Supplementary Information for:

Efficient synthesis of enantiopure amines from alcohols using resting *E. coli* cells and ammonia

Joseline A. Houwman, Tanja Knaus, Magda Costa, Francesco G. Mutti*

Van't Hoff Institute for Molecular Sciences (HIMS-Biocat), University of Amsterdam, Science Park 904, The Netherlands

* Corresponding author: f.mutti@uva.nl

Table of contents

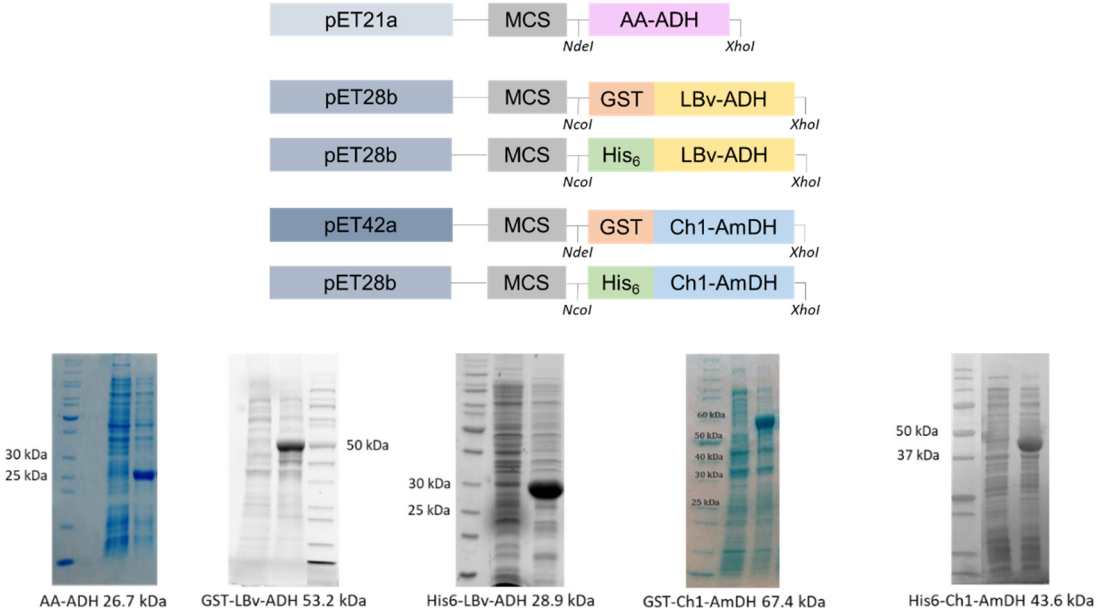
1. List of abbreviations

cww cell wet weight

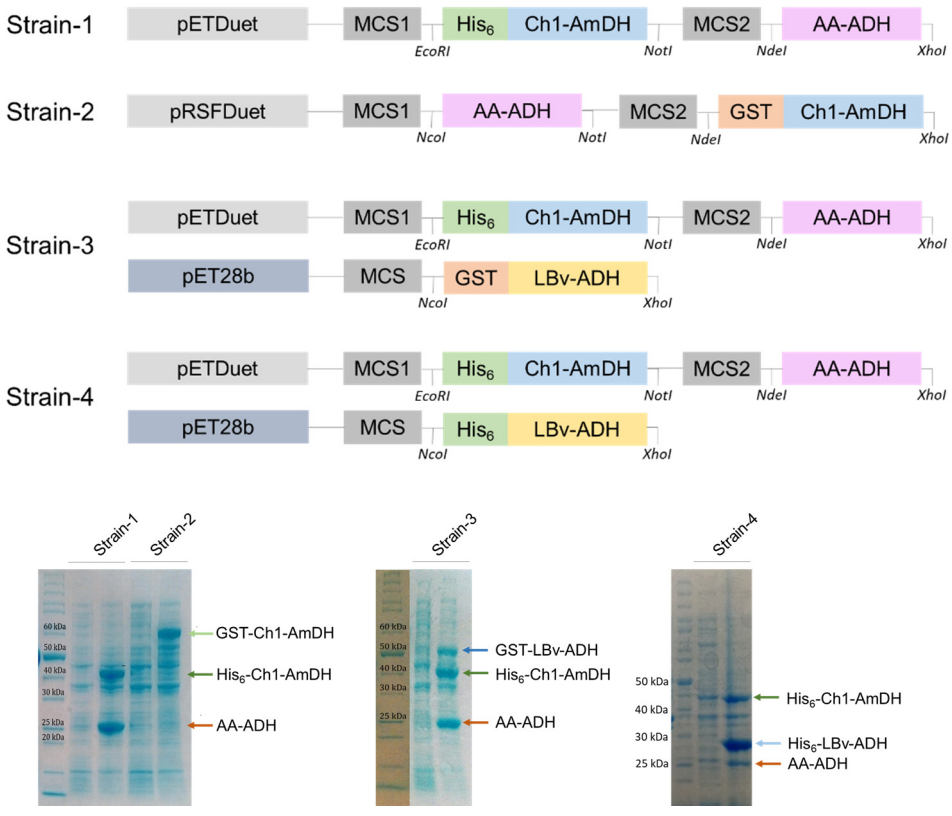
cdw cell dry weight

2. Engineered E. coli strains used in this study

2.1. Single gene constructs and single enzyme expressions



2.2. Multiple gene constructs and multiple enzymes expressions



3. Biocatalytic reactions

Preparation of resting cell suspension was performed as described in the general optimized procedure.

3.1. Preliminary experiments with Strain 3 and 4

Biocatalytic reactions were performed using NH_4Cl/NH_3 buffer (1M, pH 8.7) buffer containing NAD^+ 1 mM, glucose 20 mM, *E. coli* cells (60 mg/mL cww), substrate (20 mM) in 2 mL Eppendorf tubes. Reactions were incubated at 30 °C and 170 rpm for 48 hours.

Table S1. Results for the preliminary *activity tests* using Strain 3 and Strain 4.

Strain	Substrate	Alcohol (%)	Ketone (%)	Amine (%)
Strain-3 (containing GST-LBv-ADH)	(*S*)-2-hexanol	13	35	52
	(*S*)-2-hexanol	16	33	51
	rac-2-hexanol	16	39	45
	rac-2-hexanol	14	44	42
Strain-4 (containing NHis-LBv-ADH)	(*S*)-2-hexanol	11	58	31
	(*S*)-2-hexanol	11	62	27
	rac-2-hexanol	8	66	24
	rac-2-hexanol	10	60	30

3.2. Comparison between lyophilized and resting cells with and without the addition of NAD^+ and glucose

Biocatalytic reactions using resting cells consisted of 1 mL cell suspension in ammonium chloride buffer (70 mg mL^{-1} *E. coli* (AA-Ch1) cells (cww), 20 mM (*S*)-**2a**) in 2 mL Eppendorf tubes. Additionally either 1 mM NAD^+ or 20 mg mL^{-1} glucose was added. Reactions were incubated at 30 °C and 170 rpm for 48 hours.

Biocatalytic reactions using lyophilized cells consisted of 1 mL cell suspension in ammonium chloride buffer (20 mg mL^{-1} *E. coli* (AA-Ch1) cells (cdw), 20 mM (*S*)-**2a**) in 2 mL Eppendorf tubes. Additionally either 1 mM NAD^+ or 20 mg mL^{-1} glucose was added. Reactions were incubated at 30 °C and 170 rpm for 48 hours.

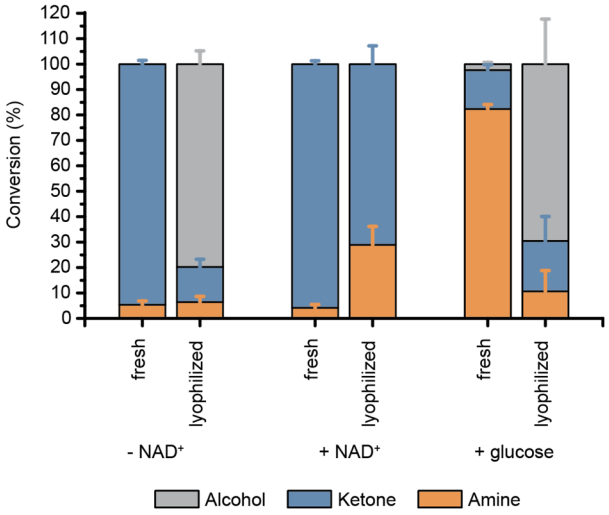


Figure S1. To investigate possible conditions for exploiting the alcohol bioamination using AA-ADH and Ch1-AmDH without the need to purify these enzymes, resting (70 mg mL^{-1} cww) and lyophilized cells (20 mg mL^{-1} cdw) overexpressing the dehydrogenases were tested under various conditions with our model substrate (S)-2-hexanol ((S)-**2a**, 20 mM) in NH_4Cl/NH_3 buffer (1 M, pH 8.7). Conversion of (S)-**2a** by resting (fresh) or lyophilized cells of *E. coli* (Ch1-AA) in either: absence or presence of NAD^+ (1 mM), or addition of glucose (20 mg mL^{-1}). Error bars indicate the standard deviation.

3.3. Optimum ratio between substrate and glucose

Biocatalytic reactions consisted of 1 mL cell suspension in ammonium chloride buffer (60 mg mL^{-1} *E. coli* (AA-Ch1) cells (cww), 20 mM (*S*)-**2a**) in 2 mL Eppendorf tubes. The substrate:glucose ratio was 1:0, 1:0.2, 1:0.4, 1:0.6, 1:0.8, 1:1, 1:2, 1:3, 1:4, 1:5, 1:7.5 or 1:10, respectively. Reactions were incubated at 30 °C and 170 rpm for 24 hours. Work-up was carried out as previously reported and the results are shown in the main paper in Figure 2A.

3.4. Consistent conversion of rac-2-hexanol by E. coli (LBv-AA-Ch1)

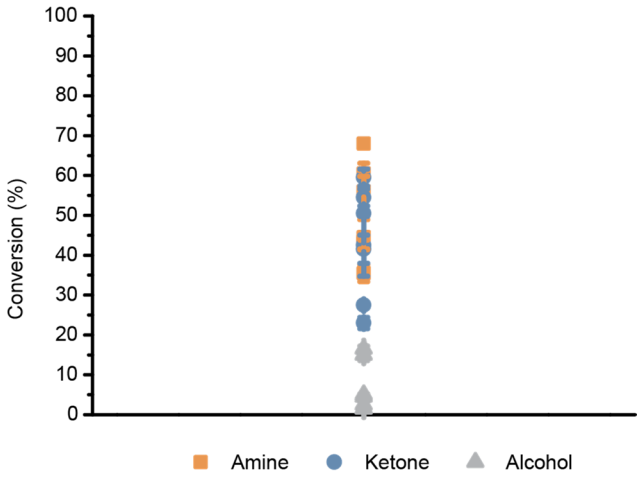


Figure S2. Independently grown batches of *E. coli* (Ch1-AA-LBv) cells (60 mg mL^{-1} cww) show consistent conversion of rac-**2a** (20 mM) to **2c** in reactions run at 1:1 substrate:glucose ratio and over 24 hours.

3.5. Amount of cells needed

Biocatalytic reactions consisted of 1 mL cell suspension in ammonium chloride buffer (*E. coli* (AA-Ch1) cells, 20 mM glucose, 20 mM (*S*)-**2a**) in 2 mL Eppendorf tubes. The cww was 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 mg mL^{-1} cells, respectively. Reactions were incubated at 30 °C and 170 rpm for 24 hours. Work-up was carried out as previously reported and the results are shown in the main paper in Figure 2B.

3.6. Quantitative accurate analytical determination of conversion using "Method of standard addition"

In this work, we also investigated the efficiency of extraction procedures when using resting *E. coli* cells and validated that all the components of the reaction mixture (substrates, intermediates and products) can be extracted quantitatively with the optimized procedure. In practice, the components of the reaction (substrates, intermediates and products) were incubated either *i)* with resting cells in reaction buffer, or *ii)* just in reaction buffer. Then, we tried to extract them. The results from extraction of compounds from samples prepared under both conditions (with or without cells) were comparable (data not shown).

In general, the results described in Figure S3 and Table S2 show that quantitative extraction of all components with EtOAc from the aqueous mixture containing the suspension of *E. coli* cells is possible. In fact, the analytical yield (determined with the "method of standard addition of product to the reaction") and the conversion (determined by conventional integration of GC-FID chromatograms) differed less than 4% from each other for the reaction at 20 mM of (*S*)-**2a** (see Table S1). The deviation was slightly higher for

biotransformations at 30 mM substrate concentration, but the average value still deviated less than 2%. Therefore, we conclude that extraction of reaction components with EtOAc is quantitative (within a reasonably low experimental error). Furthermore, the measurement of absolute conversions by integration of the obtained GC-FID traces are accurate enough for the bioamination of alcohols with *E. coli* whole-cells conducted under our optimized reaction conditions (60 mg mL^{-1} *cww*), particularly in the range of substrate concentrations mainly applied in this work.

Biocatalytic reactions consisted of 14 mL cell suspension in NH_4Cl/NH_3 buffer (60 mg mL^{-1} *E. coli* (AA-Ch1) cells (cww), 20 or 30 mM glucose, 20 mM or 30 mM (*S*)-**2a** in 30 mL glass vials. Reactions were incubated at 30 °C and 170 rpm for 24 hours. After incubation, the reaction mixture was divided into 12 aliquots of 1 mL each. A set of analytical samples was prepared by standard addition of compound (*R*)-**2c** at different concentrations (0, 5, 10 or 15 mM). Three independent samples were prepared for each concentration. After addition, the samples were incubated for 30 min. Work-up was carried out as previously reported.

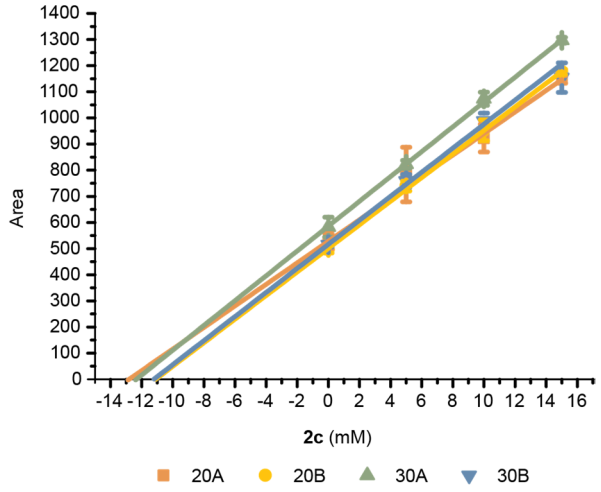


Figure S3. Standard addition of 0, 5, 10 or 15 mM of (*R*)-**2c** to biotransformations conducted at 20 or 30 mM concentration of (*S*)-**2a** catalyzed by *E. coli* (AA-Ch1) (60 mg mL-1 cww). The method can be efficiently used to calculate the absolute amount of (*R*)-**2c** formed.

Table S2. Absolute amount of (*R*)-**2c** formed from biotransformation of 20 or 30 mM (*S*)-**2a** by *E. coli* (AA-Ch1) calculated from the method of standard addition and derived from the conversion measured by GC-FID.

Substrate added (mM)	Amine formed (determined by method of Standard Addition) (mM)	Amine formed (determined from direct integration of GC-traces) (mM)*	Difference (mM)	Relative difference (%)
20	12.8	12.4 (61 % ± 0.6)	+0.4	+3.1
	11.2	11.6 (58 % ± 0)	+0.4	+3.6
30	12.2	13.8 (46 % ± 0)	+1.6	+13.1
	12.4	11.4 (37.7 ± 0.6)	-1	-8.1

* In brackets, it is reported the original conversion value in % as calculated by direct integration of GC-FID traces. Each value of conversion in %, with related standard deviation, is the average from triplicate measurements.

3.7. Maximum amount of amine formed

Biocatalytic reactions consisted of 1 mL cell suspension in ammonium chloride buffer (60 mg mL^{-1} *E. coli* (AA-Ch1) cells (cww), 1:1 substrate:glucose ratio) in 2 mL Eppendorf tubes. (*S*)-**2a** concentration was 10, 20,

30, 40 or 50 mM, respectively. Reactions were incubated at 30 °C and 170 rpm for 24 hours. Work-up was carried out as previously reported.

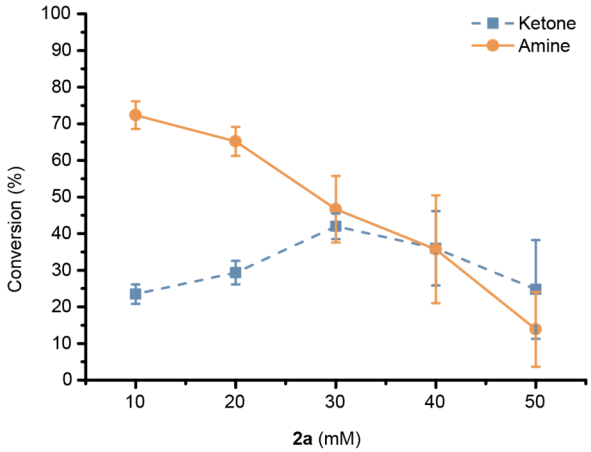


Figure S4. Conversion of (S)-**2a** (varied concentrations: 10-50 mM) to **2c** employing E. coli (Ch1-AA) cells (60 mg mL^{-1}, cww) in NH_4Cl/NH_3 buffer (1 M, pH 8.7). Error bars indicate standard deviation.

3.8. Reaction rate at high substrate concentrations and survival of cells

Biocatalytic reactions consisted of 1 mL cell suspension in ammonium chloride buffer (60 mg mL^{-1} *E. coli* (AA-Ch1) cells (cww), 1:1 substrate:glucose ratio) in 2 mL Eppendorf tubes. (*S*)-**2a** concentration was 20, 40 or 50 mM, respectively. Reactions were incubated at 30 °C and 170 rpm. Individual reactions were stopped after 1, 2, 3, 4, 7, 16, 24, 30 or 48 hours.

Before quenching and extraction of the samples as described in the general procedure, 10 µL from each sample were taken and diluted with ammonium chloride buffer. 50 µL of an appropriate dilution (final dilution 1x, 20x or 2000x) were plated on LB-agar containing ampicillin (100 mg L^{-1}). Plates were incubated overnight at 37 °C. The following day, the colonies on the plate were counted and the colony forming units (CFU) per mg cells were calculated with following equation:

$$N \cdot D_f \cdot V_s \cdot c_s$$

With *N* the number of colonies on the plate, D_f the final dilution factor, V_s the sample volume removed for plating (10 µL) and c_s the concentration of cells in the original sample (60 mg mL^{-1}).

The results are shown in the main paper in Figure 3B.

3.9. Initial substrate scope

Biocatalytic reactions consisted of 1 mL cell suspension in ammonium chloride buffer (60 mg mL^{-1} cells (cww), 20 mg mL^{-1} glucose and 20 mM substrate (*S*)-**1a**, (*S*)-**2a**, (*S*)-**3a**, (*S*)-**4a** and (*S*)-**5a**) in 2 mL Eppendorf tubes. . Reactions were incubated at 30 °C and 170 rpm for 48 hours and the work-up was carried out as described in the main paper. The results are shown in the main paper in Figure 3C.

3.10. Bioamination at varied substrate concentrations

Biocatalytic reactions consisted of 1 mL cell suspension in ammonium chloride buffer (60 mg mL^{-1} *E. coli* (AA-Ch1) cells (cww)) in 2 mL Eppendorf tubes. Glucose concentration was in a 1:1 substrate:glucose ratio. Substrates (*S*)-**1a**, (*S*)-**2a**, (*S*)-**3a**, (*S*)-**4a** or (*S*)-**5a** were tested in concentrations of 1, 5 or 10 mM. Reactions

were incubated at 30 °C and 170 rpm for 24 hours and the work-up was carried out as described in the main paper.

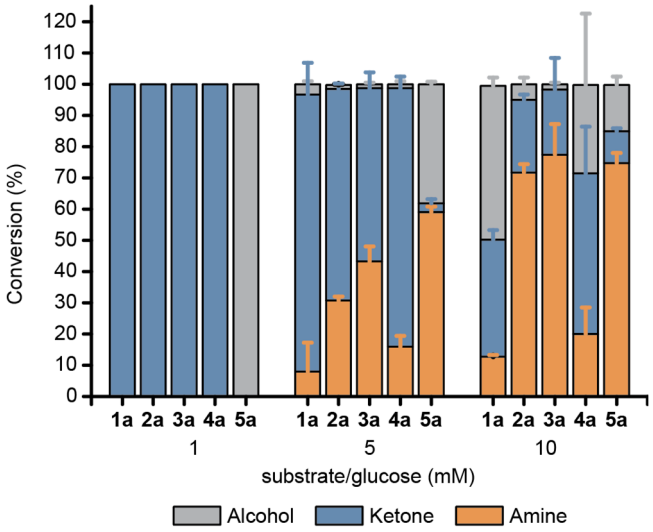


Figure S5. Conversion of (S)-**1**-**5a** in NH_4Cl/NH_3 buffer (1 M, pH 8.7) by *E. coli* (AA-Ch1) (60 mg mL^{-1} cww) for various substrate concentrations (1-10 mM) and a fixed 1:1 substrate:glucose ratio. Error bars indicate standard deviation.

3.11. Minimal inhibitory concentration assays with E. coli (AA-Ch1)

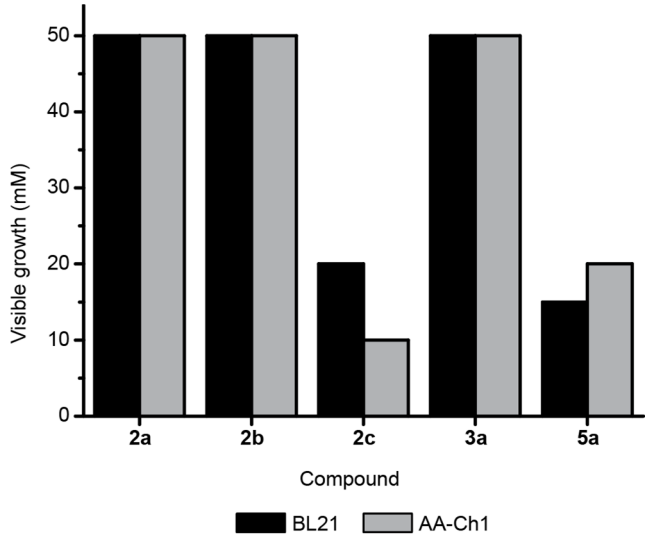


Figure S6. Determination of toxicity through minimal inhibitory concentration (MIC) assays for *E. coli* cells (devoid of plasmid) and E. coli (AA-Ch1) cells for certain compounds.

3.12. Effect of enantiopure or racemic substrate

Biocatalytic reactions consisted of 1 mL cell suspension in ammonium chloride buffer (60 mg mL^{-1} *E. coli* (LBv-AA-Ch1) cells (cww), 20 mM glucose, 20 mM substrate) in 2 mL Eppendorf tubes. Substrates tested were *rac*-**2a**, (*S*)-**2a**, *rac*-**5a**, (*S*)-**5a** and (*R*)-**5a**. Reactions were incubated at 30 °C and 170 rpm. Individual

reactions were stopped after 1, 2, 3, 4, 6 or 24 hours, respectively. Work-up was carried out as previously reported. The results are shown in the main paper in Figure 6.

3.13. Influence of co-solvents on amine product toxicity

Biotransformations consisted of 1 mL cell suspension in ammonium chloride buffer (60 mg mL^{-1} *E. coli* (AA-Ch1) cells (cww), 20 mM glucose) and 0, 0.05, 0.1 or 0.5 mL of *n*-hexadecane (C16) in 4 mL glass vials. Substrates (*S*)-**3a** and (*S*)-**4a** were tested with substrate concentrations of 1, 5, 10 or 20 mM. Reactions were incubated at 30 °C and 170 rpm for 24 hours. The results are shown in the main paper in Figure 5A and B.

4. Analytical methods, determination of absolute configuration and GC chromatograms

4.1. Analytical methods

Conversions and enantiomeric excesses (*ee*) were determined by GC using a 7890A GC system (Agilent Technologies), equipped with a FID detector and H_2 as carrier gas with a DB-1701 column (conversion) from Agilent (60 m, 250 µm,0.25 µm, or 30 m, 250 µm,0.25 µm) or a Varian Chrompack Chiracel Dex-CB column (*ee*) (25 m, 320 µm,0.25 µm).

DB1701-30m-A: constant pressure 13.5 psi, $T_{injector}$ 300 °C, split ratio 40:1, $T_{initial}$ 80 °C, hold 6.5 min; gradient 10 °C min^{-1} up to 160 °C, hold 5 min; gradient 20 °C min^{-1} up to 200 °C, hold 2 min; gradient 20 °C min^{-1} up to 300 °C, hold 1 min.

DB1701-30m-B: constant pressure 6.9 psi, $T_{injector}$ 250 °C, split ratio 40:1, $T_{initial}$ 60 °C, hold 6.5 min; gradient 20 °C min^{-1} up to 100 °C, hold 1 min; gradient 20 °C min^{-1} up to 280 °C, hold 1 min.

DB1701-60m: constant pressure 13.5 psi, $T_{injector}$ 250 °C, split ratio 40:1, $T_{initial}$ 80 °C, hold 6.5 min; gradient 5 °C min^{-1} up to 160 °C, hold 2 min; gradient 20 °C min^{-1} up to 280 °C, hold 1 min.

DEX-CB-A: constant flow 1.4 mL min^{-1}, $T_{injector}$ 200 °C, split ratio 40:1, $T_{initial}$ 100 °C, hold 2 min; gradient 1 °C min^{-1} up to 130 °C, hold 5 min; gradient 10 °C min^{-1} up to 170 °C, hold 10 min; gradient 10 °C min^{-1} up to 180 °C, hold 1 min.

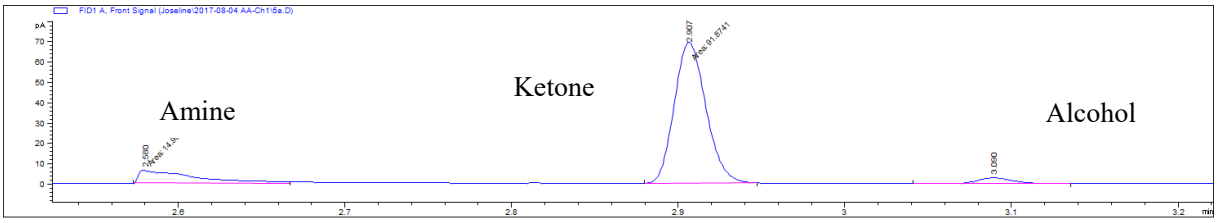
DEX-CB-B: constant flow 1.4 ml min^{-1}, $T_{injector}$ 200 °C, split ratio 40:1, $T_{initial}$ 100 °C, hold 2 min; gradient 1 °C min^{-1} up to 118 °C, hold 5 min; gradient 10 °C min^{-1} up to 170 °C, hold 10 min; gradient 10 °C min^{-1} up to 180 °C, hold 1 min.

Compound	Retention time [min]	Compound	Retention time [min]	Compound	Retention time [min]	Method
1a	3.1	**1b**	2.9	**1c**	2.5	DB1701-30m-B
2a	4.9	**2b**	4.5	**2c**	3.6	DB1701-30m-B
3a	2.5	**3b**	2.4	**3c**	1.9	DB1701-30m-A
4a	4.2	**4b**	3.9	**4c**	3.0	DB1701-30m-A
5a	21.7	**5b**	21.6	**5c**	19.9	DB1701-60m

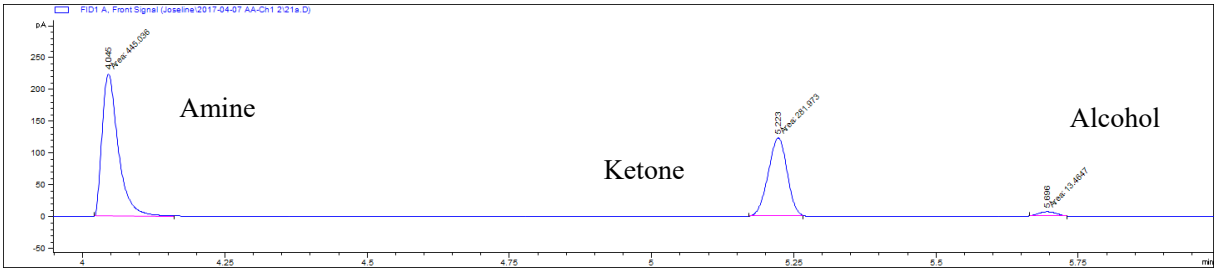
Compound	Retention time [min]	Compound	Retention time [min]	Method
(*S*)-**1c**	5.6	(*R*)-**1c**	5.9	DEX-CB-B
(*S*)-**2c**	8.4	(*R*)-**2c**	8.8	DEX-CB-B
(*S*)-**3c**	12.7	(*R*)-**3c**	13.3	DEX-CB-A
(*S*)-**4c**	18.6	(*R*)-**4c**	19.4	DEX-CB-A
(*S*)-**2a**	2.4	(*R*)-**2a**	2.5	DEX-CB-B
(*S*)-**5a**	9.8	(*R*)-**5a**	9.9	DEX-CB-A

4.2. Chromatograms for the determination of conversions

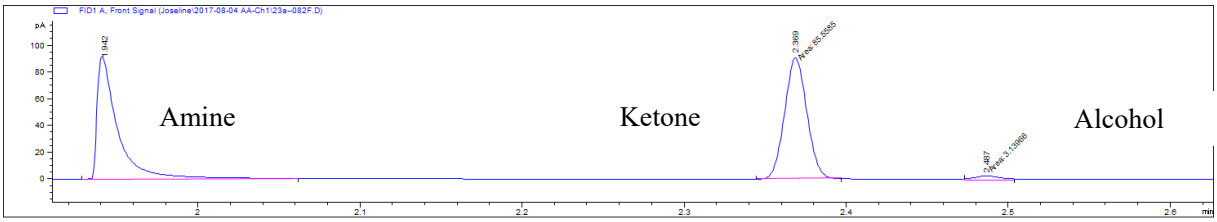
Conversion of (*S*)-2-pentanol to (*R*)-2-aminopentane



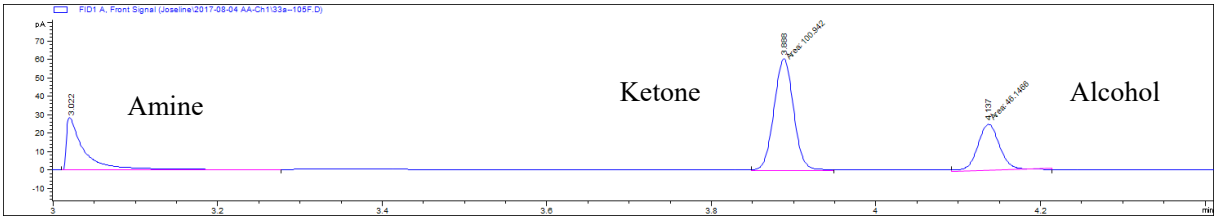
Conversion of (*S*)-2-hexanol to (*R*)-2-aminohexane



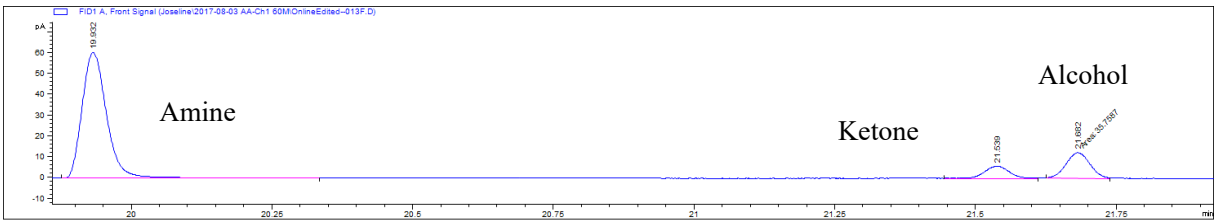
Conversion of (*S*)-2-heptanol to (*R*)-2-aminoheptane



Conversion of (*S*)-2-octanol to (*R*)-2-aminooctane

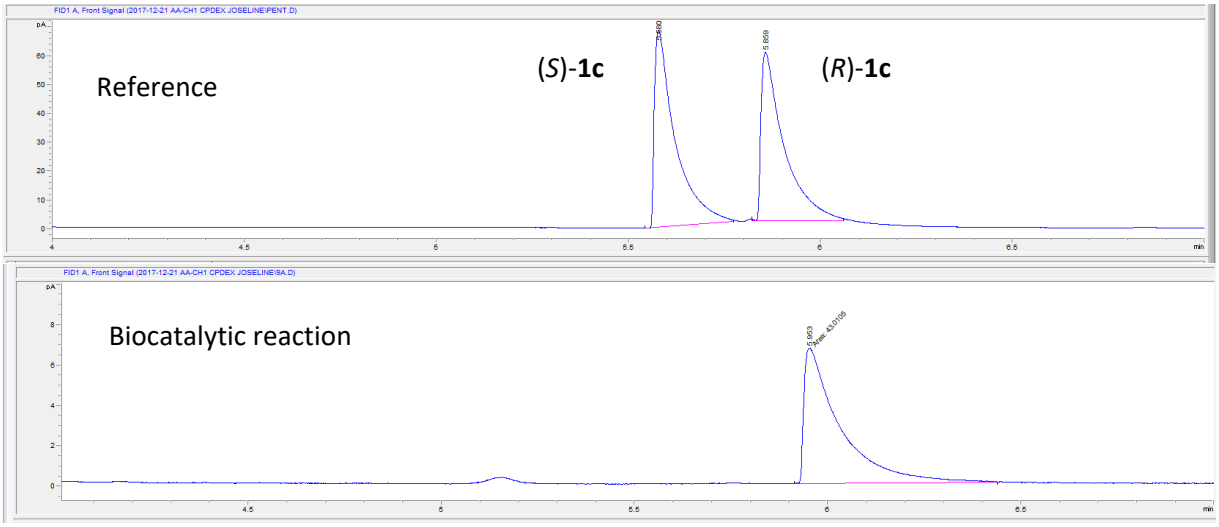


Conversion of (*S*)-1-phenyl-propan-2-ol to (*R*)-1-phenyl-2-aminopropane (measured with DB-1701-60m length column)

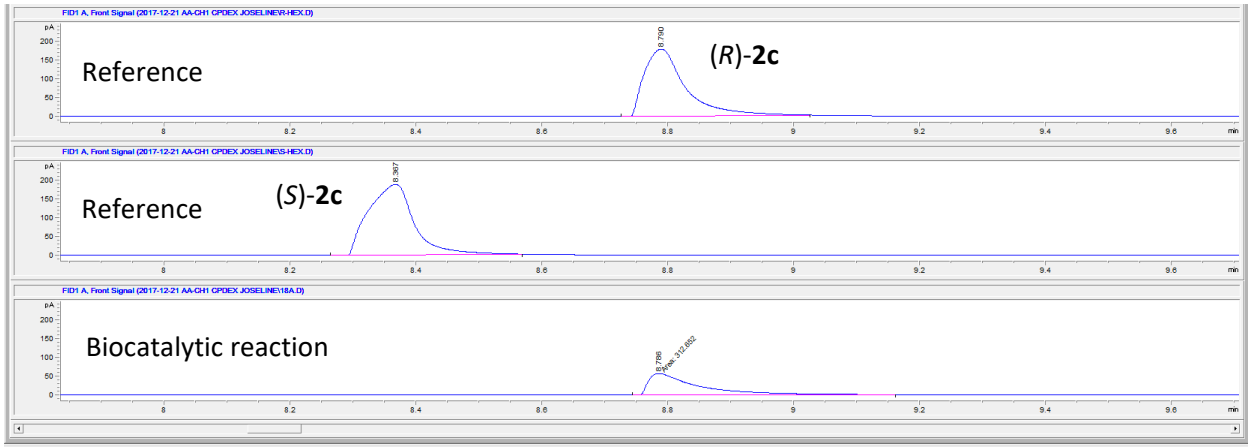


4.3. Chromatograms for the determination of the enantiomeric excess

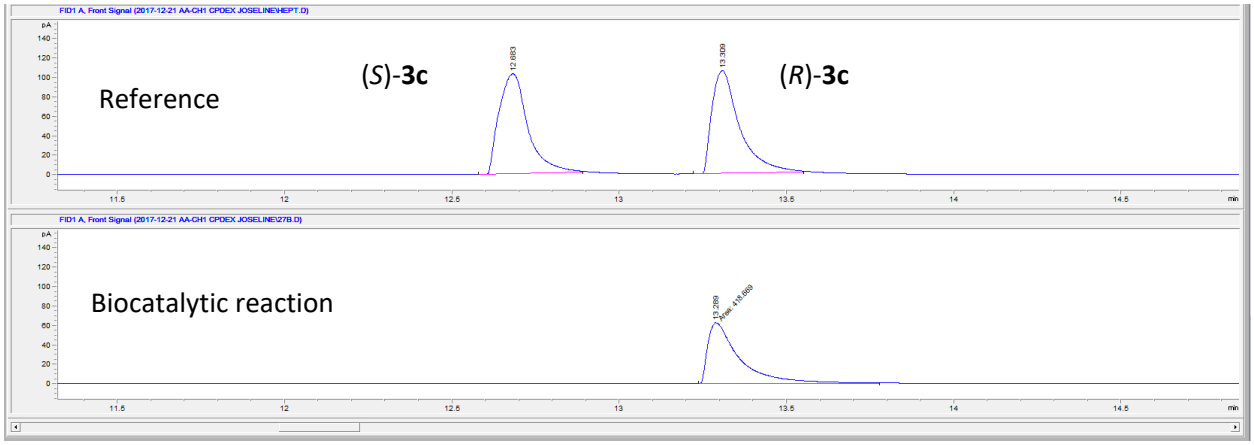
2-Aminopentane



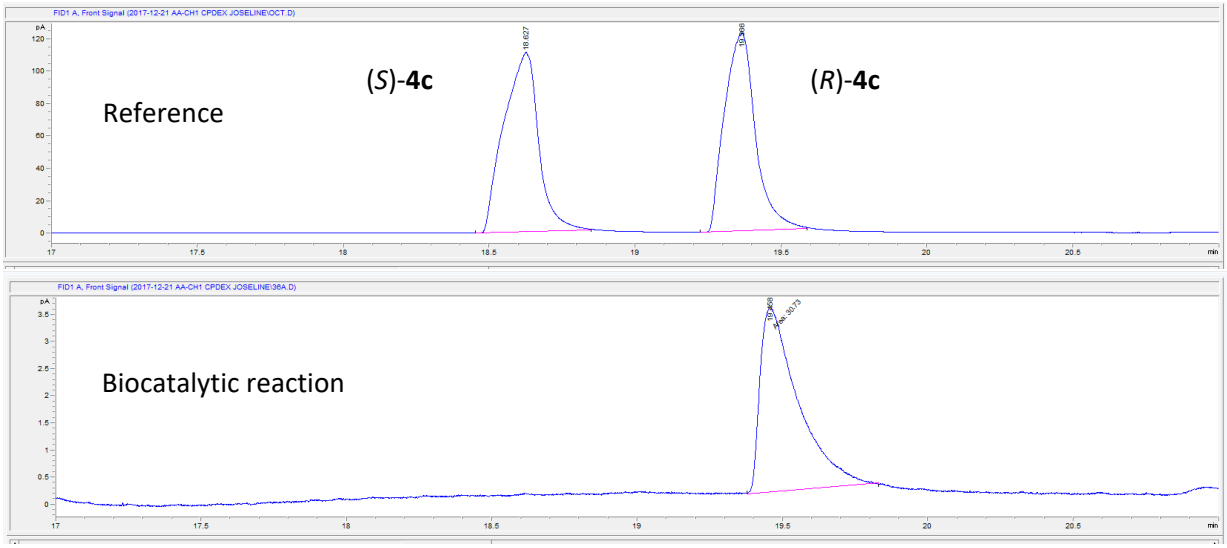
2-Aminohexane



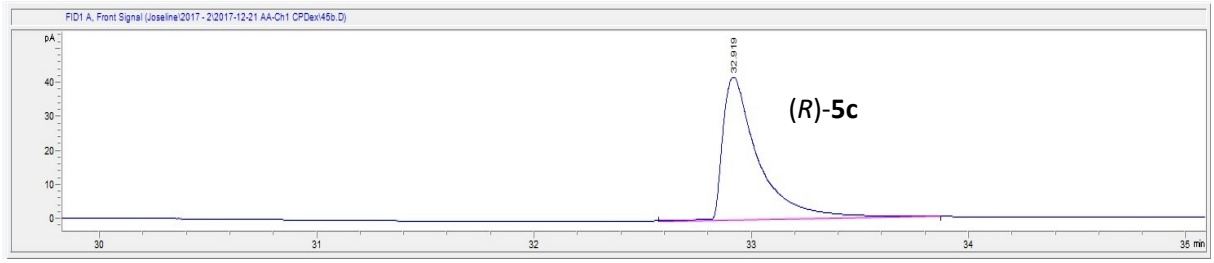
2-Aminoheptane



2-Aminooctane



(*R*)-1-phenyl-2-aminopropane (for reference racemic mixture, see F. G. Mutti et al., *Science* 2015, 349, 1525.



5. NMR Spectrum of preparative scale reaction