Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting information

Regio- and Stereoselective Multi-enzymatic Aminohydroxylation of β-methylstyrene using Dioxygen, Ammonia and Formate

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1. Abbreviations

ADH	Alcohol dehydrogenase
EH	Epoxide hydrolase
SMO	Styrene monooxygenase
BDHA	2,3-butanediol dehydrogenase
AmDH	Amine dehydrogenase
NOx	NADH-dependent oxidase from *Streptococcus mutans*
YcnD	NADPH-dependent oxidase from *Bacillus subtilis*
FDH	Formate dehydrogenase
KPi	Potassium phosphate buffer
$NAD(P)^+$	Nicotinamide adenine dinucleotide (phosphate)
FAD	Flavin adenine dinucleotide
HCOONa	Sodium formate
MTBE	*tert*-butyl methyl ether
EtOAc	Ethyl acetate
PE	Petroleum ether
TMSCN	Trimethylsilyl cyanide
$HCOONH_4$	Ammonium formate
Tris-HCl	Tris(hydroxymethyl)aminomethane buffer
GITC	2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate
ee	enantiomeric excess
de	diastereomeric excess
n.d.	not detected
n.m.	not measured

2. General information

Trans-1-Phenyl-1-propene *(trans*-β-methylstyrene, *trans*-**1**), *cis*-1-phenyl-1-propene *(cis*-β-methylstyrene, *cis*-**1**), 1-phenylpropane-1,2-dione (**7**), GITC and FAD were purchased from TCI chemicals. (2*R,*3*R*)-(+)-2-Methyl-3-phenyloxirane *(*2*R,*3*R*-**2**), (2*S,*3*S*)-(−)-2-Methyl-3-phenyloxirane (2*S,*3*S*-**2**), (1*S,*2*R*)-(+)- 2-Amino-1-phenyl-1-propanol (1*S,*2*R*-**5**), (1*R,*2*S*)-(-)-2-Amino-1-phenyl-1-propanol *(*1*R,*2*S*-**5**) and catalase from bovine liver (lyophilized powder, >10000 U mg^{-1} of protein) were purchased from Sigma Aldrich. Nicotinamide cofactor (NAD^+) was purchased from Melford Biolaboratories (Chelsworth, Ipswich, UK).

All chemicals and solvents were used without further purification.

The amino alcohol references (1*S,*2*S/*1*R*-2*R)*-2-amino-1-phenyl-1-propanol [1*S,*2*S/*1*R,*2*R*-**5**], (1*R,*2*S*)-1-amino-1-phenylpropan-2-ol [1*R,*2*S*-**9**], (1*S,*2*R*)-1-amino-1-phenylpropan-2-ol [1*S,*2*R*-**9**], (1*S,*2*S/*1*R*-2*R)*-1-amino-1-phenylpropan-2-ol [1*S,*2*S/*1*R,*2*R*-**9**], 1-phenylpropane-1,2-diol [diol **3** as a mixture of all the four possible isomers], and *rac*-1-hydroxy-1-phenylpropan-2-one [*rac*-**4**] were chemically synthesized as reported in section 6.

^1H NMR spectra were recorded on a Brucker (400 MHz) spectrometer in $CDCl_3$. All signals are expressed as ppm down field from tetramethylsilane.

3. Enzymes used in this study

Table S1: Source and expression conditions for enzymes used in this study. For the definition of enzyme classes, see abbreviations (section 1)

Name	Source/Comment	Plasmid	Tag	Expression/Purification	Used form	Ref
Fus-SMO	Fused SMO from *Pseudomonas* sp. VLB120	pET28b	N-His_6	Ref. [1]	lyophilized whole cells	[1]
Fus-SMO (1) + Cb-FDH (2)	Fused SMO coexpressed with FDH	pET28b/pET21a	N-His_6 (1)/No-Tag (2)	Ref. [1]	lyophilized whole cells	[1]
Sp(S)-EH	*Sphingomonas sp.* HXN200	pET28b	N-His_6	1 mM IPTG, 25 °C 16 h	lyophilized whole cells	[2]
St(R)-EH	*Solanum tuberosum*	pET28b	N-His_6	1 mM IPTG, 25 °C 16 h	lyophilized whole cells	[2]
Rs-ADH	*Ralstonia sp.*	pET28b	N-Strep	0.5 mM IPTG, 25 °C 16 h	lyophilized whole cells	[3]
Aa-ADH	*Aromatoleum aromaticum*	pET28b	N-His_6	Ref. [4]	purified	[5]
Pp-ADH	*Paracoccus pantotrophus* DSM 11072	pMS470/ pEamTA	C-His_6	2 mM IPTG, 25 °C, 16 h	lyophilized whole cells	[6]
Sy-ADH	*Shingobium yanoikuyae DSM 6900*	pET26b	C-Strep	0.5 mM IPTG, 25 °C 16 h	lyophilized whole cells	[7]
Te-ADH-v1	I86A variant from *Thermoanaerobacter ethanolicus*	pET21a	C-Strep	0.5 mM IPTG, 25 °C 16 h	lyophilized whole cells	[8]
Te-ADH-v2	W110A variant from *Thermoanaerobacter ethanolicus*	pET42a	GST	0.5 mM IPTG, 25 °C 16 h	lyophilized whole cells	[8]
Te-ADH-v3	I86A W110A variant from *Thermoanaerobacter ethanolicus*	pET42a	GST	0.5 mM IPTG, 25 °C 16 h	lyophilized whole cells	[8]
Lb-ADH	*Lactobacillus brevis*	pET21a	no Tag	0.5 mM IPTG, 25 °C 16 h	purified	[9]
Lbv-ADH	variant from *Lactobacillus brevis*	pET21a	no Tag	[10]	purified	[11]
Ls-ADH	*Leifsonia sp.*	pET21a	no Tag	0.5 mM IPTG, 25 °C 16 h	lyophilized whole cells and purified	[12]
Bs-BDHA	*Bacillus subtilis* BGSC1A1	pET28b	N-His_6	0.5 mM IPTG, 25 °C 16 h	purified	[13]
Ch1-AmDH	Chimeric AmDH	pET28b	N-His_6	Ref. [10]	purified	[14]
Rs-PhAmDH	variant from *Rhodoccoccus sp.*	pET28b	N-His_6	Ref. [15]	purified	[16]
Cb-FDH	*Candida boidinii*	pET28b	N-His_6	Ref. [15]	purified	[15]
YcnD	NAD(P)H-dependent oxidase from *Bacillus subtilis*	pET21a	no Tag	Ref. [8]	purified	[17]
NOx	NADH-dependent oxidase from *Streptococcus mutans*	pET21a	C-His_6	Ref. [18]	purified	[19]

Expression of the enzymes: For recombinant expression, 800 mL of LB medium supplemented with the appropriate antibiotic (100 µg mL^{-1} ampicillin or 50 µg mL^{-1} kanamycin) were inoculated with 15 mL of an overnight culture harbouring the desired vector with genes for the expression of the enzymes. *E. coli* BL21 DE3 cells was used as host organism.

Cells were grown at 37 °C until an OD_{600} of 0.6 to 1 was reached and expression of protein was induced by the addition of IPTG. Protein expression was carried out overnight and after harvesting of the cells (4 °C, 4500 rpm, 10 min), the remaining cell pellets were washed with buffer (for lyophilized cells: 50 mM Tris-HCl buffer, pH 8.0 for ADHs and 50 mM KPi, pH 8.0 for EHs; or lysis buffer for enzymes that were subsequently purified by affinity chromatography).

Purification by Nickel affinity chromatography: His_6-tagged proteins were resuspended in lysis buffer (50 mM KH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) prior to cell disruption and protein purification was performed by Ni-NTA affinity chromatography using pre-packed Ni-NTA HisTrap HP columns (GE Healthcare), previously equilibrated with lysis buffer. After loading of the filtered lysate, the column was washed with sufficient amounts of wash buffer (50 mM KH_2PO_4, 300 mM NaCl, 25 mM imidazole, pH 8.0), and bound protein was recovered with elution buffer (50 mM KH_2PO_4, 300 mM NaCl, 200 mM imidazole, pH 8.0). Purity was analysed by SDS-PAGE and fractions showing >95% purity were combined and dialyzed overnight against Tris-HCl buffer (6 L, pH 8.0, 20 mM). The enzyme solutions were concentrated and their concentration was determined spectrophotometrically based on their extinction coefficient at 280 nm.

Purification by anion exchange chromatography: Proteins devoid of a tag were purified by anion exchange chromatography using a HiPrepQ HP 16/10 column (GE Healthcare). The lysate was dissolved in start buffer (20 mM Tris-HCl, pH 8.0) and after cell disruption and centrifugation loaded onto the column. The elution of the proteins was performed with a gradient between start buffer and elution buffer (20 mM Tris-HCl, 1 M NaCl, pH 8.0). After SDS-PAGE, fractions containing the desired proteins in a sufficient purity were pooled, dialyzed against 50 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.0) overnight, and concentrated. The final concentration was determined at 280 nm by UV-vis spectroscopy.

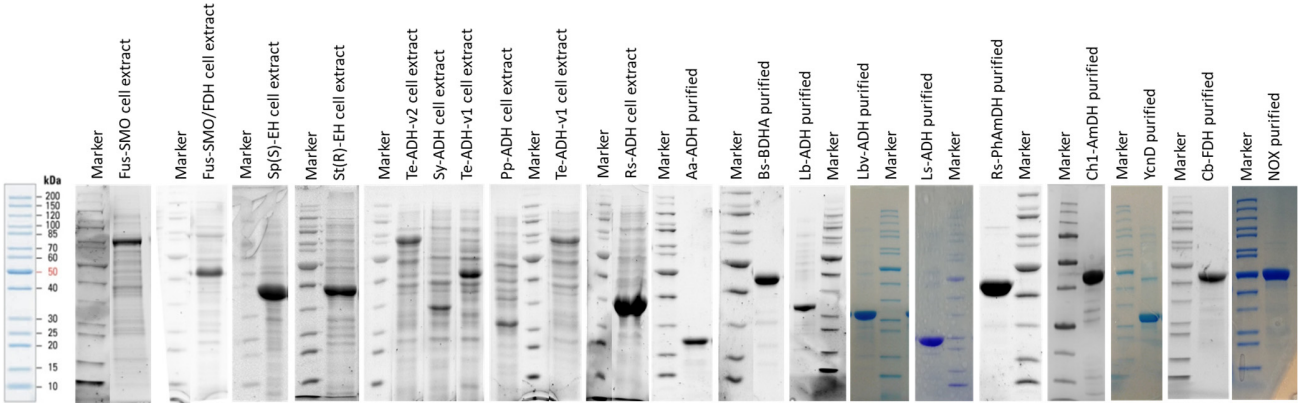
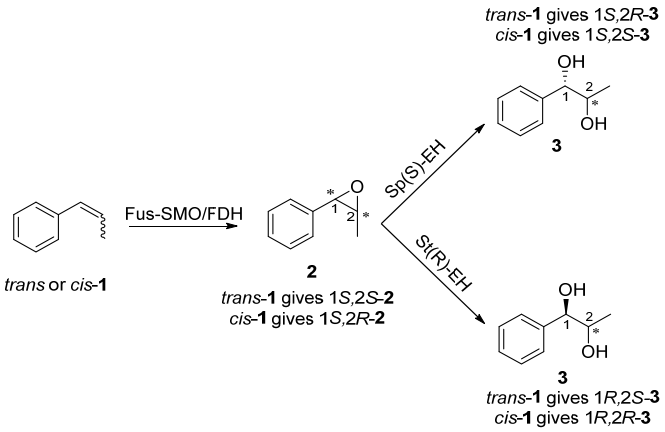


Figure S1: SDS-PAGEs of lyophilized whole cells (crude cell extract before lyophilization of the cells) or purified enzymes. Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific).

4. One-pot cascade for the dihydroxylation of *trans or cis*-1 to optically active 3

4.1. Analytical scale: preliminary investigation on the stereo-complementarity of two EHs [2]



General remark: The concentrations of coenzymes, co-substrate and recycling enzyme are always calculated on the volume of the aqueous phase, whereas the concentration of the substrate is referred to the organic phase.

Reaction conditions**:** Lyophilized *E.coli* cells co-expressing Fus-SMO (10 mg) and either Sp(S)-EH or St(R)-EH (10 mg) were rehydrated in KPi buffer (0.5 mL, 50 mM, pH 8.0) in 4 mL glass vials. After that, the cofactor NAD^+ (1 mM, 0.05 eq.), HCOONa (100 mM, 5 eq.), purified Cb-FDH (10 µM) and FAD (50 µM) were added. Heptane (0.5 mL; 1:1 volumetric ratio with the buffer) was used as biphasic solvent. Finally, the biocatalytic reactions were initiated by the addition of *trans or cis*-**1** (20 mM). The reactions were incubated at 30 °C and 180 rpm on an orbital shaker for 24 h. After saturation of the aqueous phase with solid NaCl, the organic compounds were extracted with MTBE (2 x 250 µL). The combined organic phases were dried over $MgSO_4$. Conversions were measured by GC-FID, while *ee* [%] and *de* [%] were determined by HPLC. The results are summarized in Table S2**.**

Table S2: One-pot cascade for the conversion [%] of *trans or cis*-**1** (20 mM) to optically active **3** catalysed by lyophilised whole cells expressing Fus-SMO in combination with either Sp(S)-EH or St(R)-EH. The stereochemistry of the diols (**3**) was determined accurately by HPLC analysis.

Substrate	Enzymatic system	Conversion [%][a]	*er* [%][b]	*dr* [%][b]	Product distribution			
					1*R,*2*R*-**3** **A**	1*S,*2*S*-**3** **B**	1*R,*2*S*-**3** **C**	1*S,*2*R*-**3** **D**
trans-**1**	Fus-SMO/Sp(S)-EH	99.3 ± 0.3	>99.9 (1*S,*2*R*-**3**)	>99.9	n.d.	n.d.	0.1	99.9
trans-**1**	Fus-SMO/St(R)-EH	86.3 ± 8.8	99.7 (1*R,*2*S*-**3**)	99.3	0.5	0.2	99.0	0.3
cis-**1**	Fus-SMO/Sp(S)-EH	52.5 ± 1.5	94.4 (1*S,*2*S*-**3**)	97.8	5.5	92.5	0.1	2.0
cis-**1**	Fus-SMO/St(R)-EH	70.8 ± 0.3	99.8 (1*R,*2*R*-**3**)	99.0	98.8	0.2	n.d.	1

[a] GC-FID; [b] HPLC; average of two samples

*4.2. Preparative scale: enzymatic synthesis of enantiomerically pure **3***

General remark: The concentrations of coenzymes and co-substrate are always calculated on the volume of the aqueous phase, whereas the concentration of the substrate is referred to the organic phase.

Reaction conditions: Lyophilized *E.coli* cells co-expressing Fus-SMO/Cb-FDH (250 mg, 5 mg mL^{-1}) were rehydrated in KPi buffer (50 mL, 50 mM, pH 8.0) in a baffled Erlenmeyer flask (500 mL). After that, NAD^+ (1 mM), HCOONa (5 eq.), FAD (50 µM) and catalase (0.1 mg mL^{-1}) were added. Heptane (50 mL; 1:1 volumetric ratio with the buffer) was used as biphasic solvent. Finally, the biocatalytic reactions were initiated by the addition of substrate *trans* or *cis*-**1** (50 mM, 2.5 mmol). The reactions were incubated at 30 °C and 200 rpm on an orbital shaker. After 6 hours, lyophilized *E.coli* cells expressing either Sp(S)-EH or St(R)-EH (1 g, 20 mg mL^{-1}) were added and the reactions were further incubated at 30 °C and 170 rpm on an orbital shaker for 30 h. Heptane was removed, the aqueous phase was saturated with solid NaCl and the organic compounds extracted with MTBE (3 x 25 mL). After drying over $MgSO_4$, the organic phase was removed under reduced pressure. The conversions and purity of the isolated products were determined by GC-FID, while *ee* [%] and *de* [%] were analysed by HPLC (Table S3).

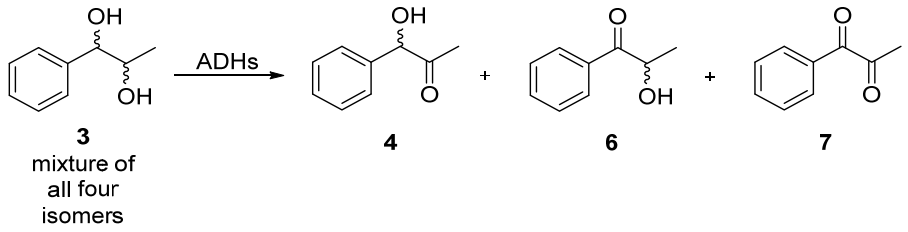
Table S3: Conversion [%] of *trans* or *cis*-**1** to optically active **3** in a biocatalytic cascade that combines Fus-SMO/Cb-FDH and two stereo-complementary EHs.

Substrate	EH	Conversion [%][a]	Isolated yield [%]	Purity [%][a]	*er* [%][b]	*dr* [%][b]
trans-**1**	Sp(S)-EH	>99	85 (323 mg)	>98	99:1 (1*S,2R*-**3**)	>99.5:<0.5
trans-**1**	St(R)-EH	>99	78 (297 mg)	>97	>99.5:<0.5 (1*R,2S*-**3**)	>99.5:<0.5
cis-**1**	Sp(S)-EH	>99	83 (316 mg)	98	95.5:4.5 (1*S,2S*-**3**)	>99.5:<0.5
cis-**1**	St(R)-EH	>99	79 (301 mg)	>97	>99.5:<0.5 (1*R,2R*-**3**)	>99.5:<0.5

[a]determined by GC-FID; [b] analysed by HPLC

5. Screening of NAD(P)$^+$-dependent ADHs for the bio-catalytic oxidation of 1-phenylpropane-1,2-diol (3)

The aim of this experiment was to determine the regio-and stereo-selectivity of a panel of ADHs (6 NAD$^+$-dependent and 5-NADP$^+$-dependent ADHs) for the oxidation of diol **3** as test substrate (mixture of all four possible stereoisomers). The results for the screening are summarized in Table S4.



3 mixture of all four isomers — **4** — **6** — **7**

Table S4: Oxidation [%] of diol**3** by NAD(P)$^+$-dependent ADHs.

Entry	ADHs	Coenzyme	4 [%][b]	6 [%][b]	7 [%][b]	Catalyst form[c]	Accepted diol isomers[a]
1	Aa-ADH	NAD$^+$	31	20	5	Purified	*1R,2R/1S,2S/1R,2S*
2	Lbv-ADH	NAD$^+$	4	4	2	Purified	-
3	Sy-ADH	NAD$^+$	<1	<1	<1	Lyophilized whole cells	-
4	Pp-ADH	NAD$^+$	<1	<1	<1	Lyophilized whole cells	-
5	Bs-BDHA	NAD$^+$	36 ± 3	22 ± 2	4 ± 1	Lyophilized whole cells	*1S,2R/1R,2S*
6	Ls-ADH	NAD$^+$	15 ± 1	9	2	Lyophilized whole cells	*1S,2R/1R,2R*
7	Lb-ADH	NADP$^+$	8	7	2	Purified	-
8	Te-ADH-v1	NADP$^+$	<1	<1	<1	Lyophilized whole cells	-
9	Te-ADH-v2	NADP$^+$	3 ± 2	1 ± 1	<1	Lyophilized whole cells	-
10	Te-ADH-v3	NADP$^+$	<1	<1	<1	Lyophilized whole cells	-
11	Rs-ADH	NADP$^+$	<1	<1	<1	Lyophilized whole cells	-

[a] This refers to the preference of the enzyme for the conversion of *diol* **3** which is a mixture of the four possible stereoisomers as described in this column; [b] average of two samples; [c] see section 3 (Table S1) for more details.

Reaction conditions for the biocatalytic oxidation of diol **3** using lyophilized *E.coli* cells expressing one of the following NAD(P)$^+$-dependent ADHs: Sy-ADH, Pp-ADH, Bs-BDHA, Te-ADH-v1, Te-ADH-v2, Te-ADH-v3 and *Rs*-ADH.

Lyophilized *E.coli* cells (20 mg mL^{-1}) were rehydrated in an Eppendorf tube (1.5 mL) in Tris-HCl buffer (0.5 mL, pH 7.5, 50 mM) containing NAD(P)$^+$ (1 mM, 0.1 eq.) and diol **3** (10 mM). NOx (0.5 µM) for NAD$^+$-dependent ADHs or YcnD (5 µM) for NADP$^+$-dependent ADHs, respectively, was also added for cofactor regeneration. The mixtures were incubated at 30 °C, 170 rpm for 24 h on an orbital shaker and, after saturation of the aqueous layer with solid NaCl, the organic compounds were extracted with MTBE (2 x 250 µL). The organic layers were dried over $MgSO_4$ and analysed by GC-FID and HPLC.

Reaction conditions for the biocatalytic oxidation of diol **3** using lyophilized *E.coli* cells expressing the NAD$^+$-dependent Ls-ADH.

Lyophilized *E.coli* cells (10 mg mL^{-1}) were rehydrated in an Eppendorf tube (2 mL) in KPi buffer (1 mL, pH 6.5, 100 mM) containing NAD$^+$ (1 mM, 0.1 eq.) and diol **3** (10 mM). NOx (0.5 µM) was also added for cofactor regeneration. The mixture

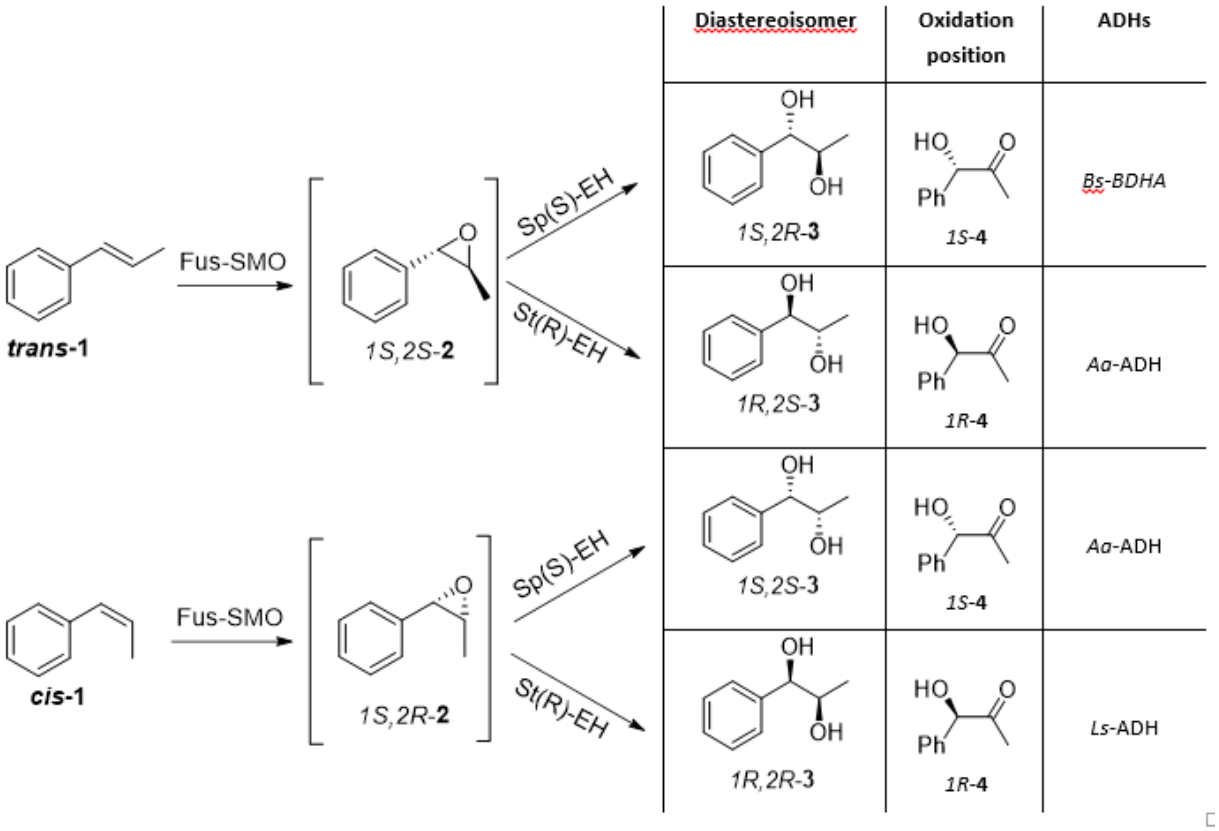
was incubated at 40 °C, 170 rpm for 24 h on an orbital shaker. After saturation of the aqueous layer with solid NaCl, extraction was performed with MTBE (2 x 500 µL). The organic layer was dried over $MgSO_4$ and analysed by GC-FID and HPLC.

General reaction conditions for the bio-oxidation of diol **3** by NAD(P)$^+$-dependent ADHs using one of the following purified ADHs: Lbv-ADH, Aa-ADH and Lb-ADH.

Tris-HCl buffer (0.5 mL, pH 7.5, 50 mM) was added to an Eppendorf tube (1.5 mL) containing NAD^+ or $NADP^+$ (1 mM, 0.1 eq.) and diol **3** (10 mM). NOx (0.5 µM) for NAD^+-dependent ADHs or YncD (5 µM) for $NADP^+$-dependent ADHs, respectively, was also added. As last, the tested ADH (50 µM) was added. The mixture was incubated at 30 °C, 170 rpm for 24 h on an orbital shaker. After saturation of the aqueous layer with solid NaCl, extraction was performed with MTBE (2 x 250 µL). The organic layer was dried over $MgSO_4$ and analysed by GC-FID and HPLC.

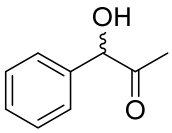
Three ADHs, namely Aa-ADH, Bs-BDHA and Ls-ADH, turned out to be suitable candidates for the regio-selective oxidation of the four diastereoisomers of diol **3**. Figure S2 depicts a graphical summary of the preferred selectivity of the ADHs within our synthetic strategy along with a general view on the conceived synthetic routes to obtain intermediate **4** in enantiomerically pure form.

Figure S2. Overview on the preferred regio- and stereo-selectivity of various ADHs deduced from the conversion of diol **3** (used as a mixture of all four possible isomers).



6. Chemical synthesis of substrates and reference compounds

*6.1. Chemical synthesis of rac-1-hydroxy-1-phenylpropan-2-one (rac-**4**) [20]*

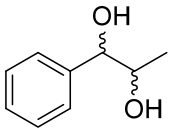


(i) To a 100 mL round bottom flask, benzaldehyde (9.4 mmol, 1 g) and K_2CO_3 (6.5 mmol, 900 mg) were added. After that, TMSCN (12.25 mmol, 1.2 g, 1.3 eq.) was added and the reaction mixture was stirred at room temperature. The progress of the reaction was followed by GC-MS. After completion, the excess of K_2CO_3 was removed by filtration, followed by washing with DCM. The solvent was removed under reduced pressure and a brown oil was obtained (1.68 g, 87% yield, >95% purity based on ^{1}H-NMR analysis), which was then directly used in the next step. The crude product was analysed by ^{1}H-NMR (400 MHz in $CDCl_3$, δ ppm): 7.54-7.46 (m, 2H, aromatics); 7.46-7.39 (m, 3H, aromatics); 5.51 (s, 1H, CH); 0.25 (s, 3H, CH_3).

(ii) A solution of MeMgI (15.6 mmol, 3 M solution in ethyl ether) in dry Et_2O (26 mL) was placed in a 100 mL dry round-bottom flask that was equipped with a condenser and a dropping funnel. The apparatus was kept under nitrogen atmosphere. The crude protected mandelonitrile (1.6 g, 7.8 mmol), obtained in the previous step, was dissolved in dry Et_2O (10 mL) and added dropwise using a dropping funnel. The reaction mixture was stirred and refluxed for 24 h; then, it was cooled to room temperature, poured into ice containing concentrated H_2SO_4 (98%, 1.65 mL in 42 g ice) and stirred further at room temperature for 6.5 h. Afterwards, the layers were separated and the water phase was extracted with Et_2O (2 x 50 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The desired product was identified by GC-MS and purified by flash column chromatography on silica gel (hexane:EtOAc 8:2; $Rf_{product}$=0.4), affording 30% yield. The isolated product was analysed by GC-MS (m/z 150), GC-FID and ^{1}H-NMR. The combination of these analytical data correlates with a mixture of ca. 90% *rac*-**4** and ca. 10% of 1-phenylpropane-1,2-dione (**7**).

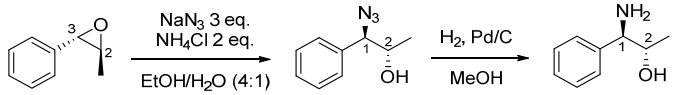
^{1}H-NMR (400 MHz in $CDCl_3$; δ ppm): 7.42-7.27 (m, 5H aromatics); 4.32 (s, broad, 1H, OH); 5.08 (s, 1H, CH); 2.07 (s, 3H, CH_3).

*6.2. Chemical synthesis of 1-phenylpropane-1,2-diol (diol **3**) as a mixture of all four possible stereoisomers*

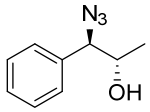


In a round-bottom flask cooled on ice, $NaBH_4$ (13.5 mmol, 511 mg) was dissolved in MeOH (10 mL). Then, **7** (3.37 mmol, 500 mg) was added. The mixture was stirred at room temperature for 4 h. After completion of the reaction monitored by TLC analysis (PE/EtOAc 1:1; $Rf_{product}$ = 0.4), the solvent was removed under reduced pressure. The crude oil was washed with KPi buffer (50 mM, pH 4-5). The resulting aqueous layer was saturated with solid NaCl and extracted with MTBE (3 x 20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The product was isolated as a white solid (470 mg, 92%) and analysed by GC-FID (>99% pure; HPLC (*d.r.*: 5:5:45:45 [RR:SS:RS:SR]), GC-MS (m/z 152) and ^{1}H-NMR (400 MHz in $CDCl_3$; only ppm (δ) of the main diastereomer reported): 7.38-7.34 (t, *J* = 4.2 Hz, 5H; CH aromatics); 4.7 (d, *J* = 4.3 Hz, 1H, CH); 4.07-3.99 (m, 1H, CH); 1.10 (d, *J* = 6.4 Hz, 3H, CH_3).

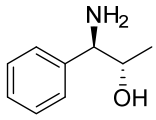
*6.3. Chemical synthesis of 1R,2S-1-amino-1-phenylpropan-2-ol (1R,2S-**9**)*



2*S*,3*S*-**2** 1*R*,2*S*-**11** 1*R*,2*S*-**9**

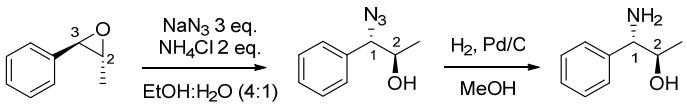


(i) Nucleophilic ring opening of 2*S*,3*S*-2-methyl-3-phenyloxirane (2*S*,3*S*-**2**) by NaN_3 [21]: In a 50 mL two-neck round-bottom flask, the epoxide 2*S,*3*S*-**2** (200 mg, 1.5 mmol) was dissolved in a mixture of EtOH/H_2O (4:1, 10 mL total volume) followed by addition of NaN_3 (290 mg, 4.5 mmol, 3 eq.) and NH_4Cl (160 mg, 3 mmol, 2 eq.). The reaction was heated up to 60 °C and stirred for 22 h. Upon completion, the organic compounds were extracted with MTBE (3 x 10 mL). The organic layers were combined, washed with H_2O (1 x 20 mL) and then with brine (1 x 20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The isolated product was obtained in 83% yield (ca. 220 mg) as a brown liquid and analysed by GC-MS. The crude product was used in the next step without further purification (>99 purity by GC-MS analysis; m/z 177). $Rf_{(2S,3S\text{-}\mathbf{2})}$ = 0.8 (PE/EtOAc 1:1); $Rf_{(1R,2S\text{-}\mathbf{11})}$ = 0.7 (PE/EtOAc 1:1)

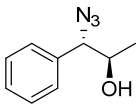


(ii) Chemical hydrogenation of 1*R*,2*S*-1-azido-1-phenylpropan-2-ol (1*R*,2*S*-**11**)**:** In a 25 mL one-neck round bottom flask, Pd/C (10% wt., 15 mg) was added followed by MeOH (5 mL). Then 1*R,*2*S*-**11** (110 mg, 0.564 mmol) was added as a solution in MeOH (3 mL). The flask was sealed with a septum and hydrogen atmosphere was supplied with a balloon. The reaction mixture was stirred at room temperature. No full conversion was achieved after 21 h and more catalyst was added (20% wt.).The reaction did not go to completion even after 48 h according to TLC analysis. After the workup, the product was isolated in pure form. The reaction mixture was filtered over a celite pad and concentrated under reduced pressure to yield a sticky clear yellow gel (52 mg, 61%, *de* >99%). The isolated product was analysed by GC-MS (m/z 151) and ^{1}H-NMR (>95% purity). $Rf_{(1R,2S\text{-}\mathbf{11})}$ = 0.88 (DCM/MeOH 3:1); $Rf_{(1R,2S\text{-}\mathbf{9})}$ = 0.3 (DCM/MeOH 3:1). ^{1}H-NMR ($CDCl_3$, δ ppm, 400 MHz): 7.36-7.34 (m, 5H), 4.04-3.97 (m, 2H), 1.05 (d, *J* = 6.0 Hz, 3H).

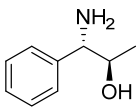
*6.4. Chemical synthesis of 1S,2R-**9***



2*R*,3*R*-**2** 1*S*,2*R*-**11** 1*S*,2*R*-**9**



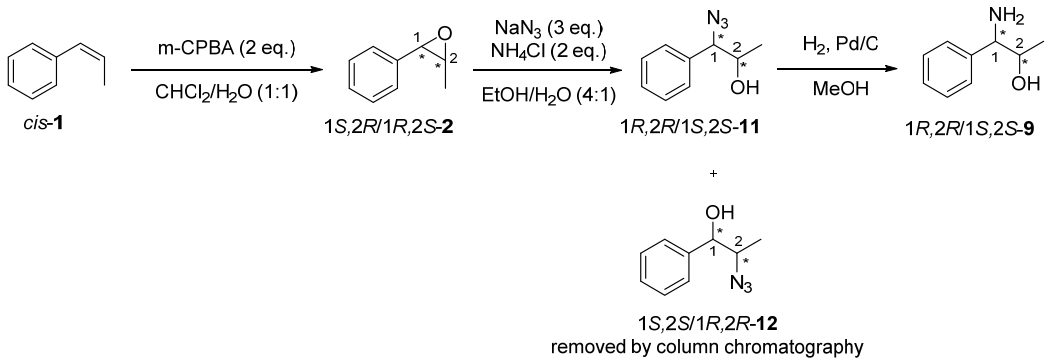
(i) Nucleophilic ring opening of 2*R*,3*R*-**2** by NaN_3[21]: In a 50 mL two-neck round-bottom flask, the epoxide 2*R,*3*R*-**2** (200 mg, 1.5 mmol) was dissolved in a mixture of EtOH/H_2O (4:1, 10 mL total volume) followed by addition of NaN_3 (290 mg, 4.5 mmol, 3 eq.) and NH_4Cl (160 mg, 3 mmol, 2 eq.). The reaction was heated up to 60 °C and stirred for 22 h. Upon completion, the organic compounds were extracted with MTBE (3 x 10 mL). The organic layers were combined, washed with H_2O (1 x 20 mL) and then with brine (1 x 20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The isolated product was obtained as a brown liquid (85%) and analysed by GC-MS. The crude product was used in the next step without further purification (>97% purity). $Rf_{(2R,3R\text{-}\mathbf{2})}$ = 0.8 (PE/EtOAc 1:1); $Rf_{(1S,2R\text{-}\mathbf{11})}$ = 0.7 (PE/EtOAc 1:1)

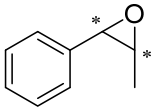


(ii) Chemical hydrogenation of 1*S*,2*R*-**11:** In a 25 mL one-neck round bottom flask, Pd/C (35% wt., 50 mg) was added followed by MeOH (5 mL). Then, substrate 1*S,*2*R*-**11** (140 mg, 0.790 mmol) was added as a solution in MeOH (3 mL). The flask was sealed with a septum and hydrogen atmosphere was supplied with a balloon. The reaction mixture was stirred at room temperature for 24 h, filtered over a celite pad and concentrated under reduced pressure to yield a sticky clear yellow gel (63 mg, 53%, *de* >99%). The isolated product was

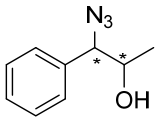
analysed by GC-MS and ^{1}H-NMR. No further purification was required (>95% purity). $Rf_{(1S,2R\text{-}\mathbf{11})}$ = 0.9 (DCM/MeOH 3:1); $Rf_{(1S,2R\text{-}\mathbf{9})}$ = 0.22 (DCM/MeOH 3:1). ^{1}H-NMR ($CDCl_3$, δ ppm, 400 MHz): 7.37-7.27 (m, 5H), 3.99-3.94 (m, 2H), 1.05 (d, *J* = 6.1 Hz, 3H).

6.5. *Chemical synthesis of* 1*S,*2*S/*1*R,*2*R*-***9*** *as racemic mixture*

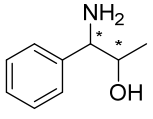




(i) Chemical epoxidation of *cis*-β-methylstyrene (*cis*-**1)** [22]: In a 25 mL round bottom flask, DCM (4 mL) and H_2O (4 mL) were added followed by substrate *cis*-**1** (150 mg, 1.27 mmol) and cooled down to 0 °C. Then *m*-CPBA (438 mg, 2.54 mmol, 2 eq.) was added in portions over 20 min and the reaction mixture was stirred for 18 h at room temperature. Upon completion, the mixture was quenched with a saturated solution of K_2CO_3 (5 mL) and the organic compounds extracted with MTBE (3 x 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to yield the desired epoxide product as a colourless liquid (97% yield, 168 mg). The isolated product was analysed by GC-MS (m/z 134). No further purification was required (>98% purity). $Rf_{\mathbf{cis\text{-}1}}$ = 0.75 (PE/EtOAc 1:1); $Rf_{(1S,2R/1R,2S\text{-}\mathbf{2})}$ = 0.45 (PE/EtOAc 1:1)



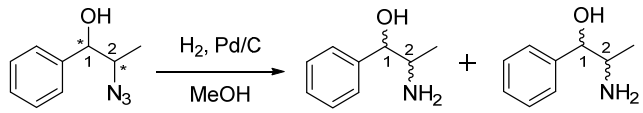
(ii) Nucleophilic ring opening of 1*S,*2*R/*1*R,*2*S*-**2** by NaN_3 [21]: In a 25 mL two-neck round-bottom flask, the epoxide 1*S,*2*R/*1*R,*2*S*-**2** (160 mg, 1.17 mmol) was dissolved in a mixture of EtOH/H_2O (4:1, 8 mL total volume) followed by addition of NaN_3 (229 mg, 3.5 mmol, 3 eq.) and NH_4Cl (126 mg, 2.34 mmol, 2 eq.). The reaction was heated up to 60 °C and stirred for 22 h. No full conversion was obtained even after further addition of NaN_3 and stirring for longer time. Therefore, the organic compounds were extracted with MTBE (3 x 15 mL). The organic layers were combined, washed with H_2O (1 x 20 mL) and then with brine (1 x 50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product (169 mg) was isolated as a mixture of three compounds as confirmed by TLC and GC-MS analysis. The mixture was then purified by flash column chromatography (PE/EtOAc 5:1) to yield 1*S,*2*S/*1*R,*2*R*-**11** as a brown liquid (53.6 mg) and (1*S,*2*S/*1*R,*2*R*)-2-azido-1-phenylpropan-1-ol (1*S,*2*S/*1*R,*2*R*-**12**) as a dark yellow liquid (49.8 mg). The two azido alcohol isomers were analysed by GC-MS and confirmed by ^{1}H-NMR. $Rf_{\mathbf{2}}$ = 0.55 (PE/EtOAc 5:1); $Rf_{(1S,2S/1R,2R\text{-}\mathbf{11})}$ = 0.2 (PE/EtOAc 5:1); $Rf_{(1S,2S/1R,2R\text{-}\mathbf{12})}$ = 0.4 (PE/EtOAc 5:1). ^{1}H-NMR 1*S,*2*S/*1*R,*2*R*-**11** ($CDCl_3$, δ ppm, 400 MHz): 7.43-7.35 (m, 3H, CH aromatics), 7.32-7.30 (m, 2H, CH aromatics), 4.31 (d, *J* = 8.0 Hz, 1H, CH), 3.89 (p, *J* = 6.4 Hz, 1H, CH), 2.39 (broad, 1H, OH), 1.04 (d, *J* = 6.3 Hz, 3H, CH_3).



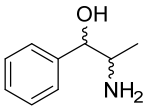
(iii) Chemical hydrogenation of 1*S*,2*S*/1*R*,2*R*-**11:** In a 25 mL one-neck round bottom flask, Pd/C (30% wt., 16 mg) was added followed by MeOH (2.5 mL). Then 1*S,*2*S/*1*R,*2*R*-**11** (54 mg, 0.305 mmol) was added as a solution in MeOH (1 mL). The flask was sealed with a septum and hydrogen atmosphere was supplied

with a balloon. The reaction mixture was stirred at room temperature for 24 h, then filtered over a celite pad and concentrated under reduced pressure to yield an off-white solid (45.6 mg, 99.6%, de >98%). The isolated product was analysed by GC-MS (m/z 151) and ^{1}H-NMR. No further purification was required (>95% purity). $Rf_{(1S,2S/1R,2R\text{-}\mathbf{11})}$ = 0.89 (DCM/MeOH 3:1); $Rf_{(1S,2S/1R,2R\text{-}\mathbf{9})}$ = 0.22 (DCM/MeOH 3:1). ^{1}H-NMR 1*S*,2*S*/1*R*,2*R*-**9** ($CDCl_3$, δ ppm, 400 MHz): 7.36-7.28 (m, 5H, CH aromatics), 3.94-3.87 (m, 1H, CH), 3.78 (d, *J* = 8.8 Hz, 1H, CH), 1.02 (d, *J* = 6.1 Hz, 3H, CH_3).

*6.6. Chemical synthesis of 2-amino-1-phenyl-1-propanol (1S,2S-**5** and 1R,2R-**5**) as a racemic mixture*



1*S*,2*S*/1*R*,2*R*-**12** 1*S*,2*S*/1*R*,2*R*-**5** 1*S*,2*R*/1*R*,2*S*-**5**



(i) Chemical hydrogenation of 1*S*,2*S*/1*R*,2*R*-**12**: In a 25 mL one-neck round bottom flask, Pd/C (30% wt., 15 mg) was added followed by MeOH (2 mL). Then 1*S*,2*S*/1*R*,2*R*-**12** (50 mg, 0.282 mmol) was added as a solution in MeOH (1 mL). The flask was sealed with a septum and hydrogen atmosphere was supplied with a balloon. The reaction mixture was stirred at room temperature for 24 h, then filtered over a celite pad and concentrated under vacuum to yield an off-white solid (24.7 mg, 58%, de >51%). The isolated product was analysed by GC-MS (m/z 151). No further purification was required (>95% purity). $Rf_{(1S,2S/1R,2R\text{-}\mathbf{12})}$ = 0.64 (DCM/MeOH 3:1); $Rf_{\mathbf{5}}$ = 0.18 (DCM/MeOH 3:1).

^{1}H-NMR 1*S*,2*S*/1*R*,2*R*-**12** ($CDCl_3$, δ ppm, 400 MHz): 7.40-7.31 (m, 5H, CH aromatics), 4.48 (d, *J* = 7.4 Hz, 1H, CH), 3.68 (p, *J* = 6.7 Hz, 1H, CH), 2.47 (broad, 1H, OH), 1.13 (d, *J* = 6.7 Hz, 3H).

^{1}H-NMR 1*S*,2*S*/1*R*,2*R*-**5** ($CDCl_3$, δ ppm, 400 MHz): 7.33-7.32 (m, 5H, aromatics), 4.97 (broad, 1H, OH), 4.31 (d, *J* = 6.6 Hz, 1H, CH), 3.12-3.07 (m, 1H, CH), 2.06 (broad, 2H, NH_2), 1.04 (d, *J* = 6.2 Hz, 3H, CH_3).

7. One pot hydrogen-borrowing amination of enantiopure or enantioenriched diols 3

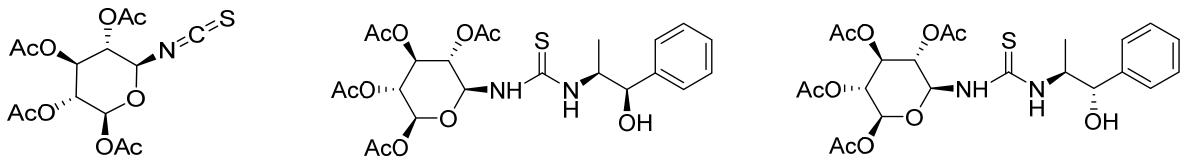
This section describes the screened conditions for the optimization of the hydrogen-borrowing-cascade for the amination of enantiomerically pure or enantiomerically enriched substrates **3**.

7.1. General experimental procedure for the biocatalytic hydrogen-borrowing amination

In an Eppendorf tube (1.5 mL), $HCOONH_4$ buffer (0.5 mL, pH 8.5, 1 M) and NAD^+ (1 mM) were added followed by purified ADH (varied concentrations) and AmDH (varied concentrations). Enantiomerically pure or enantioenriched diol **3** (5 to 30 mM) was added as last. The mixture was incubated at 30 °C (otherwise stated), 170 rpm for 48 h on an orbital shaker and, after that, quenched with 10 M KOH (100 µL). The aqueous layer was saturated with solid NaCl and the organic compounds extracted with MTBE (1 x 500 µL). The organic layer was dried over $MgSO_4$ and analysed by GC-FID and/or GC-MS to determine the conversion, while diastereomeric and enantiomeric excesses were analysed by HPLC after derivatization with a chiral reagent (GITC) as reported in section 7.2. All experiments were performed in duplicate and the results reported are the average of two samples.

7.2. Derivatization of the amino alcohols with GITC to determine the ee/de by RP-HPLC [23]

The aqueous reaction mixture (20 µL) was dissolved in acetonitrile (180 µL) to yield a final concentration of 0.5 mM. Then, GITC (2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate) (1.5 mM) and Et_3N (1.5 mM) were added as a solution in acetonitrile (200 µL). The mixture was incubated at room temperature at 1000 rpm for 35 min. Before injection into the RP-HPLC, the samples were centrifuged and filtered if required.



GITC *GITC*-1*R*,2*R*-**5** **GITC**-1*S*,2*R*-**5**

Figure S3: Structures of GITC (left) and GITC-derivatized amino alcohols obtained in this study (centre and right).

7.3. Hydrogen-borrowing amination of 1*S*,2*S*-**3**

7.3.1. Influence of the temperature for the hydrogen-borrowing amination of 1*S*,2*S*-**3**:

The influence of the temperature (20, 30, 40 and 50 °C) for the one-pot HB-amination of 1*S*,2*S*-**3** (5 mM) was investigated combining Aa-ADH (50 µM) with AmDH (50 µM, either Ch1-AmDH or Rs-PhAmDH) as described in 7.1. The reactions were run for 48 h in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) in the presence of NAD^+ (1 mM).

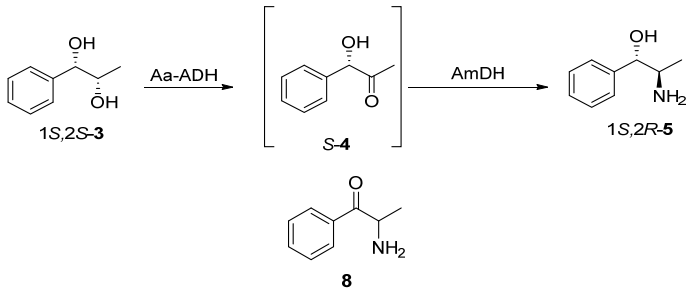


Table S5: Conversions [%] for 1*S,2S*-**3** (5 mM) at different temperatures by combining Aa-ADH (50 µM) and AmDH (50 µM)

Enzymatic system	T [°C]	Conversion [%][a]	1*S,2R*-5 [%][a]	8 [%][a][b]	Unidentified [%][a][b]
Aa-ADH/Ch1-AmDH	20	96 ± 2	87 ± 1	4 ± <1	5 ± 1
Aa-ADH/Rs-PhAmDH	20	49 ± 1	45 ± 1	3 ± <1	2 ± <1
Aa-ADH/Ch1-AmDH	30	>99	89 ± 2	8 ± 2	4 ± <1
Aa-ADH/Rs-PhAmDH	30	>99	89 ± <1	6 ± <1	6 ± <1
Aa-ADH/Ch1-AmDH	40	89 ± 1	81 ± 2	5 ± <1	2 ± <1
Aa-ADH/Rs-PhAmDH	40	86 ± 1	79 ± 1	4 ± <1	3 ± <1
Aa-ADH/Ch1-AmDH	50	>99	91 ± 1	6 ± <1	3 ± <1
Aa-ADH/Rs-PhAmDH	50	92 ± 1	85 ± 1	5 ± <1	2 ± <1

[a] Determined by GC-FID; [b] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID); *er* and *dr* were not determined at this stage.

7.3.2. Initial optimization of the ADH:AmDH molar ratio for the hydrogen-borrowing amination of 1S,2S-3

Three different ADH:AmDH ratios/concentrations (ADH:AmDH 10:50; 20:50; 24:60 in µM) were investigated with the aim of increasing the conversion and analytical yield of desired product for the HB-amination of 1*S,2S*-**3**. The biocatalytic reactions were performed at 30 °C as described in 7.1.

Table S6: Conversions [%] for 1*S,2S*-**3** (5 mM) by combining different ratios of Aa-ADH and AmDH at 30 °C

Enzymatic system	ADH:AmDH [µM]	Conversion [%][a]	1*S,2R*-5 [%][a]	*er* [%][b]	*dr* [%][b] [SS:RR:RS:SR]	8 [%][a][c]	Unidentified [%][a][c]
Aa-ADH/Ch1-AmDH	10:50	80 ± 5	74 ± 4	n.m.	n.m.	4 ± 0.2	2 ± <1
Aa-ADH/Rs-PhAmDH	10:50	61 ± 6	55 ± 6	n.m.	n.m.	4 ± 0.2	2 ± <1
Aa-ADH/Ch1-AmDH	20:50	89 ± 1	87 ± 1	>99.5	0:5:0:95	n.d.	2 ± <1
Aa-ADH/Rs-PhAmDH	20:50	68 ± 3	66 ± 3	>99.5	0:5:0:95	n.d.	2 ± <1
Aa-ADH/Ch1-AmDH	24:60	>99	86 ± 6	>99.5	0:4:0:96	6 ± 3	8 ± 1
Aa-ADH/Rs-PhAmDH	24:60	>99	87 ± 5	>99.5	0:5:0:95	6 ± 2	8 ± 1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

Note: *the fact that the diastereomeric ratio of the product* 1*S,2R-**5** is not absolutely perfect must not be attributed to the hydrogen-borrowing amination step. In fact, the enantiomeric ratio of the diol substrate* 1*S,2S-**3**—obtained in the previous enzymatic step—was not perfect (95.5:4.5) and that reflected on the subsequent amination step.*

7.3.3. Further optimization of the hydrogen-borrowing amination of 1S,2S-3

The screening at higher substrate concentration (10 to 30 mM) was performed with the best conditions disclosed so far: Aa-ADH (20 µM), Ch1-AmDH (50 µM) in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) at 30 °C for 48 h as described in 7.1.

Table S7: Conversion [%] for 1*S,2S*-**3** (varied concentration: 10 to 30 mM) at 30 °C combining Aa-ADH (24 µM) and Ch1-AmDH (60 µM)

1*S,2S*-3 [mM]	**Conversion [%][a]**	**1*S,2R*-5 [%][a]**	***er* [%][b]**	***dr* [%][b] [SS:RR:RS:SR]**	**8 [%][a][c]**	**Unidentified [%][a][c]**
10	69.9 ± 0.3	69 ± <1	>99.5	0.8:3.5:0:95.7	<1	1 ± <1
15	51.9 ± 2.0	51 ± 2	>99.5	0:2.5:0:97.5	<1	1 ± <1
20	42.2 ± 0.6	41 ± 1	>99.5	0:2.3:0:97.7	<1	1 ± <1
25	36.2 ± 0.1	36 ± <1	>99.5	0:2.4:0:97.6	<1	1 ± <1
30	33.5 ± 0.9	33 ± 1	>99.5	0:1.8:0:98.2	<1	1 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

Note: *the fact that the diastereomeric ratio of the product* 1*S,2R-**5** is not absolutely perfect must not be attributed to the hydrogen-borrowing amination step. In fact, the enantiomeric ratio of the diol substrate* 1*S,2S-**3**—obtained in the previous enzymatic step—was not perfect (95.5:4.5) and that reflected on the subsequent amination step.*

Additional experiments were performed with varied concentrations of substrate (5-20 mM) and enzymes in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) at 30 °C for 48 h as described in 7.1.

Table S8: Conversion [%] for 1*S,2S*-**3** (varied concentration: 5 to 20 mM) at 30 °C using varied enzyme concentrations (Aa-ADH/Ch1-AmDH)

1*S,2S*-3[mM]	**ADH:AmDH [µM]**	**Conversion [%][a]**	**1*S,2R*-5 [%][a]**	***er* [%][b]**	***dr* [%][b] [SS:RR:RS:SR]**	**8 [%][a][c]**	**Unidentified [%][a][c]**
10	48:120	78 ± 1	75 ± 1	>99.5	0:6:0:94	1 ± <1	3 ± <1
10	72:180	85	82	>99.5	0:6:0:94	1	2
15	72:180	69 ± 1	67 ± 1	>99.5	0:6:0:94	1 ± <1	2 ± <1
5	60:24	>99 ± <1	94 ± 5.9	>99.5	2:6:0:92	n.d.	6 ± 1
10	60:24	>99 ± <1	96 ± 2	>99.5	2:5:0:93	1 ± <1	3 ± <1
20	60:24	>99 ± <1	97 ± 1	>99.5	1:4:0:95	<1	2 ± <1
5	70:35	>99 ± <1	96 ± <1	>99.5	2:7:0:91	<1	4 ± <1
10	70:35	>99 ± <1	97 ± 1	>99.5	2:6:0:92	<1	3 ± <1
20	70:35	>99 ± <1	98 ± <1	>99.5	1:4:0:95	1 ± <1	2 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

Note: *the fact that the diastereomeric ratio of the product 1S,2R-**5** is not absolutely perfect must not be attributed to the hydrogen-borrowing amination step. In fact, the enantiomeric ratio of the diol substrate* 1*S,2S-**3**—obtained in the previous enzymatic step—was not perfect (95.5:4.5) and that reflected on the subsequent amination step.*

*7.3.4. Time study for the one pot hydrogen-borrowing amination of 1S,2S-**3** under optimised conditions*

A time study for the amination of 1*S,*2*S*-**3** (20 mM) with the optimized conditions determined before was performed: Aa-ADH (70 µM) in combination with Ch1-AmDH (35 µM) in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) and in the presence of NAD^+ (1 mM) as described in 7.1. The reactions were stopped at different time points (1, 3, 5, 7, 16, 20, 24, 30, 42 and 48 h).

Table S9: Time study for the one pot hydrogen-borrowing amination of 1*S,*2*S*-**3** (20 mM) by combining Aa-ADH (70 µM) and Ch1-AmDH (35 µM) in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL)

Time [h]	Conversion [%][a]	1*S,*2*R*-5 [%][a]	8 [%][a][b]	Unidentified [%][a][b]
1	2	1	<1	<1
3	7	7	<1	<1
5	16	15	<1	<1
7	23	22	1	<1
16	62	60	2	<1
20	75	72	2	<1
24	87	84	2	1
30	88	85	2	<1
42	>99	97	2	<1
48	>99	98	2	<1

[a] Determined by GC-FID analysis; [b] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

*7.4. Hydrogen-borrowing amination of 1S,2R-**3***

Two different ADHs (Ls-ADH and Bs-BDHA; 50 µM) were combined with two AmDHs (Ch1-AmDH and Rs-PhAmDH; 50 µM) in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) at 30 °C for 48 hours. The reactions were performed as described in 7.1.

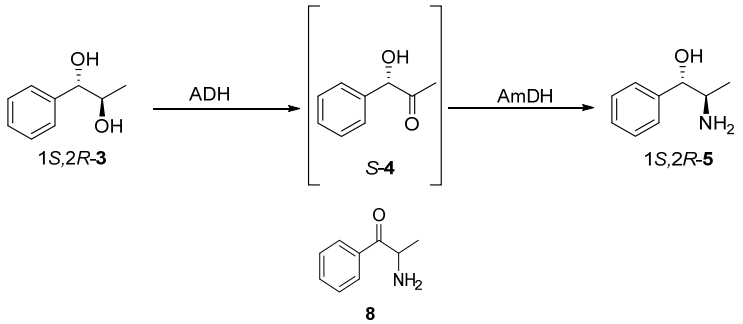


Table S10: Conversion [%] for 1*S,*2*R*-**3** (5 mM) at 30 °C with 50:50 µM ADH:AmDH

Enzymatic system	Conversion [%][a]	1*S,*2*R*-5 [%][a]	*er* [%][b]	*dr* [%][b] [SS:RR:RS:SR]	8 [%][a][c]	Unidentified [%][a][c]
Ls-ADH/Ch1-AmDH	96 ± 1	89 ± 1	>99.5	0:4:0:96.0	3 ± <1	4 ± <1
Ls-ADH/Rs-PhAmDH	65 ± 1	59 ± 1	>99.5	0:5.5:0:94.5	2 ± <1	3 ± <1
Bs-BDHA/Ch1-AmDH	98 ± 2	91 ± 3	>99.5	0:4.5:0:95.5	2 ± <1	4 ± <1
Bs-BDHA/Rs-PhAmDH	95 ± 1	90 ± 1	>99.5	0:3.9:0:96.1	2 ± <1	4 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

Note: *the fact that the diastereomeric ratio of the product* 1*S,*2*R*-***5*** *is not absolutely perfect must not be attributed to the hydrogen-borrowing amination step. In fact, the enantiomeric ratio of the diol substrate* 1*S,*2*R*-***3****—obtained in the previous enzymatic step—was not perfect (99:1) and that reflected on the subsequent amination step.*

7.4.1. Increase in substrate concentration for the hydrogen-borrowing amination of 1*S,*2*R*-**3**

The biocatalytic reactions were carried out with substrate 1*S,*2*R*-**3** (varied concentration: 10 to 30 mM) in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) in the presence of NAD^+ (1 mM) and by combining Bs-BDHA (50 μM) and Ch1-AmDH (50 μM) at 30 °C for 48 hours as described in 7.1.

Table S11: Conversion [%] for 1*S,*2*R*-**3** (varied concentration: 10 to 30 mM) at 30 °C combining Bs-BDHA (50 μM) and Ch1-AmDH (50 μM).

1*S,2R*-3 [mM]	**Conversion [%][a]**	**1*S,2R*-5 [%][a]**	***er* [%][b]**	***dr* [%][b] [SS:RR:RS:SR]**	**8 [%][a][c]**	**Unidentified [%][a][c]**
10	>99	92 ± 2	>99.5	0:2.9:0:97.1	2 ± <1	6 ± <1
15	99 ± 1	95 ± 1	>99.5	0:2.4:0:97.6	1 ± <1	3 ± <1
20	95 ± 2	91 ± 1	>99.5	0:2.2:0:97.8	2 ± 1	2 ± <1
25	89 ± 1	87± 1	>99.5	0:2.2:0:97.8	1 ± <1	2 ± <1
30	82 ± 1	80 ± 1	>99.5	0:2.2:0:97.8	1 ± <1	1 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

Note: *the fact that the diastereomeric ratio of the product* 1*S,*2*R*-***5*** *is not absolutely perfect must not be attributed to the hydrogen-borrowing amination step. In fact, the enantiomeric ratio of the diol substrate* 1*S,*2*R*-***3****—obtained in the previous enzymatic step—was not perfect (99:1) and that reflected on the subsequent amination step.*

7.4.2. Further optimization of the hydrogen-borrowing amination of 1*S,*2*R*-**3**

Further experiments were performed by varying the substrate as well as enzyme concentrations (Table S12) according to 7.1.

Table S12: Conversion [%] for 1*S,*2*R*-**3** at 30 °C after further optimization of enzyme and substrate concentrations using Bs-BDHA in combination with Ch1-AmDH

1*S,*2*R*-3 [mM]	Enzyme ratio [µM]	Conversion [%][a]	1*S,*2*R*-5 [%][a]	*er* [%][b]	*dr* [%][b] [SS:RR:RS:SR]	8 [%][a][c]	Unidentified [%][a][c]
10	24:60	94 ± <1	89 ± <1	>99.5	0:2.5:0:97.5	1 ± <1	4 ± <1
15	24:60	90 ± 1	87 ± 1	>99.5	0:2.1:0:97.9	1 ± <1	2 ± <1
20	24:60	79 ± <1	77 ± <1	>99.5	0:2:0:98	1 ± <1	2 ± <1
25	24:60	72 ± <1	71 ± <1	>99.5	0:2:0:98	1 ± <1	1 ± <1
30	24:60	65 ± 2	63 ± 2	>99.5	0:2.1:0:97.9	1 ± <1	1 ± <1
10	60:24	97 ± 1	93 ± 1	>99.5	0:3:0:97	1 ± <1	3 ± <1
10	70:35	97 ± <1	93 ± <1	>99.5	0:3:0:97	1 ± <1	3 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

Note: *the fact that the diastereomeric ratio of the product* 1*S,*2*R*-***5*** *is not absolutely perfect must not be attributed to the hydrogen-borrowing amination step. In fact, the enantiomeric ratio of the diol substrate* 1*S,*2*R*-***3****—obtained in the previous enzymatic step—was not perfect (99:1) and that reflected on the subsequent amination step.*

7.5. Hydrogen-borrowing amination of 1*R,*2*R*-***3***

7.5.1. Influence of the temperature for the hydrogen-borrowing amination of 1*R,*2*R*-**3**

The influence of the temperature (30, 40 and 50 °C) for the HB-amination of 1*R,*2*R*-**3** (5 mM) was investigated combining Ls-ADH (50 µM) with AmDH (50 µM, Ch1-AmDH or Rs-PhAmDH). The reactions were run for 48 h in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) in the presence of NAD^+ (1 mM) as described in 7.1.

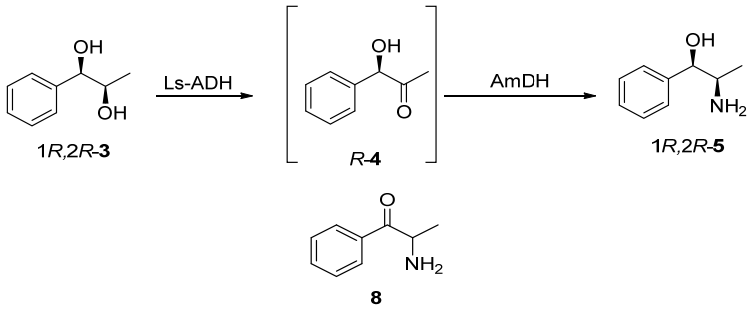


Table S13: Conversions [%] for 1*R,*2*R*-**3** (5 mM) at different temperatures by combining Ls-ADH and AmDH

Enzymatic system	T [°C]	Conversion [%][a]	1*R*,2*R*-5 [%][a]	*er* [%][b]	*dr* [%] [SS:RR/RS:SR]	8 [%][a][c]	Unidentified [%][a][c]
Ls-ADH/Ch1-AmDH	30	>99 ± <1	93 ± 1	>99.5	0:>99.5/0:0[b]	2 ± <1	5 ± <1
Ls-ADH/Rs-PhAmDH	30	90 ± 6	84 ± 5	>99.5	0:>99.5/0:0[b]	1 ± <1	5 ± 1
Ls-ADH/Ch1-AmDH	40	89 ± 1	82 ± 2	n.m.	77/23[a]	4 ± <1	3 ± <1
Ls-ADH/Rs-PhAmDH	40	66 ± 1	63 ± 2	n.m.	73/27[a]	2 ± <1	2 ± <1
Ls-ADH/Ch1-AmDH	50	19 ± 3	18 ± 3	n.m.	48.5/51.5[a]	1 ± <1	1 ± <1
Ls-ADH/Rs-PhAmDH	50	17 ± 1	16 ± 1	n.m.	61/39[a]	<1	1 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; "0" means that the other diastereomers and enantiomer were never observed. [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

7.5.2. Increase in the substrate concentration for the hydrogen-borrowing amination of 1R,2R-3

The biocatalytic reactions were carried out as described in 7.1 with substrate 1*R,*2*R*-**3** (10 to 30 mM) in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) in the presence of NAD^+ (1 mM) and by combining Ls-ADH (50 µM) and Ch1-AmDH (50 µM) at 30 °C for 48 h as described in section 7.1.

Table S14: Conversion [%] for 1*R,*2*R*-**3** (varied concentrations: 10-30 mM) at 30 °C combining Ls-ADH (50 µM) and Ch1-AmDH (50 µM)

1*R*,2*R*-3 [mM]	Conversion [%][a]	1*R*,2*R*-5 [%][a]	*er* [%][b]	*dr* [%][b] [SS:RR:RS:SR]	8 [%][a][c]	Unidentified [%][a][c]
10	93 ± 2	92 ± 4	>99.5	0:>99.5:0:0	1 ± <1	1 ± <1
15	96 ± 1	93 ± 1	>99.5	0:>99.5:0:0	<1	2 ± <1
20	95 ± <1	93 ± <1	>99.5	0:>99.5:0:0	1 ± <1	2 ± <1
25	91 ± 1	89 ± <1	>99.5	0:>99.5:0:0	<1	2 ± <1
30	89 ± 1	87 ± 1	>99.5	0:>99.5:0:0	1 ± <1	1 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; "0" means that the other diastereomers and enantiomer were never observed; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

7.5.3. Further optimization of the hydrogen-borrowing amination of 1*R*,2*R*-**3**

The ratio between Ls-ADH and Ch1-AmDH was optimized at various substrate concentrations as shown in subsequent table. Biocatalytic reactions were performed as described in 7.1 .

Table S15: Conversion [%] for 1*R*,2*R*-**3** (varied concentrations: 5 to 20 mM) at 30 °C combining Ls-ADH and Ch1-AmDH at various concentrations.

1*R*,2*R*-3 [mM]	ADH:AmDH [μM]	Conversion [%][a]	1*R*,2*R*-5 [%][a]	*er* [%][b]	*dr* [%][b] [SS:RR:RS:SR]	8 [%][a][c]	Unidentified [%][a][c]
20	24:60	>99	98 ± <1	>99.5	0:>99.5:0:0	<1	1 ± <1
20	50:60	>99	98 ± <1	>99.5	0:>99.5:0:0	1 ± <1	1 ± <1
20	35:70	>99	99 ± <1	>99.5	0:>99.5:0:0	<1	1 ± <1
20	60:24	99 ± 2	96 ± 2	>99.5	0:>99.5:0:0	1 ± <1	2 ± <1
20	70:35	>99	98 ± 1	>99.5	0:>99.5:0:0	1 ± <1	2 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; "0" means that the other diastereomers and enantiomer were never observed; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

7.6. Hydrogen-borrowing amination of 1*R*,2*S*-***3***

Two different ADHs (Aa-ADH and Bs-BDHA; 50 μM) were combined with two AmDHs (Ch1-AmDH and Rs-PhAmDH; 50 μM) in $HCOONH_4$ buffer (pH 8.5, 1 M, 0.5 mL) at 30 °C for 48 hours as described in 7.1.

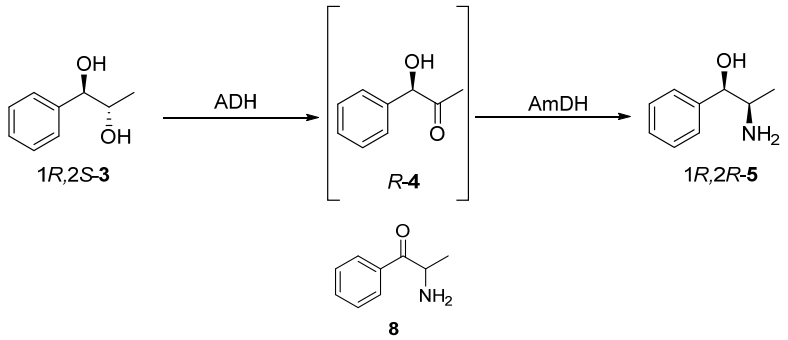


Table S16: Conversion [%] for 1*R*,2*S*-**3** (5 mM) at 30 °C with 50:50 μM ADH:AmDH.

Enzymatic system	Conversion [%][a]	1*R*,2*R*-5 [%][a]	*er* [%][b]	*dr* [%][b]	8 [%][a][c]	Unidentified [%][a][c]
Aa-ADH/Ch1-AmDH	80 ± 5	75 ± 5	>99.5	0:>99.5:0:0	2 ± <1	4 ± 1
Aa-ADH/Rs-PhAmDH	69 ± 5	63 ± 3	>99.5	0:>99.5:0:0	2 ± 1	4 ± 1
Bs-BDHA/Ch1-AmDH	18 ± <1	16 ± 1	>99.5	0:>99.5:0:0	1 ± <1	1 ± <1
Bs-BDHA/Rs-PhAmDH	16 ± <1	14 ± 1	>99.5	0:>99.5:0:0	1 ± <1	1 ± <1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; "0" means that the other diastereomers and enantiomer were never observed; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

*7.6.1. Increase in the substrate concentration for the hydrogen-borrowing amination of 1R,2S-**3***

Table S17: Conversion [%] for 1*R,*2*S*-**3** (10 mM) at 30 °C with 50:50 µM ADH:AmDH

1*R*,2*S*-3 [mM]	**Conversion [%][a]**	**1*R*,2*R*-5 [%][a]**	***er* [%][b]**	***dr* [%][b] [SS:RR:SR:RS]**	**8 [%][a][c]**	**Unidentified [%][a][c]**
10	58 ± 1	57 ± 1	>99.5	0:>99.5:0:0	1 ± <1	<1

[a] Determined by GC-FID analysis; [b] Determined by RP-HPLC after derivatization with a chiral reagent; "0" means that the other diastereomers and enantiomer were never observed; [c] This may come, at least in part, from decomposition of the amino alcohols product (also present in the reference compound purchased by Sigma Aldrich upon injection in GC-MS or GC-FID).

*7.7. One pot biocatalytic hydrogen-borrowing amination of enantiomerically pure or enantiomerically enriched diols **3** in semi-preparative scale (100 mg)*

The one pot hydrogen-borrowing amination on semi-preparative scale was performed for two out of the four enantiomerically pure or enantiomerically enriched diols **3** as reported in Table S18, applying the optimum conditions obtained in the sections before. Conversions were analysed by GC-FID while enantiomeric as well as diastereomeric ratios were determined by RP-HPLC after derivatization with GITC. Furthermore, the isolated products were characterized by ^{1}H-NMR and analysed by GC-FID and NP-HPLC. Representative NMR spectra and GC/HPLC chromatograms for each substrate are reported in paragraph 9.

Reaction conditions for the preparative scale biocatalytic hydrogen-borrowing amination of 1*R*,2*R*-**3** to yield 1*R*,2*R*-**5**: To a 100 mL Erlenmeyer flask, $HCOONH_4$ buffer (33 mL final volume, pH 8.5, 1 M), 1*R,*2*R*-**3** (20 mM, 105 mg, 0.690 mmol), Ls-ADH (24 µM), Ch1-AmDH (60 µM) and NAD^+ (1 mM) were added. The mixture was incubated at 30 °C and 170 rpm on an orbital shaker for 48 h. The reaction was quenched with KOH (4 mL, 10 M). The aqueous layers were saturated with solid NaCl and the extraction was performed with MTBE (3 x 20 mL). The organic layers were then dried over $MgSO_4$ and concentrated under reduced pressure.

Reaction conditions for the preparative scale biocatalytic hydrogen-borrowing amination of 1*S*,2*R*-**3** to yield 1*S*,2*R*-**5**: To a 100 mL Erlenmeyer flask, $HCOONH_4$ buffer (44 mL final volume, pH 8.5, 1 M), 1*S,*2*R*-**3** (15 mM, 102 mg, 0.670 mmol), Bs-BDHA (50 µM), Ch1-AmDH (50 µM) and NAD^+ (1 mM) were added. The mixture was incubated at 30 °C and 170 rpm on an orbital shaker for 48 h. The reaction was quenched with KOH (5 mL, 10 M), the aqueous layers were saturated with solid NaCl and the extraction was performed with MTBE (4 x 20 mL). The organic layers were then dried over $MgSO_4$ and concentrated under reduced pressure.

Table S18: Overview for the one pot hydrogen-borrowing amination of enantiomerically pure or enantiomerically enriched diols **3** in semi-preparative scale in $HCOONH_4$ buffer (pH 8.5, 1 M) at 30 °C for 48 h. Ch1-AmDH was used as aminating enzyme in all cases.

Substrate	**Conc. [mM]**	**ADH**	**ADH:AmDH [µM]**	**Conv. [%][b]**	**Isolated yield [mg]**	**Isolated yield [%]**	**Purity [%][b]**	***er* [%][a]**	***dr*[%][b] [SS:RR:RS:SR]**
1*R,*2*R*-**3**	20 (105 mg)	Ls-ADH	24:60	>94	77	74	>98	>99.5 (1*R,*2*R*-**5**)	0:>99.5:0:0
1*S,*2*R*-**3**	15 (102 mg)	Bs-BDHA	50:50	>99	75	74	>95	>99.5 (1*S,*2*R*-**5**)	0:2.3:0:97.7

[a] determined by RP-HPLC after derivatization with a chiral compound; [b] determined by GC-FID analysis and further confirmed by NP-HPLC analysis and ^{1}H-NMR; "0" means that the other diastereomers and enantiomer were never observed.

8. Analytical methods

8.1. Determination of the conversion by GC-FID

Note: *For the analysis of the outcome of the biocatalytic hydrogen-borrowing cascade, various analytical techniques were used. The formation of the targeted optically active amino alcohols* ***5*** *was initially investigated by using GC-FID. However, under certain conditions, both diol substrate and amino alcohol products tend to partly decompose at elevated temperature during GC injection, thus resulting in possible formation of benzaldehyde. Nevertheless, we could exclude any chemical formation of benzaldehyde during the reaction as benzaldehyde was never observed when the samples were analysed by normal-phase HPLC analysis. Finally, reverse-phase HPLC (C18 column) was used for the determination of the enantiomeric as well as diastereomeric ratio. In order to do so, the amino alcohol products were derivatized with a chiral reagent (GITC) (section 7.2) and compared with the chemically synthesized references.*

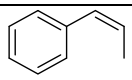
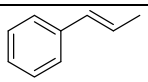
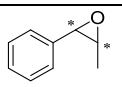
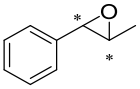
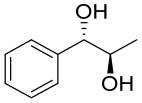
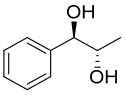
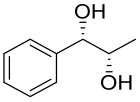
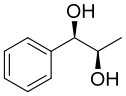
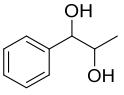
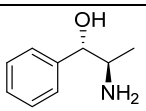
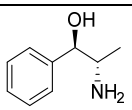
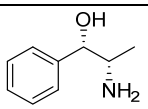
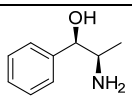
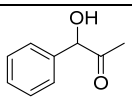
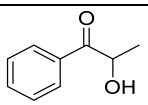
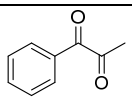
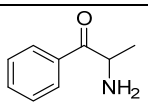
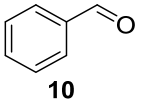
Method A: Column: Agilent J&W DB1701 (30 m, 250 µm, 0.25 µm). Carrier gas: H_2

Parameter: T injector 250 °C; constant pressure 6.9 psi; temperature program: 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 280 °C, hold 1 min.

Method B: Column: Agilent J&W DB1701 (30 m, 250 µm, 0.25 µm). Carrier gas: H_2

Parameter: T injector 250 °C; constant pressure 6.9 psi; temperature program: 80 °C, hold 6.5 min; gradient 5 °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 280 °C, hold 4 min.

Table S19: Retention times [min] for the compound analysed by GC-FID

Compound	[min]	Method	Compound	[min]	Method	Compound	[min]	Method
 *cis*-**1**	6.8	A	 *trans*-**1**	8.1	A	 *1S,2R*-**2**	10.8	A
 *1S,2S*-**2**	11.4	A	 *1S,2R*-**3**	18.9 23.8	A B	 *1R,2S*-**3**	18.9 23.8	A B
 *1S,2S*-**3**	18.7 23.6	A B	 *1R,2R*-**3**	18.7 23.6	A B	 **3** mixture of all four possible isomers	Two pairs of enantiomers [SS/RR:SR/RS] 18.7:18.9	A
 *1S,2R*-**5**	17.4 21.1	A B	 *1R,2S*-**5**	17.4 21.1	A B	 *1S,2S*-**5**	17.3 20.9	A B
 *1R,2R*-**5**	17.3 20.9	A B	 **4**	15.6 19.2	A B	 **6**	15.8 19.4	A B
 **7**	13.6 16.6	A B	 **8**	16.8 19.9	A B	 **10**	8.3 8.5	A B

8.2. General GC-MS method

Method C: Column Agilent DB-1701 (30 m, 250 µm, 0.25 µm); injector temperature 250 °C; constant pressure 71.8 kPa; temperature program: 80 °C/hold 6.5 min; 160 °C/rate 10 °C min^{-1}/hold 5 min; 200 °C/rate 20 °C min^{-1}/hold 2 min; 280 °C/rate 20 °C min^{-1}/hold 1 min.

8.3. Method for the determination of the enantiomeric excess and diastereomeric excess by HPLC

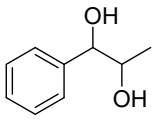
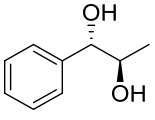
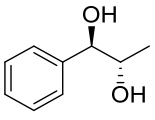
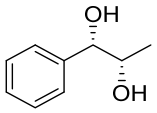
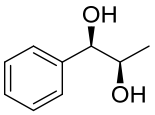
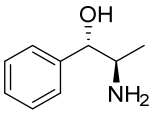
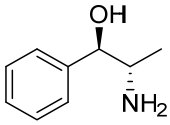
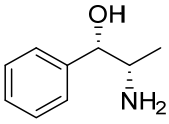
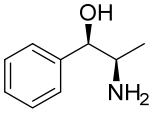
Method D: Column Chiralcel-OD (0.46 cm x 25 cm)

HPLC program: constant oven temperature 10 °C; constant pressure 14 bar; eluent composition: Hexane:Isopropanol 97:3, 1 mL/min, detection at 210 nm.

Method E: Column: Nucleosil C_{18} HD (0.46 cm x 25 cm)

HPLC program: constant oven temperature 30 °C; eluent composition: isocratic MeOH + 0.1% TFA/MilliQ + 0.1% TFA 50:50; flow rate: 1 mL min^{-1}, detection at 248 nm.

Table S20: Retention times for compounds analysed by HPLC

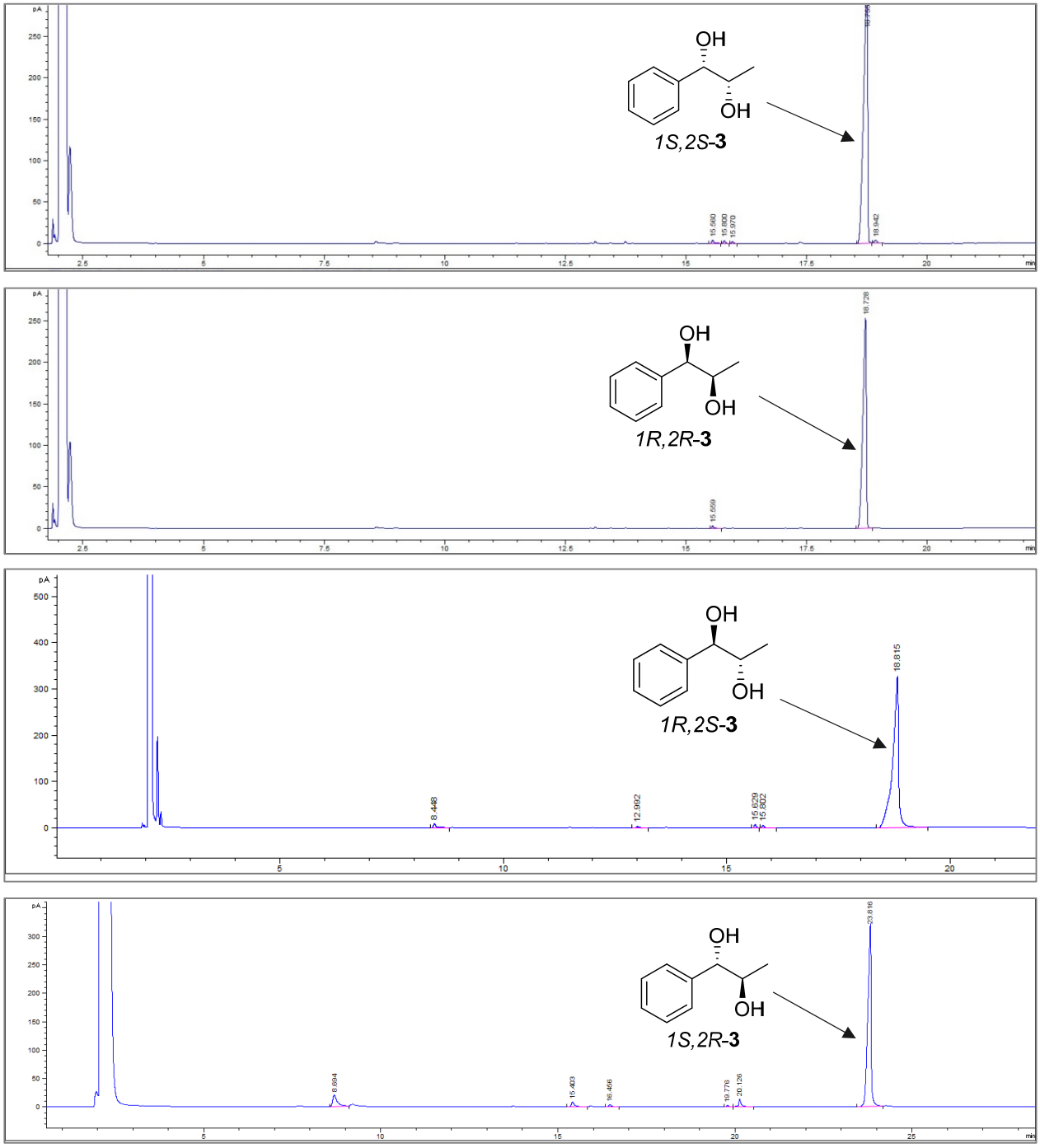
Compound	Retention time [min]	method	Compound	Retention time [min]	method	Compound	Retention time [min]	method
 **3**	**4 peaks** 33.4/33.7 35.3/35.6 39.1/43.1 41.1/45.4	D	 *1S,2R*-**3**	39.1/43.1	D	 *1R,2S*-**3**	41.1/45.4	D
 *1S,2S*-**3**	35.3/35.6	D	 *1R,2R*-**3**	33.4/33.7	D	 *1S,2R*-**5**	38.1[a]	E
 *1R,2S*-**5**	26.7[a]	E	 *1S,2S*-**5**	18.6[a]	E	 *1R,2R*-**5**	20.7[a]	E

[a] after derivatization with GITC (see paragraph 7.2).

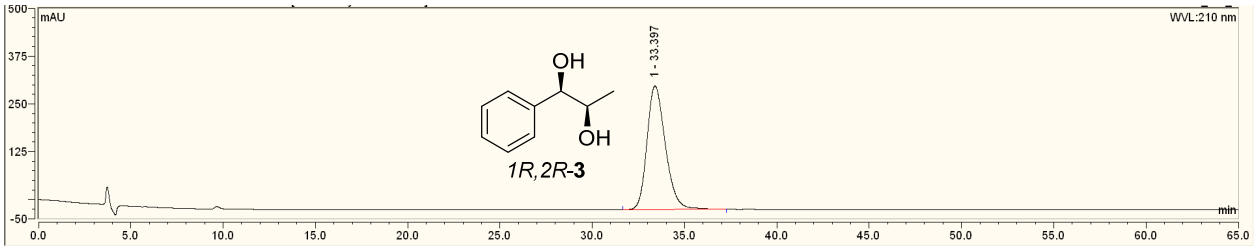
The absolute configuration of the products were identified by comparison with authentic optically active reference compounds that were either purchased or chemically synthesised.

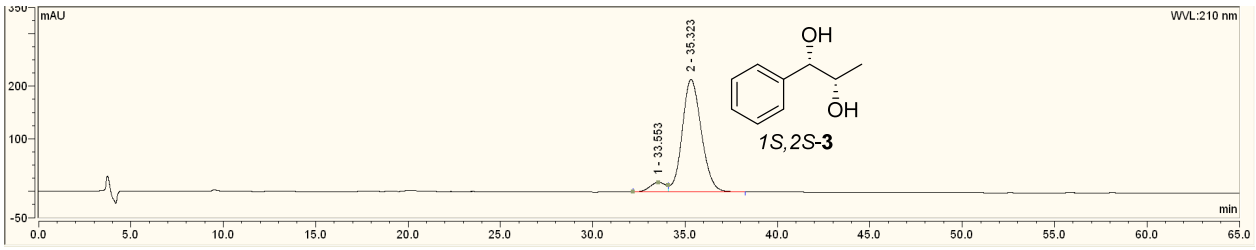
9. GC and HPLC chromatograms and NMR spectra

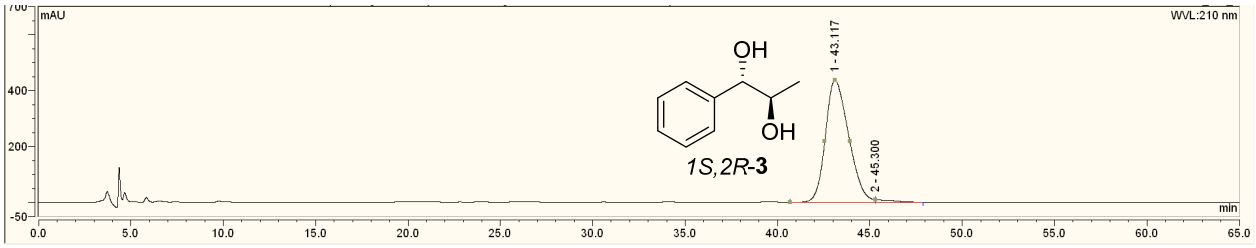
9.1. *GC-FID chromatograms of the enzymatic one pot dihydroxylation of trans* or *cis-**1**: formation of **3** on semi-preparative scale*

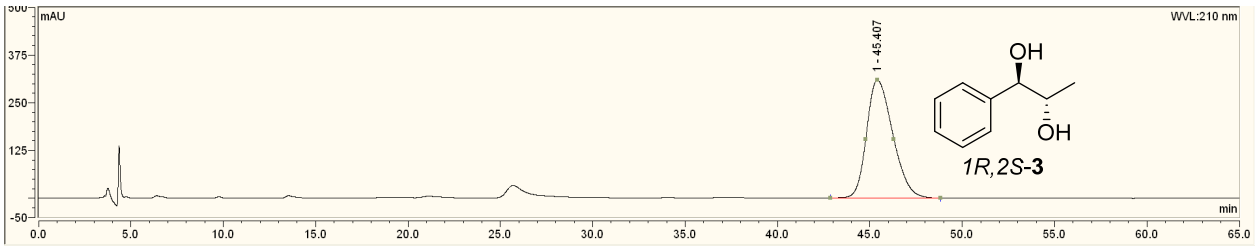


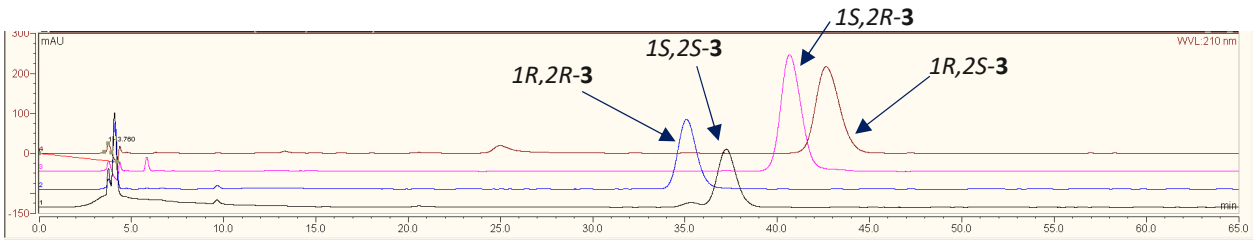
*9.2. HPLC chromatograms of the enzymatic one pot dihydroxylation of trans or cis-**1**: formation of **3** on preparative scale*

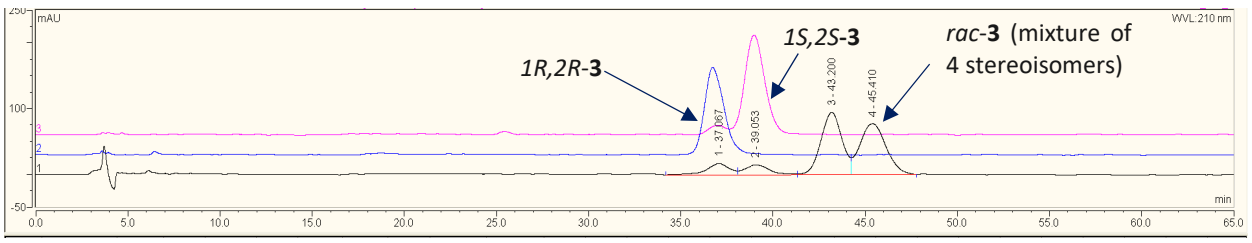


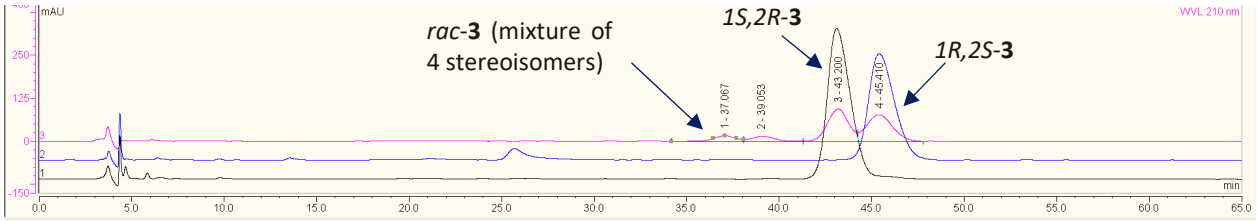






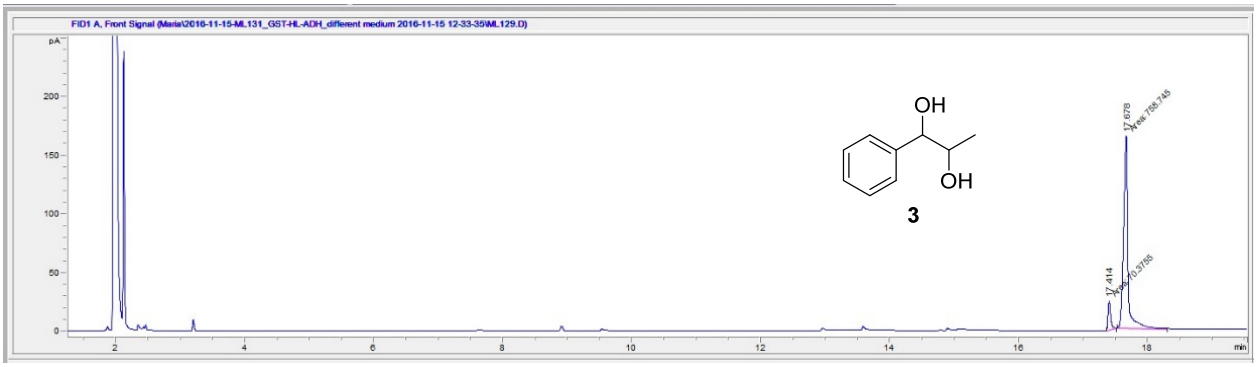




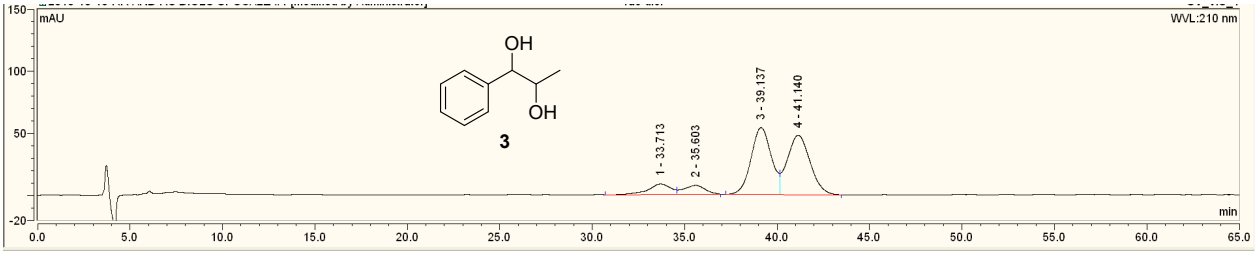


*9.3. Chromatograms of the chemically synthesized diol **3** as a mixture of all the four possible stereoisomers*

GC-FID chromatogram of the chemically synthesized diol **3**



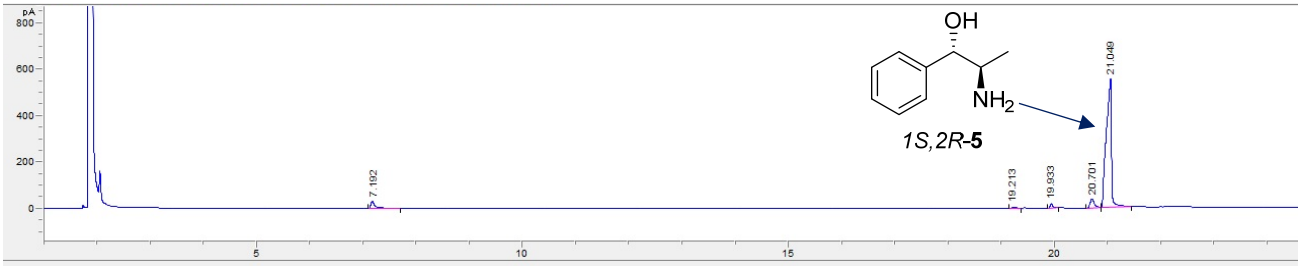
HPLC chromatogram: separation of all the 4 isomers of the chemically synthesized diol **3**



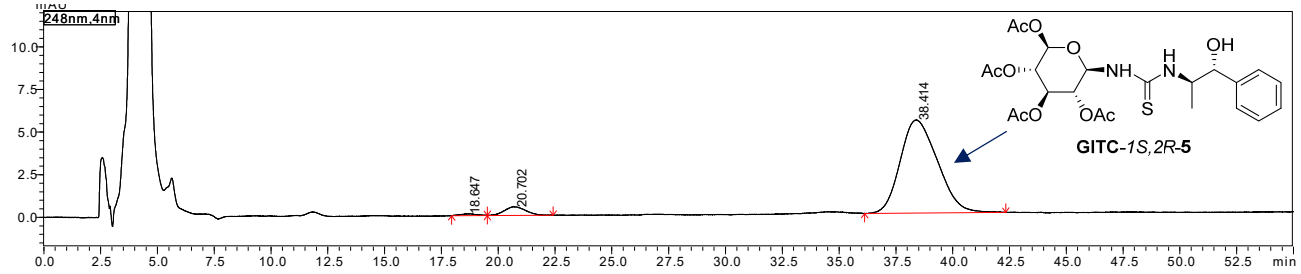
Isomer mixture diol 3	Peak Number	Retention time [min]	[%]	Comments
1*R*,2*R*	1	33.7	5	Couple of enantiomers
1*S*,2*S*	2	35.6	5	
1*S*,2*R*	3	39.1	45	Couple of enantiomers
1*R*,2*S*	4	41.1	45	

*9.4. GC-FID and RP-HPLC analysis for the one pot hydrogen-borrowing-amination of the diols **3** on semi-preparative scale*

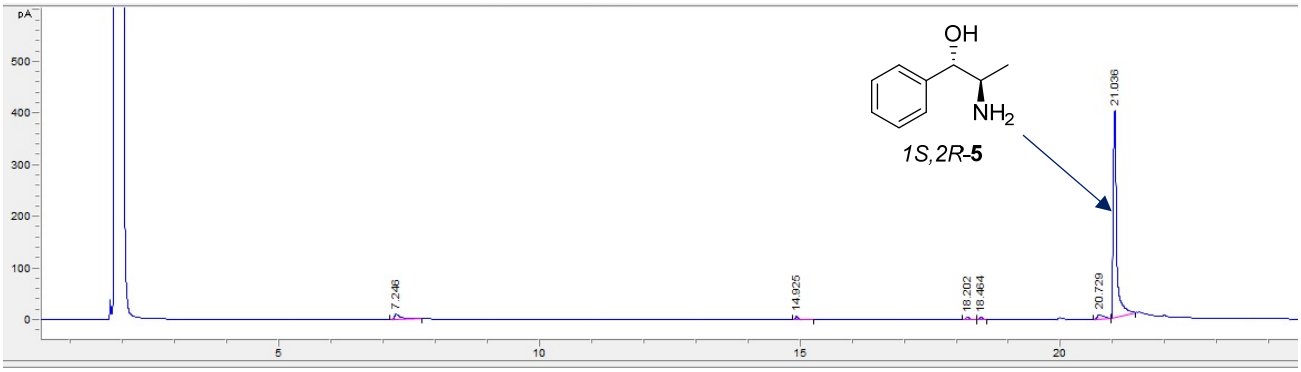
GC-FID chromatogram for the conversion of *1S,2S*-**3** to *1S,2R*-**5** by Aa-ADH in combination with Ch1-AmDH (analytical scale)



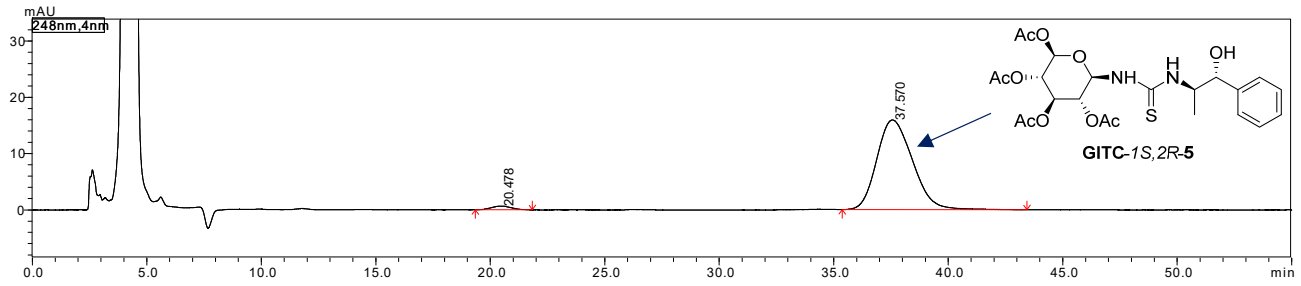
RP-HPLC chromatogram of *1S,2S*-**3** to *1S,2R*-**5** by Aa-ADH in combination with Ch1-AmDH after derivatization with GITC (analytical scale)



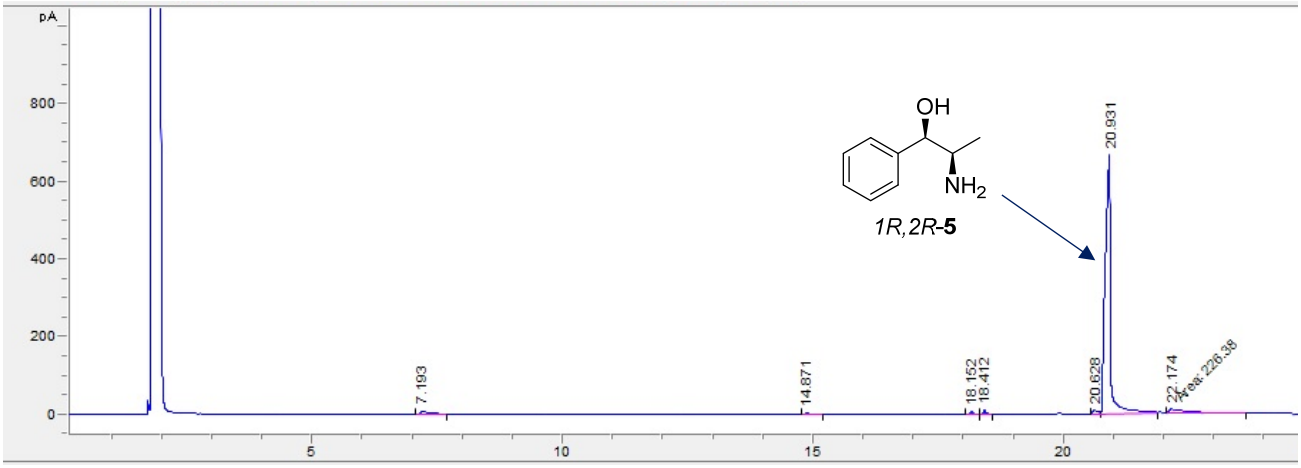
GC-FID chromatogram for the conversion of *1S,2R*-**3** to *1S,2R*-**5** by Bs-BDHA in combination with Ch1-AmDH



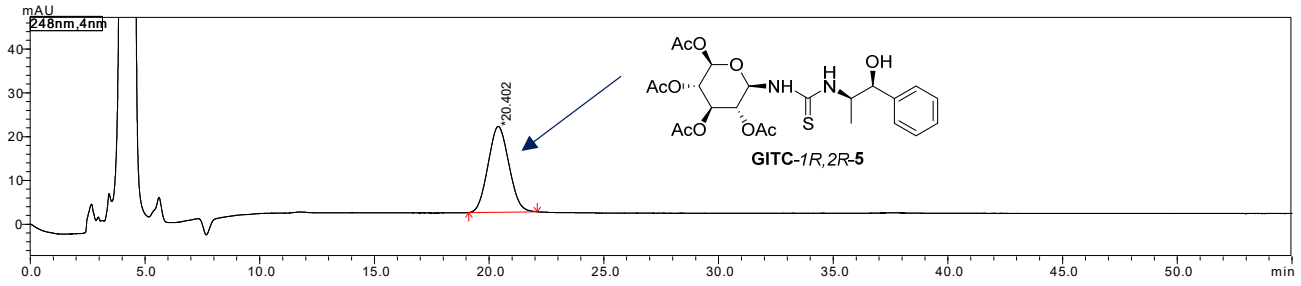
RP-HPLC chromatogram of *1S,2R*-**3** to *1S,2R*-**5** by Bs-BDHA in combination with Ch1-AmDH after derivatization with GITC



GC-FID chromatogram for the conversion of *1R,2R*-**3** to *1R,2R*-**5** by Ls-ADH in combination with Ch1-AmDH

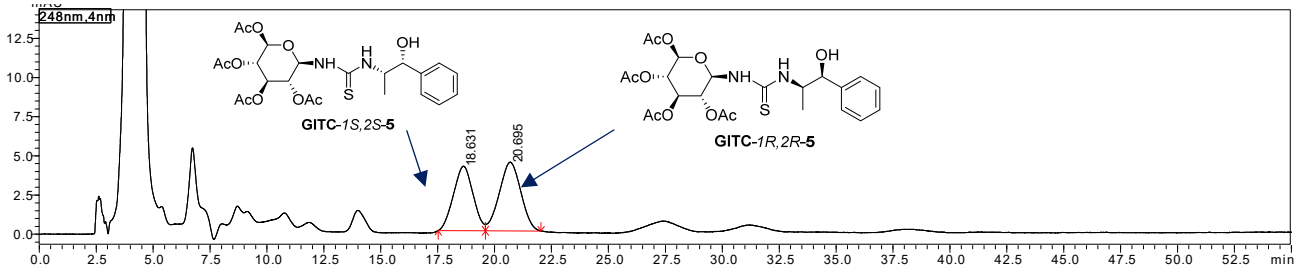


RP-HPLC chromatogram of 1*R,2R*-**3** to *1R,2R*-**5** by Ls-ADH in combination with Ch1-AmDH after derivatization with GITC

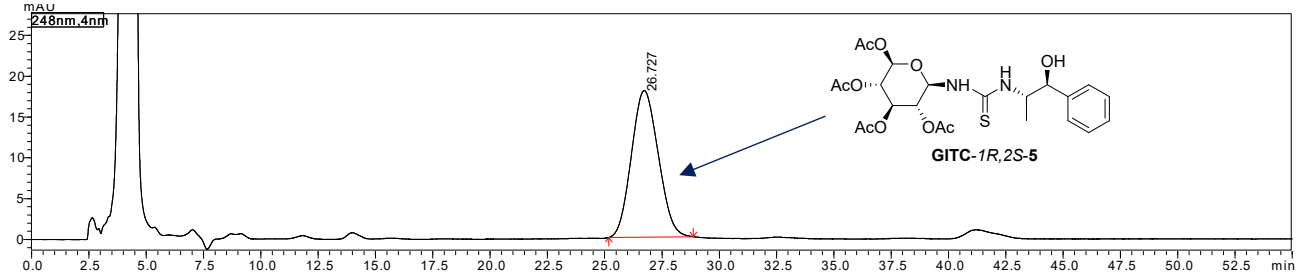


9.5. HPLC chromatograms of the derivatized amino alcohol references

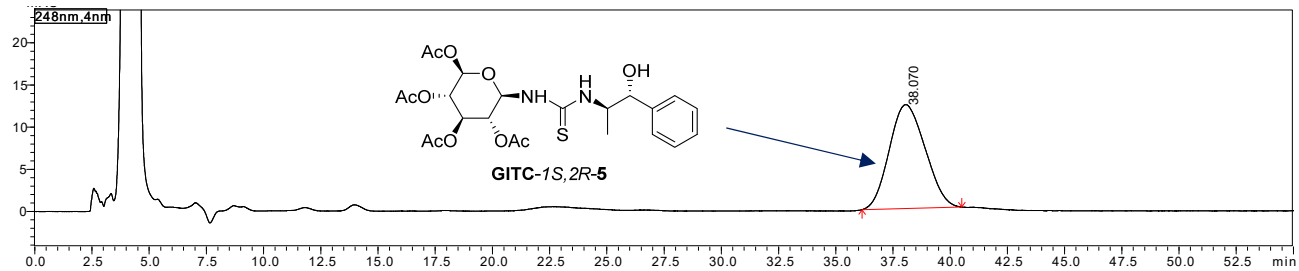
RP-HPLC chromatogram of the chemically synthesized racemic mixture *1S,2S/1R,2R*-**5** after derivatization with GITC



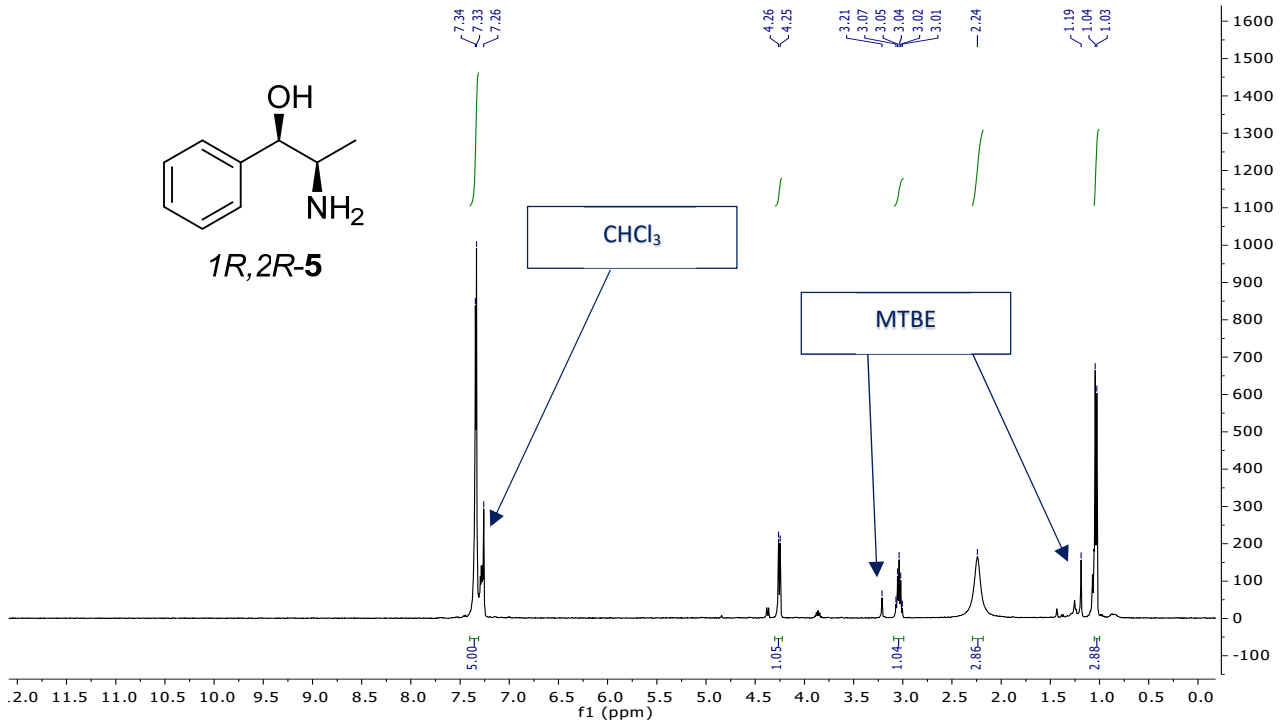
RP-HPLC chromatogram of *1R,2S*-**5** purchased by Sigma Aldrich after derivatization with GITC

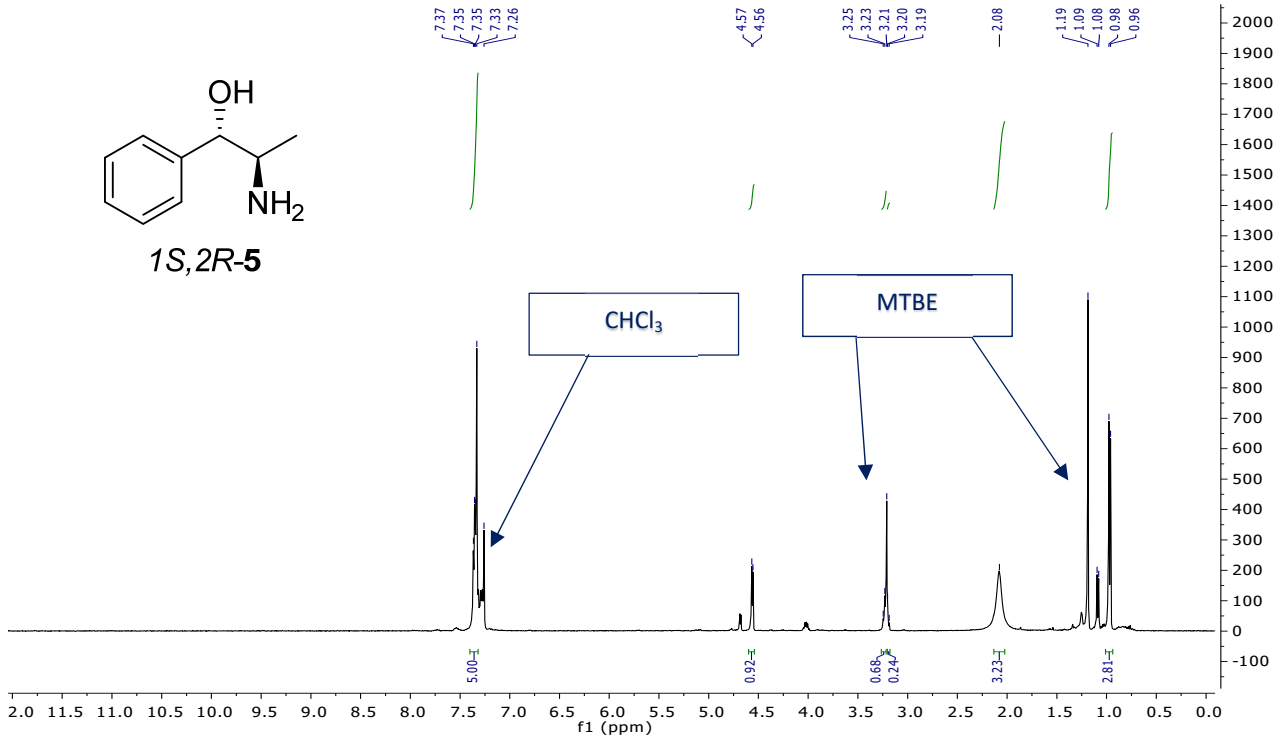


RP-HPLC chromatogram of *1S,2R*-**5** purchased by Sigma Aldrich after derivatization with GITC



*9.6. [1]H-NMR spectra of enzymatically synthesized 1R,2R-**5** and 1S,2R-**5** ($CDCl_3$, 400 MHz)*





10. Estimation and comparison of simple E-factor (sEF) and solvent demand among the herein described and other methodologies for the synthesis of phenylpropanolamines

Table S21 shows a comparison of estimated values of simple E-factor and solvent demand among the enzymatic approach describe in this work, another enzymatic approach and two chemical methods involving catalytic steps. Table S21 shows the advantage of using biocatalytic cascades rather than other chemical methods for the synthesis of phenylpropanolamines (PPAs, **5**).

Though the approach by Sehl *et al.* towards the preparation of 1*R*,2*R*-**5** turned out to have a lower E factor (4 vs. 10-11), it cannot be directly compared to our study since:

1) A different starting material (benzaldehyde vs. β-methylstyrenes) was used. Consequently, the method by Sehl *et al.* consists of a one-pot two-enzyme route. Conversely, our study comprises a total of five enzymes within two separated cascade reactions.

2) The data for the method by Sehl *et al.* are calculated for a reaction on analytical scale for which a conversion was measured but the product was not isolated.

3) The biocatalysts were used in different forms (e.g., purified enzymes, lyophilized free cells extract or lyophilized whole cells) and therefore the mass of biocatalysts used is not entirely comparable (i.e., in the first step of our process we have used lyophilized whole cells that contribute significantly to the waste mass).

By considering these factors, we deem the actual sEF values of the two biocatalytic processes to be essentially equivalent.

Regarding the two chemical methods, the E-factor were in the range between 50 and 120; hence, from 5 to 12-fold higher than our approach. Moreover, the waste generated for the preparation of the required catalysts for these reaction has not been considered in this calculation as these data could not be accessed in a reliable manner. Furthermore, in terms of selectivity, we obtained compound **5** as individual isomers (either 1*R*,2*R*-**5** or 1*S*,2*R*-**5**) therefore with very high *er* and *dr*. On the other hand, as shown in table S21, the two chemical methods could lead only to a mixture of isomers and low yield (as in the case of Legnani *et al.*) or high yield but low enantioselectivity (Minakata *et al.*).

Table S21. Simplified E-factor (sEF) and solvent demand calculations for various methods for the synthesis of chiral **5**

System	sEF[a][b]	Solvent demand [mL $mg^{-1}_{product}$]	Stereoselectivity
This work	10-11	1.8-2.3	1*R*,2*R*-**5** (>99.5:<0.5 *er* and *dr*) 1*S*,2*R*-**5** (>99.5:<0.5 *er*; 2.3:97.7 *dr*)
Sehl *et al.*[24]	4	0.8	1*R*,2*R*-**5** (*ee* >99%; *de* >98%)
Legnani *et al*.[25]	52-80	4.2-6.9	*ee* n.a. *dr* = 1.2:1.0 [*RR*/*SS*:*SR*/*RS*] *dr* = 1.0:3.0 [*RR*/*SS*:*SR*/*RS*]
Minakata *et al*.[26]	116	5.0	1*R*,2*R*-**5** (*ee* 86%; *de* n.a.)

[a] Generated waste for the preparation of catalyst was not included; [b] Reaction buffers and other solvents were not included here, but calculated apart (see solvent demand); n.a. means not available.

11. References

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