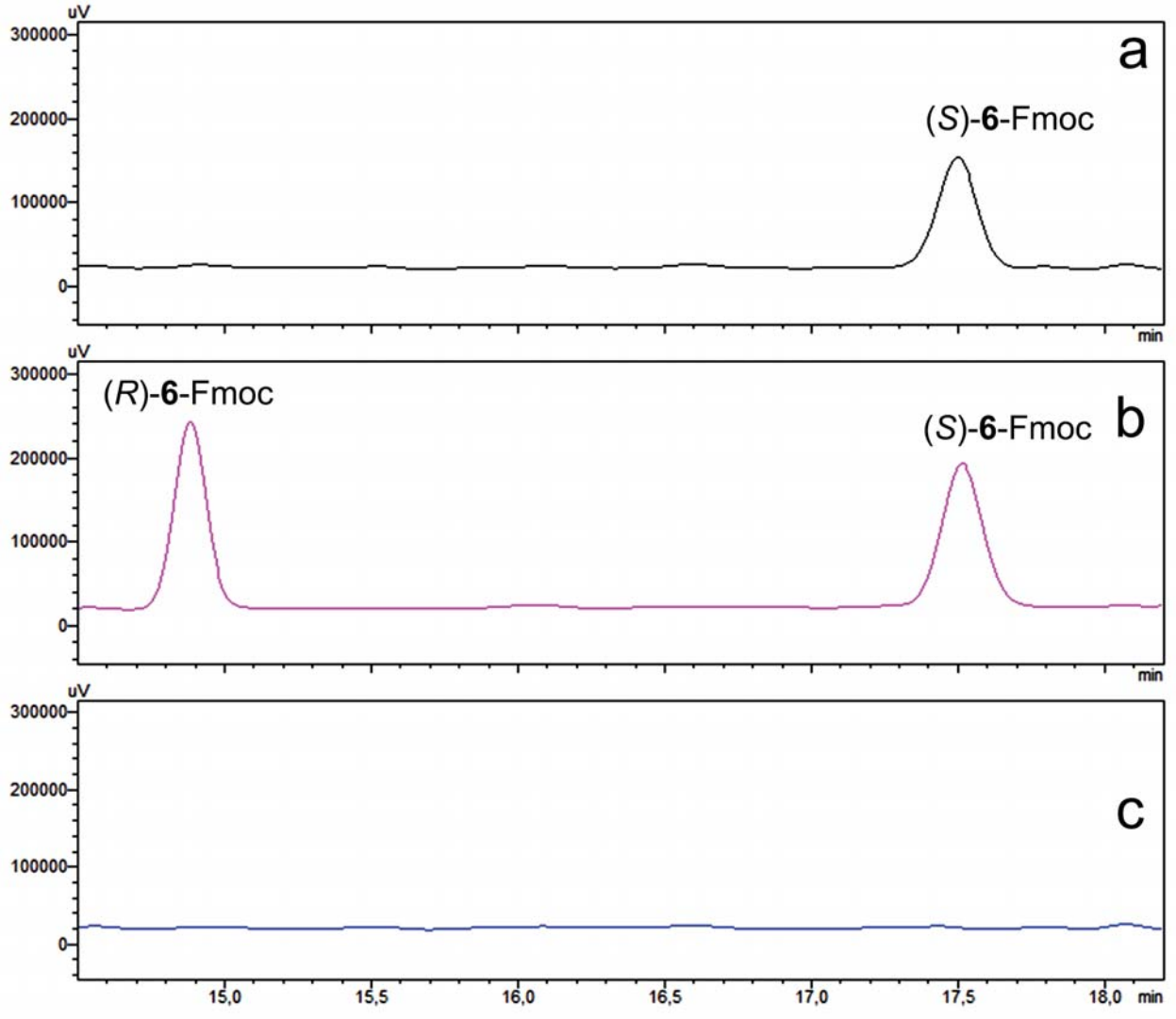

Figure S27. Chiral HPLC of (*S*)-**6** and (*R*)-**6** after Fmoc derivatization: **a**) purified product (*S*)-**6**-Fmoc after ATA-117-Rd11 catalyzed kinetic resolution of *rac*-**6**; **b**) reference sample of *rac*-**6**-Fmoc; c) control sample lacking the amine **6** after Fmoc derivatization. HPLC was performed with binary gradient elution program and the mobile phase consisting of 0.1% (v/v) formic acid and 0.1% (v/v) formic acid in acetonitrile.

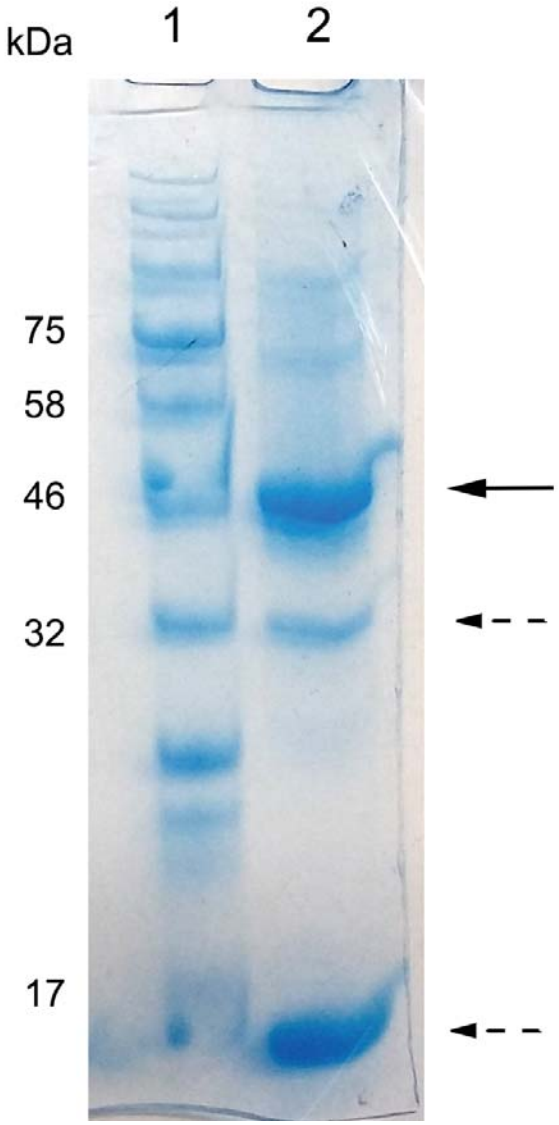

Figure S28. SDS-PAGE of heterologously expressed *Cv*-ATA L59A. Lane 1, molecular weight marker; lane 2, total soluble protein extract from E. coli expressing *Cv*-ATA L59A. The target protein is marked with the solid arrow. The lysate components, DNAse I (31 kDa) and lysozyme (14 kDa) are marked with dotted arrows.

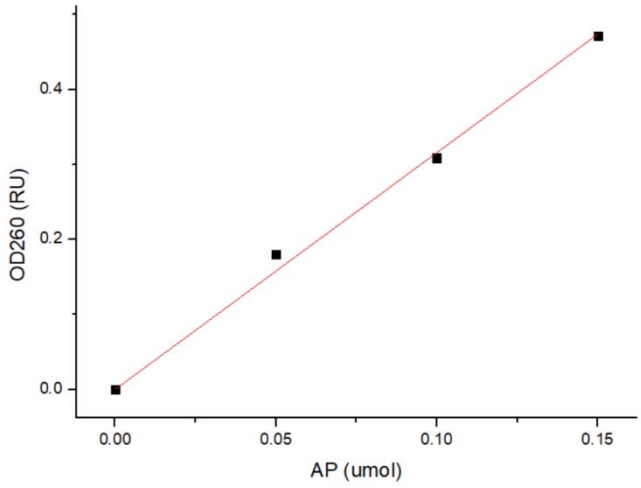

Figure S29. Calibration curve built for the standard solutions of **1** (0.00-0.75 mM) in the reaction mixture (total volume 200 µl) containing 0.75-0.00 mM **2**, 0.1 mM PLP and 1 mg mL^{-1} CFE of *E. coli* harbouring the vector without TA gene. The value of the slope is 3.15761, and the value of the adjusted R-squared is 0.99797.

S3. References

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